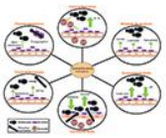
Virulence



Special Focus Section: Mycobiome

ISSN: 2150-5594 (Print) 2150-5608 (Online) Journal homepage: https://www.tandfonline.com/loi/kvir20

Fungi in the healthy human gastrointestinal tract

Heather E. Hallen-Adams & Mallory J. Suhr

To cite this article: Heather E. Hallen-Adams & Mallory J. Suhr (2017) Fungi in the healthy human gastrointestinal tract, Virulence, 8:3, 352-358, DOI: 10.1080/21505594.2016.1247140

To link to this article: https://doi.org/10.1080/21505594.2016.1247140

VIRULENCE
2017, VOL. 8, NO. 3, 352–358
http://dx.doi.org/10.1080/21505594.2016.1247140

Taylor & Francis
Taylor & Francis Group

REVIEW

Fungi in the healthy human gastrointestinal tract

Heather E. Hallen-Adams and Mallory J. Suhr

Department of Food Science and Technology, University of Nebraska, Lincoln, NE, USA

ABSTRACT

Many species of fungi have been detected in the healthy human gut; however, nearly half of all taxa reported have only been found in one sample or one study. Fungi capable of growing in and colonizing the gut are limited to a small number of species, mostly *Candida* yeasts and yeasts in the family Dipodascaceae (*Galactomyces, Geotrichum, Saprochaete*). *Malassezia* and the filamentous fungus *Cladosporium* are potential colonizers; more work is needed to clarify their role. Other commonly-detected fungi come from the diet or environment but either cannot or do not colonize (*Penicillium* and *Debaryomyces* species, which are common on fermented foods but cannot grow at human body temperature), while still others have dietary or environmental sources (*Saccharomyces cerevisiae*, a fermentation agent and sometime probiotic; *Aspergillus* species, ubiquitous molds) yet are likely to impact gut ecology. The gut mycobiome appears less stable than the bacterial microbiome, and is likely subject to environmental factors.

ARTICLE HISTORY
Received 20 July 2016
Revised 7 September 2016
Accepted 8 September 2016

KEYWORDS
Candida; *Cladosporium*; dietary fungi; Dipodascaceae; gut fungal ecology; gut mycobiome; gut mycobiota; *Malassezia*; yeasts

Introduction

The gastrointestinal tract of an animal provides an attractive niche for organisms which have evolved to withstand its unique challenges. Nutrients ingested by the host are in steady supply and obviate the need to seek food. In the intestines, the outer mucus layer allows colonization and provides a nutrient rich habitat, while the dense inner layer segregates colonizing microbes from the dense concentration of immune cells in the intestinal epithelium.[1] To take advantage of these resources, organisms must be equipped to tolerate gut conditions: absence of oxygen; physiological temperatures (in mammals); peristaltic contractions and consequent movement of GI contents; variable pH, from the highly acidic stomach to the alkaline intestinal mucosa.

For most years since the development and acceptance of the germ theory, studies of human-associated microbes focused on pathogens.[2] While the existence of commensal and mutualistic relationships between humans and their gut microbiota has been known for well over a century (see, e.g., refs[3,4]), culture-based methods precluded study of any but the most tractable organisms. The development of culture-independent PCR-based methods revolutionized the study of the whole, healthy, human microbiome.[2] At the turn of the century, several large scale international healthy human microbiome projects were initiated.[5-7] With the decrease in cost and rise in capacity of next-generation sequencing technologies, microbiome projects are now more feasible to the single investigator. The contribution of the microbiome to overall well-being is now widely accepted and studied.

The healthy human gut microbiome contains members of all domains of life, with Eukarya primarily represented by the fungi and, in some populations, protists, notably *Blastocystis*.[8,9] The fungal component—the mycobiome—has received little attention compared with bacteria, but steady work by a number of researchers has produced a mature discipline, as evidenced by this special issue. Several important reviews have been published in this decade. Specifically, whole-body perspectives of the human mycobiome are provided by Cui and colleagues,[10] Huffnagle and Noverr,[11] Seed,[12] and Underhill and Iliev,[13] while the gut mycobiota is reviewed by Ianiro and colleagues,[14] Kirschner and colleagues,[15] and Suhr and Hallen-Adams.[16] The role of the gut mycobiota in disease is reviewed by Moyes and Naglik,[17] Wang and colleagues,[18] Gouba and Drancourt,[19] Mukherjee and colleagues,[20] and Richard and colleagues.[21] In this paper, we focus on the gut mycobiome of healthy humans, with a particular emphasis on the relatively few fungi that are widely distributed in human gut samples. We will also discuss the contributions of diet and the environment to gut fungal composition, and the stability of the gut mycobiome over time.

CONTACT Heather E. Hallen-Adams hhallen-adams2@unl.edu Food Science and Technology, University of Nebraska-Lincoln, 1901 N. 21st St., Lincoln, NE 68588, USA.

Many fungi have been reported from the human gut, but few are common. From 36 studies spanning 1917 to 2015 and using a broad array of culture-based and non-culture-based methods, 267 distinct, valid species were detailed.[16] (Taxa identified only to genus, or as "undescribed," in the studies were not included in this tally.) Two hundred species, or nearly 75%, were only reported in one study. A further 37 species were reported in 2 studies, and only 15 were reported in 5 or more studies. When studies involve multiple samples a similar trend is observed: many species may be observed, but a majority are only detected in a single sample. The studies from our own lab identified 97 distinct fungal taxa in 85 samples from 60 subjects.[22,23] Forty-eight taxa were limited to a single sample, while 14 occurred in 10 samples or more. The most commonly reported genera and species of gut fungi are given in Table 1. Most rarely detected taxa are unlikely to play a role in gut ecology or host health, for reasons discussed below.

Table 1. Most commonly-detected fungi in gut mycobiome studies.

Taxon[a]	# studies (%)[b]	# samples (%)[c]	References
Candida	**32 (86%)**	**68 (80%)**	
Candida albicans	26 (70%)	18 (21%)	3,9,22,23,31-33,41-44,46,54-57,59,60-63,65,66,68,69
Candida tropicalis	17 (46%)	57 (67%)	3,22,23,29,30-33,42,44,51,54,55,60,61,62,68
Candida parapsilosis	13 (35%)	2 (2%)	9,22,30-33,42,55,60,61,63,67,68,69
Candida glabrata	12 (32%)	0	3,31,41,43,44,46,54,55,59,60,61,63
Candida krusei	10 (27%)	0	3,23,31-33,44,45,54,55,60,63
Candida lusitaniae	6 (16%)	0	30,33,43,54,55,60
Saccharomyces	**20 (54%)**	**5 (6%)**	
Saccharomyces cerevisiae	20 (54%)	5 (6%)	9,22,23,30,33,34,41-45,51,54,58,59,61-64,69
Penicillium	**14 (38%)**	**17 (20%)**	9,22,42,69d
Penicillium aff. commune	10 (27%)	10 (12%)	23,29,31,41,43,44,51,58,62,67
Aspergillus	**12 (32%)**	**20 (24%)**	22,23,42,43,51,58,62
Aspergillus aff. versicolor	5 (14%)	0	9,29,31,44,67
Cryptococcus	**10 (27%)**	**3 (4%)**	3,22,23,30,31,41,42,60-62
Malassezia	**11 (30%)**	**21 (25%)**	69
Malassezia globosa	8 (22%)	1 (1%)	23,29,30,34,42,43,45,58
Malassezia restricta	7 (19%)	20 (24%)	22,23,29,30,43,45,58
Malassezia pachydermatis	6 (16%)	1 (1%)	23,29,43,44,45,58
Cladosporium	**10 (27%)**	**15 (18%)**	
Cladosporium aff. herbarum	10 (27%)	18 (21%)	22,23,30,31,32,41,43,58,61,67
Galactomyces	**8 (22%)**	**8 (9%)**	60
Galactomyces geotrichum	7 (19%)	7 (8%)	9,22,29,41,43,44,45
Debaryomyces	**8 (22%)**	**18 (21%)**	
Debaryomyces hansenii	7 (19%)	18 (21%)	22,23,30,34,43,54,59
Trichosporon	**6 (17%)**	**8 (9%)**	22,30,33,41,43,45

Notes.
[a]**Bold type** and left justified refers to the genus as a whole; right justified *italics* indicates individual species.
[b]Based on 37 papers giving species-level identifications, published between 1917–2016.
[c]Based on 85 samples published in refs.[22,23]
[d]References for genus are only given if they are distinct from species references for that genus.

Categories of gut fungi

Fungi detected in the human gut can be split into resident and non-resident. As a minimum requirement, a resident (or autochthonous) fungus must be able to grow at 37°C to colonize the gut. For a few species of the wide and diverse yeast genus *Candida*, the mammalian digestive tract can be considered the primary niche. Species including *Candida albicans, C. tropicalis, C. parapsilosis*, and *C. glabrata* may all be found as natural, asymptomatic components of the human microbiome, and published estimates of *C. albicans* carriage in healthy individuals range from 30–60%.[24] Furthermore, many of these species do not appear to have a niche apart from living mammals; absent a source of contamination, they will not be found in significant concentrations in the air, or in soil, or in food.[25,26] *Malassezia* is another genus of yeasts whose primary niche is the mammalian microbiome; in fact, *Malassezia* species have lost the ability to synthesize their own lipids, so they are dependent on a host for their nutritional needs.[27] The known niche for *Malassezia* is skin, where it is the predominant fungal genus detected in 11 of 14 body sites (the exceptions all being on the foot; ref.[28]). *Malassezia* has also been reported in significant abundance in fecal samples and may play a role in the gut.[29,30,23] To determine whether any *Malassezia* is indigenous to the gut would require more invasive sampling techniques than the standard fecal collection, to eliminate the possibility of inoculation of the feces by skin flora.

Two further groups of fungi reported repeatedly in gut fungal studies likely do not have the gut as their primary niche but may be considered potential colonizers: *Cladosporium*, and yeasts in the Dipodascaceae (includes *Geotrichum/Saprochaete* and *Galactomyces*). *Cladosporium*, along with *Aspergillus* and *Penicillium* (discussed below) is a filamentous fungus, or mold; the other fungi commonly detected in the gut are all yeasts. *Cladosporium* has been reported in the healthy human GI tract (ileum as well as fecal samples) since 1969,[31] and was widespread in the microbiota of Apollo astronauts.[32] In the Dipodascaceae, yeasts in the genus *Galactomyces* have been reported in 22% of gut fungal studies, with *Galactomyces geotrichum* reported in 19% (see Table 1). A species related, but not identical, to *Geotrichum gigas* in the Dipodascaceae was detected in 54% of 69 samples by Hallen-Adams and colleagues,[22] but has only been reported from this study. *Cladosporium* and Dipodascaceae yeasts most likely enter the gut from environmental

sources, but their occurrence is sufficiently common that some degree of colonization cannot be ruled out.

Saccharomyces cerevisiae – bakers' and brewers' yeast – in the gut presumably originates in food.[33,34] We would not consider *S. cerevisiae* an autochthonous gut organism nor a true human commensal (although it is often called commensal in the literature); it is a domesticated species of fermentations whose "wild" niche is associated with plants. However, the ability of strains to grow at 37°C and the opportunities for repeated introduction render it among the most commonly detected fungi in fecal samples, and it likely contributes to gut microbial ecology. Probiotic *S. cerevisiae* ("*S. boulardii*") does not persist for more than 5 d after administration stops in healthy subjects,[35] but has given rise to an increasing incidence of *Saccharomyces* fungemia when co-morbidities are present.[36] Many *Aspergillus* species fall into a similar category – they survive human physiological temperatures, but are much more commonly reported in environmental (soil, air, plant matter) than in gut samples, and are presumably of environmental origin.[37,38] Like *S. cerevisiae*, the potential for these species to persist and respond to the gut environment (see below) means they may play a role in the gut, regardless of preferred niche.

Other commonly-detected fungi include *Debaryomyces hansenii* and *Penicillium* aff. *commune*, foodborne species incapable of growing at 37°C.[39,40] Finally, there is the long tail of rarely-detected fungi which make up most of the species richness in gut mycobiota studies but whose presence in human fecal samples is strictly incidental and cannot be assumed to influence the gut ecology. These include edible mushrooms,[23] plant pathogens (widely reported; see, e.g. refs.[41-43]), xerophiles (*Wallemia sebi* and *W. muriae*),[44] wood decay fungi,[22,45,46] and other organisms whose growth and nutrition requirements preclude any lasting role in the mammalian GI tract.

Influence of diet on gut fungi

The gut microbiome has been presumed sterile until birth, although detection of bacteria in amniotic fluid and in the meconium have raised recent challenges to that assumption.[47] Starting at birth and throughout life, the primary route for microbes to enter the gastrointestinal tract is via ingestion,[48] although inhalation can play a role as well. The first fungi detected in the infant gut are Saccharomycetalean yeasts, especially *Candida* species; these are presumed to be transmitted by the mother as *Candida* species are common inhabitants of the skin and vaginal mucosa as well as the colon.[49]

Providing both a means of entrance for microorganisms to the gut, and a major source of nutrients for established microbes, the diet is an obvious influence on gut microbial composition. David and colleagues found broad, reproducible, dietary-induced changes in the gut microbiome depending on whether volunteers consumed a plant- or an animal-based diet.[34] While bacterial composition showed a clear response to nutrient availability (carbohydrates/fiber vs. proteins and fats), fungal composition appeared to be driven by food colonization. Notably, the same species of fungi were detected in participant fecal samples and in cheese fed to those participants.[34] Hoffmann and colleagues found that *Candida* abundance was positively correlated with recent consumption of carbohydrates and negatively correlated with total saturated fatty acids.[50] Recent consumption of short chain fatty acids drove down the abundance of *Aspergillus*.[50] Ukhanova et al. found a decrease in *Candida* and *Penicillium* related to almond and pistachio consumption.[51]

Finally, Suhr and colleagues examined 16 samples from 15 vegetarians, while Hallen-Adams et al. used the same methodology in the same laboratory to isolate and sequence fungal DNA from 69 samples from 45 people on a conventional Western diet.[22,23] The distribution of fungi differed considerably between the 2 groups (Table 2). Plant pathogenic *Fusarium* was detected in all but 2 samples from vegetarians (14 samples; 88%), while it was only detected in 2 samples from participants on conventional diets (3%). *Malassezia* and (presumed) foodborne *Penicillium* and *Aspergillus* were also present in more than 50% of vegetarian samples but much rarer in conventional diet samples. Common fungi were also proportionally more common in vegetarian samples; the top 5 genera were detected in 88, 81, 75, 68 and 63% of samples, respectively, while the top 5 genera in conventional diet samples were detected in 84, 46, 16, 16 and 12%. These differences are not due solely to the smaller sample set for vegetarians; *Fusarium, Malassezia, Penicillium* and *Aspergillus* were detected in a higher number of vegetarian than conventional diet samples. Conversely, David and colleagues found a significant enrichment of *Penicillium* in participants on an animal-based diet than

Table 2. Most common taxa in vegetarian and conventional diet samples.

Genus	Vegetarian	Conventional
Fusarium	14 (88%)	2 (3%)
Candida	10 (63%)	58 (84%)
Malassezia	13 (81%)	8 (12%)
Penicillium	12 (75%)	1 (1%)
Aspergillus	11 (68%)	4 (6%)
Geotrichum	ND	32 (46%)
Pichia	1 (6%)	11 (16%)
Cladosporium	4 (25%)	11 (16%)

Note. Data from refs.[22,23]

on a plant-based diet (and an overall enrichment in fungal transcripts and CFUs on the animal-based diet);[34] however, their controlled animal-based diet was rich in cheeses, including Camembert and blue which are processed with *Penicillium* and were absent from the plant-based diet. The vegetarian subjects in Suhr et al.[22] included lacto-ovo vegetarians, who consume dairy.

Studies to date provide intriguing hints about the role of diet in influencing the gut mycobiome, but broad conclusions are precluded by the limited number of studies and the differing methodologies. To date, only David et al. have conducted a controlled diet study;[34] their findings were sufficiently dramatic to demonstrate the benefit of such studies. Additional studies, involving multiple locations and populations and incorporating detailed dietary information, would be valuable in clarifying the impact of diet on fungi. Environmental sampling for studies taking place in circumscribed environments, such as hospitals, and sampling of participants' skin and oral mycobiome, could suggest other sources of gut fungi.

Stability of the gut mycobiome

While the stability of the bacterial microbiome is now well-documented (e.g., ref.[52]), the situation in fungi is less clear. Comparatively few studies have genus- or species-level fungal data for multiple samples from the same subject over time, and those who have addressed the issue have reached differing conclusions. Our own impression, based on samples at 2 time points each from 24 participants, is that the composition of the gut mycobiome is not particularly stable; we found the same fungus at both time points less than 20% of the time.[22] Given the high proportion of rare, incidental taxa detected in fecal samples, we eliminated these taxa from consideration, which led to a slight increase; commonly-detected gut fungi were detected in both time points 27% of the time. In this dataset only the most common fungus, *Candida tropicalis*, was present at both time points a majority of the time (58%).

Mouse studies have shown variability over time,[53] leading Underhill and Iliev to generalize that "(t)his suggests that commensal fungal populations are more variable than those of bacteria and that they may be influenced by fungi in the environment."[13] Anderson, writing near the advent of gut mycobiota studies observed "(t)he blastomycetes [=yeasts] must, for the present be regarded as accidental ingredients of the feces, since in the same case observed at different periods they vary so extraordinarily"[3] Possibly in contradiction of the foregoing, Cohen and colleagues report "(t)he stability of the mycofloral pattern of the small intestine was demonstrated in 5 subjects who were resampled 5 to 9 months after the initial studies; the fungal pattern was qualitatively and quantitatively unchanged,"[31] however, these authors sampled from multiple parts of the GI tract and specified the small intestine in their observations, while other studies have been limited to the feces. More studies, with samples collected over multiple time points, are needed to address the matter of gut mycobiome persistence.

Concluding remarks

Multiple studies have now provided a baseline snapshot of the gut mycobiome in healthy individuals. While more studies and more samples would address some outstanding questions (are there geographical differences in mycobiome? Differences based on age, gender or other demographic factors?), we can begin to make some broad generalizations about which groups of fungi are likely to be of importance to the host and the overall microbiome. As far as possible, new studies should take diet and the environment into account, which can be as simple a matter as requesting a dietary log and including one or more relevant skin (hands and face may come into contact with food and contribute organisms which are then swallowed, while elbow or foot samples are not likely to) and oral sample from participants. Sequencing multiple samples over time from as large a pool of participants as possible would be highly desirable; at present, mycobiome studies lag far behind bacterial microbiome studies in terms of numbers and consequent statistical power.

Many unanswered questions remain about those fungi whose niche is the gut: what are they doing in a healthy host, and would their absence be detrimental? Are species interchangeable, i.e., is there any effect in replacing *Candida albicans* with *C. tropicalis* or *C. parapsilosis* in an individual host? Do species, or strains of the same species, compete and, if so, are there predictable outcomes? – Given the importance of *Candida* yeasts as opportunistic pathogens, answering these questions could provide insight into limiting or preventing yeast infections or candidiasis. Whether there are gut-resident strains or species of *Malassezia* or *Cladosporium* remains to be answered as well.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported in part by the USDA National Institute of Food and Agriculture Hatch Project NEB-31-136, to Heather Hallen-Adams.

References

[1] Faderl M, Noti M, Corazza N, Mueller C. Keeping bugs in check: The mucus layer as a critical component in maintaining intestinal homeostasis. IUBMB Life 2015; 67:275-85; PMID:25914114; http://dx.doi.org/10.1002/iub.1374

[2] The NIH HMP Working Group, Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, Bonazzi V, McEwen JE, Wetterstrand KA, et al. The NIH Human Microbiome Project. Genome Res 2009; 19:2317-23; PMID:19819907; http://dx.doi.org/10.1101/gr.096651.109

[3] Anderson HW. Yeast-like fungi of the human gastrointestinal tract. J Infect Dis 1917; 21:341-86; http://dx.doi.org/10.1093/infdis/21.4.341

[4] Rogers LA, Clark WM, Lubs HA. The characteristics of bacteria of the colon type occurring in human feces. J Bacteriol 1918; 3:231-52; PMID:16558790

[5] Blaut M, Collins MD, Welling GW, Doré J, van Loo J, de Vos W. Molecular biological methods for studying the gut microbiota: the EU human gut flora project. Brit J Nutr 2002; 87 Suppl 2:S203-11; PMID:12088520; http://dx.doi.org/10.1079/BJN/2002539

[6] Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett C, Knight R, Gordon JI. The human microbiome project: exploring the microbial part of ourselves in a changing world. Nature 2007; 449:804-10; PMID:17943116; http://dx.doi.org/10.1038/nature06244

[7] Robles-Alonso V, Guarner F. From basic to applied research: lessons from the human microbiome projects. J Clin Gastroenterol 2014; 48 Suppl 1:S3-4; PMID:25291122; http://dx.doi.org/10.1097/MCG.0000000000000242

[8] Rajilic-Stojanovic M, Smidt H, de Vos WM. Diversity of the human gastrointestinal tract microbiota revisited. Environ Microbiol 2007; 9:2125-36; PMID:17686012; http://dx.doi.org/10.1111/j.1462-2920.2007.01369.x

[9] Scanlan PD, Marchesi JR. Micro-eukaryotic diversity of the human distal gut microbiota: qualitative assessment using culture-dependent and independent analysis of feces. ISMA J 2008; 2:1183-93; http://dx.doi.org/10.1038/ismej.2008.76

[10] Cui L, Morris A, Ghedin E. The human mycobiome in health and disease. Genome Med 2013; 5:63; PMID:23899327; http://dx.doi.org/10.1186/gm467

[11] Huffnagle G, Noverr M. The emerging world of the fungal microbiome. Trends Microbiol 2013; 21:334-41; PMID:23685069; http://dx.doi.org/10.1016/j.tim.2013.04.002

[12] Seed PC. The human mycobiome. Cold Spring Harb Perspect Med 2014; 5:a019810; PMID:25384764; http://dx.doi.org/10.1101/cshperspect.a019810

[13] Underhill D, Iliev I. The mycobiota: interactions between commensal fungi and the host immune system. Nat Rev Immunol 2014; 14:405-16; PMID:24854590; http://dx.doi.org/10.1038/nri3684

[14] Ianiro G, Bruno G, Lopetuso L, Beghella FB, Laterza L, D'Aversa F, Gigante G, Cammarota G, Gasbarrini A. Role of yeasts in healthy and impaired gut microbiota: the gut mycome. Curr Phar Des 2014; 20:4565-69; http://dx.doi.org/10.2174/1381612811319660723

[15] Kirschner R, Hsu T, Tuan NN, Chen CL, Huang SL. Characterization of fungal and bacterial components in gut/fecal microbiome. Curr Drug Metab 2015; 16:272-83; PMID:26264196; http://dx.doi.org/10.2174/1389200216666150812124625

[16] Suhr MJ, Hallen-Adams HE. The human gut mycobiome: pitfalls and potentials – a mycologist's perspective. Mycologia 2015; 107:1057-73; PMID:26354806; http://dx.doi.org/10.3852/15-147

[17] Moyes DL, Naglik JR. The mycobiome: influencing IBD severity. Cell Host Microbe 2012; 11:551-2; PMID:22704612; http://dx.doi.org/10.1016/j.chom.2012.05.009

[18] Wang ZK, Yang YS, Stefka AT, Sun G, Peng LH. Review article: fungal microbiota and digestive diseases. Aliment Pharmacol Ther 2014; 39:751-66; PMID:24612332; http://dx.doi.org/10.1111/apt.12665

[19] Gouba N, Drancourt M. Digestive tract mycobiota: a source of infection. Med Mal Infect 2015; 45:9-16; PMID:25684583; http://dx.doi.org/10.1016/j.medmal.2015.01.007

[20] Mukherjee PK, Sendid B, Hoarau G, Colombel JF, Poulain D, Ghannoum MA. Mycobiota in gastrointestinal diseases. Nat Rev Gastroenterol Hepatol 2015; 12:77-87; PMID:25385227; http://dx.doi.org/10.1038/nrgastro.2014.188

[21] Richard ML, Lamas B, Liguori G, Hoffmann TW, Sokol H. Gut fungal microbiota: the Yin and Yang of inflammatory bowel disease. Inflamm Bowel Dis 2015; 21:656-65; PMID:25545379; http://dx.doi.org/10.1097/MIB.0000000000000261

[22] Hallen-Adams HE, Kachman SD, Kim J, Legge RM, Martínez I. Fungi inhabiting the healthy human gastrointestinal tract: a diverse and dynamic community. Fungal Ecol 2015; 15:9-17; http://dx.doi.org/10.1016/j.funeco.2015.01.006

[23] Suhr MJ, Banjara N, Hallen-Adams HE. Sequence-based methods for detecting and evaluating the human gut mycobiome. Lett Appl Microbiol 2016; 62:209-15; PMID:26669281; http://dx.doi.org/10.1111/lam.12539

[24] Moran G, Coleman D, Sullivan D. An introduction to the medically important *Candida* species. In *Candida and Candidiasis*, 2nd Edition, Calderone RA, Clancy CJ (eds.) 2012; Washington, DC: ASM Press. pp. 11-25; http://dx.doi.org/10.1128/9781555817176.ch2

[25] Vogel C, Rogerson A, Schatz S, Laubach H, Tallman A, Fell J. Prevalence of yeasts in beach sand at three bathing beaches in South Florida. Water Res 2007; 41:1915-20; PMID:17382990; http://dx.doi.org/10.1016/j.watres.2007.02.010

[26] Saleh HA, Moawad AA, El-Hariri M, Refai MK. Prevalence of yeasts in human, animals and soil sample at El-Fayoum Governorate in Egypt. Int J Microbiol Res 2011; 2:233-9.

[27] Gupta AK, Batra R, Bluhm R, Boekhout T, Dawson TL, Jr. Skin diseases associated with *Malassezia* species. J Am Acad Dermatol 2004; 51:785-98; PMID:15523360; http://dx.doi.org/10.1016/j.jaad.2003.12.034

[28] Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, Schoenfeld D, Nomicos E, Park M, NIH Intramural Sequencing Center Comparative Sequencing Program, Kong HH, Segre JA. Topographic diversity of fungal and bacterial communities in human skin. Nature 2013; 498:367-70; PMID:23698366; http://dx.doi.org/10.1038/nature12171

[29] Gouba N, Raoult D, Drancourt M. Plant and fungal diversity in gut microbiota as revealed by molecular and culture investigations. PLoS One 2013; 8:e59474; PMID:23555039; http://dx.doi.org/10.1371/journal.pone.0059474

[30] Cano RJ, Rivera-Perez J, Toranzos GA, Santiago-Rodrigues TM, Narganes-Storde YM, Chanlatte-Baik L, García-Roldán E, Bunkley-Williams L, Massey SE. Paleomicrobiology: revealing fecal microbiomes of ancient indigenous cultures. PLoS One 2014; 9:e106833; PMID:25207979; http://dx.doi.org/10.1371/journal.pone.0106833

[31] Cohen R, Roth FJ, Delgado E, Ahearn DG, Kalser MH. Fungal flora of the normal human small and large intestine. N Engl J Med 1969; 280:638-41; PMID:5764842; http://dx.doi.org/10.1056/NEJM196903202801204

[32] Taylor GR, Henney MR, Ellis WL. Changes in the fungal autoflora of Apollo astronauts. Appl Microbiol 1973; 26:804-13; PMID:4762399

[33] Angebault C, Djossou F, Abélanet S, Permal E, Ben Soltana M, Diancourt L, Bouchier C, Woerther PL, Catzeflis F, Andremont A, et al. *Candida albicans* is not always the preferential yeast colonizing humans: a study in Wayampi Amerindians. J Infect Dis 2013; 208:1705-16; PMID:23904289; http://dx.doi.org/10.1093/infdis/jit389

[34] David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014; 505:559-63; PMID:24336217; http://dx.doi.org/10.1038/nature12820

[35] Moré MI, Swidsinski A. *Saccharomyces boulardii* CNCM I-745 supports regeneration of the intestinal microbiota after diarrheic dysbiosis – a review. Clin Exp Gastroenterol 2015; 8:237-55; http://dx.doi.org/10.2147/CEG.S85574

[36] Doron S, Snydman DR. Risk and safety of probiotics. Clin Infect Dis 2015; 60(Suppl 2):S129-34; PMID:25922398; http://dx.doi.org/10.1093/cid/civ085

[37] O'Gorman CM, Fuller HT. Prevalence of culturable airborne spores of selected allergenic and pathogenic fungi in outdoor air. Atmos Env 2008; 42:4355-68; http://dx.doi.org/10.1016/j.atmosenv.2008.01.009

[38] Mortensen KL, Mellado E, Lass-Flörl C, Rodriguez-Tudela JL, Johansen HK, Arendrup MC. Environmental study of azole-resistant *Aspergillus fumigatus* and other aspergilli in Austria, Denmark, and Spain. Antimicrob Agents Chemother 2010; 54:4545-9; PMID:20805399; http://dx.doi.org/10.1128/AAC.00692-10

[39] Desnos-Olliveir M, Ragon M, Robert V, Raoux D, Gantier JC, Dromer F. *Debaryomyces hansenii* (*Candida famata*), a rare human fungal pathogen often misidentified as *Pichia guilliermondii* (*Candida guilliermondii*). J Clin Microbiol 2008; 46:3237-42; PMID:18701668; http://dx.doi.org/10.1128/JCM.01451-08

[40] Banjara N, Suhr MJ, Hallen-Adams HE. Diversity of yeast and mold species from a variety of cheese types. Curr Microbiol 2015; 70:792-800; PMID:25694357; http://dx.doi.org/10.1007/s00284-015-0790-1

[41] Ott SJ, Kühbacher T, Musfeldt M, Rosenstiel P, Hellmig S, Rehman A, Drews O, Weichert W, Timmis KN, Schreiber S. Fungi and inflammatory bowel diseases: alterations of composition and diversity. Scand J Gastroenterol 2008; 43:831-41; PMID:18584522; http://dx.doi.org/10.1080/00365520801935434

[42] Li Q, Wang C, Zhang Q, Tang C, Li N, Ruan B, Li J. Use of 18S ribosomal DNA polymerase chain reaction-denaturing gradient gel electrophoresis to study composition of fungal community in two patients with intestinal transplants. Hum Pathol 2012; 43:1273-81; PMID:22305239; http://dx.doi.org/10.1016/j.humpath.2011.09.017

[43] Gouba N, Raoult D, Drancourt M. Eukaryote culturomics of the gut reveals new species. PLoS One 2014; 9:e106994; PMID:25210972; http://dx.doi.org/10.1371/journal.pone.0106994

[44] Chen Y, Chen Z, Guo R, Chen N, Lu H, Huang S, Wang J, Li L. Correlation between gastrointestinal fungi and varying degrees of chronic hepatitis B virus infection. Diagn Microbiol Infect Dis 2011; 70:492-98; PMID:20846815; http://dx.doi.org/10.1016/j.diagmicrobio.2010.04.005

[45] Hamad I, Sokhna C, Raoult D, Bittar F. Molecular detection of eukaryotes in a single human stool sample from Senegal. PLoS One 2012; 7:e40888; PMID:22808282; http://dx.doi.org/10.1371/journal.pone.0040888

[46] Stewart CJ, Nelson A, Scribbins D, Marrs ECL, Lanyon C, Perry JD, Embleton ND, Cummings SP, Berrington JE. Bacterial and fungal viability in the preterm gut: NEC and sepsis. Arch Dis Child Fetal Neonatal Ed 2013; 98:F298-303; PMID:23426613; http://dx.doi.org/10.1136/archdischild-2012-302119

[47] Neu J. The microbiome during pregnancy and early postnatal life. Semin Fetal Neonatal Med 2016; [epub ahead of print]; http://dx.doi.org/10.1016/j.siny.2016.05.001

[48] Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, van den Brandt PA, Stobberingh EE. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics 2006; 118:511-21; PMID:16882802; http://dx.doi.org/10.1542/peds.2005-2824

[49] Bliss JM, Basavegowda KP, Watson WJ, Sheikh AU, Ryan RM. Vertical and horizontal transmission of *Candida albicans* in very low birth weight infants using DNA fingerprinting techniques. Pediatr Infect Dis J 2008; 27:231-5; PMID:18277930; http://dx.doi.org/10.1097/INF.0b013e31815bb69d

[50] Hoffmann C, Dollive S, Grunberg S, Chen J, Li H, Wu GD, Lewis JD, Bushman FD. Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. PLoS One 2013; 8:e66019; PMID:23799070; http://dx.doi.org/10.1371/journal.pone.0066019

[51] Ukhanova M, Wang X, Baer DJ, Novotny JA, Fredborg M, Mai V. Effects of almond and pistachio consumption on gut microbiota composition in a randomized crossover human feeding study. Br J Nutr 2014; 111:2146-52; PMID:24642201; http://dx.doi.org/10.1017/S0007114514000385

[52] Lozupone CA, Stombaugh JI, Gordon JL, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature 2012; 489:220-30; PMID:22972295; http://dx.doi.org/10.1038/nature11550

[53] Dolive S, Chen YY, Grunberg S, Bittinger K, Hoffmann C, Vandivier L, Cuff C, Lewis JD, Wu GD, Bushman FD. Fungi of the murine gut: episodic variation and proliferation during antibiotic treatment. PLoS One 2013; 8:e71806; PMID:23977147; http://dx.doi.org/10.1371/journal.pone.0071806

[54] Agirbasli H, Özcan SA, Gedikoğlu G. Fecal fungal flora of pediatric healthy volunteers and immunosuppressed patients. Mycopathologia 2005; 159:515-20; PMID:15983737; http://dx.doi.org/10.1007/s11046-005-3451-2

[55] Biasoli MS, Tosello ME, Magaró HM. Adherence of *Candida* strains isolated from the human gastrointestinal tract. Mycoses 2002; 45:465-9; PMID:12472722

[56] Finegold SM, Attebery HR, Sutter VL. Effect of diet on human fecal flora: comparison of Japanese and American diets. Am J Clin Nutr 1974; 27:1456-69; PMID:4432829

[57] Finegold SM, Sutter VL, Sugihara PT, Elder HA, Lehmann SM, Phillips RL. Fecal microbial flora in Seventh Day Adventist populations and control subjects. Am J Clin Nutr 1977; 30:1781-92; PMID:920638

[58] Gouba N, Raoult D, Drancourt M. Gut microeukaryotes during anorexia nervosa: a case report. BMC Res Notes 2014; 7:33; PMID:24418238; http://dx.doi.org/10.1186/1756-0500-7-33

[59] Jobst D, Kraft K. *Candida* species in stool, symptoms and complaints in general practice – a cross-sectional study of 308 outpatients. Mycoses 2006; 49:415-20; PMID:16922795; http://dx.doi.org/10.1111/j.1439-0507.2006.01244.x

[60] Khatib R, Riederer KM, Ramanathan J, Baran J, Jr. Fecal fungal flora in healthy volunteers and inpatients. Mycoses 2001; 44:151-6; PMID:11486452; http://dx.doi.org/10.1046/j.1439-0507.2001.00639.x

[61] LaTuga MS, Ellis JC, Cotton CM, Goldberg RN, Wynn JL, Jackson RB, Seed PC. Beyond bacteria: a study of the enteric microbial consortium in extremely low birth weight infants. PLoS One 2011; 6:e27858; PMID:22174751; http://dx.doi.org/10.1371/journal.pone.0027858

[62] Li Q, Wang C, Tang C, He Q, Li N, Li J. Dysbiosis of gut fungal microbiota is associated with mucosal inflammation in Crohn's disease. J Clin Gastroenterol 2014; 48:513-23; PMID:24275714

[63] Macura AB, Witalis J. Fungi isolated from the stool in patients with gastrointestinal disorders in 2005–2009. Przegl Epidemiol 2010; 634:313-7.

[64] Nam YD, Chang HW, Kim KH, Roh SW, Kim MS, Jung MJ, Lee SW, Kim JY, Yoon JH, Bae JW. Bacterial, archael and eukaryal diversity in the intestines of Korean people. J Microbiol 2008; 46:491-501; PMID:18974948; http://dx.doi.org/10.1007/s12275-008-0199-7

[65] Pandey PK, Siddharth J, Verma P, Bavdekar A, Patole MS, Shouche YS. Molecular typing of fecal eukaryotic microbiota of human infants and their respective mothers. J Biosci 2012; 37:221-6; PMID:22581327; http://dx.doi.org/10.1007/s12038-012-9197-3

[66] Soyucen E, Gulcan A, Aktuglu-Zeybek AC, Onal H, Kiykim E, Aydin A. Differences in the gut microbiota of healthy children and those with type I diabetes. Pediatr Int 2014; 56:336-43; PMID:24475780; ; http://dx.doi.org/10.1111/ped.12243

[67] Taylor GR, Kropp KD, Molina TC. Nine-year microflora study of an isolator-maintained immunodeficient child. Appl Env Microbiol 1985; 50:1349-56.

[68] von Rosenvinge EC, Song Y, White JR, Maddox C, Blanchard T, Fricke WF. Immune status, antibiotic medication and pH are associated with changes in the stomach fluid microbiota. ISMA J 2013; 7:1354-66; http://dx.doi.org/10.1038/ismej.2013.33

[69] Sokol H, Leducq V, Aschard H, Pham HP, Jegou S, Landman C, Cohen D, Liguori G, Bourrier A, Nion-Larmurier I, et al. Fungal microbiota dysbiosis in IBD. Gut 2016; [epub ahead of print]; http://dx.doi.org/10.1136/gutjnl-2015-310746