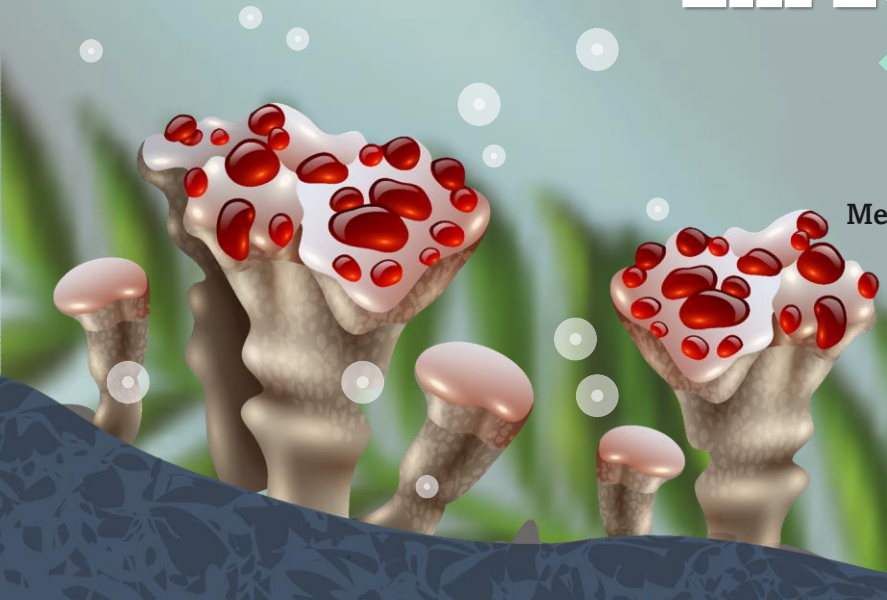
NATIONAL FUNGUS DAY OF EGYPT
20TH OF FEBRUARY

THE HIDDEN WORLD WITHIN:

EXPLORING THE MYCOBIOTA



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MMIA

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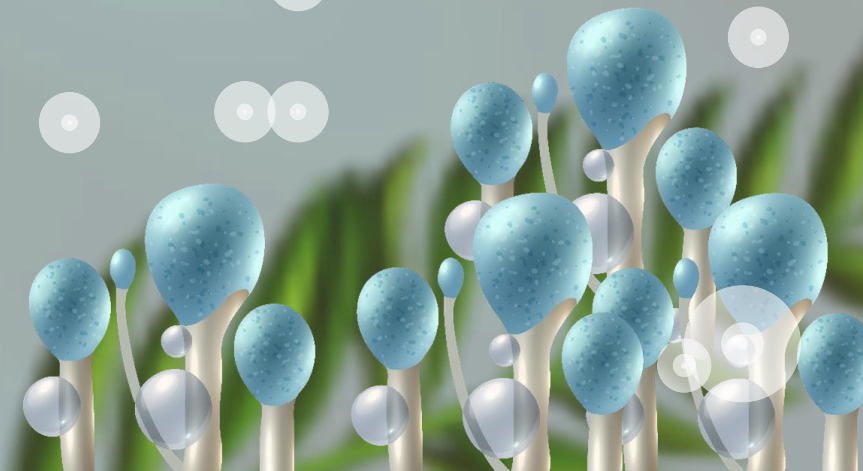
INTRODUCTION

What is the Mycobiota?



- **The term “microbiota” invokes images of mucosal surfaces densely populated with bacteria.**

- **However, research from this past decade has started to complete the picture by focusing on largely neglected components of the microbiota: archaea, fungi, protozoa, & viruses.**

- **The community of commensal fungi, the mycobiota, interacts with commensal bacteria & the host, influencing overall body health and homeostasis.**

- **Fungi are microeukaryotes which their presence in humans was historically thought to be only associated with pathogenesis.**

- **It was recently proved they can be isolated from humans in the absence of disease.**

- **Investigation of intestinal commensal-host symbiosis places emphasis on gut bacteria while neglecting gut fungi due to their lower abundance (0.01–0.1% of gut microbiome).**

- **In the period of 2008–2018, there have been almost 100 times more peer-reviewed publications on microbiota than on mycobiota.**

- **Notably, despite relatively small number of gut fungi, they profoundly affect intestinal homeostasis & barrier function.**

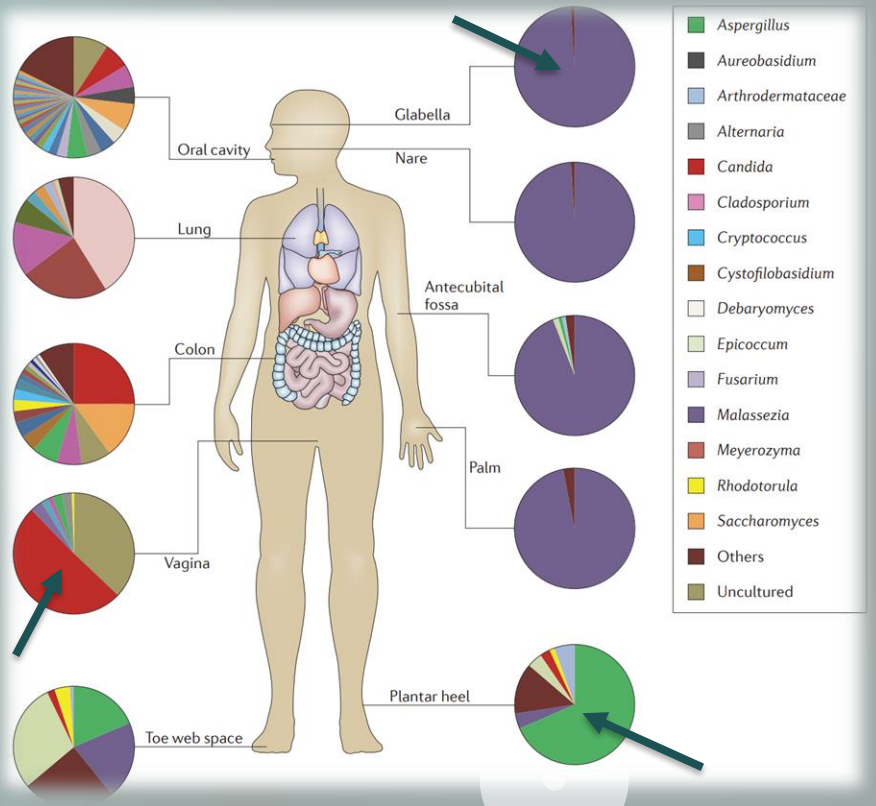
- **They also interact with the host immune system, impacting mucosal immune responses & inflammation.**

- **Though there is still relatively poor understanding of the influence of gut mycobiota on host's health & disease, imbalance in gut mycobiota has been linked to various gastrointestinal & systemic diseases.**

- **Complex populations of fungi have been found associated with the skin & all mucosal surfaces of the healthy human body, especially the GIT.**

- **The fungal populations that are found on mucosal surfaces tend to be more diverse than those on the skin.**

- **In general, *Malassezia spp., Aspergillus spp., & Candida spp.* are among the most fungal Mycobiota isolated from healthy individuals.**



FACTORS INFLUENCING THE MYCOBIOME

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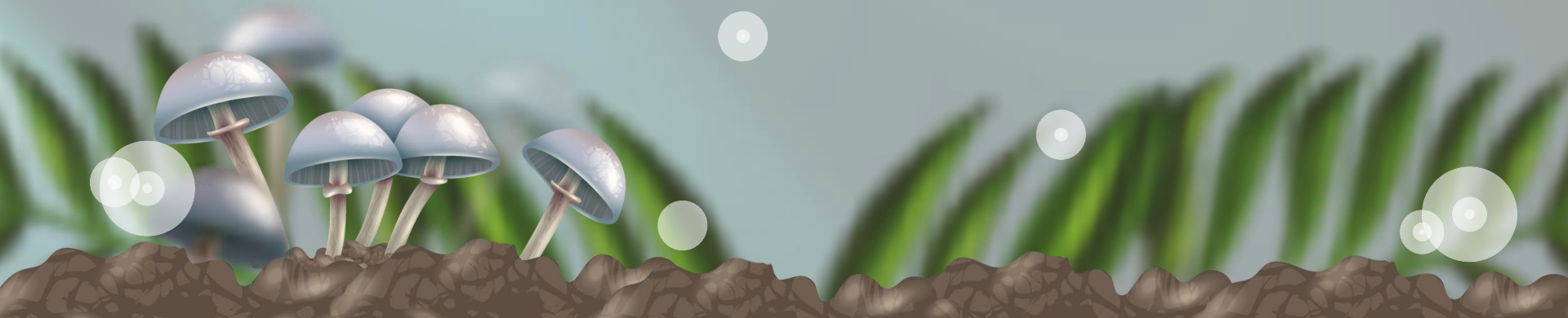

- **Compared to the bacterial microbiome, the mycobiome seems to be less consistent & less stable over time.**

- **The mycobiome is primarily shaped by delivery mode, diet, body weight, sex, age, medication, immune responses, the environment & geographical localization.**

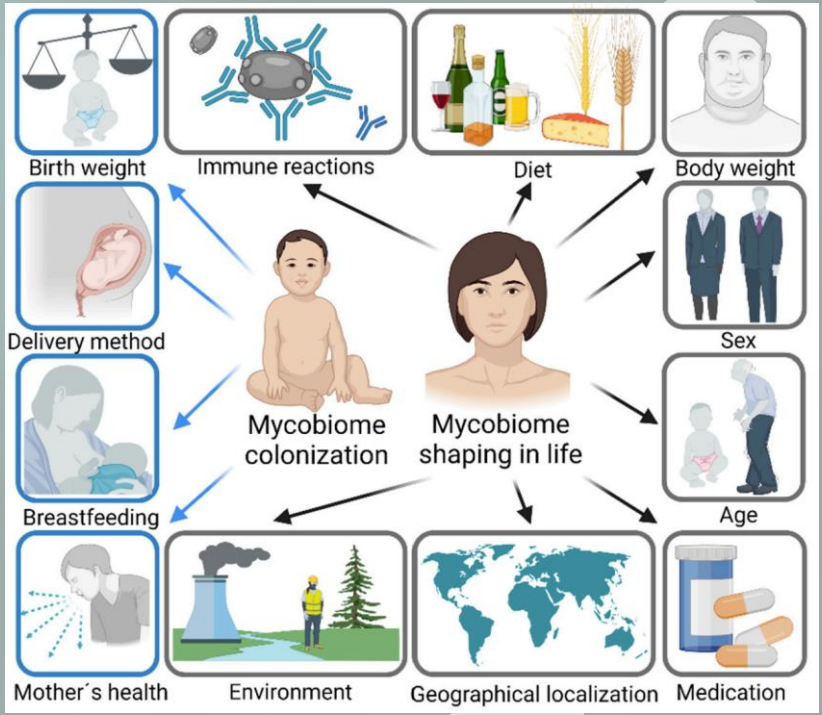

Table 1 Factors affecting the composition of gut mycobiota

Factors			Composition of gut mycobiota	References
Delivery method	→	Natural birth	Fungi from mother's genital tract ↑ Russulales ↑	[37, 40–42]
	→	C-section	Fungi from maternal skin and surroundings ↑ Saccharomycetales ↑	
Gestational age	→	Preterm infants	Fungal diversity ↓ Saccharomycetales ↑ *Candida* ↑	[37]
	→	Term infants	Polyporales ↑ Russulales ↑ *Stereum* ↑ *Malassezia* ↑	
Environment		Mice from Jackson Laboratory's & Services (JAX)	Basidiomycota ↑	[18, 43]
Season				

- **Naturally born infants are more likely to get *Candida spp.* from maternal genital tract, whereas infants born after cesarean delivery are more likely to get *Malassezia spp.* from maternal skin.**
- **Preterm delivery is specifically associated with *Candida spp.*, while term delivery is associated with increased *Malassezia spp.* colonization.**

Diet and nutrition

Gender

Metabolic disorder

Maternal antibiotic exp

Species

Table 2. The list of food products colonized or contaminated by food-borne microscopic fungi.

Food Product	Microscopic Fungi	References
Fruit and vegetables		
Fresh citrus and grape	*Candida prunicola*, *Pichia fermentans*	[66]
Fresh apple, plum and pear	*Saccharomyces cerevisiae*, *Pichia kluyveri*, *Pichia kudriavzevii*, *Galactomyces candidus*, *Hanseniaspora uvarum*, *Hanseniaspora guilliermondii*	[67]
Peeled fruit salads	*Candida* sp., *Debaryomyces* sp., *Rhodotorula* sp., *Penicillium* sp., *Cladosporium* sp.	[68]
Dried fruit	*Cladosporium* sp., *Aspergillus niger*, *Aspergillus tubingiensis*, *Penicillium palitans*	[68,69]
Various fresh vegetables (salad, tomato, cucumber, green inion, lettuce, spinach, etc.)	*Geotrichum* sp., *Alternaria* sp., *Cladosporium* sp., *Penicillium* sp.	[68,70]
Dairy		
Various cheeses (Blue cheese, Camembert, Cheddar)	*Penicillium* sp., *Candida* sp., *Scopulariopsis* sp.	[64]
Acidophilus milk	*Saccharomyces fragilis*, *Candida pseudotropicalis*	[71,72]
Meat		
Various meats (fermented sausage, dried meat, salami, ham)	*Debaryomyces* sp., *Penicillium* sp.	[64]
Beverages		
Wine	*Hanseniaspora* sp., *Saccharomyces* sp.	[73,74]
Beer	*Brettanomyces* sp., *Saccharomyces* sp.	[73,74]
Sake	*Aspergillus* sp., *Saccharomyces* sp.	[73]
Other		
Various nuts (pecan, almond, walnut, pine nut)	*Aspergillus* sp. *Penicillium* sp., *Alternaria* sp., *Cladosporium* sp., *Rhizopus* sp., *Fusarium* sp.	[69]
Koji	*Aspergillus* sp., *Rhizopus* sp.	[75]
Soy sauce	*Aspergillus* sp., *Hansenula* sp., *Zygosaccharomyces* sp.	[73]
Steamed pastry	*Wickerhamomyces anomalus*	[76,77]

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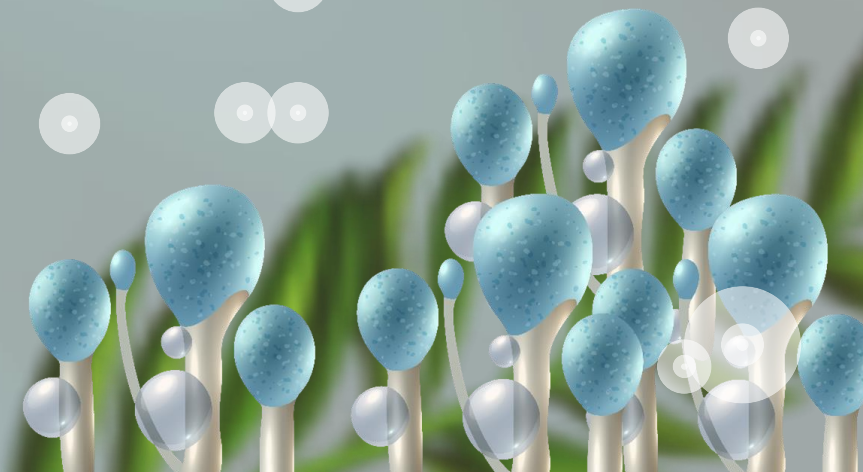
METHODOLOGIES FOR STUDYING MYCOBIOTA



- **Methodologies for studying gut mycobiome are divided into culture-dependent & culture-independent methods.**

- **Traditionally, fungal diversity was assessed using culture-dependent methods, using various culture media as SDA & CHROMagar™ *Candida* medium used for *Candida* isolation & differentiation.**

- **ID fungi plate culture with MALDI-TOF MS is suitable for identification of filamentous fungi.**

- **Though cultivation of fungi is the most direct method with visualization of fungal morphology and colony color, there are some <u>limitations in its application</u>:**
  - ✓ **During cultivation, the environmental fungi may contaminate culture & easily affect the precision of results.**
  - ✓ **It is difficult to distinguish specific species and genus of similarly looking fungi even by microscope.**
  - ✓ **Some fungi cannot be cultured.**
  - ✓ **Culture methods are very time-consuming.**

- **Culture-independent methods depends on recent advances in deep-sequencing technologies and bioinformatics analysis, which are based on the analysis of genomic DNA, shedding light on the complexity of the gut fungal communities.**

- **Generally, many culture-independent methods have been developed, such as sequencing for 18s ribosomal DNA, ITS-1 and 2, & RFLP.**

- **The advent of sequencing technology allowed us to answer important questions about fungi:**
  - ✓ **Which fungi do commensal communities comprise?**
  - ✓ **Are they transiently present or do they stably colonize?**
  - ✓ **What are their functions?**

- **Overall, there is no consensus on the optimal methodology for characterizing mycobiome.**

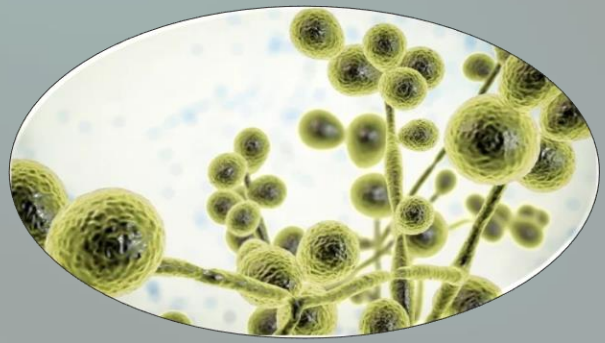
04

THE ORAL MYCOBIOTA

- ***Ghannoum et al.*** **identified a total of 101 species in the oral cavities of 20 healthy individuals.**

- **The oral mycobiome appears to be more subject specific than the oral bacteriome.**

- **Oral mycobiota comprises active & transient colonizers.**



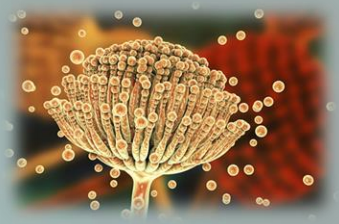
Members of the Oral Mycobiota

- **Among the most found fungi in the oral cavity are *Aspergillus spp. & Candida spp.***

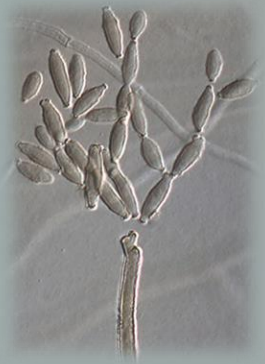
- ***Candida* infections of the oral cavity are very common. On the other hand, pathological conditions due to *Aspergillus* are rare in the oral cavity.**

- **This discrepancy might be due to the possibility that *Aspergillus* is a transient member of the oral mycobiome, acquired via diet intake or inhalation.**

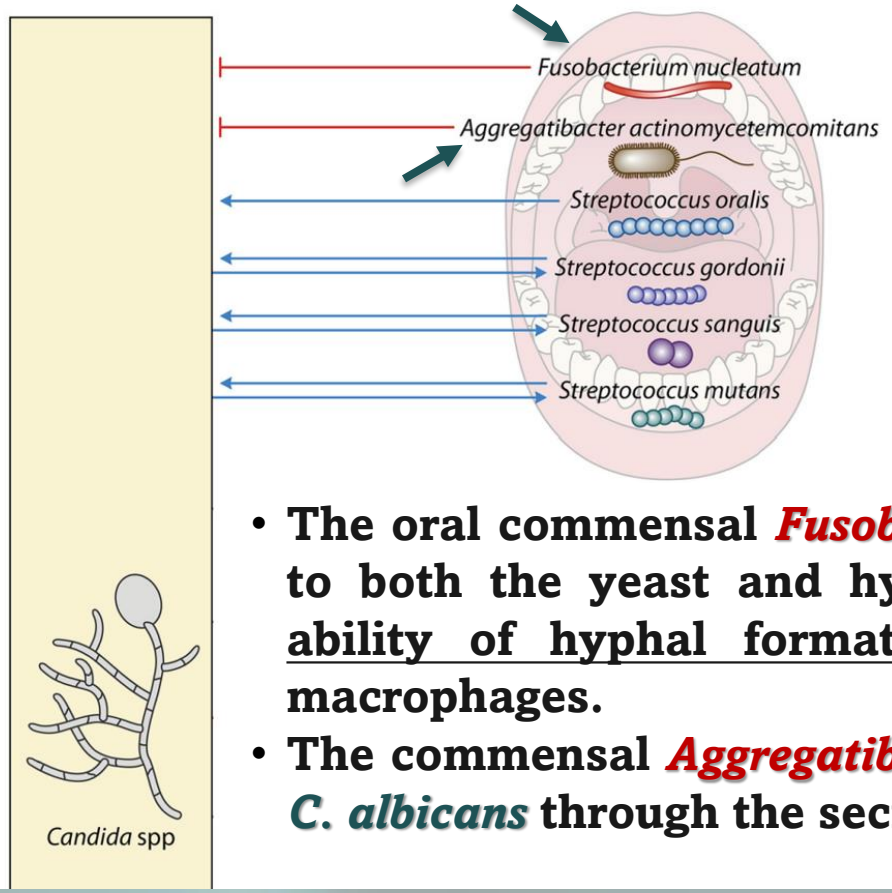
- **Other fungi of the oral mycobiota, such as *Saccharomyces, Penicillium, Malassezia, Cladosporium, & Fusarium* also represent active members of the oral microbiota.**





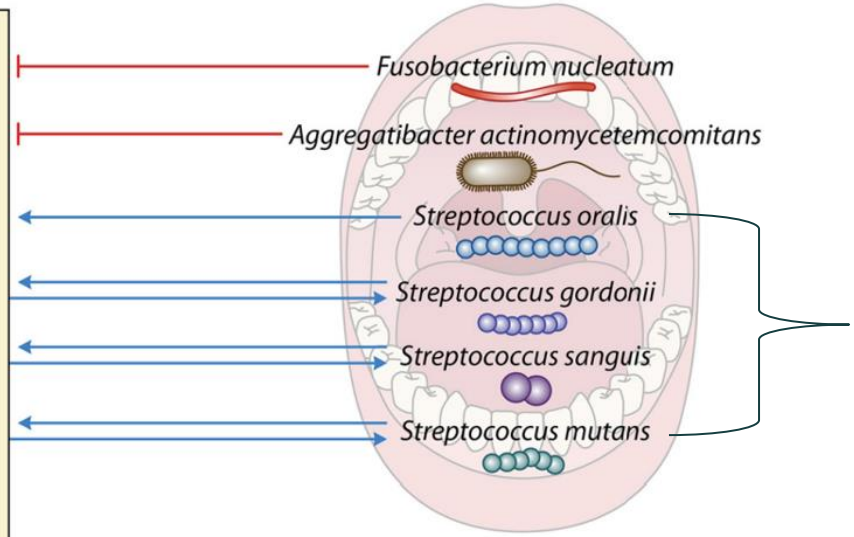

Fungal-Bacterial Interactions in the Oral Cavity

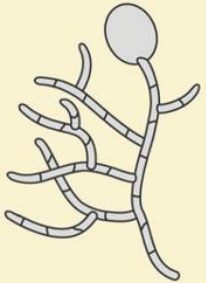


Protective Interactions

- The oral commensal ***Fusobacterium spp.*** has been shown to adhere to both the yeast and hyphal forms of ***C. albicans***, <u>limiting its ability of hyphal formation</u>, thus reducing its ability to kill macrophages.
- The commensal ***Aggregatibacter spp.*** <u>inhibits biofilm production</u> by ***C. albicans*** through the secretion of the quorum-sensing molecules.

Pathogenic Interactions




Candida spp

- **Various studies have investigated mutualistic interactions between *C. albicans* & *Streptococcus spp.* that promote infection by taking an active part in biofilm formation and plaque virulence.**
- **This is mediated by cell wall polysaccharides, salivary proteins, adhesins on the surface of *Streptococci* & receptors on surface of *Candida*.**

05

THE GUT MYCOBIOTA

Development of GUT Mycobiota

- ***Willis et al.*** **recently suggested that fungal species might be present prior to birth & that *C. albicans* specifically could be associated with preterm delivery.**
- **Additional studies have shown that vaginal delivery allows vertical transmission of *Candida spp.* from mother to infant.**
- **Infants born by C-section harbor a bacterial microbiome similar to the mother's skin microbiome, therefore they also harbor higher *Malassezia spp.* in their GIT.**

- ***Boix-Amorós et al.*** **found a core breast milk mycobiome, composed of *Malassezia, Cladosporium, & Penicillium.***
- **Accordingly, the infant gut mycobiome is initially dominated by *Malasseziales*, most likely taken up through lactation.**

- After the first 6 months of age, the infant gut mycobiome undergoes a dramatic change and is no longer dominated by *Malasseziales* but by ***Saccharomycetales*** instead.
- This change in mycobiome coincides with a change from breast milk to solid food.

- The gut microbiota further changes and matures during the development from childhood to adulthood.
- These changes are most likely **driven by the development of the immune system** and by the microorganisms that humans are exposed to through their **diet and environment**.

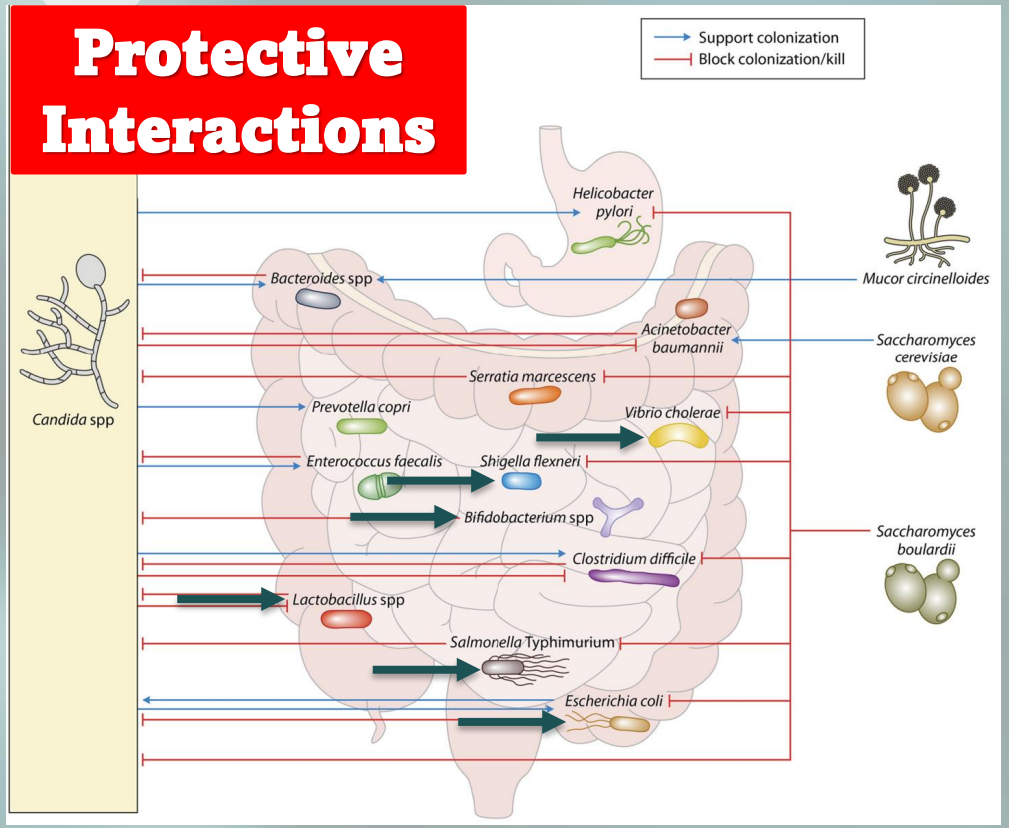
Members of GUT Mycobiota

- **Similar to the oral mycobiome, GUT mycobiota comprises <u>active & transient colonizers</u>.**

- **Several studies determined *Saccharomyces, Malassezia, & Candida* as the three most abundant genera present in the human gut .**
  - ✓ ***Saccharomyces* is ubiquitously present in the human diet.**
  - ✓ ***Malassezia* is the most abundant fungus colonizing the human skin.**
  - ✓ ***Candida* is the most identified fungus in the oral cavity.**

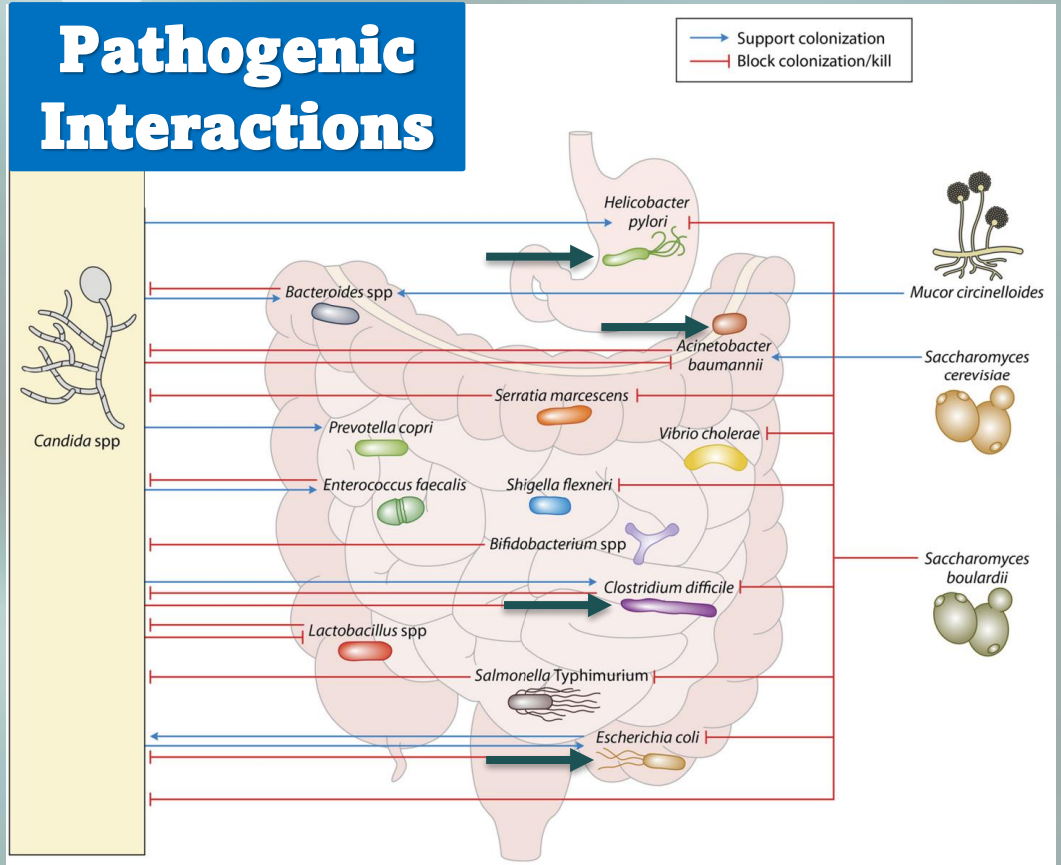
Fungal-Bacterial Interactions in the GUT

Protective Interactions



- ***Saccharomyces spp. & C. albicans*** **have been extensively studied as a potential probiotics due to its protective effect against various bacterial GI pathogens.**
- **Probiotic bacterial strains *Lactobacillus & Bifidobacterium spp.* have shown efficacy in limiting the severity of *C. albicans* infection:**
  - ✓ **Inhibit the transition to invasive hyphal form**
  - ✓ **Inhibit biofilm formation**

Pathogenic Interactions

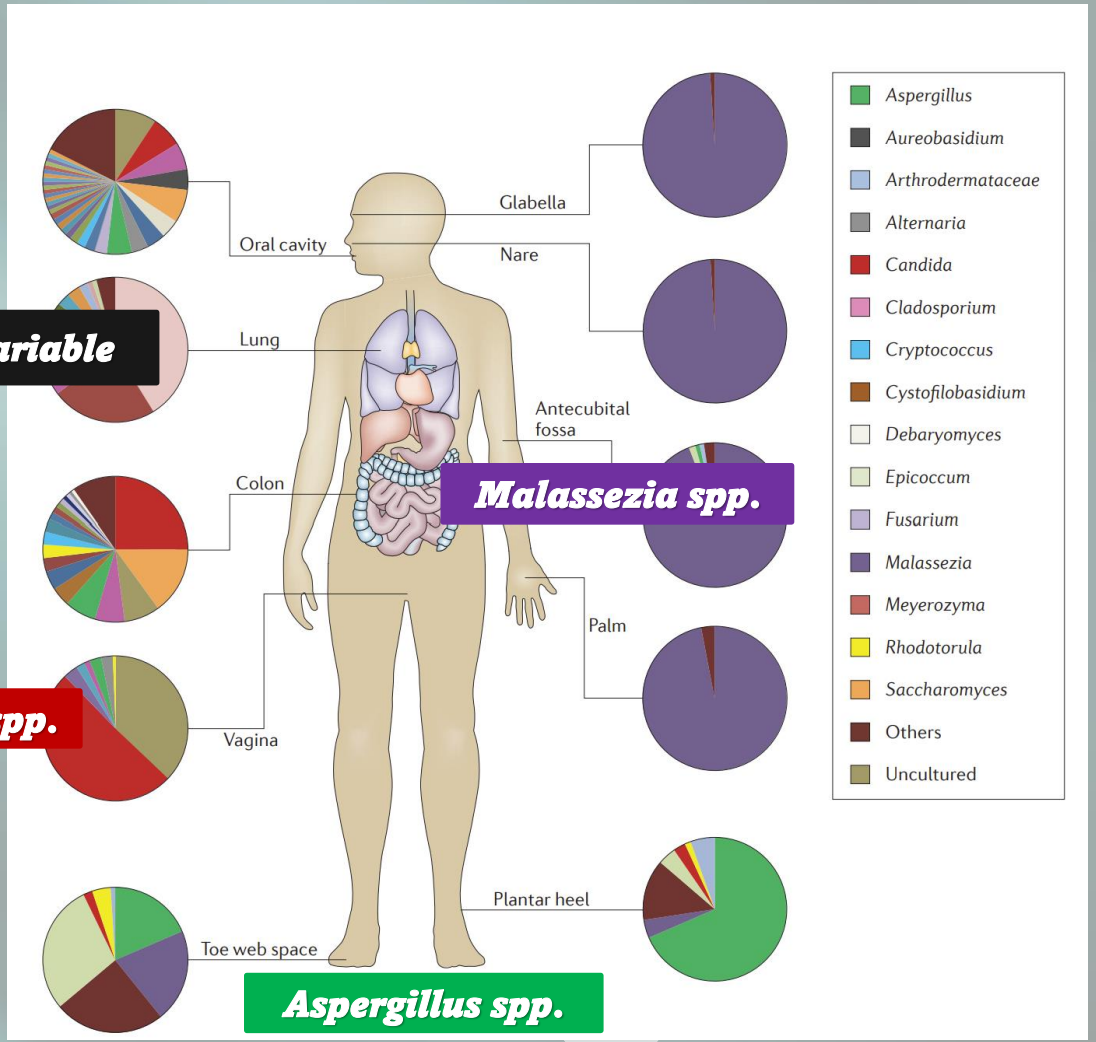


- ***Enterohemorrhagic E.coli*** **enhances** ***C. albicans*** **invasion of intestinal epithelial cells in vitro.**
- ***C. albicans*** **allows the growth of the strict anaerobe** ***C. difficile*** **under aerobic culture conditions due to the rapid reduction of dissolved oxygen in the vicinity of the yeast.**
- ***H. pylori*** **was found within vacuoles in** ***C. albicans*** **cells which provides an environment that *H. pylori* can use to survive the low pH of the stomach.**
- ***S. cerevisiae*** **enhances the growth of the opportunistic** ***Acinetobacter baumannii*** **by producing ethanol.**

06

Mycobiota at Other Body Sites

Complex & Variable



Candida spp.

Malassezia spp.

Aspergillus spp.

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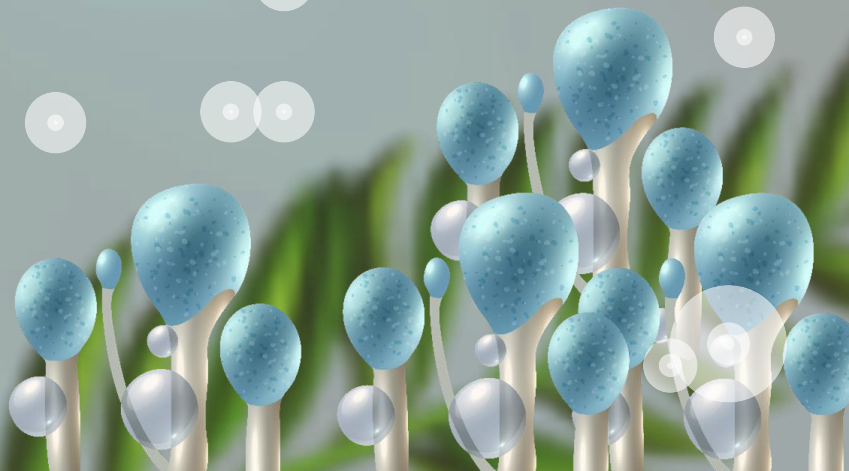
MYCOBIOME DYSBIOSIS



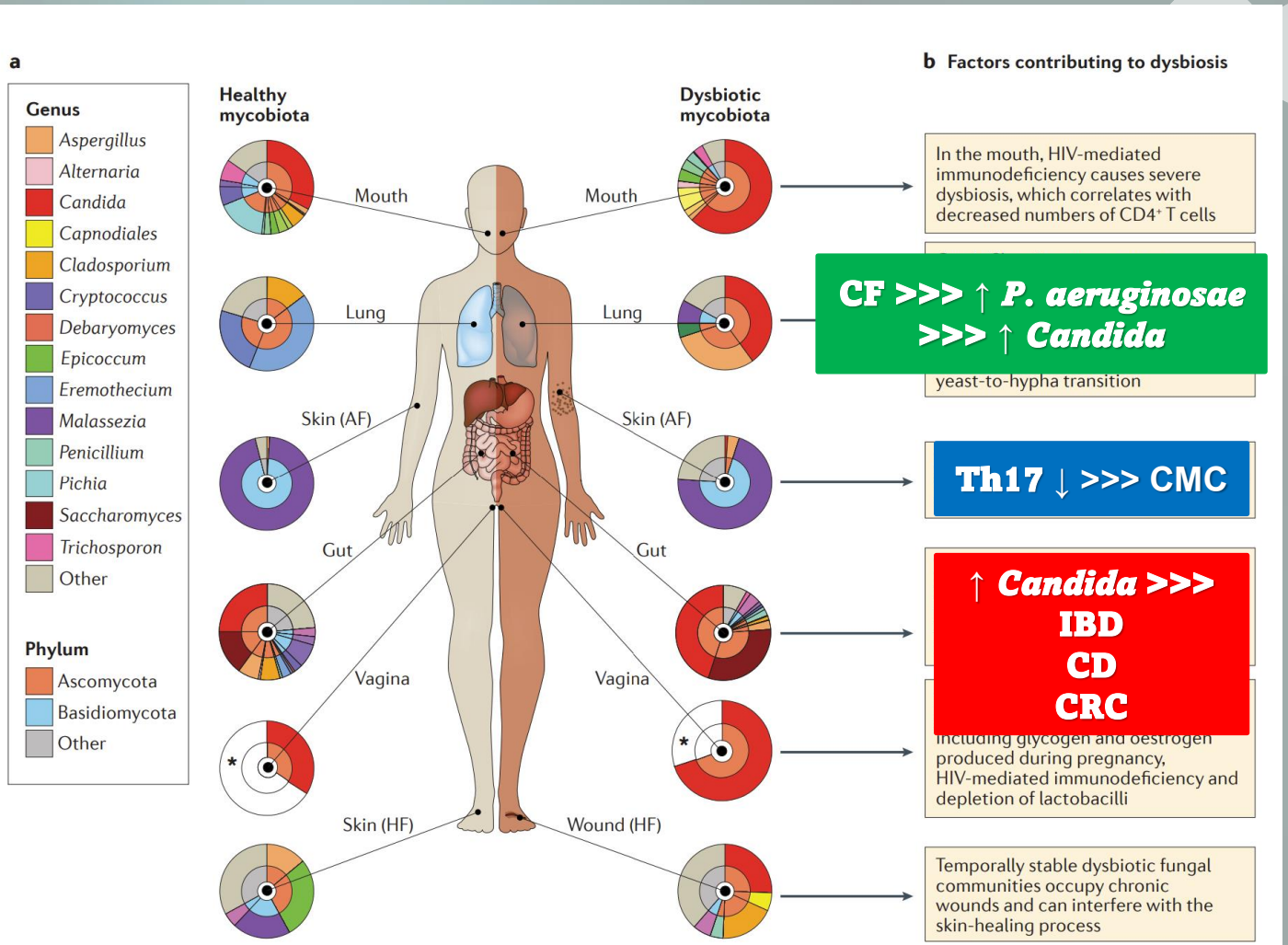


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Table 3 Association of enteral and parenteral diseases with gut mycobiota

Targets		Diseases	Related fungi	Reference
Intestinal tract	→	Inflammatory bowel disease (IBD)	Basidiomycota ↑ Ascomycota ↓ *Candida* ↑ *S. cerevisiae* ↓	[115–118]
	→	Celiac disease	*Candida*↑	[119, 120]
	→	Colon cancer	*C. tropicalis* ↑	[121, 122]
Extra-intertinal tract	Lung	Fluconazole induced Allergic airway disease (AAD)	*Candida* ↓ Aspergillus ↑ Wallemia ↑ Epicoccum ↑	[11, 114]
		Pulmonary infection	*Histoplasma capsulatum*	[123]
	Liver	Cirrhosis	Fungal detection ↑	[124]
	Kidney (possible)	Sepsis	*C. albicans*	[125, 126]
	Pancreas →	Pancreatic ductal adenocarcinoma (PDA)	*Malassezia* ↑	[127]
	Brain →	Multiple sclerosis (MS)	*Candida*	[128]
	→	Schizophrenia (SCs)	*Chaetomium* ↑	[129]

"↑" indicates increase and "↓" indicates decrease

CONCLUDING REMARKS

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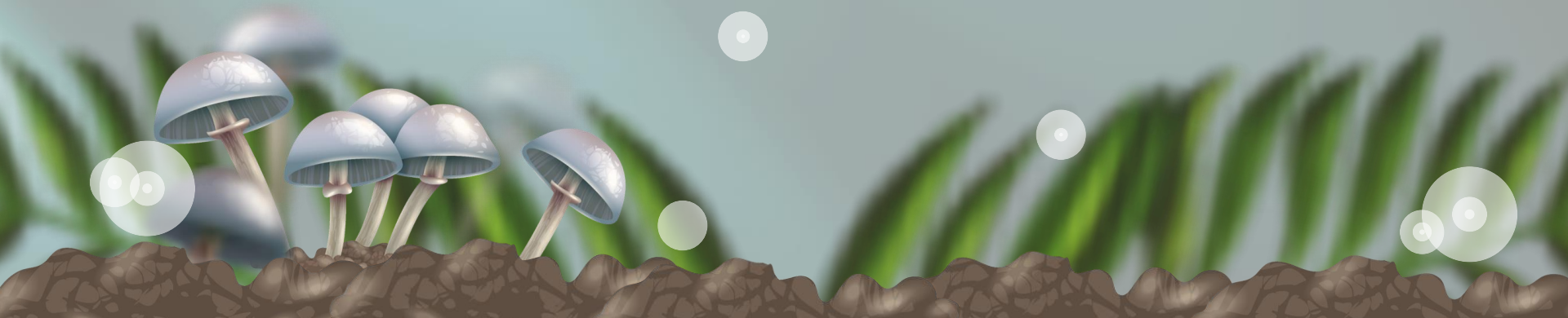
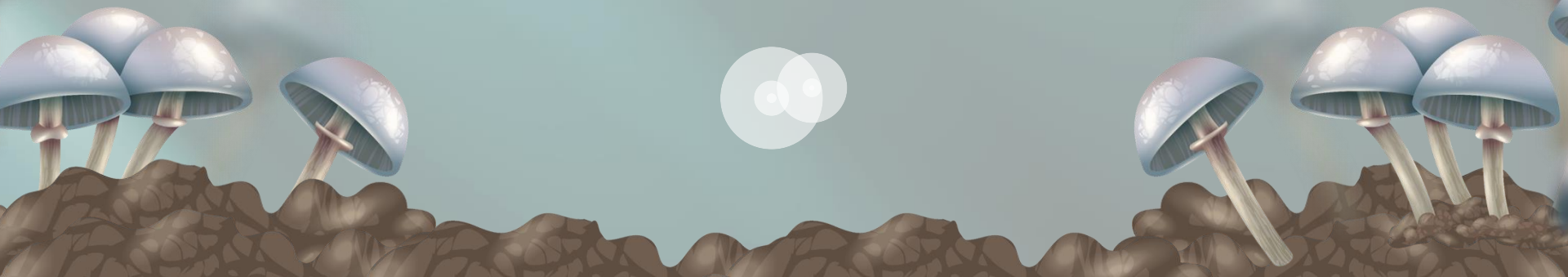


- **Mycobiome research is a rapidly expanding field, yet many questions are currently still unanswered.**

- **Due to the high inter- and intra-individual variability, it is unclear if core mycobiomes can be defined.**

- **Future research will expand our knowledge on which fungi are resident and which are transiently present in the gastrointestinal tract.**



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