Published in final edited form as:

Microbiol Spectr. 2019 January ; 7(1): . doi:10.1128/microbiolspec.GPP3-0051-2018.

The Biology of *Streptococcus mutans*

JA Lemos^{1,*}, SR Palmer², L Zeng¹, ZT Wen³, JK Kajfasz¹, IA Freires¹, J Abranches¹, and LJ Brady¹

¹Department of Oral Biology, University of Florida College of Dentistry, Gainesville, FL

²Division of Biosciences, College of Dentistry, Ohio State University, Columbus, OH

³Department of Comprehensive Dentistry and Biomaterials and Department of Microbiology, Immunology and Parasitology, Louisiana State University Health Sciences Center, New Orleans, LA.

In 1924, J. Clarke isolated an organism from carious lesions and called it *Streptococcus mutans*, because he thought the oval shaped cells observed were mutant forms of streptococci (1). However, it was in the late 1950s when *S. mutans* gained widespread attention within the scientific community and, by the mid 1960s, clinical and animal-based laboratory studies depicted *S. mutans* as an important etiologic agent in dental caries (2). The natural habitat of *S. mutans* is the human oral cavity, more specifically the dental plaque, a multispecies biofilm formed on hard surfaces of the tooth. It has been largely accepted that the cariogenic potential of *S. mutans* resides in three core attributes: (i) the ability to synthesize large quantities of extracellular polymers of glucan from sucrose that aid in the permanent colonization of hard surfaces and in the development of the extracellular polymeric matrix *in situ*, (ii) the ability to transport and metabolize a wide range of carbohydrates into organic acids (acidogenicity), and (iii) the ability to thrive under environmental stress conditions, particularly low pH (aciduricity) (3). While *S. mutans* does not act alone in the development of dental caries, studies from several laboratories have convincingly demonstrated that *S. mutans* can alter the local environment by forming an EPS-rich and low pH milieu, thereby creating a favorable niche for other acidogenic and aciduric species to thrive. As a human pathogen *S. mutans* is also implicated in sub-acute bacterial endocarditis, a life-threatening inflammation of heart valves, while a subset of strains has been linked to other extraoral pathologies such as cerebral microbleeds, IgA nephropathy and atherosclerosis.

Strains of *S. mutans* can be classified into four different serological groups (c, e, f and k) based on the composition of cell-surface rhamnose-glucose polysaccharide, with ~75% of strains isolated from dental plaque belonging to serotype c, ~ 20% to serotype e, and the remaining 5% classified as serotypes f or k (4). While biochemical and genetic approaches to dissect the biology of *S. mutans* have been used for at least the past 4 decades, the publication of the complete genome sequence of the *S. mutans* strain UA159 in 2001 (5) dramatically changed the landscape and, today, *S. mutans* is one of the best characterized

*Corresponding author: Mailing address, 1395 Center Drive, PO Box 100424, Gainesville, FL, 32610, USA, Phone: (352) 2738843, Fax: (352) 2738829, jlemos@dental.ufl.edu.

Gram-positive pathogens. In this chapter, we highlight some of the key studies that have led to our current understanding of *S. mutans* genetics, physiology and virulence. For a historical perspective and complete survey of the field, we direct the reader to the following review articles (2, 3, 6–9).

Genetic and phenotypic heterogeneity

The first *S. mutans* genome sequenced (serotype c UA159 strain) was found to contain ~ 2.0 Mb of DNA and to encode approximately 2,000 genes (5). As the cost of Next Generation Sequencing (NGS) technologies has gone down, genomes from dozens of *S. mutans* strains have been sequenced, assembled, and are now available on public databases such as the NCBI (www.ncbi.nlm.nih.gov/assembly/?term=streptococcus+mutans). This influx of genome sequences has led to an increase in comparative genomic studies focused on *S. mutans* (10–13). One of the first such studies was based on the shot-gun genome sequence from 57 geographically diverse *S. mutans* clinical isolates. This study concluded that the *S. mutans* pangenome contains a minimum of ~3,300 possible genes, and has a core genome (genes that are common to all strains) of 1,490 genes (14). This means that in any one *S. mutans* isolate, ~500 genes could be distinct from any other strain, perhaps significantly influencing virulence potential or fitness. The same group, using population demographic analysis based on single nucleotide polymorphisms of the core genes, determined that a large expansion took place in the *S. mutans* population between 3,000 and 10,000 years ago, which coincided with the advent of human agriculture and increased consumption of carbohydrates in the human host diet (14). This study also identified 73 unique core genes that are found only in *S. mutans* and not in its closest relatives, many of which are involved in carbohydrate metabolism and acid resistance (14). In a follow up study, Palmer *et al.* (15) characterized 15 of the most genetically diverse isolates of the 57 strains sequenced by Cornejo *et al.*, and found great variation in the phenotypes directly related to virulence including the ability to form biofilm in the presence of sucrose and the ability to tolerate low pH and oxidative stresses, suggesting that not all strains of *S. mutans* are equally virulent and providing a rational explanation as to why attempts to correlate carriage of certain genotypes of *S. mutans* with incidence of dental caries has proven so difficult (10, 16–18).

Work to characterize the unique core and non-core genes is ongoing and will likely lead to a greater understanding of the physiology and diversity of *S. mutans* as a species. For example one of the unique core hypothetical genes, SMu.1147, which encodes a small peptide, was found to regulate genetic competence and other traits of general importance to *S. mutans* virulence (19). In contrast, efforts to characterize the non-core genes indicate they likely provide a competitive advantage under particular circumstances. Such is the case for a galactose-specific PTS transporter found in several strains (20). Among the many non-core genes of *S. mutans* identified in recent years, those encoding the collagen-binding proteins (CBPs) Cnm and Cbm were shown to confer adhesion to collagen and laminin, invasion of endothelial and epithelial cells, and virulence in the *Galleria mellonella* invertebrate model (21, 22) as well as in rabbit and rat models of infective endocarditis (IE) (23, 24). Epidemiological studies indicate that *cnm* is present in approximately 15% of *S. mutans* isolates, whereas *cbm* is rarely found (~ 2%) (21, 22). Genes encoding CBPs have an uneven distribution among the different serotypes, and are found at higher frequency among

serotype *e*, *f* and *k* strains, but rarely present in serotype *c* strains (25). Notably, CBP⁺ *S. mutans* strains are more frequently isolated from dental plaque of individuals with bacteremia and IE suggesting a correlation between the production of these adhesins and systemic infections (26). Interestingly, the presence of *cnm* in *S. mutans* strains isolated from saliva has been linked to IgA nephropathy, cerebral microbleeds and cognitive impairment (27, 28). Finally, animal and clinical studies have suggested an association of CBPs in caries risk and severity (27, 29, 30).

Carbohydrate Metabolism

As a lactic acid bacterium (LAB) *S. mutans* relies exclusively on glycolysis for energy production (Figure 1). A signature characteristic of this organism is its ability to metabolize a large variety of carbohydrates. The genome of the UA159 type strain encodes 14 phosphoenolpyruvate-dependent sugar:phosphotransferase systems (PTS) with specificities for various mono- and di-saccharides, as well as two ATP-binding cassette (ABC) transporters involved primarily in internalizing oligosaccharides (5). Sucrose is a β 2,1-linked disaccharide composed of glucose and fructose that has proven, for a number of reasons, to be the most cariogenic of all carbohydrates. *S. mutans* has evolved multiple pathways to catabolize sucrose for acid production (31), and several glycosyltransferase enzymes (Gtfs) convert sucrose into a glue-like extracellular polymer glucan that promotes the buildup of biofilms by way of cellular attachment to dental surfaces and other oral microorganisms (7). As will be discussed in more detail below, recent multi-species modeling studies have confirmed the unique roles of Gtfs in the formation of a heterogeneous, diffusion-limiting, low-pH matrix that is conducive to both dental demineralization and the eventual dominance by acid-tolerant species (7, 32).

Carbon catabolite repression.

Most bacteria have evolved regulatory capacities that enable them to respond efficiently to changes in carbon source and thereby shift gene regulation, a phenomenon called carbon catabolite repression (CCR) (33, 34). The majority of low-GC Gram-positive bacteria depend on a LacI-type transcriptional regulator called CcpA to ensure that catabolic genes for less preferred carbohydrates are suppressed when a preferred carbohydrate (normally glucose) is present. Interestingly, *S. mutans* deviates from this paradigm in that CcpA plays a less direct role. Instead the glucose/mannose-PTS (EII^{Man}) system regulates the expression of catabolic operons required for assimilating secondary carbohydrates such as oligo- and di-saccharides (35). Inactivation of *manL*, which encodes the A and B domains of the EII^{Man} permease, results in enhanced expression of the *fruAB* and *levDEFG* (EII^{Lev}) operons. These operons contain genes which encode two different fructanases, FruAB for hydrolyzing fructose polymers (36), and a fructose/mannose-PTS (37), respectively. As a result, a *manL* mutant demonstrates improved growth on media supplemented with fructose polymers such as inulin or levan (35). CcpA and its co-factor HPr-Ser-P are also involved in this regulation (38, 39). Whereas CcpA interact directly with cognate c/s-elements called *cre* (catabolite response element) near the *fruA* promoter, ManL influences *fruAB* and *levDEFG* expression independently of CcpA (39). It is now believed that EII^{Man}-dependent glucose transport influences carbohydrate metabolism via substrate-specific signaling, which occurs

primarily at sub-mM concentrations of carbohydrate (38). However, substrate-level regulation is also influenced indirectly by CcpA under excess-carbohydrate conditions, since CcpA appears to regulate the transcription of *manL* (38, 39). A transcriptomic study of a *manL* deletion strain validated the key roles of EII^{Man} in global gene regulation (40), and transcriptional studies of a *eepA* deletion strain revealed both a central role for CcpA in controlling carbon flux and the existence of a substantial network of CcpA-independent genes (41, 42).

Catch and release.

To further explore substrate-dependent CCR in *S. mutans*, Zeng *et al.* assessed the relative contributions of various sucrolytic enzymes (31). The majority (> 95%) of sucrose encountered by *S. mutans* is believed to be internalized via the EII^{Scr} PTS system (43, 44) while the rest is likely metabolized extracellularly by the GTFs and fructosyltransferase (FTF). Enhanced transcription of the *fruAB* and EII^{Lev} operons was observed in the presence of sucrose with such activation requiring both the sucrose PTS (ScrA) and the LevQRST regulatory pathways. LevQRST is an unconventional four-component system that activates transcription of *fruAB* and EII^{Lev} in response to extracellular fructose or mannose (37, 45). Since free fructose was detected in the culture media of a *S. mutans* strain deficient in all extracellular sucrolytic enzymes after treatment with sucrose (46), it was hypothesized that following sucrose internalization and subsequent cleavage of sucrose-6-P into glucose-6-P and unphosphorylated fructose by the ScrB hydrolase, some of the fructose is released into the medium and can activate the LevQRST circuit but is insufficient to trigger CcpA-dependent CCR. Such a strategy of carbohydrate metabolism would be energetically efficient and avoid perturbing intracellular ATP levels, particularly important to a bacterium such as *S. mutans* that actively pumps protons out of the cell at the expense of ATP to maintain pH homeostasis. Interestingly fructose may not be the only carbohydrate to be released and reinternalized by *S. mutans*. Recent studies have characterized maltose and maltooligosaccharide metabolism, and demonstrated the presence of free glucose in the culture medium that is apparently reinternalized by way of the glucose-PTS EII^{Man} (47, 48). Transient expulsion of glucose was also observed when *S. mutans* was grown on a mixture of glucose and lactose (49). Considering diffusion limitations within biofilm matrices (50), hexose release may likely serve as temporary energy storage and/or as a carbohydrate source for neighboring cells that may not be actively invested in metabolizing these di-saccharides. This would thereby represent a bet-hedging strategy within the population, and also contribute toward a mutualistic relationship with other microorganisms that are closely associated with *S. mutans* (51, 52).

Biofilm formation

As a major etiological agent of human dental caries, *S. mutans* lives primarily in biofilms on the tooth surfaces, the so-called dental plaque. Strains of *S. mutans* produce up to three glucosyltransferases, GtfB, -C and -D, that utilize the glucose moiety of sucrose as the substrate to synthesize glucose polymers of glucans (also known as mutans) (Figure 1) (7). GtfB synthesizes water-insoluble glucans rich in $\alpha(1-3)$ -linkages, GtfC produces a mixture of soluble glucans rich in $\alpha(1-6)$ -linkages and insoluble glucans, and GtfD makes primarily

soluble glucans (often called dextran). These polymers, especially the α 3,1-linked water insoluble glucans, are major constituents of plaque biofilm matrices. Gtfs also bind to other oral microbes, even those that do not naturally express Gtfs, thereby converting them into *de facto* glucan producers (7). In addition, *S. mutans* encode several surface-associated glucan-binding proteins, GbpA, -B, -C and -D. Together the Gtfs, Gbps and adhesive glucans serve as an integrated scaffold for sucrose-dependent biofilm formation central to this organism's cariogenicity by promoting the local accumulation of microbial cells while forming a diffusion-limiting polymeric matrix that protects the embedded bacteria (Figure 2).

S. mutans also possesses multiple high-affinity surface adhesins that enable colonization even in the absence of sucrose. One of the most widely studied adhesins is the dual Antigen I/II, also known as P1, SpaP or PAC. This structurally complex multi-functional adhesin mediates bacterial attachment to the tooth's salivary pellicle via interactions with the host scavenger receptor glycoprotein GP340 or DMBT-1 (53–55). The AgI/II family of adhesins also interact with other bacteria and host proteins such as fibronectin and collagen (53, 56). Relative to the wild-type, a P1-deficient mutant demonstrates reduced binding to saliva or GP340-coated surfaces, aberrant biofilm formation, and reduced cariogenicity in a rat caries model (57, 58). In addition, P1 and WapA, another surface-localized adhesin, have been recently shown to form fibrillar amyloid aggregates (59). Amyloids are increasingly recognized as integral bacterial biofilm matrix components that interact with extracellular DNA (eDNA) and confer stability to the exopolysaccharide matrix (60). A *srtA* mutant lacking the only surface-anchoring sortase enzyme found in *S. mutans* is severely defective in biofilm development and does not produce amyloid suggesting fibril nucleation occurs at the cell surface (61). Treatment of *S. mutans* with known amyloid inhibitors inhibits biofilm formation via P1 and WapA-dependent mechanisms (59). In addition to P1 and WapA, a third amyloidogenic protein (SMU_63c) was recently identified. This secretory protein serves as an apparent negative regulator of biofilm cell density and genetic competence (59). Its production is associated with K^+ availability and influenced by growth phase (62).

Several cell envelope-associated proteins also contribute to *S. mutans* biofilm formation, including AtlA, RgpG, BrpA and Psr. AtlA is an autolysin whose deficiency results in decreased autolysis, longer chain length, and drastically reduces biofilm formation regardless of the carbohydrate used for growth (63). RgpG is the first enzyme of the biosynthetic pathway for rhamnose-containing glucose polymers (RGP), a major surface antigen of oral streptococci responsible for the different serotypes (64, 65). RgpG deficiency does not impair growth, but leads to a pronounced reduction of cell surface antigens and major defects in cell morphology and cell division (65). RgpG-deficient mutants display long chains of swollen “squarish” dividing cells and also form less biofilms irrespective of carbohydrate source. The BrpA and Psr proteins are members of the LytR-CpsA-Psr family of proteins widespread in Gram-positive bacteria (66–68). Deletion of *brpA* has little effect on growth but causes major impairment in biofilm formation. The *brpA* mutant is also less tolerant to acid and oxidative stresses, and more susceptible to cell envelope antimicrobial agents, all of which likely contribute to its reduced biofilm phenotype. Psr also strongly influences *S. mutans* biofilm formation and acid tolerance, but unlike BrpA, Psr deficiency does not diminish oxidative stress tolerance or resistance to cell envelope antimicrobial agents. Both BrpA and Psr exhibit ligase functions for cell wall antigens, such as the RGP,

and thereby globally impact cell envelope composition. Deficiency of either BrpA or Psr results in a reduction of cell wall antigens and their concurrent accumulation in cell-free culture medium (65). Finally, eDNA is another functional constituent of the *S. mutans* biofilm matrix – eDNA forms nanofibers that connect cells to one another and to the substratum contributing to bacterial adherence and biofilm structural integrity and stability (69).

Stress tolerance

The ability of *S. mutans* to adapt to sudden and substantial environmental changes within the dental plaque is a key attribute that contributes to its status as the major etiologic agent of dental caries. Fermentable carbohydrates consumed by the host provide a substrate for *S. mutans* and other lactic acid bacteria that ultimately results in the production of acidic end products that accumulate within the biofilm. To thrive at low pH values, *S. mutans* mounts the so-called acid tolerance response (ATR), a robust transcriptional and physiologic adaptation mechanism that encompasses the induction of pathways that contribute to cytoplasm buffering and changes in membrane fatty acid composition, ultimately protecting the cellular machinery from acid damage and contributing to the survival of the bacteria during stress (Figure 3) (3, 6, 70). Collectively, the different cellular processes that constitute the ATR contribute to the ability of *S. mutans* to maintain an intracellular pH that is more alkaline than the surrounding environment (pH) by about 0.5 to 1 pH unit (71). Environmental acidification also triggers *S. mutans* to modify the composition of the plasma membrane in a way that may alter proton permeability. These modifications are accomplished by increasing the proportion of monounsaturated fatty acids (both by incorporating exogenous fatty acids and by *de novo* synthesis) and by increasing the length of the carbon chains composing these membrane fatty acids (72). Inactivation of the gene responsible for biosynthesis of monounsaturated fatty acids in *S. mutans*, *fabM*, results in extreme sensitivity to low pH, inability to maintain pH, and reduced virulence in a rat caries model (73, 74). In addition to changes in fatty acid composition, the phospholipid cardiolipin has also emerged as an important contributor to acid tolerance, as deletion of the cardiolipin synthase (*cls*) increased acid sensitivity (75).

Alkalinization of the cytoplasm occurs either by pumping protons out of the cell or through the generation of neutralizing molecules. In *S. mutans*, the membrane-bound F₁F₀-ATPase (F-ATPase) is the primary mechanism by which protons are extruded to maintain pH homeostasis. The *S. mutans* F-ATPase is transcriptionally induced by low pH values and has an optimal pH of 6.0, which is lower than that of most streptococci associated with oral health (76, 77). Some oral streptococci utilize the urease enzyme (e.g. *Streptococcus salivarius*) or the arginine deiminase system (ADS) (e.g. *Streptococcus gordonii*) to produce the neutralizing molecules ammonia and CO₂ in order to cope with acid stress. Although these pathways are absent in *S. mutans*, the agmatine deiminase system (AgDS), analogous to the ADS, is present in this species (78, 79). The AgDS converts agmatine, a decarboxylated derivative of arginine found in dental plaque, to ammonia, CO₂, putrescine, and ATP. Though the AgDS does not appear to have a considerable impact upon environmental alkalinization, the ammonia generated internally may contribute to neutralization of the cytoplasmic pH, while the ATP generated can be used to fuel proton

extrusion via the F-ATPase (80). Malolactic fermentation (MLF) converts malate, an acid commonly found in wine and in fruits such as apples, to the less acidic lactate and to CO₂. The CO₂ product can then be used for cytoplasmic neutralization by conversion to bicarbonate via carbonic anhydrase. In *S. mutans*, transcription of the genes encoding the malolactic enzyme and permease is acid-inducible, and MLF activity was found to be optimal at an extracellular pH of 4.0 (81). Most importantly, malate was shown to protect *S. mutans* against acid killing and was associated with maintenance of ATP pools during starvation (82).

While the dental plaque environment was initially thought to be anaerobic, it is now known that the oral microbial community has a high capacity to reduce oxygen, resulting in the generation of toxic reactive oxygen species (ROS) (83). Most *S. mutans* strains are susceptible to exposure to H₂O₂, which can be generated by metabolism of other species within the dental plaque such as peroxigenic streptococci, or is contained as a component of oral hygiene and tooth bleaching products. Exposure to high levels of H₂O₂, and its more damaging breakdown products hydroxyl radical and superoxide anion, can rapidly cause irreversible cellular damage by triggering mismetallation of enzymes, damaging proteins through oxidation of sulfurous amino acids and metal-binding sites, and by disturbing DNA integrity (84). Although oral streptococci lack catalase, *S. mutans* utilizes a number of scavenging and protective systems to prevent the accumulation of toxic ROS. Among these are a manganese-dependent superoxide dismutase (SodA), an alkyl hydroperoxidase reductase (AhpCF), a thioredoxin reductase (TrxAB), and glutaredoxin system (GshA/B/R) (85). Tight control of free iron in the cell is an important aspect of minimizing exposure to ROS, as free radical formation is a direct result of the Fenton chemistry, when H₂O₂ comes into contact with ferrous iron. For this reason, the iron binding protein Dpr is a critical mediator of oxidative stress exposure in *S. mutans* (86, 87). Finally, the Spx regulators, SpxA1 and SpxA2, are responsible for the transcriptional activation of virtually every major oxidative stress response gene in *S. mutans* (85, 88). Not surprisingly, *spx* deletion strains were ill-equipped to tolerate oxidative challenges and caused fewer sulcal caries lesions in rats fed a highly cariogenic diet (85, 89, 90).

Signaling pathways

Two-component signal transduction systems (TCSTS).

TCSTS play important roles in bacterial adaptation, survival and virulence by sensing changes in the environment and altering expression of specific sets of genes to mount coordinated responses to the environmental stimuli. In TCSTS, a signal is sent by phosphotransfer between histidine sensor kinases and their cognate response regulators, triggering the response regulators to dimerize and bind to conserved DNA motifs, ultimately tuning cellular functions such as biofilm formation, stress tolerance and nutrient uptake (91). The genome of the type strain UA159 encodes 14 TCSTS and one orphan response regulator with no genetic link to a histidine kinase (92). Among those, ComDE, LevRS, VicRK and the orphan CovR regulator have been studied in some detail and shown to coordinate the expression of a number of virulence attributes. The LevRS system important for management of carbohydrate metabolism was introduced in the “carbohydrate metabolism”

session whereas the ComDE system, involved in the regulation of bacteriocin production and competence, will be discussed in the next paragraph. VicRK influences acid and oxidative stress responses and competence, and is the only TCSTS essential in *S. mutans* (93, 94). Several studies have proposed phosphotransfer crosstalk between VicRK and the LiaFSR TCSTS, which contributes to surface adhesion, mutacin production, and the ability to tolerate environmental stresses including cell envelope damage and heat shock (95–98). In addition, VicRK and CovR directly regulate genes implicated in the synthesis of and interaction with extracellular polysaccharides (7, 99). For example, transcription of *gfpB* is positively regulated by VicR (100, 101), while *gtfB*, *gtfC* and *gfpC* are repressed by CovR (99).

Quorum sensing (QS).

QS systems are communication networks that enable microbes to sense and respond to environmental conditions such as nutrient availability and population density, as signaling molecules naturally accumulate along with bacterial density. In Gram-positive bacteria, QS is coordinated by peptide pheromones, which serve as extracellular signaling molecules that trigger changes in gene expression and ultimately, activation of a coordinated response by the population (102–104). In *S. mutans*, QS systems have evolved to regulate production of bacteriocins (known in *S. mutans* as mutacins), which are peptide antibiotics used in defense against other oral microbes (105–107), and in competence (natural transformation), a transient state in which the organism is primed to take up foreign DNA (Figure 4). The competence-stimulating peptide signaling molecule, called CSP or mutacin-inducing peptide (MIP), is encoded by *comC*. Once CSP has been secreted and processed, it is recognized by the TCSTS ComDE (104, 108, 109). Activated (phosphorylated) ComE regulates mutacin production by recognizing a conserved sequence in the promoter region of its target genes (95, 110, 111). Also sharing regulatory links with ComDE in *S. mutans* is another TCSTS, CiaRH, which contributes to biofilm formation, acid tolerance, and entry into competence, possibly by interacting with CSP (112). Among the consequences of ComE activation is the stimulation of *comRS*, the QS pathway directly responsible for competence activation via the alternative sigma factor ComX (also called SigX) (113, 114). Once exported to the extracellular space, the pre-peptide ComS is presumably processed into the peptide pheromone XIP (*comX* or *sigX*-inducing peptide) (114, 115). When imported back into the cell via the oligopeptide permease system, XIP is sensed by ComR, which binds to conserved inverted repeat sequences upstream of both *comX* and *comS*, thereby causing auto-induction of the ComRS pathway and activation of ComX-regulated genes. It is worth noting that while all *S. mutans* isolates sequenced to date possess a functional ComRS-ComX system, not all strains harbor a complete ComCDE pathway, nor are all isolates equally competent (15).

The mutacins produced by *S. mutans* are categorized as either lantibiotics (broadly active against Gram-positive bacteria) or non-lantibiotics (most active against closely-related species), and production of these molecules is strain-specific (116). Mutacins provide a competitive advantage to *S. mutans* by inhibiting growth of neighboring bacterial species (117, 118). A close relationship exists between competence induction and mutacin production, as a threshold concentration of CSP activates ComDE to result in increased

mutacin production (95, 111). Adding to the layers of regulation of mutacin production are two LytTR regulatory systems, HdrRM and BrsRM, which include a membrane bound inhibitor protein that prompts the activity of the transcriptional regulator (119, 120). In this case, an overlap in the regulatory systems that govern mutacin production and competence is again observed as LytTR systems also induce competence through ComX (120, 121). A complete understanding of the environmental conditions that trigger these regulators and hence influence mutacin production is yet to be uncovered.

Another *S. mutans* QS mechanism employs the autoinducer-2 (AI-2), the collective name for a group of furanones formed as a by-product of LuxS-mediated methyl metabolism (122). The lack of species specificity of AI-2 signaling molecules, which are produced by both Gram-positive and Gram-negative organisms, facilitates interspecies communication as population growth contributes to accumulation of AI-2. In *S. mutans*, deficiency of LuxS reduces the ability of *S. mutans* to tolerate acid and oxidative stress and to form biofilms, especially during growth in sucrose (123, 124). In agreement with those phenotypes, LuxS-deficiency causes alterations in expression of a large number of genes, including those with known roles in stress tolerance response and biofilm formation (123–125). Addition of synthetic AI-2 to the growth medium can restore the phenotypes of LuxS mutants with regard to the stress tolerance response, biofilm formation, and the expression of selected genes (124, 125). To date, however, a specific receptor for AI-2 is yet to be identified (125).

Regulatory nucleotides.

The regulatory nucleotides (p)ppGpp and c-di-AMP have also been shown to modulate biofilm formation and stress responses in *S. mutans* (126–128). Guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp), collectively called (p)ppGpp, are the effector molecules of a conserved stress response referred to as the stringent response (SR) (129). Three (p)ppGpp-metabolizing enzymes have been identified in *S. mutans*; RelA is a bifunctional synthetase/hydrolase contributing to metabolism of (p)ppGpp, while two small alarmone synthetase (SAS) enzymes, RelP and RelQ, harbor only the synthetase domain (130). In *S. mutans*, changes in (p)ppGpp pools affect biofilm formation, carbohydrate metabolism and stress tolerance suggesting an overlap between circuits that govern general stress tolerance and biofilm formation. While the mechanism is not completely understood, (p)ppGpp pools also modulate competence signaling through interactions with an ABC-type transporter RcrRPQ and at least one peptide encoded within *rcrQ* (126, 131). Cyclic di-AMP (c-di-AMP) is an emerging second messenger in bacteria shown to play important roles in bacterial fitness and virulence (132). In *S. mutans*, increased c-di-AMP levels promoted biofilm formation through interactions with the VicRK-gtfB network that ultimately resulted in the transcriptional activation of *gtfB* (128).

Interspecies and cross-kingdom interactions

Interactions between *S. mutans* and certain members of the dental plaque community have been documented, and have recently been shown to exert major influence to the development and pathogenicity of plaque. These interactions can be synergistic, i.e. promote *S. mutans* growth, or antagonistic, i.e. inhibitory to *S. mutans*. A classic example of an antagonistic

interaction occurs between *S. mutans* and members of the mitis streptococci group such as *S. sanguinis* and *S. gordonii*. Specifically, several members of the mitis group secrete millimolar concentrations of H₂O₂, which can be highly inhibitory to *S. mutans* (118). On the other hand, strains of *S. mutans* not only produce mutacins that are specific against mitis streptococci, most are also generally more aciduric than species from the mitis group. This antagonistic relationship becomes more evident in dental plaque whereby there is an inverse association between the abundance of oral commensal streptococci such as *S. sanguinis* and *S. gordonii* (abundant in healthy plaque) and that of *S. mutans* (abundant in caries lesions) (133) (Figure 2).

While production of lactic acid by *S. mutans* is inhibitory to growth of many acid-sensitive oral commensals, lactic acid serves as a carbon source for *Veillonella* spp, a prevalent genus in the oral cavity (134). Thus, it is thought that *Veillonella* may act as an “acid sink” preventing the dental biofilm from reaching extremely low pH values that even aciduric organisms such as *S. mutans* may not be able to survive. This nutritional mutualism may explain the close association of *Veillonella* with streptococci in the oral cavity, which excrete lactate as a waste product of carbohydrate fermentation. In dual-species biofilms, *S. mutans* and *V. parvula* form distinctive structures and display increased resistance to chlorhexidine and other antimicrobials when compared to single species biofilms (135, 136). Using a three-species mixed culture, Liu et al. showed that the presence of *V. parvula* negated the growth inhibition of *S. mutans* caused by peroxigenic *S. gordonii* (137).

In addition to bacteria, the oral cavity is also colonized by yeasts. *Candida albicans* in particular is frequently found in the oral mucosa. Interestingly, *C. albicans* is often associated with *S. mutans* and detected in high numbers in cases of early childhood caries (ECC) (138). Recent studies have shown that the *S. mutans* GtFB enzyme binds to mannan receptors on the *C. albicans* surface leading to enhanced adherence and biofilm accumulation by *C. albicans* thereby enhancing biofilm formation and cariogenicity (139, 140). At the same time, *C. albicans* synthesizes farnesol, which enhances extracellular polysaccharide production by *S. mutans* (52). In a rat caries model, co-infection with *S. mutans* and *C. albicans* led to higher levels of microbial carriage in plaque biofilms and significantly increased the cariogenic potential of dental plaque, resulting in aggressive disease onset and rampant caries lesions (140).

***S. mutans* as a model organism: shifting long-standing paradigms**

Key advances in the field of bacteriology have come from studies with *Escherichia coli* and *Bacillus subtilis*, which represent the Gram-negative and Gram-positive bacterial paradigms, respectively. Despite the important contributions of these two species to bacteriology research, no model organism is a perfect representative of other species. In the case of Gram-positive organisms, *B. subtilis* is a free-living sporulating organism found in soil and plants but not in humans. Conversely, *S. mutans* is an obligate human pathogen with a biofilm-dependent lifestyle. It does not come as a surprise that a greater overlap in the mechanisms of gene regulation and metabolic pathways are observed between *S. mutans* and related Gram-positive pathogens that share host-associated lifestyle. Such organisms, like *S. mutans*, generally have compact genomes with low-GC contents. Like *E. coli* and *B. subtilis*,

S. mutans is highly amenable to genetic manipulation and is therefore also easy to study and control in the laboratory setting. Among closely-related streptococci, it has the most complete and sophisticated set of genetic tools that can be used in combination with simple and efficient *in vitro* and *in vivo* models to enhance our current understanding of *S. mutans* biology and beyond (141). As a result, a number of *S. mutans* investigations have challenged long-standing bacterial dogmas that were established based on work conducted with *E. coli* and *B. subtilis*. One such example is the signal recognition particle (SRP) pathway involved in co-translational protein translocation (142). The first clue that protein transport in *S. mutans* differs from the established *Escherichia coli* model, came from identification of an acid-sensitive transposon mutant in which *fth* encoding the fifty-four kilodalton homolog of the eukaryotic signal recognition particle (SRP) was insertionally inactivated. Subsequently, all three conserved bacterial elements of the co-translational translocation SRP pathway including the particle components Ffh and small 4.5S cytoplasmic RNA, along with the membrane-associated SRP receptor FtsY were proven dispensable in *S. mutans*. This was noteworthy because the SRP pathway was long believed essential for viability in all living cells. An explanation for this surprise lay in the presence in *S. mutans* of two paralogs of the YidC/Oxa/Alb family of membrane-localized chaperone insertases found in bacteria, mitochondria, and chloroplasts. Unlike Gram-negative bacteria, most Gram-positive organisms possess two, if not more, YidC homologs. Another almost universal distinguishing feature between Gram-positive and Gram-negative bacteria, also discovered in *S. mutans*, is the SRP accessory factor YlxM, which interacts both with Ffh and scRNA and influences the GTPase activity of the Ffh/FtsY heterodimer necessary for pathway component recycling once ribosome/nascent protein chain complexes are delivered to the membrane-localized SecYEG translocon (143). Another example occurred during the initial characterization of the enzyme responsible for the synthesis of (p)ppGpp in *S. mutans*, the effector molecule of the bacterial stringent response (see Signaling Pathways section). Until the work with *S. mutans*, the bifunctional enzyme RelA enzyme (also known as Rsh or Rel) was considered the sole enzymatic source of (p)ppGpp synthesis and degradation in all Firmicutes. The serendipitous finding that deletion of *relA* did not abolish (p)ppGpp production in *S. mutans* led to the discovery of two additional enzymes, RelP and RelQ, capable of synthesizing (p)ppGpp (130). Subsequent bioinformatics and genetic analysis revealed that these enzymes are ubiquitous in Firmicutes and eventually found in other bacterial species.

***S. mutans* as a therapeutic target in caries prevention.**

While caries is a polymicrobial disease, selective targeting of *S. mutans* in dental biofilms is viewed as a suitable approach for its prevention. This is mainly because the synthesis of insoluble glucans from sucrose by *S. mutans* is central for the formation of a stable biofilm matrix that facilitates bacterial colonization of the tooth surface and, at the same time, serves as a diffusion barrier helping to maintain the acidic milieu within which cariogenic bacteria thrive (144). One of the current lines of thought in dental caries prevention is that it may be possible to halt the development of cariogenic biofilms by selectively targeting *S. mutans* such that the oral microbiome associated with health is not disturbed. Most of the early research conducted in the late 1960's into the 1980's focused on the development of anti-*S.*

mutans vaccines. Despite some recent successes in animal models with vaccines containing or encoding single or a combination of antigens (145–148), emphasis on an immunoprophylactic approach has slowed somewhat due to the many challenges in obtaining long-term salivary IgA responses, safety concerns with anti-streptococci antibody cross-reactivity, and providing species-specific protection without disrupting related beneficial commensal organisms. Over the past two decades or more, a number of natural products such as propolis (149); curcumin (150); cranberry (151); or green tea extracts (152) among many others have been shown to be effective against *S. mutans* biofilms and to prevent dental caries albeit, to date, none of these products has proven to be selective towards *S. mutans*. More recently, antimicrobial peptides (AMPs), small molecules and probiotics have emerged as promising new approaches for the development of novel anticaries strategies that specifically target *S. mutans*. While AMPs kill bacteria indiscriminately, the selective killing of *S. mutans* by AMPs has been achieved by creating a synthetic peptide that combines a large spectrum AMP with the *S. mutans* specific competence-stimulating peptide (CSP) (153, 154). Phase 2 clinical trials have recently showed that a single varnish application of one such peptide achieved significant reductions of *S. mutans* in the oral cavity (155). More recently, a small molecule derived from 2-aminotriazole (designated 3F1) was also shown to selectively disperse *S. mutans* biofilms (156). Most importantly, 3F1 was shown to reduce caries without disturbing the oral microbiome in a rat caries model. An alternative approach to combat *S. mutans* and therefore prevent dental caries may be the use of alkalinogenic bacteria as a probiotics, or the use of substrates for such alkalinogenic activities, e.g. arginine, as a prebiotics. This is because some oral bacteria can neutralize plaque pH by producing large quantities of ammonia by metabolizing arginine or urea, thereby creating an environment in favor of health-associated bacteria (157). A promising candidate is a highly arginolytic streptococcal strain, designated A12, isolated from supragingival plaque of a caries-free individual (158). Not only did the A12 strain neutralize acid by metabolizing arginine, it was also shown to kill *S. mutans* by producing H₂O₂ and to interfere with signaling pathways that control bacteriocin production.

Future perspectives

Evidence accumulated over many decades have clearly shown that *S. mutans* is a major agent in dental caries vis-à-vis its capacity to orchestrate changes in the plaque microbiome via EPS and acid production. Thus, continued efforts to elucidate how *S. mutans* senses and responds to environmental cues through interconnected circuits that govern stress tolerance and biofilm formation can facilitate the identification of new targets for caries treatment and prevention. As the biology of *S. mutans* continues to be unraveled, a better understanding of the consequences of genomic and phenotypic heterogeneity among strains is an important area for development. Specifically, how *S. mutans* isolates with different properties contribute to the different stages of disease through synergistic or antagonistic interactions with the microbiome is an area largely unexplored. Future investigations should also consider the role of certain strains of *S. mutans* in systemic infections.

ACKNOWLEDGEMENTS

We thank Dr. Robert A. Burne for suggestions and critical reading of the text. The authors' work in this area was supported by NIH-NIDCR grants DE019783, DE022559, DE008007, DE021789, DE024348, DE12236, DE19452 and DE25348.

REFERENCES

- Clarke JK. 1924 On the bacterial factor in the etiology of dental caries. *Brit J Exp Pathol* 5:141–147.
- Loesche WJ. 1986 Role of *Streptococcus mutans* in human dental decay. *Microbiol Rev* 50:353–380. [PubMed: 3540569]
- Lemos JA, Burne RA. 2008 A model of efficiency: stress tolerance by *Streptococcus mutans*. *Microbiology* 154:3247–3255. [PubMed: 18957579]
- Nakano K, Ooshima T. 2009 Serotype classification of *Streptococcus mutans* and its detection outside the oral cavity. *Future Microbiol* 4:891–902. [PubMed: 19722842]
- Ajdic D, McShan WM, McLaughlin RE, Savic G, Chang J, Carson MB, Primeaux C, Tian R, Kenton S, Jia H, Lin S, Qian Y, Li S, Zhu H, Najar F, Lai H, White J, Roe BA, Ferretti JJ. 2002 Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. *Proc Natl Acad Sci USA* 99:14434–14439. [PubMed: 12397186]
- Baker JL, Faustoferri RC, Quivey RG Jr. 2017 Acid-adaptive mechanisms of *Streptococcus mutans*—the more we know, the more we don't. *Mol Oral Microbiol* 32:107–117. [PubMed: 27115703]
- Bowen WH, Koo H. 2011 Biology of *Streptococcus mutans*-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. *Caries Res* 45:69–86.
- Lemos JA, Abranches J, Burne RA. 2005 Responses of cariogenic streptococci to environmental stresses. *Curr Issues Mol Biol* 7:95–107. [PubMed: 15580782]
- Hamada S, Slade HD. 1980 Biology, immunology, and cariogenicity of *Streptococcus mutans*. *Microbiol Rev* 44:331–384. [PubMed: 6446023]
- Argimon S, Konganti K, Chen H, Alekseyenko AV, Brown S, Caufield PW. 2014 Comparative genomics of oral isolates of *Streptococcus mutans* by *in silico* genome subtraction does not reveal accessory DNA associated with severe early childhood caries. *Infect Genet Evol* 21:269–278. [PubMed: 24291226]
- Liu L, Hao T, Xie Z, Horsman GP, Chen Y. 2016 Genome mining unveils widespread natural product biosynthetic capacity in human oral microbe *Streptococcus mutans*. *Sci Rep* 6:37479. [PubMed: 27869143]
- Maruyama F, Kobata M, Kurokawa K, Nishida K, Sakurai A, Nakano K, Nomura R, Kawabata S, Ooshima T, Nakai K, Hattori M, Hamada S, Nakagawa I. 2009 Comparative genomic analyses of *Streptococcus mutans* provide insights into chromosomal shuffling and species-specific content. *BMC Genomics* 10:358. [PubMed: 19656368]
- Meng P, Lu C, Zhang Q, Lin J, Chen F. 2017 Exploring the Genomic Diversity and Cariogenic Differences of *Streptococcus mutans* Strains Through Pan-Genome and Comparative Genome Analysis. *Curr Microbiol* doi:10.1007/s00284-017-1305-z.
- Cornejo OE, Lefebure T, Bitar PD, Lang P, Richards VP, Eilertson K, Do T, Beighton D, Zeng L, Ahn SJ, Burne RA, Siepel A, Bustamante CD, Stanhope MJ. 2013 Evolutionary and population genomics of the cavity causing bacteria *Streptococcus mutans*. *Mol Biol Evol* 30:881–893. [PubMed: 23228887]
- Palmer SR, Miller JH, Abranches J, Zeng L, Lefebure T, Richards VP, Lemos JA, Stanhope MJ, Burne RA. 2013 Phenotypic heterogeneity of genomically-diverse isolates of *Streptococcus mutans*. *PLoS One* 8:e61358. [PubMed: 23613838]
- Lembo FL, Longo PL, Ota-Tsuzuki C, Rodrigues CR, Mayer MP. 2007 Genotypic and phenotypic analysis of *Streptococcus mutans* from different oral cavity sites of caries-free and caries-active children. *Oral Microbiol Immunol* 22:313–319. [PubMed: 17803628]
- Napimoga MH, Hofling JF, Klein MI, Kamiya RU, Goncalves RB. 2005 Transmission, diversity and virulence factors of *Streptococcus mutans* genotypes. *J Oral Sci* 47:59–64. [PubMed: 16050484]

18. Phattarataratip E, Olson B, Broffitt B, Qian F, Brogden KA, Drake DR, Levy SM, Banas JA. 2011 *Streptococcus mutans* strains recovered from caries-active or caries-free individuals differ in sensitivity to host antimicrobial peptides. *Mol Oral Microbiol* 26:187–199. [PubMed: 21545696]
19. Kim JN, Stanhope MJ, Burne RA. 2013 Core-gene-encoded peptide regulating virulence-associated traits in *Streptococcus mutans*. *J Bacteriol* 195:2912–2920. [PubMed: 23603743]
20. Zeng L, Xue P, Stanhope MJ, Burne RA. 2013 A galactose-specific sugar: phosphotransferase permease is prevalent in the non-core genome of *Streptococcus mutans*. *Mol Oral Microbiol* 28:292–301. [PubMed: 23421335]
21. Abranches J, Miller JH, Martinez AR, Simpson-Haidaris PJ, Burne RA, Lemos JA. 2011 The collagen-binding protein Cnm is required for *Streptococcus mutans* adherence to and intracellular invasion of human coronary artery endothelial cells. *Infect Immun* 79:2277–2284. [PubMed: 21422186]
22. Nomura R, Nakano K, Naka S, Nemoto H, Masuda K, Lapirottanakul J, Alaluusua S, Matsumoto M, Kawabata S, Ooshima T. 2012 Identification and characterization of a collagen-binding protein, Cbm, in *Streptococcus mutans*. *Mol Oral Microbiol* 27:308–323. [PubMed: 22759315]
23. Otsugu M, Nomura R, Matayoshi S, Teramoto N, Nakano K. 2017 Contribution of *Streptococcus mutans* Strains with Collagen-Binding Proteins in the Presence of Serum to the Pathogenesis of Infective Endocarditis. *Infect Immun* 85 e00401–17 [PubMed: 28947650]
24. Freires IA, Aviles-Reyes A, Kitten T, Simpson-Haidaris PJ, Swartz M, Knight PA, Rosalen PL, Lemos JA, Abranches J. 2017 Heterologous expression of *Streptococcus mutans* Cnm in *Lactococcus lactis* promotes intracellular invasion, adhesion to human cardiac tissues and virulence. *Virulence* 8:18–29. [PubMed: 27260618]
25. Lapirottanakul J, Nakano K, Nomura R, Leelataweewud P, Chalermarp N, Klaophimai A, Srisatjaluk R, Hamada S, Ooshima T. 2011 Multilocus sequence typing analysis of *Streptococcus mutans* strains with the cnm gene encoding collagen-binding adhesin. *J Med Microbiol* 60:1677–1684. [PubMed: 21680768]
26. Nomura R, Naka S, Nemoto H, Inagaki S, Taniguchi K, Ooshima T, Nakano K. 2013 Potential involvement of collagen-binding proteins of *Streptococcus mutans* in infective endocarditis. *Oral Dis* 19:387–393. [PubMed: 22998492]
27. Misaki T, Naka S, Hatakeyama R, Fukunaga A, Nomura R, Isozaki T, Nakano K. 2016 Presence of *Streptococcus mutans* strains harbouring the cnm gene correlates with dental caries status and IgA nephropathy conditions. *Sci Rep* 6:36455. [PubMed: 27811984]
28. Miyatani F, Kuriyama N, Watanabe I, Nomura R, Nakano K, Matsui D, Ozaki E, Koyama T, Nishigaki M, Yamamoto T, Mizuno T, Tamura A, Akazawa K, Takada A, Takeda K, Yamada K, Nakagawa M, Ihara M, Kanamura N, Friedland RP, Watanabe Y. 2015 Relationship between Cnm-positive *Streptococcus mutans* and cerebral microbleeds in humans. *Oral Dis* 21:886–893. [PubMed: 26205098]
29. Miller JH, Aviles-Reyes A, Scott-Anne K, Gregoire S, Watson GE, Sampson E, Progulski-Fox A, Koo H, Bowen WH, Lemos JA, Abranches J. 2015 The collagen binding protein Cnm contributes to oral colonization and cariogenicity of *Streptococcus mutans* OMZ175. *Infect Immun* 83:2001–2010. [PubMed: 25733523]
30. Esberg A, Sheng N, Marell L, Claesson R, Persson K, Boren T, Stromberg N. 2017 *Streptococcus Mutans* Adhesin Biotypes that Match and Predict Individual Caries Development. *EBioMedicine* 24:205–215. [PubMed: 28958656]
31. Zeng L, Burne RA. 2013 Comprehensive mutational analysis of sucrose-metabolizing pathways in *Streptococcus mutans* reveals novel roles for the sucrose phosphotransferase system permease. *J Bacteriol* 195:833–843. [PubMed: 23222725]
32. Xiao J, Klein MI, Falsetta ML, Lu B, Delahunty CM, Yates JR 3rd, Heydorn A, Koo H. 2012 The exopolysaccharide matrix modulates the interaction between 3D architecture and virulence of a mixed-species oral biofilm. *PLoS Pathog* 8:e1002623. [PubMed: 22496649]
33. Deutscher J 2008 The mechanisms of carbon catabolite repression in bacteria. *Curr Opin Microbiol* 11:87–93. [PubMed: 18359269]

34. Deutscher J, Francke C, Postma PW. 2006 How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. *Microbiol Mol Biol Rev* 70:939–1031. [PubMed: 17158705]
35. Abranches J, Chen YY, Burne RA. 2003 Characterization of *Streptococcus mutans* strains deficient in EIIAB^{Man} of the sugar phosphotransferase system. *Appl Environ Microbiol* 69:4760–4769. [PubMed: 12902269]
36. Burne RA, Penders JE. 1992 Characterization of the *Streptococcus mutans* GS-5 *fruA* gene encoding exo- β -D-fructosidase. *Infect Immun* 60:4621–4632. [PubMed: 1398976]
37. Zeng L, Wen ZT, Burne RA. 2006 A novel signal transduction system and feedback loop regulate fructan hydrolase gene expression in *Streptococcus mutans*. *Mol Microbiol* 62:187–200. [PubMed: 16987177]
38. Zeng L, Burne RA. 2008 Multiple sugar: phosphotransferase system permeases participate in catabolite modification of gene expression in *Streptococcus mutans*. *Mol Microbiol* 70:197–208. [PubMed: 18699864]
39. Zeng L, Burne RA. 2010 Seryl-phosphorylated HPr regulates CcpA-independent carbon catabolite repression in conjunction with PTS permeases in *Streptococcus mutans*. *Mol Microbiol* 75:1145–1158. [PubMed: 20487301]
40. Abranches J, Candella MM, Wen ZT, Baker HV, Burne RA. 2006 Different roles of EIIAB^{Man} and EIIIGlc in regulation of energy metabolism, biofilm development, and competence in *Streptococcus mutans*. *J Bacteriol* 188:3748–3756. [PubMed: 16707667]
41. Abranches J, Nascimento MM, Zeng L, Browngardt CM, Wen ZT, Rivera MF, Burne RA. 2008 CcpA regulates central metabolism and virulence gene expression in *Streptococcus mutans*. *J Bacteriol* 190:2340–2349. [PubMed: 18223086]
42. Zeng L, Choi SC, Danko CG, Siepel A, Stanhope MJ, Burne RA. 2013 Gene regulation by CcpA and catabolite repression explored by RNA-Seq in *Streptococcus mutans*. *PLoS ONE* 8:e60465. [PubMed: 23555977]
43. Chassy BM, Porter EV. 1979 Initial characterization of sucrose-6-phosphate hydrolase from *Streptococcus mutans* and its apparent identity with intracellular invertase. *Biochem Biophys Res Commun* 89:307–314. [PubMed: 224874]
44. Tanzer JM, Chassy BM, Krichevsky MI. 1971 Sucrose metabolism by *Streptococcus mutans*, SL-I. *Biochim Biophys Acta* 261:379–387. [PubMed: 4111389]
45. Zeng L, Das S, Burne RA. 2011 Genetic analysis of the functions and interactions of components of the LevQRST signal transduction complex of *Streptococcus mutans*. *PLoS ONE* 6:e17335. [PubMed: 21364902]
46. Zeng L, Burne RA. 2015 Sucrose- and fructose-specific effects on the transcriptome of *Streptococcus mutans*, as determined by RNA sequencing. *Appl Environ Microbiol* 82:146–156. [PubMed: 26475108]
47. Sato Y, Okamoto-Shibayama K, Azuma T. 2013 The *malQ* gene is essential for starch metabolism in *Streptococcus mutans*. *J Oral Microbiol* 5 doi 10.3402
48. Sato Y, Okamoto-Shibayama K, Azuma T. 2015 Glucose-PTS involvement in maltose metabolism by *Streptococcus mutans*. *Bull Tokyo Dent Coll* 56:93–103. [PubMed: 26084997]
49. Liberman ES, Bleiweis AS. 1984 Role of the phosphoenolpyruvate-dependent glucose phosphotransferase system of *Streptococcus mutans* GS5 in the regulation of lactose uptake. *Infect Immun* 43:536–542. [PubMed: 6420344]
50. Klein MI, Hwang G, Santos PH, Campanella OH, Koo H. 2015 *Streptococcus mutans*-derived extracellular matrix in cariogenic oral biofilms. *Front Cell Infect Microbiol* 5:10. [PubMed: 25763359]
51. He J, Kim D, Zhou X, Ahn SJ, Burne RA, Richards VP, Koo H. 2017 RNA-Seq reveals enhanced sugar metabolism in *Streptococcus mutans* co-cultured with *Candida albicans* within mixed-species biofilms. *Front Microbiol* 8:1036. [PubMed: 28642749]
52. Kim D, Sengupta A, Niepa TH, Lee BH, Weljie A, Freitas-Blanco VS, Murata RM, Stebe KJ, Lee D, Koo H. 2017 *Candida albicans* stimulates *Streptococcus mutans* microcolony development via cross-kingdom biofilm-derived metabolites. *Sci Rep* 7:41332. [PubMed: 28134351]

53. Brady LJ, Maddocks SE, Larson MR, Forsgren N, Persson K, Deivanayagam CC, Jenkinson HF. 2011 The changing faces of *Streptococcus* antigen I/II polypeptide family adhesins. *Mol Microbiol* 77:276–286.
54. Larson MR, Rajashankar KR, Crowley PJ, Kelly C, Mitchell TJ, Brady LJ, Deivanayagam C. 2011 Crystal structure of the C-terminal region of *Streptococcus mutans* antigen I/II and characterization of salivary agglutinin adherence domains. *J Biol Chem* 286:21657–21666. [PubMed: 21505225]
55. Jakubovics NS, Stromberg N, van Dolleweerd CJ, Kelly CG, Jenkinson HF. 2005 Differential binding specificities of oral streptococcal antigen I/II family adhesins for human or bacterial ligands. *Mol Microbiol* 55:1591–1605. [PubMed: 15720563]
56. Sullan RM, Li JK, Crowley PJ, Brady LJ, Dufrene YF. 2015 Binding forces of *Streptococcus mutans* P1 adhesin. *ACS Nano* 9:1448–1460. [PubMed: 25671413]
57. Ahn SJ, Wen ZT, Brady LJ, Burne RA. 2008 Characteristics of biofilm formation by *Streptococcus mutans* in the presence of saliva. *Infect Immun* 76:4259–4268. [PubMed: 18625741]
58. Crowley PJ, Brady LJ, Michalek SM, Bleiweis AS. 1999 Virulence of a *spaP* mutant of *Streptococcus mutans* in a gnotobiotic rat model. *Infect Immun* 67:1201–1206. [PubMed: 10024561]
59. Besingi RN, Wenderska IB, Senadheera DB, Cvitkovitch DG, Long JR, Wen ZT, Brady LJ. 2017 Functional Amyloids in *Streptococcus mutans*, their use as Targets of Biofilm Inhibition and Initial Characterization of SMU_63c. *Microbiology*. 163: 488–501 [PubMed: 28141493]
60. Taglialegna A, Lasa I, Valle J. 2016 Amyloid Structures as Biofilm Matrix Scaffolds. *J Bacteriol* 198:2579–2588. [PubMed: 27185827]
61. Oli MW, Otoo HN, Crowley PJ, Heim KP, Nascimento MM, Ramsook CB, Lipke PN, Brady LJ. 2012 Functional amyloid formation by *Streptococcus mutans*. *Microbiology* 158:2903–2916. [PubMed: 23082034]
62. Binopal G, Wenderska IB, Crowley P, Besingi RN, Senadheera DB, Jeannine Brady L, Cvitkovitch DG. 2017 K+ modulates genetic competence and the stress regulon of *Streptococcus mutans*. *Microbiology* 163:719–730. [PubMed: 28530170]
63. Brown TA Jr., Ahn SJ, Frank RN, Chen YY, Lemos JA, Burne RA. 2005 A hypothetical protein of *Streptococcus mutans* is critical for biofilm formation. *Infect Immun* 73:3147–3151. [PubMed: 15845523]
64. Yamashita Y, Shibata Y, Nakano Y, Tsuda H, Kido N, Ohta M, Koga T. 1999 A novel gene required for rhamnose-glucose polysaccharide synthesis in *Streptococcus mutans*. *J Bacteriol* 181:6556–6559. [PubMed: 10515952]
65. De A, Liao S, Bitoun JP, Roth R, Beatty WL, Wu H, Wen ZT. 2017 Deficiency of RgpG Causes Major Defects in Cell Division and Biofilm Formation, and Deficiency of LCP proteins Leads to Accumulation of Cell Wall Antigens in Culture Medium by *Streptococcus mutans*. *Appl Environ Microbiol* doi:10.1128/AEM.00928-17.
66. Wen ZT, Burne RA. 2002 Functional genomics approach to identifying genes required for biofilm development by *Streptococcus mutans*. *Appl Environ Microbiol* 68:1196–1203. [PubMed: 11872468]
67. Bitoun JP, Liao S, Yao X, Ahn S-J, Isoda R, Nguyen AH, Brady LJ, Burne RA, Abranches A, Wen ZT. 2012 BrpA is Involved in Regulation of Cell Envelope Stress Responses in *Streptococcus mutans*. *Appl Environ Microbiol* 78:2914–2922. [PubMed: 22327589]
68. Bitoun JP, Liao S, McKey BA, Yao X, Fan Y, Abranches J, Beatty WL, Wen ZT. 2013 Psr is involved in regulation of glucan production, and double deficiency of BrpA and Psr is lethal in *Streptococcus mutans*. *Microbiology* 159:493–506. [PubMed: 23288544]
69. Liao S, Klein MI, Heim KP, Fan Y, Bitoun JP, Ahn SJ, Burne RA, Koo H, Brady LJ, Wen ZT. 2014 *Streptococcus mutans* extracellular DNA is upregulated during growth in biofilms, actively released via membrane vesicles, and influenced by components of the protein secretion machinery. *J Bacteriol* 196:2355–2366. [PubMed: 24748612]
70. Matsui R, Cvitkovitch D. 2010 Acid tolerance mechanisms utilized by *Streptococcus mutans*. *Future Microbiol* 5:403–417. [PubMed: 20210551]

71. Bender GR, Sutton SV, Marquis RE. 1986 Acid tolerance, proton permeabilities, and membrane ATPases of oral streptococci. *Infect Immun* 53:331–338. [PubMed: 3015800]
72. Fozo EM, Quivey RG Jr. 2004 Shifts in the membrane fatty acid profile of *Streptococcus mutans* enhance survival in acidic environments. *Appl Environ Microbiol* 70:929–936. [PubMed: 14766573]
73. Fozo EM, Quivey RG Jr. 2004 The *fabM* gene product of *Streptococcus mutans* is responsible for the synthesis of monounsaturated fatty acids and is necessary for survival at low pH. *J Bacteriol* 186:4152–4158. [PubMed: 15205416]
74. Fozo EM, Scott-Anne K, Koo H, Quivey RG Jr. 2007 Role of unsaturated fatty acid biosynthesis in virulence of *Streptococcus mutans*. *Infect Immun* 75:1537–1539. [PubMed: 17220314]
75. MacGilvray ME, Lapek JD Jr., Friedman AE, Quivey RG Jr. 2012 Cardiolipin biosynthesis in *Streptococcus mutans* is regulated in response to external pH. *Microbiology* 158:2133–2143. [PubMed: 22628481]
76. Kuhnert WL, Zheng G, Faustoferri RC, Quivey RG Jr. 2004 The F-ATPase operon promoter of *Streptococcus mutans* is transcriptionally regulated in response to external pH. *J Bacteriol* 186:8524–8528. [PubMed: 15576803]
77. Sheng J, Marquis RE. 2006 Enhanced acid resistance of oral streptococci at lethal pH values associated with acid-tolerant catabolism and with ATP synthase activity. *FEMS Microbiol Lett* 262:93–98. [PubMed: 16907744]
78. Griswold AR, Chen YY, Burne RA. 2004 Analysis of an agmatine deiminase gene cluster in *Streptococcus mutans* UA159. *J Bacteriol* 186:1902–1904. [PubMed: 14996823]
79. Griswold AR, Jameson-Lee M, Burne RA. 2006 Regulation and physiologic significance of the agmatine deiminase system of *Streptococcus mutans* UA159. *J Bacteriol* 188:834–841. [PubMed: 16428386]
80. Liu Y, Burne RA. 2009 Multiple two-component systems of *Streptococcus mutans* regulate agmatine deiminase gene expression and stress tolerance. *J Bacteriol* 191:7363–7366. [PubMed: 19783635]
81. Lemme A, Sztajer H, Wagner-Dobler I. 2010 Characterization of *mleR*, a positive regulator of malolactic fermentation and part of the acid tolerance response in *Streptococcus mutans*. *BMC Microbiol* 10:58. [PubMed: 20178568]
82. Sheng J, Marquis RE. 2007 Malolactic fermentation by *Streptococcus mutans*. *FEMS Microbiol Lett* 272:196–201. [PubMed: 17490430]
83. Marquis RE. 1995 Oxygen metabolism, oxidative stress and acid-base physiology of dental plaque biofilms. *J Ind Microbiol* 15:198–207. [PubMed: 8519478]
84. Imlay JA. 2013 The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. *Nat Rev Microbiol* 11:443–454. [PubMed: 23712352]
85. Kajfasz JK, Ganguly T, Hardin EL, Abranches J, Lemos JA. 2017 Transcriptome responses of *Streptococcus mutans* to peroxide stress: identification of novel antioxidant pathways regulated by Spx. *Sci Rep* 7:16018. [PubMed: 29167560]
86. Galvao LC, Miller JH, Kajfasz JK, Scott-Anne K, Freires IA, Franco GC, Abranches J, Rosalen PL, Lemos JA. 2015 Transcriptional and Phenotypic Characterization of Novel Spx-Regulated Genes in *Streptococcus mutans*. *PLoS One* 10:e0124969. [PubMed: 25905865]
87. Yamamoto Y, Fukui K, Koujin N, Ohya H, Kimura K, Kamio Y. 2004 Regulation of the intracellular free iron pool by Dpr provides oxygen tolerance to *Streptococcus mutans*. *J Bacteriol* 186:5997–6002. [PubMed: 15342568]
88. Kajfasz JK, Rivera-Ramos I, Scott-Anne K, Gregoire S, Abranches J, Lemos JA. 2015 Transcription of Oxidative Stress Genes Is Directly Activated by SpxA1 and, to a Lesser Extent, by SpxA2 in *Streptococcus mutans*. *J Bacteriol* 197:2160–2170. [PubMed: 25897032]
89. Galvao LC, Rosalen PL, Rivera-Ramos I, Franco GC, Kajfasz JK, Abranches J, Bueno-Silva B, Koo H, Lemos JA. 2017 Inactivation of the *spxA1* or *spxA2* gene of *Streptococcus mutans* decreases virulence in the rat caries model. *Mol Oral Microbiol* 32:142–153. [PubMed: 27037617]
90. Kajfasz JK, Rivera-Ramos I, Abranches J, Martinez AR, Rosalen PL, Derr AM, Quivey RG, Lemos JA. 2010 Two Spx proteins modulate stress tolerance, survival, and virulence in *Streptococcus mutans*. *J Bacteriol* 192:2546–2556. [PubMed: 20233935]

91. Stock AM, Robinson VL, Goudreau PN. 2000 Two-component signal transduction. *Annu Rev Biochem* 69:183–215. [PubMed: 10966457]
92. Smith EG, Spatafora GA. 2012 Gene regulation in *S. mutans*: complex control in a complex environment. *J Dent Res* 91:133–141. [PubMed: 21743034]
93. Senadheera D, Krastel K, Mair R, Persadmehr A, Abranches J, Burne RA, Cvitkovitch DG. 2009 Inactivation of VicK affects acid production and acid survival of *Streptococcus mutans*. *J Bacteriol* 191:6415–6424. [PubMed: 19684142]
94. Senadheera MD, Lee AW, Hung DC, Spatafora GA, Goodman SD, Cvitkovitch DG. 2007 The *Streptococcus mutans* *vicX* gene product modulates *gtfB/C* expression, biofilm formation, genetic competence, and oxidative stress tolerance. *J Bacteriol* 189:1451–1458. [PubMed: 17114248]
95. Perry JA, Jones MB, Peterson SN, Cvitkovitch DG, Levesque CM. 2009 Peptide alarmone signalling triggers an auto-active bacteriocin necessary for genetic competence. *Mol Microbiol* 72:905–917. [PubMed: 19400789]
96. Tremblay YD, Lo H, Li YH, Halperin SA, Lee SF. 2009 Expression of the *Streptococcus mutans* essential two-component regulatory system VicRK is pH and growth-phase dependent and controlled by the LiaFSR three-component regulatory system. *Microbiology* 155:2856–2865. [PubMed: 19589829]
97. Chong P, Drake L, Biswas I. 2008 LiaS regulates virulence factor expression in *Streptococcus mutans*. *Infect Immun* 76:3093–3099. [PubMed: 18458070]
98. Shankar M, Mohapatra SS, Biswas S, Biswas I. 2015 Gene Regulation by the LiaSR Two-Component System in *Streptococcus mutans*. *PLoS One* 10:e0128083. [PubMed: 26020679]
99. Biswas S, Biswas I. 2006 Regulation of the glucosyltransferase (*gtfBC*) operon by CovR in *Streptococcus mutans*. *J Bacteriol* 188:988–998. [PubMed: 16428403]
100. Duque C, Stipp RN, Wang B, Smith DJ, Hofling JF, Kuramitsu HK, Duncan MJ, Mattos-Graner RO. 2011 Downregulation of GbpB, a component of the VicRK regulon, affects biofilm formation and cell surface characteristics of *Streptococcus mutans*. *Infect Immun* 79:786–796. [PubMed: 21078847]
101. Stipp RN, Boisvert H, Smith DJ, Hofling JF, Duncan MJ, Mattos-Graner RO. 2013 CovR and VicRK regulate cell surface biogenesis genes required for biofilm formation in *Streptococcus mutans*. *PLoS One* 8:e58271. [PubMed: 23554881]
102. Lyon GJ, Novick RP. 2004 Peptide signaling in *Staphylococcus aureus* and other Gram-positive bacteria. *Peptides* 25:1389–1403. [PubMed: 15374643]
103. Monnet V, Juillard V, Gardan R. 2016 Peptide conversations in Gram-positive bacteria. *Crit Rev Microbiol* 42:339–351. [PubMed: 25198780]
104. Shanker E, Federle MJ. 2017 Quorum Sensing Regulation of Competence and Bacteriocins in *Streptococcus pneumoniae* and *Streptococcus mutans*. *Genes (Basel)* 8.
105. Hamada S, Ooshima T. 1975 Inhibitory spectrum of a bacteriocinlike substance (mutacin) produced by some strains of *Streptococcus mutans*. *J Dent Res* 54:140–145. [PubMed: 1053754]
106. Kreth J, Merritt J, Shi W, Qi F. 2005 Co-ordinated bacteriocin production and competence development: a possible mechanism for taking up DNA from neighbouring species. *Mol Microbiol* 57:392–404. [PubMed: 15978073]
107. Yonezawa H, Kuramitsu HK. 2005 Genetic analysis of a unique bacteriocin, Smb, produced by *Streptococcus mutans* GS5. *Antimicrob Agents Chemother* 49:541–548. [PubMed: 15673730]
108. Hossain MS, Biswas I. 2012 An extracellular protease, SepM, generates functional competence-stimulating peptide in *Streptococcus mutans* UA159. *J Bacteriol* 194:5886–5896. [PubMed: 22923597]
109. Martin B, Quentin Y, Fichant G, Claverys JP. 2006 Independent evolution of competence regulatory cascades in streptococci? *Trends Microbiol* 14:339–345. [PubMed: 16820295]
110. Kreth J, Hung DC, Merritt J, Perry J, Zhu L, Goodman SD, Cvitkovitch DG, Shi W, Qi F. 2007 The response regulator ComE in *Streptococcus mutans* functions both as a transcription activator of mutacin production and repressor of CSP biosynthesis. *Microbiology* 153:1799–1807. [PubMed: 17526837]

111. Kreth J, Merritt J, Zhu L, Shi W, Qi F. 2006 Cell density- and ComE-dependent expression of a group of mutacin and mutacin-like genes in *Streptococcus mutans*. FEMS Microbiol Lett 265:11–17. [PubMed: 16981904]
112. Ahn SJ, Wen ZT, Burne RA. 2006 Multilevel control of competence development and stress tolerance in *Streptococcus mutans* UA159. Infect Immun 74:1631–1642. [PubMed: 16495534]
113. Hale JD, Ting YT, Jack RW, Tagg JR, Heng NC. 2005 Bacteriocin (mutacin) production by *Streptococcus mutans* genome sequence reference strain UA159: elucidation of the antimicrobial repertoire by genetic dissection. Appl Environ Microbiol 71:7613–7617. [PubMed: 16269816]
114. Mashburn-Warren L, Morrison DA, Federle MJ. 2010 A novel double-tryptophan peptide pheromone controls competence in *Streptococcus* spp. via an Rgg regulator. Mol Microbiol 78:589–606. [PubMed: 20969646]
115. Khan R, Rukke HV, Ricomini Filho AP, Fimland G, Arntzen MO, Thiede B, Petersen FC. 2012 Extracellular identification of a processed type II ComR/ComS pheromone of *Streptococcus mutans*. J Bacteriol 194:3781–3788. [PubMed: 22609914]
116. Merritt J, Qi F. 2012 The mutacins of *Streptococcus mutans*: regulation and ecology. Mol Oral Microbiol 27:57–69. [PubMed: 22394465]
117. Kamiya RU, Taiete T, Goncalves RB. 2011 Mutacins of *Streptococcus mutans*. Braz J Microbiol 42:1248–1258. [PubMed: 24031748]
118. Kreth J, Merritt J, Shi W, Qi F. 2005 Competition and coexistence between *Streptococcus mutans* and *Streptococcus sanguinis* in the dental biofilm. J Bacteriol 187:7193–7203. [PubMed: 16237003]
119. Okinaga T, Xie Z, Niu G, Qi F, Merritt J. 2010 Examination of the *hdrRM* regulon yields insight into the competence system of *Streptococcus mutans*. Mol Oral Microbiol 25:165–177. [PubMed: 20536745]
120. Okinaga T, Niu G, Xie Z, Qi F, Merritt J. 2010 The *hdrRM* operon of *Streptococcus mutans* encodes a novel regulatory system for coordinated competence development and bacteriocin production. J Bacteriol 192:1844–1852. [PubMed: 20118256]
121. Aspiras MB, Ellen RP, Cvitkovitch DG. 2004 ComX activity of *Streptococcus mutans* growing in biofilms. FEMS Microbiol Lett 238:167–174. [PubMed: 15336418]
122. Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczar I, Bassler BL, Hughson FM. 2002 Structural identification of a bacterial quorum-sensing signal containing boron. Nature 415:545–549. [PubMed: 11823863]
123. Merritt J, Kreth J, Shi W, Qi F. 2005 LuxS controls bacteriocin production in *Streptococcus mutans* through a novel regulatory component. Mol Microbiol 57:960–969. [PubMed: 16091037]
124. Wen ZT, Nguyen AH, Bitoun JP, Abranches J, Baker HV, Burne RA. 2011 Transcriptome analysis of LuxS-deficient *Streptococcus mutans* grown in biofilms. Mol Oral Microbiol 26:2–18. [PubMed: 21214869]
125. Sztajer H, Lemme A, Vilchez R, Schulz S, Geffers R, Yip CY, Levesque CM, Cvitkovitch DG, Wagner-Dobler I. 2008 Autoinducer-2-regulated genes in *Streptococcus mutans* UA159 and global metabolic effect of the *luxS* mutation. J Bacteriol 190:401–415. [PubMed: 17981981]
126. Seaton K, Ahn SJ, Sagstetter AM, Burne RA. 2011 A transcriptional regulator and ABC transporters link stress tolerance, (p)ppGpp, and genetic competence in *Streptococcus mutans*. J Bacteriol 193:862–874. [PubMed: 21148727]
127. Lemos JA, Brown TA Jr., Burne RA. 2004 Effects of RelA on key virulence properties of planktonic and biofilm populations of *Streptococcus mutans*. Infect Immun 72:1431–1440. [PubMed: 14977948]
128. Peng X, Zhang Y, Bai G, Zhou X, Wu H. 2016 Cyclic di-AMP mediates biofilm formation. Mol Microbiol 99:945–959. [PubMed: 26564551]
129. Gaca AO, Colomer-Winter C, Lemos JA. 2015 Many means to a common end: the intricacies of (p)ppGpp metabolism and its control of bacterial homeostasis. J Bacteriol 197:1146–1156. [PubMed: 25605304]
130. Lemos JA, Lin VK, Nascimento MM, Abranches J, Burne RA. 2007 Three gene products govern (p)ppGpp production by *Streptococcus mutans*. Mol Microbiol 65:1568–1581. [PubMed: 17714452]

131. Kaspar J, Kim JN, Ahn SJ, Burne RA. 2016 An Essential Role for (p)ppGpp in the Integration of Stress Tolerance, Peptide Signaling, and Competence Development in *Streptococcus mutans*. *Front Microbiol* 7:1162. [PubMed: 27516759]
132. Commichau FM, Dickmanns A, Gundlach J, Ficner R, Stulke J. 2015 A jack of all trades: the multiple roles of the unique essential second messenger cyclic di-AMP. *Mol Microbiol* 97:189–204. [PubMed: 25869574]
133. Giacaman RA, Torres S, Gomez Y, Munoz-Sandoval C, Kreth J. 2015 Correlation of *Streptococcus mutans* and *Streptococcus sanguinis* colonization and ex vivo hydrogen peroxide production in carious lesion-free and high caries adults. *Arch Oral Biol* 60:154–159. [PubMed: 25455129]
134. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, Lakshmanan A, Wade WG. 2010 The human oral microbiome. *J Bacteriol* 192:5002–5017. [PubMed: 20656903]
135. Luppens SB, Kara D, Bandounas L, Jonker MJ, Wittink FR, Bruning O, Breit TM, Ten Cate JM, Crielaard W. 2008 Effect of *Veillonella parvula* on the antimicrobial resistance and gene expression of *Streptococcus mutans* grown in a dual-species biofilm. *Oral Microbiol Immunol* 23:183–189. [PubMed: 18402603]
136. Kara D, Luppens SB, van Marle J, Ozok R, ten Cate JM. 2007 Microstructural differences between single-species and dual-species biofilms of *Streptococcus mutans* and *Veillonella parvula*, before and after exposure to chlorhexidine. *FEMS Microbiol Lett* 271:90–97. [PubMed: 17403046]
137. Liu J, Wu C, Huang IH, Merritt J, Qi F. 2011 Differential response of *Streptococcus mutans* towards friend and foe in mixed-species cultures. *Microbiology* 157:2433–2444. [PubMed: 21565931]
138. de Carvalho FG, Silva DS, Hebling J, Spolidorio LC, Spolidorio DM. 2006 Presence of mutans streptococci and *Candida* spp. in dental plaque/dentine of carious teeth and early childhood caries. *Arch Oral Biol* 51:1024–1028. [PubMed: 16890907]
139. Gregoire S, Xiao J, Silva BB, Gonzalez I, Agidi PS, Klein MI, Ambatipudi KS, Rosalen PL, Bauserman R, Waugh RE, Koo H. 2011 Role of Glucosyltransferase B in Interactions of *Candida albicans* with *Streptococcus mutans* and with an Experimental Pellicle on Hydroxyapatite Surfaces. *Appl Environ Microbiol* 77:6357–6367. [PubMed: 21803906]
140. Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S, Pai CH, Gonzalez-Begne M, Watson G, Krysan DJ, Bowen WH, Koo H. 2014 Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes virulence of plaque biofilms in vivo. *Infect Immun* 82:1968–1981. [PubMed: 24566629]
141. Lemos JA, Quivey RG Jr., Koo H, Abranches J. 2013 *Streptococcus mutans*: a new Gram-positive paradigm? *Microbiology* 159:436–445. [PubMed: 23393147]
142. Hasona A, Crowley PJ, Levesque CM, Mair RW, Cvitkovitch DG, Bleiweis AS, Brady LJ. 2005 Streptococcal viability and diminished stress tolerance in mutants lacking the signal recognition particle pathway or YidC2. *Proc Natl Acad Sci USA* 102:17466–17471. [PubMed: 16293689]
143. Williams ML, Crowley PJ, Hasona A, Brady LJ. 2014 YlxM is a newly identified accessory protein that influences the function of signal recognition particle pathway components in *Streptococcus mutans*. *J Bacteriol* 196:2043–2052. [PubMed: 24659773]
144. Bowen WH. 2016 Dental caries - not just holes in teeth! A perspective. *Mol Oral Microbiol* 31:228–233. [PubMed: 26343264]
145. Batista MT, Ferreira EL, Pereira GS, Stafford P, Maeda D, Rodrigues JF, Brady LJ, Johnston SA, Ferreira LCS, Ferreira RCC. 2017 LT adjuvant modulates epitope specificity and improves the efficacy of murine antibodies elicited by sublingual vaccination with the N-terminal domain of *Streptococcus mutans* P1. *Vaccine* 35:7273–7282. [PubMed: 29146379]
146. Cao XX, Li YH, Ye QL, Hu X, Wang TF, Fan MW. 2017 Self-assembling anticaries mucosal vaccine containing ferritin cage nanostructure and glucan-binding region of *S. mutans* glucosyltransferase effectively prevents caries formation in rodents. *Hum Vaccin Immunother* 13:2332–2340. [PubMed: 28759297]

147. Yan YH, Yu F, Zeng C, Cao LH, Zhang Z, Xu QA. 2016 CCL17 combined with CCL19 as a nasal adjuvant enhances the immunogenicity of an anti-carries DNA vaccine in rodents. *Acta Pharmacol Sin* 37:1229–1236. [PubMed: 27546005]
148. Yang J, Sun Y, Bao R, Zhou D, Yang Y, Cao Y, Yu J, Zhao B, Li Y, Yan H, Zhong M. 2017 Second-generation Flagellin-rPac Fusion Protein, KFD2-rPac, Shows High Protective Efficacy against Dental Caries with Low Potential Side Effects. *Sci Rep* 7:11191. [PubMed: 28894188]
149. Santiago KB, Piana GM, Conti BJ, Cardoso EO, Murbach Teles Andrade BF, Zanutto MR, Mores Rall VL, Fernandes A Jr., Sforcin JM. 2017 Microbiological control and antibacterial action of a propolis-containing mouthwash and control of dental plaque in humans. *Nat Prod Res* doi: 10.1080/14786419.2017.1344664:1–5.
150. Lee HJ, Kang SM, Jeong SH, Chung KH, Kim BI. 2017 Antibacterial photodynamic therapy with curcumin and Curcuma xanthorrhiza extract against *Streptococcus mutans*. *Photodiagnosis Photodyn Ther* 20:116–119. [PubMed: 28890211]
151. Koo H, Duarte S, Murata RM, Scott-Anne K, Gregoire S, Watson GE, Singh AP, Vorsa N. 2010 Influence of cranberry proanthocyanidins on formation of biofilms by *Streptococcus mutans* on saliva-coated apatitic surface and on dental caries development *in vivo*. *Caries Res* 44:116–126. [PubMed: 20234135]
152. Xu X, Zhou XD, Wu CD. 2011 The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of *Streptococcus mutans*. *Antimicrob Agents Chemother* 55:1229–1236. [PubMed: 21149622]
153. Eckert R, He J, Yarbrough DK, Qi F, Anderson MH, Shi W. 2006 Targeted killing of *Streptococcus mutans* by a pheromone-guided “smart” antimicrobial peptide. *Antimicrob Agents Chemother* 50:3651–3657. [PubMed: 17060534]
154. Guo L, McLean JS, Yang Y, Eckert R, Kaplan CW, Kyme P, Sheikh O, Varnum B, Lux R, Shi W, He X. 2015 Precision-guided antimicrobial peptide as a targeted modulator of human microbial ecology. *Proc Natl Acad Sci USA* 112:7569–7574. [PubMed: 26034276]
155. Eckert R, Sullivan R, Shi W. 2012 Targeted antimicrobial treatment to re-establish a healthy microbial flora for long-term protection. *Adv Dent Res* 24:94–97. [PubMed: 22899688]
156. Garcia SS, Blackledge MS, Michalek S, Su L, Ptacek T, Eipers P, Morrow C, Lefkowitz EJ, Melander C, Wu H. 2017 Targeting of *Streptococcus mutans* Biofilms by a Novel Small Molecule Prevents Dental Caries and Preserves the Oral Microbiome. *J Dent Res* 96:807–814. [PubMed: 28571487]
157. Burne RA, Marquis RE. 2000 Alkali production by oral bacteria and protection against dental caries. *FEMS Microbiol Lett* 193:1–6. [PubMed: 11094270]
158. Huang X, Palmer SR, Ahn SJ, Richards VP, Williams ML, Nascimento MM, Burne RA. 2016 A Highly Arginolytic *Streptococcus* Species That Potently Antagonizes *Streptococcus mutans*. *Appl Environ Microbiol* 82:2187–2201. [PubMed: 26826230]

carbohydrate with at least 3 PTS involved in fructose transport and several PTS and permeases involved in glucose transport. In the intracellular environment, carbohydrates are phosphorylated and processed to fructose-6-phosphate (Fru-6-P) and fermented by glycolysis with production of organic acids, mainly lactic acid. In addition, glucosamine-6-phosphate (GlcN-6-P) is synthesized from Fru-6-P and serves as an initial precursor for cell wall biosynthesis. Cells can synthesize an intracellular polysaccharide (IPS), a polymer of the glycogen-amylopectin type, when carbohydrates are in excess that can be stored as intracellular granules and is used as energy source reserve during starvation.

Figure 2. Biofilm formation and host-pathogens interactions in *Streptococcus mutans*.

Early colonizers (e.g. *Streptococcus gordonii*, *S. mitis*, *Actinomyces* spp. among others) attach to the tooth enamel *via* salivary proteins and start to form three-dimensional biofilms under non-cariogenic conditions. At pHs close to neutrality, production of H_2O_2 by peroxigenic bacteria and other antimicrobial products produced by oral commensals prevent the overgrowth of specific pathogens (e.g. *S. mutans*) on dental biofilms.

Glycosyltransferases (GTFs) secreted by *S. mutans* adsorb onto the enamel pellicle or bacterial surfaces. In the presence of sucrose, GTFs catabolize sucrose to produce large amounts of insoluble and soluble glucans, which contribute to the buildup of a robust extracellular polysaccharide matrix (EPS), particularly insoluble components. The EPS matrix will serve as an architectural scaffold for the biofilm structure mediating tight adherence to the tooth enamel and bacteria, as glucans provide binding sites for *S. mutans* glucan-binding proteins (GBP) and other organisms. Extracellular DNA (eDNA) is another functional constituent of the oral biofilm matrix forming nanofibers that connect cell to cell and cell to substratum contributing to biofilm structural integrity and stability. Continuous intake of sucrose by the host leads to a series of ecological and structural shifts that favor the growth of aciduric and highly acidogenic bacteria, such as *S. mutans*. These changes alter the oral biofilm metabolism in a way that copious amounts of organic acids are produced contributing to a decrease in environmental pH. Once *S. mutans* becomes dominant, the secretion of large amounts of mutacins kills nearby competitors such as peroxigenic streptococci. The constant low-pH milieu surrounding the hydroxyapatite structure of the enamel leads to demineralization and initiates the carious process.

Figure 3. Acid stress tolerance mechanisms of *Streptococcus mutans*.

The ability of *S. mutans* to catalyze fermentable dietary carbohydrates into organic acids can promptly drop the environmental pH. Exposure to sub-lethal low pH triggers an acid-adaptive response known as ATR (Acid Tolerance Response), which is a robust transcriptional and physiologic adaptation mechanism for pH homeostasis through alteration of proton permeability, generation of neutralizing molecules, and changes in membrane fatty acid composition. The membrane F₁F₀-ATPase (F-ATPase) is transcriptionally induced by low pH and serves as the primary mechanism by which protons are extruded to maintain pH homeostasis. The modifications in membrane composition refers to an increase in the proportion of monounsaturated fatty acids (UFA) over saturated fatty acids (SFA), and in the length of the carbon chains composing the membrane fatty acids. Production of neutralizing molecules, such as ammonia and CO₂, is also an important way to cope with acid stress. In *S. mutans*, the agmatine deiminase system (AgDS) converts agmatine, a decarboxylated derivative of arginine found in dental plaque, to ammonia, CO₂, putrescine, and ATP. The ammonia generated internally may contribute to cytoplasmic buffering, while the ATP generated can be used to fuel proton extrusion via the F-ATPase. A decrease in environmental pH also triggers activation of malolactic fermentation (MLF), which converts malate to the less acidic lactate and to CO₂. The CO₂ product can then be used for cytoplasmic neutralization by conversion to bicarbonate (HCO₃⁻) via carbonic anhydrase.

Figure 4. Quorum-sensing systems involved in the regulation of bacteriocin production and competence in *S. mutans*.

Cell density, nutrient availability and other environmental conditions induce the expression of *comC*, a gene encoding the competence-stimulating peptide (CSP) precursor. The CSP propeptide is cleaved off inside the cell and exported through a specific ABC transporter encoded by *comAB*. In the extracellular environment, CSP then undergoes a final post export processing mediated by the SepM protease. Upon reaching a certain threshold, mature CSP is recognized by the two-component system ComDE triggering a phosphorylation cascade. Activated (phosphorylated) ComE activates transcription of: (i) *comC* and *comDE*, creating a positive feedback loop, (ii) genes involved in mutacin production and, by a yet-to-be-determined mechanism, (iii) *comRS*, the quorum sensing pathway directly responsible for competence activation *via* the alternative sigma factor ComX. Once exported to the extracellular milieu, the pre-peptide ComS is processed into the peptide pheromone XIP (*comX*- or sigX-inducing peptide). XIP is transported back into the cell via Opp (oligopeptide permease system) and sensed by the Rgg-type regulator ComR. Activated ComR binds to both *comX* and *comS* promoters, thereby causing auto-induction of ComRS and activation of late (ComX-regulated) competence genes.