

Transmission, diversity and virulence factors of *Streptococcus mutans* genotypes

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Abstract: Dental caries is an infectious and transmissible disease, in which many genetic, environmental and behavioral risk factors interact. The mutans streptococci (MS), mainly *Streptococcus mutans* and *Streptococcus sobrinus* are the microorganisms most strongly associated with this disease. The main virulence factors associated with MS cariogenicity include adhesion, acidogenicity and acid tolerance. These properties work together to modify the physico-chemical properties of the biofilm, resulting in ecological changes in the form of increased proportions of *S. mutans* and other acidogenic and aciduric species. In addition, reports of higher numbers of *S. mutans* genotypes with increased virulence in caries-active subjects suggest the importance of microenvironmental factors in increasing the risk of caries. This review focuses on the transmission and establishment of different genotypes of *S. mutans* and the role they play in the development of dental caries. (J. Oral Sci. 47, 59-64, 2005)

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Introduction

Dental caries is a transmissible infectious disease in which mutans streptococci (MS) play the major role. As in many infectious diseases, colonization by pathogens is required before the disease can occur. MS are generally considered to be the principal etiological agent of dental caries (1).

There is a range of virulence factors important for the establishment of MS in the complex microbial community of dental biofilm. Studies of the virulence factors of *S. mutans* and their correlation with species biodiversity are fundamental to understanding the role played by colonization by different genotypes in the same individual, and the expression of characteristics that may or may not influence their virulence capacity and survival ability under different environmental conditions.

Colonization, transmissibility and stability of *S. mutans* in the oral cavity

Studies using phenotyping and/or genotyping methods strongly suggest that the mother is the major primary source of infection for children who carry *S. mutans* and/or *S. sobrinus* strains (2-10), and the saliva is the principal vehicle by which transfer of MS may occur (10-12). However, detection of genotypes that are not found in children's mothers or other family members indicates that *S. mutans* and/or *S. sobrinus* may also be acquired from other sources (5,7,8,10,12).

Furthermore, variability in transmission can be associated with children's individual susceptibilities, including the period defined as the window of infectivity (13), which was reported to occur earlier in Brazilian children (14,15); the number of erupted teeth (13,15,16); the emergence of

molars (13); the presence of enamel hypoplasia (17); sucrose consumption (14); the action of nonspecific factors of the salivary and mucosal immune systems (3); and immunological conditions in children (18).

It has been observed that children harbor one to five distinct genotypes of MS at different ages (4-7,10,12,19-21). The MS genotypic diversity in four sampling sites (saliva, tongue dorsum, alveolar ridge mucosa, dental biofilm) from children's oral cavities was shown to be homogeneous; however, the dental biofilm was an important site given the greater number of MS genotypes and strains isolated (10). Studies of the initial colonization by MS indicate that these bacteria require non-shedding tooth surfaces to become established in the oral cavity (16). More sensitive methods using DNA specific probes indicate that the retentive surfaces of the tongue dorsum may function as a reservoir for posterior tooth colonization (22).

Nevertheless, few data are available about the stability of the genotypes detected at the time of initial acquisition. Previous studies suggested that early colonizing MS strains might be stable in the mouth for many years, although some genotypes detected in childhood could not be recovered in later years (8-10,19,23).

Klein et al. (10) identified a total of 52 distinct genotypes in children, but mothers transmitted only 16 of them. However, a tendency toward effective stability of genotypes transmitted by mothers was observed (10). One explanation for this selective colonization is that the immune response to a successfully colonizing maternal genotype may interfere with colonization of other genotypes, making colonization less likely (3,18). The maternal role in infection also suggests that the contacts responsible for salivary transmission of these organisms can also provide frequent immunological exposure to bacterial antigens (18). The mechanisms of action of salivary IgA antibodies against MS include interference with their sucrose-independent and sucrose-dependent attachments to, and accumulation on, tooth surfaces, as well as possible inhibition of their metabolic activities (24).

Alaluusua et al. (25) demonstrated a high degree of homology between MS strains recovered among members of the same family, indicating both vertical and horizontal routes of transmission, and persistent colonization by early acquired MS until young adulthood. It was also shown that isolates that had similar genetic fingerprints (transmitted strains) had similar expression of glucosyltransferase (GTF). One case was reported of a similar *S. mutans* genotype colonizing two children from unrelated families attending the same Brazilian nursery (12). In addition, a study of 39 Japanese children from a day

nursery found that six of them shared the same strain type of MS (26). Taken together, these results suggest that horizontal transmission may also occur.

Some previous work has shown greater genetic variability of *S. mutans* in nursing-bottle caries than in healthy children; however, a positive relationship between caries activity and the genetic diversity of *S. mutans* is still controversial. Alaluusua et al. (4) suggested that caries-active children with high sucrose consumption carry greater ribotype diversity of MS compared with caries-free children. On the other hand, Kreulen et al. (21) showed a negative correlation between caries activity and genotypic diversity.

The ability of bacteria to survive and persist in a given environment will depend, in part, on their inherent genetic plasticity, which determines their ability to respond to fluctuating local environmental conditions or stresses (27). The microbiota resident in the oral biofilm are subjected to many variable environmental stresses, including the availability or lack of nutrients, acidic pH, and exposure to organic acids (28,29). Paddick et al. (30) showed that the proportions of MS and lactobacilli were elevated in the biofilm of caries-active subjects, while *A. naeslundii* isolates formed a significantly greater proportion of the microbiota in samples from caries-free subjects. These observations support the assertion that the biofilm samples from the two subject populations were exposed to different environments and, consequently, to different stresses.

In a recent study of young adults, Redmo-Emanuelsson et al. (31) found a maximum of seven genotypes in subjects who had previous caries experience. This study is consistent with our findings of a maximum of eight genotypes in caries-active young subjects using AP-PCR (32). The existence of several genotypes in biofilm could merely be a consequence of favorable circumstances for MS in biofilm, but it is possible that the simultaneous action of different genotypes with distinct virulence potential further increases the risk of caries. Nascimento et al. (33) found that *S. mutans* was more prevalent in coronal than root dental biofilm, but no difference was found between root and coronal caries lesions. Furthermore, the maximum number of *S. mutans* genotypes found together at a specific site was five and the same genotype could be found at more than one oral site.

Adhesion

Biofilm development occurs in two distinct phases: during the first, bacterial surface proteins interact with host or bacterial products adsorbed on the tooth surface. In the second phase, biofilm forms as bacteria accumulate by aggregation with the same or other species and produce

an extracellular polysaccharide matrix (34). Genetic differences may relate to differences in virulence between MS strains. One important characteristic of *S. mutans* in promoting caries development is the ability to adhere firmly to the tooth surface in the presence of sucrose (25), and this adherence is mediated mainly by the enzymatic action of the GTF enzymes (1,35). These enzymes are considered fundamental for the virulence of *S. mutans* in the pathogenesis of dental caries (36). Previous research has shown differences in virulence factors among *S. mutans* isolates (32,37,38). Differences in the synthesis of water insoluble-glucan (WIG) or biofilm formation between genotypes could be associated with different levels of virulence. This is important, since it has been demonstrated that WIG produced from sucrose modifies the physico-chemical properties of dental plaque, including low inorganic concentrations of calcium, phosphorus and fluoride (39) and increased porosity of the dental plaque matrix (40), making it more cariogenic.

Bacteria are known to regulate diverse physiological processes through a mechanism called quorum sensing (41). The *com* system, which controls genetic competence development in response to the concentration of the competence-stimulating peptide, is also involved in biofilm formation and the biofilm architecture of *S. mutans*. This cell-cell signaling system involves several gene products encoded by *cslAB* (*comAB*) (42) and *comCDE* (43). This quorum-sensing system functions optimally when the cells are living in actively growing biofilms (43), suggesting that the cell-cell signaling system might play a role in the formation of *S. mutans* biofilms. A recent study described the effects of mutations in several *com* loci with regard to biofilm formation. The results clearly showed that inactivation of any one of the genes encoding the components of the quorum-sensing system, in particular *comC*, results in the formation of an abnormal biofilm (44). Recent studies have shown that *luxS* is involved in global regulation of physiological functions and virulence. Also, *luxS* inactivation resulted in decreased expression of several genes that encode membrane-associated proteins, including *BrpA* and *Ffh*, which have been shown to play roles in envelope integrity and acid tolerance (45).

Other bacterial components associated with the accumulation phase of MS are proteins that bind glucan. At least three *S. mutans* glucan binding proteins (Gbp) have been identified: GbpA (46), GbpB (47), and GbpC (48).

Although over the years numerous surface or secreted products of MS have been proposed as vaccine antigen candidates, attention has recently focused on three protein antigens: the surface fibrillar adhesins known as AgI/II (or SpaP, PAc), the glucosyltransferases and the glucan-

binding proteins (49). Numerous experiments in a variety of animal models comprising rodents and primates have demonstrated the induction of salivary S-IgA and circulating IgG antibodies to MS antigens by oral or intranasal immunization with AgI/II, GTF or Gbps (24,50-52). Immunization of mice with synthetic peptides (residues 301-319) from the alanine-rich region of antigen I/II suppressed tooth colonization with *S. mutans* (53). Intranasal immunization with antigen I/II, coupled with the cholera toxin B subunit, suppressed colonization of mouse teeth by *S. mutans* (53). Although the basic principle of immune protection from dental caries caused by MS has been established in pre-clinical studies, the effective application of this approach to humans needs further refinement (54). Furthermore, in experimental infection of rats, systemic or mucosal immunization with GbpB induced protective immunity to dental caries, indicating that GbpB may be an important target for the development of caries vaccines (55).

Mutacin

The role of mutacins *in vivo* is unclear, however the antimicrobial activity of these substances may confer an ecological advantage for the producing strain in bacterial communities such as dental biofilm (56), and they may also be important for the establishment of *S. mutans in vivo* (56,57).

Mutacins are peptide or protein antibiotics that are mainly bactericidal for other bacteria of the same or closely related species, as well as for other Gram-positive microorganisms, and are likely to confer an ecological advantage in diverse bacterial communities such as dental biofilm (56). The relationship between caries activity and the higher synthesis of some virulence factors by different genotypes of *S. mutans* has been demonstrated in the literature (32,37).

The mutacin activity of *S. mutans* may facilitate the transmission of the species between mother and child and increase the ratio of this species in the dental biofilm, contributing to increased risk of caries (6). However, some studies found no association between the inhibitory spectrum of mutacins and infecting levels of MS or caries incidence, suggesting that mutacin production may not be relevant in the ability of *S. mutans* to colonize the host and induce disease (58,59).

In a recent study, Kamiya et al. (60) showed distinct mutacin production profiles between *S. mutans* isolated from caries-active and caries-free individuals, which can be related to different colonization profiles described in these individuals. Mutacins could play an important biological role in the regulation and composition of dental

biofilm due to their synergistic or antagonistic activity, suggesting that wide spectrum mutacins may be more important in the colonization and stabilization of this cariogenic species, mainly in the stable niche of highly complex microbial activity (60).

Conclusion

The identification of the source of MS transmission is essential to the development of strategies for the prevention of dental caries. The early acquisition of MS by infants occurs mainly via the mother's saliva, and probably also by other sources of transmission. Immunological interception of the initial attempts of MS to colonize the tooth surface would seem to be the preferred vaccine strategy. Successful establishment of MS in infancy appears to lead to colonization of the permanent dentition by MS and their persistence into adulthood. In addition to environmental and host factors, identification of specific pathogenic genotypes of *S. mutans* that may be more virulent colonizers might predict sites that are more susceptible to disease.

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