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MUTACINS OF *STREPTOCOCCUS MUTANS*

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ABSTRACT

The colonization and accumulation of *Streptococcus mutans* are influenced by various factors in the oral cavity, such as nutrition and hygiene conditions of the host, salivary components, cleaning power and salivary flow and characteristics related with microbial virulence factors. Among these virulence factors, the ability to synthesize glucan of adhesion, glucan-binding proteins, lactic acid and bacteriocins could modify the infection process and pathogenesis of this species in the dental biofilm. This review will describe the role of mutacins in transmission, colonization, and/or establishment of *S. mutans*, the major etiological agent of human dental caries. In addition, we will describe the method for detecting the production of these inhibitory substances *in vitro* (mutacin typing), classification and diversity of mutacins and the regulatory mechanisms related to its synthesis.

Key words: *Streptococcus mutans,* mutacin, mutacintyping

INTRODUCTION AND REVIEW

The role of mutacins in transmission, colonization and/or establishment of *S. mutans*

Most *S. mutans* strains produce mutacins (bacteriocins) that are antimicrobial peptides that can inhibit mainly the growth of bacteria genetically and environmentally related. Some mutacin-like substances present broad antimicrobial spectra and are able to inhibit the growth of indicator strains of medical interest that are resistant to some commonly used antibiotics, as well as *Enterococcus faecalis, Streptococcus pyogenes, Staphylococcus aureus* and *Staphylococcus epidermidis, Escherichia coli* and mycobacteria (28, 29, 41, 49).

The broad spectra of mutacins emphasize its importance in the future pharmacology applications, although more studies are necessary to acknowledge their use as a safe antimicrobial agent (41).

The ability to produce mutacins by *S. mutans* may confer ecological advantages to producing strains on other sensitive bacteria in diverse bacterial communities such as dental biofilm and caries (4). The antagonism probably occurs at the tooth

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surface and may be important for the stability of *S. mutans in situ*.

According to Grönroos *et al*. (16), mutacin production by *S. mutans* may promote transmission of bacteria from mother to child and may have significant role in the colonization process of this species in the oral cavity due to their selective antagonism mechanisms. Theoretically, the ability to produce broad spectrum mutacins could favor the colonization of producer *S. mutans* in the complex dental biofilm, thereby increasing the risk of caries (16).

The mechanisms of mutacins in ecologic regulation of the oral microbiota are still unknown. For Rogers (52) the o role of mutcains *in vivo* is limited, because in the same niche in the oral cavity both producing and sensitive strains of *S. mutans* were isolated.

Several *in vitro* studies with salivary enzymes (19) have not proven the enzymatic inactivation of mutacins. According to Parrot *et al.* (46) enzymatic inactivation of mutacins in the oral cavity is limited due to low concentrations of proteolytic substances in this environment.

Saliva does not seem to affect the inhibitory activity of mutacins, because several *in vivo* studies have reported the most successful colonization of highly producing strains, in the oral cavity of rats and humans (25, 58). Some groups of researchers support the idea of using mutacins in the control and prevention of dental caries (22, 25).

In 1985, Hillman *et al*. (23) observed the persistent colonization of the strain *S. mutans* JH1001, a producing strain of mutacin with high inhibitory spectra, in the oral cavity of humans, after four or more exposures to it. This experiment showed a positive correlation between the potential for infection *in vivo* and the levels of mutacin production *in vitro*. Based on the selective and ecological properties of mutacins *in vivo*, Hillman *et al*. (25) developed bacterial replacement therapy for preventing the development of dental caries in animals and humans. Basically, by constructing a mutant strain of *S. mutans* BCS3-L1, bacteriocin-producing with broad-spectrum *in vitro* (mutacin 1140) and defective in the synthesis of lactic acid, it was possible to effect the colonization of the producing strain of low cariogenicity in oral cavity of rats and adult humans. The biological activity of mutacins *in vivo* reduced the rate of caries possibly due to inhibition of more cariogenic *S. mutans* competitors in the oral cavity, however, many experiments in humans still should be made so that the hypothesis of replacement therapy will be substantiated, as well as highly safe and effective in the prevention of dental caries (25).

Although Mota Meira *et al*. (41) demonstrated, for the first time, the *in vivo* efficacy of a mutacin (B-Ny266) against an experimental intraperitoneal infection by *S. aureus* in a mouse model, the role of mutacins *in vivo* in the oral cavity is still unknown. The regulatory mechanisms involved in mutacins production, which will be described later in this review, suggest that producing strains can benefit in environments with highly complex microbial population, such as dental biofilm and caries.

Another important feature that can show the activity of mutacins in the oral cavity is based on the adsorption capacity of active molecules of bacteriocins on the surface of sensitive bacteria through specific or not specific receptors (61). This characteristic of adsorption and aggregate formation can increase the antimicrobial efficiency of mutacins *in situ* while maintaining the active molecule in cells sensitive or not adhered to the dental biofilm.

MUTACIN TYPING

About 70-100% of *S. mutans* strains can produce mutacin-like substances (5, 6, 16, 26, 53) and different from the detected frequency of production, are probably due to the use of different indicator strains and differences in culture media and conditions for testing (53).

The bacteriocin typing is based on the pattern of bacteriocins production against a set of strains, as well as the

sensitivity profile for different inhibitory substances (30, 53). This phenotypic method emerged in the 70s during the race in pursuit of global scientific epidemiological investigation of cariogenic streptococci and allowed grouping strains with similar profiles of production and / or sensitivity to bacteriocins, and made it possible to demonstrate the heterogeneity of species (30). According to Rogers (53), the high incidence of mutacin production in *S. mutans* promotes the use of phenotypic typing technique for this species.

The bacteriocin typing of *S. mutans* is called mutacin typing and according to Arbeit (3), the technique possesses the basic criteria for typing of microorganisms as well as typeabiltiy (ability to classify the isolates), reproducibility (ability to obtain the same results in repeated analysis) and discrimination (ability to differentiate unrelated strains). From the late 80s on, molecular methods with higher discriminatory capacity improved the epidemiological studies and they have been commonly applied to study the genetic diversity of bacterial species from the oral cavity, replacing the methods for phenotypic analysis.

After the discovery of the direct involvement of streptococci in the development of human dental caries (15, 32), a global scientific race began in pursuit of elucidating the process of transmission and epidemiology of the major cariogenic microorganisms.

Several scholars engaged in the discovery of possible routes of transmission of *S. mutans*, the primary etiologic agent of dental caries in order to control the disease. Early studies sought to classify *S. mutans* in sub-groups related antigenically (7). Using serotyping, Köhler & Bratthall (33) demonstrated that parents or guardians transmitted *S. mutans* to children, most of the time. The adults responsible for the transmission of bacteria through saliva and fomites, were carrying a large number of streptococci of a particular serotype in the saliva. In 1980, Hamada *et al*. (20) proved that the same serotype could occur simultaneously in mother-child pairs (a) and that transmission could be propagated repeatedly by saliva.

Currently, it is known that sub-groups may correspond antigenically related to different species. Davey & Rogers, in 1984 (12), reported that the serotyping of mutans streptococci is unsatisfactory for epidemiological purposes, particularly since *S. mutans* serotype c is most frequently isolated in children and adults in the West. From the limitations of the classification of mutans streptococci by serotyping, several groups (5, 12, 54) began to use techniques with more discriminating infraspecific power like bacteriocin typing in the epidemiological investigation of mutans streptococci.

Using this phenotypic method, Kelstrup *et al*. (30) demonstrated the heterogeneity and diversity of mutans streptococci in the same individual. Years later, Berkowitz & Jordan (5) found that the pattern of mutacins production analyzed in 120 *S. mutans* strains were similar between four mother-child pairs, suggesting a maternal transmission (vertical) of this species. In 1981, Rogers (54) reported that in 88% of families analyzed in the study, two or more members, at least one represented by a parent, shared strains of *S. mutans* with the same phenotypic.

A comparison of biotypes of *S. mutans* between different individuals led to the identification of mothers as the main source of infection of this species (5, 12), although not all maternal bacteriocintypes are transferred to their children (6).

The composition of the culture medium can influence the sensitivity and the production of bacteriocins. Rogers (53), using two types of culture medium (BHI agar and TSA) in Mutacin typing of *S. mutans* detected distinct production patterns for the same strain. Through these observations, the author suggested that more than one type of mutacins could be synthesized by the same strain of *S. mutans*. Differences in the dimensions of the inhibition zones can also be attributed to the rate of diffusion of the substance in the culture medium and the mutacin concentration in agar (46).

Most strains of *S. mutans* produce mutacins in solid media, and previous studies have demonstrated that the inhibition zones observed on agar are not associated

with the presence of bacteriophage or the production of acids (6, 46).

Currently, Mutacin typing applied to the phenotypic classification of *S. mutans* has been shown to be limited. For Eijsink et al. (14), the use of culture supernatants instead of purified bacteriocins in tests of antagonism can create restricted results as to the bacteriocin activity of strains. The use of purified bacteriocins on comparative analysis between strains producing is essential, since a bacteriocin producing strain can synthesize more than one type of inhibitory peptide (14).

Even with the limitations of the technique, the mutacin typing is still employed (60), mainly associated with molecular typing methods (16, 26) as well as in combination with studies on the ecology and microbial virulence factors (26, 28) and purification and characterization of new bacteriocins with future applications in biotechnology (40).

CLASSIFICATION AND DIVERSITY OF MUTACINS

Kelstrup & Gibbons (29) initially suggested that bacteriocins of streptococci could be distinguished and characterized based on the inhibitory spectrum of activity, the morphology of the inhibition zone and sensitivity to chloroform and various enzymes. Kelstrup & Funder-Nielsen (31) divided the bacteriocins from *S. mutans* into two groups according to their molecular weight and sensitivity to heat. The first group was inactivated with heating at 80^{o}C for 20 min and possessed a molecular mass> 10,000 Da, the second group was composed of small molecules (<10,000 Da) and heat stable. But the spectrum of activity, sensitivity to ether, chloroform and the trypsin varied considerably within the same group.

Years later, Hamada & Ooshima (19) based on physicochemical properties and sensitivity to different enzymes proposed the existence of at least two groups of mutacins, sensitive or resistant to the action of proteases and lipases. Through these results, and analyzing the diffusion capacity of mutacins in cellophane, the authors concluded that mutacins can be represented by a variety of proteins of low molecular weight or high molecular weight polypeptide associated or not with lipids and / or polysaccharides.

Subsequently, Caufield *et al*. (10) classified mutacin producing *S. mutans* in two groups, based on the spectrum of inhibition and cross-immunity. They also found that strains of group II produced mutacin type II in a liquid medium (TSB supplemented with 2% yeast extract), different strains of group I, mutacin type I producing.

Through the techniques of purification and sequencing, mutacin I (48) and mutacin II (45), previously identified by Caufield *et al*. (10), were classified as lantibiotics (class of bacteriocins with residues of methyl-lantionine or β-methyl-lantionine). Subsequently, Qi *et al*. (47) identified another lantibiotic, called mutacin III and reported the existence of a single strain of *S. mutans* (UA140) able to synthesize mutacins I (lantibiotic) and mutacins IV (non-lantibiotic) (49). Protein purification and sequencing has led to the identification characterization of other antimicrobial substances. In particular, the mutacin produced by *S. mutans* strain Ny266 (39) differs by two amino acids from those mutacin of strains *S. mutans* JH1140 (23) and *S. mutans* UA787 (mutacin III) (47).

Morency *et al*. (38) showed phenotypically the diversity of antimicrobial peptides classifying 86 mutacinogenic strains in 24 different groups (A - X) based on similarity of their spectrum inhibition against 12 oral streptococci and cross-immunity to other mutacin producing strains.

In 2002, Ali *et al*. (2) compared the phenotypic grouping by activity spectra, previously described by Morency *et al*. (38), with genotypic analysis based on the sequences of genes encoding for mutacins pro-peptides. The amino acid sequences of four characterized mutacins (B-Ny266/1140/mutacin III and mutacin II) were used to design two DNA probes in order to detect similar genes among 24 phenotypic groups from A to X described previously by Morency *et al*. (38). The first probe was based on highly-conserved amino acid sequence of *lan*A from *S. mutans* Ny266, JH 1140 and UA787 (mutacin III) and

the second probe was selected from the sequence of *mut*A coding mutacin II produced by *S. mutans* T8 strain. Only six out in 24 *S. mutans* strains tested have genes that are similar to known lantibiotic sequences, indicating a rich diversity in bacteriocins that can be explored. In addition, strains with similar structural genes presented distinct phenotypic profiles. The difference in the pattern of production of antimicrobial substances, due in part to differential diffusion of substances in the culture medium (13), for example Nisin A and its homologous bacteriocin Nisin Z, produced by *Lactococcus lactis*, differ in one amino acid, but the spectrum of activity have only 83% similarity (11).

The difficulty in classifying mutacins through its spectrum of activity or immunity is partly due to the use of culture supernatants in bacteriocin typing instead of substances previously purified. Restricted and contrasting results can be obtained, hindering comparative analysis of mutacins synthesis, since a single strain can produce more than one type of inhibitory substance (14).

Currently, the characterization of phenotypic properties of mutacins is associated with genetic characterization, which includes sequencing, identification or screening of genes related to the biosynthesis of these substances in producing strains. Studies have detected a low frequency of biosynthesis genes of characterized mutacins in different phenotypes/genotypes of *S. mutans* confirming the existence of high diversity of these inhibitory substances, not yet identified, in the cariogenic species (27, 28).

The chromosomal locations of structural genes of characterized mutacins I, II and III (47, 48) were confirmed by cloning and sequencing. Subsequently, Qi *et al*. (49) reported that the two components of mutacins IV, produced by the strain *S. mutans* UA140, are encoded by genes and *nlm*A and *nml*B, also located in chromosomal DNA.

Bacteriocins are synthesized by ribosomes as precursor peptides with a signal peptide at the N-terminus, typical of secreted proteins (42), which is cleaved concomitantly with the export of the mature peptide across the membrane (21).

The bacteriocins synthesized by several species of bacteria have very different structures and activities, however, they preserve properties in common with each other. Generally, these proteins consist of 20-60 amino acid residues with positive electric charges and can be hydrophobic and / or amphiphilic, acting in the formation of pores in cell membranes or by reducing the metabolism of sensitive bacteria (43).

In recent years, the antibacterial peptides, particularly synthesized by species of lactic acid bacteria (LAB) have attracted considerable interest in different areas of knowledge, and numerous bacteriocins of LAB have been characterized for probiotic purposes or for future use in therapy antibiotics against bacteria resistant to antibiotics currently used (40).

Based on post-translational modification, the bacteriocins of Gram-positive were classified into two groups: class I bacteriocins modified after translation or lantibiotics and class II bacteriocins unmodified lantibiotics or not. The lantibiotics are small peptides containing lantionina, β-methyl-lantionina dehidratados residues and are synthesized by ribosomes and are modified after translation (55). Among mutacins well characterized, mutacins I, II, III, 1140, B-Ny 266, and SmbA SmbB are classified as lantibiotics (39, 47, 48, 62). The mutacin type IV (consisting of two peptides of synergistic activities, and NlmA NlmB) and N were classified as non lantibiotics (4, 49).

Currently, fragments were sequenced open reading (ORFs) flanking the structural genes of mutacins IV, N, and SmbA SmbB and that is possibly related to regulation of the expression and production of their compounds (17, 18, 62) (Figure 1). The proximity of the structural genes of mutacins (*mut*N) and the signal peptide of competence (*com*C) suggests a correlation between the production of N mutacins and development of competence (17).

The analysis of Figure 1 shows that the same strain of *S. mutans* is able to synthesize more than one type of antibacterial peptides. The strain *S. mutans* UA140 yielded three fractions

with inhibitory activity after chromatographic tests, then a fraction was identified as mutacins type I (a lantibiotic), and other fractions were corresponding to peptides non lantibiotics (NlmA and NlmB) of mutacins type IV (49). It has been identified in the strain *S. mutans* GS5, the presence and expression of structural genes of two peptide lantibiotics with synergistic activities (SmbA and SmbB) (62). In our studies, through screening by PCR and expression analysis of structural genes of characterized mutacins, it was possible to identify an isolate of *S. mutans* able to produce mutacins types I and II, both broad-spectrum inhibitory (28).

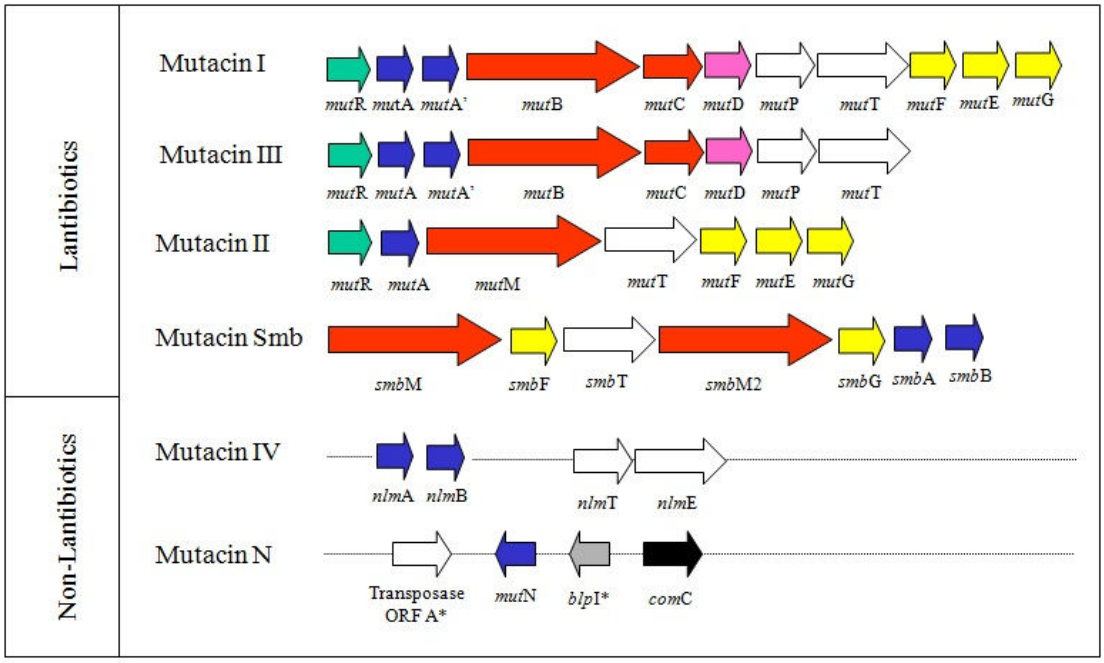

Figure 1. Organization of clusters of genes involved in biosynthesis of characterized mutacins from *Streptococcus mutans*. Genes with similar functions have the same color (green encoding regulation protein of the production, blue encoding the pro-peptide, red encoding modification proteins, white related with transport and processing, yellow encoding immunity proteins, and pink represents an additional gene - *mut*D).* Sequencing cluster biosynthesis mutacins N, involves an ORF homologous to the transposase gene *blp*I and to the bacteriocin structural gene of *Streptococcus pneumoniae.* (Figure based on 4, 17, 47, 48, 49, 62).

Robson *et al*. (51) identified the mutacin K8, a type AII lantibiotic produced by *S. mutans* K8 strain. The mutacin K8-encoding *muk* locus consists of 13 ORFs, three of which (*muk*A1, A2 and A3) have close homology to *scn*A, the structural gene encoding the *Streptococcus pyogenes* lantibiotic SA-FF22, and another (*muk*A') resembles *scn*A', an ORF in the SA-FF22 locus that has no currently assigned function. The *muk* locus may be widely distributed in *S. mutans*, since 9 (35 %) out of 26 strains tested contained at least part of the locus, although positive genes expression was not analyzed in this work. In the genome sequence of strain UA159 the *muk* locus is incomplete, the sole residual components being the ORFs encoding the putative two-component regulatory system *muk*R (SMU.1815) and *muk*K (SMU.1814), followed by two transposases (SMU.1813 and SMU.1812) and then the ORFs *muk*F (SMU.1811), *muk*E (SMU.1810) and *muk*G (SMU.1809), thought to encode putative immunity peptides. Strains such as UA159 having incomplete loci did not produce

detectable levels of mutacin K8.

Previous studies had suggested the existence of a high diversity of mutacins produced by *S. mutans* which have not been identified yet (27, 28). Analysis of the *S. mutans* genome sequence (1) revealed 10 small open reading frames with high similarity to the leader peptides of NlmA and NlmB that encode class IIb bacteriocins (nonlantibiotics), each possessing a double-glycine-type leader sequence similar to that of NlmAB (Figure 2) (18, 59). The putative bacteriocins, designed Bsm (bacteriocin *Streptococcus mutans*) ranged in size from 47 to 87 amino acids, had leader peptides from 22 to 25 amino acids, and contained a double glycine motif that could be recognized by the ComAB processing and export system (NlmTE), the same used for mutacin IV exportation. Some of the genes encoding the putative bacteriocins were located in tandem, indicating that they might act cooperatively, as it is typical for class IIb bacteriocins and these peptides would represent a large repertoire of antimicrobial substances produced by *S. mutans* (18, 59).

Kamiya *et al*. (28) detected the frequency and expression of mutacins biosynthesis genes in isolates of *S. mutans* with different mutacin producing phenotypes. Detection of frequency and expression of genes encoding mutacins types I, II, III, and IV were performed by PCR and semi-quantitative RT-PCR, respectively, using specific primers for each type of biosynthesis genes. In addition, 8 more genes, encoding putative bacteriocins, designated Bsm 283, 299, 423, 1889c, 1892c, 1896, 1906c and 1914 were also screened. The frequency of the Bsm gene expression was higher than that of characterized mutacins (I to IV). In conclusion, the high diversity of producing phenotypes associated with the high frequency of screened Bsm biosynthesis genes expression reveal a broad repertoire of genetic determinants encoding antimicrobials peptides which can act in different combinations.

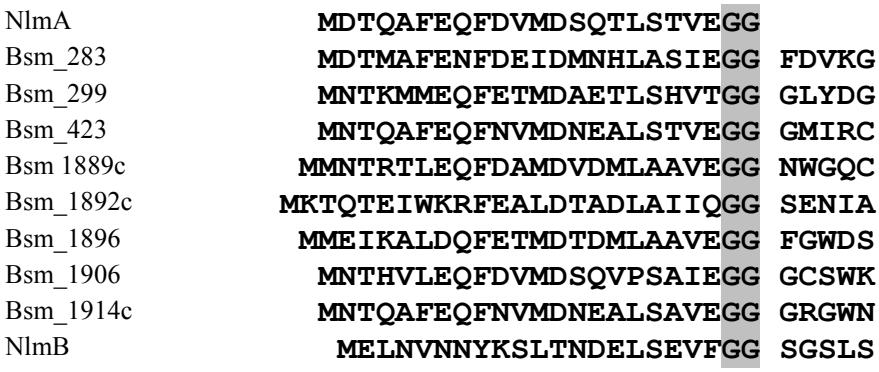

Figure 2. Clustal W sequence alignment of NlmA and NlmB (two preptides of mutacin IV) with other prepeptides sequences identified in *S. mutans* UA159 genome. These prepeptides contain the double-glycine motif. Adapted from van DER PLOEG (59) and Hale *et al*. (18).

REGULATORY MECHANISMS RELATED WITH MUTACIN PRODUCTION

The production of bacteriocins by Gram-positive bacteria is generally associated with the transition from log phase to stationary phase of bacterial growth (8) or the cell density in the culture medium (50). Such statements may suggest intense bacteriocin activity in dental biofilm, where there is high cell density and complexity.

There is evidence that the mutacins can play an important role in the protection and colonization of *S. mutans* in the oral cavity, especially in complex environments, such as dental biofilm and caries (4). The synthesis of broad-spectrum antimicrobial peptides was observed in genotypes of *S. mutans*

isolated from caries-active individuals, who have a resident microbiota more diverse and complex in relation to caries-free individuals (26). The production of these substances *in vivo* suggests ecological advantages to the producer strain by replacing the resident microbiota and prevention of invasion by exogenous microorganisms (22).

In addition, *in vitro* studies suggested greater biological activity of mutacins characterized in dental biofilm than in saliva, due to increased production of inhibitory substances by sessile colonies grown on solid media compared to planktonic cells, grown in liquid media (34). The patterns of gene expression and consequently phenotypes developed in planktonic and biofilm state are different, probably due to environmental differences which bacterial cells are exposed to (9).

Furthermore, some studies have linked the production of antimicrobial peptides with the *Quorum Sensing* System, demonstrating the increased expression of structural genes under conditions of greater cell density and control gene expression through regulatory mechanisms pertaining to this system (17, 49, 59, 62).

Conditions in the biofilm, such as high cell density and increased environmental stress, among other factors activate the *quorum sensing* system by increasing the concentration of peptide pheromones or signals that coordinate protective functions and cell survival, which include cellular mechanisms of competence, resistance to acid pH and production of mutacins (59).

Basically, the *quorum sensing* system via signal transduction is accomplished via two regulatory components consisting of a histidine protein kinase, associated with the cytoplasmic membrane, and a second regulatory protein, located in the cytoplasm. The histidine protein kinase captures the presence of peptide signals in the environment and transmits this signal through the phosphorylation of regulatory protein which activates transcription of target genes involved in adaptation, survival and virulence mechanisms (59). This system functions as a modulator of expression of different genes in response to environmental changes.

The genome of *Streptococcus mutans* harbors 13 two-component signal transduction systems (TCSTSs). Many of these two-component systems are well known to regulate several virulence-associated traits *in vitro* experiments, including genetic competence, bacteriocin production, biofilm formation, acid tolerance and stress responses.

Among mutacins well characterized, genes expressions of mutacins I, IV and Smb are controlled by the increase in cell density *(in vitro*) (59, 62). In addition, it was shown that protein kinases CiaH, ComD and LiaS components are important regulators that coordinate the expression of genes for production of these mutacins (59, 62). These regulatory mechanisms support an important means of signaling the expression of virulence factors related to the survival of producing strains especially in complex and competitive environments as the dental biofilm and caries.

Mutacin production is controlled by many genetic as well as environmental factors. While the non-lantibiotic mutacin IV is controlled by *quorum sensing* via the three-component system *com*CDE, associated with cell competence mechanism (34, 35), regulation of the lantibiotic mutacin I is much more complex and less understood.

Previous studies indicate that the proteins encoded by *cia*H and *lux*S are required for mutacin I production (37). LuxS is responsible for the synthesis of the interspecies cell signaling molecule autoinducer 2 (AI-2) (56). In addition to regulating mutacin I production, both CiaH and LuxS were also found to be involved in regulating other cellular functions in *S. mutans* such as biofilm formation and stress tolerance (36).

Tsang *et al*. (57) used a random insertional mutagenesis approach to search for genes that are associated with mutacin I production in the virulent strain UA140. A random insertional mutagenesis library consisting of 11 000 clones was constructed and screened for a mutacin-defective phenotype. Mutacin-defective clones were isolated, and their insertion sites

were determined by PCR amplification or plasmid rescue followed by sequencing. A total of twenty-five unique genes were identified. These genes can be categorized into the following functional classes: two-component sensory systems, stress responses, energy metabolism and central cellular processes. Several conserved hypothetical proteins with unknown functions were also identified. These results suggest that mutacin I production is stringently controlled by diverse and complex regulatory pathways.

In a previous study, Tsang *at al*. (57) constructed a random insertional-mutation library to screen for genes involved in regulating mutacin I production, and found 25 genes/operons that have a positive effect on mutacin I production. Later, Nguyen *et al*. (44) identified 17 genes whose mutation increased mutacin I production under repressive conditions. These genes are involved in a variety of cellular functions such as sugar transport, protein/peptide hydrolysis, amino acid and nucleotide synthesis, cell-wall metabolism, surface binding and unknown functions. These results further demonstrate an intimate and intricate connection between mutacin I production and the overall cellular homeostasis.

The regulatory mechanisms involved in mutacins production, as well as cell density, *quorum sensing* and the interspecies signaling system mediated by autoinducer molecules suggest that producing strains can benefit in environments with highly complex microbial population, such as dental biofilm and caries. In an environment such as the dental biofilm, energy expenditures may be better utilized by producing bacteriocins when the total population density is high and competitors are likely to be present. This may be particularly true for mutacins production, which requires many gene products to make a mature peptide.

CONCLUDING REMARKS

In conclusion, there is great diversity of mutacins unexplored. Many studies showed that these antimicrobial substances may promote the transmissibility of cariogenic species, as well as colonization and stability of *S. mutans* in the oral cavity. In addition, the regulatory mechanisms involved in increased mutacins production, as well as high cell density, *quorum sensing* and signaling system of auto-inducer molecules suggest that producing strains can have ecologic advantages in environments with highly complex microbial population, such as dental biofilm and caries.

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