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VIRULENCE PROPERTIES OF *STREPTOCOCCUS MUTANS*

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1. ABSTRACT

Streptococcus mutans is considered one of the primary causative agents of dental caries and can also be a source of infective endocarditis. The main virulence factors associated with cariogenicity include adhesion, acidogenicity, and acid tolerance. Each of these properties works coordinately to alter dental plaque ecology. The ecological changes are characterized by increased proportions of *S. mutans* and other species that are similarly acidogenic and aciduric. The selection for a cariogenic flora increases the magnitude of the drop in pH following the fermentation of available carbohydrate and increases the probability of enamel demineralization. This review focuses on the bacterial components that contribute to each of the major virulence properties. Further understanding of how these components work together in the development of dental caries will be aided by the recent completion of the sequence of the *S. mutans* genome and experimental designs that model the dental plaque biofilm.

2. INTRODUCTION

The bacterial colonization of human teeth begins immediately upon tooth eruption. Salivary proteins and glycoproteins coat the enamel surface forming the acquired enamel pellicle (1,2) to which primary plaque colonizers can specifically adhere (3). Most of these early colonizers are species of *Streptococcus*, though eventually dental plaque becomes home to hundreds of bacterial species (4-7). Many of these species are capable of fermenting dietary carbohydrates and secreting acid as a byproduct. These properties led Miller in 1890 to propose the chemicoparasitic theory of dental caries (8). This theory, later described as the non-specific plaque hypothesis (9), posited that the decalicification of enamel, or dental caries, was a consequence of the cumulative acid production of dental plaque bacteria. Today, many hygiene practices are still based on the principle that plaque is bad and must be removed. But because teeth are exposed to a non-sterile environment it is inevitable that they will harbor a normal flora. In many instances the presence of a normal flora is protective against colonization with more virulent bacterial species and so it seems peculiar that humans would have evolved a normal flora that predisposed them to the loss of their teeth.

In 1924 Clarke isolated a bacterial species from carious lesions that looked like a mutant form of a coccus, and so he designated it *Streptococcus mutans* (10). The association of S. mutans with dental decay was not generally recognized, however, until dental researchers in the 1960s revived interest in the organism. Since then several studies confirmed the association of S. mutans with carious lesions and longitudinal studies followed the ascendancy of *S. mutans* on sites that eventually became cariogenic (11,12). Corroborating the clinical studies were experiments in animal models like the germfree rat (13,14). This model was instrumental in highlighting the requirement for bacteria in dental decay. Mono-infected rats were used to evaluate the cariogenic potential of several plaque species and *S. mutans* claimed the top position among cariogens (15). Out of this research was born the "specific plaque hypothesis" which proposed that particular plaque species, such as *S. mutans*, were primarily responsible for dental decay (16). With advances in taxonomy it became apparent that *S. mutans*-like isolates actually represented several species (17) that collectively became known as the mutans streptococci (MS). *S. mutans* and *Streptococcus sobrinus* are considered the primary human pathogens (12).

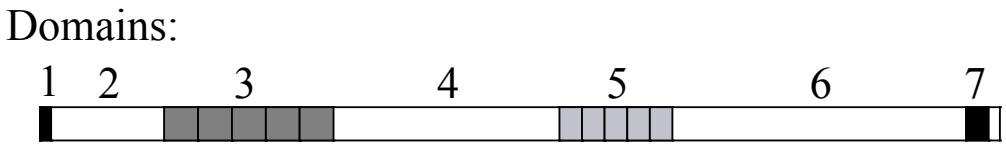

Figure 1. The antigen I/II family of proteins share seven domains. The signal peptide, 1; a highly-charged amino terminal region, 2; multiple copies of an alanine-rich repeat, 3; a variable region, 4; multiple copies of a proline-rich repeat, 5; a carboxyl terminal domain, 6; and a cell wall anchor, 7 (34-36).

Despite strong evidence for the role of the MS, there are nonetheless instances in which dental caries occur in the absence of high proportions of MS and instances where a high proportion of MS exist in the absence of caries. These anomalies form the basis for ongoing debate over the precise etiology of dental caries (18). Some investigators remain unconvinced of the specific plaque hypothesis and feel that the key factor in dental caries susceptibility is the ecological balance of metabolic groups within dental plaque (19). For example, a higher proportion of arginolytic bacteria capable of producing alkalai would neutralize the acidogenic capabilities of plaque bacteria thereby reducing the probability of demineralization (19). However, this scenario is not necessarily incompatible with a specific role for *S. mutans* and other MS. The specific plaque hypothesis does not exclude the influence of variables such as salivary flow, tooth anatomy, host immunity, genetics, oral hygiene, or contributions from other oral microbes, all of which will influence plaque ecology. For some individuals the coalescence of these variables may allow the development of dental caries with minimal contribution from MS just as the status of a host can lead to opportunistic infections with organisms that are normally harmless. It should not be surprising that dental caries, like many other diseases, could have multiple etiologies. However, a primary variable for caries occurrence has been diet, particularly sucrose consumption (12). Frequent sucrose intake has also been implicated in shaping plaque ecology by providing a substrate for the synthesis of adhesive glucans by the MS thereby promoting their colonization and accumulation (12). The acid tolerance of the MS further enhances their accumulation as well as the accumulation of other acid-tolerant species such as the lactobacilli (20). Since these species are also highly acidogenic, the introduction of any fermentable dietary carbohydrate results in a greater than normal drop in plaque pH and an increased time below the critical pH for decalcification (21,22). Given this scenario researchers have focused on the adhesion, acidogenicity, and acid-tolerant properties of the MS. Since most virulence properties are shared among the various MS, this review will focus on *S. mutans* as a paradigm for the virulence of dental caries.

3. ADHESION

The adhesion of *S. mutans* within dental plaque can be mediated via sucrose-independent and sucrose-dependent means. Sucrose-independent adhesion to salivary components within the acquired enamel pellicle may initiate the attachment process, but sucrose-dependent adhesion may be primarily responsible for establishing colonization to the tooth surface. Sucrose intake is one of the factors that correlates with the level of infant colonization (23). Adhesion to pre-formed glucan on the tooth surface may also facilitate colonization (24). *S. mutans* is most frequently transmitted to infant children from their mothers, and *S. mutans* can generally be recovered from the oral cavity following a window of infectivity (25). The ability of *S. mutans* to synthesize glucans from sucrose increases the efficiency of adhesion and enhances the proportion of *S. mutans* within dental plaque. Thus, sucrose-dependent adhesion plays a prominent role in initiating the changes in plaque ecology that can lead to dental caries.

3.1. Sucrose-Independent Adhesion

Sucrose-independent adhesion of *S. mutans* is thought to be most profoundly influenced by antigen I/II, a 185kDa surface protein. Similar proteins are found on most oral streptococci (26) and have been designated by a variety of names including P1, SpaP, Sr, PAc, and antigen B. Proteins within the antigen I/II family share structural similarity based on amino acid domains (Figure 1), but display variable functionality with respect to binding salivary agglutinins, salivary pellicle components, and other plaque bacteria (27,28). The alanine-rich and proline-rich domains are thought to be primarily responsible for the interaction between antigen I/II and salivary components (29-33).

The evidence for a role for antigen I/II in adhesion is based primarily on studying the adhesion of *S. mutans* to saliva-coated hydroxyapatite (37-39). A notable example is provided by Bowen *et al*. (40) who demonstrated that an isogenic mutant lacking P1 (antigen I/II) did not bind as well as the wild-type to saliva-coated hydroxyapatite, but bound equally well as the wild-type to saliva-coated hydroxyapatite that also contained in situ synthesized glucan. Additionally, the P1 mutant was as cariogenic as the wild-type in rats fed a high (56%) sucrose diet.

A subsequent report by Crowley *et al*. (41) that a P1 mutant was less virulent in rats fed a 5% sucrose diet might have seemed contradictory. But a reported role for antigen I/II in dentinal tubule invasion (42) provided a potential explanation for the reduction in virulence in rats fed a low sucrose diet and supported the designation of P1 as a virulence factor. Additionally, we have noted (unpublished data) that the loss of P1 results in a change in the structural architecture of *in vitro*-formed biofilms. If a similar change occurs *in vivo* it might have an influence on virulence.

3.2. Sucrose-Dependent Adhesion

The action of glucosyltransferases (GTFs) in the synthesis of glucans is the major mechanism behind sucrose-dependent adhesion. The GTFs possess a sucrase activity that results in the splitting of sucrose, the only natural substrate for the GTFs, into glucose and fructose (43). The glucose moiety is then added to a growing polymer of glucan. *S. mutans* possesses three GTFs encoded by *gtfB*, *gtfC*, and *gtfD*. Other members of the MS, for example *S. sobrinus*, harbor four genes encoding GTFs. Collectively, the GTFs synthesize both water-soluble and water-insoluble glucans. The water-soluble glucan is a predominantly linear polymer linked by alpha-1,6-glycosidic linkages that resembles dextran. The water-insoluble variety, also called mutan, has a higher degree of branching and predominantly alpha-1,3-linkages. Both types of polymers are thought to contribute to sucrose-dependent colonization and caries but the water-insoluble glucan may be of primary importance for smooth surface caries (44). Several groups have demonstrated that *S. mutans* strains inactivated in one or more *gtf* genes have diminished virulence when tested in rodent models of caries (45-47).

The ability of glucan to facilitate adhesion of *S. mutans* may be due to hydrogen bonding of the glucan polymers to both the salivary pellicle and the bacteria (48). *In vitro* in the presence of sucrose, *S. mutans* becomes coated with glucan. Presumably, sucrose-dependent adhesion can involve binding by glucan-coated bacteria, or attachment of *S. mutans* to glucan present within the dental plaque (24,49,50). This glucan could be synthesized by extracellular GTFs that bound the salivary pellicle, *S. mutans* that had previously adhered via sucrose-independent means, or perhaps by other oral streptococci. It is not known why *S. mutans* requires multiple GTFs, but there is evidence that the different GTFs have differing affinities for the bacterial surface or salivary pellicle (51), and that a particular ratio of each is necessary for optimal sucrose-dependent adhesion (52).

The basis for glucan-binding by individual bacteria is still subject to speculation. The search for a cell surface glucan receptor led to the isolation of several non-enzymatic glucan-binding proteins and confirmed the glucan-binding abilities of the GTFs. However, most of these proteins are exported and lack cell wall anchoring motifs. The glucan-binding domain (GBD) of the GTFs (53) consists of a carboxyl terminal region of amino acid repeats that is shared by at least two non-enzymatic glucan-binding proteins, GbpA (54) and GbpD (55). It is possible that another non-enzymatic glucan-binding protein, GbpC, acts as a cell surface glucan receptor, though an aggregation phenotype attributed to GbpC is only observed under certain stressful growth conditions (56). Another possibility is the wall-associated protein WapA, originally antigen A (57). Although it has not been described as a glucan-binding protein the inactivation of the *wapA* gene resulted in a reduction in aggregation and adhesion to a smooth, glass surface (58).

Alternatively, the WapA may make a contribution to sucrose-dependent adhesion indirectly. The disruption of other genetic loci also has been correlated with diminished sucrose-dependent adhesion (59). The mechanism of contribution of these loci is uncertain, but may border on the interruption of general cell housekeeping functions.

3.3. Non-enzymatic Glucan-Binding Proteins

As described above, the search for a cell surface receptor for glucan led many groups to screen *S. mutans* culture supernatants or cell extracts, often via affinity chromatography, for proteins capable of binding glucan or mediating dextran-dependent aggregation. In addition to the GTFs, non-enzymatic glucan-binding proteins (GBPs) were recovered. The first of these was GbpA (60); subsequently GbpB (61), GbpC (56), and GbpD (55) were described. Several GBPs have been noted in other MS as well. Interestingly, non-MS oral streptococci may possess one or more GTFs but non-enzymatic GBPs have not been identified among these species. Since glucan plays such a prominent role in the caries process, proteins capable of binding glucan were hypothesized to contribute to sucrose-dependent adhesion and possibly to the cohesive nature of the dental plaque biofilm. There is now evidence in support of these ideas, though strain differences and differences in model systems may delay the task of assigning a precise role to an individual GBP.

A case in point is GbpA. *S. mutans* strain UA130, engineered to no longer make GbpA, was actually more virulent within a gnotobiotic rat model fed 5% sucrose, and adhered in higher proportion to glass surfaces *in vitro*, than the wild-type (62). These results correlated with a change in the overall architecture of biofilms formed *in vitro* by the knockout strain (63). The change included a reduction in microcolony peak height perhaps indicating that GbpA adds strength to the integrity of taller microcolonies. By contrast, Matsumura *et al.* (64) inactivated the *gbpA* gene within strain MT8148 and reported both a reduction in sucrose-dependent adhesion and a reduction in caries in a specific pathogen-free rat model.

Evidence also supports a contribution from GbpC to plaque biofilm development. But, like GbpA, enigmas remain regarding the exact nature of GbpC's participation. The GbpC was reported to promote dextran-dependent aggregation but only under certain stress conditions (56). Dextran-dependent aggregation is an *in vitro* observation of clumping that occurs following the addition of exogenous dextran to a culture of bacteria. Its *in vivo* relevance may consist of the promotion of colonization via aggregation of bacteria in the presence of glucan. Sato *et al*. (65) speculate that the expression of GbpC only under defined conditions may be indicative of autoaggregation during times of nutrient limitation within the oral cavity. A clinical isolate defective in GbpC synthesis was shown to have reduced cariogenicity in rats (66). While the results of this study must be interpreted cautiously since the test strain was also defective in the *gbpA* gene, Matusumura *et al.* (64) reported that an isogenic GbpC-defective mutant was deficient in sucrose-independent adhesion, sucrose-dependent adhesion, and caries formation.

Table 1 Additional Putative Virulence Factors

Gene Product	Hypothesized Function	Virulence Testing
Ftf	Fructosyltransferase catalyzes the synthesis of fructans; perhaps an energy reserve (84)	Variable results (45, 82, 83)
FruA	Fructanase may break down fructans for energy use (84).	Loss does not alter virulence (82, 85)
DexA	Extracellular dextranase perhaps contributes to glucan synthesis or the breakdown of glucans (87, 88)	Loss associated with decreased virulence in some rat models (86).
Dlt1-4	Accumulation of intracellular polysaccharide; energy reserve (89)	Loss decreases virulence (89); over-expression increases virulence (90).

The GbpB has turned out to be identical to the immunodominant glycoprotein IDG-60 and the general stress protein GSP-781 (67-69). Loss of IDG-60 affects the stability of the cell wall resulting in a pleopmorphic shape and retarded growth rate (68). Interestingly, clinical isolates produce different levels of GbpB and the amount correlates with their biofilm-forming abilities (67).

The role of GbpD in virulence has not yet been tested.

3.4. Biofilm Formation

The traditional methods of bacterial investigation have utilized batch cultures or continuous culture vessels in which bacteria have been grown in a planktonic state. However, dental plaque is a biofilm. Many variables associated with growth in a biofilm, including adhesion, nutrient flow, and coaggregation can influence growth rate, gene expression, and quorum sensing in ways that differ from life in a planktonic environment. Consequently, recent investigations have begun to examine the expression of virulence genes within biofilms. An excellent example of the variability of gene expression in response to the environment is the expression of the *gtfB* and *gtfC* genes. Evidence suggests that these genes can be both independently transcribed and co-transcribed. Using the chloramphenicol acetyltransferase (*cat*) reporter gene, Hudson and Curtiss (70) demonstrated increased expression of the *gtfB/C* genes in response to sucrose or when bound to an artificial tooth pellicle. Burne *et al*. (71) saw a similar stimulation with sucrose in biofilms, though the time-frame of the increase and the CAT specific activity varied between 48 hr and 7-day biofilms. But a plasmid-based luciferase reporter system found no change in *gtfB* and *gtfC* expression in the presence of sucrose (72). And Fujiwara *et al*. (73) reported that sucrose reduced the expression of *gtfB* and *gtfC* when tested using batch cultures and reverse-transcription (RT)-PCR. These studies highlight the difficulty inherent in attempting to model the *in vivo* environment and trying to understand the precise contributions of even a single virulence factor.

Another approach to investigating *S. mutans* virulence has been to search for genes required for biofilm development. This approach casts a wider net than focusing on carbohydrate metabolism and has yielded some interesting results. Disruption of genes involved in various two-component and quorum sensing signaling systems have affected the biofilm-forming capacity of *S. mutans* (74-79). Similar observations have been made in other bacterial species (80,81). These data suggest that genes encoding the GTFs, and genes encoding other exported proteins directly involved in biofilm development, may be globally regulated.

3.5. Carbohydrate Metabolism

Besides the proteins and enzymes that contribute to sucrose-dependent adhesion, there exist other proteins involved in the metabolism of sucrose, glucans, or other carbohydrates that are considered potential virulence factors. These include a fructosyltransferase (Ftf), a fructanase (FruA), an extracellular dextranase (DexA), and proteins responsible for intracellular polysaccharide accumulation (Dlt1-4). Table 1 lists the proteins, their proposed functions, and results of virulence testing.

Sucrose can enter *S. mutans* via three different transport mechanisms (91). One of these, the multiple sugar metabolism system, is encoded by an operon that includes a sucrose phosphorylase (GtfA), and an intracellular dextranase (DexB) (92). This system is capable of transporting isomaltosaccharides that can be generated by the action of the extracellular dextranase DexA (91). In an analogous manner to the glucans, the Ftf can synthesize fructan from sucrose, and FruA can liberate the fructose for transport into the cell. Nevertheless, there is no firm evidence that *S. mutans* can propagate solely on a diet of extracellular glucan or fructan.

4. ACIDOGENICITY

S. mutans contains a complete glycolytic pathway and can produce lactate, formate, acetate, and ethanol as fermentation products (93). The precise distribution of fermentation products will depend on growth conditions with lactate being the major product when glucose is abundant (94). Strains deficient in lactate dehydrogenase (LDH) display reduced cariogenicity (95,96) and the absence of lactate dehydrogenase (LDH) is lethal (97). Indeed, a genetically modified strain deficient in lactate dehydrogenase is being considered for replacement therapy as a means of out-competing more cariogenic strains of *S. mutans* (98,99).

The velocity with which *S. mutans* produces acid when tested at a pH in the range from 7.0 to 5.0 exceeds that of other oral streptococci in most instances (100). The relative acidogenicity of *S. mutans* can vary from one isolate to another, and strict correlations between acidogencity and caries experience is lacking (101). Nonetheless it is generally thought that the acidogenicity of *S. mutans* leads to ecological changes in the plaque flora that includes an elevation in the proportion of *S. mutans* and other acidogenic and acid-tolerant species. This

cariogenic flora will reduce plaque pH to lower levels than will a healthy plaque flora upon the ingestion of fermentable carbohydrate, and the recovery to a neutral pH will be prolonged (12,102,103). Sustained plaque pH values below 5.4 favor the demineralization of enamel and the development of dental caries.

5. ACID-TOLERANCE

Accompanying the acidogenicity of *S. mutans* is its aciduricity or acid-tolerance. *S. mutans* retains glycolytic capabilities even at pH levels that are growth inhibitory (as low as pH 4.4) (104). As with its acidogenicity, it is the extent of its aciduricity, rather than its novelty, that distinguishes *S. mutans* from the other oral streptococci. The acid tolerance of *S. mutans* is largely mediated by an F_1F_0-ATPase proton pump but also involves adaptation with an accompanying change in gene and protein expression. Together they constitute the acid-tolerance response (ATR). *In vitro* the ATR has been shown to protect the organisms from a sub-lethal pH challenge (105) and acid shock or growth at acidic pH has been associated with changes in the expression of over 30 proteins (106,107). The full scope of the changes associated with acid tolerance is still under investigation.

Evidence is also accumulating that acid-tolerance may be aided by the synthesis of water-insoluble glucan and the formation of a biofilm. *S. mutans* cells within a biofilm were better able to survive an acid challenge than planktonically grown bacteria (108). This may be related to quorum sensing systems efficiently inducing the ATR, and physical characteristics of the biofilm. Hata and Mayanagi (109) report that the speed of diffusion of hydronium ions is proportional to the amount of water-insoluble glucan produced by *S. mutans*. These results highlight the connection between different virulence properties of *S. mutans* and indicate that the role of glucan extends beyond promoting adhesion. These differences may also reflect the basis for why the glucan-synthesizing capacity of *S. mutans* evolved differently than those for the other oral streptococci.

5.1. Maintaining Intracellular pH

As noted above, the acidification of the external environment is a consequence of the excretion of acid by-products of metabolism. External protons are capable of permeating streptococcal membranes and acidifying the cytoplasm. Glycolytic enzymes and other cellular functions are sensitive to inhibition by low intracellular pH. Therefore, the activity of the membrane-bound proton-translocating ATPase, or F-ATPase, is critical for establishing and maintaining a pH gradient across the cytoplasmic membrane. Bender *et al*. have shown that the efficiency of this enzyme at different pH values correlates well with the acid-tolerance of various oral streptococci (110). As the pH falls, there is increased activity of the *S. mutans* F-ATPase (111,112) which helps maintain a pH gradient of approximately one pH unit (111,113). At the same time the fatty acid profile of the membrane shifts, decreasing permeability to protons (114), and excretion of acidic end products increases (94).

Catabolites other than glucose may counter the effects of the F-ATPase when introduced via symport along with a proton (115). It is interesting to speculate whether the presence of an extracellular glucan-digesting dextranase can ensure a supply of glucose during times when exogenous glucose is not available.

5.2. Induction of DNA Repair

It has already been noted that several changes in gene and protein expression accompany acid shock or acid adaptation. Some of these undoubtedly complement the activity of the F-ATPase in moderating intracellular pH. But parallels to the heat shock response can also be drawn including induction of chaperones and DNA repair functions. Quivey *et al*. (116), working with a *recA* strain of *S. mutans*, observed that the mutant could be acid-adapted to UV resistance (to killing) nearly equal to that of the wild-type strain. This led the investigators to postulate the existence of a RecA-independent, acid-inducible DNA repair system. Subsequently, Hahn *et al*. (117) identified a gene encoding an alkaline phosphatase (AP) endonuclease that was induced at low pH. Insertional inactivation of the gene nearly completely abolished AP endonuclease activity in *S. mutans* (118). Evidence for the participation of other DNA repair systems, such as nucleotide excision repair, was provided by Hanna *et al*. (119) who showed that a *uvrA* mutant had diminished growth at pH 5.0. Similar to the *recA* strain, acid adaptation of the *uvrA* strain enhanced resistance to UV irradiation, but the organism could not survive exposure to a sub-lethal pH.

5.3. Other Facets of Acid Adaptation

The ability of *S. mutans* to adapt to a low pH environment can involve the induction of certain genes, repression of others, and a requirement for certain cellular components that may be constitutively expressed. The discovery of factors correlated with acid shock and adaptation will reflect the methodology employed. Random mutagenesis can be used to isolate mutants that lose acid tolerance. Two-dimensional protein gels or microarrays can be used to document semi-quantitative changes in protein expression and gene transcription, respectively. Some of these approaches have already been carried out and have focused not only on the requirements for successful acid adaptation, but also on the effect of low pH on known virulence factors (120).

Transposon mutagenesis has been used to isolate acid-sensitive mutants deficient in diacylglycerol kinase (121), lipoteichoic acid synthesis (122), and protein translocation (123). Each of these mutations seemed to affect membrane integrity and the ability to maintain a proton gradient. Diacylglycerol kinase is an enzyme involved in phospholipid turnover and therefore affects membrane structure. The *sat* (secretion and acid tolerance) locus consists of five open reading frames including homologues to the *Bacillus subtilis ylxM* and *ffh* genes. YlxM is a protein of unknown function; Ffh is similar to the eukaryotic signal recognition particle (SRP) and may be involved in the maintenance of membrane protein composition during the ATR (124,125). Unlike other bacterial species, an *ffh* mutation did not result in the loss

of cell viability. The mutant, however, possessed a two-fold reduction in F-ATPase activity at pH 5.0 relative to the parental strain, an effect thought to be related to loss of Ffh chaperone function for moving newly synthesized proteins to the cytoplasmic membrane (125). Loss of the ability to add D-alanine esters to lipoteichoic acid was associated with loss of an ATR likely due to an increase in proton permeability (122).

The effect of low pH on heat shock genes has been investigated by Burne and colleagues (126-128). Acid shock and adaptation were associated with elevated levels of message and protein for the chaperones DnaK and GroEL. Additionally, inactivation of regulatory genes *hrcA* and *clpP* from these heat shock operons led to loss of acid tolerance and altered protein expression. These data suggest that pH, like other environmental stresses, can induce a similar array of proteins to mitigate and overcome cellular damage.

Recently, Li *et al*. (76,129) have reported that intercellular signaling and quorum sensing influence the ATR. In one instance a higher cell density was associated with greater resistance to a sub-lethal pH. A soluble, protease and heat sensitive factor from high-density culture filtrates helped modulate the ATR. Disruption of the *comC*, *-D*, or *–E* genes from the competence locus also resulted in a diminished ATR (129). In another instance allelic replacement mutagenesis was used to knock out a putative two-component signaling system. The resultant mutants formed biofilms with a reduced biomass and altered architecture. Loss of the sensor protein resulted in a loss of resistance to low pH (76). These signaling systems may function in allowing the bacteria to monitor environmental pH and initiate the ATR when pH drops. Signaling mutants have been reported to alter biofilm formation in other species as well (80,81). The use of microarrays will facilitate the monitoring of global cellular changes that occur in response to changes in biofilm architecture, nutrient availability, signaling, pH, or any number of environmental variables. Systematic studies might then elucidate the contributions of individual bacterial components as well as their inter-relatedness.

6. ENDOCARDITIS

Although the primary focus of *S. mutans* virulence is dental caries, this species is also isolated from cases of infective endocarditis. Various dental procedures can lead to a transient bacteremia with oral flora organisms. About 20% of the endocarditis cases attributed to viridans streptococci are due to *S. mutans* (12,130). Whether the frequency of causation parallels the prevalence of the organism in the oral cavity, or is influenced by distinct virulence factors is uncertain. It is believed that organisms in the blood can bind to a pre-existing injury to the endothelium that might expose extracellular matrix (ECM) components such as fibronectin, laminin, and collagen. Efforts to isolate ECM binding proteins have uncovered a fibronectin binding protein of approximately 130 kDa (131), and determined that a strain lacking the major surface antigen AgI/II displays reduced binding to fibronectin, collagen and fibrinogen (132). Fibronectin binding did not diminish in strains lacking two or three GTFs (131), though glucan has previously been cited as contributing to endocarditis via fibrin adhesion and by being antiphagocytic (133). A glucan-deficient strain of *Streptococcus gordonii*, however, did not possess reduced virulence in a rat endocarditis model (134). Perhaps adhesion by a glucan-deficient *S. gordonii* is more efficient than by a glucan-deficient *S. mutans*. Alternatively, the glucan associated with *S. mutans* may function differently than glucan associated with other oral streptococci. Glucan is a major factor in sucrose-dependent adhesion of *S. mutans* to the tooth surface yet *S. salivarius* primarily colonizes oral soft tissues despite possessing five GTFs.

7. PERSPECTIVE

The primary virulence factors of *S. mutans* are its ability to utilize sucrose to promote adhesion and accumulation, its acidogenicity, and its acid tolerance. As with most host-microbe interactions, these attributes only provide the organism with pathogenic potential. The physiology of the host and the overall oral flora ecology may or may not suppress this potential.

Most measures to interfere with the development of dental caries are still non-specific and designed to reduce the plaque population. These measures, including the use of fluoridated toothpaste and mouthrinses, are very effective when done conscientiously, especially when accompanied by reduced sucrose intake. However, this ideal is often difficult to meet in children. The recent completion of the sequence of the *S. mutans* genome (93), together with model systems for the dental plaque biofilm should propel investigations examining the molecular basis of dental caries and lead to new treatments or preventative protocols that reduce the incidence of disease.

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