Plausible Drug Targets in the *Streptococcus mutans* Quorum Sensing Pathways to Combat Dental Biofilms and Associated Risks

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Abstract *Streptococcus mutans*, a Gram positive facultative anaerobe, is one among the approximately seven hundred bacterial species to exist in human buccal cavity and cause dental caries. Quorum sensing (QS) is a cell-density dependent communication process that respond to the inter/intra-species signals and elicit responses to show behavioral changes in the bacteria to an aggressive forms. In accordance to this phenomenon, the *S. mutans* also harbors a Competing Stimulating Peptide (CSP)-mediated quorum sensing, ComCDE (Two-component regulatory system) to regulate several virulence-associated traits that includes the formation of the oral biofilm (dental plaque), genetic competence and acidogenicity. The QS-mediated response of *S. mutans* adherence on tooth surface (dental plaque) imparts antibiotic resistance to the bacterium and further progresses to lead a chronic state, known as periodontitis. In recent years, the oral streptococci, *S. mutans* are not only recognized for its cariogenic potential but also well known to worsen the infective endocarditis due to its inherent ability to colonize and form biofilm on heart valves. The review significantly appreciate the increasing complexity of the CSP-mediated quorum-sensing pathway with a special emphasis to identify the plausible drug targets within the system for the development of anti-quorum drugs to control biofilm formation and associated risks.

Keywords *Streptococcus mutans* · Dental biofilms · Quorum Sensing · CSP · ComA · Multi-drug resistance

Quorum Sensing System: Deciphering ‘Dental Talk’

Streptococcus mutans, a Gram positive coccus, is an inhabitant of human buccal cavity and is also a primary causal organism of dental caries. The tooth surface act as an essential ecological niche for *S. mutans* as it is very difficult to be identified until the teeth is erupted in the buccal cavity and dissipate immediately after the teeth is lost due to infection or old age [1–3]. *S. mutans* has advanced to rely upon a biofilm “way of life” to survive and persist for longer durations in its characteristic biological community [4–6], dental biofilm, commonly known as plaque. *S. mutans* with its own or other populations tend to form oral biofilms (dental plaque) via., inter/intra-species communication known as quorum sensing system (QS) [6–8].

QS Pathways in *S. mutans*

ComCDE QS Pathway

QS in gram-positive bacteria like *S. mutans*, generally comprise three components: a signal peptide (CSP), a two-component regulatory system (TCTS) with a membrane-bound histidine kinase (HK) sensor and an intracellular response regulator (RR) [9]. Recently, it has been identified that the CSP-mediated QS system in *S. mutans* up-regulates the genes, *csfAB* (*comAB*) and *comCDE* that exhibits the phenotypic traits like genetic competence, bacteriocin production and biofilm [10, 11]. The genes *comC*, *comD*,

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and *comE* encodes a precursor of competence-stimulating peptide (CSP), the HK sensor protein, and a cognate RR, respectively [10, 12, 13]. The genes *comC* and *comDE* are closely located on the same chromosome and the peptide (CSP) is synthesized as a collective result of their gene products [14, 15]. The genes, *csIA* and *csIB*, are divergently mapped on the same chromosome also encodes a CSP-specific maturation and secretion complex factors with an ATP-binding cassette (ABC) transporter (ComA) and an accessory factor (ComB). As these factors, specifically involved in the post-translational processing of the CSP to further secrete it towards the outside of the cell, as a mature signaling peptide [16]. The QS signalling operates optimally when the cells acquire and depend solely and actively on the biofilm lifestyle as the CSP concentration reaches a threshold value [17]. Mature CSP binds to the conserved HK residue (ComD) present in the membrane, resulting in its own phosphorylation and the subsequent relay of this process to its cognate RR protein (ComE) [18]. Thus the cell-density dependent process elicits a cellular response to activate the gene loci such as *comA* and *comB* with a simultaneous feed forward circuit for *comCDE*. Also, the same response was extended to the genes, *comR* and *sigX* expression, for which the mechanism is still unknown [2].

ComRS Pathway

Interestingly, *S. mutans* possess ComCDE as well as ComRS quorum sensing pathways. The ComRS QS system is activated on sensing the extracellular tryptophan signal peptide pheromone, XIP and get internalized to the cells through a membrane-bound oligopeptide ABC transport system, Opp/Ami [2]. Further, XIP binds to a transcriptional regulator, ComR, in turn regulates the *sigX*, an alternative sigma factor SigX (ComX), to switch on the late competence genes responsible for the genetic transformation. The ComCDE operon regulates the production of antimicrobial peptides, toxins and adherence factors, whereas the functional characterization of the ComRS quorum-sensing circuit show a pre-dominant role in the genetic competence via., the regulator, SigX [19, 20].

LuxS Pathway

Previously, it has been reported that *S. mutans* interact with the other oral flora of the dental plaque to mediate interspecies communication. LuxS is reported to involve in S-adenosylmethionine catabolism and converts ribose homocysteine into homocysteine and 4,5-dihydroxy-2,3-pentanedione that act as a precursor of Autoinducer-2 (AI-2) [21–23]. The LuxS-mediated QS are well characterised to elicit interspecies communication and modulate multiple

traits crucial to establish *S. mutans* pathogenesis. So, the *S. mutans* flourish in the buccal cavity via., activation of the *luxS* gene that leads to the production of AI-2 which ensures its survival and virulence expression in multispecies environment. The researchers have shown that the *luxS*-deficient strains affect the expression of the virulence determinant to a greater extent (>50 %) and in parallel upregulate their acid-adaptive behaviours to increase their survival rate [24]. Also, the *luxS* gene is highly conserved among the Gram positive and Gram negative bacteria and may operate as a global regulator to be an essential factor for a drug target [22, 23].

S. mutans and Dental Caries

Clarke, in 1924, designated *S. mutans*, after he could isolate a bacterial species from the carious lesions and looked like a mutant form of a coccus. The relationship of *S. mutans* with dental caries was not largely perceived until dental practitioners and researchers in the 1960s revived interest in this organism. Since then, various studies confirmed the relationship of *S. mutans* with dental decay and carious lesions and longitudinal studies followed the predominance of *S. mutans* on infected sites that ultimately became cariogenic. An experimental study on the mono-infected rats revealed the cariogenic potential of the various plaque species and notably, *S. mutans* was found to be predominant among cariogens. The research has led to the “specific plaque hypothesis” and stated that the *S. mutans*, were primarily responsible for the dental decay. With the taxonomical advances, it has become apparent that the *S. mutans*-like isolates actually accounts for several species and was collectively known as the mutans streptococci (MS).

Like many other diseases, dental caries, also have multiple etiologies. However, the topmost variable for caries occurrence has been sucrose rich diet. Frequent consumption of sucrose has also been implicated in building plaque ecological niche and provide a substrate for MS to synthesize adhesive glucans to promote their own colonization and accumulation on the tooth surface [25]. The acid tolerance capability of the MS further enhances their adhesion and growth as well as the growth of other acid-tolerant species such as the lactobacilli. Due to highly acidogenic nature of these species [26], the fermentable dietary carbohydrate results in a huge drop in plaque pH than normal paving way for demineralization and decalcification of the tooth surface. The scenario also promoted researchers worldwide to focus on the adhesion, acidogenicity, and acid-tolerant properties of the MS [27].

The focus of this review is to primarily describe the quorum sensing-mediated cell–cell communication in *S. mutans* and its various components. Since most of the

virulence properties (Table 1) are shared among the various MS, the review will address on the *S. mutans* as a paradigm for the virulence of dental caries under the regulation of QS. Also the review discusses the structural and functional aspects of various quorum molecules that would provide an insight to exploit them as drug targets. The application potential of this review would also provoke the scientific community towards target based drug discovery in synthetic biology to effectively control the bacterial biofilms and its associated risks.

Plausible Drug Targets: Hindering ‘Bacterial Chat’

Signals

CSP

In *S. mutans* *comC* encodes a precursor signal peptide, a 21-amino acid Competence Stimulating Peptide (CSP) processed and exported out of the cell to elicit its response via., the ComCDE QS pathway. The formation of biofilm and virulence traits in *S. mutans* such as acidogenicity, bacteriocin production, genetic competence solely depends on the signal, CSP-mediated QS [22]. *S. mutans* has evolved various mechanisms to allow the survival and persistence of the species in a broad range of adapting to frequent environmental pressure such as acidic environment that act as a constant stress in the oral cavity to enhance the production of CSP [28]. The stress-inducible activation of CSP induces the cell death of a sub-population accounting for the altruistic nature of bacteria to be beneficial for its own population. Similarly, the native CSP of *Streptococcus pneumonia* was known to cause chronic diseases in humans such as pneumococcal meningitis and pneumonia [13]. Earlier reports shows that the *S. mutans*

JH1005 (deletion of three amino acid residues in the C-terminal domain of the peptide, CSP) has overexpressed bacteriocin and resulted in 600-fold reduction in the competence and drive a greater significance of those residues in QS signalling pathway [29]. The CSP has been reported to exist as a factor to be structurally random coiled and highly soluble nature to increase its diffusion rate in the aqueous environment [10, 30]. The randomly coiled CSP undergoes conformational change and folding upon binding to the membrane bound Histidine Kinase (HK) receptor resulting in the formation of an amphipathic α -helix with a hydrophobic face. The change in the conformation enables the CSP to show a stronger interface of its interaction with the HK receptor binding pocket via., hydrophobic interactions [29, 31]. Till date, approximately 700 species has been reported in the buccal cavity and a few cariogenic pathogens, including *S. mutans* are well characterized to mediate dental caries. So as to reduce or completely eliminate dental caries, alternate strategies has to be prioritized as a one-step forward to prevent the *S. mutans* adherence on the tooth surface without affecting the normal oral flora. In recent years, antimicrobial peptides (AMPs) have received attention of the researchers worldwide, as a novel class of antimicrobial agents because of their ability to kill a wide range of pathogenic species, including bacteria, fungi, and viruses, through various mechanism of action [9]. The AMPs are also reported to be effective against resistant pathogens with their ability to disrupt the functions of the cellular membranes and nucleic acids directly and moreover the rate to which AMP-resistant strains appears, is very low or almost negligible [32].

Similarly, a new class of pathogen-specific antimicrobials, STAMPs (selectively targeted antimicrobial peptides) were discovered against the dental pathogen, *S. mutans*. The peptides were designed to have a targeted domain (8-aminoacid region of the CSP) fused with an antimicrobial peptide domain to show a robust species

Table 1 QS controlled genes and their phenotypic traits in *S. mutans*

QS components	Regulatory gene	Group-derived benefits (Virulence factors)	References
CSP (ComC)	<i>comC/comD/comE</i>	Bacteriocins, Biofilm Formation	[10, 11, 14, 55]
XIP (ComS)	<i>comR</i>	Competence	[10, 11]
SigX (ComX)	<i>comR</i>	DNA transport, uptake and Recombination	[12, 36]
ComAB	<i>nlmAB (comAB)</i>	Mutacin IV (bacteriocins), Maturation and export of CSP outside the cell	[16, 49]
ComD	<i>comC/comD/comE</i> (TCSTS)	Class II bacteriocins and HK receptor for CSP signal	[14, 22]
immA and immB (VicRK)	<i>comC</i>	Self- immunity proteins for protection from bacteriocins, acid tolerance	[55]
Lux S	<i>luxS</i>	Interspecies communication	[39, 40]

specific activity and eliminate the *S. mutans* from multi-species biofilm without affecting the normal (non-cariogenic) streptococci [33]. Syvitski et al. [29] reported that the C-terminal truncated CSP peptides could competitively affect the QS activity and the structural motif in the C-terminal domain restores the activation of the QS signal transduction pathway [34, 35]. Further the mutational studies (Point deletion) revealed the loss of function of CSP in promoting genetic competence without affecting the binding of peptide to the receptor [34–37].

XIP

XIP (Sig X Inducing Peptide) modulates the expression of σ^x (ComX) via., ComRS signaling pathway. The *comS* encodes the XIP precursor is processed to mature and internalize the cells through the transporter, Opp/Ami. The mature signal in-turn interacts with the *comR* and activate the gene *comX* to express the factor, σ^x [36, 37]. Recent data has shown a drastic reduction of XIP levels in the *comX* (σ^x) deficient *S. mutans* strains, as it evidence the positive feed-back role of ComX in ComRS/XIP expression where its binding sites were localized either within or upstream of ComRS [38]. Earlier reports have also demonstrated that the increase in the concentration (2–4 μ M) of CSP mediated cell-killing and growth arrest of *S. mutans* [39, 40]. Likewise, the *S. mutans* on exposure to 10 μ M of synthetic XIP (sXIP) has shown the killing effect, naturally score the phenomena of exploiting the pheromones (XIP) as a potent effector molecules to treat dental caries [41].

Autoinducer-2 (AI-2)

The previous studies had established that in dental plaque, the oral streptococci and other oral bacterial species interact with each other and form an ideal system to mediate interspecies signalling and biofilm formation in a particular niche [42]. The AI-2 mediated interspecies QS response is a well-characterized cross-talk strategy, a known co-operative synergy exists in both gram-positive and gram-negative pathogens [43]. As it is evidenced from the fact that they all have a highly conserved *luxS* gene encoding autoinducer-2 (AI-2) serve the function of optimization of the virulence gene expression in a cell density dependent manner in a particular biological niche. A study carried out by Merritt et al. [44], Yoshida et al. [42] demonstrated that the mutation in the *luxS* gene of *S. mutans* leads to an altered biofilm structure and also affected the production of the bacteriocin and mutacin I. In *Escherichia coli* and *Serratia marcescens* the mutacin production was *lexA* and *recA* dependent whereas in *S. mutans*, it was solely dependent on a Lex-A like factor, IrvR [45]. Rickard et al.

[43] and McNab et al. [46] have also shown the involvement of AI-2 multispecies biofilm communities and thus, an approach leading to the suppression of *luxS* gene expression could lead an altered interspecies behavior in the oral microbial community leading to hindrance in the QS system.

Receptor and Response Regulator

ComDE

The two signal transduction system, ComDE are proven to elicit CSP-mediated responses to the neighbouring cells via., a phospho-relay mechanism and activate its target genes, *comCDE*, *comAB* and *comX* to establish biofilm, stress response and bacteriocin production. These virulent traits show an anti-bacterial effect to the other commensal species, so that their DNA released in the growth surroundings, would provide an ecological advantage to *S. mutans* to compete the natural ecosystem i.e. multi-species dental biofilms in oral cavity. Recent studies had revealed the bifunctional nature of the ComED system showing a dual response as an activator of *nlmC* and repressor of *comC*. The researchers also reveal the puzzle behind the dual opposing characteristics of ComED as *nlmC* share an intergenic region (IGSA499) with the divergently transcribed *comC* [47, 48]. Further, experiments show that the *nlmC* expression in *comD* mutant show >40-fold fold and >fourfold reduction in comparison with the wild-type under CSP-induced or un-induced state respectively [49]. Also, the ComE share higher sequence homology to the DNA binding domains of AlgR, AlgA, LytR family members and provide an insight to be exploited for developing broad-spectrum anti-biofilm compounds. Li et al. suggest that those compound would also block the quorum sensing dependent virulence properties and reduce the cariogenic properties of *S. mutans*, regardless of the fact that the organism is still active in oral biofilms [50, 51].

ABC Transporters

ComA

The gene *comAB* encodes ComA, to act in the initial step of the QS pathway of Streptococcus, consists of bacteriocin-associated ATP-binding cassette (ABC) transporters, that employs ComB as an accessory protein for specific processing and maturation of the precursor (AMS family of ABC transporters involved in maturation and secretion of molecules) CSP [16]. The ComA has three domains (1) N-terminal Peptidase domain comprising of 150-amino

acid (2) a six membrane-spanning segments of transmembrane domain (3) an ATP-binding C-terminal motif localized on the cytoplasmic face of the membrane. The functional aspects of the peptidase domain was understood specifically to cleave their cognate propeptides after the consensus Gly–Gly motif [52].

The ATP-binding cassette (ABC) transporters are the largest well characterized protein super-family from various organisms to participate in a wide array of physiological functions, that includes virulence, antigen presentation in pathogenic bacteria, multi-drug resistance and regulation of ion channels across the bacterial membranes [53]. The core structural organization of the ABC transporter consists of a two transmembrane domains (TMD) and two cytosolic nucleotide-binding domains (NBD) [53]. The sequence variation in the several membrane-spanning α -helices that form the transport pathway for TMDs reflects its complement sequence variation in their ligands for the ABC transporters and the highly conserved NBDs provide energy on ATP hydrolysis for the substrate transport [54].

In ABC exporters, a linker region with a 20 amino acids adjoins the last α -helix of TMD and the first β -strand of NBD. The highly conserved glycine residue is located near the C-terminal end of the linker and 11 amino-acid residues proceeds the adenine stacking the tyrosine residue of the NBDs [53, 55, 56]. To date, the molecular characterization of the three-dimensional structures of the four full-length ABC exporters are known to be Sav1866 from *Staphylococcus aureus*, MsbA from *Salmonella typhimurium*, and *Thermotoga maritima* TM287/288 [5, 54–57]. Whereas, the protein sequence alignment of ComA with the ABC exporters evidence that the residue Gly526 occupy a significant position in the ABC exporters [53, 58, 59].

Since, ABC transporters have been reported to play a vital role in the maturation and secretion of CSP, anti-quorum compounds targeting the vital glycine residue and blocking the ABC transporter (ComA) in *S. mutans* may pave a way to novel drug design [52]. Moreover, the family of the bacteriocin-associated ABC transporters has so far been found only in prokaryotes, the PEP domains of ComAs would be an ideal target for the development of drugs that inhibit the biofilm formation of *Streptococcus* [52] (Fig. 1). Mutations at the active site of PEP domain has resulted in the complete loss of the catalytic activity of PEP domain [16]. Based on the observation and substrate specificity of peptidase domain of ComA, we could hypothesize that the therapeutic inhibition of PEP domain of ComA will halt the maturation and secretion of CSP from *S. mutans* and thus hindering the virulence expression associated with a quorum sensing circuit of *S. mutans*.

ComX

ComX, an alternative sigma factor (σ^x) activates the late competence genes primarily involved in the processing and recombination of the foreign DNA to the chromosome of *Streptococci* [12]. In *S. pneumoniae*, the up-regulation of σ^x is induced in response to the activation of the competence cascade via., CSP-mediated ComCDE pathway. Upon activation with the CSP, the membrane-bound HK receptor integrates the signal to its cognate cytoplasmic response regulator (ComD) to further activate the alternate sigma factor, SigX (ComX) [60]. Also, its recognizing sites are well characterized to be a set of non-canonical consensus region (CIN box) that lie in the promoter regions (TACGAATA) of various ComX dependent genes, *ssbB*, *dalA*, *ccl*, *celAB*, *cAAB*, and *cinA-recA* [12, 61]. Similarly, in *S. mutans*, it is postulated that the CSP-mediated activation of σ^x , an alternate sigma factor up-regulates the expression of several competence genes that includes *comX*, *comCDE* (positive feedback loop) and other early com genes, *comW* (σ^x Stabilizing factor). ComX (σ^x) predominates to induce CSP-dependent com regulon expression and a sole key factor to resist stress responses in gram-positive bacteria, could probably be chosen as an ideal target for disarming the pathogen against its evolving trend to resist antibacterial agents.

Conclusion

Streptococcus mutans mediated dental caries is a multifactorial disease are being ignored due to its non-life threatening nature [62, 63]. An unknown fact exists as the inflammation process takes its pace slowly and gradually, it would act as a ‘silent killer’ for the patients suffering from dental caries. Therefore the untreated carious dentine can lead to the development of systemic diseases such as infective endocarditis [61–65]. Meta-analysis reports between 2003 and 2009 has shown a weak but statistically significant correlation between cardiovascular disease and dental diseases [66–68]. Based on the reports of meta-analysis, a conclusion can be drawn, that an individual with severe dental infections is at higher risk of either having or developing cardiovascular disease and rheumatoid arthritis [25, 69]. Since the association of *S. mutans* with dental caries was established, research was focused on recognizing its virulence properties along with their role in plaque formation and the progress of dental diseases. The review has highlighted the importance of QS pathway in *S. mutans* and its role in formation of biofilm along with other virulence properties. Quite a few efforts has been taken to modulate QS to reduce the production of biofilm and associated virulence factors like Hentzer and Givskov,

Fig. 1 Schematic representation of quorum sensing circuits of *S. mutans* and blocking the communication with inhibitors. Ⓢ Inhibition, ↑ Activation, ----→ Unknown mechanism

Ravichandran et al., Arya et al., Hema et al., and Wright et al., have described the application of antagonists that act as QS inhibitors to attain the inhibition of virulence genes under QS circuit to prevent the various infections caused by *Pseudomonas aeruginosa*, *Vibrio cholerae* and *Staphylococcus aureus* [28, 33, 70–76]. The approach ensures its success in the fact that, anti-quorum compounds may control virulence traits of pathogenic microbes without significant effects on viability of bacterial cells [77]. Biofilm cells have been shown to be several fold more tolerant to antibiotics than planktonic cells, and this makes it hard to treat *S. mutans* with modern medicine [78, 79]. Thus, the importance of developing an anti-quorum compounds as alternate therapy for multi-drug resistant bacteria, has positive future perspectives with respect to medicine.

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