

Special Issue: From One to Many

Ecology of the Oral Microbiome: Beyond Bacteria

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Although great strides have been made in understanding the complex bacterial community inhabiting the human oral cavity, for a variety of (mainly technical) reasons the ecological contributions of oral fungi, viruses, phages, and the candidate phyla radiation (CPR) group of ultrasmall bacteria have remained understudied. Several recent reports have illustrated the diversity and importance of these organisms in the oral cavity, while TM7x and *Candida albicans* have served as crucial paradigms for CPR species and oral fungi, respectively. A comprehensive understanding of the oral microbiota and its influence on host health and disease will require a holistic view that emphasizes interactions among different residents within the oral community, as well as their interaction with the host.

Overlooked Contributors: Ultrasmall Bacteria, Fungi, and Phage Play a Significant Role in the Ecology of the Human Oral Microbiome

Since the initial discovery of bacteria from the oral cavity by Antonie van Leeuwenhoek in the 18th century [1], the human oral microbiota has become the model system for studying multispecies microbial communities [2–4]. These indigenous microbes must engage and coevolve with their neighbors and hosts, as well as adapt to diverse and rapidly fluctuating conditions. Despite this, the microbial composition is relatively stable [5] and also displays community-level functions such as colonization resistance [6]. These characteristics require a complex level of interspecies communication, which necessitates the full arsenal of current technologies in order to wholly appreciate. As a result, until recently the study of the oral microbiota has mainly focused on bacteria due to their relative high abundance, easy detection, and cultivability. It is now apparent that the reductionist approach of studying communities via their individual components, although useful, cannot adequately describe the complex relationships that exist. Major technological and analytical advances, particularly in **cultivation-independent detection methods** (see [Glossary](#)), have made it possible to investigate communities as a whole by using and combining systems-based approaches. These studies have revealed an exceedingly more diverse oral microbial world than what was anticipated based on culture-dependent investigation. We now know that the oral cavity not only harbors enormously diverse bacterial species, but also is home to a multitude of yet-to-be cultured ultrasmall bacteria belonging to the newly classified ‘**candidate phyla radiation**’ (CPR) group [7], as well as fungi and viruses. Compared to the traditional bacterial microbiome (**bacteriome**), knowledge regarding these ‘rare’ (fungi) and ‘tiny’ (CPR group bacteria and virus/phage) residents is lagging behind. This article briefly reviews the hurdles accounting for the lag in the study of these three oral microbial subgroups, and the techniques used to overcome these obstacles. Subsequently, the discussion covers the recently described interactions between the CPR, fungi, and viruses and their bacterial hosts and neighbors, as well as their impact on

Trends

Although the study of the CPR, fungal, and viral components of the human microbiome has lagged behind that of the bacterial component, recently developed techniques provide a more inclusive view of the ecology of the human microbiome.

The parasitic relationship between the CPR group of ultrasmall bacteria and their hosts represents a novel, while likely common, interspecies interaction within the oral microbiome.

Oral bacterial–fungal interactions highlight the importance of interkingdom interactions in the pathogenesis of polymicrobial diseases.

The prevalence and diversity of lysogenic and lytic oral phages modulate community structure and functionality of the oral microbiota.

A comprehensive understanding of the impact of the oral microbiota on host health and disease requires a holistic view of intra- and cross-kingdom interactions among members of the oral microbiome.

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the overall oral microbiome and health of the human host – an exciting and rapidly emerging new frontier in the study of the host-associated microbiome. The interspecies interactions between established bacterial species will not be discussed in this review, as there is already an extensive amount of literature covering this topic. The authors refer the interested reader to several recent reviews for useful summaries on these relationships [2–4,8].

From Reductionism to Holism: Systems-Level Understanding of the Human Oral Microbiome

Investigations into microbial communities have traditionally relied on the study of individually isolated organisms in order to make inferences about the entire community. While this approach has greatly advanced microbiology in the past 400 years, the reductionist method cannot be used to sufficiently explain the complexities of the community structure. Recognizing that the whole is more than the simple sum of its parts, modern microbiologists are learning to apply 'systems thinking' to take a holistic approach to investigations. Research has transitioned from culture-dependent studies of a single species to complex *in vitro* multispecies communities [9,10] as well as the culture-independent characterization of entire *in vivo* microbiota, and from analyzing individual gene expression to meta-omics analysis. Microbiology is experiencing a new movement that emphasizes interactions between different elements within a community (Figure 1, Key Figure).

One of the fundamental questions in the study of the microbiome is: what species are present, and in what relative abundance? To that end, sequencing of the bacterial 16S rRNA gene has yielded invaluable information on the bacterial components of the oral microbiome and has allowed for the creation of the Human Oral Microbiome Database (HOMD) [11]. With this resource, researchers have access to a curated database of oral microbial sequences, streamlining analysis of 16S sequencing data. However, extensive databases such as HOMD do not yet exist for microbes other than bacteria, which can hamper complete analysis and identification efforts. This knowledge gap is consequential; as discussed in the following sections, the fungal and viral components of the oral microbiome play significant roles in modulation of the oral community and its contribution to host disease.

Compared to the bacterial component of the microbiome, the fungal component, or **mycobiome**, as it has been termed, has remained relatively understudied. Several challenges have contributed to this lag: fungi are relatively rare (<0.1% of the microbiome, based on cfu), genetic material from fungi can be difficult to isolate, and many fungal species are uncultivable using current methods. Compounding these issues further is the fact that nomenclature of fungal species and annotation of fungal genomes is confusing, and the few existing databases are fraught with redundancies and errors [12–14]. Although there is now known to be a correlation between fungi and several diseases, including inflammatory bowel syndrome, Crohn's disease, chronic respiratory diseases, and hepatitis B, this association was not discovered until the concepts of **dysbiosis** and ecological diseases had been established [12,15,16]. These paradigm shifts in the study of microbiology, along with shotgun metagenomic sequencing and sequencing of the internal transcribed spacer (ITS) region of rRNA genes in fungi (analogous to 16S rRNA sequencing in bacteria), have finally enabled recent research to give the human mycobiome the attention that it deserves.

Although viruses and phages are the most abundant biological entities on earth [17], like the mycobiome, the viral component of the human microbiome (**virome**) has been understudied compared to the bacterial element. Viruses have been especially ignored in the context of their effects on bacteria and the human microbiome in health, as there is no overt indication of their existence in the absence of symptoms. As major technical advancements have brought the human virome to light, several critical and unanticipated observations have been described. It is

Glossary

Bacteriome: the bacterial component of the microbiome.

Candidate phyla radiation (CPR): a recently identified, but highly abundant (>15% of bacteria), group of **ultrasmall** (extremely small bacteria with sizes in the nanometer range, compared to classic bacterial size on the micron scale) bacteria with reduced genomes and unusual ribosomes.

Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system: a

prokaryotic adaptive immune system that provides resistance to foreign genetic elements such as those from plasmids and phage.

Culture-independent detection methods: methods of detection that do not require laboratory growth or isolation of the organism(s).

Dysbiosis: compositional shift in the population of a particular microbial community that promotes development of an inflammatory or disease state.

Keystone species: a species with an elevated capacity to influence a microbial community relative to its abundance.

Lysogenic: viral life cycle in which the viral nucleic acid is integrated into the host genome, and dissemination of viral DNA occurs through usual host reproduction.

Lytic: viral life cycle in which progeny virions are assembled inside of the infected host and eventually cause lysis of the host and subsequent spread of virus.

Mycobiome: the fungal component of the microbiome.

Obligate symbiont: organism that absolutely requires a host species to survive and replicate.

Phage therapy: the use of phage to treat pathogenic bacterial infections.

Prophage: a bacteriophage viral genome that has integrated into the host genome.

Red Queen Hypothesis: the concept that organisms must constantly adapt and evolve to survive competition with other organisms that are also constantly adapting and evolving.

Tropism: the range of host cells and/or growth conditions for an organism.

Virion: a single viral particle.

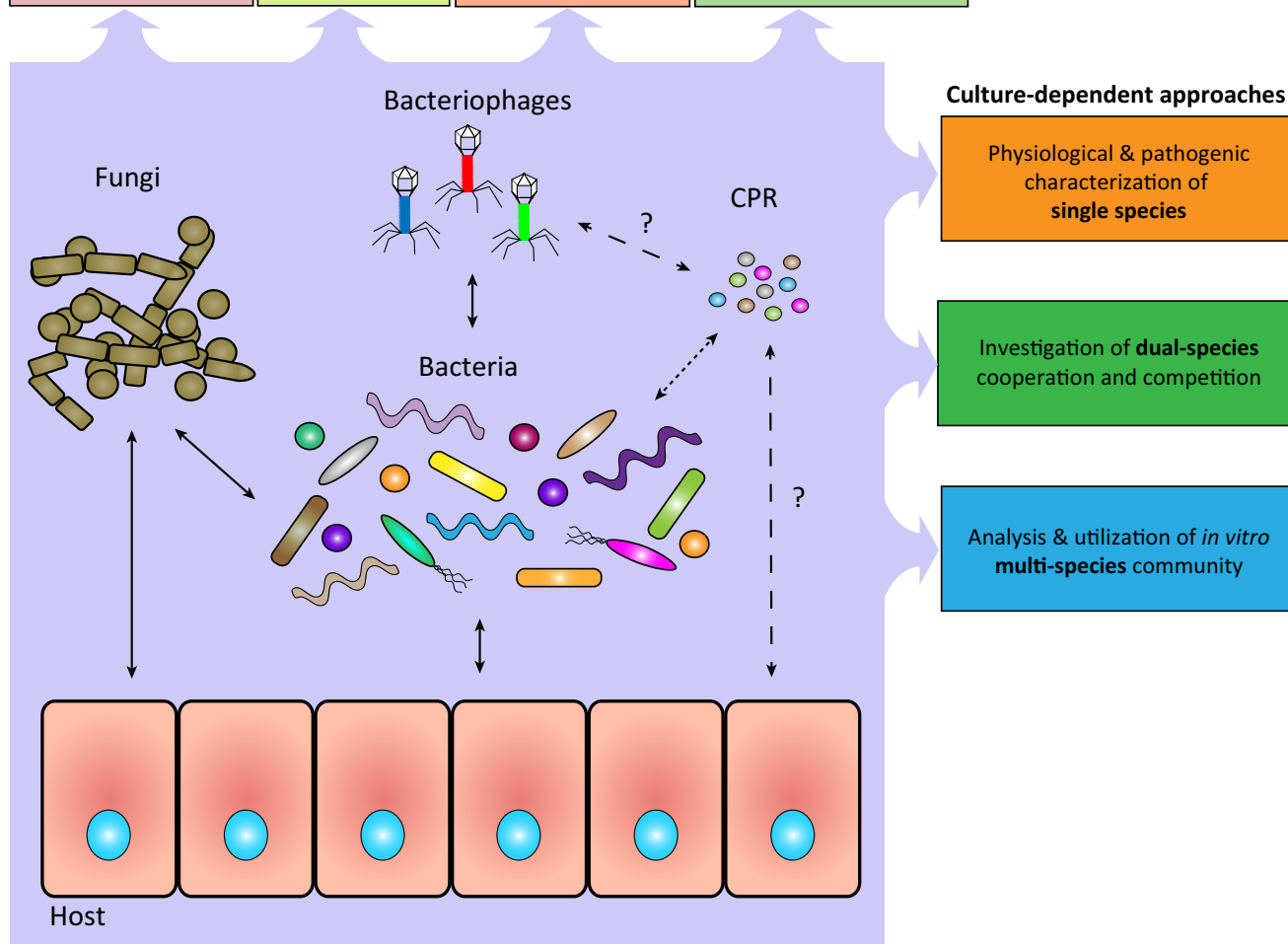
Virome: the viral component of the microbiome.

Key Figure

A Systemic Approach to the Investigation of Human Oral Microbiome

Culture-independent approaches

| Microbial profiling | Biogeography | Genetic potential | Functional genomics |
|------------------------------------|--------------|-------------------|---|
| 16S rRNA (bacteria) ITS (fungi) | CLASI-FISH | Metagenomics | Metatranscriptomics Metabolomics Proteomics |



Trends in Microbiology

Figure 1. A holistic understanding of the human microbiome requires a systemic approach to better study the interactions within and between different oral microbial groups (bacteria, candidate phyla radiation (CPR) organisms, fungi, and viruses), their impact on microbial physiology, community ecology, and host homeostasis. State-of-the-art, culture-independent methods, coupled with traditional culture-dependent approaches allow researchers to better identify the characteristics of the microbiome, such as the prevalence and biogeography of all species present, as well as the interactions and metabolic pathways critical to the community and the effects of the community on the host. The application of these approaches has revealed a highly complex oral microbial community with sophisticated and dynamic interspecies interactions, which shape and define the community structure as well as functions. Within the oral microbiome, bacterial–bacterial, bacterial–fungal and bacterial–phage interactions and their impact on the host have been extensively documented (solid arrows). Meanwhile, the improved cultivation approaches, together with crucial genetic information obtained using culture-independent methods resulted in the discovery of distinct bacterial–CPR interactions which could potentially prove to be common in host-associated microbiota (dotted arrows). Furthermore, culture-independent metagenomic analysis also suggested putative interspecies interactions, such as the presence of phage specifically targeting CPR members and direct interaction between CPR and the host (dashed arrows).

now known that healthy humans carry a large diversity of both eukaryotic viruses and prokaryotic phages [17–19]. Furthermore, human blood, once thought to be sterile in healthy individuals, was found to contain both virus and phage [17,20]. A recent review by Lecuit *et al.* metaphorically described the human virome as an iceberg, in which the visible tip was representative of the viruses causing symptoms (Ebola, HIV, influenza, etc.), which are relatively well-studied and small in number. Below the surface is the vast portion of the human virome that does not cause symptoms, is poorly studied, and the consequences of which are largely unknown [21]. Despite research and technological advances, there remain several challenges to studying the ‘bulk of the iceberg’. Although **virions** outnumber host cells roughly 10:1, viral nucleic acids are thought to make up less than 0.1% of the total nucleic acids and thus can easily be overlooked [22]. In addition, virions themselves, particularly those of unknown viruses, are difficult to purify [22]. Even after quality genome sequences are obtained, viral sequences may be completely novel and may encode proteins with no known homology, making analysis and annotation extremely difficult if not impossible [22]. Finally, identifying the host range and **tropism** of a novel virus can prove to be a significant challenge. Various techniques for overcoming these barriers are being developed and have been recently reviewed [23,24]. As momentum for the study of the human virome grows, it adds an additional layer of complexity to the microbiome.

In addition to investigating presence and abundance, community sequencing data is useful in identifying potentially interacting species through co-occurrence or co-exclusion data. By analyzing the oral mycobiome of HIV patients, Mukherjee *et al.* [25] were able to identify an antagonistic relationship between two fungal species based on their anticorrelation. This type of data can provide hypotheses for further physiological testing of potential interactions between organisms. By simultaneously sequencing the bacterial, fungal, and viral communities, novel interkingdom interactions can be identified [26].

To fully understand the relationships between the inhabitants of the oral microbial community, it is not enough to identify each organism; the complex spatial and structural organization must also be taken into account. Recently, Mark Welch *et al.* [27] combined sequencing data with spectral fluorescence imaging that allowed for the direct visualization of bacteria within dental plaque. By specifically labeling the most prevalent bacteria, the technique of CLASI-FISH (combinatorial labeling and spectral imaging-fluorescence *in situ* hybridization) revealed a highly organized structure as well as novel interbacterial interactions (Figure 1). Close proximity between organisms may suggest a metabolic or physiological relationship or dependency. This knowledge is important for understanding the microbial ecology as a whole and can aid in the discovery and cultivation of novel species.

Advanced sequencing techniques are capable of generating information on the functional activity of a microbial community. Metagenomics, which entails sequencing the entirety of the DNA from a given sample, can provide information not only on what organisms are present, but also on their functional potential through analysis of metabolic pathway genes and the use of protein-coding sequence databases [28,29]. However, DNA sequences alone do not provide any information on actual gene expression and microbial activity. Current studies in the oral cavity, and beyond, that combine metatranscriptomics and metagenomics have shown that, in many cases, the set of genes that are expressed is more important in predicting health versus disease than the species that are present [30,31].

Meta-metabolomics and -proteomics can complement sequencing data by providing higher-level functional information. Interacting organisms in a community commonly have intertwined metabolisms through cross-feeding or specialization of function. The most extreme examples of this include parasitic organisms such as TM7x, discussed in depth in the following section,

which fully rely on the metabolic pathways of the host organism [32]. Combining systems-level analyses that generate functional information with the knowledge of abundance and spatial location can provide powerful insight into the intricate microbial network of the oral microbiome, its relationship to the human host, and its potential to influence health and disease (Figure 1).

Interaction between Oral CPR Organisms and Their Bacterial Hosts

Unlike fungi and viruses, the CPR group of bacterial organisms was discovered only recently. This revelation has had an enormous impact on our view of the diversity of life on earth [7]. The previously unknown CPR group of organisms contains more than 35 phyla, and may comprise greater than 15% of the domain Bacteria! [7,33,34] CPR organisms have been found in diverse ecological niches, and share characteristics such as ultrasmall cell size, the presence of 16S rRNA gene self-splicing introns, archaeal-specific RuBisCO genes [35], and reduced genomes with the apparent absence of genes encoding a **CRISPR/Cas** bacteriophage defense-system [36]. Traits of this group also include the absence of several biosynthesis and metabolic pathways, including the electron transport chain, tricarboxylic acid cycle, amino acid and membrane biosynthesis pathways, and various ribosomal subunits [32,33,37–40]. Due to these shared properties, CPR organisms are predicted to be **obligate symbionts**. Direct evidence of their suspected symbiotic lifestyle, as well as knowledge regarding their physiology, their interaction with hosts, and their potential role in shaping the microbial community, is lacking due to their recalcitrance to *in vitro* cultivation.

TM7x: the First Cultivated CPR Species Uncovers an Obligate, Epiparasitic Interaction with an Actinomyces Host

The human microbiome project established that three CPR phyla – Gracilibacteria (GN02), Absconditabacteria (SR1), and Saccharibacteria (TM7) – are frequently detected in multiple human body sites, including the oral cavity [41,42]. Among the three human-associated CPR phyla, TM7 is the most well studied due to its association with multiple mucosal diseases such as vaginosis, inflammatory bowel disease, halitosis, and periodontitis [43–47]. TM7 is particularly prevalent in the oral cavity. Although commonly at an abundance of ~1% [48], an increase in abundance of TM7 to as high as 21% was detected in patients with various types of periodontitis [49,50]. The positive association between oral CPR members and oral diseases [51,52], highlights the capacity of ultrasmall bacteria to contribute to human disease, as well as modulate the human immune response [32].

A species of TM7, TM7x (strain TM7x HOT_952), was recently isolated from the human oral cavity together with its host bacterium, *Actinomyces odontolyticus actinosynbacter* (strain XH001) [32,53]. This is to date the only member of the entire CPR group that has been successfully cultivated and stably maintained *in vitro*. Initial studies indicate that TM7x is an obligate epiparasite that lives on the surface of its bacterial host, XH001. Like other CPR members, TM7x has an ultrasmall cell size (200–300 nm) and a reduced genome lacking essential pathways (~700 genes) [32,33]. Further studies also revealed that the epiparasitic relationship between the two species is dynamic, and can change depending on environmental conditions such as nutrient availability and oxygen concentration (Box 1). Interestingly, infection of macrophages with TM7x-associated XH001 led to a significant reduction in the production of cytokines, compared with cytokine production in macrophages infected with XH001 alone, suggesting that interactions between TM7x and XH001 are more complex than a simple metabolic dependency, and may modulate host response to oral microbiota [32,54].

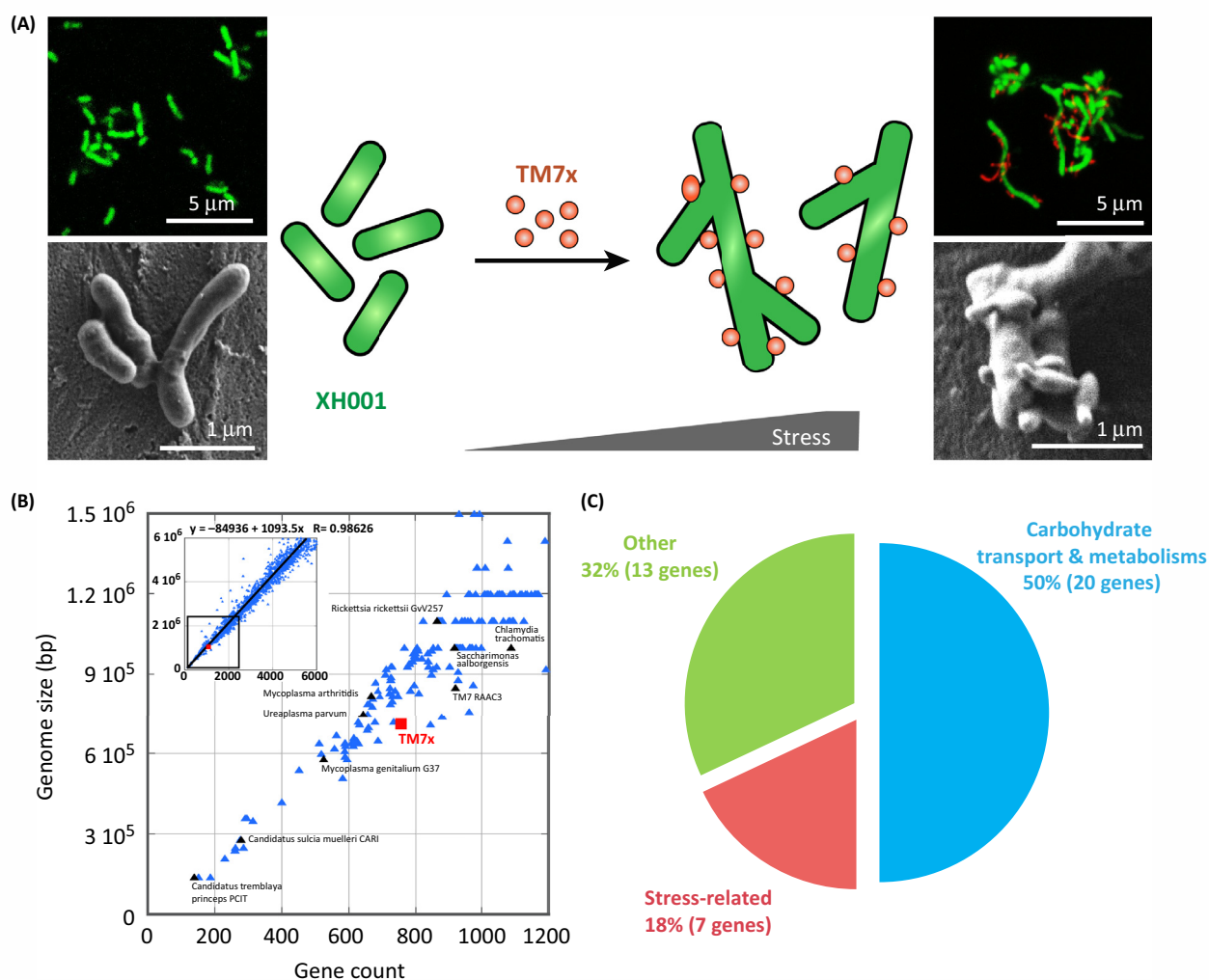
Potential Impact of CPR–Bacterial Host Interaction on Microbial Ecology

The obligate epiparasitic relationship between TM7x and XH001 represents a novel, while likely common, interspecies interaction in the oral microbiota, as suggested by the presence of a significant CPR population [55]. The distinct interspecies interactions between CPR organisms

Box 1. The Relationship between TM7x and *Actinomyces odontolyticus actinosynbacter* (XH001): A Prototype of the Parasitic Lifestyle of Candidate Phyla Radiation (CPR) Organisms

TM7x was recently isolated from the human oral cavity as an obligate epibiont that lives on the surface of its bacterial host, XH001, representing thus far the only *in vitro* cultivated and stably maintained member of the CPR group [32,89]. Isolation of pure TM7x is achieved by separating TM7x cells from host XH001 via the repeated passage of the co-culture through a 28-gauge needle, followed by filtering of the mixture through a 0.45- μm filter to collect TM7x. Isolated TM7x fails to grow, but maintains its ability to re-establish parasitic association with naïve XH001 [32,89]. During symbiotic growth under nutrient-replete conditions, TM7x mainly grows attached to the host surface as cocci with bud-like protrusions, while XH001 displays an elongated cell structure compared to the short rod-shaped naïve XH001. However, under starvation or other stress conditions, TM7x induces additional morphological changes in the host, such as clubbed ends and swollen cell body, and eventually results in host cell lysis. TM7x displays multiple cell morphotypes, ranging from cocci to short rods to filaments [89] (Figure 1A).

Whole-genome sequencing revealed that the TM7x genome has a high coding density and ranks among the smallest bacteria found to date in the human body, in both genome size (705 138 bp) and gene count (699 coding sequences) (Figure 1B). TM7x lacks genes necessary for *de novo* biosynthesis of any essential amino acid, while its host XH001 has complete biosynthesis pathways. Transcriptomics revealed a genome-wide response of XH001 when it is associated with TM7x, with about 340 XH001 genes differentially regulated. Among the most upregulated genes are those related to carbohydrate transport and metabolism, as well as general stress response (Figure 1C). Genomic and transcriptomic analysis in combination with the phenotypic data supports the hypothesis that TM7x employs a parasitic rather than mutualistic/commensal epibiotic relationship with XH001 [89]. This epiparasitic relationship is likely a life style adopted by many CPR members, which share the characteristics of small cell size and a reduced genome. Further genomic, transcriptomic, metabolomic, and pathogenesis investigations of TM7x/XH001 will lead to a better understanding of this intriguing relationship and shed light on the ecological and clinical impact of CPR organisms.



Trends in Microbiology

Figure 1. Interaction between the First Cultivated CPR Member, TM7x and Its Bacterial Host XH001. (A) Both fluorescence *in situ* hybridization and scanning electron microscopy revealed XH001 commonly has short-rod cell shape, while TM7x induces long and hyphal morphology in XH001 by inducing the stress-response genes. TM7x also displayed elongated and bud-like protrusions, in addition to cocci morphology [89]. (B) TM7x has a small genome size with reduced gene count consistent with the observed lifestyle as an obligate symbiont [89]. (C) Functional classes of the 40 most significantly upregulated genes in the transcriptomic studies in XH001 upon TM7x binding [89].

and their hosts may have a considerable impact on oral microbial ecology at various levels, ranging from direct reciprocal effects of the two species on physiology and pathogenicity, to indirect influence on the overall structure and function of the oral microbiota. It is becoming increasingly clear that the CPR group of organisms interact with the conventional microbiome, and play a critical, yet very poorly understood, role in the development of the human microbiota and disease [56,57]. As impetus for the study of ultrasmall bacteria increases, a more detailed comprehension of the relationship between TM7x and XH001 will provide a valuable prototype for understanding the unique lifestyle of the CPR group, and will shed long-awaited light on the biology of 'bacterial dark matter'.

Bacterial–Fungal Interactions in the Oral Cavity

Two landmark metagenomic studies using culture-independent identification methods showed that the healthy oral cavity contains a much more diverse array of fungal species than previously thought [58,59]. Ghannoum *et al.* reported that the healthy oral cavity was home to more than 75 genera of fungi, with *Candida*, *Cladosporium*, *Aureobasidium*, and *Aspergillus* the most abundant, found in 25–75% of subjects [58]. These findings were confirmed by the subsequent Depuy *et al.* study, which used further refined protocols to illustrate the diversity of the healthy oral mycobiome and significant variation in the fungal species present between individuals with good oral health [59]. The Depuy study also concluded that, in addition to the genera identified by the Ghannoum study, *Malassezia* spp. are a frequent member of the healthy oral mycobiome [59].

Despite being numerically underrepresented, the larger cell size of fungal species in the human microbiota indicates that they contribute a proportionally larger amount of the biomass. This greater cell size and ability to generate filamentous hyphae also places fungi in a position to create a structural 'skeleton' for fungal–bacterial multispecies biofilms. In addition, as eukaryotes, fungal species stimulate the host immune system in a distinct manner with disparate immunological outcomes compared to that of their bacterial neighbors [12]. Indeed, there is a growing body of evidence supporting the importance of immunological stimulation by fungi, in addition to bacteria, for development of a robust host immune system [12,15,60,61]. The fact that a small number of fungi can have a disproportionately large effect on the microbiota has led to fungal species being brought forward as potential 'keystone species' [62,63]. While this is indeed a tempting hypothesis, further ecological studies will be needed to confirm the status of fungal species as true keystones. This concept has most notably been proposed in the case of *Candida albicans*, which, due to its nearly ubiquitous nature and the ease of its cultivability, is an exception among the mycobiome in that it is well studied in the context of its interkingdom interactions and its importance at large to the microbiological community [62,63]. The known interactome of *C. albicans* and its relation to disease has been reviewed in depth [64], and several characterized relationships between *C. albicans* and oral bacteria are summarized in Box 2. The capability of interspecies interactions between *C. albicans* and *Streptococcus mutans* or *Streptococcus oralis* to exacerbate the severity of oral candidiasis or dental caries, respectively, as well as cooperation between *C. albicans* and *Fusobacterium nucleatum* to evade the host immune system, highlight the importance of interkingdom interactions in the pathogenesis of what are increasingly recognized as polymicrobial diseases. Going forward, the authors suggest that *C. albicans* and its partner bacteria will serve as a paradigm of fungal–bacterial interactions as new technologies improve the study of the role of the diverse group of fungal species that inhabit the human mouth.

The Human Oral Virome

In recent years, several studies have pioneered the application of next-generation sequencing techniques to elucidate the human oral virome in several contexts, including health, following long-term antibiotic use, and in association with periodontal disease [19,65–68]. The oral

virome of healthy individuals contains both eukaryotic viruses and bacteriophages. The bacteriophages are vastly greater in number, and thus will be the primary focus of this section of the review. The human oral virome is highly varied between individuals and surprisingly stable over time, even compared to the oral bacteriome [18,65,69]. Furthermore, sharing of living environment may have an important role in determining the ecology of the human oral virome, as members of the same household had remarkably similar viromes [66]. Herpesviridae, Papillomaviridae, and Anelloviridae are among the most common eukaryotic virus families present, and are asymptomatic in healthy individuals [68]. Several studies have examined the association of Herpesviruses, such as herpes simplex virus, human cytomegalovirus, and Epstein–Barr virus, with periodontal disease; however, a clear narrative regarding the influences of eukaryotic viruses on oral microbial diseases has yet to emerge [70].

Bacteriophages Modulate the Oral Microbiome through Multiple Mechanisms

The majority of the identified viruses in the oral cavity are bacteriophages, with Siphoviridae, Myoviridae, and Podoviridae as the most common phage families identified. Using homologous sequences to predict host range, Pride *et al.* identified putative phages of numerous genera covering all of the major bacterial phyla found in the oral cavity, including CPR members such as TM7 [18]. Intriguingly, the relative abundances of salivary phages and their respective putative bacterial hosts showed both direct and inverse relationships, indicating that mutualistic and antagonistic coevolutionary relationships between oral phage and their bacterial hosts exist [18].

As in many other microbial communities, phages are thought to play a significant role in shaping the oral microbiome [19]. The Siphoviridae are largely **lysogenic**, which, from a community standpoint, suggests an establishment of a dynamic equilibrium with associated host species, and also provides a huge opportunity for the transfer of genetic information. Horizontal gene

Box 2. Oral Fungal–Bacterial Interactions and Disease: *Candida albicans* As a Paradigm

A wide array of interactions involving oral bacteria and *C. albicans* have been described, and range from mutualistic to competitive, with various positive and negative impacts on host immune response and health. In addition to the three examples discussed briefly below, we refer the reader to the following reviews [64,90–93].

C. albicans* and *Streptococcus oralis

Although historically considered commensal organisms, the Mitis group of streptococci, including *S. oralis*, has been shown to cooperate with *C. albicans* to increase the severity of oral candidiasis [63,94,95]. *S. oralis* and *C. albicans* synergistically increase the level of μ -calpain, a proteolytic enzyme that degrades the E-cadherin from oral epithelial junctions [96]. This amplified structural breakdown enhances the ability of *C. albicans* to invade oral and esophageal mucosa during *S. oralis* coinfection [97] (FigureA).

C. albicans* and *Streptococcus mutans

C. albicans is frequently associated with the cariogenic organism *S. mutans*. Although there is some conflict in the literature as to the virulence of *C. albicans* in the context of dental caries [98,99], a recent report suggests that the differences observed may be due to differing growth media and conditions between the studies [100]. Regardless, several studies have confirmed that both *C. albicans* and *S. mutans* grow more robustly in a dual-species biofilm than either species alone, and that the presence of *C. albicans* induces changes in the expression of several *S. mutans* virulence factors [98,99,101]. *C. albicans* is thought to provide structural support to dental plaque by avidly binding the *S. mutans* exoenzyme GtfB, responsible for generation of the exopolysaccharide matrix, furnishing an agglutinative skeletal structure for biofilm growth [102]. The considerable increase in carious lesions observed in a *S. mutans*–*C. albicans* dual species rodent caries model, combined with frequent isolation of high numbers of both *C. albicans* and *S. mutans* from children with severe early childhood caries, suggests that, at least under certain conditions, *C. albicans* contributes significantly to the capacity of the dental plaque community to cause disease [98,103,104] (FigureB).

C. albicans* and *Fusobacterium nucleatum

Recent work showed that a direct physical interaction between *C. albicans* and *F. nucleatum* reduced both the growth of *C. albicans* as well as its capacity to produce hyphae, which is more associated with invasive candidal outgrowth than the yeast form [54]. While other species have been shown to inhibit the yeast–hyphae transition in *C. albicans*, this report was the first describing a contact-dependent and specific mechanism [54]. The attenuation of *C. albicans* hyphae caused

by this interaction actually increased the survival of *C. albicans* in the presence of macrophages, and reduced the production of proinflammatory cytokines by coinfecting macrophages. Therefore, the intriguing working hypothesis is that *C. albicans* and *F. nucleatum* work together to prevent their own outgrowth from drawing excessive attention from the host immune system, promoting a commensal rather than a dysbiotic and pathogenic way of life [54] (Figure 1C).

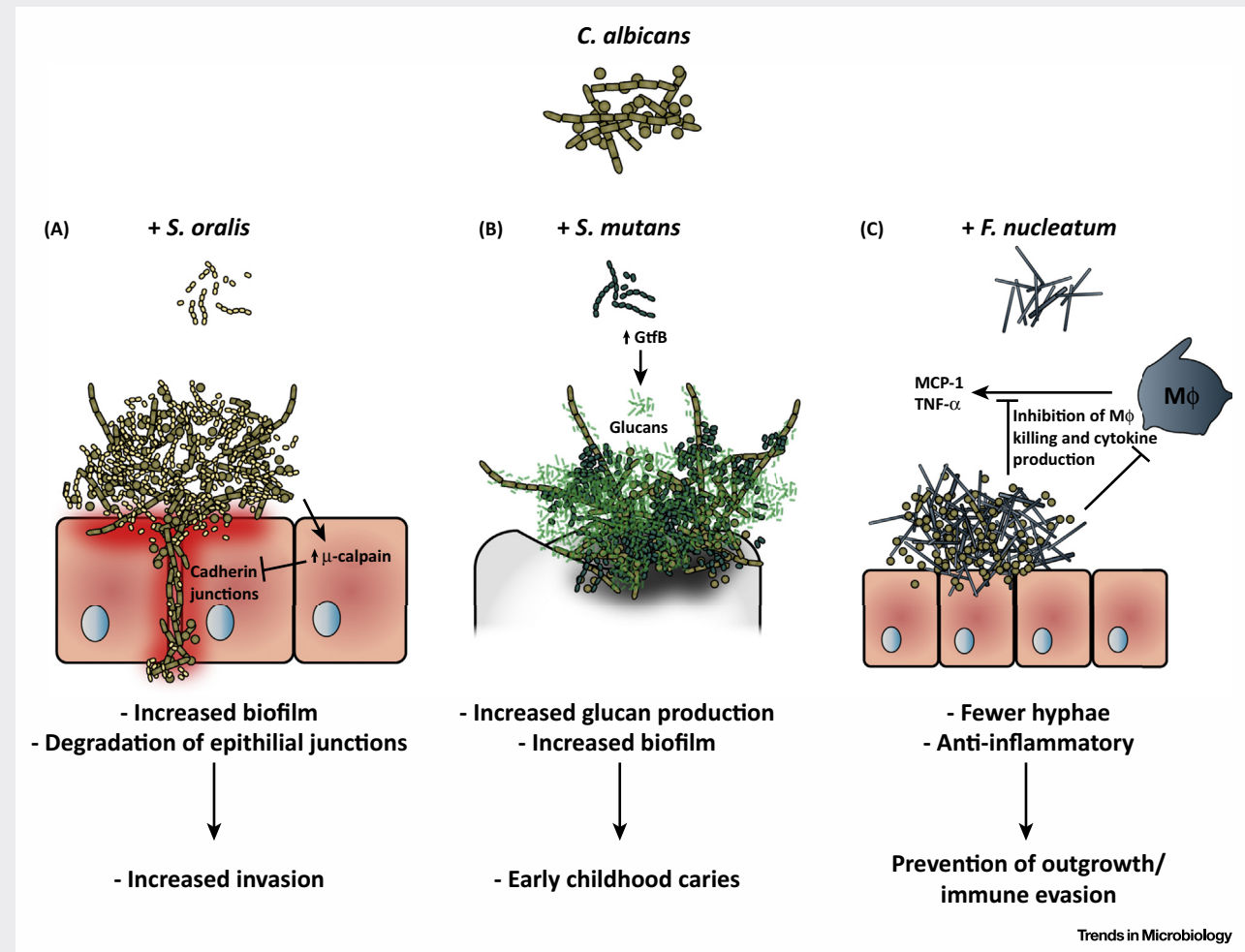


Figure 1. Three Examples of Interkingdom Interactions of *Candida albicans* and Their Effects on the Human Host. (A) Biofilms of *C. albicans* and *Streptococcus oralis* grow to a greater density than that of either species grown alone. In addition, the dual-species biofilm upregulated the host protease μ -calpain, which degrades cadherin junctions leading to increased tissue invasion by *C. albicans* and *S. oralis* [96,97]. (B) Biofilms containing both *C. albicans* and *Streptococcus mutans* grow to a greater density than that of either species grown alone and are associated with development of severe early childhood caries, due to a *C. albicans*-mediated upregulation of *S. mutans* virulence factors, including glucan production [98,103]. (C) When grown in coculture with *Fusobacterium nucleatum*, *C. albicans* is greatly inhibited in its ability to form hyphae. Further, the reduction in hyphae appears to circumvent killing and cytokine production by macrophage, indicating that the coculture may promote evasion of the host immune system [54].

transfer is thought to be particularly important in the oral cavity, as the massive diversity of organisms and large amount of extracellular DNA in biofilm matrices gives the resident species opportunity to acquire a vast array of genes [71]. Recent work has shown that phage-mediated mobile genetic elements are critical for the spread of antibiotic resistance in the oral cavity [72,73]. It is currently estimated that 60–70% of known bacterial genomes contain **prophages**, a number that is likely to increase as novel phages are sequenced. These prophages are not passive, but rather, they are active participants in shaping the bacterial community composition and biogeography, which in turn have an effect on the human host and its immune system [72,74–76].

The Myoviridae and Podoviridae are predominantly **lytic**, and rapidly eliminate their susceptible hosts. Phages are now thought to account for 20–80% of bacterial death, and therefore represent a profoundly significant bacterial limiting factor [76]. In a classic **Red Queen** dynamic, the genetic arms race invoked by coevolution of phage and host bacteria likely serves to prevent novel bacteria and phage from establishing in the oral community [75]. Several recent studies have explored CRISPRs transcribed in the oral cavity and their protective effects against invading phage [69,77,78]. On the other hand, recent investigations suggest that oral phage may employ anti-CRISPR proteins to inactivate the bacterial immune system, preventing the eradication of phage by bacteria, and allowing phage to persist within the oral microbiome [79,80]. Lytic phages have been shown to kill bacterial species that pass a particular population threshold, preventing outgrowth and dysbiosis. This 'Kill the Winner' dynamic, as well as a number of other phage–bacteria dynamics, both antagonistic and mutualistic, have been described, and have been shown to result in either positive or negative outcomes for the human host [24,75,76,81]. A recent study by Ly *et al.* showed that the human oral phage ecology in dental plaque is associated with oral health status, with a significantly higher abundance of lytic myoviruses in the subgingival plaque of individuals with periodontal disease, suggesting that phages play a critical role in modulating the microbial community and contributing to disease development [19].

Phage Therapy Revisited

The increase in attention given to the study of phage modulation of the bacterial community, coupled with the rising tide of antibiotic resistance, has led to a resurgence of interest in **phage therapy**. Phage therapy does in theory have significant advantages compared to conventional antibiotic treatment: virtually limitless diversity of potential phage and the ability of phage to rapidly overcome resistance *in situ* [82,83]. However, the capacity of phage therapy to effectively penetrate biofilms, where neighbors and polysaccharide barriers may block phage receptors, remains an area for further study and progress and is a particularly relevant issue in the oral cavity [84]. Additionally, while the specificity of phage allows it to minimally damage nontarget species, leaving the overall community intact, it requires rapid identification of the target in question, as well as the target's susceptibility to phage-mediated killing. Finally, phage therapy is likely to face significant regulatory headwinds, with issues concerning standardization, intellectual property, and safety [83,85]. Several recent reports have identified phages in the oral cavity that infect *F. nucleatum*, *S. mutans*, and *Neisseria meningitidis*, opening the possibility of therapeutic phage development against these oral pathogens [86–88]. In the future, it is likely that both antibiotics and phage will play a role in battling pathogenic infections, as supported by a number of recent studies showing synergistic effects of combined antibiotic and phage therapies (reviewed in [83]).

Just as recognition of the influence of the bacterial microbiota on the immune system and human health recast the tenets of microbial ecology, expanded insights into the interactions of viruses and phage with bacteria, as well as with the human host, are likely to revolutionize thinking about the virome and microbiome at large. Phages clearly represent an additional level of balance required for eubiosis that is still poorly understood. As the complexity of the phage–bacterial interactome is unraveled, it is likely that new mechanisms of phage resistance and bacterial immunity will be discovered and will increase our understanding of the microscopic and submicroscopic world.

Concluding Remarks

Our ability to identify and isolate oral microbial residents and decipher their extensive interactions has rapidly increased within the past decade. However, our understanding of the oral microbiota as an ecological system with diversified, interactive entities is still in its infancy (see Outstanding Questions). A comprehensive understanding of the oral microbiota and its influence on host health and disease requires a holistic view that emphasizes interactions among

Outstanding Questions

Thus far, only one member of the CPR group of organisms has been isolated and cultivated with its host bacterium. How can we achieve better isolation of CPR organisms to study their physiology, metabolism, host specificity, and pathogenicity, and, what is their impact on the oral microbiome and health?

What is the role of the diverse group of fungi, in addition to *C. albicans*, that are now known to inhabit the oral cavity during health? What bacterial species are these fungi interacting with, and what is their role in oral disease processes?

Unlike bacteria, which have a universally conserved genomic region (such as 16S rRNA), there is an enormous variety of genomic structures among viral species, making the study of virus difficult in large cohorts. What novel experimental and bioinformatic strategies can be developed to explore the role of the oral virome?

How can we develop a complex, *in vitro* multispecies model system that contains CPR, fungi, and phage, in addition to bacteria, such that we can study interspecies interaction within the oral microbiota in a comprehensive and controllable manner?

How can databases for fungal and viral/phage identification be improved and integrated with bacterial databases, such as HOMD?

different residents within the community as well as their interaction with the host. Such a deep systems-level understanding at the molecular stratum demands expansion of our knowledge from bacterial interspecies interactions to interkingdom interactions which include CPR, fungi, and viruses – a challenging task that necessitates a systems-level approach combining conventional culture-dependent methods with state-of-the-art culture-independent molecular techniques. Continued expansion of such information in the near future will greatly improve our understanding of oral microbial physiology, pathogenesis and ecology, as well as our ability to diagnose and treat microbial infections.

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