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Comparing the cariogenic species *Streptococcus sobrinus* and *S. mutans* on whole genome level

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**Background:** Two closely related species of mutans streptococci, namely *Streptococcus mutans* and *Streptococcus sobrinus*, are associated with dental caries in humans. Their acidogenic and aciduric capacity is directly associated with the cariogenic potential of these bacteria. To survive acidic and temporarily harsh conditions in the human oral cavity with hundreds of other microbial co-colonizers as competitors, both species have developed numerous mechanisms for adaptation.

**Objectives:** The recently published novel genome information for both species is used to elucidate genetic similarities but especially differences and to discuss the impact on cariogenicity of the corresponding phenotypic properties including adhesion, carbohydrate uptake and fermentation, acid tolerance, signaling by two component systems, competence, and oxidative stress resistance.

**Conclusions:** *S. sobrinus* can down-regulate the SpaA-mediated adherence to the pellicle. It has a smaller number of two-component signaling systems and bacteriocin-related genes than *S. mutans*, but all or even more immunity proteins. It lacks the central competence genes *comC*, *comS*, and *comR*. There are more genes coding for glucosyltransferases and a novel energy production pathway formed by lactate oxidase, which is not found in *S. mutans*. Both species show considerable differences in the regulation of fructan catabolism. However, both *S. mutans* and *S. sobrinus* share most of these traits and should therefore be considered as equally virulent with regard to dental caries.

**Keywords:** Mutans streptococci; comparative genomics; adhesion; sugar metabolism; two-component-systems; competence; bacteriocins; cariogenicity

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Dental caries is a complex disease that results from interactions of acidogenic/aciduric bacteria colonizing the tooth surface and the oral environment. Although other species, especially lactobacilli, bifidobacteria, and less investigated aciduric species such as *Atopobium* sp. or *Slackia exigua*, are also involved, the mutans streptococci (MS) group, in humans represented by *Streptococcus mutans* (serotypes c, e, f and k) and *Streptococcus sobrinus* (serotypes d and g) are still considered major etiological agents of dental decay. Over time, their attributed role changed from more or less true pathogens [specific plaque hypothesis (1)] to up-regulators of a sugar-triggered vicious cariogenic circle under destabilization of the homeostasis [extended caries ecological hypothesis (2, 3)] and the discussion is not finished, as outlined by Rosier et al. recently (4).

However, it is generally accepted that *S. mutans* can be more frequently isolated from carious lesions than *S. sobrinus* and is by far the most relevant cariogenic *Streptococcus* species, although we should not rule out the acidogenic properties of several non-MS (5–7). A number of epidemiological and in-vitro studies suggested that *S. sobrinus* – under circumstances yet to discover – may be even more cariogenic than *S. mutans* (8–11). In addition, clinical studies have suggested that pre-school and 15-year-old school children harboring both *S. mutans* and *S. sobrinus* had a higher incidence of dental caries than those with *S. mutans* alone (12, 13).
The virulence of MS is directly related to properties that enable these organisms to colonize and thrive on the tooth surfaces during acidic conditions. These properties include the production and regulation of adhesion proteins, glucosyltransferases (GTFs), and extracellular polysaccharides such as glucans that allow the bacteria to firmly adhere to the tooth surface in a biofilm. However, both species follow different strategies for adherence: *S. mutans* mainly using pellicle directed and specific surface antigens, *S. sobrinus* mainly using glucans and, as a consequence, both are found on different surfaces (*S. sobrinus* more buccal, *S. mutans* more occlusal). Comparing the recently explored genetic inventory of both species, this review will discuss consequences of specific genotypes for cariogenic phenotypes for each species. We focus on genes responsible for adhesion, metabolism of sugars (obtained from salivary glycoproteins and from the host diet to generate lactic acid, leading to acidogenicity), acid tolerance (ability to tolerate abundant amounts of lactic acid, leading to aciduricity), signaling, competence, bacteriocins and related immunity proteins, and oxidative stress resistance. The genetic inventory of *S. mutans* strains UA159, NN2025, 5DC8, AC4446, KK21, KK23, ATCC25175, and NCTC11060 as well as *S. sobrinus* DSM 20742 and TCI-107 was compared using the OrthoMCL software. Additional strains and data were included found in the NCBI database (http://blast.ncbi.nlm.nih.gov/) and the Human Oral Microbiome Database (HOMD at http://www.homd.org), respectively. Please refer to references 31 and 41 for details of our methods.

**Adhesion**

In several clinical studies, the role of *S. sobrinus* in relationship to caries is suggested to be additive to that of *S. mutans*. The latter is strongly associated with caries, but in situations of more severe caries, it is frequently found together with *S. sobrinus* (14–17). The acquisition of these bacteria by the susceptible host is a crucial step. The ‘infection dose’ (with respect of the ecological plaque hypothesis, we might call it ‘stress dose’) may play an important role, since more transmission, or higher bacterial counts from the source of transmission, will result in a higher chance for successful acquisition (18, 19). But when the bacterium cannot adhere in the oral cavity, the acquisition will never be successful. Therefore, adhesion to epitopes in the oral cavity is a crucial step for streptococci to become resident.

In *S. mutans*, cell surface antigen I/II (Ag/I/II), the glucan-binding region of the GTF enzymes and additional glucan-binding proteins (Gbp) have been implicated in its initial and specific adherence to saliva-coated (acquired enamel pellicle) tooth surfaces. These contact regions induce immune responses in mice effective in protection against colonization of *S. mutans* (20) and have been suggested as anti-caries vaccination agents. Cell wall proteins from the Ag/I/II family (synonyms SAI/II, PAC, PI, or SpaP; encoded by *spaP*; binding to salivary agglutinin glycoproteins, extracellular matrix molecules, and ligands of other oral bacteria) are not exclusively found in *S. mutans* but homologs have been reported in a variety of oral streptococci including *S. intermedius*, *S. gordonii*, *S. pyogenes*, and even non-oral *S. agalactiae* (21, 22). The *S. sobrinus* variant has been described as SpaA (or PAg), but with structural differences and less adhesive potential (23) possibly due to down regulation by Par, see Table 1. The Ag/I/II family is highly conserved throughout different streptococcal species and seems to be associated with the M-protein in other streptococci. It has been found that monoclonal antibodies against *S. sobrinus* SpaA are able to change the adhesion of this bacterium on hydroxyapatite disks in a triple species biofilm, suggesting a role of SpaA in the interspecies adherence in biofilms (24). In a review on streptococcal adherence factors, it has been reported that SpaA interacts with multiple host and microbial factors, from which binding to the salivary glycoprotein gp340 is most important (25). By our whole genome sequencing approach, we found another ‘adhesive protein’ encoding gene (D823_10858) in *S. sobrinus* DSM 20742. It seems to be the adherence component of an ATP binding cassette (ABC)-type Zn\(^{2+}\) and Mn\(^{2+}\) transporter system with homologs in many streptococci, including *S. mutans*, and related to the pneumococcal surface antigen PsaA (26).

Summarizing the literature, it seems that initial attachment of *S. sobrinus* to the pellicle is minimal and in a less specific manner (25, 27) but, once attached, it can, in the presence of sucrose, accumulate by massive glucan formation (7, 28). Any GTFs (and especially those secreted from *S. mutans*) present in the pellicle, promote the initial attachment of *S. sobrinus* (28, 29), explaining why *S. sobrinus* is rarely found without *S. mutans*. Different GTFs of different classes are combined to synthesize glucans. The enzyme called GTF-S synthesizes a soluble α-(1-6)-branched dextran, the enzyme called GTF-I, synthesizes an insoluble α-(1-3) rich D-mutan (7, 30). A third class (GTF-SI) does exist producing a semi-soluble glucan with mixed α-(1-6)-α-(1-3) linkages. By phenotype, *S. mutans* appears to form primarily GTF-S, whereas *S. sobrinus* has both GTF-S and GTF-I activities [see (7) for review]. In animal models, *S. sobrinus* GTF activity at slow growth rates consisted mainly of GTF-S activity (30) but at higher growth rates, such as it might occur in plaque during exposure to dietary sucrose, the proportions of GTF-I increased, resulting in more insoluble dextran (30). If this finding is extrapolated to humans, then frequent sucrose pulses allow *S. sobrinus* to accumulate on smooth surfaces via mutan production and this contributes to the increase in smooth-surface decay we indeed see in *S. sobrinus* positive subjects.
Table 1. Comparing proteins and corresponding genes involved in adherence on pellicle coated tooth surfaces between *S. mutans* [eight strains according to (31)] and *S. sobrinus* DSM 20742 and TCI-107

| Class                      | Name                          | Function                             | Eight strains\(^a\) | DSM 20742, TCI-107 |
|----------------------------|-------------------------------|--------------------------------------|----------------------|--------------------|
| Surface adhesins           | AgI/II, Spa, PA                | Specific adherence to acquired enamel pellicle | SMU.610, spaP, pac    | D823_07515, spaA, pag |
|                            | Par                           | Negative regulator of surface antigen | Absent?              | D823_01230 or D823_08637, par |
|                            | Unnamed                       | Surface adhesin, part of ABC ion transporter | SMU.1302             | D823_10858         |
| Glucosyltransferases       | Gtf-I                         | Glucosyltransferase-I (insoluble)    | SMU.1004, gtfB        | D823_05448, gtfI   |
|                            | Gtf-SI                        | Glucosyltransferase-SI               | SMU.1005, gtfC        | D823_05918, gtfS   |
|                            | Gtf-S                         | Glucosyltransferase-S (soluble)      | SMU.910, gtfD         | D823_03428, gtfS   |
|                            | Gtf-SI                        | Glucosyltransferase-S (soluble)      | Absent               | D823_01485, gtfS   |
|                            | Gtf-T                         | Glucosyltransferase-T (soluble)      | Absent               | D823_07585 or D823_10815, gtfT\(^b\) |
|                            | Gtf-U                         | Glucosyltransferase-U (soluble)      | Absent               | D823_07585 or D823_10815, gtfU\(^b\) |
|                            | Gtf                           | Glucosyltransferase                   | Absent               | D823_03433, gtf    |
| Glucan-binding proteins    | GbpA                          | Glucan-binding protein A             | SMU.2112, gbpA        | D823_05458 and D823_05463 |
|                            | GbpB                          | Glucan-binding protein B             | SMU.22, gbpB          | D823_01475         |
|                            | GbpC                          | Glucan-binding protein C             | SMU.1396, gbpC        | D823_02626 and D823_02641 |
|                            | GbpD                          | Glucan-binding protein D and lipase  | SMU.772, gbpD         | D823_00935         |

\(^a\)All genes shown are conserved for at least seven out of eight strains and the UA159 gene variant is shown as representative.

\(^b\)The exact assignment between D823_07585, D823_10815 and gtfT, gtfU was not possible.

Comparing both species on a whole genome level (Table 1) reveals new details. *S. sobrinus* DSM 20742 has seven instead of three genes encoding for GTFs, one which is insoluble (GTF-I), one intermediate (GTF-SI), two ‘regular’ soluble (GTF-S1 and -S2), and two which are α-(1-6)-branched glucan synthases [GTF-T or U respectively, see (32)], and one not further specifiable (‘GTF’). These multiple glucans apparently provide a potential by which *S. sobrinus* extends its niche from the retentive fissure site to the non-retentive smooth surfaces. It appears that GTF and glucans may play a minor role in fissure decay and perhaps no role at all where *S. mutans* is concerned. This also may explain why vaccines directed against the GTF of *S. sobrinus* (but not against the GTF of *S. mutans*) are mainly protective on smooth surfaces in animal models [reviewed by Loesche (7)].

*S. mutans* also synthesizes four Gbps: GbpA, GbpB, GbpC, and GbpD. The loss of any of the Gbps has an impact on adhesion and biofilm formation including dextran-dependent aggregation, dextranase inhibition, plaque cohesion, and perhaps cell wall synthesis (33). A whole genome comparison reveals that *S. sobrinus* has a similar repertoire in terms of Gbps (GbpA–D) but with two copies for GbpA and C encoding genes.

Uptake and metabolism of carbohydrates

Even more important than adherence is the successful growth of oral streptococci in their ecological niche. Where some years ago it was thought that pathogenic species were actively involved in acidogenicity of the ecosystem, nowadays we have more evidence that carbohydrate uptake and the resulting pH effects are, among other, the driving etiological factors, responsible for destabilization of oral biofilm homeostasis (3). MS get selected as they can compete with other species easily because of their capability to ferment many kinds of carbohydrates very efficiently. But interspecies competition is also found within MS. For instance, *S. mutans* out-competes *S. sobrinus* in the presence of the amino acid sugar *N*-acetylg glucosamine (GlcNAc) together with glucose. It is suggested that GlcNAc inhibited growth of *S. sobrinus* in media containing both glucose and GlcNAc, by competing with glucose for the glucose phosphotransferase, depleting intracellular levels of phosphoenolpyruvate, or possessing lower levels of *N*-acetyl-glucosamine-6-phosphate deactylase and/or glucosamine-6-phosphate deaminase activity (34). However, we found the deacetylase and deaminase genes in both species so that the difference might be due to transport systems [phosphotransferase system (PTS), see below] and/or promoter activity.

For *S. mutans*, the accumulation of genes related to carbohydrate uptake and metabolism was an essential evolutionary advancement contributing to the survival in the oral cavity and to the success as a caries ‘pathogen’ or – more correct – trigger. The transport of various oligosaccharides, including melibiose, raffinose, stachyose, and maltodextrins, is primarily conducted by the activity...
of ABC transporters, which include the multiple sugar metabolism (msm) and malXFGK transport systems. The predominant route for uptake of mono- and disaccharides is the phosphoenolpyruvate-sugar PTS, for review see (35). So far, we did not find essential differences in the genetic configuration related to sugar uptake between S. mutans and S. sobrinus here, as homologs for, e.g. msmG (D823_01675 in S. sobrinus), msmK (D823_07028), malX (WP_019787850.1), ptsH (D823_05408), or ptsI (WP_019775468) were found; however, this needs further in-depth investigation.

Due to their key roles in carbohydrates metabolism and energy production, glycolysis gluconeogenesis, TCA cycle and pyruvate metabolism pathways are generally considered to be highly conserved among oral bacteria. Interestingly, between other MS and S. sobrinus, differences in the central carbon metabolic pathways were found by our group (31) and shown in Fig. 1. Facultative anaerobes such as lactic acid bacteria including Streptococcus lack cytochrome oxidases of a respiratory chain and ATP required for survival and growth is generated by substrate level phosphorylation in the glycolysis pathway almost exclusively (36). Interestingly, two L-lactate oxidases (with similarity between 65 and 73% to lactate oxidases of lactobacilli) are found to be conserved in S. sobrinus (so far confirmed for strains AC153, DSM 20742, TCI-107) but are absent in all S. mutans strains. These two enzymes catalyze the reaction of L-Lactate + O2 -> Pyruvate + H2O2 and/or D-Lactate + O2 -> Pyruvate + H2O2. Indeed, three strains of S. sobrinus have been shown to produce hydrogen peroxide in vitro (37). It has been reported that in S. pneumoniae concerted action of lactate oxidase and pyruvate oxidase forms a novel energy-generation pathway by converting lactate acid to acetic acid under aerobic growth conditions (38). Because there is no pyruvate oxidase identified in S. sobrinus DSM 20742, the function of the lactate oxidases in S. sobrinus DSM 20742 should be different to that of S. pneumoniae. By a close examination we hypothesize that lactate oxidase, together with pyruvate dehydrogenase, phosphate acetyl transferase and acetate

![Fig. 1. Central metabolism pathways of mutans streptococci. The orange lines represent enzyme reactions conserved across the mutans streptococci strains compared in our recent study (31), whereas the blue lines represent enzyme reactions specifically present (solid line) or absent (dashed line) in S. sobrinus DSM 20742. Red crosses: the corresponding enzymes were not present in any strain investigated.](image-url)
kinase, could form a novel energy production pathway to convert lactate acid to acetate and simultaneously produce one additional ATP, as depicted. By doing so, the lactate oxidases of *S. sobrinus* DSM 20742 could also play a role in consuming lactate to regulate pH, which would be an advantage for *S. sobrinus* in resistance to acid stress. In addition, this pathway could replenish Acetyl-CoA, an important intermediate for the biosynthesis of fatty acids and amino acids. Furthermore, lactate oxidase and lactate dehydrogenase could form a local NAD⁺ regeneration system, which would be certainly advantageous to *S. sobrinus* DSM 20742 under aerobic growth conditions. Favored by possessing the lactate oxidases, *S. sobrinus* has the potential ability of producing H₂O₂ to kill not only competitors (oxygen sensitive *S. mutans*, oral anaerobes) but also macrophages (39), and defend its ecological niche. In contrast to the unique harboring of lactate oxidases in *S. sobrinus* DSM 20742, citrate lyase (EC 4.1.3.6), which catalyzes the cleavage of citrate into oxaloacetate and acetate, and oxaloacetate decarboxylase (EC 4.1.1.3), catalyzing the irreversible decarboxylation of oxaloacetate to pyruvate and CO₂, are not found in *S. sobrinus* DSM 20742, as shown in Fig. 1 by the blue dotted lines. The absence of citrate lyase and oxaloacetate decarboxylase implies that *S. sobrinus* DSM 20742 might lack the ability in anaerobic utilization of citrate as a substrate. The disadvantages of *S. sobrinus* DSM 20742 in citrate utilization could be offset by the novel energy production pathway from lactate to acetate, as proposed above.

**Acid tolerance and two-component signal systems**

Bacterial transduction two component systems (TCSs) play important roles by enabling cells to detect and respond to diverse changes/stresses in the environment with the pH to be one of the most important. A bacterial TCS comprises in general a trans-membrane sensor histidine kinase (HK) and a corresponding cytoplasmic response regulator (RR) encoded by genes located adjacently within the same operon, although stand-alone genes (‘orphans’) coding for HKs or RRs have also been reported (40). In a recent study, our group investigated differences in TCSs among MS including several *S. mutans* strains and *S. sobrinus* DSM 20742 (41). Totally, 18 TCS clusters comprising HK-RR pairs were identified. In Table 2, similarities and differences for both species in TCSs conserved for *S. mutans* are summarized. *S. sobrinus* DSM 20742 demonstrated deficits in the signal transduction systems related to acid tolerance and fructan catabolism (TCS-3, consisting of CovS/CovR). CovS/CovR is involved in the acid tolerance of *S. mutans* (42) and has also been reported to play a role in counteracting oxidative stress and reducing susceptibility to phagocytic killing (43). Therefore, the absence of TCS-3 can be interpreted as a selective disadvantage for *S. sobrinus* which might at least partially explain its lower prevalence and abundance in the oral cavity in general and in caries in particular.

TCS-7 (PhoR/YcbL) was shared by the eight *S. mutans* strains but was absent in *S. sobrinus*. PhoR is known for sensing environmental phosphate – which can be a limiting factor – in other species (44), but the clear function of TCS-7 in *S. mutans* is still unknown.

TCS-9 (LevRS), which affects the acid tolerance response, is also absent in *S. sobrinus* DSM 20742. In *S. mutans* UA159, the levRS gene cluster is flanked by levQ and levT, which code for two carbohydrate-binding proteins. These four genes together constitute a four-component signal transduction system *levQRST* controlling the transcription of the fructan hydrolase gene (*fruA*) and a four-gene cluster *levDEFG*, which encodes a fructose/mannose sugar-PTS located immediately downstream of *levQRST* (45). *S. sobrinus* was also found to lack the *levQ*, *levT* and *levDEFG* genes. Taking together, these findings indicate dramatic differences in the regulation of fructan catabolism and the acid tolerance response of *S. sobrinus* DSM 20742 in comparison to the *S. mutans* strains. However, other *S. sobrinus* genes (*D823_00365*, *D823_00410*) not shared with *S. mutans* and related to acid tolerance were found in strain DSM 20742.

Finally, TCS-5 (ScnKR) was found to be absent in some *S. mutans* strains and in *S. sobrinus* DSM 20742. In *S. pyogenes* ScnKR is essential for the production of a bacteriocin (SAFF22). In our recent study (41), we therefore inferred that TCS-5 might be involved in the regulation of mutacin production but not in acid tolerance.

**Development of competence**

Competence development is a complex process involving sophisticated regulatory networks that trigger the capacity of bacterial cells to take up exogenous DNA from the environment. This phenomenon is frequently encountered in bacteria of the oral cavity, e.g. *S. mutans* (46). In *S. mutans*, ComX (or SigX), an alternative sigma factor, drives the transcription of the so-called ‘late-competence genes’ required for genetic transformation. ComX activity is induced by the inputs from two types of signaling pathways, namely the competence-stimulating peptide (CSP)-dependent competence regulation system (‘Classical way’, Fig. 2, left side and Table 3) and the XIP-dependent competence regulation system (‘New way’, Fig. 2, right side, Table 3). ComX and the ‘late-competence genes’ regulated by ComX are highly conserved even between the species, indicating that all MS might have the principal ability to be induced to genetic competence. On the other hand, the upstream signaling pathways, described in-depth below, regulating the activity of ComX show large differences.

**CSP-dependent competence regulation**

In *S. mutans*, the prepeptide of CSP is encoded by comC. Whereas comC is present in all *S. mutans* strains
investigated so far, it is absent in the two annotated strains S. sobrinus DSM 20742 and TCI-107. Apart from this synthase, all genes required for CSP-dependent signaling that are found in S. mutans are also present in S. sobrinus. The membrane bound ABC-transporter (ComAB), the two-component signal transduction system ComDE, the extracellular protease SepM, which is involved in the processing of 21-CSP to the mature 18-CSP, as well as the HtrA protease, which is thought to degrade extracellular CSP (49), were all identified in S. sobrinus. But as the central comC homologue is missing in S. sobrinus, this species cannot develop competence through this signaling pathway, but what about alternatives?

**XIP-dependent competence regulation**

A new peptide regulatory system (ComSR) that is independent of CSP and directly activates ComX has been identified by Mashburn-Warren et al. (47). The novel autoinducer XIP (sigX inducing peptide) is synthesized as a prepeptide by the synthase ComS. The membrane protein that processes and exports the 17-mer ComS precursor to the active 7-mer pheromone XIP is unknown. Extracellular XIP is internalized through the peptide transporter OppD. Internalized XIP binds to the transcriptional regulator ComR, which is thereby dimerized and activated. The expression of the synthase ComS, as well as the expression of the alternative sigma-factor ComX, resulting in transcription of the complete transposome, is controlled by ComR. Deletion of the comR or comS gene completely abolished the competence in S. mutans (47). Thus, ComR is the central regulator for competence in S. mutans and is also required for CSP induced competence development. In our previous study (31), the ComSR regulatory system was identified in all of the S. mutans strains, but not in S. sobrinus DSM 20742. Accordingly, despite the presence of comX and the ‘late-competence genes’, we were not able to obtain the

### Table 2. Comparing two component systems between S. mutans [eight strains according to (31)] and S. sobrinus DSM 20742

| TCS cluster | TCS protein | Function | S. mutans | S. sobrinus |
|-------------|-------------|----------|-----------|------------|
| TCS-1       | HK-VicK     | Biofilm development, competence development, oxidative stress tolerance, acid tolerance, autolysin production, glucan metabolism, fructan metabolism | SMU.1516 | D823_04656 |
|             | RR-VicR     |          | SMU.1517 | D823_04651 |
| TCS-2       | HK-CiaH     | Sucrose-dependent biofilm formation, competence development, multiple stress response, bacteriocin production | SMU.1128 | D823_05868 |
|             | RR-CiaR     |          | SMU.1129 | D823_05873 |
| TCS-3       | HK-CovS     | Acid tolerance, hydrogen peroxide resistance, murine macrophage killing | SMU.1145c | Absent |
|             | RR-CovR     |          | SMU.1146c | Absent |
| TCS-4       | HK-KinF     | Acid tolerance, ppGpp metabolism, control of alarmone synthesis | SMU.928 | D823_08322 |
|             | RR-LirF     |          | SMU.927 | D823_08327 |
| TCS-5       | HK-ScnK     | Bacteriocin production | SMU.1814 | Absent |
|             | RR-ScnR     |          | SMU.1815 | Absent |
| TCS-6       | HK-SpaK     | Bacteriocin production, self-protection against anti-microbial peptides | SMU.660 | D823_02456 |
|             | RR-SpaR     |          | SMU.659 | D823_02461 |
| TCS-7       | HK-PhoR     | Unknown | SMU.1037c | Absent |
|             | RR-YcbL     |          | SMU.1038c | Absent |
| TCS-8       | HK-KinG     | Bacteriocin resistance, substrate transport in cell envelope stress | SMU.1009 | D823_04566 |
|             | RR-LirG     |          | SMU.1008 | D823_04561 |
| TCS-9       | HK-LevS     | Biofilm formation, acid tolerance, fructan metabolism | SMU.1965c | Absent |
|             | RR-LevR     |          | SMU.1964c | Absent |
| TCS-10      | HK-LytS     | Biofilm formation, oxidative stress tolerance, autolysis, fructan metabolism, cell wall metabolism | SMU.577 | D823_00965 |
|             | RR-LytT     |          | SMU.576 | D823_00970 |
| TCS-11      | HK-LiaS     | Biofilm formation, acid tolerance, cell envelope stress response, bacteriocin production & resistance, sucrose-dependent adherence | SMU.486 | D823_03016 |
|             | RR-LiaR     |          | SMU.487 | D823_03011 |
| TCS-12      | HK-HK11     | Unknown | SMU.1548c | D823_06808 |
|             | RR-R11      |          | SMU.1547c | D823_06803 |
| TCS-13      | HK-ComD     | Biofilm formation, quorum sensing, competence development, bacteriocin production | SMU.1916 | D823_05333 |
|             | RR-ComE     |          | SMU.1917 | D823_05328 |

Only those conserved in S. mutans are discussed for both species. For more information see ref. (41).

*aAll genes shown are conserved for at least seven out of eight strains and the UA159 gene is shown as representative. 
genetic competence state of *S. sobrinus* DSM 20742 experimentally. Interestingly, *S. sobrinus* DSM 20742 does harbor the OppD peptide transporter for import of extracellular XIP. Thus, all the genes for both signaling pathways for competence are present, with the exception of the respective autoinducer synthases *comC* and *comS*, and the essential transcriptional regulator *comR*.

**Autoinducer-independent competence regulation**

Under conditions of biofilm growth the HdrMR system, a novel two-gene regulatory system, has been shown to contribute to competence development through the activation of ComX (50, 51). Microarray analysis revealed that both regulators, ComE and HdrR, activates a large set of genes (50, 51). Recently, Xie et al. (52) identified another regulatory system in *S. mutans*, designated BrsRM, that regulates bacteriocin-related genes but also affects the HdrRM system. In our recent study, HdrR and the complete BrsRM system were found absent in *S. sobrinus*. 

**Distribution of bacteriocin-related proteins**

Bacteriocins are proteinaceous antimicrobials produced by bacteria to kill or inhibit the growth of similar or closely related bacterial strains. Bacteriocins produced by MS are named ‘mutacins’. As dental plaque, the dominating niche of MS, is a multispecies biofilm community that harbors many microorganisms, mutans group strains have developed a variety of mutacins to inhibit the growth of competitors, such as mitis group streptococci (53–55). In our previous study (31), information about known mutacins as well as mutacin-immunity proteins was collected from the NCBI (http://www.ncbi.nlm.nih.gov) and Oralgen (http://www.oralgen.lanl.gov/) databases. The collected protein sequences were used to blast against the proteomes of eight *S. mutans* strains and *S. sobrinus* DSM 20742 to see whether or not these known mutacins and corresponding immunity proteins exist in both species in a similar or different pattern. Distributions of identified mutacins and mutacin-immunity proteins are summarized in Table 4. Diversity of *Streptococcus* bacteriocins has

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**Fig. 2.** Key differences in competence-related genes between *S. mutans* and *S. sobrinus*. Those which are missing in *S. sobrinus* are crossed off. Adapted from references (47, 48).
been reported previously (56, 57). An interesting new result is that, in contrast to S. mutans
strains, S. sobrinus DSM 20742 does not possess any genes coding for mutacin
or mutacin-like proteins.

Mutacin-Smb has been identified in S. mutans and S. ratti previously (58, 59). Mutacin-K8
is an ortholog of the bacteriocin Streptococcin A-FF22 identified in group-A streptococci
(60), and its production system has also been previously identified in the S. mutans
strains K8 (61), KK23, and NN20125 (31). Possibly caused by transposase activity, the complete
mutacin-K8 production system can be disrupted leaving partial orthologs which we found in S. mutans AC4446, UA159, 5DC8,
and KK21 (31). Lantibiotic mutacins, mutacin-I (62), mutacin-II (63) and mutacin-III (64) are found in only
a few strains of S. mutans so far. Mutacin-IV, non-lantibiotic bacteriocins coded by
nlmA/B, was discovered first in S. mutans UA140 to be active against the mitis
group streptococci (65). We found nlmA/B in most S. mutans strains but not in
S. sobrinus, the latter even negative for mutacin-IV-like protein encoding
genes. Interestingly, the immunity protein for mutacin-IV (SMU.152) was identified in all mutacin-IV-negative strains
including S. sobrinus, consistent with the fact that no
inhibition phenomenon has been observed yet among
different MS strains. Mutacin-V, another non-lantibiotic

Table 3. Comparing competence development-related systems between S. mutans UA159 [and orthologs of seven additional
strains according to (31)] and S. sobrinus DSM 20742

| Group          | Name      | Function                                                      | Eight strains | DSM 20742 |
|----------------|-----------|---------------------------------------------------------------|---------------|-----------|
| Classical way  | ComC      | Competence stimulating peptide, precursor                     | SMU.1915      | Absent    |
|                | ComA/NlmT | Competence factor and non-lantibiotic mutacin transporter      | SMU.286       | SMU.1881c |
|                |           | AT-binding/permease protein                                   | D823_05343    | D823_01400|
|                | ComB/NlmE | Accessory factor for NlmT                                     | SMU.287       | D823_05923|
|                | SepM      | Cell surface-associated protease cleavage CSP                 | SMU.518       | D823_08607|
|                | ComD      | Histidine kinase                                              | SMU.1916      | D823_05333|
|                | ComE      | Response regulator                                            | SMU.1917      | D823_05328 |
|                | HtrA      | Serine protease                                               | SMU.2164      | D823_03191|
| New way        | ComS      | comX-inducing peptide (XIP) precursor                         | NC_004350.2   | Absent    |
|                |           |                                                               | (62613-62666)a|           |
| Alternative way| ComR      | ComS receptor                                                 | SMU.61        | Absent    |
|                | HdrM      | High-density responsive membrane protein                      | SMU.1855      | D823_08222|
|                | HdrR      | High-density responsive regulator                              | SMU.1854      | Absent    |
|                | BrsM      |                                                               | SMU.2081      | Absent    |
|                | BrsR      |                                                               | SMU.2080      | Absent    |
| OppD           | Oligopeptide ABC transporter                                 | SMU.258       | D823_04322|
| Late competence| ComX (SigX)| Competence-specific sigma factor                               | SMU.1997      | D823_08887|
|                | ComEA     | Competence protein                                            | SMU.625       | D823_08107|
|                | ComEC     | Competence protein; possible integral membrane protein        | SMU.626       | D823_08117|
|                | CoiA      | Competence protein CoiA                                       | SMU.644       | D823_01025|
|                | EndA      | Competence-associated membrane nuclelease (DNA-entry nuclease)| SMU.1523      | D823_09687|
|                | ComG      | Competence protein G                                          | SMU.1981c     | D823_01170|
|                | ComYD     | Competence protein ComYD                                      | SMU.1983      | D823_01160|
|                | ComYC     | Competence protein ComYC,                                     | SMU.1984      | D823_01155|
|                | CinA      | Competence damage-inducible protein A                         | SMU.2086      | D823_03558|
|                | CinYB     | Competence protein; general (type II) secretory pathway protein| SMU.1985      | D823_01150|
|                | CinYA     | Late competence protein; type II secretion system protein     | SMU.1987      | D823_01145|
|                | CinFC     | Late competence protein required for DNA uptake               | SMU.499       | D823_02981|
|                | CinFA     | Late competence protein F                                      | SMU.498       | D823_02986|
|                | CinA      | Competence damage-inducible protein A                         | SMU.2086      | D823_03593|

<sup>a</sup>All genes shown are conserved for at least seven out of eight strains and the UA159 gene variant is shown as representative.
<sup>b</sup>The gene D823_7992 is very similar to D823_5328 but found distantly on a different contig.
peptide coded by cipB, was frequently found in S. mutans strains (exceptions are, however, reference strains ATCC 15175 and NCTC 11060) but not in S. sobrinus DSM 20742. There are two homologs of mutacin-V immunity protein in S. mutans UA159, namely the product of SMU.1913 and Cipl (SMU.925) (66, 67); the latter is supposed to be the key factor of immunity. All the S. mutans strains investigated by our group together with S. sobrinus DSM 20742 possess at least one orthologous gene encoding one of the two mutacin-V immunity proteins so that they might not be inhibited by mutacin-V producing strains. Furthermore, a possible non-lantibiotic bacteriocin peptide gene (SMU.423) was found to be conserved in S. mutans and present in S. sobrinus DSM 20742. In addition, putative ComAB (NlmTE), which has been proved to be the transporter complex of mutacin IV or – more likely – for multi-type non-lantibiotic bacteriocins in S. mutans (31, 68), are identified in all S. mutans strains and S. sobrinus. It may very be possible that this bacteriocin is similar to the mutacin isolated from S. sobrinus strain MT6223 that proved to be competitive with S. mutans in rat models (69).

To summarize, S. sobrinus DSM 20742 (and confirmed in TCI-107) does not possess any genes coding for mutacin or mutacin-like proteins including Mutacin-Smb, Mutacin-K8, Mutacin-I-III, Mutacin-IV (NlmA and B), and Mutacin-V, although bacteriocin-like proteins from S. sobrinus have been found for individual strains.

However, searching among the bacteriocin/immunity genes discovered exclusively in S. sobrinus (and not in S. mutans), two immunity protein encoding genes but no additional bacteriocin-gene were identified (function in brackets): D823_05508 (immunity protein PlnI-like) and D823_03977 (putative bacteriocin immunity protein).

The accumulation and conservation of mutacin immunity proteins apparently play an important role for the survival of all MS strains and species in a bacteriocin-rich environment.

**Oxidative stress defense systems**

For protection against reactive oxygen species (such as $O_2^-$, $H_2O_2$, HO-) or adaptation to oxidative stresses, aerobes and facultative anaerobes have evolved efficient defense systems, comprising an array of antioxidant enzymes such as catalase, superoxide dismutase (SOD), alkali-hydroperoxide reductase (AhpCF), Dps-like peroxide resistance protein (Dpr), thioredoxin reductase, and glutathione reductase, which have been identified in many bacterial species. By searching for known antioxidant systems in the genomes of sequenced mutans streptococcal strains, we obtained an overview of putative oxidative defense systems (31) summarized in Table 5. Catalase, which catalyzes the decomposition of hydrogen peroxide, was never found in any of the mutans streptococcal strains but other classes of oxygen tolerance-related proteins do exist.

First, SOD, which catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, was found in all strains of our study conserved including S. sobrinus DSM 20742 and it is present in S. sobrinus TCI-107 in two copies.

Next, it has been reported that both the bi-component peroxidase system AhpF/AhpC (catalyzing the NADH-dependent reduction of organic hydroperoxides and/or $H_2O_2$ to their respective alcohol) and Dpr (a ferritin-like iron-binding protein) confer tolerance to oxidative stress in S. mutans (70). AhpF/AhpC-genes were present in all S. mutans strains, but were absent in S. sobrinus DSM 20742 (31) indicating that these genes do not form an essential peroxide tolerance system for MS. Indeed, Higuchi et al. (36) found that a double ahpF/ahpC mutant still showed the same level of peroxide tolerance as the defect could be complemented by the S. mutans-dpr gene. Dpr homologs were found in all S. mutans strains and in S. sobrinus underlining the essential function in oxygen tolerance.

Thioredoxins are a class of small redox mediator proteins known to be present in all organisms. They are involved in many important biological processes, including redox signaling. The flavor enzyme thioredoxin reductase keeps thioredoxins in the reduced state in a NADPH-dependent reaction (71). They act as electron donors
to many proteins including thiol peroxidases (72). Thioredoxin, thioredoxin reductase and thiol peroxidase, the components of the thioredoxin system, were identified in all *S. mutans* strains and in *S. sobrinus* DSM 20742. Thioredoxin family proteins (SMU.1971c and SMU.1169c) are found to be present in nearly all strains, except for *S. sobrinus* DSM 20742, which lacks any ortholog of SMU1169c (31).

Finally, glutaredoxins share many functions of thioredoxins. But they are oxidized by their corresponding substrates and reduced by glutathione (GSH) (73). The resulting oxidized glutathione (GSSG) is regenerated by glutathione reductase. Together, these components comprise the glutathione system (74). Several *S. mutans* strains possess two glutathione reductase orthologs (SMU.140 and SMU.838). In contrast, *S. sobrinus* DSM 20742 possesses an ortholog for SMU.838 but not for SMU.140, possibly leading to a reduced potential for re-generation of GSH from GSSG and weakening its oxidative resistance.

**Link between acidogenicity, aciduricity, biofilm formation, mutacin production, competence and – ultimately – cariogenicity**

The main virulence traits of *S. mutans* – adherence, acidogenicity, aciduricity, biofilm formation and mutacin production – as well as its ability to incorporate foreign DNA into its genome (genetic competence) are controlled or modulated by quorum sensing and thus depending on its own cell number but maybe also on cell numbers of cohabitants. For *S. mutans*, it has recently been shown that the complete quorum sensing system is induced by co-culture with the human pathogenic fungus *Candida albicans* (75). Additionally, both strains grow better together than as a monoculture, suggesting that this synergism may lead to enhanced cariogenicity. This could recently be confirmed in an animal model (76). Interestingly, one main cariogenic trait of *S. mutans*, the synthesis of extracellular glucans and fructans, was strongly inhibited in co-culture (75). Similar studies on *S. sobrinus* are missing but – without competence so far investigated – such a co-stimulation is not expected.

Clearly the influence of inter-species communication for caries development warrants further studies.

In summary, this work compares two main cariogenic MS on a whole genome level. Although more aciduric and acidogenic, by many other ecologically important features, *S. sobrinus* seems to be weaker in its cariogenic potential. As rapid adherence to the pellicle coated tooth surface is crucial for colonization and expressing cariogenic potential, the down-regulation of surface antigen SpaA by the negative regulator Par, which is only found in *S. sobrinus* but not in *S. mutans*, could be essential. Furthermore, the lack of genetic competence of *S. sobrinus* limits its evolutionary potential. However, we have to keep in mind that this analysis is based on about 8·20 annotated *S. mutans* strains but on only two *S. sobrinus* strains. In total we found about 470 genes in *S. sobrinus* DSM 20742 – about half of them hypothetical proteins with no allocated or known function so far – with no orthologs in *S. mutans*. Thus, *S. sobrinus* possess much more potential yet to be discovered. Finally, this comparison of genetic inventory (genotypes) might help to describe or predict phenotypes but there are more factors, especially in a very complex habitat like the human oral cavity, which

### Table 5. Comparing proteins involved in oxygen tolerance between *S. mutans* [eight strains according to (31)] and *S. sobrinus* DSM 20742

| Class                        | Name     | Function                                      | *S. mutans* | *S. sobrinus* |
|-----------------------------|----------|-----------------------------------------------|-------------|--------------|
| SOD                         | Sod      | Superoxide dismutase                          | SMU.629     | D823_08152   |
| AhpF/AhpC system            | AhpC     | Alkyl hydroperoxide reductase, subunit C      | SMU.764     | Absent       |
|                             | AhpF (Nox1) | Alkyl hydroperoxide reductase, subunit F     | SMU.765     | Absent       |
| Dpr                         | Dpr      | Peroxide resistance protein/iron binding protein | SMU.540     | D823_02352   |
| Thioredoxin system          | TrxB     | Thioredoxin reductase (NADPH)                | SMU.463     | D823_01947   |
|                             | TrxB     | Thioredoxin reductase                         | SMU.869     | D823_01550   |
|                             | TrxA     | Thioredoxin                                   | SMU.1869    | D823_06913   |
|                             | TrxH     | Thioredoxin family protein                    | SMU.1971c   | D823_06552   |
|                             | Tpx      | Thioredoxin                                   | SMU.1169c   | Absent       |
| Glutaredoxin system         | GshAB    | Glutathione biosynthesis bifunctional protein | SMU.267c    | D823_06703   |
|                             | GshR     | Glutathione reductase                         | SMU.838     | D823_04976   |
|                             | NrdH     | Glutaredoxin                                  | SMU.140     | Absent       |

*a*All genes shown are conserved for at least seven out of eight strains and the UA159 gene variant is shown as representative.*
certainly influence the true cariogenic potential of those organisms.

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References

1. Loesche WJ. Chemotherapy of dental plaque infections. Oral Sci Rev 1976; 9: 65–107.
2. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 1994; 8: 263–71.
3. Takahashi N, Nyvad B. Caries ecologies revisited: microbial dynamics and the caries process. Caries Res 2008; 42: 409–18.
4. Rosier BT, De Jager M, Zaura E, Krom BP. Historical and contemporary hypotheses on the development of oral diseases: are we there yet? Front Cell Infect Microbiol 2014; 4: 92.
5. de Soet JJ, Nyvad B, Kilian M. Strain-related acid production by oral streptococci. Caries Res 2000; 34: 486–90.
6. Carlsson P, Gandour IA, Olsson B, Rickardsson B, Abbas K. High prevalence of mutans streptococci in a population with extremely low prevalence of dental caries. Oral Microbiol Immunol 1987; 2: 121–4.
7. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev 1986; 50: 353–80.
8. de Soet JJ, Toors FA, de Graaff J. Acidogenesis by oral streptococci at different pH values. Caries Res 1989; 23: 14–17.
9. de Soet JJ, van Loveren C, Lammens AJ, Pavicic MJ, Homburg CH, ten Cate JM, et al. Differences in cariogenicity between fresh isolates of Streptococcus sobrinus and Streptococcus mutans. Caries Res 1991; 25: 116–22.
10. Okada M, Kawamura M, Oda Y, Yasuda R, Kojima T, Kurihara H. Caries prevalence associated with mutans streptococci and Streptococcus sobrinus in Japanese schoolchildren. Int J Paediatr Dent 2012; 22: 342–8.
11. Okada M, Taniguchi Y, Hayashi F, Doi T, Suzuki J, Sugai M, et al. Late established mutans streptococci in children over 3 years old. Int J Dent 2010; 2010: 723468.
12. Okada M, Soda Y, Hayashi F, Doi T, Suzuki J, Miura K, et al. Longitudinal study of dental caries incidence associated with Streptococcus mutans and Streptococcus sobrinus in pre-school children. J Med Microbiol 2005; 54: 661–65.
13. Sanchez-Acedo M, Montiel-Company JM, Dasi-Fernandez F, Almerich-Silla JM. Streptococcus mutans and Streptococcus sobrinus detection by polymerase chain reaction and their relation to dental caries in 12 and 15 year-old schoolchildren in Valencia (Spain). Med Oral Patol Oral Cir Bucal 2013; 18: 839–45.
14. Holbrook WP, Magnusdollt MO. Studies on strains of Streptococcus mutans isolated from caries-active and caries-free individuals in Iceland. J Oral Microbiol 2012; 4: 10611, doi: http://dx.doi.org/10.3402/jom.v6.2658410611
15. Lindquist B, Emilson CG. Interactions between and within Streptococcus mutans and Streptococcus sobrinus isolated from humans harboring both species. Scand J Dent Res 1991; 99: 498–504.
16. Lindquist B, Emilson CG. Dental location of Streptococcus mutans and Streptococcus sobrinus in humans harboring both species. Caries Res 1991; 25: 146–52.
17. Lindquist B, Emilson CG. Colonization of Streptococcus mutans and Streptococcus sobrinus genotypes and caries development in children to mothers harboring both species. Caries Res 2004; 38: 95–103.
18. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. J Dent Res 1993; 72: 37–45.
19. Strætemans MM, van Loveren C, de Soet JJ, de Graaff J, ten Cate JM. Colonization with mutants streptococci and lactobacilli and the caries experience of children after the age of five. J Dent Res 1998; 77: 1851–5.
20. Zhang P, Jespersgaard C, Lamberty-Mallory L, Katz J, Huang Y, Hajishengallis G, et al. Enhanced immunogenicity of a genetic chimeric protein consisting of two virulence antigens of Streptococcus mutans and protection against infection. Infect Immun 2002; 70: 6779–87.
21. Jakubovics NS, Stromberg N, van Dolleweerd CJ, Kelly CG, Jenkinson HF. Differential binding specificities of oral streptococcal antigen I/II family adhesins for human or bacterial ligands. Mol Microbiol 2005; 55: 1591–605.
22. Zhang S, Green NM, Sitkiewicz L, Lefebvre RB, Musser JM. Identification and characterization of an antigen I/II family protein produced by group A Streptococcus. Infect Immun 2006; 74: 2400–13.
23. Demuth DR, Davis CA, Corner AM, Lamont RJ, Leboy PS, Malamud D. Cloning and expression of a Streptococcus sanguis surface antigen that interacts with a human salivary agglutinin. Infect Immun 1988; 56: 2484–90.
24. van Raamsdonk M, de Soet JJ, Jones CL, de Graaff J. Effect of antibodies on the chain length and growth of Streptococcus sobrinus. Caries Res 1997; 31: 35–40.
25. Nobles AH, Lamont RJ, Jenkinson HF. Streptococcus adherence and colonization. Microbiol Mol Biol Rev 2009; 73: 407–50, table of contents.
26. Lawrence MC, Pilling PA, Epa VC, Berry AM, Oggunniyi AD, Paton JC. The crystal structure of pneumococcal surface antigen PsaA reveals a metal-binding site and a novel structure for a putative ABC-type binding protein. Structure 1998; 6: 1553–61.
27. Staat RH, Peyton JC. Adherence of oral streptococci: evidence for nonspecific adsorption to saliva-coated hydroxylapatite surfaces. Infect Immun 1984; 44: 653–9.
28. Gibbons RJ, Cohen L, Hay DI. Strains of Streptococcus mutans and Streptococcus sobrinus attach to different pellicle receptors. Infect Immun 1986; 52: 555–61.
29. Rolla G, Ciardi JE, Schultz SA. Adsorption of glucosyltransferase to saliva coated hydroxylapatite. Possible mechanism for sucrose dependent bacterial colonization of teeth. Scand J Dent Res 1983; 91: 112–17.
30. Walker GJ, Brown RA, Taylor C. Activity of Streptococcus mutans alpha-D-glucosyltransferases released under various growth conditions. J Dent Res 1984; 63: 397–400.
31. Song L, Wang W, Conrads G, Rheinberg A, Szajer H, Beck M, et al. Genetic variability of mutans streptococci revealed by whole genome sequencing. BMC Genomics 2013; 14: 430.
32. Nanbu A, Hayakawa M, Takada K, Shinozaki N, Abiko Y, Fukushima K. Production, characterization, and application of monoclonal antibodies which distinguish four glucosyltransferases.
from *Streptococcus sobrinus*. FEMS Immunol Med Microbiol 2000; 27: 9–15.

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

34. Homer KA, Patel R, Beighton D. Effects of N-acetylglucosamine on carbohydrate fermentation by *Streptococcus mutans* NCTC 10449 and *Streptococcus sobrinus* SL-1. Infect Immun 1993; 61: 295–302.

35. Moye ZD, Zeng L, Burne RA. Modification of gene expression and virulence traits in *Streptococcus mutans* in response to carbohydrate availability. Appl Environ Microbiol 2014; 80: 972–85.

36. Higuchi M, Yamamoto Y, Kamio Y. Molecular biology of oxygen tolerance in lactic acid bacteria: functions of NADH oxidases and Dpr in oxidative stress. J Biosci Bioeng 2000; 90: 484–93.

37. Garcia-Mendoza A, Liebana J, Castillo AM, de la Higuera A, Taniai H, Iida K, Seki M, Saito M, Shiota S, Nakayama H, Terao Y, Kawabata S, Okahashi N, Nakata M, Sumitomo T, GS5. Novel bacteriocin regulatory system in *Streptococcus mutans* UA159 are active against multiple streptococcal species. Appl Environ Microbiol 2011; 77: 2428–34. doi: 10.1128/AEM.02320-10.

38. Bekal-Si Ali S, Hurtubise Y, Lavoie MC, LaPointe G. Diversity of *Streptococcus mutans* bacteriocins as confirmed by DNA analysis using specific molecular probes. Gene 2002; 283: 125–31.

39. Nes IF, Diep DB, Holo H. Bacteriocin diversity in *Streptococcus and Enterococcus*. J Bacteriol 2007; 189: 1189–98.

40. Johnson DW, Tagg JR, Wannamaker LW. Production of a bacteriocine-like substance by group-A streptococci of M-type 4 and T-pattern 4. J Med Microbiol 1979; 12: 413–27.

41. Song L, Sudhakar P, Wang W, Conrads G, Brock A, Sun J, et al. A genome-wide study of two-component signal transduction systems in eight newly sequenced *Streptococcus* strains. BMC Genomics 2012; 13: 128.

42. 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.

43. 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. doi: 10.1016/j.micinf.2007.12.006.

44. Gardner SG, Johns KD, Tanner R, McCleary WR. The PhoU protein from *Escherichia coli* interacts with PhoR, PhoB, and metals to form a phosphate-signaling complex at the membrane. J Bacteriol 2014; 196: 1741–52.

45. Zeng L, Wen ZT, Burne RA. A novel signal transduction system and feedback loop regulate fructan hydrolase gene expression in *Streptococcus mutans*. Mol Microbiol 2006; 62: 187–200.

46. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ 2001; 65: 1028–37.

47. Mashburn-Warren L, Morrison DA, Federle MJ. A novel double-tryptophan peptide pheromone controls competence in *Streptococcus* spp. via an Rgg regulator. Mol Microbiol 2010; 78: 589–606. doi: 10.1111/j.1365-2958.2010.07361.x.

48. Federle MJ, Morrison DA. One if by land, two if by sea: signalling to the ranks with CSP and XIP. Mol Microbiol 2012; 86: 241–5.

49. Niu G, Xie Z, Qi F, Merritt J. The hsrRM operon of *Streptococcus mutans* encodes a novel regulatory system for coordinated competence development and bacteriocin production. J Bacteriol 2010; 192: 1844–52. doi: 10.1128/JB.01667-09.

50. Okinaga T, Xie Z, Niu G, Qi F, Merritt J. Examination of the hsrRM regulon yields insight into the competence system of *Streptococcus mutans*. Mol Oral Microbiol 2010; 25: 165–77. doi: 10.1111/j.2041-1014.2010.00574.x.

51. Xie Z, Okinaga T, Niu G, Qi F, Merritt J. Identification of a novel bacteriocin regulatory system in *Streptococcus mutans*. Mol Microbiol 2010; 78: 1431–47. doi: 10.1111/j.1365-2958.2010.07417.x.

52. Kishimoto K, Takei T, Ooshima T, Hamada S. Mutacin activity of strains isolated from children with varying levels of mutants streptococci and caries. Arch Oral Biol 1991; 36: 251–5.

53. Baba T, Schneewind O. Instruments of microbial warfare: bacteriocin synthesis, toxicity and immunity. Trends Microbiol 1998; 6: 66–71.

54. Son M, Ahn SJ, Guo Q, Burne RA, Hagen SJ. Microfluidic study of competence regulation in *Streptococcus mutans*. BMC Genomics 2012; 13: 128.

55. Hossain MS, Biswas I. Mutacins from *Streptococcus mutans* UA159 are active against multiple streptococcal species. Appl Environ Microbiol 2011; 77: 2428–34. doi: 10.1128/AEM.02320-10.

56. Baker-Si Ali S, Hurtubise Y, Lavoie MC, LaPointe G. Diversity of *Streptococcus mutans* bacteriocins as confirmed by DNA analysis using specific molecular probes. Gene 2002; 283: 125–31.

57. Nes IF, Diep DB, Holo H. Bacteriocin diversity in *Streptococcus and Enterococcus*. J Bacteriol 2007; 189: 1189–98.

58. Yonezawa H, Kuramitsu HK. Genetic analysis of a unique bacteriocin, Smh, produced by *Streptococcus mutans* GS5. Antimicrob Agents Chemother 2005; 49: 541–8.

59. Hyink O, Balakrishnan M, Tagg JR. Streptococcus rattus strain BHT produces both a class I two-component lantibiotic and a class II bacteriocin. FEMS Microbiol Lett 2005; 252: 235–41.

60. Johnson DW, Tagg JR, Wannamaker LW. Production of a bacteriocine-like substance by group-A streptococci of M-type 4 and T-pattern 4. J Med Microbiol 1979; 12: 413–27.

61. Robson CL, Wescombe PA, Klesse NA, Tagg JR. Isolation and partial characterization of the *Streptococcus mutans* type AII lantibiotic mutacin K8. Microbiology 2007; 153: 1631–41.

62. Nguyen T, Zhang Z, Huang HW, Wu C, Merritt J, Shi W, et al. Genes involved in the repression of mutacin I production in *Streptococcus mutans*. Microbiology 2009; 155: 551–6.

63. Chen P, Qi F, Novak J, Caffield PW. The specific genes for lantibiotic mutacin II biosynthesis in *Streptococcus mutans* T8 are clustered and can be transferred en bloc. Appl Environ Microbiol 1999; 65: 1356–60.

64. Qi F, Chen P, Caffield PW. Purification of mutacin III from group III *Streptococcus mutans* UA787 and genetic analyses of mutacin III biosynthesis genes. Appl Environ Microbiol 1999; 65: 3880–7.

65. Qi F, Chen P, Caffield PW. The group I strain of *Streptococcus mutans* UA140, produces both the lantibiotic mutacin I and a nonlantibiotic bacteriocin, mutacin IV. Appl Environ Microbiol 2001; 67: 15–21.

66. 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.

67. Ajdic D, McShan WM, McLaughlin RE, Savic G, Chang J, Carson MB, et al. Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. Proc Natl Acad Sci U S A 2002; 99: 14349–44.

68. Hale JD, Heng NC, Jack RW, Tagg JR. Identification of nlmTE, the locus encoding the ABC transport system required for export of nonlantibiotic mutacins in *Streptococcus mutans*. J Bacteriol 2005; 187: 5036–9.

69. Loyola-Rodriguez JP, Morisaki I, Kitamura K, Hamada S. Purification and properties of extracellular mutacin, a bacteriocin

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from *Streptococcus sobrinus*. J Gen Microbiol 1992; 138: 269–74.

70. Yamamoto Y, Higuchi M, Poole LB, Kamio Y. Role of the dpr product in oxygen tolerance in *Streptococcus mutans*. J Bacteriol 2000; 182: 3740–7.

71. Mustacich D, Powis G. Thioredoxin reductase. Biochem J 2000; 346: 1–8.

72. Arner ES, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 2000; 267: 6102–9.

73. Holmgren A. Thioredoxin and glutaredoxin systems. J Biol Chem 1989; 264: 13963–6.

74. Fernandes AP, Holmgren A. Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. Antioxid Redox Signal 2004; 6: 63–74.

75. Sztajer H, Szafranski SP, Tomasz J, Reck M, Nimitz M, Rohde M, et al. Cross-feeding and interkingdom communication in dual-species biofilms of *Streptococcus mutans* and *Candida albicans*. ISME J 2014; 8: 2256–71.

76. Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S, Pai CH, et al. Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes virulence of plaque biofilms in vivo. Infect Immun 2014; 82: 1968–81.