Anti-*Candida* activity of the human gut metabolome

Mémoire

Carlos-Enrique Garcia-Rangel

Maîtrise en biologie cellulaire et moléculaire
Maître ès sciences (M. Sc.)

Québec, Canada

Anti-*Candida* activity of the human gut metabolome

Mémoire

Carlos-Enrique Garcia-Rangel

Sous la direction de :

Adnane Sellam, directeur de recherche

Résumé

L’intestin humain contient une variété de microbes commensaux qui sont représentés par divers organismes appartenant aux trois domaines de la vie où les *Eukarya* sont essentiellement représentés par le règne des champignons. La levure commensale et opportuniste *Candida albicans* a été identifiée comme étant le champignon le plus commun dans l’intestin des humains sains. Des études récentes soutiennent que malgré leur faible abondance les levures du genre *Candida* peuvent altérer l'équilibre du microbiote et conduire à des dysbioses ou des pathologies récurrentes comme la maladie de Crohn et les colites ulcéreuses. Il a été démontré que le microbiote commensal joue un rôle essentiel dans la protection de l’intestin contre la colonisation par des bactéries pathogènes et des pathobiontes. Cependant, jusqu'à présent, on ignore si la prolifération ou la pathogénicité de *C. albicans* peuvent être contrôlées par d'autres microbiotes fécaux. Dans cette étude, nous avons démontré que le métabolome microbien de l'intestin humain exerce une activité antifongique contre *C. albicans* et d’autres levures qui résident au niveau intestinal. Ces métabolites inhibent plusieurs traits de virulence de *C. albicans* incluant la filamentation et l'invasion des cellules humaines. De plus, un crible génétique chez *C. albicans* a suggéré que TOR est la cible moléculaire de la ou des molécules antifongiques du métabolome microbien de l'intestin humain.

Abstract

The human gut contains a variety of commensal microbes which are composed of diverse organisms that belong to all three domains of life with Eukarya primarily represented by fungi. The commensal / opportunistic yeast *Candida albicans* has been reported as the most common fungus in the gut of healthy humans. Recent evidences support that, this small fraction can alter the microbiota equilibrium leading to dysbiosis and diseases like inflammatory bowel diseases. It has been demonstrated that commensal microbiota plays a critical role in the protection of the gut against colonization by other bacterial pathogens and pathobionts. However, so far, whether *C. albicans* overgrowth or pathogenicity are controlled by other fecal microbiota is not known. In this study, we showed that the human microbial gut metabolome (GM) exerts an antifungal activity against different intestinal-resident yeasts including hyphal growth and the invasion of human enterocytes of *C. albicans*. Furthermore, a genetic screen in *C. albicans* suggested that TOR is the molecular target of the antifungal molecule(s) of the GM.

Table of contents

List of tables

List of figures

List of abbreviation

%	percentage
°C	Celsius degree
µl	microliter
µm	micrometer
CD	Crohn's disease
cDNA	complementary DNA
CO_2	Carbon dioxide
CoA	coenzyme A
CT	threshold cycle
DEC60	defined microbial community of 60 strains
dNTP	deoxynucleoside triphosphate
DTT	dithiothreitol
FBS	fetal bovine serum
FDR	false-discovery rate
FPKM	fragments per kilobase of exon per million aligned fragments
g	Grams
GI	gastrointestinal
GM	gut metabolome
GRACE	Gene Replacement and Conditional Expression
h	hour
H_2	Hydrogen
HDAC	histone deacetylase
IBD	inflammatory bowel disease
LDH	lactate dehydrogenase
m	minutes
MET	microbial ecosystem therapy
mg	milligram
$MgCl_2$	Magnesium chloride
min	minutes
ml	milliliter
mM	millimolar
N_2	Nitrogen
NaCl	Sodium chloride
NCR	nitrogen catabolite repression
nm	nanometer
nt	nucleotide
OD_{490}	optical density at 490 nm
OD_{595}	optical density at 595 nm
PBS	phosphate-buffered saline

PCR	Polymerase chain reaction
PMSF	phenylmethylsulfonyl fluoride
P-S6	ribosomal protein S6
PVDF	polyvinylidene difluoride
qPCR	quantitative PCR
RNA	ribonucleic acid
RNA-seq	RNA sequencing
rRNA	Ribosomal RNA
RT	reverse transcription
s	second
SCFA	short-chain fatty acid
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
snoRNA	Small nucleolar RNA
snRNA	small nuclear RNA
TOR	target of rapamycin
tRNA	transfer RNA
U	Unit
UC	ulcerative colitis
vol	volume
wt	weight
xg	times gravity

Preface

This Master's thesis is composed by a unique chapter, written up as a scientific article which has been published in December 2017 in mSphere®, an open access journal published by the American Society of Microbiology. In this article we showed the antagonistic interkingdom interactions between *C. albicans* and common intestinal commensal bacteria and its promising therapeutic potential against gastrointestinal inflammatory diseases. I participate directly in the planning of the methodology with professor Adnane Sellam (principal investigator), Dr Faiza Tebbji (senior research associate) and indirectly with our collaborators. This article has been possible thanks to the participation of many collaborators. MSc Michelle Daigneault & Dr Emma Allen-Vercoe from the University of Guelph were in charge of growing the microbial community and harvesting their metabolome using an in-house bioreactor. Dr Ningning Liu & Dr Julia R. Köhler from Harvard Medical School made the ribosomal protein S6 (TOR effector) Western blot experiment. Meanwhile, all the antifungal and antivirulence tests, mutants screening, transcript level of yeast-to-hypha transition genes and RNA-Seq samples preparation and data analysis were performed by myself as principal author. The metabolome protective activity for host cells against fungal invasion was evaluated by myself in collaboration with Dr Faiza Tebbji.

During my Master, I also participated in the realization of some experiments for the scientific article "pH-dependent antifungal activity of valproic acid against the human fungal pathogen *Candida albicans*" which was published in the journal Frontiers in Microbiology® in October 2017 (results not presented in these thesis). Furthermore, we are currently drafting a manuscript that report the identification of small molecules that compromise *C. albicans* virulence traits as an antifungal therapeutic strategy. The paper will be submitted on January 2018.

General Introduction

Biology of *Candida albicans*.

Candida albicans was described for the first time in 1839 by Langenbeck and its name comes from the Latin words *candidus* meaning “white” and *albicare* meaning “be white”; this repetition of meaning could be due to the association with the whiteish tinge of the mucosal infections. *C.albicans* belongs to the Superkingdom Eukaryote, kingdom Fungi, subkingdom Dikarya, phylum *Ascomycota*, subphylum *Saccharomycotina*, class *Saccharomycetes*, subclass *Saccharomycetidae*, order *Saccharomycetales* [1]. The most important clinic Candida species belong to a subgroup of the family *Metschnikowiaceae* known as the CTG clade (Figure I1). The members of this clade translate the CTG codon as serine instead of leucine. This codon-usage alteration was a very important event in the evolution of *Candida* species because it has been shown to be an evolutionary accelerator and generator of phenotypic diversity [2], [3]. Another important evolutionary event in the order *Saccharomycetales* is the whole genome duplication (WGD) and is compose by species for which the genomes have undergone complete duplication like the genus *Saccharomyces*, were the most important representative is *Saccharomyces cerevisiae* (Figure I1) [4].

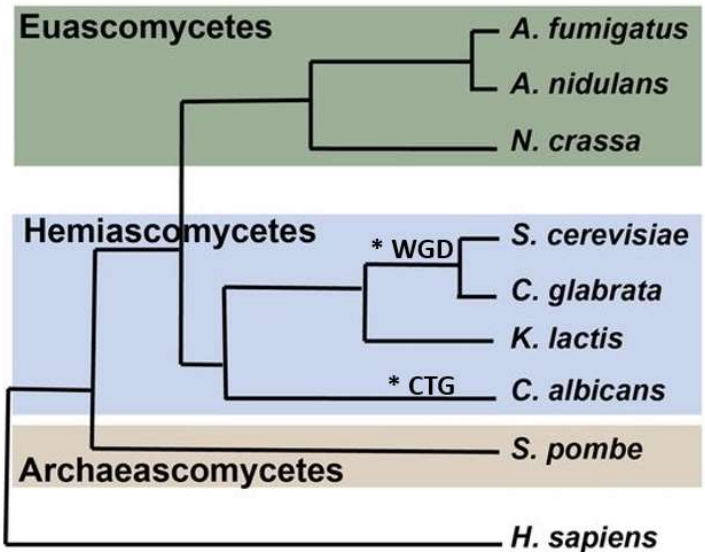

Figure I1. Classification based on phylogenetic profiles of genes and ubication of WDG and CTG clade. Modified with authorization from reference [5]

C. albicans is a diploid ascomycete yeast that is an important commensal and opportunistic pathogen in humans. It is a component of the normal human flora, colonizing primarily mucosal surfaces, gastrointestinal and genitourinary tracts, and skin without causing disease, suggesting a good adaptation to commensalism [6]. Gastrointestinal tract's colonization involves predominantly carriage of the yeast form of *C. albicans*, and furthermore it has been demonstrated that passage through mammalian gut triggers a phenotypic switch to cells termed GUT for "gastrointestinally induced transition" [7]. However, it has also been shown that in monocolonized animals, *C. albicans* remains as a commensal oval-shaped yeast [8]. This, suggest that there is no necessity to refer the GUT morphology to explain the commensal nature of *C. albicans* in the gastrointestinal tract and reinforced the premise that *C. albicans* is able to alter its shape or size in response to all the environmental conditions, growing as a budding yeast, as a pseudomycelium of adjoined and elongated cells or as true hyphae [8]–[10].

The most important feature that allows *C. albicans* as a successful pathogen is its metabolic flexibility. This feature allows the efficient use of alternate carbon sources, stress resistance, transition between yeast to hypha, biofilm formation and cell surface changes that affect its immunogenicity, to succeed in different conditions [10]. The biofilm formation is one of the major virulence traits of *C. albicans* and its matrix composition is very dynamic and depend mainly on growth conditions. The biofilm matrix formation help with the mechanical stability, adhesion, drug resistance and protection from the host immune system [11].

Although many infections involve unpleasant but non-life-threatening colonization of various surface mucosal membranes, immunosuppressed patients can fall prey to serious mucosal infections, such as oropharyngeal candidiasis in HIV patients and newborns, and lethal systemic infections or candidemia [6]. In the USA, Candida infections rank very high (4^{th}) on the list of the most common hospital acquired infections [12]. In Canada, *C. albicans* has been found responsible for up to 13% of all infections in intensive care units [13]. It has also been estimated that there is a proportion of 5,8 per 100.000 habitants cases of life-threatening invasive infections and 1.446 cases of non-life-threatening mucosal candidiasis per 100.000 habitants, being the most important the recurrent vulvovaginal candidiasis (1.403 per 100.000 habitants) followed by oropharyngeal candidiasis and esophageal candidiasis [14].

Gut Ecology.

The human gut contains a variety of commensal microbes known as microbiota and which are composed of diverse organisms that belong to all three domains of life with Eukaria primarily represented by fungi. This microbiota has more than 10 times the number of cells of the rest of the human body and also has an important role in the homeostasis and disease prevention, that’s why it could be considered as a novel and essential organ of the human body. Obligate anaerobes dominate the healthy microbiota, a number that could be two to three times greater than facultative

anaerobes and aerobes, with a low ratio of phylum *Firmicutes* to *Bacteroidetes,* high amount of nutrients and a low concentration of oxygen [15]. The host can tolerate the colonisation of opportunistic fungi in the GI tract, but defend against its invasion thanks to the immune system. When this equilibrium is broken or alterations in the gastrointestinal tract perturb the microbiota and its ratio it could lead or contribute to the pathogenesis of digestive diseases [16].

While the majority of microbiome studies have been focused on the bacterial community, the fungal component known as mycobiome has been undervalued. It is only recently that sequencing-based investigations of the gut microbial community have started to pay more attention to the fungal component [17]. This rise of interest in the mycobiome is due to importance of its interaction with the bacteriome and how these interaction is essential for a proper balance to maintain a healthy gut [18]. The composition of the intestinal microbiota and therefore the mycobiota is influenced by the genetic background of the host and other factors like dietary habits and the environment [19]. The first fungi detected in the infant gut are Saccharomycetalean yeasts, like *Candida* species, which are presumed to be transmitted by the contact of the skin and vaginal mucosa of the mother [20].

The mammalian digestive tract can be considered the primary niche of some species of the genus *Candida*, like *C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. krusei*. Genus *Candida* has been detected in 86% and *Saccharomyces cerevisiae* in 54% of 36 studies across 1917 to 2015 (Figure I2) [21]. Chronic colonization of mucosal surfaces, including the gastrointestinal (GI) tract with fungi is common, especially for Candida spp., which could be present in healthy adults in ranges from 30 to 80%. Though, when conditions permit *C. albicans*'s outgrowth and colonization could lead to infection thanks to its pathogenic potential like morphological transition (the yeast-to-hyphae transition) or biofilms formation [22]. The species of fungi present in the gut differs depending on the diet. While vegetarian diet samples are

commonly rich in plant pathogenic *Fusarium*, *Malassezia*, *Penicillium* and *Aspergillus*, conventional diet samples are rich in *Candida*, *Saccharomyces*, *Geotrichum* [23]. Furthermore, it has been proved that diet can modulate host-microbe interactions and this could be a future therapeutic approach [19].

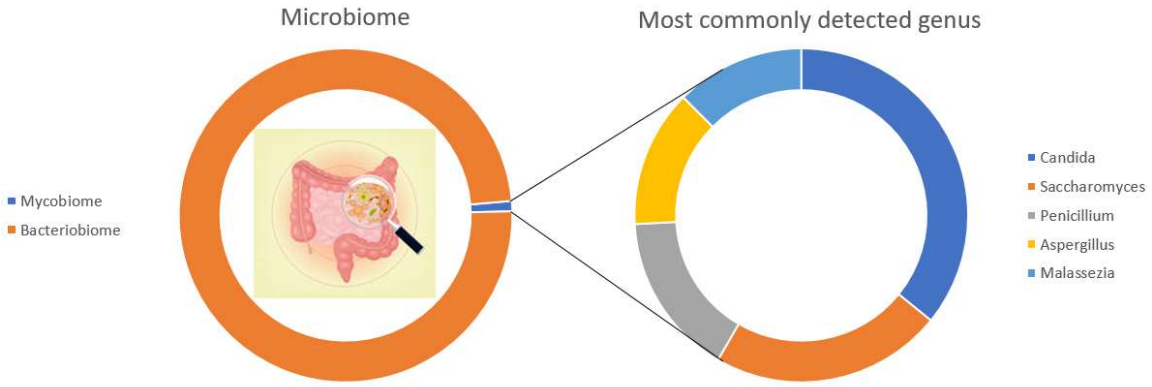

Figure I2. Most commonly detected genus in the gut mycobiome [21]

Antifungal therapy.

Current therapeutic options are limited to treatment with three longstanding antifungal classes, namely the polyenes, azoles and echinocandins [24]. These compounds target the specific fungal biological process of ergosterol metabolism (azoles and polyenes) and cell wall β-1-3-glucan synthesis (echinocandins). However, these drugs have serious side effects such as nephrotoxicity and/or create complications such as resistance due to their fungistatic rather than fungicidal characteristic [24]. Drug resistance has evolved during the years and it has become an important phenomenon that permit microorganisms to adapt and survive in their environment [25]. This evolution is mediated through mechanisms such as drug target alteration or overexpression, reduction of intracellular levels of the drug, or activation of cellular stress response pathways, which could happen simultaneously [25].

Due to the limited number of drugs, their side effects and but above all, the increase in drug resistance isolates, there is an urgent need for new strategies to identify novel protein targets for antifungal therapeutic intervention. One way to interfere with this is the use of combination therapy, this technique allows to increase killing effect and could lower individual drug dosage, minimizing host toxicity and also drug resistance [26]. Another way to interfere with drug resistance is the implementation of a relatively new but poorly exploited strategy in the antimicrobial therapy field, like targeting virulence factors for drug development. In contrast to target-focused approaches, phenotypic screens enable the identification of bioactive molecules that induce a desirable biological readout without making *a priori* assumption regarding the cellular target. Thus, targeting *C. albicans* virulence factors with small molecules without affecting commensal growth would in principle be better tolerated by the patient [25]

Interkingdom Communication

It has been shown that the mycobiome is a key component of the human microbiome. Even its low representation in comparison to the bacteriome, we can find positively correlated fungi-bacteria pairs, which means that they depend on each other for growth, probably by maintaining nutrient balance or modulating growth-related cellular pathways. We can also find negative correlated pairs that could reflect indirect mechanisms of inhibition, where one microbe hunts the nutrients needed for the other's growth or direct mechanisms involving growth inhibition mediated by secretory products [27]. Furthermore, some of these interactions (or lack of interaction) between fungi and bacteria could be associated with different diseases like fungal infections caused by the lack of competitive exclusion by resident bacteria that have a protective role against potential invasion [18] or the lack of inhibition of virulence traits [28]. Mycobiota might also interact directly or indirectly with the host immune system to generate exacerbation of some diseases like Inflammatory Bowel Disease (Table I1) [18].

Bacteria	Effect	Mechanism
Clostridium	Protective effects against *Candida*	Increase of short chain fatty acids (SCFA) to induce regulatory T cells and macrophages
Lactobacillus	Protective effects against *Candida*	Increase of SCFA to induce regulatory T cells and macrophages. Increase of indole-3-aldehyde (IAld) to stimulate IL-22 production and epithelial-mediated protection
Bacteroides	Protective effects against *Candida*	Increase of polysaccharide A to induce regulatory T cells and macrophages.
Segmented filamentous bacteria	Protective effects against *Candida*	Increase of serum amyloid A to induce T helper cell differentiation

Table I1. Major interactions between antifungal host defense and the microbiome against *Candida*. [18]

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a spectrum of intestinal inflammatory condition caused by multiple factors that could have many phenotypes and behaviours. The first classification used to confront an IBD patient are Crohn's disease (CD) and ulcerative colitis (UC) [29]. Crohn's disease had been classified phenotypically by different groups since 1988 (Rome consensus) but all agreed that, the age to see the first signs of disease, the location and the behaviour were very important variables to include in the classification. The classification of ulcerative colitis was discussed by the first time in 2005 by the Montreal consensus and the age to see the first signs of disease, the extend and the severity were important variables to include in the classification. Even if the original aim of the Montreal consensus was

to include the molecular markers into the classification, these were not included because the judged it was too soon and more studies were needed [30]. In the late 90's serological markers have been explored as alternative predictors. Some of these makers are antibodies directed against microorganism antigens. In the case of CD there are some markers like, anti-Saccharomyces cerevisiae antibodies (ASCA), antilaminarobioside carbohydrate antibodies (ALCA), antimannobioside carbohydrate antibodies (AMCA), among others. In ulcerative colitis, a strong antibody response is found against neutrophil antigens (pANCA). Although these markers were investigated as diagnostic tools, their clinical value is associated to the progression and complication of the disease [29]. Even though the pathogenesis and etiology of IBD are largely unknown, the requisite for the development of IBD is the dysbiosis or dysregulation of host/enteric microbial interactions. This dysbiosis is created by an imbalance in the microbial equilibrium, making the gut vulnerable to potentially pathogenic bacteria [31]. IBD is considered a global disease from developed countries and its prevalence in the United States is around 1-1.5 million people, prevalence is not uniform across the U.S. adult population and it has a higher prevalence among adults aged 45 years and more [32]. IBD is considered an important economic problem on healthcare services, including decreased quality of life, high hospitalization rates (8.2–17.1 per 100,000 persons with IBD annually) and direct treatment costs estimated to be more than 6.8 billion dollars in 2008 [33].

Inflammatory Bowel Disease and Mycobiota

The importance of the gut microbiota in human physiology and health through the functions encoded by their collective genome are recognized [18]. Nevertheless, the number of diseases in which the gut microbiota could play a key role has been increasing in recent years. Even if the mycobiota only represent probably 0.1% of all microbiota, it has been proved that this small percentage could alter the whole microbiota producing dysbiosis and diseases. As example, we can see how the abundance of *Candida tropicalis* is significantly higher in Crohn's Disease patients than their first-degree relatives (similar diet, ethnic and environment) and how this

alteration can be correlated with other bacteria, suggesting the existence of disease-specific inter-kingdom alterations [34]. But not always an increase or modification of the mycobiota could be negative. The mycobiota looks to have a dual action on bowel inflammation, as an example we have *Saccharomyces boulardii*, a probiotic strain that is closely related to *S. cerevisiae* which seems to exert a direct effect on proinflammatory microorganisms [35]. It has been documented that gut inflammation drives changes in the mycobiota, increasing the opportunistic pathogenic fungi and decreasing the non-pathogenic fungi, which could be observed as an increased Basidiomycota/Ascomycota ratio when it's compared with healthy controls [36]. Furthermore, there is also evidence supporting the involvement of mycobiota in IBD pathogenesis. Gut inflammation induced promotes *C. albicans* proliferation and at the same time *C. albicans* worsen the inflammation, creating a vicious circle in which IBD induces *C. albicans* proliferation, which itself enhances the inflammatory process [37]. Interactions between fungi in the mycobiome and host cells in the gut are also important because it could affect how inflammatory diseases evolve [38].

The Human Gut Microbial Metabolome Modulates Fungal Growth via the TOR Signaling Pathway.

Résumé

Candida albicans est reconnu comme étant le pathogène fongique majeur de l'Homme, mais il est également un organisme commensal qui réside dans le tractus gastro-intestinal des sujets sains. Des études récentes ont démontré que le métabolome secrété par le microbiote intestinal de l'homme représente une source intéressante de molécules bioactives avec un degré important de diversité chimique. Certaines de ces molécules bioactives pourraient détenir des propriétés antimicrobiennes et pourraient inhiber la virulence de certains organismes opportunistes. Par exemple, il a été démontré que des espèces bactériennes intestinales appartenant à la famille des *Lachnospiracea* sont capables de sécréter des molécules qui permettent d'atténuer la pathogénicité de *Salmonella* et de réprimer l'expression des gènes de virulence. Dans cette étude, nous avons essayé de savoir si le métabolome microbien de l'intestin humain contient des molécules qui peuvent favoriser le mode de vie commensal et / ou inhiber l'expression de la virulence de *C. albicans* dans l'intestin. Nous avons trouvé que les métabolites provenant des fèces humaines inhibaient la croissance de *C. albicans* ainsi que d'autres levures opportunistes. Un criblage génétique chez *C. albicans* a suggéré que la voie TOR (Target Of the Rapamycin) est la cible moléculaire de la(des) molécule(s) antifongique(s) du métabolome microbien. En outre, nous avons constaté que les métabolites du métabolome inhibent à la fois la croissance des hyphes de *C. albicans* et l'invasion des entérocytes humains. Les activités anti-croissance et anti-virulence ont été partiellement récapitulées par des métabolites secrétés respectivement par des souches *Roseburia* spp. et *Bacteroides ovatus*. Cette étude démontre que l'activité antimicrobienne du métabolome microbien de l'intestin humain peut être étendue à un pathogène eucaryote, *C. albicans*, éclairant les interactions antagonistes interrègne entre un fungus et les bactéries commensales intestinales.

Abstract

Candida albicans is well known as a major human fungal pathogen, but it is also a permanent resident of healthy gastrointestinal tracts. Recent studies have shown that the human gut microbial metabolome represents an interesting source of bioactive molecules with a significant degree of chemical diversity. Some of these bioactive molecules may have useful antivirulence activities. For instance, intestinal bacterial species belonging to the *Lachnospiraceae* family were found to secrete molecules that attenuate *Salmonella* pathogenicity and repress the expression of virulence genes. Here, we have investigated whether the microbial gut metabolome (GM) contains molecules that might promote the commensal lifestyle and/or inhibit the expression of virulence of *C. albicans* in the intestine. We found that metabolites from human feces inhibited the growth of *C. albicans* and other opportunistic yeasts. A genetic screen in *C. albicans* suggested that TOR is the molecular target of the antifungal molecule(s) of the GM. In addition, we found that the GM metabolites inhibit both *C. albicans* hyphal growth and the invasion of human enterocytes. The antigrowth and antivirulence activities were partially recapitulated by secretions from *Roseburia* spp. and *Bacteroides ovatus* strains, respectively. This study demonstrates that the antimicrobial activity of the GM can be extended to a eukaryotic pathogen, *C. albicans*, illuminating the antagonistic interkingdom interactions between a fungus and intestinal commensal bacteria.

Importance

Candida albicans is a natural component of the human microbiota but also an opportunistic pathogen that causes life-threatening infections. The human gastrointestinal tract is the main reservoir of *C. albicans*, from where systemic infections originate as a consequence of the disruption of the intestinal mucosal barrier. Recent studies provided convincing evidence that overgrowth of *C. albicans* and other related species in the gut is predominantly associated with chronic intestinal inflammatory bowel diseases. Here, we showed, for the first time, the antagonistic interkingdom interactions between *C. albicans* and common intestinal commensal bacteria. From a therapeutic perspective, administering a defined bacterial community, such as the one described here with anti-*Candida* activity, could provide potential therapeutic protection against gastrointestinal inflammatory diseases.

Keywords antifungal activity, Candida albicans, gut microbial metabolome, TOR pathway

Introduction

Candida albicans is a natural component of the human microbiota but also an opportunistic pathogen that causes life-threatening infections in immunosuppressed patients. The human gastrointestinal (GI) tract is the main reservoir of *C. albicans*, from where systemic infections originate as a consequence of the disruption of the intestinal mucosal barrier [39], [40]. While the mycobiota represent only 0.01 to 0.1% of the human microbiota [41], [42], recent evidence supports the idea that, in certain pathologies, this small fraction can alter the microbiota equilibrium, leading to dysbiosis disease. Of note, overgrowth of *C. albicans* and other *Candida* opportunistic species in the gut is predominantly associated with chronic intestinal inflammatory bowel diseases (IBDs), including Crohn's disease (CD) and ulcerative colitis (UC) [43]. CD in adults is associated with gut microbial dysbiosis characterized by a higher abundance of either *C. albicans*[44], [45], *Candida tropicalis* [34], or *Candida glabrata* [46], while pediatric patients with IBD were found to have increased amounts of *Candida parapsilosis* and *Candida guilliermondii* [47].

The gut microbiota is a complex community of microorganisms that is essential for the development of the host immune system and consequently plays a pivotal role in health and susceptibility to disease. For example, many bacterial communities directly control the activity of the immune system through the production of short-chain fatty acids (SCFAs; e.g., propionate, butyrate, and acetate) which reduce inflammation or modulate the recruitment and maturation of different immune cells [48]. Furthermore, the commensal bacterial microbiota contributes to intestinal homeostasis by directly impairing the virulence traits of many bacterial pathobionts. Recent work has demonstrated that metabolites produced by certain species of *Lachnospiraceae*, a bacterial family that is prevalent in the human gut, are able to reduce the growth and silence the expression of invasion genes of *Salmonella enterica* that are necessary to promote infection by this pathogen [49]. A similar antivirulence activity against *Salmonella* was reported for butyrate produced by many enteric bacteria, including species belonging to genera such as *Roseburia* and

Faecalibacterium [50]. In addition, commensal bacteria not only suppress the expression of virulence factors of many pathogens but can also preferentially consume nutrients that are required for the growth of competing surrounding pathobionts [51]. While convincing evidence supports the idea that the growth and virulence of bacterial pathobionts are controlled by commensal bacteria, little is known regarding how intestinal fungal opportunist species such as *C. albicans* are influenced by other microbial entities. For example, evidence supporting the overgrowth of *Candida* species as a result of the depletion of beneficial commensal microbes with antifungal properties remains unclear, particularly in the context of IBDs.

Many synergistic or antagonistic *C. albicans*-bacterium interactions in different host niches and their clinical impact on human health have been well documented [52]. However, so far, such interactions have not been reported for the GI tract in the context of either health or disease. Previous work has shown that sodium butyrate inhibited both growth and filamentation of *C. albicans* in vitro at a concentration similar to that encountered in the gut, providing indirect evidence of *C. albicans* growth control by butyrate-producing bacteria [53]. More recently, it was shown that the microbial metabolome of the mammalian gut contains a tremendous number of diverse molecules with critical biological functions [54], [55]. We therefore hypothesized that the human gut metabolome (GM) contains molecules that might promote the commensal versus the pathogenic lifestyle of *C. albicans* and other opportunistic fungi. In the current study, we show that metabolites from extracts of human feces exert an antifungal activity against *C. albicans* and other intestine-resident yeasts. This anti-*Candida* activity was recapitulated using fecal extracts derived from other human donors, suggesting a pervasive antifungal feature of these bioactive molecules. In an attempt to gain insight into the mechanism of action associated with the antifungal activity of GM, a genetic screen was undertaken. The obtained data indicated that the GM inhibits the *C. albicans* TOR pathway, a central signaling circuit that controls cellular growth in response to environmental nutrient

status in eukaryotes. We also showed that the GM has antivirulence activity through inhibition of both hyphal growth and the invasion of human colon epithelial cells by *C. albicans*. These anti-*Candida* growth and antivirulence activities were partially replicated by exposure of fungal cells to *Roseburia* spp. and *Bacteroides ovatus*, respectively. Further studies assessing the usage of these bacterial species as potential probiotics to restore the microbiota in intestinal pathologies associated with increased abundance of fungal communities represent an exciting direction for future research.

Results and discussion

The human gut metabolome exerts antifungal activity against *C. albicans*.

There is growing evidence that gut-resident microbiota control and restrain the growth of gastrointestinal pathogenic bacteria directly by inhibiting their growth or their virulence factors and thus represent a reinforcement of the human immune system [49], [56]–[58]. The antimicrobial activity of the gut microbiota is mediated by their rich secreted metabolome, which has been shown to exhibit a significant chemical diversity [49], [54], [55]. We aimed to investigate whether the GM exerts antifungal activity against the major human fungal pathogen *C. albicans*, also a resident of the GI tract. As a source of gut metabolites, we used a continuous-culture bioreactor system to grow a defined microbial community of 60 strains (DEC60) derived from the feces of a healthy human donor (donor 1) [49]. Antifungal activity of the GM on *C. albicans* clinical reference strain SC5314 was evaluated by monitoring the optical density at 595 nm (OD_{595}) of cultures exposed for 24 h to increased concentrations of the GM. A significant inhibition of *C. albicans* growth was noticed at 1% GM with 15% growth reduction (Fig. 1A and B). An equivalent inhibitory rate was obtained with filtrate from the bioreactor effluent filtered to remove cellular material, suggesting that the GM antifungal activity is mediated by secreted

molecules (Fig. 1A and B). This result demonstrated that, in addition to their activity against pathogenic bacteria, metabolites secreted by certain human gut-derived microbes possess antifungal activity against the opportunistic yeast *C. albicans*.

We also tested the GM antifungal activity on resistant *C. albicans* clinical isolates. A total of three fluconazole-resistant strains with different resistance mechanisms (see details in Table S1 in the supplemental material) were selected in addition to two echinocandin-resistant isolates. As with the *C. albicans* SC5314 sensitive strain, the GM inhibited the growth of all tested resistant clinical isolates (Fig. 1C). This demonstrates that the GM contains bioactive molecules that can potentially be used to tackle therapeutic limitations related to acquired clinical resistance to commonly used antifungals. This also suggests that the mechanisms that confer resistance to azoles and echinocandins are distinct from those that may cause resistance to antifungal gut metabolites.

The human gut metabolome exerts a broad antifungal activity against intestine-resident yeasts.

In addition to *C. albicans*, the human gut mycobiome includes other non-*albicans* opportunist *Candida* species, such as *C. glabrata, C. tropicalis, C. krusei,* and *C. parapsilosis* [21]. We tested whether the antifungal activity of the intestinal microbial metabolome can be expanded to these yeasts in addition to the foodborne gut-resident yeast *Saccharomyces cerevisiae*. The obtained data demonstrate that the GM inhibited the growth of all tested fungal species, with *C. albicans* exhibiting the highest sensitivity, followed by C*. tropicalis, C. parapsilosis, S. cerevisiae, C. glabrata,* and *C. krusei* (Fig. 1D). Thus, the GM antifungal activity is generalized to prevalent yeast residents of the gut. An obvious focus for future experimentation will be the investigation of whether the GM antifungal activity has been lost in CD and other inflammatory bowel pathologies associated with the disturbance of the fungal microbiota [59].

The inhibitory effect of the human gut metabolome is pervasive.

Since the human microbiome is very dynamic and is influenced by different conditions, including the diet and host physiology, we wanted to determine whether or not GM antifungal activity was restricted to the donor used for the above-described experiments. The activity of microbial metabolites derived from defined communities from feces of an additional donor (donor 2) in addition to the therapeutic microbial ecosystem MET-1 [57] from donor 1 was tested on *C. albicans* (Fig. 2). Exposure of the *C. albicans* SC5314 strain to 1% and 8% GM from donor 2 and MET-1 resulted in a significant growth reduction, demonstrating that the biological activity of the gut metabolome is a widespread feature. MET-1 is a therapeutic microbial ecosystem of 33 bacterial strains which has been used to cure recurrent *Clostridium difficile* infection [57]. MET-1 partially recapitulated the activity of the GM from DEC60 (donor 1), suggesting that the antifungal activity of the GM may be mediated by several other strains.

The gut metabolome inhibits *C. albicans* growth through the TOR pathway.

To gain insight into the mechanism of action of the GM associated with its antifungal activity, we screened the *C. albicans* GRACE (Gene Replacement and Conditional Expression) conditional mutant collection [60]. In solid medium, *C. albicans* growth inhibition by 8% GM is reflected by a reduction in colony size by almost 50% compared to the control assay (Fig. 3A and B). The colony size was used then as a phenotypic readout to identify genes in *C. albicans* that are required to tolerate or resist the antifungal property of the GM. Among the 2,360 unique mutants screened, a growth defect was reported and confirmed exclusively for the kog1 mutant. For this strain, growth was inhibited to 60% of that of the wild-type parental strain (not shown). Kog1 is a conserved subunit of the TOR (target of rapamycin) pathway, a central signaling circuit that controls cellular growth in response to environmental nutrient status and stress in eukaryotes [61].

Our finding suggests that the *C. albicans* TOR pathway modulates the cellular response required to tolerate the GM inhibitory effect. To examine whether the GM acts directly on the TOR pathway, the effect of GM was assessed in cells expressing a sirolimus resistance allele of the Tor1 kinase, TOR1-1 [62]. This allele encodes a mutation (S1984R) that confers complete resistance to the growth-inhibitory effect of sirolimus. The colony size of the TOR1-1/TOR1 strain treated with 8% GM was not discernible from the control experiment, suggesting that this strain is impervious to the antifungal activity of the GM (Fig. 3C and D). These data support the idea that the TOR pathway might be a direct target of the antifungal molecule(s) secreted by the gut microbiota.

To further confirm this finding, the effect of GM on the phosphorylation state of the ribosomal protein S6 (P-S6), a conserved effector of the TOR pathway in *C. albicans* [63] and other eukaryotes, was evaluated. We found that the P-S6 phosphorylation level was significantly reduced in GM-treated cells compared to the control assay (Fig. 3E). Under nutrient sufficiency, TOR activates energy-consuming biosynthetic pathways such as ribosome biogenesis, transcription, and translation [64], [65]. Oppositely, under starvation or in the presence of sirolimus, TOR activity is depleted, which prevents all processes that are energy consuming and leads the cells to enter into a quiescent state also referred to as G0 [65], [66]. Taken together, our data support a model whereby gut microbial cohabitants repress *C. albicans* TOR activity to mimic a starved environment which could lock the cells into a quiescent/G0 state and consequently lower their nutritional competitiveness in the GI tract.

The transcriptional profile of *C. albicans* cells challenged with the GM resembles nutrient starvation and TOR pathway inhibition.

As shown above, the microbiota-secreted metabolites restrained the growth of *C. albicans* through the alteration of TOR pathway activity. Therefore, we anticipate that *C. albicans* cells exposed to the GM might display a transcriptional signature

reminiscent of TOR inhibition, such as that exhibited in response to sirolimus [67]. To test this hypothesis, we used RNA sequencing (RNA-seq) to assess the transcriptome of cells treated with 8% GM for 15 min. Gene ontology analysis showed that transcripts related to biosynthesis of amino acids, including arginine, methionine, serine, aspartate, and glutamine, were induced (Table 1). Genes of the nitrogen catabolite repression (NCR) pathway, such as those for ammonium and amino acid permeases, together with the master regulator of nitrogen utilization, Gat1, and the ammonium transport regulator, Npr1 kinase, were also upregulated (Table S2). Of note, activation of both amino acid metabolism and the NCR transcripts has been previously shown to be the bona fide transcriptional signature that *C. albicans* cells and other fungi experience when growing under conditions of nitrogen starvation [68]–[72] and suggests that exposure to the GM mimics a nitrogen-depleted environment.

Carbohydrate genes comprising those for hexose transporters, carbon utilization, and trehalose metabolism were also activated by the GM (Table 1). The glycolytic genes GLK1, GLK4, and HXK2, encoding hexokinases that catalyze the phosphorylation of glucose or fructose, the first irreversible step in the intracellular metabolism of hexoses, were upregulated. Activation of sugar transporters and carbon utilization genes reflects the idea that the GM exposure might simulate a carbon-limiting environment.

Strikingly, a large proportion of GM-repressed transcripts were associated with functional categories related to protein translation, including ribosome biogenesis, structural components of the small and large subunits of the ribosome, and ribosomal noncoding gene transcription and processing (rRNA, tRNA, snRNA, and snoRNA) (Table 1; see also Table S3). Repression of translation-related genes is a universal signature observed under conditions where the TOR pathway is compromised [67] or in cells growing in nitrogen- or carbon-starved environments [68], [73]. TOR inhibition is also characterized by the transcriptional activation of

NCR and carbon utilization genes, suggesting that GM treatments in *C. albicans* recapitulate most of the transcriptional readouts caused by TOR inhibition. In the budding yeast, the TOR signaling cascade controls gene expression in response to starvation for either nitrogen or carbon. The parallels to our data set suggest that the bulk of the transcriptional response observed in *C. albicans* cells challenged with the GM is most likely the consequence of TOR pathway inhibition. RNA-seq data provided further supportive evidence for the role of secreted microbiota metabolites in inhibiting the TOR pathway to modulate *C. albicans* proliferation in the GI tract.

The human gut metabolome inhibits yeast-to-hypha transition in *C. albicans* and the inducibility of hypha-specific transcripts.

Previous studies have demonstrated that the gut commensal microbiota produces small molecules with antivirulence activity. For instance, different species of the *Lachnospiraceae* family were found to secrete molecules that attenuate *Salmonella* virulence by repressing expression of the global regulator hilA, which controls the expression of *Salmonella* pathogenicity island 1 (SPI1) pathogenicity genes, thereby compromising the pathogen's ability to invade host cells [49]. Since the gut is considered the main reservoir of *C. albicans* and the main source of systemic infection in humans [39], we hypothesized that aspects of a normal gut microbial ecosystem might be required to control *C. albicans* virulence traits. To test this hypothesis, the effect of the GM was assayed by testing the ability of *C. albicans* to form invasive hyphae in response to fetal bovine serum (FBS). Cells exposed to the GM remained responsive to FBS and formed typical hyphae; however, the average length of these hyphae was significantly reduced compared to the control (Fig. 4A). Furthermore, when exposed to 8% GM, only 80% of *C. albicans* cells had differentiated true hyphae compared to nontreated cells (Fig. 4B). At the transcriptional level, hypha-specific genes, including the cytolytic peptide toxin ECE1; the superoxide dismutase SOD5; and the adhesins ALS1, ALS3, and HWP1, were repressed in cells exposed to the GM (Fig. 4C). Taken together, these results demonstrate that gut microbiota-secreted molecules repress the yeast-to-hypha

transcriptional program, which in turn leads to the inhibition of the *C. albicans* invasive form.

The gut metabolome attenuates damage to intestinal epithelial cells caused by *C. albicans*.

Since the GM reduced the formation of invasive hyphae, we wanted to check whether it conferred a protective activity for host cells against fungal invasion. *C. albicans*-mediated damage of human colon epithelial HT-29 cells was quantified based on the lactate dehydrogenase (LDH) release assay in cells treated or not with three different concentrations of GM (1, 2, and 8%). In accordance with the inhibitory effect on invasive hyphal growth, our data showed clearly that the GM significantly reduced the damage to HT-29 enterocytes (Fig. 4D). Remarkably, the protective effect was seen even at a concentration of 1% GM (70% reduction of enterocyte invasion), which has no impact on hypha formation or elongation (data not shown). Of note, it is well known that microbiota-secreted metabolites such as propionate, acetate, and butyrate positively influence human health by contributing to protection from infection through, e.g., the active recruitment of immune cells [48], [74]. For instance, the commensal intestinal bacterium *Bacteroides thetaiotaomicron*, a fermentative anaerobe which produces several SCFAs, induces the expression of the antimicrobial peptide LL-37 in intestinal epithelial cells, in turn protecting from *C. albicans* colonization [75]. In this regard, our data suggest that the GM might have a dual action both by directly inhibiting *C. albicans* growth and virulence and by augmenting GI mucosal immunity through SCFA activation to reduce fungal infections.

Identification of microbial species secreting antifungal inhibitory molecules.

To identify the microbial species that produce an antifungal molecule(s), individual isolates from a continuous-culture bioreactor supporting the defined community MET-1 derived from donor 1 were screened for their abilities to inhibit growth of *C.*

albicans. Filtered effluents from a total of 33 isolates corresponding to 26 different bacterial species were screened (Table S4). Most of these tested effluents had little or no apparent growth inhibition properties against *C. albicans*. Notable exceptions were effluents from strains belonging to species of the genus *Roseburia*, including *R. faecis* (31FAA) and *R. intestinalis* (39FAA), which exhibited significant antifungal activity against *C. albicans* (15% and 13% growth reduction, respectively [Fig. 5A]). Effluents from further strains of *R. faecis* and *R. intestinalis* sourced from different donors were also tested, and the results confirmed a general anti-*Candida* activity originating from these species (Fig. 5A), suggesting that secreted bioactive molecules of *R. faecis* and *R. intestinalis* contribute to the antifungal activity of the GM.

Interestingly, all species of the *Roseburia* genus described to date are known to produce butyrate using the butyryl coenzyme A (CoA):acetate CoA-transferase fermentation pathway [76], [77]. Butyrate is a short-chain fatty acid histone deacetylase (HDAC) inhibitor that plays a critical role in human health [48]. However, the anti-*Candida* activity of the GM is unlikely to be related to butyrate, since the effluent of other known butyrate-producing bacteria from donor 1 did not exhibit any apparent activity against *C. albicans* (see complete listing of tested strains and their respective species in Table S5). Pure butyrate concentrations equivalent to the measured levels present in the GMs from donors 1 and 2 (0.05 to 0.25 mM in DEC60, MET-1, and MET-2) were assayed for their ability to inhibit filamentation, colony size, and the growth of *C. albicans* cells in liquid media. As expected, and previously shown [53], butyrate significantly reduced hypha formation of *C. albicans* at >20 mM; however, no apparent effect was noticed at a concentration less than 1 mM (data not shown). Neither growth nor colony size were affected by 0.1 or 0.2 mM butyrate exposure (Fig. S1). Taken together, these data suggest that butyrate alone was not responsible for the GM antifungal activity determined in this study.

While the growth-inhibitory effect of the gut-secreted metabolome was at least partially mediated by *R. faecis* and *R. intestinalis*, effluents from these bacteria had no effect on *C. albicans* filamentation (data not shown). As in the growth assay, effluents from the 33 bacterial isolates from MET-1 were tested for their antifilamentation activity. Among the tested bacteria, effluent from *Bacteroides ovatus* isolate 5MM exhibited a significant reduction of filament length (Fig. 5B). This finding was confirmed using spent medium from a further *B. ovatus* strain (S1D6FAA) isolated from donor 2 (Fig. 5C). *B. ovatus* is a common component of the resident human gut microbiota thought to play an important role in deriving energy from the indigestible dietary fibers of consumed vegetables [78]. Our finding suggests that, in the intestine, *C. albicans* virulence may be suppressed by secreted molecules from *B. ovatus*, thus promoting the commensal lifestyle of this opportunistic fungus.

In conclusion, we showed, for the first time, the antagonistic interkingdom interactions between the opportunistic fungus *C. albicans* and intestinal commensal bacteria *B. ovatus, R. intestinalis,* and *R. faecis*. Former studies had demonstrated that administration of a defined microbiota comprising *B. ovatus* in addition to *R. intestinalis and R. faecis* helped to cure patients with recurrent *Clostridium difficile* infection and had a protective role against enteric infections by *Salmonella enterica* serovar Typhimurium in a murine model [57], [58]. Furthermore, since recent work has demonstrated that the overgrowth of *C. albicans* and other non-*albicans Candida* species is associated with inflammatory bowel disease, and in particular Crohn's disease [34], [43], [45]–[47], it may be possible to administer a defined bacterial community with anti-*Candida* activity, including microbes such as *B. ovatus, R. intestinalis,* and *R. faecis*, that could provide a potential therapeutic avenue for yeast overgrowth in IBD. This study illustrated a novel biological activity of the secreted intestinal microbial metabolome, and further investigations are required to uncover the chemical nature of the anti-*Candida* molecules secreted by *B. ovatus, R. intestinalis,* and *R. faecis.*

Materials and methods

Ethics statement.

The Research Ethics Board of the University of Guelph approved the collection of healthy donor stool samples used in this study (REB no. 09AP011). Donor 1 (MET-1 and DEC60) is a healthy female, 41 years old at the time of sample collection (referred to as donor 6 in reference [57]). Donor 2 (MET-2 donor) is a healthy male, 44 years old at the time of sample collection (referred to as donor A in reference [55]).

Fungal strains and media.

The fungal reference and clinical strains used in this study are listed and described in Table S1 in the supplemental material. C. albicans and the other yeast strains were routinely maintained at 30°C on YPD (1% yeast extract, 2% peptone, 2% dextrose, with 50 mg/ml uridine) or synthetic complete (SC; 0.67% yeast nitrogen base with ammonium sulfate, 2.0% glucose, and 0.079% complete supplement mixture) medium.

Bacterial strains and media.

A subset of each donor 1 and 2 community was cultured as individual isolates to obtain culture supernatants for specific testing against *C. albicans* strains. The individual strains cultured are listed in Table S4. Each isolate was cultured in prereduced Trypticase soy broth (Oxoid) supplemented with menadione and hemin (each at 5 mg/liter) in a Ruskin Concept 400 anaerobe chamber at 37°C for 48 h under an atmosphere of 5% H_2, 5% CO_2, and 90% N_2. Supernatants were prepared by centrifuging culture for 5 min at 10,000 xg and passing the supernatant through an 0.22-μm sterile syringe filter (Sartorius).

Microbial community growth and metabolome production in bioreactors.

Fecal material or defined microbial communities were cultured in Infors Multifors bioreactor vessels (Infors AG, Switzerland) using parameters and procedures that we have previously described [79]. Briefly, 400-ml vessels were operated under continuous-culture conditions set to mimic the distal human gut: 37°C, pH 7, gently agitated, oxygen free, and fed with a constant supply of mucin (4 g/liter) and insoluble starch substrates (12 g/liter) at a flow rate of ~400 ml/day. Vessels were anaerobically maintained by constant bubbling of N_2 gas. A sample of culture from the bioreactor was withdrawn for this work once the vessel had reached steady state (21 days postinoculation for the fecal community and 10 days postinoculation from the defined communities). For this work, a fecal community was derived from a stool sample of donor 2 by obtaining a fresh fecal sample from the donor in a sealed plastic container, which was placed within the anaerobe chamber within 10 min of defecation. A 10% (wt/vol) fecal slurry was obtained by mixing 5 g of the homogenized fecal sample into 50 ml of prereduced chemostat feed medium with a stomacher (Tekmar Stomacher Lab Blender; Seward, Worthing, West Sussex, United Kingdom). Once homogenized, the fecal slurry was centrifuged (10 min, 175 xg) to remove large particulates, such as residual undigested food. The remaining supernatant was used as fecal inoculum for the bioreactor vessel. Defined communities DEC60 and MET-1 (from donor 1) were prepared for inoculation by preculture of individual isolates on fastidious anaerobe agar (FAA; Acumedia) with the addition of 5% defibrinated sheep's blood (Hemostat Laboratories), except for *Faecalibacterium prausnitzii*, for which blood was omitted. Isolates were cultured for 48 h in a Ruskin Concept 400 anaerobe chamber, as described above, and biomass was scraped directly into prereduced chemostat growth medium according to formulations indicated in Table S4 and used to inoculate the vessel. Supernatants of vessel samples were prepared by centrifuging culture for 30 min at 15,000 xg and passing the supernatant through an 0.22-μm sterile syringe filter (Sartorius).

Growth inhibition assays.

The antifungal activities of the GM and individual bacterial effluents were evaluated as follows: different fungal strains were grown overnight in YPD medium at 30°C in a shaking incubator. Cells were then suspended in fresh SC medium at an optical density at 595 nm (OD_{595}) of 0.05. A total volume of 99 µl or 92 µl of *C. albicans* culture was added to each well of a flat-bottomed 96-well plate in addition to 1 µl or 8 µl of GM, in order to get 1 and 8% GM, respectively. For controls, equivalent sterile, uninoculated bioreactor control medium was used in place of GM. The plates were incubated in the Sunrise-Tecan plate reader at 30°C under agitation, and OD_{595} readings were taken every 10 min over 24 h. All experiments were carried out in triplicate, and average values for each set were calculated for analysis.

Filamentation assay.

An overnight culture of the *C. albicans* SC5314 strain was used to inoculate 10 ml of fresh YPD at an OD_{595} of 0.05. The cells were grown for 4 h at 30°C under agitation to reach the exponential phase. To induce hypha formation, fetal bovine serum (Life Technologies) was added as a supplement at 10% and cells were incubated at 37°C under agitation for 1 h. The percentage of germ-tube-forming hyphae was determined, and the length of the generated filaments was simultaneously assessed. At least 100 fungal cells were counted per sample, and all experiments were performed in triplicate.

Genetic screen for gut metabolome-sensitive mutants.

We screened a set of 2,360 strains from the GRACE collection [60], which bear a gene deletion at one locus and an integrated tetracycline-regulated allele at the other locus. Mutant strains were grown overnight on SC medium on flat-bottomed 384-well plates and were plated on SC agar solid medium with or without GM (8%) using

a 384-well blot replicator. Colony diameter was measured using the colony imager (spImager; S&P Robotics). Mutants exhibiting more than 2-fold growth reduction based on colony diameter were considered to have been affected by treatment. Each of these mutant phenotypes was then confirmed by spreading a dilution of an overnight culture on an SC medium petri dish to resolve well-separated colonies. he average diameters of at least 50 colonies of each mutant strain as well as the parental wild-type strain were measured. The same procedure was used to measure the colony diameter of the sirolimus-resistant strain JRB12.

Intestinal epithelial cell damage assay.

Damage to the human colon epithelial cell line HT-29 (ATCC; HTB-38) was assessed using a lactate dehydrogenase (LDH) cytotoxicity detection kit (Sigma), which measures the release of LDH in the growth medium. The manufacturer's protocol was followed. HT-29 cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS to 95% confluence in a 96-well tissue culture plate and incubated at 37°C with 5% CO_2. Prepared cells were infected with 2 x 10^6 C*. albicans* SC5314 blastospores for 24 h at 37°C with 5% CO_2. Following incubation, 100 µl supernatant was removed from each experimental well and LDH activity in this supernatant was determined by measuring the absorbance at 490 nm (OD_{490}). LDH activity was calculated as the mean from at least four independent biological replicates.

Expression analysis by real-time quantitative PCR (qPCR).

Saturated overnight culture of the *C. albicans* SC5314 strain was diluted to an OD_{595} of 0.1 in 50 ml fresh SC and grown at 30°C to an OD_{595} of 0.8. The culture was then divided into two equal fractions. Fraction 1 was subjected to hypha induction by supplementing the cultures with 10% fetal bovine serum (FBS) and incubating them at 37°C for 60 min. Fraction 2 was incubated at 30°C for 60 min to encourage the maintenance of the yeast form. GM-treated (8%) and control cultures were harvested

by centrifugation at 3,000 xg for 5 min, and the pellet was rapidly frozen in liquid nitrogen. Total RNAs were extracted using the Qiagen RNeasy kit as follows: samples stored at -80°C were placed on ice, and RNeasy RLT buffer was added to pellets at a 1:1 (vol/vol) ratio of buffer to pellet. The pellet was allowed to thaw in the buffer and processed with a vortex device briefly at high speed. The resuspended pellet was placed back on ice and divided into 1-ml aliquots in 2-ml screw-cap microcentrifuge tubes containing 0.8 ml of 3-mm-diameter acid-washed glass beads. Samples were homogenized 4 times for 1 min each time in a FastPrep-24 bead beater for 60 s at a speed of 6.5 m/s. Samples were placed on ice for 5 min after each homogenization step. Following homogenization, the Qiagen RNeasy protocol was followed as recommended by the supplier. Total RNA samples were eluted in RNase-free H_2O. RNA quality and integrity were assessed using an Agilent 2100 Bioanalyzer.

cDNA was synthesized from 2 µg of total RNA using the SuperScript III (Life Technologies) reverse transcription (RT) system [1X RT buffer, 10 mM dithiothreitol, 2.5 mM $MgCl_2$, 400 nM oligo(dT)15, 1 µM random hexamers, 1 mM deoxynucleoside triphosphate (dNTP), 200 units Superscript III reverse transcriptase]. The total volume was adjusted to 20 µl, and the mixture was then incubated 60 min at 52°C. The RT reaction was terminated by heating at 85°C for 5 min. The RNA template was removed from the cDNA-RNA duplex by adding RNase H at 2 U/µl and incubating the mixture at 37°C for 20 min. Aliquots of the resulting first-strand cDNA were used for real-time PCR amplification experiments. Real-time PCR was performed using the Applied Biosystems 7500 real-time PCR system with the SYBR green PCR master mix (Qiagen) according to the manufacturer's instructions. After a 10-min denaturation at 95°C, the reaction mixtures were cycled 40 times at 95°C for 15 s, 56°C for 15 s, and 72°C for 30 s. To verify that only the specific product was amplified, a melting point analysis was performed after the last cycle by cooling samples to 55°C and then increasing the temperature to 95°C at 0.2°C per second. A single product at a specific melting temperature was found for each target. All

samples were tested in triplicate, and the mean was determined for further calculations. To evaluate the transcript level of the studied genes, the results were normalized using threshold cycle (CT) values obtained from actin (Act1, orf19.5007). The relative quantification analysis was performed using the comparative CT method [80]. Primers used for real-time quantitative PCR (qPCR) are listed in Table 2.

RNA-seq profiling.

Treatment of *C. albicans* cells with the GM and the RNA extraction procedure were performed as described for the qPCR experiment. A total of 5 µg of total RNA was used for RNA sequencing, and samples were analyzed in duplicate for all conditions. cDNA was generated from total RNA using the Clontech SMARTer PCR cDNA synthesis kit (Clontech). The resulting cDNA was fragmented using Bioruptor (Diagenode, Inc., Denville, NJ, USA), profiled using an Agilent Bioanalyzer, and subjected to Illumina library preparation using SPRIworks HT (Beckman Coulter). The quality and quantity and the size distribution of the Illumina library were determined using an Agilent Bioanalyzer. The library was then submitted for Illumina HiSeq2500 sequencing according to the standard operation. Paired-end 126-nucleotide (nt) reads were generated and checked for data quality using FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The raw RNA-seq reads for each sample were aligned to the reference *C. albicans* SC5314 genome using STAR v2.4.0 [81] with default parameters. After alignment, estimation of transcript abundance measures as fragments per kilobase of exon per million aligned fragments (FPKM) was performed using Cufflinks in the Tuxedo protocol [82]. Differentially expressed transcripts were identified using a fold change cutoff of 2 and a false-discovery rate (FDR) of less than 1%. The complete data set of the RNA-seq experiment is presented in Table S6.

Ribosomal protein S6 Western blot analyses.

Ribosomal protein S6 phosphorylation assays were performed as described previously [63]. Briefly, *C. albicans* SC5314 cells were grown overnight in YPD at 30°C with shaking, washed and resuspended in phosphate-buffered saline (PBS), and diluted into fresh SC medium to a starting OD_{595} of 0.2. Cultures were grown for 2 h before being exposed to 8% GM for an hour. Cells were then harvested by centrifugation and lysed by bead beating in buffer S6 (50 mM Tris Cl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 10% glycerol, 0.2% Nonidet P-40, 1 mM dithiothreitol [DTT], 1 mM phenylmethylsulfonyl fluoride [PMSF], cOmplete Mini EDTA-free protease inhibitor cocktail [Roche Applied Science], 0.1 mM sodium orthovanadate, 20 μM sodium glycerophosphate, 20 μM paranitrophenylphosphate, 20 μM sodium fluoride). Cell lysates were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes, which were probed for P-S6 using anti-phospho-(S/T)-Akt substrate rabbit polyclonal antibody (Cell Signaling Technology). Antitubulin rat monoclonal antibody (Abcam) was used to monitor the loading controls. Secondary antibodies used were bovine anti-rabbit antibody (Santa Cruz Biotechnology) and goat anti-rat antibody (Abcam). The blots were imaged using a Kodak Image Station 4000 MM. The presented data are representative of at least three biological replicates.

Statistical analyses.

Fungal growth data were analyzed using one-tailed, unpaired t tests.

Data availability.

The RNA-seq data are provided in Table S6 in the supplemental material

Supplemental material

Supplemental material for this article may be found at https://doi.org/10.1128/mSphere.00555-17

Acknowledgments

We thank Sandra Weber and Martine Raymond (IRIC-Université de Montréal) for providing *C. albicans* clinical strains (S2, G5, and 6692), Philippe Dufresne (Laboratoire de Santé Publique du Québec) for providing DPL-1007 and -1008, Joseph Heitman for the sirolimus-resistant TOR1-1/TOR1 strain, and Michel J. Tremblay (CRI-CHUQ, Université Laval) for the HT-29 cell line.

Work in Adnane Sellam's laboratory is supported by Fonds de Recherche du Québec-Santé (FRQS) (Établissement de jeunes chercheurs), the Canada Foundation for Innovation (CFI-34171), and the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant (06625). Adnane Sellam is a recipient of the Fonds de Recherche du Québec-Santé (FRQS) J1 salary award. J.R.K. is supported by NIH fund R01AI095305. Work in the Allen-Vercoe laboratory was supported by NSERC Discovery grant 400809, a Pulse Cluster grant (AIP-CL03), and an NIH grant (R21 AI121575-02).

E.A.-V. is cofounder and CSO of NuBiyota LLC, a company focused on the creation of novel microbial ecosystem therapeutics. The other authors declare no conflicts of interest.

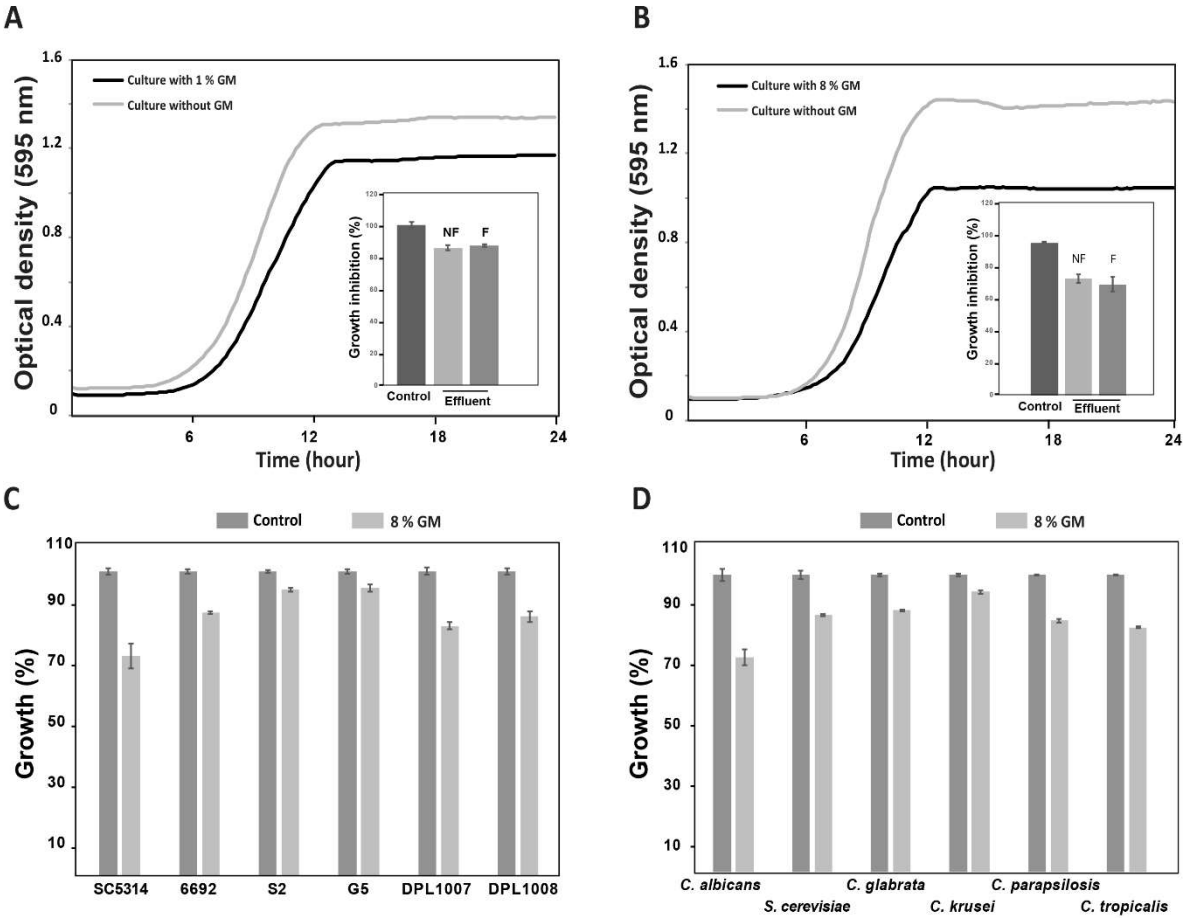

Figure 1 Antifungal activity of the gut secreted metabolome.

(A and B) The C. albicans SC5314 strain was grown in SC medium supplemented with 1% (A) and 8% (B) bioreactor effluent (DEC60 gut metabolome [GM]). Cells were grown at 30°C, and an OD595 reading was taken every 10 min. OD measurements for each GM concentration and control (cells treated with uninoculated bioreactor growth medium) are provided as the mean from triplicate assays. To determine whether the bioactive antifungal molecules were secreted by the components of the microbiota, spent culture medium was separated from bacterial cells by centrifugation followed by supernatant filtration. The histogram in each panel indicates OD595 after 24 h of exposure. (C) Antifungal activity of the GM on clinical C. albicans azole- (6692, S2, and G5) and echinocandin-resistant (DPL-1007 and DPL-1008) strains. (D) The GM inhibits the growth of representative strains of gut-resident yeasts, including *C. glabrata, C. parapsilosis, C. krusei,* and *S.*

cerevisiae. For panels C and D, cells were grown in SC medium with 8% GM or control growth medium and OD595 readings were taken after 24 h of incubation at 30°C under agitation. Results represent mean growth inhibition (percent) after 24 h of treatment of at least three replicates. NF, nonfiltered; F, filtered.

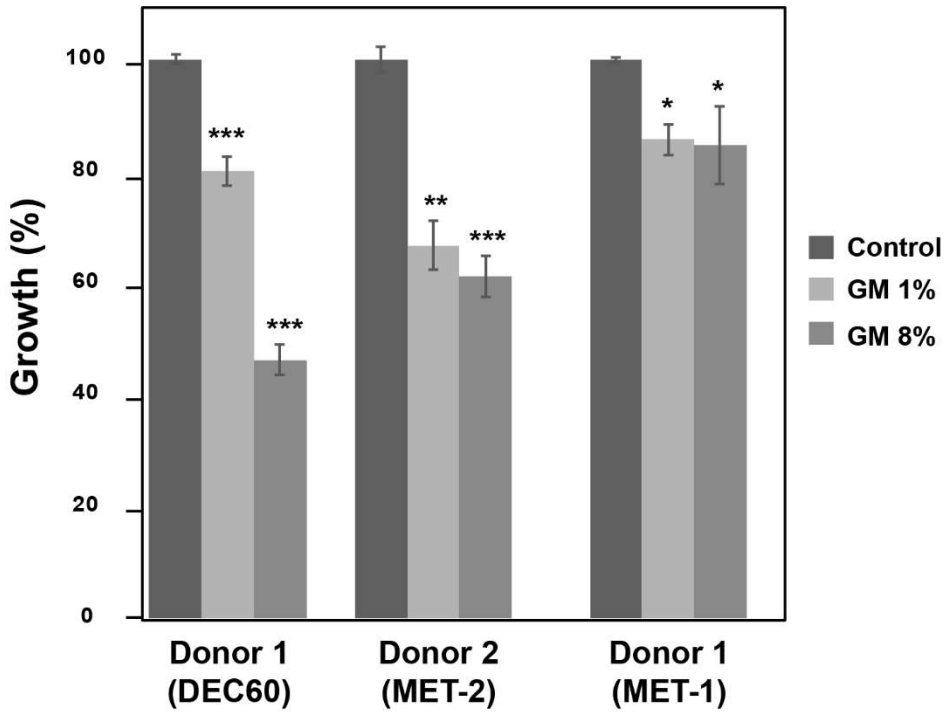

Figure 2 The inhibitory effect of the human gut metabolome is widespread.

For each human donor, results represent the average from three independent *C. albicans* cultures. *C. albicans* SC5314 cells were grown in SC medium with 1 and 8% GM or control medium, and an OD_{595} reading was taken after 24 h of incubation at 30°C under agitation. MET-1 is a defined therapeutic microbial ecosystem of 33 bacterial strains from donor 1 (DEC60). Bars show the means ± standard errors of the means. *, $P < 0.02$; **, $P < 0.01$; ***, $P < 0.0003$.

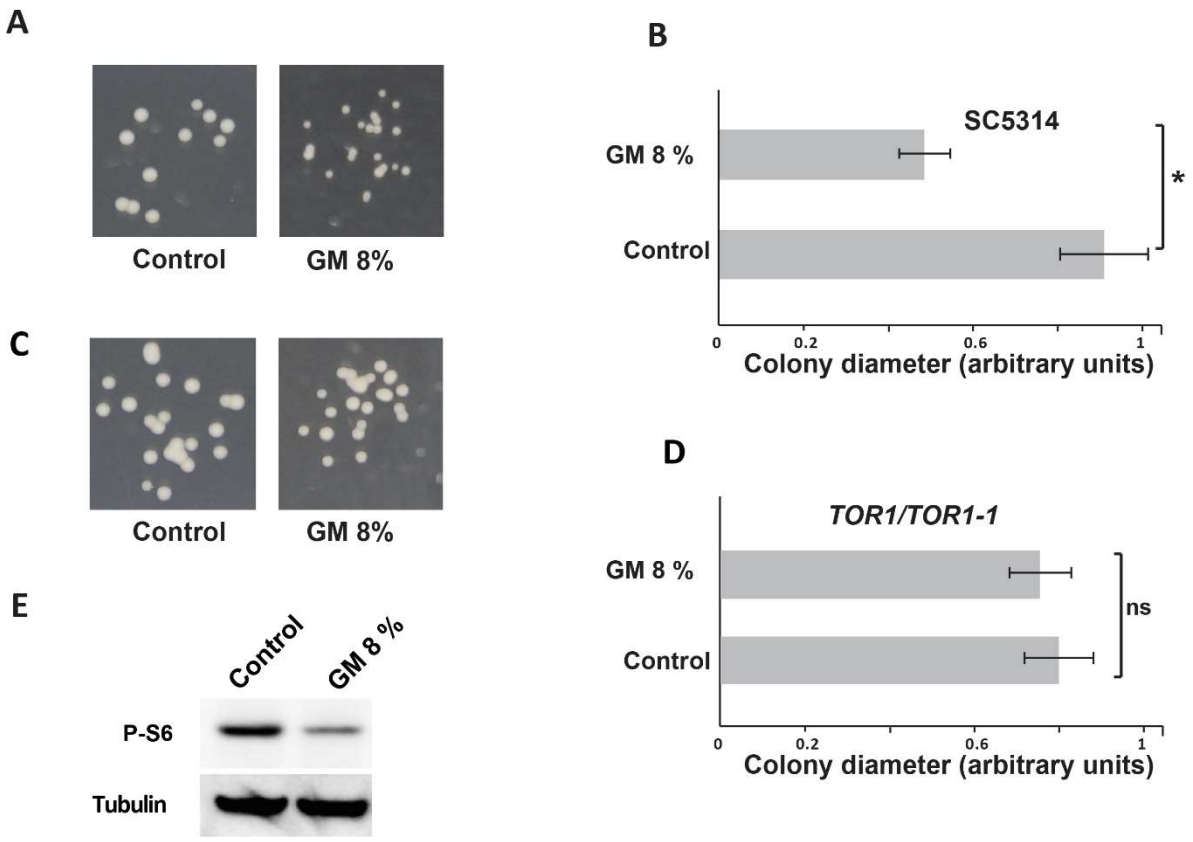

Figure 3 The gut metabolome inhibits *C. albicans* growth through the TOR pathway.

(A) In solid SC-agar medium, cells treated with 8% GM resulted in colonies with reduced size. (B) Diameters (measured in arbitrary units) of at least 50 colonies grown in SC-agar with or without GM (8%) were measured. (C) Representative image of the sirolimus-resistant TOR1-1 strain (JRB12) colony size. (D) Mean diameter of TOR1-1 colonies. In panels B and D, bars represent the means ± standard errors of the means. *, $P < 0.00001$; ns, not significant ($P > 0.12$). (E) The GM reduces the phosphorylation level of the TOR effector, ribosomal protein S6. C. albicans cells were grown in SC medium and treated for 1 h with 8% GM. The same volume of culture medium was added to the control culture. Cell lysates were probed for P-S6 and tubulin (loading control) using anti-phosphorylated-Akt substrate and antitubulin antibodies, respectively. At least three biological replicates were obtained for each experiment shown.

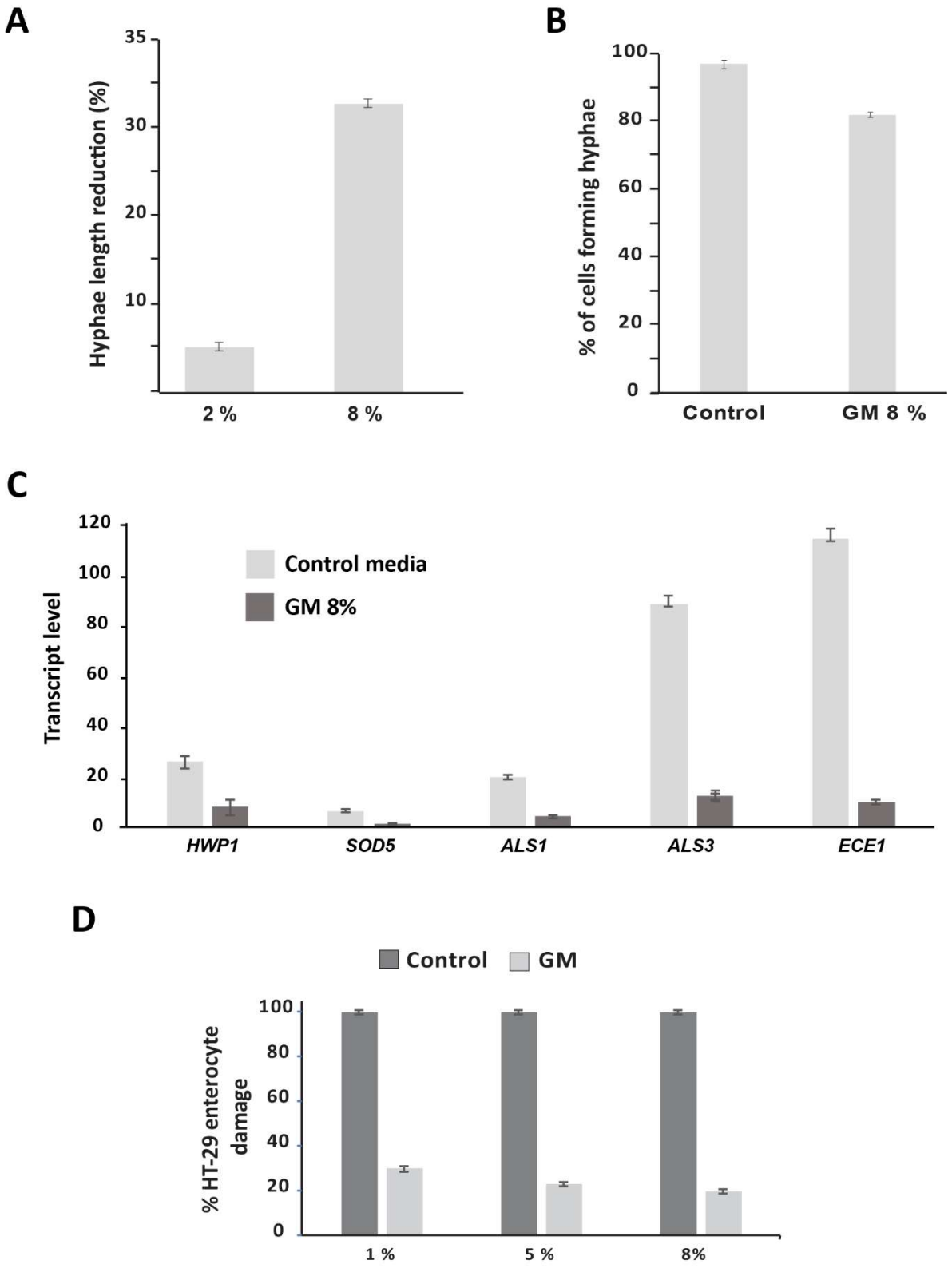

Figure 4 Antivirulence activity of the human gut metabolome.

(A and B) The GM inhibits both hypha elongation (A) and hypha formation (B). *C. albicans* SC5314 cells growing at 37°C in the presence of FBS were treated with 2 and 8% GM for 1 h. The number of germ tubes of at least 100 cells treated with GM or not was counted and compared to cells treated with the control culture medium only (B). At the same time, filament lengths were measured and results are presented as the percentage of length reduction compared to the control condition (A). The presented data are representative of three biological replicates. (C) The GM contains molecules that modulate virulence-related gene expression. Transcript levels of bona fide yeast-to-hypha transition genes, including ALS1, ALS3, ECE1, HWP1, and SOD5, were evaluated in cells exposed to 8% GM for 1 h. Transcript levels were calculated using the comparative CT method using the ACT1 gene as a reference. (D) The GM attenuates the damage to human colon epithelial cells (HT-29 cells) caused by *C. albicans*. Damage to HT-29 cells was assessed using an LDH release assay. For each GM concentration, cell damage was calculated as percentage of LDH activity of the GM-treated experiment culture relative to that of the control experiment culture (*C. albicans* invading HT-29 cells in the absence of GM). Results are expressed as the mean from three independent biological replicates.

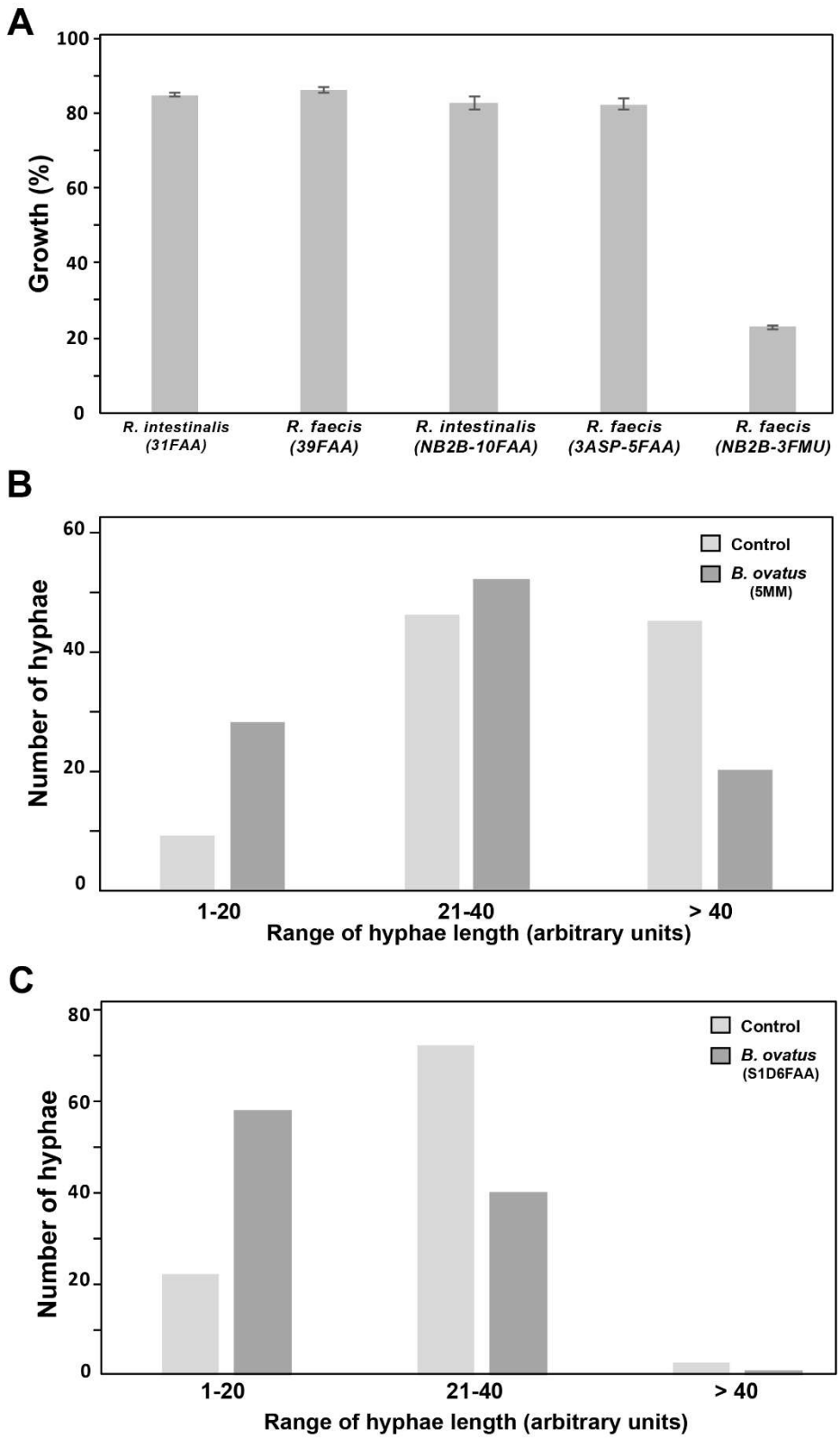

Figure 5 Identification of bacterial species producing the anti-*Candida* molecules.

(A) Both *Roseburia intestinalis* and *R. faecis* secreted molecules exert antifungal activity. *C. albicans* cells were grown in SC medium with 8% GM or control medium, and OD_{595} readings were taken after 24 h of incubation at 30°C under agitation. Results represent growth inhibition (percent) after 24 h of treatment of at least three replicates. (B and C) Both *Bacteroides ovatus* strain 5MM (B) and strain S1D6FAA (C) reduced *C. albicans* hypha elongation. A total of 100 cells with three hypha length ranges (1 to 20, 21 to 40, and >40), measured in arbitrary units, were counted. Results are presented as the number of cells with a hypha length in the corresponding range.

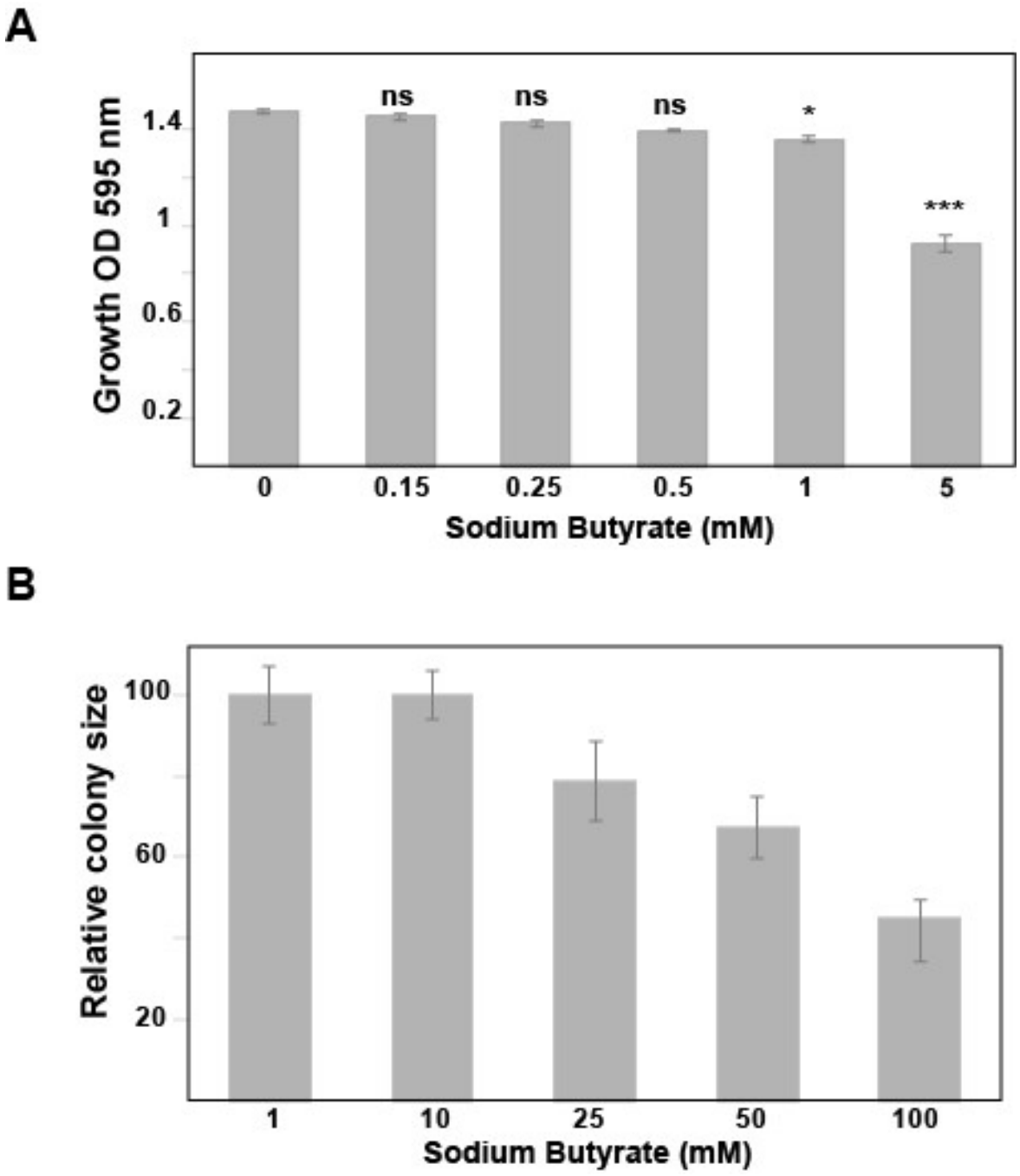

Figure S1 Effect of sodium butyrate on *C. albicans* growth and colony size.

(A) The *C. albicans* SC5314 strain was grown in SC medium supplemented with different concentrations of sodium butyrate. The histogram represents growth inhibition (%) after 24 h of treatment. (B) Effect of different concentrations of sodium butyrate on colony size. Data represent colony size reduction in percentage compared to nontreated *C. albicans* control cells.

Table 1 Gene functions and biological processes associated with *C. albicans* response to GM[b]

GO category	Gene name	*p*-value[a]
Up-regulated transcripts		
Alpha-amino acid biosynthesis	*ALT1, ARG3, ARG5,6, ARG8, CYS1, CPA1, CPA2, DFR1, GDH3, HAL21, HBR2, HOM3, IDP1, LYS12, LYS22, LYS4, MET15, MET16, MET2*	4.79e-08
Aspartate family amino acid biosynthesis	*CYS1, HAL21, HOM3, LYS12, LYS22, LYS4, MET15, MET16, MET2*	5.78e-05
Arginine metabolism	*ARG3, ARG5,6, ARG8, CAR2, CPA1, CPA2*	6e-04
Sulfur amino acid metabolism	*ARO10, C1_02970W_A, CYS1, HAL21, HOM3, LAP3, MET15, MET16, MET2*	8.4e-04
Glutamine family amino acid biosynthesis	*ARG3, ARG5,6, ARG8, CPA1, CPA2, GDH3, IDP1*	1.33e-03
Carbohydrate metabolism	*ADH2, ARA1, ATC1, GLG21, C3_03410C_A, C4_02620C_A, CIT1, DAK2, GLK1, GLK4, GRE3, HSP104, HSP21, HXK2, MDH1-1, MLS1, PGM2, PHR1, PYC2, TPS2, XKS1*	1.71e-03
Methionine metabolism	*ARO10, CYS1, HAL21, HOM3, MET15, MET16, MET2*	9.55e-03
Serine metabolism	*CYS1, DFR1, HBR2, HOM3, MET15, MET16*	7.03e-03
Trehalose metabolism	*ATC1, HSP104, HSP21, PGM2, TPS2*	5.46e-03
Alpha-amino acid catabolism	*ADH2, ALT1, ARO10, C1_08490W_A, CAR2, CPA1, LAP3*	4.12e-03
Down-regulated transcripts		
Ribosome biogenesis		
Ribosome localization	*BMS1, RRP40, BUD20, NOP9, RSA3, URB1, RRP17, LRP1, ESF1, REX4, RRB1, ECM16, RRP5, NOP12, EBP2, PUF6, NOP53, RRP46, SLX9, RRP14, NOP16, RRP43, KRI1, POP7, RSA4, RIX1, MAK11, FAF1, NUG1, SNU66, C5_04910W_A, SQT1, DHR2, TSR4, MTR4, CHR1, TMA23, IFU4, RCM1, UTP23, NAF1, NOP2, KRE33, GRC3, RRP36, TIF6, CSI2, DBP3, DBP8, DIP2, ECM1, ENP2, ERB1, FYV5, GAR1, HAS1, HIT1, IMP4, JIP5, MRT4, NHP2, NIP7, NMD3, NOC2, NOC4, NOG2,*	1.89e-70

	NOP1, NOP4, NOP5, NOP6, NOP8, NSA1, PES1, POP4, PUS7, RCL1, REI1, RPF1, RPF2, RPL7, RPP1, RRP6, RRP8, RRS1, SAS10, SDA1, SPB1, SPB4, TSR2, UTP13, UTP20, UTP21, UTP4, YTM1, YVH1	
snRNA metabolic process	*BUD20, NOP9, NOP53, SLX9, RIX1, NUG1, TIF6, ECM1, NMD3, NOG2, RPF1, RRS1, SDA1*	2.80e-07
tRNA processing	*MTR4, PUS7, RRP6, NAF1, GAR1, LRP1, NHP2*	5.84e-03
snoRNA processing	*RPP1, DUS3, TGS1, DUS1, SMM1, PUS7, POP7, SEN2, TRM5, TRM3, NOP1, DEG1, POP4*	9.45e-03
GMP metabolic process	*RPP1, RRP6, LRP1, POP7, NOP1, POP4, MTR4*	4.15e-02

[a] The P value was calculated using hypergeometric distribution, as described on the GO Term Finder website.

[b] Gene ontology (GO) analysis was performed using GO Term Finder.

Table 2 Primer sets used in real-time quantitative PCR

Gene	**5′ primer**	**3′ primer**
ACT1	GAAGCCCAATCCAAAAGA	CTTCTGGAGCAACTCTCAATTC
ALS1	CCTATCTGACTAAGACTGCACC	ACAGTTGGATTTGGCAGTGGA
ALS3	CGGTTGCGACTGCAAAGAC	GACCAACCCAAAACAGCATTCC
HWP1	CAGTTCCACTCATGCAACCATC	GCAATACCAATAATAGCAGCACCG
SOD5	ACGAGGGACACGGCAATGCT	GCGCCATTACCTTGAGGAGCAGTA
ECE-1	CCGGCATCTCTTTTAACTGG	GAGATGGCGTTCCAGATGTT

Table S1 Fungal strains used in this study.

Name	Description
Candida albicans	
SC5314 (ATCC-MYA-2876)	*C. albicans* wild-type reference strain.
6692	Azole-resistant clinical strain (overexpressing the MFS-transporter MDR1 and had a gain-of-function mutation on the transcription factor, Mrr1) isolated from mouth
S2	Fluconazole-resistant clinical strain isolated from patient with AIDS (resistance related to gain-of-function mutation of the transcription factor, Upc2 and Erg11 overexpression)
G5	Azole-resistant clinical strain (overexpressing MDR1 and harbouring a gain-of-function mutation on the transcription factor, Mrr1) isolated from oral cavity from patient with AIDS
DPL-1007	Clinical isolate resistant to echinocandin harbouring the F641S mutation on the beta-(1,3)-glucan synthase, Fks1p
DPL-1008	Clinical isolate resistant to echinocandin harbouring the F645P mutation on the beta-(1,3)-glucan synthase, Fks1p
JRB12	SC5314 TOR1-1/TOR1; A laboratory strain that is resistant to the TOR pathway inhibitor, Rapamycin.
Saccharomyces cerevisiae	
BY4741 (ATCC 4040002)	*S. cerevisiae* laboratory reference strain used as genetic background for the systematic gene disruption project
Candida tropicalis	
MY070362	Clinical susceptible strain from INSPQ (Institut National de Santé Publique, Québec, Canada)
***Candida krusei* (*Issatchenkia orientalis*)**	
ATCC6258	ATCC reference strain. Isolated from the sputum of patient with bronchomycosis
Candida parapsilosis	
ATCC90018	Reference susceptible strain used for CLSI antifungal susceptibility testing. Isolated from blood
Candida glabrata	
ATCC90030	Reference susceptible strain used for CLSI antifungal susceptibility testing. Isolated from blood

Table S2 List of GM-upregulated transcripts.

Table S2 is too lengthy to be included in this document but can be obtained upon request or downloaded at https://doi.org/10.1128/mSphere.00555-17.

Table S3 List of GM-downregulated transcripts.

Table S3 is too lengthy to be included in this document but can be obtained upon request or downloaded at https://doi.org/10.1128/mSphere.00555-17

Table S4 Bacterial strains used in this study.

Table S4 is too lengthy to be included in this document but can be obtained upon request or downloaded at https://doi.org/10.1128/mSphere.00555-17

Table S5 Butyrate-producing bacteria from MET-1 defined community.

Strain Number	Species
1FAA	*Eubacterium rectale*
29FAA	*Eubacterium rectale*
40FAA	*Fecalibacterium prausnitzii*
F1FAA	*Eubacterium eligens*
6FM	*Eubacterium rectale*
47FAA	*Eubacterium ventriosum*
18FAA	*Eubacterium rectale*
21FAA	*Clostridium* sp.
13LG	*Eubacterium limosum*
3ASP-5FAA	*Roseburia faecis*
AP34BHI	*Faecalibacterium prausnitzii*
NB2B 10 FAA	*Roseburia intestinalis*
NB2B 19 DCM	*Faecalibacterium prausnitzii*

General conclusion and perspective

The objective of my master project was to better understand the commensal lifestyle of *C. albicans* and how its phenotypic flexibility could be influenced by different cues that could provide a future therapeutic approach. The specific objective of our article was to investigate whether the microbial gut metabolome (GM) contains molecules that might promote the commensal lifestyle and/or inhibit the expression of virulence of *C. albicans* in the intestine. Our investigation showed that the microbial gut metabolome has two main effects (Figure C1).

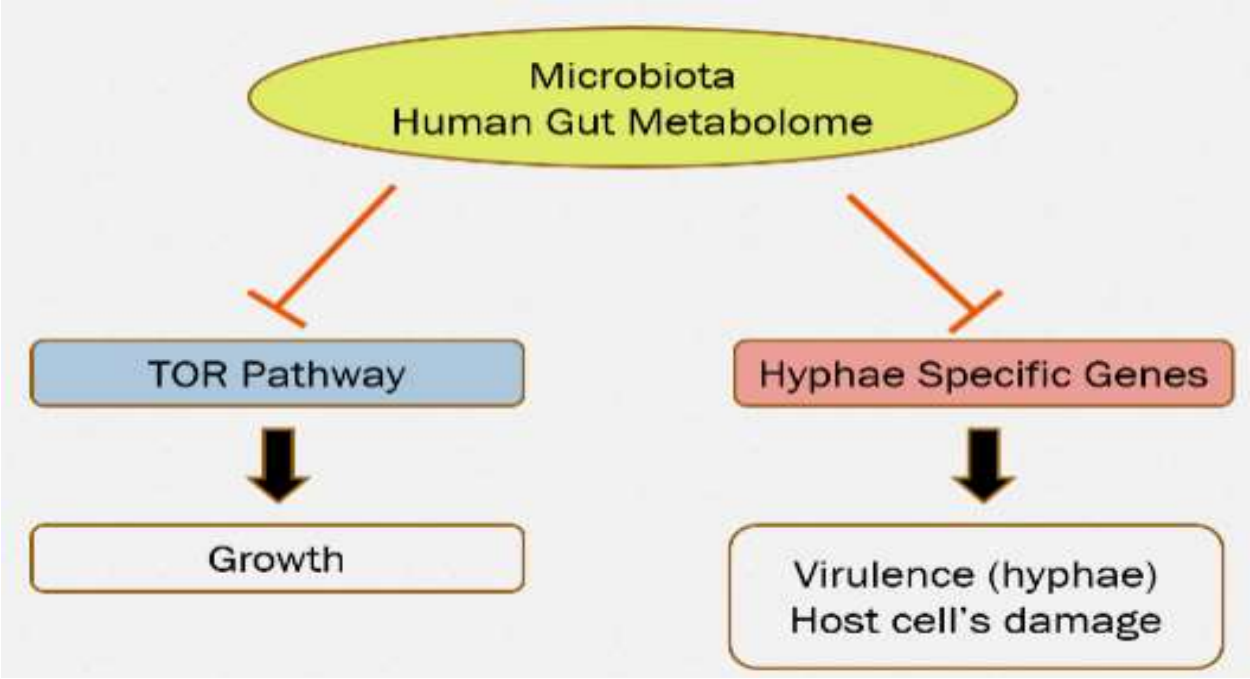

Figure C1. Effects of the human gut metabolome against *C. albicans*.

First, we have shown that the GM inhibited the growth of *C. albicans* through TOR pathway which was determined by: i) using a genetic screen that identified that the mutant of an essential component of the TOR pathway, Kog1 was hypesensitive to GM, ii) evaluation of the phosphorylation state of P-S6, a conserved effector of the

TOR pathway and iii) evaluation of the transcriptional profile of *C. albicans* in presence of GM. These results validate our hypothesis that gut microbial cohabitants repress *C. albicans* TOR activity to mimic a starved environment which could lock the cells into a quiescent state, called also G0 state, and consequently lower their nutritional competitiveness in the GI tract. Future investigations should be done to verify this hypothesis. Several histone marks such as H3K4me3, H3K36me3 and H3K79me3 are enriched in cells under G0 phase in *S. cerevisiae* and *S. pombe* [83]. Thus, it is worth checking this histone mark in *C. albicans* cells treated with the GM by western blot using commercially available antibodies against H3K4me3, H3K36me3 and H3K79me3.

The second main effect of the GM is the inhibition of the yeast-to-hyphae transition in *C. albicans*, which was demonstrated by measuring the hyphae length of cell exposed to GM or not. Furthermore, the transcription level of the hypha-specific genes was measured and we demonstrated an important reduction when compared to the control. These results validate our hypothesis that gut microbiota-secreted molecules repress the yeast-to-hypha transcriptional program, which affect the invasive form of *C. albicans*.

Another important finding during our investigation was the fact that the human gut metabolome exerts a pan-species antifungal activity, which was demonstrated by the growth inhibition of other non-*albicans* opportunist *Candida* species and gut-resident yeast, including *C. glabrata, C. parapsilosis, C. krusei,* and *S. cerevisiae*. This antifungal activity was also demonstrated on clinical *C. albicans* azole-resistant and echinocandin-resistant strains, demonstrating that the GM contains bioactive molecules that can potentially be used to tackle therapeutic limitations related to acquired clinical resistance to commonly used antifungals.

Moreover, we showed that antifungal effect of gut metabolome is common between healthy donors, even if the human microbiota is very dynamic and is influenced by different conditions. Even a Microbial Ecosystem Therapeutic (MET-1) consisting of 33 bacterial strains partially recapitulated the activity of the GM, suggesting that the antifungal activity of the GM may be mediated by several other strains or by the combination of different strains. For this reason, we individually screened the filtered effluents of the 33 isolates to evaluate their antifungal properties. Although most of them had no apparent growth inhibition properties, notable exceptions were effluents from strains belonging to species of the genus *Roseburia*, including *R. faecis* (31FAA) and *R. intestinalis* (39FAA), which exhibited significant antifungal activity against *C. albicans* suggesting that they secrete bioactive molecules that contribute to the antifungal activity of the GM. Notwithstanding, both bacteria are known as butyrate producers, the concentrations of butyrate present in the effluents were 0,05 to 0,25 mM and it has been demonstrated that butyrate could have an antifungal effect only at higher concentrations than 1mM, suggesting that butyrate alone is not responsible for the antifungal activity.

The same 33 effluents were used to evaluated the anti-filamentation activity, and the effluents responsible for the antifungal activity didn't show any representative anti-filament activity but the effluent from *Bacteroides ovatus* isolate 5MM showed significant reduction of filament length, this effect was reproduced by other *Bacteroides ovatus* effluent from another donor (S1D6FAA).

As a conclusion, we showed the antagonistic interkingdom interactions between the opportunistic fungus *C. albicans* and intestinal commensal bacteria *B. ovatus, R. intestinalis*, and *R. faecis.* This fact could open the possibility to evaluate the administration of a defined bacterial community with anti-Candida activity as potential therapeutic protection against gastrointestinal inflammatory diseases. The administration if this defined community could be done as traditional probiotics, comparing ways of administration (oral, anal or both) during one week comparing

with two control group (one heat-killed probiotic and another with only the drug vehicle). Another way to explore the impact of these bacteria on *C. albicans* could be screening the small molecules present in their effluents to target virulence factors for drug development without perturbations of the microbiota equilibrium and tackling any therapeutic limitations that could be related to acquired clinical resistance.

Given the fact that IBD diseases are associated with both fungal and bacterial microbiota dysbiosis, it is worth investigating whether the GM from UC or CD patients still have the anti-growth and the antivirulence activities reported in our study.

References

[1] N. A. R. Gow and B. Yadav, “Microbe profile: Candida albicans: A shape-changing, opportunistic pathogenic fungus of humans,” *Microbiol. (United Kingdom)*, vol. 163, no. 8, pp. 1145–1147, 2017.

[2] I. Miranda *et al.*, “A genetic code alteration is a phenotype diversity generator in the human pathogen Candida albicans,” *PLoS One*, vol. 2, no. 10, 2007.

[3] A. C. Gomes *et al.*, “A genetic code alteration generates a proteome of high diversity in the human pathogen Candida albicans,” *Genome Biol.*, vol. 8, no. 10, 2007.

[4] D. A. Fitzpatrick, M. E. Logue, J. E. Stajich, and G. Butler, “A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis,” *BMC Evol. Biol.*, vol. 6, pp. 1–15, 2006.

[5] F. Tebbji, Y. Chen, A. Sellam, and M. Whiteway, “The Genomic Landscape of the Fungus-Specific SWI/SNF Complex Subunit, Snf6, in Candida albicans,” *mSphere*, vol. 2, no. 6, pp. 1–14, 2017.

[6] J. Berman and P. E. Sudbery, “Candida albicans: A molecular revolution built on lessons from budding yeast,” *Nat. Rev. Genet.*, vol. 3, no. 12, pp. 918–932, 2002.

[7] K. Pande, C. Chen, and S. M. Noble, “Passage through the mammalian gut triggers a phenotypic switch that promotes Candida albicans commensalism,” *Nat. Genet.*, vol. 45, no. 9, pp. 1088–1091, 2013.

[8] L. Böhm, S. Torsin, S. H. Tint, M. T. Eckstein, T. Ludwig, and J. C. Pérez, “The yeast form of the fungus Candida albicans promotes persistence in the gut of gnotobiotic mice,” *PLoS Pathog.*, vol. 13, no. 10, pp. 1–26, 2017.

[9] D. R. Soll, “The role of phenotypic switching in the basic biology and pathogenesis of Candida albicans,” *J Oral Microbiol*, vol. 1, no. 23, pp. 1–13, 2014.

[10] A. da Silva Dantas *et al.*, “Cell biology of Candida albicans–host interactions,” *Curr. Opin. Microbiol.*, vol. 34, pp. 111–118, 2016.

[11] C. Pierce *et al.*, “The Candida albicans Biofilm Matrix: Composition, Structure and Function,” *J. Fungi*, vol. 3, no. 1, p. 14, 2017.

[12] P. G. Pappas *et al.*, “A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients.,” *Clin. Infect. Dis.*, vol. 37, no. 5, pp. 634–643, 2003.

[13] K. B. Laupland, A. W. Kirkpatrick, D. L. Church, T. Ross, and D. B. Gregson, “Intensive-care-unit-acquired bloodstream infections in a regional critically ill population,” *J. Hosp. Infect.*, vol. 58, no. 2, pp. 137–145, 2004.

[14] S. F. Dufresne, D. C. Cole, D. W. Denning, and D. C. Sheppard, “Serious fungal infections in Canada,” *Eur. J. Clin. Microbiol. Infect. Dis.*, vol. 36, no. 6, pp. 987–992, 2017.

[15] Y.-H. Chiu, S.-L. Lin, J.-J. Tsai, and M.-Y. Lin, “Probiotic actions on diseases: implications for therapeutic treatments.,” *Food Funct.*, vol. 5, no. 4, pp. 625–34, 2014.

[16] Z. K. Wang, Y. S. Yang, A. T. Stefka, G. Sun, and L. H. Peng, “Review article: Fungal microbiota and digestive diseases,” *Alimentary Pharmacology and Therapeutics*, vol. 39, no. 8. pp. 751–766, 2014.

[17] H. E. Hallen-Adams and M. J. Suhr, “Fungi in the healthy human gastrointestinal tract,” *Virulence*, vol. 8, no. 3, pp. 352–358, 2017.

[18] J. ten Oever and M. G. Netea, “The bacteriome-mycobiome interaction and antifungal host defense,” *European Journal of Immunology*, vol. 44, no. 11. pp. 3182–3191, 2014.

[19] R. K. Singh *et al.*, “Influence of diet on the gut microbiome and implications for human health,” *J. Transl. Med.*, vol. 15, no. 1, p. 73, 2017.

[20] J. M. Bliss, K. P. Basavegowda, W. J. Watson, A. U. Sheikh, and R. M. Ryan, “Vertical and horizontal transmission of Candida albicans in very low birth weight infants using DNA fingerprinting techniques.,” *Pediatr. Infect. Dis. J.*, vol. 27, no. 3, pp. 231–5, 2008.

[21] M. J. Suhr and H. E. Hallen-Adams, “The human gut mycobiome: pitfalls and potentials--a mycologists perspective,” *Mycologia*, vol. 107, no. 6, pp. 1057–1073, 2015.

[22] G. B. Huffnagle and M. C. Noverr, “The emerging world of the fungal microbiome,” *Trends in Microbiology*, vol. 21, no. 7. pp. 334–341, 2013.

[23] M. J. Suhr, N. Banjara, and H. E. Hallen-Adams, “Sequence-based methods for detecting and evaluating the human gut mycobiome,” *Lett. Appl. Microbiol.*, vol. 62, no. 3, pp. 209–215, 2016.

[24] R. S. Shapiro, N. Robbins, and L. E. Cowen, “Regulatory Circuitry Governing Fungal Development, Drug Resistance, and Disease,” *Microbiol. Mol. Biol. Rev.*, vol. 75, no. 2, pp. 213–267, 2011.

[25] N. Robbins, T. Caplan, and L. E. Cowen, “Molecular Evolution of Antifungal Drug Resistance,” *Annu Rev Microbiol*, vol. 8, no. 71, pp. 753–775, 2017.

[26] M. Spitzer, N. Robbins, and G. D. Wright, “Combinatorial strategies for combating invasive fungal infections,” *Virulence*, vol. 8, no. 2, pp. 169–185, 2017.

[27] P. K. Mukherjee, B. Sendid, G. Hoarau, J.-F. Colombel, D. Poulain, and M.

A. Ghannoum, “Mycobiota in gastrointestinal diseases,” *Nat. Rev. Gastroenterol. Hepatol.*, vol. 12, no. 2, pp. 77–87, 2014.

[28] D. A. Hogan, Å. ̊Vik, and R. Kolter, “A Pseudomonas aeruginosa quorum-sensing molecule influences Candida albicans morphology,” *Mol. Microbiol.*, vol. 54, no. 5, pp. 1212–1223, 2004.

[29] S. Vermeire, G. Van Assche, and P. Rutgeerts, “Classification of inflammatory bowel disease,” *Curr. Opin. Gastroenterol.*, vol. 28, no. 4, pp. 321–326, 2012.

[30] J. Satsangi, “The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications,” *Gut*, vol. 55, no. 6, pp. 749–753, 2006.

[31] A. K. DeGruttola, D. Low, A. Mizoguchi, and E. Mizoguchi, “Current Understanding of Dysbiosis in Disease in Human and Animal Models,” *Inflamm. Bowel Dis.*, vol. 22, no. 5, pp. 1137–1150, 2016.

[32] F. Shanahan and C. N. Bernstein, “The evolving epidemiology of inflammatory bowel disease,” *Curr. Opin. Gastroenterol.*, vol. 25, no. 4, pp. 301–305, 2009.

[33] J. M. Dahlhamer, E. P. Zammitti, B. W. Ward, A. G. Wheaton, and J. B. Croft, “Prevalence of Inflammatory Bowel Disease Among Adults Aged ≥ 18 Years — United States , 2015,” *Morb. Mortal. Wkly. Rep.*, vol. 65, no. 42, pp. 1166–1169, 2016.

[34] G. Hoarau *et al.*, “Bacteriome and mycobiome interactions underscore microbial dysbiosis in familial Crohn’s disease,” *MBio*, vol. 7, no. 5, 2016.

[35] M. L. Richard, B. Lamas, G. Liguori, T. W. Hoffmann, and H. Sokol, “Gut Fungal Microbiota,” *Inflamm. Bowel Dis.*, vol. 21, no. 3, pp. 656–665, 2015.

[36] C. E. Huseyin, P. W. O’Toole, P. D. Cotter, and P. D. Scanlan, “Forgotten fungi—the gut mycobiome in human health and disease,” *FEMS Microbiol. Rev.*, vol. 41, no. 4, pp. 479–511, 2017.

[37] S. Jawhara *et al.*, “Colonization of mice by Candida albicans is promoted by chemically induced colitis and augments inflammatory responses through galectin-3.,” *J. Infect. Dis.*, vol. 197, no. 7, pp. 972–80, 2008.

[38] D. L. Moyes and J. R. Naglik, “The mycobiome: Influencing IBD severity,” *Cell Host and Microbe*, vol. 11, no. 6. pp. 551–552, 2012.

[39] M. Nucci and E. Anaissie, “Revisiting the source of candidemia: skin or gut?,” *Clin. Infect. Dis.*, vol. 33, no. 12, pp. 1959–67, 2001.

[40] L. N. Miranda *et al.*, “Candida colonisation as a source for candidaemia,” *J. Hosp. Infect.*, vol. 72, no. 1, pp. 9–16, 2009.

[41] S. J. Ott *et al.*, “Fungi and inflammatory bowel diseases: Alterations of composition and diversity,” *Scand. J. Gastroenterol.*, vol. 43, no. 7, pp. 831–841, 2008.

[42] D. M. Underhill and I. D. Iliev, “The mycobiota: interactions between commensal fungi and the host immune system,” *Nat. Rev. Immunol.*, vol. 14, no. 6, pp. 405–416, 2014.

[43] Q. H. Sam, M. W. Chang, and L. Y. A. Chai, “The fungal mycobiome and its interaction with gut bacteria in the host,” *International Journal of Molecular Sciences*, vol. 18, no. 2. 2017.

[44] A. Standaert-Vitse *et al.*, “Candida albicans colonization and ASCA in familial crohn’s disease,” *Am. J. Gastroenterol.*, vol. 104, no. 7, pp. 1745–1753, 2009.

[45] H. Sokol *et al.*, “Fungal microbiota dysbiosis in IBD,” *Gut*, vol. 66, no. 6, pp. 1039–1048, 2017.

[46] G. Liguori *et al.*, “Fungal dysbiosis in mucosa-associated microbiota of Crohn’s disease patients,” *J. Crohn’s Colitis*, vol. 10, no. 3, pp. 296–305, 2016.

[47] C. Chehoud *et al.*, “Fungal signature in the gut microbiota of pediatric patients with inflammatory bowel disease,” *Inflamm. Bowel Dis.*, vol. 21, no. 8, pp. 1948–1956, 2015.

[48] G. Sharon, N. Garg, J. Debelius, R. Knight, P. C. Dorrestein, and S. K. Mazmanian, “Specialized metabolites from the microbiome in health and disease,” *Cell Metab.*, vol. 20, no. 5, pp. 719–730, 2014.

[49] L. C. M. Antunes *et al.*, “Antivirulence activity of the human gut metabolome,” *MBio*, vol. 5, no. 4, pp. e01183–e01114, 2014.

[50] I. Gantois *et al.*, “Butyrate Specifically Down-Regulates Salmonella Pathogenicity Island 1 Gene Expression Butyrate Specifically Down-Regulates Salmonella Pathogenicity Island 1 Gene Expression,” *Society*, vol. 72, no. 1, pp. 946–949, 2006.

[51] N. Kamada, G. Y. Chen, N. Inohara, and G. Núñez, “Control of pathogens and pathobionts by the gut microbiota,” *Nat. Immunol.*, vol. 14, no. 7, pp. 685–690, 2013.

[52] D. K. Morales and D. Hogan, “Candida albicans interactions with bacteria in the context of human health and disease.,” *PLoS pathogens*, vol. 6, no. 4. p. e1000886, 2010.

[53] L. N. Nguyen, L. C. L. Lopes, R. J. B. Cordero, and J. D. Nosanchuk, “Sodium butyrate inhibits pathogenic yeast growth and enhances the functions of macrophages,” *J. Antimicrob. Chemother.*, vol. 66, no. 11, pp.

2573–2580, 2011.

[54] L. C. M. Antunes, J. Han, R. B. R. Ferreira, P. Lolić, C. H. Borchers, and B. B. Finlay, “Effect of antibiotic treatment on the intestinal metabolome,” *Antimicrob. Agents Chemother.*, vol. 55, no. 4, pp. 1494–1503, 2011.

[55] S. Yen *et al.*, “Metabolomic analysis of human fecal microbiota: A comparison of feces-derived communities and defined mixed communities,” *J. Proteome Res.*, vol. 14, no. 3, pp. 1472–1482, 2015.

[56] S. L. Vogt, J. Peña-Díaz, and B. B. Finlay, “Chemical communication in the gut: Effects of microbiota-generated metabolites on gastrointestinal bacterial pathogens,” *Anaerobe*, vol. 34. pp. 106–115, 2015.

[57] E. O. Petrof *et al.*, “Stool substitute transplant therapy for the eradication of Clostridium difficile infection: ‘RePOOPulating’ the gut,” *Microbiome*, vol. 1, no. 1, p. 3, 2013.

[58] S. L. E. Martz *et al.*, “Administration of defined microbiota is protective in a murine Salmonella infection model,” *Sci. Rep.*, vol. 5, pp. 1–14, 2015.

[59] L. Cui, A. Morris, and E. Ghedin, “The human mycobiome in health and disease,” *Genome Med.*, vol. 5, no. 7, pp. 1–12, 2013.

[60] T. Roemer *et al.*, “Large-scale essential gene identification in Candida albicans and applications to antifungal drug discovery,” *Mol. Microbiol.*, vol. 50, no. 1, pp. 167–181, 2003.

[61] R. Loewith and M. N. Hall, “Target of rapamycin (TOR) in nutrient signaling and growth control,” *Genetics*, vol. 189, no. 4. pp. 1177–1201, 2011.

[62] M. C. Cruz *et al.*, “Rapamycin and less immunosuppressive analogs are toxic to Candida albicans and Cryptococcus neoformans via FKBP12-dependent inhibition of TOR,” *Antimicrob. Agents Chemother.*, vol. 45, no. 11, pp. 3162–3170, 2001.

[63] T. Chowdhury and J. R. Köhler, “Ribosomal protein S6 phosphorylation is controlled by TOR and modulated by PKA in Candida albicans,” *Mol. Microbiol.*, vol. 98, no. 2, pp. 384–402, 2015.

[64] J. L. Crespo, M. N. Hall, and L. Crespo, “Elucidating TOR Signaling and Rapamycin Action : Lessons from Saccharomyces cerevisiae Elucidating TOR Signaling and Rapamycin Action : Lessons from Saccharomyces cerevisiae,” *Microbiol. Mol. Biol. Rev.*, vol. 66, no. 4, pp. 579–591, 2002.

[65] K. Inoki, H. Ouyang, Y. Li, and K. Guan, “Signaling by Target of Rapamycin Proteins in Cell Growth Control,” *Society*, vol. 69, no. 1, pp. 79–100, 2005.

[66] J. V Gray, G. a Petsko, G. C. Johnston, D. Ringe, R. a Singer, and M. Werner-washburne, “ Sleeping Beauty : Quiescence in Saccharomyces

cerevisiae ‘ Sleeping Beauty ’: Quiescence in Saccharomyces cerevisiae †,” *Microbiol. Mol. Biol. Rev.*, vol. 68, no. 2, pp. 187–206, 2004.

[67] R. J. Bastidas, J. Heitman, and M. E. Cardenas, “The protein kinase Tor1 regulates adhesin gene expression in Candida albicans,” *PLoS Pathog.*, vol. 5, no. 2, 2009.

[68] S. Ramachandra, J. Linde, M. Brock, R. Guthke, B. Hube, and S. Brunke, “Regulatory networks controlling nitrogen sensing and uptake in Candida albicans,” *PLoS One*, vol. 9, no. 3, 2014.

[69] J. Morschhäuser, “Nitrogen regulation of morphogenesis and protease secretion in Candida albicans,” *Int. J. Med. Microbiol.*, vol. 301, no. 5, pp. 390–394, 2011.

[70] M. E. Cardenas, N. S. Cutler, M. C. Lorenz, C. J. Di Como, and J. Heitman, “The TOR signaling cascade regulates gene expression in response to nutrients,” *Genes Dev.*, vol. 13, pp. 3271–3279, 1999.

[71] J. S. Hardwick *et al.*, “Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the Tor proteins.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 96, no. 26, pp. 14866–14870, 1999.

[72] V. M. Boer, J. H. De Winde, J. T. Pronk, and M. D. W. Piper, “The genome-wide transcriptional responses of Saccharomyces cerevisiae grown on glucose in aerobic chemostat cultures limited for carbon, nitrogen, phosphorus, or sulfur,” *J. Biol. Chem.*, vol. 278, no. 5, pp. 3265–3274, 2003.

[73] A. Rodaki *et al.*, “Glucose Promotes Stress Resistance in the Fungal Pathogen Candida albicans,” *Mol. Biol. Cell*, vol. 20, no. 22, pp. 4845–4855, Nov. 2009.

[74] J. R. Brestoff and D. Artis, “Commensal bacteria at the interface of host metabolism and the immune system,” *Nat. Immunol.*, vol. 14, no. 7, pp. 676–684, 2013.

[75] D. Fan *et al.*, “Activation of HIF-1α and LL-37 by commensal bacteria inhibits Candida albicans colonization,” *Nat. Med.*, vol. 21, no. 7, pp. 808–814, 2015.

[76] P. Louis and H. J. Flint, “Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine,” *FEMS Microbiol. Lett.*, vol. 294, no. 1, pp. 1–8, 2009.

[77] S. E. Pryde, S. H. Duncan, G. L. Hold, C. S. Stewart, and H. J. Flint, “The microbiology of butyrate formation in the human colon,” *FEMS Microbiology Letters*, vol. 217, no. 2. pp. 133–139, 2002.

[78] J. Larsbrink *et al.*, “A discrete genetic locus confers xyloglucan metabolism in select human gut Bacteroidetes,” *Nature*, vol. 506, no. 7489, pp. 498–502,

2014.

[79] J. A. K. McDonald *et al.*, “Evaluation of microbial community reproducibility, stability and composition in a human distal gut chemostat model,” *J. Microbiol. Methods*, vol. 95, no. 2, pp. 167–174, 2013.

[80] T. Guillemette, A. Sellam, and P. Simoneau, “Analysis of a nonribosomal peptide synthetase gene from Alternaria brassicae and flanking genomic sequences,” *Curr. Genet.*, vol. 45, no. 4, pp. 214–224, 2004.

[81] A. Dobin *et al.*, “STAR: Ultrafast universal RNA-seq aligner,” *Bioinformatics*, vol. 29, no. 1, pp. 15–21, 2013.

[82] C. Trapnell *et al.*, “Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks,” *Nat. Protoc.*, vol. 7, no. 3, pp. 562–578, 2012.

[83] B. Roche, B. Arcangioli, and R. Martienssen, “Transcriptional reprogramming in cellular quiescence,” *RNA Biol.*, vol. 14, no. 7, pp. 843–853, 2017.