Japanese Dental Science Review (2018) 54, 22—29

Contents lists available at ScienceDirect

Japanese Dental Science Review

journal homepage: www.elsevier.com/locate/jdsr

Review Article

Role of *Streptococcus mutans* surface proteins for biofilm formation

Michiyo Matsumoto-Nakano*

Department of Pediatric Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Kita-ku, Okayama, Japan

Received 31 January 2017; received in revised form 30 June 2017; accepted 1 August 2017

KEYWORDS
Streptococcus mutans;
Surface proteins;
Biofilm;
Signal transduction

Summary *Streptococcus mutans* has been implicated as a primary causative agent of dental caries in humans. An important virulence property of the bacterium is its ability to form biofilm known as dental plaque on tooth surfaces. In addition, this organism also produces glucosyltransferases, multiple glucan-binding proteins, protein antigen c, and collagen-binding protein, surface proteins that coordinate to produce dental plaque, thus inducing dental caries. Bacteria utilize quorum-sensing systems to modulate environmental stress responses. A major mechanism of response to signals is represented by the so called two-component signal transduction system, which enables bacteria to regulate their gene expression and coordinate activities in response to environmental stress. As for *S. mutans*, a signal peptide-mediated quorum-sensing system encoded by *comCDE* has been found to be a regulatory system that responds to cell density and certain environmental stresses by excreting a peptide signal molecule termed CSP (competence-stimulating peptide). One of its principal virulence factors is production of bacteriocins (peptide antibiotics) referred to as mutacins. Two-component signal transduction systems are commonly utilized by bacteria to regulate bacteriocin gene expression and are also related to biofilm formation by *S. mutans*.

Contents

* Fax: +81 86 235 6719.
E-mail address: mnakano@cc.okayama-u.ac.jp

https://doi.org/10.1016/j.jdsr.2017.08.002

1. *Streptococcus mutans* and biofilm formation

Streptococcus mutans has been implicated as a primary causative agent of dental caries in humans [1] and one of its important virulence properties is an ability to form biofilm known as dental plaque on tooth surfaces [2]. The bacterium synthesizes adhesive glucan from sucrose by the action of glucosyltransferases (GTFs), then glucans mediate firm adherence of its cells to tooth surfaces [3]. *S. mutans* also produces multiple glucan-binding proteins (Gbp proteins), which are thought to promote adhesion [4]. Furthermore, the cell surface protein antigen c (PAc), a major surface protein of *S. mutans*, is correlated to its virulence in regard to development of dental caries, as it is known to participate in bacterial adherence to tooth surfaces via interaction with the salivary pellicle [5]. Together, these bacterial surface proteins coordinate to produce dental plaque, thus inducing dental caries.

1.1. Glucosyltransferases

S. mutans produces 3 types of GTFs (GTFB, GTFC, GTFD), whose cooperative action is essential for adherence of bacterial cells, with the highest level of sucrose-dependent cellular adhesion found at the ratio of 5:0.25:1 [6].

GTFB and GTFC, which mainly synthesize water-insoluble glucans rich in α-1,3-glucosidic linkages, are located on the cell surface, and encoded by the *gtfB* and *gtfC* genes, respectively [7,8]. On the other hand, GTFD, which synthesizes water-soluble glucans rich in α-1,6-glucosidic linkages, has been detected in culture supernatant and known to be encoded by the *gtfD* gene [9]. Each enzyme is composed of 2 functional domains, an amino-terminal catalytic domain (CAT), which binds and hydrolyzes the substrate of sucrose, and a carboxyl-terminal glucan-binding domain (GBD), which functions as an acceptor for binding glucan and also plays an important role in determining the nature of the glucan synthesized by a GTF [10—12]. In a previous study of anti-caries activities of oolong tea, high-molecular-weight polyphenols were found to have site-specific actions, thus an oolong tea fraction rich in polymeric polyphenols reduced glucan synthesis in a noncompetitive manner by targeting the *S. mutans* glucan-binding domains of GTFB and GTFD in the solution phase [13].

Simultaneous synthesis of glucans by GTFB and GTFC is essential for establishment of a matrix that enhances the coherence of bacterial cells and adherence to tooth surfaces, allowing for formation of high density biofilm [14—16]. It has been shown that the presence of highly adherent and insoluble glucans in situ increases mechanical stability by binding bacterial cells together, as well as to an apatite surface (Fig. 1). In addition to interactions with specific Gbps expressed by *S. mutans* and other oral microorganisms, these polymers are critical for maintaining the 3-dimensional structure of biofilm (Fig. 1), thereby playing a role in modulating development of cariogenic biofilm [15—17].

1.2. Glucan-binding proteins

Binding of *S. mutans* to glucans formed in situ is mediated by the presence of cell-associated GTF enzymes and non-GTF glucan-binding proteins (Gbps) [4]. This bacterial organism produces at least 4 glucan-binding proteins (Gbps); GbpA [18], GbpB [19], GbpC [20], and GbpD [21], which presumably promote its adhesion. GbpA, the first designated glucan-binding protein, contains carboxyl terminal repeats similar to the glucan-binding domain of GTF enzymes [21,22]. This protein is involved in cellular adherence to tooth surfaces, and has been shown to contribute to the cariogenicity of *S. mutans* both *in vitro* and *in vivo* [23].

GbpA contributes to development of optimal plaque biofilm, which minimizes stress on the bacterial population [24], while it also has an important role in binding proteins and exopolysaccharides for construction of biofilm and maintenance of a balanced environment, while the structure of biofilm and its tolerance to various types of stress is affected by its absence [25]. A deficiency of GbpA results in loose binding to the EPS matrix, resulting in a weak non-uniform biofilm structure (Fig. 2). Thus, GbpA has important roles as a protein for formation of firm and stable biofilm.

Alterations in biofilm structure cause harbored bacteria to be exposed to acid, making them susceptible to gene introduction, with the stress response proteins RecA, DnaK, and GroEL possibly related to that response, though the detailed mechanisms remain unclear [24,25].

GbpB has been purified and shown to be immunologically distinct from other Gbps expressed by *S. mutans* and *Streptococcus sobrinus* [19]. It was also found to be homologous with peptidoglycan hydrolases of other Gram-positive microorganisms, while results of a comparative genomic analysis of the *gbpB* region suggested a functional relationship between genes involved in cell shape and cell wall maintenance [26,27]. GbpB is considered to have some roles in the cariogenicity of *S. mutans*, as mucosal immunization has been found to induce protective immune responses against experimental dental caries [28,29].

GbpC is a cell-surface-associated protein involved in dextran-induced aggregation and is expressed only under stress conditions [20]. Although the glucan-binding domain of GbpC has not been identified, it is homologous with the AgI/II family of proteins [20]. GbpC (and possibly

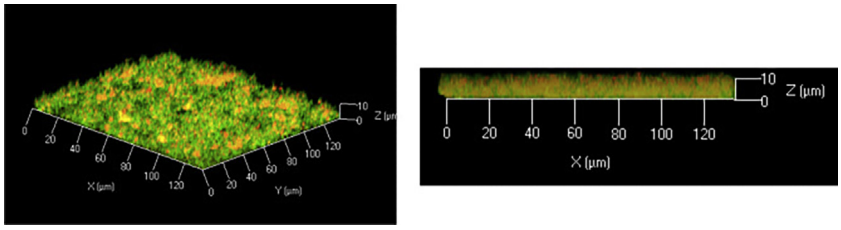

Figure 1 Representative confocal images of bacterial cells (green) and glucans (red) within biofilm formed by *S. mutans* MT8148 on tooth enamel surface in presence of 0.5% (wt/vol) sucrose.

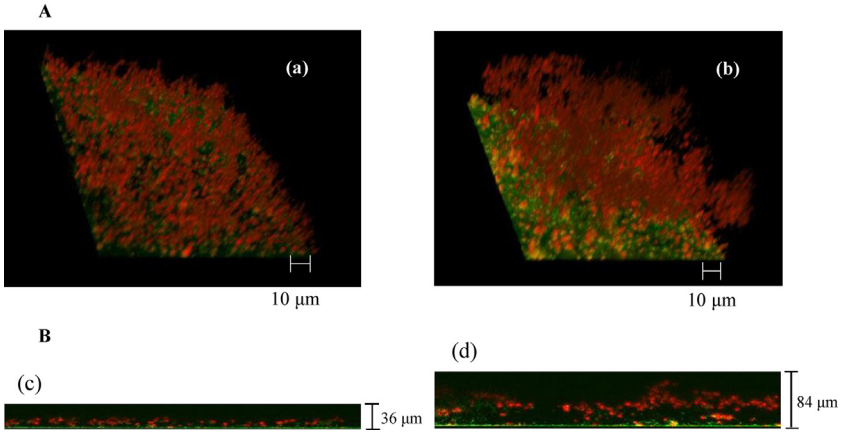

Figure 2 Biofilm analysis using confocal laser scanning microscopy. (A) Representative three-dimensional images of biofilms formed by *S. mutans* MT8148 (a) and AD1 (b) in the presence of 0.5% sucrose. (B) x—z section and z-projection of biofilm formed by MT8148 (c) and AD1 (d). *S. mutans* was stained with SYTO® 9 green fluorescent nucleic acid stain (green) and labeled-dextran (Alexa Fluor® 647; red) was used.

GbpB) is a cell-wall bound protein that may function as a cell surface glucan receptor in *S. mutans* [4]. While each of the Gbps appears to have roles in sucrose-dependent adhesion and biofilm formation by *S. mutans*, loss of cell-surface-anchored GbpC has been shown to reduce the caries-inducing properties of the bacterium [17,23]. It also plays an important role in sucrose-dependent adhesion by binding to soluble glucan synthesized by GTFD [30], indicating that GbpC is a cell-wall anchoring protein that possesses a specific binding domain for soluble glucans. Although GbpC does not contain repeat domains in regions involved in glucan-binding, the glucan-binding domain has been elucidated by bioinformatics analysis (Fig. 3) [31].

GbpD was discovered and isolated using sequence analysis of the complete, annotated sequence of the UA159 strain of *S. mutans* [21]. After cloning and sequencing harbored proteins, it was identified as a secreted Gbp with lipase activity [21]. In addition, the amino acid sequence of *gbpD* is homologous to the carboxyl terminus regions of GTF and GbpA [21]. GbpD contributes to the cohesiveness of aggregates and adhesion to tooth surfaces, and is known to be mediated by glucans, particularly dextran, similar to GbpA [21].

1.3. Protein antigen c

The cell surface protein antigen c (PAc) is one of the major surface proteins of *S. mutans* [32] and known by a number of other names, including SpaP [33], antigen I/II [34] and B [35], P1 [36], and MSL-1 [37]. PAc is known to be correlated with virulence of the organism for development of dental caries and participates in bacterial adherence to teeth via interaction with the salivary pellicle [38], which is termed sucrose-independent adhesion. The gene for PAc of *S. mutans* serotype *c* has been cloned and sequenced [32,33], and shown to include an N-terminal signal sequence, a region with a series of three 82-residue alanine-rich repeats (A-region) within the N-terminal third of the molecule, as well as a region with a series of three 39-residue proline-rich repeats (P-region) in the central portion of the molecule [32]. In addition, C-terminal sequences characteristic of wall- and membrane-spanning domains of streptococcal surface proteins have also been reported [39]. Furthermore, the A-region has a strong relationship with adhesion to tooth surfaces, while the P-region has a high affinity for PAc [40]. Several studies have reported an association of PAc with the virulence of *S. mutans* for infective endocarditis (IE) development. For example, PAc antibody titers were found to be elevated in a human subject with IE as compared with healthy subjects [41], whereas PAc was not related to endocarditis virulence in rat models in another study [42]. On the other hand, PAc has been shown to contribute to the interactions of *S. mutans* cells with fibronectin, collagen type I, and fibrinogen [43]. In addition, platelet aggregation occurring after pathogenic bacterial infection is considered to be one of the most important

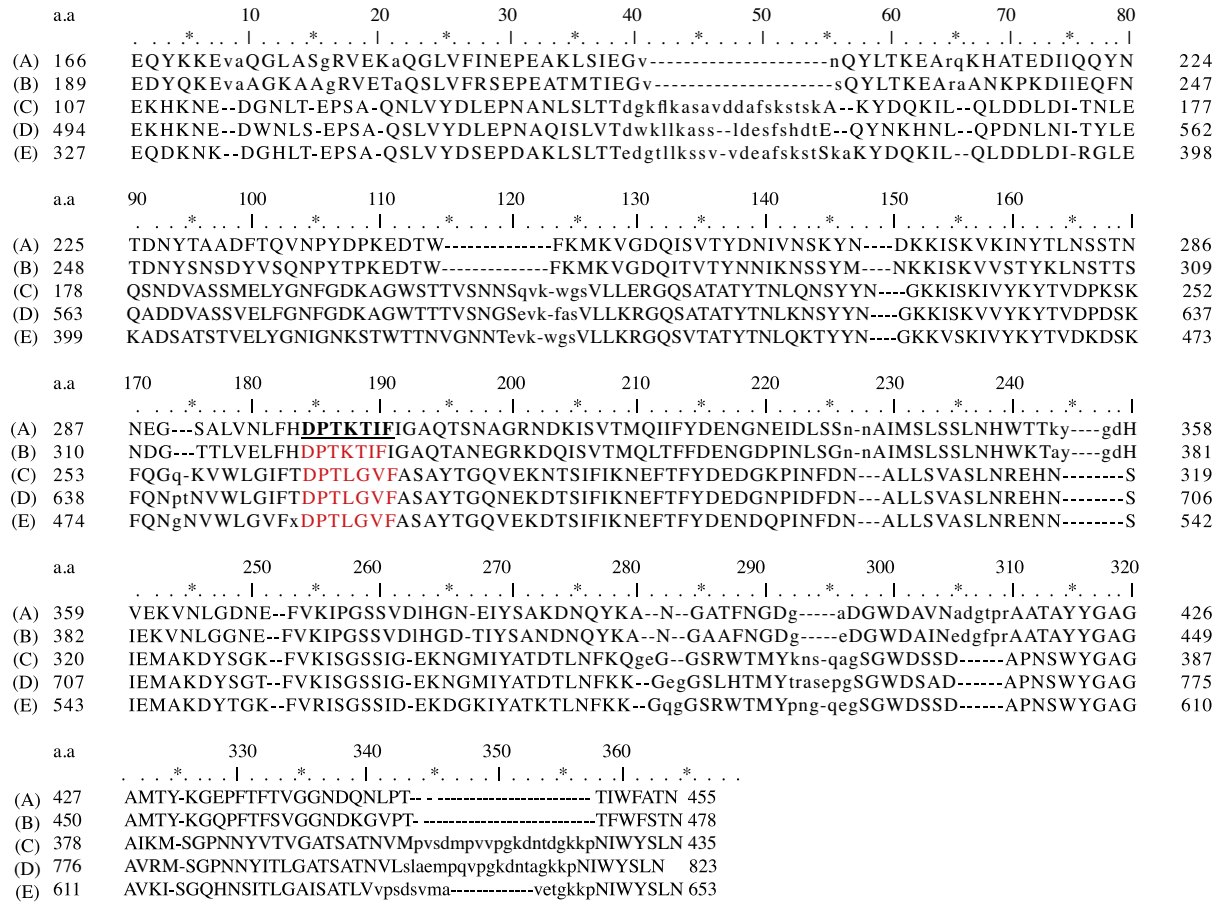

Figure 3 Results of NCBI Conserved Domains Database search of loop regions relative to the putative dextran-binding domain in GbpC. (A) Glucan-binding protein C: *S. mutans*. (B) Glucan binding protein C: *S. macacae*. (C) AgiI: *S. mutans*. (D) SpaA: *S. sobrinus*. (E) Probable cell-surface antigen I/II: *S. intermedius*. Asterisks indicate mid-points. Bold letters indicate core regions of potential dextran-binding domain sequences. Lower-case letters indicate no homology among strains.

factors in the pathogenesis of IE. As for *S. mutans*, PAc is involved in human platelet aggregation, as it binds directly to platelets [44].

1.4. Collagen-binding protein

Recently, an approximately 120-kDa Cnm protein related to the collagen-binding activity of *S. mutans* was identified, and its encoding gene was cloned and sequenced [45]. This protein consists of a collagen-binding domain, a putative B-repeat domain, and a cell-wall-anchored LPXTG motif, such as PAc. The distribution frequency of strains with the *cnm* gene among oral isolates has been estimated to be in the range of 10—20% and *cnm*-positive strains are known to possess high collagen-binding properties [45—48]. These strains have been predominantly identified in serotype *f* and *k* strains, though those are considered to be minor serotypes in the oral cavity [46,49]. The Cnm protein has also been shown to possess binding activity to type I collagen [45], a major organic component of dentin, which is regarded as advantageous for binding to exposed dentin [50]. Recently, Cnm was reported to be involved in adherence to and invasion of human coronary artery endothelial cells, indicating its possible contributions to cardiovascular infections and pathology [51]. In addition, Cnm of *S. mutans* was shown to related to deterioration caused by cerebral hemorrhage [52]. Another collagen-binding protein, Cbm, has been predominantly identified in serotype *k* strains [53] and also proposed to be a potential important factor for inducing infective endocarditis [54].

2. Important factors related to biofilm formation by *S. mutans*

Biofilm formation is initiated by interactions between planktonic bacteria and an oral surface in response to appropriate environmental signals [55—61]. *S. mutans* metabolizes carbohydrates to adhere to and form biofilm on tooth surfaces, thus allowing the pathogen to tolerate rapid and frequent environmental fluctuations such as nutrient availability, aerobic-to-anaerobic transitions, and pH changes [62,63] In addition, in response to physical and chemical signals, bacteria regulate diverse physiological processes in a cell density-dependent manner, known as quorum sensing [64], which they utilize to modulate environmental stress responses. Major participants in signal response are the so-called two-component signal transduction systems (TCSTSs), which enable bacteria to regulate their gene expression and

coordinate activities in response to environmental stress [65,66].

Two-component signal transduction systems (TCSTSs) of *S. mutans*

Several TCSTSs have been reported present in *S. mutans*. Initially, a signal peptide-mediated quorum-sensing system encoded by *comCDE* was found to function as a regulatory system that responds to cell density and certain environmental stresses by excreting a peptide signal molecule termed CSP (competence-stimulating peptide) that is encoded by the *comC* gene [67—70]. *comCDE* genes are located in the same locus and function together for generating and responding to CSP [67]. While *comC* encodes the precursor CSP, *comDE* genes encode a TCSTS comprised of a membrane-bound histidine kinase (ComD) and its cognate response regulator (ComE) [67]. When the genetic components of the *S. mutans* quorum-sensing system were initially identified, it was noted that genetic transformability was 10- to 600-fold higher in cells derived from biofilms as compared with those obtained from planktonic cultures [67]. Hence, in addition to competence development, this system is also involved in biofilm formation. To examine this assumption, mutants deficient in *comC*, *comD*, and *comE* were constructed and assayed for their ability to initiate biofilm formation. All of the mutants formed biofilms, though they lacked architectural integrity as compared to wild-type biofilm, while the *comD*- and *comE*-deficient mutant biofilms also had reduced biomass [68]. By interfering with this cell-cell signaling mechanism, biofilm formation by *S. mutans*, which utilizes quorum-sensing to control virulence, could potentially be attenuated. Another system termed HK/RR11 is involved in the relationship of *S. mutans* to acid tolerance [71]. This system consists of a membrane-associated histidine kinase (HK) protein, which senses a specific stimulus, as well as a cytoplasmic response regulator (RR) protein, which enables bacterial cells to respond to stimulus via regulation of gene expression [72]. Environmental signals are sensed by HK, resulting in autophosphorylation at a specific histidine residue, thus creating a high-energy phosphoryl group that is subsequently transferred to a specific aspartate residue within the N-terminal half of the cognate RR protein. Phosphorylation induces a conformational change in the regulatory domain resulting in activation of the RR protein, which then regulates gene expression by functioning as a DNA-binding transcriptional regulator that activates or represses genes whose products are specifically utilized to respond to the given input signal [73]. In addition, deletion of hk11 or rr11 has been reported to result in biofilm with a sponge-like architecture and composed of cells organized in very long chains [74]. Furthermore, inactivation of either *hk11* or *rr11* has been shown to lead to an abnormal biofilm phenotype, similar to that formed by a *comC* mutant, thus HK11 is suspected to be the second receptor for competence-stimulating peptide (CSP) [68]. On the other hand, an *hk11*-deficient mutant also showed reduced bacteriocin production, deficiency in transformability, and diminished ability to tolerate stress [75]. A major mechanism of signal transduction known to be widespread in bacteria is represented by TCSTSs, which enable bacteria to regulate their gene expression and coordinate activities in response to environmental stimuli [65,66,72]. In addition, the CiaR/H system has been characterized and found to play a coordinating role with the ComCDE quorum-sensing system for regulating genetic competence and stress response [76]. The *ciaH* and *ciaR* genes include a histidine kinase sensor protein and its cognate response regulator, as well as *hk11/rr11*, while activation of either *ciaR* or *ciaH* resulted in reduced biofilm biomass, whereas absence of *ciaH* alters sucrose-dependent biofilm formation [78]. In addition, CiaH signal transduction may be linked with the surface-anchored serine-protease HtrA, which is connected with CiaH on the stress response pathway [76]. That report also showed that deletion of CiaH resulted in biofilm with reduced biomass and very short chains, suggesting its role in regulating cell growth and/or cell division.

The VicRK system, which shares a high similarity to CovSR of *Streptococcus pyogenes*, has been found to regulate sucrose-dependent biofilm formation by *S. mutans* [77,79]. In addition, VicR and CovR directly regulate a panel of genes implicated in the synthesis of and interaction with extracellular polysaccharides [79—81]. CovR negatively regulates expression of the *gtfB* and *gtfC* genes by directly binding to the promoter region [80]. Also, *vicK*-deficient mutants showed defects regarding separation of daughter cells and in sucrose-dependent biofilm formation [79]. On the other hand, the ScnRK system in *S. mutans* has been shown to regulate hydrogen resistance and macrophage killing, as a study found that ScnRK contributes to hydrogen peroxide stress tolerance and enhances the resistance of *S. mutans* to killing by macrophages [82]. In addition, ScnRK, which has also been termed HK/RR3, was reported to have a modest effect on acid tolerance, while an apparent ortholog of this system in *Streptococcus pyogenes* has been found to influence the expression of bacteriocin [83,84]. Although each of these systems is related to biofilm formation, the only mechanism elucidated up to this point is gene expression of surface proteins. Additional studies are needed to clarify the related mechanisms.

2.1. Bacteriocin immunity proteins

One of the principal virulence factors *of S. mutans* is production of bacteriocins (peptide antibiotics), referred to as mutacins [85]. Although these peptide molecules are not required for growth, they may help the microorganisms that produce them to compete for limited nutrients in their environment [86]. TCSTs are commonly utilized by bacteria to regulate bacteriocin gene expression [87,88], while several CSP-induced genes have stress-related roles, or participate in synthesis and transport of bacteriocin-like peptides [69,89]. Two types of bacteriocins have been characterized, lantibiotics and non-lantibiotics. The former are lanthionine-containing small-peptide antibiotics that tend to show a wide spectrum of activities against Gram-positive bacteria, including non-producing strains of *S. mutans*, while non-lantibiotic bacteriocins, with one or two peptides, have thus far been shown to be primarily active against closely related species [90,91]. Furthermore, the non-lantibiotic group of bacteriocins contains peptides that do not require modification for their biological activities [92], whereas those in the lantibiotic group possess peptides that require posttranslational modification for their antimicrobial activities [93,94]. In *S. mutans*, the Com-

CDE TCSTS plays a direct role in regulation of a variety of mostly non-lantibiotic bacteriocins. Phosphorylated ComE then activates gene expression by its target bacteriocin promoters, which results in greatly increased bacteriocin production [69,70,89,95,96].

Producer organisms usually encode specific immunity proteins to protect themselves from the deleterious effects of their own lantibiotics, with immunity protein encoding genes often present within the same lantibiotic biosynthesis operon [97—99]. Generally, bacteriocin immunity protein (Bip) is an integral membrane proteins that confer protection against certain classes of antimicrobial agents and often enhance stress tolerance [97]. Inactivation of *bip* genes, which encode Bip, has effects on sensitivity to a variety of antimicrobial agents. Thus, the antimicrobial sensitivity of a bacterium can be modulated by a putative Bip expressed by the organism. These observations have implications regarding the evolution of *bip* protein genes as well as for potential new chemotherapeutic strategies.

Conflict of interest

The authors have no conflicts of interest to declare in regard to this study.

References

[1] Hamada S, Slade HD. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol Rev 1980;44:331—84.

[2] Kawabata S, Hamada S. Studying biofilm formation of mutans streptococci. Methods Enzymol 1999;310:513—23.

[3] Yamashita Y, Bowen WH, Burne RA, Kuramitsu HK. Role of the *Streptococcus mutans gtf* genes in caries induction in the specific-pathogen-free rat model. Infect Immun 1993;61:3811—7.

[4] Banas JA, Vickerman MM. Glucan-binding proteins of the oral streptococci. Crit Rev Oral Biol Med 2003;14:89—99.

[5] Koga T, Okahashi N, Takahashi I, Kanamoto T, Asakawa H, Iwaki M. Surface hydrophobicity, adherence, and aggregation of cell surface protein antigen mutants of *Streptococcus mutans* serotype *c*. Infect Immun 1990;58:289—96.

[6] Ooshima T, Matsumura M, Hoshino T, Kawabata S, Sobue S, Fujiwara T. Contribution of three glucosyltransferase to sucrose-dependent adherence of *Streptococcus mutans*. J Dent Res 2001;80:1672—7.

[7] Aoki H, Shiroza M, Hayakawa S, Sato S, Kuramitsu HK. Cloning of a *Streptococcus mutans* glucosyltransferase gene coding for insoluble glucan synthesis. Infect Immun 1986;53:587—94.

[8] Hanada N, Kuramitsu HK. Isolation and characterization of *Streptococcus mutans gtfC* gene, coding for synthesis of both soluble and insoluble glucans. Infect Immun 1988;56:1999—2005.

[9] Hanada N, Kuramitsu HK. Isolation and characterization of the *Streptococcus mutans gtfD* gene, coding for primer-dependent soluble glucan synthesis. Infect Immun 1989;57:2079—85.

[10] Kato C, Nakano Y, Lis M, Kuramitsu HK. Molecular genetic analysis of the catalytic site of *Streptococcus mutans* glucosyltransferase. Biochem Biophys Res Commun 1992;189:1184—8.

[11] Mooser G, Hefta SA, Paxton RJ, Shively JE, Lee TD. Isolation of a glucan-binding domain of glucosyltransferase (1,6-α-glucan synthase) from *Streptococcus sobrinus*. Infect Immun 1988;56:880—4.

[12] Wong C, Hefta SA, Paxton RJ, Shively JE, Mooser G. Size and subdomain architecture of the glucan-binding domain of sucrose: 3-α-D-glucosyltransferase from *Streptococcus mutans*. Infect Immun 1990;58:2165—70.

[13] Matsumoto M, Hamada S, Ooshima T. Molecular analysis of the inhibitory effects of oolong tea polyphenols on glucan-binding domain of recombinant glucosyltransferases from *Streptococcus mutans* MT8148. FEMS Microbiol Lett 2003;228:73—80.

[14] Tamasada M, Kawabata S, Fujiwara T, Hamada S. Synergistic effects of streptococcal glucosyltransferases on adhesive biofilm formation. J Dent Res 2004;83:874—9.

[15] Koo H, Xiao J, Klein MI, Jeon JG. Exopolysaccharides produced by *Streptococcus mutans* glucosyltransferases modulate the establishment of microcolonies within multispecies biofilms. J Bacteriol 2010;192:3024—32.

[16] Xiao J, Koo H. Structural organization and dynamics of exopolysaccharide matrix and microcolonies formation by *Streptococcus mutans* in biofilms. J Appl Microbiol 2010;108:2103—13.

[17] Lynch DJ, Fountain TL, Mazurkiewicz JE, Banas JA. Glucan-binding proteins are essential for shaping *Streptococcus mutans* biofilm architecture. FEMS Microbiol Lett 2007;268:158—65.

[18] Russell RRB, Coleman D, Dougan G. Expression of a gene for glucan-binding protein from *Streptococcus mutans* in *Escherichia coli*. J Gen Microbiol 1985;131:295—9.

[19] Smith DJ, Akita H, King WF, Taubman MA. Purification and antigenicity of a novel glucan-binding protein of *Streptococcus mutans*. Infect Immun 1994;62:2545—52.

[20] Sato Y, Yamamoto Y, Kizaki H. Cloning and sequence analysis of the *gbpC* gene encoding a novel glucan-binding protein of *Streptococcus mutans*. Infect Immun 1997;65:668—75.

[21] Shah DSH, Russell RRB. A novel glucan-binding protein with lipase activity from the oral pathogen *Streptococcus mutans*. Microbiology 2004;150:1947—56.

[22] Banas JA, Russell RRB, Ferretti JJ. Sequence analysis of the gene for the glucan-binding protein of *Streptococcus mutans* Ingbritt. Infect Immun 1990;58:667—73.

[23] Matsumoto-Nakano M, Fujita K, Ooshima T. Comparison of glucan-binding proteins in cariogenicity of *Streptococcus mutans*. Oral Microbiol Immunol 2007;22:30—5.

[24] Banas JA, Fountain TL, Mazurikiewicz JE, Sun K, Vickermann MM. *Streptococcus mutans* glucan-binding protein-A affects *Streptococcus gordonii* biofilm architecture. FEMS Microbiol Lett 2007;267:80—8.

[25] Matsumi Y, Fujita K, Takashima Y, Yanagida K, Morikawa Y, Matsumoto-Nakano M. Contribution of glucan-binding protein A to firm and stable biofilm formation by *Streptococcus mutans*. Mol Oral Microbiol 2015;30:217—26.

[26] Fujita K, Matsumoto-Nakano M, Inagaki S, Ooshima T. Biological functions of glucan-binding protein B of *Streptococcus mutans*. Oral Microbiol Immunol 2007;22:289—92.

[27] Mattos-Graner RO, Jin S, King WF, Chen T, Smith DJ, Duncan MJ. Cloning of *Streptococcus mutans* gene encoding glucan binding protein B and analysis of genetic diversity and protein production in clinical isolates. Infect Immun 2001;69:6931—41.

[28] Smith DJ, King WF, Barnes LA, Peacock Z, Taubman MA. Immunogenicity and protective immunity induced by synthetic peptides associated with putative immunodominant regions of *Streptococcus mutans* glucan-binding protein B. Infect Immun 2003;71:1179—84.

[29] Nogueira RD, Alves AC, Napimoga MH, Smith DJ, Mattos-Graner RO. Characterization of salivary immunoglobulin A responses in children heavily exposed to the oral bacterium *Streptococcus mutans*: influence of specific antigen recognition in infection. Infect Immun 2005;73:5675—84.

[30] Matsumoto M, Fujita K, Ooshima T. Binding of glucan-binding protein C to GTFD-synthesized soluble glucan in sucrose-

dependent adhesion of *Streptococcus mutans*. Oral Microbiol Immunol 2006;21:42—6.
[31] Takashima Y, Fujita K, Ardin AC, Nagayama K, Nomura R, Nakano K, et al. Characterization of the dextran-binding domain in the glucan-binding protein C of *Streptococcus mutans*. J Appl Microbiol 2015;119:1148—57.
[32] Okahashi NC, Sasakawa M, Yoshikawa S, Koga T. Cloning of a surface protein antigen gene from type *c Streptococcus mutans*. Mol Microbiol 1989;3:221—8.
[33] Lee SF, Progulske-Fox A, Bleiweis AS. Molecular cloning and expression of a *Streptococcus mutans* major surface protein antigen, P1 (I/I), in *Escherichia coli*. Infect Immun 1988;56:2114—9.
[34] Russell MW, Lehner T. Characterization of antigens extracted from cells and culture fluids of *Streptococcus mutans* serotype *c*. Arch Oral Biol 1978;23:7—15.
[35] Russell RR. Wall-associated protein antigens of *Streptococcus mutans*. J Gen Microbiol 1979;114:109—15.
[36] Forester H, Hunter N, Knox KW. Characteristics of a high molecular weight extracellular protein of *Streptococcus mutans*. J Gen Microbiol 1983;129:2779—88.
[37] Demuth DR, Lammey MS, Huck M, Lally ET, Malamud D. Comparison of *Streptococcus mutans* and *Streptococcus sanguis* receptors for human salivary agglutinin. Microb Pathog 1990;9:199—211.
[38] Koga T, Okahashi N, Takahashi I, Kanamoto T, Asakawa H, Iwaki M. Surface hydrophobicity, adherence, and aggregation of cell surface protein antigen mutants of *Streptococcus mutans* serotype *c*. Infect Immun 1990;58:289—96.
[39] Homonylo-McGavin MK, Lee SF. Role of the C terminus in antigen P1 surface localization in *Streptococcus mutans* and two related cocci. J Bacteriol 1996;178:801—7.
[40] Matsumoto-Nakano M, Tsuji M, Amano A, Ooshima T. Molecular interactions of alanine-rich and proline-rich regions of cell surface protein antigen c in *Streptococcus mutans*. Oral Micobiol Immunol 2008;23:265—70.
[41] Russell MW, Wu HY, White PL, Kilian M, Henrichsen J. Serum antibody responses to *Streptococcus mutans* antigens in humans systemically infected with oral streptococci. Oral Microbiol Immunol 1992;7:321—5.
[42] Ryd M, Schennings T, Flock M, Heimdahl A, Flock JI. *Streptococcus mutans* major adhesion surface protein, P1 (I/II), does not contribute to attachment to valvular vegetations or to the development of endocarditis in a rat model. Arch Oral Biol 1996;41:999—1002.
[43] Beg AM, Jones MN, Miller-Torbert T, Holt RG. Binding of *Streptococcus mutans* to extracellular matrix molecules and fibrinogen. Biochem Biophys Res Commun 2002;298:75—9.
[44] Matsumoto-Nakano M, Tsuji M, Inagaki S, Fujita K, Nagayama K, Nomura R, et al. Contribution of cell surface protein antigen c of *Streptococcus mutans* to platelet aggregation. Oral Microbiol Immunol 2009;24:427—30.
[45] Sato Y, Okamoto K, Kagami A, Yamamoto Y, Igarashi T, Kizaki H. *Streptococcus mutans* strains harboring collagen-binding adhesin. J Dent Res 2004;83:534—9.
[46] Nomura R, Nakano K, Taniguchi N, Lapirattanakul J, Nemoto H, Grönroos L, et al. Molecular and clinical analyses of the gene encoding the collagen-binding adhesin of *Streptococcus mutans*. J Med Microbiol 2009;58:469—75.
[47] Nakano K, Nomura R, Taniguchi N, Lapirattanakul J, Kojima A, Naka S, et al. Molecular characterization of *Streptococcus mutans* strains containing the cnm gene encoding a collagen-binding adhesin. Arch Oral Biol 2010;55:34—9.
[48] Lapirattanakul J, Nakano K, Nomura R, Leelataweewud P, Chalermsarp N, Klaophimai A, et al. Multilocus sequence typing analysis of *Streptococcus mutans* strains with the cnm gene encoding collagen-binding adhesin. J Med Microbiol 2011;60:1677—84.
[49] Lapirattanakul J, Nakano K, Nomura R, Nemot H, Kojima A, Senawongse P, et al. Detection of serotype k *Streptococcus mutans* in Thai subjects. Oral Microbiol Immunol 2009;24:431—3.
[50] Switalski LM, Butcher WG, Caufield PC, Lantz MS. Collagen mediates adhesion of *Streptococcus mutans* to human dentin. Infect Immun 1993;61:4119—25.
[51] Abranches J, Miller JH, Martinez AR, Simpson-Haidaris PJ, Burne RA. The collagen-binding protein Cnm is required for *Streptococcus mutans* adherence to and intracellular invasion of human coronary artery endothelial cells. Infect Immun 2011;79:2277—84.
[52] Nakano K, Hokamura K, Taniguchi N, Wada K, Kudo C, Nomura R, et al. The collagen-binding protein of *Streptococcus mutans* is involved in haemorrhagic stroke. Nat Commun 2011;2:485.
[53] Nomura R, Nakano K, Naka S, Nemoto H, Masuda K, Lapirattanakul J, et al. Identification and characterization of collagen-binding protein, Cbm, in *Streptococcus mutans*. Mol Oral Microbiol 2012;27:308—23.
[54] Nomura R, Naka S, Nemoto H, Inagaki S, Taniguchi K, Ooshima T, et al. Potential involvement of collagen-binding proteins of *Streptococcus mutans* in infective endocarditis. Oral Dis 2013;19:387—93.
[55] Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999;284:1318—22.
[56] Donlan RM, Costerton JW. Biofilm: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15:167—93.
[57] Kolenbrander PE. Oral microbial communities: biofilms, interactions, and genetic systems. Annu Rev Microbiol 2000;54:413—37.
[58] Kuramitsu HK. Virulence factors of mutans streptococci role of molecular genetics. Crit Rev Oral Biol Med 1993;4:159—76.
[59] O'Toole GA, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annu Rev Microbiol 2000;54:49—79.
[60] O'Toole GA, Kolter R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol Microbiol 1998;30:295—304.
[61] O'Toole GA, Kolter R. Initiation of biofilm formation in *Pseudomonas fluorescence* WCS365 proceeds via multiple, convergent signaling pathways: a genetic analysis. Mol Microbiol 1998;28:449—61.
[62] Lemos JA, Burne RA. A model of efficiency: stress tolerance by *Streptococcus mutans*. Microbiology 2008;154:3247—55.
[63] Yoshida A, Kuramitsu HK. *Streptococcus mutans* biofilm formation: utilization of a *gtfB* promoter-green fluorescent protein (*PgtfB:gfp*) construct to monitor development. Microbiology 2002;148:3385—94.
[64] Bassler BL. How bacteria talk to each other: regulation of gene expression by quorum sensing. Curr Opin Microbiol 1999;2:582—7.
[65] Barrett JF, Hoch JA. Two-component signal transduction as a target for microbial anti-infective therapy. Antimicrob Agents Chemother 1998;42:1529—36.
[66] Beier D, Gross R. Regulation of bacterial virulence by two-component systems. Curr Opin Microbiol 2006;9:143—52.
[67] Li YH, Lau PCY, Lee LH, Ellen RP, Cvitkovitch DG. Natural genetic transformation of *Streptococcus mutans* growing in biofilm. J Bacteriol 2001;183:897—908.
[68] Li YH, Tang N, Aspiras MB, Lau PCY, Lee LH, Ellen RP, et al. A quorum sensing signaling system essential for genetic competence in Streptococcus mutans is involved in biofilm formation. J Bacteriol 2002;184:2699—708.
[69] van der Ploeg JR. Regulation of bacteriocin production in *Streptococcus mutans* by the quorum-sensing system

required for development of genetic competence. J Bacteriol 2005;187:3980—9.
[70] Perry JA, Jones MB, Peterson SN, Cvitkovitch DG, Levesque CM. Peptide alarmone signalling triggers an auto-active bacteriocin necessary for genetic competence. Mol Microbiol 2009;72:905—17.
[71] Li YH, Lau PCY, Tang N, Svensater G, Ellen RP, Cvitkovitch DG. Novel two-component regulatory system involved in biofilm formation and acid resistance in *Streptococcus mutans*. J Bacteriol 2002;184:6333—42.
[72] Hoch JA. Two-component and phosphorelay signal transduction. Curr Opin Microbiol 2000;3:165—70.
[73] Stock AM, Robinson VL, Goudreau PN. Two-component signal transduction. Annu Rev Biochem 2000;69:183—215.
[74] Cvitkovitch DG, Li YH, Ellen RP. Quorum sensing and biofilm formation in streptococcal infections. J Clin Investig 2003;112:1626—32.
[75] Idone V, Brendtro Gillespie R, Kocaj S, Peterson E, Rendi M, Warren W, et al. Effect of an orphan response regulator on *Streptococcus mutans* sucrose-dependent adherence and cariogenesis. Infect Immun 2003;71:4351—60.
[76] Ahn SJ, Wen ZT, Burne RA. Multilevel control of competence development and stress tolerance in *Streptococcus mutans* UA159. Infect Immun 2006;74:1631—42.
[77] Lee SF, Delaney GD, Elkhateeb M. A two-component *covRS* regulatory system regulates expression of fructosyltransferase and a novel extracellular carbohydrate in *Streptococcus mutans*. Infect Immun 2004;72:3968—73.
[78] Qi F, Merritt J, Lux R, Shi W. Inactivation of the ciaH gene in *Streptococcus mutans* diminishes mutacin production and competence development, alters sucrose-dependent biofilm formation, and reduces stress torelance. Infect Immun 2004;72:4895—9.
[79] Senadheera MD, Guggenheim B, Spatafora GA, Huang YC, Choi J, Hung DC, et al. A VicRK signal transduction system in *Streptococcus mutans* affects *gtfBCD*, *gbpB*, and *ftf* expression, biofilm formation, and genetic competence development. J Bacteriol 2005;187:4064—76.
[80] Biswas S, Biswas I. Regulation of the glucosyltransferase (*gtfBC*) operon by CovR in *Streptococcus mutans*. J Bacteriol 2006;188:988—98.
[81] Sato Y, Yamamoto Y, Kizaki H. Construction of region-specific partial duplication mutants (merodiploid mutants) to identify the regulatory gene for the glucan-binding protein C gene in vivo in *Streptococcus mutans*. FEMS Microbiol Lett 2000;186:187—91.
[82] Chen PM, Chen HC, Ho CT, Jung CJ, Lien HT, Chen JY, et al. The two-component system ScnRK of *Streptococcus mutans* affects hydrogen peroxide resistance and murine macrophage killing. Microbes Infect 2008;10:293—301.
[83] Levesque CM, Mair RW, Perry JA, Lau PC, Li YH, Cvitkovitch DG. Systemic inactivation and phenotypic characterization of two-component systems in expression of *Streptococcus mutans* virulence properties. Lett Appl Microbiol 2007;45:398—404.
[84] McLaughlin RE, Ferretti JJ, Hynes WL. Nucleotide sequence of the streptococcin A-FF22 lantibiotic regulon: model for production of the lantibiotic SA-FF22 by strains of *Streptococcus pyogenes*. FEMS Microbiol Lett 1999;175:171—7.
[85] Hamada S, Ooshima T. Inhibitory spectrum of a bacteriocinlike substance (mutacin) produced by some strains of *Streptococcus mutans*. J Dent Res 1975;54:140—5.
[86] Vining LC. Functions of secondary metabolites. Annu Rev Microbiol 1990;44:395—427.
[87] Eijsink VG, Axelsson L, Diep DB, Havarstein LS, Holo H, Nes IF. Production of class II bacteriocins by lactic acid bacteria; an example of biological warfare and communication. Antonie van Leeuwenhoek 2002;81:639—54.
[88] Kleerebezem M. Quorum sensing control of lantibiotic production; nisin and subtilin autoregulate their own biosynthesis. Peptides 2004;25:1405—14.
[89] Yonezawa H, Kuramitsu HK. Genetic analysis of a unique bacteriocin, Smb, produced by *Streptococcus mutans* GS5. Antimicrob Agents Chemother 2005;49:541—8.
[90] Sahl HG, Jack RW, Bierbaum G. Biosynthesis and biological activities of lantibiotics with unique post-translational modifications. Eur J Biochem 1995;230:827—53.
[91] Nissen-Meyer J, Nes IF. Ribosomally synthesized antimicrobial peptides: their function, structure, biosynthesis, and mechanism of action. Arch Microbiol 1997;167:67—77.
[92] Kreth J, Merritt J, Shi W, Qi F. Co-ordinated bacteriocin production and competence development: a possible mechanism for taking up DNA from neighbouring species. Mol Microbiol 2005;57:392—404.
[93] Kreth J, Merritt J, Zhu L, Shi W, Qi F. Cell density- and ComE-dependent expression of a group of mutacin and mutacin-like genes in *Streptococcus mutans*. FEMS Microbiol Lett 2006;265:11—7.
[94] van Belkum MJ, Stiles ME. Nonlantibiotic antibacterial peptides from lactic acid bacteria. Nat Prod Rep 2000;17:323—35.
[95] Islam MR, Nishie M, Nagao J, Zendo T, Keller S, Nakayama J, et al. Ring A of nukacin ISK-1: a lipid II-binding motif for type-A(II) lantibiotic. J Am Chem Soc 2012;134:3687—90.
[96] Willey JM, van der Donk WA. Lantibiotics: peptides of diverse structure and function. Annu Rev Microbiol 2007;61:477—501.
[97] Matsumoto-Nakano M, Kuramitsu HK. Role of bacteriocin immunity proteins in the antimicrobial sensitivity of *Streptococcus mutans*. J Bacteriol 2006;188:8095—102.
[98] Chatterjee C, Paul M, Xie L, van der Donk WA. Biosynthesis and mode of action of lantibiotics. Chem Rev 2005;105:633—84.
[99] Draper LA, Ross RP, Hill C, Cotter PD. Lantibiotic immunity. Curr Protein Pept Sci 2008;9:39—49.