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Achieving Probiotic Effects via Modulating Oral Microbial Ecology

Xuesong He¹, Renate Lux¹, Howard K. Kuramitsu², Maxwell H. Anderson³, and Wenyan Shi^{1,3,*}

¹Section of Oral Biology, School of Dentistry and Department of Microbiology, Immunology and Molecular Genetics, School of Medicine, University of California, Los Angeles, California, USA

²Department of Oral Biology, School of Dental Medicine, State University of New York, Buffalo, New York, USA

³C3 Jian Inc, Inglewood, CA, USA

Abstract

Unlike many pathogens 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 or not. 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 study 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 impacting 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 (Aas et al., 2005; Paster et al., 2001; Paster et al., 2006). Clinical studies have indicated that dental caries is one 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

*Corresponding author: Mailing address: UCLA School of Dentistry, Los Angeles, CA 90095, USA, Phone: 310-825-8356. Fax: 310-794-7109. wenyuan@ucla.edu.

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 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; Clarke, 1924), 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 effectively adhere 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.

From a microbial physiology aspect, oral microbial communities are classical examples of biofilms. As initially proposed by Costerton, the behavior displayed by oral microbial organisms grown in liquid culture are very different from 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 is 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 comparable planktonic cells (Black et al., 2003; Burne et al., 1997).

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 or not (Kleinberg, 2002; Marsh, 2005). In other words, within complex biofilms, it is not merely the presence of a single organism, but the interactions between the biofilm residents that is crucial and determines 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 removing 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.

a. Probiotic approaches

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

Recently, more evidence suggests that probiotic therapy might be applied to the maintenance of oral health (Caglar et al., 2005; Meurman, 2005; Meurman et al., 2007). Classical probiotic strains, such as those that belong to the genera *Lactobacillus* and *Bifidobacterium*, have been tested for their ability to confer probiotic effect in 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 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 classical 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 suggests 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 later organisms alone. This is likely due to the ability of TOVE-R to preempt the initial colonization of teeth surface and displace the cariogenic *S. mutans* that has already colonized the teeth surface (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 only isolated from caries free human subjects, 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.

b. Other new approaches to achieve probiotic effects

While 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 to conduct traditional toxicity studies. 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 modulating existing microbial flora associated with the host, thus obtaining 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.

b-1. 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 fewer *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 mouth rinses.

b-2. 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 using experimental animal models, it appears unlikely that active immunization approaches will be further pursued 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 or not these approaches will lead to a general strategy for immunizing susceptible children against dental caries in the future.

b-3. Interference with signaling mechanisms—Several pathogenic properties of *S. mutans* are regulated by quorum sensing mechanism involving Competence Stimulating Peptide (CSP) as the signaling molecule. 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, 2003; Qi *et al.*, 2005)

b-4. Targeted antimicrobial therapy via a novel STAMP technology—Eckert *et al.* 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 Anti-Microbial Peptides (STAMPs). A “STAMP” is a fusion peptide with two moieties: a killing moiety made of a nonspecific 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), they 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 noncariogenic 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 using *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 key components has rapidly increased during the past decade. Continued expansion of such information in the future may enable an exogenous modulation of the interactions between biofilm constituents, and thereby result in novel approaches for controlling biofilm activities.

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References

- Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol* 2005;43:5721–5732. [PubMed: 16272510]
- Black C, Allan I, Ford SK, Wilson M, McNab R. Biofilm specific surface properties and protein expression in oral *Streptococcus sanguis*. *Arch Oral Biol* 2003;49:295–304. [PubMed: 15003548]
- Burne RA, Chen YM, Penders JEC. Analysis of gene expression in *Streptococcus mutans* in biofilms in vitro. *Adv Dent Res* 1997;11:100–109. [PubMed: 9524447]
- Caglar E, Kargul B, Tanboga I. Bacteriotherapy and probiotics' role on oral health. *Oral Dis* 2005;11:131–137. [PubMed: 15888102]
- Caglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with Bifidobacterium DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. *Acta Odontol Scand* 2005;63:317–320. [PubMed: 16512103]
- Caglar E, Cilder SK, Ergeneli S, Sandalli N, Twetman S. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium *Lactobacillus reuteri* ATCC 55739 by straws or tablets. *Acta Odontol Scand* 2006;64:314–318. [PubMed: 16945898]
- Clarke JK. On the bacterial factor in the etiology of dental caries. *Brit J Exp Pathol* 1924;5:141–147.
- Costerton JW, Lewandowski Z, Caldwell DE, Keber KR, Lappin-Scott HM. Microbial biofilms. *Annu Rev Microbiol* 1995;49:711–745. [PubMed: 8561477]
- Cvitkovitch DG, Li Y, Ellen RP. Quorum sensing and biofilm formation in Streptococcal infections. *J Clin Invest* 2003;112:1626–1632. [PubMed: 14660736]

- Eckert R, He J, Yarbrough DK, Qi F, Anderson MH, Shi W. Targeted killing of *Streptococcus mutans* by a pheromone-guided “Smart” antimicrobial peptide. *Antimicrob Agents Chemother* 2006;50:3651–3657. [PubMed: 17060534]
- Guarner F, Perdigon G, Coerthier G, Salminen S, Koletzko B, Morelli L. Should yoghurt cultures be considered probiotic? *Br J Nutr* 2005;93:783–786. [PubMed: 16022746]
- Harish K, Varghese T. Probiotics in humans – evidence based review. *Calicut Med J* 2006;4:e3.
- Hillman JD. Genetically modified *Streptococcus mutans* for prevention of dental caries. *Antoine van Leeuwenhoek* 2002;82:361–366.
- Jenkinson HF, Demuth DR. Structure, function and immunogenicity of streptococcal antigen I/II polypeptides. *Mol Microbiol* 1997;23:183–190. [PubMed: 9044252]
- Kelly CG, Younson JS, Hikmat BY, Todryk SM, Czisch M, Harris PI, Flindall IR, Newby C, Mallet AI, Ma JK, Lehner T. A synthetic peptide adhesion epitope as a novel antimicrobial agent. *Nat Biotechnol* 1999;17:42–47. [PubMed: 9920267]
- Kleinberg I. A mixed-bacterial ecological approach to understanding the role of oral bacteria in dental caries causation: an alternate to *Streptococcus mutans* and the specific-plaque hypothesis. *Crit Rev Oral Biol Med* 2002;13:108–125. [PubMed: 12097354]
- Koga T, Oho T, Shimzaki Y, Nakano Y. Immunization against dental caries. *Vaccine* 2002;20:2027–2044. [PubMed: 11972971]
- Marquis, RE.; Burne, RA.; Parsonsons, DT.; Casiano-Colon, AE. Arginine deiminase and alkaline generation in plaque. In: Bowen, WH.; Tabak, LA., editors. *Cariology for the nineties*. Univ. of Rochester Press; Rochester, N.Y.: 1993. p. 309-317.
- Marsh PD. Dental plaque: biological significance of a biofilm and community life-style. *J Clin Periodontol* 2005;32:7–15. [PubMed: 16128825]
- Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. *Adv Dent Res* 1994;8:263–271. [PubMed: 7865085]
- Metchnikoff, E. The prolongation of life. In: Heinemann, W., editor. *Optimistic studies*. G. P. Putnam & Sons; London: 1907. p. 1-100.
- Meurman JH. Probiotics: do they have a role in oral medicine and dentistry. *Eur J Oral Sci* 2005;113:188–196. [PubMed: 15953242]
- Meurman JH, Stamatova I. Probiotics: contributions to oral health. *Oral Dis* 2007;13:443–451. [PubMed: 17714346]
- Miller, WD. *The micro-organisms of the human mouth*. Graphische AnstaltSchuler AG; Biel, Switzerland: 1890.
- Nase L, Hatakka K, Savilahti E, Saxelin M, Ponka A, Poussa T, Korpela R, Meurman JH. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. *Caries Res* 2001;35:412–420. [PubMed: 11799281]
- Nelson, D. *A mental revolution: scientific management since Taylor*. Columbus, USA: Ohio State University Press; 1992.
- Paster BJ, Olsen I, Aas JA, Dewhirst FE. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontology* 2000 2006;42:80–87. [PubMed: 16930307]
- Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, Sahasrabudhe A, Dewhirst FE. Bacterial diversity in human subgingival plaque. *J Bacteriol* 2001;183:3770–3783. [PubMed: 11371542]
- Qi F, Kreth J, Levesque CM, Kay O, Mair RW, Shi W, Cvitkovitch DG, Goodman SD. Peptide pheromone induced cell death of *Streptococcus mutans*. *FEMS Microbiol Lett* 2005;251:321–326. [PubMed: 16165324]
- Russell MN, Childers NK, Michalek SM, Smith DJ, Taubman MA. A caries vaccine? The state of the science of immunization against dental caries. *Caries Res* 2004;38:230–235. [PubMed: 15153693]
- Tanzer JM, Kurasz AB, Clive J. Inhibition of ecological emergence of mutans streptococci naturally transmitted between rats and consequent caries inhibition by *Streptococcus salivarius* TOVE-R infection. *Infect Immun* 1985;49:76–83. [PubMed: 4008050]

Tong H, Chen W, Merritt J, Qi F, Shi W, Dong X. *Streptococcus oligofermentans* inhibits *Streptococcus mutans* through conversion of lactic acid into inhibitory H₂O₂: a possible counteroffensive strategy for interspecies competition. *Mol Microbiol* 2007;63:872–880. [PubMed: 17302806]