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Invited Medical Review

Title: Biodiversity of the Human Oral Mycobiome in Health and Disease

Running title: Human oral mycobiome

Keywords: Human microbiome, oral mycobiome, biodiversity, fungi, high throughput sequencing

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Abstract:

The organisms that colonize the human body over a lifetime are diverse, extensive and gargantuan. A fair proportion of the microbiota that constitutes this human microbiome live within our oral cavities mostly as harmonious associates causing only sporadic disease. An important core constituent of the microbiome is the mycobiome, representing various fungal genera. Up until recently, only a few species of fungi, mainly *Candida* species, were thought to constitute the human oral mycobiome. The reasons for this are manifold, although the uncultivable nature of many fungi in conventional laboratory media, and their complex genetic composition seem to be the major factors which eluded their detection over the years. Nevertheless, recent advances in computing and high throughput sequencing such as next generation sequencing (NGS) platforms have provided us a panoramic view of a totally new world of fungi that are human oral co-habitués. Their diversity is perplexing, and functionality yet to be deciphered. Here we provide a glimpse of what is currently known of the oral mycobiome, in health and disease, with some future perspectives.

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Introduction

The human body, like all other complex multicellular eukaryotes, is inhabited by a bewildering array of microbes. Bacterial constituents make up over 99 % of total microbial counts, hence termed the core microbiome, whilst the remaining, less abundant and more diverse flora forms a 'rare biosphere' (Dethlefsen, Huse, Sogin, & Relman, 2008; Sogin et al., 2006). A significant proportion of the rare biosphere, in turn is represented by fungi - the mycobiome. Despite the low abundance, the impact of the mycobiome on human health and disease is wide-ranging. For instance, it is estimated that over 600 out of 5.1 million species of fungi comprising the rare biosphere cause human disease (Blackwell, 2011; Hawksworth & Lucking, 2017) ranging from mild superficial disease to deep seated, life-threatening, systemic infections (Brown, Denning, & Levitz, 2012; Huffnagle & Noverr, 2013).

Although a vast multitude of bacteria have been recognized, classified and categorized as can be seen in the Human Microbiome Project (HMP), critical components of the rare biosphere including the mycobiome, virome (*syn.* virobiome) and parasitome have received less attention. Despite the ever-rising incidence of fungal infections, particularly due to the burgeoning immunocompromised populations worldwide, the diversity and the functionality of resident fungi in the human body is therefore an under-researched area.

Particularly relevant to the current review, the fungal component of the oral microbiome, the 'oral mycobiome' has also received scant attention. The reasons for this are manifold, the uncultivable nature of many fungi in conventional laboratory media and their complex genetic composition as eukaryotes seem to be the key reasons that may have eluded their detection over the years. Additionally, the growth of the oral mycobiome constituents, thought to comprise less than 0.1% of the oral microbiome based on conventional colony forming unit (CFU) analysis (Baker, Bor, Agnello, Shi, & He, 2017), appears to have been overshadowed by the predominant bacteria in traditional laboratory cultures of oral samples. Other reasons include, difficulties in nomenclature of fungi and annotating fungal databases and the historical redundancies and errors associated with fungal nomenclature.

What follows is a review of the data emanating from the use of newer technologies, such as NGS platforms that throw light on the composition and the physiology of the oral mycobiome. After discussing the latter attributes of the mycobiome we review the impact of the extant new information on our understanding of the oral ecosystem in various diseased states such as endodontic infections, periodontal disease, and oral squamous cell carcinomas.

Newer microbial detection technologies and the oral mycobiome

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Characterization of fungal communities including the oral mycobiome has evolved, but relatively slowly, compared with the oral bacteriome. Once genetic material-based detection technologies were introduced in early 1990s, the shortcomings of traditional culture- based clinical laboratory techniques and identification of human fungal pathogens using selective media and, biochemical assays were evident. Gene-based identification of fungi has led to the documentation of unique sequences in fungal ribosomes. The genetic loci enclosing the 18S, 5.8S, and 28S rRNA genes, internal transcribed spacer (ITS) regions between the 18S and 28S rRNA genes (ITS1 and ITS2), and the non-functional RNA coding DNA that transcribe during rRNA synthesis, have been principally targeted in recent culture independent, gene based fungal identification techniques (Hershkovitz & Lewis, 1996; Hillis & Dixon, 1991; Nilsson, Kristiansson, Ryberg, Hallenberg, & Larsson, 2008).

The most recent, state of the art next generation sequencing (NGS) technology also uses targets similar to ITS regions, and clone libraries derived from the components of rRNA (16S, 5.8S, and 28S rRNA genes). Unlike the more advanced and extensive bacterial databases, the fungal ribosomal database is still immature, although, recent inclusions tend to show the perplexing diversity of the human mycobiome, including the oral mycobiome. (Charlson et al., 2012; Delhaes et al., 2012; Gardes & Bruns, 1993; Ghannoum et al., 2010; Lindsley, Hurst, Iqbal, & Morrison, 2001). As members of the rare biosphere, the physiology, lifestyle and host pathogen interactions of a vast proportion of these recently identified uncultivable fungi are yet to be fully unravelled.

As for the oral mycobiome, there was a general consensus until recently, both by the scientific and the clinical community, that *Candida* species are the predominant opportunistic oral pathogens of clinical significance (Samaranayake, 2018). This, despite that fact that lesser known fungi other than *Candida* species were sporadically and infrequently cultured from oral samples, particularly from medically compromised individuals. Some of these infrequently isolated species included *Saccharomyces cerevisiae, Penicillium, Aspergillus, Geotrichum, Hemispora, Scopulariopsis*, and *Hormodendrum* species (Jabra-Rizk et al., 2001; Salonen et al., 2000; Schuster, 1999). However, newer mycobiome studies using NGS have recently revealed the existence of *Malassezia* species in a majority of oral samples, an organism that has rarely, if ever, isolated from the mouth. The role of *Malassezia* in oral health and disease is yet to be defined.

One of the pioneering and seminal studies that used a Multitag Pyrosequencing approach was conducted by Gahnnoum et al., (2010). Pyrosequencing analysis was the predecessor to the NGS platforms that are currently in use. The latter group for the first time described the 'basal oral mycobiome' of healthy individuals. They reported 74 culturable and 11 non-culturable fungal genera and a total of 101 different fungal species in the oral samples from 20 healthy individuals. Not unexpectedly, the most common genera isolated belonged to *Candida* species (obtained from 75% of all study subjects) followed by *Cladosporium* (65%). One half of the cohort carried *Aureobasidium* and Saccharomycetales, and notable quantities of *Aspergillus* (35%), *Fusarium* (30%), and *Cryptococcus* (20%) were also

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identified. *C. albicans* were isolated in 40% of the subjects while 15% carried *Candida parapsilosis* and *Candida tropicalis* and 5% carried *Candida khmerensis* and *Candida metapsilosis* (Ghannoum et al., 2010). The relative distribution of various *Candida* species was similar to those reported in previous studies using conventional technology (Samaranayake & MacFarlane, 1990).

Following this study, Dupuy et al., evaluated the 'human core mycobiome' (Dupuy et al., 2014) using an improved approach of pyrosequencing in order to i) overcome process-induced sequencing errors, ii) to accurately assign fungal taxonomy and iii) to develop a proper binary naming and phylogenetic classification. This was the first study to reveal the presence of *Malassezia* species, previously known to be a skin commensal and an opportunistic pathogen, as a predominant commensal of saliva. So, how did oral *Malassezia* species evade detection for virtually decades? Some reasons could be i) the necessity for additional lipids in culture media for their growth due to their incomplete fatty acid synthetic potency, ii) incubation over prolonged periods required to elicit growth in conventional mycological media and, iii) their extreme sensitivity and inability to withstand harsh protocols used in early molecular detection methods (Findley et al., 2013; Imabayashi et al., 2016; Monteiro-da-Silva, Araujo, & Sampaio-Maia, 2014; Shelburne et al., 2015; Wu et al., 2015). Hence, it is not surprising that the identity of the organism by culture-based methods eluded oral mycologists thus far.

The two pioneering studies on the oral mycobiome by Ghannoum et al., and Dupuy et al., stated above, entailed the use of comparable methodology but the results were somewhat dissimilar. Out of 13 fungi recognized by Ghannoum et al., (i.e. *Alternaria, Aspergilllus, Aureobasidium, Candida, Cladosporium, Cryptococcus,* Dothioraceae*, Eurotium, Fusarium, Glomus, Saccharomyces,* Saccharomycetales, and *Teratosphaeria),* Dupuy and colleagues did not detect *Glomus, Teratosphaeria,* Saccharomycetales and Dothioraceae in their samples. In contrast, they observed five new genera of fungi; *Malassezia, Irpex, Cytospora/Valsa, Lenzites/Trametes*, and *Sporobolomyces/Sporidiobolus* in saliva samples of all six individual participants (Dupuy et al., 2014).

Clearly, to the surprise of the oral microbiologists, the foregoing work, essentially focusing on the healthy oral mycobiome revealed an array of exotic, pathogenic fungi within the oral mycobiome. These studies were indeed the forerunners for those investigating the mycobiome of a variety of other oral diseases ranging from periodontal disease to oral carcinomas.

Technical Challenges in oral mycobiome analysis

It is clear that the culture independent microbial identification techniques have expanded our understanding of the human mycobiome. However, like any other microbial identification systems, novel molecular approaches also carry associated challenges. With the emergence of the `big data` on the oral mycobiome, there is a concomitant and an

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urgent need for a well-curated oral mycobiome database. Deposition of vague and erroneously annotated entries, redundant taxa and, sequences created as a consequence of primer artefacts in publicly available repositories such as GenBank are likely to generate unreliable outcomes during taxonomic identifications of unknown oral mycobiota (Dupuy et al., 2014; Nilsson et al., 2006).

Recently, efforts have been made to construct a niche-specific reference set to avoid inaccuracies generated using hitherto existing databases such as UNITE and Fungal ITS RefSeq Targeted Loci Project (Abarenkov et al., 2010; Findley et al., 2013; Irinyi et al., 2015; Tang, Iliev, Brown, Underhill, & Funari, 2015). Nevertheless, compounding fungal nomenclature, particularly fungal species possessing multiple names based on their morphological (sexual vs asexual), geographical and historical characteristics, is likely to complicate high throughput identification of mycobiota from the human oral mycobiome (Hibbett & Taylor, 2013; Quaedvlieg et al., 2014; Taylor, 2011).

Further, due to the wide variations in the fungal ITS amplicon lengths (~200-~600 base pairs), ITS based fungal surveys must be carried out with extreme care. This is critical to prevent erroneous bidirectional sequencing due to overlaps between forward and reverse reads and, to prevent potential bias when selecting the amplification length when calculating the quantity (Tang et al., 2015). In addition, ITS regions exhibit remarkably greater sequence variations within the same species making it difficult to generate similarity thresholds to define open taxonomic units (OTU) at a species-specific level.

Also, the interspecies variations of ITS copy number among fungi (few to ~ 250) complicate the quantitative estimation of the fungal load using universal primers (Liu et al., 2012). Species specific primers would only be useful when the copy number of the target sequences in a particular species is hitherto known as some important species in the oral mycobiome possess variable copy numbers among the strains, e.g. *C. albicans* (56-222 copies) and *A. fumigatus* (38-91 copies) (Herrera, Vallor, Gelfond, Patterson, & Wickes, 2009; Rustchenko, Curran, & Sherman, 1993). Due to such variations in ITS sequences, various other additional genes have been used to discriminate among species (multigene approach) (Hibbett & Taylor, 2013).

These challenges further point to the importance of robust novel approaches to precisely identify fungi at a species-specific level. Nevertheless, further improvements and refinements and fungal identification technology through advanced platforms, and resolution of taxonomic issues inherently extant in fungal nomenclature should help precise delineation of the oral mycobiome in the not too distant future.

Oral mycobiome and oral physiology

The fluctuations of the functionality and the dynamics of the human microbiome in response to physiological vagaries such as age and diet are well known. Similar to their

bacterial counterpart, the mycobiome also exhibits changes in response to physiological vicissitudes of the human body. For instance, fungal ITS1 sequencing of saliva samples collected from community dwelling elderly (77-99 years of age) has revealed a significant positive association of oral mycobiota with increasing age, number of missing teeth, denture wearing, and low salivary flow rates (Ikebe, Morii, Matsuda, Hata, & Nokubi, 2006). Although the artificial retention areas offered by dentures may be a major reason for this observation, it is clear that other factors such as reduced salivary flow and the quality of the mucosa associated with advancing age may have played a contributory role in the changing landscape of the oral mycobiome (Samaranayake, 2018). In another previous investigation, despite the differences in primers used, community dwelling elderly possessed lower total fungal load in their oral mycobiome compared to those institutionalized (Li et al., 2012). The reasons for this are unclear, although communal sharing of utensils and poor hygiene could be attributable to these observations. Uncultivable oral mycobiota in advancing age has not been studied and is warranted to fill this glaring gap in knowledge.

It appears that oral *Candida* species significantly vary especially in elderly from diverse geographical and ethnic backgrounds, and non-*albicans* species are estimated to be predominant in Asian populations (Falagas, Roussos, & Vardakas, 2010; Kraneveld et al., 2012; Samaranayake, 2009). For instance, one study indicates that the oral carriage of *Candida krusei* in leprosy patients in Thailand is higher than *C. albicans* (36% vs 10% control) - the most common oral yeast isolate in humans (Reichart, Samaranayake, & Philipsen, 2000). Samaranayake et al., noted on review of 44 publications in the literature for which statistics on rates of human carriage of *C. krusei* are available, the highest carriage rates to be 6.1% for the oral cavity, 10.3% for the gastrointestinal tract, and 12.5% for the vagina either in health or in disease (Samaranayake & Samaranayake, 1994). They hypothesized that the exceptionally high prevalence of *C. krusei* in the leprosy cohort, could be attributed to the institutionalization and the shared dietary regimens possibly originating from a common outlet. NGS studies of population groups in diverse geographic locales and ethnicities should be of extreme interest to obtain a clearer picture of their oral mycobiome.

In comparison to the elderly, the carriage of *Candida* in the oral cavities of infants at birth is lower and colonization rates rise with age reaching adult carriage rates in the first year of life (Kleinegger, Lockhart, Vargas, & Soll, 1996; Russell & Lay, 1973; Ward, Knights, & Gale, 2017). In another recent study, Zakaria *et al.,* noted that the oral cavities of those with lower BMI (body mass index) were more frequently colonized with *Candida dubliniensis* and *Candida glabrata* while those with lower salivary flow rates were colonized by *C. albicans* (Zakaria et al., 2017). Nonetheless, new studies using novel, culture independent analyses, such as NGS technology are needed to re-evaluate the fungal diversity in human mouth under changing physiological and chronological conditions as historical data are likely to be biased towards the yeasts that flourished in conventional laboratory culture.

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Saliva and the mycobiome

There are two recent studies in the literature investigating the salivary mycobiome. The first is the afore mentioned seminal study of Ghannoum et al., (2010) which evaluated oral rinse samples that could be considered as surrogate salivary samples, and the second is that of Monteiro-da-Silva et al., (2014) which evaluated salivary samples using a longitudinal approach.

Ghannoum et al., noted in 20 healthy adults a basal mycobiome of 74 culturable and 11 non-culturable fungal genera. Among these genera, 39 were present in only one person, 16 were present in two participants, and five were present in three individuals, while 15 genera (including non-culturable organisms) were present in ≥4 (20%) participants. *Candida* species were the most frequent followed by *Cladosporium* (65%), *Aureobasidium*, Saccharomycetales (50% for both), *Aspergillus* (35%), *Fusarium* (30%), and *Cryptococcus* (20%) (Ghannoum *et al.,* 2010).

The second study of salivary samples collected over the period of 30 weeks revealed that, despite no intraindividual variations over time, the prevalence of moulds and yeasts, 100% and 92.5% of samples respectively, was significantly higher in the oral cavities (Monteiro-da-Silva et al., 2014). The moulds were mainly *Penicillium spp.*, *Aspergillus spp.*, and *Cladosporium spp.* and yeasts were predominantly *Candida spp.* and *Rhodotorula spp*. (Monteiro-da-Silva et al., 2014). Interestingly. The latter three mould species together with *Candida* are known to be human pathogens, especially in compromised patient groups.

Salivary mycobiome studies are still in its infancy. Yet to be explored areas include the natural history and the evolution of the mycobiota from birth to adulthood in healthy and diseased individuals. On the other hand, information on the mycobiota of a few diseased states of the oral cavity are emerging as discussed below.

Mycobiome of periodontal disease

Microbiology of periodontal diseases is complex. Although there is consensus as to a few key periodontopathogens (the so-called red complex bacteria or key stone pathogens) that are involved in periodontal diseases, further work is clearly necessary to demystify the pathogenesis of this disease. There have also been recent calls that fungi may play a role in periodontal diseases (Canabarro et al., 2013). The latter group noted several yeast species such as *C. parapisilosis*, *Rhodotorula spp.*, *C. dubliniensis* and *C. tropicalis* in both healthy and periodontitis cohorts but only *C. albicans* was present in all the patients with yeast positive periodontitis.

There is one recent study by Peters et al., (2017) evaluating the mycobiome of periodontal patients. The latter workers studied patients with periodontal infections using fungal ITS1 sequencing and noted no less than 150 fungal species belonging to at least five phyla including *Ascomycota, Basidiomycota, Glomeromycota, Chytridiomycota,* together with an

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abundance of unclassified organisms (Peters, Wu, Hayes, & Ahn, 2017). Another intriguing finding was a predominance of phylum *Ascomycota* (86.5%) in both the healthy and the periodontally affected individuals. In line with previous findings, *Candida* and *Aspergillus* were isolated from all the samples (100% prevalence), and *Penicillium* (97%), *Schizophyllum* (93%), *Rhodotorula* (90%), and *Gibberella* (83%) too were frequent isolates. Not unexpectedly, there were a significant proportion of unidentified fungi as well. Interestingly, Peters *et al*., could not find a significant difference in the overall composition of the mycobiome between the periodontally affected and the healthy cohorts (Peters et al., 2017), although there was a clear association between tooth loss and increased prevalence of *Candida.* Furthermore, isolation of 14 different open reading frames (OTUs) from patients with periodontitis suggested that rare *Candida spp.* may be associated with periodontal diseases, particularly in the subgingival sites that provide favourable habitats for yeasts (Canabarro et al., 2013).

In vitro studies have shown that the keystone bacterial pathogens such as *P. gingivalis* and *A. actinomycetemcomitans* physically interact with *Candida* species. For instance, both non-vital *C. albicans* and its cell wall components (mannoprotein-b-glucan complex) enhances *P. gingivalis* invasion of oral mucosal cells via unknown mechanisms (Tamai, Sugamata, & Kiyoura, 2011). In addition, *A. actinomycetemcomitans* also binds to *C. albicans* hyphae, and yet inhibits the fungal growth through quorum sensing signalling (Bachtiar et al., 2014). Sztukowska *et al.* (2018) have recently characterized the interactions between *C. albicans* and *P. gingivalis* and demonstrated that co-adhesion mediated by specific proteins results in major changes in gene expression by *P. gingivalis*, which could serve to increase their pathogenic potential. This implies that interdomain interactions can modulate oral diseases (Sztukowska et al., 2018). Considering the fact that the etiologic flora of periodontal disease is yet to be fully unravelled, these findings give credence to those who propose that the mycobiome is a key player in the initiation and progression of periodontal infections.

Mycobiome of Endodontic infections

Although there are many studies on the bacteriome of endodontic infections (Hong et al., 2013; Tawfik, Azab, Ahmed, & Fayyad, 2018), there are scant data on its mycobiome evaluated by newer techniques uncovering the uncultivable flora. In a relatively recent study, Gomes et al., (Gomes, Fidel, Fidel, & de Moura Sarquis, 2010), isolated filamentous fungi from over one fourth of the root canals of teeth with pulp necrosis and identified *Aspergillus spp.* (*ustus, granulosus, niger, sydowii*) *Emericella quadriluniata* (sexual form of *Aspergillus*), *Penicillium* species (*implicatum, micsynvisk, lividum* and *citrionigrum*), *Fusarium* (*moniliforme* and *melanochorum*), *Aureobasidium pullulans, Exophiala jeanselmei, Eurotium amstelodame* and *Cladosporium sphaerospermum*. More recently, Persoon et al., noted (using sequenced data of ITS1 or ITS2 regions of ribosomal RNA) that *Candida* and *Malassezia* as the most frequently isolated fungi from endodontic samples collected from six infected canals (Persoon, Buijs, et al., 2017). Despite the small number of samples tested

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and the low diversity of the fungi isolated, the commonest isolates were *Candida, Cladosporium, Epicoccum, Malassezia,* and *Saccharomyces* species. These findings are akin to those of the salivary mycobiome mentioned above (Dupuy et al., 2014; Ghannoum et al., 2010) thus questioning the veracity and the integrity of their sample collection method.

In a recent systematic review of 54 select studies on fungi in endodontic infections Persoon et al., (2017) concluded an overall 7.5%prevalence of fungi in root canal infections predominated by *Candida* species (Persoon, Crielaard, & Ozok, 2017). The vast majority of these were culture-based investigations. Taken together, the foregoing points to the fact that a rich mycobiota may exist and cause, endodontic infections and a comprehensive picture of the infected root canal mycobiome awaits discovery through the use of newer technology.

Oral mycobiome in compromised patient populations

There are two studies evaluating the mycobiota in compromised patients using newer technologies, particularly in HIV disease and in leukemic patients.

Despite the wide use of ART (antiretroviral therapy), the incidence of oral candidiasis in HIV infected individuals is a relatively common finding (Patel et al., 2012; Thompson et al., 2010). Oral candidiasis is considered as a harbinger of worsening CD4+ cell counts and transition of HIV infected status to AIDS (Samaranayake, 2018). Using a pyrosequencing approach, Mukharjee et al., compared the oral mycobiomes of HIV-infected individuals with a healthy cohort (Mukherjee et al., 2014). Unlike the bacteriome of the participants, the oral mycobiome exhibited significant differences in composition between the two groups. HIV infected patients carried higher numbers of *Candida* (92%; most abundant), *Epicoccum,* and *Alternaria* while healthy individuals had an abundance of *Candida* (58%), *Pichia,* and *Fusarium*. While *Candida* and *Penicillium* were common in both groups, *Candida* occurred in higher frequencies in HIV patients. Interestingly, *Pichia* decreased in HIV infected individuals with a concomitant rise in *Candida* suggesting a potential antagonistic relationship between these fungi (Mukherjee et al., 2014). In contrast, Aas et al., noted only two fungal species, *C. albicans* and *S. cerevisiae,* in subgingival plaque in HIV-infected individuals (Aas et al., 2007). Smaller sample size and the differences in microbial isolation, detection and sequencing techniques may have led to these disparate results.

There is a single study evaluating the interactions of a compromised host, genetic polymorphisms, and the mycobiome. Shelburne *et al.,* in 2015 isolated *Malassezia velutinosus* DNA in oral samples form a leukemic patient with invasive mucormycosis and concluded that the host-microbiome-mycobiome triad is important in understanding the pathogenesis of disease complexes in compromised patients and their clinical outcomes (Shelburne et al., 2015). It is noteworthy here that host-mycobiome interactions either in the oral cavity or other regions of the human body are yet to be explored, and the current data are tentative and sparse at best.

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Oral mycobiome in oral candidiasis

In a very early seminal study Samaranayake et al., (1987) have shown, using differential media such a Pagano-Levin agar, which was the precursor of the commercially available Chrome agar, the phenomenon of multi-species oral carriage of *Candida* in a population of patients presenting in a British dental hospital. They noted that some 15% of the oral *Candida* infections in their cohort were polymicrobial in nature, and caused by mixed *Candida* species (Samaranayake, MacFarlane, & Williamson, 1987). Subsequent studies have confirmed these findings (Samaranayake, 2009). The latter study was purely based on laboratory culture and species identification through phenotypic analyses.

Imabayashi et al., (2016), using next generation sequencing of the ITS1 region, have examined the mycobiome in patients with candidal infections to explore the cohabiting fungal species They noted a relatively lower abundance of fungal species in patients with pseudomembranous oral candidiasis compared with healthy controls (Imabayashi et al., 2016). There were 45 common fungal species between the test and the control subjects. However, only 22 additional fungal species were isolated exclusively from patients with pseudomembranous candidiasis compared to 41 additional fungal species identified exclusively from the healthy individuals. Significantly higher numbers of *C. dubliniensis*, *C. parapsilosis*, *Wallumia sebi*, *Rhodosporidium babjevae*, *Candida krusei*, *Antrodiella micra*, *Cladosporium sphaerospermum*, and *Sporidiobolales* species were noted in those with pseudomembranous candidiasis whereas healthy mouths were significantly colonized by *Exophiala equina*, *Cladosporium halotolerans*, and *Agaricomycetes* species (Imabayashi et al., 2016). *Candida* species were common to both the healthy and the candidiasis group although C*. dubliniensis* were more abundant with the latter group. The authors opined that there were no marked differences in the mycobiomes of the healthy and the candidiasis groups, and the pathogenesis of the disease is likely to be an outcome of the interplay of microbial strain shifts, pathogenic potential, and host immunity thus echoing the above sentiments of Shelburne et al., (Shelburne et al., 2015).

Oral mycobiome and oral carcinoma

Although oral cancer is a multifactorial disease, there are some reports indicating that *Candida* infected oral lesions have a higher propensity for malignant transformation (Gholizadeh et al., 2016; Sankari, Gayathri, Balachander, & Malathi, 2015). The conditions that have been investigated include oral squamous cell carcinoma (OSCC), lichen planus and fibro epithelial polyps.

Recent NGS data tend to confirm this hypothesis as Perera et al., have noted a dysbiotic mycobiome with over representation by *Candida, Hannaella, and Gibberella* genera in OSCC. *Alternaria* and *Trametes* were more abundant in fibro epithelial polyps. Species-wise, *C. albicans, Candida etchellsii,* and a *Hannaella luteola*–like species were enriched in OSCC,

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while a *Hanseniaspora uvarum*–like species, *Malassezia restricta, and Aspergillus tamarii* were the most significantly abundant in fibro epithelial polyps (Perera et al., 2017). Mechanisms that entail carcinogenesis in these conditions may include the production of carcinogenic compounds by *Candida,* such as nitrosamines and/or acetaldehyde (Gainza-Cirauqui et al., 2013; Meurman & Uittamo, 2008).

Conclusion and future directions

On a cellular basis, approximately 0.1% of the microbes in the adult intestine are fungi, representing approximately 60 unique species (Hoffmann et al., 2013; Rajilic-Stojanovic & de Vos, 2014). Although equivalent data are not available for the oral mycobiome it is clear that the oral cavity offers a comfortable residence to a fair proportion of fungi. As fungi are difficult to grow in ordinary laboratory culture media, they have eluded detection and the predominant species belonging to the genus *Candida* have stolen the spotlight for decades, thus leaving the uncultivable fungi a hidden entity up until now.

The arrival of NGS platforms has elucidated the hidden world of the oral mycobiome for the very first time, and a reasonable number of research articles are now available that is incrementally demystifying the oral mycobiome, as discussed above. Yet NGS technology platforms as well as the analysis of `big data` spewing out from such research are extremely cost intensive thus limiting the sample size in most evaluations to date. However, the recent advent of microfluidics technology, is a ray of hope. The latter technology (such as Fluidygm multiplex PCR) in combination with NGS permit the analysis of up to 40 samples in a single step with major efficiency and cost savings.

There are a number of immediate and pressing questions that need addressed in the field of oral mycobiome. Broad surveys of the oral mycobiome have thus far captured snapshots of the dynamic fungal community but their chronologic evolution from infancy to adulthood through longitudinal studies are warranted. Longitudinal tracking of the early-life oral mycobiota using mother–infant pairs while monitoring health outcomes, such as allergies, and acute fungal infections such as pseudomembranous candidiasis seen in infancy should be of immense clinical interest.

The inter-kingdom exploits of fungi-bacteria in the mouth is an area awaiting further studies, not only to better characterize their structural and functional relationships, but also their combined effects on disease initiation and progression. Other questions include the impact of the fungal community on the oral immune system, and whether they are accessory pathogens in oral diseases such as various periodontal diseases or indeed caries (Pereira, Seneviratne, Koga-Ito, & Samaranayake, 2018). Finally, research is necessary to address the methodological challenges in generating fungal microbiome data, so as to generate a comprehensive and a reliable reference database including sampling of the low fungal biomass extant in the oral ecosystem.

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