Université Paris Cité

Université Paris Cité

Ecole doctorale Bio Sorbonne Paris Cité (ED562) – Infectiologie Microbiologie

Institut Pasteur

Département de Mycologie

Unité Biologie et Pathogénicité Fongiques

Influence of the microbiota on *Candida albicans* gastrointestinal colonization in healthy individuals

Par Margot DELAVY

Thèse de doctorat de Microbiologie

Dirigée par Marie-Elisabeth BOUGNOUX

Présentée et soutenue publiquement le 30/06/2023

Devant un jury composé de :

Marie-Elisabeth BOUGNOUX, MCU-PH, Université Paris Cité, directrice de thèse

David MOYES, Senior Lecturer, King's College London, rapporteur

Mathias RICHARD, Directeur de recherche, Université Paris-Saclay, rapporteur

Gérard EBERL, Professeur HDR, Université Paris Cité, examinateur

Tobias HOHL, Professeur, Weill Cornell University, examinateur

Anne PUEL, Directeur de recherche, Université Paris Cité, examinatrice

Alix COSTE, Maître d'enseignement et de recherche, Université de Lausanne, membre invité

INSTITUT PASTEUR

FunHoMic
Fungus Host Microbiota
Innovative Training Network

ACKNOWLEDGMENTS

I would like to begin by expressing my gratitude to the members of my thesis jury: David Moyes, Mathias Richard, Gérard Eberl, Tobias Hohl, Anne Puel and Alix Coste. I truly appreciate your input and the time you dedicated to being part of these final steps of my thesis. Your involvement means a lot to me. I thank Christophe d'Enfert for giving me the opportunity to be part of his amazing research team for the last four years and for his support and advice throughout my PhD. I would also like to express my profound gratitude to Marie-Elisabeth Bougnoux for her guidance and support through this PhD. You have always encouraged and supported me, even when I was stressing out. Your suggestions and our many long discussions pushed me to always go deeper in my analyses and reasoning and this really shaped my research and academic development. Working with you has taught me so much and I am so grateful!

These almost four years in Pasteur have been incredible. I never thought I would meet so many amazing people and be so well surrounded, and this is thanks to all the BPF lab members, past and present. In particular, I would like to thank Natacha and Murielle for their unwavering support and encouragements; Sophie for all her advice and for sharing her English skills, Corinne for answering all my questions about Unix and for showing me that using the Pasteur cluster was actually not so scary; Christelle for making every administrative procedure looks so easy and for solving every travel catastrophes, and of course Clara for sharing and understanding all the high and low of the PhD student's life, thank you for always being there! And of course, thank you to Mélanie, both Thierry, Colin, Serge, Nelson, Elise, Pedro, Daria, Timea, Iryna, Lasse and Laxmi for the time we shared together. I would also like to thank Lucia, Catherine, Frédérique, Guilhem, Gwenaël, Sarah and everyone from Fernbach 4th floor and/or from the Mycology department, I will miss you all.

I would also like to thank the members of the FunHoMic consortium for their support and advice. I thank Chaysavanh Manichanh and her lab at Vall d'Hebron Research Institute, and Alan Walker and his team at the Rowett Institute for welcoming me in their lab. A big thank you in particular to Zixuan for all her help with Qiime2 and for teaching me all these delicious Chinese recipes, and to Nate for showing me the tricks for growing these annoying anaerobic bugs and for making me discover the best book deals in Aberdeen.

A big thank you to all my friends that have always been there for me despite the distance. Danielle, Emilie and Elodie, I could not dream of better friends! Least but not last, I thank my family, especially my father, Florian, my grandparents and my amazing uncle and aunt, Anne-France and Vincent. I could not have done this without your unconditional support and encouragements.

Influence du microbiote sur la colonisation gastro-intestinale à *Candida albicans* chez des individus sains

RESUME

Candida albicans est une levure commensale des tractus intestinaux, génitaux et oraux de l'homme. Cette levure inoffensive chez le sujet sain peut devenir un redoutable pathogène opportuniste quand les défenses naturelles de l'hôte sont affaiblies, comme chez les patients immunodéprimés. *C. albicans* peut alors causer des infections systémiques mortelles, dont la source primaire est le portage intestinal de *C. albicans.* Au cours de cette thèse, je me suis intéressée aux facteurs qui pourraient moduler l'intensité du portage intestinal de *C. albicans* chez le sujet sain, et ainsi contribuer à contrôler le portage intestinal chez les patients à risque de développer des infections.

J'ai notamment étudié l'influence de facteurs associés au mode de vie, comme la composition du microbiote, l'alimentation et la prise d'antibiotiques, mais également le profil génétique de l'hôte sur les niveaux de portage de *C. albicans* de sujets sains.

Premièrement, j'ai comparé trois techniques d'extraction d'ADN fongiques et bactériens à partir d'échantillons fécaux. En parallèle, j'ai développé un protocole de PCR quantitative (qPCR) spécifique, pour quantifier de manière absolue la concentration de *C. albicans* présente dans les selles.

Deuxièmement, j'ai étudié l'impact des β-lactamines, une famille d'antibiotiques à large spectre, sur le mycobiote de 22 sujets sains (cohorte CEREMI). L'analyse des selles collectés avant, durant et après le traitement par antibiotiques m'a permis de décrire chronologiquement les perturbations induites. J'ai ainsi constaté que l'abondance de *C. albicans* était augmentée suite au traitement, mais avec de fortes variations inter-individuelles. Ces variations pouvaient être expliquées, en partie, par des changements de l'activité fécale des β-lactamases endogènes, des enzymes capables d'hydrolyser les β-lactamines.

Troisièmement, j'ai étudié comment le microbiote bactérien, l'alimentation et la génétique de l'hôte modulent le portage de *C. albicans* chez 695 individus sains (cohorte Milieu Intérieur). J'ai détecté un portage intestinal de *C. albicans* chez 82,9%

des sujets par qPCR, mais à des concentrations très variables. À l'aide de modèles linéaires mixtes, j'ai exploré les concentrations intestinales de *C. albicans* en fonction de la composition du microbiote intestinal et de l'alimentation des sujets. J'ai ainsi montré que *Intestinimonas butyriciproducens* était la principale espèce bactérienne dont l'abondance relative était négativement corrélée aux concentrations de *C. albicans* chez ces sujets. Cependant, je n'ai pas pu observer un effet notable du surnageant de cette bactérie sur la croissance de *C. albicans*, du moins dans les conditions testées. J'ai également noté que le régime alimentaire des sujets contribuait à la croissance de *C. albicans* ; un régime faible en sel et le fait de manger entre les repas étant associés à de plus hautes concentrations de *C. albicans*. Par ailleurs, d'un point de vue génétique, j'ai identifié 26 polymorphismes associés à la colonisation par *C. albicans.*

Finalement, j'ai constaté que les niveaux intestinaux de *C. albicans* influençaient la réponse immunitaire de l'hôte. J'ai analysé les niveaux de transcription de 546 gènes impliqués dans la réponse immunitaire de l'hôte et la concentration de 13 cytokines dans le sang des sujets, après stimulation avec des cellules de *C. albicans* et j'ai pu mettre en évidence des associations positives entre la concentration intestinale de *C. albicans* et l'expression de *NLRP3*, et les concentrations d'IL-2 et de CXCL5.

Bien que l'importance relative de certaines associations identifiées doit encore être déterminée, ces résultats offrent une meilleure compréhension des mécanismes de la colonisation de *C. albicans* chez l'hôte sain. De plus, ces résultats pourraient permettre d'ouvrir la voie à de nouvelles cibles pour des stratégies d'intervention visant à limiter la prolifération de *C. albicans.*

Mots-clés : *Candida albicans*, résistance à la colonisation, microbiote intestinal, mycobiote intestinal

Influence of the microbiota on *Candida albicans* gastrointestinal colonization in healthy individuals

ABSTRACT

Candida albicans is a commensal yeast of the intestinal, genital, and oral tracts of humans. This yeast, which is harmless in healthy individuals, can become a formidable opportunistic pathogen when the host's natural defenses are weakened, as in immunocompromised patients. *C. albicans* can then cause life-threatening systemic infections, whose primary source is *C. albicans* intestinal carriage. During this thesis, I was interested in the different factors that could modulate the intensity of *C. albicans* intestinal carriage in healthy subjects, and thus contribute to control this carriage in patients at risk of developing *C. albicans* infections.

In particular, I studied the influence of lifestyle factors, such as the microbiota composition, diet and antibiotic use, but also the host's genetic profile, on *C. albicans* intestinal levels in healthy subjects.

First, I compared three protocols for fungal and bacterial DNA extraction from fecal samples. In parallel, I developed a specific quantitative PCR (qPCR) protocol to quantify the absolute concentration of *C. albicans* in fecal samples.

Second, I studied the impact of β-lactam antibiotics, a broad-spectrum antibiotic family, on the mycobiota of 22 healthy subjects (CEREMI cohort). The analysis of fecal samples collected before, during and after antibiotic treatment allowed the chronological description of the induced perturbations. I found that the abundance of *C. albicans* was increased after treatment, but with strong interindividual variations. These variations could be explained, in part, by changes in the fecal activity of endogenous β-lactamases, enzymes capable of hydrolyzing β-lactams.

Third, I studied how the bacterial microbiota, diet, and host genetics modulate *C. albicans* carriage in 695 healthy individuals (Milieu intérieur cohort). I detected *C. albicans* intestinal carriage in 82.9% of subjects by qPCR, but with highly variable concentrations. Using linear mixed models, I explored *C. albicans* intestinal concentrations based on the composition of the gut microbiota and the diet of the

subjects. I showed that *Intestinimonas butyriciproducens* was the main bacterial species whose relative abundance was negatively correlated with *C. albicans* concentrations in these subjects. However, I could not observe a significant effect of the supernatant of this species on *C. albicans* growth, under the tested conditions. I also noted that the subjects' diet contributed to the growth of *C. albicans;* eating between meals and a salt-free diet were associated with higher *C. albicans* concentrations. Moreover, from a genetic point of view, I identified 26 polymorphisms associated with *C. albicans* colonization using genome wide associations study.

Finally, I found that *C. albicans* intestinal levels affected the host immune response. I analyzed the transcription levels of 546 immune genes and the concentration of 13 cytokines in the blood of subjects after stimulation with C*. albicans* cells and showed positive associations between the extent of *C. albicans* intestinal levels and the expression of *NLRP3*, and the concentrations of IL-2 and CXCL5.

Although the relative importance of some of the identified associations has yet to be determined, these results offer a better understanding of the mechanisms of *C. albicans* colonization in the healthy host. In addition, these results may open the door to new targets for intervention strategies aiming to curb *C. albicans* overgrowth.

Keywords: *Candida albicans*, colonization resistance, gut microbiota, gut mycobiota

RESUME SUBSTANTIEL EN FRANÇAIS

Contexte

Souvent sous-estimés, les champignons peuvent être de redoutables pathogènes. Parmi les 1,5 à 3,8 millions d'espèces fongiques existantes, on décompte plus de 300 pathogènes fongiques humains (Hawksworth and Lücking 2017). Chaque année, les pathogènes fongiques tuent plus de 1,6 millions de personnes ("Stop Neglecting Fungi" 2017), soit plus que le paludisme (United States Centers for Disease Control and Prevention 2014) et le cancer du sein (Arnold *et al.* 2022) combinés.

Candida albicans est un champignon pathogène opportuniste qui cause principalement des infections superficielles, fréquentes mais bénignes. Toutefois, quand les défenses naturelles de l'hôte sont affaiblies, comme par exemple chez les patients immunodéprimés, *C. albicans* peut causer des infections systémiques sévères, associées à des mortalités supérieures à 50% (Pappas *et al.* 2018). La source primaire de ces infections est le portage de *C. albicans* dans le tractus digestif de l'hôte. En effet, bien qu'il soit un pathogène opportuniste redoutable chez les patients immunodéprimés, *C. albicans* est également une levure commensale des tractus gastro-intestinaux, vaginaux et oraux, dont le portage est le plus souvent asymptomatique, du moins chez les individus sains (d'Enfert *et al.* 2021).

Objectifs de la thèse

Au cours de cette thèse, je me suis intéressée aux différents facteurs qui pourraient moduler l'intensité du portage intestinal de *C. albicans* chez le sujet sain, et ainsi contribuer à contrôler ce portage chez les patients à risque de développer des infections. J'ai notamment étudié l'influence de facteurs associés au mode de vie des sujets sains, comme la composition du microbiote, l'alimentation et la prise d'antibiotiques. J'ai également étudié l'effet du profil génétique de l'hôte sur les niveaux de portage *C. albicans* de sujets sains. En outre, j'ai aussi déterminé si les niveaux intestinaux de *C. albicans* pouvaient influencer la réponse immunitaire de l'hôte dans le cas d'une infection simulée.

Chapitre 1 : Comparaison et implémentation de protocoles d'extraction d'ADN et de tests qPCR pour l'analyse des micro- et mycobiotes intestinaux

Les microbiotes bactériens et fongiques (mycobiotes) intestinaux sont habituellement étudiés par des approches de séquençage à haut débit, soit par métagénomique ciblée, soit par le séquençage shotgun du génome entier. Le séquençage métagénomique ciblé repose sur l'amplification d'une région spécifique de l'ADN microbien, généralement l'ADN ribosomique 16S, pour l'ADN bactérien, ou l'ADN ribosomique 18S ou les régions ITS, pour l'ADN fongique. Le séquençage shotgun, quant à lui, est une forme de séquençage non ciblé qui repose sur le séquençage de l'ensemble des génomes composant le microbiome. Par conséquent, le séquençage shotgun offre une meilleure résolution taxonomique, permettant une distinction plus fine entre les espèces microbiennes, ou même les souches microbiennes, mais à un coût élevé (Peterson *et al.* 2021; Durazzi *et al.* 2021). De plus, cette approche est encore en cours de développement pour la caractérisation du microbiote fongique (Xie and Manichanh 2022; Lind and Pollard 2021; Marcelino *et al.* 2020). Le séquençage ciblé est moins coûteux mais donne des résultats moins précis. Le succès de ces approches dépend fortement de la qualité de l'ADN disponible (Angebault *et al.* 2018; Leigh Greathouse, Sinha, and Vogtmann 2019). Par conséquent, le choix d'un protocole d'extraction d'ADN approprié est crucial pour toute analyse du micro- ou du mycobiote. Dans le premier chapitre de cette thèse, j'ai donc comparé l'efficience de trois techniques d'extraction d'ADN fongiques et bactériens à partir d'échantillons fécaux. En parallèle, j'ai développé un protocole de PCR quantitative (qPCR) spécifique, pour quantifier de manière absolue la concentration d'ADN de *C. albicans* présente dans les selles. J'ai finalement mis au point une équation qui prend en compte l'efficacité de l'extraction d'ADN afin de convertir la quantité d'ADN de *C. albicans,* mesurée par qPCR, en un nombre de cellules.

Dans un premier temps, j'ai comparé trois protocoles d'extraction d'ADN : (i) le protocole Q, qui est la référence actuelle des études de microbiote pour l'extraction d'ADN à partir d'échantillons fécaux (Santiago *et al.* 2014), (ii) le protocole A, une méthode simplifiée du protocole Q (Yu and Morrison 2004) et (iii) le protocole FAST, un protocole basé sur un kit commercial. Les protocoles Q et A contiennent plusieurs étapes de broyages par billes ce qui permet une lyse plus efficace de la paroi fongique mais augmente drastiquement la durée requise pour compléter le protocole (8-9

heures pour le protocole Q contre 6-7 heures pour le protocole A). Le protocole FAST consiste en une procédure plus directe et peut être réalisé en moins de deux heures. Les trois protocoles ont été testés sur les 10 mêmes échantillons fécaux et j'ai évalué leur efficacité en termes de quantité d'ADN total extrait, mesuré par Qubit, de quantité d'ADN fongique extrait, mesuré par un protocole de qPCR pan-fongique, et de qualité des séquences obtenues après un séquençage métagénomique ciblé des régions ITS1 et 16S.

Dans l'ensemble, le protocole FAST était associé à de moins bons résultats que les protocoles Q et A. En effet, le protocole FAST a produit une quantité réduite d'ADN fongique, comparé aux autres protocoles, et des séquences ITS1 de moins bonne qualité, avec un fort pourcentage de séquences non-assignées au règne fongique. Les protocoles Q et A ont retournés, dans l'ensemble, des résultats satisfaisants. Toutefois, l'usage du protocole Q était associé à une diversité bactérienne réduite comparé au protocole A. Compte tenu de ces éléments et du fait que le protocole Q est plus fastidieux à réaliser, augmentant ainsi le risque d'erreurs expérimentales, j'ai décidé de sélectionner le protocole A pour toutes les extractions d'ADN à partir d'échantillons fécaux réalisées dans cette thèse.

Dans la deuxième partie de ce chapitre, j'ai implémenté dans le laboratoire deux tests qPCR pour quantifier la charge fongique totale et les niveaux de *C. albicans* présents dans les échantillons fécaux. Je les ai également optimisés pour pouvoir les utiliser soit individuellement dans des tests simplex, soit en combinaison avec un test qPCR de contrôle interne pour la détection des inhibiteurs de qPCR, ceux-ci étant fréquemment présents dans les échantillons fécaux, pouvant ainsi conduire à des résultats faussement négatifs.

Dans la dernière partie de ce chapitre, j'ai estimé l'efficacité du protocole d'extraction d'ADN sélectionné en début de chapitre en extrayant l'ADN d'échantillons fécaux non colonisés par *C. albicans* et inoculé avec des concentrations connues de cellules de *C. albicans.* Après quantification de l'ADN de *C. albicans* contenu dans les extraits d'ADN par qPCR, j'ai établi que l'extraction était associée à une efficacité moyenne de 2,79%. En me basant sur la taille du génome de *C. albicans,* le poids moléculaire d'une base d'ADN et cette efficacité d'extraction, j'ai développé l'équation suivante qui

permet de transformer la quantité d'ADN de *C. albicans,* mesurée par qPCR, en un nombre de cellules :

$$N_{cells} = \frac{DNA_m \times N_A}{M \times Genome \times E_{eff}} = 1{,}257{,}822 \times DNA_c$$

Dans laquelle N_{cells} est le nombre estimé de cellule de *C. albicans,* DNA_m est la quantité d'ADN de *C. albicans* mesurée par qPCR, en nanogramme, N_A est le nombre d'Avogadro (6.022 x 10^{23} molécules par mole), M est la masse molaire d'une paire de base d'ADN, $Genome$ est la taille du génome de *C. albicans* (26Mb), et E_{eff} est l'efficacité de l'extraction d'ADN, estimé par l'approche présentée ci-dessus (2,79%).

Chapitre 2 : l'impact d'une dysbiose du microbiote, induite par antibiotiques, sur le mycobiote et le portage intestinal de *C. albicans,* chez des individus sains

La prolifération intestinale de *C. albicans* est une condition préalable à la translocation intestinale, qui est à l'origine des infections systémiques par cette levure. Un facteur de risque bien connu pour ces infections est l'administration d'antibiotiques à large spectre (Pappas *et al.* 2018). Les antibiotiques sont des perturbateurs majeurs du microbiote intestinal, mais leur rôle sur le mycobiote reste à élucider (Burdet, Nguyen, *et al.* 2019 ; Burdet, Grall, *et al.* 2019 ; Seelbinder *et al.* 2020 ; Fouhy *et al.* 2012). Des études ont examiné l'effet des antibiotiques sur le mycobiote de la souris (Dollive *et al.* 2013 ; Fan *et al.* 2015), mais, au début de cette thèse, il n'existait aucun rapport sur le rôle direct des antibiotiques dans la prolifération de *C. albicans* chez les humains en bonne santé. Dans le deuxième chapitre de cette thèse, j'ai analysé comment le mycobiote intestinal et, en particulier *C. albicans*, était affecté par les céphalosporines de troisième génération (C3G), des antibiotiques appartenant à la famille des β-lactamines, une famille d'antibiotiques à large spectre largement utilisée en clinique, et qui sont excrétés par la bile dans l'intestin et peuvent donc affecter le mycobiote intestinal.

Nous avons suivi prospectivement des volontaires sains avant, pendant et après un traitement antibiotique par C3G (Burdet, Nguyen, *et al.* 2019 ; Burdet, Grall, *et al.* 2019). Le mycobiote des volontaires sains avant le traitement était caractérisé par une

faible richesse et homogénéité, avec une médiane de seulement 25 OTUs fongiques par échantillon et un indice de Shannon de 1,18, mais une forte diversité entre les sujets et également au sein d'un même individu, entre les différents échantillons collectés. Pour quantifier le portage de *C. albicans*, nous avons utilisé un test qPCR, plus sensible que la culture ou la métagénomique, ce qui nous a permis de déterminer l'abondance absolue de *C. albicans* alors que les approches métagénomiques ne peuvent retourner qu'une abondance relative. J'ai ainsi observé que 95,2% des sujets étaient colonisés par cette levure.

Les antibiotiques C3G n'ont pas eu d'effet sur la diversité fongique mais ont modifié le profil du mycobiote, avec une diminution de l'abondance relative de *Penicillium roqueforti* et *Debaryomyces hansenii* et une augmentation de l'abondance relative de *Saccharomyces cerevisiae* après le traitement. L'abondance relative et absolue de *C. albicans* a augmenté après le traitement, mais cette augmentation dépendait du sujet, avec de fortes variations interindividuelles. Comme une étude préliminaire suggère que l'intestin humain héberge des bactéries naturellement capables de produire des β-lactamases (Leonard *et al.* 1989), des enzymes capables d'hydrolyser les antibiotiques de type β-lactamines, j'ai émis l'hypothèse que l'activité β-lactamase pouvait varier chez les sujets après le traitement antibiotique et ainsi réduire l'impact des antibiotiques dans l'intestin de certains d'entre eux. Dans l'ensemble, l'activité β-lactamase a augmenté après le traitement antibiotique, mais certains sujets ont montré une augmentation plus forte que d'autres, et cette augmentation était négativement corrélée avec le changement des niveaux de *C. albicans* après traitement par antibiotiques.

Cela suggère que l'appauvrissement en bactéries spécifiques qui se produit à la suite du traitement antibiotique permet une croissance excessive de *C. albicans*. Des espèces bactériennes spécifiques pourraient donc être utilisées pour freiner la croissance de *C. albicans*. Cependant, peu d'études ont cherché à identifier des espèces bactériennes - ou des signatures bactériennes - ayant une activité anti-*C. albicans* potentielle. Dans cette section, j'ai cherché à identifier les bactéries dont les niveaux dans le microbiote intestinal étaient inversement corrélés à ceux de *C. albicans,* dans l'hypothèse que certaines de ces espèces pourraient limiter la colonisation intestinale de *C. albicans.* Pour identifier ces bactéries avec une potentielle activité antagoniste, j'ai recherché des corrélations de Spearman

significatives entre l'abondance absolue de *C. albicans*, déduite des données qPCR, et l'abondance relative des espèces métagénomiques intestinales, déduites des données métagénomiques shotgun obtenues à partir de tous les échantillons fécaux, et annotées au niveau des espèces. J'ai également recherché des espèces fongiques susceptibles d'inhiber la croissance de *C. albicans* : pour cela, j'ai recherché des corrélations de Spearman significatives entre l'abondance absolue de *C. albicans*, une fois encore déduite des données qPCR, et l'abondance relative des espèces fongiques intestinales, déduite des données métagénomiques ciblées ITS1 obtenues à partir de tous les échantillons des sujets CEREMI, et annotées au niveau des espèces. J'ai ainsi identifié un set de 54 espèces bactérienne et une espèce fongique, *Geotrichum candidus* (anciennement *Galactomyces candidus*), qui pourraient jouer un rôle dans la résistance de l'hôte à la colonisation intestinale par *C. albicans.* Des études supplémentaires doivent être menées afin de valider l'activité anti-*C. albicans* de ces espèces microbiennes, mais si elles sont confirmées *in vitro* et *in vivo*, ces résultats pourraient ouvrir la voie à de nouvelles stratégies d'intervention pour limiter la prolifération intestinale de *C. albicans.*

Chapitre 3 : l'influence de l'hôte et des facteurs environnementaux sur la colonisation de *C. albicans* chez les individus sains.

Il est bien connu que le microbiote intestinal et le système immunitaire de l'hôte jouent un rôle crucial dans le contrôle de la croissance de *C. albicans* dans l'intestin (Naglik *et al.* 2017; Kumamoto, Gresnigt, and Hube 2020; Ricci *et al.* 2022; Zeise, Woods, and Huffnagle 2021). Mais comme la plupart des études sont menées sur des cohortes de patients, il est nécessaire d'améliorer notre compréhension des mécanismes impliqués dans la prolifération de *C. albicans* chez le sujet humain sain. En outre, bien que plusieurs maladies génétiques ou polymorphismes génétiques communs aient été associés à une susceptibilité accrue aux infections à *C. albicans* (Ouederni *et al.* 2014; Smeekens *et al.* 2013; Puel 2020), on sait peu de choses sur les polymorphismes génétiques associés à la susceptibilité à la colonisation par *C. albicans* chez les personnes en bonne santé. Ainsi, dans le troisième chapitre de cette thèse, j'ai étudié comment la composition du microbiote bactérien et le régime alimentaire, les antécédents médicaux et l'environnement de l'hôte peuvent influencer le portage de *C. albicans* chez les adultes en bonne santé, en utilisant les données recueillies auprès de 695 volontaires sains de la cohorte Milieu Intérieur. De plus, grâce à une étude

d'association à l'échelle du génome (GWAS), j'ai recherché des facteurs génétiques associés à la susceptibilité de l'hôte à la colonisation par *C. albicans*. Enfin, compte tenu des rapports précédents sur l'importance de l'immunité innée « entraînée » dans les infections à *C. albicans*, je me suis demandé s'il y avait un avantage pour l'hôte sain à maintenir des niveaux élevés de *C. albicans* dans l'intestin. J'ai donc étudié l'interaction entre l'intensité du portage intestinal de *C. albicans* et la réponse immunitaire après la stimulation du sang de l'hôte par *C. albicans*.

Dans un premier temps, j'ai caractérisé le mycobiote de 96 sujets sains de la cohorte Milieu Intérieur par séquençage ciblé ITS2. Le mycobiote des sujets était défini par une faible richesse et homogénéité, avec un indice de Shannon médian de 2,21 mais par une forte variation de la composition entre les différents individus. Le portage de *C. albicans* a été estimé par qPCR pour 695 des sujets Milieu Intérieur, et j'ai ainsi montré que 82.9% des individus étaient colonisés par cette levure mais avec de fortes variations interindividuelles.

J'ai ensuite exploré le potentiel rôle du microbiote dans les variations de concentrations de *C. albicans.* J'ai donc recherché des espèces bactériennes susceptibles d'avoir une action antagoniste envers *C. albicans.* Pour ce faire, j'ai utilisé des modèles linéaires mixtes pour identifier des espèces bactériennes dont l'abondance relative, déduite des données métagénomiques shotgun obtenues à partir de tous les échantillons des sujets Milieu Intérieur, et annotées au niveau des espèces, était associée à l'abondance de *C. albicans,* déduite des données qPCR. J'ai ainsi montré que *Intestinimonas butyriciproducens* était la seule espèce dont l'abondance relative était négativement corrélée aux concentrations de *C. albicans* chez ces sujets. J'ai ensuite cherché à valider l'activité anti-*C. albicans* de cette espèce en testant l'effet du surnageant de la culture de *I. butyriciproducens* sur la croissance et la morphologie de *C. albicans.* Toutefois, je n'ai pas pu observer un effet notable du surnageant de cette bactérie sur la croissance de *C. albicans*, du moins dans les conditions testées.

Comme la composition du microbiote intestinal semblait avoir un impact limité sur la colonisation par *C. albicans* chez les individus sains de Milieu Intérieur, l'intensité du portage de *C. albicans* pouvait être modulé par le régime alimentaire, les antécédents médicaux des sujets ou par des facteurs environnementaux. J'ai combiné des modèles linéaires mixtes et des analyses de variances, ajustés pour l'âge, le sexe et des

variables techniques, afin d'identifier les facteurs associés à la colonisation *par C. albicans*. J'ai ainsi noté que le régime alimentaire des sujets contribuait à la croissance de *C. albicans* ; un régime faible en sel et le fait de manger entre les repas étant associés à de plus hautes concentrations de *C. albicans*. De plus, étonnamment, la concentration intestinale de *C. albicans* était fortement associée à la concentration corpusculaire moyenne d'hémoglobine des sujets.

J'ai ensuite étudié les effets de la variation génétique des sujets sur la susceptibilité à la colonisation intestinale par *C. albicans.* Par GWAS, nous avons ainsi comparé les profils génotypiques des 576 sujets Milieu Intérieur colonisés par *C. albicans* et ceux des 119 sujets Milieu Intérieur non colonisés. J'ai ainsi identifié 26 polymorphismes nucléotidiques (SNP), situés dans deux loci distincts, montrant une association potentielle avec la colonisation intestinale par *C. albicans.* Parmi ces associations, un SNP situé sur le chromosome 20, rs2870723, était caractérisé par la plus forte association avec la susceptibilité à la colonisation intestinale par *C. albicans*. Ce SNP est situé entre l'ARN5SP487, un pseudogène ribosomal 5S, et *MC3R*, un gène associé à l'obésité (Demidowich, Jun, and Yanovski 2017). Il est intéressant de noter que ce SNP est relativement proche d'*AURKA*, un gène dans lequel plusieurs SNPs ont été associés à la concentration corpusculaire moyenne d'hémoglobine, une variable que j'ai identifiée comme étant significativement associée à la colonisation par *C. albicans.*

Finalement, j'ai cherché à déterminer si les niveaux intestinaux de *C. albicans* influençaient la réponse immunitaire de l'hôte. J'ai donc analysé les niveaux de transcription de 546 gènes impliqués dans la réponse immunitaire et la concentration de 13 cytokines dans le sang des sujets, après stimulation avec des cellules de *C. albicans* ; cette analyse a révélé des associations positives entre les niveaux intestinaux de *C. albicans* et l'expression de *NLRP3* d'une part , et les concentrations d'IL-2 et de CXCL5 d'autre part, par modèles linéaires mixtes, ajustés en fonction de l'âge et du sexe des sujets, des proportions de cellules immunitaires et des variables techniques relatives aux échantillons.

Bien que l'importance relatives de certaines associations identifiées doive encore être déterminée, ces résultats offrent une meilleure compréhension des mécanismes de la colonisation de *C. albicans* chez l'hôte sain. De plus, ces résultats pourraient permettre

d’ouvrir la voie à de nouvelles cibles pour des stratégies d’intervention visant à limiter la prolifération de *C. albicans.*

TABLE OF CONTENTS

FIGURES AND TABLES

INTRODUCTION

CHAPTER 1: COMPARISON AND IMPLEMENTATION OF DNA EXTRACTION PROTOCOLS AND QPCR ASSAYS FOR THE ANALYSIS OF THE INTESTINAL MICRO- AND MYCOBIOTA

CHAPTER 2: THE IMPACT OF AN ANTIBIOTIC-INDUCED MICROBIOTA DYSBIOSIS ON THE MYCOBIOTA AND C. ALBICANS INTESTINAL CARRIAGE IN HEALTHY INDIVIDUALS.

CHAPTER 3: THE INFLUENCE OF HOST AND ENVIRONMENTAL FACTORS ON *C. ALBICANS* COLONIZATION IN HEALTHY INDIVIDUALS

ABBREVIATIONS

2GC: second generation cephalosporin

3GC: third generation cephalosporin

α-MSH: α-Melanocyte-stimulating hormone

AIDS: acquired immunodeficiency syndrome

AIRE: autoimmune regulator

AMP: anti-microbial peptides

APECED: autoimmune polyendocrinopathy, candidiasis, ectodermal dysplasy

ASC: apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain

ATP: adenosine triphosphate

AURKA: aurora kinase A

CARD: caspase recruitment domain

COVID-19: coronavirus disease 2019

C-section: cesarean section

CLR: C-type lectin receptor

CMC: chronic mucocutaneous candidiasis

CR3: complement receptor 3

DAMP: danger-associated molecular patterns

DC: dendritic cell

DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin

DOCK8: dedicator of cytokinesis

DNA: deoxyribonucleic acid

FcγR: Fcγ receptor

G-CSF: granulocyte-colony stimulating factor

GI: gastrointestinal

GUT cells: gastrointestinally induced transition cells

GWAS: genome-wide association study

HIES: hyper-IgE syndrome

HIV: human immunodeficiency viruses

Hyr1: hyphal-regulated protein 1

IBD: inflammatory bowel diseases

ICU: intensive care unit

IL: interleukin

INF: interferon

ITS: internal transcribed spacer

JAK: Janus kinase

MC3R: melanocortin 3 receptor

MDA5: melanoma differentiation-associated factor 5

MHC: major histocompatibility complex

MINCLE: macrophage inducible Ca2+-dependent lectin receptor

NET: neutrophil extracellular traps

NFκB: nuclear factor-kappa B

NGS: next-generation sequencing

NK: natural killer

NLR: NOD-like receptor

NLRC4: NOD-like receptor family CARD domain-containing protein 4

NLRP3: NOD-like receptor family pyrin domain-containing 3

NOD: nucleotide oligomerisation domain

OPC: oropharyngeal candidiasis

OTU: operational taxonomy unit

PAMP: pathogen-associated molecular pattern

Pb: base pair

PLA2G4B: phospholipase A2 Group IVB

PRR: pattern-recognition receptor

qPCR: quantitative PCR

rDNA: ribosomal DNA

RigI: retinoic-acid-inducible gene I

RLR: RigI-helicase receptor

ROS: reactive oxygen species

RVVC: recurrent vulvovaginal candidiasis

Sap6: secreted aspartyl protease 6

SHIME: Simulator of Human Intestinal Microbial Ecosystem

SNP: single nucleotide polymorphism

***Sp*:** species

STAT: signal transducer and activator of transcription

SYK: spleen tyrosine kinase

Th: lymphocyte T helper

TIR: toll-interleukin-1 receptor

TLR: toll-like receptors

TNF: tumor necrosis factors

TRIF: TIR domain-containing adaptor protein

TYK2: tyrosine kinase 2

VVC: vulvovaginal candidiasis

WOR1: white-opaque regulator 1

LIST OF PUBLICATIONS

Published

- **Margot Delavy**, Charles Burdet, Natacha Sertour, Savannah Devente, Jean-Denis Docquier, Nathalie Grall, Stevenn Volant, Amine Ghozlane, Xavier Duval, France Mentré, Christophe d'Enfert, Marie-Elisabeth Bougnoux, for the PrediRes group. (2022). A Clinical Study Provides the First Direct Evidence That Interindividual Variations in Fecal β-Lactamase Activity Affect the Gut Mycobiota Dynamics in Response to β-Lactam Antibiotics. mBio, November. https://doi.org/10.1128/MBIO.02880-22.

- Christophe d'Enfert, Ann-Kristin Kaune, Leovigildo-Rey Alaban, Sayoni Chakraborty, Nathaniel Cole, **Margot Delavy**, Daria Kosmala, Benoît Marsaux, Ricardo Fróis-Martins, Moran Morelli, Diletta Rosati, Marisa Valentine, Zixuan Xie, Yoan Emritloll, Peter A Warn, Frédéric Bequet, Marie-Elisabeth Bougnoux, Stephanie Bornes, Mark S Gresnigt, Bernhard Hube, Ilse D Jacobsen, Mélanie Legrand, Salomé Leibundgut-Landmann, Chaysavanh Manichanh, Carol A Munro, Mihai G Netea, Karla Queiroz, Karine Roget, Vincent Thomas, Claudia Thoral, Pieter Van den Abbeele, Alan W Walker, Alistair J P Brown (2020). The impact of the Fungus-Host-Microbiota interplay upon *Candida albicans* infections: current knowledge and new perspectives. FEMS Microbiology Reviews 45 (3): 1–55. https://doi.org/10.1093/femsre/fuaa060

Submitted

- **Margot Delavy,** Natacha Sertour, Christophe d'Enfert, Marie-Elisabeth Bougnoux. (submitted). Finding microbiome-based antifungal strategies: impact of OMICs approaches.

- Camille d'Humières*, **Margot Delavy***, Laurie Alla*, Farid Ichou, Emilie Gauliard, Amine Ghozlane, Florence Levenez, Nathalie Galleron, Benoit Quinquis, Nicolas Pons, Jimmy Mullaert, Antoine Bridier-Nahmias, Bénédicte Condamine, Marie Touchon, Dominique Rainteau, Antonin Lamazière, Philippe

Lesnik, Maharajah Ponnaiah, Marie Lhomme, Natacha Sertour, Savannah Devente, Jean-Denis Docquier, Marie-Elisabeth Bougnoux, Olivier Tenaillon, Mélanie Magnan, Etienne Ruppé, Nathalie Grall, Xavier Duval, Dusko Ehrlich, France Mentré, Erick Denamur, Eduardo P C Rocha, Emmanuelle Le Chatelier, Charles Burdet, for the PrediRes study group. (submitted). Perturbation and resilience of the gut microbiome up to three months after β-lactams exposure in healthy volunteers suggest an important role of endogenous β-lactamases.

PREFACE

The human body is colonized by billions of bacteria, virus, and fungi, that form the human microbiota. In particular, the gut harbors the highest concentration of microbes within the human body. These microbes play an important role in health and disease, notably through a modulation of the human physiology, metabolism, nutrition and immune functions. In turn, the gut microbiota can be modified by various factors, including the host immune response, diet, or environment. The gut microbiota also contributes to the maintenance of the homeostasis of its environment by preventing infections by various pathogenic species.

One of such species is *Candida albicans,* an opportunistic pathogen that commonly cause superficial infections. Besides, when the host immune defenses are compromised, for example after a surgery and/or in immunocompromised patients, *C. albicans* can cause severe systemic infections, which are associated to up to 50% mortality. Despite being an opportunistic pathogen, *C. albicans* is primarily a commensal yeast. It is the most frequently isolated yeast in humans and colonizes the healthy oral, vaginal and gastrointestinal tracts, therefore interacting with the thousands of microbial species sharing these niches.

Consequently, *C. albicans* ability to colonize and maintain itself in the human gut is the result of an equilibrium between the microbiota, the host, and the fungus itself. This fine-tuned balance is particularly pivotal considering that *C. albicans* intestinal colonization is the cornerstone of the initiation of an infection in at-risk patients. Therefore, understanding the factors underlying this colonization is crucial to control the proliferation of *C. albicans.*

Thorough this thesis, I will discuss the different factors that can modulate the extent of *C. albicans* carriage in healthy individuals, with a particular interest in the gut microbiota, the host environment, and the host immune and genetic profile.

INTRODUCTION

A - BURDEN OF FUNGAL INFECTIONS IN HUMANS

Although often underestimated, fungi are redoubtable disease-causing agents. Among the estimated 1.5 to 3.8 million fungal species (Hawksworth and Lücking 2017), 300 are known human pathogens (“Stop Neglecting Fungi” 2017). They are responsible for a heavy economical and sanitary burden, with more than 300 million people affected by a severe fungal-disease annually (“Stop Neglecting Fungi” 2017). With 1.6 million deaths caused by fungal infections each year (“Stop Neglecting Fungi” 2017), fungi are more frequent killers than malaria (United States Centers for Disease Control and Prevention 2014) and breast cancer (Arnold *et al.* 2022) combined. *Candida, Aspergillus, Pneumocystis* and *Cryptococcus* sp. are the most common causal agents of fatal fungal infections in humans, causing more than 90% of the reported fungal-related deaths (Brown *et al.* 2012; Bongomin *et al.* 2017, Fig. 1). In particular, species from the *Candida* genus are responsible for the majority of the invasive fungal infections, with more than 700,000 cases reported annually.

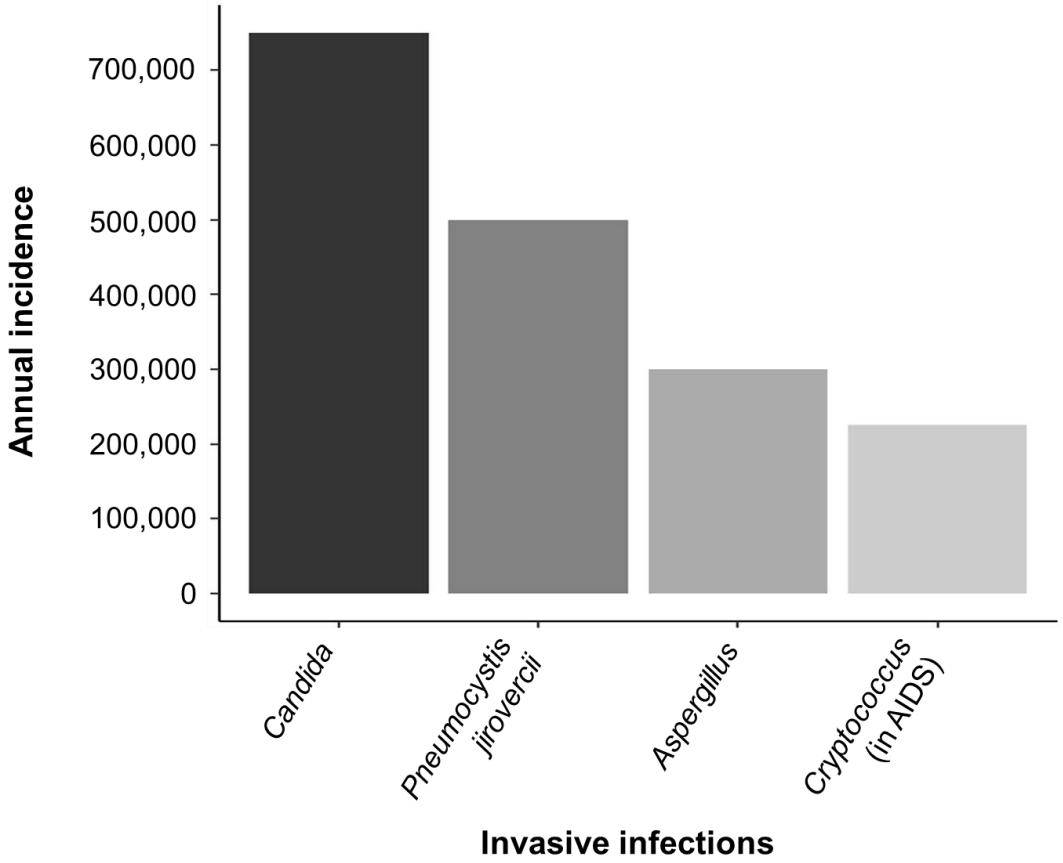


Figure 1: Worldwide annual incidence of the invasive fungal infections caused by the *Candida* sp., *Pneumocystis jirovercii, Aspergillus* sp. and *Cryptococcus* sp. *Figure adapted from Bongomin et al. 2017.*

B - *CANDIDA ALBICANS* AS A PATHOGEN

Candida albicans, an Ascomycota from the *Saccharomycetaceae* family, is the main etiologic species associated to *Candida* infections. This opportunistic pathogen is responsible for 80-90% of *Candida*-associated mucosal infections and for 40% of invasive candidiasis (Macias-Paz *et al.* 2022; Talapko *et al.* 2021).

1. Superficial infections

Candida albicans causes mainly mucosal infections, which are frequent but benign (Dadar *et al.* 2018; Brown *et al.* 2012). In particular, vulvovaginal candidiasis (VVC), which is characterized by the overgrowth of *C. albicans* in the vaginal tract and a subsequent inflammatory host response, affects 70-75% of women, at least once in their lifetime (Foxman *et al.* 2013; Yano *et al.* 2019; Rosati *et al.* 2020) and more than 100 million of women suffer from more than 3 VVC episodes each year (Bongomin *et al.* 2017). Meanwhile, more than 2 million people suffer from oropharyngeal candidiasis annually (OPC) (Bongomin *et al.* 2017; Talapko *et al.* 2021), a mucosal infection of the mouth and/or throat which is the most frequent oral opportunistic infection observed in HIV-positive patients, with many cases reported after a broad-spectrum antibiotic treatment (Vila *et al.* 2020; Samaranayake 1992).

2. Invasive infections

C. albicans is also responsible for life-threatening invasive infections, associated with up to 50% mortality (Pappas *et al.* 2018; Brown *et al.* 2012). Indeed, 700,000 invasive candidiasis are reported each year and half of them are caused by this yeast (Bongomin *et al.* 2017; Macias-Paz *et al.* 2022). Invasive candidiasis include deep-seated *Candida* infections such as intra-abdominal abscesses and peritonitis and bloodstream infections, which are referred as candidemia (Pappas *et al.* 2016). Such infections arise when host defenses are compromised, for example after a surgery, the use of a central venous catheter, dialysis, and/or in immunocompromised patients (Pappas *et al.* 2018; Zhai *et al.* 2020). *C. albicans* can then invade the bloodstream and disseminate to all internal organs such as kidney, liver and spleen (Pappas *et al.* 2018). These infections occur mainly after an intestinal overgrowth of *C. albicans* and

its subsequent translocation from the gut to the bloodstream (Zhai *et al.* 2020). Four main classes of antifungal agents are currently approved to treat *C. albicans* infections: azoles, polyenes, pyrimidine analogs and echinocandins (Sanglard 2016; Vandeputte, Ferrari, and Coste 2012; Sanguinetti, Posteraro, and Lass-Flörl 2015) and their efficiency is impaired by the rising incidence of antifungal resistance, especially when they are used in prophylaxis (Cleveland *et al.* 2012; Costa-de-oliveira and Rodrigues 2020).

C - *C. ALBICANS* AS AN INTESTINAL COMMENSAL

Despite being a harmful opportunistic pathogen, *C. albicans* is primarily a commensal fungus. It is the most frequently isolated yeast in humans and colonizes the healthy oral, vaginal and gastrointestinal (GI) tracts (Ghannoum *et al.* 2010; Nash *et al.* 2017; d'Enfert *et al.* 2021). The prevalence of *C. albicans* and the extent of its carriage in the human gut is still questioned. Indeed, *C. albicans* has been shown to be present in 40-50% of the population, by culture methods (Bougnoux *et al.* 2006; da Silva Dantas *et al.* 2016; Standaert-Vitse *et al.* 2009). However, targeted metagenomic sequencing has shown a higher prevalence of *C. albicans,* with this species being detected in 63.6% of the studied cohort (Nash *et al.* 2017). Considering that most studies are conducted in cohorts of patients and that culture and metagenomic sequencing lack in specificity and sensitivity, we still need a reliable and accurate estimate of *C. albicans* carriage and colonization in healthy individuals.

C. albicans is thought to be transmitted vertically, from mother to child. It is likely that the primo-colonization occurs at birth, especially during vaginal delivery, or in the perinatal period (Azevedo *et al.* 2023; Bliss *et al.* 2008; Bougnoux *et al.* 2006; d'Enfert 2009), with babies delivered by cesarean-section (C-section) being twice less likely to become colonized by *C. albicans,* compared to babies born vaginally (Parm *et al.* 2011). Moreover, several reports have suggested that *C. albicans* might be transmitted from mother to offspring through breastfeeding (Boix-Amorós *et al.* 2017; Azevedo *et al.* 2023; Richard and Sokol 2019). There is also evidence of intrafamilial transmission since it has been shown that members from the same family, including spouses, are frequently colonized by genetically indistinguishable or close *C. albicans* isolates (Bougnoux *et al.* 2006).

1. Adaptation to the GI tract

Contrary to what has been described for other fungal pathogens, *C. albicans* colonization is strictly associated with warm-blooded animals and an environmental reservoir for this species has yet to be found (Jabra-Rizk *et al.* 2016; Nucci and Anaissie 2001; d'Enfert *et al.* 2021). *C. albicans* has thus evolved to be particularly adapted to colonize the human body, especially the GI tract.

i. Morphogenic adaptation

One of the key factors in the ability of *C. albicans* to adapt both as a commensal and as a pathogen is its polymorphic nature. Indeed, depending on the environmental conditions, such as the physiological temperature, cell starvation, pH, CO_2 levels or presence of serum, *C. albicans* can adopt a yeast form, or become either a pseudo- or true hypha (Mayer, Wilson, and Hube 2013, Fig. 2). Yeasts are round or oval and unicellular while hyphae are tubular and multicellular. Pseudohyphae stand in between yeast and hyphae in term of morphology and harbour a long, elliptic multicellular form (Chen *et al.* 2020). The yeast form is thought to allow an easier dissemination of the fungus whereas the hyphal form enables tissue invasion (Gow, Brown, and Odds 2002; Min, Neiman, and Konopka 2020; Jacobsen and Hube 2017).

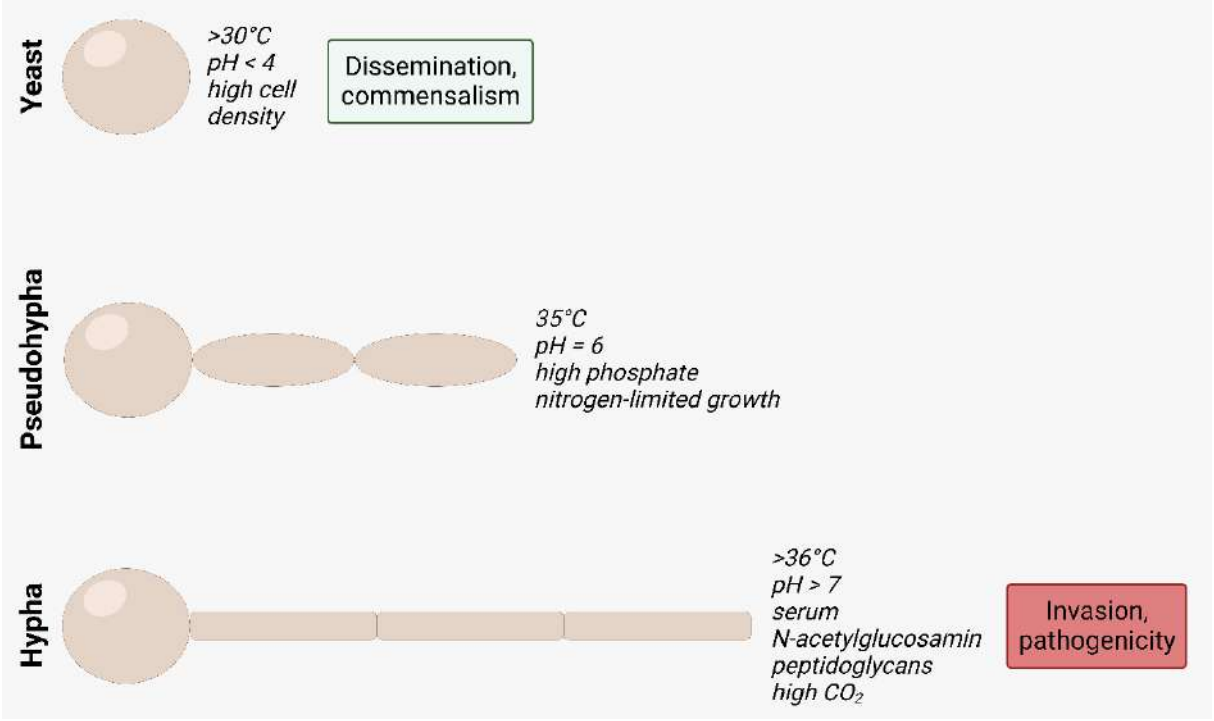


Figure 2: Scheme of *C. albicans* yeast, pseudohypha and hypha and environmental inputs triggering the transition to each form. *Figure created with Biorender.com and inspired from Chen et al. 2020 and d'Enfert et al. 2020.*

In the yeast form, *C. albicans* cells can adopt one of three non-genetic phenotypes: white, opaque or gray cells, each with a distinct cell morphology and gene expression profile (Tao *et al.* 2014). In addition, during colonization, *C. albicans* can also form Gastrointestinally Induced Transition (GUT) cells (Pande, Chen, and Noble 2013). These cells experience developmental modifications through their passage in the GI tract, resulting in a more elongated morphology than that of the yeast form usually

observed in the bloodstream (Pande, Chen, and Noble 2013). They also exhibit a distinct transcriptomic and metabolic activity, which confer them a better adaptation to the commensal state. In particular, they have been shown to display an increased adhesion to the gut mucosa (Prieto *et al.* 2017). However, this morphology has been only observed in strains over-expressing the white–opaque regulator 1 (WOR1). Thus, GUT cells have yet to be observed outside of experimental settings to confirm their role in *C. albicans* gut colonization (Rai *et al.* 2021).

On the contrary, hyphal morphology seems to be detrimental to the maintenance of *C. albicans* in the GI tract as a commensal (Tso *et al.* 2018; Witchley *et al.* 2019). Indeed, the loss of hyphal-specific transcription factors such as Ume6 or hyphal-associated factors such as secreted aspartyl protease 6 (Sap6) and hyphal-regulated gene 1 (Hyr1) have been shown to enhance *C. albicans* gut colonization (Witchley *et al.* 2019). Moreover, long-term experimental colonization results in mutated strains that tend to lose their ability to form hyphae (Tso *et al.* 2018). Interestingly, several intestinal bacteria have been shown to inhibit *C. albicans* yeast-to-hyphae transition (Cruz *et al.* 2013; Matsubara *et al.* 2016; García *et al.* 2017), which might explain why *C. albicans* yeast morphology is predominant in the GI tract (Jacobsen and Hube 2017). However, despite the fact that the yeast form seems to favor *C. albicans* GI colonization, there might be an evolutionary pressure to maintain the ability to form hyphae. Indeed, *C. albicans* has been shown to colonize the gut under both yeast and hyphal forms, and most clinical strains retain the ability to filament (Kumamoto, Gresnigt, and Hube 2020; Tso *et al.* 2018).

ii. Adaptation to hypoxia

The GI tract is mostly hypoxic although oxygen gradients exist along the length of the gut and between the lumen and the epithelium (L. Zheng, Kelly, and Colgan 2015). This usually results in a geographical repartition of the microbial species in the intestine depending on their respective oxygen tolerance (Zeise, Woods, and Huffnagle 2021).

C. albicans is a facultative anaerobe. It is thus able of both aerobic respiration and anaerobic fermentation, although its growth is enhanced in presence of oxygen. Consequently, *C. albicans* can thrive in various niches along the GI tract by adapting its metabolism to account for the surrounding oxygen levels (Burgain *et al.* 2020, Fig. 3).

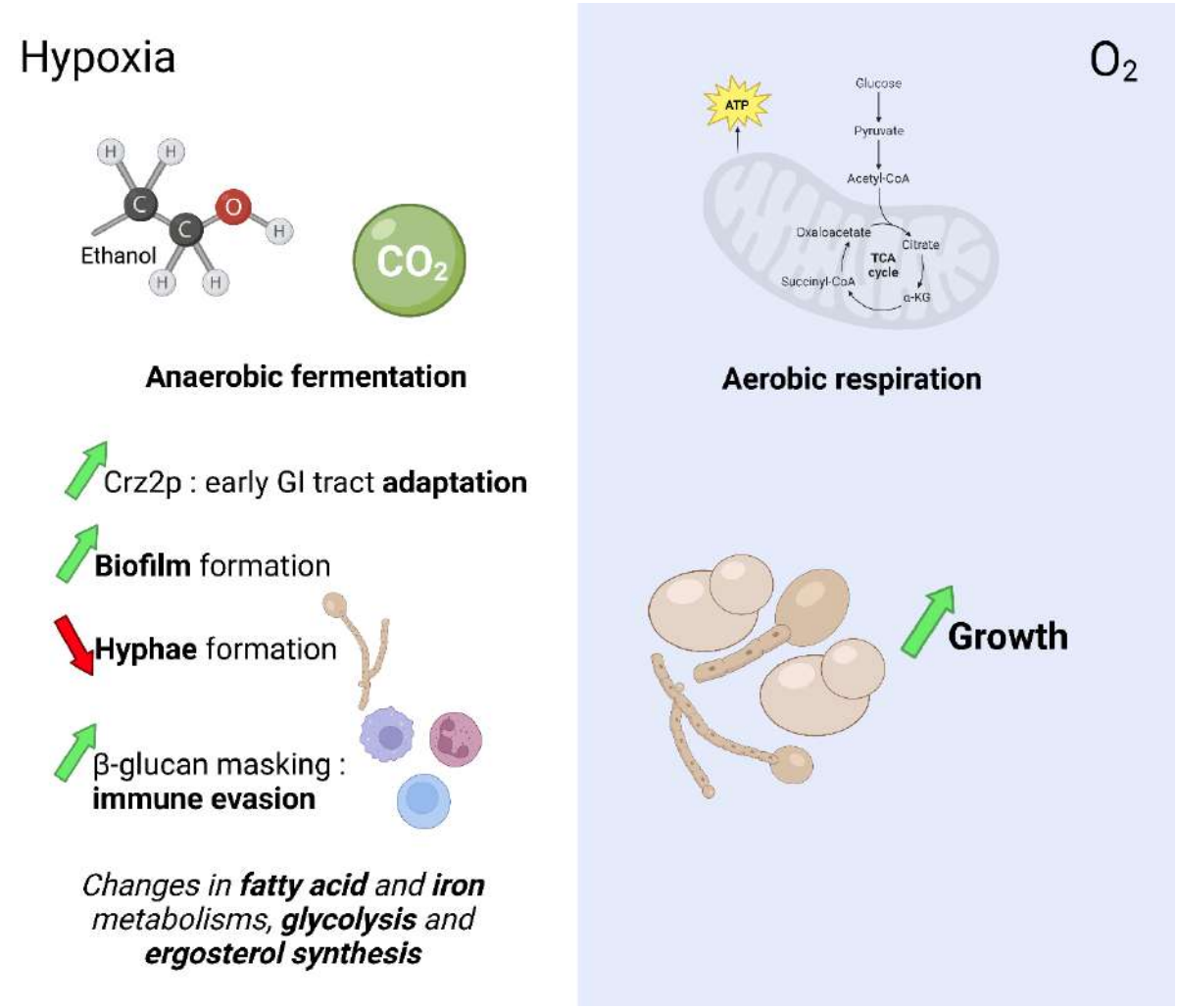


Figure 3: *C. albicans* adaptation to hypoxic conditions. *C. albicans* is able of anaerobic fermentation, under hypoxic conditions, and aerobic respiration, in the presence of oxygen. Hypoxia triggers *C. albicans* adaptation to the GI tract via Crz2 activity, biofilm formation and immune evasion through β-glucans masking. Hypoxia also decreases hyphae formation and results in altered fatty acid and iron metabolism, glycolysis and ergosterol synthesis. *C. albicans* grows optimally under aerobic conditions. *Figure created with Biorender.com.*

In particular, hypoxic conditions are required to ensure the optimal activity of Crz2p, a transcription factor that contributes to *C. albicans* adaptation during the early colonization of the GI tract (Znaidi *et al.* 2018). Low oxygen levels also trigger *C. albicans* immune evasion by promoting the masking of β-glucans, cell wall molecules usually recognized by the immune system, thus leading to an anti-*C. albicans* immune response (Pradhan *et al.* 2018). Moreover, hypoxia can induce modification in *C. albicans* fatty acid metabolism, glycolysis, iron metabolism and ergosterol synthesis (Grahl *et al.* 2012). Efg1, a crucial regulator of the hypoxic response, downregulates hyphal formation while inducing genes involved in biofilm formation (Setiadi *et al.* 2006;

Stichternoth and Ernst 2009), thus suggesting that adaptation to low oxygen levels is crucial for *C. albicans* commensal colonization (Grahl *et al.* 2012).

iii. Metabolic adaptation

C. albicans maintenance in the GI tract is dependent on the fungus access to carbon, nitrogen and phosphate sources, which are essential to maintain its metabolic functions (Zeise, Woods, and Huffnagle 2021). *C. albicans* preferred carbon source is glucose, whose concentration is particularly limited in the intestine (Kastora *et al.* 2017). However, *C. albicans* displays a highly flexible nutrient metabolism, which allows the exploitation of various alternative carbon sources. *C. albicans* is therefore well adapted to a glucose-free environment (Miramón *et al.* 2020; Miramón and Lorenz 2017; Kastora *et al.* 2017). For instance, under-sugar limiting conditions, *C. albicans* can use amino acids both as a source of carbon and nitrogen (Tripathi *et al.* 2002; Tournu *et al.* 2005; Mayer *et al.* 2012; Vylkova *et al.* 2011; Ene *et al.* 2014) and can exploit simultaneously different carbon sources, including lactate (Kumamoto, Gresnigt, and Hube 2020). In the gut, mutants unable to grow on alternative carbon sources such as lactate, citrate and glycerol are rapidly outcompeted by wild-type *C. albicans* strains (Ramírez-Zavala *et al.* 2017).

Iron metabolism also plays an important role in *C. albicans* GI tract colonization. Indeed, a decrease of iron acquisition is observed in GUT cells in response to the iron high availability observed in the gut. Iron availability also seems to act as a signalling factor for *C. albicans*, thus activating pathways involved in stress response, adhesion and biofilm formation (Fourie *et al.* 2018; Kaba *et al.* 2013; Puri *et al.* 2014).

Finally, secondary bile acids limit the growth, filamentation and adhesion of *C. albicans*, and could thus participate in the maintenance of a commensal state in the GI tract (Guinan, Villa, and Thangamani 2018). On the other hand, primary bile acids can promote the proliferation of *C. albicans* and trigger hyphae formation (Guinan and Thangamani 2018; Hsieh, Brunke, and Brock 2017).

D - *C. ALBICANS* – A MEMBER OF THE INTESTINAL MICROBIOTA

In the GI tract, *C. albicans* shares a niche with thousands of bacterial and fungal species (Nash *et al.* 2017) that play an important role in homeostasis (Ohland and Jobin 2015) and compose the gut microbiota, which includes bacteria, archaea, eukaryotes and viruses. The fungal component of the microbiota is called mycobiota.

1. The gut mycobiota

The gut mycobiota represents less than 0.1% of the total microbiota and is therefore more tedious to study than its bacterial counterpart (Underhill and Iliev 2014; Qin *et al.* 2010; Zuo *et al.* 2018). The gut mycobiota is characterized by a low α-diversity, resulting in a low richness and species homogeneity, with an average of only 10-25 different fungal species per sample and a Shannon index usually comprised between 1.2 and 1.8, depending of the cohorts studied (Nash *et al.* 2017; Chehoud *et al.* 2015; Olaisen *et al.* 2022). However, the gut mycobiota is highly variable from one individual to another, but also within the same individual, with a fungal composition that can change drastically within days in a same individual (Nash *et al.* 2017; Raimondi *et al.* 2019). This makes it particularly difficult to define a healthy “core mycobiota”. However, research agrees that the majority of the mycobiota is composed of species from the phyla Ascomycota and Basidiomycota, with a predominance of species from the phylum Ascomycota (Nash *et al.* 2017; Chehoud *et al.* 2015, Fig. 4). In particular, *Galactomyces, Penicillium, Saccharomyces* (with mainly *S. cerevisiae*), *Debaryomyces, Fusarium, Aspergillus, Malassezia* (with mainly *M. restricta*) and *Candida* (with mainly *C. albicans*) are the most frequently identified genera in mycobiota studies (Nash *et al.* 2017; Chehoud *et al.* 2015, Fig. 4). Most of the fungi detected in the GI tract are probably only transient species, brought by diet or the environment and are thus not true colonizers (Auchtung *et al.* 2018; Raimondi *et al.* 2019), which suggests a strong impact of the lifestyle on the composition of the gut mycobiota.

i. Gut mycobiota and aging

The healthy gut mycobiota has been shown to be modified with age (d’Enfert *et al.* 2021; Mishima *et al.* 2023; Schei *et al.* 2017; Wu *et al.* 2020, Fig. 4). A recent study

conducted in Japanese infants showed that new-borns had a relatively high fungal diversity that was progressively decreasing in the three first years of life. During this period, a yeast-dominant mycobiota was established whereas the relative abundance of molds progressively decreased (Mishima *et al.* 2023). Weaning appears to plays an important role in the establishment of a mature intestinal mycobiota, with differentiation of the gut mycobiota occurring after this stage (Mishima *et al.* 2023). Similarly, another study showed that the intestinal mycobiota of infants was characterized by an enrichment of *Debaryomyces hansenii* and that, with age, children acquire a mycobiota similar to their mother's (Schei *et al.* 2017). *Debaryomyces* sp. seems to be also less predominant in elderly than in young populations (L. Wu *et al.* 2020).

ii. Gut mycobiota and alimentation

Different diets have been reported to impact differently the richness or the composition of the gut mycobiota (D'Enfert *et al.* 2021, Fig. 4). For example, an increase of *Penicillium* sp. was observed in volunteers subjected to a short-term animal-based diet, whereas plant-based diet resulted in an increase of *Candida* sp. (David *et al.* 2014). Similarly, the high abundance of *Penicillium* sp. and *Debaryomyces* sp. observed in Sardinian volunteers was suggested to be associated with the high cheese consumption reported in this region (L. Wu *et al.* 2020). *Aspergillus oryzae,* a fungal species commonly used in the fermentation of soybeans during the fabrication of soy sauce, has been shown to be a common species of the Japanese healthy gut (Motooka *et al.* 2017). Finally, Wayampi Amerindians harbour higher amounts of *Candida krusei* and *S. cerevisiae* in their GI tract compared to what is usually observed in industrialized population, and these species were identified in local fruits and cachiri, a handmade manioc-fermented beverage commonly consumed by this population (Angebault *et al.* 2013). Overall, a recent study conducted on Polish volunteers showed that the consumption of chips, meats, soda, alcohol, sugar and processed food was the factor that most strongly affected the composition of the gut mycobiota (Szóstak *et al.* 2023). In particular, vegetarians had a more diverse gut mycobiota than the other participants (Szóstak *et al.* 2023).

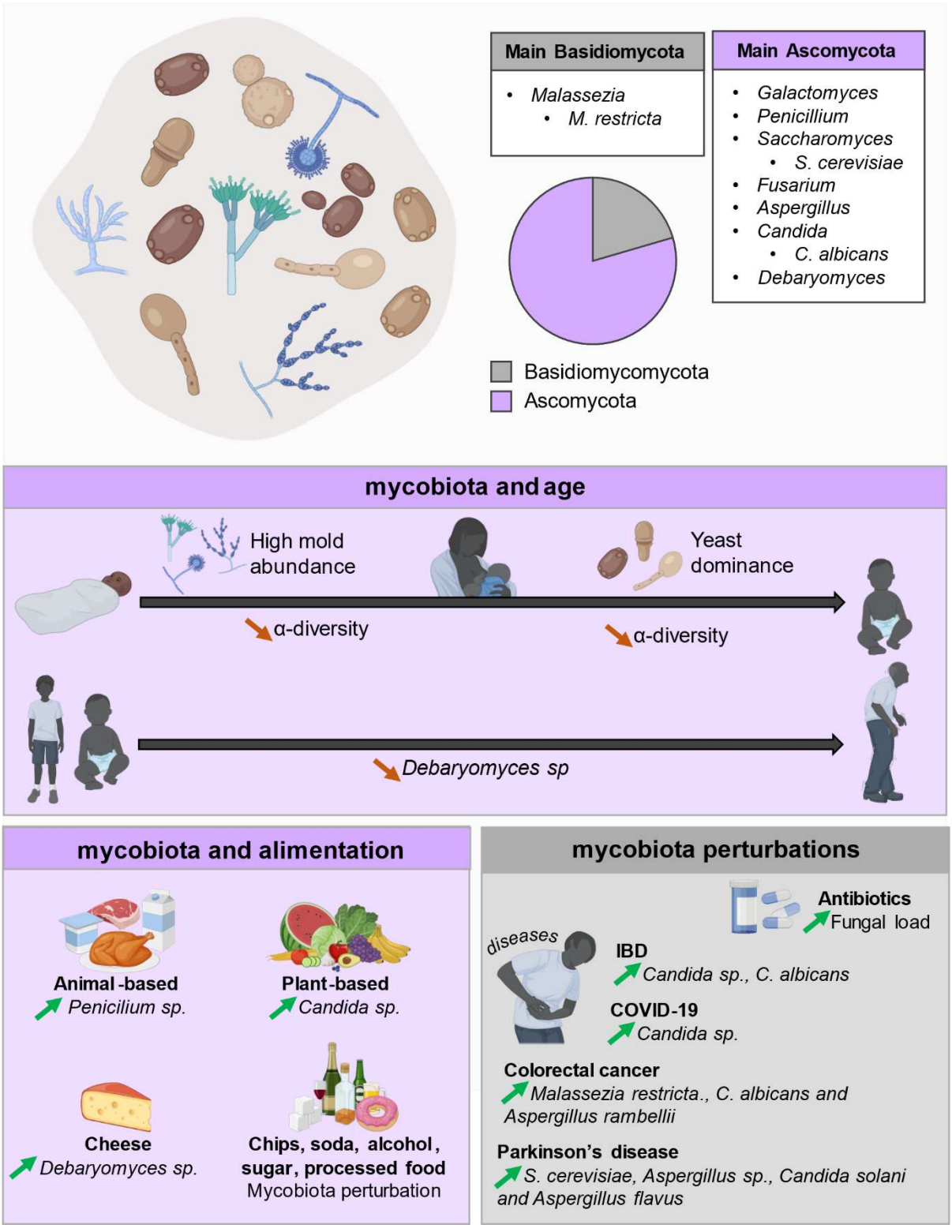


Figure 4: Gut mycobiota and factors that influence its composition. The mycobiota is composed mainly of species from the Ascomycota (purple) and Basidiomycota (gray). The pie chart represents the relative abundance of both phyla in a representative healthy gut. The lower panels summarize the effects of age, alimentation, antibiotics, and diseases on the composition of the gut mycobiota. *Figure created with Biorender.com.*

iii. Perturbations of the gut mycobiota and contribution to diseases

An healthy intestinal mycobiota can be disturbed by many factors (d'Enfert *et al.* 2021), including antibiotics (Fig. 4). By killing the resident bacteria of the gut, broad-spectrum antibiotics allow an overgrowth of the fungal component of the GI tract (Dollive *et al.* 2013). A recent study investigated the effects of 5 different antibiotics on the fungal and bacterial microbiota and showed a long-term perturbation of the fungal species profile after antibiotic exposure, with decreased relative abundance of *Saccharomyces spp., Candida parapsilosis* and *M. restricta.* Interestingly, the relative abundance of *C. albicans* tended to increase during antibiotic treatment but was decreased after it (Seelbinder *et al.* 2020).

A perturbation of the gut mycobiota has also been reported in many different diseases (Sokol *et al.* 2018; 2017; Chehoud *et al.* 2015; Chen *et al.* 2023; Gao *et al.* 2022; Maeda *et al.* 2022; Wang *et al.* 2021; d'Enfert *et al.* 2021). Patients suffering from inflammatory bowel diseases (IBD) experiment a decrease of fungal diversity and an increase of a small subset of species, especially *Candida sp.*, including *C. albicans,* which suggests that fungi might play a role in the pathogenicity of this disease (Sokol *et al.* 2018; 2017; Chehoud *et al.* 2015). Changes in the gut mycobiota have also been reported in patients suffering from Parkinson disease, with an increased abundance of *S. cerevisiae*, *Aspergillus sp.*, *Candida solani* and *Aspergillus flavus* and a decrease of *Malassezia restricta* (Chen *et al.* 2023). Severe and mild COVID-19 patients have also been shown to experience mycobiota dysbiosis, with a decrease of the mycobiota diversity and an increase of *Candida* sp. and a domination of *C. albicans* (Maeda *et al.* 2022). Finally, recent studies have shown associations between intestinal mycobiota dysbiosis and cancers. Indeed, patients with colorectal cancers harbor a specific mycobiota, with a particular increase of *M. restricta* (Gao *et al.* 2022). In another study, *C. albicans* was shown to be more abundant in patients with colorectal cancers than in healthy controls (Wang *et al.* 2021). Finally, a recent study identified several mycobiota signatures associated with colorectal cancers. In particular, *Aspergillus rambellii* abundance was not only associated with colorectal cancers but this species had a direct effect on tumor growth *in vitro* and *in vivo* (Lin *et al.* 2022), probably through the secretion of Aflatoxins, a fungal toxin with mutagens and carcinogen properties (Perrone and Gallo 2017).

2. The gut bacterial microbiota

Contrary to its fungal counterpart, the bacterial component of the microbiota has been extensively studied. The bacterial microbiota is highly diverse, and is composed in average of 200 to 250 different bacterial species, which corresponds to a Shannon index superior to 4 (Byrd *et al.* 2020; Manor *et al.* 2020). Its composition varies geographically along the GI tract, in adaptation to the physicochemical characteristics specific to each region (Fig. 5). In the small intestine, the bacterial population is less diverse than in the colon and is composed principally of fast-growing facultative anaerobes such as Proteobacteria and bacteria from the *Streptococcaceae* family (Zoetendal *et al.* 2012). Such species are well adapted to the high levels of stomach acids, the higher oxygen and bile acids concentrations and the antimicrobial molecules present in this environment (Donaldson, Lee, and Mazmanian 2015). In the colon, the microbiota becomes more diverse and is mainly composed of fermentative anaerobes. Firmicutes and Bacteroidetes are the more abundant phyla (Donaldson, Lee, and Mazmanian 2015). The colon is also the human niche with the highest bacterial concentrations, with densities that can reach up to 10^{11} cells per gram of colonic content. However, the bacterial population is not distributed evenly in the colon. The colon harbours two layers of mucus that acts as a barrier between the epithelial cells and the bacterial communities. The inner layer is nearly sterile whereas the outer layer, in direct contact with the lumen, is a rich niche for bacterial, but also fungal, colonization (Johansson, Holmén Larsson, and Hansson 2011; Johansson *et al.* 2008; Witchley *et al.* 2019). The gut bacterial microbiota varies from one person to another although slightly less than its fungal counterpart (Nash *et al.* 2017). In particular, the abundance of specific bacteria such as *Lachnospiraceae* and *Bifidobacterium* seems to have a genetic component. However, host genetic has only a minor impact on gut microbiota composition, with genetics accounting for 1.9 to 8.1% of gut microbiota variability (Scepanovic *et al.* 2018; Bonder *et al.* 2016; Goodrich *et al.* 2016; Rothschild *et al.* 2018).

i. Gut bacterial microbiota and aging

The initial structure of the gut bacterial population is strongly affected by the mode of delivery (d'Enfert *et al.* 2021; Reyman *et al.* 2019, Fig. 5). Indeed, the gut microbiota of vaginally born babies is enriched in bacterial species derived from the mother's vaginal microbiota, such as *Bifidobacterium, Lactobacillus, Prevotella* and *Atopobium*

(Dominguez-Bello *et al.* 2010). In contrast, more *Enterobacter, Haemophilus, Staphilococcus* and *Veillonella* are found the in gut of babies born by C-section (Bäckhed *et al.* 2015). After birth, the gut microbiota is shaped by the babies' diet. Infants fed exclusively on breastmilk have a higher abundance of *Bifidobacterium,* a genus comprising species able to metabolize milk oligosaccharides (Lawson *et al.* 2020; Yatsunenko *et al.* 2012; Odamaki *et al.* 2016; Hill *et al.* 2017), whereas the gut microbiota of formula-fed infants is more diverse (Lee *et al.* 2015; Klaassens *et al.* 2009). After weaning, children develop a richer microbiota that tends to remain relatively stable into adulthood (Faith *et al.* 2013), as long as no microbiota-disturbers such as antibiotics are introduced (Burdet, Grall, *et al.* 2019; Burdet, Nguyen, *et al.* 2019). Finally, a decrease in bacterial diversity and beneficial bacteria is observed in the elderly, while opportunistic bacteria become more frequent (Simon, Hollander, and McMichael 2015; C. Xu, Zhu, and Qiu 2019).

ii. Gut bacterial microbiota and alimentation

The composition of the gut microbiota is strongly influenced by diet (D'Enfert *et al.* 2021; Walker *et al.* 2011, Fig. 5). Animal-based diets, high in fat and proteins, result in higher levels of *Bacteroides, Bilophilia* and *Alistipes,* whereas people on plant-based diets have higher abundance of *Prevotella* and *Firmicutes* (G. D. Wu *et al.* 2011; Pareek *et al.* 2019; David *et al.* 2014). In addition, an unhealthy diet, with high consumption of fried foods, soda, sugary drinks, fatty sugary products, meat and/or convenience foods is associated with decreased bacterial α-diversity (Partula *et al.* 2019). In contrast, people who regularly eat fish and raw fruits have a more diverse gut microbiota (Partula *et al.* 2019).

It is well known that the gut microbiota vary between people residing in different regions or countries, or even between people living in urbanised and rural habitats (He *et al.* 2018; Schnorr *et al.* 2014; Vangay *et al.* 2018). However, rather than being due to geographical characteristics, these variations are largely explained by the difference of diets observed in these different regions (He *et al.* 2018). This could be explained by the fact that people residing in more urbanized areas or countries consume more protein, fat and processed foods, thereby reducing their α-bacterial diversity (Schnorr *et al.* 2014).

iii. Perturbations of the gut bacterial microbiota and contribution to diseases

The taxonomic composition or the diversity of the gut microbiota can be disturbed in disease or metabolic disorders (Fig. 5). For instance, IBD patients show a reduction of their microbiota diversity and a decrease in beneficial *Firmicutes* bacteria such as *Faecalibacterium prausnitzii*. In parallel, they have increased levels of *Enterobacteriaceae*, probably because these bacteria are better adapted to an inflammatory environment (Manichanh *et al.* 2006; Sokol *et al.* 2009; Pascal *et al.* 2017; Lloyd-Price *et al.* 2019). The gut microbiota is not only disturbed by an unhealthy state, but an unbalanced microbiota can promote health-related issues. The gut microbiota regulates obesity by modulating absorption of energy, appetite, fat storage, chronic inflammation, and circadian rhythms (for review, see Liu *et al.* 2021). The first evidence of the direct link between the composition of the gut microbiota and obesity was obtained from germ-free mice studies. It was thus demonstrated that transplanting the gut microbiota of overweight mice into germ-free mice resulted in a drastic increase of the transplanted mice weight, fat content and insulin resistance levels (Bäckhed *et al.* 2004). In humans, there is a decrease in bacteria usually considered as probiotics such as *Christensenellaceae, Methanobacteriales, Lactobacillus, Bifidobacteria*, and *Akkermansia* (Liu *et al.* 2021) in people suffering from obesity. In addition, perturbation of the gut microbiota might regulate Alzheimer's disease and other neurological disorders pathogenesis (Jiang *et al.* 2017; Chen *et al.* 2022). Microbiota dysbiosis is also contributing to carcinogenesis and can affect the response to treatment (Meng *et al.* 2018; Zhou, Zhou, and Fang 2021). This association is not limited to GI cancer as the bacterial gut microbiota has been shown to promote the progression of liver cancers through the activation of inflammatory and metabolic pathways (Dapito *et al.* 2012; Yoshimoto *et al.* 2013). Finally, besides its role in carcinogenesis, the gut microbiota affects the patient response to cancer treatments, such as chemotherapy and immunotherapy (Terrisse, Zitvogel, and Kroemer 2022; 2023; Routy *et al.* 2018, for review, see Helmink *et al.* 2019).

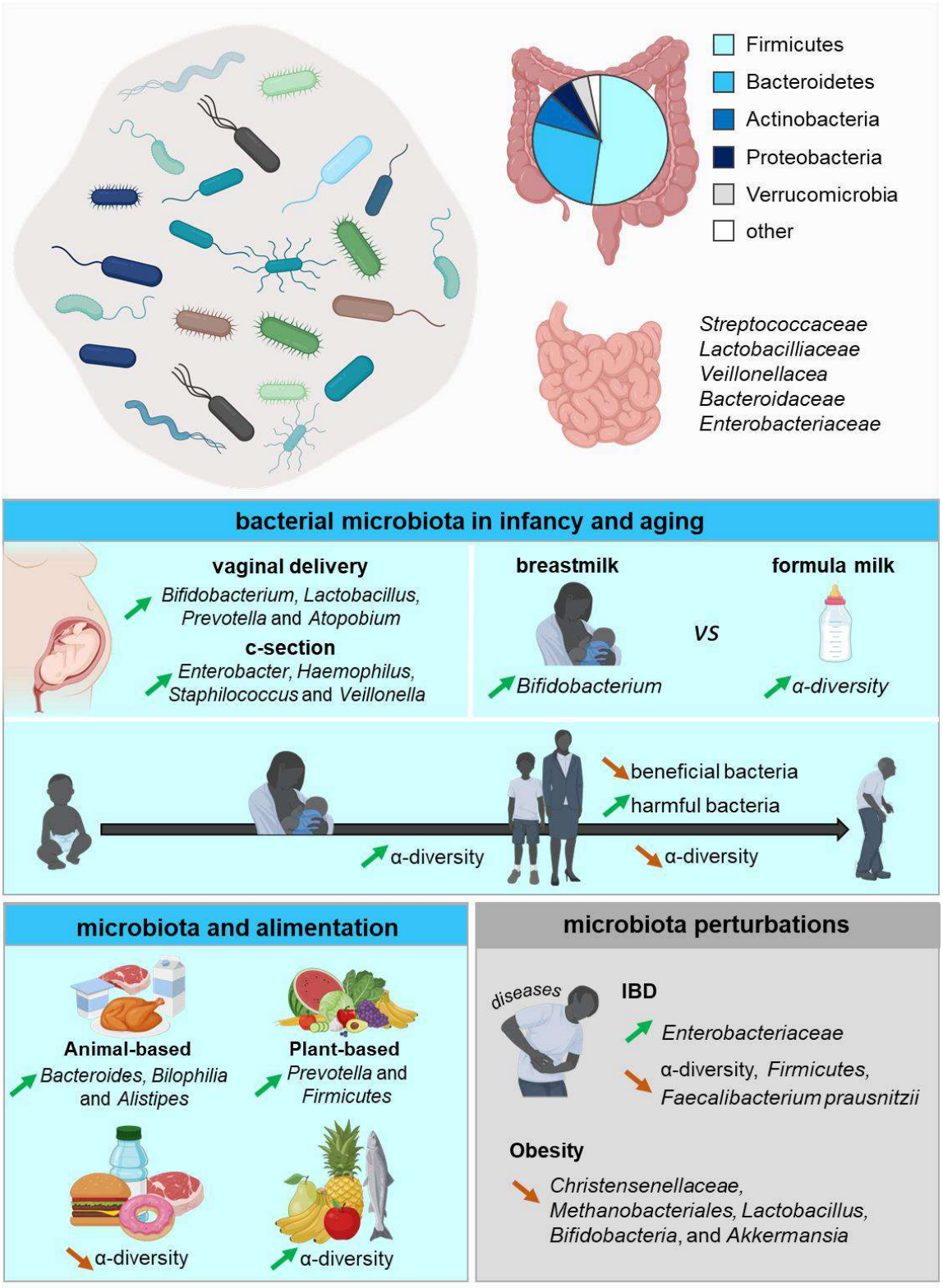


Figure 5: Gut bacterial microbiota and factors that influence its composition. The gut microbiota composition varies across the gut. The pie chart represents the relative abundance of the main phyla in a representative healthy colon. The lower panels summarize the effects of age, alimentation, and diseases on the composition of the gut bacterial microbiota. *Figure created with Biorender.com.*

3. Finding microbiome-based antifungal strategies: impact of OMICs approaches

i. Context and aim

As mentioned previously, *C. albicans* shares its anatomical niches with numerous bacterial species from the microbiota, that form together an ecosystem able to prevent infections by various pathogens, including fungi. It is thus acknowledged that specific bacterial species could be used to curb *C. albicans* growth before the emergence of an infection. In the recent years, *in silico* and experimental approaches have allowed an ever-growing understanding of the mechanisms that support, in the gut and vaginal microbiota, the host's resistance to colonization by *C. albicans*. In particular, the emergence of next-generation sequencing (NGS) and metabolomics approaches has made it possible to accumulate an ever-growing amount of data. Therefore, in my thesis, I became interested in the methods available for generating and, above all, analysing these types of data, with the aim of identifying bacterial species with antifungal potential. In this context, I wrote a literature review that is currently under review in *Trends in Microbiology*, to highlight and hopefully answer these questions. This review, presented in the section below, takes a multidimensional look on the research of microbiome-based anti-*C. albicans* strategies, by describing not only the recent literature on the experimental approaches available to identify or validate bacterial species with potential antifungal activities, but also by highlighting recent approaches based on cutting-edge NGS- and metabolomic-based technologies

ii. Submitted review

Finding microbiome-based antifungal strategies: impact of OMICs approaches

Contains: review under review in *Trends in Microbiology*

Margot Delavy[1], Natacha Sertour[1], Christophe d'Enfert[1] and Marie-Elisabeth Bougnoux[1,2]

[1] Institut Pasteur, Université Paris Cité, INRAE USC2019, Unité Biologie et Pathogénicité Fongiques, Paris, France.

[2] Assistance Publique des Hôpitaux de Paris (APHP), Hôpital Necker-Enfants-Malades, Unité de Parasitologie-Mycologie, Service de Microbiologie Clinique, Paris, France

Keywords: *Candida albicans*, microbial signatures, OMICs approaches, microbiota

Highlights

- *Candida albicans* is an opportunistic fungal pathogen that can cause superficial and invasive infections.

- 75% of women suffers from vaginal candidiasis at least once in their lifetime, 372 million women are currently suffering from recurrent vulvovaginal candidiasis and 700'000 cases of invasive candidiasis are reported yearly, with an associated mortality of 30-40%.

- Bacteria from the gut and vaginal microbiota can control *C. albicans* growth by the release of antifungal metabolites, modulation of the host immune response

and/or competition for nutrients, niches, and adhesion sites.

- Cutting-edge OMICs-based pipelines, relying on metagenomics and/or metabolomics have permitted the identification of bacterial species and metabolites with potential antifungal activities.

Abstract

In silico and experimental approaches have allowed an ever-growing understanding of the interactions within the microbiota. For instance, recently acquired data have increased knowledge of the mechanisms that support, in the gut and vaginal microbiota, the resistance to colonization by *Candida albicans*, an opportunistic pathogen whose overgrowth can initiate severe infections in immunocompromised patients. Here, we review how bacteria from the microbiota interact with *C. albicans.* We show how recent OMICs-based pipelines, using metagenomics and/or metabolomics, have identified bacterial species and metabolites modulating *C. albicans* growth. We finally discuss how the combined use of cutting-edge OMICs-based and experimental approaches could provide new means to control *C. albicans* overgrowth within the microbiota and prevent its consequences.

Interactions between *Candida albicans* and the microbiota

Candida albicans is an opportunistic pathogen that causes superficial infections such as vaginal candidiasis which affects 75% of women during their lifetime (Rosati *et al.* 2020; Denning *et al.* 2018). When the host's defenses are compromised, as in immunocompromised patients, *C. albicans* can cause systemic infections, associated to a mortality of 30-40% (Brown *et al.* 2012; Zhai *et al.* 2020; Pappas *et al.* 2018).

However, *C. albicans* is also a commensal yeast that colonizes the gastrointestinal (GI) tract of up to 95% of the healthy population (Delavy *et al.* 2022). It belongs to the healthy oral, vaginal and intestinal human microbiota and shares a niche with thousands of bacterial species (d'Enfert *et al.* 2021), some of which can prevent infections by various pathogens (Sonnenborn 2016; Deriu *et al.* 2013). Bacteria from the gut microbiota act as fungal growth regulator, the depletion of the intestinal bacterial microbiota by broad-spectrum antibiotics resulting in an increase of the fungal

burden and *C. albicans* carriage in the mice and human gut (Dollive *et al.* 2013; Delavy *et al.* 2022; Seelbinder *et al.* 2020). Specific bacteria have been shown to modulate *C. albicans* growth (for review, see (Li *et al.* 2022)). Especially, *Lactobacillus rhamnosus* strain Lcr35 inhibits *C. albicans* growth *in vitro* and *in vivo* (Coudeyras *et al.* 2008; Poupet *et al.* 2019; Dausset *et al.* 2020). Bacteria might even play a role in *C. albicans* systemic infections since immunocompromised patients experience a loss in bacterial diversity before *C. albicans* overgrowth and translocation into the bloodstream (Zhai *et al.* 2020).

Therefore, specific bacteria might be used to curb *C. albicans* growth before the emergence of an infection. However, few studies have aimed to identify new bacterial species – or bacterial signatures – with a potential anti-*C. albicans* activity. Here, we examine how bacteria might inhibit *C. albicans* growth and how recent OMICs-based studies have allowed the identification of such species. Moreover, we discuss the limitations and the experimental validations required for such approaches.

Modes-of-action of *C. albicans* inhibition: how can bacteria modulate *C. albicans* growth?

Bacteria can inhibit *C. albicans* growth through the release of metabolites, such as short-chain fatty acids (SCFA) (Nguyen *et al.* 2011; Guinan *et al.* 2019). The decrease of bacteria-derived SCFA after antibiotics exposure has been associated to an increase of *C. albicans* intestinal carriage (Guinan *et al.* 2019). Butyrate, especially, impairs *C. albicans* growth (Nguyen *et al.* 2011) and hyphae formation (García *et al.* 2017). Besides SCFA, the effect of lactate on *C. albicans* growth has been questioned, due to numerous reports of the potential anti-*C. albicans* activity of lactic acid-producing bacteria and to this metabolite presence in the vaginal niche (Liang *et al.* 2016; Jang *et al.* 2019; Zeise, Woods, and Huffnagle 2021).

A second mode-of-action for *C. albicans* growth inhibition is the stimulation of the host immune defenses by bacteria. The microbiota, especially in the gut, can train and shape the host's immune systems (D. Zheng, Liwinski, and Elinav 2020; Gensollen *et al.* 2016). Therefore, specific bacteria might modulate the host immune response against *C. albicans,* leading to a limitation of its growth. SCFA can modulate host inflammation and promote immune cells recruitment and maturation, leading to *C. albicans* reduced survival (Nguyen *et al.* 2011). Bacteria can also modulate *C. albicans*

gut colonization by activating mucosal immune effectors. For instance, *Bacteroides thetaiotaomicron* protects mice against *C. albicans* gut colonization and invasive infections by activating HIF-1α, a transcription factor expressed in intestinal epithelial cells, leading to the secretion of antimicrobial peptides (Fan *et al.* 2015). Alternatively, *Lactobacillus crispatus* activates the epithelial immune response against *C. albicans* by modulating the expressions of TLR2 and TLR4 toll-like receptors in epithelial cells, thus inducing the production of cytokines and β-defensins (Rizzo, Losacco, and Carratelli 2013).

Besides releasing metabolites with a direct effect against *C. albicans* or modulating the host immune response, commensal bacteria regulate *C. albicans* growth through competition for niches, nutrients and/or for adhesion to epithelium receptors. *Lactobacillus rhamnosus* thus protects the host against *C. albicans* infections via a competition for carbon and nitrogen sources (Alonso-Roman *et al.* 2022; Mailänder-Sánchez *et al.* 2017), and by blocking adhesion sites, thus reducing *C. albicans* ability to bind to epithelial cells (Mailänder-Sánchez *et al.* 2017).

Potential anti-*C. albicans* bacterial signatures identified by OMICs-based approaches.

Considering the various ways by which microbes can inhibit fungal growth, identifying bacterial species with a potential anti-*C. albicans* activity by experimental works alone can be tedious. OMICs-based approaches thus offer an alternative for this identification.

Two main types of OMICs-based approaches have been used to identify bacterial species associated to *C. albicans* carriage: (i) sequencing-based approaches that rely on sequencing data and consist of associating *C. albicans* carriage to the relative abundance of the bacterial species present in the same niche (Seelbinder *et al.* 2020; Xie and Manichanh 2022; Fan *et al.* 2015), and (ii) metabolomics-based approaches, in which *C. albicans* carriage or metabolic profile is associated to the metabolome of the microbial population present in the same environment (Mirhakkak *et al.* 2021; Gutierrez *et al.* 2020). In the first approach, microbial sequences are acquired with targeted metagenomics sequencing, whole-genome shotgun metagenomics sequencing, or a mix of both. Targeted metagenomics - or amplicon sequencing - relies on the amplification of a specific region, usually the ribosomal DNA (rDNA) 18S or ITS

regions for fungal DNA, and the rDNA 16S region for bacterial DNA, whereas untargeted shotgun sequencing relies on sequencing the full genomes composing the microbiome (Fig. 1).

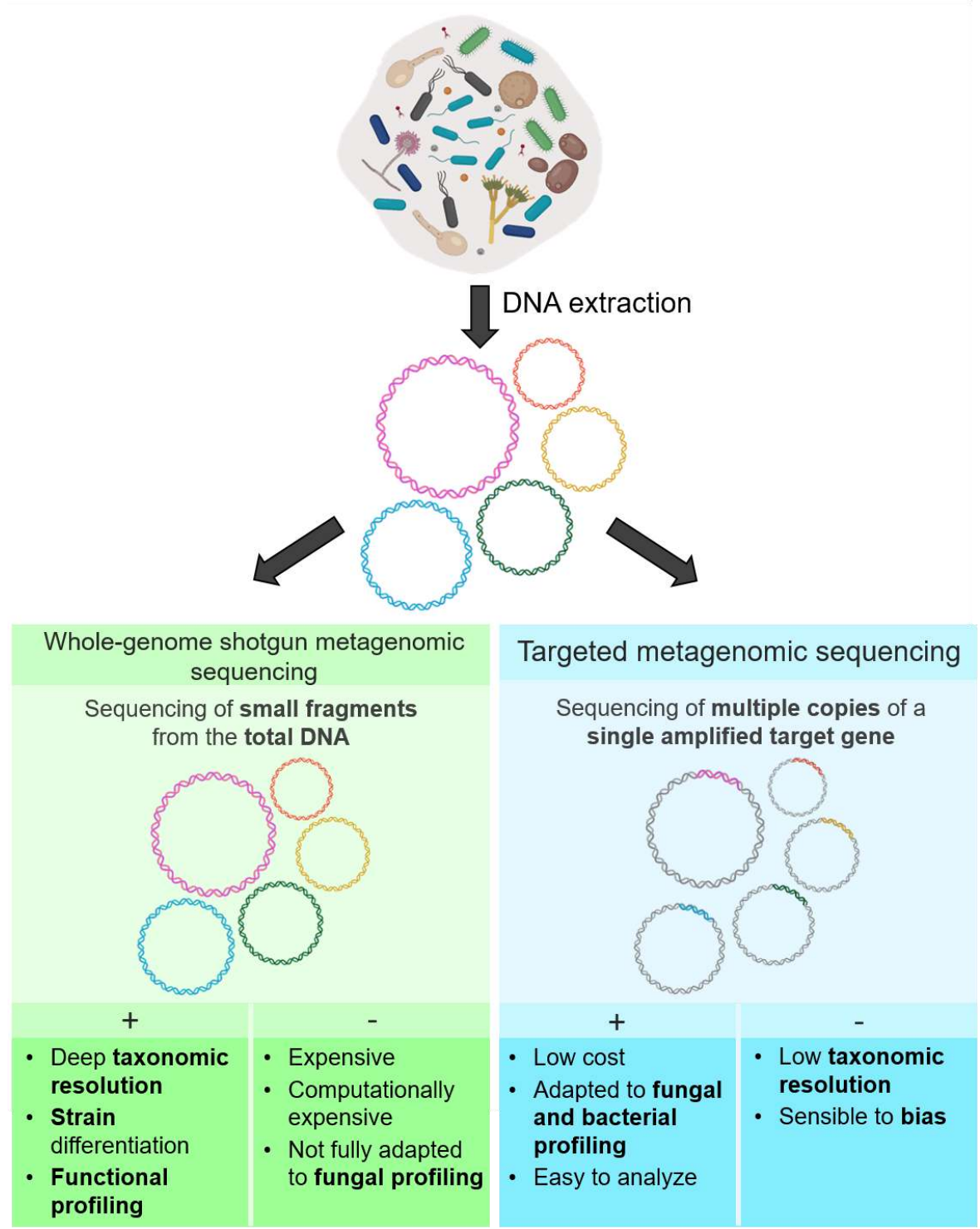


Figure Review 1: Comparison of whole-genome shotgun metagenomic sequencing and targeted metagenomic sequencing. Whole-genome shotgun sequencing consists in sequencing the full genomes present in the microbiota, whereas targeted metagenomic sequencing consists in the amplification and subsequent sequencing of a specific genomic region.

Shotgun sequencing offers a deep taxonomic resolution, allowing an easier distinction between microbial species and strains, but at a high cost (Peterson *et al.* 2021; Durazzi *et al.* 2021) and although some recent studies have proposed new pipelines, shotgun metagenomics is still at a developing stage for the characterization of the fungal microbiota (Xie and Manichanh 2022; Lind and Pollard 2021). Amplicon-based sequencing is cheaper but returns less accurate results. Moreover, the amplification of 18S and ITS regions can introduce analysis bias since rDNA copy number is highly variable across the fungal kingdom, leading to an uneven quantification of the different fungal species (Lofgren *et al.* 2019). Such variation in rDNA copy number has also been observed across bacterial taxa but at a much lower extent (Větrovský and Baldrian 2013). In the second approach, metabolomics profiles are obtained experimentally (Seelbinder *et al.* 2020; S. Han *et al.* 2021; Krautkramer, Fan, and Bäckhed 2021) or predicted *in silico* using genome-scale metabolic models (GEMs), a computational description of the metabolic pathways of a given organisms (Mirhakkak *et al.* 2021; Zampieri *et al.* 2019). GEMs have been constructed and experimentally validated for hundreds of bacteria, archaea and eukaryotes (Feist *et al.* 2009; Mirhakkak *et al.* 2021).

Xie and Manichanh study is a fitting example of the sequencing-based approach; the authors analyzed a publicly accessible set of metagenomes obtained from gut samples of healthy individuals from Spain and Denmark to generate an inter-kingdom association network, using SparCC correlations (Friedman and Alm 2012). They identified 20 bacterial species negatively associated to *C. albicans* in the Spanish cohort and 17 in the Danish cohort. Notably, in the Spanish cohort, *C. albicans* was strongly negatively correlated to *Bifidobacterium scardovii*, *Desulfovibrio fairfieldensis*, *Ruminococcus sp CAG563*, *Coprococcus catus*, and *Roseburia sp CAG309,* most of these bacteria being probable SCFA producers. However, the correlations were cohort specific.

Meanwhile, the study performed by Seelbinder *et al.* allowed the identification of potential anti-*C. albicans* bacteria by combining sequencing- and metabolomics-based approaches (Seelbinder *et al.* 2020). First, the authors integrated shotgun and ITS-targeted sequencing and created a co-abundance network, using BAnOCC (Schwager *et al.* 2017), a Bayesian method, to study the inter- and cross-kingdom interactions of the human gut upon various antibiotic treatments. They identified three bacterial

species negatively associated to *C. albicans* abundance: *Odoribacter splanchnicus, Roseburia inulinivorans* and *Eubacterium rectale*. In parallel, they searched for bacteria likely to produce metabolites with an anti-*C. albicans* activity. They established the bile acids and metabolites profiles of a subset of the subjects and searched for correlations between the abundance of each metabolite and *C. albicans* relative abundance, estimated by ITS-targeted sequencing, before associating the concentrations of the metabolites of interest with the bacterial species relative abundance, inferred from the shotgun sequencing data. The predicted inhibiting bacterial species included *Faecalibacterium prausnitzii, Bacteroides eggerthi, Alistipes obesi, Odoribacter splanchnicus, Coprococcus comes, Roseburia inulinivorans* and *Eubacterium rectale*.

In contrast, Mirhakkak *et al.* identified bacteria of interest by implementing a metabolomics-based approach complemented with a sequencing analysis (Mirhakkak *et al.* 2021). To investigate the interactions between *C. albicans* and the intestinal bacteria, they constructed a *C. albicans* GEM and coupled it to 910 published gut bacteria GEMs. They thus predicted how intestinal bacteria might modulate essential *C. albicans* metabolic pathways, and identified several potential anti-*C. albicans* bacterial signatures, including species from the *Bifidobacterium* and *Listeria* genera and species from the *Bacteriodetes* phylum, including *Alistipes putredinis.* To strengthen their findings, they performed metagenomics analyses of the gut microbiota of 24 cancers patients. By computing spearman correlations between *C. albicans* relative abundance, estimated from ITS-targeted sequencing, and the gut bacteria relative abundances, estimated by shotgun sequencing, they highlighted two bacteria with a potential antagonistic activity against *C. albicans: Barnesiella intestinihominis* and *Alistipes putredinis.*

Although several potential anti-*C. albicans* bacteria were identified in each of the studies presented above (Fig. 2), there is a poor overlap between the studies. This might be explained by the different technics and sequencing approaches used. First, the choice of a sequencing approach is crucial since different methods might return different microbial profiles, and thus different anti-*C. albicans* bacterial signatures.

Consequently, it is probably not surprising that the bacteria highlighted by Xie and Manichanh, who used shotgun sequencing to estimate both bacterial relative

abundance and *C. albicans* carriage, are distinct from the one identified in the study conducted by Seelbinder *et al.,* which used ITS-targeted sequencing to quantify *C. albicans* burden.

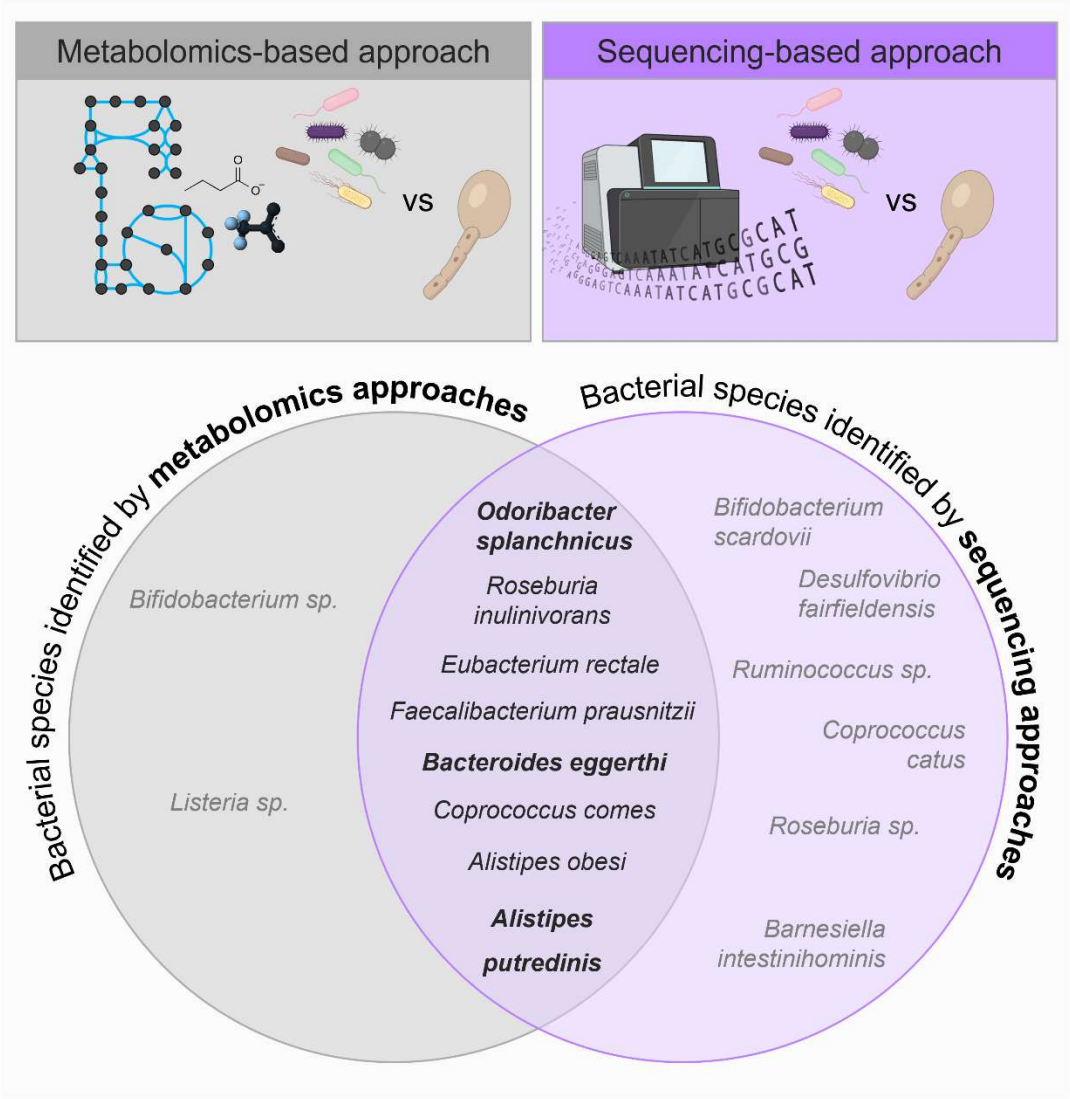


Figure Review 2: Anti-*Candida albicans* signatures identified by metabolomic-based and sequencing-based approaches. The bacterial species identified only by a sequencing-based analysis are represented in the right circle, the bacterial species identified only by a metabolomic-based analysis are represented in the left circle. Bacterial species identified both by a metabolomic- and a sequencing-based approach are represented in the intersection between the two circles. The anti-*C. albicans* activity of the bacteria highlighted in bold was validated in an in vitro assay. It should be noted that none of these species were identified in two of the presented study.

In addition, the sequencing-based approaches used by Xie and Manichanh and Seelbinder and colleagues are based on co-abundance analyses (Xie and Manichanh 2022; Seelbinder *et al.* 2020). These correlations cannot distinguish the direction of a

potential inhibition. Some of the bacteria identified might thus be inhibited by *C. albicans* rather than having themselves an antagonistic activity. Besides, a correlation does not always translate into causation and some associations identified might be coincidental. The samples and cohorts used in the analysis might also be a determining factor. All studies used fecal samples (Xie and Manichanh 2022; Seelbinder *et al.* 2020; Mirhakkak *et al.* 2021), but they were collected from different types of volunteers, with Xie and Manichanh using fecal samples from healthy volunteers whereas Seelbinder *et al.* and Mirhakkak and colleagues used fecal samples from adults treated with antibiotics and from cancer patients, respectively. Since antibiotics kill the resident bacteria of the gut, a part of the negative associations between bacterial species and *C. albicans* might results from false-positive signals, due to the overall decrease of bacterial abundance and overall increase of *C. albicans* carriage observed after antibiotics. Moreover, certain cancers are characterized by specific gut microbiota profiles (Narunsky-Haziza *et al.* 2022; Dohlman *et al.* 2022), which might also explain why the bacterial species identified by Mirhakkak and colleagues in the sequencing part of their study were not identified in the two other studies. Finally, some differences in the anti-*C. albicans* bacterial signatures identified might originate from the differences between the sequencing-based and the metabolomics-based approaches. Indeed, the use of GEMs in Mirhakkak studies highlighted the metabolic interactions between the intestinal bacteria and *C. albicans* (Mirhakkak *et al.* 2021). By highlighting anti-*C. albicans* metabolites, this approach offers a mode-of-action behind the potential antagonistic effect of the bacterial species on *C. albicans.* However, metabolic interactions represent only a subset of the mechanisms by which bacteria can modulate *C. albicans* growth. Considering the limitations associated with each study, it is essential to validate *in vitro* or *in vivo* any signature identified by OMICs approaches.

Experimental validation of anti-*C. albicans* bacterial signatures identified by OMICs-approaches: current approaches and future development.

The validation of the potential antagonistic activity of a bacterium on *C. albicans* depends on the inhibition mechanism. Thus, the choice of a validation assay is crucial. In this part, we review four approaches that have been or could be used to evaluate the antifungal activity of specific bacteria: (i) supernatant-based inhibition assays, (ii) murine models, (iii) fermentation-based systems and (iv) organs-on-a-chip.

Supernatant-based inhibition assays

A first experimental validation is the assessment of the effect of bacterial supernatants on *C. albicans* growth. This is a widespread approach to validate or screen microbial species for inhibition against *C. albicans* (Ricci *et al.* 2022; Parolin *et al.* 2022; Mirhakkak *et al.* 2021; Seelbinder *et al.* 2020) or other fungal (Mani-López, Arrioja-Bretón, and López-Malo 2022) and bacterial species (Mani-López, Arrioja-Bretón, and López-Malo 2022; Santos *et al.* 2022). In particular, Walker and colleagues have recently shown the anti-*C. albicans* activity of *Bifidobacterium adolescentis* using such approach (Ricci *et al.* 2022). Seelbinder *et al.* and Mirhakkak *et al.* both used a similar approach to validate *in vitro* at least some of the bacterial species they identified (Seelbinder *et al.* 2020; Mirhakkak *et al.* 2021). Seelbinder and colleagues measured how the culture supernatant of two of their candidate bacterial strains impacted *C. albicans* growth *in vitro* and demonstrated that sterilized supernatants from *Bacteroides eggerthii* and *Odoribacter splanchnicus* could inhibit *C. albicans* growth by 50% and 40% respectively. By a similar approach, Mirhakkak *et al.* showed that *C. albicans* growth was reduced when it was co-cultured with spent media from *Alistipes putredinis* (Mirhakkak *et al.* 2021). These *in vitro* assays, that bring bacteria or the metabolites they produce into contact with *C. albicans* in a growth medium or on epithelial cells allow the detection of an inhibition caused by the release of small metabolites and are essential to have a first understanding of their potential anti-*C. albicans* activity. However, these assays do not reproduce the complexity of *C. albicans* natural niches, especially the human gut. Ideally, the biological effect of bacterial signatures on *C. albicans* should be tested within a system that considers the interactions between these bacteria and the microbiota and their ability to efficiently colonize the target niche, since this ability can itself determine if these bacteria can modulate *C. albicans* growth. In addition, most of the bacterial species with a known anti-*C. albicans* activity are obligate anaerobes, as are more than 99% of the gut bacteria (Guarner and Malagelada 2003), whereas *C. albicans* growth is optimal in aerobic conditions (Biswas and Chaffin 2005). We thus need a system that can include an oxygen gradient, such as the one naturally found in the gut, at least for the validation of bacteria originating from this niche.

Murine models

The mouse is another desirable model to study the factors behind *C. albicans* colonization since it reproduces the main human characteristics (Neville, d'Enfert, and Bougnoux 2015). Murine models have been developed to study *C. albicans* colonization of the vaginal (Miao, Willems, and Peters 2021; Jang *et al.* 2019) and intestinal (Neville, d'Enfert, and Bougnoux 2015; Fan *et al.* 2015) tract, and the interactions between *C. albicans* and vaginal or intestinal bacteria (Fan *et al.* 2015; Jang *et al.* 2019). Mice are naturally resistant to *C. albicans* GI colonization (Fan *et al.* 2015), thus forcing the experimenter to use antibiotics or a specific diet to implement *C. albicans* in the GI tract. While this does not reproduce a healthy human gut (Neville, d'Enfert, and Bougnoux 2015), a part of the issue can be circumvented by the use of germ-free mice, since they lack a *C. albicans*-inhibitory microbiota. Using germ-free mice allows the implementation of a controlled microbiota, such as what is observed in humans. Murine models can be used to determine if a candidate anti-*C. albicans* bacterial species – or a consortium of bacteria – can prevent *C. albicans* colonization. However, identifying by which mechanisms bacteria modulate *C. albicans* growth remains a challenge, the use of living animals limiting the dynamic monitoring of *C. albicans* colonization since it relies on endpoint measurements that often require the animals sacrifice. This and the fact that animal models are a rising concern that still causes ethical issues (Kiani *et al.* 2022), might lead researchers to develop additional platforms to use instead, or in combination, with murine models.

Fermentation-based system - SHIME®

Fermentation-based systems are powerful *in vitro* tools mimicking the human gut properties. They consist in a single static (Walker *et al.* 2005) or multi-stage fermentation models (Payne *et al.* 2003; Wynne *et al.* 2004; Van Den Abbeele *et al.* 2010). Continuous fed-batch models have already been used to demonstrate the anti-*C. albicans* activity of *Lactobacillus plantarum* (Payne *et al.* 2003; Wynne *et al.* 2004).

The SHIME® (Simulator of Human Intestinal Microbial Ecosystem) is a multi-compartment semi-continuous fed-batch system, originally developed in Ghent University and currently further developed and commercialized by the company ProDigest, that simulates the different sections of the GI tract from the stomach or small intestine to the distal colon (Van Den Abbeele *et al.* 2013). It can also include a

mucosal compartment (d'Enfert *et al.* 2021; Van Den Abbeele *et al.* 2013) and a Host-Microbiota Interaction module that allow co-culturing complex bacterial communities with a monolayer of enterocyte human cells (Marzorati *et al.* 2014).

SHIME® studies have focused on the bacterial component of the gut microbiota but we can expect, before long, an application for the interactions between *C. albicans* and commensal bacteria. Indeed, in the context of the FunHoMic consortium (FunHomic consortium 2023.), ProDigest is developing a SHIME® model to assess the interplay between fungi and bacteria, with a specific focus on *C. albicans* (Marsaux and Marzorati, personal communication). Such model would be particularly relevant to study the impact of a single or a cocktail of bacteria on *C. albicans* gut colonization, since it would allow following not only the bacteria and C*. albicans* growth, but also the levels of molecules with potential anti-*C. albicans* activity secreted into the medium. In addition, such model could infer the nutrient and niche competition that could modulate the interactions between specific bacteria and *C. albicans.* Moreover, the SHIME® can be adapted to host microbial communities from specific populations, such as infants, toddlers, or adults based on the faecal samples used to set up the system (Natividad *et al.* 2022; Van den Abbeele *et al.* 2021), thus allowing testing the effects of specific bacteria on *C. albicans* colonization various populations.

Such model is evidently limited to the simulation of the GI tract niche and other models need to be developed to study *C. albicans* colonization of the vaginal and oral niches, and it does not integrate immune cells, thus limiting the identification of a potential immuno-stimulatory role of the bacteria against *C. albicans.*

Ex vivo models – organ-on-a-chip

Ex vivo models, relying on the culture of human epithelial cells, are alternative tools that could be developed to study *C. albicans* interactions with bacteria. These systems have been widely used to explore *C. albicans* colonization and interactions with mucosal surfaces (Graf *et al.* 2019; Alonso-Roman *et al.* 2022). However, they often use a single cell type, which does not reproduce the human physiology complexity. Moreover, most studies developed models highlighting *C. albicans* pathogenicity and invasion of the tissues, rather than its commensal state. An exception is the gut model developed by Graf *et al* (Graf *et al.* 2019). The authors added goblet cells within the epithelial layer to produce a mucus layer that greatly reduced *C. albicans*

pathogenicity. A bacterial community composed of *Lactobacilli* was implemented in the model, bringing a protection against *C. albicans* overgrowth and invasion (Graf *et al.* 2019). Such models are promising tools because they are relatively simple to use and cheaper than animal experiments. However, most of them lack an immune component. Fortunately, in recent years, more complex *in vitro* models, namely organ-on-a-chip, have emerged. These systems consist in a cell culture of one or several tissues contained into a microfluidic chip mimicking the key characteristic of a specific organ. Their main advantage over classical *ex vivo* cell models is their complexity, with several cell types, a tissue 3D arrangement on the chip and the integration of biomechanical cues such as intestinal peristalsis and/or oxygen gradient (Low *et al.* 2021; Ashammakhi *et al.* 2020; Leung *et al.* 2022). Moreover, they allow the inclusion of immune cells into the chip, thus simulating the host defence (Low *et al.* 2021; Ashammakhi *et al.* 2020; Leung *et al.* 2022). Gut-on-a-chip (for review, see (Ashammakhi *et al.* 2020)) and vagina-on-a-chip (Mahajan *et al.* 2022; Tantengco *et al.* 2022) systems are already available and could be used or optimised to study *C. albicans* interaction with bacteria. Organ-on-a-chip technology is also suitable for industrialization, and are currently developed by companies such as Mimetas (Mimetas 2019) and Emulate (Emulate 2023.). In the future, using commercial organ-on-a-chip to test fungal-bacterial infections might offer a promising complement or alternative to *in vitro* and *in vivo* assays.

Concluding remarks

OMICs-based approaches offer a convenient way for identifying commensal bacteria of the human GI and vaginal tracts that might modulate *C. albicans* growth. Although only a few studies have aimed to identify potential anti-*C. albicans* microbial species using such approaches, the analyses presented above contribute to a better understanding of *C. albicans* physiopathology and its interactions with the bacterial microbiota.

Although the identification of more anti-*C. albicans* bacteria is desirable, the experimental models available to study microbial interactions are still limited in their ability to reproduce accurately the multidimensionality of the human environment. Therefore, to identify such bacteria reliably, we require new or optimized experimental models, such as germ-free mice models, fermentation-based systems, such as the

SHIME®, and/or organ-on-a-chips (see Outstanding Questions Box). In parallel, it is crucial to democratize the use of multidimensional statistical tools, relying on machine-learning or deep-learning, to create complex models of microbial interactions that could explain the clearance of *C. albicans* in the microbiota. For now, OMICs-based approaches are mainly used to translate a network of microbial interactions into a simplified list of 1-to-1 interactions between a single microbial species and *C. albicans*. Although such approaches are essential to highlight microbial species with a potential anti-*C. albicans* activity, it is unlikely that a single bacterial species would have the potential to completely clear *C. albicans* from its niches. More complex statistical models would thus return a more complete overview of what is happening in the human body.

Finally, OMICs-based approaches, especially if they rely on complex algorithms, require large datasets, which are often costly and difficult to obtain. This is why we need to develop public databases of human microbiota and mycobiota, which could limit the risk of identifying cohort- or study-specific anti-*C. albicans* bacterial signatures.

Overall, although the combination of optimized *in silico* pipelines and experimental procedures could allow the identification of additional antifungal species, the current research has already identified dozens of bacterial species *in silico* with a potential anti-*C. albicans* activity, and several have been validated *in vitro*. While additional validations need to be performed to confirm the antifungal properties of these species, these discoveries might open the way to the development of a consortium of bacteria that would allow the recovery of a microbiota limiting *C. albicans* overgrowth, thus preventing the emergence or recurrence of vulvovaginal candidiasis, or life-threatening systemic infections.

Aknowledgments

We thank Massimo Marzorati and Benoît Marsaux for the fruitful discussions.

Outstanding Questions Box

- How can machine-learning and/or deep-learning approaches be used to extend our understanding of the interactions between the microbiota and *C. albicans*?
- How can OMICs-based strategies be combined to machine-learning and/or deep-learning approaches to develop a consortium of bacteria able to curb *C. albicans* growth in the intestinal and vaginal niches?
- Can OMICs-based strategies be adapted to identify bacterial species with an antagonistic activity against other fungal species, notably in the context of invasive infections or of fungi-associated cancers?
- What will be the role of public databases contained microbiota data in the context of the identification of antifungal microbial species and compounds?
- How to develop an *ex vivo* or *in vitro* model that mimics the key characteristics of the human gut, including host immune response and oxygen gradient?

E - INTERACTIONS BETWEEN *C. ALBICANS* AND THE HOST'S IMMUNITY

C. albicans maintenance in the human gut is the result of a complex interplay between this yeast and the host, in which the immune system plays a crucial role. In healthy individuals, the immune system restricts the growth of *C. albicans*, thus confining it to a commensal state. However, in the absence of an adequate immune response, proliferation of *C. albicans* becomes possible. This may be followed by a transition from yeast to hyphae, after which the hyphae may penetrate mucosal surfaces, causing damage to the underlying tissues and in the worst case, *C. albicans* may reach the bloodstream and disseminate through the body (Richardson and Moyes 2015; Brown *et al.* 2012, Fig. 6).

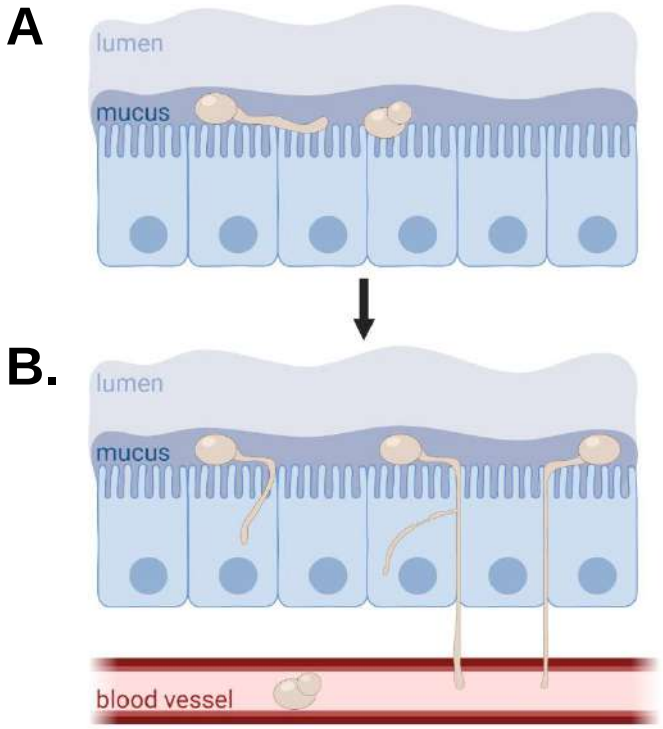


Figure 6: *C. albicans* invasion of the intestinal epithelium. (A). Adhesion and colonization to the epithelium and (B). Penetration of the hyphae into the epithelium and dissemination into the bloodstream. *Figure created with Biorender.com and inspired from Gow et al. 2012*

1. Host immune responses to *C. albicans*

C. albicans overgrowth on the mucosal surface and the subsequent invasion of the tissues is prevented by the host active surveillance and protection of the epithelial

barrier. This is possible thanks to the interplay between innate and adaptive immunity, two complementary but cooperative arms of the immune system (Richardson and Moyes 2015; d'Enfert *et al.* 2021). In addition, the epithelium, which is the primary site of interaction between *C. albicans* and the host, acts as a physical barrier by preventing a direct access to the bloodstream.

i. Innate immunity – *C. albicans* recognition

The first step in the defense against *C. albicans* infection is the recognition of the pathogen by epithelial cells and innate immune cells (Richardson, Ho, and Naglik 2018; Nikou *et al.* 2019; Verma, Gaffen, and Swidergall 2017; Naglik *et al.* 2017, Fig. 7). The myeloid cells of the innate immune system, including the monocytes, macrophages, and dendritic cells (DCs), harbour conserved pattern recognition receptors (PRRs) able to recognise specific pathogen-associated patterns (PAMPs). There are four families of PRRs that are expressed in different cell types and recognise different fungal PAMPs (Fig. 7): (i) Toll-like receptors (TLRs), (ii) C-type lectin receptors (CLRs), (iii) nucleotide oligomerisation domain (NOD)-like receptors (NLRs) and (iv) retinoic-acid-inducible gene I (RigI)-helicase receptors (RLRs).

Toll-like receptors (TLRs). The main TLRs involved in fungal recognition are TLR2, TLR4 and TLR9. TLR2 and TLR4 recognise different mannoproteins whereas TLR9 recognises fungal DNA and chitin (Naglik *et al.* 2017; Wagener *et al.* 2014). TLR-mediated PAMP recognition triggers MyD88-dependent and TRIF signalling pathways in innate immune cells, which regulate the inflammatory response (Kawasaki and Kawai 2014; Swidergall 2019).

C-type lectin receptors (CLRs). Dectin-1, Dectin-2, Macrophage inducible Ca^{2+}-dependent lectin receptor (MINCLE), dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and the mannose receptor (MR) are CLRs able to recognise *C. albicans* (Dambuza *et al.* 2017; Goyal *et al.* 2018; Swidergall 2019; Tong and Tang 2017). Dectin-1 can recognise β-glucans on *C. albicans* cell wall and trigger the spleen tyrosine kinase (SYK)/caspase activation and recruitment domain-containing 9 (CARD9) pathway (Cohen-Kedar *et al.* 2014). By activating the Nuclear Factor-kappa B (NFκB), this pathway triggers the production of cytokines and chemokines, thus activating an immune response in human intestinal epithelial cells (Cohen-Kedar *et al.* 2014). Moreover, Dectin-1 is involved in the induction of

phagocytosis and the activation of the inflammasome (Kankkunen *et al.* 2010; Swidergall 2019), which leads to the release of pro-inflammatory cytokines (Camilli *et al.* 2020). Dectin-2, MINCLE, DC-SIGN and MR recognise mannoproteins. In particular, Dectin-2 recognises α-mannans and induces the formation of Neutrophil Extracellular Traps (NET), a network of DNA fibres that bind and kill pathogens (S. Y. Wu *et al.* 2019; Papayannopoulos 2018), while Dectin-3 forms heterodimers with Dectin-2 to bind the α-mannans of *C. albicans* hyphae (Erwig and Gow 2016; Dambuza *et al.* 2017).

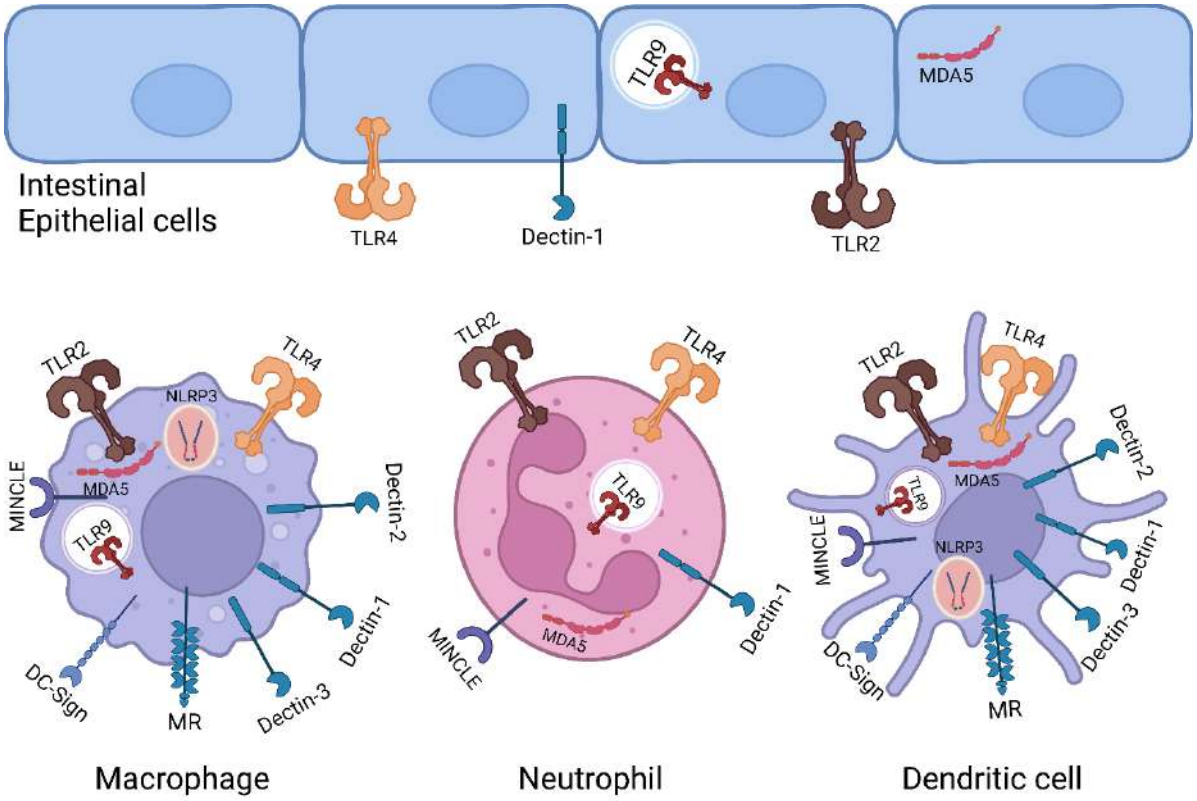


Figure 7: Pattern recognition receptors expressed in intestinal epithelial cells, macrophages, neutrophils, and dendritic cells. TLR2, TLR4 and TLR9 are toll-like receptors. Dectin-1, dectin-2, dectin-3, MINCLE, DC-Sign and MR are C-type lectin receptors. NLRP3 is a NOD-like receptor and MDA5 is a RIGI-helicase receptor. TLR: toll-like receptors, MINCLE: macrophage inducible Ca^{2+}-dependent lectin receptor**,** DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin**,** MDA5: melanoma differentiation-associated factor 5, MR: mannose receptor. NLRP3: NOD-like receptor family pyrin domain-containing 3. *Figure created with Biorender.com.*

NOD-like receptors (NLRs). NLRs interact with an adapter molecule, the apoptosis-associated speck-like protein containing a C-terminal CARD (ASC) and the inflammatory protease caspase-1, to form the inflammasome (Latz, Xiao, and Stutz

2013). In humans, *C. albicans* activates the NOD-like receptor family pyrin domain-containing 3 (NLRP3) and NOD-like receptor family CARD domain-containing protein 4 (NLRC4) (Camilli *et al.* 2020). Candidalysin, a cytolytic peptine toxin secreted by *C. albicans*, has been shown to trigger the NLRP3 inflammasome activation after priming by fungal β-glucans (Kasper *et al.* 2018; Rogiers *et al.* 2019)

RIGI-helicase receptors (RLRs). RLRs are cytoplasmic PRRs that recognize non-self RNA and activate the innate immunity (Thompson, Stone, and Gale 2016). The melanoma differentiation-associated factor 5 (MDA5) is triggered by *C. albicans* hyphae and might recognise fungal DNA (M. Jaeger *et al.* 2015).

ii. Innate immunity – anti-*C. albicans* immune response

Recognition of *C. albicans* by the innate immune system triggers a myriad of responses to clear the pathogen (Fig. 8).

Macrophages and neutrophils. Macrophages and neutrophils are key players of the innate immunity, notably by their ability to phagocytose microbial targets. Neutrophils are more abundant and offer a more rapid response than macrophages, but their lifespan is shorter. The macrophages originate from blood-circulating monocytes. They conduct a constant surveillance at the surface of the epithelium and can target microbial species by chemotaxis (Erwig and Gow 2016).

When *C. albicans* mannoproteins and/or β-glucans are recognised by PRRs such as TLR4 (Netea *et al.* 2002), MR (Porcaro *et al.* 2003; Netea *et al.* 2008) and Dectin-1 (Herre *et al.* 2004) or when phagocytic receptors such as complement receptor 3 (CR3) and Fcγ receptors (FcγR) recognize opsonised *C. albicans* (Netea *et al.* 2008), macrophages and neutrophils can engulf the fungi (Brown 2011). *C. albicans* can then be killed by the acidification of the phagolysosome, a lack of nutriment and the secretion of a cocktail of proteases, reactive oxygen species (ROS) and anti-microbial peptides (AMPs) (Miramón *et al.* 2012; Erwig and Gow 2016; G. Small *et al.* 2019). The engulfment of *C. albicans* by macrophages can trigger the yeast transition to hyphae, which activates the NLRP3 inflammasome. This leads to the release of pro-inflammatory interleukin (IL)-1β and lL-18 and thus promotes the activity of helper lymphocytes T (Th) 1 and Th17 (Kasper *et al.* 2018; van de Veerdonk *et al.* 2011; Rogiers *et al.* 2019, see *Adaptive immunity*). However, transition to hyphae allows *C. albicans* to escape the macrophages. Indeed, *C. albicans* can use pore-forming

proteins, such as candidalysin, which is released by the hyphae, and Gasdermin D, a host protein that is activated by the NLRP3 inflammasome, to cause the lysis of the macrophage (Olivier *et al.* 2022).

Besides phagocytosis, neutrophils can promote fungal clearance by the formation of NETs, as mentioned above, and through degranulation, a phenomenon in which granules containing AMPs are released and used to kill the pathogen (Rosales 2018; Gierlikowska *et al.* 2021).

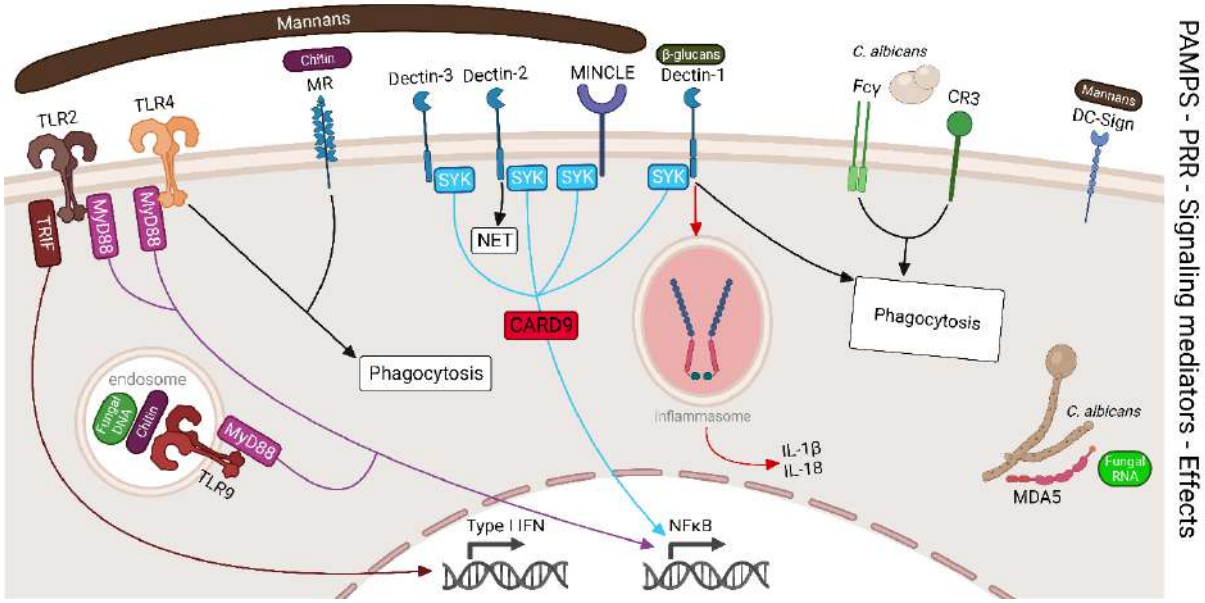


Figure 8: Pattern recognition receptors and innate immunity pathways involved in response to *C. albicans*. Fungal pathogens associated molecular pattern (PAMPs) are recognized by specific pattern recognition receptors (PRRs). The recognition of *C. albicans* by the PRRs triggers different specific responses. Toll-like receptors such as TLR2, TLR4 and TLR9 trigger the production of pro-inflammatory cytokines via the MyD88 and TRIF pathway. Dectin-1, dectin-2, dectin-3 and MINCLE induce a spleen tyrosine kinase (SYK)/ caspase activation and recruitment domain-containing 9 (CARD9) response. Dectin-1 is also involved in the activation of the inflammasome, which leads to the production of interleukin 1β (IL-1β) and IL-18. TLR4, dectin-1, Fcγ and CR3 induce phagocytosis and subsequent killing of the fungal pathogen. MR: mannose receptor, IFN: interferon, NFκB: nuclear factor- κB. *Figure created with Biorender.com and inspired from Netea et al. 2015.*

Host-cell signalling. The recognition of *C. albicans* PAMPs by PRRs also triggers host-cell signalling, which results in a cascade of effector responses, which are cell and tissue type-dependent (d'Enfert *et al.* 2021). The epithelial cells release AMPs that act against *C. albicans* in various ways. For instance, by binding to *C. albicans* carbohydrates, LL-32 can reduce *C. albicans* adhesion (Tsai *et al.* 2011; Chang *et al.* 2012), induce massive membrane disruption (Den Hertog *et al.* 2005) and lead to efflux

of ATP and small molecules (Den Hertog *et al.* 2005). Histatins have been shown to accumulate in *C. albicans* cells, inducing ROS and efflux of ions and ATP, resulting in cell death (Kumar *et al.* 2011; Koshlukova *et al.* 2000; Helmerhorst, Troxler, and Oppenheim 2001). Finally, β-defensins kill *C. albicans* by permeabilization of its cell membrane (Krishnakumari, Rangaraj, and Nagaraj 2009).

In the gut, mucins produced by goblet cells limit *C. albicans* yeast-to-hyphae transition, surface adherence and ability to form biofilm, thus reducing *C. albicans* ability to invade and damage the epithelium (Kavanaugh *et al.* 2014).

If *C. albicans* succeeds in colonizing the epithelium, the subsequent damage caused by the secretion of candidalysin by *C. albicans* hyphae leads to the release of danger-associated molecular patterns (DAMPs) or alarmins by the epithelial cells. These molecules can then signal the presence of *C. albicans* to the patrolling immune cells (Yang and Oppenheim 2009; Swidergall *et al.* 2019). In addition, epithelial cells can produce pro-inflammatory cytokines including IL-1β, IL-6, IL-8, granulocyte-colony stimulating factor (G-CSF), Tumor necrosis factor (TNF), and IL-36.

Natural killer cells. Natural killer (NK) cells play a crucial role in modulating the innate immune response and have an impact on the adaptive immune system. Human NK cells are able to recognise *C. albicans* directly. They exert antifungal activity through two main mechanisms: first through the release of cytokines such as G-CSF, interferon (IFN)-γ and TNF-α, allowing the recruitment of specialized immune cells including neutrophils, DCs, macrophages and T cells, and second, through the secretion of anti-microbial molecules (Voigt *et al.* 2014). NK cells have been shown to be particularly important in the immunocompromised host, but they seem to be redundant and even trigger a hyperinflammatory state in immunocompetent mice (Quintin *et al.* 2014).

Dendritic cells maturation and activation. DCs appear to serve as a link between the innate and adaptive immune system. They are involved in different processes of both the innate and the adaptive response. Indeed, DCs can recognise fungal pathogens, including *C. albicans*, through the PRRs expressed at their surface. They secrete cytokines, can use phagocytosis to engulf pathogen particles and induce the adaptive immune response by presenting antigens to T cells (Ramirez-Ortiz and Means 2012). Immature DCs play a surveillance role by patrolling the mucosae and can be recruited to the infection site by the chemokines and AMPs secreted by the

epithelial cells. Once recruited, DCs can directly recognise *C. albicans* PAMPs and phagocytose the fungal cells. This phagocytosis allows the degradation of fungal proteins into antigenic peptides. These fungal antigens can then be assembled on the major histocompatibility complex (MHC) class I or MHC-II and presented to naive $CD4^+$ and $CD8^+$ T cells, respectively, thereby activating the adaptive immune response (Richardson and Moyes 2015; Ramirez-Ortiz and Means 2012).

iii. Adaptive immunity - anti-*C. albicans* immune response

The adaptive immune response allows the implementation of a long-term protection through the establishment of an immunological memory. This adaptive immunity is mainly provided by T and B lymphocytes, with T lymphocytes and Th cells, in particular, supporting mucosal host defense and the innate immune response, and B lymphocytes being involved in antibody production. The adaptive immune response also has the difficult task to mediate the inflammatory response in order to maintain a balance between immune protection and levels of inflammation to limit immunopathological effects (Bacher *et al.* 2019).

Lymphocyte T response. Lymphocyte T-derived response is the main component of the mucosal immunity against *C. albicans.* $CD4^+$ T cells, also known as Th cells, are critical for a protective antifungal immunity (Borghi *et al.* 2014; Lionakis and Levitz 2018). There are four different Th subsets involved in anti-*C. albicans* immunity : Th1, Th2, Th17 and regulatory T cells (Treg), and their maturation from naive CD4+ cells is dependent on the presence of specific cytokines in the environment when the priming occurs (Richardson and Moyes 2015, Fig. 9). Th1 and Th17 secrete inflammatory cytokines, including IFN-γ and IL-17A/F for Th1, and IL-22 for Th17. IL-17A and IL-17F are responsible for the recruitment and activation of the neutrophils while IL-22 enhances the epithelial barrier function. Unsurprisingly, the inflammasome is crucial for the activation and maturation of Th1/Th17 via the secretion of IL-1β. In opposition, Th2 is mainly activated by IL-2 and is known to counter regulate the Th1/Th17 response and it thus associated with *C. albicans* persistence (Borghi *et al.* 2014; Lionakis and Levitz 2018; Richardson and Moyes 2015; Leigh *et al.* 1998). Finally, Treg ensure the maintenance of homeostasis between Th1/Th17 and Th2 responses.

Cytotoxic ($CD8^+$) T-cells might also play a role in the adaptive immune response against *C. albicans*, notably by exerting an antifungal activity against hyphae (Beno,

Stöver, and Mathews 1995; Marquis *et al.* 2006). Double positive $CD4^+CD8^+$ cells might also confer an antifungal immunity (Misme-Aucouturier *et al.* 2019).

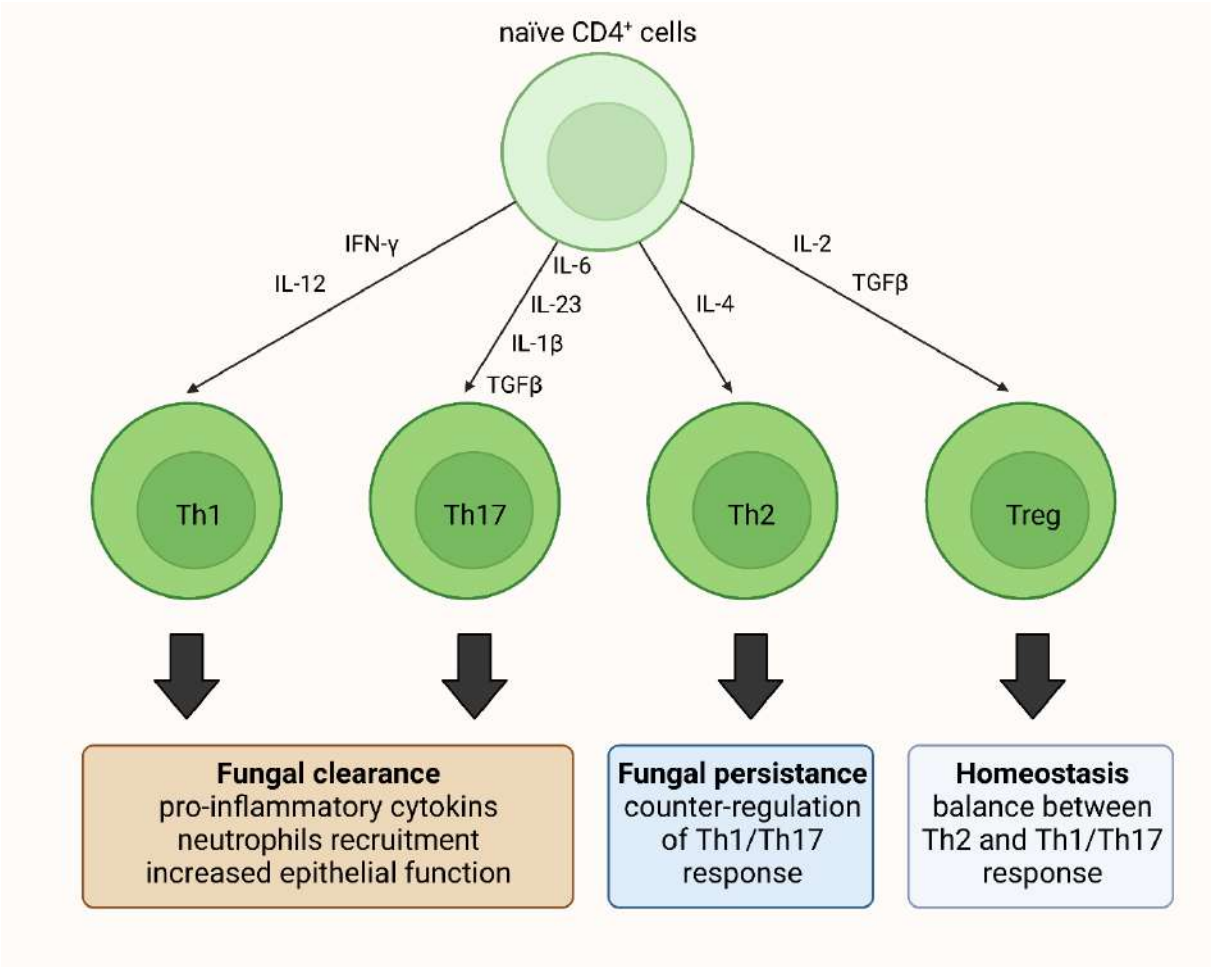


Figure 9: Cytokine-directed polarization of naive CD4+ T cell to one of the four known Th subsets. The Th1 and Th17 responses trigger the fungal clearance while the Th2 response is associated with fungal persistence. The Treg response is responsible for maintaining homeostasis between the Th1/Th17 and Th2 responses. Th: lymphocyte T helper, Treg: regulatory lymphocyte T. *Figure created with Biorender.com and adapted from Richardson and Moyes 2015.*

Antibody response. Specific anti-*C. albicans* antibodies have been detected in the blood of subjects previously exposed to the pathogen, suggesting a role for the humoral response in the protection against *C. albicans* infection (Pitarch *et al.* 2006; López-Ribot *et al.* 2004). Cell wall molecules of *C. albicans*, such as mannose, are preferential targets for antibodies due to their accessibility. In particular, vaccination with *C. albicans* mannan reduced the propensity of mice to develop invasive candidiasis (Y. Han and Cutler 1995). However, molecules that are not part of the cell

wall may also be the target of antibodies and could provide protection against invasive infection (Matthews *et al.* 1991; De Bernardis *et al.* 1997).

However, B cell deficiency does not increase the susceptibility of mice to invasive *C. albicans* infection (Balish, Jensen, and Warner 1993; Jensen, Warner, and Balish 1994; Carrow, Hector, and Domer 1984). Therefore, the contribution of the humoral response to the anti-*C. albicans* immune response appears to be relatively minor compared to that of lymphocytes T, and especially of the Th1/Th17 response (Richardson and Moyes 2015).

2. Trained immunity and *C. albicans* colonization

Although the immune system is essential to counter-act *C. albicans* infection, the interactions between *C. albicans* and the host immune system are also active during *C. albicans* colonization and promote the fungus commensal state. Indeed, several studies have shown that a long-term colonization by *C. albicans* resulted in the production of tissue-resident memory Th cells (Acosta-Rodriguez *et al.* 2007; Shao *et al.* 2019; Kirchner and LeibundGut-Landmann 2021), or specific anti-*C. albicans* antibodies (Huertas *et al.* 2017; Tansho *et al.* 2004; Bai, Liu, and Tong 2004), which conferred a protection against fungal proliferation and systemic infections. *C. albicans* colonization, in particular in the GI tract, thus appears to shape the immune system by offering a protection against systemic candidiasis but also against other pathogens such as *Staphylococcus aureus, Aspergillus fumigatus* or *Pseudomonas aeruginosa* (Shao *et al.* 2019; 2022; Tso *et al.* 2018).

This protection was thought to be completely dependent on T and B lymphocytes responses. However, similar protection against pathogen reinfection has been demonstrated in plants (Durrant and Dong 2004) and insects (Pham *et al.* 2007; Rodrigues *et al.* 2010) that lack an adaptative response, and in T- and B-cells deficient mice (Bistoni *et al.* 1986). The term “trained immunity” has therefore be proposed to explain the enhanced innate immune response that occurs after exposure to specific pathogens, such as *C. albicans*, and which results in an increased resistance to (re)infection (Netea, Quintin, and Van Der Meer 2011). It was shown that challenging mice with β-glucans increased their survival upon a *C. albicans* infection (Quintin *et al.*

2012). However, we are still lacking information on the effect of *C. albicans* carriage on the host immune response upon a *C. albicans* infection in Humans.

F - INTERACTIONS BETWEEN *C. ALBICANS* AND THE HOST GENETICS

Host-fungus interactions can be affected by genetic variations between individuals. Indeed, although medically- or disease-induced immunodeficiencies, such as HIV-induced acquired immunodeficiency syndrome (AIDS), chemotherapy-induced neutropenia or immunosuppressive therapy for organ transplantation, are recognized as major risk factors for *C. albicans* infections (Pappas *et al.* 2018; Singh, Fatima, and Hameed 2015), patients may also have rare or more common genetic variabilities that influence their susceptibility to these infections (Smeekens *et al.* 2013; Plantinga, Johnson, Scott, Joosten, *et al.* 2012; Puel 2020). Susceptibility to *C. albicans* colonization may also depend in part on host genetics (Jurevic *et al.* 2003).

1. Monogenic primary immunodeficiency disorders

Several monogenetic disorders can lead to primary immunodeficiency. These disorders are the consequence of mutations in crucial immune genes, such as signal transducer and activator of transcription 1 (*STAT1*) or *CARD9*, and are therefore associated with increased susceptibility to fungal infections (Smeekens *et al.* 2013; Plantinga, Johnson, Scott, Joosten, *et al.* 2012; Puel 2020). The most frequent monogenic disorders associated with *C. albicans* infections are chronic mucocutaneous candidiasis (CMC) and hyper-IgE syndrome (HIES) (Smeekens *et al.* 2013; Puel 2020).

i. Chronic mucocutaneous candidiasis

CMC is a severe and recurrent non-invasive infection that can affect the skin, nails and mucosal surfaces of the oral and genital tracts. It is the consequence of a defect in the Th17 immune response and can be caused by mutations in several crucial immune genes (Carey *et al.* 2019, Fig. 10).

Gain-of-function mutations in the gene encoding the STAT1 protein are the most common cause of CMC (Liu et al. 2011; Toubiana et al. 2016). STAT1 is a signaling molecule involved downstream of the type I and type II IFN receptors. Gain-of-function mutations in *STAT1* result in decreased production of IFN-γ, IL-22 and IL-17, thus altering the Th1 and Th17 adaptive immune response against *C. albicans* (Carey *et al.*

2019; Okada *et al.* 2020; van de Veerdonk, Plantinga, *et al.* 2011). Moreover, in a subset of CMC patients, defects in the autoimmune regulator *(AIRE)* gene result in autoimmune polyendocrinopathy, candidiasis, and ectodermal dysplasy (APECED), a syndrome characterized by multi-organ autoimmunity.

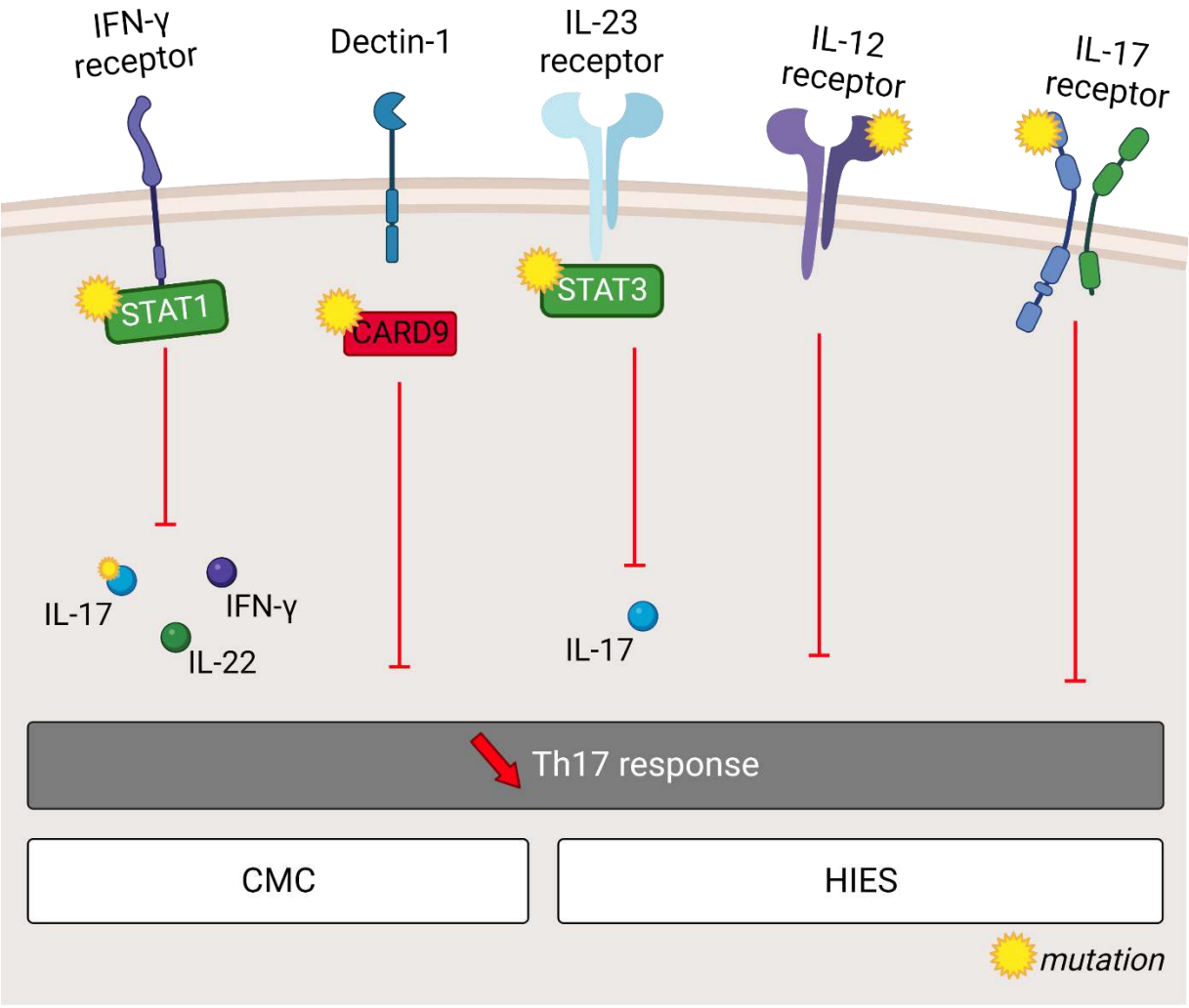


Figure 10: Overview of the main genetic defects resulting in monogenetic disorders associated with *C. albicans* infections. Mutations in *STAT1*, *IL-17RA* or *IL-17F* and *CARD9* are responsible of a reduced Th17 response, leading to chronic mucocutaneous candidiasis (CMC). Mutations in *TYK2*, *STAT3* and *DOCK8* have been associated with a reduced Th17 response, leading to hyper-IgE syndrome (HIES). Mutations are represented in yellow. *Figure created on Biorender.com and adapted from Singh, Fatima, and Hameed 2015.*

Homozygous loss-of-expression *CARD9* mutations have also been reported to increase the susceptibility to both CMC and invasive candidiasis, by almost totally depleting the Th17 response (Corvilain *et al.* 2018). Indeed, CARD9 is a central mediator of the anti-*C. albicans* immune response and trigger the production of pro-

inflammatory cytokines by DCs, thus inducing the Th17 adaptive immune response against *C. albicans* (Smeekens *et al.* 2013).

Finally, mutations in *IL-17RA* and/or *IL-17F* have been identified in several unexplained CMC cases (Puel *et al.* 2011) and IL-12Rb1 deficiency has been linked to mild signs of CMC and to an increase susceptibility to invasive candidiasis (Ouederni *et al.* 2014).

ii. Hyper-IgE syndrome

HIES, which was first described as Job's syndrome, is characterized by elevated levels of IgE in serum, eczema, skin and pulmonary infections by *Staphylococcus aureus* and recurrent mucosal infections with *C. albicans* (Davis, Schaller, and Wedgwood 1966; Smeekens *et al.* 2013). HIES can be caused by mutations in *STAT3* (Holland *et al.* 2007; Minegishi *et al.* 2007), which encodes a signaling molecule involved downstream of the IL-23 receptor, which results in reduced IL-17 production (De Beaucoudtey *et al.* 2008; Milner *et al.* 2008).

2. Common genetic polymorphisms

Mutations associated with monogenic primary immunodeficiency disorders are extremely rare and therefore cannot explain most reported *C. albicans* infections. As previously mentioned, there are several exogenous risk factors, such as a medically-induced immunosuppression, surgery, use of a central venous catheter, long-term stay in an intensive care unit (ICU) and exposure to antibiotics (Pappas *et al.* 2018; Papon, Bougnoux, and d'Enfert 2020; Zhai *et al.* 2020). In addition, more common genetic polymorphisms can be linked to increased susceptibility to different types of *C. albicans* infections (Smeekens *et al.* 2013, Table 1).

In particular, genetic polymorphisms in PRRs have been associated with increased susceptibility to fungal infections (Martin Jaeger *et al.* 2015; Smeekens *et al.* 2013). For instance, an early stop codon in *DECTIN-1* (Y238X), present in up to 8% of the European population and up to 40% of some sub-Saharan African populations (Ferwerda *et al.* 2009), has been associated with *Candida* mucosal colonization and recurrent vulvovaginal candidiasis (RVVC) (Plantinga *et al.* 2009). This association can be explained by defects in β-glucan recognition and Th17 response resulting from this mutation (Plantinga *et al.* 2009). Single nucleotide polymorphisms (SNPs) in *TLR1*, *TLR2* and *TLR4* have also been identified as increasing the risk of developing invasive

candidiasis through decreased levels of IL-8 and IFN-γ for the identified *TLR1* (Plantinga, Johnson, Scott, Van De Vosse, *et al.* 2012) and *TLR2* (Woehrle *et al.* 2008) gene polymorphism and increased IL-10 production for the identified *TLR4* SNPs (Van der Graaf *et al.* 2006). Furthermore, the risk of CMC is increased in carriers of the L412F *TLR3* polymorphism, probably due to decreased IFN-γ production (Nahum *et al.* 2011; 2012) while length polymorphisms in the NLRP3 inflammasome receptor subunit appear to increase the risk for RVVC (Lev-Sagie *et al.* 2009).

Genetic polymorphisms in several cytokines have also been identified as risk factors for *C. albicans* infections (Smeekens *et al.* 2013). Indeed, three SNPs in *IL-4* have been linked to chronic disseminated candidiasis (Choi *et al.* 2003), and one of them (-589T/C) has also been associated with increased susceptibility to RVVC (Babula *et al.* 2005). Genetic polymorphisms in *IL-10* and *IL-12b* have also been associated with persisting invasive candidiasis (Johnson *et al.* 2012).

In recent years, genome-wide association studies (GWAS) have been an essential tool to identify new common genetic polymorphisms associated with increased susceptibility to *C. albicans* infections (de Vries *et al.* 2020; Tian *et al.* 2017; Martin Jaeger *et al.* 2019). This type of untargeted analysis allows the identification of variants in genes that have not yet been reported to be involved in the host immune response, thus not only identifying new risks factors for fungal infections but also improving our understanding of fungus-host interactions. Indeed, Jaeger *et al.* identified a strong genetic association between candidemia and polymorphisms in the phospholipase A2 Group IVB (*PLA2G4B)* locus. *PLA2G4B* may be involved in the control of the phospholipid and/or arachidonate metabolism, highlighting for the first time a potential role for this gene in the anti-*C. albicans* host response (Martin Jaeger *et al.* 2019). Moreover, by integrating GWAS with RNA-sequencing, de Vries *et al.* uncovered a potential role of Ly86 in the host susceptibility to candidemia, through a differentiated monocyte migration toward the chemokine ligand 2 (CCL2) (de Vries *et al.* 2020).

However, such studies are limited by the lack of statistical power generally associated with the rather small cohorts of patients studied, making it difficult to identify SNPs with a high level of statistical significance (Manolio 2010; Chapman and Hill 2012). Furthermore, similar approaches have yet to be performed in order to identify genetic polymorphisms associated with host susceptibility (or resistance) to *C. albicans*

colonization, especially in healthy populations. Indeed, there are only few reports on genetic variants associated with *C. albicans* carriage, and they have been mainly reported in patient cohorts rather than in healthy subjects (Jurevic *et al.* 2003; Plantinga *et al.* 2009). Indeed, Plantinga *et al.* showed that the Y238X polymorphism of *DECTIN-1* was associated with *C. albicans* carriage in hematopoietic stem cell transplant patients (Plantinga *et al.* 2009) and Jurevic *et al.* identified a SNP in the gene coding for β-defensin 1 (DEFB1) associated with a higher oral *C. albicans* carriage in both type I diabetics patients and healthy controls (Jurevic *et al.* 2003). However, to the best of my knowledge, there are no reports on genetic variants associated with *C. albicans* intestinal carriage in healthy subjects. This could be due to the limited access to large cohorts of healthy patients containing both stool samples and genetic data. Furthermore, such studies rely on a reliable estimation of *C. albicans* carriage, as the use of culture is questioned due to its low sensitivity.

Table 1: Common genetic variants associated with C. albicans infections or carriage presented in this section. *Table adapted from Smeekens et al. 2013.*

Gene	SNP	Phenotype	Disease	References
Dectin-1	Y238X (rs16910526)	Decrease of IL-1β production Decrease of Th17 response	*C. albicans* colonization RVVC	(Plantinga, Johnson, Scott, Joosten, *et al.* 2012)
TLR1	R80T (rs5743611) S248N (rs4833095) I602S (rs5743618)	Decrease of IL-1β, IL-6 and IL-8 production	Increased susceptibility to candidemia	(Plantinga *et al.* 2009)
TLR2	R753Q (rs5743708)	Decrease of IFN-γ and IL-8 levels	Increased susceptibility to candidemia	(Woehrle *et al.* 2008)
TLR3	L412F (rs3775291)	Decrease of IFN-γ levels	Increased risk for CMC	(Nahum *et al.* 2011; 2012)
TLR4	D299G (rs4986790) Y399I (rs4986791)	Increase of IL-10 production	Increased susceptibility to candidemia	(Van der Graaf *et al.* 2006)
IL-4	−589T/C (rs2243250)	Increase of vaginal IL-4 levels	RVVC	(Babula *et al.* 2005)
IL-10	−1082A/G (rs1800896)	Increase of IL-10 production	Persisting candidemia	(Johnson *et al.* 2012)
1L-12B	2724INS/DEL (rs17860508)	Decrease of IFN-γ production	Persisting candidemia	(Johnson *et al.* 2012)
PLA2G4B	rs8028958	Decreased of ROS production	Increased susceptibility to candidemia	(Martin Jaeger *et al.* 2019)
LY86	rs9405943	Reduction of monocyte recruitment towards CCL2	Increased susceptibility to candidemia	(de Vries *et al.* 2020)
DEFB1	−44C/G (rs1800972)	Unknown	Oral *Candida* (including *C.* albicans) carriage	(Jurevic *et al.* 2003)

AIMS OF THE PHD THESIS

C. albicans interactions with the host and the microbiota play a central role in its ability to colonize and maintain itself in the human gut, and *C. albicans* intestinal colonization is the cornerstone of the initiation of an infection in at-risk patients. Therefore, understanding the factors underlying this colonization is crucial to control the proliferation of *C. albicans.* At the beginning of my thesis, there were only a few studies that described potential factors behind *C. albicans* colonization in healthy individuals. In particular, we were lacking an accurate estimate of *C. albicans* carriage in healthy humans, most studies having been performed either on patients, and/or using culture-based assays, that lack the sensitivity required to detect low *C. albicans* burden. Moreover, it is well known that *C. albicans* carriage can drastically vary between individuals, and these variations probably reflect variations of the host immunity and/or of the gut microbiota profile. Finally, although it was known that broad-spectrum antibiotics were a risk factor for *C. albicans* overgrowth, research had focused mainly on cohorts of patients and the understanding of the additional mechanisms involved in *C. albicans* overgrowth in healthy humans was very limited.

Therefore, my thesis aims to (i) accurately estimate the carriage of *C. albicans* in healthy subjects, (ii) explore how the bacterial microbiota, and the host diet, lifestyle, and genetic profile might modulate *C. albicans* carriage, and (iii) decipher the influence of *C. albicans* intestinal carriage on the host immune response.

To do so, I worked with two cohorts of healthy individuals: (i) the CEREMI cohort (Burdet, Nguyen, *et al.* 2019), which included 22 volunteers for which fecal samples were collected before, during and after a 3-day regimen of third generation cephalosporin (3GC) antibiotics, and (ii) the Milieu Intérieur cohort (Thomas *et al.* 2015), which included 1000 healthy individuals for which a single fecal sample was collected.

My PhD work will thus be presented in three distinct chapters:

CHAPTER 1: COMPARISON AND IMPLEMENTATION OF DNA EXTRACTION PROTOCOLS AND QPCR ASSAYS FOR THE ANALYSIS OF THE INTESTINAL MICRO- AND MYCOBIOTA

The gut micro- and mycobiota are commonly studied through high-throughput sequencing approaches, with either amplicon-targeted metagenomics or whole-genome shotgun metagenomics sequencing. Such approaches rely heavily on

the quality of the DNA recovered from fecal samples. Consequently, the choice of an appropriate DNA extraction protocol is crucial. In this first chapter, I tested three DNA extraction protocols on fecal samples and compared their efficiency in recovering high-quality fungal DNA. I thus determined that the simplified version of the protocol Q, proposed by Yu and Morrison 2004, resulted in the recovery of a relatively large amount of fungal DNA, and resulted in an overall increased bacterial α-diversity compared to the other protocols, while being relatively simple to implement. I therefore selected this protocol to perform all DNA extractions from fecal samples conducted in my thesis. In parallel, I implemented two qPCR assays to quantify the fungal burden and the levels of *C. albicans* recovered from fecal samples, and optimized them to be used either individually, in simplex assays, or combined, in duplex, with an internal control qPCR assay, for the detection of qPCR inhibitors, as these molecules are often recovered in DNA extracts from fecal samples. Finally, I developed an equation taking into account the efficacy of the DNA extraction to convert *C. albicans* DNA contents, measured by qPCR into a cell count.

CHAPTER 2: THE IMPACT OF AN ANTIBIOTIC-INDUCED MICROBIOTA DYSBIOSIS ON THE MYCOBIOTA AND *C. ALBICANS* INTESTINAL CARRIAGE IN HEALTHY INDIVIDUALS

Antibiotics are known to massively disrupt the intestinal microbiota, by killing the resident gut bacteria. However, their role on the intestinal mycobiota is still unclear. In particular, when I started my thesis, there were no report of the direct role of antibiotics in the overgrowth of *C. albicans* in healthy humans, the few studies on the subject having been performed either on mice, or on cohorts of patients. In this second chapter, I followed prospectively the 22 healthy subjects of the CEREMI cohort before, during and after they were treated with β-lactams, a family of large-spectrum antibiotics widely used in clinics that is excreted through the bile into the gut and can thus affect the intestinal mycobiota. I first characterized the mycobiota of the subjects, as well as their fungal burden and *C. albicans* carriage before antibiotic exposure. I showed that the relative and absolute abundance of *C. albicans* increased after treatment, but this increase in *C. albicans* levels was subject-dependent, with strong inter-individual variations. As the human gut hosts bacteria naturally able to produce enzymes

able to hydrolyze β-lactam antibiotics (β-lactamases), the β-lactamase activity of each subject was measured before, during and after exposure to β-lactams. I thus showed that the change of β-lactamase activity was negatively correlated with the change of *C. albicans* levels after antibiotics, which could explain part of the variability in *C. albicans* levels after antibiotic treatment. In addition, I used Spearman correlations between *C. albicans* absolute abundance, deduced from qPCR data, and the relative abundance of the gut metagenomic species, deduced from shotgun metagenomics data or the relative abundance of the gut fungal species, deduced from the ITS1-targeted metagenomics data to identify bacteria or fungi potentially inhibiting *C. albicans*.

CHAPTER 3: THE INFLUENCE OF HOST AND ENVIRONMENTAL FACTORS ON *C. ALBICANS* COLONIZATION IN HEALTHY INDIVIDUALS

As mentioned through the introduction, it is well known that the gut microbiota and the host immune system are crucial in controlling the growth of *C. albicans* in the intestine. But as most studies are conducted on cohorts of patients, we still need to better understand the mechanisms involved in the proliferation of *C. albicans* in healthy humans. Thus, in this third chapter, I investigated how bacterial microbiota composition, diet, medical history and host environment can shape the carriage of *C. albicans* in healthy adults, using data collected from 695 of the 1000 healthy volunteers from the Milieu Intérieur cohort. Moreover, although several genetic disorders or common genetic polymorphisms are known to be associated with increased susceptibility to *C. albicans* infections, little is known about genetic polymorphisms associated with the susceptibility to *C. albicans* colonization in healthy individuals. We therefore conducted a genome-wide association study to identify genetic factors associated with host susceptibility to *C. albicans* colonization. Finally, considering previous reports on the importance of trained immunity in *C. albicans* infections, I wondered whether there was an advantage to the healthy host to maintain high levels of *C. albicans* in the gut. I therefore investigated the interplay between the extent of the intestinal carriage of *C. albicans* and the host immune response upon a mimicked *C. albicans* blood infection.

Overall, the work conducted during these past years has allowed a deeper understanding of the multiple factors associated with *C. albicans* colonization. Each of the following chapters contain a detailed description of each project conducted during my thesis. The second and third chapter are presented in the form of an article, with any additional results that could not be included in the article being presented at the end of the chapter. The overall outcomes of this thesis and the resulting questions that could not be included in one of the three chapters will be discussed in the perspectives section of this thesis.

RESULTS AND DISCUSSION

A - CHAPTER 1: COMPARISON AND IMPLEMENTATION OF DNA EXTRACTION PROTOCOLS AND QPCR ASSAYS FOR THE ANALYSIS OF THE INTESTINAL MICRO- AND MYCOBIOTA

The first part of my PhD work consisted into optimizing and implementing experimental procedures, such as DNA extraction and qPCR assays, which are required to analyze the bacterial and fungal microbiota. As mentioned before, the gut micro- and mycobiota are mostly studied through high-throughput sequencing approaches and the quality of the results relies strongly on the DNA extraction protocol (Angebault *et al.* 2018; Leigh Greathouse, Sinha, and Vogtmann 2019; Gerasimidis *et al.* 2016; Fiedorová *et al.* 2019). Standardized protocols for the isolation of bacterial DNA from fecal samples have been proposed (Santiago *et al.* 2014) but such efforts remain to be conducted for the isolation of fungal DNA. DNA extraction of the intestinal fungal microbiota brings additional challenges: fungi are underrepresented in the gut, with fungal genes being accountable for less than 0.1% of the gut microbiome (Underhill and Iliev 2014; Qin *et al.* 2010) and the structure of their cell wall prevents an efficient lysis of the fungal cells during the extraction (Karakousis *et al.* 2006; Yu and Morrison 2004; Angebault *et al.* 2018). I thus needed to validate a DNA extraction protocol that returns DNA of a good enough quality to analyze both the bacterial and the fungal microbiota, so that this protocol can be used for the experimental part of this thesis.

In this work, I tested three DNA extraction protocols on fecal samples and compared their efficiency in recovering fungal DNA. In parallel, I optimized two qPCR assays to quantify the total fungal burden and *C. albicans* levels in fecal samples. Finally, I developed an equation taking into account the efficacy of the DNA extraction to convert *C. albicans* DNA contents, measured by qPCR, into cell count.

1. Comparison of protocols for the extraction of fungal DNA from fecal samples

To identify a DNA extraction protocol adapted for the analysis of the intestinal mycobiota, I conducted a pilot study aiming to compare three protocols : (i) protocol Q, the current reference for DNA extraction from fecal samples in microbiota studies (IHMS protocol Q, Santiago *et al.* 2014), (ii) protocol A, the repeated bead beating plus column method proposed by Yu and Morrison 2004, which is a simplified version of the protocol Q and (iii) protocol FAST, a kit-based protocol provided by the FASTDNA™ SPIN Kit for Soil (MP Biomedicals, USA). Protocol Q and A require several steps of bead-beating, which allow a thorough lysis of the fungal cell walls but increase the time required to complete the DNA extraction (8-9 hours and 6-7 hours, respectively) whereas protocol FAST consists into a more straight-forward procedure which can be completed under 2 hours. The protocols were performed on the same 10 fecal samples and their efficiency was assessed based on (i) the quantity of the DNA extracted, estimated by Qubit BR, (ii) the amount of fungal DNA recovered, quantified by 18S Panfungal qPCR, and (iii) the quality of the sequences obtained after 16S- and ITS1-targeted metagenomics sequencing.

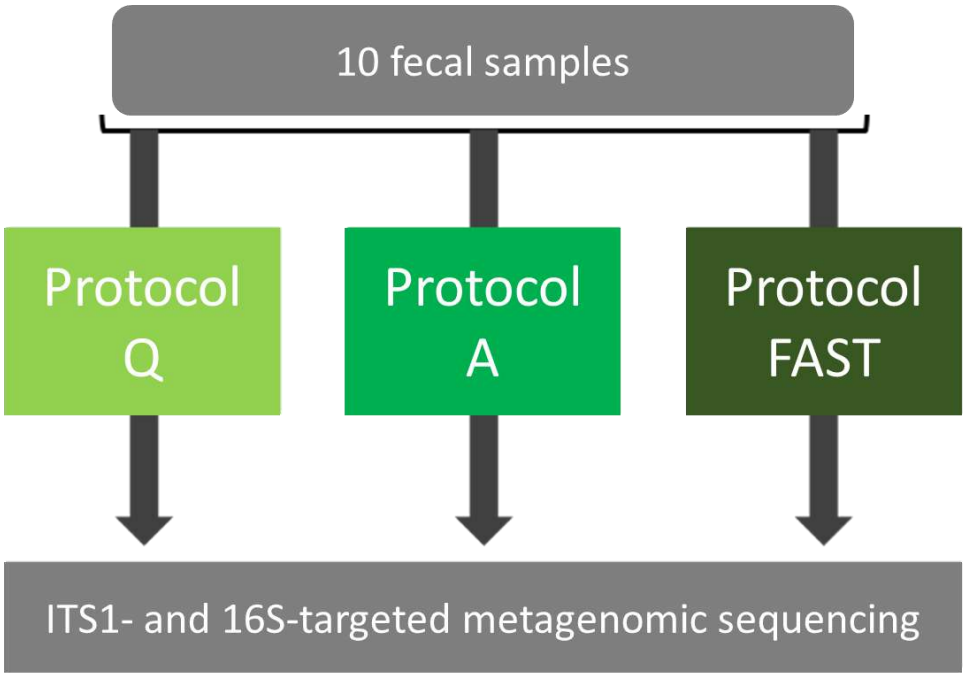


Figure 1: Experimental design of the pilot study for the comparison of fungal DNA extraction protocols from fecal samples. Protocol Q is the current reference for DNA extraction from fecal samples, Protocol A is a simplified version of the protocol Q and Protocol FAST is provided by the FASTDNA™ SPIN Kit for Soil.

i. Influence of the DNA extraction on the total and fungal DNA recovered

All three protocols yielded similar amounts of total fecal DNA (Kruskal Wallis paired test; p-value of 0.34, Fig. 2A and 2B) but differences were observed in the amount of fungal DNA recovered, with protocol FAST returning a lower concentration of fecal fungal DNA (Kruskal Wallis paired test; p-value of 0.043, Fig. 2C and 2D).

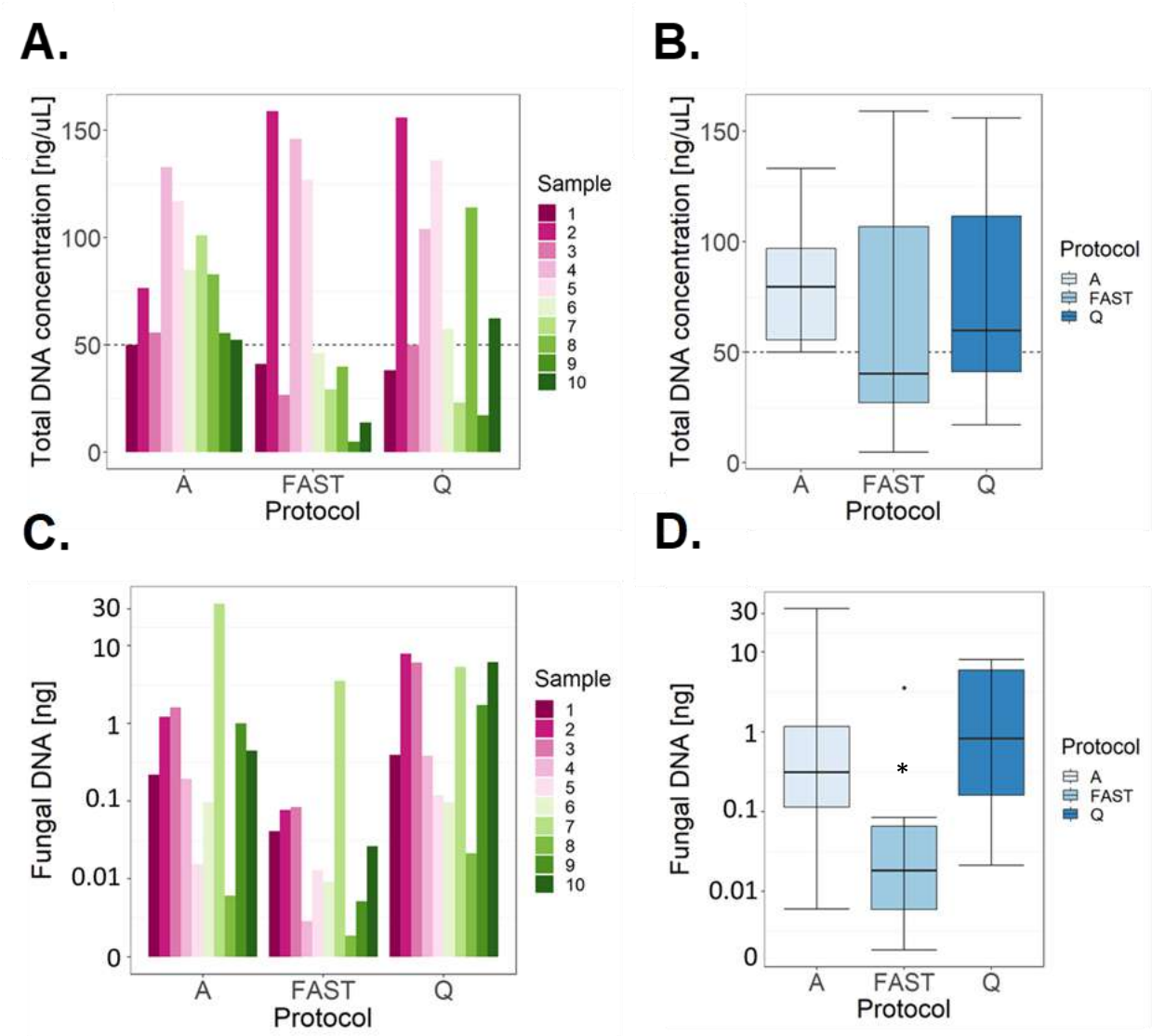


Figure 2: Comparison of DNA extraction protocols in term of quantity of DNA extracted and fungal DNA recovered. (A). Barplot of the total DNA concentration obtained for each sample extracted by each protocol. The horizontal line corresponds to the minimal DNA content required to proceed with the downstream analysis. (B) Boxplot of the total DNA concentration obtained for each protocol. The horizontal line corresponds to the minimal DNA content required to proceed with the downstream analysis. (C). Barplot of the quantity of fungal DNA recovered for each sample by each protocol. (D). Boxplot of the quantity of fungal DNA recovered by each protocol. **p-value < 0.05.*

ii. Influence of the DNA extraction on the bacterial diversity and microbiota community profile

The bacterial α-diversity, estimated by Shannon index, was similar for the samples processed by protocol A and FAST with a median of 5.34 (Kruskall-Wallis paired test; p-value of 0.19, Fig. 3A) whereas samples processed through protocol Q were characterized by a lower bacterial diversity (median of Shannon index: 5.06; Kruskall-Wallis paired test; p-value of 0.002, Fig. 3A). The bacterial community structure was comparable between the three protocols with *Firmicutes, Bacteroides, Actinobacteria* and *Proteobacteria* being the more abundant phyla in all conditions (Fig. 3B).

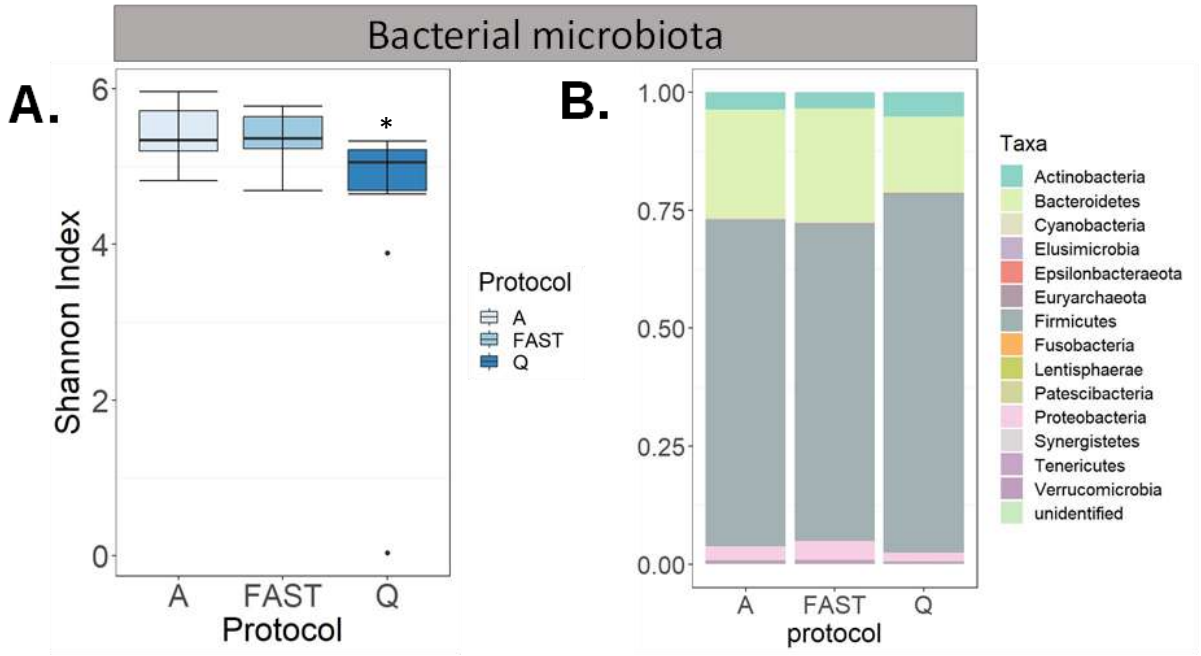


Figure 3: Comparison of DNA extraction protocols in term of bacterial α-diversity and bacterial community profile. (A) Boxplot of the Shannon Indexes obtained for each DNA extraction protocol. (B). Relative abundance of the bacterial phyla identified with each DNA extraction protocol. **p-value < 0.05.*

iii. Influence of the DNA extraction on the fungal diversity and mycobiota community profile

ITS1-targeted metagenomic sequencing resulted in a large number of unassigned sequences (i.e. sequences that cannot be assigned to the fungal kingdom), reaching up to 97.9% in sample 5 when processed with protocol FAST. This might reflect either a low quality of sequences or the presence of plant DNA that can be partly amplified by the primers used. Although more extreme values were obtained with protocol FAST, the percentage of unassigned sequences was similar among the three DNA extraction

protocols tested, suggesting that all protocols return sequences of comparable quality (Kruskall-Wallis paired test, p-value of 0.118, Fig. 4A and B). The fungal α-diversity was comparable between the samples processed with the three DNA extraction protocols, with a Shannon index median of 1.18 (Kruskall-Wallis paired test; p-value of 0.094, Fig. 4C). Overall, the same main fungal genera were identified with all DNA extraction protocols with similar relative abundance (Fig. 4D), suggesting that none of the protocols introduce bias in fungal taxonomy.

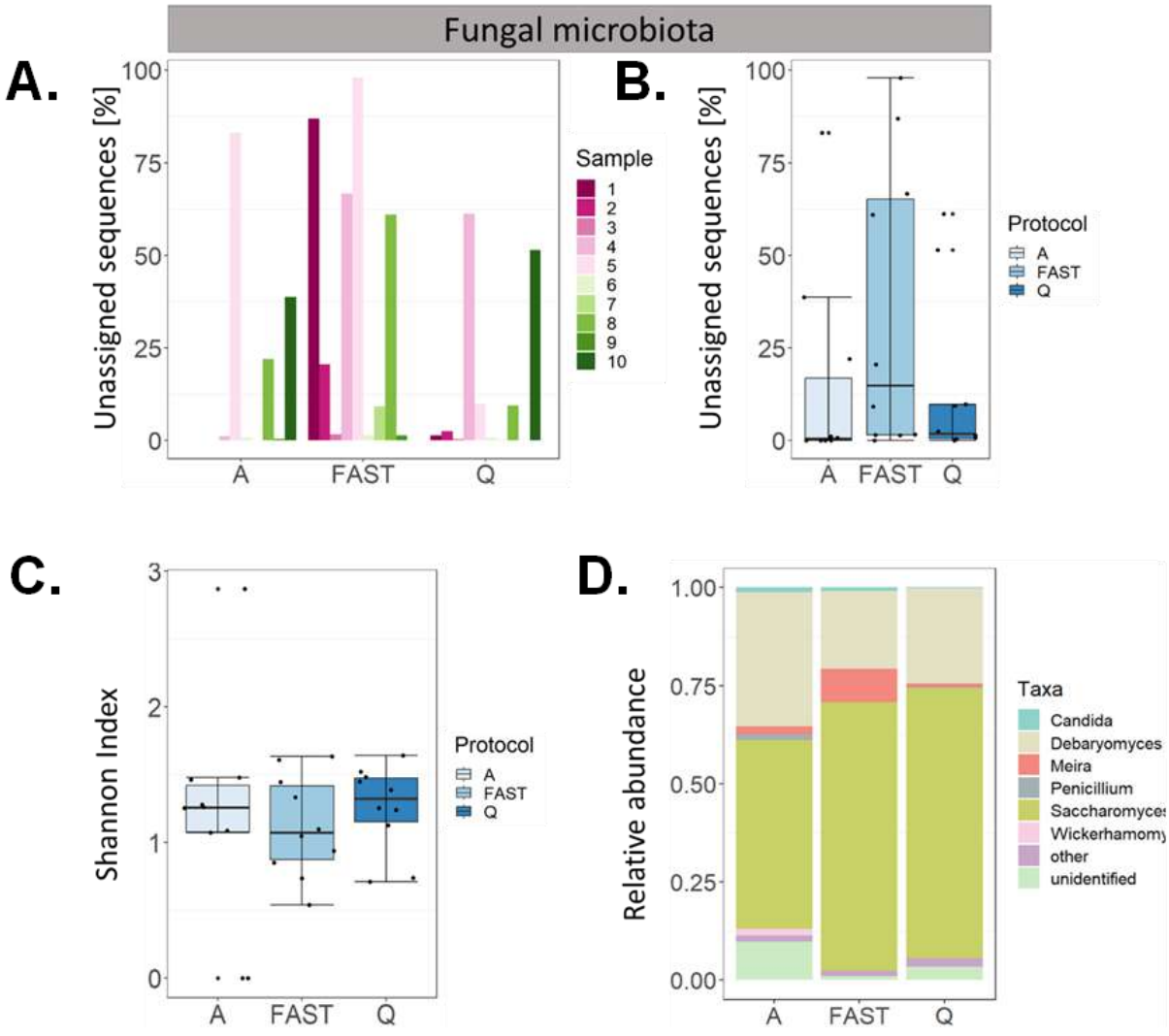


Figure 4: Comparison of DNA extraction protocols in term of fungal α-diversity and community profile. (A) Barplot of the percentage of unassigned sequences obtained for each sample with each DNA extraction protocol. (B). Boxplot of the percentage of unassigned sequences obtained with each protocol. (C) Boxplot of the Shannon Indexes obtained for each DNA extraction protocol. (D). Relative abundance of the fungal genera identified with each DNA extraction protocol.

Overall, protocol FAST performed more poorly than protocol A and Q, with a reduced amount of fungal DNA recovered and a higher, although not significant, percentage of unassigned sequences. Considering this and the fact that protocol Q returned a lower bacterial α-diversity and is more tedious to perform than protocol A, thus increasing the risk of experimental errors, I decided to select protocol A for all DNA extractions from fecal samples conducted in this thesis.

2. Optimization of qPCR assays for the quantification of fungal and *C. albicans* DNA

Once the DNA extracts were obtained, I needed a reliable and sensitive method for the quantification of the total fungal burden and levels of *C. albicans* in fecal samples. Culture methods are not optimal as they are tedious to implement on a large number of samples and not all fungi are cultivable. Moreover, these methods can only detect high levels of *C. albicans*. Targeted metagenomics approaches are more sensitive than culture, but the primers used for amplification of rDNA regions may introduce a taxonomic bias by uneven amplification of specific fungal taxa (Thielemann *et al.* 2022; Bellemain *et al.* 2010). Therefore, I opted for two qPCR assays: a 18S Panfungal qPCR, described by Liu *et al.* 2012, for the quantification of fungal DNA, and a *C. albicans*-specific qPCR assay adapted from Guiver, Levi, and Oppenheim 2001, for the estimation of *C. albicans* carriage.

i. Identification of qPCR inhibitors

qPCR assays are sensitive to qPCR inhibitors, that prevent DNA amplification, leading to false negative results (Valentine-Thon 2002), and qPCR inhibitors are frequently found in fecal samples (Oikarinen *et al.* 2009). In our preliminary tests, the DNA extractions from fecal samples occasionally resulted in colored DNA extracts, that were associated with a lower fungal DNA amplification (Fig. 5). However, some non-colored DNA extracts also returned negative results that were not coherent with either culture or sequencing data. Since the color of the samples alone did not allow a sensitive detection of qPCR inhibitors, I implemented an internal control qPCR assay to detect specifically qPCR inhibition in the DNA extracts. Using the Universal Exogenous qPCR Positive Control for TaqMan® Assay with a Cy®5-QXL®670 Probe system (Eurogentec, Belgium), a qPCR inhibition was detected in 120 of the 266 DNA extracts tested at a

1:10 dilution and increasing the dilution factor to 1:100 reversed the qPCR inhibition in 111 of the 120 DNA extracts containing inhibitors (Fig. 6).

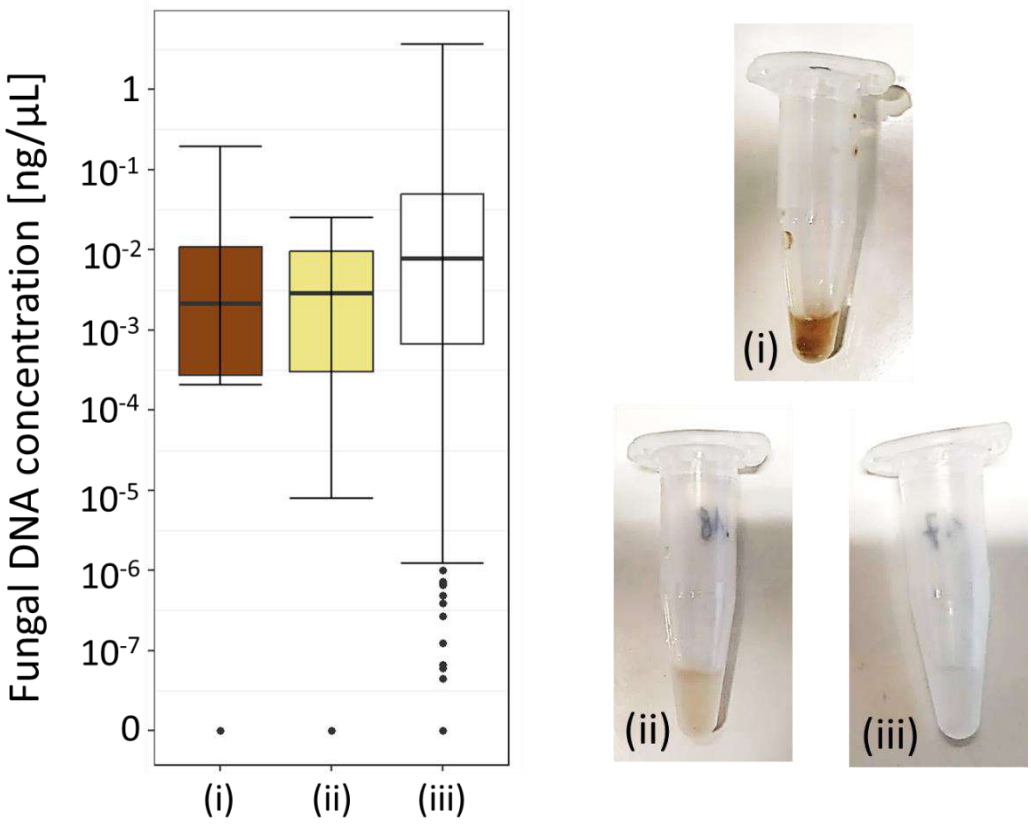


Figure 5: Coloration of the DNA extracts is associated with a lower fungal DNA concentration. Boxplot of the fungal DNA concentrations obtained in (i) the strongly colored DNA extracts, (ii) the slightly colored DNA extracts and (iii) the non-colored DNA extracts.

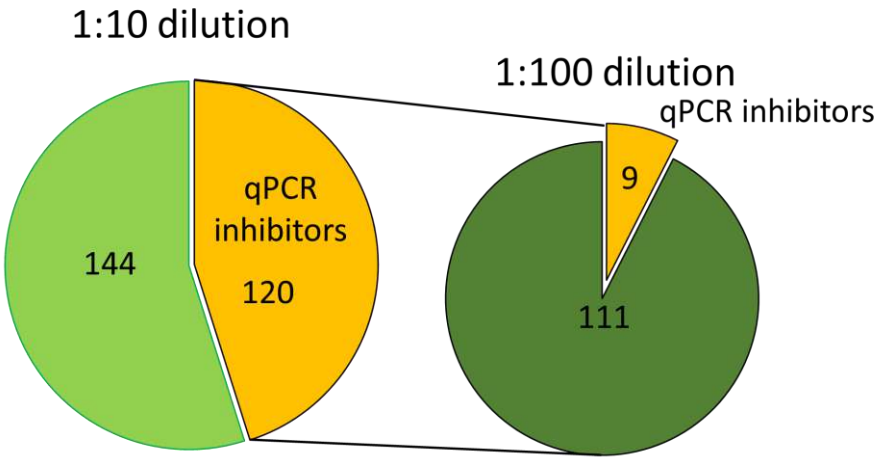


Figure 6: Proportion of DNA extracts with qPCR inhibitors among the DNA extracts diluted to 1:10 (left) or 1:100 (right).

Although this assay was sensitive and easy to implement, it required all DNA extracts to be subjected to another qPCR assay. Considering the large amount of fecal samples analyzed in my PhD work, I had to combine this internal qPCR control with the *C. albicans* qPCR assay and/or the 18S Panfungal qPCR assay, in order to reduce the cost and time required to perform fungal and *C. albicans* quantification. However, as the three qPCR assays were characterized by different annealing temperatures: 60°C for the internal control qPCR assay, 65°C for the 18S Panfungal qPCR and 62°C for the *C. albicans* qPCR assay, I had to optimize the qPCR conditions.

ii. Optimization of a duplex *C. albicans*-internal control qPCR assay

In order to perform the *C. albicans* qPCR assay at an annealing temperature of 60°C, I first optimized the concentration of the qPCR primers. I compared the qPCR efficiency with the primers at 50nM, 300nM, 400nM and 900nM and the Taqman probe at a fixed concentration of 100nM (Fig. 7).

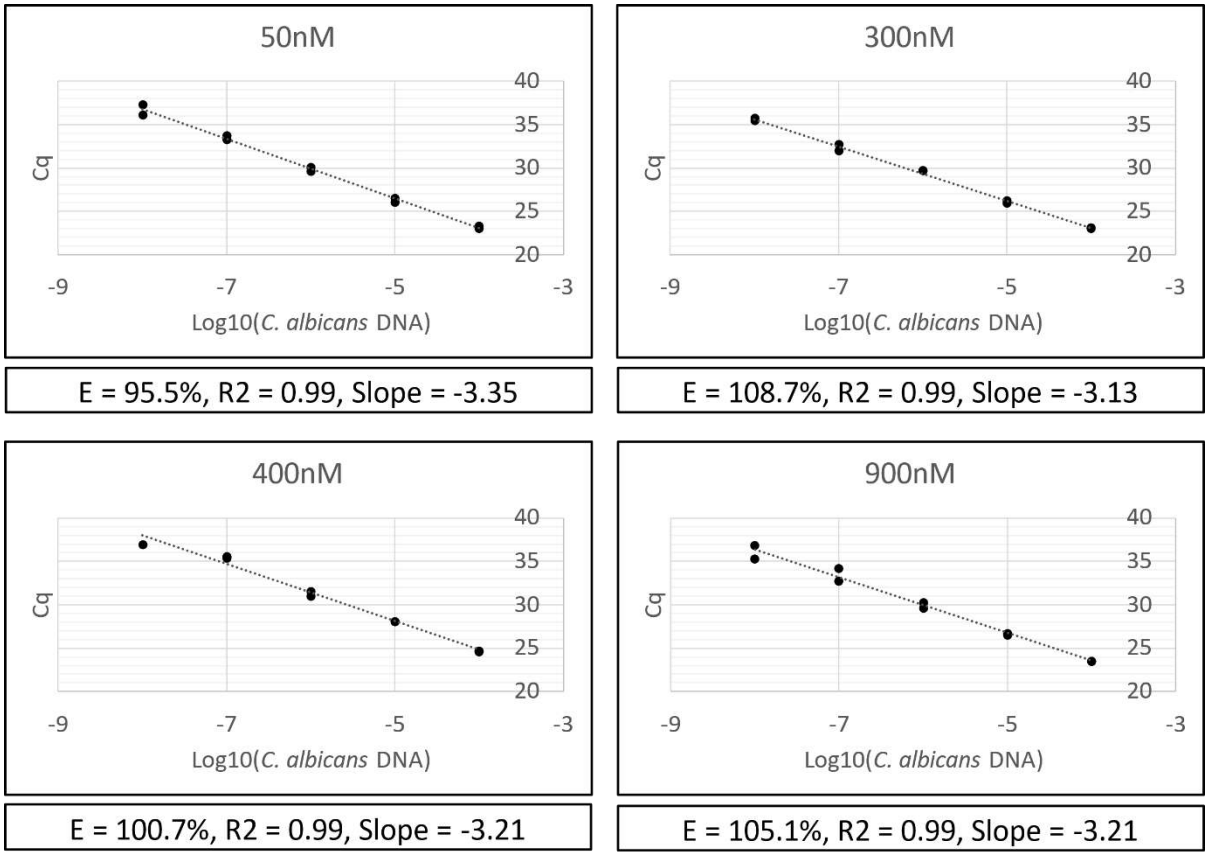


Figure 7: Comparison of four primers concentrations on the efficiency of a *C. albicans* qPCR assay. qPCR standard curves. E = efficiency, R2 = coefficient of correlation. All reactions were conducted at an annealing temperature of 60°C with a Taqman probe concentration of 100nM. *C. albicans* DNA concentrations are given in ng/µL.

All primers concentrations tested were associated with sufficient efficiency, but using a primer concentration of 400nM resulted in the reaction with the closest efficiency to 100% and was therefore retained for further analysis. With the primer concentration set at 400nM, I then compared qPCR efficiency with TaqMan probe concentrations of 50nM, 100nM and 250nM (Fig. 8). The assays with 50nM and 100nM probe both gave a sufficient efficiency, but the assay with 100nM probe had better reproducibility between technical replicates and this probe concentration was therefore retained. Once the *C. albicans* qPCR assay optimized to be efficient at an annealing temperature of 60°C, I combined the *C. albicans* qPCR and the internal control qPCR assays in a duplex assay. The duplex qPCR assay gave similar results to those obtained with the two simplex assays, with a slight increase in resistance to qPCR inhibitors in the duplex which was therefore validated for use (Table 1).

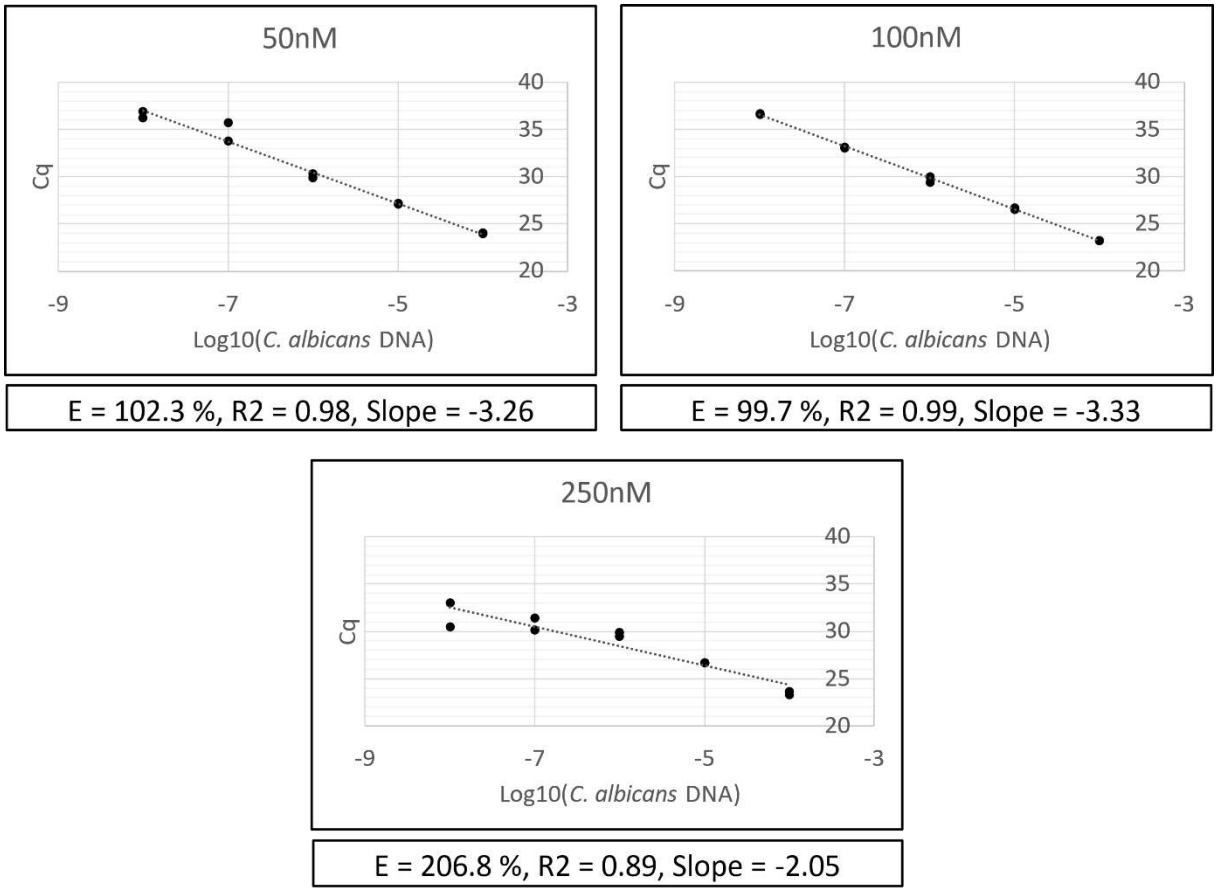


Figure 8: Comparison of three probe concentrations on the efficiency of a *C. albicans* qPCR assay. qPCR standard curves. E = efficiency, R2 = coefficient of correlation. All reactions were conducted at an annealing temperature of 60°C with primers concentration of 400nM. *C. albicans* DNA concentrations are represented in ng/µL.

Table 1: Comparison of the two simplex *C. albicans* and internal control qPCR assays with the duplex assay combining both qPCR.

		Original protocol simplex		Optimized protocol duplex	
	Sample	Cq IPC	*C. albicans* DNA [ng/uL]	Cq IPC	*C. albicans* DNA [ng/uL]
1/10 dilution	A	24.92	1.13E-03	23.41	6.41E-04
	B	23.97	1.14E-03	24.87	8.30E-04
	C	26.87	0.00E+00	27.42	0.00E+00
	D	27.51	0.00E+00	28.01	0.00E+00
	E	No Amplification	0.00E+00	No Amplification	9.24E-07
	F	No Amplification	0.00E+00	27.32	1.42E-04
	G	No Amplification	0.00E+00	No Amplification	0.00E+00
	H	No Amplification	0.00E+00	No Amplification	0.00E+00
	I	No Amplification	0.00E+00	No Amplification	0.00E+00

iii. Optimization of a 18S Panfungal qPCR assay

Since the 18S Panfungal qPCR assay had an annealing temperature of 65°C, five more degrees than for the internal control qPCR assay, I compared the efficiency of this assay on a gradient of annealing temperatures ranging from 65°C to 60°C. As the tested temperatures did not seem to affect the efficiency of the qPCR assay (Fig. 9), I conserved an annealing temperature of 60°C. Once the 18S Panfungal qPCR assay was confirmed to be efficient at an annealing temperature of 60°C, I combined this qPCR and the internal control qPCR assays in a duplex assay. The duplex qPCR assay gave similar results than those obtained with the two simplex assays and was therefore validated for use (Table 2).

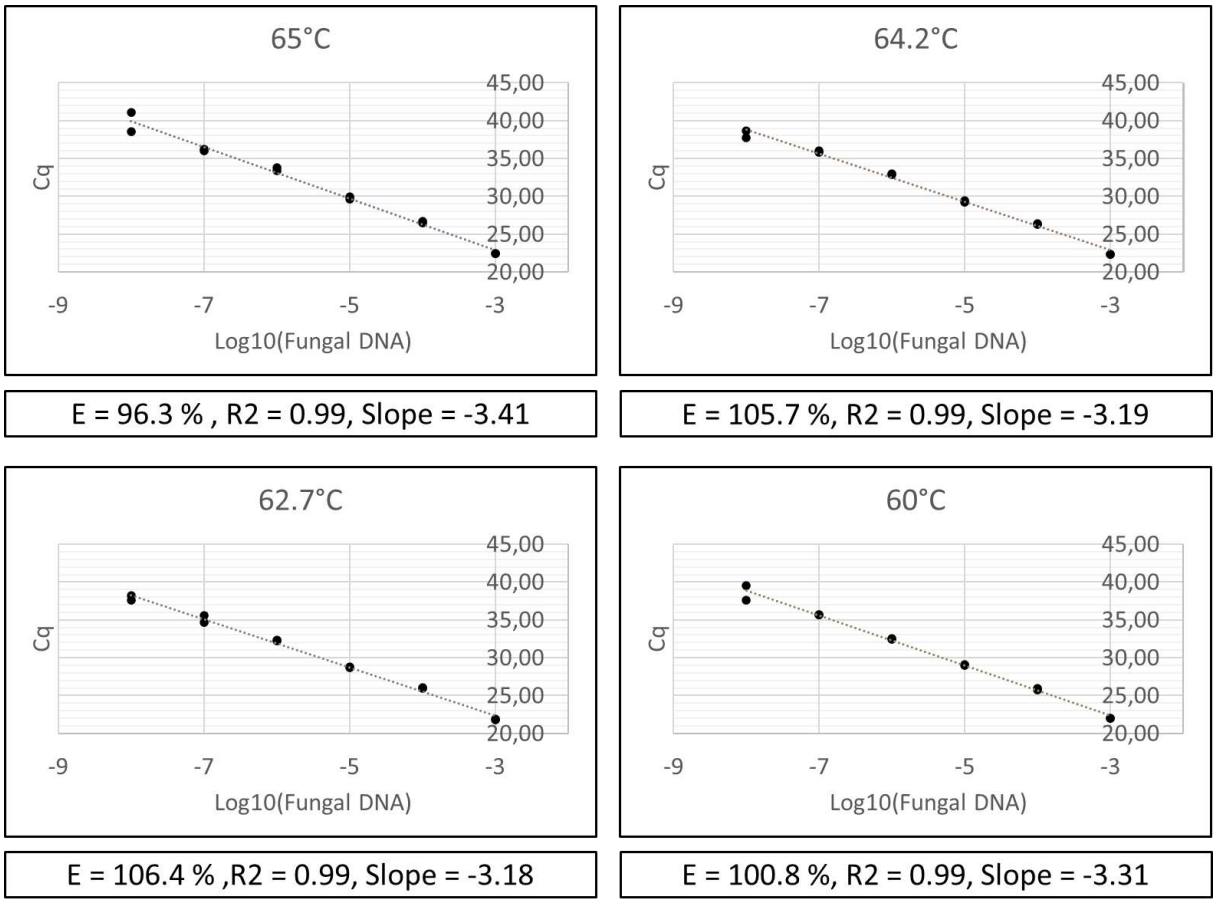


Figure 9: Comparison of a gradient on annealing temperature on the efficiency of a 18S Panfungal qPCR assay. qPCR standard curves. E = efficiency, R2 = coefficient of correlation. Fungal DNA concentrations are represented in ng/µL

Table 2: Comparison of the two simplex 18S Panfungal qPCR and internal control qPCR assays with the duplex assay combining both qPCR.

		Original protocol (simplex)		Optimized protocol (duplex)	
	Sample	Cq IPC	Fungal DNA [ng/uL]	Cq IPC	Fungal DNA [ng/uL]
1/100 dilution	A	NA	6.80E-01	25.12	2.24E-02
	B	NA	2.45E+00	26.2	1.09E+00
	C	NA	1.54E-02	27.21	7.22E-03
	D	NA	1.59E-02	27.33	5.09E-03
	E	24.73	1.99E-01	24.49	5.61E-02
	F	26.93	1.91E-02	27.11	1.18E-02
	G	28.12	0.00E+00	28	0.00E+00
	H	27.18	2.38E-02	27.11	5.21E-02
	I	No Amplification	0.00E+00	No Amplification	0.00E+00

3. From DNA content to cell count: assessment of *C. albicans* cell count in fecal samples from qPCR data

When *C. albicans* carriage is measured by qPCR assay using a quantification standard, the result is a DNA concentration, usually expressed in nanogram per microliter. The DNA content can then be transformed into a cell number. In theory, the number of cells initially present in a sample can be estimated based on the DNA content quantified by qPCR, the size of the *C. albicans* genome and the molecular weight of a DNA molecule, using the following formula:

$$N_{cells} = \frac{DNA_m \times N_A}{M \times Genome} = 35{,}093.24 \times DNA_c$$

Where N_{cells} is the number of estimated *C. albicans* cells, DNA_m is *C. albicans* DNA content measured by qPCR, in nanogram, N_A is Avogadro's number (i.e. 6.022 x 10^{23} molecules/mole), M is the molar mass of a DNA base pair (bp) (i.e. 6.6 x 10^{11} ng/mole) and $Genome$ is the size of *C. albicans* genome (i.e. 26Mb).

However, this equation returns the number of estimated cells present in a sample without considering the efficiency of the DNA extraction; a significant amount of DNA being lost during any DNA extraction protocol. Therefore, to estimate the number of cells in a fecal sample, we need to know the proportion of DNA recovered after extraction.

To do so, I spiked aliquots from two independent fecal samples, in which no *C. albicans* DNA had been detected by qPCR, with 10^6, 10^5, 10^4 and 10^3 *C. albicans* cells and measured, by qPCR, the amount of *C. albicans* DNA recovered after DNA extraction. I was thus able to compare the theorical number of *C. albicans* – as quantified by qPCR and transformed with the above equation – and the number of cells actually spiked in the fecal sample. In average, only 2.79% (min: 0.28%, max: 6.74%) of the total *C. albicans* content was recovered after DNA extraction (Fig. 10). Therefore, for a fecal sample containing 1000 *C. albicans* cells, an average of only 8 x 10^{-4} ng of DNA is recovered, corresponding to 28 *C. albicans* cells. Considering the limit of detection of the *C. albicans* qPCR (10^{-5} ng of *C. albicans* DNA per fecal sample, see *Materials and Methods*), we can therefore detect *C. albicans* DNA in fecal samples containing a

minimum of around 100 cells per gram of stool, thus allowing a more sensitive detection than cultures methods.

Thus, to take into account the efficiency of the DNA extraction, the above equation can be adapted to convert *C. albicans* DNA content, measured by qPCR, to the number of *C. albicans* cells initially present in a fecal sample.

$$N_{cells} = \frac{DNA_m \times N_A}{M \times Genome \times E_{eff}} = 1{,}257{,}822 \times DNA_c$$

Where N_{cells} is the number of estimated *C. albicans* cells, DNA_m is *C. albicans* DNA content measured by qPCR, in nanogram, N_A is Avogadro's number (i.e. 6.022 x 10^{23} molecules/mole), M is the molar mass of a DNA base pair (i.e. 6.6 x 10^{11} ng/mole), $Genome$ is the size of the *C. albicans* genome (i.e. 26Mb) and E_{eff} is the DNA extraction efficiency, estimated by the spiking approach presented above (i.e 2.79%).

Considering the variations in extraction efficiency across the samples, the cell counts estimated by this equation can only be indicative but it helps in interpreting biologically the qPCR results. Therefore, in this PhD work, I chose to express all qPCR results on ng of DNA per gram of fecal samples, in order to retain a maximum of the accuracy brought by the qPCR assays.

In summary, in this chapter I compared three DNA extraction protocols for their efficiency to extract fungal DNA from fecal samples and selected one to use in the following analysis. In addition, I implemented two qPCR assays to quantify the total fungal burden and the levels of *C. albicans* in fecal samples and optimized them to be able to use them individually, in simplex assays, or combined with an internal control qPCR assay for the detection of qPCR inhibitors. Finally, I developed an equation taking into account the efficacity of the DNA extraction to convert *C. albicans* DNA contents, measured by qPCR, into cell count.

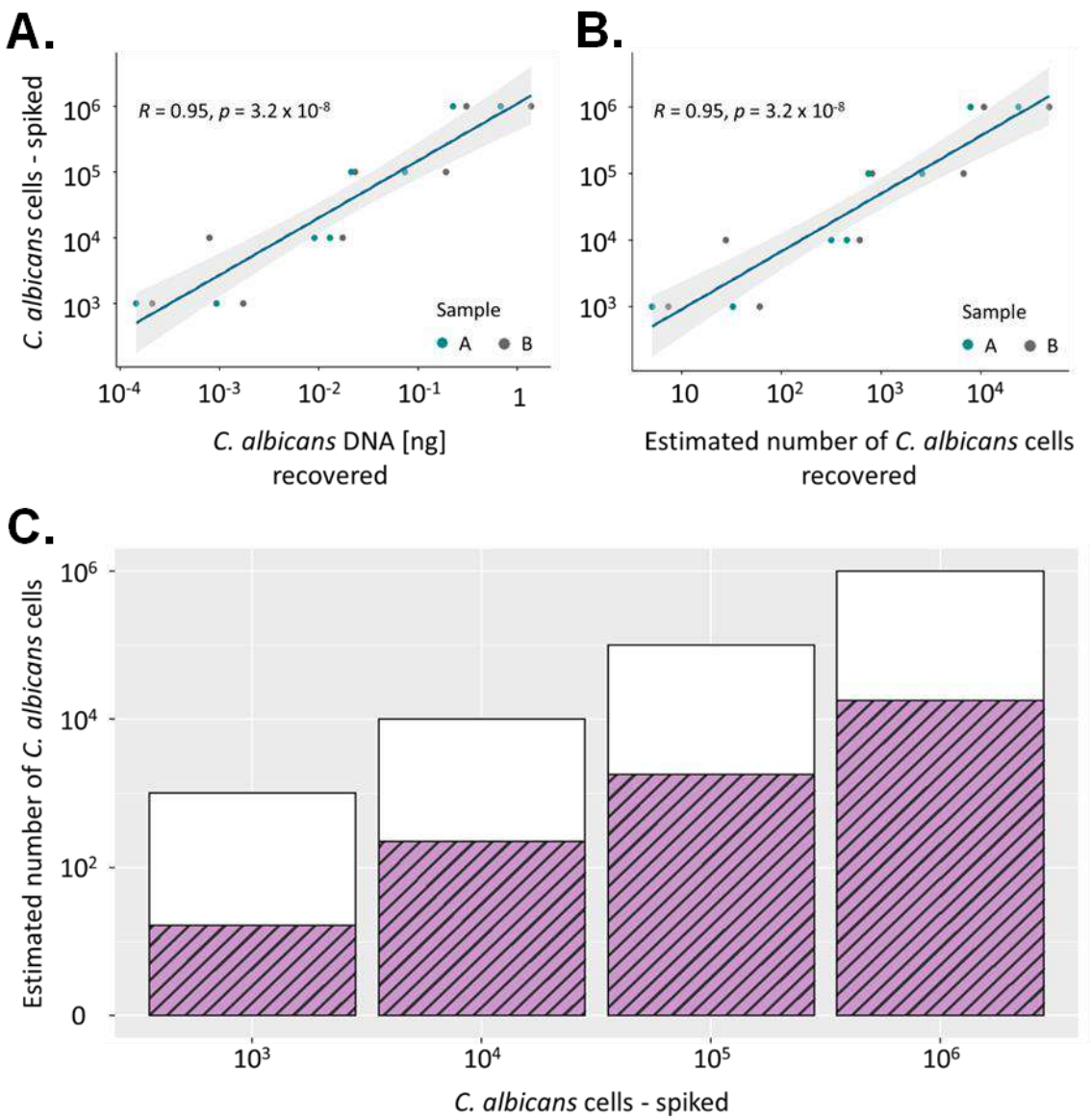


Figure 10: Estimation of *C. albicans* DNA and cells recovered after DNA extraction. (A). Correlation plot between the number of *C. albicans* cells spiked into a fecal sample and the amount of *C. albicans* DNA recovered after DNA extraction, as measured by qPCR. Regression is represented by a cyan line and the confidence interval by the gray area. Spearman's correlation coefficient (*R*) and associated p-value (*p*) are indicated at the top of the graph. (B) Correlation plot between the number of *C. albicans* cells spiked into a fecal sample and the estimated number of *C. albicans* cells recovered after DNA extraction, as measured by qPCR (see equation above). Regression is represented by a cyan line and the confidence interval by the gray area. Spearman's correlation coefficient (*R*) and associated p-value (*p*) are indicated at the top of the graph. (C) Boxplot of the efficiency of the DNA extraction associated with different amount of spiked *C. albicans* cells. Pink aeras corresponds to the recovered *C. albicans* content and white aeras to *C. albicans* content lost during the extraction.

4. Materials and Methods

Fecal samples. *Comparison of three DNA extraction protocols.* To compare the efficacy of the three DNA extraction protocols, I used five fecal samples from five healthy adults, that were collected from the ICAREB platform (Institut Pasteur, France). In addition of these samples, I used five additional fecal samples collected from five healthy subjects of the CEREMI study, a prospective, open-label and randomized clinical trial conducted from March 2016 to August 2017 in healthy adult subjects at the Clinical Investigation Center at Bichat-Claude Bernard Hospital (Paris, France) (Burdet, Nguyen, *et al.* 2019). The study is registered at ClinicalTrials.gov under identifier NCT02659033 and obtained approval from the Independent Ethics Committee Île-de-France 1 on 21 December 2015 (2015-oct-14028) and from the National Agency for Security of Medicinal Products on 24 July 2015 (150527A-41). Participants were given oral and written information and had to return signed consent before inclusion in the trial.

qPCR optimization. To optimize the qPCR assays presented in this study, we used the 266 fecal samples from healthy subjects, available with the CEREMI study.

Spiking experiment. Two fecal samples from healthy subjects, available with the CEREMI study, were used to assess the efficiency of *C. albicans* DNA extraction from fecal samples.

DNA extraction protocols. For each sample, three different genomic DNA extraction protocols were used (IHMS protocol Q, repeated bead beating plus column method and FASTDNA™ SPIN Kit, see below).

Protocol Q, International Human Microbiota Standards. For each sample, 100-250 mg of stool was processed following the recommendation of the IHMS_SOP 006 (Dore *et al.* 2015) with some minor adjustments. I added 0.1g of 0.5mm zirconia beads to the 0.3 g of 0.1mm zirconia beads recommended and performed 6 bead-beating cycles of 1 min at 6400 rpm with 5 minutes resting on ice between cycles.

Protocol A, Repeated bead beating plus column method. For each sample, 100-250 mg of stool was processed following the repeated bead beating plus column method described by Yu and Morrison, 2004 (Yu and Morrison 2004) except than a FastPrep-24TM device (MP Biomedicals, Belgium) was used instead of a Mini-Beadbeater™.

Protocol FAST, FASTDNA™ SPIN Kit (MP Biomedicals, USA). For each sample, 100-250 mg of stool was processed following the manufacturer recommendation. DNA extracts were eluted in 100µL DNase/Pyrogen-Free Water.

Total DNA quantification. Total fecal DNA levels were measured by Qubit (Invitrogen, USA) using the dsDNA Broad Range Kit (Invitrogen, USA).

16S-targeted metagenomic sequencing. Amplicon libraries, targeting the V3-V4 16S region (Klindworth *et al.* 2013) were prepared as described in the 16S Metagenomic Sequencing Library Preparation guide ("16S Metagenomic Sequencing Library Preparation", online).

ITS1-targeted metagenomic sequencing. Amplicon library, targeting the ITS1 region were prepared as described in Delavy *et al.* 2022.

Bacterial and fungal DNA sequences analyses. 16S and ITS1 sequences were analyzed with QIIME 2™ (Quantitative Insights into Microbial Ecology) (Bolyen *et al.* 2019).

16S sequences analysis. A total of 15.4 million sequences were generated from the 30 samples, with a mean of 514'750 sequences per sample. Sequences were trimmed to 300 bp and 228 bp for the forward and reverse sequences, respectively. Using the DADA2 tool (Callahan *et al.* 2016), the sequences were denoised and dereplicated into amplicons sequences variants (ASVs), and the chimeras were removed. I generated a feature table for all samples with a minimum of 6058 sequences per sample, and then subjected the feature table to taxonomic classification. The taxonomic annotation was performed using the SILVA database (release 132).

ITS1 sequences analysis. A total of 16.6 million of sequences were generated from the 30 samples, with a mean of 553'093 sequences per sample. Sequences were trimmed to 282 and 283 bp for the forward and reverse sequences, respectively. Using the DADA2 tool (Callahan *et al.* 2016), the sequences were denoised and dereplicated into ASVs, and the chimeras were removed. I generated a feature table for all samples with a minimum of 1167 sequences per sample. The taxonomic annotation was performed on the feature table using the UNITE database (rev. 8.0).

Statistical analysis. All analyses were performed on R (v. 4.1.2, Team R Development Core 2018). We used the vegan package (v. 2.6-4, Oksanen *et al.* 2019) to compute

diversity indexes and the ggplot2 package to generate the figures (v. 3.4.0, Wickham *et al.* 2018).

Quantitative PCR internal amplification control. To exclude the presence of qPCR inhibitors, samples diluted at 1:10, were submitted to the Universal Exogenous qPCR Positive Control for TaqMan® Assay (Eurogentec, Belgium), using a Cy®5-QXL®670 Probe system (Eurogentec, Belgium). Manufacturer's recommendations were followed. Samples with qPCR inhibitors at a 1:10 dilution were diluted at 1:100 and submitted to a new round of qPCR.

Quantitative PCR for detection of C. albicans DNA in human DNA samples. 10 µL of the extracted total fecal DNA, at 1:10 dilution, were used as a template for TaqMan qPCR analysis, using probe and primers described by Guiver *et al,* 2001 (Guiver, Levi, and Oppenheim 2001), for a final volume of reaction of 50 µL.

Original protocol. Primers and probes were used at 100nM and 200nM, respectively. All reactions were performed on a CFX96 Real-Time PCR system (BioRad, USA) with the following conditions: 2 min at 50°C, 10 min at 95°C, 15 s at 95°C and 1 min at 62°C, the last two steps repeated for 45 cycles. All samples were tested in duplicates.

Optimization. Primer concentration of 50nM, 300nM, 400nM and 900nM and probe concentrations of 50nM, 100nM and 250nM were tested. All reactions were performed on a CFX96 Real-Time PCR system (BioRad, USA) with the following conditions: 2 min at 50°C, 10 min at 95°C, 15 s at 95°C and 1 min at 60°C, the last two steps repeated for 45 cycles. All samples were tested in duplicates. In the optimized conditions, the limit of detection is of 10^{-8} ng/µL. Considering that the DNA extracts are prepared in a final volume of 100µL and diluted at 1:10 for the qPCR, this corresponds to a limit of detection of 10^{-5} ng of *C. albicans* DNA per fecal sample for the samples diluted at 1:10.

Quantitative PCR for detection of total fungal load in human DNA samples. Fungal DNA was quantified by TaqMan qPCR as described by Liu *et al.* 2012 (C. M. Liu *et al.* 2012) using a double dye MGB 5' 6-FAM-labelled probe (Eurogentec, Belgium). The fungal load was estimated by dividing the fungal DNA concentration by the total DNA concentration of the sample (Zuo *et al.* 2018), obtained by Qubit Broad Range protocol

Original protocol. All reactions were performed on a CFX96 Real-Time PCR system (BioRad, USA) with the following conditions: 2 min at 50°C, 10 min at 95°C, 15 s at 95°C and 1 min at 65°C, the last two steps repeated for 45 cycles. All samples were tested in two independent rounds, each time in duplicates.

Optimization. All reactions were performed on a CFX96 Real-Time PCR system (BioRad, USA) with an annealing temperature of 65°C, 64.2°C, 62.7°C and 60°C.

Spiking of fecal samples with C. albicans cells. The cell counts of an overnight culture of *C. albicans* strain SC5314 was quantified by flow cytometry using a MacsQuant Analyzer (Miltenyi Biotech, Germary). 100 mg of fecal samples were spiked with 10^6, 10^5, 10^4 and 10^3 *C. albicans* cells. The fecal samples were extracted using protocol A, (repeated bead beating plus column method). *C. albicans* DNA was quantified using the specific *C. albicans* qPCR assay described above. *C. albicans* DNA content was reverted into cell counts using the following equation, and compared to the initial number of *C. albicans* cells spiked:

$$N_{cells} = \frac{DNA_m \times N_A}{M \times Genome} = 35{,}093.24 \times DNA_c$$

Where N_{cells} is the number of estimated *C. albicans* cells, DNA_m is *C. albicans* DNA content measured by qPCR, in nanogram, N_A is Avogadro's number (i.e. 6.022 x 10^{23} molecules/mole), M is the molar mass of a base pair (bp) of DNA (i.e. 6.6 x 10^{11} ng/mole) and $Genome$ is the size of the *C. albicans* genome (i.e. 26Mb).

Statistical analysis. All analyses were performed on R (v. 4.1.2, Team R Development Core 2018). We the ggplot2 (v. 3.4.0, Wickham *et al.* 2018) and the ggpubr (v. 0.5.0, Kassambara 2022) packages to generate the figures.

B - CHAPTER 2: THE IMPACT OF AN ANTIBIOTIC-INDUCED MICROBIOTA DYSBIOSIS ON THE MYCOBIOTA AND *C. ALBICANS* INTESTINAL CARRIAGE IN HEALTHY INDIVIDUALS.

1. Context and aim

As seen in the introduction, *C. albicans* intestinal overgrowth is a prerequisite for intestinal translocation, which is at the root of systemic infections by this yeast (Zhai *et al.* 2020). A well-known risk factor for these infections is the administration of large-spectrum antibiotics (Pappas *et al.* 2018). Antibiotics are major disturbers of the intestinal microbiota but their role on the mycobiota is still unclear (Burdet, Nguyen, *et al.* 2019; Burdet, Grall, *et al.* 2019; Seelbinder *et al.* 2020; Fouhy *et al.* 2012). Studies have investigated the effect of antibiotics on mice mycobiota (Dollive *et al.* 2013; Fan *et al.* 2015) but at the beginning of this thesis, there was no report of the direct role of antibiotics in the overgrowth of *C. albicans* in healthy humans. In the study presented here, I analyzed how the gut mycobiota and, especially *C. albicans,* was affected by β-lactams, a family of large-spectrum antibiotics widely used in clinics that is excreted through the bile into the gut and can thus affect the intestinal mycobiota.

We followed prospectively healthy volunteers before, during and after a β-lactam treatment by third-generation cephalosporins (3GC) (Burdet, Nguyen, *et al.* 2019; Burdet, Grall, *et al.* 2019). The mycobiota of the healthy volunteers before the treatment was characterized by a low richness and evenness, with a median of only 25 fungal Operational Taxonomic Units (OTUs) per sample and a Shannon Index of 1.18, but a high diversity between the subjects and also within a same individual between the different samples collected. To quantify the carriage of *C. albicans*, I used a qPCR assay, which is more sensible than culture or metagenomic, and allows the determination of *C. albicans* absolute abundance, whereas metagenomic approaches can only return a relative abundance. I thus observed than 95.2% of the subjects were colonized by this yeast.

The antibiotics had no effect on the fungal diversity but the mycobiota profile was altered with a decrease of *Penicillium roqueforti* and *Debaryomyces hansenii* and an

increase of *Saccharomyces cerevisiae* relative abundances after the treatment. Both *C. albicans* relative and absolute abundance were increased after the treatment but this increase of *C. albicans* levels was subject-dependent, with strong inter-individual variations. Since a preliminary report suggested that the human gut hosts bacteria naturally able to produce β-lactamases (Leonard *et al.* 1989), enzymes able to hydrolyze β-lactam antibiotics, I hypothesized that β-lactamase activity could vary across the subjects following the antibiotic treatment and thus reduce antibiotics impact in the gut of some of them. Overall, β-lactamase activity was increased after the antibiotic treatment, but some subjects displayed a stronger increase than other, and this increase was negatively correlated with the change of *C. albicans* levels after antibiotics.

In summary, in this study I showed that *C. albicans* is present in the gut of almost all individuals, even at very low concentrations, which suggests that this species is not a facultative commensal, as it was previously hypothesized. Moreover, I highlighted an overall increase of the fungal load and *C. albicans* levels after 3CG treatment, but the degree to which *C. albicans* populations increased was subject-dependent. The same antibiotic treatment can therefore disturb the gut mycobiota and *C. albicans* carriage differently, depending on the subject receiving it. Taken together, the results presented in this study bring a new understanding of the factors behind the proliferation of *C. albicans* after exposure to antibiotics, which could lead to the identification of new ways to prevent potentially life-threatening secondary infections caused by this pathogen.

2. Research article: A Clinical Study Provides the First Direct Evidence That Interindividual Variations in Fecal β-Lactamase Activity Affect the Gut Mycobiota Dynamics in Response to β-Lactam Antibiotics

Contains: Article published in mBio (30/11/2022)

Margot Delavy[a], Charles Burdet[b,c], Natacha Sertour[a], Savannah Devente[d], Jean-Denis Docquier[d], Nathalie Grall[b], Stevenn Volant[e], Amine Ghozlane[e], Xavier Duval[b,f], France Mentré[b,c], Christophe d'Enfert[a], Marie-Elisabeth Bougnoux[a,g,#], on behalf of the PrediRes study group

[a] Institut Pasteur, Université Paris Cité, INRAE USC2019, Unité Biologie et Pathogénicité Fongiques, Paris, France

[b] Université Paris Cité, IAME, INSERM, Paris, France

[c] AP-HP, Département d'Epidémiologie, Biostatistique et Recherche Clinique, Hôpital Bichat, Paris, France

[d] Università di Siena, Dipartimento di Biotecnologie Mediche, Siena, Italy

[e] Institut Pasteur, Université Paris Cité, Hub de Bio-informatique et Biostatistique, Paris, France

[f] INSERM Centre d'Investigation clinique 1425, Paris, France

[g] Unité de Parasitologie-Mycologie, Service de Microbiologie Clinique, Hôpital Necker-Enfants-Malades, Assistance Publique des Hôpitaux de Paris (APHP), Paris, France.

Running Head: β-lactams and gut mycobiota dynamic in healthy subjects

i. Abstract

Antibiotics disturb the intestinal bacterial microbiota, leading to gut dysbiosis and an increased risk for the overgrowth of opportunistic pathogens. It is not fully understood to what extent antibiotics affect the fungal fraction of the intestinal microbiota, the mycobiota. There is no report of the direct role of antibiotics in the overgrowth in healthy humans of the opportunistic pathogenic yeast *Candida albicans*. Here, we have explored the gut mycobiota of 22 healthy subjects before, during, and up to 6 months after a 3-day regimen of third-generation cephalosporins (3GCs). Using ITS1-targeted metagenomics, we highlighted the strong intra- and interindividual diversity of the healthy gut mycobiota. With a specific quantitative approach, we showed that *C. albicans* prevalence was much higher than previously reported, with all subjects but one being carriers of *C. albicans*, although with highly variable burdens. 3GCs significantly altered the mycobiota composition and the fungal load was increased both at short and long term. Both *C. albicans* relative and absolute abundances were increased but 3GCs did not reduce intersubject variability. Variations in *C. albicans* burden in response to 3GC treatment could be partly explained by changes in the levels of endogenous fecal β-lactamase activity, with subjects characterized by a high increase of β-lactamase activity displaying a lower increase of *C. albicans* levels. A same antibiotic treatment might thus affect differentially the gut mycobiota and *C. albicans* carriage, depending on the treated subject, suggesting a need to adjust the current risk factors for *C. albicans* overgrowth after a β-lactam treatment.

ii. Importance

Fungal infections are redoubtable healthcare-associated complications in immunocompromised patients. Particularly, the commensal intestinal yeast *Candida albicans* causes invasive infections in intensive care patients and is, therefore, associated with high mortality. These infections are preceded by an intestinal expansion of *C. albicans* before its translocation into the bloodstream. Antibiotics are a well-known risk factor for *C. albicans* overgrowth but the impact of antibiotic-induced dysbiosis on the human gut mycobiota—the fungal microbiota—and the understanding of the mechanisms involved in *C. albicans* overgrowth in humans are very limited. Our study shows that antibiotics increase the fungal proportion in the gut and disturb the fungal composition, especially *C. albicans*, in a subject-dependent manner. Indeed,

variations across subjects in *C. albicans* burden in response to β-lactam treatment could be partly explained by changes in the levels of endogenous fecal β-lactamase activity. This highlighted a potential new key factor for *C. albicans* overgrowth. Thus, the significance of our research is in providing a better understanding of the factors behind *C. albicans* intestinal overgrowth, which might lead to new means to prevent life-threatening secondary infections.

Keywords: antibiotics, *Candida albicans*, gut mycobiota, healthy individuals, beta-lactamases

iii. Introduction

Interest in the role of the gut microbiota in health and disease is rising (Krajmalnik-Brown *et al.* 2015; Milani *et al.* 2016; T *et al.* 2020; Turnbaugh *et al.* 2007) and the role of antibiotics as major disturbers of the microbiota healthy state has been largely studied (Burdet, Nguyen, *et al.* 2019; Seelbinder *et al.* 2020; Fouhy *et al.* 2012; Burdet, Grall, *et al.* 2019). By killing the resident bacteria of the gut, broad-spectrum antibiotics reduce bacterial diversity in the gastrointestinal (GI) tract and decrease the abundance of beneficial bacteria (Burdet, Nguyen, *et al.* 2019; Fouhy *et al.* 2012). They also alter the gut microbiota interaction network, thus contributing to the overgrowth of opportunistic pathogens (Seelbinder *et al.* 2020; Smits *et al.* 2016). More alarmingly, the prolonged use of antibiotics may promote antibiotic resistance (Blair *et al.* 2015). For example, β-lactam exposure can lead to the selection of specific gut bacteria able to produce β-lactamases, enzymes that can hydrolyze β-lactam antibiotics, leading to an overall increase in antibiotic resistance (Leonard *et al.* 1989; Niehus *et al.* 2020).

While the bacterial microbiota is extensively studied, less attention has been paid to the mycobiota – the fungal part of the microbiota – and to the consequences that antibiotic-induced dysbiosis may have on the fungal communities of the gut. It is now well established that fungi can rapidly proliferate in the GI tract of mice after removal of gut bacteria by antibiotics (Dollive *et al.* 2013). The mouse GI tract is not naturally colonized by the opportunistic pathogen *Candida albicans* and antibiotics have been used to trigger such colonization (Fan *et al.* 2015), suggesting that they clear specific bacteria able to inhibit *C. albicans* growth in the mouse GI tract (Mirhakkak *et al.* 2021). Yet, we need more information about the impact of an antibiotic-induced dysbiosis on the healthy human gut mycobiota and specifically *C. albicans*. Since *C. albicans*

systemic infections are responsible for thousands of deaths each year (Brown *et al.* 2012) and since antibiotics are a well-known risk factor for these infections (Pappas *et al.* 2018), we need to better understand the mechanisms of *C. albicans* overgrowth in the human gut, upon antibiotic treatment.

In this work, we prospectively followed two parallel groups of 11 healthy subjects each, before, during and after they were treated intravenously with either cefotaxime or ceftriaxone, two third generation cephalosporin (3GC) antibiotics that share a similar activity spectrum (Burdet, Grall, *et al.* 2019). We quantified the levels of *C. albicans* carriage in all subjects and characterized their healthy mycobiota and its variability during the two-week period preceding antibiotic administration. Then, we analyzed the changes in terms of fungal diversity, fungal burden, community profile and *C. albicans* levels, occurring in the mycobiota after antibiotics were administrated, both at short and long term. Finally, we monitored the level of fecal β-lactamase activity, which is known to modulate the intensity of the post-3GC intestinal dysbiosis, and we correlated the changes in β-lactamase activity with the impact of 3GCs on *C. albicans* carriage.

iv. Results

The gut mycobiota of healthy subjects is highly dynamic and variable. To study the healthy mycobiota, we used fecal samples collected from each of 22 healthy volunteers at D-15, D-7 and D-1, before antibiotic administration (see Methods). In total, 54 fecal samples (1-3 available per subject) were available for analyses.

First, we assessed the fungal load, *i.e.* the ratio between the fungal DNA concentration and the total fecal DNA (see Materials and Methods). The mycobiota represented a very small fraction of the total microbiota in healthy subjects (median fungal load: $7.9x10^{-6}$, min: $6.7x10^{-10}$, max: $1.5x10^{-3}$, Supplementary Figure 1A).

Using ITS1 sequencing, we further characterized the mycobiota composition of the 22 subjects during the two weeks preceding 3GC exposure. We identified 233 different OTUs, 182 OTUs (78.1%) being annotated at the phylum level, 167 (71.7%) at the genus level and 123 (52.8%) at the species level. Overall, the 167 OTUs annotated at the genus level and the 123 OTUs annotated at the species level represented 99.7% and 91.2% of the total number of sequences, respectively.

Ascomycota was the most abundant phylum (mean relative abundance of 77.9%), followed by Basidiomycota (21.9%, Supplementary Figure 1B). Sixty-two fungal

genera were identified in at least two samples, with eight reaching a mean relative abundance across subjects above 1% (Supplementary Figure 1C). Ninety-five species were identified in at least two samples, nine reaching a mean relative abundance across subjects above 1% (Table 1).

Table 1: Prevalence of the main fungal species in healthy subjects and in their fecal samples, estimated by ITS1 sequencing.

Main fungal species	Prevalence[a] Fecal samples (%) (N=54)	Healthy subjects (%) (N=22)
Vanrija humicola	98.2	100.0
Galactomyces candidus	92.6	95.5
Saccharomyces cerevisiae	88.9	95.5
Candida parapsilosis	88.9	95.5
Penicillium roqueforti	72.2	90.9
Cutaneotrichosporon curvatum	87.0	86.4
Malassezia restricta	88.8	77.3
Candida albicans	75.9	72.7
Debaryomyces hansenii	68.5	59.1

[a]A species is considered present in a sample if its relative abundance is above 0.1%. A species is considered present in a subject if it is present in at least one sample between −D15 and −D1.

The taxa relative abundances were highly variable between individuals and across time (Figure 1A), with *Galactomyces candidus* being the most disparately represented taxa, with a relative abundance varying from 0 to 99.2% depending on the sample.

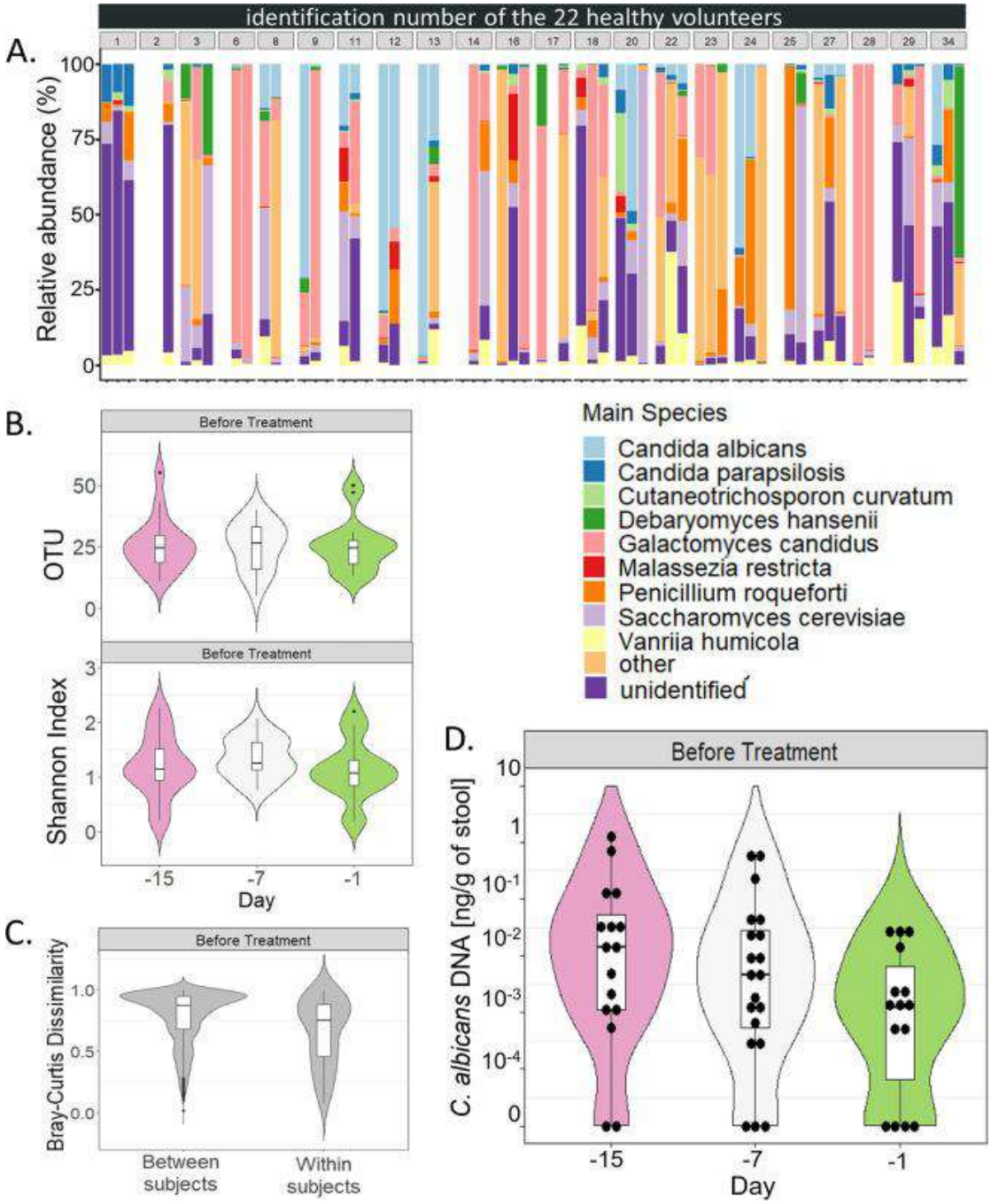


Figure 1: Dynamic of the mycobiota characteristics in 22 healthy individuals during a 2-week period. (A) Fungal species relative abundances at 1-week apart time points for 22 healthy subjects. For each subject, barplots are ordered by time (−D15, −D7, −D1 before antibiotics). Represented species reached a mean relative abundance across subjects above 1%. (B) Alpha diversity: violin plot of the number of OTUs and of the Shannon index values at 1-week apart time points for 22 healthy subjects. (C) Beta diversity: Bray-Curtis dissimilarity values between samples donated by different subjects (between subjects) and between samples donated by the same subjects (within subjects) for ITS1 sequencing data. Values range from 0 to 1, with 0 being the least dissimilar and 1 being the most dissimilar. (D) Violin and boxplots of the *C. albicans* DNA levels at 1-week apart time points for 22 healthy subjects.

Each dot represents a sample. *For all panels, the upper whiskers extend from the hinge to the largest value below 1.5× the interquartile range, and the lower whiskers extend from the hinge to the smallest value above 1.5× the interquartile range.*

We identified a median of only 25 OTUs per sample (min: 5, max: 55, Figure 1B), corresponding to a median Shannon Index of 1.18 (min: 0.18, max: 2.26, Figure 1B), reflecting a low richness and evenness within each sample. Unlike this low α-diversity, we observed a high β-diversity, which quantifies the level of dissimilarity between samples, with a median Bray-Curtis dissimilarity index of 0.87 between the subjects (min: 0.02 max: 1.00, Figure 1C). We also followed the variations occurring overtime during the 2-week period preceding 3GC exposure. The within subjects' diversity, measured between the samples collected from the same subject at different time, was almost as high as the between subject diversity, with a Bray-Curtis dissimilarity index of 0.75 (min: 0.08, max: 0.99, Figure 1C, Supplementary Figure 2).

We quantified the levels of fecal *C. albicans* in these volunteers, by determining the absolute abundance of *C. albicans*, using specific qPCR. We detected *C. albicans* DNA at least once between D-15 and D-1 in 20/21 subjects (95.2%) before 3GC administration. In total, 42/51 samples analyzed were positive for *C. albicans* (82.4%) and in these samples, *C. albicans* DNA levels ranged from $2.8x10^{-4}$ to 1.26 ng/g of stool, with a median of $2.x10^{-3}$ ng/g of stool (Figure 1D). In comparison, by using ITS1 sequencing data and culture methods, we could detect *C. albicans* in only 16/22 (72.7%, Table 1) and in 7/22 (15.8%) subjects, respectively.

Cefotaxime and ceftriaxone exposure increases the fungal load and disturbs the gut mycobiota composition. To measure how much the antibiotic treatment affected the gut mycobiota, we compared its features, including the fungal load, genera and species composition, and *C. albicans* absolute levels, at baseline (D0) with those during and after antibiotics (Figure 2A). Data collected at D-1 were used as baseline. If missing, D-7 data were used instead (See Supplementary Figure 3).

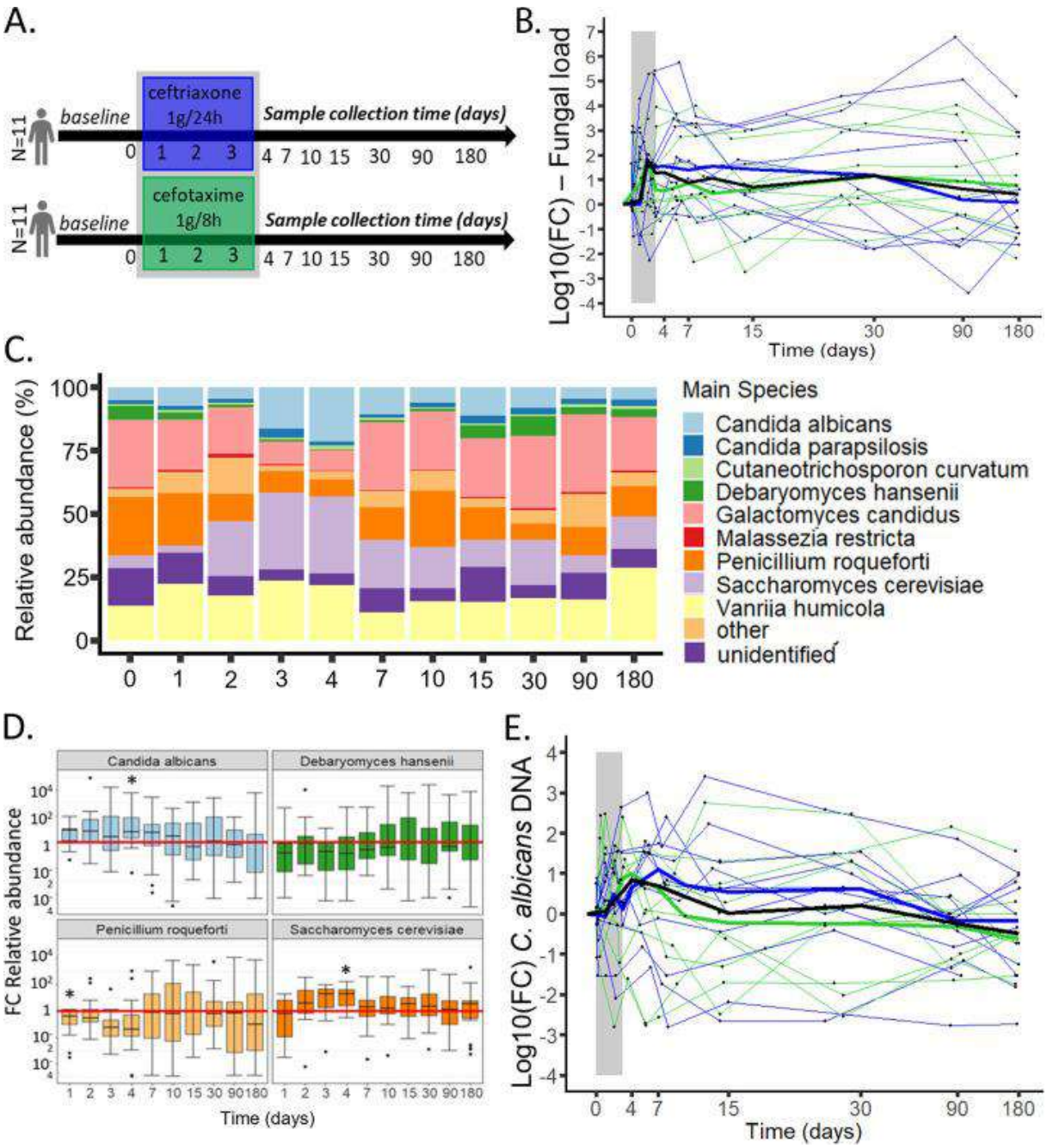


Figure 2: Impact of 3-day cefotaxime and ceftriaxone IV treatment on the gut mycobiota of 22 healthy subjects followed for a 6-month period. (A) Study design. (B) Log10 (foldchange [FC]) of the fungal load following ceftriaxone and cefotaxime treatment (gray area). Thin lines represent the subjects; thicker lines represent the medians at each day for each treatment group (blue and green) and for all subjects (black). (C) Main fungal species distribution following ceftriaxone and cefotaxime treatment. (D) Distribution of the relative abundance log10 (FC) AUCs for *Candida albicans, Debaryomyces hansenii, Penicillium roqueforti,* and *Saccharomyces cerevisiae*, highlighting the duration and the amplitude of the perturbations **q value < 0.05, Wilcoxon t-test, false-discovery rate correction. Upper whiskers extend from the hinge to the largest value below 1.5× the interquartile range, and the lower whiskers extend from the hinge to the smallest value above 1.5× the interquartile range.* (E) Log10 (FC) of *C. albicans* DNA levels following ceftriaxone and cefotaxime treatment (gray

area). Thin lines represent the subjects; thicker lines represent the medians at each day for each treatment group (blue and green) and for all subjects (black).

We used two metrics to estimate changes during and after antibiotic administration: the Areas Under the Curve (AUCs) of the mycobiota characteristics' changes from D0, and the changes from D0 of the mycobiota characteristics, for each subject, at different time points between D1 and D180. The first metric allows the aggregation of both the duration of the changes and their amplitude whereas the second allows the detection of more punctual variations.

We observed a general long-term increase of the fungal load in the 22 subjects early after the start of the antibiotic treatment. The fungal load significantly increased immediately after the start of antibiotics, independently of the antibiotic used, with a positive AUCs for all calculated periods between D0 and D2 and D0 and D90 (Wilcoxon test; p-values of 0.008, 0.017, 0.040, 0.014, 0.009, 0.005, 0.006 and 0.048, respectively) with a maximal 62.3-fold increase at D2 (min: 0.02, max: 1.8 $x10^4$; Wilcoxon test; p-value of 0.007, Figure 2B, Supplementary Figure 4B, Supplementary Table 1). No difference was observed between the subjects treated with ceftriaxone and those treated with cefotaxime (Figure 2B, Supplementary Table 2).

At D15, we observed a slight increase of the number of fungal OTUs, compared to D0 (Wilcoxon test; p-value of 0.030, Supplementary Table 1) but not of the Shannon Index (Wilcoxon test; p-value of 0.47), suggesting that the fungal α-diversity is not strongly impacted by the antibiotics. No difference was observed between the subjects treated with ceftriaxone or those treated with cefotaxime, and this for all fungal diversity indices studied (Supplementary Table 2).

Three genera were significantly impacted by the antibiotics: *Debaryomyces* sp, *Penicillium* sp. and *Saccharomyces* sp. (Supplementary Figure 4A and B, Supplementary Table 1). *Debaryomyces* sp. were significantly decreased immediately after the start of the treatment, with negative AUCs between D0 and D3 (Wilcoxon test; q-value of 0.02) and a maximal but not significant 12.5-fold drop at D3 (min: 0.09, max: $1.7x10^5$, Wilcoxon test; q-value of 0.08). *Penicillium* sp. were also decreased immediately after the start of the treatment, with negative AUCs between D0 and D2 and up to D0 and D7 (Wilcoxon test; q-values of 0.01, 0.005, 0.003 and 0.0002, respectively) with a maximal 21.4-fold decrease at D4 (min: 0.81, max: 776.2; Wilcoxon test; q-value of 0.0008). On the contrary, *Saccharomyces* sp. relative abundance was

punctually increased at D4 (median: 19.5-fold increase, min: 0.32, max: 169.8), compared to baseline (Wilcoxon test; q-value of 0.01), before returning to basal levels. No significant difference between the subjects of the two treatment groups was observed at any day, for all genera tested (Supplementary Table 2).

In addition, at the species level, four taxa were significantly affected by 3GC treatment: *S. cerevisiae, D. hansenii, P. roqueforti* and *C. albicans* (Figure 2C, Supplementary Table 1). *D. hansenii* was decreased for the period D0D3, with a corresponding negative AUC (Wilcoxon test; q-value of 0.047, Supplementary Figure 4C) and *P. roqueforti* was punctually reduced after the treatment with a 2.4-fold drop at D1 (min: 0.74, max: $3.3x10^3$; Wilcoxon test; q-value of 0.026, Figure 2D). By contrast, *C. albicans* and *S. cerevisiae* relative abundance displayed a 9.8-fold and 19.5-fold raise at D4, respectively (*C. albicans:* max: $1.1x10^5$, min: 0.004; Wilcoxon test; q-value of 0.04, *S. cerevisiae:* max: 169.8, min: 0.32 Wilcoxon test; q-value of 0.026, Supplementary Figure 4C). As for the genera, no significant difference between the subjects of the two groups was observed for any species (Supplementary Table 2).

Not only *C. albicans* relative abundance but also its absolute abundance was punctually increased after antibiotics. Indeed, 3GC administration led to a punctual raise of *C. albicans* DNA levels on the D0D4 period (Wilcoxon test, q-value of 0.047) with a maximal 2.1-fold increase at D2 (min: 0.03, max: 288.4; Wilcoxon test; p-value of 0.02, Figure 2E), when measured by qPCR. However, this increase of *C. albicans* DNA levels was subject-dependent. For example, subject 1 displayed an impressive increase of *C. albicans* DNA, with a maximal 2521.3-fold raise at D15 whereas *C. albicans* DNA levels were reduced in subject 12 after the treatment. No difference was observed between the two groups of treatment at any days (Figure 2E, Supplementary Table 2).

Change in β-lactamase activity levels as a key parameter for C. albicans overgrowth in the GI tract after 3rd generation cephalosporin administration. β-lactamase activity was measured in each fecal sample by dosing the NFC-hydrolyzing activity. This activity was heterogenous between subjects before antibiotics, ranging from 2.40 to 1240 nmol/ min·g of stool, with no difference between the two groups that received either ceftriaxone or cefotaxime (Wilcoxon test, p-value: 0.78). Globally, β-lactamase activity was significantly increased after 3GC administration for all the

periods calculated between D0D3 and D0D180 (Wilcoxon test; p-values of 0.040, 0.006, 0.002, 0.0008, 0.0003, 0.0006, 0.0007 and 0.03, respectively), with a maximal 2.25-fold increase at D7 (Figure 3A). However, we observed two types of behavior in the D0D10 AUC of the change in β-lactamase activity, with some subjects displaying a high increase of the β-lactamase activity after antibiotic treatment (up to a 28-fold rise) whereas others showed no change or even a decrease of this activity (up to a 7-fold decrease). Therefore, we split the 22 subjects in two groups, the “High” group was characterized by a strong increase of the fecal β-lactamase activity (AUC D0D10 >= 2.36),

whereas the “Low” group had a lower increase or even a decrease of this activity (AUC D0D10 < 2.36, Figure 3B). Changes of *C. albicans* DNA levels were significantly different between subjects of the “Low” and “High” groups, both for the D0D10 period and at D4 and D30 (Wilcoxon test; p-value of 0.02, 0.02 and 0.007, respectively). At D4 and D30, *C. albicans* DNA levels were significantly increased in the group “Low”, whereas no change was detected in the group “High” (D4: Wilcoxon test; p-value of 0.008 and 0.84, respectively; D30: Wilcoxon test; p-value of 0.023 and 0.26, respectively, Figure 3C).

Finally, we showed a highly significant negative interaction between the D0D10 AUC of the change in β-lactamase activity and the D0D10 AUC of the change in *C. albicans* DNA levels (Spearman correlation; R: -0.59, p-value: 0.009, Figure 3D). No such correlation could be found between the D0D10 AUC of the fungal load and the D0D10 AUC of the change in β-lactamase activity (Spearman correlation; R: -0.25, p-value: 0.3).

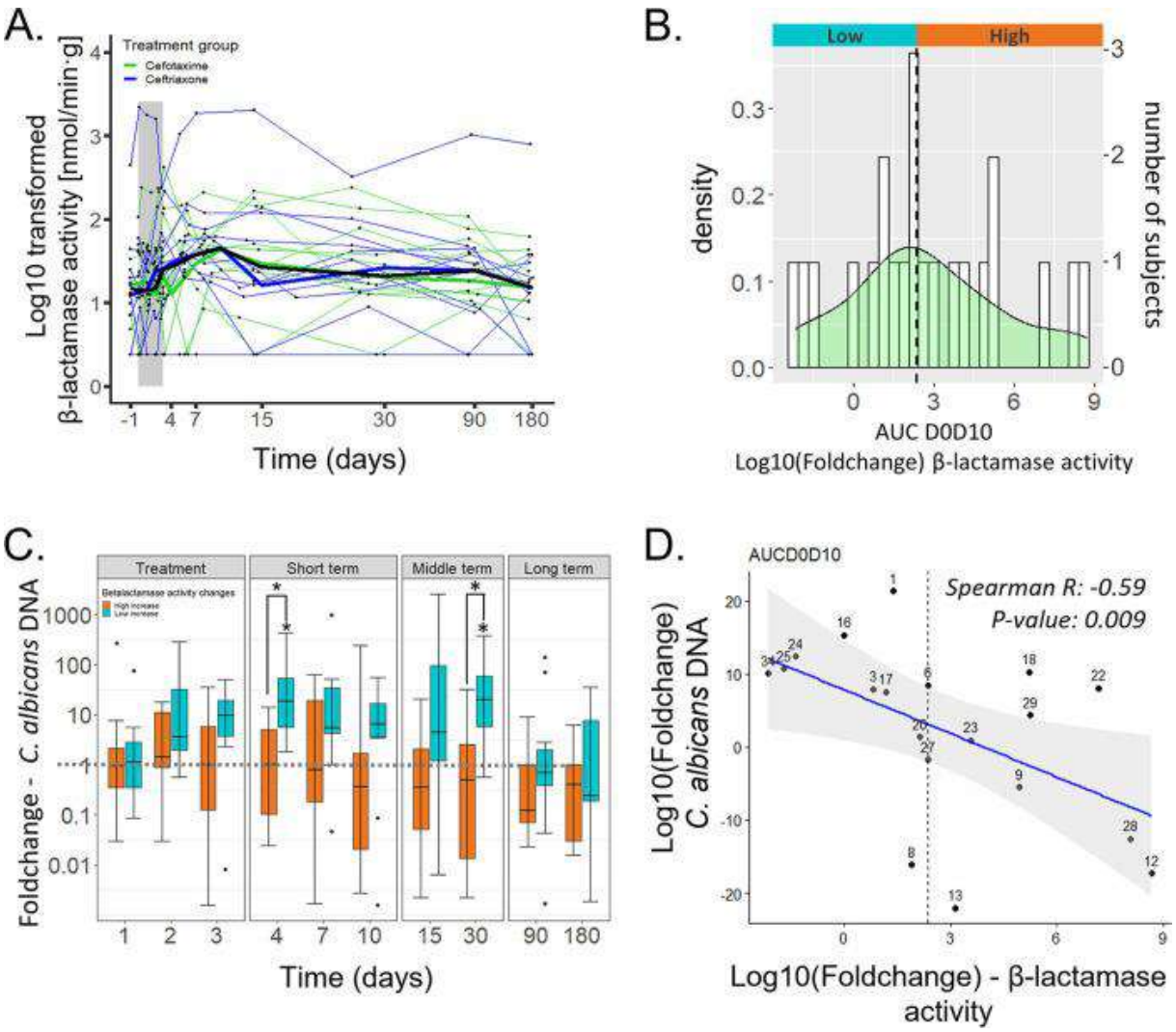


Figure 3: Change in β-lactamases activity levels as a key parameter for *Candida albicans* proliferation in the gut after third-generation cephalosporin administration. (A) β-lactamases activity before, during, and after the antibiotic treatment. Thin lines represent the subjects; thicker lines represent the medians at each day for each treatment group (ceftriaxone, blue; cefotaxime, green) and for all subjects (black). (B) Distribution of the D0 to D10 AUCs of the change of β-lactamases activity in 22 healthy subjects. The density distribution is represented by the green curve and the number of subjects for each range of AUC values are represented by the white histogram. The group "high" (orange) regroups the subjects with a D0 to D10 AUC above the median (black dashed line) and the group "low" (blue) regroups the subjects with a D0 to D10 AUC below the median. (C) Boxplots of *C. albicans* DNA levels log10 (FC) following ceftriaxone and cefotaxime treatment. Orange boxplots indicates values for the subjects from the group "high" of and blue boxplots represents the values for the subjects from the group "low." *Upper whiskers extend from the hinge to the largest value below 1.5× the interquartile range, and the lower whiskers extend from the hinge to the smallest value above 1.5× the interquartile range.* (D) Correlation plot of the log10 (FC) AUCs of *C. albicans* DNA levels and β-lactamases activity for the D0 to D10 period. Regression is represented by a blue line and the confidence interval by the gray area. Subjects are designated by their ID number.

v. Discussion

In this study, we explored the impact of β-lactam antibiotics on the human gut mycobiota by performing a targeted metagenomic analysis of the mycobiota of healthy subjects before, during and after 3GC exposure. 3GC strongly affected the mycobiota, especially *C. albicans* carriage, with wide inter-subject variations that were not related to the type of 3CG they received. We identified the changes of fecal β-lactamase activity after treatment as a potential key factor regulating *C. albicans* overgrowth, with subjects characterized by a low increase of β-lactamase activity displaying a stronger increase of *C. albicans* levels following antibiotics. This regulation is likely mediated by a differential impact of antibiotics on the endogenous gut bacteria, according to differences in the occurrence of β-lactamase-producing bacteria in the microbiota. Briefly, a microbiota rich in β-lactamase-producing bacteria would favor 3GC hydrolysis, reduced antibiotic-induced microbiota dysbiosis and reduced *C. albicans* overgrowth. In contrast, a microbiota poor in β-lactamase-producing bacteria would allow 3GC maintenance, high antibiotic-induced microbiota dysbiosis and high *C. albicans* overgrowth. This phenomenon may explain the so-called *C. albicans* colonization resistance experienced by some individuals. Such colonization resistance has been the subject of an old and preliminary report (Leonard *et al.* 1989) but has not been further explored until this present study. Overall these results are coherent with the hypothesis stating that specific intestinal bacteria or their metabolites regulate *C. albicans* overgrowth (Seelbinder *et al.* 2020; d'Enfert *et al.* 2021; Fan *et al.* 2015; Mirhakkak *et al.* 2021; Leonardi *et al.* 2020). Our results attest that the same antibiotic regimen may affect differentially the microbiota and consequently lead to different risks of *C. albicans* overgrowth depending on the subject that receives it. The current paradigm stating that antibiotics are systematically a risk factor for *C. albicans* overgrowth should thus be adjusted for treatments based on β-lactams antibiotics. Monitoring fecal β-lactamase activity during and after a β-lactams antibiotic treatment could be an accurate predictor of the actual risk of a later increase of *C. albicans* burden.

As importantly, we found that *C. albicans* was present, in varying quantity, in the gut of almost every of the healthy subjects. This study is the first to use a qPCR method to quantify and follow *C. albicans* carriage, allowing an increasingly specific detection. Using more traditional assays, the prevalence of *C. albicans* in these subjects was

much lower and close to what has been previously reported (Bougnoux *et al.* 2006; Nash *et al.* 2017; da Silva Dantas *et al.* 2016). This suggests that our results reflect the reality of what is the true presence of *C. albicans* in the gut of healthy humans. If confirmed in a larger study, this might indicate that *C. albicans* is not a facultative commensal as previously thought, but that it is able to maintain itself in the gut of most individuals, even at very low concentration.

That almost all subjects in this study were colonized by *C. albicans* renders our cohort particularly adapted to follow the effects of antibiotic treatment on *C. albicans* carriage. Moreover, contrary to what has been done in other studies (Seelbinder *et al.* 2020), our focus on a single antibiotic family allows a precise understanding on how 3GC, a largely used antibiotics family, acts on the human gut mycobiota. This allowed us to show that 3GC strongly affect the gut mycobiota, with a global increase of the fungal load, as well as a punctual perturbation of several fungal species and genera, including *C. albicans.* Indeed, both *C. albicans* relative and absolute abundances were increased after the start of antibiotics. This is particularly concerning since a recent report showed that the administration of β-lactam antibiotics leads to increased virulence of *C. albicans* (Tan *et al.* 2021). By killing Gram-negative commensal bacteria, β-lactams cause the release of a large amount of peptidoglycans, which can then induce *C. albicans* hyphal growth, an essential virulence factor of this species (Tan *et al.* 2021). Moreover, a recent study showed that exposure to non-3GC broad-spectrum antibiotics not only promotes susceptibility to *C. albicans* systemic infection in mice, but also increases the mortality, through an impairment of the lymphocyte-dependent IL-17A- and GM-CSF-mediated response (Drummond *et al.* 2022). Therefore, *C. albicans* can not only growth in patients treated with 3GCs but its disruptive abilities might also be increased. This can be particularly problematic, not only for immunosuppressed patients, but also for those with inflammatory bowel disease that are already carrying higher levels of *C. albicans* (Sokol *et al.* 2017; Sendid *et al.* 2008) in their gut.

Overall, most of the mycobiota perturbations following 3GC treatment were subject-dependent, with some subjects more impacted than others. This is not particularly surprising considering the within- and between- subjects Bray-Curtis dissimilarity observed pre-treatment. This has already been reported (Nash *et al.* 2017) and suggests that the largest part of the fecal mycobiota is made of transient species

brought by the diet, such as *D. hansenii*, which is commonly found in cheese (Fröhlich-Wyder, Arias-Roth, and Jakob 2019), or potentially by the respiration of spores of filamentous fungi, which can then be swallowed, such as *Penicillium* sp. This hypothesis is supported by a recent study showing that diet-associated fungi are recovered with low relative abundances in mucosal surgical-recovered samples, highlighting the differences observed in the gut mycobiota depending on its spatial organization across the GI tract (Leonardi *et al.* 2022). Moreover, associated fungi strongly contribute to the fungal biomass of the fecal microbiota (Doron *et al.* 2021). Our results would also indicate that contrary to *Penicillium sp* or *D. hansenii, C. albicans* main reservoir is indeed humans, which would explain why an environmental reservoir for this species has yet to be found (Jabra-Rizk *et al.* 2016; Nucci and Anaissie 2001; d'Enfert *et al.* 2021).

Finally, fungi represented only a small fraction of the total microbiota based on total and fungal DNA quantification. This underrepresentation of the fungal community in the human gut has already been reported (Qin *et al.* 2010), but the authors did not quantify the exact proportion of the mycobiota. More recently, the fungal load of twenty-four healthy subjects was estimated, with results very similar to ours (Zuo *et al.* 2018). Finally, Doron *et al.,* confirmed that the fungal biomass was low within the gut microbiota, representing only 1-2% of the microbial biomass of the gut (Doron *et al.* 2021). However, to our knowledge, this present study is the first to assess the day-to-day variation of the fungal load in healthy individuals.

Taken together, this study offers a better understanding of the factors behind *C. albicans* overgrowth after antibiotics. We showed that a same antibiotic treatment may disturb differentially the gut microbiota, depending on the subject that receives it. This highlights the importance of a more personalized use of antifungal prophylaxis and helps limiting the selection of fungi resistant to antifungal drugs in patients at high risk of invasive candidiasis, such as intensive care unit or haemato-oncology patients.

vi. Materials and Methods

CEREMI cohort. In this study, we used fecal samples from the CEREMI study, a prospective, open-label and randomized clinical trial conducted from March 2016 to August 2017 in healthy adult subjects at the Clinical Investigation Center at Bichat-Claude Bernard Hospital (Paris, France) (Burdet, Grall, *et al.* 2019). Participants were

given oral and written information and had to return signed consent before inclusion in the trial. For more information about the clinical trial, see Burdet *et al.* 2019 (Burdet, Grall, *et al.* 2019).

The 22 included subjects were randomized in a 1:1 ratio and were treated for 3 days with either ceftriaxone (1 g/24 h) or cefotaxime (1 g/8 h). 3GC were administrated as a 30-min intravenous infusion using an automatic high-precision infusion pump.

Fecal samples were collected before treatment at D -15, -7 and -1, during treatment at D1, 2 and 3 and after treatment at D4, 7, 10, 15, 30, 90 and 180. Fecal samples were stored at -80°C.

Fungal DNA extraction from fecal samples. For each sample, 250mg of stool was processed following the repeated bead beating plus column method described by Yu and Morrison, 2004 (Yu and Morrison 2004), except than a FastPrep-24™ device (MP Biomedicals, Belgium) was used instead of a Mini-Beadbeater™.

Total fecal DNA levels were measured by Qubit (Invitrogen, USA) using the dsDNA Broad Range Kit (Invitrogen, USA). Samples for which this concentration was below 50 ng/µL were excluded from the analysis.

ITS1 sequencing. We prepared amplicon libraries, targeting the ITS1 region, using ITS1F and ITS2 primers (Cui, Morris, and Ghedin 2013; GARDES and BRUNS 1993). Amplicon were generated by PCR using a 96-well thermal cycler in the following conditions: 95°C for 3 min, 25 cycles of 95°C for 30 secs, 55°C for 30 secs and 72°C for 30 secs, 72°C for 5 min and cooling at 4°C. Amplicons were purified with AMPure XP (Beckman Coulter, USA) as described in the 16S Metagenomic Sequencing Library Preparation guide (Illumina 2013). Adapter were attached using Nextera XT Index Kit (Illumina, France) and the index PCRs were performed in the following conditions: 95°C for 3 min, 8 cycles of 95°C for 30 secs, 55°C for 30 secs and 72°C for 30 secs, 72°C for 5 min and cooling at 4°C. Barcoded PCR products were purified with AMPure XP (Beckman Coulter, USA) and verified and quantified on a Bioanalyzer DNA 1000 chip (Agilent, USA). Samples were normalized at 4nM and pooled into a library, using 5µL of each diluted sample. A PhiX sequencing control was prepared following the manufacturer's instructions. The libraries were sequenced in 300 bp paired-end using the MiSeq Reagent Kit V3 on Illumina MiSeq platform (Illumina, Evry, France).

OTU building process and taxonomic assignment. We recovered 8’819’635 amplicons from ITS1 region. After removal of singletons and chimera amplicons using the SHAMAN pipeline (Volant *et al.* 2020), we clustered the 56’634 remaining amplicons in OTUs using a cut-off value of 97% similarity. 4648 OTUs were thus obtained and 551 of them could be aligned against fungal sequences of the UNITE database. On these 551 fungal OTUs, 340 were present in at least two fecal samples and were conserved for the downstream analyses. We performed a first round of annotation on SHAMAN against the UNITE database (rev. 8.0) and then a second round against a more recent release of UNITE (rev. 8.2). The OTUs we could not annotate were submitted to a classic BLASTN. Only hits matched with a similarity above 97% to reference genomes were conserved. The abundances and weighted non-null normalized counts tables were generated with SHAMAN (Volant *et al.* 2020).

Quantitative PCR for detection of total fungal load in human DNA samples. Fungal DNA was quantified by TaqMan qPCR as described by Liu *et al.* 2012 (C. M. Liu *et al.* 2012) using a double dye MGB 5' 6-FAM-labelled probe (Eurogentec, Belgium). All reactions were performed on a CFX96 Real-Time PCR system (BioRad, USA) with the following conditions: 2 min at 50°C, 10 min at 95°C, 15 s at 95°C and 1 min at 65°C, the last two steps repeated for 45 cycles. All samples were tested in two independent rounds, each time in duplicates.

The fungal load was estimated by dividing the fungal DNA concentration by the total DNA concentration of the sample (Zuo *et al.* 2018), obtained by Qubit Broad Range protocol

Quantitative PCR for detection of *C. albicans* DNA in human DNA samples. 7.5 µL of the extracted total fecal DNA, at 1:10 dilution, were used as a template for TaqMan qPCR analysis, using probe and primers described by Guiver *et al,* 2001 (Guiver, Levi, and Oppenheim 2001), at 0.1µM and 0.2 µM, respectively. All reactions were performed on a CFX96 Real-Time PCR system (BioRad, USA) with the following conditions: 2 min at 50°C, 10 min at 95°C, 15 s at 95°C and 1 min at 62°C, the last two steps repeated for 45 cycles. All samples were tested in two independent rounds, each time in duplicates.

Quantitative PCR amplification control. To exclude the presence of qPCR inhibitors, samples diluted at 1:10, were submitted to the Universal Exogenous qPCR Positive

Control for TaqMan® Assay (Eurogentec, Belgium), using a Cy®5-QXL®670 Probe system (Eurogentec, Belgium). Manufacturer's recommendations were followed.

Culture of the fecal samples. A 10µL loop of fecal samples was mixed with 100µL H_20 and plated on a *Candida* CHROMAGAR medium plate (BioMérieux, France). Potential *C. albicans* colonies were further tested by MALDI-TOF MS (Brucker, USA) to confirm the identification.

Measure of the β-lactamase activity. Fecal β-lactamase activity was quantified by measuring the hydrolysis of nitrocephin, a chromogenic cephalosporin. Activity was measured at least in duplicate.

Fecal samples (stored at 65°C) were thawed 30 min on ice. 140-380 mg of fecal sample were mixed with 5ml/g of stool HZn buffer (50 mM (2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.5, supplemented with 50 µM ZnSO4) and agitated for 1 hour. Samples were centrifuged twice at 4°C (15 min and 30 min). 3-20µL of the obtained supernatant were mixed with 100µM nitrocefin (Cayman Chemical, USA) and HZn buffer to reach a final volume of 200µL. Samples were incubated 20min at room temperature in a 1:1 ratio with HZn buffer. Nitrocefin hydrolysis was monitored in EnVision microplate reader (Perkin Elmer, USA) at a 482nm wavelength. SpectraPlate-96 (Perkin-Elmer, USA) using an automated liquid handling Janus Integrator system (Perkin Elmer, USA) was used to conduct the assays.

B-lactamase activity was normalized to one gram of fecal sample and to 1-cm pathlength. Detection threshold was set at a cut-off value of 4.8 nmol/min·g of fecal sample.

Biostatistical analyses. All analyses were performed on R (version 4.0.2, (R Core Team 2023.)). We used the vegan package (v. 2.5-6, (Oksanen *et al.* 2019)) to compute diversity indexes and ggplot2 package to generate the figures (v. 3.3.5, (Wickham *et al.* 2018))

We used samples collected at D-1 before treatment as baseline, called D0. If this sample was missing for a subject, sample collected at D-7 before treatment was used instead. If both samples were missing, sample collected at D-15 before treatment was used as D0 sample.

We calculated the change from baseline of the fungal load, *C. albicans* DNA absolute abundance, the relative abundance of the fungal genera and species and β-lactamase activity. Null values were replaced by the minimal non-null value of the given variables divided by two, to allow a log10 transformation. Only the fungal genera and species reaching a maximal relative abundance superior to 1% for at least half of the subjects were analyzed. We calculated the AUCs using the R package MESS (v. 0.5.7, (Ekstrom 2014)) for each period from D0D2 up to D0D180 based on the normalized changes from baseline and the actual time and date of stool collection.

For all analyses, we used bilateral nonparametric Wilcoxon exact tests. We used a type I error of 0.05 and corrected the p-values for multi-testing using False Discovery Rate correction.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. The Supplementary Tables 1 and 2 are available online: https://journals.asm.org/doi/10.1128/mbio.02880-22.

Acknowledgement

This work was supported by grants from: Agence Nationale de la Recherche (FunComPath ANR-14-IFEC-0004 and PrediRes ANR-16-CE15-0022); the French Government's Investissement d'Avenir program (Laboratoire d'Excellence Integrative Biology of Emerging Infectious Diseases [ANR10-LABX-62-IBEID]); the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie action, Innovative Training Network (FunHoMic; grant N°812969) and Assistance Publique – Hôpitaux de Paris (CEREMI CRC13-179).

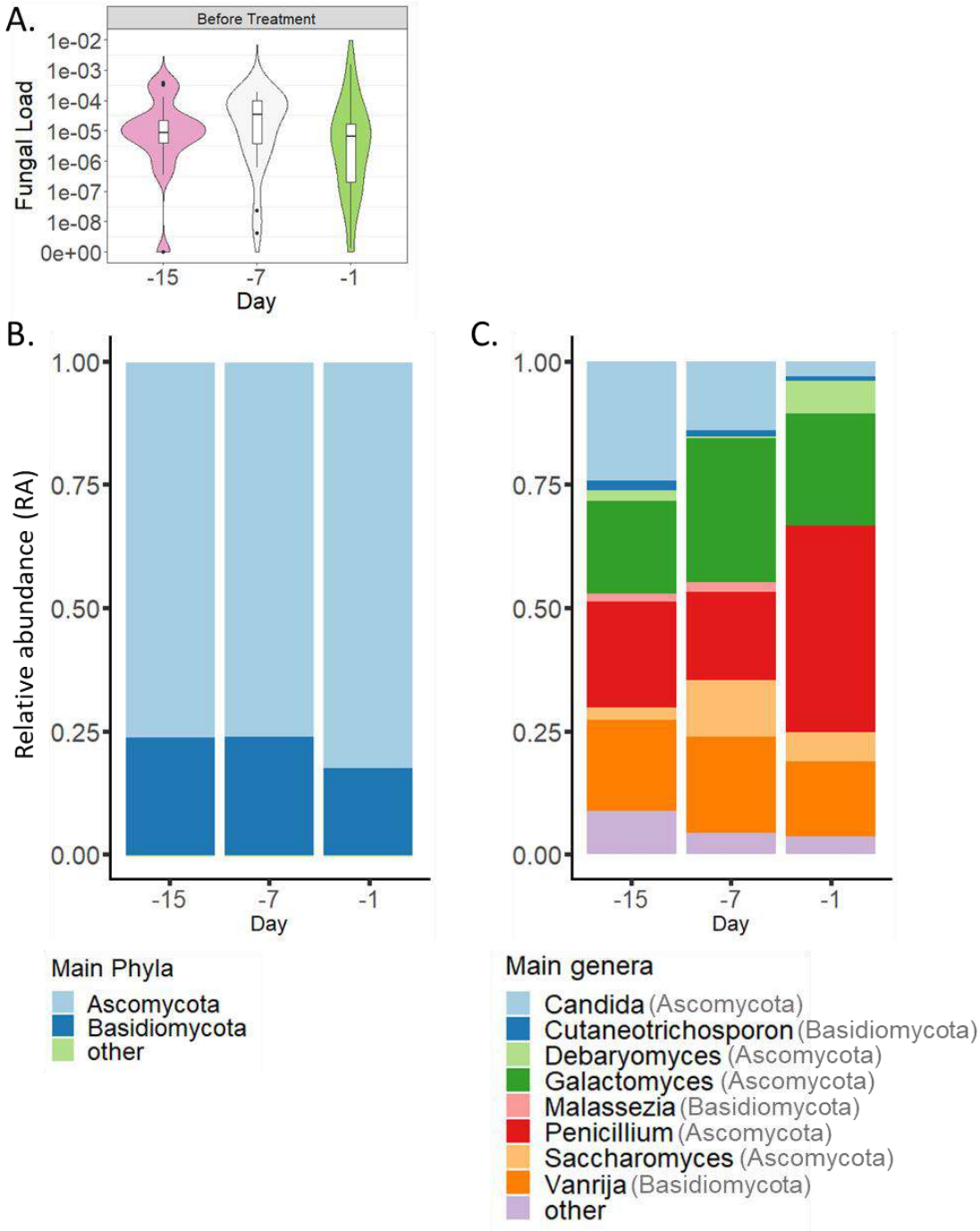
We thank Antoine Andremont and David Skurnik for the fruitful discussions.

We thank the Institut Pasteur Biomics platform (Marc Monot and Laurence Motreff) and the Institut Pasteur Bioinformatics and Biostatistics Hub for their help for generating and analyzing the sequencing data.

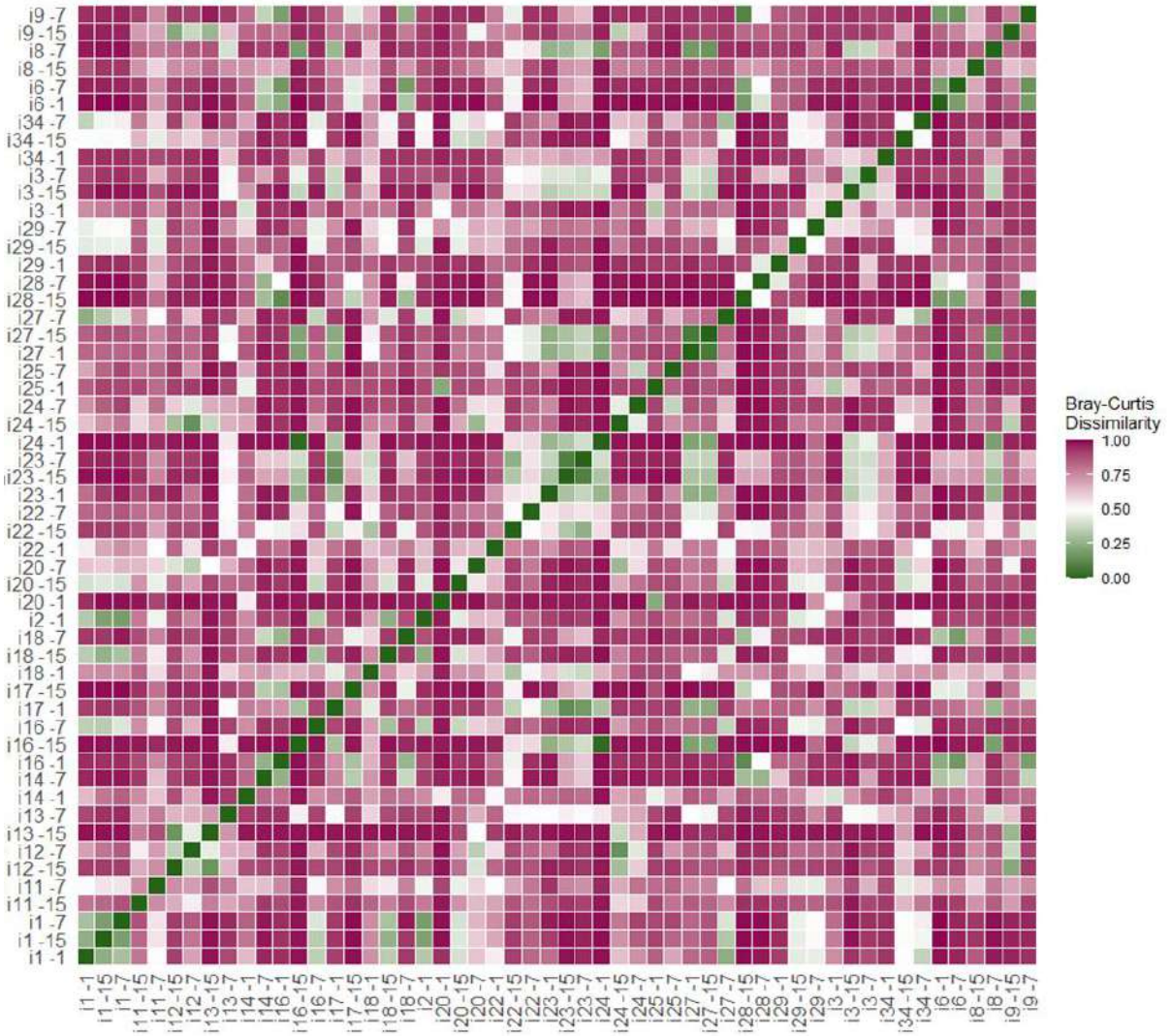
For the PrediRes study group:
Charles Burdet (INSERM, Université de Paris APHP - Bichat Hospital), Erick Denamur (INSERM, Université de Paris), Xavier Duval (INSERM, Université de Paris, APHP -

Bichat Hospital), Dusko Ehrlich (INRA Metagenopolis), France Mentré (INSERM, Université de Paris , APHP - Bichat Hospital), Eduardo P. C. Rocha (Institut Pasteur), Laurie Alla (INRA Metagenopolis), Emmanuelle Lechatelier (INRA Metagenopolis), Florence Levenez (INRA Metagenopolis), Nicolas Pons (INRA Metagenopolis), Benoît Quinquis (INRA Metagenopolis), Khadija Bourabha (INSERM), Antoine Bridier Nahmias (INSERM, Université de Paris), Olivier Clermont (INSERM, Université de Paris), Mélanie Magnan (INSERM, Université de Paris), Olivier Tenaillon (INSERM, Université de Paris), Camille d'Humières (INSERM, Université de Paris, APHP - Bichat Hospital, Institut Pasteur), Amandine Perrin (Institut Pasteur), Marie Touchon (Institut Pasteur), Dominique Rainteau (INSERM, Université Pierre et Marie Curie, APHP – Saint Antoine Hospital), Farid Ichou (ICAN), Philippe Lesnik (ICAN), Jimmy Mullaert (INSERM, Université de Paris, APHP – Bichat Hospital), Thu Thuy Nguyen (INSERM).

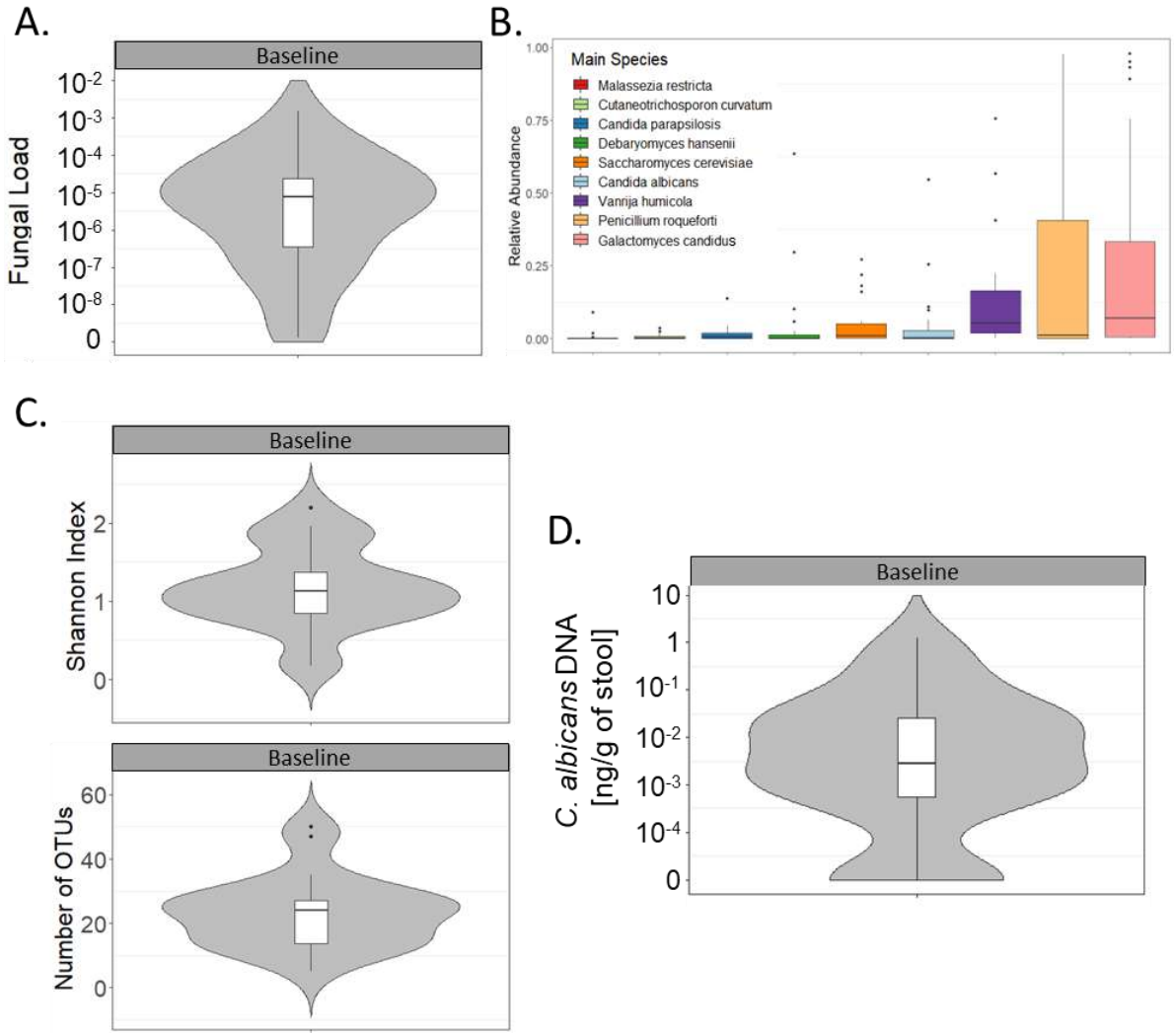
vii. Supplementary Figures



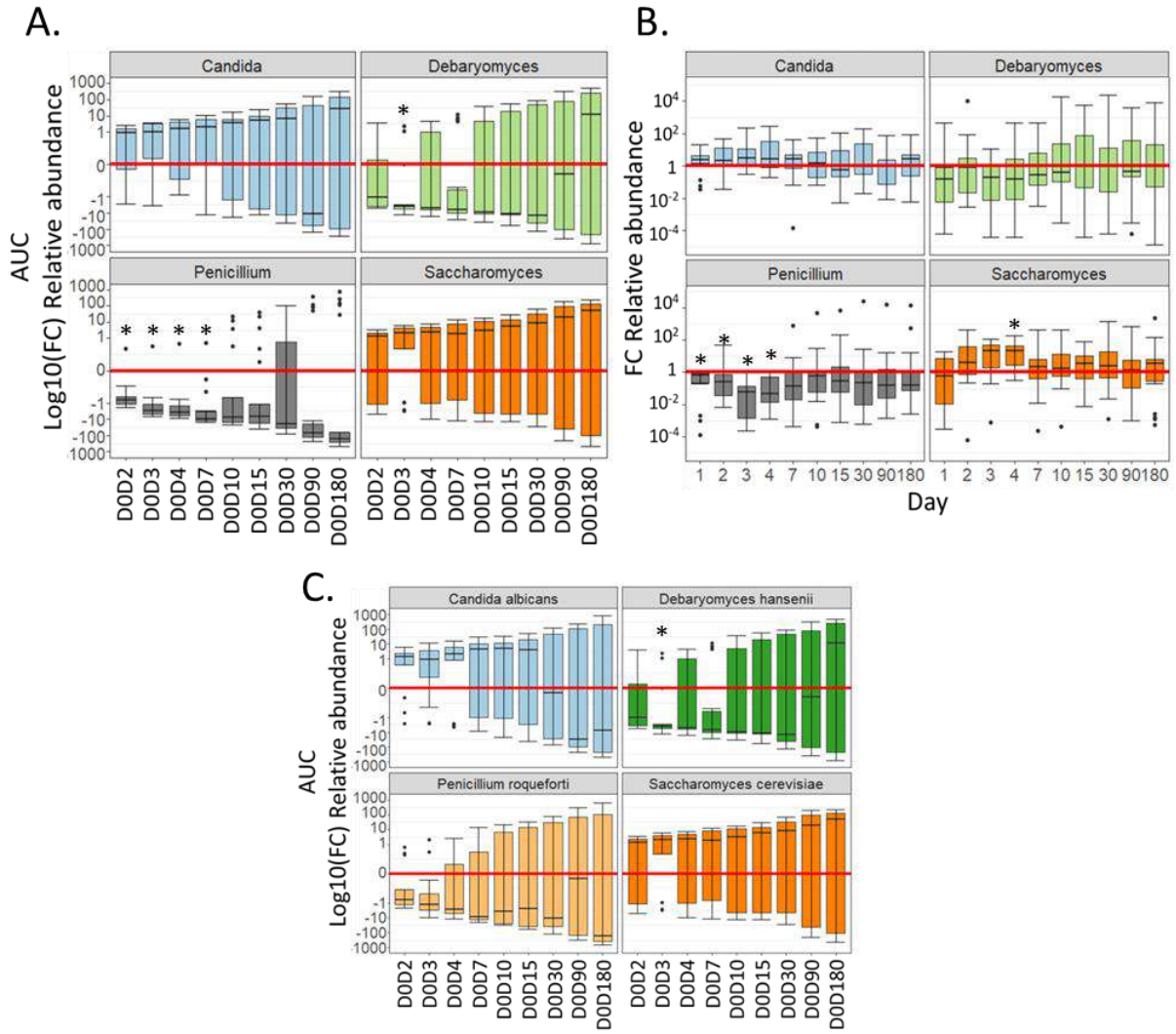
Supplementary Figure 1: Phyla and genera composition of the healthy mycobiota in 22 healthy subjects during a 2-weeks period. (A) Violin plot of the fungal load (fungal 18S rRNA DNA relative to the total fecal DNA) at 1-week apart time points for 22 healthy subjects. (B) Barplots of the average relative abundance of the main fungal phyla at 1-week apart time points for 22 healthy subjects. (C) Barplots of the average relative abundance of the main fungal genera at 1-week apart time points for 22 healthy subjects.



Supplementary Figure 2: Heatmap of the Bray-Curtis dissimilarity between the 54 fecal samples for ITS1 sequencing data. Each square represents the comparison between two samples. Bray-Curtis dissimilarity values are ranged from 0 to 1, with 0 being the least dissimilar and 1 being the most dissimilar. 1 to 3 samples are analyzed per individual (i) and are grouped together (green boxes).



Supplementary Figure 3: Mycobiota characteristics at baseline (D0) for 22 healthy subjects. (A) Violin plot of the fungal load observed among the 22 healthy subjects' samples collected at baseline. (B) Boxplot of the main fungal species the relative abundance observed among the 22 healthy subjects at baseline. (C) Alpha diversity: violin plot of the number of OTUs and of the Shannon index values observed among the 22 healthy subjects' samples collected at baseline. (D) Violin plot of the *C. albicans* DNA concentrations observed among the 22 healthy subjects' samples collected at baseline. *For all figures, the upper whiskers extend from the hinge to the largest value below 1.5× the interquartile range, and the lower whiskers extend from the hinge to the smallest value above 1.5× the interquartile range.*



Supplementary Figure 4: Impact of the antibiotic treatment on the gut mycobiota genera composition. (A) Distribution of the relative abundance log10 (FC) AUCs for *Candida sp.*, *Debaryomyces spp., Penicillium sp.*, and *Saccharomyces sp*., highlighting the duration and the amplitude of the perturbations. (B) Distribution of the relative abundance foldchanges (FC) *Candida sp., Debaryomyces sp., Penicillium sp*., and *Saccharomyces sp.*, highlighting punctual perturbations. (C) Distribution of the relative abundance log10 (FC) AUCs *for Candida albicans, Debaryomyces hansenii, Penicillium roqueforti*, and *Saccharomyces cerevisiae*, highlighting the duration and the amplitude of the perturbations. *For all figures, the upper whiskers extend from the hinge to the largest value below 1.5× the interquartile range, and the lower whiskers extend from the hinge to the smallest value above 1.5× the interquartile range. *q value < 0.05, Wilcoxon t-test, false-discovery rate correction.*

viii. Supplementary Materials.

Table S1 and Table S2 are available online at the addresses indicated below. They can also be accessed through the QR Codes below.

Table S1: Weighted areas under the curves (AUC) and changes from baseline of the mycobiota features.

https://journals.asm.org/doi/suppl/10.1128/mbio.02880-22/suppl_file/mbio.02880-22-

Table S2: Weighted areas under the curves (AUC) and changes from baseline of the mycobiota features according to the treatment group.

https://journals.asm.org/doi/suppl/10.1128/mbio.02880-22/suppl_file/mbio.02880-22-

3. Additional results

As presented in the introduction, *C. albicans* co-exists with hundreds of different bacterial species in the intestinal niche and these bacteria play an important role in microbiota homeostasis (for review, see Ohland and Jobin 2015). As seen in the previous section, I demonstrated that a depletion of the endogenous bacteria of the gut by broad-spectrum antibiotics results in an overall increase of *C. albicans* levels in the gut and the extent of this increase is directly associated with the change of β-lactamase activity following the antibiotic treatment. This association likely occurs because of a differential effect of the β-lactams on the residing bacteria of the gut, depending on differences in the occurrence of β-lactamase-producing bacteria in the microbiota. Indeed, in the subjects from the CEREMI cohort, the extent of intestinal bacterial dysbiosis, estimated by Shannon Index, was associated with the change in β-lactamase activity after 3GC exposure. The subjects displaying a low increase of β-lactamase activity were characterized by a stronger change in bacterial α-diversity at D3, and thus to a stronger bacterial dysbiosis than the subjects displaying a high increase of β-lactamase activity (Wilcoxon test; p-value of 0.041, Fig. 4).

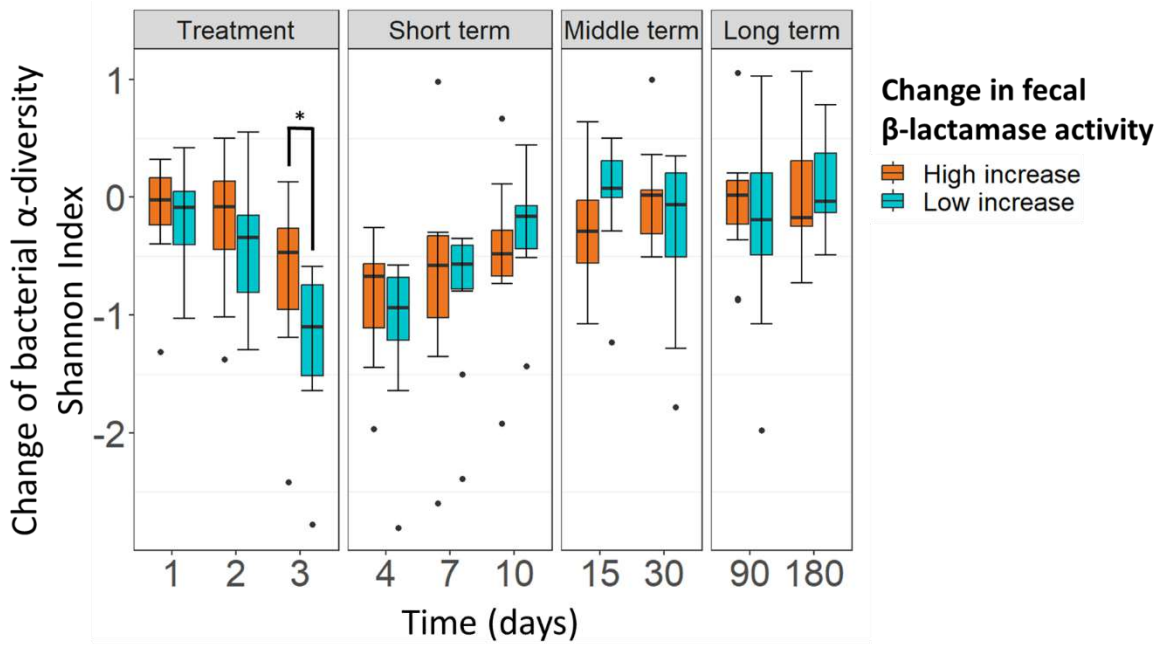


Figure 4: Change of bacterial α-diversity following 3GC exposure, depending on the change in fecal β-lactamase activity. Boxplot of the change of bacterial α-diversity, measured by Shannon Index, following ceftriaxone and cefotaxime treatment. Orange boxplots indicates values for the subjects characterized by a high increase of β-lactamase activity (group “high”) and blue boxplots represent the values for the subjects characterized by a low increase of β-lactamase activity (group “low”). Upper whiskers extend from the hinge to the

largest value below 1.5× the interquartile range, and the lower whiskers extend from the hinge to the smallest value above 1.5× the interquartile range. * *p-value < 0.05.*

This suggests that depletion of specific bacteria following antibiotic treatment allows overgrowth of *C. albicans* populations. Specific bacterial species might thus be used to curb *C. albicans* growth. However, few studies have aimed to identify bacterial species – or bacterial signatures – with a potential anti-*C. albicans* activity. In this section, I aimed to identify bacteria whose levels in the gut microbiota were inversely correlated with those of *C. albicans* and I hypothesized that some of these species might limit *C. albicans* intestinal colonization.

To identify potential *C. albicans* inhibiting bacteria, or anti-*C. albicans* bacterial signatures, I searched for significant Spearman correlations between *C. albicans* absolute abundance, deduced from qPCR data, and the relative abundance of the gut metagenomic species, deduced from shotgun metagenomics data obtained from all samples of the CEREMI subjects and annotated at the species level. I also searched for fungal species with the potential of inhibiting *C. albicans* growth by looking for significant Spearman correlations between *C. albicans* absolute abundance, once again deduced from qPCR data, and the relative abundance of the gut fungal species, deduced from the ITS1-targeted metagenomics data obtained from all samples of the CEREMI subjects and annotated at the species level (Fig. 5).

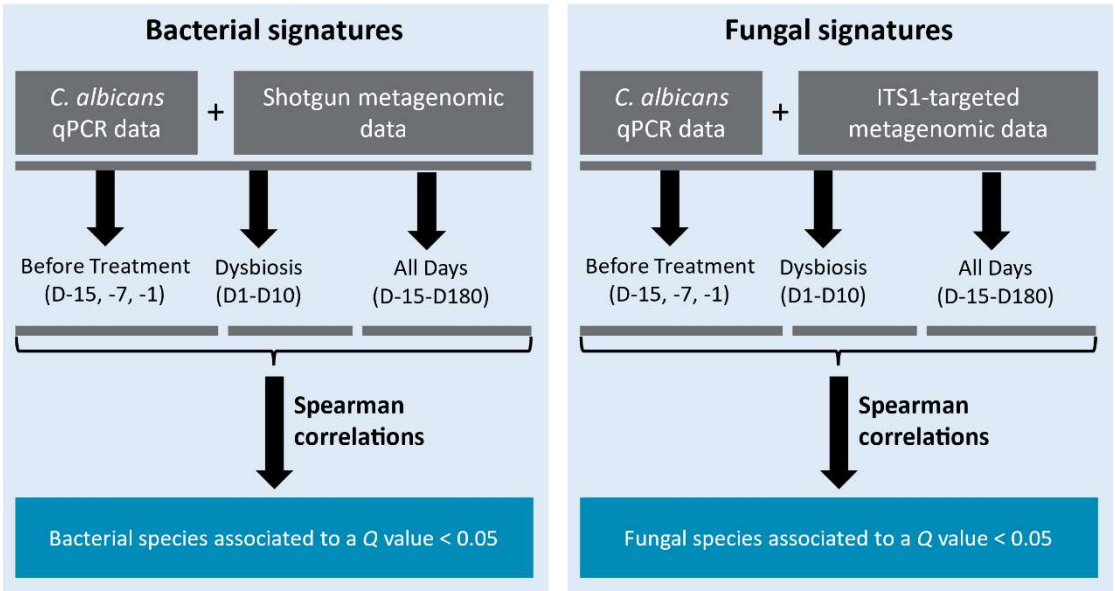


Figure 5: Schematic representation of the statistical pipeline used for the identification of bacterial or fungal species associated with *C. albicans* in the CEREMI subjects.

To take into account the specificity of the CEREMI cohort, I split the samples in three different subsets: (i) the samples collected before the antibiotic treatment, (ii) the samples collected between D1 and D10 after the start of the antibiotic treatment, which corresponds to the period at which the bacterial dysbiosis was the strongest, and (iii) all samples available, independently of the time of collection. Seventy-four microbial species were identified as being significantly associated with *C. albicans* carriage (Fig. 6).

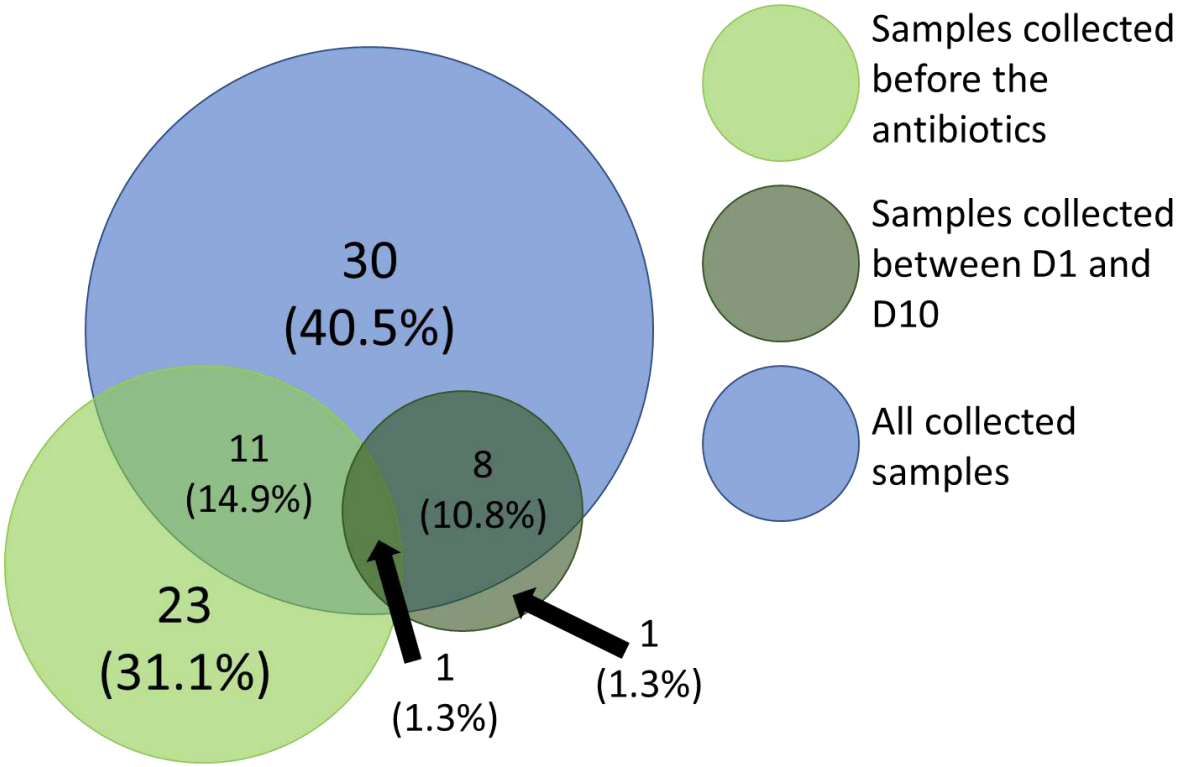


Figure 6: Venn diagrams of the bacterial species correlated to *C. albicans* carriage depending on the subset of samples used for the analysis. The numbers correspond to the number of bacterial species identified in each subset or group of subsets. The percentage is calculated on the total number of bacterial species identified.

Fifty-four were negatively correlated to *C. albicans* carriages, indicating a potential antagonistic activity against *C. albicans* (Table 2) and 20 were positively correlated to *C. albicans* carriage, highlighting a potential stimulatory effect on *C. albicans* growth (Table 3). Among the potential anti-*C. albicans* signatures, *Eisenbergiella tayi,* a SCFA-producer bacillus of the *Lachnospiraceae* family that has been described for the first time in 2014 (Amir *et al.* 2014), was the only species to be identified in all three subsets of samples. *Geotrichum candidum* (formerly, *Galactomyces candidus*) was the only fungal species to be significantly associated with *C. albicans,* with 5 OTUs

annotated as this species being negatively correlated to *C. albicans* carriage (Spearman correlation; median R coefficient of -0.22; *q* value of 0.045, Table 4). Unsurprisingly, we also identified a strong association between *C. albicans* intestinal carriage, measured by qPCR, *C. albicans* intestinal relative abundance, estimated by ITS1 metagenomic sequencing (Spearman's correlation; p-value of 2.56×10^{-35}).

Table 2: List of the potential anti-*C. albicans* bacterial species. The species are organized alphabetically. The samples subset indicates in which samples the association was identified (All Samples: all samples collected, BT: samples collected before the antibiotic treatment, D1D10: the samples collected between day 1 and 10 after antibiotic exposure. P-values were corrected by false-discovery rate.

Microbial species	Samples subset	R spearman	*P* value	*q* value
Adlercreutzia equolifaciens	All Samples	-0.28	1.67E-05	4.84E-04
	BT	-0.40	3.95E-03	3.78E-02
Alistipes ihumii	All Samples	-0.18	6.65E-03	4.40E-02
Alistipes obesi	All Samples	-0.29	8.86E-06	3.52E-04
Alistipes putredinis	All Samples	-0.29	1.06E-05	3.55E-04
	BT	-0.43	1.53E-03	2.12E-02
Alistipes shahii	All Samples	-0.33	4.56E-07	4.83E-05
	D1D10	-0.37	1.70E-04	8.35E-03
Alistipes timonensis	All Samples	-0.24	2.30E-04	3.48E-03
Anaerotruncus colihominis	BT	-0.61	2.21E-06	3.21E-04
	All Samples	-0.24	2.79E-04	4.03E-03
Bacteroides clarus	All Samples	-0.23	5.22E-04	6.39E-03
Bacteroides intestinalis	All Samples	-0.20	2.93E-03	2.17E-02
Bacteroides massiliensis	All Samples	-0.30	3.06E-06	1.78E-04
	D1D10	-0.32	1.38E-03	4.04E-02
Bacteroides salyersiae	All Samples	-0.19	4.61E-03	3.19E-02
Bacteroides stercoris	All Samples	-0.20	1.87E-03	1.61E-02
Barnesiella intestinihominis	BT	-0.46	7.52E-04	1.23E-02

Bifidobacterium pseudocatenulatum	All Samples	-0.23	3.31E-04	4.39E-03
Blautia hydrogenotrophica	BT	-0.46	7.30E-04	1.23E-02
Blautia massiliensis	All Samples	-0.18	6.86E-03	4.40E-02
Blautia obeum	All Samples	-0.25	1.50E-04	2.77E-03
	D1D10	-0.38	8.89E-05	8.35E-03
Blautia producta	BT	-0.42	2.15E-03	2.41E-02
Butyricimonas virosa	All Samples	-0.30	2.55E-06	1.78E-04
Cloacibacillus porcorum	All Samples	-0.24	1.95E-04	3.11E-03
Clostridium bolteae	BT	-0.55	3.42E-05	1.99E-03
Clostridium citroniae	BT	-0.51	1.45E-04	5.27E-03
Clostridium clostridioforme	BT	-0.43	1.67E-03	2.12E-02
Clostridium lavalense	BT	-0.53	5.83E-05	2.81E-03
Clostridium phoceensis	BT	-0.48	3.80E-04	9.34E-03
Clostridium symbiosum	BT	-0.59	6.07E-06	4.83E-04
Collinsella tanakaei	All Samples	-0.23	3.26E-04	4.39E-03
Desulfovibrio piger	All Samples	-0.25	1.57E-04	2.77E-03
	D1D10	-0.39	5.66E-05	8.26E-03
Dielma fastidiosa	All Samples	-0.21	1.09E-03	1.11E-02
Dorea longicatena	All Samples	-0.18	6.96E-03	4.40E-02
Eisenbergiella tayi	All Samples	-0.38	2.08E-09	3.30E-07
	D1D10	-0.37	1.66E-04	8.35E-03
	BT	-0.49	2.89E-04	8.40E-03
Erysipelatoclostridium ramosum	BT	-0.39	4.63E-03	4.21E-02
Escherichia coli	All Samples	-0.19	3.96E-03	2.80E-02
Eubacterium eligens	All Samples	-0.20	2.32E-03	1.84E-02
Eubacterium siraeum	All Samples	-0.18	6.82E-03	4.40E-02

Faecalibacterium prausnitzii	All Samples	-0.27	3.36E-05	8.22E-04
	D1D10	-0.37	1.72E-04	8.35E-03
Flavonifractor plautii	BT	-0.47	5.46E-04	1.22E-02
Holdemania filiformis	All Samples	-0.39	1.13E-09	3.30E-07
	D1D10	-0.39	5.42E-05	8.26E-03
Hungatella effluvii	BT	-0.46	7.82E-04	1.23E-02
	All Samples	-0.21	1.40E-03	1.31E-02
Hungatella hathewayi	BT	-0.58	6.64E-06	4.83E-04
	All Samples	-0.20	2.78E-03	2.10E-02
Intestinimonas butyriciproducens	All Samples	-0.25	1.24E-04	2.63E-03
Intestinimonas massiliensis	All Samples	-0.21	1.36E-03	1.31E-02
Methanobrevibacter smithii	All Samples	-0.22	6.17E-04	7.01E-03
Negativibacillus massiliensis	All Samples	-0.24	1.96E-04	3.11E-03
	D1D10	-0.34	5.72E-04	2.39E-02
Odoribacter splanchnicus	All Samples	-0.18	7.05E-03	4.40E-02
Parabacteroides distasonis	BT	-0.38	5.88E-03	4.89E-02
Parabacteroides merdae	All Samples	-0.29	1.12E-05	3.55E-04
	BT	-0.42	2.26E-03	2.43E-02
Roseburia hominis	All Samples	-0.23	5.85E-04	6.89E-03
Roseburia inulinivorans	All Samples	-0.19	3.51E-03	2.54E-02
Ruminococcus gnavus	BT	-0.40	3.28E-03	3.30E-02
Ruminococcus torques	All Samples	-0.20	2.30E-03	1.84E-02
	BT	-0.41	2.92E-03	3.03E-02
Ruthenibacterium lactatiformans	BT	-0.45	8.04E-04	1.23E-02
	All Samples	-0.20	2.09E-03	1.75E-02
Sellimonas intestinalis	BT	-0.46	5.89E-04	1.22E-02

Victivallis vadensis	All Samples	-0.25	1.37E-04	2.73E-03
	D1D10	-0.33	7.27E-04	2.65E-02

Table 3: List of the microbial species positively associated with *C. albicans* carriage. The species are organized alphabetically. The samples subset indicates in which samples the association was identified (All Samples: all samples collected, BT: samples collected before the antibiotic treatment, D1D10: the samples collected between day 1 and 10 after antibiotic exposure. P-values were corrected by false-discovery rate. Species highlighted in bold characters were identified in several subsets.

Microbial species	Samples subset	R spearman	*P* value	*q* value
Acidaminococcus intestini	All Samples	0.27	3.12E-05	8.22E-04
Bacteroides vulgatus	D1D10	0.32	1.17E-03	3.80E-02
Bifidobacterium angulatum	BT	0.53	6.76E-05	2.81E-03
	All Samples	0.21	1.75E-03	1.54E-02
Bifidobacterium longum	All Samples	0.22	6.84E-04	7.50E-03
Blastocystis sp. *Subtype 1*	BT	0.64	4.67E-07	1.36E-04
	All Samples	0.30	3.37E-06	1.78E-04
Butyrivibrio crossotus	BT	0.43	1.85E-03	2.15E-02
Clostridium saudiense	BT	0.48	3.85E-04	9.34E-03
Collinsella bouchesdurhonensis	All Samples	0.21	1.31E-03	1.31E-02
Coprococcus catus	BT	0.46	6.26E-04	1.22E-02
Dialister invisus	All Samples	0.20	2.55E-03	1.98E-02
Duodenibacillus massiliensis	All Samples	0.22	8.07E-04	8.56E-03
Fenollaria massiliensis	BT	0.44	1.25E-03	1.82E-02
Haemophilus parainfluenzae	BT	0.40	4.02E-03	3.78E-02
Holdemanella biformis	BT	0.49	2.57E-04	8.32E-03
Methanosphaera stadtmanae	BT	0.43	1.67E-03	2.12E-02
Prevotella stercorea	BT	0.43	1.80E-03	2.15E-02

Streptococcus parasanguinis	All Samples	0.25	1.22E-04	2.63E-03
Turicibacter sanguinis	BT	0.38	5.58E-03	4.77E-02
Veillonella atypica	All Samples	0.23	4.75E-04	6.04E-03
Veillonella infantium	All Samples	0.29	6.89E-06	3.13E-04
	BT	0.39	5.02E-03	4.43E-02

Table 4: List of the fungal OTUs associated with *C. albicans* carriage. All five OTUs were annotated as *Geotrichum candidum*. The samples subset indicates in which subset the fungal OTU was identified (All Samples: all samples collected, BT: samples collected before the antibiotic treatment, D1D10: the samples collected between day 1 and D10 after antibiotic exposure. P-values were corrected by false-discovery rate.

	Fungal OTU	Samples subset	R spearman	*P* value	*q* value
Geotrichum candidum	**OTU_1**	All Samples	-0.21	0.0012	0.045
	OTU_2069	All Samples	-0.23	0.0005	0.045
	OTU_3579	All Samples	-0.22	0.0009	0.045
	OTU_4047	All Samples	-0.22	0.0011	0.045
	OTU_561	All Samples	-0.23	0.0005	0.045

Since statistical analyses such as spearman correlations cannot distinguish the direction of the potential inhibition, some of the bacteria identified might be inhibited by *C. albicans* rather than having an antagonistic activity against it. I therefore decided to decipher further the association between *C. albicans* and *Eisenbergiella tayi* and *Geotrichum candidum* since *E. tayi* was the only bacteria to be identified in all three subsets and *G. candidum* was the only fungal species with a potential anti-*C. albicans* activity. *E. tayi* relative abundance was significantly reduced directly after 3GC exposure between D1 and D2 (Wilcoxon test; p-value of 0.007 and 0.005, respectively), just before the increase observed for *C. albicans* DNA levels (Fig. 7A).

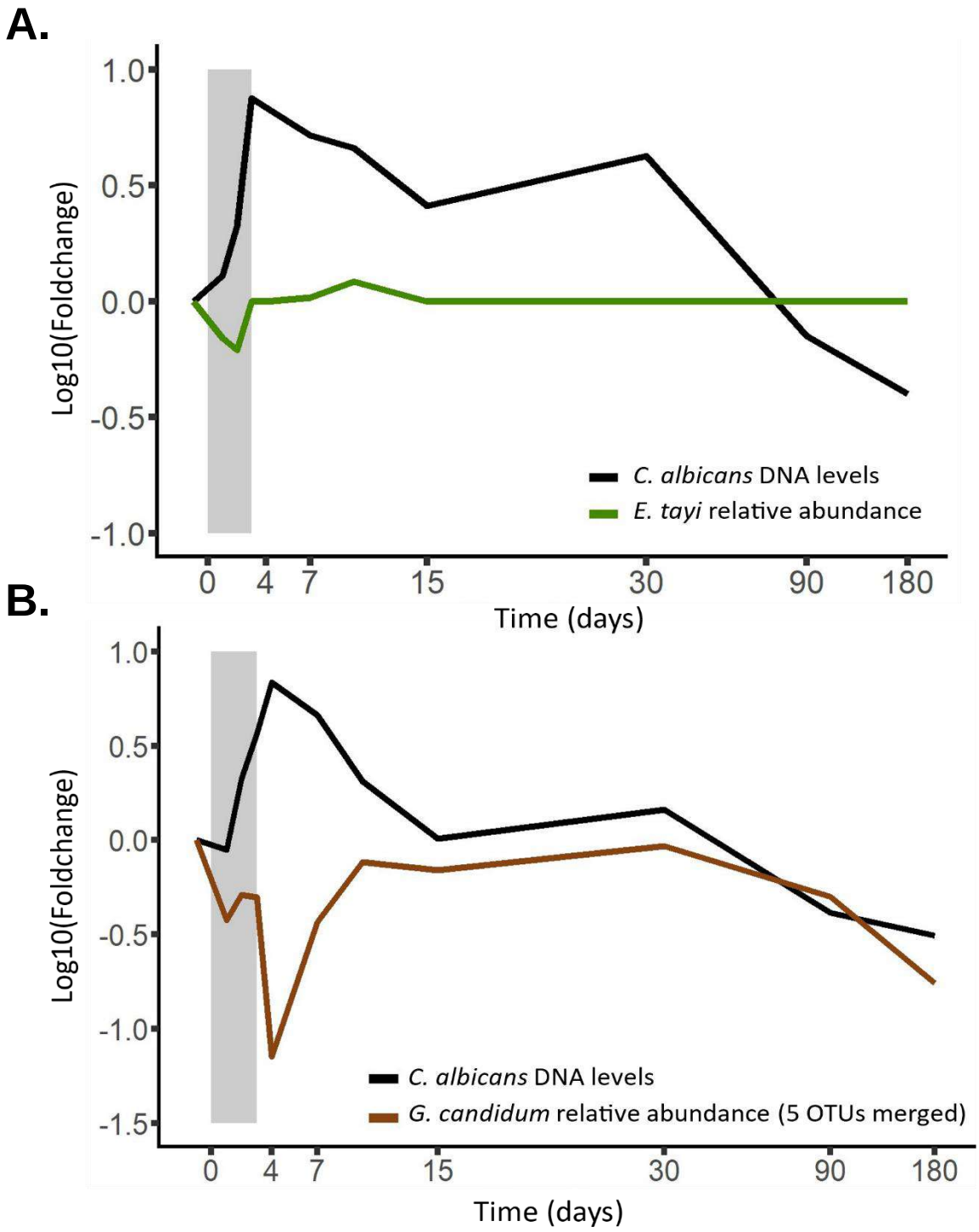


Figure 7: *Dynamics of C. albicans, Eisenbergiella tayi* and *Geotrichum candidum* after 3GC exposure. (A) Log10(Foldchange) of *C. albicans* DNA levels and *E. tayi* relative abundance during and after 3GC exposure, compared to baseline levels. The lines represent the medians of values for each species, at each day. (B). Log10(Foldchange) of *C. albicans* DNA levels and *G. candidum* relative abundance during and after 3GC exposure, compared to baseline levels. The lines represent the medians of values for each species, at each day.

Thus, the reduction of *E. tayi* relative abundance might be associated with *C. albicans* increase, which might indicate that *E. tayi* plays a potential role in curbing *C. albicans* growth in the healthy gut. It has to be noted that *E. tayi* relative abundance was independent from the change in β-lactam activity. Indeed, *E. tayi* relative abundance was similar between the subjects displaying a high increase of β-lactam activity after the treatment and the one displaying a lower increase or even a decrease of this activity, and this both during the dysbiosis period (between D1 and D10, Wilcoxon test; p-value of 0.62) and before 3GC exposure (Wilcoxon test; p-value of 0.49). It has to be noted that *E. tayi* susceptibility to 3GC has not be decribed, however, this species has been shown to be susceptible to cefoxitin, a second generation cephalosporin (2GC), *in vitro* (Bernard *et al.* 2017).

To study *G. candidum* association with *C. albicans,* I merged the relative abundances of the five *G. candidum* OTUs identified as negatively correlated to *C. albicans* (OTU_1, OTU_561, OTU_2069, OTU_3579 and OTU_4047*G. G. candidum* relative abundance decreased between D3 and D4 after antibiotics (Wilcoxon test; p-value of 0.020 and 0.010, respectively), so at the same period for which the rise of *C. albicans* carriage was observed, suggesting a potential interaction between the two fungal species.

In summary, the characterization of the mycobiota and of *C. albicans* carriage before, during and after a broad-spectrum antibiotic treatment has revealed that *C. albicans* is a highly prevalent yeast, present in the gut of the majority of the population, even at very low concentrations. Its abundance was increased after 3CG exposure but with wide inter-subject variations and these variations were directly associated with the change of β-lactamase activity, suggesting that the intestinal microbiota composition plays an important role in controlling *C. albicans* growth. The analysis of the co-abundance of *C. albicans* and the microbial species present in the human gut highlighted a set of 54 bacterial species, including *E. tayi,* and one fungal species, *G. candidum*, with a potential key role in *C. albicans* gut colonization resistance.

4. Additional materials and methods

DNA extraction of fecal samples and shotgun metagenomic sequencing. DNA extraction was performed for all fecal samples following IHMS SOP P7 (Dore *et al.* 2015). DNA content was measured using Qubit Fluorometric Quantitation (ThermoFisher Scientific, Waltham, MA, USA) and DNA quality was assessed by DNA size profiling on a Fragment Analyzer (Agilent Technologies, Santa Clara, CA, USA). The library was built with 3 µg of high molecular weight DNA (>10 kbp). DNA was sheared into fragments of approximately 150 bp using an ultrasonicator (Covaris, Woburn, MA, USA) and the DNA fragment library was constructed using Purified and amplified DNA fragment libraries were sequenced using the Ion Proton Sequencer (ThermoFisher Scientific, Waltham, MA, USA), generating 22.2 ± 1.8 million reads of 150 bp (on average) per sample.

Microbial gene count table. The METEOR software was used to create the gene count table (Pons *et al.* 2010). First, reads were filtered for low-quality by AlienTrimmer (Criscuolo and Brisse 2013). Reads that aligned to the human genome with an identity superior to 95% were also discarded. The remaining reads were trimmed to 80 bases and mapped to the Integrated Gut Catalogue 2 (IGC2)(Wen *et al.* 2017), comprising 10.4 million of genes, using Bowtie2 (Langmead and Salzberg 2012). The reads mapped to a unique gene in the catalogue were attributed to their corresponding genes. The reads that mapped with the same alignment score to multiple genes in the catalogue were attributed according to the ratio of their unique mapping counts of the captured genes. The resulting count table was further processed using the R package MetaOMineR v1.31 (Le Chatelier *et al.* 2013). Read counts were then ‘rarefied’ using 20M high quality reads (a threshold chosen to include all samples) using a random sampling procedure without replacement, this in order to decrease technical bias due to different sequencing depth and avoid any artifacts of sample size on low-abundance genes. The downsized matrix was finally normalized by dividing gene read counts per gene length x100, as a proxy of gene coverage. Since gut microbiota has been found to be enriched in species from the oral cavity upon antibiotic treatment (Hildebrand *et al.* 2019),the same process was repeated on an oral microbiota catalogue of 8.4 million genes (Le Chatelier *et al.* 2021).

Metagenomic Species profiles. The IGC2 and the oral catalogues were organized into 1990 and 853 Metagenomic Species (MGS, cluster of co-abundant genes),

respectively, using MSPminer (Le Chatelier *et al.* 2021; Plaza Onate *et al.* 2021; Oñate *et al.* 2019). After removing the MGS present in both the IGC2 and the oral catalogues, 2741 MGS were remaining. The relative abundance of an MGS was computed as the mean abundance of its 100 ‘marker’ genes (that is, the genes that correlate the most altogether). If less than 10% of ‘marker’ genes were seen in a sample, the abundance of the MGS was set to 0. MGS abundance profiles were finally normalized to estimate the proportion of each species in the microbiota, so that the sum of all the MGS abundance was equal to 1.

Biostatistical analysis. All analyses were performed on R (v. 4.1.2, Team R Development Core 2018). I used the vegan package (v. 2.6-4, Oksanen *et al.* 2019) to compute diversity indexes, the psych package (v. 2.2-9, Revelle 2022) to compute the spearman correlations and the ggplot2 package to generate the figures (v. 3.4.0, Wickham *et al.* 2018). Null values of *C. albicans* DNA levels and of the relative abundances of the MGS and fungal species were replaced by the minimal non-null value of the given variables divided by two, to allow a log10 transformation. I used a type I error of 0.05 and corrected the p-values for multi-testing using false discovery rate correction.

C - CHAPTER 3: THE INFLUENCE OF HOST AND ENVIRONMENTAL FACTORS ON *C. ALBICANS* COLONIZATION IN HEALTHY INDIVIDUALS.

Contains: Article in preparation

Margot Delavy[a], Natacha Sertour[a], Etienne Patin[b], Emmanuelle Le Chatelier[c], Florian Dubois[d], Violaine Saint-André[e], Lluis Quintana-Murci[b], Darragh Duffy[d], Christophe d'Enfert[a], Marie-Elisabeth Bougnoux[a,f]

[a] Institut Pasteur, Université Paris Cité INRAE, Unité Biologie et Pathogénicité Fongiques, Paris, France

[b] Institut Pasteur, Université Paris Cité, CNRS UMR2000, Human Evolutionary Genetics Unit, Paris, France

[c] MGP MetaGénoPolis, INRA, Université Paris-Saclay, Jouy-en-Josas, France

[d] Institut Pasteur, Université Paris Cité, Translational Immunology Unit, Paris, France, [e] Institut Pasteur, Université Paris Cité, Bioinformatics and Biostatistics Hub, Paris, France

[f] APHP, Hôpital Necker-Enfants-Malades, Service de Microbiologie Clinique, Unité de Parasitologie-Mycologie, Paris, France

1. Abstract

Candida albicans is a commensal yeast present in the gut of most healthy individuals but with highly variable concentrations. However, little is known on the interactions between the host factors and the extend of such concentrations.

We investigated how the microbiota, and the host's diet and genetics modulated *C. albicans* carriage, in 695 healthy individuals from the Milieu Intérieur cohort. *C. albicans* intestinal carriage was detected in 82.9% of the subjects by quantitative PCR. Using linear mixed models and multiway-ANOVA, we explored *C. albicans* intestinal

levels with regards to the subject gut microbiota composition and diet. We thus showed that *Intestinimonas butyriciproducens,* a SCFA-producer bacteria, was the only species whose relative abundance was negatively correlated with *C. albicans* concentrations. Diet contributed to *C. albicans* growth, with eating between meals and a low sodium diet being associated with higher *C. albicans* carriages. Also, by Genome-Wide Association Study, we highlighted 26 SNPs associated with *C. albicans* colonization.

In addition, we found that *C. albicans* intestinal levels influenced the host immune response. We analyzed the transcription levels of 546 immune genes and the concentration of 13 cytokines in the subject blood, after stimulation with *C. albicans* cells and showed positive associations between the extent of *C. albicans* intestinal levels and *NLRP3* expression, and IL-2 and CXCL5 concentrations.

The relative importance of the associations that we identified are still to be determined. Nevertheless, these finding possibly open the way for new intervention strategies to curb *C. albicans* intestinal overgrowth.

2. Introduction

Candida albicans is an opportunistic pathogen that causes superficial infections, such as vulvovaginal candidiasis, an infection which will affect 75% of premenopausal women at least once in their lifetime (Foxman *et al.* 2013; Rosati *et al.* 2020; Yano *et al.* 2019). In addition, when the host defenses are compromised, for example in immunocompromised patients, *C. albicans* can translocate from the gut to the bloodstream and cause systemic infections that are associated with up to 50% mortality (Pappas *et al.* 2018; Zhai *et al.* 2020; Brown *et al.* 2012).

But despite being an opportunistic pathogen, *C. albicans* is primarily a commensal yeast of the gastrointestinal (GI) tract that colonizes up to 95% of the population, in various degree (Nash *et al.* 2017; Delavy *et al.* 2022). As an intestinal yeast, *C. albicans* co-exists in the gut with hundreds of microbial species that compose the intestinal microbiota (Nash *et al.* 2017; Auchtung *et al.* 2018). The microbiota is likely to play a role in controlling *C. albicans* growth in the GI tract, since in mice, the depletion of the bacterial microbiota by antibiotics is necessary to induce a colonization by *C. albicans,* mice displaying a natural colonization resistance to this yeast (Fan *et*

al. 2015). Moreover, in humans, a disruption of the bacterial microbiota by an antibiotic treatment results in an overall increase of *C. albicans* carriage (Delavy *et al.* 2022; Seelbinder *et al.* 2020), making thus antibiotics a risk factor for *C. albicans* intestinal overgrowth. However, we are still lacking a thorough understanding of the other factors influencing *C. albicans* intestinal colonization.

In this study, we investigate how the composition of the bacterial and fungal microbiota and the host's diet, medical history and environment can shape *C. albicans* carriage in healthy adults, using data collected from 695 healthy volunteers from the Milieu Intérieur cohort (Thomas *et al.* 2015). In parallel, we conducted a genome-wide association study (GWAS) to identify genetic factors associated with the host susceptibility to *C. albicans* colonization. Finally, we investigated the interplay between the extent of *C. albicans* intestinal carriage and the host immune response upon a mimicked *C. albicans* blood infection.

3. Results

Characterization of the mycobiota of a large cohort of healthy subjects. To describe the main characteristics of the healthy intestinal mycobiota, we analyzed a fecal sample from each of 695 healthy adults from the Milieu Intérieur cohort. First, we quantified the fungal load, which is the ratio between the fungal DNA concentration and the total fecal DNA concentration of a given sample (see Materials and Methods). Fungi represented only a small fraction of the total intestinal microbiota with a median fungal load of 4.0×10^{-6} (min: 9.0×10^{-10}, max: 6.26×10^{-4}, Fig. 1A).

Using ITS2-targeted metagenomic sequencing, we further characterized the mycobiota of 96 of the Milieu Intérieur healthy subjects. The mycobiota of the subjects was characterized by a rather low richness and evenness, with a median Shannon Index of 2.21 (min: 0.17, max: 3.76, Fig. 1B). In contrast, the mycobiota was highly dissimilar between the subjects, with a median β-diversity, estimated by Bray-Curtis dissimilarity index, of 0.92 (min: 0.01, max: 9.99, Fig. 1C).

In total, ITS2 sequence analyses resulted in 1061 fungal amplicon sequence variants (ASVs). 1057/1061 (99.6%) were annotated at the phylum level, 909 (85.7%) at the genus level and 831 (78.3%) at the species level. Overall, the 909 ASVs annotated at the genus level and the 831 ASVs annotated at the species level represented 99.7%

and 99.3% of the total number of sequences, respectively. Most of the fungal ASVs belonged to the Ascomycota (mean relative abundance of 95.5%), the Basidiomycota (3.3%), the Mucormycota (0.8%) or the Chytridiomycota (0.2%, Supplementary Fig. 1). Among the 492 fungal species identified, 27 were present in more than 50% of the samples, the most abundant species being *Saccharomyces cerevisiae, Geotrichum candidum, Fusarium incarnatum, Fusarium equiseti, Malassezia restricta* and *Penicillium roqueforti* (Fig. 1D). Two hundred and eighty-two fungal genera were identified among which *Geotrichum, Saccharomyces, Penicillium, Aspergillus, Botryotrichum, Fusarium, Pseudogymnoascus, Malassezia, Candida* and *Clasdosporium* were the more dominant taxa, and were detected in at least 75% of the subjects (Fig. 1E).

Using a qPCR assay, we quantified *C. albicans* absolute abundance in the fecal samples of these volunteers. We detected *C. albicans* DNA in 576/695 subjects (82.9%), in a concentration ranging from 2.7×10^{-5} to 0.78 ng/g of stool, with a median of 8.95×10^{-4} (Fig. 1F). In comparison, ITS2-targeted metagenomic sequencing detected *C. albicans* DNA in only 53/96 samples (55.2%). *C. albicans* carriage and colonization rates were similar between sexes (Wilcoxon test; p-value of 0.44, Chi-square test; p-value of 0.59, Fig. 2A) but we observed dissimilarities based on the age of the volunteers both for the colonization rates and *C. albicans* levels (One-way ANOVA; p-value of 0.008, Chi-square test; p-value of 0.025, Fig. 2B). Only 73.9% of the subjects between 50 and 59 were colonized with *C. albicans,* whereas 85.2% of the rest of the cohort was colonized by this yeast. We also observed differences in *C. albicans* carriage between the age groups among the subjects that were colonized by *C. albicans.* Indeed, subjects between 20 and 29 were carrying in average higher intestinal *C. albicans* levels than subjects from the 40-49 (Tukey HSD; p-value of 0.025) and 50-59 (Tukey HSD; p-value of 0.039) age groups.

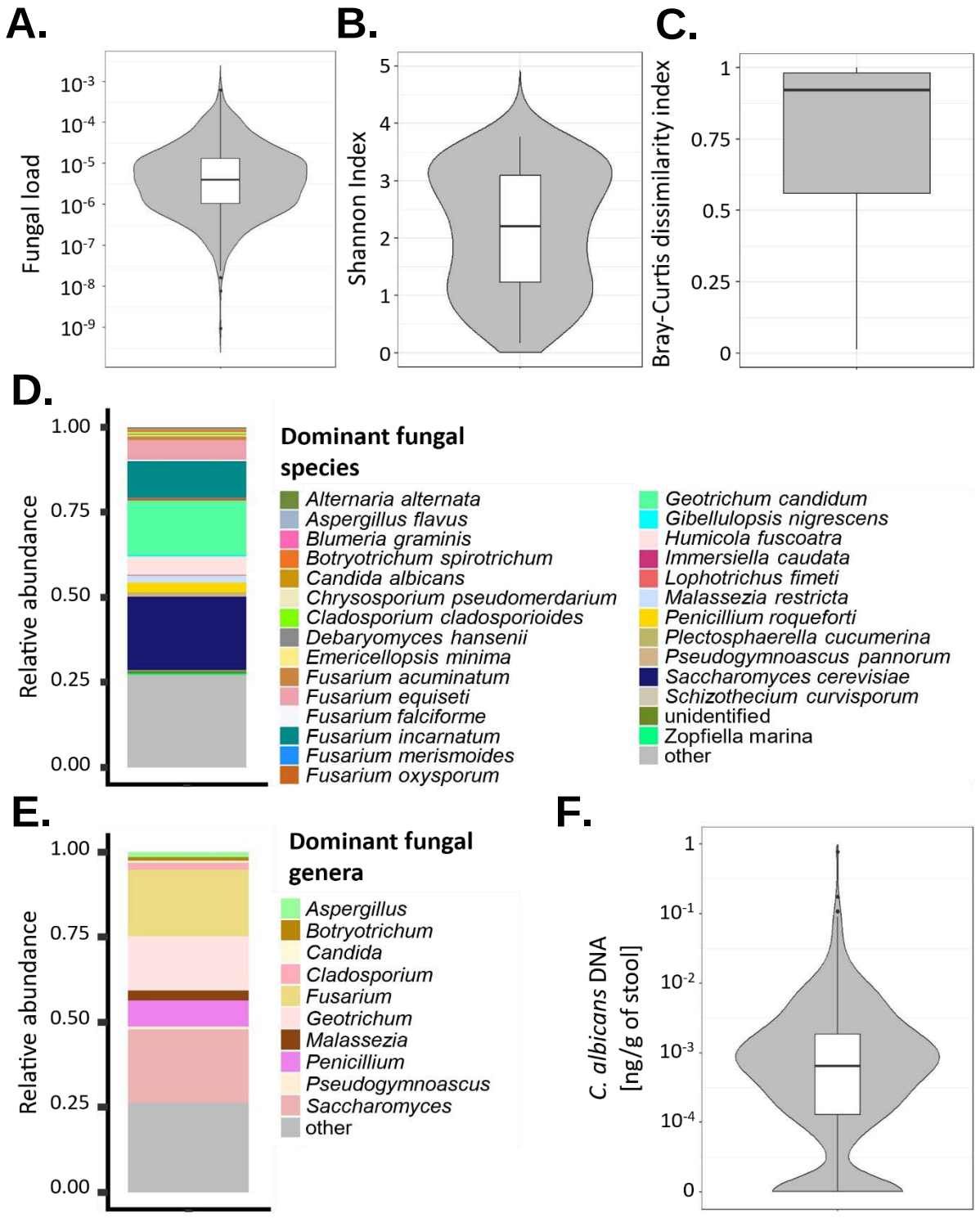


Figure 1: Mycobiota characteristic of healthy subjects. (A). Violin plot of the fungal load observed in the 695 healthy subject fecal samples. (B). Alpha diversity : violin plot of the Shannon Index obtained for 96 healthy subjects' fecal samples. (C). Beta diversity: Bray-Curtis dissimilarity values between samples donated by different subjects for ITS2 sequencing data obtained for 96 healthy subjects. Values range from 0 to 1, with 0 being the least dissimilar and 1 being the most dissimilar. (D). Barplot of the mean relative abundances of the fungal species that are detected in at least 50% of the subjects. (E). Barplot of the mean relative

abundances of the fungal genera that are detected in at least 75% of the subjects. (F) Violin plot of *C. albicans* DNA levels observed in the 695 healthy subject fecal samples.

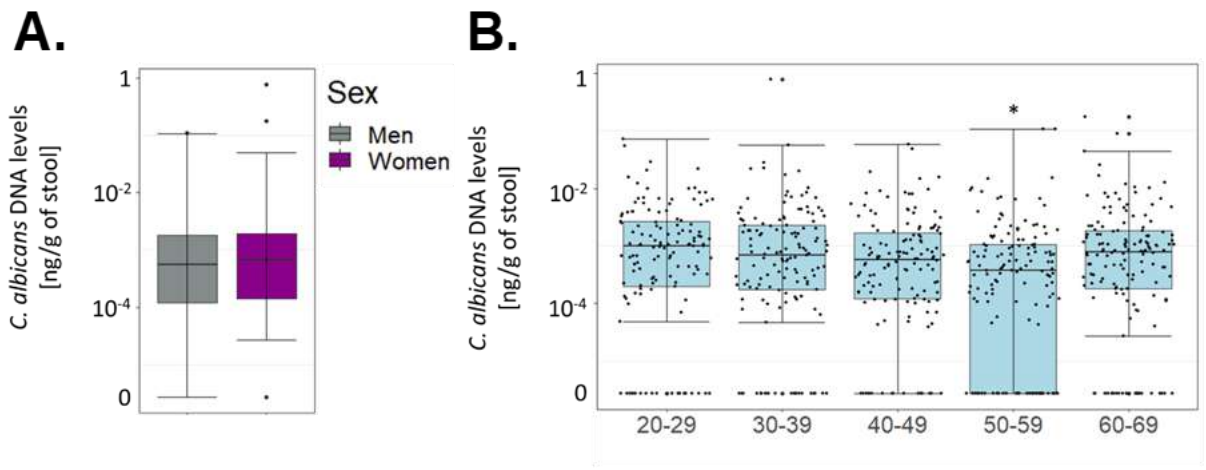


Figure 2: Effect of the age and sex of the subjects on *C. albicans* carriage. (A). Boxplot of the distribution of *C. albicans* DNA levels in male and female subjects. (B). Boxplot of the distribution of *C. albicans* DNA levels depending on the age of the subjects.

Identification of microbial species with a potential anti-C. albicans activity. Considering the wide variations of *C. albicans* carriage observed in the Milieu Intérieur healthy subjects, we hypothesized that the composition of the microbiota could explain a part of these variations and we aimed to identify bacterial species that were associated with *C. albicans* carriage in the gut and could thus either inhibit or, to the contrary, promote *C. albicans* growth in the human gut. By taking advantage of the whole genome shotgun metagenomic sequences available with Milieu Intérieur (Byrd *et al.* 2020), we used MaAsLin2 (Microbiome Multivariable Associations with Linear Models), to search for associations between *C. albicans* levels, deduced from qPCR quantification, and bacterial species abundance, deduced from the shotgun metagenomics data and annotated at the species level. On the 231 metagenomic species analyzed, *Intestinimonas butyriciproducens* was the only species to be significantly associated with *C. albicans,* highlighting a possible anti-*C. albicans* activity of this bacteria (MaAsLin2; q-value of 0.029, association coefficient of -0.11).

Since *Intestinimonas butyriciproducens* has been described as a producer of butyrate (Kläring *et al.* 2013), a molecule known to inhibit *C. albicans* growth and hyphae production (Nguyen *et al.* 2011; Noverr and Huffnagle 2004), we tested the effect of this species supernatant on *C. albicans* growth and morphogenesis. We also tested the effect of the supernatant of several additional bacterial species: *Bacteroides*

massiliensis, Bacteroides ndongoniae, Coprobacter secundus, Enorma massiliensis, Pseudoflavonifractor capillosus, Lactococcus lactis and *Roseburia intestinalis.* Since *L. lactis* is a facultative anaerobe, *L. lactis* supernatant was tested after it was grown in anaerobic and in aerobic conditions. We used *Bifidobacterium adolescentis,* strain L2-32, as a *C. albicans* inhibition control since this strain has been recently shown to have a strong inhibitory effect on both *C. albicans* growth and morphology (Ricci *et al.* 2022).

All these bacteria were short-chain fatty acid (SCFA) producers, but the concentration and type of SCFA varied greatly between the species (Fig. 3A). Unsurprisingly, *B. adolescentis* was the largest producer of SCFA, especially acetate, whereas *R. intestinalis* was the species that produced the largest amount of butyrate with an average of 42.5mM (Fig. 3A). However, except for *B. adolescentis,* none of the tested species' supernatants were able to inhibit *C. albicans* growth (Fig. 3B). None of the studied bacterial species' supernatant had an effect on *C. albicans* morphology, as observed by microscopy, except for *R. intestinalis.* Indeed, we observed a slight reduction of *C. albicans* hyphae formation in contact to *R. intestinalis* supernatant (Supplementary Fig. 2).

Diet, medical and environmental factors have a limited impact on* C. albicans *growth. Since the gut microbiota composition seems to have a limited impact on *C. albicans* colonization, we then investigated if a specific alimentation, the medical history, or a combination of environmental factors might shape *C. albicans* carriage in the human gut. We combined linear mixed models and multiway ANOVA, adjusted for age, sex and technical variables, to identify diet, medical and environmental factors associated with *C. albicans* colonization and to the extent of *C. albicans* intestinal carriage in the colonized subjects (see Materials and Methods). In total, we analyzed 201 variables including 12 demographic variables (i.e. age, sex, physical activity, housing conditions, etc.), 46 diet-related variables (i.e. consumption frequency of major food groups, number of meals per day, etc.), 6 basic physiological measures (i.e. weight, BMI, blood pressure, etc.), 70 variables relative to the subject or subject's family medical history, 44 laboratory measures (i.e. creatinine, gamma GT, serology, etc.) and 30 variables relative to the subjects' sleeping, drugs and smocking habits as well as socio-professional information. The list of the 201 variables analyzed is available in the supplementary data (Supplementary Table 1).

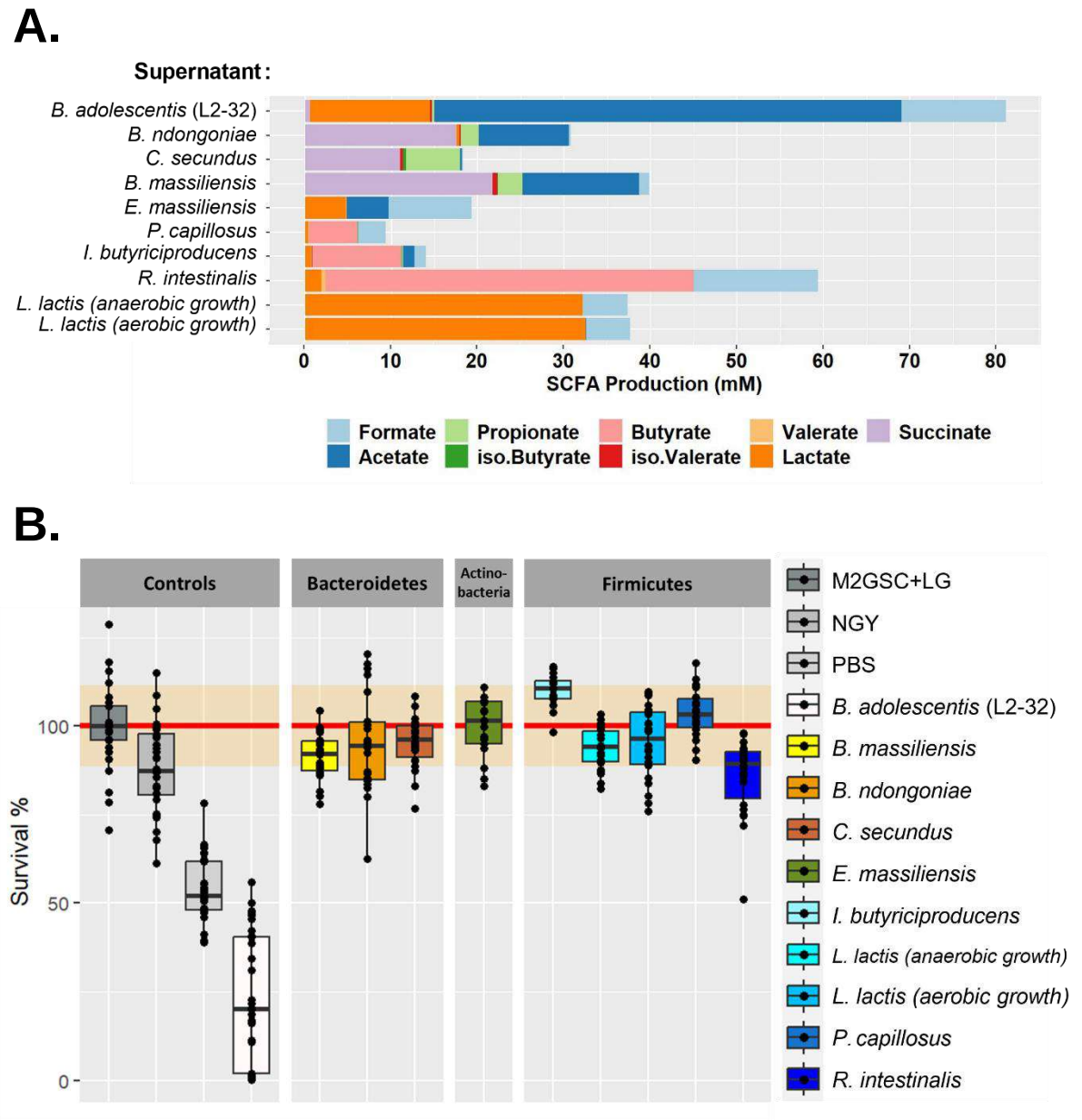


Figure 3: Characterization of bacterial species supernatants and their effect on *C. albicans* growth. (A). Characterization of the short-chain fatty acids (SCFA) content of each of the bacterial species tested. The SCFA were quantified in the species supernatant and normalized with the growth medium. (B). Boxplot representing the effect of bacterial culture supernatants on the survival of *C. albicans,* strain SC5314 after 24h of exposure, relative to the control growth in M2GSC medium (red line, confidence intervals are represented in orange). NGY: *C. albicans* growth in NGY medium, PBS: *C. albicans* growth in PBS.

Diet slightly impacted *C. albicans* growth, with the subjects' salt consumption being negatively associated with *C. albicans* carriage (q-value of 0.0047, Fig. 4A), while the subjects' snacking habits (q-value of 0.016, Fig. 4B) were associated with a higher *C. albicans* intestinal carriage. Surprisingly, the only biological-related variable we identified in this analysis was the mean corpuscular hemoglobin concentration of the

subjects, that was strongly associated with both *C. albicans* DNA levels and *C. albicans* colonization (q-value of 0.00064 and 0.0010, respectively, Fig. 4C). However, we did not observe any impact of environmental variables on *C. albicans* intestinal levels or colonization.

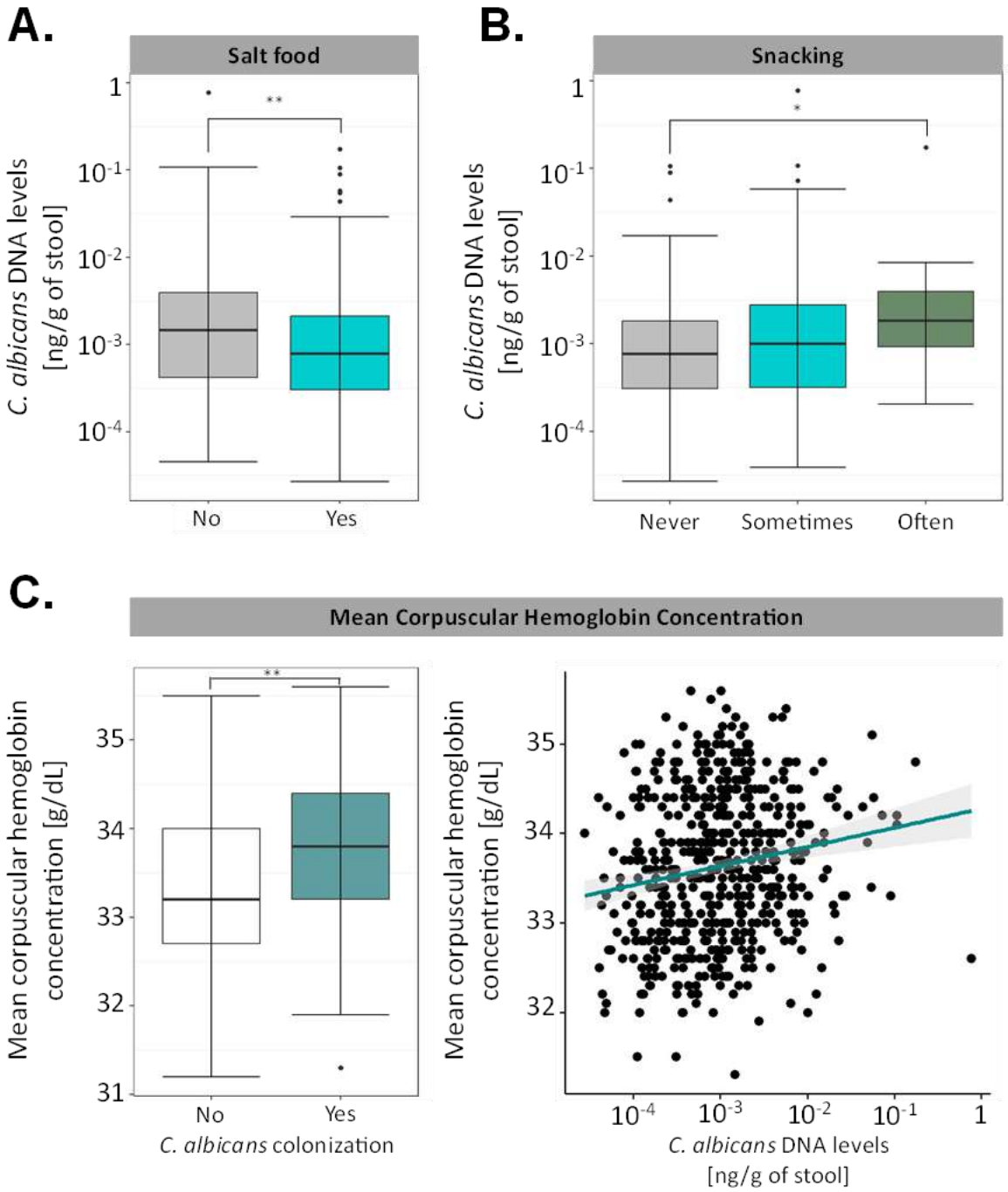


Figure 4: Diet and medical factors have a limited impact on *C. albicans* intestinal carriage and colonization. (A). Boxplot of the variation of *C. albicans* DNA levels according to the salting habits of the subjects. (B). Boxplot of the variation of *C. albicans* DNA levels according to the salting habits of the subjects. (C). Association between *C. albicans* intestinal carriage and colonization and the mean corpuscular hemoglobin concentration. (Left) Boxplot

of the distribution of the mean corpuscular hemoglobin concentration depending on *C. albicans* colonization state. (Right) Scatterplot of the mean corpuscular hemoglobin concentration relative to intestinal *C. albicans* DNA levels. The regression line is represented in green, and the interval of confidence in grey). ** p-value < 0.05, ** p-value < 0.005.*

Genome-wide association study identifies a* C. albicans *gastrointestinal colonization susceptibility locus on chromosome 20. To investigate the effects of genetic variants on *C. albicans* gut colonization susceptibility, we compared the genotype profiles (Patin *et al.* 2018) of the 576 Milieu Intérieur subjects that were colonized by *C. albicans* to those of the 119 subjects that were not. After quality control (Patin *et al.* 2018) and genotype imputation, we obtained a total of 5'677'102 single-nucleotide polymorphisms (SNPs) which were tested for association with *C. albicans* intestinal colonization state by linear mixed models. The models were adjusted by age, sex, smoking habits, and the genetic relatedness among subjects as was described by Patin *et al.* 2018. We thus identified 26 SNPs in two independent loci that showed a potential association with *C. albicans* colonization (p-value < 1.00×10^{-6}, Fig. 5A).

Among these associations, a SNP on chromosome 20, rs2870723 showed the strongest association with susceptibility to *C. albicans* intestinal colonization (p-value of 3.43×10^{-7}, β coefficient of -0.224). Although rs2870723 genotypes were associated with *C. albicans* intestinal levels when considering all 695 subjects (Fig. 6A; One-way ANOVA; p-value of 2.72×10^{-7}), this was not the case when considering only the 576 subjects colonized by *C. albicans* (Fig. 6A; One-way ANOVA; p-value of 0.10). Therefore, rs2870723 seems to be only associated with the host susceptibility to *C. albicans* colonization and not to the extent of *C. albicans* carriage, in colonized subjects.

Rs2870723 is located between *RNA5SP487*, a 5S Ribosomal pseudogene and *MC3R* (Fig. 5B), a gene that encodes for a melanocortin receptor, whose mutations have been previously associated to obesity (Koya *et al.* 2018; Mencarelli *et al.* 2011; Zegers *et al.* 2011; Lee *et al.* 2016). Interestingly, this SNP was also located relatively close to the Aurora Kinase A (*AURKA*) gene (Fig. 5B), in which several SNPs have been associated with the mean corpuscular hemoglobin concentration, a variable that we identified as being significantly associated with *C. albicans* colonization (summary statistics were downloaded from the NHGRI-EBI GWAS Catalog (Sollis *et al.* 2023) on 19/01/2023 for study GCST90002322 and GCST90002326 (Chen *et al.* 2020).

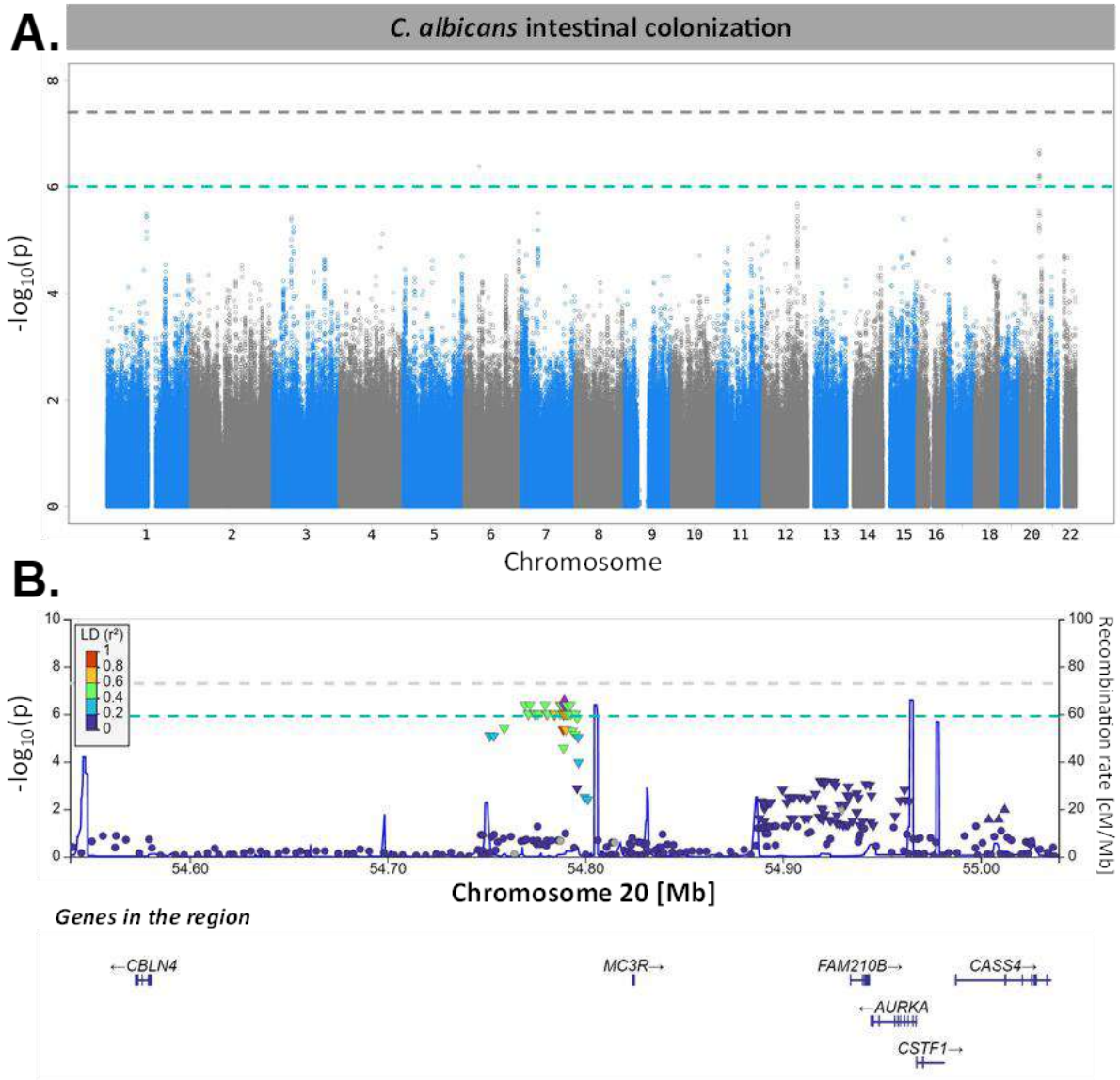


Figure 5: *MC3R* locus is associated with *C. albicans* intestinal colonization susceptibility. (A). Manhattan plot of single-nucleotide polymorphisms (SNPs) associated with *C. albicans* intestinal colonization susceptibility, identified by the genome-wide association study (GWAS) conducted on the 695 subjects of Milieu Intérieur. The x-axis represents the chromosomal position, and the y-axis represents the $-\log_{10}$(p-values) associated with each SNP. The green line represents the suggestive threshold for association (p-value < 1.00×10^{-6}). The grey line represents a threshold of 5.00×10^{-8}. (B). The regional association plot for the *C. albicans* intestinal colonization-associated SNP, rs2870723 (purple diamond). Each dot represents a SNP, the color of the dots corresponds to the linkage disequilibrium of the neighboring SNPs with the top SNP. The x-axis represents the chromosomal position, the left y-axis represents the $-\log_{10}$(p-values) associated with each SNP (dots) and the right y-axis represents the recombination rate (blue line) occurring in each position of the locus.

Higher* C. albicans *intestinal levels are associated with the immune response upon* C. albicans *blood stimulation. As seen in the previous part of this study, the factors behind *C. albicans* colonization in healthy individuals are multifactorial and several medical, diet-related, or genetic factors might contribute into shaping the extent

of *C. albicans* carriage. Since more than 80% of the healthy population is colonized by *C. albicans* even though this yeast can become highly pathogenic in immunocompromised patients, we wondered if there was an advantage to the host to maintain relatively high levels of *C. albicans* in the gut. In particular, we hypothesized that a high carriage of *C. albicans* could contribute to the host protection in case of systemic infection by this yeast.

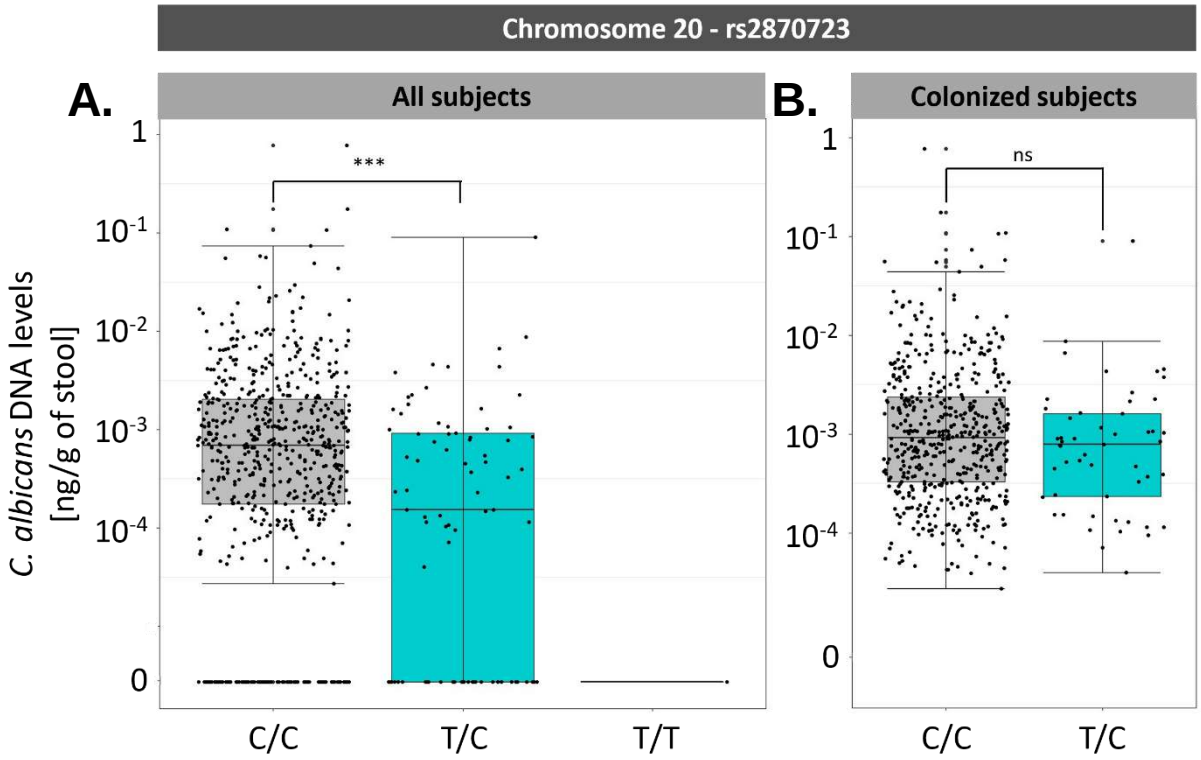


Figure 6: Association between rs2870723 genotypes and the levels of *C. albicans* intestinal carriage. (A) Boxplot of the variation of *C. albicans* DNA levels according to the rs2870723 genotype of the 694 subjects. (B) Boxplot of the variation of *C. albicans* DNA levels according to the rs2870723 genotype of the 574 subjects colonized with *C. albicans. *** p-value < 0.0005, ns non-significant.*

To test this hypothesis, we searched for associations between the extent of *C. albicans* carriage in the subjects colonized by this yeast and the level of expression of 546 immune genes and the production of 13 cytokines in the subjects' whole blood, this before and after blood stimulation with heat-killed *C. albicans* cells. To do so, we used linear mixed models adjusted for the subject age, sex, immune cells proportions and technical variables. In addition, the models were adjusted for the genotype of the rs12567990 SNP, since it has been shown that this SNP, located at the *CR1* locus,

regulates the immune gene expression upon *C. albicans* blood stimulation in the subjects from Milieu Intérieur (Piasecka *et al.* 2018).

None of the 546 genes and 13 cytokines were associated with the extent of *C. albicans* carriage in non-stimulated conditions, suggesting that *C. albicans* intestinal carriage is independent of the immune response in normal conditions. However, we identified positive associations between the extent of *C. albicans* intestinal carriage and the expression level of *NLRP3* (q-value of 0.036) and the concentration of IL-2 (q-value of 0.00059) and CXCL5 (q-value of 0.029) upon blood stimulation with *C. albicans* heat-killed cells (Fig. 7A). *NLRP3* response upon *C. albicans* stimulation was mainly driven by the proportion of monocytes, *C. albicans* intestinal carriage explaining only 2.86 % of the variance (Fig. 7B), while *CXCL5* response was largely driven by the age and sex of the subjects (1.28 % of the variance explained by *C. albicans* intestinal carriage, Fig. 7B). However, IL-2 production upon *C. albicans* blood stimulation was mainly driven by the extent of *C. albicans* carriage, which was associated with the highest percentage of variance (3.39 %, Fig. 7B). It has to be noted that these associations were specific to the stimulation of the blood with *C. albicans.* Indeed, *C. albicans* intestinal levels were not associated with either *NLRP3* expression or IL-2 and CXCL5 levels upon the subjects blood stimulation with *Escherichia coli* (*NLRP3*: q-value of 0.17, IL-2: q-value of 0.60, CXCL5: q-value of 0.46)*, Staphylococcus aureus* (*NLRP3*: q-value of 0.61), *Bacillus* Calmette-Guérin (BCG, q-value of 0.45, 0.24 and 0.24), influenza A live virus (q-value of 0.58, 0.99 and 0.99) or the staphylococcal enterotoxin B superantigen (q-value of 0.35, 0.87 and 0.28).

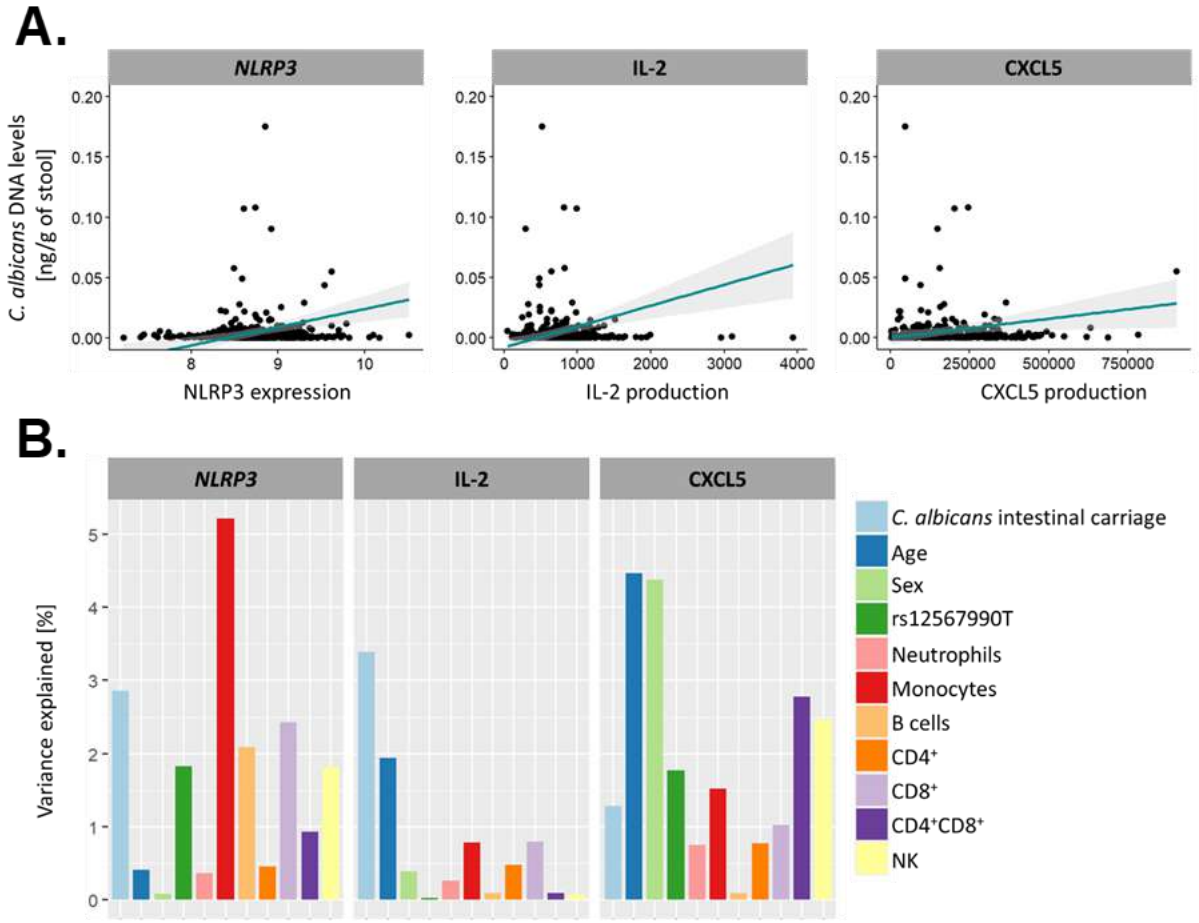


Figure 7: The extent of *C. albicans* intestinal carriage is associated with the expression levels of NLRP3 and the concentration of IL-2 and CXCL5 upon *C. albicans* blood stimulation. (A). Scatter plots of the association between *C. albicans* intestinal DNA levels and the expression of NLRP3, and the concentration of IL-2 and CXCL5 upon *C. albicans* stimulation. The regression line is represented in green and the interval of confidence in grey). (B) Proportion of the expression and concentration variance explained by *C. albicans* intestinal carriage, age, sex, genetics, and proportions of immune cells for NLRP3, IL-2 and CXCL5, in response to *C. albicans* blood stimulation.

4. Discussion

In this study, we explored different factors that could modulate the presence, or the intensity *of C. albicans* intestinal carriage in healthy subjects. First, we showed that *C. albicans* was present, with wide inter-subject variability, in the gut of 82.9% of the healthy subjects. This variability could not be explained by the subjects' gender since men and women carried similar intestinal levels of *C. albicans*. Interestingly, subjects aged between 50 and 59 years were less colonized than the other individuals, suggesting an effect of age on *C. albicans* intestinal colonization. However, considering that all subjects were recruited at the same period, this decrease in *C. albicans* colonization might be associated with this specific generation rather than being age-related. This study is the first to assess *C. albicans* carriage, using a qPCR approach, in such a large population. A recent study published by our group already reported a high carriage of *C. albicans* in healthy adults (Delavy *et al.* 2022), and our work thus confirms that *C. albicans* might not be a facultative commensal, as was previously thought, but that it is able to maintain itself in the GI tract of most individuals, often at very low levels.

The low incidence of host resistance to *C. albicans* colonization might have a genetic component. Indeed, we identified 26 SNPs, located in two independent loci, that are associated with *C. albicans* colonization. In particular, rs2870723, a SNP located in the chromosome 20, was strongly associated with the host susceptibility to *C. albicans* colonization but not to the levels of *C. albicans* carriage in the subjects colonized by this yeast. This SNP is located between *RNA5SP487,* an RNA 5S pseudogene that is not widely described, and *MC3R,* a gene encoding for a melanocortin receptor. Considering the phenomenon of Gene Linkage Disequilibrium, referring to the fact that alleles from closely located genes are more likely to be transmitted together than alleles from distant genes, it might be interesting to explore further the neighboring genes. Indeed, in this study, we tested 5'677'102 SNPs which represent only a fraction of the total number of existing SNPs. Moreover, the tag SNP – the SNPs on microarray typically used for genotyping in GWAS studies – are usually selected because they have a large amount of linkage disequilibrium with neighboring SNPs, thus serving as surrogates for the genomic regions that contain unmeasured SNPs (Stram 2004a; Ding and Kullo 2007; Schaid, Chen, and Larson 2018b).

Considering that mutations in *MC3R* have been associated with obesity in genetics (Koya *et al.* 2018; Mencarelli *et al.* 2011; Zegers *et al.* 2011) and *in vivo* (Lee *et al.* 2016) studies*,* it is possible that this genetic association with *C. albicans* colonization results from an indirect interaction with the subjects' weight. Especially since *C. albicans* have been reported to be more abundant in overweight people (García-Gamboa *et al.* 2021). Moreover, polymorphisms in *MC3R* have been associated with an increased susceptibility to tuberculosis, probably through a mediation of the inflammatory response (P. Xu *et al.* 2020; Park *et al.* 2014; Hashemi *et al.* 2013). It is thus possible that the SNPs identified result in a differentiated inflammatory and/or inhibitory response against *C. albicans*.

This interaction between neighboring genes is likely considering the fact that a SNP in the *AUKRA* gene, another neighboring gene of rs2870723*,* has been associated with the mean corpuscular hemoglobin concentration in previous GWAS studies (Sollis *et al.* 2023; Chen *et al.* 2020), a variable that we found associated with *C. albicans* intestinal carriage and colonization susceptibility.

As importantly, we found that the subjects microbiota composition might contribute to *C. albicans* carriage, by highlighting *Intestinimonas butyriciproducens,* a SCFA-producing bacteria, as a potential anti-*C. albicans* signature. Since we were not able to show an effect of this bacteria supernatant on *C. albicans* growth or morphology, it is possible that the conditions we used were not optimum for this bacteria growth and subsequent release of SCFA or other antifungal metabolites. Indeed, our analyses showed a relative low concentration of SCFA in the supernatant of this bacteria, which could be increased in alternative growth conditions. Moreover, the release of antifungal metabolites such as SCFA is only one method by which microbial species can inhibit fungal growth. Indeed, a bacterial species can also inhibit *C. albicans* growth by modulating the host immune response (Rizzo, Losacco, and Carratelli 2013; Nguyen *et al.* 2011; Fan *et al.* 2015) or through competition for niches, adhesion sites and/or nutrients (Alonso-Roman *et al.* 2022; Mailänder-Sánchez *et al.* 2017; Basson 2000). Finally, *C. albicans* inhibition by *Intestinimonas butyriciproducens,* might be strain specific, as it is has been reported for *Bifidobacterium adolescentis* (Ricci *et al.* 2022)*.*

In this study, the subjects that reported having a low sodium diet and those that reported frequent snacking between meals were carrying higher intestinal levels of *C.*

albicans, in average. This is not surprising since diet is known to strongly modulate the composition of the gut mycobiota (Szóstak *et al.* 2023; Motooka *et al.* 2017; G. D. Wu *et al.* 2011; Angebault *et al.* 2013) and several diet-related factors have been shown to affect the composition of the gut microbiota of the Milieu Intérieur subjects (Partula *et al.* 2019). The association between the snacking habits of the subjects and *C. albicans* high intestinal carriage is thus easily explained since snacking is often associated with the consumption of overall “unhealthy” food with high sugar and fat contents, and that these factors have been frequently associated with higher *C. albicans* carriage (Szóstak *et al.* 2023). The association of a low sodium diet with *C. albicans* carriage might seem more surprising, however, a high salt intake has been previously linked to an increase of Th17/IL-17 immune response that impacted the composition of the gut microbiota (Wilck *et al.* 2017).

Finally, we showed that harboring high levels of *C. albicans* in the gut might be an advantage for the host, as the extent of intestinal *C. albicans* carriage is significantly associated with the expression of *NLRP3* and the levels of IL-2 and CXCL5 upon a simulated *C. albicans* blood infection. NLRP3 inflammasome plays a crucial role in the clearance of *C. albicans,* since its activation triggers the release of IL-18 and IL-1β, two pro-inflammatory cytokines that promotes the Th1 and Th17 immune response (Vonk *et al.* 2006; Hise *et al.* 2009), while CXCL5 drives neutrophil recruitment in the Th17 immune response (Disteldorf *et al.* 2015; Conti and Gaffen 2015). However, IL-2 activates Treg, and therefore maintain the equilibrium between inflammatory and anti-inflammatory responses (Leigh *et al.* 1998; Richardson and Moyes 2015).

These results are thus coherent with previous reports stating that previous challenge with fungal components, such as β-glucans, results in an increased survival of mice upon a fungal infections (Quintin *et al.* 2012) and that *C. albicans* intestinal colonization confers a protection against systemic infections in mice (Huertas *et al.* 2017). In a similar context, recent studies have shown that mucosae-associated fungi might play a protective role for the host by reinforcing the intestinal epithelial function and thus preventing infection (Leonardi *et al.* 2022). Considering that cell wall peptidoglycans from the gut bacterial microbiota can cross the intestinal epithelial barrier and have been shown to disseminate in the blood of mice (Wheeler *et al.* 2023), it is possible that a similar phenomenon occurs with *C. albicans* cell wall molecules, such as β-glucans. Subjects highly colonized with *C. albicans* would therefore be more likely to

have fungal particles crossing the gut barrier, thus allowing the host to develop a anti-*C. albicans* immunity.

Taken together, these results offer a better understanding of the mechanisms of *C. albicans* colonization in the healthy host. Although, the relative importance of some of the associations that we identified remain to be further explored, these findings pave the way for new intervention strategies to curb the intestinal proliferation of *C. albicans*, and thus prevent the emergence of life-threatening infections in high-risk patients.

5. Materials & Methods

The Milieu Intérieur cohort. One thousand healthy volunteers – 500 men and 500 women – were recruited in the Rennes area (Ille-et-Vilaine, Bretagne, France) by BioTrial (Rennes, France) between September 2012 and August 2013. The age of the subjects was evenly distributed between 20 and 69 years, with 200 people in each decade of life. To minimize the influence of population substructure, the study was restricted to individuals of self-reported metropolitan French origin for three generations (i.e., with parents and grandparents born in continental France). Participant were selected based on strict inclusion and exclusion criteria (Thomas *et al.* 2015). In short, the subjects had no evidence of severe, chronic and/or recurrent pathology, and their body mass index (BMI) was limited to ≥18.5 and ≤32 kg/m^2. In addition, subjects were excluded from the study if they (i) had been treated with antibiotics within the last 3 months prior to inclusion, (ii) were HIV or hepatitis C seropositive,(iii) reported having travelled to tropical or subtropical countries in the 6 months prior to inclusion, (iv) vaccinated shortly before inclusion, (v) were alcohol abusers, (vi) were on a diet prescribed by a doctor or a dietician for medical reasons or (vii) with food intolerance or allergy. Moreover, only pre- or postmenopausal women were included in order to avoid the influence of hormonal fluctuations in women during the perimenopausal phase.

The clinical study was approved by the Comité de Protection des Personnes–Ouest 6 on June 13, 2012, and by the French Agence Nationale de Securité du Médicament on June 22, 2012, and was performed in accordance with the Declaration of Helsinki. The study was sponsored by the Institut Pasteur (Pasteur ID-RCB no. 2012-A00238-35) and conducted as a single-center study without any investigational product. The original protocol is registered under ClinicalTrials.gov (study number NCT01699893).

Informed consent was obtained from the participants after the nature and possible consequences of the studies had been explained to them. The samples and data used in this study were formally established as the Milieu Interieur biocollection (NCT03905993), with approvals by the Comité de Protection des Personnes – Sud Mediterranée and the Commission nationale de l'informatique et des libertés on April 11, 2018.

Fecal DNA extraction. For each sample, 100-250 mg of stool was processed following the repeated bead beating plus column method described by Yu and Morrison, 2004 (Yu and Morrison 2004) except than a Bullet Blender (NextAdvance, Troy, NY, USA) was used instead of a Mini-Beadbeater™. DNA samples were eluted in 100 µL of double distilled H_2O. Total fecal DNA levels were measured by Qubit (Invitrogen, USA) using the dsDNA Broad Range Kit (Invitrogen, USA). Only DNA extract with a total DNA concentration above 50 ng/µL and in which fungal DNA was detected by Panfungal qPCR (see below) were retained for analysis, leading to a total of 695 samples that were further analyzed.

Quantitative PCR for detection of total fungal load in human DNA samples. Fungal DNA was quantified by TaqMan qPCR as described by Liu *et al.* 2012 (C. M. Liu *et al.* 2012) using a double dye MGB 5' 6-FAM-labelled probe (Eurogentec, Belgium). All reactions were performed on a CFX96 Real-Time PCR system (BioRad, USA) with the following conditions: 2 min at 50°C, 10 min at 95°C, 15 s at 95°C and 1 min at 60°C, the last two steps repeated for 45 cycles. All samples were tested in two independent rounds, each time in duplicates. The presence of qPCR inhibitors was determined with a duplex internal control - *C. albicans* qPCR assay, as described below.

The fungal load was estimated by dividing the fungal DNA concentration by the total DNA concentration of the sample (Zuo *et al.* 2018), obtained by Qubit Broad Range protocol.

Duplex quantitative PCR for detection of C. albicans DNA in human DNA samples and internal amplification control. 10 µL of a 1:10 dilution of the extracted total fecal DNA were used as a template for TaqMan qPCR analysis, using *C. albicans* probe and primers described by Guiver *et al,* 2001 (Guiver, Levi, and Oppenheim 2001) in combination to the Cy®5-QXL®670 Probe system of the Universal Exogenous qPCR

Positive Control for TaqMan® Assay (Eurogentec, Belgium), in order to identify samples with qPCR inhibitors.

C. albicans primers and probes were used at 100nM and 400nM, respectively. All reactions were performed on a CFX96 Real-Time PCR system (BioRad, USA) with the following conditions: 2 min at 50°C, 10 min at 95°C, 15 s at 95°C and 1 min at 60°C, the last two steps repeated for 45 cycles. All samples were quantified in two independent rounds, each time in duplicates. Samples with qPCR inhibitors at a 1:10 dilution were diluted at 1:100 and submitted to a new round of qPCR. A detection threshold of 10^{-8} ng/µL of DNA was used for this assay.

ITS2-targeted metagenomic sequencing. Library construction, quality control and sequencing were performed by Novogene (Beijing, China). PCR amplification of ITS2 regions was performed by using ITS3/ITS4 primers (White *et al.* 1990), using Novogene pipeline. The PCR products were selected by 2% agarose gel electrophoresis. PCR products from each sample were pooled, end-repaired, A-tailed and further ligated with Illumina adapters, according to manufacturer's recommendation. The library was quantified with Qubit and real-time PCR, and the size distribution estimated with a bioanalyzer. Quantified libraries were pooled and sequenced on Illumina platforms (Novogene, Beijing, China), according to effective library concentration and data amount required. Paired-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Quality filtering on the raw reads was performed under specific filtering conditions (Novogene, Beijing, China) to obtain the high-quality clean reads. Paired-end clean reads were merged using FLASH (Magoč and Salzberg 2011) based on the reads overlap.

ITS2-targeted metagenomic sequences analysis. ITS2 sequences were analyzed with QIIME 2^{TM} (Quantitative Insights into Microbial Ecology) (Bolyen *et al.* 2019). A total of 14.1 million sequences were generated from 100 samples, with a mean of 140,892 sequences per sample. The sequences were trimmed to 222 bp for the forward and reverse sequences, respectively. Using the DADA2 tool (Callahan *et al.* 2016), the sequences were denoised and dereplicated into amplicon sequence variants (ASVs), and the chimeras were removed. Four samples with less than 10,000 sequences were removed from the analyses. 2480 ASVs were recovered, and we

generated a feature table for all remaining samples. The taxonomic annotation was performed on the feature table using the UNITE database (rev. 9.0). ASVs not annotated as fungi were filtered out, leading to a total of 1061 ASVs. ASVs that could not be annotated at the species levels were submitted to a second round of annotation against the UNITE database and to a classic BLASTN. Only hits matched with a similarity above 97% to reference genomes were conserved. The abundance and DESeq2 normalized count tables were generated with SHAMAN (Volant *et al.* 2020).

Shotgun metagenomic sequencing. Shotgun metagenomic sequences were obtained as described in Byrd *et al.* 2020.

Shotgun metagenomic sequences analysis.

Microbial gene count table. Gene count table were obtained using the METEOR software suite (Pons *et al.* 2010) that relies on Bowtie2 for read mapping. First, reads were filtered for low-quality by AlienTrimmer (Criscuolo and Brisse 2013) and reads that aligned to the human genome GRCh38-p13 release (identity > 95%) were also discarded. The remaining reads were trimmed to 80 bases and mapped to the Integrated Gut Catalogue 2 comprising 10.4 million genes (IGC2) (Plaza Onate *et al.* 2021), and the 8.4 million oral microbial gene catalog (Le Chatelier *et al.* 2021). The unique mapped reads (reads mapped to a unique gene in the catalogue) were attributed to their corresponding genes. The shared reads (reads that mapped with the same alignment score to multiple genes in the catalogue) were attributed according to the ratio of their unique mapping counts of the captured genes. The resulting count table was further processed using the R package MetaOMineR v1.31 (Le Chatelier *et al.* 2013). To decrease technical bias due to different sequencing depths and avoid any artifact of sample size on low-abundance genes, read counts were ‘rarefied’ using 20M high-quality reads using a random sampling procedure without replacement. The downsized matrix was finally normalized according to gene length and transformed into a frequency matrix (FPKM normalization).

Metagenomic Species (MGS) abundance profiles. The IGC2 and the oral catalogues were organized into 1990 and 853 Metagenomic Species (MGS, cluster of co-abundant genes), respectively, using MSPminer (Oñate *et al.* 2019; Le Chatelier *et al.* 2021). After removing duplicated MGS (i.e., MGS present in both catalogues), we were left with 2741 MGS. The relative abundance of an MGS was computed as the mean

abundance of its 100 ‘marker’ genes (that is, the genes that correlate the most altogether). If less than 10% of ‘marker’ genes were seen in a sample, the abundance of the MGS was set to 0**.**

Culturing of gut anaerobic bacteria. The anaerobic bacterial species tested included isolates from the Rowett Institute (Aberdeen, UK) strain collection or isolated purchased from DSMZ (Braunschweig, Germany) (Supplementary Table 2). The strains were revived anaerobically in Hungate tubes containing M2GSC medium supplemented with 1% liquid gold and incubated overnight at 37°C in a static 5% CO_2 incubator (NuAire, Plymouth, MN, USA). “Liquid gold” is the name given to fermenter run off which is collected after the addition of fecal slurry to a complex medium with the aim to simulating the human colon (Walker *et al.* 2005; Duncan *et al.* 2003). A spectrophotometer (Novaspec II, Amersham BioSciences UK Ltd., Little Chalfont, UK) was used to monitor cell growth by measuring the cultures optical density at 650 nm (OD_{650}).

Quantification of short-chain fatty acids and lactate in gut bacterial culture supernatants by capillary gas chromatography. Samples were analyzed as described in Ricci *et al.* 2022. Briefly, 1 mL of bacterial culture supernatant was filtered sterilized and added to a Sorvall screw-capped tube. 50 µL of 0.1M 2-ethylbutyric acid was added as an internal standard. A double step extraction of organic acids was performed with 0.5 mL of HCl and 2 mL of diethyl ether per sample. Tertiary butyldimethylsilyl were quantified using a capillary gas chromatography device (Agilent 6890; Agilent Technologies, Santa Clara). To control the quality of the organic acid extraction, an external standard, composed of known concentration of acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid, n-valeric acid, sodium formate, lithium lactate and sodium succinate, was analyzed with the samples in each gas chromatography run.

Assessment of C. albicans growth inhibition by gut bacterial supernatant. *C. albicans* strain SC5314 was grown overnight in NGY medium. *C. albicans* cells were then diluted to a 1:100 concentration in fresh NGY medium. In parallel, gut bacteria of interest were grown anaerobically overnight at 37°C in Hungate tube with M2GSC medium supplemented with 1% of liquid gold. Bacterial cultures were centrifuged to collect the supernatant. To remove any residual bacteria, each supernatant was then

filter-sterilized by being transferred through a 0.2 µm syringe-driven filter units (Millex, Merck Millipore Ltd, Kenilworth, NJ, USA).

One hundred µL of 1:100 diluted *C. albicans* culture was transferred to 96-well microtitre plates (CoStar, Washington, WA, USA) and incubated anaerobically for 24 h in a temperature-controlled plate reader at 37°C (Epoch 2 Microplate Spectrophotometer, BioTek, Swindon, UK) with 100 µL of bacterial culture supernatant. Fresh NGY medium, M2GSC medium supplemented with 1% of liquid gold and PBS were used as control to assess *C. albicans* growth. For each condition and each technical replicates, *C. albicans* growth was estimated by subtracting the optical density at 600nm obtained at time 0 from the one measured after 24 h. *C. albicans* growth in fresh M2GSC + 1% liquid gold was used as reference, thus corresponding to a 100% survival of the yeast. The experiment was performed in 3 biological replicates, each in 6 technical replicates.

Assessment of the effects of the gut bacterial supernatants on C. albicans morphology. *C. albicans* cells were collected after the 24h of incubation with the filter-sterilized bacterial supernatant and deposited into microscopy slides. To highlight potential bacterial contamination, each plate was Gram stained. Plates were observed under a Leica CME light microscope (Leica Microsystems, Germany) at 100x and 1000x magnification. For each biological replicate, the number of hyphae of two technical replicates was assessed visually, in five different sectors of the microscopy plates, at 1000x magnification.

Dietary, medical, environmental, and demographic data. Multiple dietary, medical, environmental and demographic data were collected for each Milieu Intérieur subject in an electronic case report form (Thomas *et al.* 2015). Subjects reported their family medical history, medical history, birth place, smoking and sleeping habits, etc., and completed a food-frequency questionnaire (Partula *et al.* 2019). In addition, clinical chemistry, hematologic and serologic assessment were performed on the blood of the subjects at the at the certified Laboratoire de Biologie Médicale, Centre Eugene Marquis (Rennes, France) (Byrd *et al.* 2020). After manual curation of the variables that displayed a near zero variance, 201 variables were analyzed for their association with *C. albicans* intestinal colonization.

Genotyping, Quality Control, and Imputation. The 1000 subjects of the Milieu Intérieur cohort were genotyped by the HumanOmniExpress-24 BeadChip (Illumina, California), as described by Patin *et al.* 2018 (Patin *et al.* 2018). SNPs that (i) were unmapped on dgSNP138, (ii) were duplicated, (iii) had a low genotype clustering quality (GenTrain score < 0.35), (iv) had a call rate inferior to 99%, (v) that were monomorphic, (vi) were on a sex chromosome, and/or (vii) were in Hardy-Winberg disequilibrium (p-value < 10^{-7}) were removed from the analyses. Possible pairs of genetically related subjects were detected as described in Patin *et al.* 2018 (Patin *et al.* 2018). Genotype imputation was then performed as presented in Patin *et al.* 2018.

Genome-wide association analysis. We conducted the GWAS analyses for *C. albicans* intestinal colonization state (576 colonized and 119 non-colonized) with the linear mixed model implemented in GEMMA (Zhou and Stephens 2014), a mixed model that allows the control of genetic relatedness among donors. For each chromosome, a genetic relatedness matrix (GRM) was estimated based on the 21 other chromosomes ('leave-one-chromosome' approach, Yang *et al.* 2014). Both genome-wide association analyses were conducted using as covariate the age, sex and smoking habit of the subjects.

Whole-Blood TruCulture Stimulation - Gene expression and proteomics analysis. TruCulture tubes were prepared in two batches (A and B) with heat-killed *C. albicans* cells (Invivogen, San Diego, USA) in 2mL buffered media. *C. albicans* blood stimulation was performed with 1mL of whole blood, for 22 hours, as previously described (Piasecka *et al.* 2018; Duffy *et al.* 2014). Gene expression analysis was conducted as previously described (Piasecka *et al.* 2018; Urrutia *et al.* 2016). The levels of cytokines present in the whole blood was measured by Luminex *x*MAP technology, as described previously (Duffy *et al.* 2014).

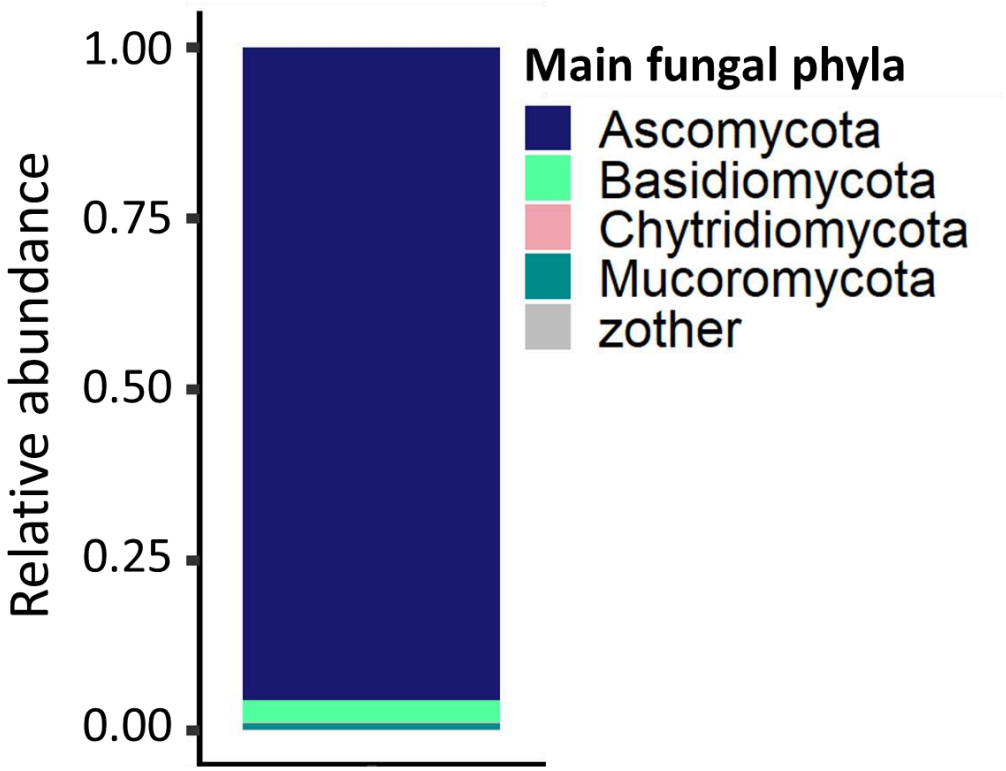
Biostatistical analyses. All analyses were performed on R (v. 4.1.2, Team R Development Core 2018). We used the vegan package (v. 2.6-4, Oksanen *et al.* 2019) to compute diversity indexes, the Maaslin2 package (v. 1.6-0, Mallick *et al.* 2021) to identify association between *C. albicans* carriage and the microbiota composition, the caret package to compute the near zero variances of the studied variables, (v. 6.0-93, Kuhn 2008) and the ggplot2 package to generate the figures (v. 3.4.0, Wickham *et al.* 2018).

*Identification of bacterial species with a potential anti-*C. albicans *activity.* Null values of *C. albicans* DNA levels and of the relative abundances of the MGS and fungal species were replaced by the minimal non-null value of the given variable divided by two, to allow a log10 transformation. MGS with a near zero variance (Kuhn 2008; 2005) were filtered out from the analyses. MaAsLin2 (Mallick *et al.* 2021) was used to search for association between *C. albicans* levels, deduced from qPCR quantification, and bacterial species abundance, deduced from the shotgun metagenomics data and annotated at the species level. Age and sex of subjects were set as random effects in the analysis. We used a type I error of 0.05 and corrected the p-values for multi-testing using false discovery rate correction.

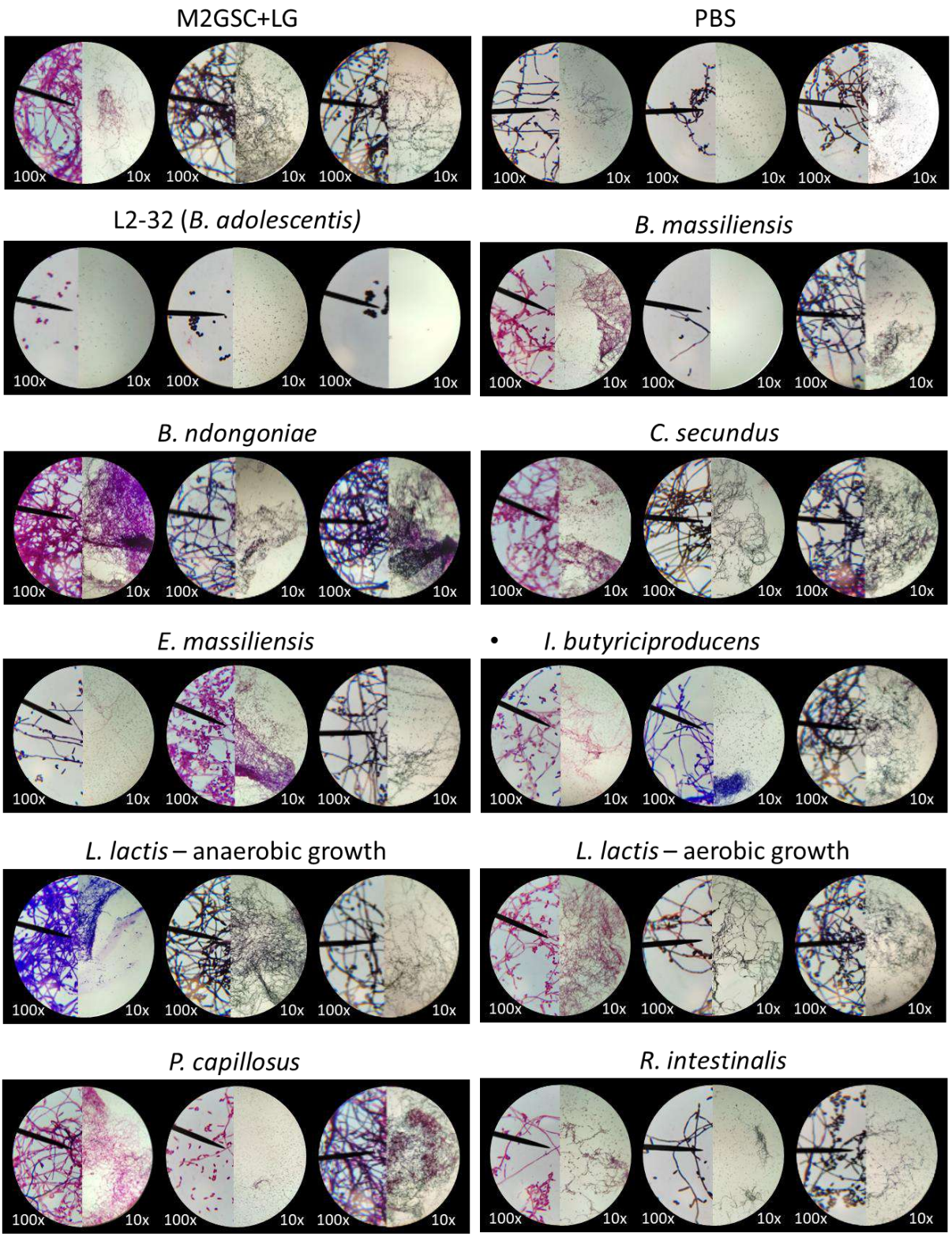
Identification of diet, medical and environmental variables associated C. albicans *intestinal carriage.* After manual curation of the variables that displayed a near zero variance, 236 variables were analyzed individually for their association with *C. albicans* intestinal colonization. We used linear mixed models and multiway ANOVA, adjusted for the age, sex and date of collection of the fecal samples, to predict either the state of *C. albicans* gut colonization of the subjects or the extent of *C. albicans* intestinal carriage. We controlled all analyses for FDR to take into account multiple testing and we used a threshold of FDR < 0.05.

Identification of associations between the extent of C. albicans intestinal carriage and the subject's immune response. To predict the expression of each gene and the concentration of each cytokine based on *C. albicans* carriage, we used linear mixed models adjusted for the subject age, sex, immune cells proportion, genotype at the rs12567990 SNP and the batch of TruCulture tube used, before and after *C. albicans* blood stimulation. We analyzed a total of 13 cytokines and 546 genes. The 546 genes analyzed have been previously showed to be differentially expressed upon *C. albicans* blood stimulation (Piasecka *et al.* 2018). The analyses were controlled for FDR to consider multiple testing and a threshold of FDR < 0.05 was used.

6. Supplementary Materials



Supplementary Figure 1: Phyla composition of healthy subjects. Barplot of the average relative abundance of the main fungal phyla for 96 healthy subjects. The fungal phyla represented have a mean relative abundance above 0.01%



Supplementary Figure 2: Gram staining and light microscopy of *C. albicans* SC5314 cells after 24h of exposure to the supernatant of bacterial isolates. The bacterial growth medium, M2GSC+LG, was used as media control, phosphate buffered saline (PBS) solution was used as no-media control and supernatant of *Bifidobacterium adolescentis* strain L2-32, was used as a filamentation inhibition control.

Supplementary Table 1: List of the diet, medical and lifestyle variables analyzed for an association with *C. albicans* carriage and colonization.

VARIABLE NAME	PANEL	DESCRIPTION	TYPE	VALUE
ABDOCM.V0	Basic physiological measurements	Abdominal circumference Visit 1	double	cm
ACCOUCH	Personal medical history at birth	Delivery time	binary	0=Born at term; 1=Premature
AGE	Demographics	Age in years	integer	years
ALB.V0	Laboratory measure	Biochemistry: Albumin	double	g/L
ALCF	Food and nutrition	Glasses of spirit per week	integer	glasses/week
ALCOOL	Food and nutrition	Alcohol	integer	30, 12, 4, 2, 0, per day
ALIM	Personal medical history at birth	Mode of feeding at birth	binary	0=Maternal breastfeeding; 1=Formula milk
ALLER	Family medical history	Allergic disease	binary	0=No; 1=Yes
ALP.V0	Laboratory measure	Biochemistry: Alkaline phosphatases	double	IU/L
ALT.V0	Laboratory measure	Biochemistry: ALAT	double	IU/L
AMIDI	Food and nutrition	Eat only in the afternoon	binary	0 = no, 1 = yes
APPET	Sleep habits, drug habits, and psychological problems	Little or too much appetite, last 2 weeks	integer	0, 3, 8, 14 days the last two weeks
APPLOC	Food and nutrition	Essential oils by local application	integer	0, 1, 5, 10, 30 times per month
AST.V0	Laboratory measure	Biochemistry: ASAT	double	IU/L
ATTAQUE	Family medical history	Cerebral accident, hemorrhage or congestion	binary	0=No; 1=Yes

AUTCFIL	Family medical history	Relationship to Other cancer	categorical	0=no relationship; 1=Father; 2=Mother; 3=Brother; 4=Sister; 5=Father & Mother; 6=Father & Brother; 9=Mother & Sister; NA=missing data
BASO.V0	Laboratory measure	Hematology: Basophils	double	G/L
BICARB.V0	Laboratory measure	Biochemistry: Bicarbonates	double	mmol/L
BIERE	Food and nutrition	Glasses of beer/cider per week	integer	glasses/week
BILI.V0	Laboratory measure	Biochemistry: Total bilirubin	double	µmol/L
BLANC	Biometrics	Hair graying	binary	0=no; 1=yes
BLANCA	Biometrics	Age at which hair started graying	integer	years
BMI.V0	Basic physiological measurements	BMI (kg/m^2) Visit 1	double	kg/m^2
BRUIT	Socio-professional information	Exposure to noise	categorical	0=No exposure; 1=Past exposure; 2=Current exposure
CAUTRE	Family medical history	Other cancer	binary	0=No; 1=Yes
CA.V0	Laboratory measure	Biochemistry: Calcium	double	mmol/L
CCOLON	Family medical history	Colon/rectum cancer	binary	0=No; 1=Yes
CHARCU	Food and nutrition	Cooked and cured meats (ham, salami, pate etc.)	integer	0, 1, 5, 10, 30, 60 times per month
CHEVEUX	Biometrics	Hair color	integer	1=Black; 2=Dark brown; 3=Light brown; 4=Red; 5=Blond; 6=Other
CHOL.V0	Laboratory measure	Biochemistry: Total cholesterol	double	mmol/L
CL.V0	Laboratory measure	Biochemistry: Chloride	double	mmol/L
CMSEQ	Comcomitant drug treatment	Subject takes concomitant drug treatment(s)	integer	Number of different drug treatments

CMV.V0	Laboratory measure	Serology: CMV	binary	0=negative; 1=positive
CONCEN	Sleep habits, drug habits, and psychological problems	Difficulty concentrating on things like reading the newspaper or watching the television, last 2 weeks	integer	0, 3, 8, 14 days the last two weeks
CORREC	Biometrics	Corrective lenses worn	binary	0=no; 1=yes
CORRECP	Biometrics	Type of corrective lenses	binary	1=glasses; 2=contact lenses
CREAT.V0	Laboratory measure	Biochemistry: Creatinine	double	µmol/L
CRP.V0	Laboratory measure	Biochemistry: CRP	double	mg/L
CRUD	Food and nutrition	Raw vegetables	integer	0, 1, 5, 10, 30, 60 times per month
CSEIN	Family medical history	Breast cancer	binary	0=No; 1=Yes
CSP	Socio-professional information	Socio-professional category, if ever employed	categorical	1=Farmer; 2=Artisan, tradesman, company director; 3=Senior executive, independent profession; 4=Middle management; 5=Employee; 6=Labourer; 7=Other (e.g. artist, clergy, soldier, policeman); NA=missing data; NaN=not applicable
CUISIN	Food and nutrition	Cooking	integer	1, 5, 20, times per month
DEJ	Food and nutrition	Eats lunch	integer	0 = always, 1 = not always, 2 = never
DESS	Food and nutrition	Desserts (cream desserts, ice cream, cream cakes, etc.)	integer	0, 1, 5, 10, 30, 60 times per month
DIABETE	Family medical history	Diabetes	binary	0=No; 1=Yes

DIABP1.V0	Basic physiological measurements	Diastolic measure 1 (mmHg) Visit 1	double	mmHg
DIABP2.V0	Basic physiological measurements	Diastolic measure 2 (mmHg) Visit 1	double	mmHg
DIAFIL	Family medical history	Relationship to Diabetes	categorical	0=no relationship; 1=Father; 2=Mother; 3=Brother; 4=Sister; 5=Father & Mother; 6=Father & Brother; 8=Mother & Brother; NA=missing data
DIFF	Sleep habits, drug habits, and psychological problems	Difficulties falling asleep or staying asleep, or sleeping too much, last 2 weeks	integer	0, 3, 8, 14 days the last two weeks
DIFFATM	Food and nutrition	Essential oils by diffusion into the atmosphere	integer	0, 1, 5, 10, 30 times per month
DINER	Food and nutrition	Eats dinner	integer	0 = always, 1 = not always, 2 = never
DORDIF	Sleep habits, drug habits, and psychological problems	Does the subject often find it difficult to fall asleep or to remain asleep?	integer	0,1,2,3 = Never, Sometimes, occasionally, most of the time, all the time
DORH.T1	Sleep habits, drug habits, and psychological problems	Hours of sleep in decimal	double	hours/day
DORLUM	Sleep habits, drug habits, and psychological problems	On average, how much light enters the subject’s bedroom while he/she is asleep?	integer	0,1,2 = Never, Sometimes, occasionally, most of the time, all the time

EMPLOIP	Socio-professional information	Category of unemployment	categorical	1=Student; 2=Looking for first job; 3=Unemployed; 4=Housewife/husband; 5=Retired; NA=missing data; NaN=not applicable
EMPLOIS	Socio-professional information	Steady job	binary	0=No; 1=Yes
EOS.V0	Laboratory measure	Hematology: Eosinophils	double	G/L
FASTF	Food and nutrition	Fast-food restaurants	integer	0, 1, 5, 20 times per month
FATI	Sleep habits, drug habits, and psychological problems	Feeling tired or having little energy, last 2 weeks	integer	0, 3, 8, 14 days the last two weeks
FCRUS	Food and nutrition	Raw fruit	integer	0, 1, 5, 10, 30, 60 times per month
FCUITS	Food and nutrition	Cooked fruit (stewed fruit etc.)	integer	0, 1, 5, 10, 30, 60 times per month
FC.V0	Basic physiological measurements	Heart rate (bpm) Visit 1	double	bpm
FECUL	Food and nutrition	Starchy foods (pasta, rice, potatoes etc.)	integer	0, 1, 5, 10, 30, 60 times per month
FRITS	Food and nutrition	Fried products (chips, crisps, doughnuts, nuggets, cordon bleu, etc.) or pasties/pies	integer	0, 1, 5, 10, 30, 60 times per month
FROM	Food and nutrition	Cheese	integer	0, 1, 5, 10, 30, 60 times per month
FSH.V0	Laboratory measure	Biochemistry: Follicle stimulating hormone	double	U/L
GFR.V0	Laboratory measure	Biochemistry: Glomerular filtration rate	double	mL/min/1.73m2

GGT.V0	Laboratory measure	Biochemistry: Gamma GT	double	IU/L
GLUC.V0	Laboratory measure	Biochemistry: Fasting glycaemia	double	mmol/L
GRIGN	Food and nutrition	Nibble between meals	categorical	0 = never, 1 = sometimes, 2 = often
GRIPPE.V1	Laboratory measure	Serology: Influenza	binary	0=negative; 1=positive
GROSS	Visit scheme	Pregnancy urine test carried out	binary	0=no; 1=yes
HASCHICH	Sleep habits, drug habits, and psychological problems	Hashish	integer	0 = never, 1 = rarely, 2 = regularly
HCT.V0	Laboratory measure	Hematology: Hematocrit	double	RATIO
HDL.V0	Laboratory measure	Biochemistry: HDL	double	mmol/L
HEIGHT.V0	Basic physiological measurements	Height (cm) Visit 1	double	cm
HGB.V0	Laboratory measure	Hematology: Hemoglobin	double	g/dL
HYPERT	Family medical history	Arterial hypertension	binary	0=No; 1=Yes
HYPFIL	Family medical history	Relationship to Arterial hypertension	categorical	0=no relationship; 1=Father; 2=Mother; 3=Brother; 4=Sister; 5=Father & Mother; 6=Father & Brother; 7=Father & Sister; 8=Mother & Brother; 9=Mother & Sister; 10=Father & Mother & Sister
IGA.V1	Laboratory measure	Immunology: IgA	double	g/l
IGE.V1	Laboratory measure	Immunology: IgE	double	UI/ml
IGG.V1	Laboratory measure	Immunology: IgG	double	g/l
IGM.V1	Laboratory measure	Immunology: IgM	double	g/l

INACT	Socio-professional information	Time without professional activity, if unemployed or retired	categorical	0=Never worked; 1=0-1 year; 2=1-3 years; 3=3 and more years; NA=missing data
INFARC	Family medical history	Myocardial infarction	binary	0=No; 1=Yes
INFFIL	Family medical history	Relationship to Myocardial infarction	categorical	0=no relationship; 1=Father; 2=Mother; 3=Brother; 5=Father & Mother; 6=Father & Brother
K.V0	Laboratory measure	Biochemistry: Potassium	double	mmol/L
LAIT	Food and nutrition	Dairy products (milk, yoghurt etc.)	integer	0, 1, 5, 10, 30, 60 times per month
LDL.V0	Laboratory measure	Biochemistry: LDL	double	mmol/L
LEGC	Food and nutrition	Cooked vegetables	integer	0, 1, 5, 10, 30, 60 times per month
LEGS	Food and nutrition	Dried pulses (lentils, chickpeas, split peas etc.)	integer	0, 1, 5, 10, 30, 60 times per month
LOG	Demographics	Does the subject own his/her housing?	binary	1=landlord; 2=leaser
LYM.V0	Laboratory measure	Hematology: Lymphocytes	double	G/L
MATIN	Food and nutrition	Eat only in the morning	binary	0 = no, 1 = yes
MCHC.V0	Laboratory measure	Hematology: Mean corpuscular hemoglobin concentration	double	g/L
MCH.V0	Laboratory measure	Hematology: Mean corpuscular hemoglobin	double	pg
MCV.V0	Laboratory measure	Hematology: Mean corpuscular volume	double	µm3

MEDACT.LOCALCON	Comcomitant drug treatment	Subject takes a drug treatment whose action is: contraception (local)	binary	0=no; 1=yes
MEDACT.ORALCONT	Comcomitant drug treatment	Subject takes a drug treatment whose action is: oral contraception	binary	0=no; 1=yes
MEDCAT	Comcomitant drug treatment	General category of drug treatment(s)	categorical	1=female sex hormons; 2=cardiovascular treatment; 3=thyroid hormon replacement; 4=Miscellaneous
MEDIND.CONTRACE	Comcomitant drug treatment	Subject takes a drug treatment whose indication is: Contraception	binary	0=no; 1=yes
METABOSCORE	Laboratory measure	Metabolic score, estimated as described in Thomas et al., Clin Immunol 2015	integer	Number of risk factors for the Metabolic Syndrome, ranging from 0 to 5
MHCAT10.MHTESTYN2	Medical history	Cat:10: Tonsillectomy	binary	0=No; 1=Yes
MHCAT10.MHTESTYN3	Medical history	Cat:10: Appendicectomy	binary	0=No; 1=Yes
MHCAT10.MHTESTYN9	Medical history	Cat:10: Other	binary	0=No; 1=Yes
MHCAT10.ORTHO	Medical history	Cat:10: Orthopedic and maxillofacial surgery	binary	0=No; 1=Yes
MHCAT10.REPRO	Medical history	Cat:10: Reproductive system surgery	categorical	0=No; 1=Yes; 2=Yes, significant surgery (ovariectomy, ectopic testis)
MHCAT10.TEETH	Medical history	Cat:10: Teeth extraction	binary	0=No; 1=Yes
MHCAT10.VASC	Medical history	Cat:10: Vascular system surgery	categorical	0=No; 1=Yes

MHCAT10.VISC	Medical history	Cat:10: Visceral system surgery	categorical	0=No; 1=Yes; 2=Yes, significant surgery (cholecystectomy, thyroidectomy)
MHCAT11.MHTESTYN1	Medical history	Cat:11: Measles	binary	0=No; 1=Yes
MHCAT11.MHTESTYN3	Medical history	Cat:11 :Hepatitis B	binary	0=No; 1=Yes
MHCAT11.MHTESTYN5	Medical history	Cat:11: Flu	binary	0=No; 1=Yes
MHCAT8.MHTESTYN1	Medical history	Cat:8: Measles	binary	0=No; 1=Yes, antecedent
MHCAT8.MHTESTYN2	Medical history	Cat:8: Rubella	binary	0=No; 1=Yes, antecedent
MHCAT8.MHTESTYN3	Medical history	Cat:8: Chicken pox	categorical	0=No; 1=Yes, antecedent; 2=Yes, current w/o treatment
MHCAT8.MHTESTYN4	Medical history	Cat:8: Mumps	binary	0=No; 1=Yes, antecedent
MHCAT8.MHTESTYN9	Medical history	Cat:8: Other	binary	0=No; 1=Yes, antecedent
MHCATYN10	Medical history	Surgical interventions (Cat:10)	binary	0=No; 1=Yes
MHCATYN8	Medical history	Childhood diseases (Cat:8)	binary	0=No; 1=Yes
MIN	Food and nutrition	Minerals only	integer	0, 1, 5, 10, 30 times per month
MONO.V0	Laboratory measure	Hematology: Monocytes	double	G/L
MULTI	Food and nutrition	Multiminerals and multivitamins	integer	0, 1, 5, 10, 30 times per month
MUMPS	Vaccination history	Vaccination against mumps	binary	0=No; 1=Yes
NAISSP	Personal medical history at birth	Weight at birth	double	kg
NAISST	Personal medical history at birth	Length at birth	double	cm
NBYLPSEXP	Smoking habits	Number of years since last secondhand smoking exposure	integer	Years
NBYLTABAC	Smoking habits	Number of years since last smoke	integer	Years

NBYPSEXP	Smoking habits	Number of years exposed to secondhand smoking	integer	Years
NBYTABAC	Smoking habits	Number of years smoking	integer	Years
NCOLL	Food and nutrition	Eats no snacks	binary	0 = no, 1 = yes
NEUT.V0	Laboratory measure	Hematology: Neutrophils	double	G/L
NIVETUD	Socio-professional information	Level of education	categorical	1=No diploma; 2=Primary school certificate; 3=CAP, BEP, Brevet de colleges; 4=Baccalaureat; 5=Higher education, cycle 1 (DUT, BTS, DEUG, L2); 6=Higher education, cycle 2 and 3 (L3, M1, M2, PhD)
NVILLES	Geographic origin	Number of place(s) of residence (French department) before the age of 13	integer	places
OEUFS	Food and nutrition	Eggs	integer	0, 1, 5, 10, 30, 60 times per month
PAIN	Food and nutrition	Bread (one baguette ~250 g)	integer	400, 250, 125, 50, grams of bread per day
PDEJ	Food and nutrition	Eats breakfast	integer	0 = always, 1 = not always, 2 = never
PERCEP	Sleep habits, drug habits, and psychological problems	Poor self-image, or you think that you are a loser or have not achieved your own expectations or those of your family, last 2 weeks	integer	0, 3, 8, 14 days the last two weeks
PHOS.V0	Laboratory measure	Biochemistry: Phosphate	double	mmol/L

PHYSDUR	Demographics	Duration of professional physical activity	categorical	1=1 to 20 minutes per day; 2=21 to 60 minutes per day; 3=1 to 2 hours per day; 4=More than 2 hours per day
PHYSDUR.T1	Demographics	Duration of professional physical activity	double	proportion of 8h working day
PHYSJ	Demographics	Days per week of physical activity during leisure	integer	days per week
PHYSL	Demographics	Physical activity during leisure	integer	1=Little or no physical activity; 2=Moderate physical activity; 3=Intense physical activity
PHYSP	Demographics	Professional physical activity	integer	1=none; 2=moderate; 3=considerable
PHYST	Demographics	Hours per day of physical activity during leisure	double	hours per day
PLAIS	Sleep habits, drug habits, and psychological problems	Lack of interest or pleasure in doing things, last 2 weeks	integer	0, 3, 8, 14 days the last two weeks
PLATC	Food and nutrition	Ready meals	integer	0, 1, 5, 10, 30, 60 times per month
PLAT.V0	Laboratory measure	Hematology: Platelets	double	G/L
PLVTSEDT.V1.T1	Visit scheme	Hour at which stool sample was taken	double	hours
PLVTSEYN.V1.T1	Visit scheme	Date at which stool sample was taken, in days since 09-01-2012	integer	days
POISSON	Food and nutrition	Fish	integer	0, 1, 5, 10, 30, 60 times per month

POUSS	Socio-professional information	Exposure to dust	categorical	0=No exposure; 1=Past exposure; 2=Current exposure; NA=Unknown
PRODALL1	Food and nutrition	Uses reduced sugar products	binary	0 = no, 1 = yes
PRODALL2	Food and nutrition	Uses reduced fat products	binary	0 = no, 1 = yes
PRODALL3	Food and nutrition	Uses reduced salt products	binary	0 = no, 1 = yes
PRODALL4	Food and nutrition	Uses light products	binary	0 = no, 1 = yes
PROT.V0	Laboratory measure	Biochemistry: Total proteins	double	g/L
PSEXP	Smoking habits	Secondhand smoking	categorical	0 = never been exposed, 1 = exposed in past, 2 = currently exposed
PTOX	Socio-professional information	Exposure to toxic products	categorical	0=No exposure; 1=Past exposure; 2=Current exposure; NA=Unknown
RBC.V0	Laboratory measure	Hematology: Erythrocytes	double	T/L
REPASH	Food and nutrition	Do you have regular mealtimes during workdays	binary	0 = have regular mealtimes, 1 = no
RESTAU	Food and nutrition	Restaurants other than work, or at friends' houses	integer	1, 5, 20, times per month
REVENUS	Socio-professional information	Net monthly income of the household (EUR)	integer	1=0-1000€; 2=1001-2000€; 3=2001-3000€; 4=3001-4000€; 5=4001-5000€; 6=5001€ and more; NA=missing data
RUBELLA	Vaccination history	Vaccination against rubella	binary	0=No; 1=Yes; NA=missing data
SEL	Food and nutrition	Salt consumption habits	binary	1 = doesn't salt food, 2 often salts food
SEX	Demographics	Sex	binary	1=male; 2=female

SODAS	Food and nutrition	Sodas and other sugary drinks (other than diet drinks low in sugar)	integer	0, 1, 5, 10, 30, 60 times per month
SODIUM.V0	Laboratory measure	Biochemistry: Sodium	double	mmol/L
SOIREE	Food and nutrition	Eat only during the evening or night	binary	0 = no, 1 = yes
STRESS	Sleep habits, drug habits, and psychological problems	Major Negative Life event, loss of loved one etc., last 12 month	binary	0 = no, 1 = yes
SUCR	Food and nutrition	Sweet things (chocolate, sweets, honey, jam etc.)	integer	0, 1, 5, 10, 30, 60 times per month
SYSBP1.V0	Basic physiological measurements	Systolic measure 1 (mmHg) Visit 1	double	mmHg
SYSBP2.V0	Basic physiological measurements	Systolic measure 2 (mmHg) Visit 1	double	mmHg
TABAC.T1	Smoking habits	Smoking tobacco?	categorical	Non-smoker = 0, Ex-Smoker = 1, Smoker = 2
TEMP.V0	Basic physiological measurements	Ear temperature (°C) Visit 1	double	°C
TOTNROFCIGS	Smoking habits	Total number of cigarettes smoked	integer	Cigarettes
TRAVJ	Socio-professional information	Working time	categorical	1=Exclusively during the day; 2=Exclusively during the night; 3=Without fixed hours; NA=missing data; NaN=not applicable
TRAVT	Socio-professional information	Type of employment	binary	0=Part time; 1=Full time
TRIG.V0	Laboratory measure	Biochemistry: Triglycerides	double	mmol/L

TRIS	Sleep habits, drug habits, and psychological problems	Feeling sad, depressed, or despairing, last 2 weeks	integer	0, 3, 8, 14 days the last two weeks
URATE.V0	Laboratory measure	Biochemistry: Uric Acid	double	µmol/L
UREA.V0	Laboratory measure	Biochemistry: Urea	double	mmol/L
VIANDE	Food and nutrition	Meat	integer	0, 1, 5, 10, 30, 60 times per month
VIENN	Food and nutrition	Pastries and sweet breads (croissants, brioches, pains au chocolat, etc.)	integer	0, 1, 5, 10, 30, 60 times per month
VILLENPOP	Geographic origin	Number of inhabitants in the place of birth	integer	inhabitants
VILLENPOP20	Geographic origin	Number of inhabitants in the place of birth larger than 20,000	binary	0=no; 1=yes
VILLESPOP	Geographic origin	Average number of inhabitants in the place(s) of residence before the age of 13	double	inhabitants
VILLESPOP20	Geographic origin	Number of inhabitants in at least one place of residence before the age of 13 larger than 20,000	binary	0=no; 1=yes
VIN	Food and nutrition	Glasses of wine per week	integer	glasses/week
VISIT2	Visit scheme	Presence at follow-up visit	binary	0=not done; 1=done
VIT	Demographics	Subject shares housing with...	categorical	1=Alone with no children; 2=Alone with children; 3=With a partner but no children; 4=With a partner and children
VITA	Food and nutrition	Vitamins only	integer	0, 1, 5, 10, 30 times per month

VIT.COUPLE	Demographics	Subject shares housing with his/her partner	binary	0=no; 1=yes
VIT.ENFANTS	Demographics	Subject shares housing with his/her children	binary	0=no; 1=yes
VOIE	Personal medical history at birth	Route of delivery	binary	0=Vaginal delivery; 1=Caesarean section
VORALE	Food and nutrition	Essential oils by oral route	integer	0, 1, 5, 10, 30 times per month
WBC.V0	Laboratory measure	Hematology: Leucocytes	double	G/L
WEIGHT.V0	Basic physiological measurements	Weight (kg) Visit 1	double	kg
WHOOPING.COUGH	Vaccination history	Vaccination against whooping cough	binary	0=no; 1=yes
YELLOW.FEVER	Vaccination history	Vaccination against yellow fever	binary	0=no; 1=yes
YEUX	Biometrics	Eye color	integer	1=Gray; 2=Blue; 3=Green/Hazel green; 4=Hazel brown; 5=Light brown; 6=Dark brown; 7=Other

Supplementary Table 2: List of the bacterial strains whose supernatant was tested against *C. albicans*. *Bifidobacterium adolescentis* was used as an inhibition control.

Strain	Species
DSM26588	*Intestinimonas butyriciproducens*
DSM17679	*Bacteroides massiliensis*
DSM103636	*Bacteroides ndongoniae*
DSM28864	*Coprobacter secundus*
DSM25476	*Enorma massiliensis*
DSM23940	*Pseudoflavonifractor capillosus*
IL14-03	*Lactococcus lactis*
DSM14610	*Roseburia intestinalis*
L2-32	*Bifidobacterium adolescentis*

D - DISCUSSION AND PERSPECTIVES

C. albicans pathogenicity has been, and still is, extensively studied. However, less attention has been paid to the factors that allow this yeast to colonize the human body as a commensal. As one of the most prevalent yeast of the healthy human gut, the ability of *C. albicans* to colonize and maintain itself in the intestinal environment depends strongly on its interactions with the host and the microbiota.

Thorough my PhD, I explored the impact of hundreds of host factors on *C. albicans* gastrointestinal colonization, including bacterial microbiota composition, lifestyle and diet factors, and the host immune and genetic profile. To do so, I had the chance to access two remarkable cohorts of healthy individuals: the CEREMI (Burdet, Nguyen, *et al.* 2019; Burdet, Grall, *et al.* 2019) and Milieu Intérieur (Thomas *et al.* 2015) cohorts. Since the second and third chapters of this thesis have been discussed individually, this section will focus on the overall impact and perspective of each main result.

Characterization of the mycobiota of healthy individuals

In this thesis, I characterized the mycobiota of the CEREMI subjects and of a subset of Milieu Intérieur subjects.

There was an overlap between the main fungal species identified between the two cohorts, with *Saccharomyces cerevisiae, Penicillium roqueforti* and *Geotrichum candidum* (formerly *Galactomyces candidus*) and *C. albicans* being identified in most of the subjects of both cohorts. However, I still observed major differences in term of mycobiota composition between the subjects from the two cohorts. This might be explained by the different sequencing and analyses approaches used in the two studies. For the CEREMI cohort, I used ITS1-targeted metagenomic sequencing while I used ITS2-targeted metagenomic sequencing for the Milieu Intérieur cohort. Since it is known that the choice of the sequencing region influences strongly on the fungal species recovered (Frau *et al.* 2019; Hoggard *et al.* 2018; Thielemann *et al.* 2022; Bellemain *et al.* 2010), the differences we observed are not surprising. Moreover, when I started my thesis, I used SHAMAN to analyze the sequencing data (Volant *et al.* 2020). Although efficient, SHAMAN does not allow fine-tuning since the user cannot modify most of the pipeline. Therefore, for the Milieu Intérieur cohort, I analyzed the sequences with Qiime2 (Bolyen *et al.* 2019). One major difference between the two analyses is that SHAMAN compute fungal OTUs while Qiime2 allows the generation

of ASVs. Since an ASV approach identify unique, exact fungal sequences whereas OTUs are a cluster of similar sequences – which, although genetically similar, might not even belong to the same biological species – ASV approach returns more accurate results (Callahan, McMurdie, and Holmes 2017), which might explain the higher α-diversity and number of fungal species I identified in the Milieu Intérieur subjects.

Overall, both approaches highlighted the high variability of the healthy gut mycobiota. It is likely that this variation originates from the fact that most fungi are probably not true colonizers of the human gut, but rather transient species brought by food and environment. This hypothesis could be further explored by determining how the Milieu Intérieur subject's diet and lifestyle can modulate the composition and diversity of the healthy gut mycobiota.

C. albicans carriage in healthy individuals

A central step of this PhD was to assess *C. albicans* carriage in the 22 volunteers of the CEREMI cohort and the 695 volunteers of the Milieu Intérieur cohort, since almost all presented results depend on this quantification. Using a qPCR assay, I showed that *C. albicans* was present in varying quantity in the gut of 95% of the CEREMI subjects and 82.9% of the Milieu Intérieur subjects. These values are far above what has been previously reported in previous studies that used more traditional assays such as culturomics or ITS-targeted metagenomics (Bougnoux *et al.* 2006; Nash *et al.* 2017; da Silva Dantas *et al.* 2016). This is not surprising since most of the subjects in both cohorts displayed relatively low amount of *C. albicans* carriage, thus highlighting the importance of using highly sensitive methods, such as qPCR, to reliably quantify *C. albicans* carriage.

One could wonder about the differences in the prevalence of *C. albicans* colonization between the two cohorts; the subjects from CEREMI being more often colonized that those of Milieu Intérieur. For each of the CEREMI subjects, I quantified *C. albicans* carriage in up to three fecal samples collected before the antibiotic treatment and considered a subject as colonized if *C. albicans* DNA was detected in at least one of these samples. In contrast, I had access to a single fecal sample for each Milieu Intérieur subject. Therefore, in the Milieu Intérieur cohort, samples with *C. albicans* DNA levels close to the qPCR detection threshold might have been seen as *C. albicans*-negative. However, having several fecal samples per volunteers, as in the

CEREMI cohort, increases the probability to have at least one sample of a low colonized subject reaching the qPCR detection threshold. This limit brought by the detection threshold of the qPCR, in addition to the low efficiency of the DNA extraction protocol we used, might have led us to underestimate *C. albicans* intestinal carriage in the subjects of the Milieu Intérieur cohort. However, a qPCR quantification still detects more sensitively *C. albicans* in fecal samples than culture or sequencing approaches.

Overall, these results indicate that *C. albicans* intestinal colonization is highly common. *C. albicans* might thus not be a facultative commensal, as previously thought, but it is instead able to maintain itself at very low levels in most individuals. This is of particular concern since it suggests that most of the population is susceptible to develop an invasive infection if subjected to immunosuppression and/or other risk factors.

Impact of an antibiotic-induced microbiota dysbiosis on *C. albicans* intestinal carriage in healthy individuals.

In the second chapter of this thesis, we followed prospectively healthy volunteers before, during and after a β-lactam treatment by 3GC and investigated the effects of antibiotic exposure on *C. albicans* overgrowth. I showed that *C. albicans* levels were significantly increased after antibiotic treatment, but with wide variations between subjects, which did not depend on the type of 3GC the subjects received. Part of these variations could be explained by the change of endogenous fecal β-lactamase activity, which thus emerges as a new potential key factor regulating *C. albicans* proliferation. This regulation is likely due to differences in the abundance of β-lactamase-producing bacteria in the microbiota of the subjects, resulting in a differential impact of the 3GC on the endogenous gut bacteria. A microbiota rich in β-lactamase-producing bacteria would thus favor 3GC hydrolysis, resulting in a reduction in antibiotic-induced microbiota dysbiosis and a reduction in *C. albicans* proliferation. These results support the hypothesis that specific bacterial species or metabolites are able to control *C. albicans* growth in the gut and may be responsible of the so-called host colonization resistance to *C. albicans* (Seelbinder *et al.* 2020; d'Enfert *et al.* 2021; Fan *et al.* 2015; Mirhakkak *et al.* 2021; Leonardi *et al.* 2020).

Our results demonstrate that, depending on the subject receiving it, treatment with the same antibiotic may differentially affect the gut microbiota, which in turn would lead to different degrees of *C. albicans* overgrowth. This challenges the current paradigm

stating that antibiotics are systematically a risk factor for *C. albicans* proliferation, and it should therefore be adjusted for antibiotic treatments based on β-lactams. Instead, monitoring fecal β-lactamase activity during and after a β-lactam antibiotic therapy could be an accurate predictor of the actual risk of subsequent increase of *C. albicans* burden in ICU patients.

Identification of bacterial species with a potential antagonistic activity against *C. albicans*

In the second and third chapters of this thesis, I identified bacterial species whose abundance was negatively correlated with the extent of *C. albicans* carriage. In the subjects of the CEREMI cohort, I identified 54 bacterial species with a potential anti-*C. albicans* activity, thus much more than in the Milieu Intérieur subjects, in whom *Intestinimonas butyriciproducens,* a SCFA-producing bacterium, was the only identified species negatively associated with *C. albicans.* This difference in the number of bacterial species highlighted in both cohorts could be explained by the fact that the CEREMI subjects were treated with antibiotics, and thus harbored a depleted microbiota after the treatment. Therefore, some of the negative associations observed between bacteria and *C. albicans* could result from false-positive signals, due to the overall decrease of bacterial abundance and overall increase of *C. albicans* carriage observed after antibiotics. Moreover, in Milieu Intérieur, due to the large number of volunteers, I was able to use mixed and linear models, such as MaAsLin2 (Mallick *et al.* 2021), to search for associations, while I used Spearman correlation to search for bacteria of interest in the CEREMI subjects. These models, that take into account subject age and gender are likely to yield more accurate results than conventional rank correlations, for which such random effects cannot be computed.

Interestingly, *I. butyriciproducens* was identified as a potential anti-*C. albicans* signature not only in the Milieu Intérieur cohort, but also in the CEREMI cohort. Considering the generally low overlap in the identification of microbial species with potential antagonistic activity against *C. albicans* between studies, or even between cohorts (see *Review: Finding microbiome-based antifungal strategies: impact of OMICs approaches*), these associations may actually reflect the ability of this bacteria to control *C. albicans* growth in the human gut. Although I could not demonstrate any effect of the supernatant of this bacterium on the growth or morphology of *C. albicans*,

this species could limit the growth of *C. albicans* by another mechanism, such as modulating the host immune response (Rizzo, Losacco, and Carratelli 2013; Nguyen *et al.* 2011; Fan *et al.* 2015) or through competition for niches, adhesion sites and/or nutrients (Alonso-Roman *et al.* 2022; Mailänder-Sánchez *et al.* 2017; Basson 2000). Moreover, inhibition of *C. albicans* by *I. butyriciproducens,* could be strain specific, as it is has been reported for *B. adolescentis* (Ricci *et al.* 2022).

Therefore, the potential antagonistic activity of *I. butyriciproducens* should be further explored, using more complex *in vitro* and *ex vivo* experimental platforms, as for instance fermentation-based systems such as the SHIME® (Van Den Abbeele *et al.* 2010; Marzorati *et al.* 2014), or organs-on-a-chip, or using *in vivo* models such as murine models of *C. albicans* colonization. This could therefore pave the way for the development of new live biotherapeutic products that could curb the proliferation of *C. albicans*, even before the emergence of a superficial or invasive infection.

Impact of the host diet on *C. albicans* intestinal carriage

In the third chapter of this thesis, I investigated the associations of 201 medical, environmental, and diet-associated variables with the extent of *C. albicans* intestinal carriage in the Milieu Intérieur healthy volunteers. I showed that host diet contributed to the growth of *C. albicans* in the human gut. Indeed, subjects who reported a low sodium diet and those who reported frequent snacking between meals were characterized, in average, by a higher intestinal carriage of *C. albicans*. This is not surprising since diet is known to strongly modulate the fungal composition of the gut microbiota (Szóstak *et al.* 2023; Motooka *et al.* 2017; Wu *et al.* 2011; Angebault *et al.* 2013). Moreover, several diet-related factors have been shown to affect the composition of the gut bacterial microbiota of the Milieu Intérieur subjects (Partula *et al.* 2019). However, in the latter study, the impact of salt consumption and snacking on the gut microbiota composition has not been investigated (Partula *et al.* 2019).

The highlighted association between the subjects' snacking habits and increased intestinal carriage of *C. albicans* is therefore easily understandable since snacking is often associated with the consumption of processed, "unhealthy" high-sugar and high-fat foods, foods that have been associated with higher carriage of *C. albicans* (Szóstak *et al.* 2023). By contrast, the association of a high-sodium diet with reduced *C. albicans* carriage might seem more coincidental. However, high salt intakes have previously

been linked to an increased Th17/IL-17 immune response that significantly affected the composition of the gut microbiota (Wilck *et al.* 2017). Therefore, *C. albicans* growth could be more efficiently limited in subjects consuming higher levels of salt, either through a modification of the gut microbiota that could, in turn, affects *C. albicans* growth, or directly through the host immune response.

The relative low number of diet-related variables we identified in this study is questionable. In particular, I did not find any significant associations between the extent of *C. albicans* intestinal levels and consumption of sugary and/or fatty foods although these factors have been previously shown to increase *C. albicans* intestinal carriage (Szóstak *et al.* 2023; Motooka *et al.* 2017; G. D. Wu *et al.* 2011). This could be explained by the relatively low consumption of this type of food by the subjects of the studied cohort. Indeed, only 7% of the Milieu Intérieur subjects reported eating sweets such as chocolate, honey or jam, at least once daily and 88.5% of the studied subjects reported eating fried foods such as potato chips or French fries less than once a week. This also raises questions about the limitations of studies based on self-reported information. Indeed, it has been shown previously that, when asked about their habits, people tend to overestimate their healthy behaviours (such as fruits and vegetable consumption, physical activities, etc.) and underestimate their less desirable habits (such as consumption of “unhealthy” food, alcohol, drugs, etc.), and this consciously, or not (Mossavar-Rahmani *et al.* 2013; Teh *et al.* 2023; Adams *et al.* 2005). This so-called social desirability bias (Crowne and Marlowe 1960) must therefore be considered when analysing these data.

However, although the relative importance of the diet-related associations identified still need to be evaluated in another cohort, these results provide insight into how host diet may be involved in *C. albicans* growth. Diet could thus be considered to limit the risk of *C. albicans* proliferation in at risk patients.

Identification of a *C. albicans* intestinal colonization susceptibility locus by Genome-Wide Association Studies

In the third chapter of this thesis, we investigated the impact of genetic variability of the Milieu Intérieur subjects on their susceptibility to *C. albicans* intestinal colonization. Using GWAS, I identified 26 SNPs, located in two independent loci that were associated with *C. albicans* gut colonization. In particular, rs2870723, a SNP located

in the chromosome 20, was most strongly associated with the host susceptibility to *C. albicans* colonization. Rs2870723 is located between *RNA5SP487,* an RNA 5S pseudogene that has not been widely described, and *MC3R,* a gene that encodes for a melanocortin receptor (Koya *et al.* 2018).

In GWAS, we must consider Gene Linkage Disequilibrium. This phenomenon, which refers to the nonrandom association of alleles from different genes, resulting from their proximity on a chromosome and the resulting co-heritance, explains that alleles of close genes are more likely to be transmitted together than alleles of distant genes. Considering this, it might be interesting to further explore the genes closest to rs2870723. Indeed, in this study, we tested about 5 million SNPs, which is only a fraction of the total number of existing SNPs. Moreover, the SNPs on microarray typically used for genotyping in GWAS studies are usually selected because they exhibit high linkage disequilibrium with neighboring SNPs, thus serving as surrogates for the genomic regions that contain unmeasured SNPs (Stram 2004b; Ding and Kullo 2007; Schaid, Chen, and Larson 2018a). Therefore, we further examined *MC3R,* to see if the SNPs we identified could result from an association with this gene.

Mutations in *MC3R* have been associated with obesity in genetics studies, including GWAS (Koya *et al.* 2018; Mencarelli *et al.* 2011; Zegers *et al.* 2011), and have been confirmed by murine models (Lee *et al.* 2016). Therefore, this genetic association with *C. albicans* colonization could be the consequence of an indirect interaction with the weight of subjects, especially since overweight people tend to carry higher levels of *C. albicans* in their gut (García-Gamboa *et al.* 2021). However, in this study, I did not identify any association between *C. albicans* colonization, or carriage, and subject weight and we might thus consider a more direct role of the *MC3R* locus.

The melanocortin receptor encoded by *MC3R* is expressed in various tissues, including the gut, and has for agonist several hormones including α-MSH. This hormone is a potent anti-inflammatory peptide that interacts with intestinal epithelial cells (Váradi *et al.* 2017) and has been shown to significantly reduce *C. albicans* growth and hyphae formation *in vitro* (Grieco *et al.* 2003; Cutuli *et al.* 2000). Therefore, this genetic association between this locus and *C. albicans* colonization could be related to the anti-*C. albicans* activity of MC3R activating hormone.

Besides, specific genetic polymorphisms in *MC3R* have also been associated with an increased susceptibility to tuberculosis, which has been explained by a differential mediation of the host inflammatory response (P. Xu *et al.* 2020; Park *et al.* 2014; Hashemi *et al.* 2013). It is therefore possible that the SNPs we identified as associated with *C. albicans* colonization are also associated with a differentiated host inflammatory and/or inhibitory response against *C. albicans*.

These interactions with neighboring genes such as *MC3R* are not unlikely if we consider that a SNP in the *AUKRA* gene, another gene close to the identified SNPs, has been associated with the mean corpuscular hemoglobin concentration in previous GWAS studies (Sollis *et al.* 2023; Chen *et al.* 2020); a variable that I found to be associated with *C. albicans* intestinal carriage and host susceptibility to colonization.

Overall, our results suggest that *C. albicans* colonization may be determined, at least in part, by the host genetics. However, the locus we identified does not seem to be involved in the extent of *C. albicans* carriage, in colonized subjects, since we did not observe any significant differences in intestinal *C. albicans* levels based on the subjects rs2870723 genotypes. Considering that *C. albicans* primo-colonization is most likely occurring at birth, during vaginal delivery, or in the perinatal period, also through breastfeeding (Azevedo *et al.* 2023; Bliss *et al.* 2008; Bougnoux *et al.* 2006; d'Enfert 2009; Boix-Amorós *et al.* 2017), it is possible that our results do not reflect a genetic association, but rather a maternal association, with the genetics playing the role of a confounding variable. Indeed, children from uncolonized mothers share 50% of their genetic material and are less likely to be colonized by *C. albicans*. The SNPs identified therefore need to be validated in replication cohorts that would, ideally, follow families.

Impact of *C. albicans* intestinal levels on the host immune response upon a simulated *C. albicans* infection.

Finally, I showed that high intestinal levels of *C. albicans* resulted in a higher expression of *NLRP3* and in higher IL-2 and CXCL5 levels upon a simulated *C. albicans* blood infection. NLRP3 inflammasome plays a crucial role in the clearance of *C. albicans* by initiating a pro-inflammatory response (Vonk *et al.* 2006; Hise *et al.* 2009), while CXCL5 drives neutrophils recruitment in the Th17/IL-17 immune response

(Disteldorf *et al.* 2015; Conti and Gaffen 2015). In contrast, IL-2 activates the Treg response, therefore

+ regulating the inflammation (Leigh *et al.* 1998; Richardson and Moyes 2015).

Intestinal *C. albicans* levels were not associated with either *NLRP3* expression or IL-2 and CXCL5 levels when the subjects' blood was stimulated with other pathogens, including *Escherichia coli, Staphylococcus aureus,* BCG and influenza A virus. This suggests that this differential immune response in the subjects heavily colonized with *C. albicans* occurred specifically in response to *C. albicans* stimulation.

As this part of the study was strictly descriptive and based on statistical analyses, the effects of a higher *C. albicans* carriage on the host immune system need to be confirmed, for example, using murine models. We could thus colonize the GI tract of mice with different levels of *C. albicans* and quantify the expression of *NLRP3* and the levels of IL-2 and CXCL5 in their blood, after a challenge with *C. albicans* cells, either *in vitro* or *in vivo.* If confirmed, this would suggest that high intestinal carriage of *C. albicans* induces a stronger immune response when the host is infected with this yeast. This phenomenon of trained immunity would thus be consistent with reports stating that previous challenges with fungal particles, such as β-glucans, lead to an increased survival of mice upon a fungal infection (Quintin *et al.* 2012) and that intestinal colonization of *C. albicans* confers protection against systemic infections in mice (Huertas *et al.* 2017).

Throughout this thesis, I had the opportunity to explore the influence of hundreds of variables, including lifestyle factors such as the microbiota composition, diet, medical history, and antibiotics use, but also the host genetic and immune profile on *C. albicans* intestinal carriage in healthy subjects. Therefore, although the relative relevance of some of the associations I identified has yet to be determined, this work offers a better understanding of the mechanisms of *C. albicans* colonization in the healthy host and could pave the way for the development of new intervention strategies to curb *C. albicans* growth. Overall, each factor I identified contributes in a relatively limited way to the overall host susceptibility to *C. albicans* intestinal colonization. This highlights the complexity of the gut system, where there is a multitude of interactions between *Candida albicans*, the microbiota and the host, leading to the maintenance or, on the contrary, to the clearance of the fungus.

This work was made possible because I had the opportunity to have access to two independent and complementary cohorts of healthy adults. However, much remains to be explored to fully understand the factors and mechanisms underlying *C. albicans* colonization of the healthy gut. Notably, I have worked with two French cohorts so we can wonder whether analysis of cohorts of subjects from other origins would reveal different factors.

The small numbers of subjects not colonized by *C. albicans* had the unfortunate consequence of limiting the statistical analyses I was able to perform, because of a resulting lack of statistical power. Hopefully, the rise of public databases, gathering information and samples of thousands of individuals, of multiple nationalities will allow the use of more powerful computational tools, such as machine-learning, or even deep-learning.

REFERENCES

Abbeele, Pieter Van Den, Clara Belzer, Margot Goossens, Michiel Kleerebezem, Willem M. De Vos, Olivier Thas, Rosemarie De Weirdt, Frederiek Maarten Kerckhof, and Tom Van De Wiele. 2013. "Butyrate-Producing *Clostridium* Cluster XIVa Species Specifically Colonize Mucins in an in Vitro Gut Model." *ISME Journal* 7 (5): 949–61. https://doi.org/10.1038/ismej.2012.158.

Abbeele, Pieter Van Den, Charlotte Grootaert, Massimo Marzorati, Sam Possemiers, Willy Verstraete, Philippe Gérard, Sylvie Rabot, *et al.* 2010. "Microbial Community Development in a Dynamic Gut Model Is Reproducible, Colon Region Specific, and Selective for Bacteroidetes and *Clostridium* Cluster IX." *Applied and Environmental Microbiology* 76 (15): 5237–46. https://doi.org/10.1128/AEM.00759-10.

Abbeele, Pieter Van den, Norbert Sprenger, Jonas Ghyselinck, Benoît Marsaux, Massimo Marzorati, and Florence Rochat. 2021. "A Comparison of the in Vitro Effects of 2'fucosyllactose and Lactose on the Composition and Activity of Gut Microbiota from Infants and Toddlers." *Nutrients* 13 (3): 1–23. https://doi.org/10.3390/nu13030726.

Acosta-Rodriguez, Eva V., Laura Rivino, Jens Geginat, David Jarrossay, Marco Gattorno, Antonio Lanzavecchia, Federica Sallusto, and Giorgio Napolitani. 2007. "Surface Phenotype and Antigenic Specificity of Human Interleukin 17-Producing T Helper Memory Cells." *Nature Immunology* 8 (6): 639–46. https://doi.org/10.1038/ni1467.

Adams, Swann Arp, Charles E. Matthews, Cara B. Ebbeling, Charity G. Moore, Joan E. Cunningham, Jeanette Fulton, and James R. Hebert. 2005. "The Effect of Social Desirability and Social Approval on Self-Reports of Physical Activity." *American Journal of Epidemiology* 161 (4): 389–98. https://doi.org/10.1093/aje/kwi054.

Alonso-Roman, Raquel, Antonia Last, Mohammad H. Mirhakkak, Jakob L. Sprague, Lars Möller, Peter Großmann, Katja Graf, *et al.* 2022. "*Lactobacillus rhamnosus* Colonisation Antagonizes *Candida albicans* by Forcing Metabolic Adaptations That Compromise Pathogenicity." *Nature Communications* 13 (1). https://doi.org/10.1038/s41467-022-30661-5.

Amir, Itay, Philippe Bouvet, Christine Legeay, Uri Gophna, and Abraham Weinberger. 2014. "*Eisenbergiella tayi* Gen. Nov., Sp. Nov., Isolated from Human Blood." *International Journal of Systematic and Evolutionary Microbiology* 64: 907–14. https://doi.org/10.1099/IJS.0.057331-0.

Angebault, Cécile, Félix Djossou, Sophie Abélanet, Emmanuelle Permal, Mouna Ben Soltana, Laure Diancourt, Christiane Bouchier, *et al.* 2013. "*Candida albicans* Is Not Always the Preferential Yeast Colonizing Humans: A Study In Wayampi Amerindians." *Journal of Infectious Diseases* 208 (10): 1705–16. https://doi.org/10.1093/infdis/jit389.

Angebault, Cécile, Amine Ghozlane, Stevenn Volant, Françoise Botterel, Christophe D'Enfert, and Marie Elisabeth Bougnoux. 2018. "Combined Bacterial and Fungal Intestinal Microbiota Analyses: Impact of Storage Conditions and DNA Extraction Protocols." *PLoS ONE* 13 (8): e0201174. https://doi.org/10.1371/journal.pone.0201174.

Arnold, Melina, Eileen Morgan, Harriet Rumgay, Allini Mafra, Deependra Singh, Mathieu

Laversanne, Jerome Vignat, *et al.* 2022. “Current and Future Burden of Breast Cancer: Global Statistics for 2020 and 2040.” *Breast* 66 (December): 15–23. https://doi.org/10.1016/j.breast.2022.08.010.

Ashammakhi, Nureddin, Rohollah Nasiri, Natan Roberto de Barros, Peyton Tebon, Jai Thakor, Marcus Goudie, Amir Shamloo, Martin G. Martin, and Ali Khademhosseni. 2020. “Gut-on-a-Chip: Current Progress and Future Opportunities.” *Biomaterials* 255 (October): 120196. https://doi.org/10.1016/j.biomaterials.2020.120196.

Auchtung, Thomas A., Tatiana Y. Fofanova, Christopher J. Stewart, Andrea K. Nash, Matthew C. Wong, Jonathan R. Gesell, Jennifer M. Auchtung, Nadim J. Ajami, and Joseph F. Petrosino. 2018. “Investigating Colonization of the Healthy Adult Gastrointestinal Tract by Fungi.” *MSphere* 3 (2). https://doi.org/10.1128/msphere.00092-18.

Azevedo, Maria João, Ricardo Araujo, Joana Campos, Carla Campos, Ana Filipa Ferreira, Inês Falcão-Pires, Carla Ramalho, Egija Zaura, Eugénia Pinto, and Benedita Sampaio-Maia. 2023. “Vertical Transmission and Antifungal Susceptibility Profile of Yeast Isolates from the Oral Cavity, Gut, and Breastmilk of Mother–Child Pairs in Early Life.” *International Journal of Molecular Sciences* 24 (2): 1449. https://doi.org/10.3390/ijms24021449.

Babula, Oksana, Gunta Lazdane, Juta Kroiča, Iara M. Linhares, William J. Ledger, and Steven S. Witkin. 2005. “Frequency of Interleukin-4 (IL-4) -589 Gene Polymorphism and Vaginal Concentrations of IL-4, Nitric Oxide, and Mannose-Binding Lectin in Women with Recurrent Vulvovaginal Candidiasis.” *Clinical Infectious Diseases* 40 (9): 1258–62. https://doi.org/10.1086/429246.

Bacher, Petra, Thordis Hohnstein, Eva Beerbaum, Marie Röcker, Matthew G. Blango, Svenja Kaufmann, Jobst Röhmel, *et al.* 2019. “Human Anti-Fungal Th17 Immunity and Pathology Rely on Cross-Reactivity against *Candida albicans*.” *Cell* 176 (6): 1340-1355.e15. https://doi.org/10.1016/j.cell.2019.01.041.

Bäckhed, Fredrik, Hao Ding, Ting Wang, Lora V. Hooper, Young Koh Gou, Andras Nagy, Clay F. Semenkovich, and Jeffrey I. Gordon. 2004. “The Gut Microbiota as an Environmental Factor That Regulates Fat Storage.” *Proceedings of the National Academy of Sciences of the United States of America* 101 (44): 15718–23. https://doi.org/10.1073/pnas.0407076101.

Bäckhed, Fredrik, Josefine Roswall, Yangqing Peng, Qiang Feng, Huijue Jia, Petia Kovatcheva-Datchary, Yin Li, *et al.* 2015. “Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life.” *Cell Host and Microbe* 17 (5): 690–703. https://doi.org/10.1016/j.chom.2015.04.004.

Bai, Xiao Dong, Xian Hua Liu, and Qing Ying Tong. 2004. “Intestinal Colonization with *Candida albicans* and Mucosal Immunity.” *World Journal of Gastroenterology* 10 (14): 2124–26. https://doi.org/10.3748/wjg.v10.i14.2124.

Balish, Edward, Jean Jensen, and Thomas Warner. 1993. “Resistance of SCID Mice to *Candida albicans* Administered Intravenously or Colonizing the Gut: Role of Polymorphonuclear Leukocytes and Macrophages.” *Journal of Infectious Diseases* 167 (4): 912–19. https://doi.org/10.1093/infdis/167.4.912.

Basson, N. J. 2000. “Competition for Glucose between *Candida albicans* and Oral Bacteria Grown in Mixed Culture in a Chemostat.” *Journal of Medical Microbiology* 49 (11): 969–75. https://doi.org/10.1099/0022-1317-49-11-969.

Beaucoudtey, Ludovic De, Anne Puel, Orchidée Filipe-Santos, Aurélie Cobat, Pegah Ghandil, Maya Chrabieh, Jacqueline Felnberg, *et al.* 2008. “Mutations in *STAT3* and *IL12RB1* Impair the Development of Human IL-17-Producing T Cells.” *Journal of Experimental Medicine* 205 (7): 1543–50. https://doi.org/10.1084/jem.20080321.

Bellemain, Eva, Tor Carlsen, Christian Brochmann, Eric Coissac, Pierre Taberlet, and Håvard Kauserud. 2010. “ITS as an Environmental DNA Barcode for Fungi: An in Silico Approach Reveals Potential PCR Biases.” *BMC Microbiology* 10: 189. https://doi.org/10.1186/1471-2180-10-189.

Beno, D W, A G Stöver, and H L Mathews. 1995. “Growth Inhibition of *Candida albicans* Hyphae by CD8+ Lymphocytes.” *The Journal of Immunology* 154 (10): 5273–81. https://doi.org/10.4049/jimmunol.154.10.5273.

Bernard, Kathryn, Tamara Burdz, Deborah Wiebe, Brittany M. Balcewich, Tina Zimmerman, Philippe Lagacé-Wiens, Linda M.N. Hoang, and Anne Marie Bernier. 2017. “Characterization of Isolates of *Eisenbergiella tayi*, a Strictly Anaerobic Gram-Stain Variable Bacillus Recovered from Human Clinical Materials in Canada.” *Anaerobe* 44 (April): 128–32. https://doi.org/10.1016/J.ANAEROBE.2017.03.005.

Bernardis, Flavia De, Maria Boccanera, Daniela Adriani, Elisabetta Spreghini, Giorgio Santoni, and Antonio Cassone. 1997. “Protective Role of Antimannan and Anti-Aspartyl Proteinase Antibodies in an Experimental Model of *Candida albicans* Vaginitis in Rats.” *Infection and Immunity* 65 (8): 3399–3405. https://doi.org/10.1128/iai.65.8.3399-3405.1997.

Bistoni, F., A. Vecchiarelli, E. Cenci, P. Puccetti, P. Marconi, and A. Cassone. 1986. “Evidence for Macrophage-Mediated Protection against Lethal *Candida albicans* Infection.” *Infection and Immunity* 51 (2): 668–74. https://doi.org/10.1128/iai.51.2.668-674.1986.

Biswas, Swarajit K., and W. La Jean Chaffin. 2005. “Anaerobic Growth of *Candida albicans* Does Not Support Biofilm Formation under Similar Conditions Used for Aerobic Biofilm.” *Current Microbiology* 51 (2): 100–104. https://doi.org/10.1007/s00284-005-4552-3.

Blair, Jessica M.A., Mark A. Webber, Alison J. Baylay, David O. Ogbolu, and Laura J.V. Piddock. 2015. “Molecular Mechanisms of Antibiotic Resistance.” *Nature Reviews Microbiology*. Nat Rev Microbiol. https://doi.org/10.1038/nrmicro3380.

Bliss, Joseph M., Kumar P. Basavegowda, Wendy J. Watson, Asad U. Sheikh, and Rita M. Ryan. 2008. “Vertical and Horizontal Transmission of *Candida albicans* in Very Low Birth Weight Infants Using DNA Fingerprinting Techniques.” *Pediatric Infectious Disease Journal* 27 (3): 231–35. https://doi.org/10.1097/INF.0b013e31815bb69d.

Boix-Amorós, Alba, Cecilia Martinez-Costa, Amparo Querol, Maria Carmen Collado, and Alex Mira. 2017. “Multiple Approaches Detect the Presence of Fungi in Human Breastmilk Samples from Healthy Mothers.” *Scientific Reports* 7 (1). https://doi.org/10.1038/s41598-017-13270-x.

Bolyen, Evan, Jai Ram Rideout, Matthew R. Dillon, Nicholas A. Bokulich, Christian C. Abnet, Gabriel A. Al-Ghalith, Harriet Alexander, *et al.* 2019. “Reproducible, Interactive, Scalable

and Extensible Microbiome Data Science Using QIIME 2." *Nature Biotechnology* 37 (8): 852–57. https://doi.org/10.1038/s41587-019-0209-9.

Bonder, Marc Jan, Alexander Kurilshikov, Ettje F. Tigchelaar, Zlatan Mujagic, Floris Imhann, Arnau Vich Vila, Patrick Deelen, *et al.* 2016. "The Effect of Host Genetics on the Gut Microbiome." *Nature Genetics* 48 (11): 1407–12. https://doi.org/10.1038/ng.3663.

Bongomin, Felix, Sara Gago, Rita O. Oladele, and David W. Denning. 2017. "Global and Multi-National Prevalence of Fungal Diseases—Estimate Precision." *Journal of Fungi* 3 (4): 57. https://doi.org/10.3390/jof3040057.

Borghi, Monica, Giorgia Renga, Matteo Puccetti, Vasileios Oikonomou, Melissa Palmieri, Claudia Galosi, Andrea Bartoli, and Luigina Romani. 2014. "Antifungal Th Immunity: Growing up in Family." *Frontiers in Immunology*. Front Immunol. https://doi.org/10.3389/fimmu.2014.00506.

Bougnoux, M. E., D. Diogo, N. François, B. Sendid, S. Veirmeire, J. F. Colombel, C. Bouchier, H. Van Kruiningen, C. D'Enfert, and D. Poulain. 2006. "Multilocus Sequence Typing Reveals Intrafamilial Transmission and Microevolutions of *Candida albicans* Isolates from the Human Digestive Tract." *Journal of Clinical Microbiology* 44 (5): 1810–20. https://doi.org/10.1128/JCM.44.5.1810-1820.2006.

Brown, Gordon D. 2011. "Innate Antifungal Immunity: The Key Role of Phagocytes." *Annual Review of Immunology* 29 (1): 1–21. https://doi.org/10.1146/annurev-immunol-030409-101229.

Brown, Gordon D., David W. Denning, Neil A.R. Gow, Stuart M. Levitz, Mihai G. Netea, and Theodore C. White. 2012. "Hidden Killers: Human Fungal Infections." *Science Translational Medicine* 4 (165): 165rv13-165rv13. https://doi.org/10.1126/scitranslmed.3004404.

Burdet, Charles, Nathalie Grall, Morgane Linard, Antoine Bridier-Nahmias, Michèle Benhayoun, Khadija Bourabha, Mélanie Magnan, *et al.* 2019. "Ceftriaxone and Cefotaxime Have Similar Effects on the Intestinal Microbiota in Human Volunteers Treated by Standard-Dose Regimens." *Antimicrobial Agents and Chemotherapy* 63 (6). https://doi.org/10.1128/AAC.02244-18.

Burdet, Charles, Thu Thuy Nguyen, Xavier Duval, Stéphanie Ferreira, Antoine Andremont, Jérémie Guedj, and France Mentré. 2019. "Impact of Antibiotic Gut Exposure on the Temporal Changes in Microbiome Diversity." *Antimicrobial Agents and Chemotherapy* 63 (10). https://doi.org/10.1128/AAC.00820-19.

Burgain, Anaïs, Faiza Tebbji, Inès Khemiri, and Adnane Sellam. 2020. "Metabolic Reprogramming in the Opportunistic Yeast *Candida albicans* in Response to Hypoxia." *MSphere* 5 (1). https://doi.org/10.1128/msphere.00913-19.

Byrd, Allyson L., Menghan Liu, Kei E. Fujimura, Svetlana Lyalina, Deepti R. Nagarkar, Bruno Charbit, Jacob Bergstedt, *et al.* 2020. "Gut Microbiome Stability and Dynamics in Healthy Donors and Patients with Non-Gastrointestinal Cancers." *Journal of Experimental Medicine* 218 (1). https://doi.org/10.1084/JEM.20200606.

Callahan, Benjamin J., Paul J. McMurdie, and Susan P. Holmes. 2017. "Exact Sequence Variants Should Replace Operational Taxonomic Units in Marker-Gene Data Analysis."

The ISME Journal 2017 11:12 11 (12): 2639–43. https://doi.org/10.1038/ismej.2017.119.

Callahan, Benjamin J., Paul J. McMurdie, Michael J. Rosen, Andrew W. Han, Amy Jo A. Johnson, and Susan P. Holmes. 2016. “DADA2: High-Resolution Sample Inference from Illumina Amplicon Data.” *Nature Methods* 13 (7): 581–83. https://doi.org/10.1038/nmeth.3869.

Camilli, Giorgio, James S. Griffiths, Jemima Ho, Jonathan P. Richardson, and Julian R. Naglik. 2020. “Some like It Hot: *Candida* Activation of Inflammasomes.” *PLoS Pathogens* 16 (10). https://doi.org/10.1371/journal.ppat.1008975.

Carey, Barbara, Jonathan Lambourne, Stephen Porter, and Tim Hodgson. 2019. "Chronic Mucocutaneous Candidiasis Due to Gain-of-Function Mutation in *STAT1"*. *Oral Diseases*. Vol. 25. https://doi.org/10.1111/odi.12881.

Carrow, Emily W., R. F. Hector, and Judith E. Domer. 1984. “Immunodeficient CBA/N Mice Respond Effectively to *Candida albicans*.” *Clinical Immunology and Immunopathology* 33 (3): 371–80. https://doi.org/10.1016/0090-1229(84)90308-8.

Chang, Hao Teng, Pei Wen Tsai, Hsin Hui Huang, Yu Shu Liu, Tzu Shan Chien, and Chung Yu Lan. 2012. “LL37 and HBD-3 Elevate the β-1,3-Exoglucanase Activity of *Candida albicans* Xog1p, Resulting in Reduced Fungal Adhesion to Plastic.” *Biochemical Journal* 441 (3): 963–70. https://doi.org/10.1042/BJ20111454.

Chapman, Stephen J., and Adrian V.S. Hill. 2012. “Human Genetic Susceptibility to Infectious Disease.” *Nature Reviews Genetics* 13 (3): 175–88. https://doi.org/10.1038/nrg3114.

Chatelier, Emmanuelle Le, Mathieu Almeida, Florian Plaza Oñate, Nicolas Pons, Franck Gauthier, Amine Ghozlane, Stanislav Dusko Ehrlich, Elizabeth Witherden, and David Gomez-Cabrero. 2021. “A Catalog of Genes and Species of the Human Oral Microbiota.” https://doi.org/10.15454/WQ4UTV.

Chatelier, Emmanuelle Le, Trine Nielsen, Junjie Qin, Edi Prifti, Falk Hildebrand, Gwen Falony, Mathieu Almeida, *et al.* 2013. “Richness of Human Gut Microbiome Correlates with Metabolic Markers.” *Nature* 500 (7464): 541–46. https://doi.org/10.1038/nature12506.

Chehoud, Christel, Lindsey G. Albenberg, Colleen Judge, Christian Hoffmann, Stephanie Grunberg, Kyle Bittinger, Robert N. Baldassano, James D. Lewis, Frederic D. Bushman, and Gary D. Wu. 2015. “Fungal Signature in the Gut Microbiota of Pediatric Patients with Inflammatory Bowel Disease.” *Inflammatory Bowel Diseases* 21 (8): 1948–56. https://doi.org/10.1097/MIB.0000000000000454.

Chen, Chun, Jianming Liao, Yiyuan Xia, Xia Liu, Rheinallt Jones, John Haran, Beth McCormick, Timothy Robert Sampson, Ashfaqul Alam, and Keqiang Ye. 2022. “Gut Microbiota Regulate Alzheimer’s Disease Pathologies and Cognitive Disorders via PUFA-Associated Neuroinflammation.” *Gut* 71 (11). https://doi.org/10.1136/gutjnl-2021-326269.

Chen, Hui, Xuedong Zhou, Biao Ren, and Lei Cheng. 2020. “The Regulation of Hyphae Growth in *Candida albicans*.” *Virulence* 11 (1): 337–48. https://doi.org/10.1080/21505594.2020.1748930.

Chen, Ming Huei, Laura M. Raffield, Abdou Mousas, Saori Sakaue, Jennifer E. Huffman, Arden Moscati, Bhavi Trivedi, *et al.* 2020. “Trans-Ethnic and Ancestry-Specific Blood-Cell

Genetics in 746,667 Individuals from 5 Global Populations." *Cell* 182 (5): 1198-1213.e14. https://doi.org/10.1016/j.cell.2020.06.045.

Chen, Ying, Li-Ying Zhang, Yi Fang, Chuan Li, Dan-Dan Xia, Gong Zhang, Yi Wen, *et al.* 2023. " Elevated Serum Anti- *Saccharomyces cerevisiae* Antibody Accompanied by Gut Mycobiota Dysbiosis as a Biomarker of Diagnosis in Patients with de Novo Parkinson's Disease ." *European Journal of Neurology*, January. https://doi.org/10.1111/ene.15711.

Choi, Eun Hwa, Charles B. Foster, James G. Taylor, Hans Christian Erichsen, Renee A. Chen, Thomas J. Walsh, Veli Jukka Anttila, Tapani Ruutu, Aarno Palotie, and Stephen J. Chanock. 2003. "Association between Chronic Disseminated Candidiasis in Adult Acute Leukemia and Common *IL4* Promoter Haplotypes." *Journal of Infectious Diseases* 187 (7): 1153–56. https://doi.org/10.1086/368345.

Cleveland, Angela Ahlquist, Monica M. Farley, Lee H. Harrison, Betsy Stein, Rosemary Hollick, Shawn R. Lockhart, Shelley S. Magill, Gordana Derado, Benjamin J. Park, and Tom M. Chiller. 2012. "Changes in Incidence and Antifungal Drug Resistance in Candidemia: Results from Population-Based Laboratory Surveillance in Atlanta and Baltimore, 2008-2011." *Clinical Infectious Diseases* 55 (10): 1352–61. https://doi.org/10.1093/cid/cis697.

Cohen-Kedar, Sarit, Liran Baram, Hofit Elad, Eli Brazowski, Hanan Guzner-Gur, and Iris Dotan. 2014. "Human Intestinal Epithelial Cells Respond to β-Glucans via Dectin-1 and Syk." *European Journal of Immunology* 44 (12): 3729–40. https://doi.org/10.1002/eji.201444876.

Conti, Heather R., and Sarah L. Gaffen. 2015. " IL-17–Mediated Immunity to the Opportunistic Fungal Pathogen *Candida albicans* ." *The Journal of Immunology* 195 (3): 780–88. https://doi.org/10.4049/jimmunol.1500909.

Core R Team. 2019. "A Language and Environment for Statistical Computing." *R Foundation for Statistical Computing* 2: https://www.R--project.org. http://www.r-project.org.

Corvilain, Emilie, Jean Laurent Casanova, and Anne Puel. 2018. "Inherited CARD9 Deficiency: Invasive Disease Caused by Ascomycete Fungi in Previously Healthy Children and Adults." *Journal of Clinical Immunology* 38 (6): 656–93. https://doi.org/10.1007/S10875-018-0539-2

Costa-de-oliveira, Sofia, and Acácio G. Rodrigues. 2020. "*Candida albicans* Antifungal Resistance and Tolerance in Bloodstream Infections: The Triad Yeast-Host-Antifungal." *Microorganisms*. https://doi.org/10.3390/microorganisms8020154.

Coudeyras, Sophie, Gwendoline Jugie, Marion Vermerie, and Christiane Forestier. 2008. "Adhesion of Human Probiotic *Lactobacillus rhamnosus* to Cervical and Vaginal Cells and Interaction with Vaginosis-Associated Pathogens." *Infectious Diseases in Obstetrics and Gynecology* 2008. https://doi.org/10.1155/2008/549640.

Criscuolo, Alexis, and Sylvain Brisse. 2013. "AlienTrimmer: A Tool to Quickly and Accurately Trim off Multiple Short Contaminant Sequences from High-Throughput Sequencing Reads." *Genomics* 102 (5–6): 500–506. https://doi.org/10.1016/j.ygeno.2013.07.011.

Crowne, Douglas P., and David Marlowe. 1960. "A New Scale of Social Desirability Independent of Psychopathology." *Journal of Consulting Psychology* 24 (4): 349–54. https://doi.org/10.1037/h0047358.

Cruz, Melissa R., Carrie E. Graham, Bryce C. Gagliano, Michael C. Lorenz, and Danielle A. Garsin. 2013. "*Enterococcus faecalis* Inhibits Hyphal Morphogenesis and Virulence of *Candida albicans*." *Infection and Immunity* 81 (1): 189–200. https://doi.org/10.1128/IAI.00914-12.

Cui, Lijia, Alison Morris, and Elodie Ghedin. 2013. "The Human Mycobiome in Health and Disease." *Genome Medicine*. BioMed Central. https://doi.org/10.1186/gm467.

Cutuli, M., S. Cristiani, J. M. Lipton, and A. Catania. 2000. "Antimicrobial Effects of α-MSH Peptides." *Journal of Leukocyte Biology* 67 (2): 233–39. https://doi.org/10.1002/jlb.67.2.233.

d'Enfert, Christophe. 2009. "Hidden Killers: Persistence of Opportunistic Fungal Pathogens in the Human Host." *Current Opinion in Microbiology* 12 (4): 358–64. https://doi.org/10.1016/j.mib.2009.05.008.

d'Enfert, Christophe, Ann Kristin Kaune, Leovigildo Rey Alaban, Sayoni Chakraborty, Nathaniel Cole, Margot Delavy, Daria Kosmala, *et al.* 2021. "The Impact of the Fungus-Host-Microbiota Interplay upon *Candida albicans* Infections: Current Knowledge and New Perspectives." *FEMS Microbiology Reviews* 45 (3): 1–55. https://doi.org/10.1093/femsre/fuaa060.

Dadar, Maryam, Ruchi Tiwari, Kumaragurubaran Karthik, Sandip Chakraborty, Youcef Shahali, and Kuldeep Dhama. 2018. "*Candida albicans* - Biology, Molecular Characterization, Pathogenicity, and Advances in Diagnosis and Control – An Update." *Microbial Pathogenesis* 117 (April): 128–38. https://doi.org/10.1016/j.micpath.2018.02.028.

Dambuza, I. M., S. M. Levitz, M. G. Netea, and G. D. Brown. 2017. "Fungal Recognition and Host Defense Mechanisms." *Microbiology Spectrum* 5 (4). https://doi.org/10.1128/microbiolspec.funk-0050-2016.

Dapito, Dianne H., Ali Mencin, Geum Youn Gwak, Jean Philippe Pradere, Myoung Kuk Jang, Ingmar Mederacke, Jorge M. Caviglia, *et al.* 2012. "Promotion of Hepatocellular Carcinoma by the Intestinal Microbiota and TLR4." *Cancer Cell* 21 (4). https://doi.org/10.1016/j.ccr.2012.02.007.

Dausset, Caroline, Stéphanie Bornes, Sylvie Miquel, Nathalie Kondjoyan, Magaly Angenieux, Laurence Nakusi, Philippe Veisseire, *et al.* 2020. "Identification of Sulfur Components Enhancing the Anti-*Candida* Effect of *Lactobacillus rhamnosus* Lcr35." *Scientific Reports* 10 (1). https://doi.org/10.1038/s41598-020-74027-7.

David, Lawrence A., Corinne F. Maurice, Rachel N. Carmody, David B. Gootenberg, Julie E. Button, Benjamin E. Wolfe, Alisha V. Ling, *et al.* 2014. "Diet Rapidly and Reproducibly Alters the Human Gut Microbiome." *Nature* 505 (7484): 559–63. https://doi.org/10.1038/nature12820.

Davis, S. D., J. Schaller, and R. J. Wedgwood. 1966. "Job's Syndrome. Recurrent, 'Cold', Staphylococcal Abscesses." *Lancet* 1 (7445): 1013–15. https://doi.org/10.1016/s0140-6736(66)90119-x.

Delavy, Margot, Charles Burdet, Natacha Sertour, Savannah Devente, Jean Denis Docquier, Nathalie Grall, Stevenn Volant, *et al.* 2022. "A Clinical Study Provides the First Direct

Evidence That Interindividual Variations in Fecal β-Lactamase Activity Affect the Gut Mycobiota Dynamics in Response to β-Lactam Antibiotics." *mBio* 13 (6). https://doi.org/10.1128/mbio.02880-22.

Demidowich, Andrew P., Joo Yun Jun, and Jack A. Yanovski. 2017. "Polymorphisms and Mutations in the Melanocortin-3 Receptor and Their Relation to Human Obesity." *Biochimica et Biophysica Acta - Molecular Basis of Disease*. https://doi.org/10.1016/j.bbadis.2017.03.018.

Denning, David W., Matthew Kneale, Jack D. Sobel, and Riina Rautemaa-Richardson. 2018. "Global Burden of Recurrent Vulvovaginal Candidiasis: A Systematic Review." *The Lancet. Infectious Diseases* 18 (11): e339–47. https://doi.org/10.1016/S1473-3099(18)30103-8.

Deriu, Elisa, Janet Z. Liu, Milad Pezeshki, Robert A. Edwards, Roxanna J. Ochoa, Heidi Contreras, Stephen J. Libby, Ferric C. Fang, and Manuela Raffatellu. 2013. "Probiotic Bacteria Reduce Salmonella Typhimurium Intestinal Colonization by Competing for Iron." *Cell Host and Microbe* 14 (1): 26–37. https://doi.org/10.1016/j.chom.2013.06.007.

Ding, Keyue, and Iftikhar J. Kullo. 2007. "Methods for the Selection of Tagging SNPs: A Comparison of Tagging Efficiency and Performance." *European Journal of Human Genetics* 15 (2): 228–36. https://doi.org/10.1038/sj.ejhg.5201755.

Disteldorf, Erik M., Christian F. Krebs, Hans Joachim Paust, Jan Eric Turner, Geraldine Nouailles, André Tittel, Catherine Meyer-Schwesinger, *et al.* 2015. "CXCL5 Drives Neutrophil Recruitment in TH17-Mediated GN." *Journal of the American Society of Nephrology* 26 (1): 55–66. https://doi.org/10.1681/ASN.2013101061.

Dohlman, Anders B., Jared Klug, Marissa Mesko, Iris H. Gao, Steven M. Lipkin, Xiling Shen, and Iliyan D. Iliev. 2022. "A Pan-Cancer Mycobiome Analysis Reveals Fungal Involvement in Gastrointestinal and Lung Tumors." *Cell* 185 (20): 3807-3822.e12. https://doi.org/10.1016/j.cell.2022.09.015.

Dollive, Serena, Ying Yu Chen, Stephanie Grunberg, Kyle Bittinger, Christian Hoffmann, Lee Vandivier, Christopher Cuff, James D. Lewis, Gary D. Wu, and Frederic D. Bushman. 2013. "Fungi of the Murine Gut: Episodic Variation and Proliferation during Antibiotic Treatment." *PloS One* 8 (8): 71806. https://doi.org/10.1371/journal.pone.0071806.

Dominguez-Bello, Maria G., Elizabeth K. Costello, Monica Contreras, Magda Magris, Glida Hidalgo, Noah Fierer, and Rob Knight. 2010. "Delivery Mode Shapes the Acquisition and Structure of the Initial Microbiota across Multiple Body Habitats in Newborns." *Proceedings of the National Academy of Sciences of the United States of America* 107 (26): 11971–75. https://doi.org/10.1073/pnas.1002601107.

Donaldson, Gregory P., S. Melanie Lee, and Sarkis K. Mazmanian. 2015. "Gut Biogeography of the Bacterial Microbiota." *Nature Reviews Microbiology* 14 (1): 20–32. https://doi.org/10.1038/nrmicro3552.

Dore, J., S.D. Ehrlich, F. Levenez, E. Pelletier, A. Alberti, L. Bertrand, P. Bork, *et al.* 2015. "IHMS_SOP 07 V1: Standard Operating Procedure for Fecal Samples DNA Extraction, Protocol H." *International Human Microbiome Standards*. http://www.microbiome-standards.org.

Doron, Itai, Irina Leonardi, Xin V. Li, William D. Fiers, Alexa Semon, Meghan Bialt-DeCelie, Mélanie Migaud, *et al.* 2021. “Human Gut Mycobiota Tune Immunity via CARD9-Dependent Induction of Anti-Fungal IgG Antibodies.” *Cell* 184 (4): 1017-1031.e14. https://doi.org/10.1016/j.cell.2021.01.016.

Drummond, Rebecca A., Jigar V. Desai, Emily E. Ricotta, Muthulekha Swamydas, Clay Deming, Sean Conlan, Mariam Quinones, *et al.* 2022. “Long-Term Antibiotic Exposure Promotes Mortality after Systemic Fungal Infection by Driving Lymphocyte Dysfunction and Systemic Escape of Commensal Bacteria.” *Cell Host and Microbe* 30 (7): 1020-1033.e6. https://doi.org/10.1016/j.chom.2022.04.013.

Duffy, Darragh, Vincent Rouilly, Valentina Libri, Milena Hasan, Benoit Beitz, Mikael David, Alejandra Urrutia, *et al.* 2014. “Functional Analysis via Standardized Whole-Blood Stimulation Systems Defines the Boundaries of a Healthy Immune Response to Complex Stimuli.” *Immunity* 40 (3): 436–50. https://doi.org/10.1016/j.immuni.2014.03.002.

Duncan, Sylvia H., Karen P. Scott, Alan G. Ramsay, Hermie J.M. Harmsen, Gjalt W. Welling, Colin S. Stewart, and Harry J. Flint. 2003. “Effects of Alternative Dietary Substrates on Competition between Human Colonic Bacteria in an Anaerobic Fermentor System.” *Applied and Environmental Microbiology* 69 (2): 1136–42. https://doi.org/10.1128/AEM.69.2.1136-1142.2003.

Durazzi, Francesco, Claudia Sala, Gastone Castellani, Gerardo Manfreda, Daniel Remondini, and Alessandra De Cesare. 2021. “Comparison between 16S RRNA and Shotgun Sequencing Data for the Taxonomic Characterization of the Gut Microbiota.” *Scientific Reports* 11 (1): 1–10. https://doi.org/10.1038/s41598-021-82726-y.

Durrant, W. E., and X. Dong. 2004. “Systemic Acquired Resistance.” *Annual Review of Phytopathology* 42: 185–209. https://doi.org/10.1146/annurev.phyto.42.040803.140421.

Ekstrom, Claus. 2014. “MESS: Miscellaneous Esoteric Statistical Scripts. R Package Version 0.3-2,” July. http://cran.r-project.org/package=MESS.

Emulate. 2023. “Organ-on-a-Chip Technology for Drug Discovery & Development.” Accessed March 9, 2023. https://emulatebio.com/.

Ene, Iuliana V., Sascha Brunke, Alistair J.P. Brown, and Bernhard Hube. 2014. “Metabolism in Fungal Pathogenesis.” *Cold Spring Harbor Perspectives in Medicine* 4 (12). https://doi.org/10.1101/cshperspect.a019695.

Erwig, Lars P., and Neil A.R. Gow. 2016. “Interactions of Fungal Pathogens with Phagocytes.” *Nature Reviews Microbiology* 14 (3): 163–76. https://doi.org/10.1038/nrmicro.2015.21.

Faith, Jeremiah J., Janaki L. Guruge, Mark Charbonneau, Sathish Subramanian, Henning Seedorf, Andrew L. Goodman, Jose C. Clemente, *et al.* 2013. “The Long-Term Stability of the Human Gut Microbiota.” *Science* 341 (6141). https://doi.org/10.1126/science.1237439.

Fan, Di, Laura A. Coughlin, Megan M. Neubauer, Jiwoong Kim, Min Soo Kim, Xiaowei Zhan, Tiffany R. Simms-Waldrip, Yang Xie, Lora V. Hooper, and Andrew Y. Koh. 2015. “Activation of HIF-1α and LL-37 by Commensal Bacteria Inhibits *Candida albicans* Colonization.” *Nature Medicine*. Nat Med. https://doi.org/10.1038/nm.3871.

Feist, Adam M., Markus J. Herrgård, Ines Thiele, Jennie L. Reed, and Bernhard Palsson. 2009. "Reconstruction of Biochemical Networks in Microorganisms." *Nature Reviews Microbiology* 7 (2): 129–43. https://doi.org/10.1038/nrmicro1949.

Ferwerda, Bart, Gerben Ferwerda, Theo S. Plantinga, Janet A. Willment, Annemiek B. van Spriel, Hanka Venselaar, Clara C. Elbers, *et al.* 2009. "Human Dectin-1 Deficiency and Mucocutaneous Fungal Infections." *New England Journal of Medicine* 361 (18): 1760–67. https://doi.org/10.1056/nejmoa0901053.

Fiedorová, Kristýna, Matěj Radvanský, Eva Němcová, Hana Grombiříková, Juraj Bosák, Michaela Černochová, Matej Lexa, David Šmajs, and Tomáš Freiberger. 2019. "The Impact of DNA Extraction Methods on Stool Bacterial and Fungal Microbiota Community Recovery." *Frontiers in Physiology* 10 (April): 821. https://doi.org/10.3389/fmicb.2019.00821.

Fouhy, Fiona, Caitriona M. Guinane, Seamus Hussey, Rebecca Wall, C. Anthony Ryan, Eugene M. Dempsey, Brendan Murphy, *et al.* 2012. "High-Throughput Sequencing Reveals the Incomplete, Short-Term Recovery of Infant Gut Microbiota Following Parenteral Antibiotic Treatment with Ampicillin and Gentamicin." *Antimicrobial Agents and Chemotherapy* 56 (11): 5811–20. https://doi.org/10.1128/AAC.00789-12.

Fourie, Ruan, Oluwasegun O. Kuloyo, Bonang M. Mochochoko, Jacobus Albertyn, and Carolina H. Pohl. 2018. *Iron at the Centre of Candida albicans Interactions*. *Frontiers in Cellular and Infection Microbiology*. Vol. 8. Frontiers Media S.A. https://doi.org/10.3389/fcimb.2018.00185.

Foxman, Betsy, Ryan Muraglia, Jean Paul Dietz, Jack D. Sobel, and Julian Wagner. 2013. "Prevalence of Recurrent Vulvovaginal Candidiasis in 5 European Countries and the United States: Results from an Internet Panel Survey." *Journal of Lower Genital Tract Disease* 17 (3): 340–45. https://doi.org/10.1097/LGT.0b013e318273e8cf.

Frau, Alessandra, John G. Kenny, Luca Lenzi, Barry J. Campbell, Umer Z. Ijaz, Carrie A. Duckworth, Michael D. Burkitt, *et al.* 2019. "DNA Extraction and Amplicon Production Strategies Deeply Influence the Outcome of Gut Mycobiome Studies." *Scientific Reports 2019 9:1* 9 (1): 1–17. https://doi.org/10.1038/s41598-019-44974-x.

Friedman, Jonathan, and Eric J. Alm. 2012. "Inferring Correlation Networks from Genomic Survey Data." *PLoS Computational Biology* 8 (9): e1002687. https://doi.org/10.1371/journal.pcbi.1002687.

Fröhlich-Wyder, Marie Therese, Emmanuelle Arias-Roth, and Ernst Jakob. 2019. "Cheese Yeasts." *Yeast* 36 (3): 129–41. https://doi.org/10.1002/yea.3368.

FunHomic consortium. 2023. "FunHoMic." Accessed March 9, 2023. https://www.funhomic.eu/.

G. Small, Annabelle, Jovanka R. King, Deborah A. Rathjen, and Antonio Ferrante. 2019. "The Role of Phagocytes in Immunity to *Candida albicans*." In *Candida albicans*. IntechOpen. https://doi.org/10.5772/intechopen.80683.

Gao, Renyuan, Kai Xia, Minkang Wu, Hui Zhong, Jing Sun, Yin Zhu, Linsheng Huang, *et al.* 2022. "Alterations of Gut Mycobiota Profiles in Adenoma and Colorectal Cancer." *Frontiers in Cellular and Infection Microbiology* 12 (February).

https://doi.org/10.3389/fcimb.2022.839435.

García-Gamboa, Ricardo, Manuel R. Kirchmayr, Misael Sebastian Gradilla-Hernández, Vicente Pérez-Brocal, Andrés Moya, and Marisela González-Avila. 2021. “The Intestinal Mycobiota and Its Relationship with Overweight, Obesity and Nutritional Aspects.” *Journal of Human Nutrition and Dietetics* 34 (4): 645–55. https://doi.org/10.1111/jhn.12864.

García, Carlos, Faiza Tebbji, Michelle Daigneault, Ning-Ning Liu, Julia R. Köhler, Emma Allen-Vercoe, and Adnane Sellam. 2017. “The Human Gut Microbial Metabolome Modulates Fungal Growth via the TOR Signaling Pathway.” *MSphere* 2 (6). https://doi.org/10.1128/msphere.00555-17.

GARDES, M., and T. D. BRUNS. 1993. “ITS Primers with Enhanced Specificity for Basidiomycetes - Application to the Identification of Mycorrhizae and Rusts.” *Molecular Ecology* 2 (2): 113–18. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x.

Gensollen, Thomas, Shankar S. Iyer, Dennis L. Kasper, and Richard S. Blumberg. 2016. “How Colonization by Microbiota in Early Life Shapes the Immune System.” *Science* 352 (6285): 539–44. https://doi.org/10.1126/science.aad9378.

Gerasimidis, Konstantinos, Martin Bertz, Christopher Quince, Katja Brunner, Alanna Bruce, Emilie Combet, Szymon Calus, Nick Loman, and Umer Zeeshan Ijaz. 2016. “The Effect of DNA Extraction Methodology on Gut Microbiota Research Applications.” *BMC Research Notes* 9 (1): 1–10. https://doi.org/10.1186/s13104-016-2171-7.

Ghannoum, Mahmoud A., Richard J. Jurevic, Pranab K. Mukherjee, Fan Cui, Masoumeh Sikaroodi, Ammar Naqvi, and Patrick M. Gillevet. 2010. “Characterization of the Oral Fungal Microbiome (Mycobiome) in Healthy Individuals.” *PLoS Pathogens* 6 (1). https://doi.org/10.1371/journal.ppat.1000713.

Gierlikowska, Barbara, Albert Stachura, Wojciech Gierlikowski, and Urszula Demkow. 2021. “Phagocytosis, Degranulation and Extracellular Traps Release by Neutrophils—The Current Knowledge, Pharmacological Modulation and Future Prospects.” *Frontiers in Pharmacology*.. https://doi.org/10.3389/fphar.2021.666732.

Goodrich, Julia K., Emily R. Davenport, Michelle Beaumont, Matthew A. Jackson, Rob Knight, Carole Ober, Tim D. Spector, Jordana T. Bell, Andrew G. Clark, and Ruth E. Ley. 2016. “Genetic Determinants of the Gut Microbiome in UK Twins.” *Cell Host and Microbe* 19 (5): 731–43. https://doi.org/10.1016/j.chom.2016.04.017.

Gow, Neil A.R., Alistair J.P. Brown, and Frank C. Odds. 2002. “Fungal Morphogenesis and Host Invasion.” *Current Opinion in Microbiology* 5 (4): 366–71. https://doi.org/10.1016/S1369-5274(02)00338-7.

Gow, Neil A.R., Frank L. Van De Veerdonk, Alistair J.P. Brown, and Mihai G. Netea. 2012. “*Candida albicans* Morphogenesis and Host Defence: Discriminating Invasion from Colonization.” *Nature Reviews Microbiology* 10 (2): 112–22. https://doi.org/10.1038/nrmicro2711.

Goyal, Surabhi, Juan Camilo Castrillón-Betancur, Esther Klaile, and Hortense Slevogt. 2018. “The Interaction of Human Pathogenic Fungi with C-Type Lectin Receptors.” *Frontiers in Immunology* 9. https://doi.org/10.3389/fimmu.2018.01261.

Graaf, Chantal A.A. Van der, Mihai G. Netea, Servaas A. Morré, Martin Den Heijer, Paul E. Verweij, Jos W.M. Van der Meer, and Bart Jan Kullberg. 2006. “Toll-like Receptor 4 Asp299Gly/Thr399Ile Polymorphisms Are a Risk Factor for *Candida* Bloodstream Infection.” *European Cytokine Network* 17 (1): 29–34.

Graf, Katja, Antonia Last, Rena Gratz, Stefanie Allert, Susanne Linde, Martin Westermann, Marko Gröger, Alexander S. Mosig, Mark S. Gresnigt, and Bernhard Hube. 2019. “Keeping Candida Commensal: How Lactobacilli Antagonize Pathogenicity of *Candida albicans* in an in Vitro Gut Model.” *DMM Disease Models and Mechanisms* 12 (9). https://doi.org/10.1242/dmm.039719.

Grahl, Nora, Kelly M. Shepardson, Dawoon Chung, and Robert A. Cramer. 2012. "Hypoxia and Fungal Pathogenesis: To Air or Not to Air?" *Eukaryotic Cell*. Vol. 11. https://doi.org/10.1128/EC.00031-12.

Grieco, Paolo, Claudia Rossi, Gualtiero Colombo, Stefano Gatti, Ettore Novellino, James M. Lipton, and Anna Catania. 2003. “Novel α-Melanocyte Stimulating Hormone Peptide Analogues with High Candidacidal Activity.” *Journal of Medicinal Chemistry* 46 (5): 850–55. https://doi.org/10.1021/jm0204338.

Guarner, Francisco, and Juan R. Malagelada. 2003. “Gut Flora in Health and Disease.” *Lancet* 361 (9356): 512–19. https://doi.org/10.1016/S0140-6736(03)12489-0.

Guinan, Jack, and Shankar Thangamani. 2018. “Antibiotic-Induced Alterations in Taurocholic Acid Levels Promote Gastrointestinal Colonization of *Candida albicans*.” *FEMS Microbiology Letters* 365 (18): 196. https://doi.org/10.1093/femsle/fny196.

Guinan, Jack, Pedro Villa, and Shankar Thangamani. 2018. “Secondary Bile Acids Inhibit *Candida albicans* Growth and Morphogenesis.” *Pathogens and Disease* 76 (3): 38. https://doi.org/10.1093/femspd/fty038.

Guinan, Jack, Shaohua Wang, Tony R. Hazbun, Hariom Yadav, and Shankar Thangamani. 2019. “Antibiotic-Induced Decreases in the Levels of Microbial-Derived Short-Chain Fatty Acids Correlate with Increased Gastrointestinal Colonization of *Candida albicans*.” *Scientific Reports* 9 (1): 1–11. https://doi.org/10.1038/s41598-019-45467-7.

Guiver, M., K. Levi, and B. A. Oppenheim. 2001. “Rapid Identification of *Candida* Species by TaqMan PCR.” *Journal of Clinical Pathology* 54 (5): 362–66. https://doi.org/10.1136/jcp.54.5.362.

Gutierrez, Daniel, Anthony Weinstock, Vijay C. Antharam, Haiwei Gu, Paniz Jasbi, Xiaojian Shi, Blake Dirks, *et al.* 2020. “Antibiotic-Induced Gut Metabolome and Microbiome Alterations Increase the Susceptibility to *Candida albicans* Colonization in the Gastrointestinal Tract.” *FEMS Microbiology Ecology* 96 (1). https://doi.org/10.1093/femsec/fiz187.

Han, Shuo, Will Van Treuren, Curt R. Fischer, Bryan D. Merrill, Brian C. DeFelice, Juan M. Sanchez, Steven K. Higginbottom, *et al.* 2021. “A Metabolomics Pipeline for the Mechanistic Interrogation of the Gut Microbiome.” *Nature* 595 (7867): 415–20. https://doi.org/10.1038/s41586-021-03707-9.

Han, Y., and J. E. Cutler. 1995. “Antibody Response That Protects against Disseminated Candidiasis.” *Infection and Immunity* 63 (7): 2714–19.

https://doi.org/10.1128/iai.63.7.2714-2719.1995.

Hashemi, M., E. Eskandari-Nasab, A. Moazeni-Roodi, M. Naderi, B. Sharifi-Mood, and M. Taheri. 2013. “Association of *CTSZ* rs34069356 and *MC3R* rs6127698 Gene Polymorphisms with Pulmonary Tuberculosis.” *International Journal of Tuberculosis and Lung Disease* 17 (9): 1224–28. https://doi.org/10.5588/ijtld.12.0762.

Hawksworth, David L., and Robert Lücking. 2017. “Fungal Diversity Revisited: 2.2 to 3.8 Million Species.” *Microbiology Spectrum* 5 (4). https://doi.org/10.1128/microbiolspec.funk-0052-2016.

He, Yan, Wei Wu, Hui Min Zheng, Pan Li, Daniel McDonald, Hua Fang Sheng, Mu Xuan Chen, *et al.* 2018. “Regional Variation Limits Applications of Healthy Gut Microbiome Reference Ranges and Disease Models.” *Nature Medicine* 24 (10): 1532–35. https://doi.org/10.1038/s41591-018-0164-x.

Helmerhorst, E. J., R. F. Troxler, and F. G. Oppenheim. 2001. “The Human Salivary Peptide Histatin 5 Exerts Its Antifungal Activity through the Formation of Reactive Oxygen Species.” *Proceedings of the National Academy of Sciences of the United States of America* 98 (25): 14637–42. https://doi.org/10.1073/pnas.141366998.

Helmink, Beth A., M. A.Wadud Khan, Amanda Hermann, Vancheswaran Gopalakrishnan, and Jennifer A. Wargo. 2019. “The Microbiome, Cancer, and Cancer Therapy.” *Nature Medicine*. https://doi.org/10.1038/s41591-019-0377-7.

Herre, Jurgen, Andrew S.J. Marshall, Emmanuelle Caron, Alexander D. Edwards, David L. Williams, Edina Schweighoffer, Victor Tybulewicz, Caetano Reis E Sousa, Siamon Gordon, and Gordon D. Brown. 2004. “Dectin-1 Uses Novel Mechanisms for Yeast Phagocytosis in Macrophages.” *Blood* 104 (13): 4038–45. https://doi.org/10.1182/blood-2004-03-1140.

Hertog, Alice L. Den, Jan Van Marle, Henk A. Van Veen, Wim Van’t Hof, Jan G.M. Bolscher, Enno C.I. Veerman, and Arie V. Nieuw Amerongen. 2005. “Candidacidal Effects of Two Antimicrobial Peptides: Histatin 5 Causes Small Membrane Defects, but LL-37 Causes Massive Disruption of the Cell Membrane.” *Biochemical Journal* 388 (2): 689–95. https://doi.org/10.1042/BJ20042099.

Hildebrand, Falk, Lucas Moitinho-Silva, Sonja Blasche, Martin T. Jahn, Toni Ingolf Gossmann, Jaime Huerta-Cepas, Rajna Hercog, *et al.* 2019. “Antibiotics-Induced Monodominance of a Novel Gut Bacterial Order.” *Gut* 68 (10): 1781–90. https://doi.org/10.1136/gutjnl-2018-317715.

Hill, Cian J., Denise B. Lynch, Kiera Murphy, Marynka Ulaszewska, Ian B. Jeffery, Carol Anne O’Shea, Claire Watkins, *et al.* 2017. “Evolution of Gut Microbiota Composition from Birth to 24 Weeks in the INFANTMET Cohort.” *Microbiome* 5 (1). https://doi.org/10.1186/s40168-016-0213-y.

Hise, Amy G., Jeffrey Tomalka, Sandhya Ganesan, Krupen Patel, Brian A. Hall, Gordon D. Brown, and Katherine A. Fitzgerald. 2009. “An Essential Role for the NLRP3 Inflammasome in Host Defense against the Human Fungal Pathogen *Candida albicans*.” *Cell Host and Microbe* 5 (5): 487–97. https://doi.org/10.1016/j.chom.2009.05.002.

Hoggard, Michael, Anna Vesty, Giselle Wong, Johanna M. Montgomery, Chantelle Fourie,

Richard G. Douglas, Kristi Biswas, and Michael W. Taylor. 2018. “Characterizing the Human Mycobiota: A Comparison of Small Subunit RRNA, ITS1, ITS2, and Large Subunit RRNA Genomic Targets.” *Frontiers in Microbiology* 9 (SEP): 2208. https://doi.org/10.3389/FMICB.2018.02208/BIBTEX.

Holland, Steven M., Frank R. DeLeo, Houda Z. Elloumi, Amy P. Hsu, Gulbu Uzel, Nina Brodsky, Alexandra F. Freeman, *et al.* 2007. “ *STAT3* Mutations in the Hyper-IgE Syndrome .” *New England Journal of Medicine* 357 (16): 1608–19. https://doi.org/10.1056/nejmoa073687.

Hsieh, Shih Hung, Sascha Brunke, and Matthias Brock. 2017. “Encapsulation of Antifungals in Micelles Protects *Candida albicans* during Gall-Bladder Infection.” *Frontiers in Microbiology* 8 (FEB): 117. https://doi.org/10.3389/fmicb.2017.00117.

Huertas, Blanca, Daniel Prieto, Aida Pitarch, Concha Gil, Jesús Pla, and Rosalía Díez-Orejas. 2017. “Serum Antibody Profile during Colonization of the Mouse Gut by *Candida albicans*: Relevance for Protection during Systemic Infection.” *Journal of Proteome Research* 16 (1): 335–45. https://doi.org/10.1021/acs.jproteome.6b00383.

Illumina. 2013. *16S Metagenomic Sequencing Library*. *Illumina.Com*. http://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf.

Jabra-Rizk, Mary Ann, Eric F. Kong, Christina Tsui, M. Hong Nguyen, Cornelius J. Clancy, Paul L. Fidel, and Mairi Noverr. 2016. “*Candida albicans* Pathogenesis: Fitting within the Host-Microbe Damage Response Framework.” *Infection and Immunity* 84 (10): 2724–39. https://doi.org/10.1128/IAI.00469-16.

Jacobsen, Ilse D., and Bernhard Hube. 2017. “*Candida albicans* Morphology: Still in Focus.” *Expert Review of Anti-Infective Therapy* 15 (4): 327–30. https://doi.org/10.1080/14787210.2017.1290524.

Jaeger, M., R. van der Lee, S. C. Cheng, M. D. Johnson, V. Kumar, A. Ng, T. S. Plantinga, *et al.* 2015. “The RIG-I-like Helicase Receptor MDA5 (*IFIH1*) Is Involved in the Host Defense against *Candida* Infections.” *European Journal of Clinical Microbiology and Infectious Diseases* 34 (5): 963–74. https://doi.org/10.1007/s10096-014-2309-2.

Jaeger, Martin, Vasiliki Matzaraki, Raúl Aguirre-Gamboa, Mark S. Gresnigt, Xiaojing Chu, Melissa D. Johnson, Marije Oosting, *et al.* 2019. “A Genome-Wide Functional Genomics Approach Identifies Susceptibility Pathways to Fungal Bloodstream Infection in Humans.” *Journal of Infectious Diseases* 220 (5): 862–72. https://doi.org/10.1093/infdis/jiz206.

Jaeger, Martin, Mark H.T. Stappers, Leo A.B. Joosten, Inge C. Gyssens, and Mihai G. Netea. 2015. “Genetic Variation in Pattern Recognition Receptors: Functional Consequences and Susceptibility to Infectious Disease.” *Future Microbiology* 10 (6): 989–1008. https://doi.org/10.2217/fmb.15.37.

Jang, Sung Jae, Kyeongju Lee, Bomi Kwon, Hyun Ju You, and Gwang Pyo Ko. 2019. “Vaginal Lactobacilli Inhibit Growth and Hyphae Formation of *Candida albicans*” 9 (1): 1–9. https://www.nature.com/articles/s41598-019-44579-4.

Jensen, Jean, Thomas Warner, and Edward Balish. 1994. “The Role of Phagocytic Cells in

Resistance to Disseminated Candidiasis in Granulocytopenic Mice." *Journal of Infectious Diseases* 170 (4): 900–905. https://doi.org/10.1093/infdis/170.4.900.

Jiang, Chunmei, Guangning Li, Pengru Huang, Zhou Liu, and Bin Zhao. 2017. "The Gut Microbiota and Alzheimer's Disease." *Journal of Alzheimer's Disease* 58 (1): 1–15. https://doi.org/10.3233/JAD-161141.

Johansson, Malin E.V., Jessica M. Holmén Larsson, and Gunnar C. Hansson. 2011. "The Two Mucus Layers of Colon Are Organized by the MUC2 Mucin, Whereas the Outer Layer Is a Legislator of Host-Microbial Interactions." *Proceedings of the National Academy of Sciences of the United States of America* 108 (SUPPL. 1): 4659–65. https://doi.org/10.1073/pnas.1006451107.

Johansson, Malin E.V., Mia Phillipson, Joel Petersson, Anna Velcich, Lena Holm, and Gunnar C. Hansson. 2008. "The Inner of the Two Muc2 Mucin-Dependent Mucus Layers in Colon Is Devoid of Bacteria." *Proceedings of the National Academy of Sciences of the United States of America* 105 (39): 15064–69. https://doi.org/10.1073/pnas.0803124105.

Johnson, Melissa D., Theo S. Plantinga, Esther Van De Vosse, Digna R. Velez Edwards, P. Brian Smith, Barbara D. Alexander, John C. Yang, *et al.* 2012. "Cytokine Gene Polymorphisms and the Outcome of Invasive Candidiasis: A Prospective Cohort Study." *Clinical Infectious Diseases* 54 (4): 502–10. https://doi.org/10.1093/cid/cir827.

Jurevic, Richard J., Mai Bai, Robert B. Chadwick, Theodore C. White, and Beverly A. Dale. 2003. "Single-Nucleotide Polymorphisms (SNPs) in Human β-Defensin 1: High-Throughput SNP Assays and Association with *Candida*Carriage in Type I Diabetics and Nondiabetic Controls." *Journal of Clinical Microbiology* 41 (1): 90–96. https://doi.org/10.1128/JCM.41.1.90-96.2003.

Kaba, Hani E.J., Manfred Nimtz, Peter P. Müller, and Ursula Bilitewski. 2013. "Involvement of the Mitogen Activated Protein Kinase Hog1p in the Response of *Candida albicans* to Iron Availability." *BMC Microbiology* 13 (1): 1–15. https://doi.org/10.1186/1471-2180-13-16.

Kankkunen, Päivi, Laura Teirilä, Johanna Rintahaka, Harri Alenius, Henrik Wolff, and Sampsa Matikainen. 2010. "(1,3)-β-Glucans Activate Both Dectin-1 and NLRP3 Inflammasome in Human Macrophages." *The Journal of Immunology* 184 (11): 6335–42. https://doi.org/10.4049/jimmunol.0903019.

Karakousis, A., L. Tan, D. Ellis, H. Alexiou, and P. J. Wormald. 2006. "An Assessment of the Efficiency of Fungal DNA Extraction Methods for Maximizing the Detection of Medically Important Fungi Using PCR." *Journal of Microbiological Methods* 65 (1): 38–48. https://doi.org/10.1016/j.mimet.2005.06.008.

Kasper, Lydia, Annika König, Paul Albert Koenig, Mark S. Gresnigt, Johannes Westman, Rebecca A. Drummond, Michail S. Lionakis, *et al.* 2018. "The Fungal Peptide Toxin Candidalysin Activates the NLRP3 Inflammasome and Causes Cytolysis in Mononuclear Phagocytes." *Nature Communications* 9 (1): 1–20. https://doi.org/10.1038/s41467-018-06607-1.

Kassambara, Alboukadel. 2022. "'ggplot2' Based Publication Ready Plots Version." *Preprints*, 1–184. https://rpkgs.datanovia.com/ggpubr/.

Kastora, Stavroula L., Carmen Herrero-de-Dios, Gabriela M. Avelar, Carol A. Munro, and

Alistair J.P. Brown. 2017. “Sfp1 and Rtg3 Reciprocally Modulate Carbon Source-Conditional Stress Adaptation in the Pathogenic Yeast *Candida albicans*.” *Molecular Microbiology* 105 (4): 620–36. https://doi.org/10.1111/mmi.13722.

Kavanaugh, Nicole L., Angela Q. Zhang, Clarissa J. Nobile, Alexander D. Johnson, and Katharina Ribbeck. 2014. “Mucins Suppress Virulence Traits of *Candida albicans*.” Edited by Judith Berman. *MBio* 5 (6). https://doi.org/10.1128/mBio.01911-14.

Kawasaki, Takumi, and Taro Kawai. 2014. “Toll-like Receptor Signaling Pathways.” *Frontiers in Immunology* 5 (SEP). https://doi.org/10.3389/fimmu.2014.00461.

Kiani, Aysha Karim, Derek Pheby, Gary Henehan, Richard Brown, Paul Sieving, Peter Sykora, Robert Marks, *et al.* 2022. “Ethical Considerations Regarding Animal Experimentation.” *Journal of Preventive Medicine and Hygiene* 63 (2): E255–66. https://doi.org/10.15167/2421-4248/jpmh2022.63.2S3.2768.

Kirchner, Florian R., and Salomé LeibundGut-Landmann. 2021. “Tissue-Resident Memory Th17 Cells Maintain Stable Fungal Commensalism in the Oral Mucosa.” *Mucosal Immunology* 14 (2): 455–67. https://doi.org/10.1038/s41385-020-0327-1.

Klaassens, Eline S., Rolf J. Boesten, Monique Haarman, Jan Knol, Frank H. Schuren, Elaine E. Vaughan, and Willem M. De Vos. 2009. “Mixed-Species Genomic Microarray Analysis of Fecal Samples Reveals Differential Transcriptional Responses of Bifidobacteria in Breast- And Formula-Fed Infants.” *Applied and Environmental Microbiology* 75 (9): 2668–76. https://doi.org/10.1128/AEM.02492-08.

Kläring, Karoline, Laura Hanske, Nam Bui, Cédric Charrier, Michael Blaut, Dirk Haller, Caroline M. Plugge, and Thomas Clavel. 2013. “*Intestinimonas butyriciproducens* Gen. Nov., Sp. Nov., a Butyrate-Producing Bacterium from the Mouse Intestine.” *International Journal of Systematic and Evolutionary Microbiology* 63 (PART 12): 4606–12. https://doi.org/10.1099/ijs.0.051441-0.

Klindworth, Anna, Elmar Pruesse, Timmy Schweer, Jörg Peplies, Christian Quast, Matthias Horn, and Frank Oliver Glöckner. 2013. “Evaluation of General 16S Ribosomal RNA Gene PCR Primers for Classical and Next-Generation Sequencing-Based Diversity Studies.” *Nucleic Acids Research* 41 (1): e1. https://doi.org/10.1093/nar/gks808.

Koshlukova, Svetlana E., Marcelo W.B. Araujo, Didi Baev, and Mira Edgerton. 2000. “Released ATP Is an Extracellular Cytotoxic Mediator in Salivary Histatin 5-Induced Killing of *Candida albicans*.” *Infection and Immunity* 68 (12): 6848–56. https://doi.org/10.1128/IAI.68.12.6848-6856.2000.

Koya, Charita, Tsung Yu, Carol Strong, and Meng Che Tsai. 2018. “Association between Two Common Missense Substitutions, Thr6Lys and Val81Ile, in *MC3R* Gene and Childhood Obesity: A Meta-Analysis.” *Childhood Obesity* 14 (4): 218–26. https://doi.org/10.1089/chi.2017.0265.

Krajmalnik-Brown, Rosa, Catherine Lozupone, Dae-Wook Kang, and James B. Adams. 2015. “Gut Bacteria in Children with Autism Spectrum Disorders: Challenges and Promise of Studying How a Complex Community Influences a Complex Disease.” *Microbial Ecology in Health & Disease* 26 (0): 26914. https://doi.org/10.3402/mehd.v26.26914.

Krautkramer, Kimberly A., Jing Fan, and Fredrik Bäckhed. 2021. “Gut Microbial Metabolites as

Multi-Kingdom Intermediates." *Nature Reviews Microbiology* 19 (2): 77–94. https://doi.org/10.1038/s41579-020-0438-4.

Krishnakumari, Viswanatha, Nandini Rangaraj, and Ramakrishnan Nagaraj. 2009. "Antifungal Activities of Human Beta-Defensins HBD-1 to HBD-3 and Their C-Terminal Analogs Phd1 to Phd3." *Antimicrobial Agents and Chemotherapy* 53 (1): 256–60. https://doi.org/10.1128/AAC.00470-08.

Kuhn. 2005. "A Short Introduction to the Caret Package. Vieena, Austria." *R Foundation for Statistical Computing*, 1–10.

Kuhn, Max. 2008. "Caret Package." *Journal Of Statistical Software* 28 (5): 1–26. http://www.jstatsoft.org/v28/i05/paper.

Kumamoto, Carol A., Mark S. Gresnigt, and Bernhard Hube. 2020. "The Gut, the Bad and the Harmless: *Candida albicans* as a Commensal and Opportunistic Pathogen in the Intestine." *Current Opinion in Microbiology* 56 (August): 7–15. https://doi.org/10.1016/j.mib.2020.05.006.

Kumar, Rohitashw, Sonia Chadha, Darpan Saraswat, Jashanjot Singh Bajwa, Rui A. Li, Heather R. Conti, and Mira Edgerton. 2011. "Histatin 5 Uptake by *Candida albicans* Utilizes Polyamine Transporters Dur3 and Dur31 Proteins." *Journal of Biological Chemistry* 286 (51): 43748–58. https://doi.org/10.1074/jbc.M111.311175.

Langmead, Ben, and Steven L. Salzberg. 2012. "Fast Gapped-Read Alignment with Bowtie 2." *Nature Methods* 9 (4): 357–59. https://doi.org/10.1038/nmeth.1923.

Latz, Eicke, T. Sam Xiao, and Andrea Stutz. 2013. "Activation and Regulation of the Inflammasomes." *Nature Reviews Immunology*. Nat Rev Immunol. https://doi.org/10.1038/nri3452.

Lawson, Melissa A.E., Ian J. O'Neill, Magdalena Kujawska, Sree Gowrinadh Javvadi, Anisha Wijeyesekera, Zak Flegg, Lisa Chalklen, and Lindsay J. Hall. 2020. "Breast Milk-Derived Human Milk Oligosaccharides Promote Bifidobacterium Interactions within a Single Ecosystem." *ISME Journal* 14 (2): 635–48. https://doi.org/10.1038/s41396-019-0553-2.

Lee, Bonggi, Jashin Koo, Joo Yun Jun, Oksana Gavrilova, Yongjun Lee, Arnold Y. Seo, Dezmond C. Taylor-Douglas, *et al.* 2016. "A Mouse Model for a Partially Inactive Obesity-Associated Human *MC3R* Variant." *Nature Communications* 7. https://doi.org/10.1038/NCOMMS10522.

Lee, Sang A., Ji Ye Lim, Bong Soo Kim, Su Jin Cho, Nak Yon Kim, Ok Bin Kim, and Yuri Kim. 2015. "Comparison of the Gut Microbiota Profile in Breast-Fed and Formula-Fed Korean Infants Using Pyrosequencing." *Nutrition Research and Practice* 9 (3): 242–48. https://doi.org/10.4162/nrp.2015.9.3.242.

Leigh Greathouse, K., Rashmi Sinha, and Emily Vogtmann. 2019. "DNA Extraction for Human Microbiome Studies: The Issue of Standardization." *Genome Biology*. Genome Biol. https://doi.org/10.1186/s13059-019-1843-8.

Leigh, Janet E., Chad Steele, Floyd L. Wormley, Wei Luo, Rebecca A. Clark, William Gallaher, and Paul L. Fidel. 1998. "Th1/Th2 Cytokine Expression in Saliva of HIV-Positive and HIV-Negative Individuals: A Pilot Study in HIV-Positive Individuals with Oropharyngeal

Candidiasis." *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* 19 (4): 373–80. https://doi.org/10.1097/00042560-199812010-00008.

Leonard, Florence, Antoine Andremont, Bernard Leclerq, Roger Labia, and Cyrille Tancrede. 1989. "Use of β-Lactamase-Producing Anaerobes to Prevent Ceftriaxone From Degrading Intestinal Resistance to Colonization." *Journal of Infectious Diseases* 160 (2): 274–80. https://doi.org/10.1093/infdis/160.2.274.

Leonardi, Irina, Iris H. Gao, Woan Yu Lin, Megan Allen, Xin V. Li, William D. Fiers, Meghan Bialt De Celie, *et al.* 2022. "Mucosal Fungi Promote Gut Barrier Function and Social Behavior via Type 17 Immunity." *Cell* 185 (5): 831-846.e14. https://doi.org/10.1016/j.cell.2022.01.017.

Leonardi, Irina, Sudarshan Paramsothy, Itai Doron, Alexa Semon, Nadeem O. Kaakoush, Jose C. Clemente, Jeremiah J. Faith, *et al.* 2020. "Fungal Trans-Kingdom Dynamics Linked to Responsiveness to Fecal Microbiota Transplantation (FMT) Therapy in Ulcerative Colitis." *Cell Host and Microbe* 27 (5): 823-829.e3. https://doi.org/10.1016/j.chom.2020.03.006.

Leung, Chak Ming, Pim de Haan, Kacey Ronaldson-Bouchard, Ge Ah Kim, Jihoon Ko, Hoon Suk Rho, Zhu Chen, *et al.* 2022. "A Guide to the Organ-on-a-Chip." *Nature Reviews Methods Primers* 2 (1): 1–29. https://doi.org/10.1038/s43586-022-00118-6.

Lev-Sagie, Ahinoam, Diana Prus, Iara M. Linhares, Yuval Lavy, William J. Ledger, and Steven S. Witkin. 2009. "Polymorphism in a Gene Coding for the Inflammasome Component NALP3 and Recurrent Vulvovaginal Candidiasis in Women with Vulvar Vestibulitis Syndrome." *American Journal of Obstetrics and Gynecology* 200 (3): 303.e1-303.e6. https://doi.org/10.1016/j.ajog.2008.10.039.

Li, Honghua, Jinpeng Yang, Xinwan Zhang, Xiuli Xu, Fuhang Song, and Hehe Li. 2022. "Biocontrol of *Candida albicans* by Antagonistic Microorganisms and Bioactive Compounds." *Antibiotics* 11 (9). https://doi.org/10.3390/antibiotics11091238.

Liang, Weihong, Guobo Guan, Yu Dai, Chengjun Cao, Li Tao, Han Du, Clarissa J. Nobile, Jin Zhong, and Guanghua Huang. 2016. "Lactic Acid Bacteria Differentially Regulate Filamentation in Two Heritable Cell Types of the Human Fungal Pathogen *Candida albicans*." *Molecular Microbiology* 102 (3): 506–19. https://doi.org/10.1111/mmi.13475.

Lin, Yufeng, Harry Cheuk Hay Lau, Yali Liu, Xing Kang, Yiwei Wang, Nick Lung Ngai Ting, Thomas Ngai Yeung Kwong, *et al.* 2022. "Altered Mycobiota Signatures and Enriched Pathogenic *Aspergillus rambellii* Are Associated With Colorectal Cancer Based on Multicohort Fecal Metagenomic Analyses." *Gastroenterology* 163 (4). https://doi.org/10.1053/j.gastro.2022.06.038.

Lind, Abigail L., and Katherine S. Pollard. 2021. "Accurate and Sensitive Detection of Microbial Eukaryotes from Whole Metagenome Shotgun Sequencing." *Microbiome* 9 (1). https://doi.org/10.1186/s40168-021-01015-y.

Lionakis, Michail S., and Stuart M. Levitz. 2018. "Host Control of Fungal Infections: Lessons from Basic Studies and Human Cohorts." *Annual Review of Immunology*. Annu Rev Immunol. https://doi.org/10.1146/annurev-immunol-042617-053318.

Liu, Bing Nan, Xiao Tong Liu, Zi Han Liang, and Ji Hui Wang. 2021. "Gut Microbiota in Obesity." *World Journal of Gastroenterology*. World J Gastroenterol.

https://doi.org/10.3748/wjg.v27.i25.3837.

Liu, Cindy M., Sergey Kachur, Michael G. Dwan, Alison G. Abraham, Maliha Aziz, Po Ren Hsueh, Yu Tsung Huang, *et al.* 2012. “FungiQuant: A Broad-Coverage Fungal Quantitative Real-Time PCR Assay.” *BMC Microbiology* 12. https://doi.org/10.1186/1471-2180-12-255.

Liu, Luyan, Satoshi Okada, Xiao Fei Kong, Alexandra Y. Kreins, Sophie Cypowyj, Avinash Abhyankar, Julie Toubiana, et al. 2011. “Gain-of-Function Human *STAT1* Mutations Impair IL-17 Immunity and Underlie Chronic Mucocutaneous Candidiasis.” *Journal of Experimental Medicine* 208 (18). https://doi.org/10.1084/jem.20110958.

Lloyd-Price, Jason, Cesar Arze, Ashwin N. Ananthakrishnan, Melanie Schirmer, Julian Avila-Pacheco, Tiffany W. Poon, Elizabeth Andrews, *et al.* 2019. “Multi-Omics of the Gut Microbial Ecosystem in Inflammatory Bowel Diseases.” *Nature* 569 (7758): 655–62. https://doi.org/10.1038/s41586-019-1237-9.

Lofgren, Lotus A., Jessie K. Uehling, Sara Branco, Thomas D. Bruns, Francis Martin, and Peter G. Kennedy. 2019. “Genome-Based Estimates of Fungal RDNA Copy Number Variation across Phylogenetic Scales and Ecological Lifestyles.” *Molecular Ecology* 28 (4): 721–30. https://doi.org/10.1111/MEC.14995.

López-Ribot, José L., Manuel Casanova, Amelia Murgui, and José P. Martínez. 2004. “Antibody Response to *Candida albicans* Cell Wall Antigens.” *FEMS Immunology and Medical Microbiology* 41 (3): 187–96. https://doi.org/10.1016/j.femsim.2004.03.012.

Low, Lucie A., Christine Mummery, Brian R. Berridge, Christopher P. Austin, and Danilo A. Tagle. 2021. “Organs-on-Chips: Into the next Decade.” *Nature Reviews Drug Discovery* 20 (5): 345–61. https://doi.org/10.1038/s41573-020-0079-3.

Macias-Paz, Ignacio Uriel, Salvador Pérez-Hernández, Alejandra Tavera-Tapia, Juan Pedro Luna-Arias, José Eugenio Guerra-Cárdenas, and Elizabeth Reyna-Beltrán. 2022. “*Candida albicans* the Main Opportunistic Pathogenic Fungus in Humans.” *Revista Argentina de Microbiologia*. https://doi.org/10.1016/J.RAM.2022.08.003.

Maeda, Yuichi, Daisuke Motooka, Takahiro Kawasaki, Hiroya Oki, Yoshimi Noda, Yuichi Adachi, Takayuki Niitsu, *et al.* 2022. “Longitudinal Alterations of the Gut Mycobiota and Microbiota on COVID-19 Severity.” *BMC Infectious Diseases* 22 (1): 1–13. https://doi.org/10.1186/s12879-022-07358-7.

Magoč, Tanja, and Steven L. Salzberg. 2011. “FLASH: Fast Length Adjustment of Short Reads to Improve Genome Assemblies.” *Bioinformatics (Oxford, England)* 27 (21): 2957–63. https://doi.org/10.1093/BIOINFORMATICS/BTR507.

Mahajan, Gautam, Erin Doherty, Tania To, Arlene Sutherland, Jennifer Grant, Abidemi Junaid, Aakanksha Gulati, *et al.* 2022. “Vaginal Microbiome-Host Interactions Modeled in a Human Vagina-on-a-Chip.” *Microbiome* 10 (1): 201. https://doi.org/10.1186/s40168-022-01400-1.

Mailänder-Sánchez, Daniela, Christina Braunsdorf, Christian Grumaz, Christoph Müller, Stefan Lorenz, Philip Stevens, Jeanette Wagener, *et al.* 2017. “Antifungal Defense of Probiotic *Lactobacillus rhamnosus* GG Is Mediated by Blocking Adhesion and Nutrient Depletion.” *PLoS ONE* 12 (10). https://doi.org/10.1371/journal.pone.0184438.

Mallick, Himel, Ali Rahnavard, Lauren J. McIver, Siyuan Ma, Yancong Zhang, Long H. Nguyen, Timothy L. Tickle, *et al.* 2021. "Multivariable Association Discovery in Population-Scale Meta-Omics Studies." *PLoS Computational Biology* 17 (11). https://doi.org/10.1371/journal.pcbi.1009442.

Mani-López, Emma, Daniela Arrioja-Bretón, and Aurelio López-Malo. 2022. "The Impacts of Antimicrobial and Antifungal Activity of Cell-Free Supernatants from Lactic Acid Bacteria *in vitro* and Foods." *Comprehensive Reviews in Food Science and Food Safety* 21 (1): 604–41. https://doi.org/10.1111/1541-4337.12872.

Manichanh, C., L. Rigottier-Gois, E. Bonnaud, K. Gloux, E. Pelletier, L. Frangeul, R. Nalin, *et al.* 2006. "Reduced Diversity of Faecal Microbiota in Crohn's Disease Revealed by a Metagenomic Approach." *Gut* 55 (2): 205–11. https://doi.org/10.1136/gut.2005.073817.

Manolio, Teri A. 2010. "Genomewide Association Studies and Assessment of the Risk of Disease." *New England Journal of Medicine* 363 (2): 166–76. https://doi.org/10.1056/nejmra0905980.

Manor, Ohad, Chengzhen L. Dai, Sergey A. Kornilov, Brett Smith, Nathan D. Price, Jennifer C. Lovejoy, Sean M. Gibbons, and Andrew T. Magis. 2020. "Health and Disease Markers Correlate with Gut Microbiome Composition across Thousands of People." *Nature Communications* 11 (1): 1–12. https://doi.org/10.1038/s41467-020-18871-1.

Marcelino, Vanessa R., Philip T.L.C. Clausen, Jan P. Buchmann, Michelle Wille, Jonathan R. Iredell, Wieland Meyer, Ole Lund, Tania C. Sorrell, and Edward C. Holmes. 2020. "CCMetagen: Comprehensive and Accurate Identification of Eukaryotes and Prokaryotes in Metagenomic Data." *Genome Biology* 21 (1): 1–15. https://doi.org/10.1186/s13059-020-02014-2.

Marquis, Miriam, Daniel Lewandowski, Véronique Dugas, Francine Aumont, Serge Sénéchal, Paul Jolicoeur, Zaher Hanna, and Louis De Repentigny. 2006. "CD8+ T Cells but Not Polymorphonuclear Leukocytes Are Required to Limit Chronic Oral Carriage of *Candida albicans* in Transgenic Mice Expressing Human Immunodeficiency Virus Type 1." *Infection and Immunity* 74 (4): 2382–91. https://doi.org/10.1128/IAI.74.4.2382-2391.2006.

Marzorati, Massimo, Barbara Vanhoecke, Tine De Ryck, Mehdi Sadaghian Sadabad, Iris Pinheiro, Sam Possemiers, Pieter Van Den Abbeele, *et al.* 2014. "The HMI™ Module: A New Tool to Study the Host-Microbiota Interaction in the Human Gastrointestinal Tract *in vitro*." *BMC Microbiology* 14 (1). https://doi.org/10.1186/1471-2180-14-133.

Matsubara, Victor Haruo, Yi Wang, H. M.H.N. Bandara, Marcia Pinto Alves Mayer, and Lakshman P. Samaranayake. 2016. "Probiotic Lactobacilli Inhibit Early Stages of *Candida albicans* Biofilm Development by Reducing Their Growth, Cell Adhesion, and Filamentation." *Applied Microbiology and Biotechnology* 100 (14): 6415–26. https://doi.org/10.1007/s00253-016-7527-3.

Matthews, R C, J P Burnie, D Howat, T Rowland, and F Walton. 1991. "Autoantibody to Heat-Shock Protein 90 Can Mediate Protection against Systemic Candidosis." *Immunology* 74 (1): 20–24. http://www.ncbi.nlm.nih.gov/pubmed/1718852%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1384665.

Mayer, François L., Duncan Wilson, and Bernhard Hube. 2013. "*Candida albicans* Pathogenicity Mechanisms." *Virulence* 4 (2): 119–28. https://doi.org/10.4161/viru.22913.

Mayer, François L., Duncan Wilson, Ilse D. Jacobsen, Pedro Miramón, Katharina Große, and Bernhard Hube. 2012. "The Novel *Candida albicans* Transporter Dur31 Is a Multi-Stage Pathogenicity Factor." *PLoS Pathogens* 8 (3). https://doi.org/10.1371/journal.ppat.1002592.

Mencarelli, Monica, Beatrice Dubern, Rohia Alili, Sabrina Maestrini, Lina Benajiba, Mariantonella Tagliaferri, Pilar Galan, *et al.* 2011. "Rare Melanocortin-3 Receptor Mutations with in Vitro Functional Consequences Are Associated with Human Obesity." *Human Molecular Genetics* 20 (2): 392–99. https://doi.org/10.1093/hmg/ddq472.

Meng, Changting, Chunmei Bai, Thomas D. Brown, Leroy E. Hood, and Qiang Tian. 2018. "Human Gut Microbiota and Gastrointestinal Cancer." *Genomics, Proteomics and Bioinformatics* 16 (1): 33–49. https://doi.org/10.1016/j.gpb.2017.06.002.

Miao, Jian, Hubertine M.E. Willems, and Brian M. Peters. 2021. "Exogenous Reproductive Hormones nor *Candida albicans* Colonization Alter the near Neutral Mouse Vaginal PH." *Infection and Immunity* 89 (2). https://doi.org/10.1128/IAI.00550-20.

Milani, Christian, Andrea Ticinesi, Jacoline Gerritsen, Antonio Nouvenne, Gabriele Andrea Lugli, Leonardo Mancabelli, Francesca Turroni, *et al.* 2016. "Gut Microbiota Composition and *Clostridium difficile* Infection in Hospitalized Elderly Individuals: A Metagenomic Study." *Scientific Reports* 6 (May): 25945. https://doi.org/10.1038/srep25945.

Milner, Joshua D., Jason M. Brenchley, Arian Laurence, Alexandra F. Freeman, Brenna J. Hill, Kevin M. Elias, Yuka Kanno, *et al.* 2008. "Impaired Th17 Cell Differentiation in Subjects with Autosomal Dominant Hyper-IgE Syndrome." *Nature* 452 (7188): 773–76. https://doi.org/10.1038/nature06764.

Mimetas. 2019. "Human Tissue Models for Better Therapies." 2019. https://www.mimetas.com/en/home/.

Min, Kyunghun, Aaron M. Neiman, and James B. Konopka. 2020. "Fungal Pathogens: Shape-Shifting Invaders." *Trends in Microbiology* 28 (11): 922–33. https://doi.org/10.1016/j.tim.2020.05.001.

Minegishi, Yoshiyuki, Masako Saito, Shigeru Tsuchiya, Ikuya Tsuge, Hidetoshi Takada, Toshiro Hara, Nobuaki Kawamura, *et al.* 2007. "Dominant-Negative Mutations in the DNA-Binding Domain of STAT3 Cause Hyper-IgE Syndrome." *Nature* 448 (7157): 1058–62. https://doi.org/10.1038/nature06096.

Miramón, Pedro, Christine Dunker, Hanna Windecker, Iryna M. Bohovych, Alistair J.P. Brown, Oliver Kurzai, and Bernhard Hube. 2012. "Cellular Responses of *Candida albicans* to Phagocytosis and the Extracellular Activities of Neutrophils Are Critical to Counteract Carbohydrate Starvation, Oxidative and Nitrosative Stress." *PLoS ONE* 7 (12). https://doi.org/10.1371/journal.pone.0052850.

Miramón, Pedro, and Michael C. Lorenz. 2017. *A Feast for Candida: Metabolic Plasticity Confers an Edge for Virulence*. *PLoS Pathogens*. Vol. 13. Public Library of Science. https://doi.org/10.1371/journal.ppat.1006144.

Miramón, Pedro, Andrew W. Pountain, Ambro van Hoof, and Michael C. Lorenz. 2020. “The Paralogous Transcription Factors Stp1 and Stp2 of *Candida albicans* Have Distinct Functions in Nutrient Acquisition and Host Interaction.” *Infection and Immunity* 88 (5). https://doi.org/10.1128/IAI.00763-19.

Mirhakkak, Mohammad H., Sascha Schäuble, Tilman E. Klassert, Sascha Brunke, Philipp Brandt, Daniel Loos, Ruben V. Uribe, *et al.* 2021. “Metabolic Modeling Predicts Specific Gut Bacteria as Key Determinants for *Candida albicans* Colonization Levels.” *ISME Journal* 15 (5): 1257–70. https://doi.org/10.1038/s41396-020-00848-z.

Mishima, Riko, Masaru Tanaka, Rie Momoda, Masafumi Sanefuji, Seiichi Morokuma, Masanobu Ogawa, Kiyoko Kato, and Jiro Nakayama. 2023. “Longitudinal Gut Mycobiota Changes in Japanese Infants during First Three Years of Life.” *Journal of Bioscience and Bioengineering*, February. https://doi.org/10.1016/j.jbiosc.2023.01.007.

Misme-Aucouturier, Barbara, Adel Touahri, Marjorie Albassier, Francine Jotereau, Patrice Le Pape, and Nidia Alvarez-Rueda. 2019. “Double Positive CD4+CD8+ T Cells Are Part of the Adaptive Immune Response against *Candida albicans*.” *Human Immunology* 80 (12): 999–1005. https://doi.org/10.1016/j.humimm.2019.09.008.

Mossavar-Rahmani, Yasmin, Lesley F. Tinker, Ying Huang, Marian L. Neuhouser, Susan E. McCann, Rebecca A. Seguin, Mara Z. Vitolins, J. David Curb, and Ross L. Prentice. 2013. “Factors Relating to Eating Style, Social Desirability, Body Image and Eating Meals at Home Increase the Precision of Calibration Equations Correcting Self-Report Measures of Diet Using Recovery Biomarkers: Findings from the Women’s Health Initiative.” *Nutrition Journal* 12 (1): 1–14. https://doi.org/10.1186/1475-2891-12-63.

Motooka, Daisuke, Kosuke Fujimoto, Reiko Tanaka, Takashi Yaguchi, Kazuyoshi Gotoh, Yuichi Maeda, Yoki Furuta, *et al.* 2017. “Fungal ITS1 Deep-Sequencing Strategies to Reconstruct the Composition of a 26-Species Community and Evaluation of the Gut Mycobiota of Healthy Japanese Individuals.” *Frontiers in Microbiology* 8 (FEB). https://doi.org/10.3389/fmicb.2017.00238.

Naglik, Julian R., Annika König, Bernhard Hube, and Sarah L. Gaffen. 2017. “*Candida albicans*–Epithelial Interactions and Induction of Mucosal Innate Immunity.” *Current Opinion in Microbiology* 40 (December): 104–12. https://doi.org/10.1016/j.mib.2017.10.030.

Nahum, Amit, Harjit Dadi, Andrea Bates, and Chaim M. Roifman. 2011. “The L412F Variant of Toll-like Receptor 3 (*TLR3*) Is Associated with Cutaneous Candidiasis, Increased Susceptibility to Cytomegalovirus, and Autoimmunity.” *Journal of Allergy and Clinical Immunology* 127 (2): 528–31. https://doi.org/10.1016/j.jaci.2010.09.031.

———. 2012. “The Biological Significance of TLR3 Variant, L412F, in Conferring Susceptibility to Cutaneous Candidiasis, CMV and Autoimmunity.” *Autoimmunity Reviews* 11 (5): 341–47. https://doi.org/10.1016/j.autrev.2011.10.007.

Narunsky-Haziza, Lian, Gregory D. Sepich-Poore, Ilana Livyatan, Omer Asraf, Cameron Martino, Deborah Nejman, Nancy Gavert, *et al.* 2022. “Pan-Cancer Analyses Reveal Cancer-Type-Specific Fungal Ecologies and Bacteriome Interactions.” *Cell* 185 (20): 3789-3806.e17. https://doi.org/10.1016/J.CELL.2022.09.005.

Nash, Andrea K., Thomas A. Auchtung, Matthew C. Wong, Daniel P. Smith, Jonathan R. Gesell, Matthew C. Ross, Christopher J. Stewart, *et al.* 2017. “The Gut Mycobiome of the Human Microbiome Project Healthy Cohort.” *Microbiome* 5 (1): 153. https://doi.org/10.1186/S40168-017-0373-4.

Natividad, Jane Mea, Benoît Marsaux, Clara Lucia Garcia Rodenas, Andreas Rytz, Gies Vandevijver, Massimo Marzorati, Pieter Van den Abbeele, Marta Calatayud, and Florence Rochat. 2022. “Human Milk Oligosaccharides and Lactose Differentially Affect Infant Gut Microbiota and Intestinal Barrier In Vitro.” *Nutrients* 14 (12): 2546. https://doi.org/10.3390/nu14122546.

Netea, Mihai G., Gordon D. Brown, Bart Jan Kullberg, and Neil A.R. Gow. 2008. “An Integrated Model of the Recognition of *Candida albicans* by the Innate Immune System.” *Nature Reviews Microbiology*. Nature Publishing Group. https://doi.org/10.1038/nrmicro1815.

Netea, Mihai G., Chantal A.A. Van der Graaf, Alieke G. Vonk, Ineke Verschueren, Jos W.M. Van der Meet, and Bart Jan Kullberg. 2002. “The Role of Toll-like Receptor (TLR) 2 and TLR4 in the Host Defense against Disseminated Candidiasis.” *Journal of Infectious Diseases* 185 (10): 1483–89. https://doi.org/10.1086/340511.

Netea, Mihai G., Leo A.B. Joosten, Jos W.M. Van Der Meer, Bart Jan Kullberg, and Frank L. Van De Veerdonk. 2015. “Immune Defence against *Candida* Fungal Infections.” *Nature Reviews Immunology 2015 15:10* 15 (10): 630–42. https://doi.org/10.1038/nri3897.

Netea, Mihai G., Jessica Quintin, and Jos W.M. Van Der Meer. 2011. “Trained Immunity: A Memory for Innate Host Defense.” *Cell Host and Microbe* 9 (5): 355–61. https://doi.org/10.1016/j.chom.2011.04.006.

Neville, B. Anne, Christophe d’Enfert, and Marie-Elisabeth Bougnoux. 2015. “*Candida Albicans* Commensalism in the Gastrointestinal Tract.” Edited by Carol Munro. *FEMS Yeast Research* 15 (7): fov081. https://doi.org/10.1093/femsyr/fov081.

Nguyen, Long Nam, Livia Cristina Liporagi Lopes, Radames J.B. Cordero, and Joshua D. Nosanchuk. 2011. “Sodium Butyrate Inhibits Pathogenic Yeast Growth and Enhances the Functions of Macrophages.” *Journal of Antimicrobial Chemotherapy* 66 (11): 2573–80. https://doi.org/10.1093/jac/dkr358.

Niehus, Rene, Esther van Kleef, Yin Mo, Agata Turlej-Rogacka, Christine Lammens, Yehuda Carmeli, Herman Goossens, *et al.* 2020. “Quantifying Antibiotic Impact on Within-Patient Dynamics of Extended-Spectrum β-Lactamase Resistance.” *ELife* 9 (May): 1–20. https://doi.org/10.7554/eLife.49206.

Nikou, Spyridoula Angeliki, Nessim Kichik, Rhys Brown, Nicole O. Ponde, Jemima Ho, Julian R. Naglik, and Jonathan P. Richardson. 2019. “*Candida albicans* Interactions with Mucosal Surfaces during Health and Disease.” *Pathogens* 8 (2): 53. https://doi.org/10.3390/pathogens8020053.

Noverr, Mairi C., and Gary B. Huffnagle. 2004. “Regulation of *Candida albicans* Morphogenesis by Fatty Acid Metabolites.” *Infection and Immunity* 72 (11): 6206–10. https://doi.org/10.1128/IAI.72.11.6206-6210.2004.

Nucci, Marcio, and Elias Anaissie. 2001. “Revisiting the Source of Candidemia: Skin or Gut?” *Clinical Infectious Diseases* 33 (12): 1959–67. https://doi.org/10.1086/323759.

Odamaki, Toshitaka, Kumiko Kato, Hirosuke Sugahara, Nanami Hashikura, Sachiko Takahashi, Jin Zhong Xiao, Fumiaki Abe, and Ro Osawa. 2016. “Age-Related Changes in Gut Microbiota Composition from Newborn to Centenarian: A Cross-Sectional Study.” *BMC Microbiology* 16 (1). https://doi.org/10.1186/s12866-016-0708-5.

Ohland, Christina L., and Christian Jobin. 2015. “Microbial Activities and Intestinal Homeostasis: A Delicate Balance Between Health and Disease.” *Cellular and Molecular Gastroenterology and Hepatology* 1 (1): 28–40. https://doi.org/10.1016/j.jcmgh.2014.11.004.

Oikarinen, Sami, Sisko Tauriainen, Hanna Viskari, Olli Simell, Mikael Knip, Suvi Virtanen, and Heikki Hyöty. 2009. “PCR Inhibition in Stool Samples in Relation to Age of Infants.” *Journal of Clinical Virology* 44 (3): 211–14. https://doi.org/10.1016/j.jcv.2008.12.017.

Okada, Satoshi, Takaki Asano, Kunihiko Moriya, Stephanie Boisson-Dupuis, Masao Kobayashi, Jean Laurent Casanova, and Anne Puel. 2020. “Human STAT1 Gain-of-Function Heterozygous Mutations: Chronic Mucocutaneous Candidiasis and Type I Interferonopathy.” *Journal of Clinical Immunology*. https://doi.org/10.1007/s10875-020-00847-x.

Oksanen, Author Jari, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan Mcglinn, Peter R Minchin, *et al.* 2019. “Package ‘ Vegan .’” *R Package Library*. https://cran.r-project.org/web/packages/vegan/index.html.

Olaisen, Maya, Mathias L. Richard, Vidar Beisvåg, Atle van Beelen Granlund, Elin S. Røyset, Olivier Rué, Tom Christian Martinsen, Arne Kristian Sandvik, Harry Sokol, and Reidar Fossmark. 2022. “The Ileal Fungal Microbiota Is Altered in Crohn’s Disease and Is Associated with the Disease Course.” *Frontiers in Medicine* 9: 2893. https://doi.org/10.3389/fmed.2022.868812.

Olivier, Françios A.B., Volker Hilsenstein, Harshini Weerasinghe, Ashley Weir, Sebastian Hughes, Simon Crawford, James E. Vince, Michael J. Hickey, and Ana Traven. 2022. “The Escape of *Candida albicans* from Macrophages Is Enabled by the Fungal Toxin Candidalysin and Two Host Cell Death Pathways.” *Cell Reports* 40 (12): 111374. https://doi.org/10.1016/j.celrep.2022.111374.

Oñate, Florian Plaza, Emmanuelle Le Chatelier, Mathieu Almeida, Alessandra C.L. Cervino, Franck Gauthier, Frédéric Magoulès, S. Dusko Ehrlich, and Matthieu Pichaud. 2019. “MSPminer: Abundance-Based Reconstitution of Microbial Pan-Genomes from Shotgun Metagenomic Data.” *Bioinformatics* 35 (9): 1544–52. https://doi.org/10.1093/bioinformatics/bty830.

Ouederni, Monia, Ozden Sanal, Aydan Ikincioğullari, Ilhan Tezcan, Figen Dogu, Ithaisa Sologuren, Sigifredo Pedraza-Sánchez, *et al.* 2014. “Clinical Features of Candidiasis in Patients with Inherited Interleukin 12 Receptor B1 Deficiency.” *Clinical Infectious Diseases* 58 (2): 204–13. https://doi.org/10.1093/cid/cit722.

Pande, Kalyan, Changbin Chen, and Suzanne M. Noble. 2013. “Passage through the Mammalian Gut Triggers a Phenotypic Switch That Promotes *Candida albicans* Commensalism.” *Nature Genetics* 45 (9): 1088–91. https://doi.org/10.1038/ng.2710.

Papayannopoulos, Venizelos. 2018. “Neutrophil Extracellular Traps in Immunity and Disease.”

Nature Reviews Immunology 18 (2): 134–47. https://doi.org/10.1038/nri.2017.105.

Papon, Nicolas, Marie Elisabeth Bougnoux, and Christophe d'Enfert. 2020. "Tracing the Origin of Invasive Fungal Infections." *Trends in Microbiology*. Trends Microbiol. https://doi.org/10.1016/j.tim.2020.01.007.

Pappas, Peter G., Michail S. Lionakis, Maiken Cavling Arendrup, Luis Ostrosky-Zeichner, and Bart Jan Kullberg. 2018. "Invasive Candidiasis." *Nature Reviews Disease Primers* 4 (May). https://doi.org/10.1038/nrdp.2018.26.

Pappas, Peter G, Carol A Kauffman, David R Andes, Cornelius J Clancy, Kieren A Marr, Luis Ostrosky-Zeichner, Annette C Reboli, *et al.* 2016. "Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America" *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* 62 (4): e1--e50. https://doi.org/10.1093/cid/civ933.

Pareek, Siddhika, Takashi Kurakawa, Bhabatosh Das, Daisuke Motooka, Shuuichi Nakaya, Temsunaro Rongsen-Chandola, Nidhi Goyal, *et al.* 2019. "Comparison of Japanese and Indian Intestinal Microbiota Shows Diet-Dependent Interaction between Bacteria and Fungi." *Npj Biofilms and Microbiomes* 5 (1). https://doi.org/10.1038/s41522-019-0110-9.

Park, Byung Lae, Lyoung Hyo Kim, Suhg Namgoong, Ji On Kim, Jason Yongha Kim, Hun Soo Chang, Jong Sook Park, *et al.* 2014. "Association Analysis of Melanocortin 3 Receptor Polymorphisms with the Risk of Pulmonary Tuberculosis." *Lung* 192 (6): 857–62. https://doi.org/10.1007/s00408-014-9625-2.

Parm, Ulle, Tuuli Metsvaht, Epp Sepp, Mari Liis Ilmoja, Heti Pisarev, Merit Pauskar, and Irja Lutsar. 2011. "Risk Factors Associated with Gut and Nasopharyngeal Colonization by Common Gram-Negative Species and Yeasts in Neonatal Intensive Care Units Patients." *Early Human Development* 87 (6): 391–99. https://doi.org/10.1016/j.earlhumdev.2011.02.007.

Parolin, Carola, Vanessa Croatti, Barbara Giordani, and Beatrice Vitali. 2022. "Vaginal Lactobacillus Impair *Candida*Dimorphic Switching and Biofilm Formation." *Microorganisms* 10 (10). https://doi.org/10.3390/microorganisms10102091.

Partula, Valentin, Stanislas Mondot, Marion J. Torres, Emmanuelle Kesse-Guyot, Mélanie Deschasaux, Karen Assmann, Paule Latino-Martel, *et al.* 2019. "Associations between Usual Diet and Gut Microbiota Composition: Results from the Milieu Intérieur Cross-Sectional Study." *American Journal of Clinical Nutrition* 109 (5): 1472–83. https://doi.org/10.1093/ajcn/nqz029.

Pascal, Victoria, Marta Pozuelo, Natalia Borruel, Francesc Casellas, David Campos, Alba Santiago, Xavier Martinez, *et al.* 2017. "A Microbial Signature for Crohn's Disease." *Gut* 66 (5): 813–22. https://doi.org/10.1136/gutjnl-2016-313235.

Patin, Etienne, Milena Hasan, Jacob Bergstedt, Vincent Rouilly, Valentina Libri, Alejandra Urrutia, Cécile Alanio, *et al.* 2018. "Natural Variation in the Parameters of Innate Immune Cells Is Preferentially Driven by Genetic Factors Resource." *Nature Immunology* 19 (3): 302–14. https://doi.org/10.1038/s41590-018-0049-7.

Payne, Samantha, Glenn Gibson, Anthony Wynne, Barry Hudspith, Jonathan Brostoff, and Kieran Tuohy. 2003. "In Vitro Studies on Colonization Resistance of the Human Gut

Microbiota to *Candida albicans* and the Effects of Tetracycline and *Lactobacillus plantarum* LPK." *Current Issues in Intestinal Microbiology* 4 (1): 1–8. https://europepmc.org/article/med/12691257.

Perrone, Giancarlo, and Antonia Gallo. 2017. "Aspergillus Species and Their Associated Mycotoxins." *Methods in Molecular Biology* 1542: 33–49. https://doi.org/10.1007/978-1-4939-6707-0_3.

Peterson, Danielle, Kevin S. Bonham, Sophie Rowland, Cassandra W. Pattanayak, Vanja Klepac-Ceraj, Sean C.L. Deoni, Viren D'Sa, *et al.* 2021. "Comparative Analysis of 16S RRNA Gene and Metagenome Sequencing in Pediatric Gut Microbiomes." *Frontiers in Microbiology* 12 (July): 670336. https://doi.org/10.3389/fmicb.2021.670336.

Pham, Linh N., Marc S. Dionne, Mimi Shirasu-Hiza, and David S. Schneider. 2007. "A Specific Primed Immune Response in Drosophila Is Dependent on Phagocytes." *PLoS Pathogens* 3 (3). https://doi.org/10.1371/journal.ppat.0030026.

Piasecka, Barbara, Darragh Duffy, Alejandra Urrutia, Hélène Quach, Etienne Patin, Céline Posseme, Jacob Bergstedt, *et al.* 2018. "Distinctive Roles of Age, Sex, and Genetics in Shaping Transcriptional Variation of Human Immune Responses to Microbial Challenges." *Proceedings of the National Academy of Sciences of the United States of America* 115 (3): E488–97. https://doi.org/10.1073/pnas.1714765115.

Pitarch, Aida, Antonio Jiménez, César Nombela, and Concha Gil. 2006. "Decoding Seroligical Response to *Candida*Cell Wall Immunome into Novel Diagnostic, Prognostic, and Therapeutic Candidates for Systemic Candidiasis by Proteomic and Bioinformatic Analyses." *Molecular and Cellular Proteomics* 5 (1): 79–96. https://doi.org/10.1074/mcp.M500243-MCP200.

Plantinga, Theo S., Melissa D. Johnson, William K. Scott, Leo A.B. Joosten, Jos W.M. Van Der Meer, John R. Perfect, Bart Jan Kullberg, and Mihai G. Netea. 2012. "Human Genetic Susceptibility to *Candida* Infections." *Medical Mycology*. Oxford Academic. https://doi.org/10.3109/13693786.2012.690902.

Plantinga, Theo S., Melissa D. Johnson, William K. Scott, Esther Van De Vosse, Digna R. Velez Edwards, P. Brian Smith, Barbara D. Alexander, *et al.* 2012. "Toll-like Receptor 1 Polymorphisms Increase Susceptibility to Candidemia." *Journal of Infectious Diseases* 205 (6): 934–43. https://doi.org/10.1093/infdis/jir867.

Plantinga, Theo S., Walter J.F.M. Van Der Velden, Bart Ferwerda, Annemiek B. Van Spriel, Gosse Adema, Ton Feuth, J. Peter Donnelly, *et al.* 2009. "Early Stop Polymorphism in Human *DECTIN-1* Is Associated with Increased *Candida* Colonization in Hematopoietic Stem Cell Transplant Recipients." *Clinical Infectious Diseases* 49 (5): 724–32. https://doi.org/10.1086/604714.

Plaza Onate, Florian, Nicolas Pons, Franck Gauthier, Mathieu Almeida, Stanislav Dusko Ehrlich, and Emmanuelle Le Chatelier. 2021. "Updated Metagenomic Species Pan-Genomes (MSPs) of the Human Gastrointestinal Microbiota." https://entrepot.recherche.data.gouv.fr/dataset.xhtml?persistentId=doi:10.15454/FLANUP%0Ahttps://doi.org/10.15454/FLANUP.

Pons, Nicolas, Jean-Michel Batto, Sean Kennedy, Mathieu Almeida, Fouad Boumezbeur,

Bouziane Moumen, Pierre Leonard, *et al.* 2010. *METEOR -a Plateform for Quantitative Metagenomic Profiling of Complex Ecosystems*.

Porcaro, Isabelle, Michel Vidal, Sylvie Jouvert, Philip D. Stahl, and Jean Giaimis. 2003. " Mannose Receptor Contribution to *Candida albicans* Phagocytosis by Murine E-Clone J774 Macrophages ." *Journal of Leukocyte Biology* 74 (2): 206–15. https://doi.org/10.1189/jlb.1202608.

Poupet, Cyril, Taous Saraoui, Philippe Veisseire, Muriel Bonnet, Caroline Dausset, Marylise Gachinat, Olivier Camarès, Christophe Chassard, Adrien Nivoliez, and Stéphanie Bornes. 2019. "*Lactobacillus rhamnosus* Lcr35 as an Effective Treatment for Preventing *Candida albicans* Infection in the Invertebrate Model *Caenorhabditis elegans*: First Mechanistic Insights." *PloS One* 14 (11). https://doi.org/10.1371/JOURNAL.PONE.0216184.

Pradhan, Arnab, Gabriela M. Avelar, Judith M. Bain, Delma S. Childers, Daniel E. Larcombe, Mihai G. Netea, Elena Shekhova, *et al.* 2018. "Hypoxia Promotes Immune Evasion by Triggering β-Glucan Masking on the *Candida albicans* Cell Surface via Mitochondrial and CAMP-Protein Kinase A Signaling." *MBio* 9 (6). https://doi.org/10.1128/mBio.01318-18.

Prieto, Daniel, Elvira Román, Rebeca Alonso-Monge, and Jesús Pla. 2017. "Overexpression of the Transcriptional Regulator *WOR1* Increases Susceptibility to Bile Salts and Adhesion to the Mouse Gut Mucosa in *Candida albicans*." *Frontiers in Cellular and Infection Microbiology* 7 (SEP): 389. https://doi.org/10.3389/fcimb.2017.00389.

Puel, Anne. 2020. "Human Inborn Errors of Immunity Underlying Superficial or Invasive Candidiasis." *Human Genetics*. Springer. https://doi.org/10.1007/s00439-020-02141-7.

Puel, Anne, Sophie Cypowyj, Jacinta Bustamante, Jill F. Wright, Luyan Liu, Hye Kyung Lim, Mélanie Migaud, *et al.* 2011. "Chronic Mucocutaneous Candidiasis in Humans with Inborn Errors of Interleukin-17 Immunity." *Science* 332 (6025): 65–68. https://doi.org/10.1126/science.1200439.

Puri, Sumant, William K.M. Lai, Jason M. Rizzo, Michael J. Buck, and Mira Edgerton. 2014. "Iron-Responsive Chromatin Remodelling and MAPK Signalling Enhance Adhesion in *Candida albicans*." *Molecular Microbiology* 93 (2): 291–305. https://doi.org/10.1111/mmi.12659.

Qin, Junjie, Ruiqiang Li, Jeroen Raes, Manimozhiyan Arumugam, Kristoffer Solvsten Burgdorf, Chaysavanh Manichanh, Trine Nielsen, *et al.* 2010. "A Human Gut Microbial Gene Catalogue Established by Metagenomic Sequencing." *Nature* 464 (7285): 59–65. https://doi.org/10.1038/nature08821.

Quintin, Jessica, Sadia Saeed, Joost H.A. Martens, Evangelos J. Giamarellos-Bourboulis, Daniela C. Ifrim, Colin Logie, Liesbeth Jacobs, *et al.* 2012. "*Candida albicans* Infection Affords Protection against Reinfection via Functional Reprogramming of Monocytes." *Cell Host and Microbe* 12 (2): 223–32. https://doi.org/10.1016/j.chom.2012.06.006.

Quintin, Jessica, Jessica Voigt, Robbert van der Voort, Ilse D. Jacobsen, Ineke Verschueren, Bernhard Hube, Evangelos J. Giamarellos-Bourboulis, *et al.* 2014. "Differential Role of NK Cells against *Candida albicans* Infection in Immunocompetent or Immunocompromised Mice." *European Journal of Immunology* 44 (8): 2405–14. https://doi.org/10.1002/eji.201343828.

R Core Team. 2023. “R: A Language and Environment for Statistical Computing.” Accessed September 10, 2019. https://www.gbif.org/tool/81287/r-a-language-and-environment-for-statistical-computing.

Rai, Laxmi Shanker, Lasse Van Wijlick, Marie Elisabeth Bougnoux, Sophie Bachellier-Bassi, and Christophe d’Enfert. 2021. “Regulators of Commensal and Pathogenic Life-Styles of an Opportunistic Fungus—*Candida albicans*.” *Yeast* 38 (4): 243–50. https://doi.org/10.1002/yea.3550.

Raimondi, Stefano, Alberto Amaretti, Caterina Gozzoli, Marta Simone, Lucia Righini, Francesco Candeliere, Paola Brun, *et al.* 2019. “Longitudinal Survey of Fungi in the Human Gut: ITS Profiling, Phenotyping, and Colonization.” *Frontiers in Microbiology* 10. https://doi.org/10.3389/FMICB.2019.01575.

Ramirez-Ortiz, Zaida G., and Terry K. Means. 2012. “The Role of Dendritic Cells in the Innate Recognition of Pathogenic Fungi (*A. fumigatus*, *C. neoformans* and *C. albicans*).” *Virulence* 3 (7). https://doi.org/10.4161/viru.22295.

Ramírez-Zavala, Bernardo, Austin Mottola, Julia Haubenreißer, Sabrina Schneider, Stefanie Allert, Sascha Brunke, Knut Ohlsen, Bernhard Hube, and Joachim Morschhäuser. 2017. “The Snf1-Activating Kinase Sak1 Is a Key Regulator of Metabolic Adaptation and *in vivo* Fitness of *Candida albicans*.” *Molecular Microbiology* 104 (6): 989–1007. https://doi.org/10.1111/mmi.13674.

Revelle, William. 2021. “Psych: Procedures for Psychological, Psychometric, and Personality Research.” Comprehensive R Archive Network (CRAN). https://cran.r-project.org/web/packages/psych/psych.pdf.

Reyman, Marta, Marlies A. van Houten, Debbie van Baarle, Astrid A.T.M. Bosch, Wing Ho Man, Mei Ling J.N. Chu, Kayleigh Arp, *et al.* 2019. “Impact of Delivery Mode-Associated Gut Microbiota Dynamics on Health in the First Year of Life.” *Nature Communications* 10 (1). https://doi.org/10.1038/s41467-019-13014-7.

Ricci, Liviana, Joanna Mackie, Gillian E. Donachie, Ambre Chapuis, Kristýna Mezerová, Megan D. Lenardon, Alistair J.P. Brown, Sylvia H. Duncan, and Alan W. Walker. 2022. “Human Gut Bifidobacteria Inhibit the Growth of the Opportunistic Fungal Pathogen *Candida albicans*.” *FEMS Microbiology Ecology* 98 (10). https://doi.org/10.1093/femsec/fiac095.

Richard, Mathias L., and Harry Sokol. 2019. “The Gut Mycobiota: Insights into Analysis, Environmental Interactions and Role in Gastrointestinal Diseases.” *Nature Reviews Gastroenterology & Hepatology* 16 (6): 331–45. https://doi.org/10.1038/s41575-019-0121-2.

Richardson, Jonathan P., Jemima Ho, and Julian R. Naglik. 2018. “*Candida*–Epithelial Interactions.” *Journal of Fungi* 4 (1): 22. https://doi.org/10.3390/jof4010022.

Richardson, Jonathan P., and David L. Moyes. 2015. “Adaptive Immune Responses to *Candida albicans* Infection.” *Virulence* 6 (4): 327–37. https://doi.org/10.1080/21505594.2015.1004977.

Rizzo, Antonietta, Antonio Losacco, and Caterina Romano Carratelli. 2013. “*LactobacilluscCrispatus* Modulates Epithelial Cell Defense against *Candida albicans*

through Toll-like Receptors 2 and 4, Interleukin 8 and Human β-Defensins 2 and 3." *Immunology Letters* 156 (1–2): 102–9. https://doi.org/10.1016/j.imlet.2013.08.013.

Rodrigues, Janneth, Fábio André Brayner, Luiz Carlos Alves, Rajnikant Dixit, and Carolina Barillas-Mury. 2010. "Hemocyte Differentiation Mediates Innate Immune Memory in *Anopheles gambiae* Mosquitoes." *Science* 329 (5997): 1353–55. https://doi.org/10.1126/science.1190689.

Rogiers, Ona, Ulrika C. Frising, Soňa Kucharíková, Mary Ann Jabra-Rizk, Geert van Loo, Patrick Van Dijck, and Andy Wullaert. 2019. "Candidalysin Crucially Contributes to Nlrp3 Inflammasome Activation by *Candida albicans* Hyphae." *MBio* 10 (1). https://doi.org/10.1128/mBio.02221-18.

Rosales, Carlos. 2018. "Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types?" *Frontiers in Physiology*. Frontiers Media SA. https://doi.org/10.3389/fphys.2018.00113.

Rosati, Diletta, Mariolina Bruno, Martin Jaeger, Jaap Ten Oever, and Mihai G. Netea. 2020. "Recurrent Vulvovaginal Candidiasis: An Immunological Perspective." *Microorganisms*. Microorganisms. https://doi.org/10.3390/microorganisms8020144.

Rothschild, Daphna, Omer Weissbrod, Elad Barkan, Alexander Kurilshikov, Tal Korem, David Zeevi, Paul I. Costea, *et al.* 2018. "Environment Dominates over Host Genetics in Shaping Human Gut Microbiota." *Nature* 555 (7695): 210–15. https://doi.org/10.1038/nature25973.

Routy, Bertrand, Emmanuelle Le Chatelier, Lisa Derosa, Connie P.M. Duong, Maryam Tidjani Alou, Romain Daillère, Aurélie Fluckiger, *et al.* 2018. "Gut Microbiome Influences Efficacy of PD-1-Based Immunotherapy against Epithelial Tumors." *Science* 359 (6371). https://doi.org/10.1126/science.aan3706.

Samaranayake, Lakshman P. 1992. "Oral Mycoses in HIV Infection." *Oral Surgery, Oral Medicine, Oral Pathology* 73 (2): 171–80. https://doi.org/10.1016/0030-4220(92)90191-R.

Sanglard, Dominique. 2016. "Emerging Threats in Antifungal-Resistant Fungal Pathogens." *Frontiers in Medicine* 3: 11. https://doi.org/10.3389/fmed.2016.00011.

Sanguinetti, Maurizio, Brunella Posteraro, and Cornelia Lass-Flörl. 2015. "Antifungal Drug Resistance among *Candida* Species: Mechanisms and Clinical Impact." *Mycoses* 58 (S2): 2–13. https://doi.org/10.1111/myc.12330.

Santiago, Alba, Suchita Panda, Griet Mengels, Xavier Martinez, Fernando Azpiroz, Joel Dore, Francisco Guarner, and Chaysavanh Manichanh. 2014. "Processing Faecal Samples: A Step Forward for Standards in Microbial Community Analysis." *BMC Microbiology* 14 (1): 1–9. https://doi.org/10.1186/1471-2180-14-112.

Santos, Ana Carolina Costa, Serena Mares Malta, Raquel Cristina Cavalcanti Dantas, Nina Dias Coelho Rocha, Vasco Ariston de Carvalho Azevedo, and Carlos Ueira-Vieira. 2022. "Antimicrobial Activity of Supernatants Produced by Bacteria Isolated from Brazilian Stingless Bee's Larval Food." *BMC Microbiology* 22 (1): 1–9. https://doi.org/10.1186/s12866-022-02548-4.

Scepanovic, Petar, Cécile Alanio, Christian Hammer, Flavia Hodel, Jacob Bergstedt, Etienne Patin, Christian W. Thorball, *et al.* 2018. “Human Genetic Variants and Age Are the Strongest Predictors of Humoral Immune Responses to Common Pathogens and Vaccines.” *Genome Medicine* 10 (1): 59. https://doi.org/10.1186/s13073-018-0568-8.

Schaid, Daniel J., Wenan Chen, and Nicholas B. Larson. 2018a. “From Genome-Wide Associations to Candidate Causal Variants by Statistical Fine-Mapping.” *Nature Reviews Genetics 2018 19:8* 19 (8): 491–504. https://doi.org/10.1038/s41576-018-0016-z.

———. 2018b. “From Genome-Wide Associations to Candidate Causal Variants by Statistical Fine-Mapping.” *Nature Reviews Genetics 2018 19:8* 19 (8): 491–504. https://doi.org/10.1038/s41576-018-0016-z.

Schei, Kasper, Ekaterina Avershina, Torbjørn Øien, Knut Rudi, Turid Follestad, Saideh Salamati, and Rønnaug Astri Ødegård. 2017. “Early Gut Mycobiota and Mother-Offspring Transfer.” *Microbiome* 5 (1): 107. https://doi.org/10.1186/s40168-017-0319-x.

Schnorr, Stephanie L., Marco Candela, Simone Rampelli, Manuela Centanni, Clarissa Consolandi, Giulia Basaglia, Silvia Turroni, *et al.* 2014. “Gut Microbiome of the Hadza Hunter-Gatherers.” *Nature Communications* 5 (April). https://doi.org/10.1038/ncomms4654.

Schwager, Emma, Himel Mallick, Steffen Ventz, and Curtis Huttenhower. 2017. “A Bayesian Method for Detecting Pairwise Associations in Compositional Data.” *PLoS Computational Biology* 13 (11). https://doi.org/10.1371/JOURNAL.PCBI.1005852.

Seelbinder, Bastian, Jiarui Chen, Sascha Brunke, Ruben Vazquez-Uribe, Rakesh Santhaman, Anne Christin Meyer, Felipe Senne De Oliveira Lino, *et al.* 2020. “Antibiotics Create a Shift from Mutualism to Competition in Human Gut Communities with a Longer-Lasting Impact on Fungi than Bacteria.” *Microbiome* 8 (1): 133. https://doi.org/10.1186/s40168-020-00899-6.

Sendid, B., N. Dotan, S. Nseir, C. Savaux, P. Vandewalle, A. Standaert, F. Zerimech, *et al.* 2008. “Antibodies against Glucan, Chitin, and Saccharomyces Cerevisiae Mannan as New Biomarkers of *Candida albicans* Infection That Complement Tests Based on *C. albicans* Mannan.” *Clinical and Vaccine Immunology* 15 (12): 1868–77. https://doi.org/10.1128/CVI.00200-08.

Setiadi, Eleonora R., Thomas Doedt, Fabien Cottier, Christine Noffz, and Joachim F. Ernst. 2006. “Transcriptional Response of *Candida albicans* to Hypoxia: Linkage of Oxygen Sensing and Efg1p-Regulatory Networks.” *Journal of Molecular Biology* 361 (3): 399–411. https://doi.org/10.1016/j.jmb.2006.06.040.

Shao, Tzu Yu, W. X.Gladys Ang, Tony T. Jiang, Felicia Scaggs Huang, Heidi Andersen, Jeremy M. Kinder, Giang Pham, *et al.* 2019. “Commensal *Candida albicans* Positively Calibrates Systemic Th17 Immunological Responses.” *Cell Host and Microbe* 25 (3): 404-417.e6. https://doi.org/10.1016/j.chom.2019.02.004.

Shao, Tzu Yu, Pallavi Kakade, Jessica N. Witchley, Corey Frazer, Kathryn L. Murray, Iuliana V. Ene, David B. Haslam, *et al.* 2022. “*Candida albicans* Oscillating *UME6* Expression during Intestinal Colonization Primes Systemic Th17 Protective Immunity.” *Cell Reports* 39 (7). https://doi.org/10.1016/j.celrep.2022.110837.

Silva Dantas, Alessandra da, Kathy K. Lee, Ingrida Raziunaite, Katja Schaefer, Jeanette Wagener, Bhawna Yadav, and Neil AR Gow. 2016. “Cell Biology of *Candida albicans*–Host Interactions.” *Current Opinion in Microbiology*. Curr Opin Microbiol. https://doi.org/10.1016/j.mib.2016.08.006.

Simon, A. Katharina, Georg A. Hollander, and Andrew McMichael. 2015. “Evolution of the Immune System in Humans from Infancy to Old Age.” *Proceedings of the Royal Society B: Biological Sciences* 282 (1821). https://doi.org/10.1098/rspb.2014.3085.

Singh, Shweta, Zeeshan Fatima, and Saif Hameed. 2015. “Predisposing Factors Endorsing *Candida* Infections.” *Infezioni in Medicina*.

Smeekens, Sanne P., Frank L. van de Veerdonk, Bart Jan Kullberg, and Mihai G. Netea. 2013. “Genetic Susceptibility to *Candida* Infections.” *EMBO Molecular Medicine*. John Wiley & Sons, Ltd. https://doi.org/10.1002/emmm.201201678.

Smits, Wiep Klaas, Dena Lyras, D. Borden Lacy, Mark H. Wilcox, and Ed J. Kuijper. 2016. “*Clostridium difficile* Infection.” *Nature Reviews Disease Primers* 2 (April): 1–20. https://doi.org/10.1038/nrdp.2016.20.

Sokol, Harry, Sarah Jegou, Claire McQuitty, Marjolene Straub, Valentin Leducq, Cecilia Landman, Julien Kirchgesner, *et al.* 2018. “Specificities of the Intestinal Microbiota in Patients with Inflammatory Bowel Disease and *Clostridium difficile* Infection.” *Gut Microbes* 9 (1): 55–60. https://doi.org/10.1080/19490976.2017.1361092.

Sokol, Harry, Valentin Leducq, Hugues Aschard, Hang Phuong Pham, Sarah Jegou, Cecilia Landman, David Cohen, *et al.* 2017. “Fungal Microbiota Dysbiosis in IBD.” *Gut* 66 (6): 1039–48. https://doi.org/10.1136/gutjnl-2015-310746.

Sokol, Harry, P. Seksik, J. P. Furet, O. Firmesse, I. Nion-Larmurier, L. Beaugerie, J. Cosnes, G. Corthier, P. Marteau, and J. Doraé. 2009. “Low Counts of *Faecalibacterium prausnitzii* in Colitis Microbiota.” *Inflammatory Bowel Diseases* 15 (8): 1183–89. https://doi.org/10.1002/ibd.20903.

Sollis, Elliot, Abayomi Mosaku, Ala Abid, Annalisa Buniello, Maria Cerezo, Laurent Gil, Tudor Groza, *et al.* 2023. “The NHGRI-EBI GWAS Catalog: Knowledgebase and Deposition Resource.” *Nucleic Acids Research* 51 (D1): D977–85. https://doi.org/10.1093/nar/gkac1010.

Sonnenborn, Ulrich. 2016. “*Escherichia coli* Strain Nissle 1917-from Bench to Bedside and Back: History of a Special *Escherichia coli* Strain with Probiotic Properties.” *FEMS Microbiology Letters*. https://doi.org/10.1093/femsle/fnw212.

Standaert-Vitse, Annie, Boualem Sendid, Marie Joossens, Nadine François, Peggy Vandewalle-El Khoury, Julien Branche, Herbert Van Kruiningen, *et al.* 2009. “*Candida albicans* Colonization and ASCA in Familial Crohn’s Disease.” *American Journal of Gastroenterology* 104 (7): 1745–53. https://doi.org/10.1038/ajg.2009.225.

Stichternoth, Catrin, and Joachim F. Ernst. 2009. “Hypoxic Adaptation by Efg1 Regulates Biofilm Formation by *Candida albicans*.” *Applied and Environmental Microbiology* 75 (11): 3663–72. https://doi.org/10.1128/AEM.00098-09.

“Stop Neglecting Fungi.” 2017. *Nature Microbiology*.

https://doi.org/10.1038/nmicrobiol.2017.120.

Stram, Daniel O. 2004a. “Tag SNP Selection for Association Studies.” *Genetic Epidemiology* 27 (4): 365–74. https://doi.org/10.1002/GEPI.20028.

Swidergall, Marc. 2019. “*Candida albicans* at Host Barrier Sites: Pattern Recognition Receptors and Beyond.” *Pathogens* 8 (1): 40. https://doi.org/10.3390/pathogens8010040.

Swidergall, Marc, Mina Khalaji, Norma V. Solis, David L. Moyes, Rebecca A. Drummond, Bernhard Hube, Michail S. Lionakis, Craig Murdoch, Scott G. Filler, and Julian R. Naglik. 2019. “Candidalysin Is Required for Neutrophil Recruitment and Virulence During Systemic *Candida albicans* Infection.” *Journal of Infectious Diseases* 220 (9): 1477–88. https://doi.org/10.1093/infdis/jiz322.

Szóstak, Natalia, Luiza Handschuh, Anna Samelak-Czajka, Katarzyna Tomela, Marcin Schmidt, Łukasz Pruss, Kaja Milanowska-Zabel, Piotr Kozlowski, and Anna Philips. 2023. “Host Factors Associated with Gut Mycobiome Structure.” *mSystems*, February, e0098622. https://doi.org/10.1128/msystems.00986-22.

Talapko, Jasminka, Martina Juzbašić, Tatjana Matijević, Emina Pustijanac, Sanja Bekić, Ivan Kotris, and Ivana Škrlec. 2021. “*Candida albicans*—The Virulence Factors and Clinical Manifestations of Infection.” *Journal of Fungi 2021, Vol. 7, Page 79* 7 (2): 79. https://doi.org/10.3390/JOF7020079.

Tan, Chew Teng, Xiaoli Xu, Yuan Qiao, and Yue Wang. 2021. “A Peptidoglycan Storm Caused by β-Lactam Antibiotic’s Action on Host Microbiota Drives *Candida albicans* Infection” 12 (1): 1–13. https://doi.org/10.1038/s41467-021-22845-2.

Tansho, Shigeru, Shigeru Abe, Hiroko Ishibashi, Masayasu Mitsuya, Kayoko Wada, Tatsuo Ikeda, Nobuo Suegara, Osamu Koshio, Yasuo Ono, and Hideyo Yamaguchi. 2004. “Production of Anti-*Candida* Antibodies in Mice with Gut Colonization of *Candida albicans*.” *Mediators of Inflammation* 13 (3): 189–93. https://doi.org/10.1080/09511920410001713510.

Tantengco, Ourlad Alzeus G., Lauren S. Richardson, Enkhtuya Radnaa, Ananth Kumar Kammala, Sungjin Kim, Paul Mark B. Medina, Arum Han, and Ramkumar Menon. 2022. “Modeling Ascending *Ureaplasma parvum* Infection through the Female Reproductive Tract Using Vagina-Cervix-Decidua-Organ-on-a-Chip and Feto-Maternal Interface-Organ-on-a-Chip.” *FASEB Journal* 36 (10). https://doi.org/10.1096/fj.202200872R.

Tao, Li, Han Du, Guobo Guan, Yu Dai, Clarissa J. Nobile, Weihong Liang, Chengjun Cao, Qiuyu Zhang, Jin Zhong, and Guanghua Huang. 2014. “Discovery of a ‘White-Gray-Opaque’ Tristable Phenotypic Switching System in *Candida albicans*: Roles of Non-Genetic Diversity in Host Adaptation.” *PLoS Biology* 12 (4): e1001830. https://doi.org/10.1371/journal.pbio.1001830.

Teh, Wen Lin, Edimansyah Abdin, Asharani P.V, Fiona Devi Siva Kumar, Kumarasan Roystonn, Peizhi Wang, Saleha Shafie, *et al.* 2023. “Measuring Social Desirability Bias in a Multi-Ethnic Cohort Sample: Its Relationship with Self-Reported Physical Activity, Dietary Habits, and Factor Structure.” *BMC Public Health* 23 (1): 1–10. https://doi.org/10.1186/s12889-023-15309-3.

Terrisse, Safae, Laurence Zitvogel, and Guido Kroemer. 2022. “Effects of the Intestinal

Microbiota on Prostate Cancer Treatment by Androgen Deprivation Therapy." *Microbial Cell*. https://doi.org/10.15698/mic2022.12.787.

Terrisse, Safae, Laurence Zitvogel, and Guido Kroemer. 2023. "Impact of Microbiota on Breast Cancer Hormone Therapy." *Cell Stress* 7 (3). https://doi.org/10.15698/cst2023.03.277.

Thielemann, Nadja, Michaela Herz, Oliver Kurzai, and Ronny Martin. 2022. "Analyzing the Human Gut Mycobiome – A Short Guide for Beginners." *Computational and Structural Biotechnology Journal*. Elsevier. https://doi.org/10.1016/j.csbj.2022.01.008.

Thomas, Stéphanie, Vincent Rouilly, Etienne Patin, Cécile Alanio, Annick Dubois, Cécile Delval, Louis Guillaume Marquier, *et al.* 2015. "The Milieu Intérieur Study - An Integrative Approach for Study of Human Immunological Variance." *Clinical Immunology* 157 (2): 277–93. https://doi.org/10.1016/j.clim.2014.12.004.

Thompson, Ahna B, Amy EL Stone, and Michael J Gale. 2016. "Identifying the Interactome of the RIG-I-like Receptor LGP2." *The Journal of Immunology* 196: 203.18-203.18. https://doi.org/10.4049/jimmunol.196.supp.203.18.

Tian, Chao, Bethann S. Hromatka, Amy K. Kiefer, Nicholas Eriksson, Suzanne M. Noble, Joyce Y. Tung, and David A. Hinds. 2017. "Genome-Wide Association and HLA Region Fine-Mapping Studies Identify Susceptibility Loci for Multiple Common Infections." *Nature Communications* 8 (1): 1–13. https://doi.org/10.1038/s41467-017-00257-5.

Tong, Yiqing, and Jianguo Tang. 2017. "*Candida albicans* Infection and Intestinal Immunity." *Microbiological Research*. Urban & Fischer. https://doi.org/10.1016/j.micres.2017.02.002.

Toubiana, Julie, Satoshi Okada, Julia Hiller, Matias Oleastro, Macarena Lagos Gomez, Juan Carlos Aldave Becerra, Marie Ouachée-Chardin, *et al.* 2016. "Heterozygous *STAT1* Gain-of-Function Mutations Underlie an Unexpectedly Broad Clinical Phenotype." *Blood* 127 (25): 3154–64. https://doi.org/10.1182/blood-2015-11-679902.

Tournu, Hélène, Gyanendra Tripathi, Gwyneth Bertram, Susan Macaskill, Abigail Mavor, Louise Walker, Frank C. Odds, Neil A.R. Gow, and Alistair J.P. Brown. 2005. "Global Role of the Protein Kinase Gcn2 in the Human Pathogen *Candida albicans*." *Eukaryotic Cell* 4 (10): 1687–96. https://doi.org/10.1128/EC.4.10.1687-1696.2005.

Tripathi, Gyanendra, Carolyn Wiltshire, Susan Macaskill, Helene Tournu, Susan Budge, and Alistair J.P. Brown. 2002. "Gcn4 Co-Ordinates Morphogenetic and Metabolic Responses to Amino Acid Starvation in *Candida albicans*." *EMBO Journal* 21 (20): 5448–56. https://doi.org/10.1093/emboj/cdf507.

Tsai, Pei Wen, Cheng Yao Yang, Hao Teng Chang, and Chung Yu Lan. 2011. "Human Antimicrobial Peptide LL-37 Inhibits Adhesion of *Candida albicans* by Interacting with Yeast Cell-Wall Carbohydrates." *PLoS ONE* 6 (3). https://doi.org/10.1371/journal.pone.0017755.

Tso, Gloria Hoi Wan, Jose Antonio Reales-Calderon, Alrina Shin Min Tan, Xiao Hui Sem, Giang Thi Thu Le, Tze Guan Tan, Ghee Chuan Lai, *et al.* 2018. "Experimental Evolution of a Fungal Pathogen into a Gut Symbiont." *Science* 362 (6414): 589–95. https://doi.org/10.1126/science.aat0537.

Turnbaugh, Peter J., Ruth E. Ley, Micah Hamady, Claire M. Fraser-Liggett, Rob Knight, and

Jeffrey I. Gordon. 2007. “The Human Microbiome Project.” *Nature 2007 449:7164* 449 (7164): 804–10. https://doi.org/10.1038/nature06244.

Underhill, David M., and Iliyan D. Iliev. 2014. “The Mycobiota: Interactions between Commensal Fungi and the Host Immune System.” *Nature Reviews Immunology*. NIH Public Access. https://doi.org/10.1038/nri3684.

United States Centers for Disease Control and Prevention. 2014. “Impact of Malaria.” Malaria Worldwide. 2014. http://www.cdc.gov/malaria/malaria_worldwide/impact.html.

Urrutia, Alejandra, Darragh Duffy, Vincent Rouilly, Céline Posseme, Raouf Djebali, Gabriel Illanes, Valentina Libri, *et al.* 2016. “Standardized Whole-Blood Transcriptional Profiling Enables the Deconvolution of Complex Induced Immune Responses.” *Cell Reports* 16 (10): 2777–91. https://doi.org/10.1016/j.celrep.2016.08.011.

Valentine-Thon, Elizabeth. 2002. “Quality Control in Nucleic Acid Testing - Where Do We Stand?” In *Journal of Clinical Virology*, 25:13–21. Elsevier. https://doi.org/10.1016/s1386-6532(02)00196-8.

Vandeputte, Patrick, Selene Ferrari, and Alix T. Coste. 2012. “Antifungal Resistance and New Strategies to Control Fungal Infections.” *International Journal of Microbiology* 2012 (December): 713687. https://doi.org/10.1155/2012/713687.

Vangay, Pajau, Abigail J. Johnson, Tonya L. Ward, Gabriel A. Al-Ghalith, Robin R. Shields-Cutler, Benjamin M. Hillmann, Sarah K. Lucas, *et al.* 2018. “US Immigration Westernizes the Human Gut Microbiome.” *Cell* 175 (4): 962-972.e10. https://doi.org/10.1016/j.cell.2018.10.029.

Váradi, Judit, András Harazin, Ferenc Fenyvesi, Katalin Réti-Nagy, Péter Gogolák, György Vámosi, Ildikó Bácskay, *et al.* 2017. “Alpha-Melanocyte Stimulating Hormone Protects against Cytokine-Induced Barrier Damage in Caco-2 Intestinal Epithelial Monolayers.” *PLoS ONE* 12 (1). https://doi.org/10.1371/journal.pone.0170537.

Veerdonk, Frank L. van de, Leo A.B. Joosten, Patrick J. Shaw, Sanne P. Smeekens, R. K.Subbarao Malireddi, Jos W.M. van der Meer, Bart Jan Kullberg, Mihai G. Netea, and Thirumala Devi Kanneganti. 2011. “The Inflammasome Drives Protective Th1 and Th17 Cellular Responses in Disseminated Candidiasis.” *European Journal of Immunology* 41 (8): 2260–68. https://doi.org/10.1002/eji.201041226.

Veerdonk, Frank L. van de, Theo S. Plantinga, Alexander Hoischen, Sanne P. Smeekens, Leo A.B. Joosten, Christian Gilissen, Peer Arts, *et al.* 2011. “*STAT1* Mutations in Autosomal Dominant Chronic Mucocutaneous Candidiasis .” *New England Journal of Medicine* 365 (1): 54–61. https://doi.org/10.1056/nejmoa1100102.

Verma, Akash, Sarah L. Gaffen, and Marc Swidergall. 2017. “Innate Immunity to Mucosal *Candida* Infections.” *Journal of Fungi* 3 (4): 60. https://doi.org/10.3390/jof3040060.

Větrovský, Tomáš, and Petr Baldrian. 2013. “The Variability of the 16S rRNA Gene in Bacterial Genomes and Its Consequences for Bacterial Community Analyses.” *PLoS ONE* 8 (2). https://doi.org/10.1371/JOURNAL.PONE.0057923.

Vila, Taissa, Ahmed S. Sultan, Daniel Montelongo-Jauregui, and Mary Ann Jabra-Rizk. 2020. “Oral Candidiasis: A Disease of Opportunity.” *Journal of Fungi*. J Fungi (Basel).

https://doi.org/10.3390/jof6010015.

Voigt, Jessica, Kerstin Hünniger, Maria Bouzani, Ilse D. Jacobsen, Dagmar Barz, Bernhard Hube, Jürgen Löffler, and Oliver Kurzai. 2014. “Human Natural Killer Cells Acting as Phagocytes against *Candida albicans* and Mounting an Inflammatory Response That Modulates Neutrophil Antifungal Activity.” In *Journal of Infectious Diseases*, 209:616–26. Oxford Academic. https://doi.org/10.1093/infdis/jit574.

Volant, Stevenn, Pierre Lechat, Perrine Woringer, Laurence Motreff, Pascal Campagne, Christophe Malabat, Sean Kennedy, and Amine Ghozlane. 2020. “SHAMAN: A User-Friendly Website for Metataxonomic Analysis from Raw Reads to Statistical Analysis.” *BMC Bioinformatics* 21 (1). https://doi.org/10.1186/s12859-020-03666-4.

Vonk, Alieke G., Mihai G. Netea, Johan H. Van Krieken, Yoichiro Iwakura, Jos W.M. Van Der Meer, and Bart Jan Kullberg. 2006. “Endogenous Interleukin (IL)-1α and IL-1β Are Crucial for Host Defense against Disseminated Candidiasis.” *Journal of Infectious Diseases* 193 (10): 1419–26. https://doi.org/10.1086/503363.

Vries, Dylan H. de, Vasiliki Matzaraki, Olivier B. Bakker, Harm Brugge, Harm Jan Westra, Mihai G. Netea, Lude Franke, Vinod Kumar, and Monique G.P. van der Wijst. 2020. “Integrating GWAS with Bulk and Single-Cell RNA-Sequencing Reveals a Role for *LY86* in the Anti-*Candida* Host Response.” *PLoS Pathogens* 16 (4): e1008408. https://doi.org/10.1371/journal.ppat.1008408.

Vylkova, Slavena, Aaron J. Carman, Heather A. Danhof, John R. Collette, Huaijin Zhou, and Michael C. Lorenz. 2011. “The Fungal Pathogen *Candida albicans* Autoinduces Hyphal Morphogenesis by Raising Extracellular pH.” *MBio* 2 (3). https://doi.org/10.1128/mBio.00055-11.

Wagener, Jeanette, R. K.Subbarao Malireddi, Megan D. Lenardon, Martin Köberle, Simon Vautier, Donna M. MacCallum, Tilo Biedermann, *et al.* 2014. “Fungal Chitin Dampens Inflammation through IL-10 Induction Mediated by NOD2 and TLR9 Activation.” *PLoS Pathogens* 10 (4). https://doi.org/10.1371/journal.ppat.1004050.

Walker, Alan W., Sylvia H. Duncan, E. Carol McWilliam Leitch, Matthew W. Child, and Harry J. Flint. 2005. “PH and Peptide Supply Can Radically Alter Bacterial Populations and Short-Chain Fatty Acid Ratios within Microbial Communities from the Human Colon.” *Applied and Environmental Microbiology* 71 (7): 3692–3700. https://doi.org/10.1128/AEM.71.7.3692-3700.2005.

Walker, Alan W., Jennifer Ince, Sylvia H. Duncan, Lucy M. Webster, Grietje Holtrop, Xiaolei Ze, David Brown, *et al.* 2011. “Dominant and Diet-Responsive Groups of Bacteria within the Human Colonic Microbiota.” *ISME Journal* 5 (2): 220–30. https://doi.org/10.1038/ismej.2010.118.

Wang, Yu, Yahui Ren, Yongming Huang, Xiangnan Yu, Yiming Yang, Di Wang, Liang Shi, Kaixiong Tao, Guobin Wang, and Ke Wu. 2021. “Fungal Dysbiosis of the Gut Microbiota Is Associated with Colorectal Cancer in Chinese Patients.” *American Journal of Translational Research* 13 (10): 11287–301. /pmc/articles/PMC8581944/.

Wen, Chengping, Zhijun Zheng, Tiejuan Shao, Lin Liu, Zhijun Xie, Emmanuelle Le Chatelier, Zhixing He, *et al.* 2017. “Quantitative Metagenomics Reveals Unique Gut Microbiome

Biomarkers in Ankylosing Spondylitis." *Genome Biology* 18 (1). https://doi.org/10.1186/s13059-017-1271-6.

Wheeler, Richard, Paulo André Dias Bastos, Olivier Disson, Aline Rifflet, Ilana Gabanyi, Julia Spielbauer, Marion Bérard, Marc Lecuit, and Ivo Gomperts Boneca. 2023. "Microbiota-Induced Active Translocation of Peptidoglycan across the Intestinal Barrier Dictates Its within-Host Dissemination." Proceedings of the National Academy of Sciences of the United States of America 120 (4): e2209936120. https://doi.org/10.1073/PNAS.2209936120/SUPPL_FILE/PNAS.2209936120.SAPP.PDF.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. "Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics." In *PCR Protocols*, 315–22. Academic Press. https://doi.org/10.1016/b978-0-12-372180-8.50042-1.

Wickham, Hadley, Winston Chang, Lionel Henry, Thomas Lin Pedersen, Kohske Takahashi, Claus Wilke, Kara Woo, and RStudio. 2018. "Ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics." https://cran.r-project.org/package=ggplot2.

Wilck, Nicola, Mariana G. Matus, Sean M. Kearney, Scott W. Olesen, Kristoffer Forslund, Hendrik Bartolomaeus, Stefanie Haase, *et al.* 2017. "Salt-Responsive Gut Commensal Modulates Th17 Axis and Disease." *Nature* 551 (7682): 585–89. https://doi.org/10.1038/nature24628.

Witchley, Jessica N., Pallavi Penumetcha, Nina V. Abon, Carol A. Woolford, Aaron P. Mitchell, and Suzanne M. Noble. 2019. "*Candida albicans* Morphogenesis Programs Control the Balance between Gut Commensalism and Invasive Infection." *Cell Host and Microbe* 25 (3): 432-443.e6. https://doi.org/10.1016/j.chom.2019.02.008.

Woehrle, Tobias, Weidong Du, Achim Goetz, Hsin Yun Hsu, Thomas O. Joos, Manfred Weiss, Ute Bauer, Uwe B. Brueckner, and E. Marion Schneider. 2008. "Pathogen Specific Cytokine Release Reveals an Effect of TLR2 Arg753Gln during *Candida* Sepsis in Humans." *Cytokine* 41 (3): 322–29. https://doi.org/10.1016/j.cyto.2007.12.006.

Wu, Gary D., Jun Chen, Christian Hoffmann, Kyle Bittinger, Ying Yu Chen, Sue A. Keilbaugh, Meenakshi Bewtra, *et al.* 2011. "Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes." *Science* 334 (6052): 105–8. https://doi.org/10.1126/science.1208344.

Wu, Lu, Tiansheng Zeng, Massimo Deligios, Luciano Milanesi, Morgan G. I. Langille, Angelo Zinellu, Salvatore Rubino, Ciriaco Carru, and David J. Kelvin. 2020. "Age-Related Variation of Bacterial and Fungal Communities in Different Body Habitats across the Young, Elderly, and Centenarians in Sardinia." *MSphere* 5 (1). https://doi.org/10.1128/msphere.00558-19.

Wu, Sheng Yang, Chia Lin Weng, Min Jhen Jheng, Hung Wei Kan, Sung Tsang Hsieh, Fu Tong Liu, and Betty A. Wu-Hsieh. 2019. "*Candida albicans* Triggers NADPH Oxidaseindependent Neutrophil Extracellular Traps through Dectin-2." *PLoS Pathogens* 15 (11). https://doi.org/10.1371/journal.ppat.1008096.

Wynne, Anthony G., Anne L. McCartney, Jonathan Brostoff, Barry N. Hudspith, and Glenn R. Gibson. 2004. "An *in vitro* Assessment of the Effects of Broad-Spectrum Antibiotics on the Human Gut Microflora and Concomitant Isolation of a *Lactobacillus plantarum* with

Anti-*Candida* Activities." *Anaerobe* 10 (3): 165–69. https://doi.org/10.1016/j.anaerobe.2004.03.002.

Xie, Zixuan, and Chaysavanh Manichanh. 2022. "FunOMIC: Pipeline with Built-in Fungal Taxonomic and Functional Databases for Human Mycobiome Profiling." *Computational and Structural Biotechnology Journal* 20 (January): 3685–94. https://doi.org/10.1016/j.csbj.2022.07.010.

Xu, Congmin, Huaiqiu Zhu, and Peng Qiu. 2019. "Aging Progression of Human Gut Microbiota." *BMC Microbiology* 19 (1). https://doi.org/10.1186/s12866-019-1616-2.

Xu, Peng, Qi le Gao, Yun jia Wang, Chao feng Guo, Ming xing Tang, Shao hua Liu, Ang Deng, Yu xiang Wang, Yan bing Li, and Hong qi Zhang. 2020. "Rs6127698 Polymorphism in the *MC3R* Gene and Susceptibility to Multifocal Tuberculosis in Southern Chinese Han Population." *Infection, Genetics and Evolution* 82 (August). https://doi.org/10.1016/j.meegid.2020.104292.

Yang, D., and J. J. Oppenheim. 2009. "Alarmins and Antimicrobial Immunity." *Medical Mycology*. Med Mycol. https://doi.org/10.1080/13693780902721416.

Yang, Jian, Noah A. Zaitlen, Michael E. Goddard, Peter M. Visscher, and Alkes L. Price. 2014. "Advantages and Pitfalls in the Application of Mixed-Model Association Methods." *Nature Genetics* 46 (2): 100–106. https://doi.org/10.1038/ng.2876.

Yano, Junko, Jack D. Sobel, Paul Nyirjesy, Ryan Sobel, Valerie L. Williams, Qingzhao Yu, Mairi C. Noverr, and Paul L. Fidel. 2019. "Current Patient Perspectives of Vulvovaginal Candidiasis: Incidence, Symptoms, Management and Post-Treatment Outcomes." *BMC Women's Health* 19 (1). https://doi.org/10.1186/s12905-019-0748-8.

Yatsunenko, Tanya, Federico E. Rey, Mark J. Manary, Indi Trehan, Maria Gloria Dominguez-Bello, Monica Contreras, Magda Magris, *et al.* 2012. "Human Gut Microbiome Viewed across Age and Geography." *Nature* 486 (7402): 222–27. https://doi.org/10.1038/nature11053.

Yoshimoto, Shin, Tze Mun Loo, Koji Atarashi, Hiroaki Kanda, Seidai Sato, Seiichi Oyadomari, Yoichiro Iwakura, *et al.* 2013. "Obesity-Induced Gut Microbial Metabolite Promotes Liver Cancer through Senescence Secretome." *Nature* 499 (7456). https://doi.org/10.1038/nature12347.

Yu, Zhongtang, and Mark Morrison. 2004. "Improved Extraction of PCR-Quality Community DNA from Digesta and Fecal Samples." *BioTechniques* 36 (5): 808–12. https://doi.org/10.2144/04365st04.

Zampieri, Guido, Supreeta Vijayakumar, Elisabeth Yaneske, and Claudio Angione. 2019. "Machine and Deep Learning Meet Genome-Scale Metabolic Modeling." *PLoS Computational Biology* 15 (7): e1007084. https://doi.org/10.1371/journal.pcbi.1007084.

Zegers, Doreen, Sigri Beckers, Fenna De Freitas, Armand V. Peeters, Ilse L. Mertens, Stijn L. Verhulst, Raoul P. Rooman, *et al.* 2011. "Identification of Three Novel Genetic Variants in the Melanocortin-3 Receptor of Obese Children." *Obesity* 19 (1): 152–59. https://doi.org/10.1038/oby.2010.127.

Zeise, Karen D., Robert J. Woods, and Gary B. Huffnagle. 2021. "Interplay between *Candida*

albicans and Lactic Acid Bacteria in the Gastrointestinal Tract: Impact on Colonization Resistance, Microbial Carriage, Opportunistic Infection, and Host Immunity." *Clinical Microbiology Reviews* 34 (4). https://doi.org/10.1128/CMR.00323-20.

Zhai, Bing, Mihaela Ola, Thierry Rolling, Nicholas L. Tosini, Sari Joshowitz, Eric R. Littmann, Luigi A. Amoretti, *et al.* 2020. "High-Resolution Mycobiota Analysis Reveals Dynamic Intestinal Translocation Preceding Invasive Candidiasis." *Nature Medicine*. Nat Med. https://doi.org/10.1038/s41591-019-0709-7.

Zheng, Danping, Timur Liwinski, and Eran Elinav. 2020. "Interaction between Microbiota and Immunity in Health and Disease." *Cell Research* 30 (6): 492–506. https://doi.org/10.1038/s41422-020-0332-7.

Zheng, Leon, Caleb J. Kelly, and Sean P. Colgan. 2015. "Physiologic Hypoxia and Oxygen Homeostasis in the Healthy Intestine. A Review in the Theme: Cellular Responses to Hypoxia." *American Journal of Physiology - Cell Physiology* 309 (6): C350–60. https://doi.org/10.1152/ajpcell.00191.2015.

Zhou, Cheng Bei, Yi Lu Zhou, and Jing Yuan Fang. 2021. "Gut Microbiota in Cancer Immune Response and Immunotherapy." *Trends in Cancer* 7 (7): 647–60. https://doi.org/10.1016/j.trecan.2021.01.010.

Zhou, Xiang, and Matthew Stephens. 2014. "Efficient Multivariate Linear Mixed Model Algorithms for Genome-Wide Association Studies." *Nature Methods* 11 (4): 407–9. https://doi.org/10.1038/nmeth.2848.

Znaidi, Sadri, Lasse van Wijlick, Arturo Hernández-Cervantes, Natacha Sertour, Jean Luc Desseyn, Frédéric Vincent, Ralitsa Atanassova, *et al.* 2018. "Systematic Gene Overexpression in *Candida albicans* Identifies a Regulator of Early Adaptation to the Mammalian Gut." *Cellular Microbiology* 20 (11): 12890. https://doi.org/10.1111/cmi.12890.

Zoetendal, Erwin G., Jeroen Raes, Bartholomeus Van Den Bogert, Manimozhiyan Arumugam, Carien C. Booijink, Freddy J. Troost, Peer Bork, Michiel Wels, Willem M. De Vos, and Michiel Kleerebezem. 2012. "The Human Small Intestinal Microbiota Is Driven by Rapid Uptake and Conversion of Simple Carbohydrates." *ISME Journal* 6 (7): 1415–26. https://doi.org/10.1038/ismej.2011.212.

Zuo, Tao, Sunny H. Wong, Chun Pan Cheung, Kelvin Lam, Rashid Lui, Kitty Cheung, Fen Zhang, *et al.* 2018. "Gut Fungal Dysbiosis Correlates with Reduced Efficacy of Fecal Microbiota Transplantation in *Clostridium difficile* Infection." *Nature Communications* 9 (1). https://doi.org/10.1038/s41467-018-06103-6.

Zuo, Tao, Fen Zhang , Grace C.Y. Lui, Yun Kit Yeoh, Amy Y.L. Li , Hui Zhan, Yating Wan, *et al.* 2020. "Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization." *Gastroenterology* 159 (3): 944-955.e8. https://pubmed.ncbi.nlm.nih.gov/32442562/.

APPENDIX

Appendix 1:

A Clinical Study Provides the First Direct Evidence That Interindividual Variations in Fecal β-Lactamase Activity Affect the Gut Mycobiota Dynamics in Response to β-Lactam Antibiotics

Margot DELAVY, Charles BURDET, Natacha SERTOUR, Savannah DEVENTE, Jean-Denis DOCQUIER, Nathalie GRALL, Stevenn VOLANT, Amine GHOZLANE, Xavier DUVAL, France MENTRÉ, Christophe D'ENFERT, Marie-Elisabeth BOUGNOUX, for the PrediRes group

mBio 2022, 13 (6). https://doi.org/10.1128/mbio.02880-22.

AMERICAN SOCIETY FOR MICROBIOLOGY | mBio®

RESEARCH ARTICLE

A Clinical Study Provides the First Direct Evidence That Interindividual Variations in Fecal β-Lactamase Activity Affect the Gut Mycobiota Dynamics in Response to β-Lactam Antibiotics

Margot Delavy,[a] Charles Burdet,[b,c] Natacha Sertour,[a] Savannah Devente,[d] Jean-Denis Docquier,[d] Nathalie Grall,[b] Stevenn Volant,[e] Amine Ghozlane,[e] Xavier Duval,[b,f] France Mentré,[b,c] Christophe d'Enfert,[a] Marie-Elisabeth Bougnoux,[a,g] on behalf of the PrediRes Study Group

[a]Institut Pasteur, Université Paris Cité, INRAE USC2019, Unité Biologie et Pathogénicité Fongiques, Paris, France
[b]Université Paris Cité, IAME, INSERM, Paris, France
[c]AP-HP, Département d'Epidémiologie, Biostatistique et Recherche Clinique, Hôpital Bichat, Paris, France
[d]Università di Siena, Dipartimento di Biotecnologie Mediche, Siena, Italy
[e]Institut Pasteur, Université Paris Cité, Bioinformatics and Biostatistics Hub, Paris, France
[f]Clinical investigation center, INSERM 1425, IAME, Hôpital Bichat, Assistance Publique des Hôpitaux de Paris (APHP), Université Paris Cité, Paris, France
[g]Unité de Parasitologie-Mycologie, Service de Microbiologie Clinique, Hôpital Necker-Enfants-Malades, Assistance Publique des Hôpitaux de Paris (APHP), Paris, France

ABSTRACT Antibiotics disturb the intestinal bacterial microbiota, leading to gut dysbiosis and an increased risk for the overgrowth of opportunistic pathogens. It is not fully understood to what extent antibiotics affect the fungal fraction of the intestinal microbiota, the mycobiota. There is no report of the direct role of antibiotics in the overgrowth in healthy humans of the opportunistic pathogenic yeast *Candida albicans*. Here, we have explored the gut mycobiota of 22 healthy subjects before, during, and up to 6 months after a 3-day regimen of third-generation cephalosporins (3GCs). Using ITS1-targeted metagenomics, we highlighted the strong intra- and interindividual diversity of the healthy gut mycobiota. With a specific quantitative approach, we showed that *C. albicans* prevalence was much higher than previously reported, with all subjects but one being carriers of *C. albicans*, although with highly variable burdens. 3GCs significantly altered the mycobiota composition and the fungal load was increased both at short and long term. Both *C. albicans* relative and absolute abundances were increased but 3GCs did not reduce intersubject variability. Variations in *C. albicans* burden in response to 3GC treatment could be partly explained by changes in the levels of endogenous fecal β-lactamase activity, with subjects characterized by a high increase of β-lactamase activity displaying a lower increase of *C. albicans* levels. A same antibiotic treatment might thus affect differentially the gut mycobiota and *C. albicans* carriage, depending on the treated subject, suggesting a need to adjust the current risk factors for *C. albicans* overgrowth after a β-lactam treatment.

IMPORTANCE Fungal infections are redoubtable healthcare-associated complications in immunocompromised patients. Particularly, the commensal intestinal yeast *Candida albicans* causes invasive infections in intensive care patients and is, therefore, associated with high mortality. These infections are preceded by an intestinal expansion of *C. albicans* before its translocation into the bloodstream. Antibiotics are a well-known risk factor for *C. albicans* overgrowth but the impact of antibiotic-induced dysbiosis on the human gut mycobiota—the fungal microbiota—and the understanding of the mechanisms involved in *C. albicans* overgrowth in humans are very limited. Our study shows that antibiotics increase the fungal proportion in the gut and disturb the fungal composition, especially *C. albicans*, in a subject-dependent manner. Indeed, variations across subjects in *C. albicans*

Editor Joseph Heitman, Duke University

Address correspondence to Marie-Elisabeth Bougnoux, bougnoux@pasteur.fr.

The authors declare no conflict of interest.

This article is a direct contribution from Christophe d'Enfert, a Fellow of the American Academy of Microbiology, who arranged for and secured reviews by Scott Filler, UCLA, and Iliyan Iliev, Cornell University.

Received 17 October 2022
Accepted 21 October 2022
Published 30 November 2022

burden in response to β-lactam treatment could be partly explained by changes in the levels of endogenous fecal β-lactamase activity. This highlighted a potential new key factor for *C. albicans* overgrowth. Thus, the significance of our research is in providing a better understanding of the factors behind *C. albicans* intestinal overgrowth, which might lead to new means to prevent life-threatening secondary infections.

KEYWORDS antibiotics, *Candida albicans*, gut mycobiota, healthy individuals, beta-lactamases

Interest in the role of the gut microbiota in health and disease is rising (1–4) and the role of antibiotics as major disturbers of the microbiota healthy state has been largely studied (5–8). By killing the resident bacteria of the gut, broad-spectrum antibiotics reduce bacterial diversity in the gastrointestinal (GI) tract and decrease the abundance of beneficial bacteria (5, 7). They also alter the gut microbiota interaction network, thus contributing to the overgrowth of opportunistic pathogens (6, 9). More alarmingly, the prolonged use of antibiotics may promote antibiotic resistance (10). For example, β-lactam exposure can lead to the selection of specific gut bacteria able to produce β-lactamases, enzymes that can hydrolyze β-lactam antibiotics, leading to an overall increase in antibiotic resistance (11, 12).

While the bacterial microbiota is extensively studied, less attention has been paid to the mycobiota—the fungal part of the microbiota—and to the consequences that antibiotic-induced dysbiosis may have on the fungal communities of the gut. It is now well established that fungi can rapidly proliferate in the GI tract of mice after removal of gut bacteria by antibiotics (13). The GI tract of mice is not naturally colonized by the opportunistic pathogen *Candida albicans* and antibiotics have been used to trigger such colonization (14), suggesting that they clear specific bacteria able to inhibit *C. albicans* growth in the mouse GI tract (15). Yet, we need more information about the impact of an antibiotic-induced dysbiosis on the healthy human gut mycobiota and specifically *C. albicans*. Because *C. albicans* systemic infections are responsible for thousands of deaths each year (16) and antibiotics are a well-known risk factor for these infections (17), we need to better understand the mechanisms of *C. albicans* overgrowth in the human gut, upon antibiotic treatment.

In this work, we prospectively followed two parallel groups of 11 healthy subjects each, before, during, and after they were treated intravenously with either cefotaxime or ceftriaxone, two third-generation cephalosporin (3GC) antibiotics that share a similar activity spectrum (8). We quantified the levels of *C. albicans* carriage in all subjects and characterized their healthy mycobiota and its variability during the 2-week period preceding antibiotic administration. Then, we analyzed the changes in terms of fungal diversity, fungal burden, community profile, and *C. albicans* levels, occurring in the mycobiota after antibiotics were administered, both at short and long term. Finally, we monitored the level of fecal β-lactamase activity, which is known to modulate the intensity of the post-3GC intestinal dysbiosis, and we correlated the changes in β-lactamase activity with the impact of 3GCs on *C. albicans* carriage.

RESULTS

The gut mycobiota of healthy subjects is highly dynamic and variable. To study the healthy mycobiota, we used fecal samples collected from each of 22 healthy volunteers at day −15 (−D15), −D7, and −D1, before antibiotic administration (see Materials and Methods). In total, 54 fecal samples (one to three available per subject) were available for analyses.

First, we assessed the fungal load, i.e., the ratio between the fungal DNA concentration and the total fecal DNA (see Materials and Methods). The mycobiota represented a very small fraction of the total microbiota in healthy subjects (median fungal load: 7.9×10^{-6}, min: 6.7×10^{-10}, max: 1.5×10^{-3}, Fig. S1A).

Using ITS1 sequencing, we further characterized the mycobiota composition of the 22 subjects during the 2 weeks preceding 3GC exposure. We identified 233 different

TABLE 1 Prevalence of the main fungal species in healthy subjects and in their fecal samples, estimated by ITS1 sequencing

Main fungal species	Prevalence[a]	
	Fecal samples (%) ($N = 54$)	Healthy subjects (%) ($N = 22$)
Vanrija humicola	98.2	100.0
Galactomyces candidus	92.6	95.5
Saccharomyces cerevisiae	88.9	95.5
Candida parapsilosis	88.9	95.5
Penicillium roqueforti	72.2	90.9
Cutaneotrichosporon curvatum	87.0	86.4
Malassezia restricta	88.8	77.3
Candida albicans	75.9	72.7
Debaryomyces hansenii	68.5	59.1

[a]A species is considered present in a sample if its relative abundance is above 0.1%. A species is considered present in a subject if it is present in at least one sample between −D15 and −D1.

OTUs, 182 OTUs (78.1%) being annotated at the phylum level, 167 (71.7%) at the genus level, and 123 (52.8%) at the species level. Overall, the 167 OTUs annotated at the genus level and the 123 OTUs annotated at the species level represented 99.7% and 91.2% of the total number of sequences, respectively.

Ascomycota was the most abundant phylum (mean relative abundance of 77.9%), followed by Basidiomycota (21.9%; Fig. S1B). Sixty-two fungal genera were identified in at least two samples, with eight reaching a mean relative abundance across subjects above 1% (Fig. S1C). Ninety-five species were identified in at least two samples, nine reaching a mean relative abundance across subjects above 1% (Table 1). The taxa relative abundances were highly variable between individuals and across time (Fig. 1A), with *Galactomyces candidus* being the most disparately represented taxa, with a relative abundance varying from 0% to 99.2% depending on the sample.

We identified a median of only 25 OTUs per sample (min: 5, max: 55, Fig. 1B), corresponding to a median Shannon Index of 1.18 (min: 0.18, max: 2.26, Fig. 1B), reflecting a low richness and evenness within each sample. Unlike this low α-diversity, we observed a high β-diversity, which quantifies the level of dissimilarity between samples, with a median Bray-Curtis dissimilarity index of 0.87 between the subjects (min: 0.02 max: 1.00, Fig. 1C). We also followed the variations occurring overtime during the 2-week period preceding 3GC exposure. The within subjects' diversity, measured between the samples collected from the same subject at different time, was almost as high as the between subject diversity, with a Bray-Curtis dissimilarity index of 0.75 (min: 0.08, max: 0.99, Fig. 1C; Fig. S2).

We quantified the levels of fecal *C. albicans* in these volunteers by determining the absolute abundance of *C. albicans* using specific qPCR. We detected *C. albicans* DNA at least once between −D15 and −D1 in 20/21 subjects (95.2%) before 3GC administration. In total, 42/51 samples analyzed were positive for *C. albicans* (82.4%). In these samples, *C. albicans* DNA levels ranged from 2.8×10^{-4} to 1.26 ng/g of stool, with a median of 9.4×10^{-3} ng/g of stool (Fig. 1D). In comparison, by using ITS1 sequencing data and culture methods, we could detect *C. albicans* in only 16/22 (72.7%; Table 1) and in 7/22 (15.8%) subjects, respectively.

Cefotaxime and ceftriaxone exposure increases the fungal load and disturbs the gut mycobiota composition. To measure how much the antibiotic treatment affected the gut mycobiota, we compared its features, including the fungal load, genera and species composition, and *C. albicans* absolute levels, at baseline (D0) with those during and after antibiotics (Fig. 2A). Data collected at −D1 were used as baseline. If missing, −D7 data were used instead (see Fig. S3).

We used two metrics to estimate changes during and after antibiotic administration: the areas under the curve (AUCs) of the mycobiota characteristics' changes from D0, and the changes from D0 of the mycobiota characteristics, for each subject, at

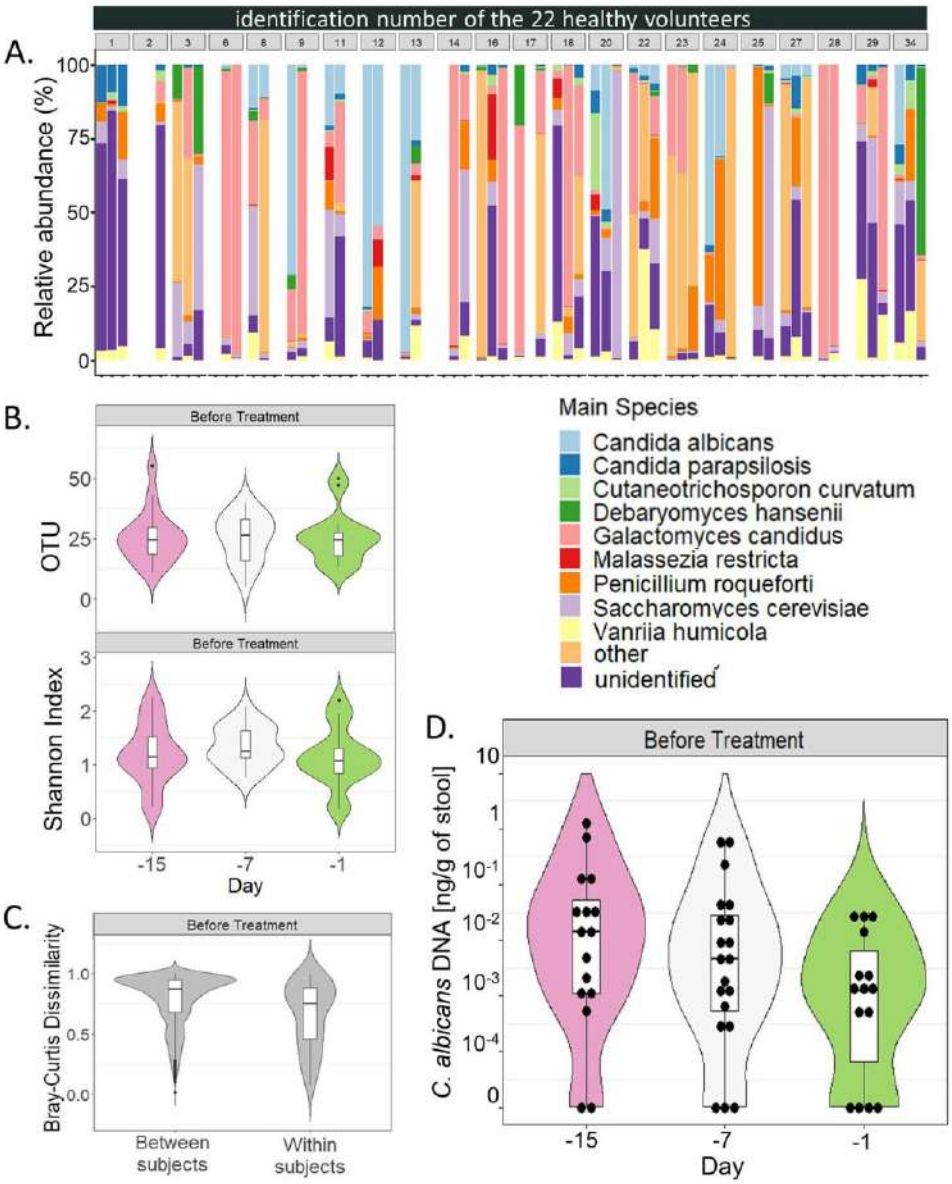


FIG 1 Dynamic of the mycobiota characteristics in 22 healthy individuals during a 2-week period. (A) Fungal species relative abundances at 1-week apart time points for 22 healthy subjects. For each subject, barplot are ordered by time (−D15, −D7, −D1 before antibiotics). Represented species reached a mean relative abundance across subjects above 1%. (B) Alpha diversity: violin plot of the number of OTUs and of the Shannon index values at 1-week apart time points for 22 healthy subjects. (C) Beta diversity: Bray-Curtis dissimilarity values between samples donated by different subjects (between subjects) and between samples donated by the same subjects (within subjects) for ITS1 sequencing data. Values range from 0 to 1, with 0 being the least dissimilar and 1 being the most dissimilar. (D) Violin and boxplots of the *C. albicans* DNA levels at 1-week apart time points for 22 healthy subjects. Each dot represents a sample. For all panels, the upper whiskers extend from the hinge to the largest value below 1.5× the interquartile range, and the lower whiskers extend from the hinge to the smallest value above 1.5× the interquartile range.

different time points between D1 and D180. The first metric allows the aggregation of both the duration of the changes and their amplitude whereas the second allows the detection of more punctual variations.

We observed a general long-term increase of the fungal load in the 22 subjects early after the start of the antibiotic treatment. The fungal load significantly increased

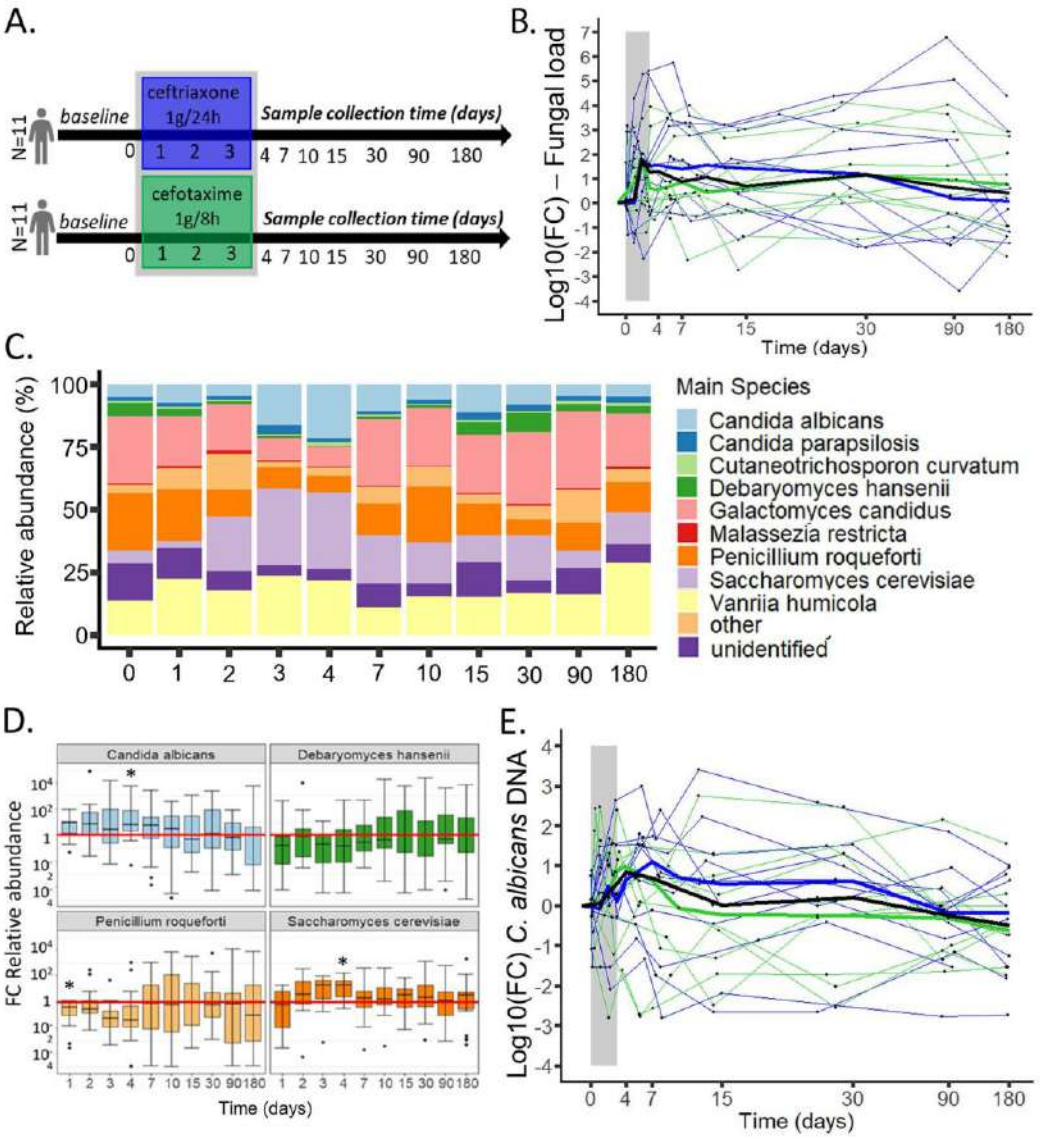


FIG 2 Impact of 3-day cefotaxime and ceftriaxone IV treatment on the gut mycobiota of 22 healthy subjects followed for a 6-month period. (A) Study design. (B) Log_{10} (foldchange [FC]) of the fungal load following ceftriaxone and cefotaxime treatment (gray area). Thin lines represent the subjects, thicker lines represent the medians at each day for each treatment group (blue and green) and for all subjects (black). (C) Main fungal species distribution following ceftriaxone and cefotaxime treatment. (D) Distribution of the relative abundance log_{10} (FC) AUCs for *Candida albicans*, *Debaryomyces hansenii*, *Penicillium roqueforti*, and *Saccharomyces cerevisiae*, highlighting the duration and the amplitude of the perturbations *q value < 0.05, Wilcoxon t-test, false-discovery rate correction. Upper whiskers extend from the hinge to the largest value below 1.5× the interquartile range, and the lower whiskers extend from the hinge to the smallest value above 1.5× the interquartile range. (E) Log_{10} (FC) of *C. albicans* DNA levels following ceftriaxone and cefotaxime treatment (gray area). Thin lines represent the subjects, thicker lines represent the medians at each day for each treatment group (blue and green) and for all subjects (black).

immediately after the start of antibiotics, independently of the antibiotic used, with a positive AUCs for all calculated periods between D0 and D2, and D0 and D90 (Wilcoxon test; *P* values of 0.008, 0.017, 0.040, 0.014, 0.009, 0.005, 0.006, and 0.048, respectively) with a maximal 62.3-fold increase at D2 (min: 0.02, max: 1.8×10^4; Wilcoxon test; *P* value of 0.007; Fig. 2B; Fig. S4B; Table S1). No difference was observed between the subjects treated with ceftriaxone and those treated with cefotaxime (Fig. 2B; Table S2).

At D15, we observed a slight increase of the number of fungal OTUs, compared with D0 (Wilcoxon test; *P* value of 0.030; Table S1) but not of the Shannon Index (Wilcoxon test; *P* value of 0.47), suggesting that the fungal α-diversity is not strongly impacted by the antibiotics. No difference was observed between the subjects treated with ceftriaxone or those treated with cefotaxime, and this for all fungal diversity indices studied (Table S2).

Three genera were significantly impacted by the antibiotics *Debaryomyces* sp., *Penicillium* sp., and *Saccharomyces* sp. (Fig. S4A and B; Table S1). *Debaryomyces* sp. were significantly decreased immediately after the start of the treatment, with negative AUCs between D0 and D3 (Wilcoxon test; *q* value of 0.02) and a maximal but not significant 12.5-fold drop at D3 (min: 0.09, max: 1.7×10^5, Wilcoxon test; *q* value of 0.08). *Penicillium* sp. were also decreased immediately after the start of the treatment, with negative AUCs between D0 and D2 and up to D0 and D7 (Wilcoxon test; *q* values of 0.01, 0.005, 0.003, and 0.0002, respectively) with a maximal 21.4-fold decrease at D4 (min: 0.81, max: 776.2; Wilcoxon test; *q* value of 0.0008). On the contrary, *Saccharomyces* sp. relative abundance was punctually increased at D4 (median: 19.5-fold increase, min: 0.32, max: 169.8), compared with baseline (Wilcoxon test; *q* value of 0.01), before returning to basal levels. No significant difference between the subjects of the two treatment groups was observed at any day, for all genera tested (Table S2).

In addition, at the species level, four taxa were significantly affected by 3GC treatment: *S. cerevisiae*, *D. hansenii*, *P. roqueforti*, and *C. albicans* (Fig. 2C; Table S1). *D. hansenii* was decreased for the period D0 to D3, with a corresponding negative AUC (Wilcoxon test; *q* value of 0.047; Fig. S4C) and *P. roqueforti* was punctually reduced after the treatment with a 2.4-fold drop at D1 (min: 0.74, max: 3.3×10^3; Wilcoxon test; *q* value of 0.026; Fig. 2D). In contrast, *C. albicans* and *S. cerevisiae* relative abundance displayed a 9.8-fold and 19.5-fold raise at D4, respectively (*C. albicans:* max: 1.1×10^5, min: 0.004; Wilcoxon test; *q* value of 0.04, *S. cerevisiae:* max: 169.8, min: 0.32 Wilcoxon test; *q* value of 0.026; Fig. S4C). As for the genera, no significant difference between the subjects of the two groups was observed for any species (Table S2).

Not only *C. albicans* relative abundance but also its absolute abundance was punctually increased after antibiotics. Indeed, 3GC administration led to a punctual raise of *C. albicans* DNA levels on the D0 to D4 period (Wilcoxon test, *q* value of 0.047) with a maximal 2.1-fold increase at D2 (min: 0.03, max: 288.4; Wilcoxon test; *P* value of 0.02; Fig. 2E), when measured by qPCR. However, this increase of *C. albicans* DNA levels was subject-dependent. For example, subject 1 displayed an impressive increase of *C. albicans* DNA, with a maximal 2,521.3-fold raise at D15 whereas *C. albicans* DNA levels were reduced in subject 12 after the treatment. No difference was observed between the two groups of treatment at any days (Fig. 2E; Table S2).

Change in β-lactamase activity levels as a key parameter for *C. albicans* overgrowth in the GI tract after third-generation cephalosporin administration. β-lactamase activity was measured in each fecal sample by dosing the NFC-hydrolyzing activity. This activity was heterogenous between subjects before antibiotics, ranging from 2.40 to 1,240 nmol/min·g of stool, with no difference between the two groups that received either ceftriaxone or cefotaxime (Wilcoxon test, *P* value: 0.78). Globally, β-lactamase activity was significantly increased after 3GC administration for all the periods calculated between D0 to D3 and D0 to D180 (Wilcoxon test; *P* values of 0.040, 0.006, 0.002, 0.0008, 0.0003, 0.0006, 0.0007, and 0.03, respectively), with a maximal 2.25-fold increase at D7 (Fig. 3A). However, we observed two types of behavior in the D0 to D10 AUC of the change in β-lactamase activity, with some subjects displaying a high increase of the β-lactamase activity after antibiotic treatment (up to a 28-fold rise) whereas others showed no change or even a decrease of this activity (up to a 7-fold decrease). Therefore, we split the 22 subjects in two groups; the "high" group was characterized by a strong increase of the fecal β-lactamase activity (AUC D0 to D10 $\geq$ 2.36), whereas the "low" group had a lower increase or even a decrease of this activity (AUC D0 to D10 $<$ 2.36; Fig. 3B). Changes of *C. albicans* DNA levels were significantly different between subjects of the "low" and "high" groups, both for the D0 to D10 period and at D4 and D30 (Wilcoxon test; *P* value of 0.02, 0.02, and 0.007, respectively). At D4 and D30,

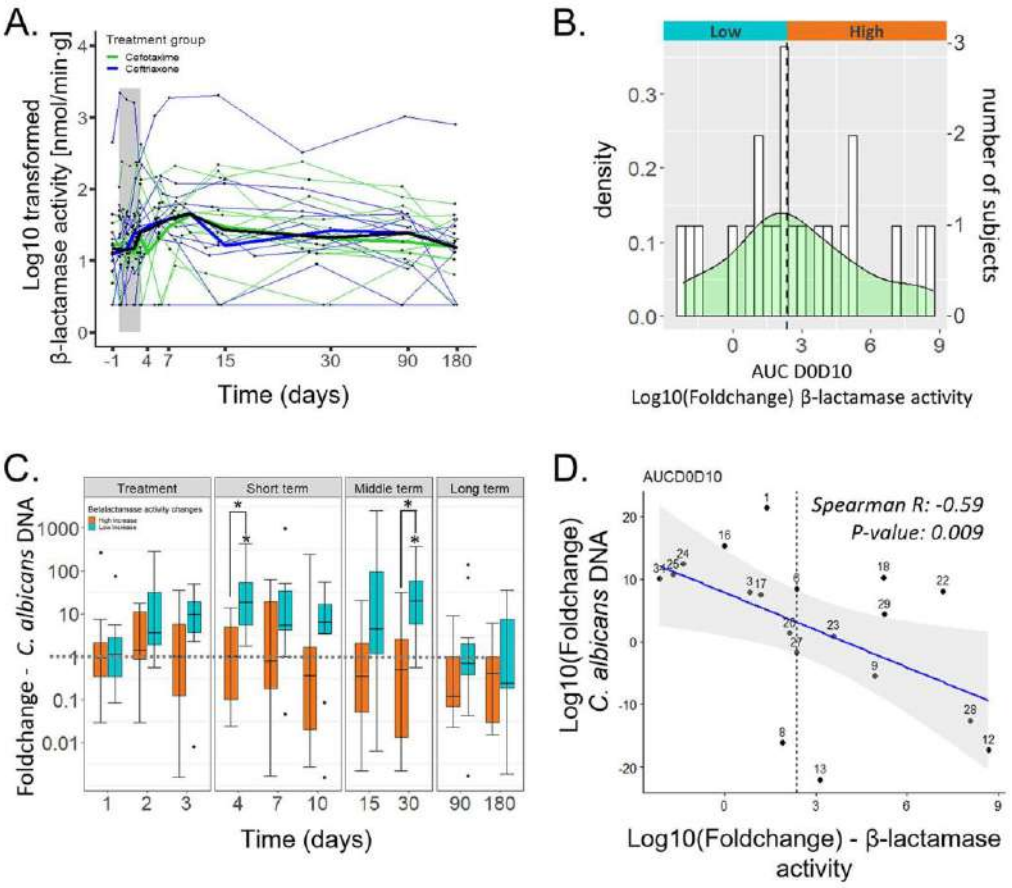


FIG 3 Change in β-lactamases activity levels as a key parameter for *C. albicans* proliferation in the gut after third-generation cephalosporin administration. (A) β-lactamases activity before, during, and after the antibiotic treatment. Thin lines represent the subjects; thicker lines represent the medians at each day for each treatment group (ceftriaxone, blue; cefotaxime, green) and for all subjects (black). (B) Distribution of the D0 to D10 AUCs of the change of β-lactamases activity in 22 healthy subjects. The density distribution is represented by the green curve and the number of subjects for each range of AUC values are represented by the white histogram. The group "high" (orange) regroups the subjects with a D0 to D10 AUC above the median (black dashed line) and the group "low" (blue) regroups the subjects with a D0 to D10 AUC below the median. (C) Boxplots of *C. albicans* DNA levels $\log_{10}$ (FC) following ceftriaxone and cefotaxime treatment. Orange boxplots indicates values for the subjects from the group "high" of and blue boxplots represents the values for the subjects from the group "low." Upper whiskers extend from the hinge to the largest value below 1.5× the interquartile range, and the lower whiskers extend from the hinge to the smallest value above 1.5× the interquartile range. (D) Correlation plot of the $\log_{10}$ (FC) AUCs of *C. albicans* DNA levels and β-lactamases activity for the D0 to D10 period. Regression is represented by a blue line and the confidence interval by the gray area. Subjects are designated by their ID number.

C. albicans DNA levels were significantly increased in the group "low," whereas no change was detected in the group "high" (D4: Wilcoxon test; *P* value of 0.008 and 0.84, respectively; D30: Wilcoxon test; *P* value of 0.023 and 0.26, respectively; Fig. 3C).

Finally, we showed a highly significant negative interaction between the D0 to D10 AUC of the change in β-lactamase activity and the D0 to D10 AUC of the change in *C. albicans* DNA levels (Spearman correlation; R: −0.59, *P* value: 0.009; Fig. 3D). No such correlation could be found between the D0 to D10 AUC of the fungal load and the D0 to D10 AUC of the change in β-lactamase activity (Spearman correlation; R: −0.25, *P* value: 0.3).

DISCUSSION

In this study, we explored the impact of β-lactam antibiotics on the human gut mycobiota by performing a targeted metagenomic analysis of the mycobiota of healthy subjects before, during, and after 3GC exposure. 3GC strongly affected the

mycobiota, especially *C. albicans* carriage, with wide intersubject variations that were not related to the type of 3CG they received. We identified the changes of fecal β-lactamase activity after treatment as a potential key factor regulating *C. albicans* overgrowth, with subjects characterized by a low increase of β-lactamase activity displaying a stronger increase of *C. albicans* levels following antibiotics. This regulation is likely mediated by a differential impact of antibiotics on the endogenous gut bacteria, according to differences in the occurrence of β-lactamase-producing bacteria in the microbiota. Briefly, a microbiota rich in β-lactamase-producing bacteria would favor 3GC hydrolysis, reduced antibiotic-induced microbiota dysbiosis, and reduced *C. albicans* overgrowth. In contrast, a microbiota poor in β-lactamase-producing bacteria would allow 3GC maintenance, high antibiotic-induced microbiota dysbiosis, and high *C. albicans* overgrowth. This phenomenon may explain the so-called *C. albicans* colonization resistance experienced by some individuals. Such colonization resistance has been the subject of an old and preliminary report (11) but has not been further explored until this present study. Overall, these results are coherent with the hypothesis stating that specific intestinal bacteria or their metabolites regulate *C. albicans* overgrowth (6, 14, 15, 18, 19). Our results attest that the same antibiotic regimen may affect differentially the microbiota and consequently lead to different risks of *C. albicans* overgrowth depending on the subject that receives it. The current paradigm stating that antibiotics are systematically a risk factor for *C. albicans* overgrowth should thus be adjusted for treatments based on β-lactams antibiotics. Monitoring fecal β-lactamase activity during and after a β-lactams antibiotic treatment could be an accurate predictor of the actual risk of a later increase of *C. albicans* burden.

As importantly, we found that *C. albicans* was present, in varying quantity, in the gut of almost every healthy subject. This study is the first to use a qPCR method to quantify and follow *C. albicans* carriage, allowing an increasingly specific detection. Using more traditional assays, the prevalence of *C. albicans* in these subjects was much lower and close to what has been previously reported (20–22). This suggests that our results reflect the reality of what is the true presence of *C. albicans* in the gut of healthy humans. If confirmed in a larger study, this might indicate that *C. albicans* is not a facultative commensal as previously thought, but that it is able to maintain itself in the gut of most individuals, even at very low concentration.

That almost all subjects in this study were colonized by *C. albicans* renders our cohort particularly adapted to follow the effects of antibiotic treatment on *C. albicans* carriage. Moreover, contrary to what has been done in other studies (6), our focus on a single antibiotic family allows a precise understanding on how 3GC, a largely used antibiotics family, acts on the human gut mycobiota. This allowed us to show that 3GC strongly affect the gut mycobiota, with a global increase of the fungal load, as well as a punctual perturbation of several fungal species and genera, including *C. albicans*. Indeed, both *C. albicans* relative and absolute abundances were increased after the start of antibiotics. This is particularly concerning since a recent report showed that the administration of β-lactam antibiotics leads to increased virulence of *C. albicans* (23). By killing Gram-negative commensal bacteria, β-lactams cause the release of a large amount of peptidoglycans, which can then induce *C. albicans* hyphal growth, an essential virulence factor of this species (23). Moreover, a recent study showed that exposure to non-3GC broad-spectrum antibiotics not only promotes susceptibility to *C. albicans* systemic infection in mice, but also increases the mortality, through an impairment of the lymphocyte-dependent IL-17A- and GM-CSF-mediated response (24). Therefore, *C. albicans* cannot only growth in patients treated with 3GCs, but its disruptive abilities might also be increased. This can be particularly problematic, not only for immunosuppressed patients, but also for those with inflammatory bowel disease that are already carrying higher levels of *C. albicans* (25, 26) in their gut.

Overall, most of the mycobiota perturbations following 3GC treatment were subject-dependent, with some subjects more impacted than others. This is not particularly surprising considering the within- and between-subjects Bray-Curtis dissimilarity observed

pretreatment. This has already been reported (21) and suggests that the largest part of the fecal mycobiota is made of transient species brought by the diet, such as *D. hansenii*, which is commonly found in cheese (27), or potentially by the respiration of spores of filamentous fungi, which can then be swallowed, such as *Penicillium* sp. This hypothesis is supported by a recent study showing that diet-associated fungi are recovered with low relative abundances in mucosal surgical-recovered samples, highlighting the differences observed in the gut mycobiota depending on its spatial organization across the GI tract (28). Moreover, associated fungi strongly contribute to the fungal biomass of the fecal microbiota (29). Our results would also indicate that contrary to *Penicillium* sp. or *D. hansenii*, *C. albicans* main reservoir is indeed humans, which would explain why an environmental reservoir for this species has yet to be found (18, 30, 31).

Finally, fungi represented only a small fraction of the total microbiota based on total and fungal DNA quantification. This underrepresentation of the fungal community in the human gut has already been reported (32), but the authors did not quantify the exact proportion of the mycobiota. More recently, the fungal load of 24 healthy subjects was estimated, with results very similar to ours (33). Finally, Doron et al. confirmed that the fungal biomass was low within the gut microbiota, representing only 1% to 2% of the microbial biomass of the gut (29). However, to our knowledge, this present study is the first to assess the day-to-day variation of the fungal load in healthy individuals.

Taken together, this study offers a better understanding of the factors behind *C. albicans* overgrowth after antibiotics. We showed that a same antibiotic treatment may disturb differentially the gut microbiota, depending on the subject that receives it. This highlights the importance of a more personalized use of antifungal prophylaxis, and helps limiting the selection of fungi resistant to antifungal drugs in patients at high risk of invasive candidiasis, such as intensive care unit or haemato-oncology patients.

MATERIALS AND METHODS

CEREMI cohort. In this study, we used fecal samples from the CEREMI study, a prospective, open-label, and randomized clinical trial conducted from March 2016 to August 2017 in healthy adult subjects at the Clinical Investigation Center at Bichat-Claude Bernard Hospital (Paris, France) (8). Participants were given oral and written information and had to return signed consent before inclusion in the trial. For more information about the clinical trial, see Burdet et al. (8).

The 22 included subjects were randomized in a 1:1 ratio and were treated for 3 days with either ceftriaxone (1 g/24 h) or cefotaxime (1 g/8 h). 3GC were administered as a 30-min intravenous infusion using an automatic high-precision infusion pump.

Fecal samples were collected before treatment at −D15, −D7, and −D1; during treatment at D1, D2, and D3; and after treatment at D4, D7, D10, D15, D30, D90, and D180. Fecal samples were stored at −80°C.

Fungal DNA extraction from fecal samples. For each sample, 250 mg of stool was processed following the repeated bead beating plus column method described by Yu and Morrison (34), except than a FastPrep-24 device (MP Biomedicals, Belgium) was used instead of a Mini-Beadbeater.

Total fecal DNA levels were measured by Qubit (Invitrogen, USA) using the dsDNA Broad Range Kit (Invitrogen, USA). Samples for which this concentration was below 50 ng/μL were excluded from the analysis.

ITS1 sequencing. We prepared amplicon libraries, targeting the ITS1 region, using ITS1F and ITS2 primers (35, 36). Amplicon were generated by PCR using a 96-well thermal cycler in the following conditions: 95°C for 3 min, 25 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and 72°C for 5 min, and cooling at 4°C. Amplicons were purified with AMPure XP (Beckman Coulter, USA) as described in the 16S Metagenomic Sequencing Library Preparation guide (37). Adapter were attached using Nextera XT Index Kit (Illumina, France) and the index PCRs were performed in the following conditions: 95°C for 3 min, eight cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and 72°C for 5 min, and cooling at 4°C. Barcoded PCR products were purified with AMPure XP (Beckman Coulter, USA) and verified and quantified on a Bioanalyzer DNA 1000 chip (Agilent, USA). Samples were normalized at 4 nM and pooled into a library, using 5 μL of each diluted sample. A PhiX sequencing control was prepared following the manufacturer's instructions. The libraries were sequenced in 300-bp paired-end using the MiSeq reagent kit V3 on Illumina MiSeq platform (Illumina, Evry, France).

OTU building process and taxonomic assignment. We recovered 8,819,635 amplicons from ITS1 region. After removal of singletons and chimera amplicons using the SHAMAN pipeline (38), we clustered the 56,634 remaining amplicons in OTUs using a cut-off value of 97% similarity. Thus, 4,648 OTUs were obtained and 551 of them could be aligned against fungal sequences of the UNITE database. On

these 551 fungal OTUs, 340 were present in at least two fecal samples and were conserved for the downstream analyses. We performed a first round of annotation on SHAMAN against the UNITE database (rev. 8.0) and then a second round against a more recent release of UNITE (rev. 8.2). The OTUs we could not annotate were submitted to a classic BLASTN. Only hits matched with a similarity above 97% to reference genomes were conserved. The abundances and weighted non-null normalized counts tables were generated with SHAMAN (38).

Quantitative PCR for detection of total fungal load in human DNA samples. Fungal DNA was quantified by TaqMan qPCR as described by Liu et al. (39) using a double dye MGB 5′ 6-FAM-labeled probe (Eurogentec, Belgium). All reactions were performed on a CFX96 real-time PCR system (Bio-Rad, USA) with the following conditions: 2 min at 50°C, 10 min at 95°C, 15 s at 95°C, and 1 min at 65°C, the last two steps repeated for 45 cycles. All samples were tested in two independent rounds, each time in duplicates.

The fungal load was estimated by dividing the fungal DNA concentration by the total DNA concentration of the sample (33), obtained by Qubit Broad Range protocol.

Quantitative PCR for detection of *C. albicans* DNA in human DNA samples. At 1:10 dilution, 7.5 μL of the extracted total fecal DNA were used as a template for TaqMan qPCR analysis, using probe and primers described by Guiver et al. (40), at 0.1 μM and 0.2 μM, respectively. All reactions were performed on a CFX96 real-time PCR system (Bio-Rad, USA) with the following conditions: 2 min at 50°C, 10 min at 95°C, 15 s at 95°C, and 1 min at 62°C; the last two steps repeated for 45 cycles. All samples were tested in two independent rounds, each time in duplicates.

Quantitative PCR amplification control. To exclude the presence of qPCR inhibitors, samples diluted at 1:10, were submitted to the Universal Exogenous qPCR Positive Control for TaqMan Assay (Eurogentec, Belgium), using a Cy5-QXL 670 Probe system (Eurogentec, Belgium). Manufacturer's recommendations were followed.

Culture of the fecal samples. A 10-μL loop of fecal samples was mixed with 100 μL H_2O and plated on a *Candida* CHROMAGAR medium plate (bioMérieux, France). Potential *C. albicans* colonies were further tested by MALDI-TOF MS (Brucker, USA) to confirm the identification.

Measure of the β-lactamase activity. Fecal β-lactamase activity was quantified by measuring the hydrolysis of nitrocephin, a chromogenic cephalosporin. Activity was measured at least in duplicate.

Fecal samples (stored at 65°C) were thawed 30 min on ice. Then, 140 mg to 380 mg of each fecal sample was mixed with 5 mL/g of stool HZn buffer (50 mM (2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.5, supplemented with 50 μM ZnSO4) and agitated for 1 h. Samples were centrifuged twice at 4°C (15 min and 30 min). Then, 3 to 20μL of the obtained supernatant were mixed with 100 μM nitrocefin (Cayman Chemical, USA) and HZn buffer to reach a final volume of 200 μL. Samples were incubated 20 min at room temperature in a 1:1 ratio with HZn buffer. Nitrocefin hydrolysis was monitored in EnVision microplate reader (Perkin Elmer, USA) at a 482-nm wavelength. SpectraPlate-96 (Perkin-Elmer, USA) using an automated liquid handling Janus Integrator system (Perkin Elmer, USA) was used to conduct the assays.

β-lactamase activity was normalized to one gram of fecal sample and to 1-cm pathlength. Detection threshold was set at a cut-off value of 4.8 nmol/min-g of fecal sample.

Biostatistical analyses. All analyses were performed on R (version 4.0.2 [41]). We used the vegan package (v.2.5-6 [42]) to compute diversity indexes and ggplot2 package to generate the figures (v. 3.3.5 [43]).

We used samples collected at −D1 before treatment as baseline, called D0. If this sample was missing for a subject, sample collected at −D7 before treatment was used instead. If both samples were missing, sample collected at −D15 before treatment was used as D0 sample.

We calculated the change from baseline of the fungal load, *C. albicans* DNA absolute abundance, the relative abundance of the fungal genera, and species and β-lactamase activity. Null values were replaced by the minimal non-null value of the given variables divided by two, to allow a $\log_{10}$ transformation. Only the fungal genera and species reaching a maximal relative abundance superior to 1% for at least half of the subjects were analyzed. We calculated the AUCs using the R package MESS (v. 0.5.7, [44]) for each period from D0 to D2 up to D0 to D180 based on the normalized changes from baseline and the actual time and date of stool collection.

For all analyses, we used bilateral nonparametric Wilcoxon exact tests. We used a type I error of 0.05 and corrected the *P* values for multitesting using false discovery rate correction.

Data availability. The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, TIF file, 1.6 MB.

FIG S2, TIF file, 1 MB.

FIG S3, TIF file, 0.9 MB.

FIG S4, TIF file, 2 MB.

TABLE S1, XLSX file, 0.02 MB.

TABLE S2, XLSX file, 0.02 MB.

ACKNOWLEDGMENTS

This work was supported by grants from Agence Nationale de la Recherche (FunComPath ANR-14-IFEC-0004 and PrediRes ANR-16-CE15-0022); the French Government's Investissement d'Avenir program (Laboratoire d'Excellence Integrative Biology of Emerging Infectious Diseases [ANR10-LABX-62-IBEID]); the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie action, Innovative Training Network (FunHoMic; Grant No. 812969) and Assistance Publique – Hôpitaux de Paris (CEREMI CRC13-179).

We thank Antoine Andremont and David Skurnik for the fruitful discussions.

We thank the Institut Pasteur Biomics platform (Marc Monot and Laurence Motreff) and the Institut Pasteur Bioinformatics and Biostatistics Hub for their help for generating and analyzing the sequencing data.

For the PrediRes study group: Charles Burdet (INSERM, Université de Paris APHP-Bichat Hospital), Erick Denamur (INSERM, Université de Paris), Xavier Duval (INSERM, Université de Paris, APHP-Bichat Hospital), Dusko Ehrlich (INRA Metagenopolis), France Mentré (INSERM, Université de Paris, APHP-Bichat Hospital), Eduardo P. C. Rocha (Institut Pasteur), Laurie Alla (INRA Metagenopolis), Emmanuelle Lechatelier (INRA Metagenopolis), Florence Levenez (INRA Metagenopolis), Nicolas Pons (INRA Metagenopolis), Benoît Quinquis (INRA Metagenopolis), Khadija Bourabha (INSERM), Antoine Bridier Nahmias (INSERM, Université de Paris), Olivier Clermont (INSERM, Université de Paris), Mélanie Magnan (INSERM, Université de Paris), Olivier Tenaillon (INSERM, Université de Paris), Camille d'Humières (INSERM, Université de Paris, APHP-Bichat Hospital, Institut Pasteur), Amandine Perrin (Institut Pasteur), Marie Touchon (Institut Pasteur), Dominique Rainteau (INSERM, Université Pierre et Marie Curie, APHP – Saint Antoine Hospital), Farid Ichou (ICAN), Philippe Lesnik (ICAN), Jimmy Mullaert (INSERM, Université de Paris, APHP – Bichat Hospital), Thu Thuy Nguyen (INSERM).

REFERENCES

1. Krajmalnik-Brown R, Lozupone C, Kang D-W, Adams JB. 2015. Gut bacteria in children with autism spectrum disorders: challenges and promise of studying how a complex community influences a complex disease. Microb Ecol Heal Dis 26.
2. Milani C, Ticinesi A, Gerritsen J, Nouvenne A, Lugli GA, Mancabelli L, Turroni F, Duranti S, Mangifesta M, Viappiani A, Ferrario C, Maggio M, Lauretani F, De Vos W, van Sinderen D, Meschi T, Ventura M. 2016. Gut microbiota composition and Clostridium difficile infection in hospitalized elderly individuals: a metagenomic study. Sci Rep 6:25945. https://doi.org/10.1038/srep25945.
3. Zuo T, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, Wan Y, Chung ACK, Cheung CP, Chen N, Lai CKC, Chen Z, Tso EYK, Fung KSC, Chan V, Ling L, Joynt G, Hui DSC, Chan FKL, Chan PKS, Ng SC. 2020. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. Gastroenterology 159:944–955.e8. https://doi.org/10.1053/j.gastro.2020.05.048.
4. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. 2007. The Human Microbiome Project. Nat 449:804–810. https://doi.org/10.1038/nature06244.
5. Burdet C, Nguyen TT, Duval X, Ferreira S, Andremont A, Guedj J, Mentré F, Ait-Ilalne B, Alavoine L, Duval X, Ecobichon JL, Ilic-Habensus E, Laparra A, Nisus ME, Ralaimazava P, Raine S, Tubiana S, Vignali V, Chachaty E, the DAV132-CL-1002 Study Group. 2019. Impact of antibiotic gut exposure on the temporal changes in microbiome diversity. Antimicrob Agents Chemother 63. https://doi.org/10.1128/AAC.00820-19.
6. Seelbinder B, Chen J, Brunke S, Vazquez-Uribe R, Santhaman R, Meyer AC, De Oliveira Lino FS, Chan KF, Loos D, Imamovic L, Tsang CC, Lam RPK, Sridhar S, Kang K, Hube B, Woo PCY, Sommer MOA, Panagiotou G. 2020. Antibiotics create a shift from mutualism to competition in human gut communities with a longer-lasting impact on fungi than bacteria. Microbiome 8:133. https://doi.org/10.1186/s40168-020-00899-6.
7. Fouhy F, Guinane CM, Hussey S, Wall R, Ryan CA, Dempsey EM, Murphy B, Ross RP, Fitzgerald GF, Stanton C, Cotter PD. 2012. High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. Antimicrob Agents Chemother 56:5811–5820. https://doi.org/10.1128/AAC.00789-12.
8. Burdet C, Grall N, Linard M, Bridier-Nahmias A, Benhayoun M, Bourabha K, Magnan M, Clermont O, d'Humières C, Tenaillon O, Denamur E, Massias L, Tubiana S, Alavoine L, Andremont A, Mentré F, Duval X, Andremont A, Burdet C, Duval X, Fantin B, Grall N, Marcault E, Massias L, Mentré F, Tubiana S, Alavoine L, Benhayoun M, Djerdjour F, Ecobichon J-L, Ilic-Habensus E, Laparra A, Mandic M, Nisus ME, Raine S, Ralaimazava P, Vignali V, Andremont A, d'Humières C, Grall N, Riberty J, Bourabha K, Nahmias AB, Clermont O, Denamur E, Magnan M, Tenaillon O, Massias L, Marcault E, Schneider M, for the CEREMI Group, et al. 2019. Ceftriaxone and cefotaxime have similar effects on the intestinal microbiota in human volunteers treated by standard-dose regimens. Antimicrob Agents Chemother 63. https://doi.org/10.1128/AAC.02244-18.
9. Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. 2016. Clostridium difficile infection. Nat Rev Dis Primers 2:16020. https://doi.org/10.1038/nrdp.2016.20.
10. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. 2015. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13:42–51. https://doi.org/10.1038/nrmicro3380.
11. Leonard F, Andremont A, Leclerq B, Labia R, Tancrede C. 1989. Use of β-lactamase-producing anaerobes to prevent ceftriaxone from degrading intestinal resistance to colonization. J Infect Dis 160:274–280. https://doi.org/10.1093/infdis/160.2.274.
12. Niehus R, van Kleef E, Mo Y, Turlej-Rogacka A, Lammens C, Carmeli Y, Goossens H, Tacconelli E, Carevic B, Preotescu L, Malhotra-Kumar S, Cooper BS. 2020. Quantifying antibiotic impact on within-patient dynamics of extended-spectrum beta-lactamase resistance. Elife 9:1–20. https://doi.org/10.7554/eLife.49206.
13. Dollive S, Chen Y-Y, Grunberg S, Bittinger K, Hoffmann C, Vandivier L, Cuff C, Lewis JD, Wu GD, Bushman FD. 2013. Fungi of the murine gut: episodic variation and proliferation during antibiotic treatment. PLoS One 8:e71806. https://doi.org/10.1371/journal.pone.0071806.
14. Fan D, Coughlin LA, Neubauer MM, Kim J, Kim MS, Zhan X, Simms-Waldrip TR, Xie Y, Hooper LV, Koh AY. 2015. Activation of HIF-1α and LL-37 by commensal bacteria inhibits Candida albicans colonization. Nat Med 21:808–814. https://doi.org/10.1038/nm.3871.

15. Mirhakkak MH, Schäuble S, Klassert TE, Brunke S, Brandt P, Loos D, Uribe RV, Senne de Oliveira Lino F, Ni Y, Vylkova S, Slevogt H, Hube B, Weiss GJ, Sommer MOA, Panagiotou G. 2021. Metabolic modeling predicts specific gut bacteria as key determinants for Candida albicans colonization levels. ISME J 15:1257–1270. https://doi.org/10.1038/s41396-020-00848-z.
16. Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC. 2012. Hidden killers: human fungal infections. Sci Transl Med 4:165rv13.
17. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. 2018. Invasive candidiasis. Nat Rev Dis Prim 4.
18. d'Enfert C, Kaune AK, Alaban LR, Chakraborty S, Cole N, Delavy M, Kosmala D, Marsaux B, Fróis-Martins R, Morelli M, Rosati D, Valentine M, Xie Z, Emritloll Y, Warn PA, Bequet F, Bougnoux ME, Bornes S, Gresnigt MS, Hube B, Jacobsen ID, Legrand M, Leibundgut-Landmann S, Manichanh C, Munro CA, Netea MG, Queiroz K, Roget K, Thomas V, Thoral C, Van den Abbeele P, Walker AW, Brown AJP. 2021. The impact of the fungus-host-microbiota interplay upon Candida albicans infections: current knowledge and new perspectives. FEMS Microbiol Rev 45.
19. Leonardi I, Paramsothy S, Doron I, Semon A, Kaakoush NO, Clemente JC, Faith JJ, Borody TJ, Mitchell HM, Colombel JF, Kamm MA, Iliev ID. 2020. Fungal trans-kingdom dynamics linked to responsiveness to fecal microbiota transplantation (FMT) therapy in ulcerative colitis. Cell Host Microbe 27:823–829.e3. https://doi.org/10.1016/j.chom.2020.03.006.
20. Bougnoux M-E, Diogo D, François N, Sendid B, Veirmeire S, Colombel JF, Bouchier C, Van Kruiningen H, d'Enfert C, Poulain D. 2006. Multilocus sequence typing reveals intrafamilial transmission and microevolutions of Candida albicans isolates from the human digestive tract. J Clin Microbiol 44:1810–1820. https://doi.org/10.1128/JCM.44.5.1810-1820.2006.
21. Nash AK, Auchtung TA, Wong MC, Smith DP, Gesell JR, Ross MC, Stewart CJ, Metcalf GA, Muzny DM, Gibbs RA, Ajami NJ, Petrosino JF. 2017. The gut mycobiome of the Human Microbiome Project healthy cohort. Microbiome 5:153. https://doi.org/10.1186/s40168-017-0373-4.
22. da Silva Dantas A, Lee KK, Raziunaite I, Schaefer K, Wagener J, Yadav B, Gow NA. 2016. Cell biology of Candida albicans–host interactions. Curr Opin Microbiol Curr Opin Microbiol 34:111–118. https://doi.org/10.1016/j.mib.2016.08.006.
23. Tan CT, Xu X, Qiao Y, Wang Y. 2021. A peptidoglycan storm caused by β-lactam antibiotic's action on host microbiota drives Candida albicans infection. Nat Commun 12. https://doi.org/10.1038/s41467-021-22845-2.
24. Drummond RA, Desai JV, Ricotta EE, Swamydas M, Deming C, Conlan S, Quinones M, Matei-Rascu V, Sherif L, Lecky D, Lee CCR, Green NM, Collins N, Zelazny AM, Prevots DR, Bending D, Withers D, Belkaid Y, Segre JA, Lionakis MS. 2022. Long-term antibiotic exposure promotes mortality after systemic fungal infection by driving lymphocyte dysfunction and systemic escape of commensal bacteria. Cell Host Microbe 30:1020–1033.e6. https://doi.org/10.1016/j.chom.2022.04.013.
25. Sokol H, Leducq V, Aschard H, Pham HP, Jegou S, Landman C, Cohen D, Liguori G, Bourrier A, Nion-Larmurier I, Cosnes J, Seksik P, Langella P, Skurnik D, Richard ML, Beaugerie L. 2017. Fungal microbiota dysbiosis in IBD. Gut 66:1039–1048. https://doi.org/10.1136/gutjnl-2015-310746.
26. Sendid B, Dotan N, Nseir S, Savaux C, Vandewalle P, Standaert A, Zerimech F, Guery BP, Dukler A, Colombe JF, Poulain D. 2008. Antibodies against glucan, chitin, and Saccharomyces cerevisiae mannan as new biomarkers of Candida albicans infection that complement tests based on C. albicans mannan. Clin Vaccine Immunol 15:1868–1877. https://doi.org/10.1128/CVI.00200-08.
27. Fröhlich-Wyder MT, Arias-Roth E, Jakob E. 2019. Cheese yeasts. Yeast 36:129–141. https://doi.org/10.1002/yea.3368.
28. Leonardi I, Gao IH, Lin WY, Allen M, Li XV, Fiers WD, De Celie MB, Putzel GG, Yantiss RK, Johncilla M, Colak D, Iliev ID. 2022. Mucosal fungi promote gut barrier function and social behavior via Type 17 immunity. Cell 185:831–846.e14. https://doi.org/10.1016/j.cell.2022.01.017.
29. Doron I, Leonardi I, Li XV, Fiers WD, Semon A, Bialt-DeCelie M, Migaud M, Gao IH, Lin WY, Kusakabe T, Puel A, Iliev ID. 2021. Human gut mycobiota tune immunity via CARD9-dependent induction of anti-fungal IgG antibodies. Cell 184:1017–1031.e14. https://doi.org/10.1016/j.cell.2021.01.016.
30. Jabra-Rizk MA, Kong EF, Tsui C, Nguyen MH, Clancy CJ, Fidel PL, Noverr M. 2016. Candida albicans pathogenesis: fitting within the host-microbe damage response framework. Infect Immun 84:2724–2739. https://doi.org/10.1128/IAI.00469-16.
31. Nucci M, Anaissie E. 2001. Revisiting the source of candidemia: skin or gut? Clin Infect Dis 33:1959–1967. https://doi.org/10.1086/323759.
32. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, MetaHIT Consortium, et al. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65. https://doi.org/10.1038/nature08821.
33. Zuo T, Wong SH, Cheung CP, Lam K, Lui R, Cheung K, Zhang F, Tang W, Ching JYL, Wu JCY, Chan PKS, Sung JJY, Yu J, Chan FKL, Ng SC. 2018. Gut fungal dysbiosis correlates with reduced efficacy of fecal microbiota transplantation in Clostridium difficile infection. Nat Commun 9. https://doi.org/10.1038/s41467-018-06103-6.
34. Yu Z, Morrison M. 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques 36:808–812. https://doi.org/10.2144/04365ST04.
35. Cui L, Morris A, Ghedin E. 2013. The human mycobiome in health and disease. Genome Med 5:63. https://doi.org/10.1186/gm467.
36. Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118. https://doi.org/10.1111/j.1365-294x.1993.tb00005.x.
37. 16S Metagenomic Sequencing Library Preparation. https://emea.support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html#. Retrieved 25 August 2021.
38. Volant S, Lechat P, Woringer P, Motreff L, Campagne P, Malabat C, Kennedy S, Ghozlane A. 2020. SHAMAN: a user-friendly website for metataxonomic analysis from raw reads to statistical analysis. BMC Bioinformatics 21. https://doi.org/10.1186/s12859-020-03666-4.
39. Liu CM, Kachur S, Dwan MG, Abraham AG, Aziz M, Hsueh PR, Huang YT, Busch JD, Lamit LJ, Gehring CA, Keim P, Price LB. 2012. FungiQuant: a broad-coverage fungal quantitative real-time PCR assay. BMC Microbiol 12. https://doi.org/10.1186/1471-2180-12-56.
40. Guiver M, Levi K, Oppenheim BA. 2001. Rapid identification of candida species by TaqMan PCR. J Clin Pathol 54:362–366. https://doi.org/10.1136/jcp.54.5.362.
41. R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
42. Oksanen AJ, Blanchet FG, Friendly M, Kindt R, Legendre P, Mcglinn D, Minchin PR, Hara RBO, Simpson GL, Solymos P, Stevens MHH, Szoecs E. 2019. Package 'vegan.' R Packag Libr. https://CRAN.R-project.org/package=vegan.
43. Wickham H, Chang W, Henry L, Pedersen TL, Takahashi K, Wilke C, Woo K. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. https://ggplot2.tidyverse.org.
44. Ekstrøm CT. 2020. Miscellaneous esoteric statistical scripts [R package MESS version 0.5.7]. https://CRAN.R-project.org/package=MESS.

Appendix 2:

The Impact of the Fungus-Host-Microbiota Interplay upon *Candida albicans* Infections: Current Knowledge and New Perspectives.

Christophe D'ENFERT, Ann-Kristin KAUNE, Leovigildo-Rey ALABAN, Sayoni CHAKRABORTY, Nathaniel COLE, Margot DELAVY, Daria Kosmala, Benoît Marsaux, Ricardo FRÓIS-MARTINS, Moran MORELLI, Diletta ROSATI, Marisa VALENTINE, Zixuan XIE, Yoan EMRITLOLL, Peter A WARN, Frédéric BEQUET, Marie-Elisabeth BOUGNOUX, Stephanie BORNES, Mark S GRESNIGT, Bernhard HUBE, Ilse D JACOBSEN, Mélanie LEGRAND, Salomé LEIBUNDGUT-LANDMANN, Chaysavanh MANICHANH, Carol A MUNRO, Mihai G NETEA, Karla QUEIROZ, Karine ROGET, Vincent THOMAS, Claudia THORAL, Pieter VAN DEN ABBEELE, Alan W WALKER, Alistair J P BROWN

FEMS Microbiology Reviews 2020, 45 (3): 1–55.
https://doi.org/10.1093/femsre/fuaa060

FEMS Microbiology Reviews, fuaa060, 45, 2021, 1–55

doi: 10.1093/femsre/fuaa060
Advance Access Publication Date: 24 November 2020
Review Article

REVIEW ARTICLE

The impact of the Fungus-Host-Microbiota interplay upon *Candida albicans* infections: current knowledge and new perspectives

Christophe d'Enfert[1,*,†], Ann-Kristin Kaune[2], Leovigildo-Rey Alaban[3,4], Sayoni Chakraborty[5,6], Nathaniel Cole[7], Margot Delavy[1,4], Daria Kosmala[1,4], Benoît Marsaux[8,9], Ricardo Fróis-Martins[10,11], Moran Morelli[12], Diletta Rosati[13,‡], Marisa Valentine[5], Zixuan Xie[14], Yoan Emritloll[1], Peter A. Warn[15], Frédéric Bequet[3], Marie-Elisabeth Bougnoux[1], Stephanie Bornes[16], Mark S. Gresnigt[5], Bernhard Hube[5], Ilse D. Jacobsen[5], Mélanie Legrand[1], Salomé Leibundgut-Landmann[10,11], Chaysavanh Manichanh[14], Carol A. Munro[2], Mihai G. Netea[13], Karla Queiroz[12], Karine Roget[17], Vincent Thomas[3], Claudia Thoral[17], Pieter Van den Abbeele[8], Alan W. Walker[7] and Alistair J. P. Brown[18,*,§]

[1]Unité Biologie et Pathogénicité Fongiques, Département de Mycologie, Institut Pasteur, USC 2019 INRA, 25, rue du Docteur Roux, 75015 Paris, France, [2]Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Ashgrove Road West, Foresterhill, Aberdeen AB25 2ZD, UK, [3]BIOASTER Microbiology Technology Institute, 40 avenue Tony Garnier, 69007 Lyon, France, [4]Université de Paris, Sorbonne Paris Cité, 25, rue du Docteur Roux, 75015 Paris, France, [5]Microbial Immunology Research Group, Emmy Noether Junior Research Group Adaptive Pathogenicity Strategies, and the Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Beutenbergstraße 11a, 07745 Jena, Germany, [6]Institute of Microbiology, Friedrich Schiller University, Neugasse 25, 07743 Jena, Germany, [7]Gut Microbiology Group, Rowett Institute, University of Aberdeen, Ashgrove Road West, Foresterhill, Aberdeen AB25 2ZD, UK, [8]ProDigest BV, Technologiepark 94, B-9052 Gent, Belgium, [9]Center for Microbial Ecology and Technology (CMET), Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links, 9000 Ghent, Belgium, [10]Immunology Section, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 266a, Zurich 8057, Switzerland, [11]Institute of Experimental Immunology, University of Zurich, Winterthurerstrasse 190, Zürich 8057, Switzerland, [12]Mimetas, Biopartner Building 2, J.H. Oortweg 19, 2333 CH Leiden, The Netherlands, [13]Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Geert Grooteplein 28, 6525 GA Nijmegen, The

Received: 3 October 2020; **Accepted:** 18 November 2020

Netherlands, [14]Gut Microbiome Group, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Passeig Vall d'Hebron 119–129, 08035 Barcelona, Spain, [15]Magic Bullet Consulting, Biddlecombe House, Ugbrook, Chudleigh Devon, TQ130AD, UK, [16]Université Clermont Auvergne, INRAE, VetAgro Sup, UMRF0545, 20 Côte de Reyne, 15000 Aurillac, France, [17]NEXBIOME Therapeutics, 22 allée Alan Turing, 63000 Clermont-Ferrand, France and [18]MRC Centre for Medical Mycology, Department of Biosciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, UK

*Corresponding authors: Unité Biologie et Pathogénicité Fongiques, Département de Mycologie, Institut Pasteur, 25–28 rue du Docteur Roux, 75015 Paris, France. E-mail: christophe.denfert@pasteur.fr; MRC Centre for Medical Mycology, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, UK. E-mail: a.j.p.brown@exeter.ac.uk

One sentence summary: The complexity and variability of FunHoMic interactions between the fungal pathogen, its human host and the Microbiota strongly influence the development and outcomes of the superficial and systemic *Candida albicans* infections that plague human health worldwide.

Editor: Bart Thomma

†Christophe d'Enfert, http://orcid.org/0000-0002-6235-3886
‡Diletta Rosati, http://orcid.org/0000-0003-2992-2503
§Alistair J.P. Brown, http://orcid.org/0000-0003-1406-4251

ABSTRACT

Candida albicans is a major fungal pathogen of humans. It exists as a commensal in the oral cavity, gut or genital tract of most individuals, constrained by the local microbiota, epithelial barriers and immune defences. Their perturbation can lead to fungal outgrowth and the development of mucosal infections such as oropharyngeal or vulvovaginal candidiasis, and patients with compromised immunity are susceptible to life-threatening systemic infections. The importance of the interplay between fungus, host and microbiota in driving the transition from *C. albicans* commensalism to pathogenicity is widely appreciated. However, the complexity of these interactions, and the significant impact of fungal, host and microbiota variability upon disease severity and outcome, are less well understood. Therefore, we summarise the features of the fungus that promote infection, and how genetic variation between clinical isolates influences pathogenicity. We discuss antifungal immunity, how this differs between mucosae, and how individual variation influences a person's susceptibility to infection. Also, we describe factors that influence the composition of gut, oral and vaginal microbiotas, and how these affect fungal colonisation and antifungal immunity. We argue that a detailed understanding of these variables, which underlie fungal-host-microbiota interactions, will present opportunities for directed antifungal therapies that benefit vulnerable patients.

Keywords: *Candida*; *Candida* infections; antifungal immunity; microbiota; mycobiota; fungus-host-microbiota interactions; patient variability; fungal variability; microbiota variability

INTRODUCTION

Fungal pathogens have a major global impact upon human health. Estimates suggest that, at any given time, over a quarter of the world's population have a fungal infection of the skin, that 75% of women suffer at least one episode of vulvovaginal candidiasis during their lifetime, and that over a million people die each year from an invasive fungal infection (Brown *et al.* 2012). Mortality rates for those suffering systemic fungal infections are unacceptably high, reaching 50% in many cases. This is because fungal infections are often difficult to diagnose, and are particularly challenging to treat (Perlroth, Choi and Spellberg 2007; Brown *et al.* 2012; Köhler, Casadevall and Perfect 2014). There is a clear and urgent medical need for more accurate diagnostics, for safer and more effective antifungal drugs, and for host-directed therapies. The search for antifungal drug targets is somewhat constrained by the fact that, as eukaryotes, fungi share fundamental mechanisms of cell growth and division with humans. The search for diagnostic markers that can distinguish infection from fungal commensalism is especially challenging. Therefore, the development of potent new clinical tools is dependent upon a comprehensive understanding of fungal pathogenicity and antifungal immunity.

Candida species are amongst the top fungal killers (Brown *et al.* 2012). Of these, *Candida albicans* remains the most common cause of life-threatening systemic candidiasis, although the frequent prophylactic use of azole antifungal drugs has led to the emergence of other *Candida* species with intrinsic resistance to these drugs (Nguyen *et al.* 1996; Silva *et al.* 2012; Chowdhary, Sharma and Meis 2017). Nevertheless, in this review we focus on *C. albicans*, because a combination of three main factors arguably makes this species unique amongst fungal pathogens: (a) its lifestyle as both a commensal and potent pathogen; (b) the range and frequency of infections that it causes; and (c) its pathobiology has been studied in greater depth than most other fungal pathogens.

Candida albicans is an opportunistic pathogen that exists as a commensal in most individuals, and is a frequent cause of mucosal and systemic infections (See *The Fungus*). Unlike most fungal pathogens, *C. albicans* is generally considered to be obligately associated with warm-blooded animals (Odds 1988). Environmental isolates of *C. albicans* continue to be reported (Bensasson *et al.* 2019; Maciel *et al.* 2019; Opulente *et al.* 2019). However, although the existence of an environmental reservoir cannot be excluded, it is apparently not necessary for human colonisation.

Candida albicans is transmitted vertically from mother to child, and infections arise predominantly from the endogenous

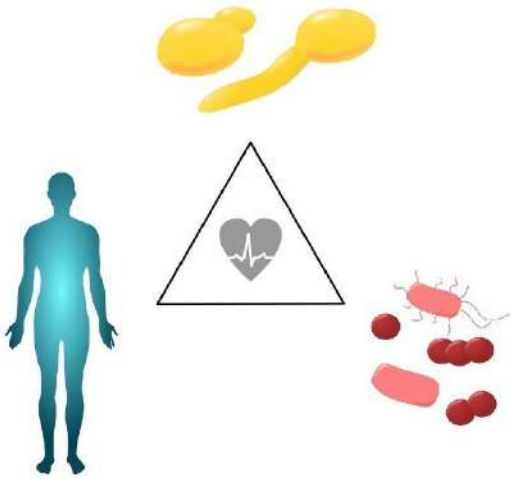


Figure 1. Three-way interactions between the fungus, the host and the local microbiota strongly influence the likelihood and severity of *C. albicans* infections. See text.

microbiota rather than other sources (d Enfert 2009; Miranda *et al.* 2009; Zhai *et al.* 2020) (see *The Microbiota*). This contrasts with other major pathogens such as *Aspergillus*, *Cryptococcus* and *Histoplasma* species, which are fundamentally environmental fungi that have evolved traits that promote pathogenicity in humans, possibly through their transient passage in niches that have similarities with those encountered in the human host, for example, their association with rodents or contact and evasion of amoebic predation in the environment (Steenbergen, Shuman and Casadevall 2001; Malliaris, Steenbergen and Casadevall 2004; Van Waeyenberghe *et al.* 2013; Hillmann *et al.* 2015). *Pneumocystis jirovecii* is obligately associated with humans, but this major pathogen differs from *C. albicans* in that it is unable to thrive outside its host (Liu, Fahle and Kovacs 2018). Consequently, key aspects of *Pneumocystis jirovecii* biology remain unexplored. The lifestyle of *C. albicans* even differs considerably from its distant cousin, *C.* (Brunke and Hube 2013; Kasper, Seider and Hube 2015). Genetic evidence suggests that, although it is often presumed to be a human commensal such as *C. albicans*, *C. glabrata* seems to be only secondarily associated with humans and is likely to have environmental reservoirs (Gabaldón and Fairhead 2019).

The biology, epidemiology, pathogenicity and immunology of *C. albicans* have been studied in greater depth than for any other fungal pathogen. This depth of knowledge provides a strong platform for studies of the relationships between the fungal pathogen, host immunity and local microbiota that lie at the heart of fungal infection (Casadevall and Pirofski 1999, 2003, 2015; Jabra-Rizk *et al.* 2016) (Fig. 1). Other major fungal pathogens infect humans by different routes to *C. albicans*, but many principles that are emerging for *C. albicans* may be applicable to these pathogens. Therefore, we present underlying principles of *C. albicans* colonisation and infection, antifungal immune defences, and the protective properties of the local microbiota in the gastrointestinal (GI) tract, oral cavity and vagina. We also address the variability that influences the *Fungus-Host-Microbiota* interplay and how this impacts infection. A detailed understanding of this tripartite interplay is essential to optimise therapeutic strategies for individual patients (d Enfert 2009; Pirofski and Casadevall 2020).

THE FUNGUS

C. albicans commensalism and pathogenicity

C. albicans frequently inhabits the oral, vaginal and GI mucosa of healthy individuals as a harmless commensal (Ghannoum *et al.* 2010; Drell *et al.* 2013; Nash *et al.* 2017) (Fig. 2). Indeed, *C. albicans* is present on the mucosa of most people in most human populations (Neville, d Enfert and Bougnoux 2015; Prieto *et al.* 2016; Mishra and Koh 2018). However, this fungus can cause infections if the local microbiota becomes perturbed, normal tissue barriers are weakened or immune defences become compromised.

Mucosal infections, characterised by fungal colonisation (i.e. overgrowth) associated with an inflammatory host response, are extremely common and can have a major impact upon the quality of life for many individuals (Fig. 2). For instance, most women of reproductive age (75%) will experience at least one episode of VVC ('thrush') in their lifetime, and up to 9% suffer from recurrent VVC, as defined by multiple episodes of vaginitis per annum (Foxman *et al.* 2013; Yano *et al.* 2019; Rosati, Bruno, Jaeger, Ten Oever *et al.* 2020). Risk factors for VVC include high estrogen levels, the use of oral contraceptives and uncontrolled diabetes. However, episodes can be idiopathic (i.e. of unknown cause) and VVC, unlike oral candidiasis, can occur in apparently healthy individuals (see *Innate antifungal responses*).

Oropharyngeal candidiasis (OPC) can broadly be classified into three main conditions, namely acute, chronic and chronic mucocutaneous candidiasis syndromes (Vila *et al.* 2020) (Fig. 2). Predisposing factors include nutritional deficiencies, local dysbiosis, salivary hypo-function, smoking, wearing dentures and dysfunctional T-cell immunity due to genetic alterations or other infections. Indeed, OPC is the most frequently diagnosed oral opportunistic infection in HIV-positive individuals and many acute cases are caused by broad-spectrum antibiotic treatments (Samaranayake 1992; Vila *et al.* 2020).

Life-threatening systemic *C. albicans* infections can arise when the fungus enters the bloodstream (Fig. 2). Candidaemia is the fourth most common nosocomial bloodstream infection in North America (Pfaller and Diekema 2010), but the incidence of invasive candidiasis in European countries is generally lower (Meyer *et al.* 2013; Yapar 2014). The presence of a central venous catheter, dialysis, antibiotic treatment, lengthy stays in intensive care units (ICUs), recent major surgery, and receiving total parenteral nutrition are among the predisposing factors for systemic candidiasis (Pappas *et al.* 2018). Most disseminated infections arise from *Candida* escaping the patient's own GI tract (Miranda *et al.* 2009; Gouba and Drancourt 2015; Zhai *et al.* 2020). Systemic infections arise when host defences are compromised by, for example, damage to the intestinal barrier (e.g. surgery or trauma), medically induced immunosuppression (corticosteroids or chemotherapy-induced neutropenia), or the use of broad-spectrum antibiotics (Pappas *et al.* 2018). A combination of these factors is typically needed to allow *C. albicans* to translocate from the gut (Koh *et al.* 2008; Papon, Bougnoux and d Enfert 2020). Once in the blood, *C. albicans* can disseminate to almost all organs including kidney, liver, and spleen (Pappas *et al.* 2018). The mortality rate for these infections, which varies across geographical regions, is reported to lie between 10% and 47% despite the availability of antifungal therapies (Brown *et al.* 2012). This is unacceptably high.

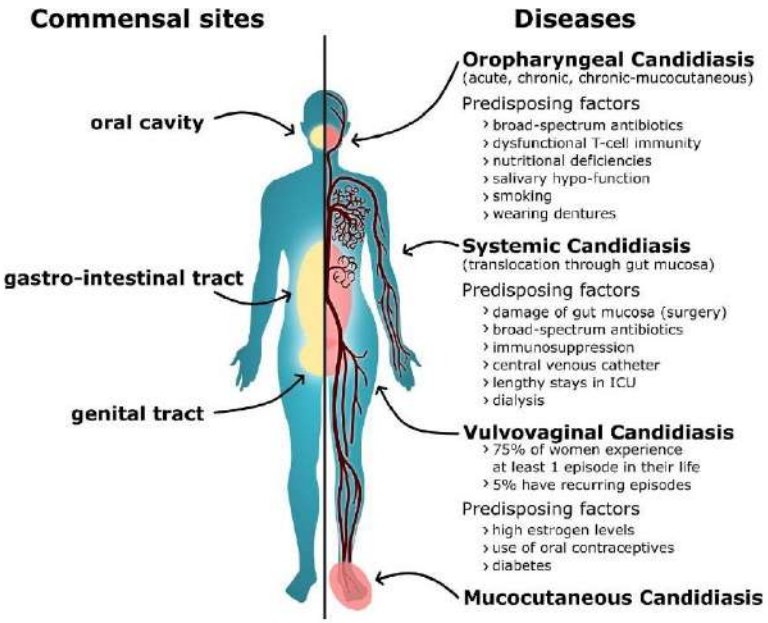


Figure 2. Sites of *C. albicans* commensalism and disease on the human body. Sites of *C. albicans* commensalism (left side) include the oral cavity, gastrointestinal tract (gut) and the genital tract. *C. albicans* can infect these sites (right side) to cause oropharyngeal or vulvovaginal candidiasis. *C. albicans* can also cause systemic infections of the blood and internal organs, which often arise *via* translocation of *C. albicans* from the gut into the bloodstream. *Candida albicans* also causes mucocutaneous infections of the skin and nails. Factors that predispose individuals to such infections are listed. See text.

Clearly, knowledge about the factors and conditions that promote *C. albicans* commensalism or opportunism is important for an understanding of the mechanisms that underlie the transition from commensalism to pathogenicity. Much work has focussed on the virulence factors and fitness attributes that promote *C. albicans* infection (see *Virulence Factors* and *Fitness attributes*). However, the pathogenesis of *C. albicans* also depends on the host site of colonisation (Fidel *et al.* 2020). *Candida albicans* asymptomatically inhabits the oral mucosa and only causes infection when host defences are weakened. In contrast, *C. albicans* is an immunoreactive coloniser during vulvovaginal infection, eliciting host damage *via* a hyperactive immune response. Meanwhile, systemic infections are mostly nosocomial and are generally associated with predisposing conditions. The fungus is able to cause these different types of infection by tuning the expression of its arsenal of virulence factors and fitness attributes to the local niche.

Virulence factors

Cellular polymorphism

The polymorphic nature of *C. albicans* is integral to both commensalism and pathogenesis. This fungus is able to switch reversibly between different growth forms and morphologies (Noble, Gianetti and Witchley 2017) (Fig. 3). Depending upon the environmental conditions, *C. albicans* can grow as unicellular yeast cells, pseudohyphae, or true hyphae that lack invaginations at septal junctions (Sudbery, Gow and Berman 2004). Also, depending on the presence of certain environmental cues, *C. albicans* can undergo phenotypic switching to interchange reversibly between white, grey and opaque phenotypes, each of which displays distinct yeast cell and colony morphologies, and gene expression profiles. Furthermore, a gastrointestinally induced transition (GUT) phenotype has been described for *C. albicans* cells that ectopically overexpress the Wor1 regulator which, together with Efg1, controls white-grey-opaque switching (Pande, Chen and Noble 2013). Phenotypic switching is a strictly regulated process that seems to be associated with commensalism, host niche adaptation, mating, immune evasion and virulence (Miller and Johnson 2002; Morschhäuser 2010; Pande, Chen and Noble 2013; Xie *et al.* 2013; Tao *et al.* 2014). Finally, *C. albicans* can differentiate to form chlamydospores, enlarged thick-walled cells, under nutrient limitation, low temperature and microaerophilia (Staib and Morschhäuser 2007; Böttcher *et al.* 2016) (Fig. 3).

Both yeast and hyphal morphologies are necessary for the full virulence of *C. albicans* (Lo *et al.* 1997; Murad *et al.* 2001; Saville *et al.* 2003; Jacobsen *et al.* 2012) (Fig. 4). However, it is generally thought that yeast cells are well suited to dissemination, and hyphal cells to tissue invasion (Gow, Brown and Odds 2002). The yeast-to-hypha transition is accompanied by an extensive change in gene expression profile, in cell wall structure, and by the expression of many virulence factors (Jacobsen *et al.* 2012; Mayer, Wilson and Hube 2013; Chen *et al.* 2020). The change in morphology can be triggered by many environmental factors present in host niches, such as physiological temperatures (>36°C), starvation, an ambient pH of >7, the presence of serum, N-acetylglucosamine, or elevated CO_2 levels (Mayer, Wilson and Hube 2013). Furthermore, hyphal development is triggered by the bacterial cell wall component, peptidoglycan (Xu *et al.* 2008), which is of particular relevance to fungus-host-microbiota interactions. Not surprisingly given the complexity of environmental

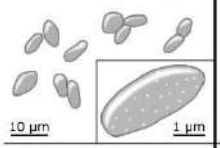
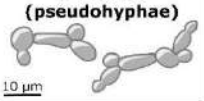
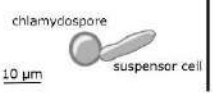
Morphology	Characteristics	Inducing factors	Importance	References
Yeast (white) 	round/oval, unicellular	<30°C, pH<4, cell density >10^7	commensalism, dissemination during systemic infection	Gow et al, 2002 PMID: 12160854, Sudbery et al, 2004 PMID: 15223059
Yeast (grey) 	ellipsoid, smallest cell type, unicellular	nutrient-rich medium (YEPD), passage through GI-tract	skin, cutaneous infection	Tao et al, 2014 PMID: 24691005, Liang et al, 2019 PMID: 30824263
Yeast (opaque) 	ellipsoid, unicellular, surface pimples	>5% CO_2, N-acetylglucosamine, acidic pH	mating	Tao et al, 2014 PMID: 24691005, Miller and Johnson, 2002 PMID: 12176317, Huang et al, 2010 PMID: 19200725, Sun et al, 2015 PMID: 26342021
Yeast (GUT) 	ellipsoid, unicellular, no pimples	genetic: ectopic *WOR1* expression	commensalism, metabolism adapted to nutrients available in the gut	Pande et al, 2013 PMID: 23892606
Filaments (hyphae) 	multicellular, tube-shaped, no indentations at septa	>36 °C, starvation, pH>7, serum, N-acetylglucosamine, peptidoglycan, high CO_2 levels	tissue invasion	Mayer et al, 2013 PMID: 23302789, Gow et al, 2002 PMID: 12160854, Xu et al, 2008 PMID: 18621008
Filaments (pseudohyphae) 	multicellular, indented cell-cell connections	high phosphate, pH=6, 35°C, nitrogen-limited growth on solid medium	found in infected lesions	Sudbery et al, 2004 PMID: 15223059
Chlamydospores 	large, round shape, thick cell wall, at the end of suspensor cell	starvation, complex sugars, 24-28°C, darkness, microaerophilic growth	diagnosis; biological function unknown	Staib et al 2007 PMID: 17302741

Figure 3. *Candida albicans* is polymorphic, displaying a range of cellular growth forms. *C. albicans* yeast cells can undergo phenotypic switching between white, grey and opaque growth forms that present with different shapes and cell surface characteristics (Gow, Brown and Odds 2002; Sudbery, Gow and Berman 2004; Xu *et al.* 2008; Huang *et al.* 2009; Mayer, Wilson and Hube 2013; Tao *et al.* 2014; Sun *et al.* 2015). These forms are induced in response to different environmental inputs, and hence are associated with different types of infection (Gow, Brown and Odds 2002). Significantly, the opaque form is associated with efficient mating in *C. albicans* (Miller and Johnson 2002), with grey cells displaying an intermediate mating competence between opaque and white cells (Tao *et al.* 2014). The gastrointestinally induced transition (GUT) phenotype is observed in *C. albicans* cells that ectopically express WOR1 (Pande, Chen and Noble 2013), a key regulator of commensalism. The transition from (white) yeast cells to pseudohyphae or hyphae is stimulated by a wide variety of environmental inputs, which include elevated temperatures, pH and peptidoglycan. Pseudohyphae can be distinguished from hyphae on the basis of the position of the septal junction between a mother yeast cell and its filamentous daughter, and by the presence of invaginations at these septal junctions in pseudohyphae, but not hyphae (Merson-Davies and Odds 1989; Sudbery 2001; Sudbery, Gow and Berman 2004). *Candida albicans* can be induced to form chlamydospores under specific environmental conditions (Jansons and Nickerson 1970), but the biological significance of this growth form remains obscure (Staib and Morschhäuser 2007). See text.

inputs and cellular outputs, yeast-hypha morphogenesis is regulated by a complex signalling network that includes the cAMP-protein kinase A, Efg1, Cph1, Czf1, Hog1 and Nrg1 pathways (Basso *et al.* 2019; Kadosh 2019; Kornitzer 2019).

During experimental colonisation of the murine GI tract, *C. albicans* was found to thrive in the yeast form (Vautier *et al.* 2015). The basis for the predominance of the yeast morphology during gut colonisation remains unclear, but unknown selective pressures favour growth in the yeast form during experimental GI colonisation in mice during GI dysbiosis (Tso *et al.* 2018). Furthermore, mucus covering the epithelium, tight junctions between epithelial cells, and the lamina propria serve as physical barriers that limit *C. albicans* translocation and dissemination from the gut (Yan, Yang and Tang 2013; Arevalo and Nobile 2020). Mucin, the main component of mucus, prevents hyphal formation (Kavanaugh *et al.* 2014) and reduces the adherence of *C. albicans* to epithelial cells (de Repentigny *et al.* 2000). Similarly, saliva can exert anti-*Candida* effects in the oral cavity (Hibino *et al.* 2009) (see *Oral cavity*). More recent work suggests that filamentous forms can exist in certain parts of the GI tract where the microenvironment favours hyphal development (Witchley *et al.* 2019). Only under certain circumstances, for example when a

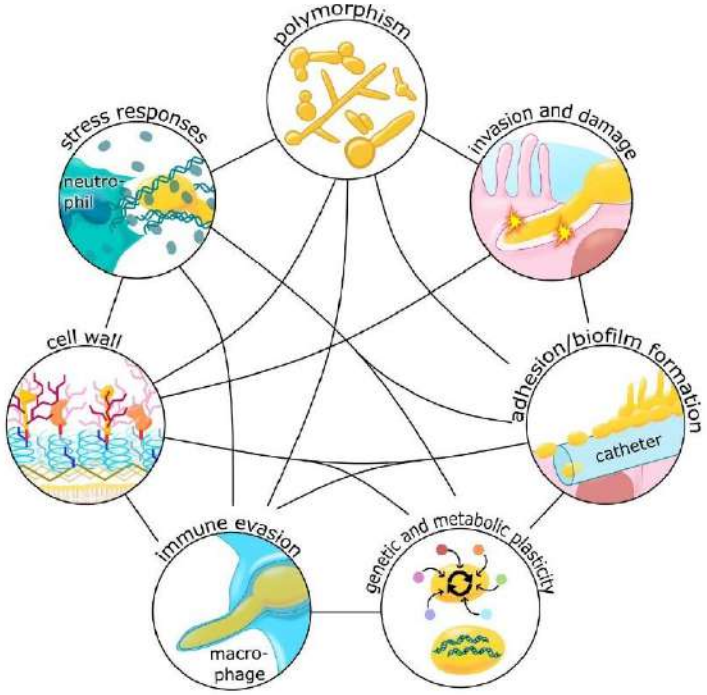


Figure 4. A combination of virulence factors and fitness attributes promote *C. albicans* virulence. *Polymorphism*: The ability of *C. albicans* to undergo morphological transitions allows it to adapt to different growth conditions, adhere to biotic and abiotic surfaces, invade cells and tissue, and escape from immune cells. *Invasion and damage*: A combination of induced endocytosis and active penetration promote fungal invasion of host tissues, and the accumulation of the toxin, candidalysin, in the invasion pocket leads to pore formation and host cell damage. *Adhesion/biofilm formation*: The battery of adhesins promotes fungal adhesions to biological and abiotic surfaces, which can lead to the development of biofilms, for example on medical devices such as catheters. *Genetic and metabolic plasticity*: *Candida albicans* displays a high degree of metabolic flexibility, which allows it to adapt rapidly to diverse host niches. This fungus also displays great genetic plasticity, which permits rapid evolutionary adaptation to selective pressures and stresses such as exposure to antifungal drugs. *Stress responses*: *Candida albicans* activates robust stress responses following exposure to host imposed stresses, including ROS and RNS, which enhances fungal survival following immune attack, for example. *Cell wall*: As well as maintaining cell morphology, the robust cell wall provides protection against host-imposed stresses including changes in osmolarity. *Immune evasion*: *Candida albicans* has evolved a variety of immune evasion strategies that include the modulation of PAMP exposure at the cell surface to evade immune recognition, and phagocytic escape mechanisms to evade killing by innate immune cells. See text.

perturbed microbiota and a compromised immune system lose control over *C. albicans* growth (see *The Host* and *The Microbiota*), the fungus can switch from commensalism to pathogenicity (Gow *et al.* 2011).

Significantly, the host can exploit the yeast-to-hypha transition to discriminate between colonisation and infection. This involves a biphasic innate immune response at the epithelial barrier (Moyes *et al.* 2010; Roselletti *et al.* 2019). The first signalling event is triggered by fungal cell wall components, notably β-glucans and mannans, irrespective of cell morphology (Moyes *et al.* 2010). The second, danger response, is only induced once a high fungal burden is achieved, hypha formation occurs, and the hypha-associated toxin candidalysin is expressed (see *Host damage*) (Moyes et al. 2010, 2016). This leads to the secretion of pro-inflammatory cytokines and phagocyte infiltration, which promote fungal clearance. In addition, phagocytes can distinguish hyphae from yeast cells based on the shorter cell wall mannan fibrils of hyphal cells (Cheng *et al.* 2011). Macrophages also respond to hyphal load, in part through the degree of metabolic competition between host and pathogen, displaying reduced activation of the NLRP3-inflammasome pathway at low hyphal burdens (Tucey *et al.* 2020; Westman *et al.* 2020). Thus, while hypha formation is critical for invasion (see *Invasion mechanisms*), the host has developed mechanisms to recognise the invasive form of *C. albicans*. Therefore, hypha formation seems to be detrimental for *C. albicans* commensalism.

Adhesion to abiotic and biotic surfaces

Candida albicans cells can adhere to each other as well as to host cells and abiotic surfaces, such as catheters or dental implants, which promotes colonisation and the formation of biofilms (de Groot *et al.* 2013; Lohse *et al.* 2018) (Fig. 4). *Candida albicans* forms hyphae upon sensing contact to a surface (Kumamoto 2008) and hyphae express specific adhesins that promote adhesion to such surfaces (de Groot *et al.* 2013).

The Agglutinin-Like Sequence (*ALS*) genes represent one family of adhesins in *C. albicans*, some of which are morphogenetically regulated (Hoyer and Cota 2016). Analogous adhesin families are present in other pathogenic and non-pathogenic fungi (Butler *et al.* 2009). Als adhesins have a three-domain structure: the N-terminal ligand-binding domain (Lin *et al.* 2014); internal tandem repeats; and the C-terminal domain, which binds the cell wall *via* a modified glycosylphosphatidylinisotol (GPI)-anchor. In *C. albicans*, the *ALS* gene family has nine mem-

bers, each of which displays a high degree of variability between alleles and strains, particularly in the length of the central repetitive domain (Hoyer and Cota 2016). Als3, the best-studied Als family member, has multiple functions. It binds heterogenous ligands including cadherins, ferritin and a *Streptococcus gordonii* surface protein (Phan *et al.* 2007; Almeida *et al.* 2008; Bamford *et al.* 2015). Als3 also acts as an invasin that promotes fungal invasion of host cells (Phan *et al.* 2007) and iron assimilation (Almeida *et al.* 2008). This makes Als3 an asset for the fungus during infection, but also a potential target for anti-*Candida* therapies (Edwards *et al.* 2018; Marc *et al.* 2018; Kioshima *et al.* 2019).

The hyphal wall protein 1 (Hwp1), is specifically expressed during hyphal growth (Staab, Ferrer and Sundstrom 1996), and is the founding member of a second family of five adhesins in *C. albicans* (de Groot *et al.* 2013). Members of the Hwp family are required for both virulence and mating. The N-terminus of Hwp1 is enriched in glutamine residues that become cross-linked to the host extracellular matrix by host transglutaminases (Staab *et al.* 1999). In contrast, Yeast wall protein 1 (Ywp1) appears to counteract adhesion leading to the release of yeast cells from surfaces, which might promote fungal dissemination during systemic candidiasis (Granger 2012).

A third family of putative adhesins is encoded by the twelve-member *HYR* gene family (de Groot *et al.* 2013). The founding member of this family, *HYR1*, like *ALS3* and *HWP1*, is expressed during hyphal development (Bailey *et al.* 1996). This *HYR* family has been less well characterised than the *ALS* and *HWP* families. Nevertheless, it adds to the adhesins that *C. albicans* expresses to promote robust adhesion to each other, abiotic surfaces or the host.

The cell wall

Both cellular polymorphism and adhesion are intimately associated with the *C. albicans* cell wall, the organelle that maintains the morphology of the *C. albicans* cell and that supplies the scaffold for most adhesin proteins (Klis, de Groot and Hellingwerf 2001; de Groot *et al.* 2004; Gow, Latge and Munro 2017) (Fig. 4). The cell wall also provides osmotic stability and protects against environmental stresses. It is robust in exerting control of cell shape, and yet elastic during responses to acute osmotic stress (Ene *et al.* 2015). Furthermore, the cell wall is a highly flexible organelle, in that it displays a high capacity to adapt and remodel itself in response to environmental challenges or antifungal drugs (Sosinska *et al.* 2008; Ene *et al.* 2012; Childers *et al.* 2019).

The *C. albicans* cell wall is a two-layered structure. The inner layer consists of chitin, β-1,3- and β-1,6-glucans and mannoproteins. The outer layer is enriched in mannan fibrils that are anchored to mannoproteins cross-linked to the inner layer of the wall (Kapteyn *et al.* 2000; Gow *et al.* 2011; Gow, Latge and Munro 2017). Chitin comprises about 2%–3% of the mass of the yeast cell wall, but represents an important structural component that is essential for the integrity of the cell wall. The main structural polysaccharide of the *C. albicans* cell wall is β-glucan, which accounts for 50%–60% of the mass of the yeast cell wall (Shepherd 1987; Klis, de Groot and Hellingwerf 2001). The β-1,3-glucan network provides the platform for covalent attachment of chitin, β-1,6-glucan and mannoproteins.

Two main classes of cell wall mannoproteins have been defined in *C. albicans*. GPI-anchored proteins are the more abundant class. As their name suggests, these are linked *via* modified GPI anchors to β-1,6-glucan which, in turn, are covalently attached to β-1,3-glucan (Kapteyn *et al.* 2000). Pir proteins (proteins with internal repeats) are covalently attached to β-1,3-glucan directly (Kapteyn *et al.* 2000). *C. albicans* cell wall mannoproteins contribute 30–40% of the mass of the yeast cell wall (Kapteyn *et al.* 2000) and are adorned with *N*- and/or *O*-linked oligosaccharides. The *O*-linked oligosaccharides are often linked to serine-threonine-rich repeats (e.g. in ALS adhesins: see *Adhesion to abiotic and biotic surfaces*) and are thought to confer rod-like structures to these domains (Gatti *et al.* 1994). *N*-linked mannans are highly branched structures that form the fibrils in the outer layer of the wall (Gow, Latge and Munro 2017; Childers *et al.* 2019). The functions of about 70% of cell wall mannoproteins remain obscure, but some are known or suspected to be involved in the infection process (De Groot, Ram and Klis 2005; Richard and Plaine 2007).

The cell wall is an attractive target for antifungal therapy because it is essential for fungal viability and not present on human cells. Consequently, β-1,3-glucan synthesis is the target for a major class of antifungal drugs in clinical use—the echinocandins (Odds, Brown and Gow 2003). Significantly, in the context of this review, the cell wall is also the first point of direct contact with the host, and therefore a prime target for immune recognition (see *Fungal recognition*) (Netea *et al.* 2008; Erwig and Gow 2016).

Biofilm formation

Candida albicans can form florid biofilms on biological surfaces and also abiotic surfaces such as catheters, dentures and prosthetic joints (Fig. 4). Biofilms are a common source of nosocomial infection (Ramage *et al.* 2005; Nobile and Johnson 2015), and they increase therapeutic challenges by enhancing the resistance to antifungal drugs (Taff *et al.* 2013).

Biofilm formation is initiated by adhesion of *C. albicans* cells to the surface (see *Adhesion to abiotic and biotic surfaces*). Surface contact stimulates hyphal growth (see *Cellular polymorphism*), the development of the biofilm and the production of extracellular matrix, and the biofilm matures into an organised and robust structure (Nobile and Johnson 2015). Biofilm formation is a complex process that is controlled by a network of transcription factors and that integrates the expression of adhesins, cellular morphogenesis and the production of extracellular matrix. Accordingly, biofilm formation is controlled by a complex transcriptional network of over 1000 genes (Finkel and Mitchell 2011; Nobile *et al.* 2012; Lohse *et al.* 2018). These target genes include members of the *ALS* family, which are essential for biofilm formation and enhance aggregation between fungal cells *via* amyloid formation (Dehullu *et al.* 2019; Vida Ho *et al.* 2019).

Biofilm maturation is followed by the dispersal of yeast cells from the biofilm, which promotes fungal dissemination. *Candida albicans* cells dispersed from biofilms are distinct from planktonically grown yeast. These dispersed cells are transcriptionally reprogrammed to utilise alternative carbon sources and they acquire nutrients, such as zinc and amino acids, with higher efficiency (Uppuluri *et al.* 2018).

Candida albicans clinical isolates display a high degree of heterogeneity with respect to their capacity to form biofilms and the underlying regulatory network (Sherry *et al.* 2017; Huang *et al.* 2019), and biofilm-forming ability has been associated with high mortality rates in patients (Rajendran *et al.* 2016). In the clinical setting, the situation is further complicated by the formation of multispecies biofilms. For example, *C. albicans* is commonly associated with *Streptococcus* and *Actinomyces* species in dental samples, with *Lactobacillus* species in vaginal specimens, and with *Pseudomonas* in the lungs of cystic fibrosis patients

(Hogan, Vik and Kolter 2004; Falagas, Betsi and Athanasiou 2006; Bamford *et al.* 2009; Bandara *et al.* 2009; Cruz *et al.* 2013; Bamford *et al.* 2015) (see *Synergistic and antagonistic interactions between kingdoms*). These inter-kingdom associations affect *C. albicans* growth, morphogenesis and drug resistance (Hogan, Vik and Kolter 2004).

Invasion mechanisms

The invasion of host cells and tissues provides an effective strategy to access more nutrients, avoid competition with other members of the microbiota, and potentially escape antimicrobial treatment (Fig. 4). Two distinct routes for the invasion of epithelia and endothelia are known for *C. albicans*: induced endocytosis and active penetration (Dalle *et al.* 2010; Wächtler *et al.* 2012). Induced endocytosis is mediated by the fungal proteins Ssa1 and Als3 (the adhesin-invasin, mentioned above), both of which are present on the cell wall. These proteins bind to E- and N- cadherins on epithelial and endothelial cells, as well as to the epithelial growth factor receptor of oral epithelial cells, to induce the uptake of fungal cells through remodelling of the host cytoskeleton (Phan *et al.* 2007; Moreno-Ruiz *et al.* 2009; Sun *et al.* 2010; Solis *et al.* 2017). Active penetration is achieved through the growth of hyphae into host tissue. This is the dominant route of fungal invasion into oral epithelial cells and the only observed route in enterocytes (Dalle *et al.* 2010; Wächtler *et al.* 2012).

As stated, the GI tract is a major reservoir for resident *C. albicans* (Nucci and Anaissie 2001; Gouba and Drancourt 2015), and hence fungal translocation across intestinal barriers is a common source of systemic candidiasis. This translocation can be promoted by injury, GI pathologies or medical interventions. Nevertheless, the translocation of *C. albicans* cells through enterocytes in a transcellular manner, and subsequent necrotic host cell death, is a major mechanism by which the fungus crosses the epithelial barrier (Allert *et al.* 2018). *C. albicans* directs physical force against cell membranes to stretch and rupture host cell membranes *via* a combination of hyphal growth and secreted virulence factors (Wächtler *et al.* 2012). Meanwhile, host cells employ several mechanisms to expand and repair membranes to limit this damage (Westman, Hube and Fairn 2019). This leads to the formation of the so-called 'invasion pocket' where the invading hypha is surrounded by host membrane (Moyes *et al.* 2016). The confined space around the hypha, within the invasion pocket, permits the accumulation of *C. albicans* secreted virulence factors to high local concentrations that cause further damage and stress to the host (Dalle *et al.* 2010; Moyes *et al.* 2016; Allert *et al.* 2018).

Host damage

The ability to damage host cells provides *C. albicans* with access to cytoplasmic nutrients, and the fungus possesses an extensive weaponry to impose damage (Fig. 4). Damaging factors that accumulate in the invasion pocket include secreted hydrolases such as phospholipase B1, lipases and secreted aspartic proteases (Saps) that degrade host membranes, proteins and extracellular matrix releasing nutrients (Mukherjee *et al.* 2001; Naglik, Challacombe and Hube 2003; Schofield *et al.* 2005). *Candida albicans* also expresses candidalysin—a pore forming α-helical peptide toxin that is encoded by the *ECE1* gene (Moyes *et al.* 2016). Pores formed in the host cell membrane by candidalysin probably leak cytoplasmic contents into the invasion pocket, thereby providing additional nutrients for the fungus. This may include access to essential micronutrients such as iron and zinc. Specific proteins bind these micronutrients, which are then endocytosed or transported across the fungal cell membrane *via* specific transporters. For example, members of the Rbt5-family transport heme across the cell wall (Kuznets *et al.* 2014; Nasser *et al.* 2016). Also, zinc is acquired *via* the zincophore Pra1 (pH-regulated antigen 1), which is released into the extracellular space and then, when loaded with zinc, is transported back into the fungus by the zinc transporter Ztr1 (Citiulo *et al.* 2012).

Fitness attributes

Fitness attributes are factors that promote fungal virulence by enhancing the physiological robustness of the fungus in host niches, rather than by interacting directly with the host. In *C. albicans*, fitness attributes include metabolic flexibility combined with potent nutrient acquisition systems, and robust stress response mechanisms (Mayer, Wilson and Hube 2013; Brown, Budge *et al.* 2014; Brown, Brown, *et al.* 2014). These promote the success of *C. albicans* both as a commensal and as a pathogen of humans.

Flexible metabolic adaptation

Metabolic adaptability is critical during *C. albicans* transitions between commensalism and pathogenicity (Fig. 4). This was highlighted by an elegant screen for regulatory circuitry that drives the commensal and pathogenic states in *C. albicans* (Pérez, Kumamoto and Johnson 2013). Much of this circuitry is involved in the regulation of metabolism. Metabolic regulation in *C. albicans* is integrated with the control of virulence factors and stress resistance through major regulatory hubs such as Efg1, Tup1, Nrg1, Hog1 and Gcn4 (Murad *et al.* 2001; Tripathi *et al.* 2002; Doedt *et al.* 2004; Alonso-Monge *et al.* 2009). Therefore, metabolic adaptation is essential for commensalism and virulence, and is intimately linked with other pathogenicity traits (Mayer, Wilson and Hube 2013; Brown, Brown, *et al.* 2014).

Glucose is a preferred carbon source for *C. albicans*, but under glucose-limiting conditions, such as in the colon or after entrapment in the phagosome, *C. albicans* tunes its metabolism to feed on alternative carbon sources (Lorenz, Bender and Fink 2004; Barelle *et al.* 2006). Even when glucose becomes available, *C. albicans* can simultaneously utilise alternative carbon sources through multiple pathways (Sandai *et al.* 2012; Childers *et al.* 2016). This metabolic flexibility allows the fungus to adapt to contrasting host niches. Significantly, it also influences the tolerance of *C. albicans* to antifungal drugs and environmental stresses (Ene *et al.* 2012). For example, growth on lactate protects against osmotic and cell wall stresses while utilisation of amino acids and N-acetylglucosamine (GlcNAc) increases fungal resistance to reactive oxygen and nitrogen species (ROS and RNS, respectively) (Williams and Lorenz 2020). These alternative carbon sources appear to serve as niche-specific signals that prime the fungus for impending challenges, pointing to the dexterity of *C. albicans* not only to adapt, but also to anticipate, local stress conditions (Brown, Budge *et al.* 2014; Alistair J P Brown *et al.* 2019; Williams and Lorenz 2020). The metabolic flexibility of *C. albicans* extends well beyond carbon metabolism to include nitrogen, phosphate and micronutrient assimilation (Lorenz, Bender and Fink 2004; Yin *et al.* 2004; Vylkova *et al.* 2011; Ene *et al.* 2014; Ikeh *et al.* 2016).

Micronutrients, such as iron and zinc, are essential for structural integrity and physiological processes in *C. albicans*. However, in response to infection, through a process called nutritional immunity, the host limits the availability of these micronutrients and exposes the fungus to toxic levels of other species such as copper ions (Noble 2013; Potrykus *et al.* 2013;

Mackie et al. 2016; Sprenger et al. 2018). In response, the fungus activates efficient micronutrient acquisition strategies. High affinity iron uptake involving a cyclic iron reduction pathway (iron reductase, multicopper ferroxidase and iron permease) is activated to take over from low affinity ferritin-iron uptake *via* the protein Als3, which is operational in hyphae during iron-replete conditions (Wilson, Naglik and Hube 2016; Bairwa, Hee Jung and Kronstad 2017). *Candida albicans* can also assimilate iron from heme and hemoglobin using Common in Fungal Extracellular Membrane (CFEM) proteins, and can scavenge siderophores synthesised by other microorganisms using the Arn1/Sit1 ferrichrome transporter (Bairwa, Hee Jung and Kronstad 2017). Transcriptional circuitry involving Sef1, Sfu1 and Hap43 control iron homeostasis by activating iron assimilation mechanisms when iron is limiting, and by repressing iron uptake when it is in excess (Chen et al. 2011; Noble 2013). *Candida albicans* utilises two uptake mechanisms to scavenge zinc. The first, which operates mainly at acidic pHs, involves uptake *via* the Zrt2 transporter into the cytoplasm (Crawford et al. 2018). The second, which is functional at neutral pHs, entails zincophore-mediated zinc scavenging through a secreted protein, Pra1 and uptake *via* the transporter Zrt1 (Citiulo et al. 2012; Wilson 2015; Crawford et al. 2018). *C. albicans* responds to zinc limitation by forming goliath cells (enlarged and spherical yeasts that exhibit enhanced adhesion) and avoids zinc toxicity by rapidly compartmentalizing zinc in storage vacuoles called zincosomes (Malavia et al. 2017; Crawford et al. 2018).

Robust stress responses

Fungal pathogens generally display robust responses to certain stresses, particularly oxidative stress (Brown et al. 2017) (Fig. 4). *Candida albicans* is resistant to significantly higher levels of ROS than its distant cousin, *Saccharomyces cerevisiae* (Jamieson, Stephen and Terrière 1996; Nikolaou et al. 2009) and this helps the fungus to counter toxic ROS produced by innate immune cells, before and during phagocytic attack (Miramón et al. 2012). *C. albicans* and other fungal pathogens counteract acute exogenous oxidative stresses by inducing genes involved in ROS detoxification (e.g. catalase and superoxide dismutases), the synthesis of antioxidants (e.g. glutathione and thioredoxin), and the repair of ROS-mediated damage to DNA, proteins and lipids (Enjalbert, Nantel and Whiteway 2003, Enjalbert et al. 2006; Znaidi et al. 2009). The inactivation of key regulators of the response in *C. albicans* (Cap1, Skn7 and Hog1) compromises oxidative stress resistance (Alarco and Raymond 1999; Singh et al. 2004; Smith et al. 2004). Virulence is attenuated by the inactivation of the Hog1 stress activated protein kinase (Alonso-Monge et al. 1999; Cheetham et al. 2011), but only to a minor extent by the loss of Cap1 or Skn7 (Singh et al. 2004; Jain et al. 2013). The overexpression of catalase, which detoxifies hydrogen peroxide, enhances oxidative stress resistance *in vitro*, and yet, counterintuitively, reduces the virulence of *C. albicans* (Román et al. 2016; Pradhan et al. 2017). This is because overexpression of this abundant ferroprotein places an undue demand for the essential micronutrient, iron, under iron limiting conditions *in vivo* (Pradhan et al. 2017). Clearly, numerous and potentially opposing, selective pressures must be balanced to optimise fungal fitness in a particular host niche.

While much attention has focussed on oxidative stress, *C. albicans* faces other forms of environmental stress in the host, including nitrosative, osmotic and thermal stresses. Innate immune cells expose *C. albicans* to RNS) in an attempt to kill and clear the fungus. *C. albicans* responds by activating genes involved in RNS detoxification (such as the flavohemoglobin, Yhb1), glutathione synthesis and recycling, and the repair of RNS-mediated damage (Hromatka, Noble and Johnson 2005; Tillmann et al. 2015). The response to nitrosative stress is driven by the transcription factor Cta4 and Hog1 (Chiranand et al. 2008; Herrero-de-Dios et al. 2018). The inactivation of *YHB1*, *CTA4* or *HOG1* attenuates nitrosative stress resistance and virulence (Alonso-Monge et al. 1999; Hromatka, Noble and Johnson 2005; Chiranand et al. 2008; Cheetham et al. 2011; Miramón et al. 2012).

Candida albicans cells thrive in niches with different osmolarities (e.g. on skin, in the oral cavity or GI tract), and yet must maintain osmo-homeostasis to grow. Hypo- and hyper-osmotic challenges are countered by modulating the levels of intracellular osmolytes. For example, *C. albicans* upregulates the synthesis and accumulation of glycerol and arabitol in response to hyperosmotic challenges (San José et al. 1996; Kayingo and Wong 2005). This response is regulated at both transcriptional and post-transcriptional levels by the evolutionarily conserved Hog1 MAP kinase signalling pathway (Smith et al. 2004; Enjalbert et al. 2006).

Candida albicans must also restore and maintain proteostasis in the face of thermal challenges, even within the mammalian host (Nicholls et al. 2011). Even mild increases in temperature lead to activation of the so-called heat shock response (Leach, Tyc et al. 2012), which is regulated by an autoregulatory circuit involving the heat shock transcription factor (Hsf1) and heat shock protein 90 (Hsp90) (Leach, Budge et al. 2012). The response involves the induction of functions involved in protein refolding and protein degradation to repair or recycle damaged proteins (Nicholls et al. 2009; Leach et al. 2016). The heat shock response is integrated with key virulence attributes in *C. albicans* such as yeast-hypha morphogenesis, adhesion and the ability to damage epithelial cells (Shapiro et al. 2009; Leach et al. 2016). Consequently, the inactivation of the response attenuates virulence (Nicholls et al. 2011).

Candida albicans can thrive over an extremely wide range of ambient pHs, from pH 2 to 10 (Vylkova et al. 2011). pH responses are particularly relevant given the ability of *C. albicans* to colonise host niches with contrasting pHs such as the vagina (acidic), GI tract (acidic to mildly alkaline) and blood (neutral). These pH responses, which are regulated in part by the evolutionarily conserved Rim101 pathway (Davis, Wilson and Mitchell 2000), are tightly integrated with metabolic adaptation, nutrient acquisition and morphogenesis (Davis et al. 2000). Yeast-hypha morphogenesis in *C. albicans* is regulated in response to ambient pH (Buffo, Herman and Soll 1984; Porta et al. 1999; Chen et al. 2020; Villa et al. 2020). Ambient pH also affects trace metal solubility, and consequently, micronutrient assimilation strategies in *C. albicans* are regulated in response to pH (Noble 2013; Wilson 2015; Crawford et al. 2018). Significantly, *C. albicans* is not simply reactive to pH: it can proactively alkalinise its microenvironment through the catabolism of polyamines and amino acids, leading to the release of ammonia and/or CO_2 (Mayer et al. 2012; Vylkova and Lorenz 2014; Danhof et al. 2016; Vylkova 2017). Interestingly, lactate production by a co-commensal in the oral cavity, *Streptococcus mutans*, provides carboxylic acid substrates that are sufficient to promote *C. albicans*-mediated alkalinisation of the microenvironment (Danhof et al. 2016; Willems et al. 2016).

Immune evasion

Immune evasion can be viewed as an additional type of fitness attribute because it promotes the physiological robustness of the fungus in the host (Fig. 4). *Candida albicans* has evolved a variety of mechanisms through which it can reduce recognition by immune cells, decrease the efficacy of antimicrobial killing

mechanisms, escape immune cells following engulfment, and manipulate the immune system (see *Innate antifungal responses* and *Fungal countermeasures* for more detail). During co-evolution with its host, *C. albicans* has even developed mechanisms by which it can anticipate, and protect itself against, imminent immune attack.

Clearly, *C. albicans* possesses an array of powerful fitness attributes through which this fungus tunes its physiology to counter environmental challenges presented by the host. Significantly, the fungus not only adapts to host-defined conditions, but can also anticipate impending challenges, and actively modulate its microenvironment.

Candida albicans epidemiology and variability

The flexibility of *C. albicans*, which underlies its success as a commensal and a pathogen, is also reflected at the genetic level (Fig. 4). Clinical isolates of *C. albicans* are generally diploid, with a haploid genome size of 16 Mb, organised into eight chromosomes. However, isolates display high levels of sequence heterozygosity between homologous chromosomes (Selmecki, Forche and Berman 2006; Ford *et al.* 2014; Hirakawa *et al.* 2015) and a high degree of genome plasticity driven by ploidy changes, karyotypic variations due to partial and whole chromosome aneuploidies, point mutations, short and long-range loss of heterozygosity (LOH) events and copy number variations (Chibana, Beckerman and Magee 2000; Selmecki, Forche and Berman 2006; Ford *et al.* 2014; Hirakawa *et al.* 2015; Ropars *et al.* 2018; Sitterlé *et al.* 2019). Furthermore, haploid and tetraploid strains have been observed both *in vitro* and *in vivo* (Hull, Raisner and Johnson 2000; Magee and Magee 2000; Hickman *et al.* 2013).

Multilocus sequence typing (MLST) and genome sequencing studies have revealed that *C. albicans* isolates are distributed amongst at least 23 genetic clusters (1–18, A-E) (Bougnoux *et al.* 2006; Odds *et al.* 2007; Odds 2010; Ropars *et al.* 2018). In general, there are no clear phenotypic associations with these clusters (Bougnoux *et al.* 2006; MacCallum *et al.* 2009). However, some clusters do exhibit geographical enrichment (Odds *et al.* 2007; MacCallum *et al.* 2009; Shin *et al.* 2011), suggesting independent recent evolutionary histories for these clusters. Cluster 13 is somewhat exceptional in that it represents a highly clonal lineage of isolates that exhibit low heterozygosity (Ropars *et al.* 2018). Isolates in cluster 13 are distributed worldwide (Fakhim *et al.* 2020), despite being called *Candida africana* strains (Tietz *et al.* 2001). They are isolated predominantly from the genital niche and display unusual morphological and phenotypic features that include slow growth, an inability to produce chlamydospores and assimilate aminosugars, and decreased virulence (Tietz *et al.* 2001; Romeo, De Leo and Criseo 2011; Borman *et al.* 2013). In contrast to other *C. albicans* clusters, cluster 13 isolates harbour a unique pattern of single nucleotide polymorphisms (SNPs) and a significantly lower level of heterozygosity (Ropars *et al.* 2018). In addition, in cluster 13 isolates, genes important for morphogenesis and virulence have undergone pseudogenisation, which probably explains the decreased virulence and apparent genital niche restriction of these isolates (Ropars *et al.* 2018).

Once thought to be an asexual obligate diploid organism, *C. albicans* has been shown to undergo a parasexual cycle (Magee and Magee 2000; Bennett and Johnson 2003; Ene and Bennett 2014). The majority of *C. albicans* diploid strains are incapable of mating, being heterozygous at the mating type-like (*MTL*) locus. However, mating can occur mainly between strains that have become homozygous at the *MTL* locus on chromosome 5, and have complementary *MTL* genotypes (i.e. are *MTLa/a* and *MTLα/α*). Additionally, mating in *C. albicans* is also dependent on a phenotypic switch from the mainly sterile 'white' phenotype to the mating competent 'opaque' phenotype (Miller and Johnson 2002). Mating between competent isolates of opposite mating-type results in tetraploid cells. These can subsequently undergo concerted chromosome loss, which can restore the diploid state in a meiosis-independent manner (Bennett and Johnson 2003; Hickman *et al.* 2015). However, this process yields diverse intermediate aneuploid states (Hickman *et al.* 2015). Hence, this mode of parasexual reproduction provides a means of generating genetic and phenotypic diversity in *C. albicans* (Forche *et al.* 2008; Hickman *et al.* 2015). Indeed, recombination has been shown to occur three orders of magnitude more frequently during concerted chromosome loss than during mitosis (Anderson *et al.* 2019). Interestingly, recombination during concerted chromosome loss is highly dependent on two meiosis-specific genes, *SPO11* and *REC8* (Forche *et al.* 2008; Anderson *et al.* 2019). The involvement of meiosis-specific genes in concerted chromosome loss has led to the suggestion that this process '*blurs the boundaries*' between meiosis and mitosis, and that this '*parameiosis*' might provide insight into the evolution of meiosis (Anderson *et al.* 2019).

The view that the parasexual cycle rarely occurs in the host is supported by population genetics, which shows that *C. albicans* populations are predominantly clonal (Pujol *et al.* 1993; McManus and Coleman 2014). Nevertheless, the conservation of mating genes suggests that this process is associated with an evolutionary advantage. Furthermore, because the parasexual cycle is stimulated by environmental stress, it may be a diversity-enhancing process that enhances adaptation and survival under hostile conditions (Selmecki, Forche and Berman 2010; Zhang *et al.* 2015; Hirakawa *et al.* 2017; Popp *et al.* 2019). This idea is corroborated by evidence of recombination and gene flow in natural isolates, despite the largely clonal structures of *C. albicans* populations (Odds *et al.* 2007; Bougnoux *et al.* 2008; Zhang *et al.* 2015; Ropars *et al.* 2018). This could explain why *C. albicans* isolates maintain a high degree of genetic diversity despite their predominantly clonal reproduction.

The diversity of *C. albicans* populations has arisen partly through changes in ploidy and aneuploidy. These mechanisms have provided *C. albicans* with a means of evolving rapidly in response to environmental challenges (Selmecki, Forche and Berman 2006; Diogo *et al.* 2009; Bennett, Forche and Berman 2014). The association of genome rearrangements with antifungal resistance acquisition has been well documented, with genomes of antifungal-resistant strains often exhibiting copy number variations and chromosome aneuploidies (Selmecki, Forche and Berman 2010). Indeed, a striking example of segmental aneuploidy was reported in fluconazole resistant strains, consisting of an isochromosome composed of the two left arms of chromosome 5 (Selmecki, Forche and Berman 2006, Selmecki *et al.* 2008). Trisomy of chromosome 2 or R has also been reported to enhance antifungal drug resistance in *C. albicans* (Xingxing Li *et al.* 2015; Yang *et al.* 2019). Large-scale chromosome rearrangements occur in *C. albicans* as an adaptation mechanism in both oral and GI niches (Ene *et al.* 2018; Forche *et al.* 2018). Similar observations have been made in isolates collected from a single human individual (Sitterlé *et al.* 2019). Genome sequencing of clinical isolates from patients that received antifungal therapy revealed that eight of the 21 isolates underwent karyotypic changes, with the majority being trisomic for chromosome 4 or

7 (Hirakawa et al. 2015). However, a more recent study of 182 clinical isolates might suggest that both segmental and whole chromosome aneuploidies are relatively infrequent events (Ropars et al. 2018). Changes in ploidy are known to provide a selective advantage under stress conditions, but can confer long-term fitness defects when grown under nonselective conditions, as illustrated by decreased growth and virulence (Hickman et al. 2015, 2013; Hirakawa et al. 2015). Therefore, the extent to which these events are observed in the genomes of *C. albicans* isolates must reflect the frequency of these types of genetic event and the nature of the selective pressures that these isolates recently faced.

Diversity has also arisen through high rates of mutation at the nucleotide level (SNPs, insertions and deletions). *Candida albicans* isolates display high levels of natural heterozygosity, with one heterozygous SNP occurring per 200–300 bp on average (Jones et al. 2004; Butler et al. 2009; Hirakawa et al. 2015; Ropars et al. 2018). The levels of heterozygosity are influenced by large LOH events, which can affect all chromosomes and are common in *C. albicans* isolates. LOH events are significantly elevated under stress conditions, such as exposure to antifungal agents, heat or oxidative stress (Forche et al. 2011; Ropars et al. 2018). Rapid phenotypic and genetic changes have been observed in various infection and colonisation models as well as in clinical isolates (Forche, May and Magee 2005; Bougnoux et al. 2006, 2009; Cheng et al. 2007; Bougnoux et al. 2008; Diogo et al. 2009; Lüttich et al. 2013; Ene et al. 2018; Forche et al. 2018; Sitterlé et al. 2020). This microevolution is driven primarily by *de novo* base substitution and short-range LOH events (Ene et al. 2018), and can clearly impact the relationship between fungus and host (Wartenberg et al. 2014; Tso et al. 2018; Liang and Bennett 2019) as well as resistance to antifungal therapy (Coste et al. 2006; Ford et al. 2014).

THE HOST

Mammals are constantly exposed to microbes on the skin and mucosal surfaces of the GI, respiratory and reproductive tracts. Therefore, epithelial surfaces in the mucosal tissues represent primary sites of interaction between *C. albicans* and the host (Lim et al. 2012). To prevent microbial overgrowth on the epithelial barriers and microbial invasion of tissues, the host actively surveys and protects its barrier surfaces *via* two distinct, complementary and cooperating branches of the immune system: innate and adaptive immunity (Fig. 5). As well as forming a physical barrier, epithelial cells contribute to the host response through active recognition of microbes and evaluation of their pathogenic potential. This is complemented by myeloid cells of the innate immune system, which exploit evolutionarily conserved pattern recognition receptors (PRRs) to recognise microbial pathogen-associated molecular patterns (PAMPs). Recognition of PAMPs by PRRs triggers phagocytosis of the microbial target and/or antimicrobial effector responses with the purpose to eradicate the pathogen. In addition, the innate immune system, and dendritic cells (DCs) in particular, activate the adaptive immune system. T helper (Th) cells are activated in an antigen-specific manner to coordinate epithelial defenses, improve innate immune function, activate antibody responses, and ultimately control the fungal load and resolve inflammation. Through the development of immunological memory, adaptive immunity provides long-lasting protection against microbes. We address the cellular and molecular mechanisms of innate and adaptive immunity that provide critical protection against *C. albicans* infection at epithelial barriers where interactions between the fungus, host and microbiota play out. These interactions are dependent on tissue type and are influenced by variations between individuals that affect susceptibility to fungal infection.

Innate immunity

Fungal recognition

The innate immune system is the first line of defense against *C. albicans* infection (Fig. 5). Epithelial cells (Richardson, Ho and Naglik 2018; Nikou et al. 2019; Swidergall 2019) combine with innate immune cells (Naglik et al. 2017; Verma, Gaffen and Swidergall 2017; Richardson et al. 2019) to provide this defense system, initiating anti-*Candida* immunity in response to fungal recognition.

Tissue-resident phagocytes, such as macrophages and DCs, are crucial in maintaining mucosal homeostasis (Ramirez-Ortiz and Means 2012; Xu and Shinohara 2017; Watanabe et al. 2019). However, innate immune cell populations differ between tissues, resulting in tissue-specific variation in the induction of innate and adaptive immune responses (see *Variability in the immune response*). Following hypha formation and *C. albicans* invasion, neutrophils and monocytes are rapidly recruited to the site of infection to mediate pathogen clearance through various antifungal responses (see *Antifungal response*) (Richardson et al. 2019).

Myeloid cells recognise specific microbial PAMPs using specific PRRs that fall into four main families: Toll-like receptors (TLRs), C-type lectin receptors (CLRs), nucleotide oligomerisation domain (NOD)-like receptors (NLRs) and RigI-helicase receptors (RLRs). CLRs are critical for fungal recognition (Hardison and Brown 2012). Several types of CLR recognise *C. albicans*, including Dectin-1, Dectin-2, Mincle, DC-Sign, and the mannose receptor (MR) (Hardison and Brown 2012; Dambuza et al. 2017; Goyal et al. 2018; Swidergall 2019). Dectin-1 recognises fungal β-glucans, which triggers the Card9-Syk pathway, leading to Nuclear Factor-kappa B (NFκB) activation and consequent cytokine and chemokine release (Drummond et al. 2011). In addition, dectin-1 induces phagocytosis and inflammasome activation (Kankkunen et al. 2010; Goodridge, Underhill and Touret 2012; Swidergall 2019). Dectin-2 recognises α-mannans (McGreal et al. 2006; Saijo et al. 2010) and induces the formation of Neutrophil Extracellular Traps (NETs) after recognising unopsonised *C. albicans* cells (Wu et al. 2019). In addition, Dectin-2 forms heterodimers with Dectin-3 and binds α-mannans on the surfaces of *C. albicans* hyphae (Zhu et al. 2013). Mannans are also recognised by Mincle, DC-Sign and the MR (Hardison and Brown 2012; Erwig and Gow 2016; Dambuza et al. 2017).

TLR-mediated PAMP recognition activates MyD88-dependent and TRIF signalling pathways in innate immune cells to regulate the inflammatory response (Kawasaki and Kawai 2014; Swidergall 2019). TLR2 and TLR4 recognise mannoproteins, while TLR9 recognises fungal DNA (Naglik et al. 2017). In addition, together with TLR9, the cytosolic NLR receptor NOD2 senses chitin particles (Wagener et al. 2014). NOD2-mediated recognition of chitin was found to down-regulate inflammatory responses (Wagener et al. 2014), which explains why NOD2 was initially described as being redundant for the induction of inflammatory responses against *C. albicans* (van der Graaf et al. 2006; van de Veerdonk et al. 2009). Recently, the epithelial Ephrin type-A receptor 2 (EphA2) was described as a non-classical PRR that recognises β-glucan (Swidergall et al. 2018). This receptor is expressed on neutrophils and stimulates antifungal activity during oropharyngeal

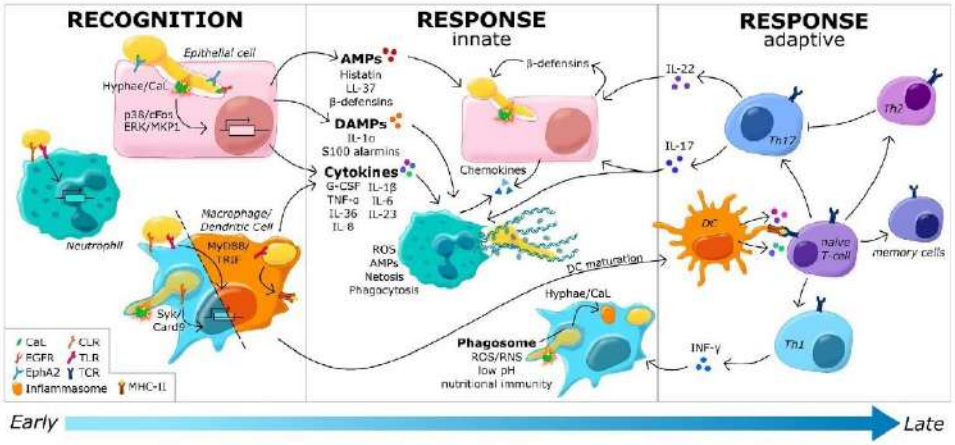


Figure 5. Immune recognition of, and immune responses against, *C. albicans*. *Candida albicans* yeast and hyphal cells are recognised by neutrophils, macrophages and dendritic cells *via* pattern recognition receptors (see key). This recognition activates the expression and release of proinflammatory cytokines and chemokines that promote the recruitment of macrophages and neutrophils to the site of infection. Epithelial cells respond to hypha formation and the subsequent secretion of candidalysin by the fungus, by activating the expression and release of AMPs, DAMPs, chemokines and cytokines via p38/cFos and ERK/MKP1 signalling. The AMPs attenuate fungal growth and invasion, while DAMPs and cytokines promote inflammation. Myeloid cells promote fungal killing and clearance through a combination of phagocytosis and NETosis in the case of neutrophils. Fungal recognition leads to the maturation of dendritic cells, and their surface presentation of fungal antigens to naïve T-cells, which stimulates adaptive immunity. The interactions between antigen-presenting dendritic cells and naïve T-cells induces T-cell activation and differentiation into various effector T cell subsets that regulate mucosal immunity largely via IL-17 and IL-22 secretion, and stimulate macrophages via IFN-γ. See text.

candidiasis (OPC) (Swidergall, Solis *et al.* 2019). Meanwhile, the melanoma differentiation-associated factor 5 (MDA5), a member of the RIG-I-like receptor (RLR) family that senses viral RNA, has been reported to also trigger an antifungal immune response, although its ligand remains obscure (Jaeger, van der Lee *et al.* 2015) (Table 1).

PRRs involved in the recognition of *C. albicans* by myeloid cells have been well characterised (above), but less is known about epithelial cell PRRs that recognise *C. albicans*. Epithelial cells use several types of PRR to sense *C. albicans*, including TLR2, TLR4, dectin-1 and EphA2 (Weindl *et al.* 2007; Décanis, Savignac and Rouabhia 2009; Cohen-Kedar *et al.* 2014; Swidergall *et al.* 2018). Despite its primordial role in the recognition of *C. albicans* by myeloid cells, dectin-1 is thought to play a limited role in epithelial cells (Moyes *et al.* 2010; Verma *et al.* 2017; Richardson, Ho and Naglik 2018). Rather, sensing of fungal β-glucans by epithelial cells is achieved mainly by EphA2, which activates MAPK and STAT3 signalling to induce the secretion of inflammatory cytokines and antimicrobial peptides by oral epithelial cells (Swidergall *et al.* 2018). PRR expression patterns vary amongst epithelial cell types and this, together with differential myeloid cell types, contributes to niche-specific variations in mucosal responses against *C. albicans* (Nikou *et al.* 2019; Swidergall 2019) (see *Tissue-specific immune responses*).

Epithelial cells can be activated by the *C. albicans* peptide toxin, candidalysin, as well as through PRR-PAMP interactions. This cytolytic peptide damages epithelial cells and activates the epithelial growth factor receptor (EGFR) (Jemima Ho *et al.* 2019). This, in turn, activates p38/cFos and ERK/MKP1 signalling, leading to the initiation of various effector responses (see *Innate antifungal responses*). The epithelial response to candidalysin is particularly relevant to the transition of *C. albicans* from commensalism to pathogenicity, because candidalysin is synthesised during hyphal growth and accumulates in the invasion pocket as the fungus invades the epithelial surface (Moyes *et al.* 2016) (see *Invasion mechanisms*). This response to candidalysin endows epithelial cells with the ability to respond to the invasive hyphal form of *C. albicans*, rather than its relatively benign commensal state (Moyes *et al.* 2010; Naglik *et al.* 2017).

Innate antifungal responses

Following recognition of *C. albicans* by phagocytic receptors, phagocytes such as neutrophils and macrophages can engulf the target *C. albicans* cell by phagocytosis, the purpose being to entrap and kill the pathogen (Brown 2011) (Fig. 5). Phagocytosis involves rapid reorganisation of the plasma membrane and cytoskeleton, and the imposition of mechanical force to engulf the fungal cell and entrap it within a phagosome (Ostrowski, Grinstein and Freeman 2016; Huse 2017). The phagosome then undergoes a series of plasma-membrane phosphoinositide- and Rab-dependent membrane fusion and fission events with endolysosomal compartments that promote the assimilation of microbicidal and lytic enzymes, and the progressive acidification of the organelle, to form the mature phagolysosome (Brown 2011; Fairn and Grinstein 2012; Miramón, Kasper and Hube 2013; Erwig and Gow 2016; Walpole, Grinstein and Westman 2018). In an attempt to kill the fungus, the phagocyte exposes its fungal cargo to a low pH, a nutrient limiting microenvironment and a potent mix of proteases, reactive chemical species ROS and RNS, cation fluxes and AMPs (Lorenz, Bender and Fink 2004; Brown 2011; Miramón, Kasper and Hube 2013; Erwig and Gow 2016). However, these skirmishes between phagocyte and fungus do not always achieve fungal clearance. This is because *C. albicans* has evolved molecular mechanisms that help it to evade phagocytic recognition, escape the phagocyte following engulfment, and resist phagocytic killing mechanisms (Austermeier *et al.* 2020) (see *Fitness attributes* and *Immune evasion*).

PAMP-PRR interactions activate host cell signalling, which in turn, induces a myriad of effector responses that are specific to the cell and tissue type (Brown *et al.* 2002; Roeder *et al.* 2004).

Table 1. Pattern recognition receptors in epithelial and innate immune cells that recognise *C. albicans* pathogen-associated molecular patterns.

PRR family	PRR	Fungal PAMP	Expressed in	Reference
TLRs	TLR2	Phospholipomannans	Neutrophils, macrophages, DCs, Epithelial cells (oral, vaginal, intestinal)	(Kurt-Jones *et al.* 2002; Jouault *et al.* 2003; Fazeli, Bruce and Anumba 2005; Décanis, Savignac and Rouabhia 2009; McClure and Massari 2014)
	TLR4	O-linked mannans	Neutrophils, monocyte, macrophages, DCs, epithelial cells (oral, vaginal, intestinal)	(Netea *et al.* 2006; Hyung Sook Kim *et al.* 2016; Fazeli, Bruce and Anumba 2005; Weindl *et al.* 2007; McClure and Massari 2014)
	TLR9	Fungal DNA Chitin	DCs, Neutrophils, macrophages, epithelial cells (oral, vaginal, intestinal)	(Miyazato *et al.* 2009; Kasperkovitz *et al.* 2011; McClure and Massari 2014; Wagener *et al.* 2014)
CLRs	Dectin-1	β-glucans	Macrophages, monocytes, neutrophils, DCs, epithelial cells (oral, intestinal)	(Brown and Gordon 2001; Brown *et al.* 2002; Taylor *et al.* 2002; Ariizumi, Shen, Shikano, Xu *et al.* 2000; Cohen-Kedar *et al.* 2014)
	Dectin-2	Mannoproteins (α-mannans)	Macrophages, DCs	(Taylor *et al.* 2005; Ariizumi, Shen, Shikano, Ritter, *et al.* 2000)
	Dectin-3	Mannoproteins (α-mannans)	Macrophages,	(Zhu *et al.* 2013)
	DC SIGN	Mannoproteins	Macrophages, DCs	(Cambi *et al.* 2003; Rappocciolo *et al.* 2006)
	Mincle	Mannoproteins	Neutrophils, macrophages, DCs	(Wells *et al.* 2008; Vijayan *et al.* 2012; Martínez-López *et al.* 2019)
	MR	Mannoproteins Chitin	DCs, macrophages	(van de Veerdonk *et al.* 2009; Martinez-Pomares 2012; Wagener *et al.* 2014)
NA	EphA2	β-glucans	Oral epithelial cells, neutrophils	(Swidergall, Solis, *et al.* 2019)
	Galectin-3	β-mannosides	Monocytes, macrophages, DCs, neutrophils, epithelial cells	(Jouault *et al.* 2006)
RLRs	MDA5	Unknown	Monocytes, DCs, macrophages, epithelial cells	(Plato, Hardison and Brown 2015)
NLRs	NOD2	Chitin	Monocytes, DCs, macrophages	(Wagener *et al.* 2014)

Epithelial cells secrete antimicrobial peptides (AMPs) such as LL-37, histatins and β-defensins. These AMPs exert their antifungal effects by a variety of mechanisms that include binding to the fungal cell wall or permeabilizing the fungal plasma membrane (Krishnakumari, Rangaraj and Nagaraj 2009; Chang *et al.* 2012; Swidergall and Ernst 2014). In the oral epithelium, nitric oxide and human-β-defensin (hBD)-2 production contribute to the early defensive response following direct contact with *C. albicans* and intra-epithelial invasion (Casaroto *et al.* 2019). In the GI tract, mucins produced by goblet cells suppress the yeast-hypha transition, surface adhesion and biofilm formation of *C. albicans*, thereby minimizing the capacity of the fungus to attach to, invade, and damage the epithelium (Kavanaugh *et al.* 2014) (see *Virulence factors*).

When *C. albicans* does manage to colonise the epithelium, the fungal toxin, candidalysin, plays a central role in triggering downstream responses (Kasper *et al.* 2018; Jemima Ho *et al.* 2019; Swidergall, Khalaji *et al.* 2019). The damage caused by candidalysin causes epithelial cells to passively alert professional immune cells through their release of danger-associated molecular patterns (DAMPs) or alarmins (Yang and Oppenheim 2009). For example, S100 alarmins produced by the vaginal epithelium are a potent driver of neutrophil influx during vaginitis in a murine model of infection (Yano *et al.* 2010, 2014). Similarly, damage to oral epithelial cells results in their release of the alarmin IL-1α, which triggers the neutrophil response to *C. albicans* in the oral mucosa *via* IL-1 signalling (Dongari-Bagtzoglou, Kashleva and Villar 2004; Altmeier *et al.* 2016). Epithelial cells also produce pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, G-CSF, TNF, and IL-36 (Villar *et al.* 2005; Verma *et al.* 2018). IL-8 acts as a chemoattractant that mobilises neutrophils from the circulation to the infection site. These neutrophils engage the fungus directly. They also engage in cross talk with local epithelial cells *via* TNF, thereby promoting TLR4-mediated signalling in the epithelium to enhance protection against fungal invasion and cell damage during oral candidiasis (Weindl *et al.* 2007).

Neutrophils are central players in antifungal defences due to their rapid activation of the fungicidal oxidative burst, (Peltroche-Llacsahuanga *et al.* 2000), their formation of NETs (Kenno *et al.* 2016), and their release of AMPs *via* degranulation (Urban *et al.* 2009) (Fig 5). Mice with *C. albicans* colonisation in their GI tract display enhanced neutrophil responsiveness and fungus-specific CD4 + T-cell responses during systemic candidiasis (Shao *et al.* 2019). This contrasts with observations during VVC in humans and mice, where fungal susceptibility is associated with uncontrolled inflammation and neutrophil influx (Black *et al.* 1998; Fidel *et al.* 2004). These observations reinforce the context-dependent nature of local immune responses.

Macrophages contribute to fungal clearance through their uptake of fungal cells, displaying a greater phagocytic capacity, but lower uptake rate, than polymorphonuclear leukocytes, (PMNs) (Rudkin *et al.* 2013) (Fig. 5). The hyphal form of *C. albicans* is relatively resistant to phagocytosis (Lewis *et al.* 2012). Nevertheless, macrophages still engulf portions of the hyphae, which can become trapped in 'frustrated phagosomes' (Maxson *et al.* 2018). After phagocytosis by macrophages, *C. albicans* yeast cells can undergo morphogenesis to generate hyphae. The yeast-hypha transition activates the NOD-and pyrin domain-containing protein 3 (NLRP3) inflammasome. This is essential for the release by the macrophage of pro-inflammatory IL-18 and IL-1β, which further promote Th1/Th17 activity during infection (Joly *et al.* 2009; van de Veerdonk, Joosten *et al.* 2011; Kasper *et al.* 2018). However, hyphal development within the phagolysosome

can help *C. albicans* evade macrophage killing by inducing pyroptosis, rupture and death of the macrophage *in vitro* (Vázquez-Torres and Balish 1997; Uwamahoro *et al.* 2014; Wellington *et al.* 2014; Kasper *et al.* 2018; O'Meara *et al.* 2018; Westman *et al.* 2018; Austermeier *et al.* 2020). Nevertheless, macrophages provide an important contribution to antifungal defences during systemic infection. For example, the functionality of resident renal macrophages, which is dependent on expression of the chemokine receptor CX3CR1, is important for controlling *C. albicans* in the early stages of a systemic infection, and for survival of the host (Lionakis *et al.* 2013). Similarly, microglia play an important role in antifungal immunity in the central nervous system, promoting neutrophil recruitment *via* candidalysin induced IL-1β and CXCL1 signalling (Drummond *et al.* 2019).

Mast cells modulate the antifungal potency of macrophages. Activated mast cells enhance macrophage functionality by improving their crawling ability and chemotaxis in response to *C. albicans* stimulation (De Zuani *et al.* 2018). Meanwhile, resting mast cells inhibit the phagocytosis of *C. albicans* by macrophages, which suggests a role for mast cells in the maintenance of commensalism (De Zuani *et al.* 2018). Inflammatory monocytes expressing CCR2 and Ly6C also contribute to fungal clearance during the early stages of systemic infection. Clearance is enhanced in the kidney and brain, but less so in the liver and spleen, indicating an organ-specific role for these monocytes during disseminated infection (Ngo *et al.* 2014).

Fungal countermeasures

During co-evolution of fungus and host, the antifungal responses of the immune system have imposed strong selective pressures upon *C. albicans* to evade these responses. Consequently, the fitness of the fungus *in vivo* has been enhanced by the development of a variety of fungal countermeasures that promote immune evasion and manipulation (Underhill 2007; Marcos *et al.* 2016).

A number of the fitness attributes and virulence factors, described above, promote immune evasion (see *Virulence factors* and *Fitness attributes*). For example, the formation of biofilms shields *C. albicans* cells from immunological attack (Kernien *et al.* 2017). The ability of *C. albicans* to resist pH extremes and to actively resist phagolysosomal acidification reduces the antifungal potency of phagocytes (Vylkova *et al.* 2011; Bain, Gow and Erwig 2015; Vylkova and Lorenz 2017; Westman *et al.* 2018). Also, the activation of robust oxidative and nitrosative stress responses provides a degree of protection against the toxic ROS and RNS generated by innate immune cells (Miramón *et al.* 2012). These responses include secreted and cell wall bound ROS detoxifying enzymes that help to counter immune attack (Crowe *et al.* 2003; Fradin *et al.* 2005; Dantas *et al.* 2015). However, *C. albicans* is sensitive to certain combinations of stress encountered within the phagosome (Kaloriti *et al.* 2014; Kos *et al.* 2016).

Hypha formation reduces the exposure of *C. albicans* to phagocytic killing because lengthy hyphal cells are harder to engulf, and hyphae have been reported to display lower levels of the inflammatory MAMP, β-1,3-glucan, at their cell surface (Gantner, Simmons and Underhill 2005; Bain *et al.* 2014; Mukaremera *et al.* 2017). Furthermore, *C. albicans* can undergo yeast-hypha morphogenesis following phagocytosis by macrophages, rupturing the phagosome and eventually leading to host cell death and fungal escape (Lewis *et al.* 2012; Ermert *et al.* 2013; Vylkova and Lorenz 2017). Indeed, the fungus is capable of triggering pyroptosis, inflammasome activation and cell death in a macrophage that has engulfed it (Uwamahoro *et al.* 2014; Wellington *et al.* 2014; O'Meara *et al.* 2015; Kasper *et al.* 2018), and can also induce host cell death through metabolic competition for glucose (Tucey *et al.* 2018; Tucey *et al.* 2020). Like other fungal pathogens, *C. albicans* may also escape the macrophage without lysing the host cell (Bain *et al.* 2012), although this mode of escape is thought to be rare.

Members of the secreted aspartic protease family (Sap1-3) promote immune evasion by degrading complement proteins (C3b, C4b, C5) thereby reducing the inhibitory potential of the complement system (Gropp *et al.* 2009). *Candida albicans* also expresses complement binding proteins at its cell surface that reduce the efficacy of the complement system (Poltermann *et al.* 2007; Zipfel and Skerka 2009). Pra1, which promotes zinc assimilation in *C. albicans* (see *Fitness attributes*), also interacts with complement regulators and plasminogen. In addition, Pra1 was the first protein described to bind to C4BP, which regulates the classical and lectin complement pathways and avoids C3b and C4b deposition on the fungal surface when captured by *C. albicans*, impeding complement cascade progression (Luo *et al.* 2009, 2011; Zipfel, Hallström and Riesbeck 2013). Furthermore, *C. albicans* secretes prostaglandins that modulate host immunity by downregulating chemokine and TNF production (Noverr *et al.* 2001). On the other hand, host immune mediators such as IFNγ, IL-17, TNF and PGE_2 influence *C. albicans* growth, filamentation and biofilm formation (Kalo-Klein and Witkin 1990; Noverr and Huffnagle 2004; Zelante *et al.* 2012; Rocha *et al.* 2017).

More recently, it was found that *C. albicans* yeast cells can evade phagocytic recognition by actively masking β-1,3-glucan at their cell surface. Interestingly the fungus exploits host signals, such as lactate, hypoxia, iron limitation and ambient pH, to modulate its β-1,3-glucan exposure (Ballou *et al.* 2016; Sherrington *et al.* 2017; Lopes *et al.* 2018; Pradhan *et al.* 2018; Cottier *et al.* 2019; Pradhan *et al.* 2019). Reducing the levels of β-1,3-glucan exposure leads to the attenuation of anti-*Candida* immune responses (Ballou *et al.* 2016; Sherrington *et al.* 2017; Lopes *et al.* 2018; Pradhan et al. 2018, 2019) and promotes disease progression (Lopes *et al.* 2018). Indeed, the fungus appears to use these host signals to anticipate impending immune attack and to protect itself by activating immune evasion mechanisms (Alistair J P Brown *et al.* 2019). These, and other anticipatory responses (Rodaki *et al.* 2009; Brunke and Hube 2014), provide strong evidence for the co-evolution of *C. albicans* with its host (Brown, Larcombe and Pradhan 2020).

Adaptive immunity

The adaptive immune system evolved to establish long-term protection through its ability to generate immunological memory (Fig. 5). The key role played by this arm of the immune system in providing surveillance of commensal organisms is reflected in the fungal dysbiosis that occurs in the absence of adaptive immunity (Lanternier, Cypowyj *et al.* 2013). The adaptive immune system involves B and T cells. B cells are essential for the production of antibodies, whereas T helper (Th) cells provide essential support for mucosal host defense and the innate immune response.

Candida albicans-specific antibodies are detectable in individuals that have been exposed to the fungus (Swoboda *et al.* 1993; López-Ribot *et al.* 2004; Pitarch *et al.* 2006). Their role in the control of fungal colonisation remains unclear, although the presence of anti-*C. albicans* antibodies might provide protection to mice against a potentially lethal systemic challenge (Matthews *et al.* 1991), as does gut colonisation through the development of pronounced anti-*C. albicans* IgG levels (Huertas *et al.* 2017). For

some time, it has been thought that antibodies may have diagnostic as well as therapeutic value (Matthews *et al.* 1988). Recent studies have reinforced their diagnostic potential (Wang *et al.* 2020), and recombinant anti-*C. albicans* antibodies have been shown to display therapeutic potential in preclinical models of infection by improving phagocytosis (Rudkin *et al.* 2018).

T cells exist as various subtypes that contribute differentially to antifungal immunity (Borghi *et al.* 2014; Verma *et al.* 2014; Lionakis and Levitz 2018) (Fig. 5). Among CD4$^+$ T cells, Th1 and Th17 cells promote the phagocytic clearance of fungal cells through the release of inflammatory cytokines such as IFN-γ and IL-17A/F, respectively, and these T cell subsets are critical for protective antifungal immunity. On the other hand, Th2 cells counter-regulate Th1 and Th17 responses, which can favour fungal persistence and promote allergic manifestations. Regulatory T cells (Tregs) maintain the homeostatic balance between these responses and limit inflammation as the infection is cleared. Th17 cells represent a major subset, and Th1 and Th2 cells minor subsets, of the human *C. albicans*-specific T helper cell population (Becattini *et al.* 2015; Bacher *et al.* 2019). However, additional T helper cell subsets have been described more recently (Eyerich *et al.* 2011; Becker *et al.* 2016). Moreover, T helper cells express plasticity. For example, *C. albicans*-specific Th17 cells can adopt the ability to produce additional cytokines, such as the Th1 prototypic cytokine IFN-γ (Zielinski *et al.* 2012). Cytotoxic (CD8$^-$) T-cells may also play a role in anti-*Candida* immunity (Beno, Stöver and Mathews 1995; Marquis *et al.* 2006).

The major protective role of Th17 cells in antifungal immunity is illustrated by the strong association of human defects in this T cell compartment and IL-17 signalling with uncontrolled fungal growth on mucosal surfaces and the skin (Puel *et al.* 2011; Ling *et al.* 2015; Li *et al.* 2017; Puel 2020). Consistently, mice with defects in the IL-17 signalling pathway display a reduced ability to cope with *C. albicans* administered *via* oropharyngeal or epicutaneous routes (Conti *et al.* 2009; Gladiator *et al.* 2013; Kashem, Igyarto *et al.* 2015), while IFN-γ-producing Th1 cells may have a disease-promoting effect (Igyártó *et al.* 2011). Also, the expansion of fungus-specific Th1 and Th17 cells in response to mucosal colonisation enhances protection against subsequent systemic *C. albicans* infections in mice (Romani *et al.* 1994; Shao *et al.* 2019). However, T cell- and IL-17-defects do not alter susceptibility to systemic infection in humans (Lionakis 2014).

CD4 + T cells are characterised by their ability to respond in an antigen-specific manner. Antigen-specific activation of (naïve) T cells depends on their interactions with DCs that present antigen on MHC-II molecules, and provide co-stimulatory and polarising cytokine signals. DCs are divisible into several subsets, most of which reside in peripheral tissues in close proximity to the microbiota where they interact with *C. albicans*. In response to PRR-mediated activation, DCs undergo a maturation program and migrate to the draining lymph nodes, where they encounter, activate, and prime antigen-specific T cells. This process relies on a tightly coordinated interplay between the innate and adaptive immune system (Fig. 5). The priming of T cells comprises of three steps. First, the recognition of peptide-MHC-II complexes by T cells *via* their T cell receptor (TCR) defines the antigen-specificity of the response. Second, this interaction is supported by adhesion and co-stimulatory molecules, which are induced at the cell surface of DCs in response to microbial stimulation, and these form an immune synapse that stimulates T cell proliferation. Third, the cytokine microenvironment directs the T cell differentiation towards distinct Th lineages *via* STAT (signal transducer and activator of transcription) signalling and the induction of fate-determining transcription factors (Wüthrich, Deepe and Klein 2012).

While antigens and the polarisation-inducing microbial signals are functionally distinct, the physical connection between antigen and PAMP enhances the efficiency of the T cell activation and differentiation process. Some of the few naturally processed and presented *C. albicans* antigens identified so far are glycosylated cell wall proteins, such as Mp65 (Pietrella *et al.* 2008) and Als3 (Bär *et al.* 2012). These mannoproteins can therefore serve concomitantly as a source of MHC-II antigen cargo as well as PAMPs. Such antigens support the coordinated process of antigen presentation and T cell polarisation.

The process of DC maturation is shaped by the specific PRR pathways that become activated in DCs following a microbial encounter (Fig. 5). This then determines the profile of cytokines that are produced, and hence directs the fate of the Th cell polarisation. Fungal cell wall components such as mannans and β-1,3-glucans trigger Syk- and CARD9-dependent cytokine signatures characterised by IL-23, IL-6, and IL-1β, which collectively instruct Th17 differentiation (LeibundGut-Landmann *et al.* 2007; Robinson *et al.* 2009; Saijo *et al.* 2010). IL-6 and IL-1β, together with TGF-β in mice, drive the commitment of Th17 cells, while IL-23 promotes lineage maintenance in a STAT3- and RORγt-dependent manner (Korn *et al.* 2009). Th17 cell differentiation is further modulated by the antigen dose and by tissue-specific cues.

Th17 cells produce the IL-17 family of effector cytokines: IL-17A and IL-17F as well as IL-22. These cytokines act primarily on epithelial cells and control the expression of genes linked to antimicrobial defense and tissue repair (Conti et al. 2009, 2016). IL-17 can also play an important role in promoting neutrophil recruitment (Liang *et al.* 2007), although, in the oral mucosa, the neutrophil response against *C. albicans* is largely independent of IL-17 (K Trautwein-Weidner *et al.* 2015). Instead, it depends on IL-1 and chemokines produced by epithelial cells in response to virulent *C. albicans* strains (Altmeier *et al.* 2016). While the functions of IL-17A and IL-17F are related, they do play non-redundant roles in host defense (Gladiator *et al.* 2013; Whibley *et al.* 2016). Similar to IL-17A and IL-17F, IL-22 also induces AMPs and contributes to fungal control (Liang *et al.* 2006). However, in contrast to IL-17A and IL-17F, defects in the IL-22 pathway have a minor impact on fungal control in experimentally infected mice (Conti *et al.* 2009; De Luca *et al.* 2013). Lately, IL-22 and IL-17A/F have been found to function nonredundantly during OPC, and IL-22 was shown to regulate the responsiveness of the epithelium to IL-17 (Aggor *et al.* 2020).

CD4 + T cells are the major source of IL-17 during responses to *C. albicans* at barrier tissues, but other sources may also contribute. CD8+ $\alpha\beta$ T cells can produce IL-17 in response to *C. albicans* (and other fungi), and these cells may play a compensatory role in the absence of CD4 + T cells (Nanjappa *et al.* 2012; Hernández-Santos *et al.* 2013). Moreover, innate lymphocytes and innate lymphoid cells (ILCs) can generate IL-17 (Cua and Tato 2010; Gladiator *et al.* 2013). In experimental models of oral infection, where naïve mice were exposed to a virulent *C. albicans* strain, the antifungal response was characterised by induc-

tion of IL-17 in ILCs, $\gamma\delta$ T cells and a tissue-resident population of $\alpha\beta$ T cells that respond in a TCR-independent manner (Sparber *et al.* 2018; Conti, Peterson *et al.* 2014; Kashem, Riedl *et al.* 2015; Verma *et al.* 2017). These three cellular subsets act in a partially redundant manner (Conti, Peterson *et al.* 2014; Gladiator *et al.* 2013). Therefore, although small in size, the IL-17-producing ILC population can compensate for the absence of $\alpha\beta$ and $\gamma\delta$ T cells during acute OPC (Gladiator *et al.* 2013). The extent to which innate sources of IL-17 contribute to antifungal defense in humans to maintain host-fungus homeostasis is not yet clear.

As a result of their exposure to *C. albicans* in the microbiota, most humans produce *C. albicans*-specific memory Th17 cells. In the circulation, these cells display the phenotype of effector memory T cells, which can respond rapidly to fungal exposure (Acosta-Rodriguez *et al.* 2007). In the skin, their expression of CD69 and CD103 characterises these *C. albicans*-specific memory Th17 cells as tissue-resident memory cells (Park *et al.* 2018). The maintenance of *C. albicans*-specific T cells is dependent on the persistence of the fungus in the host (Park *et al.* 2018; Shao *et al.* 2019; Kirchner and LeibundGut-Landmann 2020). The relevance of tissue-resident memory T cells for local immunosurveillance of *C. albicans* in barrier tissues was confirmed recently in a model of stable *C. albicans* commensalism, where tissue-resident memory T cells were sufficient to prevent fungal overgrowth (Kirchner and LeibundGut-Landmann 2020). The relationship between circulating and tissue-resident memory T cells directed against *C. albicans* remains to be determined, although their shared T cell receptor sequences suggest a common origin for both populations of memory Th17 cells (Park *et al.* 2018). Clearly, IL-17 immunity plays an important protective role in antifungal immunity. However, IL-17 signalling also has pathogenic potential, such as in the context of some autoimmune disorders (Eyerich, Dimartino and Cavani 2017) (see *Immunopathology in candidiasis*).

FoxP3 + IL-2Rα(CD25+) regulatory T cells (Tregs) are key mediators of immune regulation that provide endogenous regulatory mechanisms that can prevent potentially harmful immune responses. These Tregs confer immune tolerance through the expression of IL-10 and TGF-β, the consumption of IL-2, and the expression of inhibitory receptors that target T cells directly or indirectly *via* modulation of DC functionality (Romano *et al.* 2019). Furthermore, Tregs are developmentally linked to Th17 as they can promote Th17 differentiation by consumption of IL-2 (a cytokine that constrains Th17 differentiation) and, in mice, by providing TGF-β (which promotes Th17 polarisation) (Pandiyan *et al.* 2011). While Tregs directed against *C. albicans* are largely expanded in the physiological T cell repertoire in humans (Bacher *et al.* 2014), their contribution to the maintenance of stable *C. albicans* homeostasis in barrier tissues remains unclear. In a murine model of *C. albicans* commensalism, Tregs were dispensable for stable fungal colonisation and an absence of Tregs did not result in dysregulation of the antifungal Th17 response (Kirchner *et al.* 2019). Instead, the kynurenine pathway, which regulates tryptophan catabolism, might contribute to antifungal tolerance and limit inflammation in mucosal tissues (De Luca *et al.* 2013).

To summarise, a combination of epidemiological data, association studies in human primary immunodeficiency (PID) syndromes, *in vitro* challenges with primary human cells, and experiments in various mouse models of superficial candidiasis, have highlighted the importance of Th17 immunity during long-term colonisation of barrier tissues by *C. albicans*, and the fine lines between fungal commensalism and pathogenicity, and health and disease.

Tissue-specific variability of the mucosal immune response

Candida albicans colonises and causes infections in a range of different tissues, each of which characterised by a different architecture, nutrient supply, metabolic environment, and immune cell composition. Consequently, distinct host defense mechanisms against *C. albicans* exist in each tissue. Adaptive T cell immunity predominates in fungal control at the skin and most mucosal barriers (except for the vaginal mucosa), whereas innate myeloid cell-mediated mechanisms dominate the immune response to systemic infection (Lionakis 2014). Neutrophils and inflammatory monocytes have also been linked to antifungal immunity in barrier tissues. This notion has arisen primarily from experiments involving acute infections of previously *C. albicans*-naïve mice with highly virulent *C. albicans* strains, which trigger a strong inflammatory response and tissue damage. Under such conditions, inflammatory leukocytes (primarily neutrophils) are rapidly recruited to the infected tissues (Conti *et al.* 2009; K Trautwein-Weidner *et al.* 2015; Bai *et al.* 2020) where they prevent deep tissue invasion and mediate the rapid elimination of *C. albicans* (K Trautwein-Weidner *et al.* 2015). In contrast, *C. albicans* colonisation of barrier tissues is not generally accompanied by tissue inflammation (Schönherr *et al.* 2017), just as fungal commensalism in healthy individuals is not associated with inflammation.

In the vaginal mucosa, pathogenesis is thought to arise largely as a consequence of neutrophil-mediated immunopathology rather than a defect in T cell immunity (Fidel *et al.* 2004; Giraldo *et al.* 2012; Rosati, Bruno, Jaeger, Kullberg *et al.* 2020). Symptomatic infection correlates with elevated infiltration of neutrophils that are not able to limit the fungal burden (Yano, Noverr and Fidel 2017; Ardizzoni *et al.* 2020).

In contrast to the vaginal mucosa, where Th17 cells do not provide a major protective contribution, Th17 immunity is crucial for controlling the commensal colonisation of *C. albicans* on the skin and the mucosa of the oral cavity and GI tract (Sparber and LeibundGut-Landmann 2019). Experiments in mice have shown that the mechanisms of Th17 induction vary depending on the tissue. This is probably due to differences in the composition of antigen-presenting cells between the different tissues. Langerhans cells (LCs) are the predominant DC subset in the skin epidermis, but this cell type only represents a fraction of the DC population in the oral and vaginal epithelium (Hovav 2018). In the skin, LCs prime *C. albicans*-specific Th17 cells (Kashem, Igyarto *et al.* 2015), but they appear dispensable in the oral mucosa where conventional migratory DCs and monocyte-derived inflammatory DCs execute this task (Kerstin Trautwein-Weidner *et al.* 2015). In the gut, CX3CR1 + mononuclear phagocytes are essential for the initiation of adaptive immunity against *C. albicans* (Leonardi *et al.* 2018). Meanwhile, in the vaginal mucosa, plasmacytoid DCs may dominate and instruct a primarily tolerogenic response (LeBlanc, Barousse and Fidel 2006). Therefore, DCs are central coordinators of antifungal immunity. This relates not only to T cell activation in barrier tissues, but also to systemic candidiasis where DCs are indispensable for organising neutrophil-mediated innate immunity (Whitney *et al.* 2014).

Explanatory Box 1: Immunopathology
Neutrophils are amongst the first immune cells to be recruited from the bloodstream to the site of infection or tissue injury. Their recruitment is a multi-step process initiated by changes in the endothelium, and is induced by inflammatory mediators secreted by epithelial and tissue-resident immune cells (Kolaczkowska and Kubes 2013). At the site of infection, neutrophils clear pathogens through a combination of mechanisms including phagocytosis, degranulation, and NET formation (Selders *et al.* 2017; Rosales 2018). However, the secretion of ROS, proteolytic enzymes and AMPs by neutrophils can also lead to tissue injury and collateral damage (Wang 2018). Neutrophils die during the process of NETosis and release their nuclear and cytoplasmic contents. This can result in the presentation of auto-antigens and the production of pro-inflammatory cytokines, DAMPs and alarmins (Wang 2018; Wilgus 2018). DAMPs induce further neutrophil recruitment (Pittman and Kubes 2013), promoting a hyperinflammatory loop that, if not dampened by anti-inflammatory mechanisms, can exaggerate inflammation and tissue damage (Tisoncik *et al.* 2012). The adaptive immune system also mediates immunopathology *via* T cells, and Th17 cells in particular. *C. albicans*-specific Th17 cells promote inflammation and mediate immunopathological effects (Bacher *et al.* 2019; Shao *et al.* 2019; Hurabielle *et al.* 2020). Key anti-inflammatory mechanisms are mediated by Treg cells, myeloid suppressor cells, and anti-inflammatory molecules such as IL-1-family cytokines (IL-1Ra, IL-37, IL-38, IL-36Ra), IL-10, α1-antitrypsin, soluble cytokine receptors, and cytokine binding proteins (Netea *et al.* 2017; Dinarello 2018). The resolution of inflammation is an active process comprising of numerous signalling pathways that inhibit the inflammatory loop and limit tissue injury, as well as promoting pathogen clearance (Netea *et al.* 2017).

Immunopathology in candidiasis

The innate and adaptive immune responses provide essential protection against mucosal and life-threatening systemic infections, but uncontrolled inflammation can contribute to disease by causing immunopathology (Explanatory Box 1). There is a balance between immune protection and immunopathology. Using mouse models of systemic candidiasis, some investigators found that type I interferons promote fatal immunopathology through the recruitment and activation of inflammatory monocytes and neutrophils (Majer *et al.* 2012), whereas others observed reduced survival and concluded that type I interferons are crucial for immunity against *C. albicans* (del Fresno *et al.* 2013). Neutrophil accumulation in the kidneys and lung has been shown to cause immunopathology and organ failure in murine models (Lionakis *et al.* 2011; Desai and Lionakis 2018; Lee *et al.* 2018). During VVC in mice and humans, candidalysin-induced mucosal damage allows DAMPs and proinflammatory cytokine secretion, which promotes neutrophil recruitment and the exacerbation of inflammation (Richardson *et al.* 2018). Moreover, activation of the NLRP3 inflammasome and unrestrained IL-1β production can induce a hyperinflammatory state at the vaginal mucosa and acute symptoms of VVC (Rosati, Bruno, Jaeger, Ten Oever *et al.* 2020). This is influenced by endogenous anti-inflammatory mediators and environmental conditions (Rosati, Bruno, Jaeger, Ten Oever *et al.* 2020) such as short-chain fatty acids (SCFAs) derived from resident bacteria, which also play a crucial role in the immunopathology of oral candidiasis in mice (Bhaskaran *et al.* 2018). Th17 polarisation associated with intestinal *C. albicans* colonisation can be deemed as protective as it can cross-protect against systemic disease (Shao *et al.* 2019). However, these specific Th17 cells also contribute to allergic airway inflammation (Bacher *et al.* 2019; Shao *et al.* 2019) through cross-reactivity to the lung pathogen *Aspergillus fumigatus* (Bacher *et al.* 2019). *C. albicans*-specific Th17 cells can also promote inflammation in the skin and thereby contribute to psoriaform pathology (Hurabielle *et al.* 2020).

Several endogenous mechanisms regulate inflammation to maintain the balance between immune protection and immunopathology (Netea *et al.* 2017). The neutrophil response protects against *C. albicans* by inducing neutrophil chemokines (Mengesha and Conti 2017; Sparber and LeibundGut-Landmann 2019), but these also promote inflammation. The IL-1 family of cytokines, which drive neutrophil responses (Altmeier *et al.* 2016; Verma *et al.* 2018), are regulated by endogenous anti-inflammatory cytokines. For example, IL-37 compromises protection against systemic infection by reducing neutrophil influx (van de Veerdonk *et al.* 2014), but the capacity to reduce this influx potentially makes IL-37 a key player for preventing immunopathology. Other endogenous regulators include IL-1Ra, which neutralises IL-1 signalling and dampens NLRP3 Inflammasome activity, thereby contributing to reduced immunopathology (Borghi *et al.* 2015). The anti-inflammatory cytokines IL-36Ra and IL-38 can also attenuate the *C. albicans*-induced Th17 response (van de Veerdonk *et al.* 2012).

Clearly, molecules that target the IL-17 and IL-1 signalling pathways may have potential therapeutic value as treatments for immunopathology associated with candidiasis. Targeting the NLRP3 inflammasome has also been suggested as a potential strategy to ameliorate inflammation during VVC (Bruno *et al.* 2015; Richardson *et al.* 2018). However, the fine balance between protection and pathology must be deciphered before the accurate therapeutic modulation of these pathways can be achieved. Furthermore, the role and therapeutic applications of immunomodulators such as Indoleamine-pyrrole 2,3-dioxygenase 1 (IDO1), an enzyme producing tolerogenic kynurenines (De Luca *et al.* 2013), should be further evaluated.

Trained Immunity

The classical paradigm of host immune defense is based on the ability of the innate immune system to provide short term protection, combined with the capacity of adaptive immunity to mount immunological memory and provide long-lasting protection against the same pathogen. A growing body of evidence now shows that the innate immune system is able to generate immunological memory, independently of adaptive immunity. This phenomenon, which is termed *'trained immunity'*, has been described in invertebrates, plants, and mammals (Kurtz and Franz 2003; Durrant and Dong 2004; Netea, Quintin and van der Meer 2011), and is based on functional reprogramming of innate immune cells.

Candida albicans, and individual components of its cell wall, are potent immune modulators (see *Cell wall*). Even in mice that are deficient in T and B lymphocytes (i.e. lack adaptive immunity), an initial non-lethal exposure to *C. albicans* provides protection against a subsequent *C. albicans* infection (Bistoni

et al. 1986). This resistance to re-infection was described as a macrophage-dependent mechanism associated with enhanced production of the proinflammatory cytokines TNF, IFN-γ, and IL-1β (Vecchiarelli et al. 1989). Moreover, protection was not restricted to disseminated candidiasis: cross-protection to unrelated pathogens such as *Staphylococcus aureus* was also induced (Bistoni et al. 1986; Netea, Quintin and van der Meer 2011). Further studies demonstrated that stimulation with *C. albicans* or β-glucan, leading to activation of the dectin-1/PI3K-Akt-mTOR axis (Quintin et al. 2012; Cheng et al. 2014), elicits epigenetic remodeling of the transcriptional repertoire (Saeed et al. 2014). This leads to a shift in immune cell metabolism from oxidative phosphorylation to aerobic glycolysis (the Warburg effect) (Cheng et al. 2014), and enhanced pro-inflammatory cytokine production (Quintin 2019; Netea et al. 2020). Further studies revealed that β-glucan-primed monocytes differentiate into macrophages that display highly active metabolic activity and increased glucose consumption (Leonhardt et al. 2018). Interestingly, *C. albicans*-induced trained immunity is defective in chronic mucocutaneous candidiasis (CMC) patients, indicating that STAT-1 signalling is involved in the induction of trained immunity (Ifrim et al. 2015).

The induction of trained immunity depends strongly on the nature of the ligand and the PRR that is activated. For example, while TLR4 activation by lipopolysaccharide (LPS) can lead to a state of immunotolerance or immunoparalysis that compromises antifungal host defense (Grondman et al. 2019), the TLR4 agonist, monophosphoryl lipid A (MPLA), has been recently reported as an inducer of trained immunity (Fensterheim et al. 2018). Immunotolerance in sepsis patients increases the risk of secondary infections, including candidiasis (Otto et al. 2011). Conversely, trained immunity induced by *C. albicans* can enhance protection against sepsis in mice (Cheng et al. 2014). In addition, *C. albicans* colonisation of the GI tract provides protection against a variety of systemic pathogens (Tso et al. 2018). Therefore, the temporary transcriptional and metabolic rewiring *via* β-glucan-administration might provide a strategy to revert the LPS-induced tolerance of innate immune cells. Indeed, pharmacological targeting in myeloid cells, for example, by inhibition of the phosphatase SHIP-1 (Saz-Leal et al. 2018) or the IRG1-itaconate-SDH axis (Domínguez-Andrés et al. 2019), could play a pivotal role in harnessing beneficial effects of trained immunity (Mulder et al. 2019).

Variability amongst individuals

Variation between individuals influences the host-fungus interaction and susceptibility to fungal infection. The identification of candidate genetic traits is, therefore, pivotal for the selection of patients that would benefit from host-directed therapy or antifungal prophylaxis.

The effectiveness of a person's anti-fungal immune response is severely impaired if they acquire an immunocompromised status, for example through HIV-induced AIDS, neutropenia induced by cytostatic therapy, or immunosuppressive therapy during organ transplantation. Furthermore, certain genetic variations compromise the efficacy of immune pathways and exert strong detrimental effects upon antifungal immunity. Genetic susceptibility to fungal infection has been comprehensively studied and reviewed (Lionakis 2012). Mutations in *STAT1*, for instance, predispose individuals to CMC (Puel et al. 2011; van de Veerdonk, Plantinga et al. 2011). Also, inborn errors in Th17 or CARD9 immunity are associated with recurrent mucosal and invasive candidiasis, respectively (Puel 2020). Interestingly, genetic immunodeficiencies often lead to different susceptibilities to fungal infections of the mucosal surfaces, skin, and nails. Similarly, HIV patients develop oropharyngeal candidiasis (OPC) more often than vaginal infections (VVC) (Fidel 2002). This is consistent with the existence of distinct anti-*Candida* immune mechanisms in different mucosal niches (see *Tissue-specific variability of the immune response*).

Many genetic polymorphisms in PRRs have been associated with impaired antifungal host defense (Jaeger, Stappers et al. 2015). For instance, SNPs in the *TLR1* and *TLR4* genes increase the risk of candidaemia (Plantinga, Johnson et al. 2012; Van der Graaf et al. 2006), and a variable number tandem repeat (VNTR) polymorphism in the *NLRP3* gene is associated with increased susceptibility to VVC (Jaeger et al. 2016). Susceptibility to mucosal or systemic candidiasis varies depending on the nature of the receptor or effector molecule that is mutated. For example, a homozygous mutation in the dectin-1 gene is more likely to predispose the individual to CMC (Ferwerda et al. 2009), whereas defects in CARD9 result in systemic, mucosal, and subcutaneous candidiasis (Drewniak et al. 2013; Lionakis and Holland 2013; Lanternier, Pathan et al. 2013). Interestingly, CARD9-deficient individuals are prone to fungal proliferation in the central nervous system (CNS), but not in the kidney, spleen, or liver (Drummond et al. 2015), which highlights an organ-specific CARD9-dependent immune mechanism, such as IL-1β/CXCL1-mediated neutrophil recruitment by microglial cells (Drummond et al. 2019).

Genome-wide association studies (GWAS) have been performed to identify genetic polymorphisms associated with susceptibility to infectious diseases (Newport and Finan 2011), and overviews of comprehensive multi-*omic* systems approaches towards an understanding of host-fungal interactions have been published (Horn et al. 2012; Culibrk, Croft and Tebbutt 2016). The first GWAS analysis for fungal infections identified three novel risk loci associated with increased susceptibility and severity of candidaemia: *CD58*, *LCE4A-C1orf68*, and *TAGAP* (Kumar et al. 2014). Although GWAS is an ideal approach for the identification of novel genetic associations with susceptibility to fungal infections, it is difficult to achieve a high level of statistical significance ($<5 \times 10^{-8}$) with the generally small cohorts of candidaemia patients available (Manolio 2010; Chapman and Hill 2012). Hence, the power of GWAS can be enhanced by combining the outputs with systems biology, transcriptomics and available knowledge of immunology and microbiology, to pinpoint disease-associated genetic determinants. The functional validation of putative hits in an independent cohort can underline the relevance of newly identified genetic associations. For instance, the integration of gene expression data and functional genomics revealed the importance of type I IFNs in the host response against *C. albicans* (Smeekens et al. 2013; Jaeger, van der Lee et al. 2015). Also, using a computational approach based on publicly available transcript profiling data sets, *MALT1*, *SERPINE1*, *ICAM1*, *IL8*, and *IL1A* were discovered as common immune response-inducing genes during fungal infection (Kidane, Lawrence and Murali 2013). Furthermore, combining genetic data from candidaemia cohorts with immune-profiling of *C. albicans*-stimulated cells, the *MAP3K8* and *SERPINA1* genes were shown to contribute to candidaemia susceptibility (Matzaraki et al. 2017). Mapping genetic determinants to variability in transcription or cytokine levels can lead to the identification of expression quantitative trait loci (eQTL) or cytokine-quantitative trait loci (cQTL), respectively (i.e. the genetic variation associated with different levels of transcriptional and cytokine responses). The analysis of eQTLs is leading to an understanding of how human genetic variation

affects the anti-*Candida* host response and of the populations of cells involved in the clearance of the pathogen (de Vries *et al.* 2020). The investigation of cQTL datasets revealed SIGLEC15 as a susceptibility factor for RVVC (M Jaeger *et al.* 2019) as well as susceptibility pathways, such as lipid homeostasis and inflammation, that affect the response of monocytes to fungal bloodstream infections (Martin Jaeger *et al.* 2019).

In addition to genetic variability, external factors such as broad-spectrum antibiotics or immunosuppression regimens negatively influence the microbial community. This, in turn, affects metabolic homeostasis (Zarrinpar *et al.* 2018) and host resistance to both antibiotic-resistant microbes and fungal pathogens (Ubeda and Pamer 2012). For instance, preexposure to antibiotics not only increases *Candida* colonisation levels in the GI tract, but also facilitates disruption of the mucosal barrier and leads to *C. albicans* bloodstream infections (Das *et al.* 2011; Gutierrez *et al.* 2020). This could be due to the loss of protection from microbiota-derived metabolites, such as short-chain fatty acids (Guinan *et al.* 2019; Gutierrez *et al.* 2020). Moreover, antibiotic-induced dysbiosis can reduce pro-inflammatory cytokine production towards LPS stimulation (Lankelma *et al.* 2016). Thus, supplementation with probiotics may represent a useful strategy to counterbalance the negative effects of antibiotic-induced therapy and improve the host immune response against fungal infections (Ubeda and Pamer 2012).

THE MICROBIOTA

Gastrointestinal (GI) tract

The collection of microbes that colonises the GI tract is termed the 'gut microbiota', and is composed of bacteria, archaea, eukaryotic microbes and viruses. Thousands of microbial strains have been detected in the human gut, and these microbes can be important contributors to human health and disease (Fig. 6). For example, the gut microbiota plays key roles in nutrition (by degrading dietary components that would otherwise pass through the GI tract undigested), in host immune development and maintenance, and in protecting the host against pathogenic microbes, including *C. albicans*. This latter process, termed '*colonisation resistance*', is multifactorial. It involves both microbe-microbe interactions (such as competition for nutrients, niches and binding sites, and the release of antimicrobial substances), and host-microbe interactions (whereby the microbiota can stimulate the host's immune system or strengthen the gut epithelial barrier against invading pathogens) (Lawley and Walker 2013). Significantly, an individual's degree of colonisation resistance is thought to be strongly influenced by the composition of their gut microbiota, with some individuals being more intrinsically resistant to infection than others (Ubeda *et al.* 2010).

Most gut microbiota studies have focussed on the bacterial component, which accounts for the greatest proportion of biomass present by far (Qin *et al.* 2010; Arumugam *et al.* 2011). These gut bacteria display both antagonistic and mutualistic relationships with *C. albicans* and other members of the fungal community (mycobiota) that help to maintain homeostasis in the human GI tract. Fungi represent just ~0.1% of the GI tract biosphere, which makes the fungal mycobiota more challenging to study than the bacterial microbiota (Underhill and Iliev 2014). Also, mechanical lysis steps are best employed during DNA extraction to recover reasonable quantities of fungal DNA (Angebault *et al.* 2018), and because different extraction and sequencing methods have been used, gut mycobiota compositional analyses are difficult to compare between studies (Bellemain *et al.* 2010; Tedersoo *et al.* 2015; Tedersoo and Lindahl 2016; Huseyin *et al.* 2017; Angebault *et al.* 2018). Many mycobiota studies provide information about genera (e.g. *Candida*) rather than species (e.g. *C. albicans*). This section discusses causes of variability in the bacterial microbiota of the human GI tract and, where possible, the impact upon the GI mycobiota (fungal microbiota), and *C. albicans* in particular.

Variability along the GI tract

The compartments of the human GI tract, including the small intestine, caecum and large intestine (colon), have variable physiology and, as a result, each harbours distinct microbial communities (Fig. 6). Compared to the colon, the small intestine contains comparatively high levels of stomach acids, oxygen and antimicrobials, and is characterised by a short transit time. The small intestine also contains higher concentrations of bile acids, which are bactericidal to certain microbial species (Donaldson, Lee and Mazmanian 2016). Accordingly, the microbial community of the small intestine is less diverse than the colonic microbiota, and tends to be dominated by fast-growing facultative anaerobes such as streptococci and Proteobacteria that have the ability to adhere to epithelia or mucus (Zoetendal *et al.* 2012).

Moving from the small intestine, the caecum is the gateway to the colon. In the caecum, relatively long transit times and the prevailing environmental conditions favour the growth of fermentative anaerobes that can degrade complex polysaccharides, notably members of the Firmicutes and Bacteroidetes phyla (Donaldson, Lee and Mazmanian 2016). In contrast to the small intestine, the structure and physiology of the colon allows the survival of a more dense and diverse bacterial community, which can reach densities of up to 10^{11} cells per gram of colonic contents, one of the highest microbial concentrations in nature. The colon contains two layers of mucus, secreted by goblet cells, which separate colonic epithelial cells from the bacterial mass. The inner, firmly attached mucus layer is nearly sterile, whereas the outer layer is in direct contact with the luminal contents and can be a rich niche for microbial colonisation (Johansson et al. 2008, 2010). The luminal and mucosal compartments of the colon are often colonised by different profiles of bacterial populations. In a mouse model, *C. albicans* cells are visible in the lumen as well as the mucus layer (Witchley *et al.* 2019).

The fungal component of the GI tract is less diverse than its bacterial counterpart (Chehoud *et al.* 2015; Nash *et al.* 2017). Levels of fungal colonisation are lower in the small intestine, compared to the oral cavity and colon (Schulze and Sonnenborn 2009). Nevertheless, *C. albicans* can colonise the stomach, small intestine, caecum, and colon of mice (Witchley *et al.* 2019). The human intestinal mycobiota is characterised by a high inter- and intra-individual variability, which makes it difficult to define a 'normal' or healthy GI mycobiota composition (Nash *et al.* 2017; Raimondi *et al.* 2019). However, Ascomycota and Basidiomycota represent the two dominant phyla in the GI tract (Chehoud *et al.* 2015; Nash *et al.* 2017). The most frequently identified genera are *Candida* (e.g. *C. albicans*), *Saccharomyces* (e.g. *S. cerevisiae*), *Galactomyces*, *Penicillium*, *Aspergillus*, *Malasezzia* and *Debaryomyces*. Some of these fungi may not be true colonisers of the GI tract, but transient species that are brought by food or the environment. Consequently, an individual's lifestyle has a strong influence on their GI mycobiota and its variability (Auchtung *et al.* 2018; Raimondi *et al.* 2019).

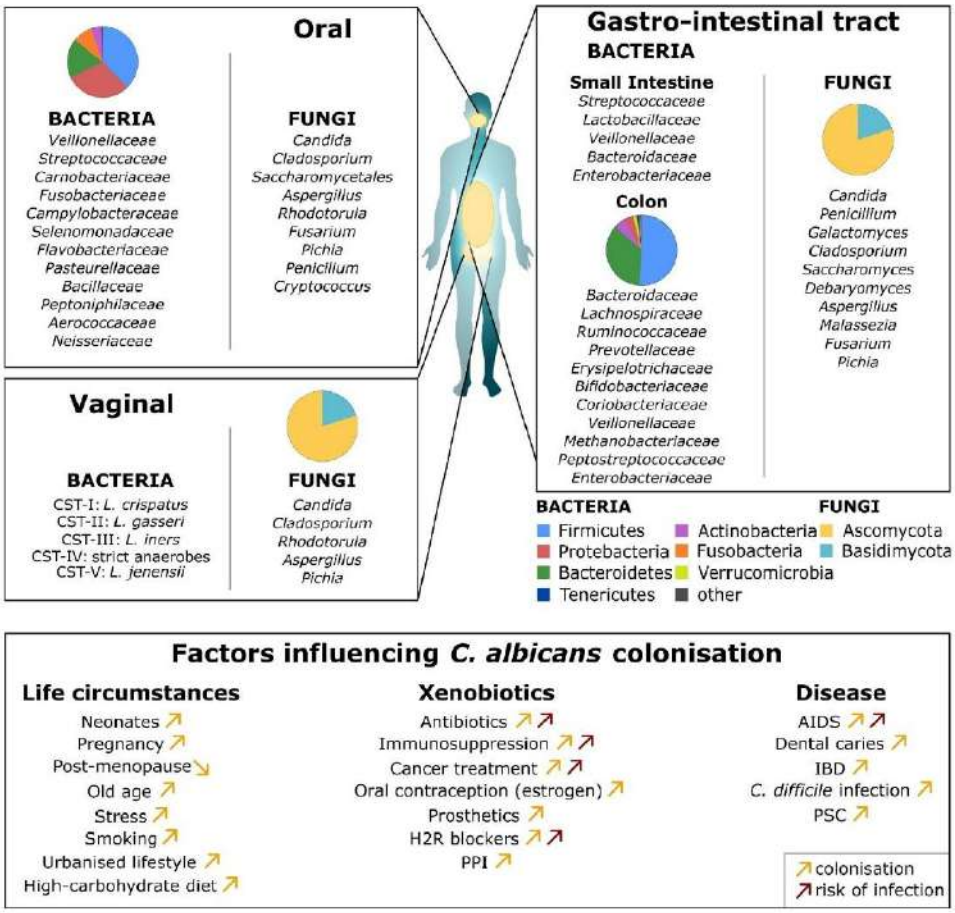


Figure 6. Oral, vaginal and GI microbiota, and factors that influence *C. albicans* colonisation of these body sites. The major microbial groups (family level for bacteria and genus level for fungi) of the healthy oral cavity (only for bacteria) (Bik *et al.* 2010; Dewhirst *et al.* 2010), GI tract (Booijink *et al.* 2010; Arumugam *et al.* 2011; Zhou *et al.* 2013; Villmones *et al.* 2018) and vagina (Human Microbiome Project Consortium 2012) are listed in decreasing order of abundance. Pie charts indicate the relative abundance of the phyla in a representative healthy oral cavity and colon (see key for colour code). The fungal component of the oral microbiota is extremely variable, and many fungi present in this compartment are likely to be transient (see text). Therefore, for the oral cavity, the fungal genera are not presented in descending order of abundance, and no pie chart is provided. The lower panel summarises factors that influence the degree of *C. albicans* colonisation (yellow) and likelihood of infection (brown arrows) of these mucosal surfaces: arrows up, increased likelihood of colonisation/infection; arrows down, decreased likelihood of colonisation/infection. See text.

Variability between individuals

Each individual has a distinct intestinal microbiota composition and structure. Many factors such as birth delivery mode, environmental exposure to colonising microbes, host genetics, host diet and lifestyle contribute to the unique nature of a given individual's intestinal bacterial and fungal microbiota (Qin *et al.* 2010; Salonen *et al.* 2014; Mehta *et al.* 2018), including their carriage of *C. albicans* (Neville, d Enfert and Bougnoux 2015) (Fig. 6).

Variability across lifespan

The mode of childbirth strongly affects the initial structure of the gut microbial community in neonates (Reyman *et al.* 2019). Bacteria such as *Enterobacter*, *Haemophilus*, *Staphylococcus* and *Veillonella* species are found in relatively high abundances in the faecal microbiota of babies born *via* caesarean (C)-section (Bäckhed *et al.* 2015). In contrast, the faecal microbiota of vaginally-delivered babies is enriched in *Bifidobacterium*, *Lactobacillus*, *Prevotella* and *Atopobium* spp., which are typically derived from the vagina of mothers (Dominguez-Bello *et al.* 2010). Babies delivered vaginally appear to be twice as likely to become colonised by *C. albicans* compared to those born by C-section (Parm *et al.* 2011).

Following birth, the main driver of gut microbiota composition is infant diet (breastfeeding *versus* formula milk). The colon of infants that are exclusively breast-fed is characterised by high numbers of milk oligosaccharide-utilising *Bifidobacterium* species (Yatsunenko *et al.* 2012; Odamaki *et al.* 2016; Hill *et al.* 2017), whereas formula-fed infants tend to possess more diverse microbiota that are less dominated by bifidobacteria (Rubaltelli *et al.* 1998; Klaassens *et al.* 2009; Lee *et al.* 2015). Following the introduction of solid food, alpha-diversity (i.e. the variation

of microbes in a single sample) increases and the microbiota transitions towards an adult-like composition (Yatsunenko *et al.* 2012; Schei *et al.* 2017). Little is known about *C. albicans* GI primocolonisation, but this species has been detected in newborns and infants (Bliss *et al.* 2008; Schei *et al.* 2017) and in maternal milk, suggesting that breastfeeding could be a source of colonisation (Boix-Amorós *et al.* 2017).

Once established, the adult bacterial microbiota is generally considered to be quite stable over many decades (Faith *et al.* 2013), albeit allowing for temporary disruptions by factors such as antibiotic treatment or inflammation. Every adult has a distinct microbiota at the species/strain level. Nevertheless, in the mature adult gut, obligate anaerobes generally predominate, the microbiota typically containing high levels of Firmicutes and Bacteroidetes spp. (Qin *et al.* 2010). There is significant variation between studies (see *Variability due to diet and geography*), but *Candida* spp. are thought to be present in the GI tracts of over half of adults (Odds 2010; Hoffmann *et al.* 2013). However, the fungal mycobiota of the adult GI tract appears to be less stable than the bacterial microbiota (Dollive *et al.* 2013).

Further changes in the microbiota occur in the elderly (generally over 65), possibly associated with altered dietary habits and living environments, reduced metabolism and immune function, and increased antibiotic usage (Lovat 1996; Simon, Hollander and McMichael 2015). Accumulating evidence suggests that old age can be associated with a decrease in 'beneficial' bacteria and an increase in 'harmful' species (Xu, Zhu and Qiu 2019), potentially making the elderly population more susceptible to *C. albicans* colonisation (Kauffman 2001; Miranda *et al.* 2009).

Variability arising from host genetics, diet and geography

The composition of the gut microbiota might also be shaped to some degree by the genetics of the individual, although recent work has shown that this only has a minor impact (between 1.9% and 8.1% of gut microbiota variability) compared to other factors such as environmental exposure and host diet (Rothschild *et al.* 2018) Studies of twins have indicated that certain types of bacteria might be more influenced by host genetics than others. For example, the *Christensenellaceae* family is more likely to be influenced by host genetics, while Bacteroidetes carriage is most likely shaped by environmental factors (Simões *et al.* 2013; Goodrich *et al.* 2014).

An individual's dietary choices have major impacts on the composition of their bacterial community, and diet is therefore an important driver of inter-individual variation (Walker *et al.* 2011; David, Maurice *et al.* 2014). The main energy sources for colonic microbiota are complex plant fibres. These can be recalcitrant to degradation by host enzymes in the small intestine and therefore pass into the colon relatively intact. Additional energy sources include residual peptides and host secretions such as mucus (Derrien *et al.* 2004, 2008; Hai Li *et al.* 2015; Van den Abbeele *et al.* 2010; Lukovac *et al.* 2014; Van Herreweghen *et al.* 2017; Van den Abbeele, Gérard *et al.* 2011; Tramontano *et al.* 2018). The type and availability of these various nutrients exert selective effects on numerous groups of bacteria. The starkest difference is between animal-based (high fat and protein content) and plant-based diets (rich in plant polysaccharides). Plant-based diets lead to increases in *Prevotella* species and Firmicutes, whereas animal-based/lower fibre diets stimulate an increase in *Bacteroides* and bile-tolerant species such as *Bilophilia* and *Alistipes* (Wu *et al.* 2011; David, Maurice *et al.* 2014; De Filippis *et al.* 2016; Pareek *et al.* 2019).

Differences in dietary habits between people inhabiting different regions of the world (He *et al.* 2018), and between those living in urbanised versus rural settings, are considered to be a main driver of geographical variation in microbiota composition. Indeed, the migration of people from rural to westernised settings greatly impacts the composition of their resident intestinal microbiota (Vangay *et al.* 2018). Those living in less urbanised societies typically consume greater amounts of dietary fibre and less meat and processed foods. Consequently, they tend to have a greater predominance and prevalence of more specialist fibre-degrading bacteria in their gut. In contrast, people living in cities or more urbanised countries, are characterised by their consumption of more refined, high protein and high fat diets, and they harbour microbial communities with reduced diversity (Schnorr *et al.* 2014).

Geographic location also affects the prevailing mycobiota, especially as many of the fungi detected in the GI tract are not true colonisers, but only transient species brought in as a result of different diets/environmental exposures (Auchtung *et al.* 2018; Raimondi *et al.* 2019). For example, *Aspergillus oryzae*, a species used to ferment soybeans to make soy sauce, is often present in the guts of healthy Japanese (Motooka *et al.* 2017). Also, the relatively high abundance of *Penicillium* and *Debaryomyces* spp. in Sardinian volunteers has been linked with high levels of cheese consumption in this region (Wu *et al.* 2020). Interestingly, the GI tracts of Wayampi Amerindians harbour a relatively high abundance of *Candida krusei* and *S. cerevisiae*, and less *C. albicans*, compared to individuals with more industrialised lifestyles (Angebault *et al.* 2013). However, the carriage of *Candida* spp. in the gut has been negatively associated with amino acid-, protein-, and fatty acid-rich diets (Hoffmann *et al.* 2013), which are characteristic of urbanised societies, suggesting that geographically-related factors other than diet may also affect the likelihood of *Candida* carriage in the gut. These may include exposure to environmental stressors and pollutants, including antibiotics (Jin *et al.* 2017; Karl *et al.* 2018) (see below).

Variability due to lifestyle and xenobiotics

A range of non-dietary lifestyle factors can also impact the gut microbiota and its resilience against invading pathogens. For example, exposure to stress is thought to lower the numbers of potentially beneficial gut bacteria such as *Lactobacillus* spp., and this has been postulated to have multiple effects on colonic motor activity *via* the gut-brain axis (Grenham *et al.* 2011; Galley *et al.* 2014; Murakami *et al.* 2017). Therefore, *Lactobacillus* spp. have been proposed as candidates for probiotic intervention (Bravo *et al.* 2011). Interestingly, preliminary studies have shown that *Lactobacillus* might reduce *C. albicans* overgrowth (Drutz 1992; Ceresa *et al.* 2015; Morais *et al.* 2017), and reductions in the prevalence of *Lactobacillus* spp. in the gut may be associated with stress-induced candidiasis (Meyer, Goettlicher and Mendling 2006; Akimoto-Gunther *et al.* 2016). Indeed, *L. rhamnosus* has been shown to reduce the capity of *C. albicans* to damage epithelial barriers and translocate into the 'bloodstream' in an intestine-on-chip model (Graf *et al.* 2019; Maurer *et al.* 2019).

Many xenobiotics interact with, and influence, the gut microbiota. In turn, these may increase the risk of developing opportunistic infections by disrupting colonisation resistance. Antibiotics have been the most studied xenobiotics. In addition to treating the aetiological agent of a disease, long-term broad-spectrum antibiotics can exert collateral damage upon beneficial indigenous gut bacteria (Dethlefsen *et al.* 2008; Fouhy *et al.* 2012; Burdet *et al.* 2019). This can have the unintended effect of suppressing colonisation resistance, leading to the outgrowth of opportunistic pathogens. This includes *C. albicans*, as antibiotic treatments permit persistent *C. albicans* colonisation of the

GI tract in mice that are normally resistant to colonisation (Fan *et al.* 2015). Several studies have attempted to define the mechanisms underlying this outgrowth (Guinan *et al.* 2019; Gutierrez *et al.* 2020; Zhai *et al.* 2020). Cefoperazone-treated mice display reduced levels of the short-chain fatty acids generated by the gut microbiota, which enhances *C. albicans* growth, morphogenesis and biofilm formation (Guinan *et al.* 2019). On the other hand, the outgrowth of *C. albicans* in antibiotic-treated mice has been linked to increased levels of carbohydrates, sugar alcohols and primary bile acids as well as decreases in carboxylic acids and secondary bile acids (Gutierrez *et al.* 2020). Although the effect of antibiotics on the gut mycobiota of healthy humans remains largely unknown, the administration of antibiotics to immunocompromised patients has been associated with decreases in the diversity of the gut microbiota and marked expansions in the burdens of pathogenic *Candida* species (Zhai *et al.* 2020). The extent of overall microbiota recovery after cessation of antibiotic treatment depends on the spectrum of activity of the antibiotic, the length of time it was administered, and the underlying composition of the baseline gut microbiota. In general, the microbiota appears to be reasonably resilient to short courses of certain antibiotics, displaying an ability to recover after treatment with, for example, ciprofloxacin (Pop *et al.* 2016) or azithromycin (Wei *et al.* 2018). However, recovery is not always complete (Dethlefsen *et al.* 2008; Fouhy *et al.* 2012).

Attention has also turned towards the susceptibility of the microbiota to non-antibiotic xenobiotics, many of which are commonly used drugs (Jackson *et al.* 2018; Maier *et al.* 2018; Vich Vila *et al.* 2020). Proton pump inhibitors (PPIs) have been the most studied non-antibiotic xenobiotics (Jackson *et al.* 2018; Vich Vila *et al.* 2020). Some evidence suggests that the use of PPIs increases the risk of *Candida* colonisation in intensive care patients (Mojazi Amiri *et al.* 2012; Jacobs *et al.* 2015). Histamine-2 receptor blockers also disturb colonisation resistance against opportunistic infections, primarily *C. albicans* (Saiman *et al.* 2001).

Variability associated with illness

Perturbations in the GI tract microbiota are associated with a multitude of disorders such as Inflammatory Bowel Disease (IBD), diabetes, obesity, colorectal cancer and cirrhosis. IBD includes conditions such as Crohn's disease (CD) and ulcerative colitis (UC). These diseases can further drive variability within the gut microbiota. Increasing evidence suggests that people suffering from some of these conditions display even more inter-individual variability than healthy controls (Zaneveld, McMinds and Vega Thurber 2017). IBD patients tend to have reduced overall microbiota diversity with decreased prevalence of potentially beneficial Firmicutes lineages such as *Faecalibacterium prausnitzii*. They also have increased levels of opportunistic pathogens such as *Enterobacteriaceae*, which are better able to thrive in an inflammatory environment than many other obligately anaerobic gut commensals (Manichanh *et al.* 2006; Sokol *et al.* 2009; Pascal *et al.* 2017; Franzosa *et al.* 2019; Lloyd-Price *et al.* 2019). IBD patients often show a disequilibrium in the diversity of bacteria and fungi in their GI tracts (Wheeler *et al.* 2016), which suggested that *Candida* spp. might also play a role in IBD pathogenesis. *C. albicans*, and the *Candida* genus in general, are more abundant in IBD patients (Ott *et al.* 2008; Kumamoto 2011; Chehoud *et al.* 2015; Sokol *et al.* 2017). Recent data indicate that *Malassezia*, rather than *Candida*, is associated which Crohn's disease (Limon *et al.* 2019). Nevertheless, a positive clinical response to faecal microbiota transplantation in ulcerative colitis patients has been associated with high levels of *Candida* spp. colonisation before treatment and decreased *Candida* abundance in the gut following treatment (Leonardi *et al.* 2020).

Patients with primary sclerosing cholangitis (PSC) also harbour decreased bacterial diversity, while the fungal diversity in their GI tract is increased (Lemoinne *et al.* 2020). Patients with *Clostridioides difficile* infections that have received a faecal microbiota transplant, often show reduced fungal diversity and *C. albicans* outgrowth in their gut. Indeed, a high abundance of *C. albicans* in the donor's gut might compromise the success of the faecal transplantation (Zuo *et al.* 2018). Alcoholic hepatitis has been associated with an increase in the abundance of *Candida* spp. in the gut mycobiota and a decrease of fungal diversity (Lang *et al.* 2020), while an outgrowth of *Candida* spp. has also been observed in children suffering from autistic spectrum disorders (Strati *et al.* 2017). Therefore, changes in the gut mycobiota are associated with, and potentially contribute to, a wide range of pathologies.

Oral cavity

Defining the core oral microbiota for a healthy individual is complicated by the fact that the oral cavity is a primary entry point for microbes in food and from the environment. Thus, microbes identified in the oral cavity may be transient, and washed out through saliva before having any impact upon health, rather than being active colonisers of this niche. Nevertheless, the oral cavity does harbour the second largest microbiota, in terms of diversity, compared to other body sites (Zhou *et al.* 2013) (Fig. 6).

Variability between individuals

Many of the factors that contribute to the variability of GI tract microbiota have a similar impact upon the microbiota present at other body sites, such as the oral environment (Fig. 6).

Variability across lifespan

The development of the oral microbiota in infants is influenced by their mode of delivery (Lif Holgerson *et al.* 2011; Dzidic *et al.* 2018). Infants born by C-section initially have more oral colonisers, such as *Staphylococcus*, *Corynebacterium* and *Propionibacterium* spp., which are derived from human skin (Dominguez-Bello *et al.* 2010). In contrast, babies born vaginally have bacterial communities reflecting their mothers' vaginal bacterial communities, dominated by *Lactobacillus*, *Prevotella* and *Sneathia* spp. *Candida* spp. are identified more frequently in the oral microbiota of newborns that were vaginally born, especially by mothers whose vagina was colonised by *Candida* (Al-Rusan, Darwazeh and Lataifeh 2017).

After 6 months of age, the impact of delivery mode is gradually eliminated as microbial patterns converge to that observed for older individuals. The oral microbial communities then evolve together over time with the host. This applies to both the oral bacterial and fungal microbiota. However, no consistent pattern has emerged for fungal colonisation, with conflicting results observed between studies (Baley *et al.* 1986; Caramalac *et al.* 2007; Farmaki *et al.* 2007; Bliss *et al.* 2008; Siavoshi *et al.* 2013; Filippidi *et al.* 2014; Stecksén-Blicks *et al.* 2015; Ward *et al.* 2018). Some studies have suggested vertical transmission from mother to child (Caramalac *et al.* 2007; Filippidi *et al.* 2014). Other studies consider breastmilk to be a source of fungal colonisation, with *Malassezia* (44%), *Candida* (19%) and *Saccharomyces* (12%) being the main taxa detected within one month of birth (Boix-Amorós *et al.* 2017). However, once again, no consistent pattern has emerged (Darwazeh and al-Bashir 1995; Matee *et al.* 1996; Mattos-Graner *et al.* 2001; Kadir, Uygun and Akyüz 2005;

Neves et al. 2015; Stecksén-Blicks et al. 2015). The development of the oral mycobiota can also be influenced by nail biting and finger sucking, which might enhance the colonisation by microbes usually found on the skin (e.g. *Malassezia* spp.) (Dupuy et al. 2014), and the use of pacifiers has also been correlated with increased fungal colonisation (Darwazeh and al-Bashir 1995; Mattos-Graner et al. 2001; Zöllner and Jorge 2003).

By the age of three, children have developed a complex oral microbial community, although they carry higher levels than older children of *Pseudomonadaceae, Moraxellaceae* and *Enterobacteriaceae*, which are not usually associated with healthy commensal oral microbiota (Crielaard et al. 2011). The oral bacterial microbiota of healthy adults is marked by increased proportions of Bacteroidetes (*Prevotella* spp.), Spirochaetes, Actinobacteria and Firmicutes (Keijser et al. 2008; Crielaard et al. 2011). The fungal taxa most frequently isolated from the oral cavity are *Candida* spp. and *S. cerevisiae* (foodborne) (Baley et al. 1986; Darwazeh and al-Bashir 1995; Matee et al. 1996; Mattos-Graner et al. 2001; Zöllner and Jorge 2003; Kadir, Uygun and Akyüz 2005; Farmaki et al. 2007; Filippidi et al. 2014; Neves et al. 2015; Ward et al. 2018). *C. albicans* is the *Candida* species most frequently isolated from the oral cavity, although other species such as *C. tropicalis, C. krusei, C. kefyr* and *C. glabrata* have also been detected.

After maturation of the microbiota, the oral cavity is thought to have the most stable microbial profile among all body sites (Zhou et al. 2013). Several studies have analysed temporal variation in the salivary microbiota (Caporaso et al. 2011; David, Materna et al. 2014; Flores et al. 2014; Belstrøm et al. 2016). This revealed high variability in the relative abundances of taxa, with, for instance, greater stability in individuals harbouring a more diverse tongue community (Flores et al. 2014). As with the GI tract microbiota, there is evidence that the oral microbiota can be influenced by birthplace and current geographic residence (Xu and Mitchell 2003; Wang et al. 2013).

Supragingival, tongue and salivary communities display strong inter-individual and inter-site differences (Hall et al. 2017). Among all the anatomical sites of the oral cavity, the supragingival plaque community is distinct from that of the tongue plaque and the saliva, with high similarity between the tongue and saliva. The supragingival plaque harbours a bacterial community with much lower diversity compared with that of the tongue and the salivary communities. Saliva has the highest number of bacterial taxa while supragingival plaque has the lowest. Hall and co-workers (Hall et al. 2017) identified 26 core taxa, belonging to five phyla (Actinobacteria, Bacteroidetes, Firmicutes, Fusobacterium, and Proteobacteria), across all sites. Few taxa were shared among all sites.

Variability arising from diet

Dietary factors contribute to the variation of oral microbial communities. In general, foods are swallowed quickly after a short period of mastication. Nevertheless, the introduction or sudden lack of certain nutrients can cause shifts in the oral microbiota (Adler et al. 2013; Zheng et al. 2015). For example, microbes that contribute to folate biosynthesis, such as *Streptococcus*, increase after long-term deprivation of fresh fruit and vegetables, which are rich in folic acid (Zheng et al. 2015). Vegetarians and non-vegetarians display similar rates of *C. albicans* carriage in their oral microflora (Patil et al. 2017). Fungi are introduced to the oral cavity *via* food and drink. Therefore, fungi commonly derived from fermented beverages such as beer are often isolated from the oral cavity (Fan et al. 2018).

Variability associated with illness

The most common oral conditions include tooth decay, periodontal disease and oral cancer. While many intestinal diseases have been associated with gut dysbiosis, there is still debate as to whether oral diseases are correlated with oral microbial diversity. For example, periodontal disease patients display more diverse and complex oral microbial communities than peri-implantitis patients (Kumar et al. 2012; Liu et al. 2012). Nevertheless, pathogenic *Streptococcus* spp. promote caries by lowering oral pH, which results in the demineralisation of enamel (Ajdić et al. 2002; Mei et al. 2013; Ito et al. 2019). Interactions between bacteria and fungi are likely to be of relevance to oral health. Interestingly, a study assessing *Candida* load and the bacterial composition of saliva in a Dutch cohort revealed that a low diversity of salivary microbiota characterised by dominant acidogenic bacilli (streptococci and lactobacilli) is positively correlated with elevated *Candida* burdens and possible overgrowth (Kraneveld et al. 2012). However, only certain diseases correlate with fungal colonisation of the oral cavity. These include, but are not limited to, HIV/AIDS (Cassone and Cauda 2012), cancer treatments (Silk 2014), dental caries (Falsetta et al. 2014) and oral lesions (ulcerations, nodules or granulomas) (Muzyka and Epifanio 2013). All of these conditions are linked either to the creation of novel niches that are not naturally present, or to perturbation of immune function. They are often correlated with *Candida* overgrowth (*C. albicans* in 70–80% cases), leading to oropharangeal candidiasis, particularly in immunocompromised individuals (Millsop and Fazel 2016).

Vaginal mucosa

Variability between individuals

Interactions between the resident microbiota and *C. albicans* in the vaginal tract are important for pathogenesis (Fig. 6). The vaginal bacterial microbiota of healthy reproductive-age women is generally dominated by *Lactobacillus* spp. (Ravel et al. 2011). Lactic acid production by these bacteria contributes to a healthy vaginal pH that is commonly lower than 4.5 (Ravel et al. 2011). The vaginal bacterial microbiota can be further sub-classified into five main community state types (CSTs): CST-I (*Lactobacillus crispatus*-dominated); CST-II (*L. gasseri*-dominated); CST-III (*L. iners*-dominated); and CST-V (*L. jensenii*-dominated) (Ravel et al. 2011). The CST-IV state is extremely diverse compared to the other types, comprising anaerobes and species linked to bacterial vaginosis (BV). CST-IV has been further divided into subgroups: CST-IVA (containing some lactobacilli); CST-IVB (high prevalence of *Atopobium* spp.); CST-IVC (*Gardnerella* subgroup A-dominated); and CST-IVD (*Gardnerella* subgroup C-associated) (Gajer et al. 2012; Albert et al. 2015). Women can transition between these CST states, for example, during menses (Gajer et al. 2012). CST-I has been associated with *C. albicans* colonisation (Sarah E Brown et al. 2019), but more studies are required to fully understand the complexity of the vaginal microbiota and its potential association with disease.

Less is known about the mycobiota of the human vagina. Culture-dependent studies indicate that *C. albicans* is the most abundant fungal species, although its abundance has been shown to vary according to lifestyle, age, ethnicity, hygiene habits and contraceptive methods (Fischer 2010; Wei, Feng and Luo 2010; Fischer and Bradford 2011; Shaaban et al. 2015; Donders et al. 2017, 2018). Indeed, intrauterine contraceptive systems have been reported to be associated with a rise in *C. albicans* colonisation, while progesterone-only pills result in lower rates of colonisation (Donders et al. 2017, 2018). However, due to

the relatively low sensitivity of conventional culture approaches, these studies may underestimate the true fungal diversity of the vagina (Guo *et al.* 2012; Drell *et al.* 2013). Using 18S rRNA gene sequencing, Guo and co-workers demonstrated that the healthy vaginal mycobiota was mainly composed of *Ascomycota* (~70% relative abundance), with the *Candida* genus dominating, and *C. albicans* as the main species. *Basidiomycota* were also detected, but with a lower proportional abundance (Guo *et al.* 2012). These results were confirmed using ITS1 pyrosequencing (Drell *et al.* 2013). Taking these findings together, the most abundant fungi in the healthy vaginal tract appear to be *C. albicans*, *S. cerevisiae* and *C. tropicalis* (Guo *et al.* 2012). Recent data indicate an association between the type of *Lactobacillus* species present and the likelihood of *Candida* colonisation (Tortelli *et al.* 2020).

Variability associated with age and pregnancy

The vaginal bacterial microbiota is influenced by oestrogen levels and is most stable when these are high (Gajer *et al.* 2012) (Fig. 6). Prepuberty is characterised by a bacterial microbiota comprised of anaerobes, diphtheroids, lactobacilli, streptococci, *Staphylococcus epidermidis*, and *Escherichia coli* (Hammerschlag *et al.* 1978). During puberty, increased oestrogen stimulates thickening of the glycogen-rich vaginal epithelium and establishes a vaginal microbiota dominated by lactobacilli (Miller *et al.* 2016). High oestrogen levels in reproductive women create unique features for the vaginal mucosa (Kalia, Singh and Kaur 2020), inducing a tolerogenic immune repertoire through immunomodulation of the neutrophil response (Willems *et al.* 2020). When glycogen is degraded by host α-amylases, products such as maltose and maltotriose foster the growth of *Lactobacillus*, leading to a reduced vaginal pH (Spear *et al.* 2014).

Lactobacillus spp. typically dominate the vaginal microbiota during pregnancy, and increased levels of these bacteria were reported in *Lactobacillus*-dominated CSTs compared to non-pregnant women (Aagaard *et al.* 2012; Romero *et al.* 2014; MacIntyre *et al.* 2015; Freitas *et al.* 2017). During pregnancy, the microbiota is also characterised by a lower occurrence of *Mollicutes*, and by members of the orders Clostridiales, Bacteroidales, and Actinomycetales (Aagaard *et al.* 2012; Freitas *et al.* 2017). Sampling six weeks postpartum revealed that bacterial diversity increases following birth and the vaginal microbiota readily assumes CST-IV (MacIntyre *et al.* 2015).

Fluctuations in oestrogen levels probably underlie variations in the abundance of *C. albicans*. Indeed, oestrogen injection is required to promote *C. albicans* colonisation of the vagina in rats and mice (Cheng, Yeater and Hoyer 2006). This is probably linked to the stimulatory effects of oestrogen upon *C. albicans* morphogenesis (White and Larsen 1997; Tarry *et al.* 2005). In humans, rising oestrogen levels during pregnancy has also been associated with an increase in *C. albicans* colonisation (Goplerud, Ohm and Galask 1976), which can potentially lead to premature birth (Roberts *et al.* 2011).

Postmenopausal women are also more likely to display a CST-IV microbiota (Brotman, Shardell *et al.* 2014). Menopausal women frequently experience a loss of lactobacilli and an increased vaginal pH (Brotman, Shardell *et al.* 2014; Gliniewicz *et al.* 2019). This elevation in vaginal pH, combined with an increase in vaginal glycogen levels, may contribute to the reduced incidence of VVC observed after the menopause (Hillier and Lau 1997; Spinillo *et al.* 1997). The reduced levels of oestrogen may also explain the decreased rates of VVC in postmenopausal women (Nwokolo and Boag 2000). Consequently, hormone replacement therapy (HRT) is a risk factor for VVC in these women (Fischer and Bradford 2011). HRT can restore a *Lactobacillus* dominated microbiota, similar to that of premenopausal women (Gliniewicz *et al.* 2019), and this treatment increases the likelihood of postmenopausal women succumbing to VVC (Fischer and Bradford 2011). However, more comprehensive studies are needed for a better understanding of the relationship between the overall vaginal mycobiota and health and disease.

Variability relating to geography and ethnicity

Independent of geography, the vaginal microbiota of women is dominated by *Lactobacillus* (Anukam *et al.* 2006; Shi *et al.* 2009; Zhou *et al.* 2010; Ravel *et al.* 2011; Pendharkar *et al.* 2013; Albert *et al.* 2015; Madhivanan *et al.* 2015). Nevertheless, the dominating species of the *Lactobacillus* genus may differ between geographical regions. Similar rates of vaginal colonisation by *Candida* spp. (11–17%) have been reported for asymptomatic women from European, South American and Middle Eastern countries (Gonçalves *et al.* 2016). However, VVC rates differ significantly for symptomatic women around the world, ranging from 12% to 57%, and most cases are caused by *C. albicans* (Gonçalves *et al.* 2016).

Regarding ethnic differences, Asian and Caucasian women from North America are mainly colonised by *Lactobacillus* spp. (CST-I, II, III and V), whereas black and Hispanic women are more likely to be colonised by CST-IV communities (Ravel *et al.* 2011). Women of African, American and European ancestry are more likely to be colonised by *L. iners* and *L. crispatus*, respectively (Fettweis *et al.* 2014). However, the basis for these differences is not clear (Gupta, Kakkar and Bhushan 2019). The evidence for different VVC rates between ethnic groups is limited (Wei, Feng and Luo 2010). Further studies would be required to define whether significant differences exist, and to parse apart the basis for any apparent differences.

Variability arising from lifestyle and xenobiotics

A number of factors influence the vaginal microbiota and, consequently, may predispose women to infection or aid in preventing infection (Fig. 6). The effects of antibiotics on the microbiota of women with vaginal infections are well studied. Metronidazole treatment of women with BV has been shown to increase prevalence of *Lactobacillus* spp. (Mayer *et al.* 2015). Similarly, the vaginal microbiota becomes dominated by *L. iners* when azithromycin is administered to treat *Chlamydia trachomatis* (Tamarelle *et al.* 2020). The composition of the lactobacilli community can shift in response to antibiotics, since vaginal *Lactobacillus* spp. have varying antibiotic sensitivity profiles (Melkumyan *et al.* 2015). Pregnant women frequently become colonised with *Staphylococcus* when receiving antibiotic treatment (Stokholm *et al.* 2014). It is well known that antibiotic treatments predispose individuals to VVC if they are already colonised with *Candida* spp. (Sobel 2007).

Not much is known regarding the impact of diet on the vaginal bacterial microbiota. Individuals consuming fibre-rich diets are less likely to have BV-associated microbiota (Shivakoti *et al.* 2020). Ingestion of micronutrients such as the zwitterion betaine (an osmolyte and methyl donor) may result in a microbiota that is predominantly lactobacilli (Tuddenham *et al.* 2019). In addition, smoking reduces vaginal lactobacilli (potentially *via* amines, anti-oestrogenic effects and bacteriophage induction) and increases the probability of acquiring a CST-IV microbiota (Brotman, He *et al.* 2014). As the vaginal microbiota influences the vaginal mycobiota (Sobel 2007), these effects are likely to influence *C. albicans* colonisation.

Translational opportunities

Given the impact that the microbiota appears to have on susceptibility to *Candida* infections, there are clear potential therapeutic benefits to bolstering our microbial communities at various body sites. Probiotics provide a means of altering the microbiota. Probiotics are defined as '*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*' (Hill *et al.* 2014). Currently, robust evidence for clinical efficacy is limited to a relatively narrow set of conditions. However, there is clear potential to widen this applicability to IBD, for example (Rondanelli *et al.* 2017). Interactions between the microbiota and host are thought to play key roles in *Candida* colonisation and pathogenesis, and therefore, live biotherapeutic products (LBPs) that exert anti-*Candida* effects are worthy of further study. (LBPs are products containing live microbes that are used to prevent or treat a medical condition.) Some are under development (Poupet *et al.* 2019). These have the potential to dramatically ameliorate the economic and health burden imposed by this fungus and reduce the risk of vulnerable individuals to *Candida* infections. Several types of patient cohort may benefit from such an approach.

Premature neonates are among those most at risk of developing systemic *Candida* infections. Compared to full-term healthy babies, premature newborns have an altered microbiota and can be colonised by opportunistic pathogens (Hill *et al.* 2017; Korpela *et al.* 2018). In addition to weakened colonisation resistance, their immature immune system places them at risk of late-onset sepsis caused by *C. albicans*, which has colonised their GI tract through vertical transmission from their mothers, or from the hospital environment (Bliss *et al.* 2008). Supplementation with a *Lactobacillus* probiotic (*L. rhamnosus* and *L. reuteri*) results in lower GI tract colonisation of *Candida* spp. compared to controls (Manzoni *et al.* 2006; Romeo *et al.* 2011). *L. reuteri* was found to be as effective as the antifungal nystatin in preventing candidaemia (Oncel *et al.* 2015). Replacing prophylactic antifungal treatments with LBP-based therapy would have the advantage of reducing the selection for antifungal drug resistance. Additionally, LBPs may benefit premature neonates by preventing GI symptoms such as regurgitation, vomiting and abdominal distension (Indrio *et al.* 2008; Rougé *et al.* 2009; Romeo *et al.* 2011), while reducing hospitalisation time (Romeo *et al.* 2011). Consequently, the use of LBPs for premature neonates may help decrease their risk of developing nosocomial infections.

HIV positive individuals are another group at high risk of developing *C. albicans* infections, especially oropharyngeal candidiasis (Patil *et al.* 2018). Administration of probiotic strains with anti-*Candida* activity could prevent the development of such infections by reducing levels of fungal colonisation (Hu *et al.* 2013).

Studies have shown that LBPs are more successful than placebos at preventing recurrence of vulvovaginal candidiasis (Vladareanu *et al.* 2018). Furthermore, therapies that combine LBPs with azoles have been shown to improve drug efficacy by restoring the local bacterial community (Kovachev and Vatcheva-Dobrevska 2015; Russo *et al.* 2019). Increasing the efficacy of the antifungal drug in this way may allow drug doses to be reduced.

Taken together, current evidence is encouraging and suggests that probiotics can be used to prevent *C. albicans* infections in vulnerable cohorts. However, further studies are required to identify optimal LBP candidates, and to understand the underlying mechanisms of action that result in clinical efficacy.

THE *FUNGUS-HOST-MICROBIOTA* INTERPLAY

The previous sections describe the multifaceted nature of *C. albicans* interactions with the host, how antifungal immunity influences these interactions, and how the microbiota is closely related to host physiology and impacts *C. albicans* colonisation. These tripartite interactions between host, fungus and microbiota are incredibly complex and strongly influence the likelihood and outcomes of *Candida* infections. Here we discuss the nature of interdependencies within the fungus-host-microbiota interplay and their impact on health and *Candida* infections, in particular.

Synergistic and antagonistic interactions between kingdoms

Many researchers have focused on antagonistic interactions between *C. albicans* and bacteria species because these could potentially be exploited in therapeutic approaches (see *Impact of a changing microbiota on the Fungus-Host-Microbiota interplay* and *Translational opportunities*). However, it is estimated that approximately 30% of all *Candida* bloodstream infections are polymicrobial and involve both fungi and bacteria (Klotz *et al.* 2007). This suggests that synergism can occur between *C. albicans* and certain bacterial species (Fig. 7). [In this context, 'synergism' describes polymicrobial interactions during which one microorganism promotes colonisation or infection by another (Brogden, Guthmiller and Taylor 2005)].

Candida albicans interacts with different types of resident microorganisms depending on the body site (see *The Microbiota*). *Candida albicans* synergises with various streptococcal species that are abundant in the oral cavity, through physical interactions that enhance bacterial growth and adhesion, lead to more pronounced biofilm formation and, in some cases, increase fungal invasion (Silverman *et al.* 2010; Diaz *et al.* 2012; Metwalli *et al.* 2013; Xu *et al.* 2016; Koo, Andes and Krysan 2018; Vila *et al.* 2020). Molecules involved in these physical interactions include bacterial adhesins (Holmes, McNab and Jenkinson 1996; Silverman *et al.* 2010), specific fungal surface proteins (Holmes, McNab and Jenkinson 1996; Dutton *et al.* 2014; Xu *et al.* 2017), and components of the extracellular polysaccharide matrix produced in biofilms (Falsetta *et al.* 2014). Communication within mixed biofilms involves bacterial and fungal quorum sensing systems that influence the expression of virulence factors and bacterial metabolism (Sztajer *et al.* 2014; He *et al.* 2017; Kim *et al.* 2017). In addition to this direct synergism, streptococcal co-infection stimulates complex immune reactions that promote the expression of proinflammatory cytokines and enhanced tissue inflammation in a murine model of *C. albicans* thrush (Xu *et al.* 2014). The clinical importance of these synergistic interactions is suggested by co-colonisation of bacteria with fungi in oral diseases such as childhood caries, periodontitis, and denture stomatitis (O'Donnell *et al.* 2015). Furthermore, a causal relationship between bacterial-fungal co-infection and disease severity has been demonstrated for caries in a rat model (Falsetta *et al.* 2014). Fungal colonisation also affects oral microbiota composition, which encourages invasive infection (Bertolini *et al.* 2019).

The synergistic cross-kingdom interactions between *C. albicans* and *Staphylococcus aureus* have been comparatively well studied (Carolus, Van Dyck and Van Dijck 2019) (Fig. 7). *Staphylococcus aureus* can bind to *C. albicans* hyphae, which indirectly enhances the attachment of the bacterium to abiotic surfaces and promotes the formation of mixed biofilms with increased resistance to antimicrobial compounds (Shirtliff, Peters and

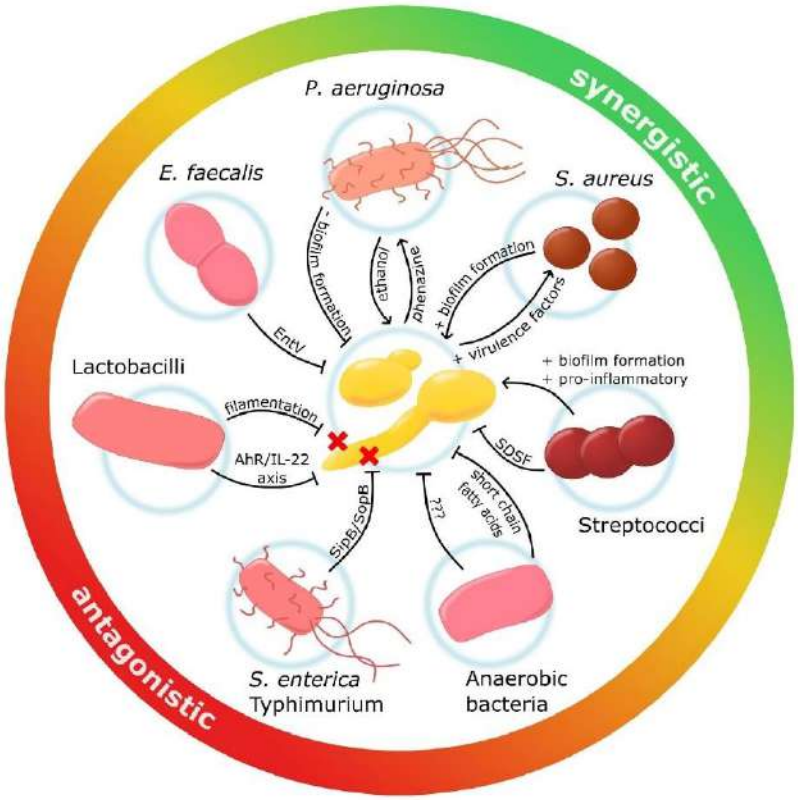


Figure 7. The interplay between *C. albicans* and certain bacterial present in human microbiotas. The growth of *C. albicans* in mucosal niches is generally constrained by the local bacterial microbiota *via* colonisation resistance. However, specific interactions with certain bacteria present in the vagina, oral cavity and/or GI tract influence the growth and/or virulence of *C. albicans* more directly. These interactions can be antagonistic (i.e. reduce the growth and virulence of the fungus) or synergistic (i.e. enhance the growth or virulence of the fungus). Anaerobic bacteria antagonise *C. albicans* colonisation by mechanisms that include the production of short chain fatty acids. *S. enterica* Typhimurium kills *C. albicans* hyphae by injecting effectors into the fungus via the SopB translocase. Lactobacilli antagonise *C. albicans* colonisation acidifying the local environment which reduces filamentation, and by generating metabolites that enhance IL-22-mediated immunity. *E. faecalis* blocks yeast-hypha morphogenesis and biofilm formation using *EntV*. Interactions between *C. albicans* and *P. aeruginosa* mutually enhance their virulence *via* cross-talk involving ethanol production by the fungus, which promotes toxic phenazine production by the bacterium, and this in turn promotes alcohol production by *C. albicans*. *S. aureus* binds *C. albicans* hyphae and promotes biofilm formation and antimicrobial resistance. Furthermore, co-infection with *S. aureus* significantly enhances the lethality of *C. albicans*. Streptococci block the formation of *C. albicans* hyphae via the diffusible factor, SDSF, but potentially co-exist as commensals with *C. albicans*. See text.

Jabra-Rizk 2009; Harriott and Noverr 2010; Peters *et al.* 2010; Harriott and Noverr 2011; Kong *et al.* 2016; Kean *et al.* 2017). Even more striking, though, is the enhanced lethality observed following co-infection in a mouse model (Peters and Noverr 2013). A synergistic enhancement of virulence occurs, independent of the ability of *C. albicans* to form filaments (Nash *et al.* 2016). This synergy is driven by an augmented host immune response (Nash *et al.* 2016, 2014; Peters and Noverr 2013). In addition, the presence of *C. albicans* increases the expression of staphylococcal virulence factors by modifying the environment (Todd *et al.* 2019; Todd, Noverr and Peters 2019). Significantly, this synergistic virulence depends on the *Candida* species involved, and was not observed for the closely related, but less virulent species, *Candida dubliniensis*.

Some relationships are difficult to place within a dichotomous scheme of synergism or antagonism. In some cases, diffusible molecules underlie inter-kingdom interactions (Deveau *et al.* 2018) as well as microbiota-induced immunomodulation of the host (Blacher *et al.* 2017). Most bacterial molecules target *C. albicans* virulence factors. *Salmonella enterica* serovar Typhimurium uses SipB translocase to inject SopB effectors and induce killing of the fungal hypha (Kim and Mylonakis 2011). *Enterococcus faecalis* restricts biofilm development by preventing yeast-to-hypha transition *via* the bacteriocin inhibitor *EntV* (Graham *et al.* 2017). *Streptococcus mutans* prevents hypha formation by targeting HWP1 (a hyphal-specific gene) using Streptococcus Diffusible Signal Factor (SDSF), a fatty acid. (Vílchez *et al.* 2010). These molecules do not act on the yeast form, indicating a potential propensity for commensal co-existence.

The host responds to, and influences, some fungal-bacterial interactions. This has been observed for interactions between *C. albicans* and *Pseudomonas aeruginosa*, which engage in an interactive molecular dialogue that leads to mutual enhancement of their virulence (Chen *et al.* 2014) (Fig. 7). *C. albicans* produces ethanol, which favours the production of more toxic classes of phenazines by *P. aeruginosa*, such as pyocyanin, phenazine methosulfate and phenazine-1-carboxylate. As well as inhibiting filamentation and biofilm formation, these phenazines

induce *C. albicans* to produce more ethanol by compromising mitochondrial functionality (Morales *et al.* 2013; Lindsay *et al.* 2014). Ethanol reduces the ability of macrophages to clear *P. aeruginosa* (Greenberg *et al.* 1999), while phenazines cause damage to respiratory epithelial tissues (Rada and Leto 2013). The mammalian host contributes actively to this interplay by responding to phenazines *via* the Aryl hydrocarbon Receptor (AhR) to enhance antimicrobial defences (Moura-Alves *et al.* 2014). Significantly, there is a strong association between ethanol production by *Candida* and the development of oral cancer (Alnuaimi *et al.* 2016).

The mammalian AhR is a multi-class receptor that modulates disease resistance (by activating IL-17A/IL-22 production) and disease tolerance (*via* TGF β activated Treg cell differentiation) (Cheng *et al.* 2010; Zelante *et al.* 2013; Bessede *et al.* 2014) (see *Adaptive immunity*). AhR functions within the indoleamine 2,3-dioxygenase 1 (IDO1)-catalysed pathway that converts tryptophan to L-kynurenine (Bessede *et al.* 2014). This pathway plays a dual role as microbial commensals use it to enhance host resistance, while pathogenic populations exploit it to dampen immune responses (Cheng *et al.* 2010; Zelante *et al.* 2013) (Fig. 7). Lactobacilli can switch their usage of carbon sources from sugar to tryptophan and utilise this pathway to initiate strain- and location-specific host effects that protect against *C. albicans* infection (Zelante *et al.* 2013). *L. reuteri* produces indole-3-aldehyde (3-IAld), which binds AhR and triggers the production of IL-22 in the gut. Meanwhile, *L. acidophilus* utilises the AhR/IL-22 axis against *C. albicans* in the vagina. However, *C. albicans* is able to switch tryptophan degradation mechanisms from L-kynurenine to 5-hydroxyltryptophan, which inhibits IL-17 production and impairs the host response against infections (Cheng *et al.* 2010). These examples illustrate the complexity of interactions between the fungus, the host and the microbiota.

Impact of a changing microbiota on the Fungus-Host-Microbiota interplay

The host microbiota contributes to anti-*Candida* defences through colonisation resistance. Consequently, perturbations of the healthy oral, gut and vaginal microbiota can predispose the host to *C. albicans* infection (see *The Microbiota*) (Fig. 8). However, *C. albicans* is not a passive player in these interactions (see *The Fungus*). For example, the fungus actively promotes oral microbiota perturbations under conditions of immunosuppression by increasing the prevalence of enterococci, which negatively impacts the integrity of the epithelial barrier and enhances *C. albicans* invasion (Bertolini *et al.* 2019). The complexity of interactions between the fungus, host and microbiota are also evident in the vagina, where high oestrogen levels promote *Lactobacillus* spp. colonisation, and affect *C. albicans* morphogenesis, thereby influencing the risk of *C. albicans* colonisation (see *Vaginal mucosa*). Colonisation resistance against *C. albicans* arises though a number of synergistic mechanisms, many of which target fungal virulence traits or modulate the host's response.

Several members of the gut microbiota that contribute to colonisation resistance against *C. albicans* produce short chain fatty acids (SCFAs). Antibiotic treatment leads to a reduction in colonic SCFAs and, consequently, an increase in the susceptibility of mice to *C. albicans* infection (Noverr and Huffnagle 2004; Guinan *et al.* 2019). Butyrate, in particular, has a profound impact on *C. albicans* growth, biofilm and hypha formation (Nguyen *et al.* 2011; Guinan *et al.* 2019). In the colon, such effects are unlikely to be mediated by weak acid stress (Ramsdale *et al.* 2008) given that the ambient pH of this niche is above the pKa for this SCFA. Rather, butyrate perturbs iron homeostasis (Cottier *et al.* 2015) and inhibits the metabolic activity of the fungus (Nguyen *et al.* 2011; Guinan *et al.* 2019) *via* Mig1 regulation of *HGT16*, which encodes a putative glucose transporter (Cottier *et al.* 2017). *In vitro* studies have demonstrated that SCFAs impair *C. albicans* morphogenesis and biofilm formation, in part by reducing the ambient pH (Noverr and Huffnagle 2004; Nguyen *et al.* 2011; Guinan *et al.* 2019). Similarly, lactic acid, generated by lactobacilli, maintains an acidic vaginal pH that inhibits *C. albicans* morphogenesis (Köhler, Assefa and Reid 2012). Although *Lactobacillus* spp. also produce hydrogen peroxide, it is believed that lactic acid is the main basis for anti-*Candida* activity in the vagina (Strus *et al.* 2005; Köhler, Assefa and Reid 2012).

Both hypha and biofilm formation promote the pathogenicity of *C. albicans*. The formation of *C. albicans* biofilms depends upon the yeast-to-hypha transition, and is a significant clinical challenge (see *Virulence factors*). Some members of the microbiota have been shown to hinder *C. albicans* morphogenesis and biofilm formation *via* secreted enzymes (Allonsius *et al.* 2019) or other products (Jarosz *et al.* 2009; Vílchez *et al.* 2010; James *et al.* 2016; Oliveira *et al.* 2016; Hager *et al.* 2019; Jang *et al.* 2019). Nevertheless, *C. albicans* can form polymicrobial biofilms with some members of the oral and gut microbiota, which display elevated drug and host resistance and can strongly influence clinical outcomes (Harriott and Noverr 2011; Fox *et al.* 2014; Cavalcanti *et al.* 2015).

Competition for adhesion sites and nutrients, especially glucose, by members of the microbiota also contributes to colonisation resistance against *C. albicans* in the gut, vagina and oral cavity (Boris *et al.* 1998; Basson 2000; Graf *et al.* 2019). *L. rhamnosus* GG is a common gut and oral isolate (Ahrné *et al.* 1998) that has been shown to prevent *C. albicans*-induced damage and invasion through both nutrient depletion and blocking of adhesion sites (Mailänder-Sánchez *et al.* 2017). *L. rhamnosus* can also reduce *C. albicans* proliferation and induce shedding from the epithelial barrier, thereby preventing invasion of the fungus into the tissue (Graf *et al.* 2019). Interactions between the microbiota, including microbial-associated factors, and the host immune system help to regulate *C. albicans* and prevent dissemination.

The microbiota also influences the colonisation of *C. albicans* indirectly by influencing host functionality. Macrophages exposed to microbiota-produced butyrate are more efficient at phagocytosing *C. albicans* cells, and they produce increased levels of nitric oxide, which enhances eradication of the pathogen (Nguyen *et al.* 2011). In response to butyrate generated by the microbiota, colon epithelial cells express the AMP, LL-37 (Schauber *et al.* 2003), which exerts candidacidal effects (see *Innate antifungal responses*). These host cells also activate LL-37 production in response to microbiota-induced hypoxia *via* HIF-1α (Hypoxia Induced Factor 1α) (Fan *et al.* 2015). *Blautia producta* and *Bacteroides thetaiotaomicron*, both common members of the human gut microbiota, promote colonisation resistance and eliminate *C. albicans* by stimulating LL-37 production in mice (Fan *et al.* 2015). Colonisation resistance against *C. albicans* is also provided by IL-22, which is produced by the host and induced by lactobacilli (Zelante *et al.* 2013). In addition, *L. rhamnosus* GG modulates the inflammatory response of epithelial cells by reducing IL-1α and GM-CSF production (Mailänder-Sánchez *et al.* 2017). By limiting the *C. albicans*-induced proinflammatory response of vaginal cells (with the exception of IL-1α and

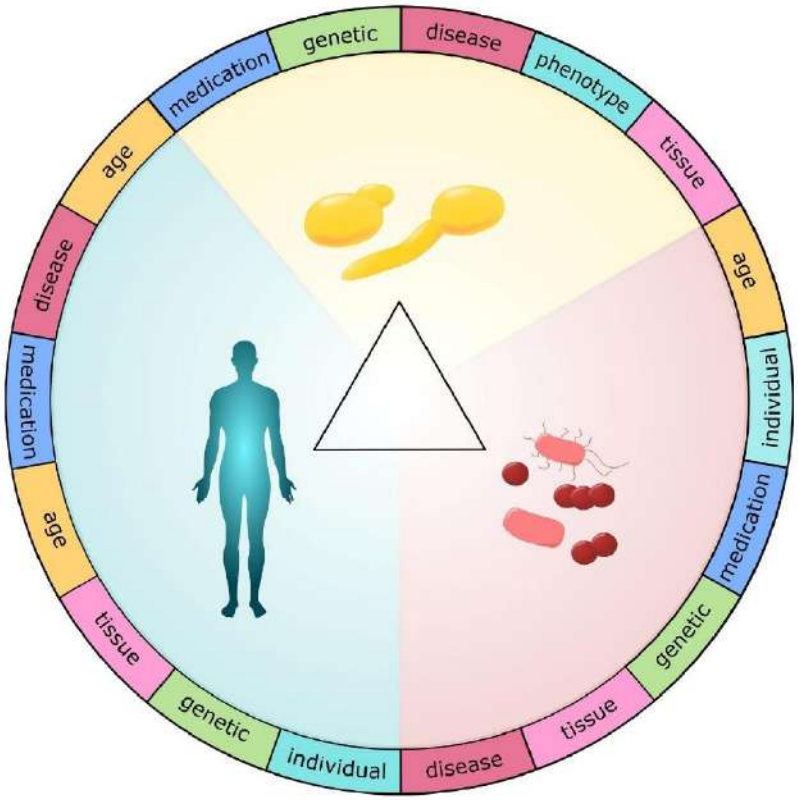


Figure 8. The complexity of fungus-host-microbiota interactions is dramatically increased by variability between *C. albicans* clinical isolates, between individuals, and in their microbiotas. Fungal variability arises through significant genetic and phenotypic variation between clinical isolates of *C. albicans*. The immune-competence of individuals varies significantly depending on their genetics, age and lifestyle. Furthermore, the microbiotas of the GI tract, oral cavity and vagina can each vary dramatically, depending on the age and health of the individual, and their diet, possible medications and life circumstances. Therefore, variation in each of the three elements of the fungus-host-microbiota interplay strongly influences the susceptibility of an individual to *C. albicans* infection as well as the outcome of that infection. See text.

IL-1β), lactobacilli can alleviate symptomatic vulvovaginal candidiasis while sustaining an antifungal immune response (Wagner and Johnson 2012). Similarly, *L. crispatus* reduces epithelial TLR2/4 expression and IL-8 levels in the presence of *C. albicans*, but maintains antifungal activity by increasing β-defensin production (Rizzo, Losacco and Carratelli 2013). Clearly, the changes in the microbiota strongly influence the iterative interactions between fungus, host and microbiota. Specific probiotic bacteria, including *Bifidobacterium breve, L. rhamnosus*, and *Lactobacillus casei* can also modulate specific PRR ligand- and *C. albicans*-induced cytokine responses (Plantinga, van Bergenhenegouwen *et al.* 2012).

Impact of patient variability upon the *Fungus-Host-Microbiota* interplay

The nature of an individual affects the types and outcomes of fungal-microbiota interactions directly and indirectly by: (i) genetic determinants that influence immune responses; (ii) personal environment and lifestyle, which affect the microbiota and (iii) iatrogenic interventions that target the microbiota or host response (Fig. 8). As outlined above, the microbiota is critical for colonisation resistance, leading to host protection, but on the other hand, certain combinations of opportunistic pathogens synergise to promote infection (see *Synergistic and antagonistic interactions between kingdoms*). It is well known that diet strongly influences the human gut microbiota (David, Maurice *et al.* 2014; Jeziorek, Frej-Mądrzak and Choroszy-Król 2019). Fundamental differences in diet, and possibly also exposure to microbes, are the most likely reason for the observed differences in *C. albicans* colonisation rates between industrialised and rural countries, which can differ by over 10-fold (Angebault *et al.* 2013) (see *Gastrointestinal (GI) tract*). Antibiotic treatments are probably the most common iatrogenic intervention that directly affects the microbiota, and one of the main predisposing factors to candidiasis in general. More specific iatrogenic factors include oral contraceptives and dental prostheses, which alter the local mucosal environment and thereby promote vaginal and oral candidiasis, respectively (Mothibe and Patel 2017; Jacob *et al.* 2018).

A healthy immune system is crucial for protection against fungal infections (see *The Host*). Individuals vary in their susceptibility to *C. albicans* infection because of genetic differences that affect susceptibility, and the existence of coexisting morbidities in some individuals. Genetic variations in key receptors or molecular effectors have been shown to increase the

risk of *Candida* infections (see *Variability amongst individuals*). For instance, monogenic primary immunodeficiency syndromes highly predispose an individual to haematogenously disseminated candidiasis and mucosal candidiasis (e.g. OPC, skin, nails). However, the genetic mutations defined to date do not explain the observed variation in susceptibility to candidiasis within not-at-risk subjects. Phenotypic variation occurs also in healthy individuals. For instance, if primary immune cells from healthy immunocompetent individuals are challenged with *C. albicans in vitro*, different outcomes are observed due to variation in their immune cell populations (Misme-Aucouturier *et al.* 2017). This can arise through genetic variation at the CR1 locus, which encodes a master regulator of *C. albicans*-specific immune responses (Piasecka *et al.* 2018). Thus, inter-individual variability in innate and adaptive responses against *Candida* spp. are likely to influence the degree of host-mediated damage during infection (Alvarez-Rueda *et al.* 2020). Consequently, understanding the basis of subject-to-subject diversity, and how this affects *Candida* pathogenicity, is likely to prove important for prevention and therapeutic strategies.

Comorbidities and treatment of other diseases can also affect a patient's susceptibility to *C. albicans* infection (see *C. albicans commensalism and pathogenicity*). Uncontrolled diabetes, for example, favours both bacterial and yeast infections due to metabolic alterations and impaired antimicrobial activity (Rodrigues, Rodrigues and Henriques 2019). Admission to an ICU, medical surgery, hematopoietic stem cell transplantation, and the use of external devices are independent risk factors for candidaemia and, together with the duration of hospitalisation, affect the mortality rates for candidiasis infections (Ortega *et al.* 2005; Das *et al.* 2011; Falcone *et al.* 2017; Poissy *et al.* 2020). These patients are commonly immunocompromised, either as a result of their primary disease, or through treatment. For example, OPC is a hallmark of HIV positive individuals and cancer patients (Samaranayake 1992; Redding *et al.* 1999). Moreover, as mentioned, dysregulated innate immunity is associated with failure to clear *Candida* spp., for example in neutropenic patients or neutrophil-related disorders (Nucci *et al.* 1997; Desai and Lionakis 2018). Glucocorticoids (Fan *et al.* 2012) and chemotherapy (Teoh and Pavelka 2016) weaken the host defence and increase the risk for invasive candidiasis.

Impact of fungal variability on the *Fungus-Host-Microbiota* interplay

Variability in *C. albicans*-host relationships is driven by the fungus as well as the host and its microbiota (Fig. 8). Clinical isolates of *C. albicans* display a high degree of genetic and phenotypic diversity (see *Candida albicans epidemiology and variability*). This fungal diversity can be observed at the genetic level (Tavanti *et al.* 2006; Cavalieri *et al.* 2017; Schönherr *et al.* 2017) as well as the transcriptional level (Thewes *et al.* 2008). The variation impacts multifarious aspects of *C. albicans* biology, such as stress and nutrient responses, and virulence properties such as morphogenesis, adhesion and invasion, that consequently, influence initial host-pathogen interactions, as well as colonisation and infection (see *The Fungus*). Therefore, it is not surprising that fungal variation affects the fitness of a given *C. albicans* strain in the host, and also disease outcome (Thewes *et al.* 2007, 2008; Cavalieri *et al.* 2017; Schönherr *et al.* 2017; Kirchner *et al.* 2019).

In principle, *C. albicans* strains can be classified on the basis of their virulence, rather than their epidemiological relationship. Comparative studies of various *C. albicans* isolates have identified genes whose expression or lack of expression strongly influences the virulence potential of these strains. Examples include *EFG1*, encoding a key transcription factor involved in morphogenesis (Hirakawa *et al.* 2015), and *DFG16*, encoding a pH sensor (Thewes *et al.* 2007). Strains displaying reduced expression of *EFG1* or *DFG16* display reduced virulence in mouse models of systemic infection (Thewes *et al.* 2007; Hirakawa *et al.* 2015). Even the development of hemizygosity at the EFG1 locus is sufficient to promote commensalism, rather than pathogenicity, in *C. albicans* (Liang *et al.* 2019).

A number of studies have highlighted the significance of variabilities between *C. albicans* isolates. The three *C. albicans* isolates, SC5314, 101 and ATCC10231 are all able to form hyphae. Nevertheless, SC5314 displays enhanced tissue invasion compared to ATCC10231 and 101 (Thewes *et al.* 2007; Schönherr *et al.* 2017), resulting in higher virulence. The strain SC5314 triggers rapid neutrophil recruitment and the production of proinflammatory cytokines leading to fungal clearance of the oral mucosa. In contrast, strains with lower virulence induce slower and weaker immune responses, which lead to fungal persistence (Schönherr *et al.* 2017). Similar results have been observed in a murine model of vaginal colonisation, where a less immunostimulatory *C. albicans* strain is able to persist over five weeks, in contrast to SC5314, which is cleared by week three (Rahman *et al.* 2007). The genetic background of *C. albicans* also influences survival in the phagosome (Tavanti *et al.* 2006; Cavalieri *et al.* 2017), the relative importance of specific PRRs for fungal clearance *in vivo* (Marakalala *et al.* 2013) and even the polarisation of the immune response (Cavalieri *et al.* 2017). Clearly, the intraspecies diversity of *C. albicans* has major consequences for the outcome of host-pathogen interactions.

NEW CHALLENGES

Elaborating the complexity of the microbiota

Meta-omics

The ability to define the complexity of relevant microbiotas rapidly and accurately represents a major challenge. It is vital that we address this challenge to establish phenotypic associations with specific members of the microbial community. Meta-omics, which refers to culture-independent functional and sequence-based analysis of the collective microbial genomes, transcriptomes, proteomes or metabolomes in a given sample (Handelsman *et al.* 1998; Riesenfeld, Schloss and Handelsman 2004), includes a powerful set of approaches to achieve this.

To date, DNA sequence-based studies have often been based on the analysis of amplicons generated from the microbial community with specific primers that are typically targeted towards bacterial 16S ribosomal RNA genes, and fungal 18S rRNA genes or internal transcribed spacer (ITS) regions. These approaches can provide comprehensive overviews of microbiota compositions, without directly assessing functional capabilities. However, the continuing development of DNA sequencing and genome analysis bioinformatics tools is permitting more widespread use of full shotgun metagenomics instead. This approach does not rely on amplification of marker genes since the extracted DNA is sequenced directly. The approach is more expensive and computationally intensive than marker gene sequencing because it requires sequencing to be carried out at a much higher depth. However, it has the advantages of avoiding biases associated with the amplification step, and generating

information on both the function and composition of microbiotas, thereby providing information at much greater resolution (Walker *et al.* 2014; Almeida *et al.* 2019). For example, a recent metagenomic analysis of the human gut microbiota from over eleven thousand individuals identified 1952 candidate bacterial species that have not yet been cultured and increased the known phylogenetic diversity of the gut microbiota almost three-fold (Almeida *et al.* 2019). Furthermore, these new genomes were estimated to encode hundreds of new biosynthetic gene clusters, revealing valuable clues about the potential functionalities of these novel candidate species.

In principle, full shotgun metagenomics can also be applied to mycobiota studies, but further advances are essential before fungal metagenomes can be analyzed more accurately. In particular, the lack of non-redundant and comprehensive fungal databases presents one of the most significant limitations. The accuracy of sequence classification depends fundamentally on the quality of the reference database, and, due to the large number of microbial species that have not yet been identified or genome sequenced, the existing databases are incomplete. This problem is gradually being lessened however, by the continual addition of genomes from newly isolated species (see *Culturomics*) and metagenome-assembled genomes (MAGs), which can provide reasonably accurate draft genomes for uncultured organisms.

Metagenomics can also be complemented by other -omic approaches to increase the power of meta-analyses. For example, metagenomics is being combined with meta-transcriptomic [i.e. the combined transcriptomes of the microbial community as a whole (Martinez *et al.* 2016; Franzosa *et al.* 2018)], proteomic (Van Belkum *et al.* 2018; Zhou *et al.* 2019), and metabolomic data sets (Smirnov *et al.* 2016; Yachida *et al.* 2019). Major software challenges must be addressed to improve the efficiency with which these different data sets can be integrated. For example, linking a metabolic gene to its transcript is relatively straightforward, but linking these to the corresponding enzyme and metabolic reaction is less so. Nevertheless, studies such as these are enhancing the associations between the composition of the gut microbiota and disease state for numerous conditions, including cancer, diabetes and inflammatory bowel diseases (Erickson *et al.* 2012; Smirnov *et al.* 2016; Zhang *et al.* 2018; Yachida *et al.* 2019; Zhou *et al.* 2019). These technologies have the potential to revolutionise our understanding of fungus-host-microbiota interactions and, as a result, our ability to develop personalised therapeutic strategies for individuals at risk of life-threatening fungal infections.

Culturomics

In the early 2010s, the use of high throughput culturing coupled to MALDI-ToF mass spectrometry (MS) revolutionised clinical microbiology (Seng *et al.* 2009; Bizzini *et al.* 2010; van Veen, Claas and Kuijper 2010). This has since been termed '*culturomics*' (Lagier *et al.* 2012). In brief, culturomics can identify atypical bacteria by combining multiple culture conditions (Beijerinck 1901; Weinstein 1996) with MALDI-ToF MS and 16S rRNA gene sequencing. The pioneering study used 212 culture conditions (Lagier *et al.* 2012), which was subsequently reduced to 18 conditions (Lagier *et al.* 2015) and recently the overall workload has been further reduced (Chang *et al.* 2019). Culturomics permits the identification of microbial minorities present at concentrations lower than 1e + 05 CFU/mL, which can encompass up to 65% of bacterial species in a given sample (Lagier *et al.* 2012). This not only enables a better description of the bacterial diversity (Dubourg *et al.* 2014), but also provides viable microbes for downstream analysis. Downstream characterisations of the new species can include pathogenic potential, metabolic functionality and interactions with other residents of the microbiota studied.

Newly identified species whose genomes have been sequenced can be used to identify previously found, yet unidentified, operational taxonomic units (OTUs), thus filling gaps in sequence-based analyses (Rinke *et al.* 2013; Lagier *et al.* 2016). Between 2015, when 2172 different species cultured from different human body sites were reported (Hugon *et al.* 2015), and 2018, 288 new species were isolated by culturomics (Bilen *et al.* 2018). Therefore, culturomics and metagenomics are complementary techniques, with an overlap as small as 15% of detected species in a given sample (Lagier et al. 2012, 2015; Pfleiderer *et al.* 2013; Dubourg *et al.* 2014; Mailhe *et al.* 2018).

Models for the experimental dissection of *Fungus-Host-Microbiota* interactions

Model experimental systems are essential for the detailed mechanistic dissection of disease establishment and progression in humans. Models of fungal infection can simulate the process with some degree of accuracy, but they never recapitulate human infections perfectly. Therefore, selecting an appropriate model is a crucial step that requires consideration of many parameters, such as similarity to the human situation, cost, workload, throughput and ethical concerns (Maccallum 2012; Brunke *et al.* 2015; Poupet *et al.* 2020). It is important to reconsider the relevance of a model to the human condition, and to clearly define the limitations of the model as well as the aspects of the human infection that are recapitulated by the model.

Mucosa simulating models

Rodents, particularly mouse models, have been used extensively to study vaginal and oral candidiasis (Rahman *et al.* 2007; Solis and Filler 2012; Cassone and Sobel 2016), as well as systemic candidiasis (MacCallum and Odds 2005; Szabo and MacCallum 2011; Brunke *et al.* 2015), allowing investigators to better understand the pathogenicity of *C. albicans*. However, there are significant differences between the immune systems of mice and humans (Mestas and Hughes 2004). Also, most laboratory mice are not naturally colonised by *C. albicans* and therefore do not develop a primed immunity to this opportunistic pathogen (Cassone and Sobel 2016). Moreover, the GI microbiota established in laboratory rodents generally mediates colonisation resistance against *C. albicans*. Thus, antibiotic treatment is required for prolonged high-level colonisation of the murine gut by *C. albicans* (Conti, Huppler *et al.* 2014). Oral models generally focus on infection, rather than colonisation, and immunosuppressive treatment is usually required to induce OPC (Solis and Filler 2012). Also, to study VVC in mice, oestrogen treatment is necessary to facilitate vaginal colonisation by *C. albicans* (Cassone and Sobel 2016).

Alternative *in vivo* mammalian models have been used to study Fungus-Host-Microbiota interactions, such as piglets and non-human primates, which are naturally colonised by *C. albicans*. However, these are cost- and labour-intensive and present ethical challenges (Steele, Ratterree and Fidel 1999; Cassone and Sobel 2016; M Jaeger *et al.* 2019). Under these circumstances, model hosts of lower phylogenetic or ontogenetic stage can provide alternative platforms to study *C. albicans* infections. Non-mammalian models that have been exploited to study *C. albicans* pathogenesis include a chorio-allantoic membrane chicken embryo model, zebrafish, nematodes and insects (Brennan *et al.*

2002; Gow et al. 2003; Jacobsen et al. 2011; Tobin, May and Wheeler 2012; Brunke et al. 2015).

In vitro cell culture systems also provide useful models of *C. albicans* infection. These are less expensive, provide higher throughput and present fewer ethical concerns compared to *in vivo* models. For example, static cell culture models that mimic *C. albicans* interactions with intestinal epithelial cells have been used to dissect processes involved in translocation through intestinal barriers (Allert et al. 2018). Also, *in vitro* circulatory *C. albicans*-endothelium interaction models have been used to study endothelial adhesion events under conditions of physiological blood pressure (Wilson and Hube 2010). Reconstituted Human Epithelium (RHE) uses Transwell® technology to form polarised epithelia and allows easy access to the apical and basolateral compartments for infection studies (Schaller et al. 2006). Such models closely recapitulate the histology of normal vaginal and oral mucosae and relevant aspects of innate immune responses (Schaller et al. 2005; Yadev et al. 2011) and can mimic epithelial interactions with phagocytes (Weindl et al. 2007). However, RHE models do have limitations, such as the lack of supporting cell types, the absence of mucins, non-constant desquamation, and the overgrowth of microbes due to static conditions (Tabatabaei, Moharamzadeh and Tayebi 2020). These limitations need to be addressed to gain accurate views of fungal infection.

Recently, human cell lines were incorporated into an oral-mucosa-on-a-chip model to study host-microbiota interactions (Rahimi et al. 2018). Also, oral mucosa organoids, which recapitulate the original tissue genetically, histologically and functionally, have been established (Driehuis et al. 2019). In principle, these organoids could be developed to integrate, for example, supporting cells and saliva, to further enhance their relevance to the natural oral mucosa. Organ-on-a-chip models of vaginal infection are under development (https://gtr.ukri.org/projects?ref=studentship-1818626; https://ncats.nih.gov/tissuechip/chip/female). Ideally, these models would include iron restriction and hypoxia as these conditions are known to influence the behaviour of *C. albicans* (Moosa et al. 2004; Sosinska et al. 2008; Rastogi et al. 2016; Pradhan et al. 2018, 2019). In the future, organ-on-a-chip models of oral and vaginal infection will exploit microfluidic platforms to combine patient-derived primary cells and microbes to represent donor variability and permit the development of predictive and potentially personalized infection models.

Gut simulating models

Models are also critical for the experimental dissection of host-microbiota interactions in the GI tract. These include organoid models as well as specialised fermentation systems (Fehlbaum et al. 2015; Park et al. 2017; Bein et al. 2018; Pearce et al. 2018; Pham et al. 2019), but the co-culturing of human and microbial cells remains a technical challenge. *L. rhamnosus* has been shown recently to modulate *C. albicans* pathogenicity in a commensal-like co-culture model (Graf et al. 2019). A similar model, involving co-culture of intestinal epithelial cells and M-cells, revealed that *C. albicans* translocate preferentially through the M-cell (Albac et al. 2016). These models are high-throughput, cost-efficient and able to recapitulate epithelial cell diversity by co-culturing different epithelial cell types. Nevertheless, such models do not provide the complex tissue architecture of the intestinal epithelium *in vivo* and, due to the static conditions, they only offer a short assay window before rapid microbial overgrowth occurs (Albac et al. 2016; Park et al. 2017; Pearce et al. 2018; Graf et al. 2019).

On-chip technologies permit the culture of human cells under perfusion, enabling their differentiation into a polarised columnar epithelium (Hyun Jung Kim et al. 2016; Trietsch et al. 2017). This has been extended to develop an immunocompetent intestine-on-a-chip model using caco-2 epithelial cells, endothelial cells and peripheral blood mononuclear cells (PBMCs) to study the interaction between *C. albicans* and probiotic *L. rhamnosus* (Maurer et al. 2019). Although this model already provides three-dimensional structures that resemble organotypic microanatomical structures and mimic microphysiological niches of the human intestine, further improvements to increase mucosa cellular diversity and mucus production are possible (Pan et al. 2015; Pearce et al. 2018). In the future, long-term cultures of patient-derived intestinal organoids may be feasible, which opens new avenues for the development of gut models that are even more physiologically relevant (Sato et al. 2009; Mottawea et al. 2019). Patient-derived ileal organoids and faecal samples have been used to culture a complex microbiota in an anaerobic gut-on-a-chip model for up to 5 days (Jalili-Firoozinezhad et al. 2019). This type of model is important because it permits the analysis of donor variability and potentially allows the development of personalised therapies.

Fermentation-based models provide powerful *in vitro* tools that permit the dissection of microbial processes in the human GI tract. Static batch fermentations with faecal inocula are the simplest and most frequently used models (Walker et al. 2005). These have provided a powerful first approach to study bacterial-fungal interactions and to screen novel therapeutics, but they do not recapitulate the richness of GI compartments (Hillman et al. 2017). Therefore, multicompartmental models have been developed (Guerra et al. 2012; Venema and van den Abbeele 2013). These often contain three-stage culture reactors (Gibson, Cummings and Macfarlane 1988) that can reproduce differences between proximal (acidic, carbohydrate-rich) and distal colonic regions (neutral, carbohydrate-depleted). The multicompartmental M-SHIME system is a powerful tool that permits the analysis of complex, rich and relatively stable microbial communities within GI compartments from the stomach to descending colon (Van de Wiele et al. 2015; Molly, Vande Woestyne and Verstraete 1993). This model has been used to study bacterial-bacterial interactions and the impact of diet and drugs on these interactions (Sivieri et al. 2014; Van den Abbeele et al. 2016; Marzorati et al. 2017; Rivière et al. 2018; Lambrecht et al. 2019).

Despite their power, these fermentation systems have rarely been used to examine fungal- bacterial interactions. In two studies, *C. albicans* colonisation and outgrowth was shown to be strongly correlated with antibiotic treatment, but mitigated by *L. plantarum* (Payne et al. 2003; Wynne et al. 2004). However, more recent work has shown that it is important to include the mucus layer to properly simulate the human gut environment *in vitro* (Van den Abbeele, Van de Wiele et al. 2011; Van den Abbeele, Gérard et al. 2011; Van den Abbeele et al. 2013). Indeed, the presence of mucus influenced interactions between the yeast *Saccharomyces boulardii* and *L. rhamnosus* GG and their ability to limit the outgrowth of toxigenic *E. coli* (Moens et al. 2019). Therefore, a M-SHIME-based system that includes a mucus-rich environment (Van den Abbeele et al. 2012) would seem most appropriate for the dissection of *C. albicans*-microbiota interactions in the GI tract.

SUMMARY AND OUTLOOK

To summarise, it is clear that the interactions between *C. albicans*, the human host, and the local microbiota have a major impact upon the likelihood of mucosal and systemic infections and the severity of these infections. It is also apparent that these fungus-host-microbiota interactions are dynamic, iterative and enormously complex (Fig. 8). This immense complexity is increased further by the genetic and phenotypic variation within the species of *C. albicans*, and by numerous factors that contribute to the variability of individuals and their microbiotas. Yet this complexity must be addressed and defined if the research community is to develop: (i) sensitive and accurate diagnostics capable of distinguishing *C. albicans* infection from commensalism, and at an early stage when the infection is more amenable to therapy; (ii) novel and efficacious anti-fungal therapies that complement the limited antifungal drugs that are currently available, and that address the problematic emergence of drug resistance and drug resistant species; (iii) tests that quickly establish whether a particular patient is at risk of developing severe candidaemia or recurrent candidiasis and (iv) personalised therapeutic strategies that address the specific make-up and needs of the individual patient.

Despite these challenges, our increased understanding of antifungal immunity and responses is offering potential immunotherapeutic opportunities (De Luca *et al.* 2013; Davidson, Netea and Kullberg 2018). Effective anti-*Candida* vaccines, which have proven elusive for so long, are now in sight (Cassone 2015; De Bernardis *et al.* 2018; Edwards *et al.* 2018). Our deeper comprehension of fungal immune evasion strategies affords the potential to block these phenotypes and thereby enhance the efficacy of natural antifungal immunity mechanisms (Childers *et al.* 2020). Significantly, the dramatic expansion in genomic and phenotypic datasets for clinical isolates of *C. albicans* is providing a much clearer picture of the nature of fungal variability and in-patient evolution (Selmecki, Forche and Berman 2006; Ford *et al.* 2014; Hirakawa *et al.* 2015; Ropars *et al.* 2018; Sitterlé *et al.* 2019). This information is vital because it will reveal ways in which this microevolution might be inhibited or exploited therapeutically. This information will also highlight essential fungal processes that are less prone to variability and hence present better therapeutic targets. Rapid advances in metagenomics and culturomics are highlighting fungal-bacterial associations between the gut microbiota that are likely to yield useful prognostic tools for patients at risk of systemic candidiasis (Yachida *et al.* 2019). Our increased knowledge of local fungus-microbiota interactions is facilitating the development of probiotic therapies to address VVC, OPC and *C. albicans* colonisation of the GI tract (Romeo *et al.* 2011; Hu *et al.* 2013; Morais *et al.* 2017; Vladareanu *et al.* 2018). In the future, the availability of effective probiotics should help to reduce our dependence on antifungal drugs while, at the same time, enhancing antifungal immunity (Ubeda and Pamer 2012).

How relevant are these points to other fungal pathogens of humans, such as *Aspergillus*, *Cryptococcus*, *Pneumocystis* and other pathogenic *Candida* species? Pathogenic *Aspergillus* and *Cryptococcus* species are environmental fungi that infect humans *via* the lung. Therefore, while fundamental principles relating to local antifungal immunity, immunotherapy and microbiota-mediated colonisation resistance are clearly of relevance (Armstrong-James *et al.* 2017; Dumas *et al.* 2018; Hernández-Santos *et al.* 2018; Drummond and Lionakis 2019; Maschirow, Suttorp and Opitz 2019; Warris, Bercusson and Armstrong-James 2019), the specific details will differ significantly. *Pneumocystis jirovecii* also infects the lung, but this fungus is an intracellular parasite that is obligately associated with its human host. In this case, the lung microbiota has not been particularly informative in distinguishing infected from uninfected patients (Kehrmann *et al.* 2017). Although other *Candida* pathogens may infect from environmental reservoirs, these species cause similar types of infection to *C. albicans*, and therefore the points raised in this review will be of general relevance to these species. However, some differences in tissue tropism and patient type exist between species (Sullivan *et al.* 1995; Silva *et al.* 2012; Pammi *et al.* 2013), and some species differ in their immune avoidance strategies (Brunke and Hube 2013; Kasper, Seider and Hube 2015). Nevertheless, the importance of the general principles discussed in this *C. albicans*-oriented review cannot be understated, most notably the major impact of variability in the fungus, the individual host, and the local microbiota upon disease severity and outcome (Carvalho *et al.* 2010; Farrer *et al.* 2015; Hube 2015; Ballard *et al.* 2018; Stone *et al.* 2019; Vandeplassche *et al.* 2019).

ACKNOWLEDGMENTS

We thank our friends and colleagues in the medical mycology, fungal immunology and microbiota fields for many thought-provoking discussions.

FUNDING

We received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie action, Innovative Training Network: FunHoMic; grant N° 812969. CdE received funding from the French Government 'Investissement d'Avenir' program (Laboratoire d'Excellence Integrative Biology of Emerging Infectious Diseases, ANR-10-LABX-62-IBEID), the Agence Nationale de la Recherche (ERA-Net Infect-ERA, FUNCOMPATH, ANR-14-IFEC-0004), the EU Horizon2020 consortium 'Host-Directed Medicine in invasive FUNgal infections'—HDM-FUN (Grant Agreement 847507). SLL and CdE received funding from the Swiss National Science Foundation (Sinergia program, #CRSII5_173863). BIOASTER received funding from the French Government 'Investissement d'Avenir' program (Grant No. ANR-10-AIRT-03). MSG was supported by a Humboldt Research Fellowship for Postdoctoral Researchers by the Alexander von Humboldt-Foundation and the Deutsche Forschungsgemeinschaft (DFG) Emmy Noether Program (project no. 434385622/GR 5617/1-1). BH was supported by the Deutsche Forschungsgemeinschaft (DFG) project Hu 532/20-1, project C1 within the Collaborative Research Centre (CRC)/Transregio 124 FungiNet and the Balance of the Microverse Cluster under Germany´s Excellence Strategy—EXC 2051–Project-ID 390713860, the EU Horizon2020 consortium 'Host-Directed Medicine in invasive FUNgal infections'—HDM-FUN (Grant Agreement 847507), the Leibniz Association Campus InfectoOptics SAS-2015-HKI-LWC and the Wellcome Trust (215599/Z/19/Z). IDJ was supported by the Deutsche Forschungsgemeinschaft (DFG) project C5 within the Collaborative Research Centre (CRC)/Transregio 124 FungiNet and the Balance of the Microverse Cluster under Germany´s Excellence Strategy—EXC 2051–Project-ID 390713860, the Leibniz Association Campus InfectoOptics SAS-2015-HKI-LWC and the Wellcome Trust (Grant 215599/Z/19/Z). CM received funding from the the Instituto de Salud Carlos III/FEDER. MGN was supported by an ERC Advanced Grant (#833247) and

a Spinoza grant of the Netherlands Organization for Scientific Research. CAM was supported by EU Horizon2020 consortium 'Host-Directed Medicine in invasive FUNgal infections'—HDM-FUN (Grant Agreement 847507) and the Wellcome Trust Strategic Award for Medical Mycology and Fungal Immunology (097377/Z/11/Z). AWW receives core funding support from the Scottish Government's Rural and Environment Science and Analytical Services (RESAS). AJPB was supported by a programme grant from the UK Medical Research Council (MR/M026663/1) and by the Medical Research Council Centre for Medical Mycology at the University of Exeter (MR/N006364/1).

Conflicts of Interest. None declared.

REFERENCES

Aagaard K, Riehle K, Ma K *et al.* A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PLoS One* 2012;**7**:e36466.

Acosta-Rodriguez EV, Rivino L, Geginat J *et al.* Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat Immunol* 2007;**8**:639–46.

Adler CJ, Dobney K, Weyrich LS *et al.* Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nat Genet* 2013;**45**:450–5, 455e1.

Aggor FEY, Break TJ, Trevejo-Nuñez G *et al.* Oral epithelial IL-22/STAT3 signaling licenses IL-17-mediated immunity to oral mucosal candidiasis. *Sci Immunol* 2020;**5**.

Ahrné S, Nobaek S, Jeppsson B *et al.* The normal Lactobacillus flora of healthy human rectal and oral mucosa. *J Appl Microbiol* 1998;**85**:88–94.

Ajdić D, McShan WM, McLaughlin RE *et al.* Genome sequence of Streptococcus mutans UA159, a cariogenic dental pathogen. *Proc Natl Acad Sci USA* 2002;**99**:14434–9.

Akimoto-Gunther L, Bonfim-Mendonça P, de S *et al.* Highlights regarding host predisposing factors to recurrent vulvovaginal candidiasis: chronic stress and reduced antioxidant capacity. *PLoS One* 2016;**11**:e0158870.

Al-Rusan RM, Darwazeh AMG, Lataifeh IM. The relationship of Candida colonization of the oral and vaginal mucosae of mothers and oral mucosae of their newborns at birth. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2017;**123**:459–63.

Alarco AM, Raymond M. The bZip transcription factor Cap1p is involved in multidrug resistance and oxidative stress response in Candida albicans. *J Bacteriol* 1999;**181**:700–8.

Albac S *et al.* Candida albicans is able to use M cells as a portal of entry across the intestinal barrier in vitro. *Cell Microbiol* 2016;**18**:195–210.

Albert AYK *et al.* A Study of the Vaginal Microbiome in Healthy Canadian Women Utilizing cpn60-Based Molecular Profiling Reveals Distinct Gardnerella Subgroup Community State Types. *PLoS One* 2015;**10**:e0135620.

Allert S *et al.* Candida albicans-Induced Epithelial Damage Mediates Translocation through Intestinal Barriers. *MBio* 2018;**9**.

Allonsius CN, Vandenheuvel D, Oerlemans EFM *et al.* Inhibition of Candida albicans morphogenesis by chitinase from Lactobacillus rhamnosus GG. *Sci Rep* 2019;**9**:2900.

Almeida A, Mitchell AL, Boland M *et al.* A new genomic blueprint of the human gut microbiota. *Nature* 2019;**568**:499–504.

Almeida RS, Brunke S, Albrecht A *et al.* the hyphal-associated adhesin and invasin Als3 of Candida albicans mediates iron acquisition from host ferritin. *PLoS Pathog* 2008;**4**:e1000217.

Alnuaimi AD, Ramdzan AN, Wiesenfeld D *et al.* Candida virulence and ethanol-derived acetaldehyde production in oral cancer and non-cancer subjects. *Oral Dis* 2016;**22**:805–14.

Alonso-Monge R, Carvaihlo S, Nombela C *et al.* The Hog1 MAP kinase controls respiratory metabolism in the fungal pathogen Candida albicans. *Microbiology (Reading, Engl)* 2009;**155**:413–23.

Alonso-Monge R, Navarro-García F, Molero G *et al.* Role of the mitogen-activated protein kinase Hog1p in morphogenesis and virulence of Candida albicans. *J Bacteriol* 1999;**181**:3058–68.

Altmeier S, Toska A, Sparber F *et al.* IL-1 Coordinates the Neutrophil Response to C. albicans in the Oral Mucosa. *PLoS Pathog* 2016;**12**:e1005882.

Alvarez-Rueda N, Rouges C, Touahri A *et al.* In vitro immune responses of human PBMCs against Candida albicans reveals fungal and leucocyte phenotypes associated with fungal persistence. *Sci Rep* 2020;**10**:6211.

Anderson MZ, Thomson GJ, Hirakawa MP *et al.* A "parameiosis" drives depolyploidization and homologous recombination in Candida albicans. *Nat Commun* 2019;**10**:4388.

Angebault C, Ghozlane A, Volant S *et al.* Combined bacterial and fungal intestinal microbiota analyses: Impact of storage conditions and DNA extraction protocols. *PLoS One* 2018;**13**:e0201174.

Angebault C *et al.* Candida albicans is not always the preferential yeast colonizing humans: a study in Wayampi Amerindians. *J Infect Dis* 2013;**208**:1705–16.

Anukam KC, Osazuwa EO, Ahonkhai I *et al.* Lactobacillus vaginal microbiota of women attending a reproductive health care service in Benin city, Nigeria. *Sex Transm Dis* 2006;**33**:59–62.

Ardizzoni A *et al.* Perinuclear Anti-Neutrophil Cytoplasmic Antibodies (pANCA) Impair Neutrophil Candidacidal Activity and Are Increased in the Cellular Fraction of Vaginal Samples from Women with Vulvovaginal Candidiasis. *J Fungi (Basel)* 2020;**6**.

Arevalo AV, Nobile CJ. Interactions of microorganisms with host mucins: a focus on Candida albicans. *FEMS Microbiol Rev* 2020.

Ariizumi K, Shen GL, Shikano S *et al.* Cloning of a second dendritic cell-associated C-type lectin (dectin-2) and its alternatively spliced isoforms. *J Biol Chem* 2000;**275**:11957–63.

Ariizumi K, Shen GL, Shikano S *et al.* Identification of a novel, dendritic cell-associated molecule, dectin-1, by subtractive cDNA cloning. *J Biol Chem* 2000;**275**:20157–67.

Armstrong-James D, Brown GD, Netea MG *et al.* Immunotherapeutic approaches to treatment of fungal diseases. *Lancet Infect Dis* 2017;**17**:e393–402.

Arumugam M *et al.* Enterotypes of the human gut microbiome. *Nature* 2011;**473**:174–80.

Auchtung TA, Fofanova TY, Stewart CJ *et al.* Investigating colonization of the healthy adult gastrointestinal tract by fungi. *mSphere* 2018;**3**.

Austermeier S, Kasper L, Westman J *et al.* I want to break free - macrophage strategies to recognize and kill Candida albicans, and fungal counter-strategies to escape. *Curr Opin Microbiol* 2020;**58**:15–23.

Bacher P *et al.* Antigen-specific expansion of human regulatory T cells as a major tolerance mechanism against mucosal fungi. *Mucosal Immunol* 2014;**7**:916–28.

Bacher P *et al.* Human Anti-fungal Th17 Immunity and Pathology Rely on Cross-Reactivity against Candida albicans. *Cell* 2019;**176**:1340–55. e15.

Bailey DA, Feldmann PJ, Bovey M *et al.* The Candida albicans HYR1 gene, which is activated in response to hyphal development, belongs to a gene family encoding yeast cell wall proteins. *J Bacteriol* 1996;**178**:5353–60.

Bain J, Gow NAR, Erwig L-P. Novel insights into host-fungal pathogen interactions derived from live-cell imaging. *Semin Immunopathol* 2015;**37**:131–9.

Bain JM, Lewis LE, Okai B *et al.* Non-lytic expulsion/exocytosis of Candida albicans from macrophages. *Fungal Genet Biol* 2012;**49**:677–8.

Bain JM *et al.* Candida albicans hypha formation and mannan masking of β-glucan inhibit macrophage phagosome maturation. *MBio* 2014;**5**:e01874.

Bairwa G, Hee Jung W, Kronstad JW. Iron acquisition in fungal pathogens of humans. *Metallomics* 2017;**9**:215–27.

Bai W, Wang Q, Deng Z *et al.* TRAF1 suppresses antifungal immunity through CXCL1-mediated neutrophil recruitment during Candida albicans intradermal infection. *Cell Commun Signal* 2020;**18**:30.

Baley JE, Kliegman RM, Boxerbaum B *et al.* Fungal colonization in the very low birth weight infant. *Pediatrics* 1986;**78**:225–32.

Ballard E, Melchers WJG, Zoll J *et al.* In-host microevolution of Aspergillus fumigatus: A phenotypic and genotypic analysis. *Fungal Genet Biol* 2018;**113**:1–13.

Ballou ER *et al.* Lactate signalling regulates fungal β-glucan masking and immune evasion. *Nat Microbiol* 2016;**2**:16238.

Bamford CV, d Mello A, Nobbs AH *et al.* Streptococcus gordonii modulates Candida albicans biofilm formation through intergeneric communication. *Infect Immun* 2009;**77**:3696–704.

Bamford CV, Nobbs AH, Barbour ME *et al.* Functional regions of Candida albicans hyphal cell wall protein Als3 that determine interaction with the oral bacterium Streptococcus gordonii. *Microbiology (Reading, Engl)* 2015;**161**:18–29.

Bandara HMHN, Yau JYY, Watt RM *et al.* Escherichia coli and its lipopolysaccharide modulate in vitro Candida biofilm formation. *J Med Microbiol* 2009;**58**:1623–31.

Barelle CJ, Priest CL, Maccallum DM *et al.* Niche-specific regulation of central metabolic pathways in a fungal pathogen. *Cell Microbiol* 2006;**8**:961–71.

Basson NJ. Competition for glucose between Candida albicans and oral bacteria grown in mixed culture in a chemostat. *J Med Microbiol* 2000;**49**:969–75.

Basso V, d Enfert C, Znaidi S *et al.* From Genes to Networks: The Regulatory Circuitry Controlling Candida albicans Morphogenesis. *Curr Top Microbiol Immunol* 2019;**422**:61–99.

Becattini S *et al.* T cell immunity. Functional heterogeneity of human memory CD4+ T cell clones primed by pathogens or vaccines. *Science* 2015;**347**:400–6.

Becker KL, Rösler B, Wang X *et al.* Th2 and Th9 responses in patients with chronic mucocutaneous candidiasis and hyper-IgE syndrome. *Clin Exp Allergy* 2016;**46**:1564–74.

Beijerinck MW. Über oligonitrophile Mikroben. *Centralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung II* 1901;**7**:561–82.

Bein A, Shin W, Jalili-Firoozinezhad S *et al.* Microfluidic Organ-on-a-Chip Models of Human Intestine. *Cell Mol Gastroenterol Hepatol* 2018;**5**:659–68.

Bellemain E, Carlsen T, Brochmann C *et al.* ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. *BMC Microbiol* 2010;**10**:189.

Belstrøm D, Holmstrup P, Bardow A *et al.* Temporal stability of the salivary microbiota in oral health. *PLoS One* 2016;**11**:e0147472.

Bennett RJ, Forche A, Berman J. Rapid mechanisms for generating genome diversity: whole ploidy shifts, aneuploidy, and loss of heterozygosity. *Cold Spring Harb Perspect Med* 2014;**4**.

Bennett RJ, Johnson AD. Completion of a parasexual cycle in Candida albicans by induced chromosome loss in tetraploid strains. *EMBO J* 2003;**22**:2505–15.

Beno DW, Stöver AG, Mathews HL. Growth inhibition of Candida albicans hyphae by CD8+ lymphocytes. *J Immunol* 1995;**154**:5273–81.

Bensasson D, Dicks J, Ludwig JM *et al.* Diverse Lineages of Candida albicans Live on Old Oaks. *Genetics* 2019;**211**:277–88.

Bertolini M, Ranjan A, Thompson A *et al.* Candida albicans induces mucosal bacterial dysbiosis that promotes invasive infection. *PLoS Pathog* 2019;**15**:e1007717.

Bessede A *et al.* Aryl hydrocarbon receptor control of a disease tolerance defence pathway. *Nature* 2014;**511**:184–90.

Bhaskaran N, Quigley C, Paw C *et al.* Role of short chain fatty acids in controlling tregs and immunopathology during mucosal infection. *Front Microbiol* 2018;**9**:1995.

Bik EM *et al.* Bacterial diversity in the oral cavity of 10 healthy individuals. *ISME J* 2010;**4**:962–74.

Bilen M, Dufour J-C, Lagier J-C *et al.* The contribution of culturomics to the repertoire of isolated human bacterial and archaeal species. *Microbiome* 2018;**6**:94.

Bistoni F, Vecchiarelli A, Cenci E *et al.* Evidence for macrophage-mediated protection against lethal Candida albicans infection. *Infect Immun* 1986;**51**:668–74.

Bizzini A, Durussel C, Bille J *et al.* Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. *J Clin Microbiol* 2010;**48**:1549–54.

Blacher E, Levy M, Tatirovsky E *et al.* Microbiome-Modulated Metabolites at the Interface of Host Immunity. *J Immunol* 2017;**198**:572–80.

Black CA, Eyers FM, Russell A *et al.* Acute neutropenia decreases inflammation associated with murine vaginal candidiasis but has no effect on the course of infection. *Infect Immun* 1998;**66**:1273–5.

Bliss JM, Basavegowda KP, Watson WJ *et al.* Vertical and horizontal transmission of Candida albicans in very low birth weight infants using DNA fingerprinting techniques. *Pediatr Infect Dis J* 2008;**27**:231–5.

Boix-Amorós A, Martinez-Costa C, Querol A *et al.* Multiple Approaches Detect the Presence of Fungi in Human Breastmilk Samples from Healthy Mothers. *Sci Rep* 2017;**7**:13016.

Booijink CCGM, El-Aidy S, Rajilić-Stojanović M *et al.* High temporal and inter-individual variation detected in the human ileal microbiota. *Environ Microbiol* 2010;**12**:3213–27.

Borghi M, Renga G, Puccetti M *et al.* Antifungal th immunity: growing up in family. *Front Immunol* 2014;**5**:506.

Borghi M *et al.* Pathogenic NLRP3 Inflammasome Activity during Candida Infection Is Negatively Regulated by IL-22 via Activation of NLRC4 and IL-1Ra. *Cell Host Microbe* 2015;**18**:198–209.

Boris S, Suárez JE, Vázquez F *et al.* Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. *Infect Immun* 1998;**66**:1985–9.

Borman AM, Szekely A, Linton CJ *et al.* Epidemiology, antifungal susceptibility, and pathogenicity of Candida africana isolates from the United Kingdom. *J Clin Microbiol* 2013;**51**:967–72.

Bougnoux M-E, Pujol C, Diogo D *et al.* Mating is rare within as well as between clades of the human pathogen Candida albicans. *Fungal Genet Biol* 2008;**45**:221–31.

Bougnoux ME et al. Multilocus sequence typing reveals intrafamilial transmission and microevolutions of Candida albicans isolates from the human digestive tract. *J Clin Microbiol* 2006;**44**:1810–20.

Bravo JA, Forsythe P, Chew MV et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci USA* 2011;**108**:16050–5.

Brennan M, Thomas DY, Whiteway M et al. Correlation between virulence of Candida albicans mutants in mice and Galleria mellonella larvae. *FEMS Immunol Med Microbiol* 2002;**34**:153–7.

Brogden KA, Guthmiller JM, Taylor CE. Human polymicrobial infections. *Lancet* 2005;**365**:253–5.

Brotman RM, He X, Gajer P et al. Association between cigarette smoking and the vaginal microbiota: a pilot study. *BMC Infect Dis* 2014;14:471.

Brotman RM, Shardell MD et al. Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. *Menopause* 2014;**21**:450–8.

Brown AJP, Brown GD, Netea MG et al. Metabolism impacts upon Candida immunogenicity and pathogenicity at multiple levels. *Trends Microbiol* 2014;**22**:614–22.

Brown AJP, Budge S et al. Stress adaptation in a pathogenic fungus. *J Exp Biol* 2014;**217**:144–55.

Brown AJP, Cowen LE, di Pietro A et al. Stress Adaptation. *Microbiol Spectr* 2017;**5**.

Brown AJP, Gow NAR, Warris A et al. Memory in fungal pathogens promotes immune evasion, colonisation, and infection. *Trends Microbiol* 2019;**27**:219–30.

Brown AJP, Larcombe DE, Pradhan A. Thoughts on the evolution of Core Environmental Responses in yeasts. *Fungal Biol* 2020;**124**:475–81.

Brown GD, Denning DW, Gow NAR et al. Hidden killers: human fungal infections. *Sci Transl Med* 2012;**4**: 165rv13.

Brown GD, Gordon S. Immune recognition. A new receptor for beta-glucans. *Nature* 2001;**413**:36–37.

Brown GD, Taylor PR, Reid DM et al. Dectin-1 is a major beta-glucan receptor on macrophages. *J Exp Med* 2002;**196**:407–12.

Brown GD. Innate antifungal immunity: the key role of phagocytes. *Annu Rev Immunol* 2011;**29**:1–21.

Brown SE et al. The Vaginal Microbiota and Behavioral Factors Associated With Genital Candida albicans Detection in Reproductive-Age Women. *Sex Transm Dis* 2019;**46**:753–8.

Brunke S, Hube B. Adaptive prediction as a strategy in microbial infections. *PLoS Pathog* 2014;**10**:e1004356.

Brunke S, Hube B. Two unlike cousins: Candida albicans and C. glabrata infection strategies. *Cell Microbiol* 2013;**15**:701–8.

Brunke S et al. Of mice, flies–and men? Comparing fungal infection models for large-scale screening efforts. *Dis Model Mech* 2015;**8**:473–86.

Bruno VM, Shetty AC, Yano J et al. Transcriptomic analysis of vulvovaginal candidiasis identifies a role for the NLRP3 inflammasome. *MBio* 2015;**6**.

Buffo J, Herman MA, Soll DR. A characterization of pH-regulated dimorphism in Candida albicans. *Mycopathologia* 1984;**85**: 21–30.

Burdet C et al. Ceftriaxone and Cefotaxime Have Similar Effects on the Intestinal Microbiota in Human Volunteers Treated by Standard-Dose Regimens. *Antimicrob Agents Chemother* 2019;**63**.

Butler G et al. Evolution of pathogenicity and sexual reproduction in eight Candida genomes. *Nature* 2009;**459**:657–62.

Bäckhed F et al. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell Host Microbe* 2015;**17**:690–703.

Bär E, Gladiator A, Bastidas S et al. A novel Th cell epitope of Candida albicans mediates protection from fungal infection. *J Immunol* 2012;**188**:5636–43.

Böttcher B, Pöllath C, Staib P et al. Candida species Rewired Hyphae Developmental Programs for Chlamydospore Formation. *Front Microbiol* 2016;**7**:1697.

Cambi A et al. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for Candida albicans on dendritic cells. *Eur J Immunol* 2003;**33**:532–8.

Caporaso JG et al. Moving pictures of the human microbiome. *Genome Biol* 2011;**12**:R50.

Caramalac DA, da Silva Ruiz L, de Batista GCM et al. Candida isolated from vaginal mucosa of mothers and oral mucosa of neonates: occurrence and biotypes concordance. *Pediatr Infect Dis J* 2007;**26**:553–7.

Carolus H, Van Dyck K, Van Dijck P. Candida albicans and Staphylococcus Species: A Threatening Twosome. *Front Microbiol* 2019;**10**:2162.

Carvalho A, Cunha C, Pasqualotto AC et al. Genetic variability of innate immunity impacts human susceptibility to fungal diseases. *Int J Infect Dis* 2010;**14**:e460–8.

Casadevall A, Pirofski L-A. Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. *Infect Immun* 1999;**67**:3703–13.

Casadevall A, Pirofski L-A. The damage-response framework of microbial pathogenesis. *Nat Rev Microbiol* 2003;**1**:17–24.

Casadevall A, Pirofski L-A. What is a host? Incorporating the microbiota into the damage-response framework. *Infect Immun* 2015;**83**:2–7.

Casaroto AR et al. Candida albicans-Cell Interactions Activate Innate Immune Defense in Human Palate Epithelial Primary Cells via Nitric Oxide (NO) and β-Defensin 2 (hBD-2). *Cells* 2019;**8**.

Cassone A, Cauda R. Candida and candidiasis in HIV-infected patients: where commensalism, opportunistic behavior and frank pathogenicity lose their borders. *AIDS* 2012;**26**:1457–72.

Cassone A, Sobel JD. Experimental models of vaginal candidiasis and their relevance to human candidiasis. *Infect Immun* 2016;**84**:1255–61.

Cassone A. Vulvovaginal Candida albicans infections: pathogenesis, immunity and vaccine prospects. *BJOG* 2015;**122**:785–94.

Cavalcanti YW et al. Virulence and pathogenicity of Candida albicans is enhanced in biofilms containing oral bacteria. *Biofouling* 2015;**31**:27–38.

Cavalieri D et al. Genomic and Phenotypic Variation in Morphogenetic Networks of TwoCandida albicansIsolates Subtends Their Different Pathogenic Potential. *Front Immunol* 2017;**8**:1997.

Ceresa C, Tessarolo F, Caola I et al. Inhibition of Candida albicans adhesion on medical-grade silicone by a Lactobacillus-derived biosurfactant. *J Appl Microbiol* 2015;**118**:1116–25.

Chang H-T, Tsai P-W, Huang H-H et al. LL37 and hBD-3 elevate the β-1,3-exoglucanase activity of Candida albicans Xog1p, resulting in reduced fungal adhesion to plastic. *Biochem J* 2012;**441**:963–70.

Chang Y et al. Optimization of culturomics strategy in human fecal samples. *Front Microbiol* 2019;**10**:2891.

Chapman SJ, Hill AVS. Human genetic susceptibility to infectious disease. *Nat Rev Genet* 2012;**13**:175–88.

Cheetham J, MacCallum DM, Doris KS *et al.* MAPKKK-independent regulation of the Hog1 stress-activated protein kinase in Candida albicans. *J Biol Chem* 2011;**286**:42002–16.
Chehoud C *et al.* Fungal signature in the gut microbiota of pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2015;**21**:1948–56.
Chen AI *et al.* Candida albicans ethanol stimulates Pseudomonas aeruginosa WspR-controlled biofilm formation as part of a cyclic relationship involving phenazines. *PLoS Pathog* 2014;**10**:e1004480.
Chen C, Pande K, French SD *et al.* An iron homeostasis regulatory circuit with reciprocal roles in Candida albicans commensalism and pathogenesis. *Cell Host Microbe* 2011;**10**:118–35.
Cheng G, Yeater KM, Hoyer LL. Cellular and molecular biology of Candida albicans estrogen response. *Eukaryotic Cell* 2006;**5**:180–91.
Cheng S, Clancy CJ, Zhang Z *et al.* Uncoupling of oxidative phosphorylation enables Candida albicans to resist killing by phagocytes and persist in tissue. *Cell Microbiol* 2007;**9**:492–501.
Cheng S-C, van de Veerdonk F, Smeekens S *et al.* Candida albicans dampens host defense by downregulating IL-17 production. *J Immunol* 2010;**185**:2450–57.
Cheng S-C *et al.* mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* 2014;**345**:1250684.
Cheng S-C *et al.* The dectin-1/inflammasome pathway is responsible for the induction of protective T-helper 17 responses that discriminate between yeasts and hyphae of Candida albicans. *J Leukoc Biol* 2011;**90**:357–66.
Chen H, Zhou X, Ren B *et al.* The regulation of hyphae growth in Candida albicans. *Virulence* 2020;**11**:337–48.
Chibana H, Beckerman JL, Magee PT. Fine-resolution physical mapping of genomic diversity in Candida albicans. *Genome Res* 2000;**10**:1865–77.
Childers DS, Avelar GM, Bain JM *et al.* Epitope shaving promotes fungal immune evasion. *MBio* 2020;**11**.
Childers DS, Avelar GM, Bain JM *et al.* Impact of the Environment upon the Candida albicans Cell Wall and Resultant Effects upon Immune Surveillance. *Curr Top Microbiol Immunol* 2019.
Childers DS *et al.* The rewiring of ubiquitination targets in a pathogenic yeast promotes metabolic flexibility, host colonization and virulence. *PLoS Pathog* 2016;**12**:e1005566.
Chiranand W, McLeod I, Zhou H *et al.* CTA4 transcription factor mediates induction of nitrosative stress response in Candida albicans. *Eukaryotic Cell* 2008;**7**:268–78.
Chowdhary A, Sharma C, Meis JF. Candida auris: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. *PLoS Pathog* 2017;**13**:e1006290.
Citiulo F, Jacobsen ID, Miramón P *et al.* Candida albicans scavenges host zinc via Pra1 during endothelial invasion. *PLoS Pathog* 2012;**8**:e1002777.
Cohen-Kedar S, Baram L, Elad H *et al.* Human intestinal epithelial cells respond to β-glucans via Dectin-1 and Syk. *Eur J Immunol* 2014;**44**:3729–40.
Conti HR, Huppler AR, Whibley N *et al.* Animal models for candidiasis. *Curr Protoc Immunol* 2014;**105**:19.6.1–19.6.17.
Conti HR, Peterson AC *et al.* Oral-resident natural Th17 cells and $\gamma\delta$ T cells control opportunistic Candida albicans infections. *J Exp Med* 2014;**211**:2075–84.
Conti HR *et al.* IL-17 Receptor Signaling in Oral Epithelial Cells Is Critical for Protection against Oropharyngeal Candidiasis. *Cell Host Microbe* 2016;**20**:606–17.
Conti HR *et al.* Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. *J Exp Med* 2009;**206**:299–311.
Coste A, Turner V, Ischer F *et al.* A mutation in Tac1p, a transcription factor regulating CDR1 and CDR2, is coupled with loss of heterozygosity at chromosome 5 to mediate antifungal resistance in Candida albicans. *Genetics* 2006;**172**:2139–56.
Cottier F, Tan ASM, Chen J *et al.* The transcriptional stress response of Candida albicans to weak organic acids. *G3 (Bethesda)* 2015;**5**:497–505.
Cottier F, Tan ASM, Yurieva M *et al.* The Transcriptional Response of Candida albicans to Weak Organic Acids, Carbon Source, and MIG1 Inactivation Unveils a Role for HGT16 in Mediating the Fungistatic Effect of Acetic Acid. *G3 (Bethesda)* 2017;**7**:3597–604.
Cottier F *et al.* Remasking of Candida albicans β-Glucan in Response to Environmental pH Is Regulated by Quorum Sensing. *MBio* 2019;**10**.
Crawford AC *et al.* Biphasic zinc compartmentalisation in a human fungal pathogen. *PLoS Pathog* 2018;**14**:e1007013.
Crielaard W, Zaura E, Schuller AA *et al.* Exploring the oral microbiota of children at various developmental stages of their dentition in the relation to their oral health. *BMC Med Genomics* 2011;**4**:22.
Crowe JD, Sievwright IK, Auld GC *et al.* Candida albicans binds human plasminogen: identification of eight plasminogen-binding proteins. *Mol Microbiol* 2003;**47**:1637–51.
Cruz MR, Graham CE, Gagliano BC *et al.* Enterococcus faecalis inhibits hyphal morphogenesis and virulence of Candida albicans. *Infect Immun* 2013;**81**:189–200.
Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. *Nat Rev Immunol* 2010;**10**:479–89.
Culibrk L, Croft CA, Tebbutt SJ. Systems Biology Approaches for Host-Fungal Interactions: An Expanding Multi-Omics Frontier. *OMICS* 2016;**20**:127–38.
d'Enfert C. Hidden killers: persistence of opportunistic fungal pathogens in the human host. *Curr Opin Microbiol* 2009;**12**:358–64.
Dalle F, Wächtler B, L'Ollivier C *et al.* Cellular interactions of Candida albicans with human oral epithelial cells and enterocytes. *Cell Microbiol* 2010;**12**:248–71.
Dambuza IM, Levitz SM, Netea MG *et al.* Fungal recognition and host defense mechanisms. *Microbiol Spectr* 2017;**5**.
Danhof HA, Vylkova S, Vesely EM *et al.* Robust Extracellular pH Modulation by Candida albicans during Growth in Carboxylic Acids. *MBio* 2016;**7**.
Dantas A da S, Day A, Ikeh M *et al.* Oxidative stress responses in the human fungal pathogen, Candida albicans. *Biomolecules* 2015;**5**:142–65.
Darwazeh AM, al-Bashir A. Oral candidal flora in healthy infants. *J Oral Pathol Med* 1995;**24**:361–4.
Das I, Nightingale P, Patel M *et al.* Epidemiology, clinical characteristics, and outcome of candidemia: experience in a tertiary referral center in the UK. *Int J Infect Dis* 2011;**15**:e759–63.
David LA, Materna AC, Friedman J *et al.* Host lifestyle affects human microbiota on daily timescales. *Genome Biol* 2014;**15**:R89.
David LA, Maurice CF *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;**505**:559–63.
Davidson L, Netea MG, Kullberg BJ. Patient Susceptibility to Candidiasis-A Potential for Adjunctive Immunotherapy. *J Fungi (Basel)* 2018;**4**.

Davis D, Edwards JE, Mitchell AP *et al.* Candida albicans RIM101 pH response pathway is required for host-pathogen interactions. *Infect Immun* 2000;**68**:5953–9.

Davis D, Wilson RB, Mitchell AP. RIM101-dependent and-independent pathways govern pH responses in Candida albicans. *Mol Cell Biol* 2000;**20**:971–8.

De Bernardis F, Graziani S, Tirelli F *et al.* Candida vaginitis: virulence, host response and vaccine prospects. *Med Mycol* 2018;**56**:26–31.

De Filippis F *et al.* High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut* 2016;**65**:1812–21.

de Groot PWJ, Bader O, de Boer AD *et al.* Adhesins in human fungal pathogens: glue with plenty of stick. *Eukaryotic Cell* 2013;**12**:470–81.

de Groot PWJ, de Boer AD, Cunningham J *et al.* Proteomic analysis of Candida albicans cell walls reveals covalently bound carbohydrate-active enzymes and adhesins. *Eukaryotic Cell* 2004;**3**:955–65.

De Groot PWJ, Ram AF, Klis FM. Features and functions of covalently linked proteins in fungal cell walls. *Fungal Genet Biol* 2005;**42**:657–75.

Dehullu J, Valotteau C, Herman-Bausier P *et al.* Fluidic Force Microscopy Demonstrates That Homophilic Adhesion by Candida albicans Als Proteins Is Mediated by Amyloid Bonds between Cells. *Nano Lett* 2019;**19**:3846–53.

Del Fresno C, Soulat D, Roth S *et al.* Interferon-β production via Dectin-1-Syk-IRF5 signaling in dendritic cells is crucial for immunity to C. albicans. *Immunity* 2013;**38**:1176–86.

De Luca A *et al.* IL-22 and IDO1 affect immunity and tolerance to murine and human vaginal candidiasis. *PLoS Pathog* 2013;**9**:e1003486.

de Repentigny L, Aumont F, Bernard K *et al.* Characterization of binding of Candida albicans to small intestinal mucin and its role in adherence to mucosal epithelial cells. *Infect Immun* 2000;**68**:3172–9.

Derrien M, Collado MC, Ben-Amor K *et al.* The Mucin degrader Akkermansia muciniphila is an abundant resident of the human intestinal tract. *Appl Environ Microbiol* 2008;**74**:1646–8.

Derrien M, Vaughan EE, Plugge CM *et al.* Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int J Syst Evol Microbiol* 2004;**54**:1469–76.

Desai JV, Lionakis MS. The role of neutrophils in host defense against invasive fungal infections. *Curr Clin Microbiol Rep* 2018;**5**:181–9.

Dethlefsen L, Huse S, Sogin ML *et al.* The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 2008;**6**:e280.

Deveau A *et al.* Bacterial-fungal interactions: ecology, mechanisms and challenges. *FEMS Microbiol Rev* 2018;**42**:335–52.

de Vries DH, Matzaraki V, Bakker OB *et al.* Integrating GWAS with bulk and single-cell RNA-sequencing reveals a role for LY86 in the anti-Candida host response. *PLoS Pathog* 2020;**16**:e1008408.

Dewhirst FE, Chen T, Izard J *et al.* The human oral microbiome. *J Bacteriol* 2010;**192**:5002–17.

De Zuani M, Paolicelli G, Zelante T *et al.* Mast Cells Respond to Candida albicans Infections and Modulate Macrophages Phagocytosis of the Fungus. *Front Immunol* 2018;**9**:2829.

Diaz PI, Xie Z, Sobue T *et al.* Synergistic interaction between Candida albicans and commensal oral streptococci in a novel in vitro mucosal model. *Infect Immun* 2012;**80**:620–32.

Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev* 2018;**281**:8–27.

Diogo D, Bouchier C, d Enfert C *et al.* Loss of heterozygosity in commensal isolates of the asexual diploid yeast Candida albicans. *Fungal Genet Biol* 2009;**46**:159–68.

Doedt T, Krishnamurthy S, Bockmühl DP *et al.* APSES proteins regulate morphogenesis and metabolism in Candida albicans. *Mol Biol Cell* 2004;**15**:3167–80.

Dollive S *et al.* Fungi of the murine gut: episodic variation and proliferation during antibiotic treatment. *PLoS One* 2013;**8**:e71806.

Dominguez-Bello MG, Costello EK, Contreras M *et al.* Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci USA* 2010;**107**:11971–5.

Domínguez-Andrés J *et al.* The itaconate pathway is a central regulatory node linking innate immune tolerance and trained immunity. *Cell Metab* 2019;**29**:211–20.e5.

Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* 2016;**14**:20–32.

Donders G, Bellen G, Janssens D *et al.* Influence of contraceptive choice on vaginal bacterial and fungal microflora. *Eur J Clin Microbiol Infect Dis* 2017;**36**:43–48.

Donders GGG, Bellen G, Ruban K *et al.* Short- and long-term influence of the levonorgestrel-releasing intrauterine system (Mirena®) on vaginal microbiota and Candida. *J Med Microbiol* 2018;**67**:308–13.

Dongari-Bagtzoglou A, Kashleva H, Villar CC. Bioactive interleukin-1alpha is cytolytically released from Candida albicans-infected oral epithelial cells. *Med Mycol* 2004;**42**:531–41.

Drell T *et al.* Characterization of the vaginal micro- and mycobiome in asymptomatic reproductive-age Estonian women. *PLoS One* 2013;**8**:e54379.

Drewniak A *et al.* Invasive fungal infection and impaired neutrophil killing in human CARD9 deficiency. *Blood* 2013;**121**:2385–92.

Driehuis E *et al.* Oral mucosal organoids as a potential platform for personalized cancer therapy. *Cancer Discov* 2019;**9**:852–71.

Drummond RA, Lionakis MS. Organ-specific mechanisms linking innate and adaptive antifungal immunity. *Semin Cell Dev Biol* 2019;**89**:78–90.

Drummond RA, Saijo S, Iwakura Y *et al.* The role of Syk/CARD9 coupled C-type lectins in antifungal immunity. *Eur J Immunol* 2011;**41**:276–81.

Drummond RA *et al.* CARD9+ microglia promote antifungal immunity via IL-1β- and CXCL1-mediated neutrophil recruitment. *Nat Immunol* 2019;**20**:559–70.

Drummond RA *et al.* CARD9-Dependent Neutrophil Recruitment Protects against Fungal Invasion of the Central Nervous System. *PLoS Pathog* 2015;**11**:e1005293.

Drutz DJ. Lactobacillus prophylaxis for Candida vaginitis. *Ann Intern Med* 1992;**116**:419–20.

Dubourg G *et al.* Culturomics and pyrosequencing evidence of the reduction in gut microbiota diversity in patients with broad-spectrum antibiotics. *Int J Antimicrob Agents* 2014;**44**:117–24.

Dumas A, Bernard L, Poquet Y *et al.* The role of the lung microbiota and the gut-lung axis in respiratory infectious diseases. *Cell Microbiol* 2018;**20**:e12966.

Dupuy AK, David MS, Li L *et al.* Redefining the human oral mycobiome with improved practices in amplicon-based taxonomy: discovery of Malassezia as a prominent commensal. *PLoS One* 2014;**9**:e90899.

Durrant WE, Dong X. Systemic acquired resistance. *Annu Rev Phytopathol* 2004;**42**:185–209.

Dutton LC, Nobbs AH, Jepson K *et al.* O-mannosylation in Candida albicans enables development of interkingdom biofilm communities. *MBio* 2014;**5**:e00911.

Dzidic M, Collado MC, Abrahamsson T *et al.* Oral microbiome development during childhood: an ecological succession influenced by postnatal factors and associated with tooth decay. *ISME J* 2018;**12**:2292–306.

Décanis N, Savignac K, Rouabhia M. Farnesol promotes epithelial cell defense against Candida albicans through Toll-like receptor 2 expression, interleukin-6 and human beta-defensin 2 production. *Cytokine* 2009;**45**:132–40.

Edwards JE *et al.* A Fungal Immunotherapeutic Vaccine (NDV-3A) for Treatment of Recurrent Vulvovaginal Candidiasis-A Phase 2 Randomized, Double-Blind, Placebo-Controlled Trial. *Clin Infect Dis* 2018;**66**:1928–36.

Ene IV, Adya AK, Wehmeier S *et al.* Host carbon sources modulate cell wall architecture, drug resistance and virulence in a fungal pathogen. *Cell Microbiol* 2012;**14**:1319–35.

Ene IV, Bennett RJ. The cryptic sexual strategies of human fungal pathogens. *Nat Rev Microbiol* 2014;**12**:239–51.

Ene IV, Brunke S, Brown AJP *et al.* Metabolism in fungal pathogenesis. *Cold Spring Harb Perspect Med* 2014;**4**:a019695.

Ene IV, Farrer RA, Hirakawa MP *et al.* Global analysis of mutations driving microevolution of a heterozygous diploid fungal pathogen. *Proc Natl Acad Sci USA* 2018;**115**:E8688–97.

Ene IV, Walker LA, Schiavone M *et al.* Cell wall remodeling enzymes modulate fungal cell wall elasticity and osmotic stress resistance. *MBio* 2015;**6**:e00986.

Enjalbert B, Nantel A, Whiteway M. Stress-induced gene expression in Candida albicans: absence of a general stress response. *Mol Biol Cell* 2003;**14**:1460–67.

Enjalbert B, Smith DA, Cornell MJ *et al.* Role of the Hog1 stress-activated protein kinase in the global transcriptional response to stress in the fungal pathogen Candida albicans. *Mol Biol Cell* 2006;**17**:1018–32.

Erickson AR *et al.* Integrated metagenomics/metaproteomics reveals human host-microbiota signatures of Crohn's disease. *PLoS One* 2012;**7**:e49138.

Ermert D, Niemiec MJ, Röhm M *et al.* Candida albicans escapes from mouse neutrophils. *J Leukoc Biol* 2013;**94**:223–36.

Erwig LP, Gow NAR. Interactions of fungal pathogens with phagocytes. *Nat Rev Microbiol* 2016;**14**:163–76.

Eyerich K, Dimartino V, Cavani A. IL-17 and IL-22 in immunity: Driving protection and pathology. *Eur J Immunol* 2017;**47**:607–14.

Eyerich S *et al.* IL-22 and TNF-α represent a key cytokine combination for epidermal integrity during infection with Candida albicans. *Eur J Immunol* 2011;**41**:1894–901.

Fairn GD, Grinstein S. How nascent phagosomes mature to become phagolysosomes. *Trends Immunol* 2012;**33**:397–405.

Faith JJ *et al.* The long-term stability of the human gut microbiota. *Science* 2013;**341**:1237439.

Fakhim H, Vaezi A, Javidnia J *et al.* Candida africana vulvovaginitis: Prevalence and geographical distribution. *J Mycol Med* 2020;100966.

Falagas ME, Betsi GI, Athanasiou S. Probiotics for prevention of recurrent vulvovaginal candidiasis: a review. *J Antimicrob Chemother* 2006;**58**:266–72.

Falcone M *et al.* Assessment of risk factors for candidemia in non-neutropenic patients hospitalized in Internal Medicine wards: A multicenter study. *Eur J Intern Med* 2017;**41**:33–38.

Falsetta ML *et al.* Symbiotic relationship between Streptococcus mutans and Candida albicans synergizes virulence of plaque biofilms in vivo. *Infect Immun* 2014;**82**:1968–81.

Fan D *et al.* Activation of HIF-1α and LL-37 by commensal bacteria inhibits Candida albicans colonization. *Nat Med* 2015;**21**:808–14.

Fan X *et al.* Drinking alcohol is associated with variation in the human oral microbiome in a large study of American adults. *Microbiome* 2018;**6**:59.

Fan Y-C, Li W-G, Zheng M-H *et al.* Invasive fungal infection in patients with systemic lupus erythematosus: experience from a single institute of Northern China. *Gene* 2012;**506**:184–7.

Farmaki E *et al.* Fungal colonization in the neonatal intensive care unit: risk factors, drug susceptibility, and association with invasive fungal infections. *Am J Perinatol* 2007;**24**:127–35.

Farrer RA *et al.* Genome Evolution and Innovation across the Four Major Lineages of Cryptococcus gattii. *MBio* 2015;**6**:e00868–15.

Fazeli A, Bruce C, Anumba DO. Characterization of Toll-like receptors in the female reproductive tract in humans. *Hum Reprod* 2005;**20**:1372–8.

Fehlbaum S, Chassard C, Haug MC *et al.* Design and Investigation of PolyFermS In Vitro Continuous Fermentation Models Inoculated with Immobilized Fecal Microbiota Mimicking the Elderly Colon. *PLoS One* 2015;**10**:e0142793.

Fensterheim BA *et al.* The TLR4 agonist monophosphoryl lipid A drives broad resistance to infection via dynamic reprogramming of macrophage metabolism. *J Immunol* 2018;**200**:3777–89.

Ferwerda B *et al.* Human dectin-1 deficiency and mucocutaneous fungal infections. *N Engl J Med* 2009;**361**:1760–7.

Fettweis JM *et al.* Differences in vaginal microbiome in African American women versus women of European ancestry. *Microbiology (Reading, Engl)* 2014;**160**:2272–82.

Fidel PL, Barousse M, Espinosa T *et al.* An intravaginal live Candida challenge in humans leads to new hypotheses for the immunopathogenesis of vulvovaginal candidiasis. *Infect Immun* 2004;**72**:2939–46.

Fidel PL, Yano J, Esher SK *et al.* Applying the Host-Microbe Damage Response Framework to Candida Pathogenesis: Current and Prospective Strategies to Reduce Damage. *J Fungi (Basel)* 2020;**6**.

Fidel PL. Distinct protective host defenses against oral and vaginal candidiasis. *Med Mycol* 2002;**40**:359–75.

Filippidi A, Galanakis E, Maraki S *et al.* The effect of maternal flora on Candida colonisation in the neonate. *Mycoses* 2014;**57**:43–48.

Finkel JS, Mitchell AP. Genetic control of Candida albicans biofilm development. *Nat Rev Microbiol* 2011;**9**:109–18.

Fischer G, Bradford J. Vulvovaginal candidiasis in postmenopausal women: the role of hormone replacement therapy. *J Low Genit Tract Dis* 2011;**15**:263–7.

Fischer G. Chronic vulvitis in pre-pubertal girls. *Australas J Dermatol* 2010;**51**:118–23.

Flores GE *et al.* Temporal variability is a personalized feature of the human microbiome. *Genome Biol* 2014;**15**:531.

Forche A, Abbey D, Pisithkul T *et al.* Stress alters rates and types of loss of heterozygosity in Candida albicans. *MBio* 2011;**2**.

Forche A, Alby K, Schaefer D *et al.* The parasexual cycle in Candida albicans provides an alternative pathway to meiosis for the formation of recombinant strains. *PLoS Biol* 2008;**6**:e110.

Forche A, Magee PT, Selmecki A *et al.* Evolution in Candida albicans populations during a single passage through a mouse host. *Genetics* 2009;**182**:799–811.

Forche A, May G, Magee PT. Demonstration of loss of heterozygosity by single-nucleotide polymorphism microarray analysis and alterations in strain morphology in Candida albicans strains during infection. *Eukaryotic Cell* 2005;**4**:156–65.

Forche A *et al.* Rapid Phenotypic and Genotypic Diversification After Exposure to the Oral Host Niche in Candida albicans. *Genetics* 2018;**209**:725–41.

Ford CB *et al.* The evolution of drug resistance in clinical isolates of Candida albicans. *Elife* 2014;**4**:e00662.

Fouhy F *et al.* High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. *Antimicrob Agents Chemother* 2012;**56**:5811–20.

Fox EP, Cowley ES, Nobile CJ *et al.* Anaerobic bacteria grow within Candida albicans biofilms and induce biofilm formation in suspension cultures. *Curr Biol* 2014;**24**:2411–6.

Foxman B, Muraglia R, Dietz J-P *et al.* Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: results from an internet panel survey. *J Low Genit Tract Dis* 2013;**17**:340–45.

Fradin C, De Groot P, MacCallum D *et al.* Granulocytes govern the transcriptional response, morphology and proliferation of Candida albicans in human blood. *Mol Microbiol* 2005;**56**:397–415.

Franzosa EA *et al.* Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat Microbiol* 2019;**4**:293–305.

Franzosa EA *et al.* Species-level functional profiling of metagenomes and metatranscriptomes. *Nat Methods* 2018;**15**:962–8.

Freitas AC, Chaban B, Bocking A *et al.* The vaginal microbiome of pregnant women is less rich and diverse, with lower prevalence of Mollicutes, compared to non-pregnant women. *Sci Rep* 2017;**7**:9212.

Gabaldón T, Fairhead C. Genomes shed light on the secret life of Candida glabrata: not so asexual, not so commensal. *Curr Genet* 2019;**65**:93–98.

Gajer P *et al.* Temporal dynamics of the human vaginal microbiota. *Sci Transl Med* 2012;**4**: 132ra52.

Galley JD, Nelson MC, Yu Z *et al.* Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. *BMC Microbiol* 2014;**14**:189.

Gantner BN, Simmons RM, Underhill DM. Dectin-1 mediates macrophage recognition of Candida albicans yeast but not filaments. *EMBO J* 2005;**24**:1277–86.

Gatti E, Popolo L, Vai M *et al.* O-linked oligosaccharides in yeast glycosyl phosphatidylinositol-anchored protein gp115 are clustered in a serine-rich region not essential for its function. *J Biol Chem* 1994;**269**:19695–700.

Ghannoum MA, Jurevic RJ, Mukherjee PK *et al.* Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. *PLoS Pathog* 2010;**6**:e1000713.

Gibson GR, Cummings JH, Macfarlane GT. Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. *Appl Environ Microbiol* 1988;**54**:2750–55.

Giraldo PC, de Carvalho JBJ, do Amaral RLG *et al.* Identification of immune cells by flow cytometry in vaginal lavages from women with vulvovaginitis and normal microflora. *Am J Reprod Immunol* 2012;**67**:198–205.

Gladiator A, Wangler N, Trautwein-Weidner K *et al.* Cutting edge: IL-17-secreting innate lymphoid cells are essential for host defense against fungal infection. *J Immunol* 2013;**190**:521–5.

Gliniewicz K, Schneider GM, Ridenhour BJ *et al.* Comparison of the vaginal microbiomes of premenopausal and postmenopausal women. *Front Microbiol* 2019;**10**:193.

Gonçalves B, Ferreira C, Alves CT *et al.* Vulvovaginal candidiasis: Epidemiology, microbiology and risk factors. *Crit Rev Microbiol* 2016;**42**:905–27.

Goodrich JK *et al.* Human genetics shape the gut microbiome. *Cell* 2014;**159**:789–99.

Goodridge HS, Underhill DM, Touret N. Mechanisms of Fc receptor and dectin-1 activation for phagocytosis. *Traffic* 2012;**13**:1062–71.

Goplerud CP, Ohm MJ, Galask RP. Aerobic and anaerobic flora of the cervix during pregnancy and the puerperium. *Am J Obstet Gynecol* 1976;**126**:858–68.

Gouba N, Drancourt M. Digestive tract mycobiota: a source of infection. *Med Mal Infect* 2015;**45**:9–16.

Gow NAR, Brown AJP, Odds FC. Fungal morphogenesis and host invasion. *Curr Opin Microbiol* 2002;**5**:366–71.

Gow NAR, Knox Y, Munro CA *et al.* Infection of chick chorioallantoic membrane (CAM) as a model for invasive hyphal growth and pathogenesis of Candida albicans. *Med Mycol* 2003;**41**:331–8.

Gow NAR, Latge J-P, Munro CA. The fungal cell wall: structure, biosynthesis, and function. *Microbiol Spectr* 2017;**5**.

Gow NAR, van de Veerdonk FL, Brown AJP *et al.* Candida albicans morphogenesis and host defence: discriminating invasion from colonization. *Nat Rev Microbiol* 2011;**10**:112–22.

Goyal S, Castrillón-Betancur JC, Klaile E *et al.* The Interaction of Human Pathogenic Fungi With C-Type Lectin Receptors. *Front Immunol* 2018;**9**:1261.

Graf K *et al.* Keeping Candida commensal: how lactobacilli antagonize pathogenicity of Candida albicans in an in vitro gut model. *Dis Model Mech* 2019;**12**.

Graham CE, Cruz MR, Garsin DA *et al.* Enterococcus faecalis bacteriocin EntV inhibits hyphal morphogenesis, biofilm formation, and virulence of Candida albicans. *Proc Natl Acad Sci USA* 2017;**114**:4507–12.

Granger BL. Insight into the antiadhesive effect of yeast wall protein 1 of Candida albicans. *Eukaryotic Cell* 2012;**11**:795–805.

Greenberg SS, Zhao X, Hua L *et al.* Ethanol inhibits lung clearance of Pseudomonas aeruginosa by a neutrophil and nitric oxide-dependent mechanism, in vivo. *Alcohol Clin Exp Res* 1999;**23**:735–44.

Grenham S, Clarke G, Cryan JF *et al.* Brain-gut-microbe communication in health and disease. *Front Physiol* 2011;**2**:94.

Grondman I *et al.* Frontline Science: Endotoxin-induced immunotolerance is associated with loss of monocyte metabolic plasticity and reduction of oxidative burst. *J Leukoc Biol* 2019;**106**:11–25.

Gropp K, Schild L, Schindler S *et al.* The yeast Candida albicans evades human complement attack by secretion of aspartic proteases. *Mol Immunol* 2009;**47**:465–75.

Guerra A, Etienne-Mesmin L, Livrelli V *et al.* Relevance and challenges in modeling human gastric and small intestinal digestion. *Trends Biotechnol* 2012;**30**:591–600.

Guinan J, Wang S, Hazbun TR *et al.* Antibiotic-induced decreases in the levels of microbial-derived short-chain fatty acids correlate with increased gastrointestinal colonization of Candida albicans. *Sci Rep* 2019;**9**:8872.

Guo R, Zheng N, Lu H *et al.* Increased diversity of fungal flora in the vagina of patients with recurrent vaginal candidiasis and allergic rhinitis. *Microb Ecol* 2012;**64**:918–27.

Gupta S, Kakkar V, Bhushan I. Crosstalk between Vaginal Microbiome and Female Health: A review. *Microb Pathog* 2019;**136**:103696.

Gutierrez D *et al.* Antibiotic-induced gut metabolome and microbiome alterations increase the susceptibility to Candida albicans colonization in the gastrointestinal tract. *FEMS Microbiol Ecol* 2020;**96**.

Hager CL, Isham N, Schrom KP *et al.* Effects of a Novel Probiotic Combination on Pathogenic Bacterial-Fungal Polymicrobial Biofilms. *MBio* 2019;**10**.

Hall MW, Singh N, Ng KF *et al.* Inter-personal diversity and temporal dynamics of dental, tongue, and salivary microbiota in the healthy oral cavity. *npj Biofilms and Microbiomes* 2017;**3**:2.

Hammerschlag MR, Alpert S, Onderdonk AB *et al.* Anaerobic microflora of the vagina in children. *Am J Obstet Gynecol* 1978;**131**:853–6.

Handelsman J, Rondon MR, Brady SF *et al.* Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chem Biol* 1998;**5**:R245–9.

Hardison SE, Brown GD. C-type lectin receptors orchestrate antifungal immunity. *Nat Immunol* 2012;**13**:817–22.

Harriott MM, Noverr MC. Ability of Candida albicans mutants to induce Staphylococcus aureus vancomycin resistance during polymicrobial biofilm formation. *Antimicrob Agents Chemother* 2010;**54**:3746–55.

Harriott MM, Noverr MC. Importance of Candida-bacterial polymicrobial biofilms in disease. *Trends Microbiol* 2011;**19**:557–63.

He J, Kim D, Zhou X *et al.* RNA-Seq Reveals Enhanced Sugar Metabolism in Streptococcus mutans Co-cultured with Candida albicans within Mixed-Species Biofilms. *Front Microbiol* 2017;**8**:1036.

Hernández-Santos N, Huppler AR, Peterson AC *et al.* Th17 cells confer long-term adaptive immunity to oral mucosal Candida albicans infections. *Mucosal Immunol* 2013;**6**:900–10.

Hernández-Santos N, Wiesner DL, Fites JS *et al.* Lung Epithelial Cells Coordinate Innate Lymphocytes and Immunity against Pulmonary Fungal Infection. *Cell Host Microbe* 2018;**23**:511–22.e5.

Herrero-de-Dios C, Day AM, Tillmann AT *et al.* Redox Regulation, Rather than Stress-Induced Phosphorylation, of a Hog1 Mitogen-Activated Protein Kinase Modulates Its Nitrosative-Stress-Specific Outputs. *MBio* 2018;**9**.

He Y *et al.* Regional variation limits applications of healthy gut microbiome reference ranges and disease models. *Nat Med* 2018;**24**:1532–5.

Hibino K, Samaranayake LP, Hägg U *et al.* The role of salivary factors in persistent oral carriage of Candida in humans. *Arch Oral Biol* 2009;**54**:678–83.

Hickman MA, Paulson C, Dudley A *et al.* Parasexual Ploidy Reduction Drives Population Heterogeneity Through Random and Transient Aneuploidy in Candida albicans. *Genetics* 2015;**200**:781–94.

Hickman MA *et al.* The "obligate diploid" Candida albicans forms mating-competent haploids. *Nature* 2013;**494**:55–59.

Hill C *et al.* Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014;**11**:506–14.

Hill CJ *et al.* Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome* 2017;**5**:4.

Hillier SL, Lau RJ. Vaginal microflora in postmenopausal women who have not received estrogen replacement therapy. *Clin Infect Dis* 1997;**25 Suppl 2**:S123–6.

Hillman ET, Lu H, Yao T *et al.* Microbial Ecology along the Gastrointestinal Tract. *Microbes Environ* 2017;**32**:300–13.

Hillmann F, Novohradská S, Mattern DJ *et al.* Virulence determinants of the human pathogenic fungus Aspergillus fumigatus protect against soil amoeba predation. *Environ Microbiol* 2015;**17**:2858–69.

Hirakawa MP, Chyou DE, Huang D *et al.* Parasex Generates Phenotypic Diversity de Novo and Impacts Drug Resistance and Virulence in Candida albicans. *Genetics* 2017;**207**:1195–211.

Hirakawa MP *et al.* Genetic and phenotypic intra-species variation in Candida albicans. *Genome Res* 2015;**25**:413–25.

Hoffmann C, Dollive S, Grunberg S *et al.* Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. *PLoS One* 2013;**8**:e66019.

Hogan DA, Vik A, Kolter R. A Pseudomonas aeruginosa quorum-sensing molecule influences Candida albicans morphology. *Mol Microbiol* 2004;**54**:1212–23.

Ho Jemima *et al.* Candidalysin activates innate epithelial immune responses via epidermal growth factor receptor. *Nat Commun* 2019;**10**:2297.

Holmes AR, McNab R, Jenkinson HF. Candida albicans binding to the oral bacterium Streptococcus gordonii involves multiple adhesin-receptor interactions. *Infect Immun* 1996;**64**:4680–5.

Horn F, Heinekamp T, Kniemeyer O *et al.* Systems biology of fungal infection. *Front Microbiol* 2012;**3**:108.

Hovav A-H. Mucosal and Skin Langerhans Cells - Nurture Calls. *Trends Immunol* 2018;**39**:788–800.

Ho Vida, Herman-Bausier P, Shaw C *et al.* An Amyloid Core Sequence in the Major Candida albicans Adhesin Als1p Mediates Cell-Cell Adhesion. *MBio* 2019;**10**.

Hoyer LL, Cota E. Candida albicans Agglutinin-Like Sequence (Als) Family Vignettes: A Review of Als Protein Structure and Function. *Front Microbiol* 2016;**7**:280.

Hromatka BS, Noble SM, Johnson AD. Transcriptional response of Candida albicans to nitric oxide and the role of the YHB1 gene in nitrosative stress and virulence. *Mol Biol Cell* 2005;**16**:4814–26.

Huang G, Srikantha T, Sahni N *et al.* CO(2) regulates white-to-opaque switching in Candida albicans. *Curr Biol* 2009;**19**:330–4.

Huang MY, Woolford CA, May G *et al.* Circuit diversification in a biofilm regulatory network. *PLoS Pathog* 2019;**15**:e1007787.

Hube B. Virulence profile: Bernhard Hube. *Virulence* 2015;**6**:523–5.

Huertas B, Prieto D, Pitarch A *et al.* Serum Antibody Profile during Colonization of the Mouse Gut by Candida albicans: Relevance for Protection during Systemic Infection. *J Proteome Res* 2017;**16**:335–45.

Hugon P, Dufour J-C, Colson P *et al.* A comprehensive repertoire of prokaryotic species identified in human beings. *Lancet Infect Dis* 2015;**15**:1211–19.

Hu H, Merenstein DJ, Wang C *et al.* Impact of eating probiotic yogurt on colonization by Candida species of the oral and vaginal mucosa in HIV-infected and HIV-uninfected women. *Mycopathologia* 2013;**176**:175–81.

Hull CM, Raisner RM, Johnson AD. Evidence for mating of the "asexual" yeast Candida albicans in a mammalian host. *Science* 2000;**289**:307–10.

Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* 2012;**486**:207–14.

Hurabielle C et al. Immunity to commensal skin fungi promotes psoriasiform skin inflammation. Proc Natl Acad Sci USA 2020;117:16465–74.
Huse M. Mechanical forces in the immune system. Nat Rev Immunol 2017;17:679–90.
Huseyin CE, Rubio RC, O'Sullivan O et al. The fungal frontier: A comparative analysis of methods used in the study of the human gut mycobiome. Front Microbiol 2017;8:1432.
Ifrim DC, Quintin J, Meerstein-Kessel L et al. Defective trained immunity in patients with STAT-1-dependent chronic mucocutaneaous candidiasis. Clin Exp Immunol 2015;181:434–40.
Igyártó BZ et al. Skin-resident murine dendritic cell subsets promote distinct and opposing antigen-specific T helper cell responses. Immunity 2011;35:260–72.
Ikeh MAC et al. Pho4 mediates phosphate acquisition in Candida albicans and is vital for stress resistance and metal homeostasis. Mol Biol Cell 2016;27:2784–801.
Indrio F, Riezzo G, Raimondi F et al. The effects of probiotics on feeding tolerance, bowel habits, and gastrointestinal motility in preterm newborns. J Pediatr 2008;152:801–6.
Ito S et al. Specific strains of Streptococcus mutans, a pathogen of dental caries, in the tonsils, are associated with IgA nephropathy. Sci Rep 2019;9:20130.
Jabra-Rizk MA, Kong EF, Tsui C et al. Candida albicans Pathogenesis: Fitting within the Host-Microbe Damage Response Framework. Infect Immun 2016;84:2724–39.
Jackson MA et al. Gut microbiota associations with common diseases and prescription medications in a population-based cohort. Nat Commun 2018;9:2655.
Jacob L, John M, Kalder M et al. Prevalence of vulvovaginal candidiasis in gynecological practices in Germany: A retrospective study of 954,186 patients. Curr Med Mycol 2018;4:6–11.
Jacobs DM, Beyda ND, Asuphon O et al. Host factors and clinical outcomes of Candida colonization in critically ill patients. Mycopathologia 2015;179:87–93.
Jacobsen ID, Grosse K, Berndt A et al. Pathogenesis of Candida albicans infections in the alternative chorio-allantoic membrane chicken embryo model resembles systemic murine infections. PLoS One 2011;6:e19741.
Jacobsen ID, Wilson D, Wächtler B et al. Candida albicans dimorphism as a therapeutic target. Expert Rev Anti Infect Ther 2012;10:85–93.
Jaeger M, Stappers MHT, Joosten LAB et al. Genetic variation in pattern recognition receptors: functional consequences and susceptibility to infectious disease. Future Microbiol 2015;10:989–1008.
Jaeger M, van der Lee R et al. The RIG-I-like helicase receptor MDA5 (IFIH1) is involved in the host defense against Candida infections. Eur J Clin Microbiol Infect Dis 2015;34:963–74.
Jaeger Martin et al. A Genome-Wide Functional Genomics Approach Identifies Susceptibility Pathways to Fungal Bloodstream Infection in Humans. J Infect Dis 2019;220:862–72.
Jaeger M et al. Association of a variable number tandem repeat in the NLRP3 gene in women with susceptibility to RVVC. Eur J Clin Microbiol Infect Dis 2016;35:797–801.
Jaeger M et al. A systems genomics approach identifies SIGLEC15 as a susceptibility factor in recurrent vulvovaginal candidiasis. Sci Transl Med 2019;11.
Jain C, Pastor K, Gonzalez AY et al. The role of Candida albicans AP-1 protein against host derived ROS in in vivo models of infection. Virulence 2013;4:67–76.
Jalili-Firoozinezhad S et al. A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip. Nat Biomed Eng 2019;3:520–31.
James KM, MacDonald KW, Chanyi RM et al. Inhibition of Candida albicans biofilm formation and modulation of gene expression by probiotic cells and supernatant. J Med Microbiol 2016;65:328–36.
Jamieson DJ, Stephen DW, Terrière EC. Analysis of the adaptive oxidative stress response of Candida albicans. FEMS Microbiol Lett 1996;138:83–88.
Jang SJ, Lee K, Kwon B et al. Vaginal lactobacilli inhibit growth and hyphae formation of Candida albicans. Sci Rep 2019;9:8121.
Jansons VK, Nickerson WJ. Induction, morphogenesis, and germination of the chlamydospore of Candida albicans. J Bacteriol 1970;104:910–21.
Jarosz LM, Deng DM, van der Mei HC et al. Streptococcus mutans competence-stimulating peptide inhibits Candida albicans hypha formation. Eukaryotic Cell 2009;8:1658–64.
Jeziorek M, Frej-Mądrzak M, Choroszy-Król I. The influence of diet on gastrointestinal Candida spp. colonization and the susceptibility of Candida spp. to antifungal drugs. Rocz Panstw Zakl Hig 2019;70:195–200.
Jin Y, Wu S, Zeng Z et al. Effects of environmental pollutants on gut microbiota. Environ Pollut 2017;222:1–9.
Johansson MEV, Gustafsson JK, Sjöberg KE et al. Bacteria penetrate the inner mucus layer before inflammation in the dextran sulfate colitis model. PLoS One 2010;5:e12238.
Johansson MEV, Phillipson M, Petersson J et al. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci USA 2008;105:15064–9.
Joly S, Ma N, Sadler JJ et al. Cutting edge: Candida albicans hyphae formation triggers activation of the Nlrp3 inflammasome. J Immunol 2009;183:3578–81.
Jones T et al. The diploid genome sequence of Candida albicans. Proc Natl Acad Sci USA 2004;101:7329–34.
Jouault T, El Abed-El Behi M, Martínez-Esparza M et al. Specific recognition of Candida albicans by macrophages requires galectin-3 to discriminate Saccharomyces cerevisiae and needs association with TLR2 for signaling. J Immunol 2006;177:4679–87.
Jouault T, Ibata-Ombetta S, Takeuchi O et al. Candida albicans phospholipomannan is sensed through toll-like receptors. J Infect Dis 2003;188:165–72.
Kadir T, Uygun B, Akyüz S. Prevalence of Candida species in Turkish children: relationship between dietary intake and carriage. Arch Oral Biol 2005;50:33–37.
Kadosh D. Regulatory mechanisms controlling morphology and pathogenesis in Candida albicans. Curr Opin Microbiol 2019;52:27–34.
Kalia N, Singh J, Kaur M. Microbiota in vaginal health and pathogenesis of recurrent vulvovaginal infections: a critical review. Ann Clin Microbiol Antimicrob 2020;19:5.
Kalo-Klein A, Witkin SS. Prostaglandin E2 enhances and gamma interferon inhibits germ tube formation in Candida albicans. Infect Immun 1990;58:260–2.
Kaloriti D et al. Mechanisms underlying the exquisite sensitivity of Candida albicans to combinatorial cationic and oxidative stress that enhances the potent fungicidal activity of phagocytes. MBio 2014;5:e01334–14.
Kankkunen P, Teirilä L, Rintahaka J et al. (1,3)-beta-glucans activate both dectin-1 and NLRP3 inflammasome in human macrophages. J Immunol 2010;184:6335–42.
Kapteyn JC, Hoyer LL, Hecht JE et al. The cell wall architecture of Candida albicans wild-type cells and cell wall-defective mutants. Mol Microbiol 2000;35:601–11.

Karl JP, Hatch AM, Arcidiacono SM *et al.* Effects of psychological, environmental and physical stressors on the gut microbiota. *Front Microbiol* 2018;**9**:2013.

Kashem SW, Igyarto BZ *et al.* Candida albicans morphology and dendritic cell subsets determine T helper cell differentiation. *Immunity* 2015;**42**:356–66.

Kashem SW, Riedl MS, Yao C *et al.* Nociceptive Sensory Fibers Drive Interleukin-23 Production from CD301b+ Dermal Dendritic Cells and Drive Protective Cutaneous Immunity. *Immunity* 2015;**43**:515–26.

Kasperkovitz PV, Khan NS, Tam JM *et al.* Toll-like receptor 9 modulates macrophage antifungal effector function during innate recognition of Candida albicans and Saccharomyces cerevisiae. *Infect Immun* 2011;**79**:4858–67.

Kasper L, Seider K, Hube B. Intracellular survival of Candida glabrata in macrophages: immune evasion and persistence. *FEMS Yeast Res* 2015;**15**: fov042.

Kasper L *et al.* The fungal peptide toxin Candidalysin activates the NLRP3 inflammasome and causes cytolysis in mononuclear phagocytes. *Nat Commun* 2018;**9**:4260.

Kauffman CA. Fungal infections in older adults. *Clin Infect Dis* 2001;**33**:550–5.

Kavanaugh NL, Zhang AQ, Nobile CJ *et al.* Mucins suppress virulence traits of Candida albicans. *MBio* 2014;**5**:e01911.

Kawasaki T, Kawai T. Toll-like receptor signaling pathways. *Front Immunol* 2014;**5**:461.

Kayingo G, Wong B. The MAP kinase Hog1p differentially regulates stress-induced production and accumulation of glycerol and D-arabitol in Candida albicans. *Microbiology (Reading, Engl)* 2005;**151**:2987–99.

Kean R *et al.* Candida albicans Mycofilms Support Staphylococcus aureus Colonization and Enhances Miconazole Resistance in Dual-Species Interactions. *Front Microbiol* 2017;**8**:258.

Kehrmann J *et al.* The lung microbiome in patients with pneumocystosis. *BMC Pulm Med* 2017;**17**:170.

Keijser BJF, Zaura E, Huse SM *et al.* Pyrosequencing analysis of the oral microflora of healthy adults. *J Dent Res* 2008;**87**: 1016–20.

Kenno S, Perito S, Mosci P *et al.* Autophagy and Reactive Oxygen Species Are Involved in Neutrophil Extracellular Traps Release Induced by C. albicans Morphotypes. *Front Microbiol* 2016;**7**:879.

Kernien JF, Snarr BD, Sheppard DC *et al.* The Interface between Fungal Biofilms and Innate Immunity. *Front Immunol* 2017;**8**:1968.

Kidane YH, Lawrence C, Murali TM. Computational approaches for discovery of common immunomodulators in fungal infections: towards broad-spectrum immunotherapeutic interventions. *BMC Microbiol* 2013;**13**:224.

Kim D *et al.* Candida albicans stimulates Streptococcus mutans microcolony development via cross-kingdom biofilm-derived metabolites. *Sci Rep* 2017;**7**:41332.

Kim Hyung Sook *et al.* Curdlan activates dendritic cells through dectin-1 and toll-like receptor 4 signaling. *Int Immunopharmacol* 2016;**39**:71–78.

Kim Hyun Jung, Li H, Collins JJ *et al.* Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *Proc Natl Acad Sci USA* 2016;**113**:E7–15.

Kim Y, Mylonakis E. Killing of Candida albicans filaments by Salmonella enterica serovar Typhimurium is mediated by sopB effectors, parts of a type III secretion system. *Eukaryotic Cell* 2011;**10**:782–90.

Kioshima ES, Shinobu-Mesquita CS, Abadio AKR *et al.* Selection of potential anti-adhesion drugs by in silico approaches targeted to ALS3 from Candida albicans. *Biotechnol Lett* 2019;**41**:1391–401.

Kirchner FR, LeibundGut-Landmann S. Tissue-resident memory Th17 cells maintain stable fungal commensalism in the oral mucosa. *Mucosal Immunol* 2020.

Kirchner FR *et al.* Persistence of Candida albicans in the Oral Mucosa Induces a Curbed Inflammatory Host Response That Is Independent of Immunosuppression. *Front Immunol* 2019;**10**:330.

Klaassens ES, Boesten RJ, Haarman M *et al.* Mixed-species genomic microarray analysis of fecal samples reveals differential transcriptional responses of bifidobacteria in breast- and formula-fed infants. *Appl Environ Microbiol* 2009;**75**: 2668–76.

Klis FM, de Groot P, Hellingwerf K. Molecular organization of the cell wall of Candida albicans. *Med Mycol* 2001;**39 Suppl** 1:1–8.

Klotz SA, Chasin BS, Powell B *et al.* Polymicrobial bloodstream infections involving Candida species: analysis of patients and review of the literature. *Diagn Microbiol Infect Dis* 2007;**59**:401–6.

Koh AY, Köhler JR, Coggshall KT *et al.* Mucosal damage and neutropenia are required for Candida albicans dissemination. *PLoS Pathog* 2008;**4**:e35.

Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol* 2013;**13**: 159–75.

Kong EF, Tsui C, Kucharíková S *et al.* Commensal Protection of Staphylococcus aureus against Antimicrobials by Candida albicans Biofilm Matrix. *MBio* 2016;**7**.

Koo H, Andes DR, Krysan DJ. Candida-streptococcal interactions in biofilm-associated oral diseases. *PLoS Pathog* 2018;**14**:e1007342.

Kornitzer D. Regulation of Candida albicans Hyphal Morphogenesis by Endogenous Signals. *J Fungi (Basel)* 2019;**5**.

Korn T, Bettelli E, Oukka M *et al.* IL-17 and Th17 Cells. *Annu Rev Immunol* 2009;**27**:485–517.

Korpela K *et al.* Intestinal microbiota development and gestational age in preterm neonates. *Sci Rep* 2018;**8**:2453.

Kos I, Patterson MJ, Znaidi S *et al.* Mechanisms Underlying the Delayed Activation of the Cap1 Transcription Factor in Candida albicans following Combinatorial Oxidative and Cationic Stress Important for Phagocytic Potency. *MBio* 2016;**7**:e00331.

Kovachev SM, Vatcheva-Dobrevska RS. Local Probiotic Therapy for Vaginal Candida albicans Infections. *Probiotics Antimicrob Proteins* 2015;**7**:38–44.

Kraneveld EA, Buijs MJ, Bonder MJ *et al.* The relation between oral Candida load and bacterial microbiome profiles in Dutch older adults. *PLoS One* 2012;**7**:e42770.

Krishnakumari V, Rangaraj N, Nagaraj R. Antifungal activities of human beta-defensins HBD-1 to HBD-3 and their C-terminal analogs Phd1 to Phd3. *Antimicrob Agents Chemother* 2009;**53**:256–60.

Kumamoto CA. Inflammation and gastrointestinal Candida colonization. *Curr Opin Microbiol* 2011;**14**:386–91.

Kumamoto CA. Molecular mechanisms of mechanosensing and their roles in fungal contact sensing. *Nat Rev Microbiol* 2008;**6**:667–73.

Kumar PS, Mason MR, Brooker MR *et al.* Pyrosequencing reveals unique microbial signatures associated with healthy and failing dental implants. *J Clin Periodontol* 2012;**39**:425–33.

Kumar V et al. Immunochip SNP array identifies novel genetic variants conferring susceptibility to candidaemia. *Nat Commun* 2014;**5**:4675.

Kurt-Jones EA, Mandell L, Whitney C et al. Role of toll-like receptor 2 (TLR2) in neutrophil activation: GM-CSF enhances TLR2 expression and TLR2-mediated interleukin 8 responses in neutrophils. *Blood* 2002;**100**:1860–68.

Kurtz J, Franz K. Innate defence: evidence for memory in invertebrate immunity. *Nature* 2003;**425**:37–38.

Kuznets G et al. A relay network of extracellular heme-binding proteins drives C. albicans iron acquisition from hemoglobin. *PLoS Pathog* 2014;**10**:e1004407.

Köhler GA, Assefa S, Reid G. Probiotic interference of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 with the opportunistic fungal pathogen Candida albicans. *Infect Dis Obstet Gynecol* 2012;**2012**:636474.

Köhler JR, Casadevall A, Perfect J. The spectrum of fungi that infects humans. *Cold Spring Harb Perspect Med* 2014;**5**:a019273.

Lagier J-C, Hugon P, Khelaifia S et al. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. *Clin Microbiol Rev* 2015;**28**: 237–64.

Lagier J-C et al. Culture of previously uncultured members of the human gut microbiota by culturomics. *Nat Microbiol* 2016;**1**:16203.

Lagier JC et al. Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect* 2012;**18**:1185–93.

Lambrecht E, Van Coillie E, Van Meervenne E et al. Commensal E. coli rapidly transfer antibiotic resistance genes to human intestinal microbiota in the Mucosal Simulator of the Human Intestinal Microbial Ecosystem (M-SHIME). *Int J Food Microbiol* 2019;**311**:108357.

Lang S et al. Intestinal fungal dysbiosis and systemic immune response to fungi in patients with alcoholic hepatitis. *Hepatology* 2020;**71**:522–38.

Lankelma JM, Belzer C, Hoogendijk AJ et al. Antibiotic-Induced Gut Microbiota Disruption Decreases TNF-α Release by Mononuclear Cells in Healthy Adults. *Clin Transl Gastroenterol* 2016;**7**:e186.

Lanternier F, Cypowyj S, Picard C et al. Primary immunodeficiencies underlying fungal infections. *Curr Opin Pediatr* 2013;**25**:736–47.

Lanternier F, Pathan S et al. Deep dermatophytosis and inherited CARD9 deficiency. *N Engl J Med* 2013;**369**:1704–14.

Lawley TD, Walker AW. Intestinal colonization resistance. *Immunology* 2013;**138**:1–11.

Leach MD, Budge S, Walker L et al. Hsp90 orchestrates transcriptional regulation by Hsf1 and cell wall remodelling by MAPK signalling during thermal adaptation in a pathogenic yeast. *PLoS Pathog* 2012;**8**:e1003069.

Leach MD, Tyc KM, Brown AJP et al. Modelling the regulation of thermal adaptation in Candida albicans, a major fungal pathogen of humans. *PLoS One* 2012;**7**:e32467.

Leach MD et al. Hsf1 and Hsp90 orchestrate temperature-dependent global transcriptional remodelling and chromatin architecture in Candida albicans. *Nat Commun* 2016; **7**:11704.

LeBlanc DM, Barousse MM, Fidel PL. Role for dendritic cells in immunoregulation during experimental vaginal candidiasis. *Infect Immun* 2006;**74**:3213–21.

Lee EKS et al. Leukotriene B4-Mediated Neutrophil Recruitment Causes Pulmonary Capillaritis during Lethal Fungal Sepsis. *Cell Host Microbe* 2018;**23**:121–33.e4.

Lee SA, Lim JY, Kim B-S et al. Comparison of the gut microbiota profile in breast-fed and formula-fed Korean infants using pyrosequencing. *Nutr Res Pract* 2015;**9**:242–8.

LeibundGut-Landmann S et al. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol* 2007;**8**:630–8.

Lemoinne S et al. Fungi participate in the dysbiosis of gut microbiota in patients with primary sclerosing cholangitis. *Gut* 2020;**69**:92–102.

Leonardi I et al. CX3CR1+ mononuclear phagocytes control immunity to intestinal fungi. *Science* 2018;**359**:232–6.

Leonardi I et al. Fungal Trans-kingdom Dynamics Linked to Responsiveness to Fecal Microbiota Transplantation (FMT) Therapy in Ulcerative Colitis. *Cell Host Microbe* 2020;**27**:823–829.e3.

Leonhardt J et al. Candida albicans β-Glucan Differentiates Human Monocytes Into a Specific Subset of Macrophages. *Front Immunol* 2018;**9**:2818.

Lewis LE, Bain JM, Lowes C et al. Stage specific assessment of Candida albicans phagocytosis by macrophages identifies cell wall composition and morphogenesis as key determinants. *PLoS Pathog* 2012;**8**:e1002578.

Liang S-H, Anderson MZ, Hirakawa MP et al. Hemizygosity enables a mutational transition governing fungal virulence and commensalism. *Cell Host Microbe* 2019;**25**:418–431.e6.

Liang S-H, Bennett RJ. The Impact of Gene Dosage and Heterozygosity on The Diploid Pathobiont Candida albicans. *J Fungi (Basel)* 2019;**6**.

Liang SC, Tan X-Y, Luxenberg DP et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006;**203**:2271–9.

Liang SC et al. An IL-17F/A heterodimer protein is produced by mouse Th17 cells and induces airway neutrophil recruitment. *J Immunol* 2007;**179**:7791–9.

Lif Holgerson P, Harnevik L, Hernell O et al. Mode of birth delivery affects oral microbiota in infants. *J Dent Res* 2011;**90**:1183–8.

Li Hai et al. The outer mucus layer hosts a distinct intestinal microbial niche. *Nat Commun* 2015;**6**:8292.

Li J, Vinh DC, Casanova J-L et al. Inborn errors of immunity underlying fungal diseases in otherwise healthy individuals. *Curr Opin Microbiol* 2017;**40**:46–57.

Lim CSY, Rosli R, Seow HF et al. Candida and invasive candidiasis: back to basics. *Eur J Clin Microbiol Infect Dis* 2012;**31**:21–31.

Limon JJ et al. Malassezia Is Associated with Crohn's Disease and Exacerbates Colitis in Mouse Models. *Cell Host Microbe* 2019;**25**:377–388.e6.

Lindsay AK, Morales DK, Liu Z et al. Analysis of Candida albicans mutants defective in the Cdk8 module of mediator reveal links between metabolism and biofilm formation. *PLoS Genet* 2014;**10**:e1004567.

Ling Y et al. Inherited IL-17RC deficiency in patients with chronic mucocutaneous candidiasis. *J Exp Med* 2015;**212**:619–31.

Lin J, Oh S-H, Jones R et al. The peptide-binding cavity is essential for Als3-mediated adhesion of Candida albicans to human cells. *J Biol Chem* 2014;**289**:18401–12.

Lionakis MS, Holland SM. CARD9: at the intersection of mucosal and systemic antifungal immunity. *Blood* 2013;**121**:2377–8.

Lionakis MS, Levitz SM. Host Control of Fungal Infections: Lessons from Basic Studies and Human Cohorts. *Annu Rev Immunol* 2018;**36**:157–91.

Lionakis MS, Lim JK, Lee C-CR et al. Organ-specific innate immune responses in a mouse model of invasive candidiasis. *J Innate Immun* 2011;**3**:180–99.

Lionakis MS. Genetic susceptibility to fungal infections in humans. *Curr Fungal Infect Rep* 2012;**6**:11–22.
Lionakis MS. New insights into innate immune control of systemic candidiasis. *Med Mycol* 2014;**52**:555–64.
Lionakis MS et al. CX3CR1-dependent renal macrophage survival promotes Candida control and host survival. *J Clin Invest* 2013;**123**:5035–51.
Liu B et al. Deep sequencing of the oral microbiome reveals signatures of periodontal disease. *PLoS One* 2012;**7**:e37919.
Liu Y, Fahle GA, Kovacs JA. Inability to Culture Pneumocystis jirovecii. *MBio* 2018;**9**.
Li X, Yang F, Li D et al. Trisomy of chromosome R confers resistance to triazoles in Candida albicans. *Med Mycol* 2015;**53**:302–9.
Lloyd-Price J et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 2019;**569**:655–62.
Lo HJ, Köhler JR, DiDomenico B et al. Nonfilamentous C. albicans mutants are avirulent. *Cell* 1997;**90**:939–49.
Lohse MB, Gulati M, Johnson AD et al. Development and regulation of single- and multi-species Candida albicans biofilms. *Nat Rev Microbiol* 2018;**16**:19–31.
Lopes JP, Stylianou M, Backman E et al. Evasion of Immune Surveillance in Low Oxygen Environments Enhances Candida albicans Virulence. *MBio* 2018;**9**.
Lorenz MC, Bender JA, Fink GR. Transcriptional response of Candida albicans upon internalization by macrophages. *Eukaryotic Cell* 2004;**3**:1076–87.
Lovat LB. Age related changes in gut physiology and nutritional status. *Gut* 1996;**38**:306–9.
Lukovac S, Belzer C, Pellis L et al. Differential modulation by Akkermansia muciniphila and Faecalibacterium prausnitzii of host peripheral lipid metabolism and histone acetylation in mouse gut organoids. *MBio* 2014;**5**.
Luo S, Blom AM, Rupp S et al. The pH-regulated antigen 1 of Candida albicans binds the human complement inhibitor C4b-binding protein and mediates fungal complement evasion. *J Biol Chem* 2011;**286**:8021–9.
Luo S, Poltermann S, Kunert A et al. Immune evasion of the human pathogenic yeast Candida albicans: Pra1 is a Factor H, FHL-1 and plasminogen binding surface protein. *Mol Immunol* 2009;**47**:541–50.
López-Ribot JL, Casanova M, Murgui A et al. Antibody response to Candida albicans cell wall antigens. *FEMS Immunol Med Microbiol* 2004;**41**:187–96.
Lüttich A, Brunke S, Hube B et al. Serial passaging of Candida albicans in systemic murine infection suggests that the wild type strain SC5314 is well adapted to the murine kidney. *PLoS One* 2013;**8**:e64482.
MacCallum DM, Castillo L, Nather K et al. Property differences among the four major Candida albicans strain clades. *Eukaryotic Cell* 2009;**8**:373–87.
MacCallum DM, Odds FC. Temporal events in the intravenous challenge model for experimental Candida albicans infections in female mice. *Mycoses* 2005;**48**:151–61.
Maccallum DM. Hosting infection: experimental models to assay Candida virulence. *Int J Microbiol* 2012;**2012**:363764.
Maciel NO et al. Occurrence, antifungal susceptibility, and virulence factors of opportunistic yeasts isolated from Brazilian beaches. *Mem Inst Oswaldo Cruz* 2019;**114**:e180566.
MacIntyre DA et al. The vaginal microbiome during pregnancy and the postpartum period in a European population. *Sci Rep* 2015;**5**:8988.
Mackie J, Szabo EK, Urgast DS et al. Host-Imposed Copper Poisoning Impacts Fungal Micronutrient Acquisition during Systemic Candida albicans Infections. *PLoS One* 2016;**11**:e0158683.
Madhivanan P et al. Identification of culturable vaginal Lactobacillus species among reproductive age women in Mysore, India. *J Med Microbiol* 2015;**64**:636–41.
Magee BB, Magee PT. Induction of mating in Candida albicans by construction of MTLa and MTLalpha strains. *Science* 2000;**289**:310–13.
Maier L et al. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* 2018;**555**:623–8.
Mailhe M et al. Repertoire of the gut microbiota from stomach to colon using culturomics and next-generation sequencing. *BMC Microbiol* 2018;**18**:157.
Mailänder-Sánchez D et al. Antifungal defense of probiotic Lactobacillus rhamnosus GG is mediated by blocking adhesion and nutrient depletion. *PLoS One* 2017;**12**:e0184438.
Majer O, Bourgeois C, Zwolanek F et al. Type I interferons promote fatal immunopathology by regulating inflammatory monocytes and neutrophils during Candida infections. *PLoS Pathog* 2012;**8**:e1002811.
Malavia D, Lehtovirta-Morley LE, Alamir O et al. Zinc Limitation Induces a Hyper-Adherent Goliath Phenotype in Candida albicans. *Front Microbiol* 2017;**8**:2238.
Malliaris SD, Steenbergen JN, Casadevall A. Cryptococcus neoformans var. gattii can exploit Acanthamoeba castellanii for growth. *Med Mycol* 2004;**42**:149–58.
Manichanh C et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut* 2006;**55**:205–11.
Manolio TA. Genomewide association studies and assessment of the risk of disease. *N Engl J Med* 2010;**363**:166–76.
Manzoni P, Mostert M, Leonessa ML et al. Oral supplementation with Lactobacillus casei subspecies rhamnosus prevents enteric colonization by Candida species in preterm neonates: a randomized study. *Clin Infect Dis* 2006;**42**:1735–42.
Marakalala MJ et al. Differential adaptation of Candida albicans in vivo modulates immune recognition by dectin-1. *PLoS Pathog* 2013;**9**:e1003315.
Marc G, Araniciu C, Oniga SD et al. New N-(oxazolylmethyl)-thiazolidinedione Active against Candida albicans Biofilm: Potential Als Proteins Inhibitors. *Molecules* 2018;**23**.
Marcos CM et al. Anti-Immune Strategies of Pathogenic Fungi. *Front Cell Infect Microbiol* 2016;**6**:142.
Marquis M, Lewandowski D, Dugas V et al. CD8+ T cells but not polymorphonuclear leukocytes are required to limit chronic oral carriage of Candida albicans in transgenic mice expressing human immunodeficiency virus type 1. *Infect Immun* 2006;**74**:2382–91.
Martinez-Pomares L. The mannose receptor. *J Leukoc Biol* 2012;**92**:1177–86.
Martinez X, Pozuelo M, Pascal V et al. MetaTrans: an open-source pipeline for metatranscriptomics. *Sci Rep* 2016;**6**:26447.
Martínez-López M et al. Microbiota Sensing by Mincle-Syk Axis in Dendritic Cells Regulates Interleukin-17 and -22 Production and Promotes Intestinal Barrier Integrity. *Immunity* 2019;**50**:446–461.e9.
Marzorati M, Vilchez-Vargas R, Bussche JV et al. High-fiber and high-protein diets shape different gut microbial communities, which ecologically behave similarly under stress conditions, as shown in a gastrointestinal simulator. *Mol Nutr Food Res* 2017;**61**.
Maschirow L, Suttorp N, Opitz B. Microbiota-Dependent Regulation of Antimicrobial Immunity in the Lung. *Am J Respir Cell Mol Biol* 2019;**61**:284–9.

Matee MI, Samaranayake LP, Scheutz F *et al.* Biotypes of oral Candida albicans isolates in a Tanzanian child population. *APMIS* 1996;**104**:623–8.

Matthews R, Burnie J, Smith D *et al.* Candida and AIDS: evidence for protective antibody. *Lancet* 1988;**2**:263–6.

Matthews RC, Burnie JP, Howat D *et al.* Autoantibody to heat-shock protein 90 can mediate protection against systemic candidosis. *Immunology* 1991;**74**:20–24.

Mattos-Graner RO, de Moraes AB, Rontani RM *et al.* Relation of oral yeast infection in Brazilian infants and use of a pacifier. *ASDC J Dent Child* 2001;**68**:10.

Matzaraki V *et al.* An integrative genomics approach identifies novel pathways that influence candidaemia susceptibility. *PLoS One* 2017;**12**:e0180824.

Maurer M *et al.* A three-dimensional immunocompetent intestine-on-chip model as in vitro platform for functional and microbial interaction studies. *Biomaterials* 2019;**220**:119396.

Maxson ME, Naj X, O'Meara TR *et al.* Integrin-based diffusion barrier separates membrane domains enabling the formation of microbiostatic frustrated phagosomes. *Elife* 2018;**7**.

Mayer BT, Srinivasan S, Fiedler TL *et al.* Rapid and profound shifts in the vaginal microbiota following antibiotic treatment for bacterial vaginosis. *J Infect Dis* 2015;**212**:793–802.

Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. *Virulence* 2013;**4**:119–28.

Mayer FL, Wilson D, Jacobsen ID *et al.* The novel Candida albicans transporter Dur31 Is a multi-stage pathogenicity factor. *PLoS Pathog* 2012;**8**:e1002592.

McClure R, Massari P. TLR-Dependent Human Mucosal Epithelial Cell Responses to Microbial Pathogens. *Front Immunol* 2014;**5**:386.

McGreal EP, Rosas M, Brown GD *et al.* The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. *Glycobiology* 2006;**16**:422–30.

McManus BA, Coleman DC. Molecular epidemiology, phylogeny and evolution of Candida albicans. *Infect Genet Evol* 2014;**21**:166–78.

Mehta RS *et al.* Stability of the human faecal microbiome in a cohort of adult men. *Nat Microbiol* 2018;**3**:347–55.

Mei M-L, Chu C-H, Low K-H *et al.* Caries arresting effect of silver diamine fluoride on dentine carious lesion with S. mutans and L. acidophilus dual-species cariogenic biofilm. *Med Oral Patol Oral Cir Bucal* 2013;**18**:e824–31.

Melkumyan AR, Priputnevich TV, Ankirskaya AS *et al.* Effects of antibiotic treatment on the lactobacillus composition of vaginal microbiota. *Bull Exp Biol Med* 2015;**158**:766–8.

Mengesha BG, Conti HR. The Role of IL-17 in Protection against Mucosal Candida Infections. *J Fungi (Basel)* 2017;**3**.

Merson-Davies LA, Odds FC. A morphology index for characterization of cell shape in Candida albicans. *J Gen Microbiol* 1989;**135**:3143–52.

Mestas J, Hughes CCW. Of mice and not men: differences between mouse and human immunology. *J Immunol* 2004;**172**:2731–8.

Metwalli KH, Khan SA, Krom BP *et al.* Streptococcus mutans, Candida albicans, and the human mouth: a sticky situation. *PLoS Pathog* 2013;**9**:e1003616.

Meyer E, Geffers C, Gastmeier P *et al.* No increase in primary nosocomial candidemia in 682 German intensive care units during 2006 to 2011. *Euro Surveill* 2013;**18**.

Meyer H, Goettlicher S, Mendling W. Stress as a cause of chronic recurrent vulvovaginal candidosis and the effectiveness of the conventional antimycotic therapy. *Mycoses* 2006;**49**: 202–9.

Miller EA, Beasley DE, Dunn RR *et al.* Lactobacilli dominance and vaginal ph: why is the human vaginal microbiome unique? *Front Microbiol* 2016;**7**:1936.

Miller MG, Johnson AD. White-opaque switching in Candida albicans is controlled by mating-type locus homeodomain proteins and allows efficient mating. *Cell* 2002;**110**:293–302.

Millsop JW, Fazel N. Oral candidiasis. *Clin Dermatol* 2016;**34**:487–94.

Miramón P, Dunker C, Windecker H *et al.* Cellular responses of Candida albicans to phagocytosis and the extracellular activities of neutrophils are critical to counteract carbohydrate starvation, oxidative and nitrosative stress. *PLoS One* 2012;**7**:e52850.

Miramón P, Kasper L, Hube B. Thriving within the host: Candida spp. interactions with phagocytic cells. *Med Microbiol Immunol* 2013;**202**:183–95.

Miranda LN *et al.* Candida colonisation as a source for candidaemia. *J Hosp Infect* 2009;**72**:9–16.

Mishra AA, Koh AY. Adaptation of Candida albicans during gastrointestinal tract colonization. *Curr Clin Microbiol Rep* 2018;**5**:165–72.

Misme-Aucouturier B, Albassier M, Alvarez-Rueda N *et al.* Specific Human and Candida Cellular Interactions Lead to Controlled or Persistent Infection Outcomes during Granuloma-Like Formation. *Infect Immun* 2017;**85**.

Miyazato A *et al.* Toll-like receptor 9-dependent activation of myeloid dendritic cells by Deoxynucleic acids from Candida albicans. *Infect Immun* 2009;**77**:3056–64.

Moens F, Duysburgh C, van den Abbeele P *et al.* Lactobacillus rhamnosus GG and Saccharomyces cerevisiae boulardii exert synergistic antipathogenic activity in vitro against enterotoxigenic Escherichia coli. *Benef Microbes* 2019;**10**:923–35.

Mojazi Amiri H, Frandah W, Colmer-Hamood J *et al.* Risk factors of Candida colonization in the oropharynx of patients admitted to an intensive care unit. *J Mycol Med* 2012;**22**:301–7.

Molly K, Vande Woestyne M, Verstraete W. Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. *Appl Microbiol Biotechnol* 1993;**39**:254–8.

Moosa M-YS, Sobel JD, Elhalis H *et al.* Fungicidal activity of fluconazole against Candida albicans in a synthetic vagina-simulative medium. *Antimicrob Agents Chemother* 2004;**48**:161–7.

Morais IMC, Cordeiro AL, Teixeira GS *et al.* Biological and physicochemical properties of biosurfactants produced by Lactobacillus jensenii P6A and Lactobacillus gasseri P65. *Microb Cell Fact* 2017;**16**:155.

Morales DK, Grahl N, Okegbe C *et al.* Control of Candida albicans metabolism and biofilm formation by Pseudomonas aeruginosa phenazines. *MBio* 2013;**4**:e00526–12.

Moreno-Ruiz E, Galán-Díez M, Zhu W *et al.* Candida albicans internalization by host cells is mediated by a clathrin-dependent mechanism. *Cell Microbiol* 2009;**11**:1179–89.

Morschhäuser J. Regulation of white-opaque switching in Candida albicans. *Med Microbiol Immunol* 2010;**199**:165–72.

Mothibe JV, Patel M. Pathogenic characteristics of Candida albicans isolated from oral cavities of denture wearers and cancer patients wearing oral prostheses. *Microb Pathog* 2017;**110**:128–34.

Motooka D *et al.* Fungal ITS1 Deep-Sequencing Strategies to Reconstruct the Composition of a 26-Species Community

and Evaluation of the Gut Mycobiota of Healthy Japanese Individuals. *Front Microbiol* 2017;**8**:238.
Mottawea W, Butcher J, Li J *et al.* The mucosal-luminal interface: an ideal sample to study the mucosa-associated microbiota and the intestinal microbial biogeography. *Pediatr Res* 2019;**85**:895–903.
Moura-Alves P *et al.* AhR sensing of bacterial pigments regulates antibacterial defence. *Nature* 2014;**512**:387–92.
Moyes DL *et al.* A biphasic innate immune MAPK response discriminates between the yeast and hyphal forms of Candida albicans in epithelial cells. *Cell Host Microbe* 2010;**8**:225–35.
Moyes DL *et al.* Candidalysin is a fungal peptide toxin critical for mucosal infection. *Nature* 2016;**532**:64–68.
Mukaremera L, Lee KK, Mora-Montes HM *et al.* Candida albicans Yeast, Pseudohyphal, and Hyphal Morphogenesis Differentially Affects Immune Recognition. *Front Immunol* 2017;**8**:629.
Mukherjee PK, Seshan KR, Leidich SD *et al.* Reintroduction of the PLB1 gene into Candida albicans restores virulence in vivo. *Microbiology (Reading, Engl)* 2001;**147**:2585–97.
Mulder WJM, Ochando J, Joosten LAB *et al.* Therapeutic targeting of trained immunity. *Nat Rev Drug Discov* 2019;**18**:553–66.
Murad AM, d'Enfert C, Gaillardin C *et al.* Transcript profiling in Candida albicans reveals new cellular functions for the transcriptional repressors CaTup1, CaMig1 and CaNrg1. *Mol Microbiol* 2001;**42**:981–93.
Murad AM, Leng P, Straffon M *et al.* NRG1 represses yeast-hypha morphogenesis and hypha-specific gene expression in Candida albicans. *EMBO J.* 2001;**20**:4742–52.
Murakami T *et al.* Changes in intestinal motility and gut microbiota composition in a rat stress model. *Digestion* 2017;**95**:55–60.
Muzyka BC, Epifanio RN. Update on oral fungal infections. *Dent Clin North Am* 2013;**57**:561–81.
Naglik JR, Challacombe SJ, Hube B. Candida albicans secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol Rev* 2003;**67**:400–28, table of contents.
Naglik JR, König A, Hube B *et al.* Candida albicans-epithelial interactions and induction of mucosal innate immunity. *Curr Opin Microbiol* 2017;**40**:104–12.
Nanjappa SG, Heninger E, Wüthrich M *et al.* Tc17 cells mediate vaccine immunity against lethal fungal pneumonia in immune deficient hosts lacking CD4+ T cells. *PLoS Pathog* 2012;**8**:e1002771.
Nash AK *et al.* The gut mycobiome of the Human Microbiome Project healthy cohort. *Microbiome* 2017;**5**:153.
Nash EE, Peters BM, Fidel PL *et al.* Morphology-Independent Virulence of Candida Species during Polymicrobial Intra-abdominal Infections with Staphylococcus aureus. *Infect Immun* 2016;**84**:90–98.
Nash EE, Peters BM, Palmer GE *et al.* Morphogenesis is not required for Candida albicans-Staphylococcus aureus intra-abdominal infection-mediated dissemination and lethal sepsis. *Infect Immun* 2014;**82**:3426–35.
Nasser L, Weissman Z, Pinsky M *et al.* Structural basis of haem-iron acquisition by fungal pathogens. *Nat Microbiol* 2016;**1**:16156.
Netea MG, Brown GD, Kullberg BJ *et al.* An integrated model of the recognition of Candida albicans by the innate immune system. *Nat Rev Microbiol* 2008;**6**:67–78.
Netea MG, Quintin J, van der Meer JWM. Trained immunity: a memory for innate host defense. *Cell Host Microbe* 2011;**9**:355–61.
Netea MG *et al.* A guiding map for inflammation. *Nat Immunol* 2017;**18**:826–31.
Netea MG *et al.* Defining trained immunity and its role in health and disease. *Nat Rev Immunol* 2020;**20**:375–88.
Netea MG *et al.* Immune sensing of Candida albicans requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. *J Clin Invest* 2006;**116**:1642–50.
Neves AB, Lobo LA, Pinto KC *et al.* Comparison between Clinical Aspects and Salivary Microbial Profile of Children with and without Early Childhood Caries: A Preliminary Study. *J Clin Pediatr Dent* 2015;**39**:209–14.
Neville BA, d'Enfert C, Bougnoux M-E. Candida albicans commensalism in the gastrointestinal tract. *FEMS Yeast Res* 2015;**15**.
Newport MJ, Finan C. Genome-wide association studies and susceptibility to infectious diseases. *Brief Funct Genomics* 2011;**10**:98–107.
Ngo LY, Kasahara S, Kumasaka DK *et al.* Inflammatory monocytes mediate early and organ-specific innate defense during systemic candidiasis. *J Infect Dis* 2014;**209**:109–19.
Nguyen LN, Lopes LCL, Cordero RJB *et al.* Sodium butyrate inhibits pathogenic yeast growth and enhances the functions of macrophages. *J Antimicrob Chemother* 2011;**66**:2573–80.
Nguyen MH, Peacock JE, Morris AJ *et al.* The changing face of candidemia: emergence of non-Candida albicans species and antifungal resistance. *Am J Med* 1996;**100**:617–23.
Nicholls S, Leach MD, Priest CL *et al.* Role of the heat shock transcription factor, Hsf1, in a major fungal pathogen that is obligately associated with warm-blooded animals. *Mol Microbiol* 2009;**74**:844–61.
Nicholls S, MacCallum DM, Kaffarnik FAR *et al.* Activation of the heat shock transcription factor Hsf1 is essential for the full virulence of the fungal pathogen Candida albicans. *Fungal Genet Biol* 2011;**48**:297–305.
Nikolaou E, Agrafioti I, Stumpf M *et al.* Phylogenetic diversity of stress signalling pathways in fungi. *BMC Evol Biol* 2009;**9**:44.
Nikou S-A, Kichik N, Brown R *et al.* Candida albicans Interactions with Mucosal Surfaces during Health and Disease. *Pathogens* 2019;**8**.
Nobile CJ, Fox EP, Nett JE *et al.* A recently evolved transcriptional network controls biofilm development in Candida albicans. *Cell* 2012;**148**:126–38.
Nobile CJ, Johnson AD. Candida albicans Biofilms and Human Disease. *Annu Rev Microbiol* 2015;**69**:71–92.
Noble SM, Gianetti BA, Witchley JN. Candida albicans cell-type switching and functional plasticity in the mammalian host. *Nat Rev Microbiol* 2017;**15**:96–108.
Noble SM. Candida albicans specializations for iron homeostasis: from commensalism to virulence. *Curr Opin Microbiol* 2013;**16**:708–15.
Noverr MC, Huffnagle GB. Regulation of Candida albicans morphogenesis by fatty acid metabolites. *Infect Immun* 2004;**72**:6206–10.
Noverr MC, Phare SM, Toews GB *et al.* Pathogenic yeasts Cryptococcus neoformans and Candida albicans produce immunomodulatory prostaglandins. *Infect Immun* 2001;**69**:2957–63.
Nucci M, Anaissie E. Revisiting the source of candidemia: skin or gut? *Clin Infect Dis* 2001;**33**:1959–67.
Nucci M, Colombo AL, Spector N *et al.* Breakthrough candidemia in neutropenic patients. *Clin Infect Dis* 1997;**24**:275–6.
Nwokolo NC, Boag FC. Chronic vaginal candidiasis. Management in the postmenopausal patient. *Drugs Aging* 2000;**16**:335–9.

O'Donnell LE, Millhouse E, Sherry L *et al.* Polymicrobial Candida biofilms: friends and foe in the oral cavity. *FEMS Yeast Res* 2015;**15**.

O'Meara TR, Veri AO, Ketela T *et al.* Global analysis of fungal morphology exposes mechanisms of host cell escape. *Nat Commun* 2015;**6**:6741.

O'Meara TR *et al.* High-Throughput Screening Identifies Genes Required for Candida albicans Induction of Macrophage Pyroptosis. *MBio* 2018;**9**.

Odamaki T, Kato K, Sugahara H *et al.* Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiol* 2016;**16**:90.

Odds FC, Brown AJP, Gow NAR. Antifungal agents: mechanisms of action. *Trends Microbiol* 2003;**11**:272–9.

Odds FC. *Candida and candidosis: a review and bibliography*. 2nd ed. London: Baillière Tindall, 1988.

Odds FC. Molecular phylogenetics and epidemiology of Candida albicans. *Future Microbiol* 2010;**5**:67–79.

Odds FC *et al.* Molecular phylogenetics of Candida albicans. *Eukaryotic Cell* 2007;**6**:1041–52.

Oliveira VMC, Santos SSF, Silva CRG *et al.* Lactobacillus is able to alter the virulence and the sensitivity profile of Candida albicans. *J Appl Microbiol* 2016;**121**:1737–44.

Oncel MY *et al.* Comparison of Lactobacillus reuteri and nystatin prophylaxis on Candida colonization and infection in very low birth weight infants. *J Matern Fetal Neonatal Med* 2015;**28**:1790–4.

Opulente DA *et al.* Pathogenic budding yeasts isolated outside of clinical settings. *FEMS Yeast Res* 2019;**19**.

Ortega M, Rovira M, Almela M *et al.* Bacterial and fungal bloodstream isolates from 796 hematopoietic stem cell transplant recipients between 1991 and 2000. *Ann Hematol* 2005;**84**: 40–6.

Ostrowski PP, Grinstein S, Freeman SA. Diffusion barriers, mechanical forces, and the biophysics of phagocytosis. *Dev Cell* 2016;**38**:135–46.

Otto GP, Sossdorf M, Claus RA *et al.* The late phase of sepsis is characterized by an increased microbiological burden and death rate. *Crit Care* 2011;**15**:R183.

Ott SJ *et al.* Fungi and inflammatory bowel diseases: Alterations of composition and diversity. *Scand J Gastroenterol* 2008;**43**:831–41.

Pammi M, Holland L, Butler G *et al.* Candida parapsilosis is a significant neonatal pathogen: a systematic review and meta-analysis. *Pediatr Infect Dis J* 2013;**32**:e206–16.

Pande K, Chen C, Noble SM. Passage through the mammalian gut triggers a phenotypic switch that promotes Candida albicans commensalism. *Nat Genet* 2013;**45**:1088–91.

Pandiyan P, Conti HR, Zheng L *et al.* CD4(+)CD25(+)Foxp3(+) regulatory T cells promote Th17 cells in vitro and enhance host resistance in mouse Candida albicans Th17 cell infection model. *Immunity* 2011;**34**:422–34.

Pan F, Han L, Zhang Y *et al.* Optimization of Caco-2 and HT29 co-culture in vitro cell models for permeability studies. *Int J Food Sci Nutr* 2015;**66**:680–5.

Papon N, Bougnoux M-E, d'Enfert C. Tracing the origin of invasive fungal infections. *Trends Microbiol* 2020;**28**:240–2.

Pappas PG, Lionakis MS, Arendrup MC *et al.* Invasive candidiasis. *Nat Rev Dis Primers* 2018;**4**:18026.

Pareek S *et al.* Comparison of Japanese and Indian intestinal microbiota shows diet-dependent interaction between bacteria and fungi. *npj Biofilms and Microbiomes* 2019; 5:37.

Park CO *et al.* Staged development of long-lived T-cell receptor $\alpha\beta$ TH17 resident memory T-cell population to Candida albicans after skin infection. *J Allergy Clin Immunol* 2018;**142**:647–62.

Park G-S, Park MH, Shin W *et al.* Emulating Host-Microbiome Ecosystem of Human Gastrointestinal Tract in Vitro. *Stem Cell Rev and Rep* 2017;**13**:321–34.

Parm U, Metsvaht T, Sepp E *et al.* Risk factors associated with gut and nasopharyngeal colonization by common Gram-negative species and yeasts in neonatal intensive care units patients. *Early Hum Dev* 2011;**87**:391–9.

Pascal V *et al.* A microbial signature for Crohn's disease. *Gut* 2017;**66**:813–22.

Patil S, Majumdar B, Sarode SC *et al.* Oropharyngeal Candidosis in HIV-Infected Patients-An Update. *Front Microbiol* 2018;**9**:980.

Patil S, Rao RS, Raj AT *et al.* Oral Candidal Carriage in Subjects with Pure Vegetarian and Mixed Dietary Habits. *J Clin Diagn Res* 2017;**11**:ZC22–4.

Payne S, Gibson G, Wynne A *et al.* In vitro studies on colonization resistance of the human gut microbiota to Candida albicans and the effects of tetracycline and Lactobacillus plantarum LPK. *Curr Issues Intest Microbiol* 2003;**4**:1–8.

Pearce SC, Coia HG, Karl JP *et al.* Intestinal in vitro and ex vivo Models to Study Host-Microbiome Interactions and Acute Stressors. *Front Physiol* 2018;**9**:1584.

Peltroche-Llacsahuanga H, Schnitzler N, Schmidt S *et al.* Phagocytosis, oxidative burst, and killing of Candida dubliniensis and Candida albicans by human neutrophils. *FEMS Microbiol Lett* 2000;**191**:151–5.

Pendharkar S, Magopane T, Larsson P-G *et al.* Identification and characterisation of vaginal lactobacilli from South African women. *BMC Infect Dis* 2013;**13**:43.

Perlroth J, Choi B, Spellberg B. Nosocomial fungal infections: epidemiology, diagnosis, and treatment. *Med Mycol* 2007;**45**: 321–46.

Peters BM, Jabra-Rizk MA, Scheper MA *et al.* Microbial interactions and differential protein expression in Staphylococcus aureus -Candida albicans dual-species biofilms. *FEMS Immunol Med Microbiol* 2010;**59**:493–503.

Peters BM, Noverr MC. Candida albicans-Staphylococcus aureus polymicrobial peritonitis modulates host innate immunity. *Infect Immun* 2013;**81**:2178–89.

Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. *Crit Rev Microbiol* 2010;**36**:1–53.

Pfleiderer A, Lagier JC, Armougom F *et al.* Culturomics identified 11 new bacterial species from a single anorexia nervosa stool sample. *Eur J Clin Microbiol Infect Dis* 2013;**32**:1471–81.

Pham VT, Chassard C, Rifa E *et al.* Lactate metabolism is strongly modulated by fecal inoculum, ph, and retention time in polyferms continuous colonic fermentation models mimicking young infant proximal colon. *mSystems* 2019;**4**.

Phan QT, Myers CL, Fu Y *et al.* Als3 is a Candida albicans invasin that binds to cadherins and induces endocytosis by host cells. *PLoS Biol* 2007;**5**:e64.

Piasecka B *et al.* Distinctive roles of age, sex, and genetics in shaping transcriptional variation of human immune responses to microbial challenges. *Proc Natl Acad Sci USA* 2018;**115**:E488–97.

Pietrella D, Lupo P, Rachini A *et al.* A Candida albicans mannoprotein deprived of its mannan moiety is efficiently taken up and processed by human dendritic cells and induces T-cell activation without stimulating proinflammatory cytokine production. *Infect Immun* 2008;**76**:4359–67.

Pirofski L-A, Casadevall A. Antimicrobial Therapy in the Context of the Damage-Response Framework: the Prospect of Optimizing Therapy by Reducing Host Damage. *Antimicrob Agents Chemother* 2020;**64**.

Pitarch A, Jiménez A, Nombela C *et al.* Decoding serological response to Candida cell wall immunome into novel diagnostic, prognostic, and therapeutic candidates for systemic candidiasis by proteomic and bioinformatic analyses. *Mol Cell Proteomics* 2006;**5**:79–96.

Pittman K, Kubes P. Damage-associated molecular patterns control neutrophil recruitment. *J Innate Immun* 2013;**5**:315–23.

Plantinga TS, Johnson MD *et al.* Toll-like receptor 1 polymorphisms increase susceptibility to candidemia. *J Infect Dis* 2012;**205**:934–43.

Plantinga TS, van Bergenhenegouwen J, Jacobs C *et al.* Modulation of Toll-like receptor ligands and Candida albicans-induced cytokine responses by specific probiotics. *Cytokine* 2012;**59**:159–65.

Plato A, Hardison SE, Brown GD. Pattern recognition receptors in antifungal immunity. *Semin Immunopathol* 2015;**37**:97–106.

Poissy J *et al.* Risk factors for candidemia: a prospective matched case-control study. *Crit Care* 2020;**24**:109.

Poltermann S, Kunert A, von der Heide M *et al.* Gpm1p is a factor H-, FHL-1-, and plasminogen-binding surface protein of Candida albicans. *J Biol Chem* 2007;**282**:37537–44.

Pop M *et al.* Individual-specific changes in the human gut microbiota after challenge with enterotoxigenic Escherichia coli and subsequent ciprofloxacin treatment. *BMC Genomics* 2016;**17**:440.

Popp C, Ramírez-Zavala B, Schwanfelder S *et al.* Evolution of Fluconazole-Resistant Candida albicans Strains by Drug-Induced Mating Competence and Parasexual Recombination. *MBio* 2019;**10**.

Porta A, Ramon AM, Fonzi WA. PRR1, a homolog of Aspergillus nidulans palF, controls pH-dependent gene expression and filamentation in Candida albicans. *J Bacteriol* 1999;**181**:7516–23.

Potrykus J, Stead D, Maccallum DM *et al.* Fungal iron availability during deep seated candidiasis is defined by a complex interplay involving systemic and local events. *PLoS Pathog* 2013;**9**:e1003676.

Poupet C, Chassard C, Nivoliez A *et al.* Caenorhabditis elegans, a Host to Investigate the Probiotic Properties of Beneficial Microorganisms. *Frontiers in nutrition* 2020.

Poupet C, Veisseire P, Bonnet M *et al.* Curative Treatment of Candidiasis by the Live Biotherapeutic Microorganism Lactobacillus rhamnosus Lcr35® in the Invertebrate Model Caenorhabditis elegans: First Mechanistic Insights. *Microorganisms* 2019;**8**.

Pradhan A *et al.* Elevated catalase expression in a fungal pathogen is a double-edged sword of iron. *PLoS Pathog* 2017;**13**:e1006405.

Pradhan A *et al.* Hypoxia Promotes Immune Evasion by Triggering β-Glucan Masking on the Candida albicans Cell Surface via Mitochondrial and cAMP-Protein Kinase A Signaling. *MBio* 2018;**9**.

Pradhan A *et al.* Non-canonical signalling mediates changes in fungal cell wall PAMPs that drive immune evasion. *Nat Commun* 2019;**10**:5315.

Prieto D, Correia I, Pla J *et al.* Adaptation of Candida albicans to commensalism in the gut. *Future Microbiol* 2016;**11**:567–83.

Puel A. Human inborn errors of immunity underlying superficial or invasive candidiasis. *Hum Genet* 2020;**139**:1011–22.

Puel A *et al.* Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. *Science* 2011;**332**:65–68.

Pujol C, Reynes J, Renaud F *et al.* The yeast Candida albicans has a clonal mode of reproduction in a population of infected human immunodeficiency virus-positive patients. *Proc Natl Acad Sci USA* 1993;**90**:9456–9.

Pérez JC, Kumamoto CA, Johnson AD. Candida albicans commensalism and pathogenicity are intertwined traits directed by a tightly knit transcriptional regulatory circuit. *PLoS Biol* 2013;**11**:e1001510.

Qin J *et al.* A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010;**464**:59–65.

Quintin J. Fungal mediated innate immune memory, what have we learned? *Semin Cell Dev Biol* 2019;**89**:71–77.

Quintin J *et al.* Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. *Cell Host Microbe* 2012;**12**:223–32.

Rada B, Leto TL. Pyocyanin effects on respiratory epithelium: relevance in Pseudomonas aeruginosa airway infections. *Trends Microbiol* 2013;**21**:73–81.

Rahimi C, Rahimi B, Padova D *et al.* Oral mucosa-on-a-chip to assess layer-specific responses to bacteria and dental materials. *Biomicrofluidics* 2018;**12**:054106.

Rahman D, Mistry M, Thavaraj S *et al.* Murine model of concurrent oral and vaginal Candida albicans colonization to study epithelial host-pathogen interactions. *Microbes Infect* 2007;**9**:615–22.

Raimondi S *et al.* Longitudinal survey of fungi in the human gut: ITS profiling, phenotyping, and colonization. *Front Microbiol* 2019;**10**:1575.

Rajendran R *et al.* Biofilm formation is a risk factor for mortality in patients with Candida albicans bloodstream infection-Scotland, 2012–2013. *Clin Microbiol Infect* 2016;**22**:87–93.

Ramage G, Saville SP, Thomas DP *et al.* Candida biofilms: an update. *Eukaryotic Cell* 2005;**4**:633–8.

Ramirez-Ortiz ZG, Means TK. The role of dendritic cells in the innate recognition of pathogenic fungi (A. fumigatus, C. neoformans and C. albicans). *Virulence* 2012;**3**:635–46.

Ramsdale M, Selway L, Stead D *et al.* MNL1 regulates weak acid-induced stress responses of the fungal pathogen Candida albicans. *Mol Biol Cell* 2008;**19**:4393–403.

Rappocciolo G, Jenkins FJ, Hensler HR *et al.* DC-SIGN is a receptor for human herpesvirus 8 on dendritic cells and macrophages. *J Immunol* 2006;**176**:1741–9.

Rastogi R, Su J, Mahalingam A *et al.* Engineering and characterization of simplified vaginal and seminal fluid simulants. *Contraception* 2016;**93**:337–46.

Ravel J *et al.* Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA* 2011;**108 Suppl 1**:4680–7.

Redding SW *et al.* Epidemiology of oropharyngeal Candida colonization and infection in patients receiving radiation for head and neck cancer. *J Clin Microbiol* 1999;**37**:3896–900.

Reyman M *et al.* Impact of delivery mode-associated gut microbiota dynamics on health in the first year of life. *Nat Commun* 2019;**10**:4997.

Richard ML, Plaine A. Comprehensive analysis of glycosylphosphatidylinositol-anchored proteins in Candida albicans. *Eukaryotic Cell* 2007;**6**:119–33.

Richardson JP, Ho J, Naglik JR. Candida-Epithelial Interactions. *J Fungi (Basel)* 2018;**4**.

Richardson JP, Moyes DL, Ho J *et al.* Candida innate immunity at the mucosa. *Semin Cell Dev Biol* 2019;**89**:58–70.

Richardson JP, Willems HME *et al.* Candidalysin drives epithelial signaling, neutrophil recruitment, and immunopathology at the vaginal mucosa. *Infect Immun* 2018;**86**.

Riesenfeld CS, Schloss PD, Handelsman J. Metagenomics: genomic analysis of microbial communities. *Annu Rev Genet* 2004;**38**:525–52.

Rinke C *et al.* Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 2013;**499**:431–7.

Rivière A, Selak M, Geirnaert A *et al.* Complementary Mechanisms for Degradation of Inulin-Type Fructans and Arabinoxylan Oligosaccharides among Bifidobacterial Strains Suggest Bacterial Cooperation. *Appl Environ Microbiol* 2018;**84**.

Rizzo A, Losacco A, Carratelli CR. Lactobacillus crispatus modulates epithelial cell defense against Candida albicans through Toll-like receptors 2 and 4, interleukin 8 and human β-defensins 2 and 3. *Immunol Lett* 2013;**156**:102–9.

Roberts CL, Rickard K, Kotsiou G *et al.* Treatment of asymptomatic vaginal candidiasis in pregnancy to prevent preterm birth: an open-label pilot randomized controlled trial. *BMC Pregnancy Childbirth* 2011;**11**:18.

Robinson MJ *et al.* Dectin-2 is a Syk-coupled pattern recognition receptor crucial for Th17 responses to fungal infection. *J Exp Med* 2009;**206**:2037–51.

Rocha FAC, Alves AMCV, Rocha MFG *et al.* Tumor necrosis factor prevents Candida albicans biofilm formation. *Sci Rep* 2017;**7**:1206.

Rodaki A, Bohovych IM, Enjalbert B *et al.* Glucose promotes stress resistance in the fungal pathogen Candida albicans. *Mol Biol Cell* 2009;**20**:4845–55.

Rodrigues CF, Rodrigues ME, Henriques M. Candida sp. Infections in Patients with Diabetes Mellitus. *J Clin Med* 2019;**8**.

Roeder A, Kirschning CJ, Rupec RA *et al.* Toll-like receptors and innate antifungal responses. *Trends Microbiol* 2004;**12**:44–49.

Romani L, Mencacci A, Tonnetti L *et al.* IL-12 is both required and prognostic in vivo for T helper type 1 differentiation in murine candidiasis. *J Immunol* 1994;**153**:5167–75.

Romano M, Fanelli G, Albany CJ *et al.* Past, present, and future of regulatory T cell therapy in transplantation and autoimmunity. *Front Immunol* 2019;**10**:43.

Romeo MG, Romeo DM, Trovato L *et al.* Role of probiotics in the prevention of the enteric colonization by Candida in preterm newborns: incidence of late-onset sepsis and neurological outcome. *J Perinatol* 2011;**31**:63–69.

Romeo O, De Leo F, Criseo G. Adherence ability of Candida africana: a comparative study with Candida albicans and Candida dubliniensis. *Mycoses* 2011;**54**:e57–61.

Romero R *et al.* The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome* 2014;**2**:4.

Román E, Prieto D, Martin R *et al.* Role of catalase overproduction in drug resistance and virulence in Candida albicans. *Future Microbiol* 2016;**11**:1279–97.

Rondanelli M, Faliva MA, Perna S *et al.* Using probiotics in clinical practice: Where are we now? A review of existing meta-analyses. *Gut Microbes* 2017;**8**:521–43.

Ropars J *et al.* Gene flow contributes to diversification of the major fungal pathogen Candida albicans. *Nat Commun* 2018;**9**:2253.

Rosales C. Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types? *Front Physiol* 2018;**9**:113.

Rosati D, Bruno M, Jaeger M *et al.* An exaggerated monocyte-derived cytokine response to Candida hyphae in patients with recurrent vulvovaginal candidiasis. *J Infect Dis* 2020.

Rosati D, Bruno M, Jaeger M *et al.* Recurrent vulvovaginal candidiasis: an immunological perspective. *Microorganisms* 2020;**8**.

Roselletti E, Perito S, Sabbatini S *et al.* Vaginal Epithelial Cells Discriminate Between Yeast and Hyphae of Candida albicans in Women Who Are Colonized or Have Vaginal Candidiasis. *J Infect Dis* 2019;**220**:1645–54.

Rothschild D *et al.* Environment dominates over host genetics in shaping human gut microbiota. *Nature* 2018;**555**:210–5.

Rougé C *et al.* Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr* 2009;**89**:1828–35.

Rubaltelli FF, Biadaioli R, Pecile P *et al.* Intestinal flora in breast- and bottle-fed infants. *J Perinat Med* 1998;**26**:186–91.

Rudkin FM, Bain JM, Walls C *et al.* Altered dynamics of Candida albicans phagocytosis by macrophages and PMNs when both phagocyte subsets are present. *MBio* 2013;**4**:e00810–13.

Rudkin FM *et al.* Single human B cell-derived monoclonal anti-Candida antibodies enhance phagocytosis and protect against disseminated candidiasis. *Nat Commun* 2018;**9**:5288.

Russo R, Superti F, Karadja E *et al.* Randomised clinical trial in women with Recurrent Vulvovaginal Candidiasis: Efficacy of probiotics and lactoferrin as maintenance treatment. *Mycoses* 2019;**62**:328–35.

Saeed S *et al.* Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science* 2014;**345**:1251086.

Saijo S *et al.* Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against Candida albicans. *Immunity* 2010;**32**:681–91.

Saiman L *et al.* Risk factors for Candida species colonization of neonatal intensive care unit patients. *Pediatr Infect Dis J* 2001;**20**:1119–24.

Salonen A *et al.* Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *ISME J* 2014;**8**:2218–30.

Samaranayake LP. Oral mycoses in HIV infection. *Oral Surg Oral Med Oral Pathol* 1992;**73**:171–80.

Sandai D *et al.* The evolutionary rewiring of ubiquitination targets has reprogrammed the regulation of carbon assimilation in the pathogenic yeast Candida albicans. *MBio* 2012;**3**.

San José C, Monge RA, Pérez-Díaz R *et al.* The mitogen-activated protein kinase homolog HOG1 gene controls glycerol accumulation in the pathogenic fungus Candida albicans. *J Bacteriol* 1996;**178**:5850–2.

Sato T *et al.* Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 2009;**459**:262–5.

Saville SP, Lazzell AL, Monteagudo C *et al.* Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of Candida albicans during infection. *Eukaryotic Cell* 2003;**2**:1053–60.

Saz-Leal P, Del Fresno C, Brandi P *et al.* Targeting SHIP-1 in Myeloid Cells Enhances Trained Immunity and Boosts Response to Infection. *Cell Rep* 2018;**25**:1118–26.

Schaller M, Korting HC, Borelli C *et al.* Candida albicans-secreted aspartic proteinases modify the epithelial cytokine response in an in vitro model of vaginal candidiasis. *Infect Immun* 2005;**73**:2758–65.

Schaller M, Zakikhany K, Naglik JR *et al.* Models of oral and vaginal candidiasis based on in vitro reconstituted human epithelia. *Nat Protoc* 2006;**1**:2767–73.

Schauber J *et al.* Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes: relevance of signalling pathways. *Gut* 2003;**52**:735–41.

Schei K, Avershina E, Øien T *et al.* Early gut mycobiota and mother-offspring transfer. *Microbiome* 2017;**5**:107.
Schnorr SL *et al.* Gut microbiome of the Hadza hunter-gatherers. *Nat Commun* 2014;**5**:3654.
Schofield DA, Westwater C, Warner T *et al.* Differential Candida albicans lipase gene expression during alimentary tract colonization and infection. *FEMS Microbiol Lett* 2005;**244**:359–65.
Schulze J, Sonnenborn U. Yeasts in the gut: from commensals to infectious agents. *Dtsch Arztebl Int* 2009;**106**:837–42.
Schönherr FA *et al.* The intraspecies diversity of C. albicans triggers qualitatively and temporally distinct host responses that determine the balance between commensalism and pathogenicity. *Mucosal Immunol* 2017;**10**:1335–50.
Selders GS, Fetz AE, Radic MZ *et al.* An overview of the role of neutrophils in innate immunity, inflammation and host-biomaterial integration. *Regen Biomater* 2017;**4**:55–68.
Selmecki A, Forche A, Berman J. Aneuploidy and isochromosome formation in drug-resistant Candida albicans. *Science* 2006;**313**:367–70.
Selmecki A, Forche A, Berman J. Genomic plasticity of the human fungal pathogen Candida albicans. *Eukaryotic Cell* 2010;**9**:991–1008.
Selmecki A, Gerami-Nejad M, Paulson C *et al.* An isochromosome confers drug resistance in vivo by amplification of two genes, ERG11 and TAC1. *Mol Microbiol* 2008;**68**:624–41.
Seng P, Drancourt M, Gouriet F *et al.* Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis* 2009;**49**:543–51.
Shaaban OM, Abbas AM, Moharram AM *et al.* Does vaginal douching affect the type of candidal vulvovaginal infection? *Med Mycol* 2015;**53**:817–27.
Shao T-Y *et al.* Commensal Candida albicans Positively Calibrates Systemic Th17 Immunological Responses. *Cell Host Microbe* 2019;**25**:404–17.e6.
Shapiro RS, Uppuluri P, Zaas AK *et al.* Hsp90 orchestrates temperature-dependent Candida albicans morphogenesis via Ras1-PKA signaling. *Curr Biol* 2009;**19**:621–9.
Shepherd MG. Cell envelope of Candida albicans. *Crit Rev Microbiol* 1987;**15**:7–25.
Sherrington SL, Sorsby E, Mahtey N *et al.* Adaptation of Candida albicans to environmental pH induces cell wall remodelling and enhances innate immune recognition. *PLoS Pathog* 2017;**13**:e1006403.
Sherry L, Kean R, McKloud E *et al.* Biofilms Formed by Isolates from Recurrent Vulvovaginal Candidiasis Patients Are Heterogeneous and Insensitive to Fluconazole. *Antimicrob Agents Chemother* 2017;**61**.
Shin JH *et al.* Genetic diversity among Korean Candida albicans bloodstream isolates: assessment by multilocus sequence typing and restriction endonuclease analysis of genomic DNA by use of BssHII. *J Clin Microbiol* 2011;**49**:2572–7.
Shirtliff ME, Peters BM, Jabra-Rizk MA. Cross-kingdom interactions: Candida albicans and bacteria. *FEMS Microbiol Lett* 2009;**299**:1–8.
Shivakoti R, Tuddenham S, Caulfield LE *et al.* Dietary macronutrient intake and molecular-bacterial vaginosis: Role of fiber. *Clin Nutr* 2020.
Shi Y, Chen L, Tong J *et al.* Preliminary characterization of vaginal microbiota in healthy Chinese women using cultivation-independent methods. *J Obstet Gynaecol Res* 2009;**35**:525–32.
Siavoshi F, Taghikhani A, Malekzadeh R *et al.* The role of mother's oral and vaginal yeasts in transmission of Helicobacter pylori to neonates. *Arch Iran Med* 2013;**16**:288–94.
Silk H. Diseases of the mouth. *Prim Care* 2014;**41**:75–90.
Silva S, Negri M, Henriques M *et al.* *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiol Rev* 2012;**36**:288–305.
Silverman RJ, Nobbs AH, Vickerman MM *et al.* Interaction of Candida albicans cell wall Als3 protein with Streptococcus gordonii SspB adhesin promotes development of mixed-species communities. *Infect Immun* 2010;**78**:4644–52.
Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. *Proc Biol Sci* 2015;**282**:20143085.
Simões CD, Maukonen J, Kaprio J *et al.* Habitual dietary intake is associated with stool microbiota composition in monozygotic twins. *J Nutr* 2013;**143**:417–23.
Singh P, Chauhan N, Ghosh A *et al.* SKN7 of Candida albicans: mutant construction and phenotype analysis. *Infect Immun* 2004;**72**:2390–94.
Sitterlé E, Maufrais C, Sertour N *et al.* Within-Host Genomic Diversity of Candida albicans in Healthy Carriers. *Sci Rep* 2019;**9**:2563.
Sitterlé E *et al.* Large-scale genome mining allows identification of neutral polymorphisms and novel resistance mutations in genes involved in Candida albicans resistance to azoles and echinocandins. *J Antimicrob Chemother* 2020;**75**:835–48.
Sivieri K, Morales MLV, Saad SMI *et al.* Prebiotic effect of fructooligosaccharide in the simulator of the human intestinal microbial ecosystem (SHIME® model). *J Med Food* 2014;**17**:894–901.
Smeekens SP *et al.* Functional genomics identifies type I interferon pathway as central for host defense against Candida albicans. *Nat Commun* 2013;**4**:1342.
Smirnov KS, Maier TV, Walker A *et al.* Challenges of metabolomics in human gut microbiota research. *Int J Med Microbiol* 2016;**306**:266–79.
Smith DA, Nicholls S, Morgan BA *et al.* A conserved stress-activated protein kinase regulates a core stress response in the human pathogen Candida albicans. *Mol Biol Cell* 2004;**15**:4179–90.
Sobel JD. Vulvovaginal candidosis. *Lancet* 2007;**369**:1961–71.
Sokol H *et al.* Fungal microbiota dysbiosis in IBD. *Gut* 2017;**66**:1039–48.
Sokol H *et al.* Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflamm Bowel Dis* 2009;**15**:1183–9.
Solis NV, Filler SG. Mouse model of oropharyngeal candidiasis. *Nat Protoc* 2012;**7**:637–42.
Solis NV, Swidergall M, Bruno VM *et al.* The Aryl Hydrocarbon Receptor Governs Epithelial Cell Invasion during Oropharyngeal Candidiasis. *MBio* 2017;**8**.
Sosinska GJ, de Groot PWJ, Teixeira de Mattos MJ *et al.* Hypoxic conditions and iron restriction affect the cell-wall proteome of Candida albicans grown under vagina-simulative conditions. *Microbiology (Reading, Engl)* 2008;**154**:510–20.
Sparber F, Dolowschiak T, Mertens S *et al.* Langerin+ DCs regulate innate IL-17 production in the oral mucosa during Candida albicans-mediated infection. *PLoS Pathog* 2018;**14**:e1007069.
Sparber F, LeibundGut-Landmann S. Interleukin-17 in Antifungal Immunity. *Pathogens* 2019;**8**.
Spear GT *et al.* Human α-amylase present in lower-genital-tract mucosal fluid processes glycogen to support vaginal colonization by Lactobacillus. *J Infect Dis* 2014;**210**:1019–28.

Spinillo A, Bernuzzi AM, Cevini C *et al.* The relationship of bacterial vaginosis, Candida and Trichomonas infection to symptomatic vaginitis in postmenopausal women attending a vaginitis clinic. *Maturitas* 1997;**27**:253–60.

Sprenger M, Kasper L, Hensel M *et al.* Metabolic adaptation of intracellular bacteria and fungi to macrophages. *Int J Med Microbiol* 2018;**308**:215–27.

Staab JF, Bradway SD, Fidel PL *et al.* Adhesive and mammalian transglutaminase substrate properties of Candida albicans Hwp1. *Science* 1999;**283**:1535–8.

Staab JF, Ferrer CA, Sundstrom P. Developmental expression of a tandemly repeated, proline-and glutamine-rich amino acid motif on hyphal surfaces on Candida albicans. *J Biol Chem* 1996;**271**:6298–305.

Staib P, Morschhäuser J. Chlamydospore formation in Candida albicans and Candida dubliniensis–an enigmatic developmental programme. *Mycoses* 2007;**50**:1–12.

Stecksén-Blicks C, Granström E, Silfverdal SA *et al.* Prevalence of oral Candida in the first year of life. *Mycoses* 2015;**58**:550–6.

Steele C, Ratterree M, Fidel PL. Differential susceptibility of two species of macaques to experimental vaginal candidiasis. *J Infect Dis* 1999;**180**:802–10.

Steenbergen JN, Shuman HA, Casadevall A. Cryptococcus neoformans interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. *Proc Natl Acad Sci USA* 2001;**98**:15245–50.

Stokholm J *et al.* Antibiotic use during pregnancy alters the commensal vaginal microbiota. *Clin Microbiol Infect* 2014;**20**: 629–35.

Stone NR *et al.* Dynamic ploidy changes drive fluconazole resistance in human cryptococcal meningitis. *J Clin Invest* 2019;**129**:999–1014.

Strati F *et al.* New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome* 2017;**5**:24.

Strus M, Kucharska A, Kukla G *et al.* The in vitro activity of vaginal Lactobacillus with probiotic properties against Candida. *Infect Dis Obstet Gynecol* 2005;**13**:69–75.

Sudbery P, Gow N, Berman J. The distinct morphogenic states of Candida albicans. *Trends Microbiol* 2004;**12**:317–24.

Sudbery PE. The germ tubes of Candida albicans hyphae and pseudohyphae show different patterns of septin ring localization. *Mol Microbiol* 2001;**41**:19–31.

Sullivan DJ, Westerneng TJ, Haynes KA *et al.* Candida dubliniensis sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. *Microbiology (Reading, Engl)* 1995;**141**:1507–21.

Sun JN *et al.* Host cell invasion and virulence mediated by Candida albicans Ssa1. *PLoS Pathog* 2010;**6**:e1001181.

Sun Y, Cao C, Jia W *et al.* pH Regulates White-Opaque Switching and Sexual Mating in Candida albicans. *Eukaryotic Cell* 2015;**14**:1127–34.

Swidergall M, Ernst JF. Interplay between Candida albicans and the antimicrobial peptide armory. *Eukaryotic Cell* 2014;**13**:950–7.

Swidergall M, Khalaji M *et al.* Candidalysin Is Required for Neutrophil Recruitment and Virulence During Systemic Candida albicans Infection. *J Infect Dis* 2019;**220**:1477–88.

Swidergall M, Solis NV, Lionakis MS *et al.* EphA2 is an epithelial cell pattern recognition receptor for fungal β-glucans. *Nat Microbiol* 2018;**3**:53–61.

Swidergall M, Solis NV, Wang Z *et al.* EphA2 Is a Neutrophil Receptor for Candida albicans that Stimulates Antifungal Activity during Oropharyngeal Infection. *Cell Rep* 2019;**28**:423–433.e5.

Swidergall M. Candida albicans at Host Barrier Sites: Pattern Recognition Receptors and Beyond. *Pathogens* 2019;**8**.

Swoboda RK, Bertram G, Hollander H *et al.* Glycolytic enzymes of Candida albicans are nonubiquitous immunogens during candidiasis. *Infect Immun* 1993;**61**:4263–71.

Szabo EK, MacCallum DM. The contribution of mouse models to our understanding of systemic candidiasis. *FEMS Microbiol Lett* 2011;**320**:1–8.

Sztajer H, Szafranski SP, Tomasch J *et al.* Cross-feeding and interkingdom communication in dual-species biofilms of Streptococcus mutans and Candida albicans. *ISME J* 2014;**8**:2256–71.

Tabatabaei F, Moharamzadeh K, Tayebi L. Three-Dimensional In Vitro Oral Mucosa Models of Fungal and Bacterial Infections. *Tissue Eng Part B Rev* 2020.

Taff HT, Mitchell KF, Edward JA *et al.* Mechanisms of Candida biofilm drug resistance. *Future Microbiol* 2013;**8**:1325–37.

Tamarelle J *et al.* Nonoptimal Vaginal Microbiota After Azithromycin Treatment for Chlamydia trachomatis Infection. *J Infect Dis* 2020;**221**:627–35.

Tao L *et al.* Discovery of a "white-gray-opaque" tristable phenotypic switching system in candida albicans: roles of non-genetic diversity in host adaptation. *PLoS Biol* 2014;**12**:e1001830.

Tarry W, Fisher M, Shen S *et al.* Candida albicans: the estrogen target for vaginal colonization. *J Surg Res* 2005;**129**:278–82.

Tavanti A, Campa D, Bertozzi A *et al.* Candida albicans isolates with different genomic backgrounds display a differential response to macrophage infection. *Microbes Infect* 2006;**8**: 791–800.

Taylor PR, Brown GD, Reid DM *et al.* The beta-glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. *J Immunol* 2002;**169**:3876–82.

Taylor PR, Reid DM, Heinsbroek SEM *et al.* Dectin-2 is predominantly myeloid restricted and exhibits unique activation-dependent expression on maturing inflammatory monocytes elicited in vivo. *Eur J Immunol* 2005;**35**: 2163–74.

Tedersoo L, Lindahl B. Fungal identification biases in microbiome projects. *Environ Microbiol Rep* 2016;**8**:774–9.

Tedersoo L *et al.* Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. *MC* 2015;**10**:1–43.

Teoh F, Pavelka N. How chemotherapy increases the risk of systemic candidiasis in cancer patients: current paradigm and future directions. *Pathogens* 2016;**5**.

Thewes S, Kretschmar M, Park H *et al.* In vivo and ex vivo comparative transcriptional profiling of invasive and non-invasive Candida albicans isolates identifies genes associated with tissue invasion. *Mol Microbiol* 2007;**63**: 1606–28.

Thewes S, Moran GP, Magee BB *et al.* Phenotypic screening, transcriptional profiling, and comparative genomic analysis of an invasive and non-invasive strain of Candida albicans. *BMC Microbiol* 2008;**8**:187.

Tietz HJ, Hopp M, Schmalreck A *et al.* Candida africana sp. nov., a new human pathogen or a variant of Candida albicans? *Mycoses* 2001;**44**:437–45.

Tillmann AT *et al.* Contribution of Fdh3 and Glr1 to Glutathione Redox State, Stress Adaptation and Virulence in Candida albicans. *PLoS One* 2015;**10**:e0126940.

Tisoncik JR, Korth MJ, Simmons CP *et al.* Into the eye of the cytokine storm. *Microbiol Mol Biol Rev* 2012;**76**:16–32.

Tobin DM, May RC, Wheeler RT. Zebrafish: a see-through host and a fluorescent toolbox to probe host-pathogen interaction. *PLoS Pathog* 2012;**8**:e1002349.

Todd OA, Fidel PL, Harro JM *et al.* Candida albicans Augments Staphylococcus aureus Virulence by Engaging the Staphylococcal agr Quorum Sensing System. *MBio* 2019;**10**.

Todd OA, Noverr MC, Peters BM. Candida albicans Impacts Staphylococcus aureus Alpha-Toxin Production via Extracellular Alkalinization. *mSphere* 2019;**4**.

Tortelli BA, Lewis WG, Allsworth JE *et al.* Associations between the vaginal microbiome and Candida colonization in women of reproductive age. *Am J Obstet Gynecol* 2020;**222**:471.e1–9.

Tramontano M *et al.* Nutritional preferences of human gut bacteria reveal their metabolic idiosyncrasies. *Nat Microbiol* 2018;**3**:514–22.

Trautwein-Weidner K, Gladiator A, Kirchner FR *et al.* Antigen-Specific Th17 Cells Are Primed by Distinct and Complementary Dendritic Cell Subsets in Oropharyngeal Candidiasis. *PLoS Pathog* 2015;**11**:e1005164.

Trautwein-Weidner K, Gladiator A, Nur S *et al.* IL-17-mediated antifungal defense in the oral mucosa is independent of neutrophils. *Mucosal Immunol* 2015;**8**:221–31.

Trietsch SJ *et al.* Membrane-free culture and real-time barrier integrity assessment of perfused intestinal epithelium tubes. *Nat Commun* 2017;**8**:262.

Tripathi G, Wiltshire C, Macaskill S *et al.* Gcn4 co-ordinates morphogenetic and metabolic responses to amino acid starvation in Candida albicans. *EMBO J* 2002;**21**:5448–56.

Tso GHW *et al.* Experimental evolution of a fungal pathogen into a gut symbiont. *Science* 2018;**362**:589–95.

Tucey TM, Verma J, Olivier FAB *et al.* Metabolic competition between host and pathogen dictates inflammasome responses to fungal infection. *PLoS Pathog* 2020;**16**:e1008695.

Tucey TM *et al.* Glucose Homeostasis Is Important for Immune Cell Viability during Candida Challenge and Host Survival of Systemic Fungal Infection. *Cell Metab* 2018;**27**:988–1006.e7.

Tuddenham S *et al.* Associations between dietary micronutrient intake and molecular-Bacterial Vaginosis. *Reprod Health* 2019;**16**:151.

Ubeda C, Pamer EG. Antibiotics, microbiota, and immune defense. *Trends Immunol* 2012;**33**:459–66.

Ubeda C *et al.* Vancomycin-resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *J Clin Invest* 2010;**120**:4332–41.

Underhill DM, Iliev ID. The mycobiota: interactions between commensal fungi and the host immune system. *Nat Rev Immunol* 2014;**14**:405–16.

Underhill DM. Escape Mechanisms from the Immune Response. In: Brown GD, Netea MG (eds.). *Immunology of fungal infections*. Dordrecht: Springer Netherlands, 2007, 429–42.

Uppuluri P, Acosta Zaldívar M, Anderson MZ *et al.* Candida albicans Dispersed Cells Are Developmentally Distinct from Biofilm and Planktonic Cells. *MBio* 2018;**9**.

Urban CF, Ermert D, Schmid M *et al.* Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. *PLoS Pathog* 2009;**5**:e1000639.

Uwamahoro N *et al.* The pathogen Candida albicans hijacks pyroptosis for escape from macrophages. *MBio* 2014;**5**:e00003–14.

Van Belkum A, Broadwell D, Lovern D *et al.* Proteomics and metabolomics for analysis of the dynamics of microbiota. *Expert Rev Proteomics* 2018;**15**:101–4.

Van den Abbeele P, Belzer C, Goossens M *et al.* Butyrate-producing Clostridium cluster XIVa species specifically colonize mucins in an in vitro gut model. *ISME J* 2013;**7**:949–61.

Van den Abbeele P, Gérard P *et al.* Arabinoxylans and inulin differentially modulate the mucosal and luminal gut microbiota and mucin-degradation in humanized rats. *Environ Microbiol* 2011;**13**:2667–80.

Van den Abbeele P, Marzorati M, Derde M *et al.* Arabinoxylans, inulin and Lactobacillus reuteri 1063 repress the adherent-invasive Escherichia coli from mucus in a mucosa-comprising gut model. *npj Biofilms and Microbiomes* 2016;**2**:16016.

Van den Abbeele P, Van de Wiele T, Verstraete W *et al.* The host selects mucosal and luminal associations of coevolved gut microorganisms: a novel concept. *FEMS Microbiol Rev* 2011;**35**:681–704.

Van den Abbeele P *et al.* Incorporating a mucosal environment in a dynamic gut model results in a more representative colonization by lactobacilli. *Microb Biotechnol* 2012;**5**:106–15.

Van den Abbeele P *et al.* Microbial community development in a dynamic gut model is reproducible, colon region specific, and selective for Bacteroidetes and Clostridium cluster IX. *Appl Environ Microbiol* 2010;**76**:5237–46.

Vandeplassche E, Tavernier S, Coenye T *et al.* Influence of the lung microbiome on antibiotic susceptibility of cystic fibrosis pathogens. *Eur Respir Rev* 2019;**28**.

van der Graaf CAA, Netea MG, Franke B *et al.* Nucleotide oligomerization domain 2 (Nod2) is not involved in the pattern recognition of Candida albicans. *Clin Vaccine Immunol* 2006;**13**:423–5.

Van der Graaf CAA, Netea MG, Morré SA *et al.* Toll-like receptor 4 Asp299Gly/Thr399Ile polymorphisms are a risk factor for Candida bloodstream infection. *Eur Cytokine Netw* 2006;**17**:29–34.

van de Veerdonk FL, Gresnigt MS, Oosting M *et al.* Protective host defense against disseminated candidiasis is impaired in mice expressing human interleukin-37. *Front Microbiol* 2014;**5**:762.

van de Veerdonk FL, Joosten LAB, Shaw PJ *et al.* The inflammasome drives protective Th1 and Th17 cellular responses in disseminated candidiasis. *Eur J Immunol* 2011;**41**:2260–8.

van de Veerdonk FL, Plantinga TS *et al.* STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. *N Engl J Med* 2011;**365**:54–61.

van de Veerdonk FL *et al.* IL-38 binds to the IL-36 receptor and has biological effects on immune cells similar to IL-36 receptor antagonist. *Proc Natl Acad Sci USA* 2012;**109**:3001–5.

van de Veerdonk FL *et al.* The macrophage mannose receptor induces IL-17 in response to Candida albicans. *Cell Host Microbe* 2009;**5**:329–40.

Van de Wiele T, Van den Abbeele P, Ossieur W *et al.* The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). In: Verhoeckx K, Cotter P, López-Expósito I etal (eds.). *The Impact of Food Bioactives on Health: in vitro and ex vivo models [Internet]*. Cham: Springer International Publishing, 2015, 305–17.

Vangay P *et al.* US immigration westernizes the human gut microbiome. *Cell* 2018;**175**: 962–72.e10.

Van Herreweghen F *et al.* In vitro colonisation of the distal colon by Akkermansia muciniphila is largely mucin and pH dependent. *Benef Microbes* 2017;**8**:81–96.

van Veen SQ, Claas ECJ, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. *J Clin Microbiol* 2010;**48**:900–7.

Van Waeyenberghe L, Baré J, Pasmans F *et al.* Interaction of Aspergillus fumigatus conidia with Acanthamoeba castellanii parallels macrophage-fungus interactions. *Environ Microbiol Rep* 2013;**5**:819–24.

Vautier S *et al.* Candida albicans colonization and dissemination from the murine gastrointestinal tract: the influence of morphology and Th17 immunity. *Cell Microbiol* 2015;**17**:445–50.

Vecchiarelli A, Cenci E, Puliti M *et al.* Protective immunity induced by low-virulence Candida albicans: cytokine production in the development of the anti-infectious state. *Cell Immunol* 1989;**124**:334–44.

Venema K, van den Abbeele P. Experimental models of the gut microbiome. *Best Pract Res Clin Gastroenterol* 2013;**27**:115–26.

Verma A, Wüthrich M, Deepe G *et al.* Adaptive immunity to fungi. *Cold Spring Harb Perspect Med* 2014;**5**:a019612.

Verma AH *et al.* IL-36 and IL-1/IL-17 Drive Immunity to Oral Candidiasis via Parallel Mechanisms. *J Immunol* 2018;**201**:627–34.

Verma Akash, Gaffen SL, Swidergall M. Innate immunity to mucosal candida infections. *J Fungi (Basel)* 2017;**3**.

Verma Akash H *et al.* Oral epithelial cells orchestrate innate type 17 responses to Candida albicans through the virulence factor candidalysin. *Sci Immunol* 2017;**2**.

Vich Vila A *et al.* Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nat Commun* 2020;**11**:362.

Vijayan D, Radford KJ, Beckhouse AG *et al.* Mincle polarizes human monocyte and neutrophil responses to Candida albicans. *Immunol Cell Biol* 2012;**90**:889–95.

Vila T, Sultan AS, Montelongo-Jauregui D *et al.* Oral candidiasis: A disease of opportunity. *J Fungi (Basel)* 2020;**6**.

Villar CC, Kashleva H, Mitchell AP *et al.* Invasive phenotype of Candida albicans affects the host proinflammatory response to infection. *Infect Immun* 2005;**73**:4588–95.

Villa S, Hamideh M, Weinstock A *et al.* Transcriptional control of hyphal morphogenesis in Candida albicans. *FEMS Yeast Res* 2020;**20**.

Villmones HC, Haug ES, Ulvestad E *et al.* Species level description of the human ileal bacterial microbiota. *Sci Rep* 2018;**8**:4736.

Vladareanu R, Mihu D, Mitran M *et al.* New evidence on oral L. plantarum P17630 product in women with history of recurrent vulvovaginal candidiasis (RVVC): a randomized double-blind placebo-controlled study. *Eur Rev Med Pharmacol Sci* 2018;**22**:262–7.

Vylkova S, Carman AJ, Danhof HA *et al.* The fungal pathogen Candida albicans autoinduces hyphal morphogenesis by raising extracellular pH. *MBio* 2011;**2**:e00055–11.

Vylkova S, Lorenz MC. Modulation of phagosomal pH by Candida albicans promotes hyphal morphogenesis and requires Stp2p, a regulator of amino acid transport. *PLoS Pathog* 2014;**10**:e1003995.

Vylkova S, Lorenz MC. Phagosomal Neutralization by the Fungal Pathogen Candida albicans Induces Macrophage Pyroptosis. *Infect Immun* 2017;**85**.

Vylkova S. Environmental pH modulation by pathogenic fungi as a strategy to conquer the host. *PLoS Pathog* 2017;**13**:e1006149.

Vázquez-Torres A, Balish E. Macrophages in resistance to candidiasis. *Microbiol Mol Biol Rev* 1997;**61**:170–92.

Vílchez R, Lemme A, Ballhausen B *et al.* Streptococcus mutans inhibits Candida albicans hyphal formation by the fatty acid signaling molecule trans-2-decenoic acid (SDSF). *Chembiochem* 2010;**11**:1552–62.

Wagener J *et al.* Fungal chitin dampens inflammation through IL-10 induction mediated by NOD2 and TLR9 activation. *PLoS Pathog* 2014;**10**:e1004050.

Wagner RD, Johnson SJ. Probiotic lactobacillus and estrogen effects on vaginal epithelial gene expression responses to Candida albicans. *J Biomed Sci* 2012;**19**:58.

Walker AW, Duncan SH, Louis P *et al.* Phylogeny, culturing, and metagenomics of the human gut microbiota. *Trends Microbiol* 2014;**22**:267–74.

Walker AW, Duncan SH, McWilliam Leitch EC *et al.* pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol* 2005;**71**:3692–700.

Walker AW *et al.* Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J* 2011;**5**:220–30.

Walpole GFW, Grinstein S, Westman J. The role of lipids in host-pathogen interactions. *IUBMB Life* 2018;**70**:384–92.

Wang H, Xu J, Guo H *et al.* Patterns of human oral yeast species distribution on Hainan Island in China. *Mycopathologia* 2013;**176**:359–68.

Wang J. Neutrophils in tissue injury and repair. *Cell Tissue Res* 2018;**371**:531–9.

Wang K *et al.* Diagnostic value of Candida mannan antigen and anti-mannan IgG and IgM antibodies for Candida infection. *Mycoses* 2020;**63**:181–8.

Ward TL, Dominguez-Bello MG, Heisel T *et al.* Development of the Human Mycobiome over the First Month of Life and across Body Sites. *mSystems* 2018;**3**.

Warris A, Bercusson A, Armstrong-James D. Aspergillus colonization and antifungal immunity in cystic fibrosis patients. *Med Mycol* 2019;**57**:S118–26.

Wartenberg A *et al.* Microevolution of Candida albicans in macrophages restores filamentation in a nonfilamentous mutant. *PLoS Genet* 2014;**10**:e1004824.

Watanabe S, Alexander M, Misharin AV *et al.* The role of macrophages in the resolution of inflammation. *J Clin Invest* 2019;**129**:2619–28.

Weindl G, Naglik JR, Kaesler S *et al.* Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling. *J Clin Invest* 2007;**117**:3664–72.

Weinstein MP. Current blood culture methods and systems: clinical concepts, technology, and interpretation of results. *Clin Infect Dis* 1996;**23**:40–46.

Wei S, Mortensen MS, Stokholm J *et al.* Short- and long-term impacts of azithromycin treatment on the gut microbiota in children: A double-blind, randomized, placebo-controlled trial. *EBioMedicine* 2018;**38**:265–72.

Wei Y-P, Feng J, Luo Z-C. Isolation and genotyping of vaginal non-albicans Candida spp. in women from two different ethnic groups in Lanzhou, China. *Int J Gynaecol Obstet* 2010;**110**:227–30.

Wellington M, Koselny K, Sutterwala FS *et al.* Candida albicans triggers NLRP3-mediated pyroptosis in macrophages. *Eukaryotic Cell* 2014;**13**:329–40.

Wells CA *et al.* The macrophage-inducible C-type lectin, mincle, is an essential component of the innate immune response to Candida albicans. *J Immunol* 2008;**180**:7404–13.

Westman J, Hube B, Fairn GD. Integrity under stress: Host membrane remodelling and damage by fungal pathogens. *Cell Microbiol* 2019;**21**:e13016.

Westman J, Moran GP, Mogavero S *et al.* Candida albicans Hyphal Expansion Causes Phagosomal Membrane Damage and Luminal Alkalinization. *MBio* 2018;**9**.

Westman J, Walpole GFW, Kasper L *et al.* Lysosome Fusion Maintains Phagosome Integrity during Fungal Infection. *Cell Host Microbe* 2020.

Wheeler ML et al. Immunological consequences of intestinal fungal dysbiosis. *Cell Host Microbe* 2016;**19**:865–73.

Whibley N *et al.* Antibody blockade of IL-17 family cytokines in immunity to acute murine oral mucosal candidiasis. *J Leukoc Biol* 2016;**99**:1153–64.

White S, Larsen B. Candida albicans morphogenesis is influenced by estrogen. *Cell Mol Life Sci* 1997;**53**:744–9.

Whitney PG, Bär E, Osorio F *et al.* Syk signaling in dendritic cells orchestrates innate resistance to systemic fungal infection. *PLoS Pathog* 2014;**10**:e1004276.

Wilgus TA. Alerting the body to tissue injury: The role of alarmins and DAMPs in cutaneous wound healing. *Curr Pathobiol Rep* 2018;**6**:55–60.

Willems HM, Kos K, Jabra-Rizk MA *et al.* Candida albicans in oral biofilms could prevent caries. *Pathog Dis* 2016;**74**.

Willems HME, Ahmed SS, Liu J *et al.* Vulvovaginal candidiasis: A current understanding and burning questions. *J Fungi (Basel)* 2020;**6**.

Williams RB, Lorenz MC. Multiple Alternative Carbon Pathways Combine To Promote Candida albicans Stress Resistance, Immune Interactions, and Virulence. *MBio* 2020;**11**.

Wilson D, Hube B. Hgc1 mediates dynamic Candida albicans-endothelium adhesion events during circulation. *Eukaryotic Cell* 2010;**9**:278–87.

Wilson D, Naglik JR, Hube B. The Missing Link between Candida albicans Hyphal Morphogenesis and Host Cell Damage. *PLoS Pathog* 2016;**12**:e1005867.

Wilson D. An evolutionary perspective on zinc uptake by human fungal pathogens. *Metallomics* 2015;**7**:979–85.

Witchley JN, Penumetcha P, Abon NV *et al.* Candida albicans Morphogenesis Programs Control the Balance between Gut Commensalism and Invasive Infection. *Cell Host Microbe* 2019;**25**:432–443.e6.

Wu GD *et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011;**334**:105–8.

Wu L, Zeng T, Deligios M *et al.* Age-Related Variation of Bacterial and Fungal Communities in Different Body Habitats across the Young, Elderly, and Centenarians in Sardinia. *mSphere* 2020;**5**.

Wu S-Y, Weng C-L, Jheng M-J *et al.* Candida albicans triggers NADPH oxidase-independent neutrophil extracellular traps through dectin-2. *PLoS Pathog* 2019;**15**:e1008096.

Wynne AG, McCartney AL, Brostoff J *et al.* An in vitro assessment of the effects of broad-spectrum antibiotics on the human gut microflora and concomitant isolation of a Lactobacillus plantarum with anti-Candida activities. *Anaerobe* 2004;**10**:165–9.

Wächtler B, Citiulo F, Jablonowski N *et al.* Candida albicans-epithelial interactions: dissecting the roles of active penetration, induced endocytosis and host factors on the infection process. *PLoS One* 2012;**7**:e36952.

Wüthrich M, Deepe GS, Klein B. Adaptive immunity to fungi. *Annu Rev Immunol* 2012;**30**:115–48.

Xie J *et al.* White-opaque switching in natural MTLa/α isolates of Candida albicans: evolutionary implications for roles in host adaptation, pathogenesis, and sex. *PLoS Biol* 2013;**11**:e1001525.

Xu C, Zhu H, Qiu P. Aging progression of human gut microbiota. *BMC Microbiol* 2019;**19**: 236.

Xu H, Sobue T, Bertolini M *et al.* S. oralis activates the Efg1 filamentation pathway in C. albicans to promote cross-kingdom interactions and mucosal biofilms. *Virulence* 2017;**8**:1602–17.

Xu H, Sobue T, Bertolini M *et al.* Streptococcus oralis and Candida albicans Synergistically Activate μ-Calpain to Degrade E-cadherin From Oral Epithelial Junctions. *J Infect Dis* 2016;**214**:925–34.

Xu H, Sobue T, Thompson A *et al.* Streptococcal co-infection augments Candida pathogenicity by amplifying the mucosal inflammatory response. *Cell Microbiol* 2014;**16**:214–31.

Xu J, Mitchell TG. Geographical differences in human oral yeast flora. *Clin Infect Dis* 2003;**36**:221–4.

Xu S, Shinohara ML. Tissue-Resident Macrophages in Fungal Infections. *Front Immunol* 2017;**8**:1798.

Xu X-L, Lee RTH, Fang H-M *et al.* Bacterial peptidoglycan triggers Candida albicans hyphal growth by directly activating the adenylyl cyclase Cyr1p. *Cell Host Microbe* 2008;**4**:28–39.

Yachida S *et al.* Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nat Med* 2019;**25**:968–76.

Yadev NP, Murdoch C, Saville SP *et al.* Evaluation of tissue engineered models of the oral mucosa to investigate oral candidiasis. *Microb Pathog* 2011;**50**:278–85.

Yang D, Oppenheim JJ. Alarmins and antimicrobial immunity. *Med Mycol* 2009;**47 Suppl 1**:S146–53.

Yang F, Teoh F, Tan ASM *et al.* Aneuploidy Enables Cross-Adaptation to Unrelated Drugs. *Mol Biol Evol* 2019;**36**:1768–82.

Yan L, Yang C, Tang J. Disruption of the intestinal mucosal barrier in Candida albicans infections. *Microbiol Res* 2013;**168**:389–95.

Yano J, Lilly E, Barousse M *et al.* Epithelial cell-derived S100 calcium-binding proteins as key mediators in the hallmark acute neutrophil response during Candida vaginitis. *Infect Immun* 2010;**78**:5126–37.

Yano J, Noverr MC, Fidel PL. Vaginal Heparan Sulfate Linked to Neutrophil Dysfunction in the Acute Inflammatory Response Associated with Experimental Vulvovaginal Candidiasis. *MBio* 2017;**8**.

Yano J, Palmer GE, Eberle KE *et al.* Vaginal epithelial cell-derived S100 alarmins induced by Candida albicans via pattern recognition receptor interactions are sufficient but not necessary for the acute neutrophil response during experimental vaginal candidiasis. *Infect Immun* 2014;**82**:783–92.

Yano J, Sobel JD, Nyirjesy P *et al.* Current patient perspectives of vulvovaginal candidiasis: incidence, symptoms, management and post-treatment outcomes. *BMC Womens Health* 2019;**19**:48.

Yapar N. Epidemiology and risk factors for invasive candidiasis. *Ther Clin Risk Manag* 2014;**10**:95–105.

Yatsunenko T *et al.* Human gut microbiome viewed across age and geography. *Nature* 2012;**486**:222–7.

Yin Z *et al.* Proteomic response to amino acid starvation in Candida albicans and Saccharomyces cerevisiae. *Proteomics* 2004;**4**:2425–36.

Zaneveld JR, McMinds R, Vega Thurber R. Stress and stability: applying the Anna Karenina principle to animal microbiomes. *Nat Microbiol* 2017;**2**:17121.

Zarrinpar A, Chaix A, Xu ZZ *et al.* Antibiotic-induced microbiome depletion alters metabolic homeostasis by affecting gut signaling and colonic metabolism. *Nat Commun* 2018;**9**:2872.

Zelante T *et al.* Sensing of mammalian IL-17A regulates fungal adaptation and virulence. *Nat Commun* 2012;**3**:683.

Zelante T et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* 2013;**39**:372–85.

Zhai B et al. High-resolution mycobiota analysis reveals dynamic intestinal translocation preceding invasive candidiasis. *Nat Med* 2020;**26**:59–64.

Zhang N, Magee BB, Magee PT et al. Selective Advantages of a Parasexual Cycle for the Yeast Candida albicans. *Genetics* 2015;**200**:1117–32.

Zhang X et al. Metaproteomics reveals associations between microbiome and intestinal extracellular vesicle proteins in pediatric inflammatory bowel disease. *Nat Commun* 2018;**9**:2873.

Zheng W et al. Metagenomic sequencing reveals altered metabolic pathways in the oral microbiota of sailors during a long sea voyage. *Sci Rep* 2015;**5**:9131.

Zhou W et al. Longitudinal multi-omics of host-microbe dynamics in prediabetes. *Nature* 2019;**569**:663–71.

Zhou X, Hansmann MA, Davis CC et al. The vaginal bacterial communities of Japanese women resemble those of women in other racial groups. *FEMS Immunol Med Microbiol* 2010;**58**:169–81.

Zhou Y et al. Biogeography of the ecosystems of the healthy human body. *Genome Biol* 2013;**14**:R1.

Zhu L-L, Zhao X-Q, Jiang C et al. C-type lectin receptors Dectin-3 and Dectin-2 form a heterodimeric pattern-recognition receptor for host defense against fungal infection. *Immunity* 2013;**39**:324–34.

Zielinski CE, Mele F, Aschenbrenner D et al. Pathogen-induced human TH17 cells produce IFN-γ or IL-10 and are regulated by IL-1β. *Nature* 2012;**484**:514–8.

Zipfel PF, Hallström T, Riesbeck K. Human complement control and complement evasion by pathogenic microbes–tipping the balance. *Mol Immunol* 2013;**56**:152–60.

Zipfel PF, Skerka C. Complement regulators and inhibitory proteins. *Nat Rev Immunol* 2009;**9**:729–40.

Znaidi S, Barker KS, Weber S et al. Identification of the Candida albicans Cap1p regulon. *Eukaryotic Cell* 2009;**8**:806–20.

Zoetendal EG et al. The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. *ISME J* 2012;**6**:1415–26.

Zuo T et al. Gut fungal dysbiosis correlates with reduced efficacy of fecal microbiota transplantation in Clostridium difficile infection. *Nat Commun* 2018;**9**:3663.

Zöllner MSA da C, Jorge AOC. Candida spp. occurrence in oral cavities of breastfeeding infants and in their mothers' mouths and breasts. *Pesqui Odontol Bras* 2003;**17**:151–5.

Appendix 3:

Perturbation and resilience of the gut microbiome up to three months after β-lactams exposure in healthy volunteers suggest an important role of endogenous β-lactamases

Camille d'Humières*, Margot Delavy*, Laurie Alla*, Farid Ichou, Emilie Gauliard, Amine Ghozlane, Florence Levenez, Nathalie Galleron, Benoit Quinquis, Nicolas Pons, Jimmy Mullaert, Antoine Bridier-Nahmias, Bénédicte Condamine, Marie Touchon, Dominique Rainteau, Antonin Lamazière, Philippe Lesnik, Maharajah Ponnaiah, Marie Lhomme, Natacha Sertour, Savannah Devente, Jean-Denis Docquier, Marie-Elisabeth Bougnoux, Olivier Tenaillon, Mélanie Magnan, Etienne Ruppé, Nathalie Grall, Xavier Duval, Dusko Ehrlich, France Mentré, Erick Denamur, Eduardo P. C. Rocha, Emmanuelle Le Chatelier, Charles Burdet, for the PrediRes study group.

Submitted, under review, https://doi.org/10.21203/rs.3.rs-2689371/v1 (preprint)

Perturbation and resilience of the gut microbiome up to three months after β-lactams exposure in healthy volunteers suggest an important role of endogenous β-lactamases

Camille d'Humières
Institut Pasteur
Margot Delavy
Institut Pasteur
Laurie Alla
University of Paris-Saclay
Farid Ichou
ICANomics
Emilie gauliard
Sorbonne University
Amine Ghozlane
Institut Pasteur
Florence Levenez
University of Paris-Saclay
Nathalie Galleron
University of Paris-Saclay
Benoit Quinquis
University of Paris-Saclay
Nicolas Pons
University of Paris-Saclay
Jimmy Mullaert
Université Paris-Cité
Antoine Bridier-Nahmias
Université Paris-Cité
Bénédicte Condamine
Université Paris-Cité
Marie Touchon
Institut Pasteur
Dominique Rainteau
Sorbonne University
Antonin Lamazière
Assistance Publique - Hôpitaux de Paris
Philippe Lesnik
Sorbonne University
Maharajah Ponnaiah
Institute of Cardiometabolism and Nutrition
Marie Lhomme
Institute of Cardiometabolism and Nutrition
Natacha Sertour

Institut Pasteur
Savannah Devente
University of Siena
Jean-Denis Docquier
University of Siena
Marie-Elisabeth Bougnoux
Institut Pasteur
Olivier Tenaillon
Université Paris-Cité
Mélanie Magnan
Université Paris-Cité
Etienne Ruppe
Assistance Publique - Hôpitaux de Paris
Nathalie Grall
Assistance Publique - Hôpitaux de Paris
Xavier Duval
Assistance Publique - Hôpitaux de Paris
Dusko Ehrlich
University of Paris-Saclay
France Mentre
Université Paris-Cité
erick Denamur
Université Paris-Cité
Eduardo P C Rocha
Institut Pasteur
Emmanuelle Le Chatelier
University of Paris-Saclay
Charles Burdet (charles.burdet@inserm.fr)
Université Paris-Cité

Research Article

Keywords: Antibiotics, human gut microbiota, metagenomics, metabolomics, resilience, β-lactamase

Posted Date: March 16th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2689371/v1

Abstract

Background

Antibiotics notoriously perturb the gut microbiota. We used untargeted and targeted phenotypic and genotypic approaches to study faecal samples collected up to 90 days following a 3-day course of intravenous β-lactam antibiotics in 22 healthy volunteers. We studied the changes in the bacterial, phage and fungal components of the microbiota as well as the metabolome and the β-lactamase activity of the stools. This allowed assessing their degrees of perturbation and resilience.

Results

While only two subjects had detectable concentrations of antibiotics in their faeces, suggesting important antibiotic degradation in the gut, the intravenous treatment perturbed very significantly the bacterial and phage microbiota, as well as the composition of the metabolome. In contrast, treatment impact was relatively low on the fungal microbiota. At the end of the surveillance period, we found evidence of resilience across the gut system since most components returned to a state like the initial one, even if the taxonomic composition of the bacterial microbiota changed and the dynamics of the different components over time were rarely correlated. The richness of the resistome was significantly reduced up to day 30, while a significant increase in the relative abundance of β-lactamase encoding genes was observed up to day 10, consistent with a concomitant increase in the β-lactamase activity of the microbiota. The level of β-lactamase activity at baseline was positively associated with the resilience of the metabolome content of the stools.

Conclusions

In healthy adults, antibiotics perturb all the components of the microbiota, which mostly return to its baseline state within 30 days. These data suggest an important role of endogenous β-lactamases producing anaerobes in protecting the functions of the microbiota by de-activating the antibiotics reaching the colon.

Background

The Human gut microbiome is composed of a variety of archaea, bacteria, viruses, fungi and protozoa, which have a complex relationship with their host, from mutualism or commensalism to pathogenesis [1]. Although the bacterial microbiota is relatively stable over time in healthy subjects, several factors can modify its composition, including age, lifestyle [2, 3] or the use of medication such as antibiotics, which are often absorbed or excreted through the digestive tract. [4]. Antibiotics are known to disrupt the taxonomic composition of the bacterial microbiota at different levels (from phyla to strains), and the resilience of the latter results in a partial return to the pre-treatment state [5, 6]. Antibiotics induce selection for resistance in commensal and pathogenic bacteria, contributing to the dissemination of resistant bacterial strains in the environment [7, 8]. Given the high density and diversity of bacteria in the gut, it has been suggested that the gut plays a key role in the development and spread of bacterial resistance [9]. In addition to bacteria, numerous bacterial viruses (bacteriophages or phages), fungi and protozoa are present in the gut. They may be indirectly affected by antibiotics, as competition for resources and predation leads to a complex network of interactions.

To evaluate the consequences of gut exposure to antibiotics, one must understand their long-term effects on healthy individuals. This avoids the interference of the effects of pathologies with the outcome of the bacteria-antibiotic interactions. The study of this perturbation requires the investigation of the multiple components describing the complex gut system. This includes biotic variables such as the population of specific bacteria of interest (e.g., Enterobacterales that include many pathogens), bacterial predators (phages) and competitors (fungi). Other key insights are obtained by the study of metabolites (small molecules < 1500 Da), which are intermediate or end products of cell metabolism [10]. Such metabolites are produced by the host, by the microorganisms or external sources. While most of these variables have been shown to be important in delineating the effect of antibiotics on the gut [11, 12], there is a lack of understanding of how they are associated and interact.

We recently conducted the CEREMI clinical trial [13], a study including 22 healthy subjects to understand and compare the impact of two intravenous β-lactam antibiotics, ceftriaxone and cefotaxime, on the intestinal microbiota, following standard

clinical doses administered for 3 days. Our previous analysis of 16S rRNA gene sequences showed that both antibiotics had a marked impact on the composition of the gut microbiota, but no significant differences were observed between the two antibiotics, suggesting they have the same effects. Of note, only two subjects had detectable faecal concentrations of antibiotics, suggesting antibiotic degradation by endogenous β-lactamases produced by anaerobes while they reach the colon [14]. Here, we analysed these stool samples to gain insights into the perturbation of the gut system at multiple levels. For this, we coupled shotgun sequencing methods, targeted and untargeted metabolomics approaches with phenotypic and genotypic targeted analyses of the faecal content. We focused on the bacterial, phage and fungal components of the community, along with the metabolite composition and β-lactamase activity of the stool content. We assessed the perturbations induced by both antibiotics and studied the correlations between them. The same analysis was performed for resilience, *i.e.* return to baseline state. We then evaluated whether the baseline microbiota status is associated with protection from perturbation and/or resilience following antibiotic administration. This revealed a comprehensive view of the impact of antibiotics on the gut microbiota.

Results

A multi-organism/multi-omic study of the effect of antibiotics on the gut microbiota of healthy volunteers

We administered to 22 healthy volunteers a standard 3-day course of intravenous β-lactam antibiotics partially eliminated through the intestinal route (Supplementary Figure S1). We sampled the stools of subjects (i) before (ii) during and (iii) after (up to day 90) the antibiotic treatment. We used different untargeted and targeted approaches to analyse phenotypic and genotypic characteristics of the bacterial, phage and fungal components of the microbiota, as well as the metabolic composition and β-lactamase content of the stools.

The variables obtained from these analyses were classified as 'high-dimensionality' (metagenomic analyses of the bacterial, phage and fungal microbiomes, and metabolome) or 'low-dimensionality' (richness of the bacterial, phage and fungal microbiomes, richness of the resistome and metabolome, relative abundance of the β-lactamasome, total bacterial counts, β-lactamase activity, fungal and *Candida albicans* DNA levels, cholesterol conversion and biliary acid transformation rates, see Supplementary Figure S1).

Variability of the gut microbiota components before antibiotic treatment

We first studied the between and within subjects variability of 'low-dimensionality' data' before antibiotics administration (Table 1).

Table 1

Variability of the studied gut microbiota and stool components before antibiotic treatment administration in the 22 healthy volunteers included in the CEREMI trial. n, number of subjects with available data. CV, coefficient of variation.

Component	n	Mean	Between subjects		Within subjects	
			Standard deviation	CV (%)	Standard deviation	CV (%)
Bacterial microbiota						
Bacterial counts (log_{10} CFU/g)	22	11.3	0.19	1.7	0.14	1.2
Bacterial microbiome richness (MGS/g)	22	269.7	64.69	24.0	16.01	5.9
Enterobacterales counts (log_{10} CFU/g)	22	7.5	1.37	18.3	1.00	13.3
Resistance						
Global resistome richness (copies/g)	22	820.1	159.50	19.5	69.75	8.5
B-lactamasome abundance (log_{10})	22	-0.8	0.03	3.8	0.02	2.5
B-lactamase activity (log_{10} nmol/min.g)	22	1.2	0.59	49.2	0.20	16.7
Phage microbiota						
Phage microbiome richness (contigs/g)	21	1226.6	271.81	22.2	91.37	7.5
Fungal microbiota						
Fungal load (log_{10})	21	-5.2	0.72	13.9	1.27	24.4
Fungal microbiome richness (fungal OTUs/g)	22	25.6	4.77	18.6	9.13	35.7
C. albicans DNA concentration (log_{10})	21	-5.0	0.81	16.2	0.47	9.4
Metabolome						
Metabolome richness (chemical species/g)	22	1472.7	< 0.01	0.0	98.85	6.7
Cholesterol conversion rate (log_{10})	22	-0.6	1.03	171.7	0.49	81.7
Bile acids transformation capacity (log_{10})	22	-0.1	0.09	90.0	0.06	60.0

Most variables had a higher variability between subjects than within subjects. The bacterial microbiota appeared to be relatively stable within a subject, while the richness of the bacterial microbiome and counts of Enterobacterales had a variability of approximately 20% between subjects. In line with these observations, the phage microbiota, whose composition is expected to be related to that of the bacterial microbiota, had similar variability values.

Interestingly, the the β-lactamasome abundance exhibited a very small between subject variability (4%), while the β -lactamase activity had a relatively high variability (approximately 49%). Variability of the resistome was relatively stable within a subject.

The fungal microbiota exhibited a high variability, especially within subject. Overall, the specific metabolic functions of cholesterol conversion or biliary acid transformation had the highest variability, while the richness in chemical species was very stable both between and within subjects.

Differential perturbation of the gut microbiota by antibiotics

We then enquired about the perturbation that followed antibiotic administration. The gut microbiota of most individuals was significantly disrupted over the 30 days following antibiotic administration (Fig. 1 and Supplementary Table S1). We found very few significant differences between individuals treated with ceftriaxone or cefotaxime (Supplementary Table S2), suggesting

similar effects of the two antibiotics. This fits our previous analysis [13] and led us to analyse together the two groups of individuals. The results of these analyses show that the impact of antibiotics differed markedly from one component to another (Supplementary Table S1).

Antibiotics do not affect fungi directly, but some fungal species might profit from the depletion of bacterial populations to proliferate. Punctual perturbations in the fungal load were observed up to day 30, with a global increase in the fungal load after antibiotic treatment. The concentration of *C. albicans* DNA was not significantly impacted at the studied timepoints (Supplementary Table S1) and antibiotic treatment had little impact on the variables describing the fungal components of the microbiota. Notably, we found no effect of antibiotics on the taxonomic structure nor on the richness of the fungal microbiome. Hence, the treatment seems to have had little impact on this component of the gut, at least at the time points studied here.

In contrast, and as previously observed using 16S sequences [13], the bacterial microbiome was very perturbed. Although the bacterial counts showed only a slight decrease after antibiotic treatment, the richness of the bacterial microbiome was markedly decreased, and its taxonomic composition was profoundly disrupted, with perturbation being still significant at day 90. Counts of Enterobacterales were significantly reduced up to day 10, with a maximal reduction observed on day 4, just after the end of the antibiotic treatment. The richness of the phage microbiome also decreased following antibiotic treatment, although the perturbation resolved earlier than for the bacterial microbiome. Hence, the bacterial fraction of the microbiome, and its viral predators, were very much affected by antibiotic treatment.

The lack of antibiotics in the faeces and the high perturbation observed in the bacterial fraction suggest antibiotics degradation in the gut. To analyse this, we searched for antibiotic-resistance genes in the bacterial genomes using Mustard [15]. We found that among the 19061 antibiotic resistance determinants identified, 1823 (9.6%) were genes encoding for β-lactamases: 627 from class A, 463 from classes B1-B2, 463 from class B3, 181 from class C and 89 from class D. The richness of the resistome was significantly reduced up to day 30, while a significant increase in the relative abundance of β-lactamase encoding genes was observed up to day 10, which was consistent with an increase in the β-lactamase activity of the microbiota and the lack of antibiotics in faeces.

Overall, these results indicate very different levels of perturbation caused by antibiotics on the components of the gut microbiota. This resulted in a minimal perturbation of the metabolome richness, whose composition was however profoundly disrupted and was still far from the initial state at day 90. Functions of the microbiota, such as the conversion rate of cholesterol and the capacity of the microbiota to transform primary bile acids into secondary bile acids, seem to return to baseline within 10 days. This suggests that despite a long-term perturbation of the taxonomic structure of the microbiota and its biochemical composition, the functions of the microbiota are not lost and can be restored quickly following exposure to antibiotics.

Correlation between maximal perturbations of the different gut microbiota components

To understand the relationship between each component's perturbation following antibiotic exposure, we computed for each sample and at each sampling time a distance from baseline to trace the evolution of the components over time. This distance was normalized by pre-treatment values to allow for comparisons between individuals and between variables. We defined the maximal perturbation as the maximal distance from baseline observed up to day 10. We then studied the correlations of these distances across variables to identify groups of variables that show similar patterns of perturbation. Among the 136 pairwise correlations, 17 (12.5%) were found to be statistically significant, even if their magnitude was moderate (maximal absolute value of 0.71) (Fig. 2, panel A). A cluster of significant positive correlations was observed between the maximal perturbations of the bacterial counts, bacterial microbiome richness and global resistome richness. It was also positively correlated with the β-lactamasome abundance and the β-lactamase activity, although in these cases the values were not significant. This confirms the association between the level of perturbation in the bacterial component of the microbiota and that of antibiotic-resistance genes in the bacteria.

One might have expected an association between the maximal perturbation in the phage fraction of the microbiome and other variables, especially those associated with the bacterial composition. Intriguingly, more pronounced changes in the bacterial

counts are significantly associated with a less pronounced perturbation in phage richness, i.e., when the bacterial counts decrease to a greater extent, we observe a lower change in the number of different phages contigs. One possibility is that phage induction has a role in bacterial mortality that stabilises the absolute number of phages. If so, the expected decrease in phage associated with the decrease in bacterial populations by the action of antibiotics would be compensated by increased induction rates of the prophages, which would amplify bacterial death rates.

As expected, the maximal perturbation of the metabolome was correlated to the maximal perturbation of the cholesterol conversion rate, the bile acid transformation capacity, and the bacterial taxonomic structure. Interestingly, the perturbation of the bile acid transformation capacity was correlated with the level of perturbation of the taxonomic structure of all studied components, *i.e.*, bacterial, phage, and fungal.

Finally, we found no significant perturbation in the richness or the structure of the fungal microbiome. Accordingly, it was not correlated with the other perturbations. However, the maximal perturbation of the fungal load was negatively correlated with that of the total bacterial counts, suggesting a direct inverse association between perturbations at the bacterial and at the fungal scales. Hence, fungi thrive when bacterial populations are rarefied by antibiotics, which seems to leave the taxonomic structure and richness of their population relatively unchanged. The abundance of fungi increases and is associated with significant changes in the metabolic functions of the microbiota.

Correlation between resilience of the different gut microbiota components

Once perturbations subside, systems may return to a situation close to the initial one, which we refer to as resilience. For the analysis of resilience, we computed for each variable the minimal normalized distance from baseline observed on the samples collected between day 15 and day 90. We then made correlations across all variables as described above for perturbations. Overall, 7 (5.1%) of the 136 pairwise correlations were statistically significant, and all with a moderate magnitude (maximal absolute value of 0.55) (Fig. 2, panel B). We found a cluster of positive correlations between the resilience of the bacterial microbiome richness and structure, of the β-lactamasome, of the β-lactamase activity, and of the bacterial counts. Even if only some pairwise correlations between these systems were significant, this suggests the existence of a group of variables with similar patterns of resilience associated with bacterial composition and the protection from the agents producing the perturbation (antibiotics). The resilience of the structure of the phage microbiome was also correlated with that of the bacterial microbiome, suggesting a tight association between the mechanisms of recovery of both variables. This may be caused by the arrest of prophage induction shortly after the end of antibiotic treatment, which would tightly link the recoveries of the populations of phages and bacteria.

Relationship between baseline composition, maximal perturbation and resilience of the gut microbiota components

One would expect an increase in the frequency of antibiotic-resistance genes in bacteria following exposure to antibiotics because those encoding them are more likely to survive. Indeed, the content in antibiotic-resistance conferring genes was disrupted, with an increase of genes encoding for β-lactamases, even if this was also associated with a decrease of other ARGs (Fig. 1). These changes were followed by a significant increase in the β-lactamase activity of the microbiota.

We then set out to evaluate how the initial state of the microbiota, including its content in β-lactamases encoding genes, might influence the antibiotic-induced perturbations. For this, we investigated the correlations between the initial composition of the gut microbiota (using the untransformed values of each variable at baseline) and the maximal perturbation of the variable among the 22 sampled volunteers, measured by the normalized distances from baseline. We observed few significant correlations and they had moderate absolute magnitudes (Fig. 3, panel A). In particular, the relative abundance of β-lactamase encoding genes or the β-lactamase activity were not significantly associated with a reduction in the level of perturbation of any of the studied systems. This suggests that the baseline level in β-lactamases of the microbiota does not limit the perturbation induced by antibiotics. For reasons that are unclear at this stage, the perturbation of the cholesterol conversion rate was higher when the richness of the global resistome at baseline was high. This might be an indirect effect due to the relation between resistome and

the bacterial microbiome. More expectedly, when the richness of resistance genes at the baseline was high, the increase in the frequency of *C. albicans* was lower. This suggests that bacteria more resistant to antibiotics could provide fewer niches for the expansion of the populations of *C. albicans*.

The same type of analysis was performed to study the correlation between the initial composition of the gut microbiota and the maximal resilience of the gut components (Fig. 3, panel B). We observed that the level of β-lactamase activity at baseline, but not that of the relative abundance of β-lactamase encoding genes, was positively correlated with the resilience of the bacterial microbiome and metabolome structures. The counts of Enterobacterales at baseline were negatively correlated with the resilience of the fungal microbiome richness. We also observed a cluster of correlations between variables associated with fungi: the baseline values of the fungal load, of the fungal microbiome richness and of the *C. albicans* DNA concentration and the resilience of the fungal load and *C. albicans* DNA concentration. This suggests that a complex and rich fungal microbiota might facilitate its restauration after a perturbation.

A focus on the rare subjects with antibiotics in the faeces

Ceftriaxone and cefotaxime are excreted in part through the intestinal route, which explains the perturbations found in this study, but only 2 of the 22 included subjects had detectable levels of antibiotics in faeces [13]. This is probably related to the ability of endogenous β-lactamase to hydrolyse β-lactam antibiotics in the faecal content [16, 17].

The two subjects who had detectable beta-lactam residues in the faeces, subject #16 (from the ceftriaxone treatment group) had detectable concentrations between days 2 and 7 ranging between 5.0 µg/g and 93.7 µg/g while subject #3 (from the cefotaxime treatment group) had detectable concentrations of cefotaxime in faeces at day 4 (1.6 µg/g). Of note, both showed no detectable β-lactamase activity at baseline (Supplementary Table S3). These subjects also had among the lowest bacterial richness before antibiotic treatment, with a particularly low richness in resistance-conferring genes. Intriguingly, their β-lactamasome was among the most abundant among the study participants, suggesting that these abundant resistance genes were either not expressed or non-functional.

These two subjects exhibited different levels of perturbation following antibiotic treatment: despite the absence of β-lactamase activity at baseline, subject #3 was among those with the least altered gut microbiota, while subject #16 was among those with the most disrupted (Supplementary Figure S2). The analysis of these two subjects suggests that outcomes of antibiotic therapies can be quite variable and sometimes unexpected.

Discussion

Here, we evaluated the impact of a short course of commonly used antibiotics in the hospital on the gut microbiota from healthy volunteers who had not been exposed to antibiotics for an extended period. We assessed this impact from several perspectives, analysing over 90 days the population dynamics of microorganisms, the antibiotic-resistance determinants, and some key metabolic functions, either using high throughput untargeted approaches or targeted tools. In the present report, we focused on global analysis, and in-depth analysis of individual components will be presented elsewhere.

We observed that the dynamics of disturbance over time, followed by those of resilience, are correlated for only a small part of the studied components. This indicates that, although gut microbiota can be considered as a network of interactions, some features are behaving in a similar way whereas others are not.

As expected, we observed that bacterial and phage population structures were quickly disrupted, as were the bacterial counts. The effect of antibiotics on the bacterial populations of the gut microbiota has long been known [18, 19], yet the interest in the Human gut phage population is more recent, and the existence of a characteristic healthy gut phage population has recently been suggested [20]. A few studies investigated the disruption of the phage population following antibiotics administration [21, 22]. Here, we confirmed this disruption, which was followed by a progressive return to a baseline state. This return occurred before that of the bacterial microbiome, possibly because the stabilization of the population of phages facilitates the subsequent stabilization of the bacterial fraction.

The changes in the frequency of antibiotic-resistance genes are particularly interesting to understand the impact of antibiotics in the promotion of resistant bacteria and/or the protection of the microbiota. We observed variations in the repertoire of resistance genes, and in particular at day 4 a decrease in the global resistome richness contemporaneous to an increase in the content in β-lactamase encoding genes and in the β-lactamase activity. This is contrary to what had been reported previously following the administration of a cocktail of 3 broad-spectrum antibiotics (meropenem, gentamicin and vancomycin) [6]. Intriguingly, the changes in the abundance of β-lactam resistance genes or β-lactamase activity were not associated with the changes observed in the bacterial component of the microbiota. It was however associated with an increased resilience of the metabolomic content of the microbiota, suggesting that the functional resilience of the microbiota following antibiotic-induced perturbation might be enhanced by endogenous β-lactamases. A recent study of healthy volunteers receiving widely prescribed antibiotics (ciprofloxacin, clindamycin, minocycline and amoxicillin) showed the minimal microbiome perturbance under the β-lactam amoxicillin [23], in agreement with our hypothesis.

Although the abundance of fungi increased after antibiotic exposure, their taxonomic composition was not strongly affected and we could not detect any changes in *C. albicans* DNA levels after antibiotics administration at the studied timepoints. A more detailed analysis of the mycobiota conducted by our group on the 22 subjects suggested that *C. albicans* levels increased immediately after antibiotic administration in some subjects, this increase being subject-dependant and relying largely on the variations in β-lactamase activity observed after the antibiotic treatment [24]. The perturbation of the fungal load and *C. albicans* DNA levels after antibiotics was previously reported in mice, where the fungal burden was increased after bacterial elimination by antibiotics [25]. Furthermore, antibiotics are required to allow *C. albicans* gut colonization in mice [26]. These results suggest that *C. albicans* growth could be efficiently prevented by specific bacteria residing in the human gut [27]. The decrease of specific bacterial populations induced by antibiotics would then open the way for the overgrowth of *C. albicans*. In agreement with this hypothesis, we observed that the perturbation of *C. albicans* DNA level was negatively correlated with the bacterial microbiome richness at baseline. Hence, the lack of diversity in the bacterial fraction and antibiotics favors the expansion of *C. albicans*.

Alterations of the faecal metabolome followed the trends observed for bile acid and sterol profiles. This might be explained by the interdependence of metabolites which can be either substrates or cofactors for the biosynthesis of new metabolites, as shown by well-known metabolites modulated by the gut microbiota, such as tryptophan and tryptophan-related metabolites which constitute a central hub for the production of indoles, incorporation into proteins and breakdown into kynurenine [28–30]. Disruption of both faecal bile acid and cholesterol metabolisms by antibiotics was previously reported [31, 32]. In particular, this led to an enrichment of faecal cholesterol and primary conjugated bile acids and the loss of coprostanol and secondary bile acids [33].

Collectively, our data suggest that metabolomic signatures following antibiotic treatment are primarily related to the dynamics of disruption of gut-resident structures (microbiome, phage, and fungi) over time rather than the dynamics of their recovery, consistent with their cellular or viral origins. Moreover, the baseline characteristics of metabolomic features may determine maximal disruption of resistome richness and β-lactamasome abundance revealing potential inter-domain connection.

Conclusions

Antibiotics affect multiple aspects of the gut microbiota and stool composition of healthy individuals. This leads to a change in the metabolites present in the gut, and noticeably of cholesterol and bile acids. However, the perturbation of the system does not irreversibly change it. Instead, we observed resilience at 30 days, and a positive relationship between the baseline levels of endogenous β-lactamase activity and the structure of the metabolome.

These data indicate that a normal microbiota is able to absorb the antibiotic stress, probably thanks to the β-lactamasome of anaerobes [15]. This underlines their importance in protecting the functions of the microbiota against the deleterious effects of antibiotics and paves the way for the use of either cephalosporinases released in the colon by *Bacteroides* sp [17] or powerful antibiotic adsorbent acting in the late ileum as activated charcoal [34] to combat antibiotic-induced microbial dysbiosis.

Methods And Materials

Study population and sample collection

We analyzed the samples collected during the CEREMI trial (ClinicalTrials.gov identifier NCT02659033), a prospective, randomized open-label clinical trial conducted at the Clinical Investigation Center of the Bichat-Claude Bernard Hospital (Paris, France) from March 2016 to August 2017. The trial was sponsored by Assistance Publique - Hôpitaux de Paris and approved by French Health Authorities and by the Independent Ethics Committee Île-de-France-1. All procedures were conducted in compliance with good clinical practice and the Declaration of Helsinki. Full details of the trial have been reported elsewhere [13].

Briefly, healthy volunteers of both genders aged between 18 and 65-year-old without exposure to antibiotics in the preceding 3 months nor the history of hospitalization in the last 6 months were prospectively included after obtention of their informed consent. A total of 22 healthy volunteers were randomly assigned (1:1 ratio) to receive for three days either 1 gram of ceftriaxone once a day (n = 11) or 1 gram of cefotaxime three times a day (n = 11). Antibiotic treatment was administered as 30-minute intravenous infusions. For each volunteer, 12 faecal samples were collected (Supplementary Figure S1): before the beginning of treatment at days − 15, -7 and − 1; every day during treatment at days 1, 2, and 3, and after the end of treatment at days 4, 7, 10, 15, 30 and 90.

Bacterial counts

Sample collected at days 15, -1, 4, 10, and 30 were analyzed to determine the total bacterial counts (Supplementary Figure S1). Aliquots containing 200 mg of faeces were diluted 200,000 times in a physiological solution (8.5 g/L NaCl). Samples were filtered for debris removal from faecal solutions using a sterile syringe filter (pore size 5 µm; Sartorius Stedim Biotech GmbH, Göttingen, Germany). Then, 1 mL of the microbial cell suspension obtained was stained with 1 µL SYBR Green I (1:100 dilution in dimethylsulfoxide; shaded 15 min incubation at 37°C; 10,000 concentrate, Thermo Fisher Scientific, Waltham, MA, USA). The flow cytometry analysis of the microbial cells present in the suspension was performed using a C6 Fortessa flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Fluorescence events were monitored using the FITC filter 505LP 530/30 nm and perCP filter 635LP 695/40 nm optical detectors. Forward and sideways-scattered light was also collected. The BD Accuri CFlow software was used to gate and separate the microbial fluorescence events on the FL1−FL3 density plot from the faecal sample background. A threshold value of 200 was applied to FSC/SSC light. The gated fluorescence events were evaluated on the forward−sideways density plot, to exclude remaining background events and to obtain an accurate microbial cell count.

Metagenomic analysis of the bacterial microbiome

All samples were analysed through shotgun sequencing for bacterial microbiome analysis.

DNA extraction of stool samples and shotgun sequencing

DNA extraction from aliquots of all faecal samples was performed following IHMS SOP P7 V2 (Supplementary Figure S1) [35]. DNA was quantitated using Qubit Fluorometric Quantitation (ThermoFisher Scientific, Waltham, MA, USA) and qualified using DNA size profiling on a Fragment Analyzer (Agilent Technologies, Santa Clara, CA, USA). Three µg of high molecular weight DNA (> 10 kbp) was used to build the library. Shearing of DNA into fragments of approximately 150 bp was performed using an ultrasonicator (Covaris, Woburn, MA, USA) and DNA fragment library construction was performed using the Ion Plus Fragment Library and Ion Xpress Barcode Adapters Kits (ThermoFisher Scientific, Waltham, MA, USA). Purified and amplified DNA fragment libraries were sequenced using the Ion Proton Sequencer (ThermoFisher Scientific, Waltham, MA, USA), generating 22.2 ± 1.8 million reads of 150 bp (on average) per sample.

Microbial gene count table

To create the gene count table, the METEOR software was used [36]: first, reads were filtered for low-quality by AlienTrimmer [37]. Reads that aligned to the human genome (identity > 95%) were also discarded. The remaining reads were trimmed to 80 bases and mapped to the Integrated Gut Catalogue 2 (IGC2) [38], comprising 10.4 millions genes, using Bowtie2 [39]. The unique mapped reads (reads mapped to a unique gene in the catalogue) were attributed to their corresponding genes. The shared reads (reads that mapped with the same alignment score to multiple genes in the catalogue) were attributed according to the ratio of their unique mapping counts of the captured genes. The resulting count table was further processed using the R package

MetaOMineR v1.31 [40]. To decrease technical bias due to different sequencing depths and avoid any artifacts of sample size on low-abundance genes, read counts were 'rarefied' using 20M high-quality reads (a threshold chosen to include all samples) using a random sampling procedure without replacement. The downsized matrix was finally normalized dividing gene read counts per gene length x100, as a proxy of gene coverage. Since gut microbiota has been found to be enriched in species from the oral cavity upon antibiotic treatment [41], the same process was repeated on an oral microbiota catalogue of 8.4 million genes [42].

Metagenomic Species (MGS) profiles

The IGC2 and the oral catalogues were organized into 1990 and 853 Metagenomic Species (MGS, cluster of co-abundant genes), respectively, using MSPminer [42–44]. After removing duplicated MGS (i.e., MGS present in both catalogues), we were left with 2741 MGS. The relative abundance of an MGS was computed as the mean abundance of its 100 'marker' genes (that is, the genes that correlate the most altogether). If less than 10% of 'marker' genes were seen in a sample, the abundance of the MGS was set to 0. MGS abundance profiles were finally normalized to estimate the proportion of each species in the microbiota (sum of all species abundance = 1).

Bacterial microbiome richness of each sample was evaluated as the number of unique species (MGS) identified. Bacterial microbiome structure is evaluated according to species abundance.

Determination of the Enterobacterales counts

During the CEREMI trial, faecal samples from all volunteers (Supplementary Figure S1) were stored at 4°C after emission and transmitted to the bacteriology laboratory after blinding. One hundred mg of faeces were suspended in 1 mL of brain–heart infusion broth containing 30% glycerol and stored at -80°C. Enterobacterales were counted by plating serial dilutions of broth on Drigalski agar (bioMérieux, Marcy-l'Etoile, France).

Determination of the resistome and β-lactamasome

The IGC2 and the oral catalogues were annotated for the Antibiotic Resistant Determinants (ARD) using a two steps approach. First, potential ARD homologs were selected among catalogue genes using BLASTP against Mustard antibiotic resistance determinant database (http://www.mgps.eu/Mustard) [15]. Genes with ≥ 50% identity for ≥ 90% alignment coverage were selected and tested using pairwise comparative modelling (PCM), a 3-dimensional modelling-based approach [15]. This allowed the identification of a non-redundant list of 19061 ARD from 21 families of which 5 beta-lactamase families: 627 *blaA* genes, 463 *blaB1*, 463 *blaB3*, 181 *blaC* and 89 *blaD*.

The richness of the resistome was evaluated as the number of copies of genes mapping to one of the identified ARD. The relative abundance of the β-lactamasome was computed as the proportion of copies of genes mapping to any beta-lactamase family among all copies of genes mapping to one of the identified ARD.

Determination of the β-lactamase activity

β-lactamase activity of the faecal content was analysed in all samples (Supplementary Figure S1). For extraction of faeces, samples (stored at -65°C) were thawed on ice for 30 min, where after 140–380 mg of faeces material was transferred to a 2-ml Eppendorf tube by means of a spatula. Ice-cold HZn buffer (50 mM (2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.5, supplemented with 50 µM ZnSO4) was then added to the faeces material at a concentration of 5 ml/g faeces. Samples were briefly mixed by means of vortexing and incubated horizontally for 1 hour under mild agitation. Sample were clarified by two centrifugation steps of 15 minutes and 30 minutes (4°C), respectively, in which the supernatant was transferred to a new 2-ml Eppendorf or finally 1.5-ml screw-cap tube.

Assays for determination of β-lactamase activity were performed in HZn buffer using 3–20 µL of freshly clarified faeces sample kept at 4°C. Reactions were carried out in a final volume of 200 µL with 100 µM nitrocefin (Cayman Chemical Company, Ann Arbor, MI, USA). In the first assay, 10 µL of sample was tested for the hydrolysis activity of nitrocefin. This assay was, subsequently, repeated with an adjusted sample volume if necessary. Assays were performed in 96-well microplates (SpectraPlate-96, PerkinElmer, Waltham, MA, USA) using an automated liquid handling Janus Integrator system (PerkinElmer,

Waltham, MA, USA) and nitrocefin hydrolysis was monitored spectrophotometrically at a wavelength of 482 nm (EnVision microplate reader, PerkinElmer, Waltham, MA, USA). All assays always included a buffer control to assess substrate stability.

Metagenomic analysis of the phage microbiome

The phage microbiome was analyzed in samples collected at days 15, -1, 4, 10, and 30 (Supplementary Figure S1). Phage isolation was performed using a polyethylene glycol (PEG) concentration step, as previously recommended [45]. One gram of faecal samples was weighed and homogenized in 40 mL of phosphate buffered saline (PBS) (Sigma-Aldrich, Saint-Louis, MO, USA). The sample was then agitated with a mechanic laboratory agitator for 1 hour at 4°C, centrifuged at 17,000 g for 5 min and the supernatant was filtered at 2 µm and 0.45 µm. Phages were then concentrated using PEG. One molar solid NaCl and 10% (v/v) PEG 8000 (Sigma-Aldrich, Saint-Louis, MO, USA) were dissolved into the filtered culture fluid and incubated overnight at 4°C as recommended for a constant and stable precipitation. The solutions with the phages were pelleted by centrifugation at 5,250 g for 1 hour at 4°C and re-suspended in 500 µL of SM buffer (NaCl 100mM, MgSO4.7H2O 8mM, Tris-Cl 50mM). Samples were treated with 10 U/ml of DNAse (Sigma-Aldrich, Saint-Louis, MO, USA) for 30 min at 37°C followed by 10 min at 65°C to stop the reaction. DNA was then extracted using the commercial kit "Phage DNA extraction" (Norgen biotek Corp, Thorold, ON, Canada). DNA was purified on a sephadex column (Sigma-Aldrich, Saint-Louis, MO, USA), measured with Qubit dsDNA HS Assay kit (ThermoFisher Scientific, Waltham, MA, USA), and sequenced with the Illumina HiSeq2500 PE_250 bases method using the Kit Nextera XT with an input of 1 ng DNA. The sequence reads of the six samples of the same volunteers were pooled. They were trimmed to remove the Illumina adapters and remove low-quality reads using Atropos (v1.1.18) [46] with parameters: atropos trim -m 100 –q 20,20 –trim-n. The resulting reads were assembled using SPAdes 3.15.2 [47] with the metaviralSPAdes mode. At this step we obtained 22 pools of contigs (1 pool per volunteer). Gene prediction was made using Prodigal (v2.6.3) [48] with –p meta option. We excluded genes lacking start and stop codons. In order to focus our analysis on contigs sufficiently large to study genetic contexts, we excluded contigs with less than 3 open reading frames (ORFs).

In order to create a non-redundant catalogue of contigs, the 22 pools of contigs were concatenated and clustered with cd-hit-est (v4.8.1) [49]. The sequence identity threshold was 0.95, the alignment must cover 90% of the shorter sequence and a sequence was clustered into the most similar cluster that meets the threshold. We used viralVerify 1.1 [47] to classify the non-redundant contigs as viral or non-viral, and only viral contig were selected for further analysis. Then, we mapped each sample read on the "viral non-redundant contigs catalog" using bowtie2 (v2.4.2 local –very-fastlocal options) [39] and exploited SAM files with samtools (v1.3.1 with the following commands: views, sort, index, idxstat) [50]. As a result, we obtained a matrix (matrix count) representing the number of reads of a sample (columns) mapping each contig reference catalog (rows) in the dataset. All the matrix counts were rarefied at 3 003 762 reads with the "rarefy" function of the vegan package in R [51].

The phage microbiome richness was computed as the number of phage contigs identified in each sample.

Determination of fungal load and *Candida albicans* DNA concentration

The fungal loads and *Candida albicans* DNA concentration were analysed in all available samples. For each faecal sample, 250 mg were processed using the repeated bead beating plus column protocol described elsewhere [52] (Supplementary Figure S1). A FastPrep-24™ device (MP Biomedicals, Santa Ana, CA, USA) was used instead of a Mini-BeadbeaterTM. Faecal DNA levels were quantified with the Qubit dsDNA Broad Range Kit (Invitrogen, Waltham, MA, USA) and only samples with a concentration above 50 ng/µL were considered in the analysis.

A TaqMan qPCR protocol, using a double dye MGB 5' 6-FAM-labelled probe (Eurogentec, Seraing, Belgium), with the following conditions: 2 min at 50°C, 10 min at 95°C, 15 sec at 95°C and 1 min at 65°C, the last two steps repeated for 45 cycles, was used to measure fungal DNA levels [53]. Samples were processed in two sets of duplicates, in two independent rounds. The fungal load was estimated for each sample as the ratio of the fungal DNA levels to the faecal DNA levels [54].

A TaqMan qPCR protocol in the following conditions: 2 min at 50°C, 10 min at 95°C, 15 sec at 95°C and 1 min at 62°C, the last two steps repeated for 45 cycles, was used to quantify *C. albicans* DNA levels. 7.5 µL of the extracted faecal DNA, at a 1:10 dilution, were used as a template, using probes and primers described by Guiver et al., 2001, at 0.1µM and 0.2 µM, respectively [55]. Samples were processed in two sets of duplicates, in two independent rounds.

The presence of qPCR inhibitors in the samples were verified in all samples, diluted at 1:10, using the Universal Exogenous qPCR Positive Control for TaqMan® Assay (Eurogentec, Seraing, Belgium), with a Cy®5-QXL®670 Probe system (Eurogentec, Seraing, Belgium). Manufacturer's recommendations were followed, using the target Ct > 30 option.

Targeted-metagenomic analysis of the fungal microbiome

All samples were processed to study the fungal microbiota (Supplementary Figure S1). The Internal Transcriber Spacers (ITS) 1 region was targeted for the preparation of the amplicon libraries. The amplicons were produced by PCR using the ITS1F and ITS2 primers in the following conditions [56, 57]: 95°C for 3 min, 25 cycles of 95°C for 30 secs, 55°C for 30 sec and 72°C for 30 sec, 72°C for 5 min and cooling at 4°C, and their size were verified with a Bioanalyzer DNA 1000 chip (Agilent Technologies, Santa Clara, CA, USA). The purification of the amplicon was performed with AMPure XP (Beckman Coulter, Brea, CA, USA) as described in the 16S Metagenomic Sequencing Library Preparation guide [58]. The adapters were attached with the Nextera XT Index Kit (Illumina, San Diego, CA, USA) and index PCRs were done in the following conditions: 95°C for 3 min, 8 cycles of 95°C for 30 secs, 55°C for 30 secs and 72°C for 30 secs, 72°C for 5 min and cooling at 4°C. AMPure XP (Beckman Coulter, Brea, CA, USA) was used to purify the PCR products and a Bioanalyzer DNA 1000 chip allowed their verification and their quantification. Samples concentrations were normalized at 4 nM and 5 µL of each diluted sample were pooled into a library and a PhiX sequencing control was prepared by the manufacturer's guidelines. Libraries were sequenced on Illumina MiSeq platform (Illumina, San Diego, CA, USA) with the MiSeq Reagent Kit V3 in 300bp paired-end.

The sequencing allowed the recovery of 8'819'635 amplicons from ITS1 region. The SHAMAN pipeline was used to remove the singletons and chimera amplicons, resulting in a total of 56'634 amplicons [59]. The remaining amplicons were clustered in 4648 OTUs using a cut-off value of 97% identity. 551 OTUs could be associated to fungal sequences using the Unite database and on these OTUs, 340 were present in at least two faecal samples and were kept for further analysis. A first round of annotation was performed on SHAMAN against the UNITE database (rev. 8.0) and then a second round was performed against a more recent release of UNITE (rev. 8.2). The OTUs that could not be annotated after these two rounds submitted to a classic BLASTN and only hits matched with a similarity above 97% to reference genomes were conserved. The abundances and weighted non-null normalized counts tables were generated with SHAMAN [59].

The richness of the fungal microbiome was computed as the number of unique fungal OTUs identified in each sample.

Non-targeted analysis of the metabolome

The metabolome was analyzed in all collected samples (Supplementary Figure S1). Experimental methods and parameters for the non-targeted approach were carried out by liquid chromatography and high-resolution mass spectrometry (LC-HRMS) as detailed in [60, 61]. Briefly, eight volumes of frozen acetonitrile (-20°C) containing internal standards (labelled IS mix of amino acids at 10 µg/mL) were added to 100 µL serum samples and vortexed. The resulting samples were then sonicated for 10 min and centrifuged for 2 min at 10 000 g at 4°C. Supernatants were incubated at 4°C for 1 hour for a slow protein precipitation process. Samples were centrifuged for 20 min at 20 000 g at 4°C. Supernatants were transferred to another series of tubes and then dried and stored at -80°C before LC-HRMS analyses. Pellets were diluted 3-fold and reconstituted with H2O/ACN (20/80).

Non-targeted approach experiments were performed using a HILIC phase chromatographic column, ZIC-pHILIC 5µm, 2.1 × 150 mm at 15◦C (Merck, Darmstadt, Germany), and on a UPLC Waters Acquity (Waters, Milford, MA, USA) coupled to Q-Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Processing steps were carried out using the R software [62]. Peak detection, correction, alignment and integration were processed using XCMS R package with CentWave algorithm [63, 64] and workflow4metabolomics platforms [65]. The resulted datasets were log10 normalized, filtered and cleaned based on quality control (QC) samples [66]. The features were then putatively annotated based on their mass over charge ratio (m/z) as well as retention time using a local database as described previously [67] and then validated based on MS/MS experiments. The remaining features were either characterized using public repositories [68, 69] or discarded when feature status are still unknown to remove noise and artifact signals. The relative abundance of all annotated chemical features was then summed and computed as a total signal, named 'total useful signal', for each sample. The richness of the metabolome was computed as the number of unique chemical species identified in each sample.

Analysis of the cholesterol conversion rate

The microbiota-dependent catabolism of the cholesterol in faeces was analyzed in all collected samples (Supplementary Figure S1). Sterols and stanols were extracted from faeces as follow. Faeces were weighted (~ 50 mg) and resuspended in 1% formic acid to a final concentration of 167 µg/µL. The mixture was homogenized using a Precellys Evolution instrument (Bertin Instruments, Montigny-le-Bretonneux, France) using the 'soft program'. Volume equivalent to 1 mg of dried faeces was supplemented with deuterated internal standards (cholesterol d7 and coprostanol d5) and sterols and stanols extracted with 1.2 mL of methanol/chloroform (2:1 v/v) and 320 µL deionized water. Phase separation was triggered with 400 µL chloroform and 400 µL water. The mixture was centrifuged for 10 minutes at 3600 g and the lower phase was collected and dried. Sterols and stanols were derivatized for compatibility with GC-MS analysis using 60 µL of BSTFA (with 1% TMCS). The solution was heated at 80°C for 1h, dried and resuspended in 0.1% BSTFA in cyclohexane before injection in the GC-MS. Samples were injected at 250°C in split mode and sterols/stanols separated on a 50mx0.25mm, 0.25µm DB-5MS column. Sterols/stanols were ionized using electronic impact (EI) and analyzed in SIM mode using m/z 329 as quantitative ion and m/z 368 as qualitative ion for cholesterol and m/z 370 and m/z 215 as quantitative and qualitative ions for coprostanol respectively. The rate of cholesterol conversion to coprostanol was computed as the ratio of faecal coprostanol concentration and the sum of faecal coprostanol and cholesterol concentrations.

Analysis of the biliary acid transformation

The metabolism of the biliary acids in faeces was analyzed in all collected samples (Supplementary Figure S1). All chemicals and solvents were of the highest purity available. Cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA), lithocholic acid (LCA), hyocholic acid HCA, hyodeoxicholic acid (HDCA), glyco and tauro derivatives were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). 3α-sulfate derivatives were a generous gift of J. Goto (Niigita University of Pharmacy and Applied Life Science, Niigata, Japan) and 7α-cholic acid (CA-7S) was from Cayman Chemical (Ann Arbor, MI, USA). 23-NOR-5β-cholanoic acid-3α,12α diol, all muricholic acids, glyco, tauro derivatives and iso, keto bile acids were purchased from Steraloids Inc (Newport, RI, USA). Acetic acid, ammonium carbonate, ammonium acetate and methanol were of HPLC grade and purchased from Sigma-Aldrich (Saint Quentin Fallavier, France).

Bile acid molecular species concentrations were measured by HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) as previously described with slight modification [70]. Two microlitres of an internal standard solution (23-nor-5β-cholanoic acid-3α, 12α-diol at 1 mg/ml) was added to 10–50 mg of faeces lyophilized samples using a Lyovapor L200 (Buchi, Villebon-sur-Yvette, France). For 15–20 mg lyophilized faeces samples, 2 ml of NaOH (0.1 M) was added and incubated for 1 h at 60°C before adding 4 ml of water. The solution was homogenized by two 10 s runs in an Ultra-Turrax disperser (IMLAB, Lille, France). The preanalysis cleanup procedure was achieved by centrifugation (12 000 g for 20 min) followed by solid-phase extraction using reversed-phase silica cartridges (HLB Oasis, Waters, Milford, MA, USA) and we used a 5500Q-trap (AB Sciex, Framingham, MA, USA) as mass spectrometer.

The hydrophobicity index reflects BA hydrophobicity, taking into account the concentration and the retention time of different BAs on a C18 column with a methanol gradient; lithocholic acid has the highest retention time, tauroursodeoxycholic acid-3S has the lowest.

The ability of the gut microbiota to metabolize the biliary acids was computed as the ratio of the secondary biliary acids (LCA and DCA) to the total concentration of the faecal content in biliary acids.

Data analysis

Data were classified between 'high-dimensionality' data relative to the taxonomic composition of the bacterial, phage and fungal microbiomes, and metabolome, and 'low-dimensionality' data (richness of the bacterial, phage and fungal microbiomes, richness of the resistome and metabolome, relative abundance of the β-lactamasome, total bacterial counts, β-lactamase activity, fungal load and *Candida albicans* DNA levels, cholesterol conversion rate, and biliary acid transformation).

Baseline was defined at day 0, and the baseline sample was defined as the sample obtained at day − 1. If this sample at day − 1 was not available, the sample obtained at day − 7 was considered as the baseline, or the one obtained at day − 15 if this latter was also missing.

For 'high-dimensionality' data, we computed for each subject the Spearman's correlation coefficient (s) of the taxonomic composition of the studied system between baseline and each sampling day. These correlation coefficients were used to evaluate the change from baseline of the taxonomic composition of the system. Among 'low-dimensionality' data, all variables, except those relative to the richness of the systems, were log10 transformed before analysis, and we computed the change from baseline at each sampling time as the difference of the values at each time.

In order to study the variability between subjects and within subjects for each variable before the administration of antibiotics, we analysed the 'low-dimensionality' data using a linear mixed effect model (lmer function of R package lme4), treating subjects as random effects.

In order to study the perturbation of systems, we computed a raw distance from the baseline, that increases with the extent of the perturbation of each system, regardless of the direction of the perturbation. It was calculated at each sampling time as $1 - s^2$ for 'high-dimensionality' data (with s being the Spearman's correlation coefficient as described above) and as the absolute change from baseline for 'low-dimensionality' data.

Raw distances from baseline were normalized to address the effect of intra-individual variations of the systems before the start of antibiotic treatment. Normalization was made for each subject by dividing distances from baseline by the individual average of the distances from baseline computed before the beginning of antibiotic treatment (at days − 7 and − 15). In the case of missing samples at days − 7 and − 15, the normalization was used as the median of the average raw distances computed for all other subjects.

We studied the effect of antibiotics on the gut content using both fixed endpoints (days 4, 7, 10, 30 and 90) and areas under the curve between baseline and days 10 (AUC_{D0-D10}) and 30 (AUC_{D0-D30}). Metrics used were the changes from baseline for 'low-dimensionality' data and the normalized distances for 'high-dimensionality' data. AUCs were computed using the trapezoidal rule, using the actual date and time of stool emission. AUCs were standardized using the observed delay between baseline and the actual time of collection of the day 10 (for AUC_{D0-D10}) or day 30 (for AUC_{D0-D30}) sample. We used the non-parametric Wilcoxon test to compare these metrics at fixed sampling times, the AUC_{D0-D10}, and the AUC_{D0-D30} to 0 for 'low-dimensionality' data, and to 1, 10 or 30 for 'high-dimensionality' data and their AUC_{D0-D10} AUC_{D0-D30}, respectively. We also compared the effect of the two antibiotics on the microbiota using the non-parametric Wilcoxon test. All statistical tests were bilateral, with a type-I error fixed to 0.05.

Next, we defined for each subject and system the maximal perturbation as the maximal normalized distance from baseline observed between the baseline and day 10, and maximal resilience as the minimal normalized distance from baseline observed on days 15, 30 or 90. Pairwise relations between the level of maximal perturbation for each system was investigated using Spearman's correlation coefficients and comparing them to 0. A similar analysis was performed to study the relationship between the maximal resilience of systems.

Finally, the relationship between the composition of the microbiome at baseline (studied using the 'low-dimensionality' data) and the maximal perturbation and resilience of studied systems was assessed using the Spearman's correlation coefficient and its test to 0.

Declarations

Ethics approval and consent to participate

The CEREMI trial obtained approval from the Independent Ethics Committee "Île-de-France 1" on 12/21/2015 (2015-oct-14028) and from the National Agency for Security of Medicinal Products on 07/24/2015 (150527A-41). All participants received oral

and written information, and provided signed consent before inclusion.

Consent for publication

Not applicable

Availability of data and material

The metagenomic shotgun sequencing data of bacterial microbiome are available from the European Nucleotide Archive (EMBL-EBI) under accession number PRJEB58157.

The metagenomic shotgun sequencing data of phage microbiome are available from the European Nucleotide Archive (EMBL-EBI) under accession number PRJEB58823.

The other datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests

All authors have no competing interest to declare regarding the present work

Funding

The CEREMI clinical trial was funded by the Contrat de Recherche Clinique 2013 (Assistance Publique-Hôpitaux de Paris, Département de la Recherche Clinique et du Développement, CRC13-179). The PrediRes project was funded by a grant from the French National Research Agency (ANR-16-CE15-0022-01). Analysis of the fungal microbiome was funded by grants from the French National Research Agency (FunComPath ANR-14-IFEC-0004) and the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie action, Innovative Training Network (FunHoMic; grant N° 812969). High-throughput sequencing of phage DNA was performed on the Institut Pasteur Genomics Platform, member of "France Génomique" consortium (ANR10-INBS-09-08). Analysis of the microbiome and resistome was partly funded by the Metagenopolis grant (ANR-11-DPBS-0001).

Authors' contributions

CB, FM, OT, ED, XD, DE, ER and MEB obtained funding. LA, FL, NGa, BQ, NP, DE, ELC analyzed the bacterial microbiome. NGr, MM and ED analysed the Enterobacterales. SD and JDD analyzed the beta lactamase activity. CH, MT, AG and ER analyzed the phage microbiome. MD, NS and MEB analyzed the fungal microbiome, the fungal load and *C. albicans* DNA level. PL, ML, FI and MP analyzed the cholesterol metabolism and performed the nontargeted analysis of the metabolome. DR, AL and EG analyzed the bile acids. CB, CH, MD, LA, FI, ELC and FM analyzed the data. All authors read and approved the final manuscript.

Acknowledgments

Composition of the PrediRes study group:

Scientific council: Charles Burdet (INSERM, Univ Paris Diderot, APHP - Bichat Hospital), Erick Denamur (INSERM, Univ Paris Diderot), Xavier Duval (INSERM, Univ Paris Diderot, APHP - Bichat Hospital), Dusko Ehrlich (INRA Metagenopolis), France Mentré (INSERM, Univ Paris Diderot, APHP - Bichat Hospital), Eduardo P. C. Rocha (Institut Pasteur).

Metagenomic analyses : Laurie Alla (INRAE Metagenopolis), Dusko Ehrlich (INRAE Metagenopolis), Emmanuelle Le Chatelier (INRAE Metagenopolis), Florence Levenez (INRAE Metagenopolis), Nathalie Galleron (INRAE Metagenopolis), Nicolas Pons (INRAE Metagenopolis), Benoît Quinquis (INRAE Metagenopolis).

E. coli analyses: Khadija Bourabha (INSERM), Antoine Bridier Nahmias (INSERM, Univ Paris Diderot), Olivier Clermont (INSERM, Univ Paris Diderot), Erick Denamur (INSERM, Univ Paris Diderot), Mélanie Magnan (INSERM, Univ Paris Diderot), Olivier Tenaillon

(INSERM, Univ Paris Diderot).

Phage analyses : Camille d'Humières (INSERM, Univ Paris Diderot, APHP - Bichat Hospital, Institut Pasteur), Eduardo Rocha (Institut Pasteur), Marie Touchon (Institut Pasteur).

Bile acids : Dominique Rainteau (INSERM, Univ Pierre et Marie Curie, APHP – Saint Antoine Hospital), Antonin Lamazière (INSERM, Univ Pierre et Marie Curie, APHP – Saint Antoine Hospital), Emilie Gauliard (INSERM, Univ Pierre et Marie Curie, APHP – Saint Antoine Hospital)

Untargeted and other targeted metabolomics: Farid Ichou (ICAN), Philippe Lesnik (ICAN), Marie Lhomme (ICAN)

Statistical analyses: Charles Burdet (INSERM, Univ Paris Diderot, APHP - Bichat Hospital), France Mentré (INSERM, Univ Paris Diderot, APHP - Bichat Hospital), Jimmy Mullaert (INSERM, Univ Paris Diderot, APHP - Bichat Hospital), Thu Thuy Nguyen (INSERM).

References

1. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell. 2006;124:837–48.
2. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505:559–63.
3. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y-Y, Keilbaugh SA, et al. Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. Science. 2011;334:105–8.
4. Sommer F, Bäckhed F. The gut microbiota — masters of host development and physiology. Nat Rev Microbiol. 2013;11:227–38.
5. Anthony WE, Wang B, Sukhum KV, D'Souza AW, Hink T, Cass C, et al. Acute and persistent effects of commonly used antibiotics on the gut microbiome and resistome in healthy adults. Cell Rep. 2022;39:110649.
6. Palleja A, Mikkelsen KH, Forslund SK, Kashani A, Allin KH, Nielsen T, et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. Nat Microbiol. 2018;3:1255–65.
7. Nguyen TT, Guedj J, Chachaty E, de Gunzburg J, Andremont A, Mentré F. Mathematical modeling of bacterial kinetics to predict the impact of antibiotic colonic exposure and treatment duration on the amount of resistant enterobacteria excreted. PLoS Comput Biol. 2014;10:e1003840.
8. Lim MY, Cho Y, Rho M. Diverse Distribution of Resistomes in the Human and Environmental Microbiomes. Curr Genomics. 2018;19:701–11.
9. Carlet J. The gut is the epicentre of antibiotic resistance. Antimicrob Resist Infect Control. 2012;1:39.
10. Lamichhane S, Sen P, Dickens AM, Orešič M, Bertram HC. Gut metabolome meets microbiome: A methodological perspective to understand the relationship between host and microbe. Methods San Diego Calif. 2018;149:3–12.
11. Forslund SK, Chakaroun R, Zimmermann-Kogadeeva M, Markó L, Aron-Wisnewsky J, Nielsen T, et al. Combinatorial, additive and dose-dependent drug-microbiome associations. Nature. 2021;600:500–5.
12. Haak BW, Argelaguet R, Kinsella CM, Kullberg RFJ, Lankelma JM, Deijs M, et al. Integrative Transkingdom Analysis of the Gut Microbiome in Antibiotic Perturbation and Critical Illness. mSystems. 2021;6:e01148-20.
13. Burdet C, Grall N, Linard M, Bridier-Nahmias A, Benhayoun M, Bourabha K, et al. Ceftriaxone and Cefotaxime Have Similar Effects on the Intestinal Microbiota in Human Volunteers Treated by Standard-Dose Regimens. Antimicrob Agents Chemother. 2019;63:02244-18.
14. Léonard F, Andremont A, Leclerq B, Labia R, Tancrède C. Use of beta-lactamase-producing anaerobes to prevent ceftriaxone from degrading intestinal resistance to colonization. J Infect Dis. 1989;160:274–80.
15. Ruppé E, Ghozlane A, Tap J, Pons N, Alvarez A-S, Maziers N, et al. Prediction of the intestinal resistome by a three-dimensional structure-based method. Nat Microbiol. 2019;4:112–23.

16. Stiefel U, Tima MA, Nerandzic MM. Metallo-β-Lactamase-Producing Bacteroides Species Can Shield Other Members of the Gut Microbiota from Antibiotics. Antimicrob Agents Chemother. 2015;59:650–3.
17. Stentz R, Horn N, Cross K, Salt L, Brearley C, Livermore DM, et al. Cephalosporinases associated with outer membrane vesicles released by Bacteroides spp. protect gut pathogens and commensals against β-lactam antibiotics. J Antimicrob Chemother. 2015;70:701–9.
18. Dethlefsen L, Huse S, Sogin ML, Relman DA. The Pervasive Effects of an Antibiotic on the Human Gut Microbiota, as Revealed by Deep 16S rRNA Sequencing. Eisen JA, editor. PLoS Biol. 2008;6:e280.
19. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci. 2011;108:4554–61.
20. Manrique P, Bolduc B, Walk ST, van der Oost J, de Vos WM, Young MJ. Healthy human gut phageome. Proc Natl Acad Sci U S A. 2016;113:10400–5.
21. Górska A, Peter S, Willmann M, Autenrieth I, Schlaberg R, Huson DH. Dynamics of the human gut phageome during antibiotic treatment. Comput Biol Chem. 2018;74:420–7.
22. Fernández-Orth D, Miró E, Brown-Jaque M, Rodríguez-Rubio L, Espinal P, Rodriguez-Navarro J, et al. Faecal phageome of healthy individuals: presence of antibiotic resistance genes and variations caused by ciprofloxacin treatment. J Antimicrob Chemother. 2019;74:854–64.
23. Shaw LP, Bassam H, Barnes CP, Walker AS, Klein N, Balloux F. Modelling microbiome recovery after antibiotics using a stability landscape framework. ISME J. 2019;13:1845–56.
24. Delavy M, Burdet C, Sertour N, Devente S, Docquier J-D, Grall N, et al. A Clinical Study Provides the First Direct Evidence That Interindividual Variations in Fecal β-Lactamase Activity Affect the Gut Mycobiota Dynamics in Response to β-Lactam Antibiotics. mBio. 2022;e0288022.
25. Dollive S, Chen Y-Y, Grunberg S, Bittinger K, Hoffmann C, Vandivier L, et al. Fungi of the Murine Gut: Episodic Variation and Proliferation during Antibiotic Treatment. Jacobsen ID, editor. PLoS ONE. 2013;8:e71806.
26. Fan D, Coughlin LA, Neubauer MM, Kim J, Kim MS, Zhan X, et al. Activation of HIF-1α and LL-37 by commensal bacteria inhibits Candida albicans colonization. Nat Med. 2015;21:808–14.
27. Mirhakkak MH, Schäuble S, Klassert TE, Brunke S, Brandt P, Loos D, et al. Metabolic modeling predicts specific gut bacteria as key determinants for Candida albicans colonization levels. ISME J. 2021;15:1257–70.
28. Mittal R, Debs LH, Patel AP, Nguyen D, Patel K, O'Connor G, et al. Neurotransmitters: The Critical Modulators Regulating Gut–Brain Axis. J Cell Physiol. 2017;232:2359–72.
29. Fiore A, Murray PJ. Tryptophan and indole metabolism in immune regulation. Curr Opin Immunol. 2021;70:7–14.
30. Roager HM, Licht TR. Microbial tryptophan catabolites in health and disease. Nat Commun. 2018;9:3294.
31. Antunes LCM, Han J, Ferreira RBR, Lolić P, Borchers CH, Finlay BB. Effect of antibiotic treatment on the intestinal metabolome. Antimicrob Agents Chemother. 2011;55:1494–503.
32. Lu S, Huang Q, Wei B, Chen Y. Effects of β-Lactam Antibiotics on Gut Microbiota Colonization and Metabolites in Late Preterm Infants. Curr Microbiol. 2020;77:3888–96.
33. Hashimoto S, Igimi H, Uchida K, Satoh T, Benno Y, Takeuchi N. Effects of beta-lactam antibiotics on intestinal microflora and bile acid metabolism in rats. Lipids. 1996;31:601–9.
34. de Gunzburg J, Ghozlane A, Ducher A, Le Chatelier E, Duval X, Ruppé E, et al. Protection of the Human Gut Microbiome From Antibiotics. J Infect Dis. 2018;217:628–36.
35. Doré J, Ehrlich SD, Levenez F, Pelletier E, Alberti A, Bertrand L, et al. IHMS_SOP 07 V1: Standard operating procedure for fecal samples DNA extraction, Protocol H. [Internet]. 2015 [cited 2021 Oct 18]. Available from: http://www.microbiome-standards.org
36. Pons N, Batto J-M, Kennedy S, Almeida M, Boumezbeur F, Moumen B, et al. METEOR -a plateform for quantitative metagenomic profiling of complex ecosystems. 2010. Available at: https://www.researchgate.net/profile/Pierre-Leonard/publication/264156450_METEOR_-

a_plateform_for_quantitative_metagenomic_profiling_of_complex_ecosystems/links/53cfbe870cf2fd75bc59f034/METEOR-a-plateform-for-quantitative-metagenomic-profiling-of-complex-ecosystems.pdf

37. Criscuolo A, Brisse S. AlienTrimmer: a tool to quickly and accurately trim off multiple short contaminant sequences from high-throughput sequencing reads. Genomics. 2013;102:500–6.
38. Wen C, Zheng Z, Shao T, Liu L, Xie Z, Le Chatelier E, et al. Quantitative metagenomics reveals unique gut microbiome biomarkers in ankylosing spondylitis. Genome Biol. 2017;18:142.
39. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9.
40. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;500:541–6.
41. Hildebrand F, Moitinho-Silva L, Blasche S, Jahn MT, Gossmann TI, Huerta-Cepas J, et al. Antibiotics-induced monodominance of a novel gut bacterial order. Gut. 2019;68:1781.
42. Le Chatelier E, Almeida M, Plaza Oñate F, Pons N, Gauthier F, Ghozlane A, et al. A catalog of genes and species of the human oral microbiota [Internet]. Portail Data INRAE; 2021 [cited 2021 Oct 18]. Available from: https://data.inrae.fr/citation?persistentId=doi:10.15454/WQ4UTV
43. Plaza Onate F, Pons N, Gauthier F, Almeida M, Ehrlich SD, Le Chatelier E. Updated Metagenomic Species Pan-genomes (MSPs) of the human gastrointestinal microbiota [Internet]. Portail Data INRAE; 2021 [cited 2021 Oct 18]. Available from: https://data.inrae.fr/citation?persistentId=doi:10.15454/FLANUP
44. Plaza Oñate F, Le Chatelier E, Almeida M, Cervino ACL, Gauthier F, Magoulès F, et al. MSPminer: abundance-based reconstitution of microbial pan-genomes from shotgun metagenomic data. Bioinforma Oxf Engl. 2019;35:1544–52.
45. d'Humières C, Touchon M, Dion S, Cury J, Ghozlane A, Garcia-Garcera M, et al. A simple, reproducible and cost-effective procedure to analyse gut phageome: from phage isolation to bioinformatic approach. Sci Rep. 2019;9:11331.
46. Didion JP, Martin M, Collins FS. Atropos: specific, sensitive, and speedy trimming of sequencing reads. PeerJ. 2017;5:e3720.
47. Antipov D, Raiko M, Lapidus A, Pevzner PA. Metaviral SPAdes: assembly of viruses from metagenomic data. Bioinforma Oxf Engl. 2020;36:4126–9.
48. Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010;11:119.
49. Li W, Godzik A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinforma Oxf Engl. 2006;22:1658–9.
50. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinforma Oxf Engl. 2009;25:2078–9.
51. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. Community Ecology Package [Internet]. 2020 [cited 2021 Oct 4]. Available from: https://cran.r-project.org/web/packages/vegan/vegan.pdf
52. Yu Z, Morrison M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. BioTechniques. 2004;36:808–12.
53. Liu CM, Kachur S, Dwan MG, Abraham AG, Aziz M, Hsueh P-R, et al. FungiQuant: A broad-coverage fungal quantitative real-time PCR assay. BMC Microbiol. 2012;12:255.
54. Zuo T, Wong SH, Cheung CP, Lam K, Lui R, Cheung K, et al. Gut fungal dysbiosis correlates with reduced efficacy of fecal microbiota transplantation in Clostridium difficile infection. Nat Commun. 2018;9:3663.
55. Guiver M. Rapid identification of candida species by TaqMan PCR. J Clin Pathol. 2001;54:362–6.
56. Cui L, Morris A, Ghedin E. The human mycobiome in health and disease. Genome Med. 2013;5:63.
57. Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol Ecol. 1993;2:113–8.
58. Illumina. 16S Metagenomic Sequencing Library Preparation [Internet]. [cited 2021 Aug 25]. Available from: https://emea.support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html

59. Volant S, Lechat P, Woringer P, Motreff L, Campagne P, Malabat C, et al. SHAMAN: a user-friendly website for metataxonomic analysis from raw reads to statistical analysis. BMC Bioinformatics. 2020;21:345.
60. Garali I, Adanyeguh IM, Ichou F, Perlbarg V, Seyer A, Colsch B, et al. A strategy for multimodal data integration: application to biomarkers identification in spinocerebellar ataxia. Brief Bioinform. 2018;19:1356–69.
61. Aron-Wisnewsky J, Prifti E, Belda E, Ichou F, Kayser BD, Dao MC, et al. Major microbiota dysbiosis in severe obesity: fate after bariatric surgery. Gut. 2019;68:70–82.
62. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. R Foundation for Statistical Computing; 2021. Available from: https://www.R-project.org/
63. Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Anal Chem. 2006;78:779–87.
64. Tautenhahn R, Patti GJ, Rinehart D, Siuzdak G. XCMS Online: a web-based platform to process untargeted metabolomic data. Anal Chem. 2012;84:5035–9.
65. Giacomoni F, Le Corguillé G, Monsoor M, Landi M, Pericard P, Pétéra M, et al. Workflow4Metabolomics: a collaborative research infrastructure for computational metabolomics. Bioinforma Oxf Engl. 2015;31:1493–5.
66. Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nat Protoc. 2011;6:1060–83.
67. Boudah S, Olivier M-F, Aros-Calt S, Oliveira L, Fenaille F, Tabet J-C, et al. Annotation of the human serum metabolome by coupling three liquid chromatography methods to high-resolution mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2014;966:34–47.
68. Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R, et al. HMDB 4.0: the human metabolome database for 2018. Nucleic Acids Res. 2018;46:D608–17.
69. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 2017;45:D353–61.
70. Humbert L, Maubert MA, Wolf C, Duboc H, Mahé M, Farabos D, et al. Bile acid profiling in human biological samples: Comparison of extraction procedures and application to normal and cholestatic patients. J Chromatogr B. 2012;899:135–45.

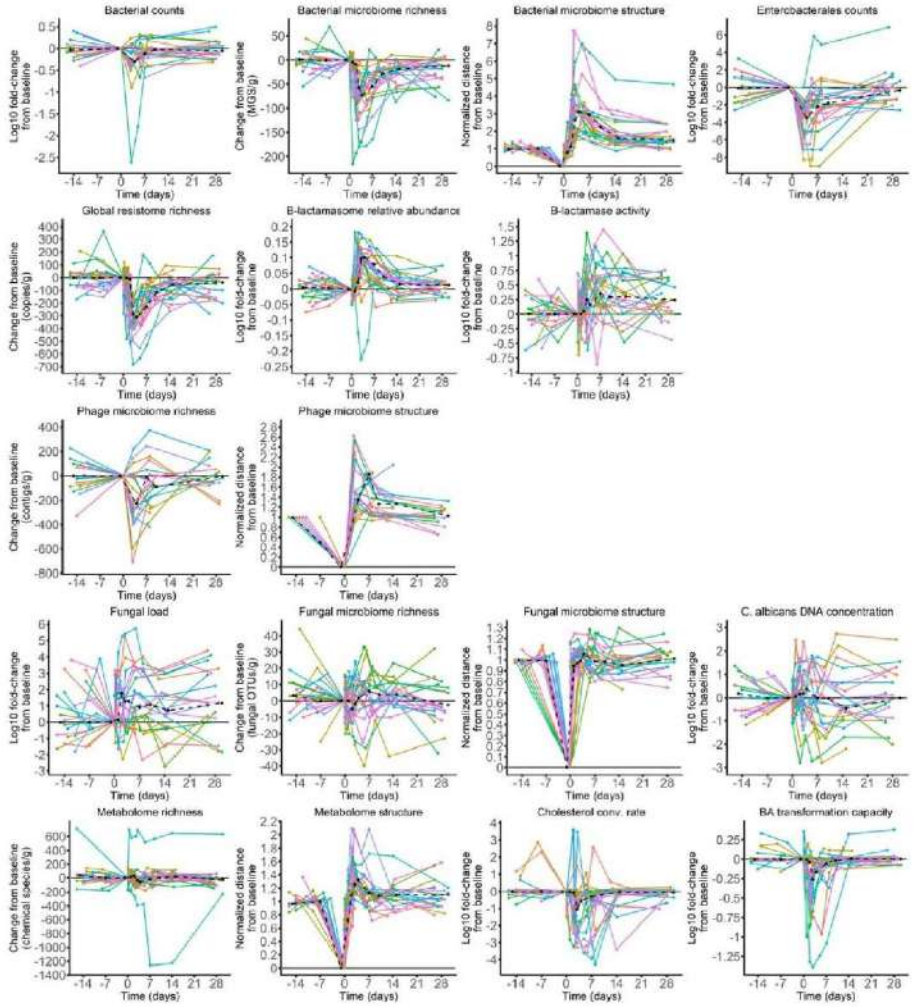

Bacterial counts
Log10 fold-change from baseline
Bacterial microbiome richness
Change from baseline (MGS/g)
Bacterial microbiome structure
Normalized distance from baseline
Enterobacterales counts
Log10 fold-change from baseline
Global resistome richness
Change from baseline (copies/g)
B-lactamasome relative abundance
Log10 fold-change from baseline
B-lactamase activity
Log10 fold-change from baseline
Phage microbiome richness
Change from baseline (contigs/g)
Phage microbiome structure
Normalized distance from baseline
Fungal load
Log10 fold-change from baseline
Fungal microbiome richness
Change from baseline (fungal OTUs/g)
Fungal microbiome structure
Normalized distance from baseline
C. albicans DNA concentration
Log10 fold-change from baseline
Metabolome richness
Change from baseline (chemical species/g)
Metabolome structure
Normalized distance from baseline
Cholesterol conv. rate
Log10 fold-change from baseline
BA transformation capacity
Log10 fold-change from baseline
Time (days)

Figure 1

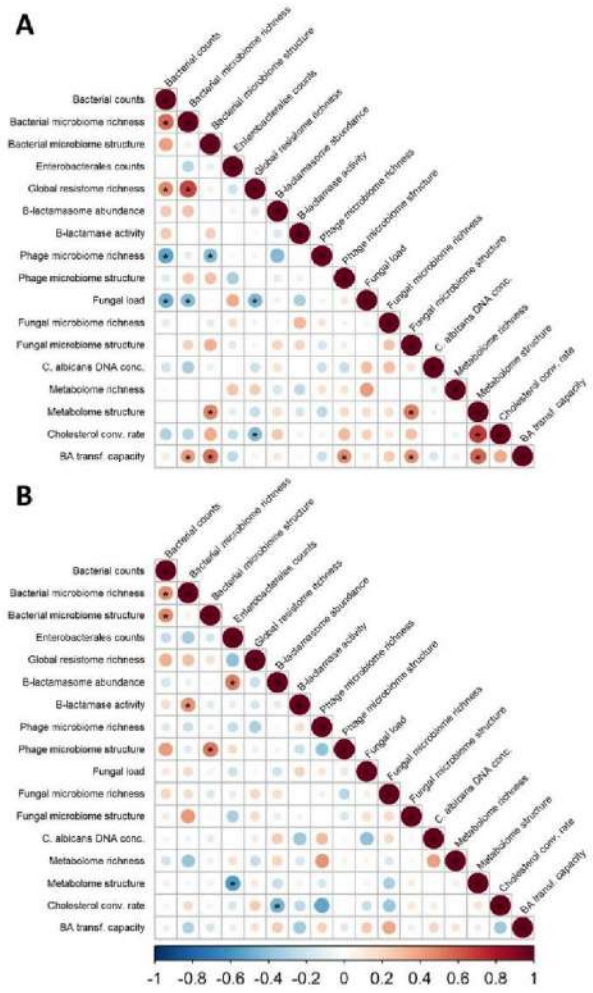

A
Bacterial counts
Bacterial microbiome richness
Bacterial microbiome structure
Enterobacterales counts
Global resistome richness
B-lactamasome abundance
B-lactamase activity
Phage microbiome richness
Phage microbiome structure
Fungal load
Fungal microbiome richness
Fungal microbiome structure
C. albicans DNA conc.
Metabolome richness
Metabolome structure
Cholesterol conv. rate
BA transf. capacity
B
-1 -0.8 -0.6 -0.4 -0.2 0 0.2 0.4 0.6 0.8 1

Figure 2

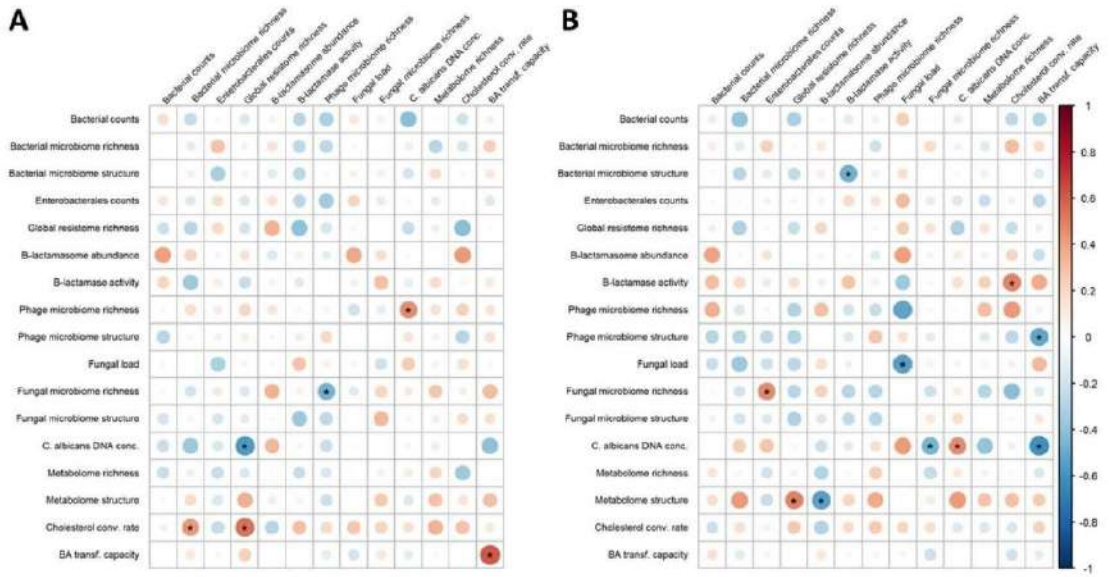


Figure 3

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 20230313SuppMat.docx