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# Achieving Probiotic Effects via Modulating Oral Microbial Ecology

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Unlike many pathogens that are “foreign” invaders, oral pathogens such as *Streptococcus mutans* are part of the “normal” oral microbial flora. While they express certain pathogenic properties, the balance of synergistic and antagonistic interactions determines whether these commensal pathogens cause damage. Recognition of these microbial ecology-based pathogenesis argues for new strategies for disease treatment and prevention. Probiotics, potentially beneficial live bacteria or yeasts, have been used to combat dental caries. This includes the application of *S. mutans* types that cannot produce acids or other bacteria that interfere with the pathogenic effects of *S. mutans*. While these approaches show therapeutic effects against *S. mutans* experimentally, the conversion into commercial products remains a challenge, due to safety and shelf-life issues. New high-tech approaches, such as quorum-sensing interference of pathogenic bacteria or targeted antimicrobial therapies, offer novel ways to achieve probiotic effects against dental caries.

## INTRODUCTION

For a long time, microbiologists took the reductionist approach to studying complex microbial communities by analyzing individual bacterial species. The strategy has been used to understand the whole by examining smaller components, and has been the hallmark of much of the industrial and scientific revolutions for the past 150 years (Nelson, 1992). While reductionism has greatly advanced microbiology, it was recognized that assembly of smaller pieces cannot explain the whole. Modern microbiologists are learning “system thinking”. From “biofilms” to “metagenomics”, microbiology is experiencing a new trend that emphasizes interactions of different elements within a microbial community. Such approaches are changing our understanding of microbial physiology and our ability to diagnose/

treat microbial infections. This trend is having an impact on oral microbiology as well.

Oral microbial communities with the common name “dental plaque” are some of the most complex microbial floras in the human body, consisting of more than 700 bacterial species (Paster *et al.*, 2001, 2006; Aas *et al.*, 2005). Clinical studies have indicated that dental caries is one of the major human diseases caused by the oral microbial flora (Marsh, 1994). For a very long time, oral microbiologists used reductionism to identify the key pathogens responsible for dental caries (Miller, 1890; Clarke, 1924; Marsh, 1994). The limitations of reductionism forced scientists to adopt concepts such as inter-species interaction, microbial community, biofilms, poly-microbial disease, etc. These new research directions have revealed new physiological functions, which result from interactions between and among different components, and might not be observed with individual organisms. These inter-species-interaction scenarios serve as the foundation for new therapeutic and preventive tools, as discussed in this review.

## UNDERSTANDING THE ORAL MICROBIAL COMMUNITY

From the initial isolation of *S. mutans* by J. Clarke in 1924 to the latest large-scale 16S rRNA/DNA-based oral bacterial studies (Aas *et al.*, 2005), the oral microbial community has been shown to be one of the most complex microbial biota in the human body. The oral microbial biofilm, conventionally called “dental plaque”, is a sophisticated microbial community with novel functions that are essential for biofilm architecture and microbial physiology (Marsh, 1994, 2005).

From a structural point of view, dental plaque shows a high degree of organization. During dental plaque formation, some oral bacteria are early colonizers that express biochemical components allowing them to adhere effectively to specific tissues (teeth or periodontal tissue). The later colonizers often contain components that enable them to adhere to the early colonizers, bringing competitive advantages. Within an established dental plaque, specific bacterial species are often found located adjacent to each other or mixed together to form unique structures that may confer adherence or growth advantages.

### Key Words

Probiotics, microbial ecology, dental caries, dental plaque, *Streptococcus mutans*.

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From a microbial physiology aspect, oral microbial communities are classic examples of biofilms. As initially proposed by Costerton, the behavior displayed by oral microbial organisms grown in liquid culture is very different from that of the same organisms grown on a solid surface or within a community such as dental plaque (Costerton *et al.*, 1995). This is of significant medical interest, since it has been well-documented that there is an increased resilience of oral bacteria within dental plaque to antimicrobial agents, relative to their planktonic susceptibility. Confirmation of these differences has been provided by investigations revealing that oral bacteria grown within biofilm showed a pattern of gene expression and protein synthesis that is distinct from that of comparable planktonic cells (Burne *et al.*, 1997; Black *et al.*, 2003).

Because of the multi-species nature of dental plaque, the oral microbial community is one of the best biofilm models for studying inter-species interactions. Based on our current knowledge, it is reasonable to assume that the interactions between the oral microbial residents may influence the properties of the whole community. For example, while the oral “pathogens” such as *S. mutans* express certain pathogenic properties (such as acid production), a dynamic balance of synergistic and antagonistic interactions with its neighboring bacteria is crucial in determining whether these pathogenic factors cause damage (Kleinberg, 2002; Marsh, 2005). In other words, within complex biofilms, it is not merely the presence of a single organism, but the interactions between and among the biofilm residents that are crucial and determine the properties of a biofilm. As an example, in the presence of nearby base-producing bacteria, *S. mutans* in dental plaque may not be pathogenic. Thus, for dental caries, it is now recognized that this disease results not solely because of the presence of *S. mutans* or any single organism in dental plaque. Rather, it is the interaction of multiple acid-producing organisms such as *S. mutans* with other biofilm residents (Kleinberg, 2002; Marsh, 2005). Such a microbial ecology-based theory serves as a new paradigm to understand the relationship between dental plaque and the host in health or disease, offering new strategies for disease treatment and prevention.

## NEW APPROACHES FOR CONTROLLING DENTAL CARIES *via* MODULATING THE ORAL MICROBIAL ECOLOGY

Current dental therapy is primarily focused on the removal of dental plaque. Since dental plaque is made of large numbers of commensal bacteria, together with a limited number of oral pathogens, such an approach may not be effective, since the “remove all or kill all” approach creates open, non-competitive surfaces for pathogens to repopulate the oral cavity. With our new understanding of the oral microbial community interactions, there is now interest in approaches that selectively inhibit oral pathogens or modulate the microbial composition of dental plaque to control community-based microbial pathogenesis. In the past several years, oral microbiology has become a leading area for developing technologies which might also be useful for managing other community-based microbial pathogenesis.

Among them, the probiotic approach has been a popular method for modulating microbial communities.

### Probiotic approaches

The term ‘probiotics’ refers to “live micro-organisms, which, when administered in adequate amounts, confer a health benefit on the host” (Guarner *et al.*, 2005). The concept of ‘probiotics’ evolved from Elie Metchnikoff’s ideas that the bacteria in fermented products could compete with microbes that are injurious to the host and thus are beneficial for health (Metchnikoff, 1907). In the past decade, there have been numerous exciting discoveries that revealed many beneficial effects resulting from the administration of probiotics, ranging from direct inhibition of pathogenic microbes to improving host immune functions (Harish and Varghese, 2006).

Recently, more evidence suggests that probiotic therapy might be applied to the maintenance of oral health (Caglar *et al.*, 2005a; Meurman, 2005; Meurman and Stamatova, 2007). Classic probiotic strains, such as those that belong to the genera *Lactobacillus* and *Bifidobacterium*, have been tested for their ability to confer probiotic effect in the oral cavity. Using randomized controlled trials, Meurman and colleagues demonstrated that long-term consumption of milk containing the probiotic *Lactobacillus rhamnosus* GG strain reduced initial caries in kindergarten children (Nase *et al.*, 2001). Caglar *et al.* also showed that administration of the probiotic bacterium *Lactobacillus reuteri* ATCC 55739 or *Bifidobacterium* DN-173 010 induced significant reduction of cariogenic *S. mutans* in saliva (Caglar *et al.*, 2005b, 2006).

In addition to the classic probiotic strains, other oral residents or genetically modified strains have also been tested for their ability to inhibit cariogenic microbes. Hillman and colleagues introduced a non-acid-producing *S. mutans* strain that produces a bacteriocin active against other *S. mutans* strains into the oral cavity to replace the naturally occurring cariogenic strains (Hillman, 2002). Both *in vitro* and animal model assessments suggest its potential in reducing *S. mutans* colonization. This approach is currently awaiting evaluation for its efficacy in humans.

Another potential probiotic approach for reducing dental caries involves the use of oral streptococci that are able to metabolize arginine or urea to ammonia (Marquis *et al.*, 1993). Since such organisms naturally occur in dental plaque, and therefore may not offer safety concerns, they could be used in probiotic approaches for controlling dental caries.

Co-infection of rats with oral streptococci *S. salivarius* TOVE-R and *S. mutans* reduced dental caries incidence relative to the latter organisms alone. This is likely due to the ability of TOVE-R to pre-empt the initial colonization of tooth surfaces and displace the cariogenic *S. mutans* that have already colonized the tooth surfaces (Tanzer *et al.*, 1985).

Various oral streptococci have mutual antagonistic effects. Implantation of specific oral streptococci or the encouragement of their growth in dental plaque may thus be considered a probiotic approach by encouraging an ecological shift. Recently, *S. oligofermentans*, a bacterium that could be isolated only from caries-free humans, was found to metabolize lactic acid into hydrogen peroxide, thus inhibiting the growth of *S. mutans*

(Tong *et al.*, 2007). This property makes it a good candidate for probiotic application.

### Other new approaches to achieve probiotic effects

While the therapeutic effects of various probiotics have been demonstrated, it has, to date, been very difficult to develop probiotic-based commercial products, since the complexity of live organisms makes it virtually impossible for traditional toxicity studies to be conducted. While arguments have been used that most organisms selected for probiotic applications exist naturally in the oral cavity, and are likely safe, these arguments lack a solid rationale, since *S. mutans* itself naturally exists in the oral cavity. Another major challenge for probiotic-based products is the instability of such products. Live organisms often have short shelf-times and require complex storage conditions.

The beneficial effects of probiotic therapy are mainly achieved through the modulation of existing microbial flora associated with the host, thus attaining a balanced and healthy microbes-host relationship. Recognizing the disadvantages of traditional probiotic approaches, microbiologists are developing novel techniques and products that do not involve live organisms, yet generate targeted effects against pathogenic factors or organisms, thus achieving similar probiotic effects.

#### Inhibiting adherence with antagonists

Since *S. mutans*' virulence is strongly associated with its adherence, reducing the adherence of *S. mutans* to the tooth's surface should create a microbial community with less *S. mutans*. For example, a cell-surface protein of *S. mutans*, termed SpaP or Ag I/II, has been identified as an adhesin which interacts with the tooth pellicle (Jenkinson and Demuth, 1997). A synthetic dodecapeptide analogue of the active binding site of SpaP has been shown to inhibit attachment of *S. mutans* to teeth both *in vivo* and *in vitro* (Kelly *et al.*, 1999). Such analogs are potential therapeutic agents that could be incorporated into toothpastes or mouthrinses.

#### Passive immunization

Another general approach for controlling bacterial pathogens has been the development of specific vaccination strategies. In the case of dental caries, this possibility has been investigated for the past four decades (Russell *et al.*, 2004). Despite promising results in experimental animal models, it appears unlikely that active immunization approaches will be pursued further in most developed countries, because of economic and ethical concerns. As an alternative to active immunization, passive immunization strategies have been proposed (Koga *et al.*, 2002). However, it remains unclear whether these approaches will lead to a general strategy for immunizing susceptible children against dental caries in the future.

#### Interference with signaling mechanisms

Several pathogenic properties of *S. mutans* are regulated by a quorum-sensing mechanism involving Competence Stimulating Peptide (CSP) as the signaling molecule. The addition of a high concentration of CSP can interfere with signaling events of

*S. mutans* and induce the death of the bacterium, thus exhibiting a potential beneficial effect against dental caries (Cvitkovitch *et al.*, 2003; Qi *et al.*, 2005).

### Targeted antimicrobial therapy via a novel STAMP technology

Eckert *et al.* (2006) reasoned that, with the exception of a limited number of pathogens, the majority of indigenous oral microorganisms are benign or beneficial. Currently available antimicrobials exhibit broad-spectrum killing properties. Indiscriminate killing of all microbes by these conventional antimicrobials disrupts the ecological balance of the indigenous microbiota, with unknown clinical consequences. These investigators formulated a new class of antimicrobials called 'Specifically Targeted Antimicrobial Peptides' (STAMPs). A STAMP is a fusion peptide with two moieties: a killing moiety, made of a non-specific antimicrobial peptide; and a targeting moiety, containing a species-specific binding peptide. The targeting moiety provides specific binding to a selected pathogen and facilitates the targeted delivery of an attached antimicrobial peptide. In one of their recently published papers (Eckert *et al.*, 2006), these investigators explored a pheromone produced by *S. mutans*, namely, CSP, as a STAMP-targeting domain to mediate *S. mutans*-specific delivery of a killing domain. They discovered that such STAMPs were potent against *S. mutans* grown in liquid or biofilm states. Further studies showed that an 8-amino-acid region within the CSP sequence was sufficient for targeted delivery of the killing domain to *S. mutans*. The STAMPs were capable of eliminating *S. mutans* from multispecies biofilms without affecting closely related non-cariogenic oral streptococci, indicating the potential of these molecules to be developed into "probiotic" antimicrobials that may selectively eliminate pathogens while preserving the protective benefits of the normal flora. This proof-of-principle demonstration with *S. mutans* suggests that it may be possible to develop other STAMPs which are specifically targeted to other biofilm pathogens.

## CONCLUSIONS

Most of the bacteria which are of dental and medical concern reside in multi-species biofilm structures, and these microbial communities exhibit properties that are dependent on how the resident organisms interact. Our ability to identify the resident organisms in biofilms and decipher the interactions between and among key components has rapidly increased during the past decade. Continued expansion of such information in the future may facilitate an exogenous modulation of the interactions between biofilm constituents, and thereby result in novel approaches for controlling biofilm activities.

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