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Review

Metagenomics and metabolomics approaches in the study of *Candida albicans* colonization of host niches: a framework for finding microbiome-based antifungal strategies

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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 fungal 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.**

Highlights

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

Seventy-five percent of women suffer 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.

The development of experimental platforms has allowed the validation and screening of new anti-*C. albicans* bacteria.

Interactions between *C. albicans* and the microbiota

C. albicans is an opportunistic fungal pathogen that causes superficial infections such as vaginal candidiasis which affects 75% of women during their lifetime [1,2]. When the host's defenses are compromised, as in immunocompromised patients, *C. albicans* can cause systemic infections, associated with a mortality of 30–40% [3–5].

However, *C. albicans* is also a commensal yeast that colonizes the gastrointestinal (GI) tract of up to 95% of the healthy population [6]. It belongs to the healthy oral, vaginal, and intestinal human microbiota and shares a niche with thousands of bacterial species [7], some of which can prevent infections by various pathogens [8,9]. For example, *Clostridium difficile* infections, which are caused by the overgrowth of this pathogen in the human gut [10–12], can be treated with fecal microbiota transplantation, an approach that has shown encouraging results for the recovery of a healthy microbiota in these patients (for review, see [12]). Another example is the *Escherichia coli* strain Nissle 1917, which has been used for years as a probiotic to inhibit the growth of opportunistic pathogens, including members from the genus *Salmonella* [8,9,13]. Bacteria from the gut microbiota also act as a fungal growth regulator, the depletion of the intestinal bacterial microbiota by broad-spectrum antibiotics resulting in an increase in the fungal burden and *C. albicans* carriage in mouse urine and the human gut [6,14,15]. Specific bacteria have been shown to modulate *C. albicans* growth (for review, see [16]). Especially, *Lactobacillus rhamnosus* strain Lcr35 inhibits *C. albicans* growth *in vitro* and *in vivo*[i] [17–19]. 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 [4].

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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.

Potential anti-*C. albicans* bacterial species identified by metabolomics and next-generation sequencing approaches

Two main types of OMICs-based approaches have been used to identify bacterial species associated with *C. albicans* carriage: (i) sequencing-based approaches that rely on sequencing data and consist of associating *C. albicans* carriage with the relative abundance of the bacterial species present in the same niche [15,20,21], and (ii) metabolomics-based approaches, in which *C. albicans* carriage or metabolic profile is associated with the metabolome of the microbial population present in the same environment [22,23]. 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 internal transcribed spacer (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 (Figure 1).

Shotgun sequencing offers a deep taxonomic resolution, allowing an easier distinction between microbial species and strains, but at a high cost [24,25] and although some recent studies have proposed new pipelines, shotgun metagenomics is still at a developing stage for the characterization of the fungal microbiota [20,26]. Amplicon-based sequencing is cheaper but returns less accurate results. Moreover, the amplification of 18S and ITS regions can introduce analysis bias since the rDNA copy number is highly variable across the fungal kingdom, leading to an uneven quantification of the different fungal species [27]. Such variation in rDNA copy number has also been observed across bacterial taxa but to a much lower extent [28]. In the second approach, metabolomics profiles are obtained experimentally [15,29,30] or predicted *in silico* using genome-scale metabolic models (GEMs), a computational description of the metabolic pathways of given organisms [22,31]. GEMs have been constructed and experimentally validated for hundreds of bacteria, archaea, and eukaryotes [22,32].

The Xie and Manichanh study [20] 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 interkingdom association network, using SparCC correlations [33]. They identified 20 bacterial species negatively associated with *C. albicans* in the Spanish cohort and 17 in the Danish cohort. Notably, in the Spanish cohort, *C. albicans* was strongly negatively correlated with *Bifidobacterium scardovii*, *Desulfovibrio fairfieldensis*, *Ruminococcus* sp. *CAG563*, *Coprococcus catus*, and *Roseburia* sp. *CAG309,* most of these bacteria being probable producers of short-chain fatty acids (SCFAs). 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 [15]. First, the authors integrated shotgun and ITS-targeted sequencing and created a co-abundance network, using BAnOCC [34], a Bayesian method, to study the inter- and cross-kingdom interactions of the human gut upon various antibiotic treatments. They identified three

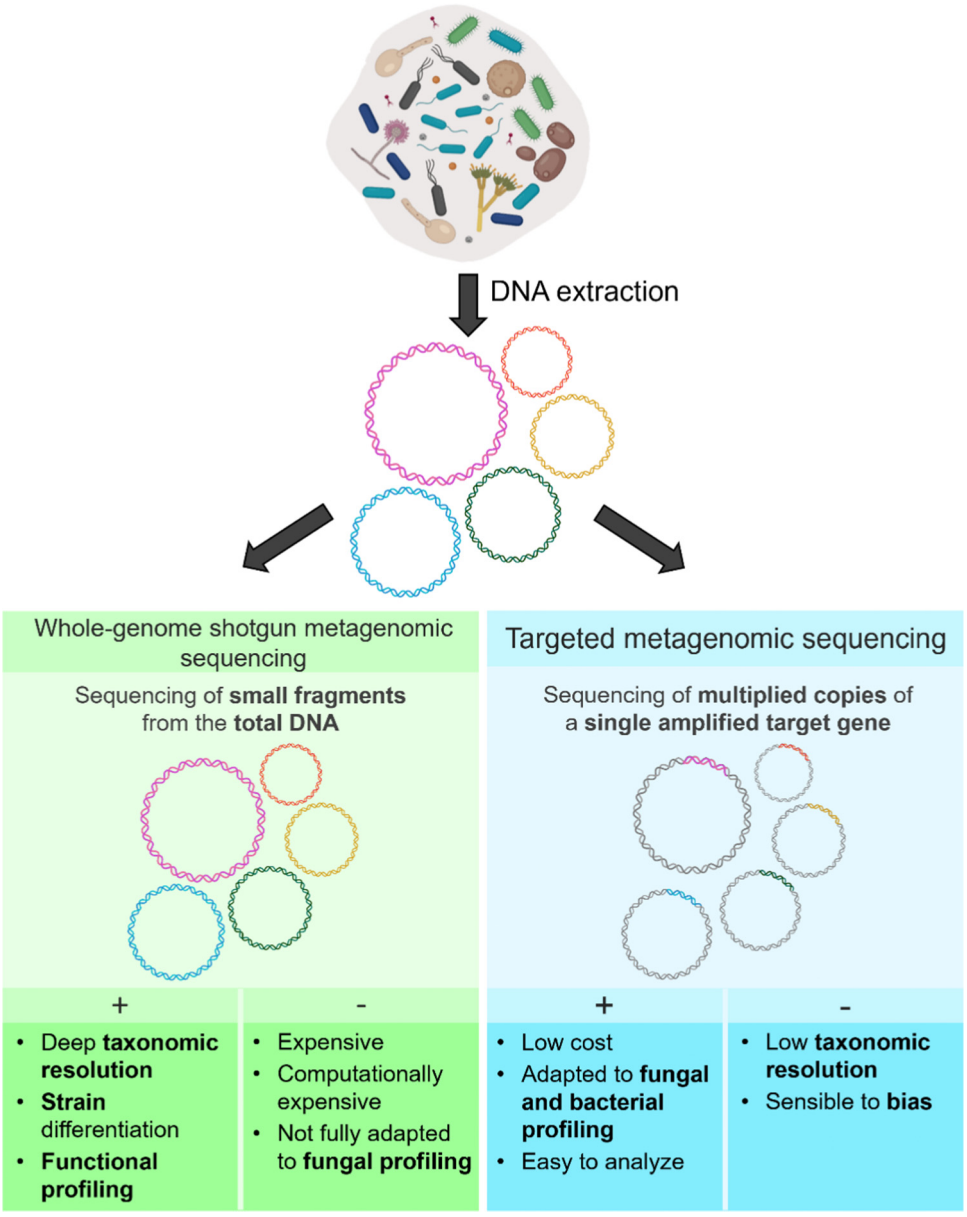

Figure 1. Comparison of whole-genome shotgun metagenomic sequencing and targeted metagenomic sequencing. Whole-genome shotgun sequencing consists in sequencing the full genomes present in the microbiota, whereas targeted metagenomic sequencing consists in the amplification and subsequent sequencing of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

bacterial species negatively associated with *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 eggerthii, Alistipes obesi, O. splanchnicus, Coprococcus comes, R. inulinivorans*, and *E. rectale*.

By contrast, Mirhakkak *et al.* identified bacteria of interest by implementing a metabolomics-based approach complemented with a sequencing analysis [22]. 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 genera *Bifidobacterium* and *Listeria* and species from the phylum Bacteriodetes, including *Alistipes putredinis.* To strengthen their findings, they performed metagenomics analyses of the gut microbiota of 24 cancer 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 *A. putredinis.*

Although several potential anti-*C. albicans* bacteria were identified in each of the studies presented above (Figure 2), there is a poor overlap between the studies. This might be explained by the different techniques 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. All of the studies presented above used shotgun sequencing to establish the subject's bacterial taxonomic profiles. However, they used different sequencing approaches to quantify *C. albicans* relative abundance. Seelbinder and colleagues and Mirhakkak and colleagues used ITS1- and ITS2-targeted sequencing, respectively, while Xie and Manichanh inferred *C. albicans* abundance in the samples using shotgun sequencing. The selection of a target region in amplicon sequencing has been shown to introduce taxonomic bias. Indeed, ITS1 sequencing tends to amplify basidiomycetes more easily, whereas ITS2 sequencing tends to favor the amplification of ascomycetes [35,36]. Moreover, it has been recently shown that ITS sequencing and shotgun sequencing result in vastly different fungal profiles, with shotgun sequencing being unable to identify most of the fungal species identified by ITS sequencing [37]. Fungi-specific pipelines can increase the amount of fungal sequences recovered from shotgun sequencing but they still need optimization to reach the levels usually recovered with ITS sequencing [20,37]. This is likely due to the low proportion of fungi among the microbiome that can be tedious to detect without a prior amplification. 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 ones identified in the study conducted by Seelbinder *et al.,* which used ITS-targeted sequencing to quantify *C. albicans* burden.

In addition, the sequencing-based approaches used by Xie and Manichanh and Seelbinder and colleagues are based on co-abundance analyses [15,20]. 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 [15,20,22], 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,

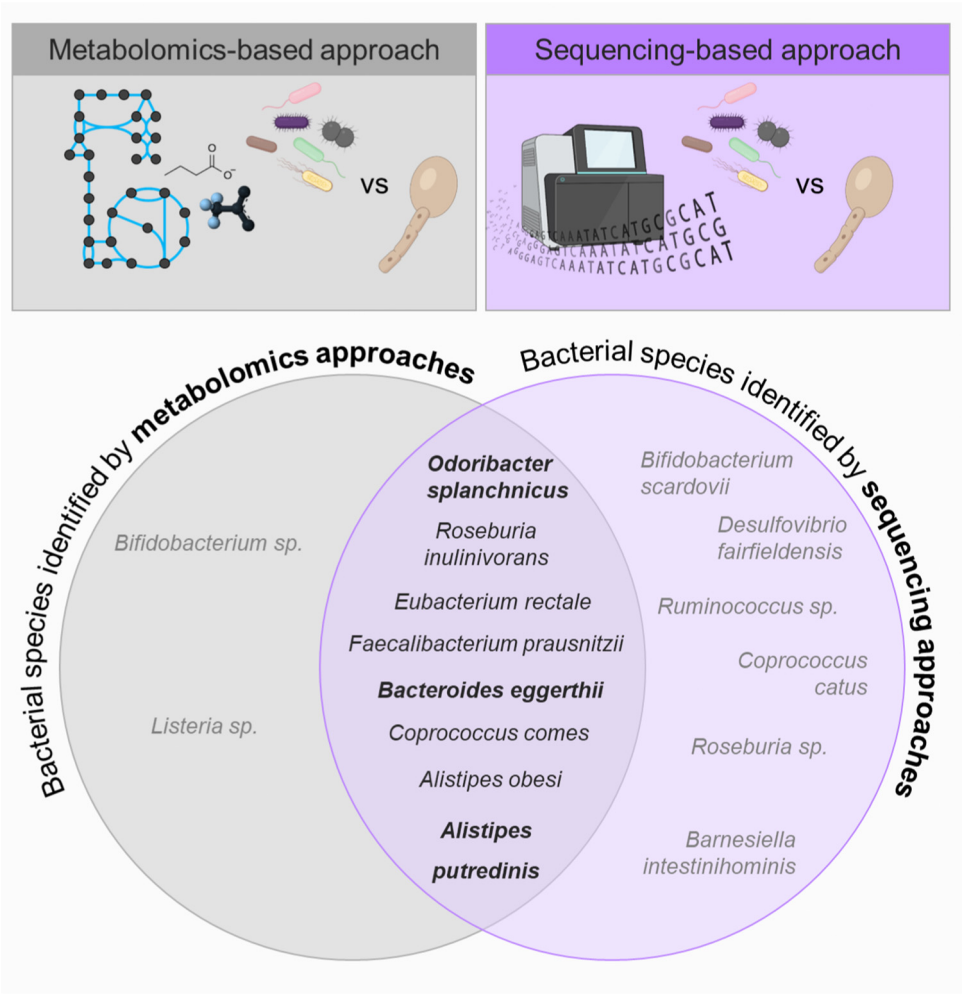

Figure 2. Anti-*Candida albicans* signatures identified by metabolomic-based and sequencing-based approaches. The bacterial species identified only by a sequencing-based analysis are represented in the right circle, the bacterial species identified only by a metabolomic-based analysis are represented 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 was identified in two of the presented studies.

respectively. Since antibiotics kill the resident bacteria of the gut, a part of the negative associations between bacterial species and *C. albicans* might result 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 [38,39], 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 the Mirhakkak's study highlighted the metabolic interactions between

the intestinal bacteria and *C. albicans* [22]. 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.

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

The validation of the potential antagonistic activity of a bacterium on *C. albicans* depends on the inhibition mechanism. Therefore, it is crucial to understand the many ways by which a bacterium can exhibit antifungal activities before attempting this validation.

Bacteria can inhibit *C. albicans* growth through the release of metabolites, such as SCFAs [40,41]. The decrease of bacteria-derived SCFAs after antibiotic exposure has been associated with an increase in *C. albicans* intestinal carriage [41]. Butyrate, especially, impairs *C. albicans* growth [40] and hyphae formation [42]. Besides SCFAs, 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 [43–45]. Besides metabolites, bacteria can also release proteins that can directly act against *C. albicans*. For instance, *Enterococcus faecalis* has been shown to inhibit *C. albicans* hyphal morphogenesis, biofilm formation, and virulence, through the release of EntV, a bacteriocin and antimicrobial peptide [46,47].

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 [48,49]. Therefore, specific bacteria might modulate the host immune response against *C. albicans*, leading to a limitation of its growth. SCFAs can modulate host inflammation and promote immune cells' recruitment and maturation, leading to *C. albicans* reduced survival [40]. 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 [21]. Alternatively, *Lactobacillus crispatus* activates the epithelial immune response against *C. albicans* by modulating the expressions of Toll-like receptors (TLRs) 2 and 4 in epithelial cells, thus inducing the production of cytokines and β-defensins [50].

Besides releasing molecules 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. *L. rhamnosus* thus protects the host against *C. albicans* infections via a competition for carbon and nitrogen sources [51,52], and by blocking adhesion sites, thus reducing *C. albicans* ability to bind to epithelial cells [52]. For a more detailed overview on the role of fungal–bacterial interactions in mediating *C. albicans* colonization and virulence, see [53].

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

Considering the wide variety of ways by which commensal bacteria can modulate *C. albicans* growth, the choice of a validation assay is crucial in order to assess reliably the anti-*C. albicans* activity of a bacterial species. In this section, 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* [15,22,54,55] or other fungal [56] and bacterial [56,57] species. In particular, Walker and colleagues have recently shown the anti-*C. albicans* activity of *Bifidobacterium adolescentis* using such an approach [54]. 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 [15,22]. 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 *B. eggerthii* and *O. 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 cocultured with spent media from *A. putredinis* [22]. 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 [58], whereas *C. albicans* growth is optimal in aerobic conditions [59]. 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 [60]. Murine models have been developed to study *C. albicans* colonization of the vaginal [44,61] and intestinal [21,60] tract, and the interactions between *C. albicans* and vaginal or intestinal bacteria [21,44]. Mice are naturally resistant to *C. albicans* GI colonization [21], 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 [60], 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 that observed in humans. Moreover, it has been recently shown that specific *C. albicans* strains, such as CHN1 and 529L, are actually able to colonize the murine gut without the removal of the gut microbiota with antibiotics [62]. Murine models can thus 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 [63], 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 [64] or multistage fermentation models [65–67]. Continuous fed-batch models have already been used to demonstrate the anti-*C. albicans* activity of *Lactobacillus plantarum* [65,66].

The SHIME® (Simulator of Human Intestinal Microbial Ecosystem) is a multicompartment semicontinuous 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 [68]. It can also include a mucosal compartment [7,68] and a host–microbiota interaction module that allows coculturing complex bacterial communities with a monolayer of enterocyte human cells [69].

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[ii], 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 a model would be particularly relevant to study the impact of a single species 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 a 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 fecal samples used to set up the system [70,71], thus allowing testing the effects of specific bacteria on *C. albicans* colonization by various populations.

Such a 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 immunostimulatory 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 [51,72]. 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.* [72]. 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 [72]. 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 of a cell culture of one or several tissues contained in 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 an oxygen gradient [73–75]. Moreover, they allow the inclusion of immune cells in the chip, thus simulating the host defense [73–75]. Gut-on-a-chip (for review, see [74]) and vagina-on-a-chip [76,77] systems are already available and could be used or optimized to study *C. albicans* interaction with bacteria. Organ-on-a-chip technology is also suitable for industrialization, and is currently being developed by companies such as Mimetas[iii] and Emulate[iv]. 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, like the SHIME®, and/or organs-on-a-chip (see Outstanding questions). In parallel, the statistical tools used for the identification of bacterial species with a potential antifungal activity are mainly used to translate a network of microbial interactions into a simplified list of one-to-one 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. Therefore, it is crucial to democratize the use of multidimensional statistical tools to create complex models of microbial interactions that could explain the host resistance or susceptibility to *C. albicans* intestinal colonization. In this context, machine learning offers a promising alternative to simpler statistical methods since such approaches can analyze simultaneously hundreds, if not thousands, of variables. They can therefore identify patterns in the gut microbiota as a whole, which would in turn return a more complete overview of what is happening in the human body. This could therefore lead to the identification of consortia of bacteria acting synergistically against *C. albicans.* Such approaches could also be extended to the research of bacterial species or molecules with broader antifungal activities. In particular, various fatty acids, produced by lactic acid bacteria, have been shown to have strong antifungal properties against various fungi, including *Mucor*, *Penicillium,* and *Aspergillus* species [78–80]. Machine-learning approaches could thus offer a convenient and inexpensive way of screening molecules, based on their chemical properties, for a potential broad antifungal activity.

Machine learning could also be used to search for fungal species with a potential antagonistic activity against *C. albicans.* Indeed, due to the low diversity and high variability of the fungal microbiota [6,81], and the fact that most fungal species are likely not to be true colonizers of the human gut but rather transient species introduced by food and/or the environment [82,83], it can be tedious to identify fungi that are strongly negatively associated with *C. albicans* abundance in the gut. However, by employing machine learning, bias-inducing parameters such as the diet of the subjects could be considered, thus increasing the likelihood of identifying true fungal signatures.

However, 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.

Outstanding questions

How can machine-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 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 microbiota data contained in public databases 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?

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Declaration of interests

No interests are declared.

Resources

[i]https://clinicaltrials.gov/ct2/show/NCT02251093

[ii]www.funhomic.eu/

[iii]www.mimetas.com/en/home/

[vi]https://emulatebio.com/

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