CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

Bacterial Interactions in Dental Biofilm Development

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ABSTRACT

Recent analyses with ribosomal RNA-based technologies have revealed the diversity of bacterial populations within dental biofilms, and have highlighted their important contributions to oral health and disease. Dental biofilms are exceedingly complex and multispecies ecosystems, where oral bacteria interact cooperatively or competitively with other members. Bacterial interactions that influence dental biofilm communities include various different mechanisms. During the early stage of biofilm formation, it is known that planktonic bacterial cells directly attach to surfaces of the oral cavity or indirectly bind to other bacterial cells that have already colonized. Adherence through co-aggregation may be critical for the temporary retention of bacteria on dental surfaces, and may facilitate eventual bacterial colonization. It is likely that metabolic communication, genetic exchange, production of inhibitory factors (*e.g.,* bacteriocins, hydrogen peroxide, etc.), and quorum-sensing are pivotal regulatory factors that determine the bacterial composition and/or metabolism. Since each bacterium can easily access a neighboring bacterial cell and its metabolites, genetic exchanges and metabolic communication may occur frequently in dental biofilms. Quorum-sensing is defined as gene regulation in response to cell density, which influences various functions, *e.g.,* virulence and bacteriocin production. In this review, we discuss these important interactions among oral bacteria within the dental biofilm communities.

KEY WORDS: dental plaque, biofilm formation, synergism, antagonism, metabolic communication, quorum-sensing, bacterial adherence, bacterial aggregation.

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INTRODUCTION

The microflora of the oral cavity is diverse, and more than 700 bacterial species have been detected (Aas *et al*., 2005; Paster *et al*., 2006; Faveri *et al*., 2008). These bacterial species are thought to play important roles in the maintenance of oral health and in the aetiology of oral diseases in humans (Socransky *et al*., 2002; Kumar *et al*., 2005). However, little is known about a beneficial roles of oral bacteria, whereas harmful behavior of certain oral bacteria in diseases is well-known. Since dental biofilms are also found in health, certain commensal bacteria may potentially exclude pathogens and allochthonous bacteria. It is probable that the mechanisms underlying this preventive ability include competition for nutrients and attachment sites (Bowden and Li, 1997; Nobbs *et al*., 2007; van Hoogmoed *et al*., 2008), and production of antimicrobial substances such as hydrogen peroxide and bacteriocins (Kreth *et al*., 2005a; Pangsomboon *et al*., 2006). In contrast, the accumulation of dental biofilms, which are accompanied by a change in bacterial composition, leads to the onset of dental diseases such as dental caries, gingivitis, periodontitis, etc. (Baehni and Takeuchi, 2003). The bacterial composition often changes from a scanty biofilm dominated by Gram-positive bacteria, usually found in healthy individuals, to an increased number of Gram-negative anaerobic rods, usually observed in periodontitis.

Recent molecular methods have revealed that almost all dental diseases are caused by dental biofilms that consist of a multispecies community (Becker *et al*., 2002; Socransky *et al*., 2002; Kumar *et al*., 2005; Brito *et al.,* 2007). The biofilm communities are complex and dynamic structures that accumulate through the sequential and ordered colonization of multiple oral bacteria (Kolenbrander *et al*., 2002). One of the most notable features of dental biofilms is that oral bacteria growing in the biofilms frequently express phenotypes that are different from those of planktonic bacteria. For instance, many bacterial species in biofilms exhibit greater tolerance to antibiotics and other environmental factors, such as pH and oxygen (Bradshaw *et al*., 1998; Sedlacek and Walker, 2007; Welin-Neilands and Svensäter, 2007). Dental biofilms are characterized by surface attachment, structural heterogeneity, complex interspecies interactions, and an extracellular matrix of polymeric substances, and are high-density micro-niches that differ dramatically from surrounding conditions. Bacterial species present in the dental biofilm communities interact cooperatively or competitively with other members. It has been shown that the bacterial interactions that influence biofilm formation, metabolic change, and physiological function involve various different mechanisms (Fig. 1.). From a physical aspect, planktonic bacterial cells attach directly to surfaces of the oral cavity or bind indirectly to other bacterial cells that have already colonized (Kolenbrander *et al*., 2002). From metabolic and physiological points of view, avenues of communication within dental biofilms are likely to include metabolic communication, genetic exchange,

quorum-sensing, etc. (Chalmers *et al*., 2008; Sedgley *et al*., 2008). These interactions as well as host-related factors, such as age, sex, immunity, heredity, and life-style, should be pivotal regulatory factors that determine bacterial composition and/or metabolism. In the present review, we discuss such significant interactions among bacteria in the dental biofilm communities. Here, we refer to bacterial adhesion to surfaces of the oral cavity, although this mechanism is relevant to bacteria-host interactions, but not to bacterial interactions, because it is essential for the development of dental biofilms, especially initial bacterial colonization.

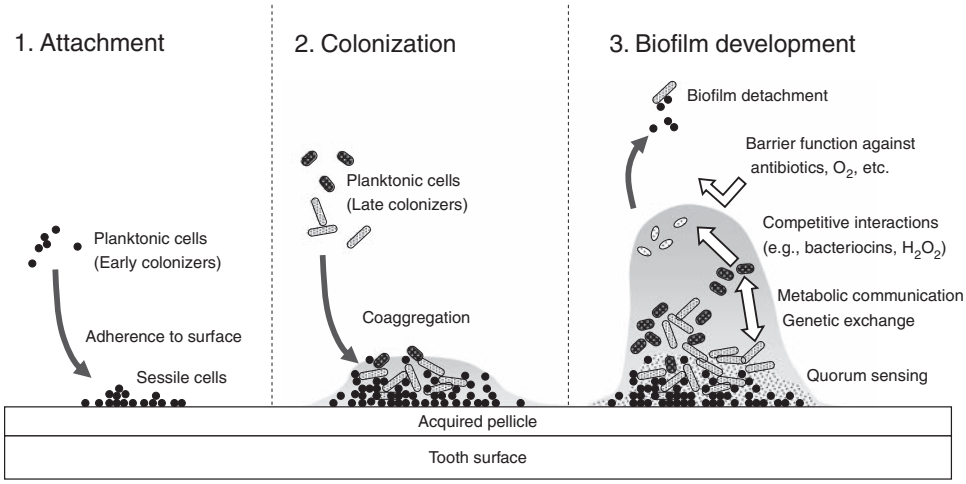

Figure 1. A diagrammatic representation of biofilm formation on the tooth surface and the potential roles of bacterial interactions. The tooth pellicle is generally colonized by early colonizers. Co-aggregation contributes to sequential binding and colonization. Bacterial interactions include metabolic communication and genetic exchange. The development of a biofilm having a high bacterial cell density increases the concentration of signaling molecules. Dental biofilms function as a barrier against deleterious factors such as antibiotics and oxygen.

ADHESION TO TOOTH SURFACES

Bacterial adhesion to and subsequent colonization of the surfaces of teeth and tissue are the first steps toward the formation of dental biofilms (Fig. 1). Tooth pellicle is a thin film that covers the tooth soon after the tooth is thoroughly cleansed, and it originates from salivary proteins. Oral bacteria such as viridans streptococci can colonize the tooth surface by binding to the complex proteinaceous pellicle (Rogers *et al*., 2001; Kolenbrander *et al*., 2002). Therefore, adhesion to the tooth pellicle is relevant to the interactions of a certain oral bacterial species with host molecules.

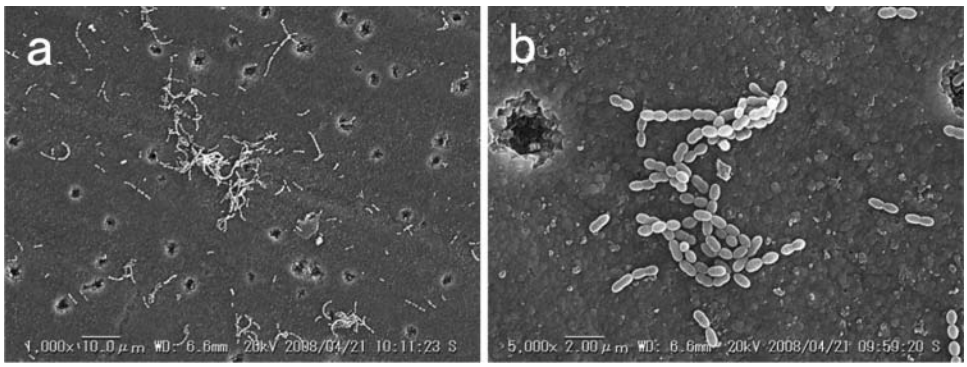

Figure 2. These images, obtained by scanning electron microscopy, demonstrate the adherence of *Streptococcus sanguinis* JCM 5708^T to saliva-coated hydroxyapatite discs. Images were taken at ×1000 **(a)** and ×5000 **(b)** magnifications (VE-9800; KEYENCE Co., Osaka, Japan).

Many oral streptococci have the ability to bind to proteins such as alpha-amylase, proline-rich proteins, and proline-rich glycoproteins, and are recognized as early colonizers. This ability may confer an advantage on the streptococci in establishing early dental plaque (Kolenbrander *et al*., 2002). *Streptococcus gordonii* is one of the early colonizers in dental biofilms. This species binds to acidic proline-rich proteins that account for 25-30% of the total proteins in saliva. The alpha-amylase-binding protein A of *S. gordonii* interacts with salivary amylase, which suggests that this interaction contributes to the attachment of *S. gordonii* to the tooth surface (Rogers *et al*., 2001). *S. sanguinis* is thought to be one of the first bacterial species to adhere selectively to and colonize saliva-coated teeth. This species generally appears in the human oral cavity after tooth eruption, and it becomes a normal inhabitant of the human mouth. *S. sanguinis* colonized the surfaces of saliva-coated hydroxyapatite discs in an *in vitro* experiment (Fig. 2). A complex enriched in secretory immunoglobulin A and alpha-amylase forms a binding site for *S. sanguinis* (Gong *et al*., 2000).

CO-AGGREGATION AMONG ORAL BACTERIA

Planktonic bacterial cells that cannot directly colonize the tooth surface may bind *via* receptors to the cell surfaces of early colonizers that adhere to the surfaces. Co-aggregation is a specific cell-to-cell reaction that occurs between distinct bacterial cells and is one of the most important mechanisms underlying oral bacterial colonization and dental biofilm formation. A previous comprehensive review has provided concrete evidence that can help identify specific interspecies interactions

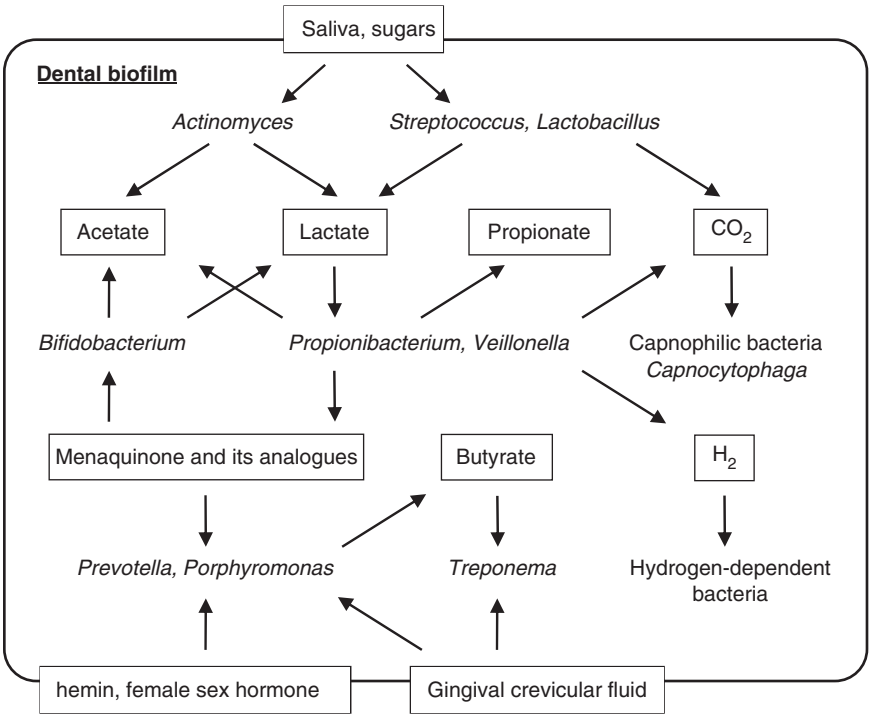

Figure 3. Illustration of representative metabolic relationships among oral bacteria within the dental biofilm communities. *Streptococcus*, *Lactobacillus*, and *Actinomyces* secrete lactate, and it is utilized by *Veillonella* and *Propionibacterium* as a carbon source (Marcotte and Lavoie, 1998; Chalmers *et al.*, 2008). *Veillonella* and *Propionibacterium* produce menaquinone and its analogues, which promote the growth of vitamin K-auxotrophic bacteria such as *Porphyromonas*, *Prevotella*, and *Bifidobacterium* (Marcotte and Lavoie, 1998; Hojo *et al.*, 2007). Carbon dioxide, produced by aerotolerant *Propionibacterium*, hetero-fermentative *Lactobacillus*, and *Veillonella*, is utilized by Capnophilic bacteria such as *Capnocytophaga* (Kapke *et al.*, 1980). A certain micro-aerophilic motile bacterium depends on hydrogen produced by other oral bacteria, *e.g.*, *Veillonella* (van Palenstein Helderman and Rosman, 1976).

in the co-aggregation among oral bacteria (Kolenbrander *et al*., 2002). According to their proposed spatio-temporal model of oral bacterial colonization, starting from the bottom layer of the dental biofilm, early colonizers bind *via* adhesins to the complementary pellicle receptors. Secondary colonizers bind to bacteria that are previously bound to the teeth. Sequential binding results in the appearance of a nascent surface that forms a bridge with the adjacent co-aggregating partner cells. For example, a typical periodontal pathogen, namely, *Porphyromonas gingivalis,* can bind to early colonizers. The long (major) fimbriae of *P. gingivalis* are composed of the FimA protein, which binds to the glyceraldehyde-3-phosphate dehydrogenase present on the surface of *S. oralis* (Maeda *et al*., 2004). The process of bridging between a co-aggregation of cells consisting of more than 3 bacterial species is very important, because it connects a few species that are not co-aggregation partners. *Fusobacterium nucleatum* can co-aggregate with many oral bacteria, including streptococci and obligate anaerobes. Therefore, this species is a key component of dental biofilms and serves as a coordinator that bridges the late and early colonizers (Kolenbrander *et al*., 2002).

Co-aggregation between *F. nucleatum* and other bacteria is a highly specific process involving interaction among the surface molecules of bacterial cells. The co-aggregation reactions between *F. nucleatum* and Gram-negative bacteria are mediated by lectin-carbohydrate interactions. For instance, the co-aggregation between *P. gingivalis* and *F. nucleatum* is mediated by a galactoside moiety on the surface of *P. gingivalis* and a lectin moiety on that of *F. nucleatum*, which is inhibited by lactose. Capsular polysaccharides and lipopolysaccharides of the *P. gingivalis* serotype K5 act as receptors mediating the co-aggregation between oral bacteria (Rosen and Sela, 2006). In contrast, although co-aggregation between *F. nucleatum* and many other Gram-positive bacteria has been observed, co-aggregation is rarely inhibited by sugars (Kolenbrander *et al*., 1989; Kang *et al*., 2005; Nagaoka *et al*., 2008). Thus, intergeneric co-aggregation between *F. nucleatum* and Gram-negative cells is quite different from that with Gram-positive bacteria.

Co-aggregation among oral bacteria is thought to contribute to not only bacterial colonization through physico-chemical mechanisms, but also to metabolic communication and genetic exchange, because each bacterium can easily access a neighboring bacterial cell and its metabolites. In a recent *in vitro* study, Chalmers *et al*. (2008) demonstrated that metabolic dependence is facilitated by intergeneric co-aggregation. *Streptococcus oralis* and *S. gordonii* co-aggregated with *Veillonella* sp. PK1910. These bacteria formed interdigitated three-species clusters when grown as a biofilm with saliva as the nutritional source. *Veillonella* sp. PK1910 grew only when streptococci were present. It has also been suggested that bacterial co-aggregation is related to the survival of obligate anaerobic bacteria in aerobic condition (Bradshaw *et al.*, 1998). It is therefore likely that intergeneric co-aggregation is an important factor in the cooperative communications among oral bacteria.

METABOLIC COMMUNICATION AMONG ORAL BACTERIA

For oral bacteria, nutrients are available from saliva, gingival crevicular fluid, food containing sugars, food debris, and metabolic products of other bacteria (Fig. 3). Metabolic communications among oral bacteria may occur through the excretion of a metabolite by one organism that can be used as a nutrient by a different organism, or through the breakdown of a substrate

by the extracellular enzymatic activity of one organism that creates biologically available substrates for different organisms (Kolenbrander *et al*., 2002). As an example, Byers *et al*. (1999) proposed that the hydrolysis of host glycoproteins by *S. oralis* and the subsequent utilization of released monosaccharides are important in the survival and persistence of this species and other oral bacteria. Similarly, the desialylation of immunoglobulin A1, the dominant isotype of antibody in the oral cavity, by oral Gram-positive rods may facilitate the proteolytic activities of other oral bacteria, and the concerted action may positively influence the survival of the bacteria in the oral community (Frandsen, 1994).

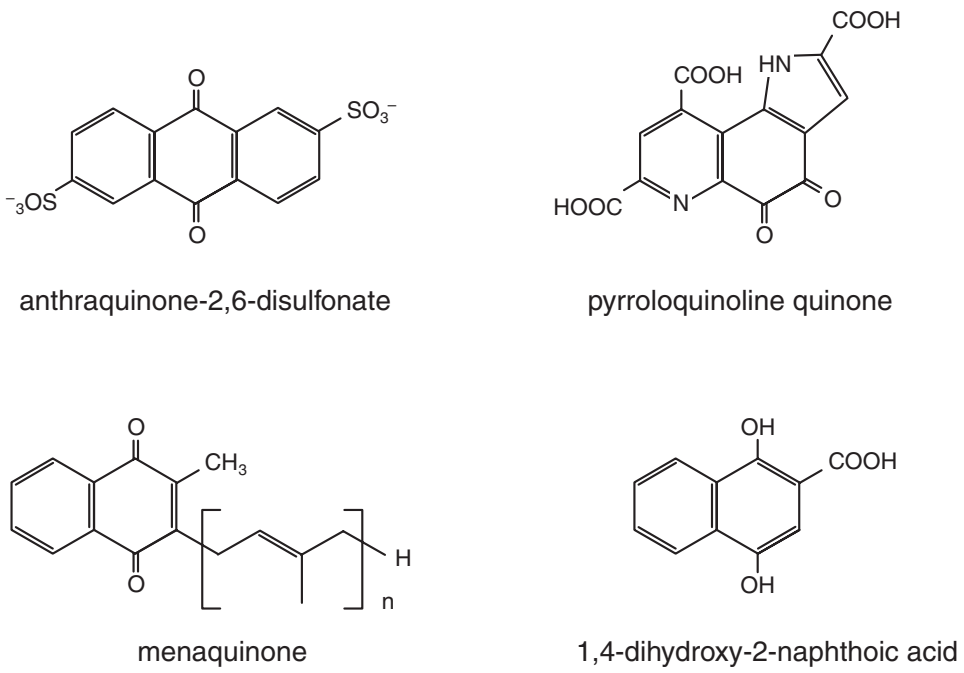

Figure 4. Structure of representative quinones and naphthoic acid that influence bacterial growth. Anthraquinone-2,6-disulfonate acts as an electron acceptor (Newman and Kolter, 2000; Mehta *et al.*, 2005). Pyrroloquinoline quinone is a component of quinoproteins such as glucose dehydrogenase and methanol dehydrogenase, and it is an effective growth factor for certain micro-organisms (Ameyama *et al.*, 1984). Menaquinone and its precursor, namely 1,4-dihydroxy-2-naphthoic acid, are also growth factors for certain micro-organisms (Marcotte and Lavoie, 1998; Isawa *et al.*, 2002; Yamamoto *et al.*, 2005, 2006).

Oral bacteria present in dental biofilms provide their metabolites as energy sources for other members. Short-chain fatty acids produced by oral bacteria are thought to be an essential carbon source for certain oral bacteria. Several studies have suggested a symbiotic association between *Streptococcus* and *Veillonella* species *via* lactic acid produced by the former (Kumar *et al*., 2005; Chalmers *et al*., 2008). In human studies, streptococci and veillonellae often occur in the same site of the oral cavity (Haffajee *et al*., 1998; Tanner *et al*., 1998; Kumar *et al*., 2005). Moreover, interestingly, these human clinical studies suggested that these genera are associated with periodontal health. Kumar *et al*. (2005) stated that the parallel relationship is not surprising in view of the fact that veillonellae utilize short-chain acids such as lactates that are secreted by Gram-positive bacteria such as streptococci. From both *in vitro* and *in vivo* studies, it is probable that the growth of veillonellae present in the oral cavity depends on lactate produced by other oral bacteria. Likewise, *P. gingivalis* and *Treponema denticola* are frequently detected together in the dental biofilms of persons with periodontitis. Growth enhancement due to the mutual symbiotic relationship between *P. gingivalis* and *T. denticola* has been described in previous studies (*e.g*., Grenier, 1992). The growth factors produced by *P. gingivalis* and *T. denticola* were identified to be short-chain fatty acids. The growth factor produced by *T. denticola* is thought to be succinic acid, which appears to be incorporated into the lipids and phospholipids present on the cell envelope of *P. gingivalis* (Lev and Milford, 1971; Lev, 1979). Moreover, the growth of *T. denticola* is stimulated by the isobutyric acid that is produced by *P. gingivalis* (Grenier, 1992). Because these 2 species can co-aggregate, their metabolites are easily accessible to each other, since they are not diluted.

Several exogenous quinones influence bacterial growth and metabolism. The structures of representative quinones that are reported to promote bacterial growth are shown in Fig. 4. Exogenous quinones are thought to act as electron acceptors in bacterial metabolism (Newman and Kolter, 2000; Yamazaki *et al*., 2002; Mehta *et al*., 2005; Yamamoto *et al*., 2005, 2006). The following is an interesting example of the role of an exogenous quinone in *Streptococcus agalactiae* that is found in the human intestine and the female urogenital tract. Yamamoto *et al*. (2005, 2006) suggested that exogenous quinone contributes to the metabolic shift from anaerobic metabolism to the respiration, and that this metabolic change was relevant to the virulence in a murine septicemia model. They proposed that *S. agalactiae* may benefit in the ecological niche by capturing menaquinone from other bacteria.

Vitamin K often has a highly stimulatory effect or is required for most *Prevotella* and *Porphyromonas* strains. Vitamin K is not synthesized in humans; therefore, it is known that auxotrophic micro-organisms that require vitamin K acquire it from the bacteria present in dental biofilm. *Veillonella* species—a ubiquitous component of dental biofilms—is thought to produce menaquinone (vitamin K_2), which is then utilized by *Prevotella* and *Porphyromonas* (Marcotte and Lavoie, 1998). Similarly, quinones and their related compounds stimulate the growth of *Bifidobacterium* (Glick *et al*., 1959; Mori *et al*., 1997; Isawa *et al*., 2002). In our study, the growth of almost all *Bifidobacterium*

strains isolated from human saliva was stimulated by vitamin K, and a *Veillonella* culture supernatant substituted for this growth factor (Hojo *et al*., 2007). However, no menaquinone was detected in the supernatant, whereas it was contained in the *Veillonella* cellular region. Therefore, the growth factor derived from the *Veillonella* culture supernatant is suggested to be an analogue of vitamin K, although it has not yet been identified. The menaquinone precursor, 1,4-dihydroxy-2-naphthoic acid, also actively promotes the growth of *P. gingivalis* and *Bifidobacterium* (Wyss, 1992; Isawa *et al*., 2002). This precursor is found in extracellular regions in the culture supernatant of *Propionibacterium* species (Isawa *et al*., 2002; Furuichi *et al*., 2007; Kouya *et al*., 2007) and *Lactococcus* species (our unpublished observations). Menaquinone is a component of the bacterial membrane; therefore, vitamin K-auxotrophic micro-organisms cannot utilize this growth factor unless the menaquinone-producing bacteria die and release it from their cell membranes. Accordingly, we propose that the extracellular vitamin K-like growth factors produced by *Propionibacterium* and *Veillonella* influence the growth of the vitamin K-auxotrophic bacteria directly.

BACTERIOCINS AND OTHER INHIBITORY METABOLITES

While cooperative interactions of nutrients and colonization exist in dental biofilm communication, close competition with antagonists must occur. Bacteriocins are likely to be a formidable weapon in the armory of an oral bacterium as it competes for the restricted nutrients and the limited foothold with other bacteria in the dental biofilm. Bacteriocins are defined as proteinaceous bactericidal substances produced by bacteria to inhibit the growth of closely related bacterial species or strains. For instance, *S. mutans* is able to produce several kinds of bacteriocins called mutacins, including lantibiotics and non-lantibiotics. The mutacins belonging to lantibiotics such as mutacins I, II, and III have a relatively wide spectrum against other streptococci and Gram-positive bacteria (Qi *et al*., 1999; Mota-Meira *et al*., 2000). In contrast, mutacins IV and V are unmodified non-lantibiotics, and mutacin IV is specifically active against members of the mitis group of oral streptococci (Qi *et al*., 2001; Hale *et al*., 2005). Mutacins may allow *S. mutans* to invade the dental biofilm community and to colonize there, because the mutacin activity of this species could be related to the prevalence and the successful establishment in the dental biofilm (Kreth *et al*., 2005a; Nes *et al*., 2007). Mutacin production is controlled by many genetic as well as environmental factors. Mutacin IV is controlled by quorum-sensing *via* the three-component system *comCDE* (Kreth *et al*., 2005b, 2006), whereas regulation of mutacin I is much more complex and less understood. In a more recent study, Nguyen *et al*. (2009) suggested that mutacin I production is related to at least 17 genes that are involved in a variety of cellular functions, such as sugar transport, protein/peptide hydrolysis, amino acid and nucleotide synthesis, cell wall metabolism, and surface binding.

Bacteriocins are typically thought to have a narrow spectrum; however, some of them produced by oral bacteria apparently have a relatively broad spectrum, such as the lantibiotics mentioned above. It has been reported that a 56-kDa novel bacteriocin produced by *Lactobacillus paracasei* HL32 inhibits the growth of *P. gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, *S. salivarius,* and *S. sanguinis* (Pangsomboon *et al.*, 2006, 2009). The bacteriocin was heat-stable, surviving at 110ºC under pressure, and possessed activity over a pH range of 6.8-8.5. Regarding ecological study of oral lactobacilli, Kõll-Klais *et al*. (2005) investigated the distribution of oral lactobacilli in persons with chronic periodontitis and periodontally healthy individuals. They reported that obligately homofermentative lactobacilli, particularly *L. gasseri*, were significantly prevalent in healthy individuals compared with those with periodontitis. Strong antimicrobial activity against *S. mutans*, *P. gingivalis*, and *P. intermedia* was detected in *L. paracasei*, *L. plantarum*, *L. rhamnosus,* and *L. salivarius*, although biochemical properties have not yet been well-characterized. Bacteriocin or bacteriocin-like activities have been documented for many other oral bacteria. Therefore, it seems reasonable to suppose that competition through bacteriocin production can occur commonly in the dental biofilm.

Several recent molecular and genetic studies by Qi *et al*. have revealed that bacteriocin production, especially mutacin production, is regulated by both genetic factors and environmental conditions such as cell density and pH (Qi *et al*., 2004; Kreth *et al*., 2005b, 2006; Merritt *et al*., 2007). A previous review (Kuramitsu *et al*., 2007) serves to understand the role of these important properties. In their words, these regulatory systems ensure that bacteriocins are produced at the right time and place so that they can be effectively used for "war and peace" activities within an oral microbial community. Further, Kuramitsu *et al*. stated that the production of, and sensitivities to, certain bacteriocin or bacteriocin-like activities among oral bacteria could enable bacteria to select their neighbors, promote the establishment of a community with specific bacterial species, and play an important role in the ecological balance of the oral ecosystem.

Based on many *in vitro* studies, other bacterial metabolites, such as hydrogen peroxide and short-chain fatty acids, are strongly suggested to be competitive factors in oral biofilms. Hydrogen peroxide production by streptococci is well-known to inhibit the growth of other bacterial species. It is suggested that hydrogen peroxide produced by *S. sanguinis* contributes to the antagonism against *S. mutans* in an oral biofilm model (Kreth *et al*., 2005a, 2008). Many oral bacteria produce large quantities of short-chain fatty acids as the end-products of carbohydrate fermentation. The production of lactic acid lowers the pH in dental biofilm, thereby having a disadvantageous effect on less aciduric oral bacteria (Dashper and Reynolds, 2000).

QUORUM-SENSING

Quorum-sensing is a process of chemical communication among bacteria; it is defined as gene regulation in response to cell density, which influences various functions, *viz*., virulence, acid tolerance, and biofilm formation. Because bacteria within biofilms reach a high cell density, quorum-sensing is considered one of the important bacterial functions. Autoinducer-2

Table. Signaling Molecules and Functions in Oral Bacteria

Signals	Bacteria	Functions	References
AI-2	*Porphyromonas gingivalis*	Hemin and iron uptake	Chung *et al.* (2001), James *et al.* (2006)
		Protease and hemaggulutinin activities	Burgess *et al.* (2002)
		Stress gene response	Yuan *et al.* (2005)
	Streptococcus mutans	Biofilm formation	Yoshida *et al.* (2005)
	S. gordonii	Carbohydorate metabolism	McNab *et al.* (2003)
	S. anginosus	Biofilm formation	Petersen *et al.* (2006)
	S. intermedius	Virulence factors	Pecharki *et al.* (2008)
	Aggregatibacter actinomycetemcomitans	Biofilm formation	Shao *et al.* (2007)
	Eikenella corrodens	Biofilm formation	Azakami *et al.* (2006)
	P. gingivalis - S. gordonii	Biofilm formation	McNab *et al.* (2003)
	S. oralis - Actinomyces naeslundii	Biofilm formation	Rickard *et al.* (2006)
CSP	*S. mutans*	Bacteriocin production, competence	van der Ploeg (2005), Kreth *et al.* (2005b)
		Biofilm formation	Li *et al.* (2001a), Aspiras *et al.* (2004)
		Acid tolerance	Li *et al.* (2001b)
	S. gordonii	Biofilm formation	Loo *et al.* (2000)
	S. intermedius	Biofilm formation	Petersen *et al.* (2004)

(AI-2) is one of the most well-known signaling molecules associated with quorum-sensing. It was initially identified in *Vibrio harveyi* (Bassler *et al.*, 1994) and subsequently in several bacterial species. The synthesis of AI-2 is catalyzed by LuxS, an enzyme encoded by the *luxS* gene. The *luxS* gene is highly conserved in the genome of a wide range of Gram-positive and Gram-negative bacteria. Many studies suggest that oral bacteria have a quorum-sensing system that depends on LuxS/AI-2 (Table). For example, a LuxS-deficient *P. gingivalis* mutant was found to produce low levels of protease. In this mutant strain, the activities of Arg-gingipain and Lys-gingipain were approximately 45% and 30% lower, respectively, than those in the parent strain, and the mutant exhibited a four-fold reduction in hemagglutinin activities (Burgess *et al.*, 2002). Moreover, altered expression of genes involved in hemin and iron uptake has been reported in LuxS-deficient mutants (Chung *et al*., 2001; James *et al*., 2006). Yuan *et al*. (2005) compared the gene expression of wild-type with that of LuxS-deficient mutant *P. gingivalis*, using a microarray technique. Their results suggested that the *luxS* gene is involved in protecting *P. gingivalis* against environmental stresses such as temperature, hydrogen peroxide, and pH.

According to recent studies (McNab *et al*., 2003; Rickard *et al*., 2006), AI-2 controls biofilm formation. The growth and biofilm-forming ability of an isogenic mutant of *S. gordonii*, generated by the insertional inactivation of the *luxS* gene, was unaffected when grown on polystyrene surfaces (McNab *et al*., 2003). In contrast, the mutant was unable to form a mixed-species biofilm with a luxS-null strain of *P. gingivalis*. Complementation of the *luxS* mutation in *S. gordonii* restored normal biofilm formation with the luxS-deficient *P. gingivalis*. The formation of a biofilm of *P. gingivalis* and *S. gordonii* requires, as an initial event, adherence mediated through the interactions between the streptococcal surface proteins of *S. gordonii* and the minor fimbriae of *P. gingivalis* (Lamont *et al*., 2002). However, the expression of the streptococcal surface proteins was not affected in the luxS-null mutant of *S. gordonii*. Hence, it is suggested that the influence of *luxS* on biofilm formation occurs subsequent to the initial adherence to the teeth (McNab *et al*., 2003).

Competence-stimulating peptide (CSP) mediates bacterial cell-to-cell signal communication. CSP-dependent quorum-sensing functions in oral bacteria are listed in the Table. CSP is a small soluble peptide having from 14 to 23 amino acid residues and is potentially produced by many species of oral streptococci (Li *et al*., 2001a). CSP is implicated in bacteriocin production, virulence, and biofilm formation. Moreover, CSP enhances genetic competence, which allows for the transport of exogenous DNA into cells (Dubnau, 1991). CSP derived from *S. mutans* has been reported to induce the bacteriocin (mutacin IV) gene, and it has been shown that *S. mutans* possibly utilizes the bacteriocin to acquire the ability to transform DNA from other streptococcal species, such as *S. gordonii,* living in the same ecological niche (Kreth *et al*., 2005b).

Further, quorum-sensing research should include the development of future therapy for oral infections. The AI-2 and CSP system has attracted attention as a target for weakening bacterial virulence by interfering with cell-to-cell communication. A new class of specifically targeted antimicrobial peptides (STAMPs) has recently been reported for use in a unique strategy (Eckert *et al*., 2006). The STAMPs have a two-sided structure. The first is a short homing sequence of CPS that can be as unique to a bacterium as a fingerprint and ensures that the STAMPs will find their target. The second is a non-specific antibacterial peptide that is linked chemically to the homing sequence and kills the targeted bacterium on delivery. It has been suggested that STAMPs, which were designed based on the CSP of *S. mutans*, are potentially capable of eliminating *S. mutans* from multispecies biofilms without affecting the closely related oral streptococci such as *S. gordonii* and *S. sanguinis* (Eckert *et al*., 2006).

BIOFILM AS A BARRIER AGAINST EXTERNAL FACTORS

In collaboration with each other, oral bacteria may confront oxygen, host immunity, and antimicrobial agents through dental

biofilm formation as a united barrier. Since the tooth surface is exposed to an aerobic environment, it is likely that oral anaerobic bacteria encounter residual amounts of oxygen in the early stages of dental biofilm development and periodontal pocket formation (Marquis, 1995). The survival of oral anaerobic bacteria depends on the specific tolerance of each species to oxygen (*i.e.,* through enzymes such as superoxide dismutase, oxidase/peroxidase, and catalase) and the bacterial interactions within the biofilm community. In an oxygenated and CO_2-depleted environment, *F. nucleatum* supports the growth of *P. gingivalis* by providing a capnophilic environment (Diaz *et al*., 2002). In a two-stage chemostat system, co-aggregation-mediated interactions between *F. nucleatum* and other species facilitated the survival of obligate anaerobic bacteria in an aerated environment (Bradshaw *et al*., 1998). When *F. nucleatum* was omitted from the inoculum, the viable cell counts of *P. gingivalis* and *P. nigrescens* in biofilm significantly decreased under aerobic conditions. The metabolism of aerobic and oxygen-tolerant species may reduce the concentration of oxygen to levels that can be detoxified by the obligate anaerobic bacteria (Marquis, 1995).

Oral bacteria that bind to the tooth surface exhibit a behavioral pattern different from that of free-floating or planktonic bacteria. The most notable difference between the oral bacteria in dental biofilms and the same strain grown planktonically is the increased tolerance of the former to antimicrobial agents in a mature biofilm. According to the data reported by Sedlacek and Walker (2007), the concentration of the antibiotic for inhibiting the growth of bacterial strains within their biofilms was approximately 250 times greater than that required when the same strains were grown planktonically. Owing to the heterogeneous nature of dental biofilms, it is thought that there are multiple tolerance mechanisms (Gilbert *et al*., 2002; Patel, 2005; Anderson and O'Toole, 2008). First, it is likely that extracellular matrix physically restricts the diffusion of antimicrobial agents. Second, it seems that slow growth within dental biofilms contributes to biocide resistance because of less sensitivity to growth-dependent killing. Third, there are resistance factors, such as drug-inactivating enzymes. For example, β-lactamase causes the degradation of β-lactam antibiotics. Therefore, retention of these enzymes in dental biofilm amplifies its barrier function. Moreover, the degradation of antibiotics by a neighboring bacterial species is of benefit to other oral bacteria that cannot produce the enzymes.

An antibiotic-sensitive bacterium may acquire the genes related to antibiotic resistance through horizontal gene transfer. There are 3 basic ways for the exchange of DNA between and among bacteria: conjugation, transduction, and transformation. In conjugation, a bacterium, the donor, transfers a conjugative plasmid to another bacterium, the recipient. In transduction, the transfer of DNA takes place with the aid of bacteriophages. In transformation, DNA that is located outside the cell is fragmented and then imported into the bacterial cell. Subsequently, the DNA replaces a piece of original DNA in the chromosome *via* recombination. The genes related to antibiotic resistance and toxins are known to be exchanged among bacteria by horizontal gene transfer (Sedgley *et al*., 2008; Chen and Novick, 2009). Conjugation regarding oral streptococci was demonstrated over 30 years ago (LeBlanc *et al*., 1978). In an *ex vivo* tooth model, the exchange of conjugative plasmid carrying erythromycin resistance between *S. gordonii* and *Enterococcus faecalis* was observed (Sedgley *et al*., 2008). Wang *et al*. (2002) suggested that an erythromycin-resistant shuttle plasmid present in *T. denticola* could be transformed into *S. gordonii* in artificial biofilms. Since each bacterium can easily contact a neighboring bacterial cell, it is reasonable to speculate that antibiotic resistance gene transfer may occur in dental biofilms.

CONCLUSION

For a decade, many microbiologists have been attracted to new emerging concepts such as polymicrobial diseases, heterogeneous biofilms, and multispecies communities. The recent advent of molecular technologies—namely, the 16S rRNA gene clone library, fluorescence *in situ* hybridization, and checkerboard DNA-DNA hybridization—has shed new light on dental biofilm research. We now have a much clearer view of the diversity of oral bacteria present in the human oral cavity. Nevertheless, the available information on dental biofilms remains limited. These technologies have allowed for a fragmented observation of these communities, but a full picture of the bacterial interactions and their functions is still lacking. Furthermore, many bacterial species detected in dental biofilms remain uncultured. To further our understanding, a combination of multiple approaches, ranging from the investigation of pure cultures and *in vitro* biofilm model systems to animal model and human investigation studies, should be undertaken. The development of technologies that enable us to analyze putative functions and metabolisms of a complete dental biofilm may be necessary. Such efforts could contribute to the elucidation of ecological constraints that govern multispecies communities, and help develop novel methods of controlling dental biofilms.

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