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REVIEW ARTICLE

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).

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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, adherence to epitopes in the oral cavity is a crucial step for streptococci to become resident.

In *S. mutans*, cell surface antigen I/II (AgI/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 AgI/II family (synonyms SAI/II, PAc, P1, 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 AgI/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.

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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

			S. mutans	*S. sobrinus*
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, *gtfSI*
	Gtf-S	Glucosyltransferase-S (soluble)	SMU.910, *gtfD*	D823_03428, *gtfS1*
	Gtf-S	Glucosyltransferase-S (soluble)	Absent	D823_01485, *gtfS2*
	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), 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*-acetylglucosamine (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 deacetylase 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 maltodextrans, 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 + O_2 → Pyruvate + H_2O_2 and/or D-Lactate + O_2 → Pyruvate + H_2O_2. 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

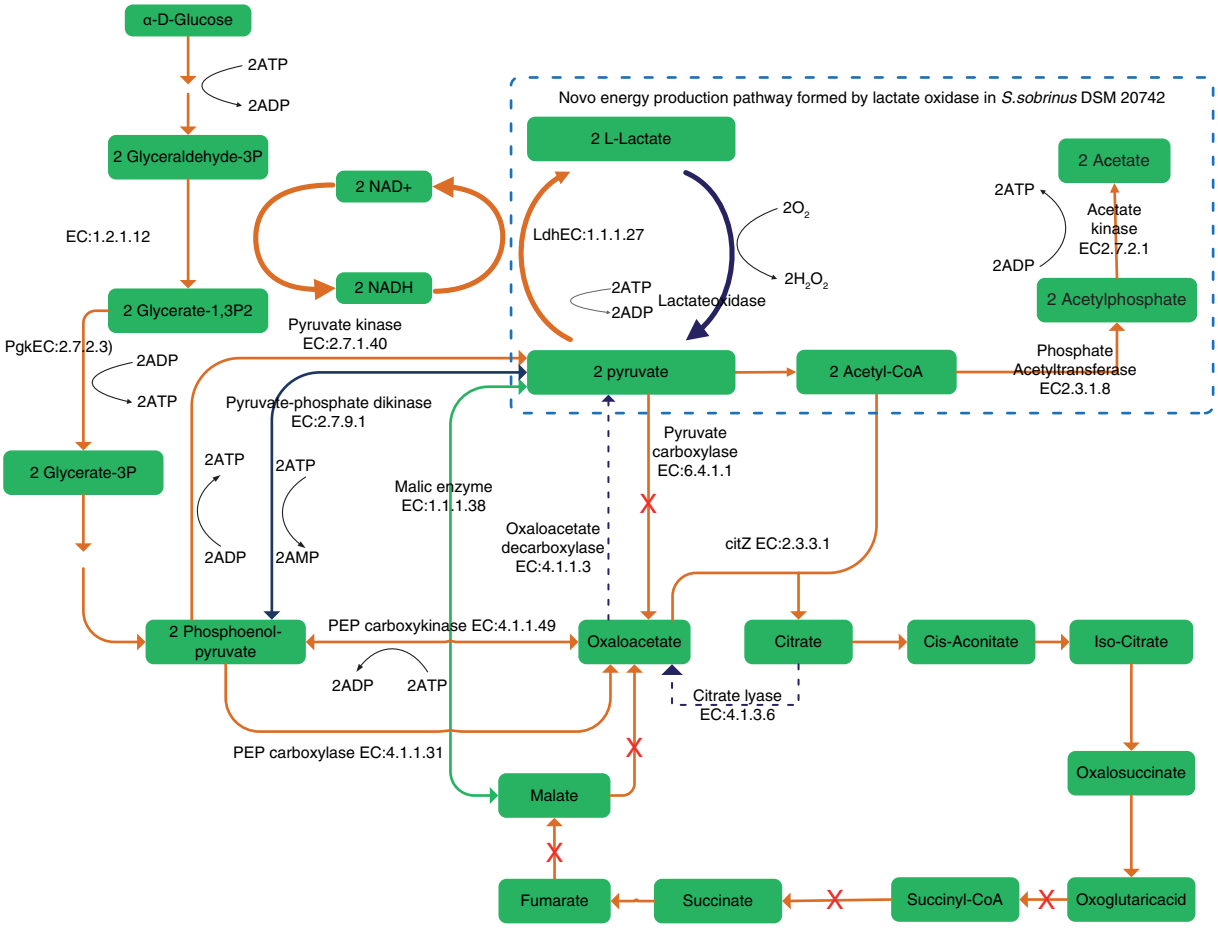

Fig. 1. Central metabolism pathways of mutans streptococci. The orange lines represent enzyme reactions conserved across the mutans streptococci strains compared in our recent study (31), whereas the blue lines represent enzyme reactions specifically present (solid line) or absent (dashed line) in *S. sobrinus* DSM 20742. Red crosses: the corresponding enzymes were not present in any strain investigated.

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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 H_2O_2 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 CO_2, 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

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

			S. mutans	*S. sobrinus*
TCS cluster	TCS protein	Function	8 strains[a]	DSM 20742
TCS-1	HK-VicK	Biofilm development, competence development, oxidative stress tolerance,	SMU.1516	D823_04656
	RR-VicR	acid tolerance, autolysin production, glucan metabolism, fructan metabolism	SMU.1517	D823_04651
TCS-2	HK-CiaH	Sucrose-dependent biofilm formation, competence development,	SMU.1128	D823_05868
	RR-CiaR	multiple stress response, bacteriocin production	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, pp(G)pp metabolism, control of alarmone synthesis	SMU.928	D823_08322
	RR-LlrF		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-LlrG		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,	SMU.577	D823_00965
	RR-LytT	fructan metabolism, cell wall metabolism	SMU.576	D823_00970
TCS-11	HK-LiaS	Biofilm formation, acid tolerance, cell envelope stress response,	SMU.486	D823_03016
	RR-LiaR	bacteriocin production & resistance, sucrose-dependent adherence	SMU.487	D823_03011
TCS-12	HK-HK11	Unknown	SMU.1548c	D823_06808
	RR-RR11		SMU.1547c	D823_06803
TCS-13	HK-ComD	Biofilm formation, quorum sensing, competence development, bacteriocin	SMU.1916	D823_05333
	RR-ComE	production	SMU.1917	D823_05328

Only those conserved in *S. mutans* are discussed for both species. For more information see ref. (41).
[a]All genes shown are conserved for at least seven out of eight strains and the UA159 gene is shown as representative.

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 transformasome, 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

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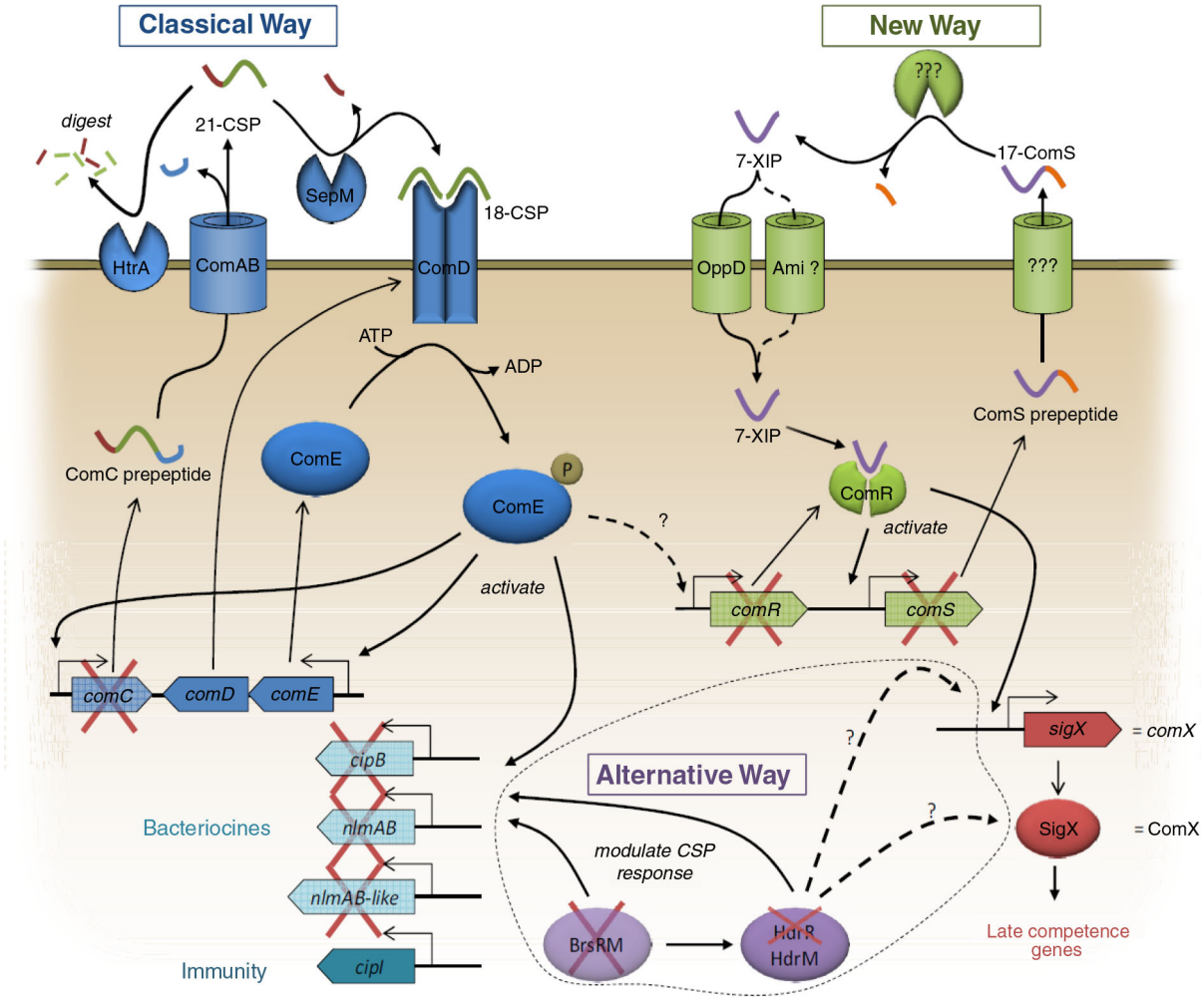

Fig. 2. Key differences in competence-related genes between *S. mutans* and *S. sobrinus*. Those which are missing in *S. sobrinus* are crossed off. Adapted from references (47, 48).

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, activate 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

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

			S. mutans	*S. sobrinus*
Group	Name	Function	Eight strains[a]	DSM 20742
Classical way	ComC	Competence stimulating peptide, precursor	SMU.1915	Absent
	ComA/NlmT	Competence factor and non-lantibiotic mutacin	SMU.286 SMU.1881c	D823_05343
		transporter ATP-binding/permease protein		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
				D823_7992[b]
	HtrA	Serine protease	SMU.2164	D823_03191
New way	ComS	*comX*-inducing peptide (XIP) precursor	NC_004350.2 (62613-62666)a	Absent
	ComR	ComS receptor	SMU.61	Absent
Alternative way	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 nuclease (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
		Possible competence-induced protein	SMU.2075c	D823_03558
	CinA	Competence damage-inducible protein A	SMU.2086	D823_03593
	ComYB	Competence protein; general (type II) secretory pathway protein	SMU.1985	D823_01150
	ComYA	Late competence protein; type II secretion system protein	SMU.1987	D823_01145
	ComFC	Late competence protein required for DNA uptake	SMU.499	D823_02981
	ComFA	Late competence protein F	SMU.498	D823_02986
	CinA	Competence damage-inducible protein A	SMU.2086	D823_03593

[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 gene D823_7992 is very similar to D823_5328 but found distantly on a different contig.

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* DSM 20742, 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

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Table 4. Comparing bacteriocin and corresponding immunity proteins between *S. mutans* [eight strains according to (31)] and *S. sobrinus* DSM 20742

	S. mutans	*S. sobrinus*
Mutacin/immunity protein	Eight strains	DSM 20742
Lantibiotic mutacins		
Mutacin-Smb	Rare	Absent
Mutacin-I	Rare	Absent
Mutacin-II	Rare	Absent
Mutacin-III	Rare	Absent
Mutacin-K8	Rare	Absent
Non-lantibiotic bacteriocins		
Mutacin-IV (NlmA)	Frequent	Absent
Mutacin-IV (NlmB)	Frequent	Absent
Mutacin-IV like (SMU.283)	Conserved	Absent
Immunity protein of Mutacin-IV	Highly conserved	Present
Mutacin-V (CipB)	Frequent	Absent
CipI, immunity protein of CipB	Very frequent	Absent
Homolog of CipI	Frequent	Present
SMU.423 (possible bacteriocin)	Conserved	Present
NlmT/ComA	Conserved	Present
ATP-binding protein of NlmTE	Frequent	Present
NlmE/ComB (accessory factor for NlmT)	Highly conserved	Present

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 CipI (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 peptidegene (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), alkylhydroperoxide 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

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

			S. mutans	*S. sobrinus*
Class	Name	Function	Eight strains[a]	DSM 20742
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_08552
		Thioredoxin family protein	SMU.1169c	Absent
	Tpx	Thiol peroxidase	SMU.924	D823_07595
Glutaredoxin system	GshAB	Glutathione biosynthesis bifunctional protein	SMU.267c	D823_06703
	GshR	Glutathione reductase	SMU.838	D823_04976
	GshR	Glutathione reductase	SMU.140	Absent
	NrdH	Glutaredoxin	SMU.669c	D823 05398

[a]All genes shown are conserved for at least seven out of eight strains and the UA159 gene variant is shown as representative.

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

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certainly influence the true cariogenic potential of those organisms.

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