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REVIEW ARTICLE

Guardian genes ensuring subsistence of oral *Streptococcus mutans*

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ABSTRACT

Despite the substantial research advancements on oral diseases, dental caries remains a major healthcare burden. A disease of microbial dysbiosis, dental caries is characterised by the formation of biofilms that assist demineralisation and destruction of the dental hard tissues. While it is well understood that this is a multi-kingdom biofilm-mediated disease, it has been elucidated that acid producing and acid tolerant bacteria play pioneering roles in the process. Specifically, *Streptococcus mutans* houses major virulence pathways that enable it to thrive in the oral cavity and cause caries. This pathogen adheres to the tooth substrate, forms biofilms, resists external stress, produces acids, kills closely related species, and survives the acid as well as the host clearance mechanisms. For an organism to be able to confer such virulence, it requires a large and complex gene network which synergise to establish disease. In this review, we have charted how these multi-faceted genes control several caries-related functions of *Streptococcus mutans*. In a futuristic thinking approach, we also briefly discuss the potential roles of omics and machine learning, to ease the study of non-functional genes that may play a major role and enable the integration of experimental data.

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Introduction

While several theories have been proposed to describe the aetiopathogenesis of dental caries, the chemo-parasitic theory is one of the most accepted (Levine and Rowles 1973). Bacteria consume carbohydrates and produce acids which dissolves the minerals of the tooth tissue and forms cavitated lesions. While the microbial aetiology is well elucidated, there are three other biological contributors to the caries process: oral hygiene, saliva, and the fermentable carbohydrate which the host consumes (Pitts et al. 2017). Thus, dental caries is a multi-factorial disease, the progression of which may be largely defined by the mineral homeostasis. Imbalances in the mineralisation process increase net demineralisation. Apart from poor hygiene, sugar consumption, lifestyle and economical attributes also favour the development of dental caries. Interestingly, more cases are reported from developed countries than developing countries, primarily because of high sugar consumption. However, epidemiological studies are yet to decipher the effect of lifestyle on caries (Pitts et al. 2017).

The dynamic and multifactorial nature of the disease makes it a salient oral disease, which also has a huge impact on the economy. Dental procedures are exceedingly expensive across the world, remaining inaccessible to people from poor social status. The global level economic impact for the dental treatment was about $544.41 billion in 2015, of which a staggering 65.53% is due to the direct costs of treatment (Listl et al. 2015; Righolt et al. 2018). As a result of this, dental caries is an impending epidemic worldwide (Marcenes et al. 2013).

Microbiology of caries

Many hypotheses have been postulated on the mechanism of the development of dental infections. The specific plaque hypothesis (SPH) describes that caries is caused by *Streptococcus mutans* (Loesche 1976). However, caries formation is not solely dependent on initiation by acidogenic species or the progression by cariogenic species. Earlier studies on infected germ/non-germ-free hamsters show that diet and food habits,

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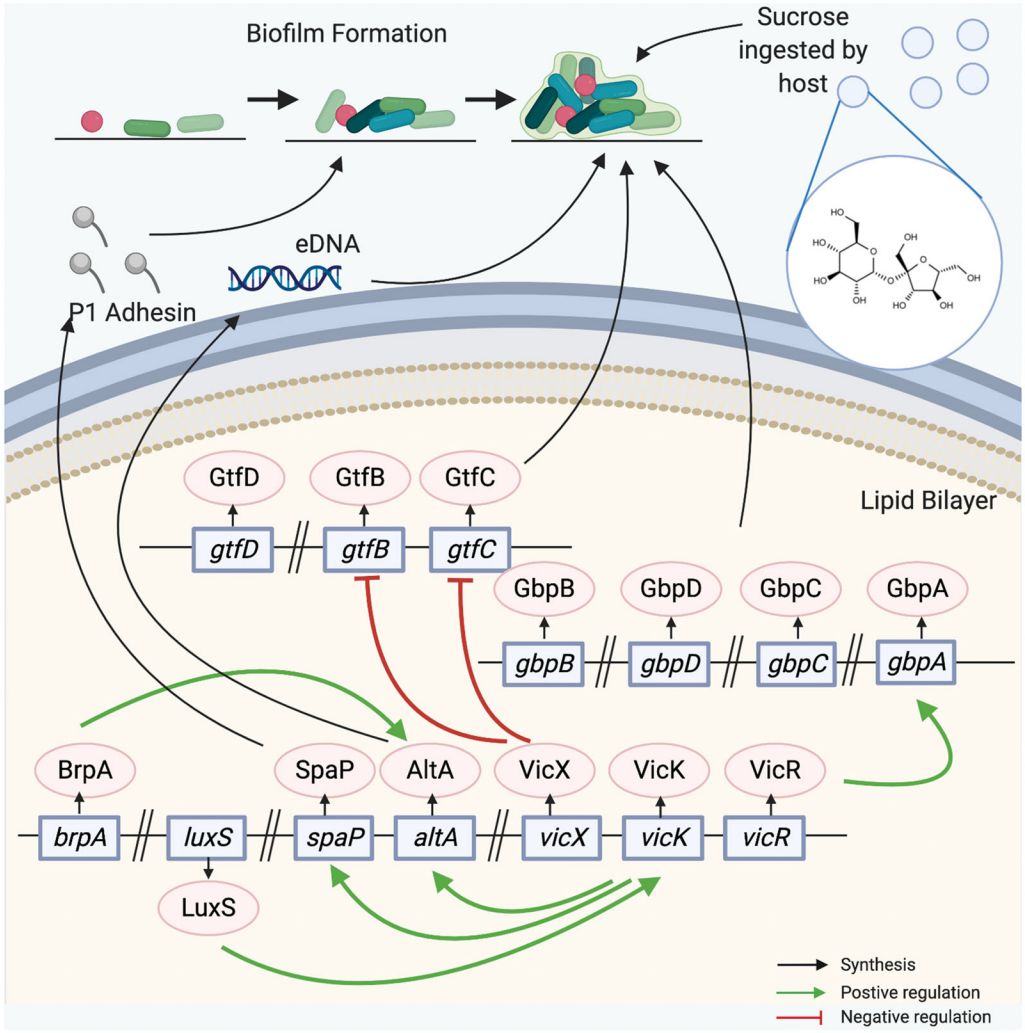

Figure 1. Biofilm formation pathways in *S. mutans*. The combined effect of the VicRK and the Gtfs genes are shown in this diagram. VicK regulates the expression of *atlA*, which results in lysis of *S. mutans* and also regulates SpaP which is crucial for sucrose- independent biofilm formation. VicR positively regulates the *gbpABCD* locus that act as a bridge between the synthesised glucans and *S.mutans* to define its biofilm structure. VicX down-regulates *gtfB* and *gtfC* results in less accumulation of water-insoluble glucan. The essential components of the biofilm are the polysaccharide, eDNA, and the adhesin proteins. Sucrose is a major contributor to biofilm formation. Images created with BioRender.com.

host factors, and inhabiting species are indicative of the aetiology of caries (Fitzgerald and Keyes 1960). Later, developments in oral microbiology revealed that the microbial homeostasis is a part of the dynamic balance of synergistic and antagonistic interactions of oral microflora. The homeostasis of the microbiome of the plaque isaffected by the environmental changes including low pH and inflammatory host response, which leads to the dysbiosis and microbiome shift, subsequently affecting the interaction between microorganisms. Thus the "ecological plaque hypothesis" proposes that alteration in the vital environmental factors could imbalance the plaque commensal flora predisposing disease site. This led to an understanding that therapies to diseases could be achieved by aiming pathogens and the processes that energise the homeostasis collapse (Bradshaw et al. 1989; Marsh et al. 1989; Marsh 1994). Sugar consumption and saliva production were identified as important factors in the disease progression, which paved the way for the ecological plaque hypothesis (EPH) (Rosier et al. 2014). Here, ecological stresses, such as acid production on sugar fermentation, create an imbalance that favours cariogenic pathogens to flourish (Marsh 1994). *S. mutans* an acidophilic organism thrives in this stressful environment and regulates certain genes to survive and proliferate (Rosier et al. 2014). As well, the cariogenic pathogen, *S. mutans* encounters several caries preventive mechanisms conferred by the saliva including the maintenance of oral pH and the antagonistic effect of the anti-cariogenic agents. Yet, the impenetrable biofilm and other virulence factors render these beneficial properties of saliva

obsolete (Lenander-Lumikari and Loimaranta 2000; Stookey 2008). However, biofilm formation by *S. mutans* is an important caries-related virulence trait. By forming tenacious biofilms, the clearance by salivary flow is negated, and the antimicrobial agents in saliva are unable to penetrate these intractable biofilms. Besides biofilm formation and acid tolerance, *S. mutans* also houses complex interlinked pathways to confer supplementary virulence factors, such as genetic competence, oxidative stress tolerance, and bacteriocin production. This review aims to highlight the dynamic nature of the disease and the major regulatory pathways involved therein. We also provide a brief insight into the OMICS approach as a future perspective.

Biofilm builders

Biofilms can form on any surface in the oral cavity, including the oral mucosa, enamel, dentine, root canals, and periodontal tissues. Furthermore, biofilm formation on prostheses such as dentures and dental implants are an issue of major concern. In general, biofilms have a dual role: they form a protective barrier from ecological stresses, yet in a stagnant dysbiotic biofilm, disease prevails. Caries development is initiated by the migration of planktonic cells to form a meshed layer, rich in glucans. This further acts as an adhesive to aid plaque formation and induce caries. The extracellular polymeric substance (EPS) forms the backbone of such biofilm, protecting it from antimicrobials and other competitive cells. The EPS amongst several components, mainly contains glucans, surface proteins, binding proteins, and eDNA (Krzyściak et al. 2014; Matsumota-Nakano 2018).

Sucrose-dependent biofilm formation is orchestrated by an interplay of glucan synthesis and glucan binding protein (Ooshima et al. 2001). While the *gtf* locus (Glycosyltransferase) is responsible for glucan production from sucrose, *gbp* locus (Glucan binding protein) binds to the synthesised glucans and determines the three-dimensional biofilm structure (Mishra et al. 2015). Genes *gtfB* and *gtfC* encodes water-insoluble α-(1$\rightarrow$3)-linked glucans, while *gtfD* encodes water-soluble glucans (Decker et al., 2014). The GTFs are structurally characterised to have 2 functional domains, a catalytic amino terminal domain (C-ATD) and a glucan binding carboxyl domain (GB-CTD). The C-ATD hydrolyses sucrose, while the GB-CTD binds to glucans and determines the chemical nature of the GTF-synthesised glucan (Mishra et al. 2015).

Glucan binding proteins act as a bridge between the synthesised glucans and *S. mutans*, thus providing a defined structure to the biofilm. The genes in the *gbp* loci - *gbpB, gbpD, gbpC,* and *gbpA*- are essential for determining this architecture. GbpA contains certain repeats of Gtf enzyme-like domains, highlighting possible co-induction and co-expression of genes (Matsumota-Nakano 2018). GbpA optimises plaque biofilm to minimise the stress on the bacteria within the biofilm sub-populations, and to facilitate subsequent interactions with adhesion proteins and the EPS. This creates a balanced environment for the cells to tolerate harsh environments (Banas et al. 2007). In coherence, recent genetic studies have demonstrated that GbpA deficient cells show less interactions with the EPS, which results in the formation of a weak, non-uniform biofilm (Matsumi et al. 2015) (Figure 1).

Comparative genome analysis of GbpB has shown structural and functional relationships with the peptidoglycan hydrolases from other Gram-positive bacteria, which is known to influence the cell shape and cell wall maintenance (Mattos-Graner et al. 2001; Fujita et al. 2007). GbpC is a cell-surface associated protein belonging to the AgI/II family, expressed in response to stress conditions and known to exert dextran induced aggregation (Sato et al., 1997). Furthermore, the annotated genome sequence of *S. mutans* UA159 reveals that the protein architecture of GbpD is homologous with the carboxyl terminal domain of GTF and GbpA. Thus, GbpD contributes to the aggregation and adhesion of *S. mutans* to the tooth surface (Shah and Russell 2004).

The cell surface protein antigen c, also known as P1, SpaP, antigen I/II, B, Pac, and MSL-1 is crucial for the formation of sucrose- independent biofilm (Russell and Lehner 1978; Russell 1979; Forester et al. 1983; Demuth et al. 1990). The PI antigen binds directly to the salivary pellicle and mediates bacterial adherence, even in the absence of sucrose (Koga et al. 1990). Structural elucidation of P1 predicted the presence of a signal peptide (1–38 aa residues), N-terminal (60–500 aa residues) and C-terminal (800–1540 aa residues) of three complete and one incomplete alanine (A) rich repeats and proline rich (P) residues, respectively (Brady et al. 2010). The A-region favours adhesion to the tooth surface, whereas the P-region has a higher binding affinity with fibronectin, collagen type I, and fibrinogen (Beg et al. 2002). It has been reported that the 120 kDa collagen-binding protein (CBP) of *S. mutans* interacts with type I collagen, which is the primary component of dentine, and with endothelial cells, thereby exemplifying its association with cardiovascular infections (Abranches et al. 2011).

To overcome the stresses induced by high levels of acid, oxidative stresses, and damage to DNA, and to maintain as well as protect the biofilm structures,

multiple two component signal transduction systems (TCSs) are involved (Kaur et al. 2015). They respond coherently to diverse physiological and environmental stresses through a cell density-dependent process of quorum sensing (QS). The primary QS based TCS system locus, *comCDE* shows >600-fold higher expression in biofilms compared to planktonic cells, demonstrating the combined action in response to the signal CSP (competence- stimulating peptide) (O'Toole and Kolter 1998). Typically, comDE genes encode a membrane-bound histidine kinase (ComD) and its cognate response regulator (ComE), respectively (Li et al. 2001). ComDE works together in response to CSP, encoded by the *comC* gene positioned in the same locus (Li et al. 2002). CSP is a known "alarmone" that triggers autolysis within a population under stress, inducing cell death, and release of the chromosomal DNA into the extracellular matrix. Genetic studies have highlighted that the *comDE* deficient strains produce biofilm with substantially reduced biomass, compared to the wild-type strain (Li et al. 2002).

Further studies have shown that the involvement of another TCS system, ScnR/Scnk is likely to have a major influence in the formation of a sponge-like biofilm architecture (Cvitkovitch et al. 2003). Interestingly, scnRK expression was unaffected by H_2O_2 and play a crucial role in counteracting oxidative stress and enhanced resistance to phagocytosis by macrophages (Chen et al. 2008). Also, in coordination with ComCDE QS system, the CiaR/H TCS system induce competence, resist the superoxide radical stress response, and enhance biofilm formation (Qi et al. 2004; Levesque et al. 2007). Further, the mutant *ciaH* regulatory system resulted in reduced biomass and short chains with a possible link in regulating cell division (Ahn et al. 2005).

VicRK is a TCS that positively regulates the gbp locus sucrose-dependent biofilm (Lee et al. 2004) and autolysis pathways. *vicK*-deficient mutants not only lacked in the formation of sucrose-dependent biofilm but were also ineffective in the separation of daughter cells (Senadheera et al. 2005). VicK regulates the expression of *atlA, autolysin* A, a component crucial for the lysis of *S. mutans,* and subsequent release of e-DNA (Sztajer et al. 2008). Multiple functionalities of VicK as a ComD receptor have been reported, implying the interdependency between ComDE and VicRK. A *vicX* mutant showed higher accumulation of glucans, encoded by water-insoluble glucans in the EPS (Sztajer et al. 2008). Deletion of a Sugar Transporter Systems Regulator (StsR) in *S. mutans* modified the expression of more than 188 genes that could be clustered with the sugar ABC and PTS transporters. StsR is a GntR transcription factor and its deletion also caused a decrease in biofilm formation and EPS production in *S. mutans* (Li et al. 2018). In fact, an investigation on the effect of *S. mutans* during infectious endocarditis with rats induced with heart valve injury revealed that dental caries were more serious in rats with heart injury. This study also showed that sucrose uptake could contribute to the colonisation of *S. mutans* in the heart tissues (Nomura et al. 2020).

Similarly, Sortase A an enzyme encoded by *srtA* facilitates adhesion and formation of biofilm. The deletion of the *srtA* resulted in the mature biofilm that was deficient in EPS and a pleiotropic modification in the transcriptome was observed in a growth-dependent manner. It was also suggested that the modulated genes could be involved in carbohydrate transport and aciduricity (Chen et al. 2019). Thus, the factors building *S.mutans* biofilm aid a first-line defence to overcome harsh environments and have a pleiotropic effect on other phenotypes such as acid production and tolerance assistants as a part of the progression of caries.

Acid production and tolerance assistants

Dental plaque bacteria produce organic acids as by-products during carbohydrate fermentation, a reaction catalysed by glycolytic enzymes. This dissolves the hydroxyapatite of enamel and dentine leading to the initiation and progression of caries (Hojo et al. 1991). The organic acids released into the environment disassociate as anions and conjugate protons, drastically reducing the pH of the environment. Incidentally, these protons also diffuse into the cytoplasm of the cells through bacterial membranes, thereby acidifying the cytoplasm. The low cytoplasmic pH is detrimental to the bacteria, as acid sensitive glycolytic enzymes may dysfunction, inhibiting ATP production, and also damaging the DNA and proteins (Quivey et al. 2001).

Evolutionarily, acid stress has forced *S. mutans* to develop several mechanisms to maintain its cytoplasmic pH and protect its cellular building blocks. The acid tolerance response (ATR) by *S. mutans* has been classified into three main categories: acute, adaptive, and transient ATR (Baker et al., 2015). As *S. mutans* carries out glycolysis at a pH lower than 4.0, the organism protects its acid sensitive glycolytic enzymes by transporting the protons across the cell membrane through the membrane associated F-ATPase (H^+-translocating ATPase) (Bender et al. 1986). The tolerance induced by glycolytic acids are stimulated not only due to environmental stimuli including potassium or magnesium levels but also by specific catabolic activities of the

mutans Streptococci species. Such acid adaptation can also be a response action to acids present within the cell as well as the environment (Belli and Marquis 1994). Another ATPase, P-type ATPase (H^+-ATPase) in *S. mutans* has also been suggested to act in conjugation with F_1F_o-ATPase to eliminate the intracellular protons produced during anaerobic metabolism (Magalhães et al. 2005). The plasma membrane of *S. mutans* consists of insoluble glucans that are composed of α -(1, 3)-linked glucose moieties that create a sieving effect, which entraps proton binding macromolecules (Hata and Mayanagi 2003). This proton entrapment in a lower pH cell surface environment triggers adaptation processes to acid. Transcriptomic analysis reveals a positive association between acid tolerance and sucrose-mediated glucans biosynthesis. Nevertheless, it was noted that the induction of ATR genes requires both low pH and glucans (Guo et al. 2015). The amino acid biosynthetic gene including *ilvC* and *ilvE* were upregulated during acid stress conditions in *S. mutans* (Len, et al., 2003). Indeed, the *ilvE* deletion mutant of *S. mutans* UA159 resulted in decreased acid tolerance in addition to defective F1-Fo ATPase activity (Santiago et al. 2012).

Also, *S. mutans* could neutralise the cytoplasmic acid by the agmatine deiminase system (AgDS) by converting agmatine to carbon dioxide, ammonia, and putrescine. This process appears to support growth by generating ATP (Griswold et al. 2006). AgDS is a two-component system encoded within an operon *aguBDAC*, that is induced in the presence of the growth inhibitor agmatine and, in the presence of acidic and heat stress (Ahn et al. 2006). It has been shown that the regulation of the AgDS system is tightly regulated by VicRK, ComDE, CiaRH, and CcpA (Liu and Burne 2009). Evaluation of *S. mutans* response to an acidic pH through genome-wide transcriptional analysis revealed the upregulation of genes belonging to a wide range of two component systems such as ComDE, LevSR, CiaHR, ScnKR, LiaSR, and Hk/Rr1037/1038. Inactivation of these TCS individually, affected the acid tolerance of *S. mutans* significantly, suggesting a coordinated control of these TCS on acid adaptation (Gong et al. 2009). The reduction of acidity by *S. mutans* is also executed by malolactic fermentation, a process of producing lactic acid through the decarboxylation of L-malate (Sheng and Marquis 2007). The genes involved in the malolactic fermentation are induced upon acid shock and the positive regulator MleR was required for maximum expression of these genes (Lemme et al. 2010).

S. mutans also possess DNA repair mechanisms to protect the DNA from pH fluctuations and reactive oxygen species that are produced as by-products of metabolism. Studies with non-functional Fpg and MutY (DNA glycosylases) and Smn (endonucleases) belonging to the DNA base excision repair mechanisms, raised the spontaneous mutation frequencies that corroborated with the ability of the mutants to survive acid and oxidative stress mediated destruction (Gonzalez et al. 2012).

The role of small RNAs (sRNA) in the regulation of acid tolerance in mutans Streptococci was initially demonstrated with the existence of sRNA L10-Leader in *S. mutans* UA159. The L10-Leader in *S. mutans* UA159 was associated with the pH level, yet not to the quantity of glucose or sucrose in the environment. *In silico* and transcriptomic analysis affirmed that the association of target mRNA of L10-Leader was involved in the acid adaptation responses of *S.mutans* (Xia et al. 2012). Investigation into 110 sRNAs in *S. mutans* under various pH levels indicated that specific sRNAs are induced at different acid stress conditions. Specifically, the levels of sRNAs progressively decreased between pH 7.5 and 5.5 and increased to some extent at pH 4.5. In fact, the expression of mRNAs was upregulated in acidic conditions (Liu et al. 2016). Genome analysis of *S. mutans* strains isolated from dental plaques of children revealed that the sRNA133474 and its target mRNA, regulate the TCS for acid adaptation. It has been suggested that the sRNA133474 may negatively regulate the expression of mRNA covR/liaR/ciaR which are major regulons of the two component systems LiaSR, CiaRH, and CovRS, critical for acid tolerance in *S.mutans* (Zhu et al. 2017). The response to acid production and tolerance by *S. mutans* is more intertwined with various systems to favour the cariogenic pathogen to acquire the inherent trait of competence.

Competence craftsmen

Competence is an innate complex transformation process in bacteria, wherein the homologous recombination of the eDNA occurs. This allows the bacteria to acquire new traits for resistance to antimicrobials, and increased survivability. The competence conferring pathway is multi-layered with multiple regulatory factors and genes. Of the 13 identified competence regulating pathways, two quorum sensing regulated pathways namely, ComRS and ComCD (or BlpHR) play a primary role (Reck et al. 2015). The most striking feature is that both extracellular signalling (ComCDE) and the intracellular signalling (ComRS) are present in *S. mutans*, conferring a selective advantage in the pathogenesis.

The quorum sensing signal peptides, CSP (competence-stimulating peptide)/mutacin inducing peptide,

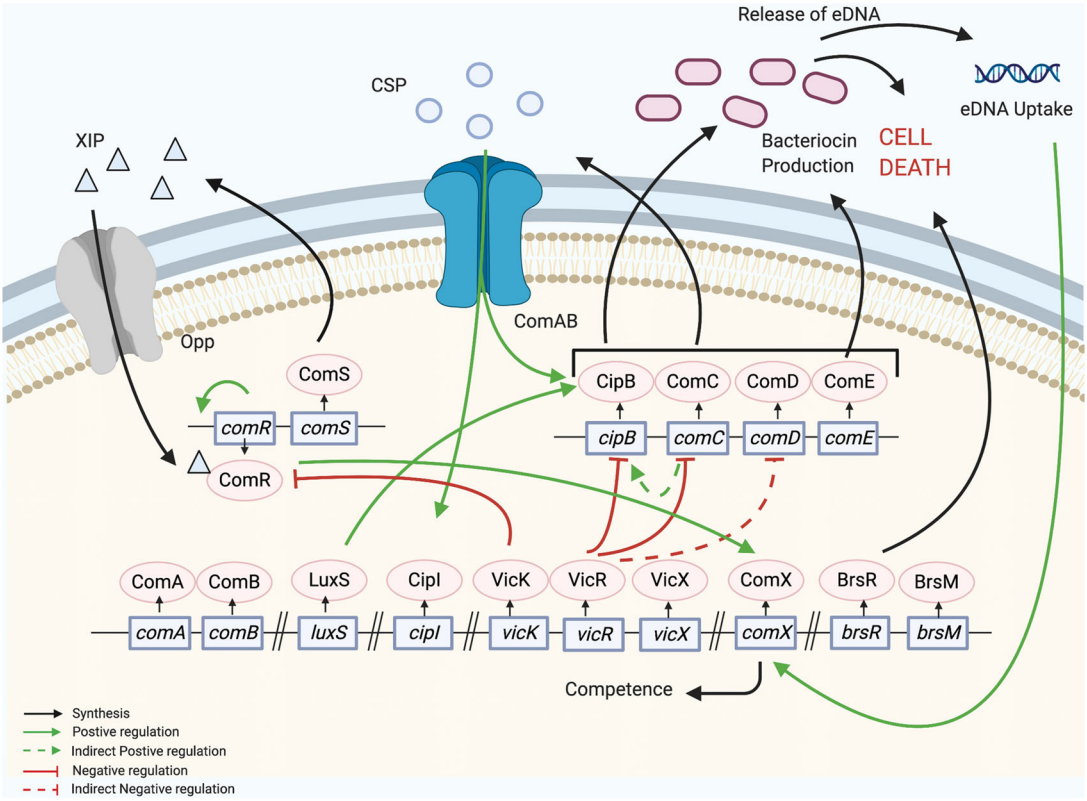

Figure 2. Competence pathways are highlighted in this diagram. The eDNA uptake is facilitated by the ComX. The Opp/Rgg transporters are responsible for the influx of XIP and CSP, respectively. CSP and XIP activate ComCD and ComRS systems, both converge in the regulation of *comX*. These QS associated signalling molecules have a positive feedback loop while also initiating a cascade of competence conferring gene up-regulation. Images created with BioRender.com.

and XIP (*sig* X-inducing peptide) are well known to activate the ComCD and ComRS system, respectively. Both these systems converge in the regulation of *comX*. The 21 amino acid residue signal peptide, CSP which is encoded by comC is exported through an ATP-binding cassette transporter. This signal peptide is later matured into 18 amino acid residue molecules by a protease, SepM (Petersen et al. 2006; Hossain and Biswas 2012). Once the TCS-ComDE senses the mature CSP, the phospho-relay mechanism is switched on through the histidine kinase, ComD. This results in autophosphorylation and subsequently transfers the phosphoryl (∼P) group to its response regulator, ComE. The phosphorylated ComE (ComE ∼ P) increases the expression of ComX/SigX that effects changes in the downstream 30 late competence genes (Shanker and Federle 2017). However, the immediate regulator of comX is the comRS system. The cognate response regulator ComR is a Rgg regulator. It binds with the mature 7 aa residue small hydrophobic peptide- XIP, that was initially processed from the pre-peptide ComS secreted outside the cell. The OppD/Ami permease facilitates the influx of the processed ComS – XIP. A positive feedback loop was observed as the ComR/XIP complex binds to the promoters of genes *comS* and *sigX*. Comparative transcriptomic analysis of *S. mutans* with other streptococci showed that the presence of peptide pheromones upregulated 27 genes belonging to ComE, ComR, and SigX regulons, and that 27 pan-streptococcal genes belonging to SigX regulon were upregulated during competence (Khan et al. 2016).

A link between regulation of competence to the regulation of cell division, cell envelope integrity monitoring, and signal transduction in *S. mutans* was established. Gene knockout experiments led to the discovery of 20 genes that substantially impacted the regulation of competence and associated phenotypes in *S. mutans*. It was shown that approximately 35 genes were required for transcription of *comX,* suggesting very limiting conditions for initiation of *comX* transcription that leads to the state of competence. Some of the products of these genes including PknB, LiaS, PspC, DltA, Rgpl, LtaS that impact the expression of *comX*, are known to engage in the biogenesis and integrity of cell wall in *S. mutans*. Mutational studies with some of these genes showed that DNA uptake diminished since the uptake of single stranded DNA is membrane associated (Shields et al. 2017). Even though exogenous CSP and

XIP activates *comX*, qualitatively different behaviours are observed in the expression of *comX* in different environments (Son et al. 2012). Activation of *com*X is a bimodal response with exogenous CSP, while XIP elicits a unimodal response. The expression of *comX* by CSP is bimodal, rendering only a fraction of cells within a population transformable. This bimodality of *comX* originates from the modulation of the transcriptional feedback loop in ComRS. In fact, the CSP upregulates *comR* by triggering the ComRS feedback system in a carbohydrate source-dependent fashion. Meanwhile, the endopeptidase PepO inhibits the ComRS feedback loop possibly through degradation of the XIP/ComS feedback signal. This eliminates *comX* bimodality and results in unimodal expression of *comX* response to CSP (Mashburn-Warren et al. 2010; Underhill et al. 2018). Indeed, a complete *comS* gene requires bimodal *comX* to respond to CSP (Figure 2).

The close association of the competence pathway with the autolysin pathway endows virulence. While the autolysin pathway works towards destroying neighbouring organisms, the competence pathway uptakes the eDNA from the lysed organisms for increased fitness. Thus, the competence system has a bidirectional regulation with the autolysin pathway (Son et al. 2015). This interlink is evidenced in the *altA* mutant strain, which shows reduced expression of competence related genes, *comD*, *comX,* and *ciaR*. Another noteworthy influencer of the Com pathway is a special TCS namely CovR. It is termed as an "orphan" TCS, where the CovS is not found in the *S. mutans* species although it is present in other streptococcal species (Dmitriev et al. 2011). The *covR* mutant strain down regulates many of the late competence genes, *comF*, *comEC,* and *comY* operon. CovR also upregulates *spaP* which is responsible for synthesis of antigen II and *patB* (a putative hemolysin) (Dmitriev et al. 2011). Also, the interlink between competence and bacteriocin production emphasises the significance of the survival fitness of *S. mutans* in a polymicrobial environment.

Bacteriocin production

In addition to bactericidal properties against closely related species, bacteriocins function as crucial factors in the pathogenesis of *S. mutans*. The presence of mutacins in *S. mutans* offers a selective advantage not only in the colonisation process but also in the transmission of bacteria from mother to child. Mutacin production is regulated by two major systems: Rgg-like regulators and LytTR regulatory system (Hossain and Biswas 2012). The lantibiotic mutacin is regulated by *mutR*, a Rgg-family regulator present in the gene cluster of the mutacin I, II, and III loci. They regulate the transcription of the mutacin operon, but the exact role remains to be elucidated. The non-lantibiotic mutacin production is regulated by the CSP- induced factors. These factors specifically interact with the DNA binding site, upstream to several bacteriocin and bacteriocin immunity related loci, *nlmC* (Hung et al. 2011). The synthesis of mutacin IV is related to *com*D which is a target for sRNA *srn225147*. Although *srn225147* exerts a two-way regulation on *com*D expression, this regulation is weak (Liu et al. 2019). In addition to the above mutacins, a close association exists between ComCDE and mutacin, CipB (mutacin V). It was shown that the mutacin, CipB has a role in the cellular processes of CSP-induced competence, autolysis, and DNA transformation (Dufour et al. 2011).

Non- lantibiotic mutacins, which are ComCDE regulated, are also regulated by an independent system, LytTR. This system consists of two major components: an inhibitor Protein (M) and a transcriptional Regulator (R). Thus far, two LytTR systems have been studied in *S. mutans*- HdrR and BrsR (Okinaga et al. 2010). Overexpression of *hdrR* results in increased mutacin production and lysis of competing strains and *brsR* is lethal to the producer (Okinaga et al. 2010). Mutational studies of *hdrM* and *bsrM* have revealed an increased expression of *hdrR* and *bsrR,* respectively. A striking commonality is that the repeat sequence of bacteriocin genes regulated by ComE is the same sequence which is identified by the LytTR regulators (Hossain and Biswas 2012). In addition to the genetic regulation, mutacin production is under the influence of the environmental cues, cell density, and nutrition. A higher cell density with minimal nutrient availability upregulates mutacin production (Merritt and Qi 2012). Thus, in a biofilm, the upregulation of bacteriocin is subsequent, thereby strongly aiding the establishment of *S. mutans* to overcome other stress-related factors.

Reprisal of S. mutans *to oxidative stress*

Commensal microbes in oral biofilms generate reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) and hydroxyl radical ($OH^·$) that increases the vulnerability of *S. mutans* to oxidative stress (Marquis 1995). Upon entering the microbial cells, H_2O_2 induces cellular damage by disrupting membrane lipids, initiating mis-metalation of enzymes, damaging proteins, and DNA integrity (Imlay 2013). H_2O_2 also reacts with the intermediates of the Fenton's reaction and generates reactive oxygen species to

damage DNA (Imlay 2013). Molecular studies have provided novel insights into the Spx (Spx1 and Spx2) antioxidant pathways in peroxide stress response mechanisms in *S. mutans* (Kajfasz et al. 2017). Notably, when the Spx function was mapped through genetic studies using isogenic mutant *spxA1* and double mutant (Δ*spxA1/*Δ*spxA2*) strains, an increased sensitivity to oxidative stress was observed. SpxA2 activates SpxA1 and directly modulates the expression of oxidative stress tolerance genes, *ahpC* (alkyl hydroperoxidase), *dpr* (iron-binding protein), *sodA* (superoxide dismutase) and the pyruvate metabolism genes, *adhABCD-lplA*, *aldB, alsS*, *pflA* (Baker et al. 2014; Galvão et al. 2015; Kajfasz et al. 2015).

The *lplA* gene encodes lipoate ligase that scavenges the lipoic acid as a part of its antioxidant property and serves as a co-factor to AoDH (dehydrogenases) for lipolytic activity (Kajfasz et al. 2015). Meanwhile, the protein products of *aldB-alsS* and *adhABCD* genetic loci are responsible to convert pyruvate to acetoin and acetoin to acetaldehyde, respectively (Packer et al. 1995). Furthermore, the pyruvate formate-lyase (PflA) encoded by *pflA* may be more crucial than lactate dehydrogenase (LDH) in pyruvate metabolism. This may be involved in ATP synthesis and NAD^+ and/or NADH recycling of pflA (Carlsson and Griffith 1974; Yamada and Carlsson 1975; Takahashi et al. 1982; Yamamoto et al. 2000). Following the above responses, it was also observed that peroxide exposure impacted *S. mutans* metabolic profile in altering the lactate, ethanol, formate, and acetoin levels (Kajfasz et al. 2017). Although pyruvate is excreted during growth as a secondary metabolite, *S. mutans* reutilises this component using LrgAB, when the primary carbon source is depleted. However, the induction of *lrgAB* during the stationary phase is modulated by hydrogen peroxide. Therefore, pyruvate may also serve as a buffer against oxidative stress (Ahn et al. 2019). Taken together, the SpxA regulon is critical for the metabolic alteration to switch on the cells to scavenge oxidants and promote biofilm formation, thereby establishing a physical barrier to endure oxidative stress (Galvão et al. 2017).

Upregulation of oxidative stress response genes *ahpC* and *gor* was observed when *S. mutans* biofilms were treated with gentamicin, vancomycin, and linezolid antibiotics. However, this response was absent in the *spxA1* mutant biofilms. As *gor* encodes the formation of glutathione (an antioxidant), exogenous addition of glutathione restored the antibiotic tolerance to *spxA1* mutant strain (Nilsson et al. 2019).

Proteomic analysis revealed a new link between a putative glycosyltransferase SMU_833 and oxidative stress. The SMU_833 regulates about 10 proteins responsible for the synthesis of mutanobactin, a peptide that is crucial for *S. mutans* to achieve tolerance against H_2O_2 and survival (Rainey et al. 2018, 2019). Global regulator CodY, in addition to regulation of survival also regulates tolerance to H_2O_2 in *S. mutans.* When exposed to hydrogen peroxide, a △*codY* strain better tolerated the stress, compared to wild type UA159, indicating that CodY negatively regulates the stress tolerance genes in *S. mutans* (Rioux and Santiago-Narvaez 2019).

Role of house-keeping genes: DNA repair system

While thriving in a pH fluctuant environment, *S. mutans* modulates the expression of genes to tolerate these harsh conditions. In addition to these genes, the hidden players in conferring the tolerance are the DNA repair systems, by maintaining the genome. Environmental responses of the DNA repair systems are well studied. During an acid influx, the cells go through a cascade of events known as the acid tolerance response (ATR) which enhances the production of acid pumps, chaperones, and DNA repair. The acidic metabolites or the reactive oxygen species cause damage to the DNA, which is later encountered by the SOS (error-prone repair) system to restore the organism's survivability. The SOS repair system responds to the DNA damage in co-ordination with a sensor/inducer RecA and a negative regulator, LexA (Wen and Burne 2004). Acidification in cariogenic *S. mutans* triggers the SOS system; while there also exists an alternate pathway to repair DNA damage in a *recA* deficient strain. The alternate pathway involves the role of a novel gene, *cinA*, which is activated via the competence genes, and it primarily repairs DNA. In the pipeline of repair of DNA damage, the Base Excision Repair (BER) proteins, such as Fgp, MutY, Smx, and Smn are involved in repairing the oxidised bases (Gonzalez et al. 2012). Thereby, *S. mutans* houses various DNA repair systems, which are involved in competence, acid, and oxidative stress tolerance and coexist in an oral biofilm community with the aid of inter-species communication controllers.

Interspecies communication controllers

Any biofilm community consists of many microcolonies that coexist and communicate through metabolites and molecules. Communication is key to the overall development and persistence of biofilms in a mutualistic manner. In the oral microbiome, the communication network is driven by small-molecule chemical signals,

autoinducer −2 (AI-2), and metabolic cue –H_2O_2. S-Adenosylhomocysteinase, a bifunctional enzyme encoded by *luxS*, which play an integral role by providing methyl groups in the methyl cycle. This is essential for the nucleic acids (DNA and RNA), certain proteins, and metabolites of several oral bacteria (Winzer et al. 2003; Vendeville et al. 2005). The universal inter-generic signal, AI-2 is a product of 4,5-dihydroxy-2,3-pentanedione (DPD) by the activated methyl cycle, and subsequent LuxS/AI-2 synthase mediated cleavage of the intermediate, S-ribosyl homocysteine (Pereira et al. 2013). It is crucial for eliciting response across species barriers for shaping multi-species biofilms in the dental plaque (Bassler et al. 1997; Chen et al. 2002; Xavier and Bassler 2005).

The foundation for a dysbiotic dental plaque is laid by *Streptococci* and certain fungal species, that provides a broader habitat for the growth, development, promotion of virulence strategies, and resistance towards host defence mechanisms (Jakubovics 2010; Kim et al. 2017; Koo et al. 2018). The complexity of the multi-species ecosystems surges when the oral microbes establish mutualistic relationships through physical interaction, exchange of metabolites, QS signal molecules, and genetic material (Bose and Santosh 2018). As polysaccharide recognition is fundamental to colonial or bacterial aggregation, *luxS* proficient strains, *S. oralis,* and *Actinomyces naeslundii*, develop thick biofilms as a part of their mutualistic relationship than when grown alone. Since the expression of *luxS* is crucial for the synthesis of AI-2 which in turn governs the biofilm formation by *Streptococci*, mutation of *luxS* gene sojourns communication between the cells. Indeed, the absence of the *luxS* gene also affects the density of the bacteria resulting in a loosely formed biofilm (Rickard et al. 2006; Huang et al. 2011; He et al. 2015).

The density of interdigitated biofilms formed by the mixed colonisers relates to the succession of oral communities and AI-2 signal concentration. In light of this concept, it has been demonstrated that the relative amount of AI-2 produced by commensal bacteria and pathogens has a certain impact on the mutualistic association with the initial colonisers, *Streptococci,* and *Actinomyces*. These species colonise the tooth enamel on their interaction with the salivary pellicle, and subsequently grow together with *Veillonella* as multispecies biofilm communities. Meanwhile, an increase in the biomass of commensals proportionately elevates the AI-2 concentration to establish a transition with the bacterial species *Fusobacterium* (Parashar et al. 2015).

Importantly, Streptococcal H_2O_2, a metabolic cue, triggers the release of DNA without involving DNA lysis. It also stabilises the biofilm structure by oxidising the macromolecules to enhance competition and communication between oral bacteria at sub-lethal concentrations (Rickard et al. 2008; Parashar et al. 2015). Microarray analyses indicate that the Gram-negative pathogen, *Aggregatibacter actinomycetemcomitans* affects the expression of two different genes, *katA* (catalase) and *apiA* (outer membrane protein) in response to H_2O_2. These factors are crucial to an increase in the likelihood of inflammation, on interaction with the human serum protein factor H and enhances the resistance demonstrated by the bacterial pathogen, against host serum mediated-killing (Federle and Bassler 2003).

Interaction is not always lucrative for both sides, as reported in studies involving *S. mutans* and *S. sanguinis*, where *S. mutans* dominates the other, through elevated mutacin and organic acid production (Mahajan et al. 2013). Certain Gram-positive bacteria possess the ability to produce antimicrobial peptides (mutacins or bacteriocins) to contest other competitive microorganisms both from closely related genera and species found in the same dental microcosm (Wang and Kuramitsu 2005; Merritt and Qi 2012). The possibility of survival/altruistic suicide of pathogenic species increases as a part of ecological balance to overcome stresses through inter-species communication networks among microbial communities.

Other influential contributors to dental caries

Mutans streptococci (MS) is an umbrella term for the streptococcal species which cause dental caries. Notably, few of the MS species with their host-specificity are *Streptococcus mutans* and *Streptococcus sobrinus* in humans, and *Streptococcus criceti* in rats (Tamura et al. 2012). *S. mutans* and *S. sobrinus* are the initial colonisers during the onset of caries. Therefore, several research groups focus on targeting the MS to prevent/treat caries. While the role of *S. mutans* in dental caries is irrefutable, its role as the "Keystone pathogens" in caries is yet to be established. Microbiological analysis of early carious and non-carious human fissures revealed that *S. mutans* encompasses about 7.3% of the total microflora in carious fissures compared to 2.3% of the non-carious fissures (Meiers et al. 1982).

The prevention, diagnosis, and treatment are wholly dependent on the understanding not just the causative agents, but also the other factors. Therefore, understanding the microbiome of caries and the influence of various host factors (age, salivary conditions, smoking, etc.,) on microbiome shifts are critical to our enhanced

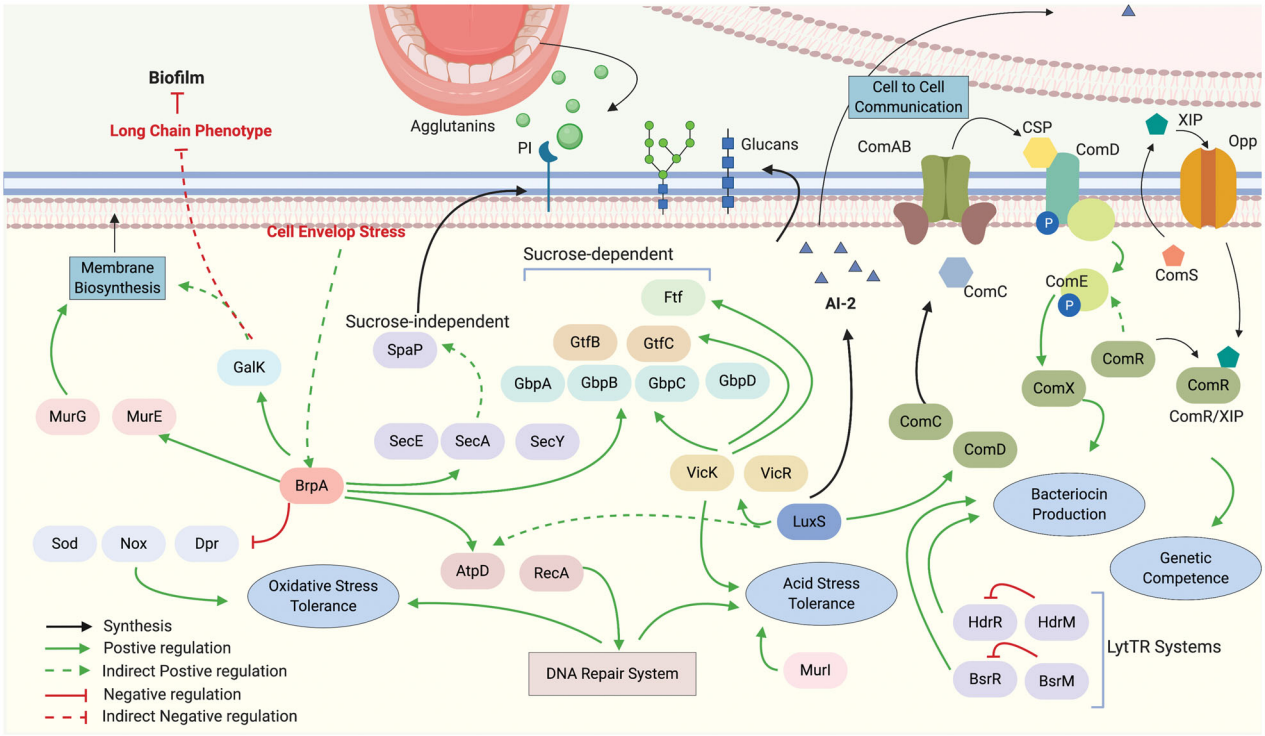

Figure 3. Overall gene network of the major virulence pathways in *S. mutans*. The cariogenic organism houses intricate pathways which regulates each other to achieve virulence and to thrive in harsh environment. The major virulence features of cariogenic *S. mutans* is the oxidative stress tolerance, acid stress tolerance, bacteriocin production, biofilm formation and genetic competence. The biofilm formation is bimodal, the *gpb* loci and *gtf* loci are part of sucrose dependent pathways, and they consume sucrose to synthesise adhesins and glucans. Also, the diagram highlights the sucrose independent pathway via SpaP, which is transported to membrane by Sec translocase. These are controlled by the BrpA protein. On the other hand, the LuxS regulates the interspecies communication while playing a role in biofilm formation and competence. The close association of bacteriocin production and competence pathways gives *S. mutans* an edge over other oral microbials. Images created with BioRender.com.

understanding of caries. But there are many confounders in the determination of the aetiology, from sampling, incidence, and process till the microbiome and genetic contributions. Microbial diversity in the plaque microflora are catalogued based on 16s rRNA sequence and operates on operational taxonomic units (OTUs). The OTUs are limited to assign the genus/species but an efficient tool to understand the complexity and functioning of oral microbial communities (Banas and Drake 2018). Yet, such an approach has not been successful in classifying oral streptococci as they are poorly differentiated by 16S rRNA gene sequences (Bishop et al. 2009).

Prospects using the OMICS approach: Keys to efficient caries control?

A vital question that needs to be answered is "*Should novel therapeutics be directed at multi-faceted genes in the hopes of diminishing the effects of related genes, or focus on host needs, including nullifying the effects of biofilm formation and acid production*?" Our review highlights that the caries-related gene network in *S. mutans* is multi-layered and complex (Figure 3). Biofilm formation, acid and oxidative stress tolerance, competence, bacteriocin production, and DNA damage repair system have common regulators and there are about 15 Two Component System (TCS) housed within the organism, that co-regulate to confer virulence (Table 1). Recent reports suggest that the bacterial metabolites (Bio-Gene clusters) such as Polyketide Synthases (PKS), Non-Ribosomal Peptide Synthases (NRPS), hybrid PKS/NRPS, and Ribosomal Synthesised and Post-translationally modified Peptides (RiPPs) play a critical role in the virulence and progression of caries (Momeni et al. 2020). In the complex ecology of the dental microbiota, *Streptococcus mutans* co-exists with periodontitis-associated species *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* amongst many others. Targeting *S. mutans* alone could lead to further dysbiosis, likely initiating gingivitis and periodontal disease (Kilian et al. 2016).

When statistical approaches, mathematical modelling, and Artificial Intelligence (AI) such as deep learning

Table 1. Two Component Systems (TCS) of *S. mutans* and the pathway which it regulates.

TCS	Gene locus	Regulatory effect on the phenotypes					References
		Biofilm	Competence	Acid tolerance	Oxidative stress tolerance	Bacteriocin production/resistance	
HK-ComD RR-ComE	*comDE*	+	+	–	–	+	Li et al.2002; van der Ploeg 2005; Ahn et al.2006; Kreth et al. 2007; Li et al. 2008; Song et al. 2012
HK-LevS RR-LevR	*levRS*	+	–	+	–	–	Zeng et al. 2006; Zeng et al.2011; Song et al. 2012
HK-VicK RR-VicR	*vicRK*	+	–	–	+	–	Liu and Burne 2009; Lee et al. 2004; Senadheera et al. 2005; Deng et al. 2007; Ahn and Burne 2007; Tremblay et al. 2009; Song et al. 2012
HK-CovS RR-CovR	*covRS*	–	+	–	–	–	Levesque et al. 2007; Chen et al. 2008; Song et al. 2012
HK-CiaH RR-CiaR	*ciaRH*	–	+	+	–	–	Song et al. 2012; Levesque et al. 2007; Ahn et al. 2006; Qi et al. 2004; Lemos and Burne 2008
HK-LytS RR-LytT	*LytST*	+	+	–	+	–	Brunskill and Bayles 1996; Kawada-Matsuo et al. 2009; Ahn et al. 2010; Song et al. 2012
HK-LiaS RR-LiaR	*LiaRS*	+	–	–	–	+	Li et al. 2002; Chong et al. 2008; Perry et al. 2008; Suntharalingam et al. 2009; Zhang and Biswas 2009; Song et al. 2012
HK-NsrS RR-NsrR	*NsrRS*	–	–	–	–	+	Kawada-Matsuo and Komatsuzawa 2017
HK-BceS RR-BceR	*BceRS*	–	–	–	–	+	Kawada- Matsuo and Komatsuzawa 2017
HK-LcrS RR-LcrR	*lcrRS*	–	–	–	–	+	Kawada-Matsuo and Komatsuzawa 2017
HK-ScnK RR-ScnR	*scnRK*	–	–	–	–	+	McLaughlin et al. 1999; Song et al. 2012
HK-SpaK RR-SpaR	*spaRK*	–	–	–	–	+	Klein et al.1993; Klein and Entian 1994; Kleerebezem et al.1997; Song et al. 2012
HK-KinF RR-LlrF	*llrFkinF*	–	–	+	–	–	Lemos and Burne 2008; Kawada-Matsuo et al. 2009; Song et al. 2012
HK-KinG RR-LlrG	*llrGkinG*	–	–	–	–	+	Tsuda et al. 2002; Ouyang et al. 2010; Song et al. 2012

*HK-Sensing Histidine Kinase; *RR- Response Regulator; +Positive influence on the phenotype; No influence on the phenotype

is applied, the basic OMICS assisted learning of the whole microbiome is tremendous, irrespective of the functionality. With advanced knowledge being achieved with every passing day, compilation and integration are key elements to this learning. Virtual and predictive technologies assisted by AI can empower meta-analysis and microbiome-wide studies. For future studies, the modus operandi could be the amalgamation of machine learning and regression modelling, as has been achieved with the gut microbiome (Armour et al. 2019). This will facilitate our understanding of the correlation between the various microorganisms in the dental microbiota and also with the microbiomes of other niches in the body. Therapeutic processes can be modified according to the specific functional aetiology in a personalised manner, for effective and efficient containment, culmination, and cure (Nath and Thaiss 2019). AI technologies will help to study patterns and repetitive behaviours which could make it easier to study, predict, and analyse non-phenotypic characteristics and features through metabolomics, proteomics, and transcriptomics. This can aid in early detection and possibly relevant, precautionary treatment against dental caries ahead in time. It would take into consideration the variations in microbiota and the handling capacity of an individual based on their microbiome. Furthermore, the development of specifically targeted novel treatments and therapies to eradicate harmful bacteria and/or mitigate virulence, may be a cost-effective, highly efficient, and game-changing approach to the management of dental caries and other oral biofilm infections.

Concluding remarks

To understand the roles of *S. mutans* in causing caries, and its role in systemic diseases such as bacteraemia and endocarditis, novel approaches are required, and one such approach is the use of OMICS platform (Figure 4). The OMICS approach goes through a phenomenon called the hype cycle. The hype cycle is a standardised pathway of a new technology that follows four major stages namely technology trigger, the peak of inflated expectation, trough of disillusionment, and plateau of productivity. Bearing in mind the recent technological developments, caries research is predicted to be in the trough of disillusionment. At such a stage, a network would serve as a base for in depth research into the pathways and target identification in caries. The assumption that one microorganism is the main "keystone" in a multifactorial disease is fallible. Most bacteria isolated from the caries site have been *S.*

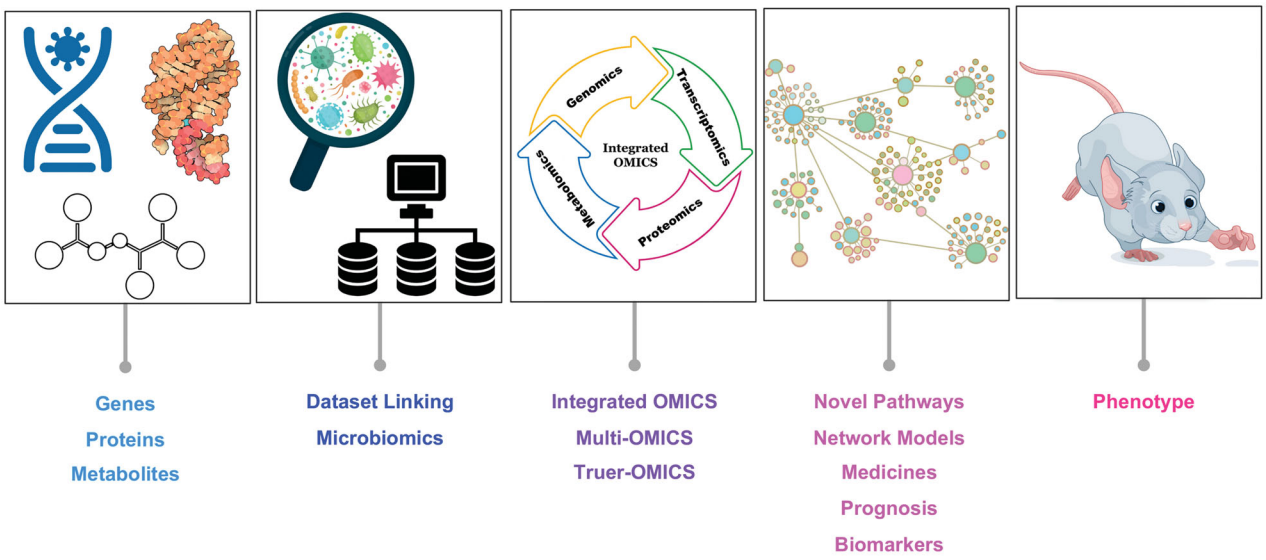

Figure 4. General layout of the OMICS approach. The genetic data is processed to phenotypic data by involving multi layers of 'omics' studies such the metabolomics and proteomics.

mutans, lactobacilli and *Actinomyces* where *S. mutans* has been indicated to be the major causative agent. However, developments in the metabolomics have revealed the sophistication in the human microbiome, and detailed study into the polymicrobial oral cavity may shed more light into the co-ordination of species for disease progression. In future cases, consideration of previous theories and disregard of the need for accounting for the effects of other co-existing microorganisms will prove inimical due to possible wrong prognosis. It is important to prevent the overlooking of these possible paths- resistance, symbiosis, synergy among others.

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References

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(6):2277–2284.

Ahn SJ, Burne RA. 2007. Effects of oxygen on biofilm formation and the AtlA autolysin of *Streptococcus mutans*. J Bacteriol. 189(17):6293–6302.

Ahn SJ, Deep K, Turner ME, Ishkov I, Waters A, Hagen SJ, Rice KC. 2019. Characterization of LrgAB as a stationary phase-specific pyruvate uptake system in *Streptococcus mutans*. BMC Microbiol. 19(1):1–15.

Ahn SJ, Lemos JA, Burne RA. 2005. Role of HtrA in growth and competence of *Streptococcus mutans* UA159. J Bacteriol. 187(9):3028–3038.

Ahn SJ, Wen ZT, Burne RA. 2006. Multilevel control of competence development and stress tolerance in *Streptococcus mutans* UA159. Infect Immun. 74(3):1631–1642.

Ahn S-J, Rice KC, Oleas J, Bayles KW, Burne RA. 2010. The *Streptococcus mutans* Cid and Lrg systems modulate virulence traits in response to multiple environmental signals. Microbiology (Reading, Engl.). 156(Pt 10):3136–3147.

Armour CR, Nayfach S, Pollard KS, Sharpton TJ. 2019. A metagenomic meta-analysis reveals functional signatures of health and disease in the human gut microbiome. MSystems. 4(4). DOI:10.1128/mSystems.00332-18.

Baker JL, Abranches J, Faustoferri RC, Hubbard CJ, Lemos JA, Courtney MA, Quivey R. Jr 2015. Transcriptional profile of glucose-shocked and acid-adapted strains of *Streptococcus mutans*. Mol Oral Microbiol. 30(6):496–517.

Baker JL, Derr AM, Karuppaiah K, MacGilvray ME, Kajfasz JK, Faustoferri RC, Rivera-Ramos I, Bitoun JP, Lemos JA, Wen ZT, et al. 2014. *Streptococcus mutans* NADH oxidase lies at the intersection of overlapping regulons controlled by oxygen and NAD + levels. J Bacteriol. 196(12):2166–2177.

Banas JA, Drake DR. 2018. Are the mutans streptococci still considered relevant to understanding the microbial etiology of dental caries? BMC Oral Health. 18(1):129.

Banas JA, Fountain TL, Mazurkiewicz JE, Sun K, Margaret VM. 2007. *Streptococcus mutans* glucan-binding protein-A affects *Streptococcus gordonii* biofilm architecture. FEMS Microbiol Lett. 267(1):80–88.

Bassler BL, Greenberg EP, Stevens AM. 1997. Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. J Bacteriol. 179(12):4043–4045.

Beg AM, Jones MN, Miller-Torbert T, Holt RG. 2002. Binding of *Streptococcus mutans* to extracellular matrix molecules and fibrinogen. Biochem Biophy Res Commun. 298(1): 75–79.

Belli WA, Marquis RE. 1994. Catabolite modification of acid tolerance of *Streptococcus mutans* GS-5. Oral Microbiol Immunol. 9(1):29–34.

Bender GR, Sutton SV, Marquis RE. 1986. Acid tolerance, proton permeabilities, and membrane ATPases of oral streptococci. Infect Immun. 53(2):331–338.

Bishop CJ, Aanensen DM, Jordan GE, Kilian M, Hanage WP, Spratt BG. 2009. Assigning strains to bacterial species via the internet. BMC Biol. 7(1):3–20.

Bose A, Santosh HN. 2018. Interspecies communication in oral biofilm. JCRI. 5(6):196–199.

Bradshaw DJ, McKee AS, Marsh PD. 1989. Effects of carbohydrate pulses and pH on population shifts within oral microbial communities in vitro. J Dent Res. 68(9): 1298–1302.

Brady LJ, Maddocks SE, Larson MR, Forsgren N, Persson K, Deivanayagam CC, Jenkinson HF. 2010. The changing faces of Streptococcus antigen I/II polypeptide family adhesins. Mol Microbiol. 77(2):276–286.

Brunskill EW, Bayles KW. 1996. Identification and molecular characterization of a putative regulatory locus that affects autolysis in *Staphylococcus aureus*. J Bacteriol. 178(3): 611–618.

Carlsson J, Griffith CJ. 1974. Fermentation products and bacterial yields in glucose-limited and nitrogen-limited cultures of streptococci. Arch Oral Biol. 19(12):1105–1109.

Chen PM, Chen HC, Ho CT, Jung CJ, Lien HT, Chen JY, Chia JS. 2008. The two-component system ScnRK of *Streptococcus mutans* affects hydrogen peroxide resistance and murine macrophage killing. Microbes Infect. 10(3): 293–301.

Chen X, Liu C, Peng X, He Y, Liu H, Song Y, Xiong K, Zou L. 2019. Sortase A-mediated modification of the *Streptococcus mutans* transcriptome and virulence traits . Mol Oral Microbiol. 34(5):219–233.

Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczer I, Bassler BL, Hughson FM. 2002. Structural identification of a bacterial quorum-sensing signal containing boron. Nature. 415(6871):545–549.

Chong P, Drake L, Biswa I. 2008. LiaS regulates virulence factor expression in *Streptococcus mutans*. Infect Immun. 76(7):3093–3099.

Cvitkovitch DG, Li YH, Ellen RP. 2003. Quorum sensing and biofilm formation in Streptococcal infections. J Clin Invest. 112(11):1626–1632.

Decker EM, Klein C, Schwindt D, Von OC. 2014. Metabolic activity of *Streptococcus mutans* biofilms and gene expression during exposure to xylitol and sucrose. Int J Oral Sci. 6(4):195–204.

Demuth DR, Lammey MS, Huck M, Lally ET, Malamud D. 1990. Comparison of *Streptococcus mutans* and *Streptococcus sanguis* receptors for human salivary agglutinin. Microb Pathog. 9(3):199–211.

Deng DM, Liu MJ, Ten CJM, Crielaard W. 2007. The VicRK system of *Streptococcus mutans* responds to oxidative stress. J Dent Res. 86(7):606–610.

Dmitriev A, Mohapatra SS, Chong P, Neely M, Biswas S, Biswas I. 2011. CovR-controlled global regulation of gene expression in *Streptococcus mutans*. PLoS One. 6(5):e20127.

Dufour D, Cordova M, Cvitkovitch DG, Lévesque CM. 2011. Regulation of the competence pathway as a novel role associated with a streptococcal bacteriocin. J Bacteriol. 193(23):6552–6559.

Federle MJ, Bassler BL. 2003. Interspecies communication in bacteria. J Clin Invest. 112(9):1291–1299.

Fitzgerald RJ, Keyes PH. 1960. Demonstration of the etiologic role of streptococci in experimental caries in the hamster. J Am Dent Assoc. 61(1):9–19.

Forester H, Hunter N, Knox KW. 1983. Characteristics of a high molecular weight extracellular protein of *Streptococcus mutans*. Microbiology. 129(9):2779–2788.

Fujita K, Matsumoto-Nakano M, Inagaki S, Ooshima T. 2007. Biological functions of glucan-binding protein B of *Streptococcus mutans* . Oral Microbiol Immunol. 22(5): 289–292.

Galvão LCC, Miller JH, Kajfasz JK, Scott-Anne K, Freires IA, Franco GCN, Abranches J, Rosalen PL, Lemos JA. 2015. Transcriptional and phenotypic characterization of novel Spx-regulated genes in *Streptococcus mutans*. PLoS One. 10(4):e0124969.

Galvão LCC, Rosalen PL, Rivera-Ramos I, Franco GCN, 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(2):142–153.

Gong Y, Tian XL, Sutherland T, Sisson G, Mai J, Ling J, Li YH. 2009. Global transcriptional analysis of acid-inducible genes in *Streptococcus mutans*: multiple two-component systems involved in acid adaptation. Microbiology (Reading, Engl). 155(Pt 10):3322–3332.

Gonzalez K, Faustoferri RC, Quivey RG. 2012. Role of DNA base excision repair in the mutability and virulence of *Streptococcus mutans*. Mol Microbiol. 85(2):361–377.

Griswold AR, Jameson-Lee M, Burne RA. 2006. Regulation and physiologic significance of the agmatine deiminase system of *Streptococcus mutans* UA159. J Bacteriol. 188(3): 834–841.

Guo L, McLean JS, Lux R, He X, Shi W. 2015. The well-coordinated linkage between acidogenicity and aciduricity via insoluble glucans on the surface of *Streptococcus mutans*. Sci Rep. 5:18015.

Hata S, Mayanagi H. 2003. Acid diffusion through extracellular polysaccharides produced by various mutants of *Streptococcus mutans*. Arch Oral Biol. 48(6):431–438.

He Z, Liang J, Tang Z, Ma R, Peng H, Huang Z. 2015. Role of the luxS gene in initial biofilm formation by *Streptococcus mutans*. J Mol Microbiol Biotechnol. 25(1):60–68.

Hojo S, Takahashi N, Yamada T. 1991. Acid profile in carious dentin. J Dent Res. 70(3):182–186.

Hossain MS, Biswas I. 2012. An extracelluar protease, SepM, generates functional competence-stimulating peptide in *Streptococcus mutans* UA159. J Bacteriol. 194(21): 5886–5896.

Huang R, Li M, Gregory RL. 2011. Bacterial interactions in dental biofilm. Virulence. 2(5):435–444.

Hung DCI, Downey JS, Ayala EA, Kreth J, Mair R, Senadheera DB, Qi F, Cvitkovitch DG, Shi W, Goodman SD. 2011. Characterization of DNA binding sites of the ComE

response regulator from *Streptococcus mutans*. J Bacteriol. 193(14):3642–3652.
Imlay JA. 2013. The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. Nat Rev Microbiol. 11(7):443–454.
Jakubovics NS. 2010. Talk of the town: interspecies communication in oral biofilms. Mol Oral Microbiol. 25(1):4–14.
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(1):1–13.
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(13):2160–2170.
Kaur G, Rajesh S, Princy SA. 2015. Plausible drug targets in the *Streptococcus mutans* quorum sensing pathways to combat dental biofilms and associated risks. Indian J Microbiol. 55(4):349–356.
Kawada-Matsuo M, Komatsuzawa H. 2017. Role of *Streptococcus mutans* two-component systems in antimicrobial peptide resistance in the oral cavity. Jpn Dent Sci Rev. 53(3):86–94.
Kawada-Matsuo M, Shibata Y, Yamashita Y. 2009. Role of two component signaling response regulators in acid tolerance of *Streptococcus mutans*. Oral Microbiol Immunol. 24(2): 173–176.
Khan R, Rukke HV, Høvik H, Åmdal HA, Chen T, Morrison DA, Petersen FC. 2016. Comprehensive transcriptome profiles of *Streptococcus mutans* UA159 map core streptococcal competence genes. mSystems. 1(2). DOI:10.1128/mSystems.00038-15.
Kilian M, Chapple ILC, Hannig M, Marsh PD, Meuric V, Pedersen AML, Tonetti MS, Wade WG, Zaura E. 2016. The oral microbiome - an update for oral healthcare professionals. Br Dent J. 221(10):657–666.
Kim D, Sengupta A, Niepa THR, Lee B-H, 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(1):41314–41332.
Kleerebezem M, Quadri LE, Kuipers OP, De Vos WM. 1997. Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria . Mol Microbiol. 24(5):895–904.
Klein C, Entian KD. 1994. Genes involved in self-protection against the lantibiotic subtilin produced by *Bacillus subtilis* ATCC 6633. Appl Environ Microbiol. 60(8):2793–2801.
Klein C, Kaletta C, Entian KD. 1993. Biosynthesis of the lantibiotic subtilin is regulated by a histidine kinase/response regulator system. Appl Environ Microbiol. 59(1):296–303.
Koga T, Okahashi N, Takahashi I, Kanamoto T, Asakawa H, Iwaki M. 1990. Surface hydrophobicity, adherence, and aggregation of cell surface protein antigen mutants of *Streptococcus mutans* serotype c. Infect Immun. 58(2): 289–296.
Koo H, Andes DR, Krysan DJ. 2018. Candida–streptococcal interactions in biofilm-associated oral diseases. PLoS Pathog. 14(12):e1007342.
Kreth J, Hung DCI, 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 (Reading, Engl). 153(Pt 6): 1799–1807.
Krzyściak W, Jurczak A, Kościelniak D, Bystrowska B, Skalniak A. 2014. The virulence of *Streptococcus mutans* and the ability to form biofilms. Eur J Clin Microbiol Infect Dis. 33(4):499–515.
Lee SF, Delaney GD, Elkhateeb M. 2004. A two-component covRS regulatory system regulates expression of fructosyltransferase and a novel extracellular carbohydrate in *Streptococcus mutans*. Infect Immun. 72(7):3968–3973.
Lemme A, Sztajer H, Wagner-Döbler I. 2010. Characterization of mleR, a positive regulator of malolactic fermentation and part of the acid tolerance response in *Streptococcus mutans*. BMC Microbiol. 10(1):58.
Lemos JA, Burne RA. 2008. A model of efficiency: stress tolerance by *Streptococcus mutans*. Microbiology (Reading, Engl). 154(Pt 11):3247–3255.
Len AC, Cordwell SJ, Harty DW, Jacques NA. 2003. Cellular and extracellular proteome analysis of *Streptococcus mutans* grown in a chemostat. Proteomics. 3(5):627–646.
Lenander-Lumikari M, Loimaranta V. 2000. Saliva and dental caries. Adv Dent Res. 14(1):40–47.
Levesque CM, Mair RW, Perry JA, Lau PCY, Li YH, Cvitkovitch DG. 2007. Systemic inactivation and phenotypic characterization of two-component systems in expression of *Streptococcus mutans* virulence properties . Lett Appl Microbiol. 45(4):398–404.
Levine RS, Rowles SL. 1973. Further studies on the remineralization of human carious dentine in vitro. Arch Oral Biol. 18(11):1351–1356.
Li YH, Lau PC, Lee JH, Ellen RP, Cvitkovitch DG. 2001. Natural genetic transformation of *streptococcus mutans* growing in biofilms. J Bacteriol. 183(3):897–908.
Li YH, Tang N, Aspiras MB, Lau PC, Lee JH, Ellen RP, Cvitkovitch DG. 2002. A quorum-sensing signaling system essential for genetic competence in *Streptococcus mutans* is involved in biofilm formation. J Bacteriol. 184(10): 2699–2708.
Li YH, Tian XL, Layton G, Norgaard C, Sisson G. 2008. Additive attenuation of virulence and cariogenic potential of *Streptococcus mutans* by simultaneous inactivation of the ComCDE quorum-sensing system and HK/RR11 two-component regulatory system. Microbiology (Reading, Engl.). 154(Pt 11):3256–3265.
Li Z, Xiang Z, Zeng J, Li Y, Li J. 2018. A GntR family transcription factor in *Streptococcus mutans* regulates biofilm formation and expression of multiple sugar transporter genes. Front Microbiol. 9:3224.
Li Y-H, Lau PCY, Tang N, Svensäter G, Ellen RP, Cvitkovitch DG. 2002. Novel two-component regulatory system involved in biofilm formation and acid resistance in *Streptococcus mutans*. JB. 184(22):6333–6342.
Listl S, Galloway J, Mossey PA, Marcenes W. 2015. Global economic impact of dental diseases. J Dent Res. 94(10): 1355–1361.
Liu Y, Burne RA. 2009. Multiple two-component systems of *Streptococcus mutans* regulate agmatine deiminase gene expression and stress tolerance. J Bacteriol. 191(23): 7363–7366.

Liu S, Li H, Guo Z, Guan J, Sun Y, Zhang K. 2019. Insight into the effect of small RNA srn225147 on mutacin IV in *Streptococcus mutans*. Indian J Microbiol. 59(4):445–450.

Liu S, Tao Y, Yu L, Zhuang P, Zhi Q, Zhou Y, Lin H. 2016. Analysis of small RNAs in *Streptococcus mutans* under acid stress—a new insight for caries research. IJMS. 17(9):1529.

Loesche WJ. 1976. Chemotherapy of dental plaque infections. Oral Sci Rev. 9:65–107.

Magalhães PP, Paulino TP, Thedei G, Jr, Ciancaglini P. 2005. Kinetic characterization of P-type membrane ATPase from *Streptococcus mutans*. Comp Biochem Physiol B, Biochem Mol Biol. 140(4):589–597.

Mahajan A, Singh B, Kashyap D, Kumar A, Mahajan P. 2013. Interspecies communication and periodontal disease. ScientificWorldJournal. 2013:765434.

Marcenes W, Kassebaum NJ, Bernabé E, Flaxman A, Naghavi M, Lopez A, Murray CJ. 2013. Global burden of oral conditions in 1990-2010: a systematic analysis. J Dent Res. 92(7): 592–597.

Marquis RE. 1995. Oxygen metabolism, oxidative stress and acid-base physiology of dental plaque biofilms. J Ind Microbiol. 15(3):198–207.

Marsh PD. 1994. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res. 8(2): 263–271.

Marsh PD, Featherstone A, McKee AS, Hallsworth AS, Robinson C, Weatherell JA, Newman HN, Pitter AF. 1989. A microbiological study of early caries of approximal surfaces in schoolchildren. J Dent Res. 68(7):1151–1154.

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(3):589–606.

Matsumi Y, Fujita K, Takashima Y, Yanagida K, Morikawa Y, Matsumoto-Nakano M. 2015. Contribution of glucan-binding protein A to firm and stable biofilm formation by *Streptococcus mutans* . Mol Oral Microbiol. 30(3):217–226.

Matsumoto-Nakano M. 2018. Role of *Streptococcus mutans* surface proteins for biofilm formation. Jpn Dent Sci Rev. 54(1):22–29.

Mattos-Graner RO, Jin S, King WF, Chen T, Smith DJ, Duncan MJ. 2001. Cloning of the *Streptococcus mutans* gene encoding glucan binding protein B and analysis of genetic diversity and protein production in clinical isolates. Infect Immun. 69(11):6931–6941.

McLaughlin RE, Ferretti JJ, Hynes WL. 1999. Nucleotide sequence of the streptococcin A-FF22 lantibiotic regulon: model for production of the lantibiotic SA-FF22 by strains of *Streptococcus pyogenes*. FEMS Microbiol Lett. 175(2): 171–177.

Meiers JC, Wirthlin MR, Shklair IL. 1982. A microbiological analysis of human early carious and non-carious fissures. J Dent Res. 61(3):460–464.

Merritt J, Qi F. 2012. The mutacins of *Streptococcus mutans*: regulation and ecology. Mol Oral Microbiol. 27(2):57–69.

Mishra A, Pandey RK, Manickam N. 2015. The significance of gtf genes in caries expression: a rapid identification of *Streptococcus mutans* from dental plaque of child patients. J Indian Soc Pedod Prev Dent. 33(2):134–137.

Momeni SS, Beno SM, Baker JL, Edlund A, Ghazal T, Childers NK, Wu H. 2020. Caries-associated biosynthetic gene clusters in *Streptococcus mutans*. J Dent Res. 99(8):969–976.

Nath K, Thaiss CA. 2019. Digitalizing the microbiome for human health. Msystems. 4(3). DOI:10.1128/mSystems.00129-19.

Nilsson M, Jakobsen TH, Givskov M, Twetman S, Tolker-Nielsen T. 2019. Oxidative stress response plays a role in antibiotic tolerance of *Streptococcus mutans* biofilms. Microbiology. 165(3):334–342.

Nomura R, Matayoshi S, Otsugu M, Kitamura T, Teramoto N, Nakano K. 2020. Contribution of severe dental caries induced by *Streptococcus mutans* to the pathogenicity of infective endocarditis. Infect Immun. 88(7):5223–5234.

O'Toole GA, Kolter R. 1998. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Mol Microbiol. 28(3):449–461.

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(7):1844–1852.

Ooshima T, Matsumura M, Hoshino T, Kawabata S, Sobue S, Fujiwara T. 2001. Contributions of three glycosyltransferases to sucrose-dependent adherence of *Streptococcus mutans* . J Dent Res. 80(7):1672–1677.

Ouyang J, Tian XL, Versey J, Wishart A, Li YH. 2010. The BceABRS four-component system regulates the bacitracin-induced cell envelope stress response in *Streptococcus mutans*. Antimicrob Agents Chemother. 54(9):3895–3906.

Packer L, Witt EH, Tritschler HJ. 1995. Alpha-lipoic acid as a biological antioxidant. Free Radic Biol Med. 19(2):227–250.

Parashar A, Parashar S, Zingade A, Gupta S, Sanikop S. 2015. Interspecies communication in oral biofilm: an ocean of information. Int J Oral Sci. 12(2):37–42.

Pereira CS, Thompson JA, Xavier KB. 2013. AI-2-mediated signalling in bacteria. FEMS Microbiol Rev. 37(2):156–181.

Perry JA, Levesque CM, Suntharaligam P, Mair RW, Bu M, Cline RT, Peterson SN, Cvitkovitch DG. 2008. Involvement of *Streptococcus mutans* regulator RR11 in oxidative stress response during biofilm growth and in the development of genetic competence. Lett Appl Microbiol. 47(5): 439–444.

Petersen FC, Fimland G, Scheie AA. 2006. Purification and functional studies of a potent modified quorum-sensing peptide and a two-peptide bacteriocin in *Streptococcus mutans* . Mol Microbiol. 61(5):1322–1334.

Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, Tagami J, Twetman S, Tsakos G, Ismail A. 2017. Dental caries. Nat Rev. 3(1):1–6.

Qi F, Merritt J, Lux R, Shi W. 2004. Inactivation of the ciaH gene in *Streptococcus mutans* diminishes mutacin production and competence development, alters sucrose-dependent biofilm formation, and reduces stress tolerance. Infect Immun. 72(8):4895–4899.

Quivey RG, Jr Kuhnert WL, Hahn K. 2001. Genetics of acid adaptation in oral streptococci. Crit Rev Oral Biol Med. 12(4):301–314.

Rainey K, Michalek SM, Wen ZT, Wu H. 2018. Glycosyltransferase-mediated biofilm matrix dynamics and virulence of *Streptococcus mutans*. Appl Environ Microbiol. 85(5). DOI:10.1128/AEM.02247-18.

Rainey K, Wilson L, Barnes S, Wu H. 2019. Quantitative proteomics uncovers the interaction between a virulence

factor and mutanobactin synthetases in *Streptococcus mutans*. MSphere. 4(5):e00429–19.
Reck M, Tomasch J, Wagner-Döbler I. 2015. The alternative sigma factor SigX controls bacteriocin synthesis and competence, the two quorum sensing regulated traits in *Streptococcus mutans*. PLoS Genet. 11(7):e1005353.
Rickard AH, Campagna SR, Kolenbrander PE. 2008. Autoinducer-2 is produced in saliva-fed flow conditions relevant to natural oral biofilms . J Appl Microbiol. 105(6): 2096–2103.
Rickard AH, Palmer RJ, Jr Blehert DS, Campagna SR, Semmelhack MF, Egland PG, Bassler BL, Kolenbrander PE. 2006. Autoinducer 2: a concentration-dependent signal for mutualistic bacterial biofilm growth. Mol Microbiol. 60(6): 1446–1456.
Righolt AJ, Jevdjevic M, Marcenes W, Listl S. 2018. Global-, regional-, and country-level economic impacts of dental diseases in 2015. J Dent Res. 97(5):501–507.
Rioux KM, Santiago-Narvaez B. 2019. The role of CodY in hydrogen peroxide tolerance and biofilm formation of *Streptococcus mutans*. Honors Program Theses. 78. https://scholarship.rollins.edu/honors/78.
Rosier BT, De Jager M, Zaura E, Krom BP. 2014. Historical and contemporary hypotheses on the development of oral diseases: are we there yet? Front Cell Infect Microbiol. 4:92.
Russell RB. 1979. Wall-associated protein antigens of *Streptococcus mutans*. Microbiology. 114(1):109–115.
Russell MW, Lehner T. 1978. Characterisation of antigens extracted from cells and culture fluids of *Streptococcus mutans* serotype c. Arch Oral Biol. 23(1):7–15.
Santiago B, MacGilvray M, Faustoferri RC, Quivey RG. 2012. The branched-chain amino acid aminotransferase encoded by ilvE is involved in acid tolerance in *Streptococcus mutans*. J Bacteriol. 194(8):2010–2019.
Sato Y, Yamamoto Y, Kizaki H. 1997. Cloning and sequence analysis of the gbpC gene encoding a novel glucan-binding protein of *Streptococcus mutans*. Infect Immun. 65(2): 668–675.
Senadheera MD, Guggenheim B, Spatafora GA, Huang YC, Choi J, Hung DC, Treglown JS, Goodman SD, Ellen RP, Cvitkovitch DG. 2005. A VicRK signal transduction system in *Streptococcus mutans* affects gtfBCD, gbpB, and ftf expression, biofilm formation, and genetic competence development. JB. 187(12):4064–4076.
Shah DS, Russell RR. 2004. A novel glucan-binding protein with lipase activity from the oral pathogen *Streptococcus mutans*. Microbiology (Reading, Engl). 150(Pt 6): 1947–1956.
Shanker E, Federle MJ. 2017. Quorum sensing regulation of competence and bacteriocins in *Streptococcus pneumoniae* and *mutans*. Genes. 8(1):15.
Sheng J, Marquis RE. 2007. Malolactic fermentation by *Streptococcus mutans*. FEMS Microbiol Lett. 272(2):196–201.
Shields RC, O'Brien G, Maricic N, Kesterson A, Grace M, Hagen SJ, Burne RA. 2017. Genome-wide screens reveal new gene products that influence genetic competence in *Streptococcus mutans*. J Bacteriol. 200(2). DOI:10.1128/JB.00508-17.
Son M, Ahn SJ, Guo Q, Burne RA, Hagen SJ. 2012. Microfluidic study of competence regulation in *Streptococcus mutans*: environmental inputs modulate bimodal and unimodal expression of comX. Mol Microbiol. 86(2):258–272.
Son M, Shields RC, Ahn SJ, Burne RA, Hagen SJ. 2015. Bidirectional signaling in the competence regulatory pathway of *Streptococcus mutans*. FEMS Microbiol Lett. 362(19): fnv159.
Song L, Sudhakar P, Wang W, Conrads G, Brock A, Sun J, Wagner-Döbler I, Zeng AP. 2012. A genome-wide study of two-component signal transduction systems in eight newly sequenced mutans streptococci strains. BMC Genomics. 13(1):128.
Stookey GK. 2008. The effect of saliva on dental caries. J Am Dent Assoc. 139:11S–17S.
Suntharalingam P, Senadheera MD, Mair RW, Lévesque CM, Cvitkovitch DG. 2009. The LiaFSR system regulates the cell envelope stress response in *Streptococcus mutans*. J Bacteriol. 191(9):2973–2984.
Sztajer H, Lemme A, Vilchez R, Schulz S, Geffers R, Yip CY, Levesque CM, Cvitkovitch DG, Wagner-Döbler I. 2008. Autoinducer-2-regulated genes in *Streptococcus mutans* UA159 and global metabolic effect of the luxS mutation. J Bacteriol. 190(1):401–415.
Takahashi S, Abbe K, Yamada T. 1982. Purification of pyruvate formate-lyase from *Streptococcus mutans* and its regulatory properties. J Bacteriol. 149(3):1034–1040.
Tamura H, Yamada A, Kato H. 2012. Characterization of *Streptococcus criceti* insertion sequence ISScr1. Genes Genet Syst. 87(3):153–160.
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 (Reading, Engl). 155(Pt 9): 2856–2865.
Tsuda H, Yamashita Y, Shibata Y, Nakano Y, Koga T. 2002. Genes involved in bacitracin resistance in *Streptococcus mutans*. Antimicrob Agents Chemother. 46(12):3756–3764.
Underhill SA, Shields RC, Kaspar JR, Haider M, Burne RA, Hagen SJ. 2018. Intracellular signaling by the comRS system in *Streptococcus mutans* genetic competence. MSphere. 3(5). DOI:10.1128/mSphere.00444-18.
van der Ploeg JR. 2005. Regulation of bacteriocin production in *Streptococcus mutans* by the quorum-sensing system required for development of genetic competence. J Bacteriol. 187(12):3980–3989.
Vendeville A, Winzer K, Heurlier K, Tang CM, Hardie KR. 2005. Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria. Nat Rev Microbiol. 3(5): 383–396.
Wang BY, Kuramitsu HK. 2005. Interactions between oral bacteria: inhibition of *Streptococcus mutans* bacteriocin production by *Streptococcus gordonii*. Appl Environ Microbiol. 71(1):354–362.
Wen ZT, Burne RA. 2004. LuxS-mediated signaling in *Streptococcus mutans* is involved in regulation of acid and oxidative stress tolerance and biofilm formation. J Bacteriol. 186(9):2682–2691.
Winzer K, Hardie KR, Williams P. 2003. LuxS and autoinducer-2: their contribution to quorum sensing and metabolism in bacteria. Adv Appl Microbiol. 53:291–396.

Xavier KB, Bassler BL. 2005. Interference with AI-2-mediated bacterial cell-cell communication. Nature. 437(7059): 750–753.

Xia L, Xia W, Li S, Li W, Liu J, Ding H, Li J, Li H, Chen Y, Su X, et al. 2012. Identification and expression of small non-coding RNA, L10-Leader, in different growth phases of *Streptococcus mutans*. Nucleic Acid Ther. 22(3):177–186.

Yamada TA, Carlsson J. 1975. Regulation of lactate dehydrogenase and change of fermentation products in streptococci. J Bacteriol. 124(1):55–61.

Yamamoto Y, Sato Y, Takahashi-Abbe S, Takahashi N, Kizaki H. 2000. Characterization of the *Streptococcus mutans* pyruvate formate-lyase (PFL)-activating enzyme gene by complementary reconstitution of the in vitro PFL-reactivating system. Infect Immun. 68(8):4773–4777.

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(2):e17335.

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(1):187–200.

Zhang J, Biswas I. 2009. A phenotypic microarray analysis of a *Streptococcus mutans* liaS mutant. Microbiology (Reading, Engl.). 155(Pt 1):61–68.

Zhu W, Liu S, Zhuang P, Liu J, Wang Y, Lin H. 2017. Characterization of acid-tolerance-associated small RNAs in clinical isolates of *Streptococcus mutans*: potential biomarkers for caries prevention. Mol Med Rep. 16(6): 9242–9250.