Candida and Other Fungal Species: Forgotten Players of Healthy Oral Microbiota

B.P. Krom, S. Kidwai and J.M. ten Cate

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CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

B.P. Krom[1]*#, S. Kidwai[1#], and J.M. ten Cate[1]

[1]Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Free University Amsterdam, Amsterdam, The Netherlands; #authors contributing equally to this work; *corresponding author, b.krom@acta.nl

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ABSTRACT

In the last half-decade or so, interest in the bacterial part of the human microbiome and its role in maintaining health have received considerable attention. Since 2009, over 300 publications have appeared describing the oral bacterial microbiome. Strikingly, fungi in the oral cavity have been studied exclusively in relation to pathologies. However, little to nothing is known about a role of fungi in establishing and maintaining a healthy oral ecology. In a healthy ecology, balance is maintained by the combined positive and negative influences between and among its members. Interactions between fungi and bacteria occur primarily at a physical and chemical level. Physical interactions are represented by (co-)adhesion and repulsion (exclusion), while chemical interactions include metabolic dependencies, quorum-sensing, and the production of antimicrobial agents. Information obtained from oral model systems and also from studies on the role of fungi in gastro-intestinal ecology indicates that fungi influence bacterial behavior through these different interactions. This review describes our current knowledge of the interactions between fungi and bacteria and aims to illustrate that further research is required to establish the role of fungi in maintaining a healthy oral cavity.

KEY WORDS: bacterium-fungus interactions, resilience, balance, healthy oral ecosystem, bacteriome, mycobiome.

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INTRODUCTION

An ecosystem is a community of various organisms within an environment that function as a unit. Within this community, each member has a role that contributes to an optimal efficiency of the ecosystem, albeit to different extents. Key players are members that significantly affect other members and, even when present in low abundance, have a significant impact on the community and its environment (Smee, 2012). Dominant species are not always key players, since abundance does not necessarily correlate with importance within the community. Elimination or inhibition of one or more key players might lead to malfunctioning or failure of the complete ecosystem. Hence, key players have a crucial role in maintaining the structure and behavior of an ecological community (see Appendix). In a healthy ecosystem, balance is maintained by the total of all interactions between its members and their environment. These members might have a positive or negative influence on the presence, numbers, and behavior of other species through chemical and/or physical interactions. The ways in which species interact with each other may be very complex. This explains why most of our current knowledge on these interactions is derived from simple model systems using 2 or at most a few microbial species.

The oral cavity hosts a complex ecosystem because it harbors several significantly different niches and because the oral cavity changes with time. Prior to birth, the oral cavity is sterile and composed only of soft mucosal surfaces, kept moist by salivary secretions. Upon passing through the birth canal, the oral cavity becomes colonized with microbes derived from the mother. *Streptococcus salivarius* is dominant at this stage and may be found in multiple niches (tongue, cheek, palate) until tooth eruption (Cephas *et al.*, 2011). The eruption of teeth is an ecological earthquake, since it introduces non-shedding hard surfaces. Introduction of these new surfaces allows other microbial species to become part of the oral ecology, in particular, but not exclusively, *S. mutans* and *S. sanguis* (Könönen *et al.*, 2002; Crielaard *et al.*, 2011). Other streptococcal species adhere strongly to, and colonize, the mucosa (Rudney *et al*., 2005) of the tongue, gums, and cheeks but not the teeth. The development of gingival crevices allows anaerobic micro-organisms to become part of the healthy oral ecology. Around puberty, *Bacteroidetes* and *Spirochetes* colonize (Keijser *et al*., 2008; Lazarevic *et al*., 2009). Later in life, loss of teeth and their replacement by dentures or implants create new 'hotspots' for microbial accumulation (Jenkinson and Lamont, 2005). Behavioral changes such as smoking, gain and (later in life) loss of manual dexterity, and hormonal changes cause the normal oral ecology to be a dynamic entity throughout lifetime. In addition to lifetime changes, shorter host-related perturbations influence the dynamics of the oral ecology. These

Table. Relative Frequency of Fungal Species in the Oral Cavity of 20 Healthy Volunteers

Species	Relative Frequency (in 20 volunteers), %
Candida	75
Cladosporium	65
Aureobasidium	50
Saccharomycetales	50
Aspergillus	35
Fusarium	30
Cryptococcus	20

Identification was based on sequencing of ITS regions in oral samples. Data obtained from Ghannoum *et al.* (2010).

may also dramatically shift the ecosystem toward an unbalanced state for a short period of time (Cephas *et al.*, 2011).

Many studies have now characterized the bacterial component in the oral cavity, both in disease and in health (Aas *et al.*, 2005; Ruby and Goldner, 2007; Filoche *et al.*, 2010; Zarco *et al.*, 2012; Weerasekera *et al.*, 2013). These 'microbiome' studies are incomplete, since they study bacteria exclusively and therefore should more accurately be referred to as 'bacteriome' studies.

In recent reviews (Jenkinson, 2011; Naglik *et al.*, 2013; Wright *et al.*, 2013), oral colonization by fungi is typically presented as a cause of disease, since their presence is generally assumed to be associated with pathologies. A possible alternative role for fungi, notably in healthy oral ecology, is disregarded. An exception to this is the landmark study by Ghannoum and co-workers (Ghannoum *et al.*, 2010) in which the presence of up to 100 fungal species (mycobiome) in healthy individuals was reported (Table). Since fungi are ubiquitously present in the oral cavity, they potentially interact with numerous oral bacterial species. A healthy oral ecology is held together by the sum of positive and negative influences between and among all microbial species. Therefore, to fully understand how a healthy oral ecology is established and maintained, we need to study interactions between fungi and bacteria. In this review, the current knowledge on fungus-bacterium interactions is described, with an emphasis on their potential contributions to a healthy oral ecology.

PHYSICAL, CHEMICAL, AND METABOLIC INTERACTIONS

Health in the oral cavity depends on the mucosal surfaces that function as a barrier to prevent the invasion of microbes. The oral ecology, with both bacteria and fungi, contributes to minimalizing growth and colonization by pathogens. Balance among all players is maintained through the interplay of physical, chemical, and metabolic interactions. The importance of these interactions in oral pathologies has been thoroughly reviewed in several recent publications (Kolenbrander *et al.*, 2010; Jenkinson, 2011; Wright *et al.*, 2013) and will therefore be discussed only briefly here. The current review focuses on the role of these interactions with respect to oral health.

Physical Interactions

Attachment of bacteria to immobilized bacteria is termed 'co-adhesion', and binding of bacteria in suspension is termed 'co-aggregation'. Adhesion is defined as attachment of microbes to a surface, *e.g.*, a tooth or mucosal surface. During early plaque formation, specific groups of bacteria accumulate on the hard surfaces at different stages (Kolenbrander, 2011). 'Early' colonizers, such as streptococci and Gram-positive rods, adhere to the pellicle-covered tooth surface through physico-chemical interactions. Co-adhesion and growth result in microcolonies (Rickard *et al.*, 2003). Next, 'late' colonizers adhere to form a mature dental plaque. *Fusobacterium nucleatum* is proposed as a "bridging" bacterium, since it co-aggregates with both early- and late-colonizers. This ordered series of events explains why, in fully established dental plaque, specific bacterial species are typically found clustered together (Kolenbrander *et al.*, 2010). Detailed analysis of supragingival plaque illustrates this grouping of particular species (Fig. 1). Physical interaction, adhesion, and co-adhesion are thus crucial processes in determining the bacterial diversity of oral microbiota.

Physical Interactions between Fungi and Bacteria

Candida spp. co-aggregate with bacteria, since they are commonly seen in so-called 'corncob' structures on teeth *in vivo* (Zijnge *et al.*, 2010). *In vitro, Candida* spp. physically interact with many oral bacteria, such as *S. mutans* (Pereira-Cenci *et al.*, 2008; Jarosz *et al.*, 2009) and *S. gordonii* (Silverman *et al.*, 2010). It can be assumed that many oral bacterial species have the ability to adhere to fungi. Fungi could act as bridging organisms similar to *F. nucleatum*. Importantly, while *S. mutans* do not readily colonize mucosal surfaces, *C. albicans* do [for review see Williams *et al.* (2013)]. Therefore, *C. albicans* might serve as a bridge between the mucosa and bacteria that normally do not adhere to mucosal surfaces. This mechanism protects these bacteria from being removed by salivary flow and swallowing. In addition, bacterial adhesion to *C. albicans* can render the bacterium less susceptible to antibiotic treatment, as shown for *Staphylococcus aureus* adhering to *C. albicans* (Harriott and Noverr, 2009). This adhesion is mediated through a cell-surface protein present exclusively on the hyphal cell wall, Als3p (Peters *et al.*, 2012) is also involved in the adhesion of *S. gordonii* to *C. albicans* (Nobbs *et al.*, 2010). Similarly, oral bacteria adhering to *Candida* might be protected against antimicrobials delivered by dentifrices. Taken together, the numerous physical interactions found between bacteria and fungi and the effects of these interactions on bacterial behavior will affect oral ecology, in not only diseased but also healthy conditions.

Chemical Interaction

Bacteria communicate through diffusible signals in a process termed 'quorum-sensing' (QS). QS is represented by the production, secretion, and sensing of secreted small molecules termed 'QS molecules'. These molecules can be specific, *i.e.*, Gram-negative bacteria can produce acylated homoserine lactones (HSL), while Gram-positive bacteria produce species-specific

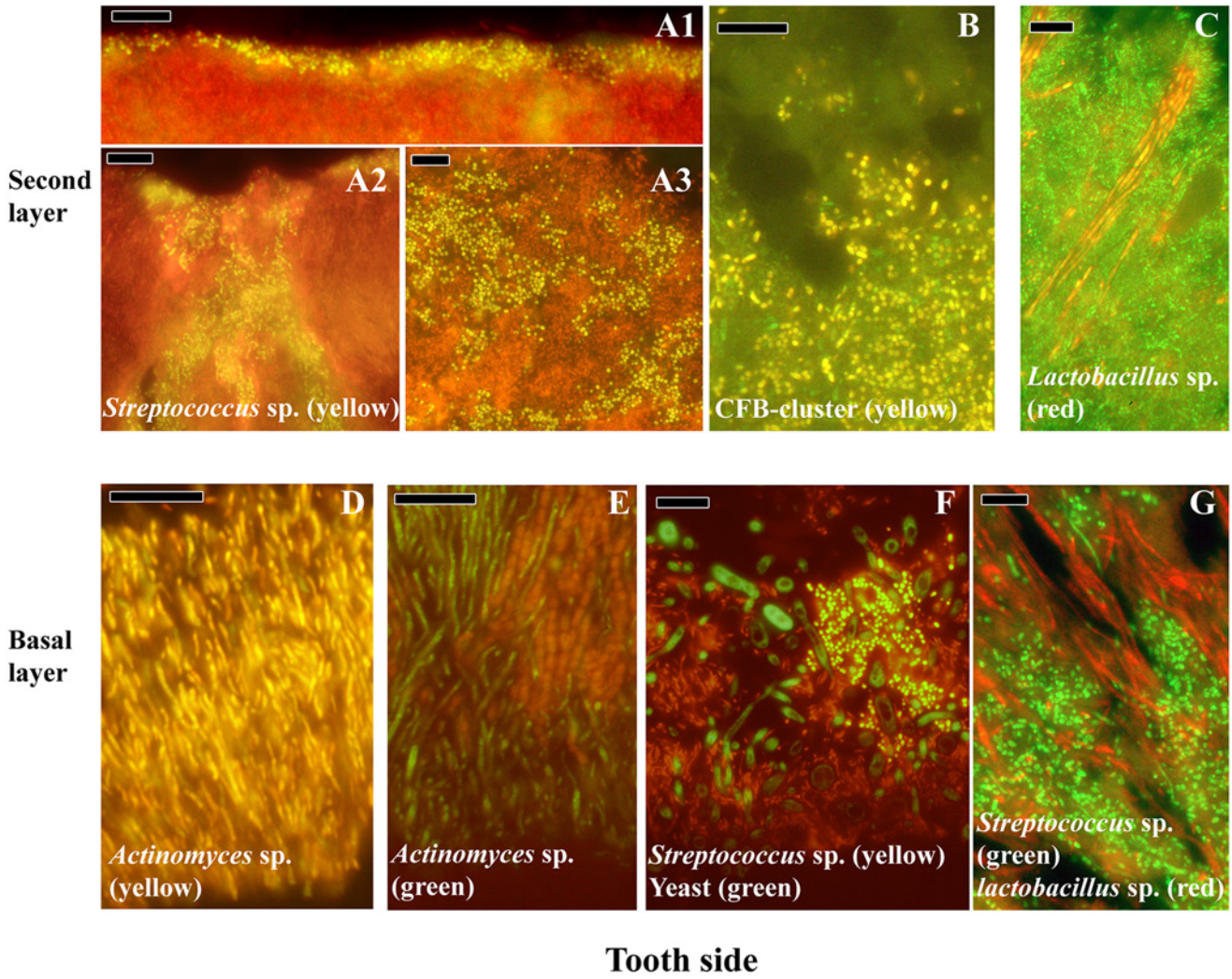

Figure 1. Localization of the most abundant species in supragingival biofilms. *Streptococcus* sp. (yellow) form a thin band on top of the biofilm **(A1)**, almost engulfing the biofilm **(A2)** or present as small cells scattered through the top layer of the biofilm **(A3).** (**B**) Cells from the CFB cluster (*Cytophaga-Flavobacterium-Bacteroides* cluster) of bacteria in the top layer of the biofilm, without defined structure. (**C**) *Lactobacillus* sp. (red) forming long strings through the top layer. (**D**) *Actinomyces* sp. (yellow) plaque attached to the tooth. (**E**) *Actinomyces* sp. (green) and cocci forming initial plaque. (**F**) Multispecies initial plaque composed of *Streptococcus* sp. (yellow), yeast cells (green), and unidentified bacteria (red). (**G**) *Streptococcus* sp. (green) and *Lactobacillus* sp. (red) forming initial plaque. Black holes might be channels through the biofilm. Panels A, B, C, E, and F are double-stained with probe EUB338 labeled with FITC or Cy3. Bars are 10 μm. Reproduced with permission from Zijnge *et al.* (2010).

small signaling peptides. Alternatively, QS molecules can be more generic, such as autoinducer-2 (AI-2), synthesized by the *LuxS* gene family. Regardless of the type of system, these QS molecules play an important role in interspecies signaling because of their extracellular nature (Jarosz *et al.*, 2011). AI-2 signaling affects biofilm formation and microbial composition within the oral cavity [reviewed in Wright *et al.* (2013)]. Recently, it was shown that AI-2 inhibitors reduced adhesion of the bridging organism *F. nucleatum* to late-colonizers such as the periodontal pathogens *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella forsythia* (Jang *et al.*, 2013). Inhibition of QS, especially interspecies communication, seems a promising new avenue for the control of bacterial proliferation and diversity of the oral cavity.

Chemical Interactions between Bacteria and Fungi

Pseudomonas aeruginosa, a Gram-negative pathogen, applies multiple QS systems, each with specific QS molecules to coordinate biofilm development, motility, cell aggregation, and exopolysaccharide production. Once a critical concentration of QS molecules is reached, these bacteria are able to damage the host while they protect themselves from the host immune system by forming a biofilm. Because of the extracellular nature of these QS molecules, neighboring species can respond to the increasing presence of *P. aeruginosa* (Jarosz *et al.*, 2011), which can adhere to and kill *C. albicans* hyphae (Hogan and Kolter, 2002; Brand *et al.*, 2008; Ovchinnikova *et al.*, 2012) through chitin-binding proteins. To protect itself, *C. albicans* reverts

from its hyphal morphology to a yeast morphology (Hogan *et al*., 2004), to which *P. aeruginosa* does not adhere (Ovchinnikova *et al.*, 2013). *C. albicans* secretes the QS molecule farnesol (Hornby *et al.*, 2001) which, in turn, inhibits *Pseudomonas* quinolone signal (PQS) production (Cugini *et al.*, 2007, 2010), needed for the expression of several virulence factors (Bjarnsholt *et al*., 2010).

Such interkingdom signaling is also seen between *C. albicans* and *S. mutans. S. mutans* secretes competence-stimulating peptide (CSP), which is involved in the competence of *S. mutans* and other streptococci. CSP secretion during early stages of growth is able to inhibit the yeast-to-hyphae switch in *C. albicans* (Jarosz *et al.*, 2009). Reduced hyphal growth has been observed in mixed-species biofilms on hydroxyapatite (Pereira-Cenci *et al*., 2008).

The cross-species communication signal AI-2, secreted by many oral bacteria that carry the *LuxS* gene, such as *Aggregatibacter actinomycetemcomitans, P. gingivalis*, and *Streptococcus* spp., is also involved in interactions within mixed *C. albicans* biofilms. For instance, interaction of *S. gordonii* with *C. albicans* enhances hyphal formation and promotes biofilm development; this effect is reduced in the *luxS* mutant (Bamford *et al.*, 2009). *S. gordonii* secretes diffusible signals partly mediated by *LuxS*. The effects of these signals might be enhanced by contact signals generated through attachment of *S. gordonii* to *C. albicans* hyphae. The potential of AI-2 and certain metabolic byproducts [*e.g.*, H_2O_2 and mutanobactin (Joyner *et al*., 2010)] to act as signaling mechanisms between *Streptococcus* and *C. albicans* might be stimulated by the close proximity (Fig. 1) of different species within a biofilm (Kolenbrander *et al.*, 2010; Zijnge *et al.*, 2010). In summary, because of the extracellular and ubiquitous nature of QS molecules, of both bacterial and fungal origin, it is clear that chemical interactions occur in the oral ecology and are involved in maintaining a healthy balance.

Metabolic Interactions

Metabolic interaction is used as an umbrella term for a variety of direct and indirect dependencies that are relatively well-studied in oral bacterial ecology. Direct metabolic dependency is often mediated by carbon source and driven by production of metabolites by one species, followed by consumption of that metabolite by another species. Recent microbiome analysis showed that *Veillonella* spp. levels were a good predictor of caries in children (Gross *et al.*, 2012). This reflects the metabolic dependency between acid-producers such as *S. mutans* and acid-consumers such as *Veillonella* spp.

Indirect metabolic dependency can be represented by removal of a toxic metabolite by one species, resulting in growth of another species. For instance, anaerobes in the oral biofilm are dependent on rapid oxygen consumption by aerobic bacteria, leading to reduced local oxygen levels that are critical for survival of anaerobic bacteria (Morales and Hogan, 2010). A similar situation pertains to bacteria that are able to increase external pH through metabolic action. This type of metabolism can counteract acid formation by acidogenic species, preventing the accumulation of toxic levels of acid. Bacteria that are sensitive to low pH are thus dependent for survival on the metabolism of these acid-neutralizing bacteria. In conclusion, both indirect and direct metabolic dependency among several oral bacterial species have been described.

Metabolic Interactions between Fungi and Bacteria

Compared with metabolic interactions between and among bacteria, the metabolic interactions between bacteria and fungi are understudied. *C. albicans* has the ability to grow under a variety of environmental conditions and on various carbon sources. Combined, this allows the fungus to survive in rapidly changing conditions. For instance, *C. albicans* can utilize sucrose and glucose as well as the non-fermentable lactate. Lactate is produced by *S. mutants*, and since *C. albicans* and *S. mutans* are commonly found together (Metwalli *et al.*, 2013), it is conceivable that *C. albicans* growth is stimulated by lactate produced by *S. mutans*, as described for *S. mutans* and *Veillonella* spp. The latter is supported by recent studies showing that both *Veillonella* spp. (Gross *et al.*, 2012) and *C. albicans* (Klinke *et al.*, 2011) were highly prevalent in caries patients with high *S. mutans* carriage. In addition, the close proximity in supragingival plaque between yeasts and cocci (Fig. 1F) points toward their beneficial co-existence (Zijnge *et al.*, 2010).

C. albicans can grow under both aerobic and anaerobic conditions. By utilizing the limited oxygen available in oral biofilms, they ensure favorable conditions for anaerobes. For instance, *C. albicans* promotes survival and colonization of *S. gordonii* by reducing oxygen tension to levels favorable for *S. gordonii. Vice versa*, H_2O_2 produced by *S. gordonii* influences morphogenesis and farnesol production of *C. albicans* (Bamford *et al.*, 2009). Glucosyl transferase (GtfB) produced and secreted by *S. mutans* binds to the hyphal surface of *C. albicans*, where it produces exopolysaccharides, and thus facilitates binding of *S. mutans* to that hyphal surface. *S. mutans* also readily adheres to yeast cell surfaces. Collectively, these conditions promote the development of mature fungal-bacterial biofilms and enable other microbial species to bind to the surrounding extracellular matrix (Morales and Hogan, 2010). The versatile nature of fungal metabolism allows for a plethora of metabolic interactions between bacteria and fungi, and, as such, it is conceivable that these interactions are involved in establishing and maintaining a healthy oral ecology.

DIVERSITY AND RESILIENCE OF ORAL MICROBIAL ECOSYSTEMS

Two aspects of ecological systems require special attention in any discussion of a healthy, balanced oral ecology: diversity and resilience. Diversity of microbiomes is defined by the Human Genome Project Consortium as the number, distribution, and richness of microbial species in an ecological niche. It demonstrates how much variety is found in a community (Human Microbiome Project, 2012). The oral ecology is generally diverse, since it is home to a large number of different microbial species: Over 500 distinct species (of which 75% are found in high proportions at healthy sites) reside in the ‘‘core oral microbiome’’ (Zaura *et al.*, 2009; Dewhirst *et al*., 2010). Diversity in the oral cavity is among the highest of all human microbial habitats (Huse *et al.*, 2012), probably because of 2 types of functional surfaces, *i.e.*, soft surfaces of the mucosa (tongue,

palate, and cheek) and hard surfaces (teeth). The various sites in the oral cavity, each with very different environmental conditions related to nutrient and oxygen availability, make a diverse ecological landscape (Simon-Soro *et al.*, 2013). Each surface and niche provide specific mechanical disruptions, nutrient sources, and oxygen availability, selecting for a variety of micro-organisms to grow, colonize, and survive (Marsh, 2012).

Microbial Diversity Affects Oral Health

It is generally accepted that a more diverse community is a more healthy community that can better respond to changes in the environment (see Appendix). A recent study reported that a higher level of microbial diversity was found in children with a healthy oral cavity in comparison with children suffering from severe tooth decay (Kanasi *et al.*, 2010). This finding suggests that species present in healthy oral ecological niches carry out specific functions which are needed to sustain microbial homeostasis and contribute in maintaining equilibrium in the ecology of the mouth (Zarco *et al.*, 2012). Conversely, microbial diversity is decreased in case of disease. For instance, the subgingival bacterial microbiome of smokers with periodontitis was significantly lower in taxonomic diversity than in non-smokers. This lower diversity corresponded with significantly higher attachment loss of the gingival tissue (Bizzarro *et al.*, 2013). Similarly, progression of caries in permanent dentition coincided with a loss of bacterial diversity (Gross *et al.*, 2010). At the moment, it is unclear whether disease is caused by loss of diversity or whether the specific niche in pathologic tissue represents a very selective environment resulting in a less diverse microbial ecology.

Fungi Influence Bacterial Diversity

Many fungi are members of the commensal microbiota and reside in various ecological niches in most healthy humans (Kim and Sudbery, 2011); they cause infections only if conditions so allow. For instance, *C. albicans* is commonly isolated from skin and mucosal surfaces of the mouth, vagina, and intestine. Little is known about the effect of fungal colonization on bacterial diversity *in vivo*. In a study of healthy Dutch elderly, Kraneveld and co-workers showed that bacteriomes with a high *Candida* load were less diverse (Kraneveld *et al*., 2012), and thus that a low-level *Candida* load corresponded with a more diverse (and thus healthier) oral microbiome. Based on 16S rDNA sequencing, they showed a shift in individuals with high *Candida* load toward a microbial composition dominance by saccharolytic and acidogenic bacteria – streptococci. Whether the change in bacterial composition was caused by increased *Candida* load or *vice versa* is currently unknown.

Other *in vivo* evidence showing an effect of fungi on microbial diversity was obtained in pioneering studies by Mason and colleagues (Mason *et al*., 2012a,b). In a murine model, they demonstrated that *C. albicans* interacts with the bacterial microbiota. This interaction not only affected microbiome composition but also, more importantly, accelerated the recovery of 10 of the 11 most dominant bacterial families after antibiotic treatment (Erb Downward *et al*., 2013). The presence of fungi in the gut influenced diversity of the microbiome and enhanced its resilience upon antibiotic treatment.

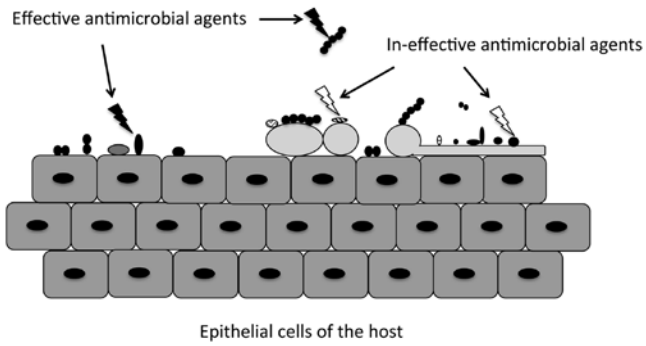

Figure 2. Physical interactions between fungi and bacteria influence the oral microbiota. First, *Candida albicans* and other oral fungi could function as bridging organisms. Certain oral bacteria preferentially adhere to *Candida*, and *Candida* adheres to epithelial cells. Consequently, different sets of bacteria adhere to *Candida*-covered epithelial cells *vs*. non-covered cells. Second, interspecies interactions influence bacterial resilience. Fungi-associated bacteria show decreased sensitivity (black *vs*. white lightning bolts) to antimicrobial agents (either antibiotics or host-derived antimicrobial peptides). In this way, fungi modulate diversity of the adherent oral microbiota.

In vitro studies on fungus-bacterium interactions relevant to the oral cavity have focused mainly on *C. albicans. C. albicans* interacts with bacteria through physical, chemical, and metabolic interactions (Jarosz *et al.*, 2011; Wright *et al.*, 2013). Many oral bacteria, such as *S. mutans* and *S. gordonii*, interact with *C. albicans*. The presence of *C. albicans*, and other fungi for that matter, could thus influence the oral bacteriome in a fashion similar to that described above for the gut microbiome (Fig. 2). *C. albicans* readily adheres to mucosal surfaces. Fungi might serve as a reservoir for bacterial recolonization of teeth after oral hygiene. For instance, *S. mutans* adheres to *C. albicans*, which could allow it to colonize mucosal surfaces such as buccal epithelial cells (Rudney *et al*., 2005), with *C. albicans* as the scaffold. Moreover, *C. albicans* has the ability to change the environmental conditions within a certain niche by increasing the pH, thus ensuring a more permissive environment for many bacterial species. Last, *C. albicans* produces secondary metabolites, such as farnesol, that are antimicrobial against certain bacteria. Farnesol was found to repress expression of virulence factors in *S. mutans* and, in combination with fluoride, to be a more effective anti-caries therapy than fluoride alone (Falsetta *et al*., 2012). Thus, fungi affect bacteria and are affected by bacteria through the production of metabolites, either as nutrients or secondary metabolites, involved in changing the environmental conditions.

Resilience

The balance of an ecosystem is frequently influenced by perturbations (disturbances in environmental or biological conditions). If such perturbations are prolonged, they may significantly affect an ecosystem, because the microbial shifts typically cause different processes and structures to dominate (Folke *et al.*, 2004). In contrast, short-term disturbances do not have a major impact on ecology because of a natural system referred to as ‘resilience’ (see Appendix). Briefly, resilience is the ability of an ecosystem to resist change (perturbation, damage, or disturbance) and the rate

of an equilibrium to reach a (new) balance following perturbation (Relman, 2012).

Resilience Affects Oral Health

In the oral cavity, resilience of the resident microbial ecology keeps the balance of a healthy mouth intact. It allows for an adequate response to various kinds of temporary disturbances related to, for instance, oral hygiene. Additionally, host-related changes such as hormone-dependent changes in salivary composition (Marsh, 2012) can also temporarily affect the ecological balance in the oral cavity. If resilience is lost, any environmental change will result in a loss of balance and, potentially, disease. For instance, Gram-positive cocci rapidly ferment certain carbohydrates to lactic acid, which results in an almost instantaneous drop in pH to values below 5.5 that compromise the growth of certain members of the ecosystem. In a resilient, hence balanced system, the pH is rapidly increased to physiological values by the action of, for instance, *Veillonella* spp. If this does not happen, acidogenic bacteria will gain control, and a cariogenic biofilm is formed. Resilience of an ecosystem is therefore related to its species diversity, since this provides a higher versatility for the community. Consequently, resilience provides the ability to respond to various types of environmental stresses in the most suitable manner and thereby retains or restores health.

Fungi Influence Resilience

Fungi potentially promote resilience by providing a protected environment for bacteria. For instance, when *S. aureus* adheres to *C. albicans*, it becomes resistant to treatment with antibiotics (Harriott and Noverr, 2009). As illustrated above, *C. albicans* changes the microbial composition of the murine gut post-antibiotic treatment. Thus, the presence of *C. albicans*, and possibly other fungi, could affect resilience of the bacterial part of the cecum ecology. In line with the above-mentioned antibiotic resistance induced by adhesion of bacteria to fungi, we envision that adhesion of oral bacteria to fungi will similarly affect the resilience of the oral ecology and thereby health.

DISCUSSION

Fungi, not only yeasts such as *Candida* spp., are members of the oral microbiota in healthy individuals and not just opportunistic pathogens of the elderly and immune-compromised. Several observations reported above support a hypothesis that fungi have a beneficial or favorable role in maintaining a healthy balance between microbes and the host. Strikingly, fungi have until now almost exclusively been investigated in relation to disease. In this review we have summarized the wealth of *in vitro* data that suggest a role for fungi in a healthy oral ecology, being an integral part of the ecosystem. The lack of direct evidence on the role of fungi in healthy oral ecology warrants support for future research. The oral microbial interactome (Jenkinson, 2011), the total of all interactions between all microbes in the oral cavity, is not complete without detailed information about bacterial-fungal interactions obtained from various systems modeling the oral cavity. This research effort requires close collaboration among multiple disciplines to effectively answer the important questions: How do fungi, not only yeasts like *Candida*, affect the oral bacterial community? How do fungi interact with the oral immune system, and what is the effect thereof on bacterial colonization?

We anticipate that, with the onset of advanced genomic analyses (*e.g.*, Next Generation Sequencing) and biochemical (metabolome and proteome analysis) and imaging (real-time microscopy) techniques and making use of reproducible, relevant oral model systems, the next 5 to 10 years will provide striking new insights into the role of fungi in the development and maintenance of a healthy oral ecosystem.

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