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Computational tools for exploring peptide-membrane interactions in gram-positive bacteria

Shreya Kumar [a,b,1], Rex Devasahayam Arokia Balaya [a,*,1], Saptami Kanekar [a], Rajesh Raju [a,b], Thottethodi Subrahmanya Keshava Prasad [b], Richard K. Kandasamy [c,d,**]

[a] Centre for Integrative Omics Data Science, Yenepoya (Deemed to be University), Mangalore 575018, India
[b] Centre for Systems Biology and Molecular Medicine, Yenepoya Research Centre, Yenepoya (Deemed to be University), Mangalore 575018, India
[c] Centre of Molecular Inflammation Research (CEMIR), and Department of Clinical and Molecular Medicine (IKOM), Norwegian University of Science and Technology, 7491 Trondheim, Norway
[d] Department of Laboratory Medicine and Pathology, Center for Individualized Medicine, Mayo Clinic, Rochester, MN 55905, USA

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ABSTRACT

The vital cellular functions in Gram-positive bacteria are controlled by signaling molecules known as quorum sensing peptides (QSPs), considered promising therapeutic interventions for bacterial infections. In the bacterial system QSPs bind to membrane-coupled receptors, which then auto-phosphorylate and activate intracellular response regulators. These response regulators induce target gene expression in bacteria. One of the most reliable trends in drug discovery research for virulence-associated molecular targets is the use of peptide drugs or new functionalities. In this perspective, computational methods act as auxiliary aids

Abbreviations: QS, Quorum Sensing; MRSA, Methicillin Resistant *S. aureus*; WHO, World Health Organization; PSM, Phenol-Soluble Modulin; AIP, Autoinducing Peptide; QSP, QS Peptides; Agr, Accessory gene regulator; QSI, QS Inhibitors; ABC, ATP-binding cassette; TCS, Two-Component Sensory; H, Histidine; H-Kinase, Histidine Kinase; D, Aspartate; SAR, Structure-Activity Relationship; PTM, Post Translational Modification; CSP, Competence Stimulating Peptide; ATP, Adenosine Triphosphate; H-phosphotransferase, Histidine Phosphotransferase; HNP, Human Neutrophil Peptide; PCP, Physicochemical Properties; QSPR, Quantitative Structure Property Relationship; QSHGM, Quorum Sensing of Human Gut Microbes; HGM, Human Gut Microbiota; QSCN, QS communication network; BLAST, Basic Local Alignment Search Tool; SVM, Support Vector Machine; ML, Machine Learning; MCC, Mathew Co-relation Coefficient; ROC, Receiver Operating Characteristic; AAC, Amino Acid Composition; CTD, Composition-Transition-Distribution; GDC, g-gap Dipeptide; OVP, Overlapping Property Features; IT, Information Theory Features; mRMR, minimum Redundancy and Maximum Relevance; SFS, Sequential Forward Search; RF, Random Forest; GBM, Gradient Boosting Machine; DT, Decision Tree; KNN, K-Nearest Neighbors; BNB, Bernoulli Naïve-Bayes; GNB, Gaussian NB; QSIM, QS Interference Molecules; BIP, Biofilm Inhibitory Peptides; AMP, Anti-Microbial Peptide; CADD, Computer-Aided Drug Design; MD, Molecular Dynamics; BFE, Binding Free Energy; PPIs, Protein-Protein Interactions; MSL, Multiple Sequence Alignment; FDA, Food and Drug Administration; SIT, Sitagliptin; TRG, Trelagliptin; OMR, Omargliptin; 3-HBA, 3–Hydroxybenzoic Acid; HAM, Hamamelitannin; RAP, RNAIII-activating protein; TRAP, Target of RAP; RIP, RNAIII-inhibiting peptide; ACD, Available Chemicals Database; PDB, Protein Data Bank; DCH, 3,3′-(3,4-dichlorobenzylidene)-bis-(4-hydroxycoumarin); MDR, Multiple Drug Resistance

* Corresponding author.
** Corresponding author at: Centre of Molecular Inflammation Research (CEMIR), and Department of Clinical and Molecular Medicine (IKOM), Norwegian University of Science and Technology, 7491 Trondheim, Norway.
E-mail addresses: rexprem@yenepoya.edu.in (R.D.A. Balaya), Kandasamy.RichardKumaran@mayo.edu (R.K. Kandasamy).
[1] Equal contribution

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for biologists, where methodologies based on machine learning and *in silico* analysis are developed as suitable tools for target peptide identification. Therefore, the development of quick and reliable computational resources to identify or predict these QSPs along with their receptors and inhibitors is receiving considerable attention. The databases such as Quorumpeps and Quorum Sensing of Human Gut Microbes (QSHGM) provide a detailed overview of the structures and functions of QSPs. The tools and algorithms such as QSPpred, QSPred-FL, iQSP, EnsembleQS and PEPred-Suite have been used for the generic prediction of QSPs and feature representation. The availability of compiled key resources for utilizing peptide features based on amino acid composition, positional preferences, and motifs as well as structural and physicochemical properties, including biofilm inhibitory peptides, can aid in elucidating the QSP and membrane receptor interactions in infectious Gram-positive pathogens. Herein, we present a comprehensive survey of diverse computational approaches that are suitable for detecting QSPs and QS interference molecules. This review highlights the utility of these methods for developing potential biomarkers against infectious Gram-positive pathogens.

Contents

1. Introduction

In the microbial community, bacterial cell-to-cell communication plays a vital role in coordinating population density dependent behaviors that support antibiotic resistance, infectivity, and symbiotic interactions. This occurs through sensing extracellular peptide signals, which are biosynthesized through divergent signaling pathways that possess significant functional chemistry due to post-translational modifications (PTMs). This signaling mechanism is referred to as quorum sensing (QS). Gram-positive bacterial species including *Bacillus subtilis, Streptococcus pyogenes, Bacillus anthracis, Bacillus cereus, Bacillus mycoides, Bacillus pseudomycoides, Bacillus thuringiensis, and Bacillus wiehenstephanensis* use peptide-based QS signaling to control critical bacterial functions such as virulence, persistence, conjugation, and competence [1,2]. One of these Gram-positive bacteria, *Staphylococcus aureus*, is primarily and extensively present in acute and chronic infections in humans and animals, and QS has a significant impact on its behavior [3,4].

Moreover, *S. aureus* ranks first in the world as the causative agent of nosocomial infections [4]. According to the World Health

Organization (WHO) reports from the 2017 global "PRIORITY PATHOGENS" list for antibiotic-resistant bacteria, the methicillin-resistant *S. aureus* (MRSA) is categorized as a human pathogen of major clinical concern and is categorized under PRIORITY 2: HIGH group (WHO priority pathogens list for R&D of new antibiotics, 2017) [5]. The *S. aureus* causes persistent biofilm infections that are associated with medical implants and devices such as catheters. The wide variety of infections associated with *S. aureus* includes osteomyelitis, endocarditis, toxic shock syndrome, as well as skin and soft tissue infections [6,7].

The complexity of *S. aureus* infections and the mechanisms utilized by the pathogen to overcome the host immune response are due to the numerous multi-functional virulence factors produced by the pathogen during the course of the infection. These virulence factors consist of exotoxins, exoenzymes, adhesion molecules/structures, superantigens, leucocidins and phenol-soluble modulins (PSMs) [8]. Due to their ability to regulate a wide variety of virulence factors, *S. aureus* is considered a significant pathogen in both acute and chronic human infections. It is capable of infecting various parts of the body and securing a niche in affected tissues [9]. The QS system is an important regulator of virulence factors associated with *S. aureus* [5]. It acts as a regulator of cell-cell communication through signaling molecules called autoinducing peptides (AIPs). In *S. aureus,* AIPs are called quorum sensing peptides (QSPs). The QSPs use the surface-located receptors of accessory gene regulatory (Agr) QS system for regulation of virulence phenotypes in *S. aureus* [10].

The screening and identification of QSPs in bacteria is time consuming and expensive. Therefore, the development of computational models for effective, rapid and high-throughput QSP identification with high prediction accuracy has potential clinical applications and drug development implications [11]. There are databases constructed to exploit structures and peptide features of QSPs and tools for predicting random peptides activity solely based on QSP sequence information are available [12]. These advanced screening approaches through bioinformatics/computational methods will help in target specific identification of QSPs. *In vitro* and *in vivo* studies have well explored the interference or inhibition of QSPs [8,13,14]. Limited QS inhibitors were developed based on the molecular structures specific for QS receptors. Understanding the interactions between proteins and the ligand binding through *In silico* analysis will help in predicting new drug targets for the QSPs. This review provides a comprehensive summary of the computational approaches available for predicting the QSPs and a comprehensive information about QS inhibitors based on target receptors which can serve as important molecules for understanding QS associated peptide-membrane interactions in disease-causing pathogens.

2. Biological role of autoinducing peptides in quorum sensing of Gram-positive bacteria

AIPs are the autoinducers or pheromones of the QS system in Gram-positive bacteria [15]. These AIPs are oligopeptides consisting of 5–16 amino acids and unusual side-chain modifications. AIPs are synthesized by the ribosomes of the bacterial cells as precursor peptides, activated and stabilized by PTMs during their release. As the bacterial cell membrane is impermeable to these AIPs, they are released via the cell-surface oligopeptide/protein transport machineries into the extracellular environment. In general, the AIP release is facilitated by a membrane associated ATP-binding cassette (ABC) transporter or a simple transporter protein [16]. The released AIPs are then detected as signals by two-component sensory (TCS) systems [17]. These AIPs bind to the specific membrane-bound histidine (H) kinase receptors [18,19], resulting in the induction of a cascade of phosphorylation events of QS regulatory proteins and regulating the expression of target genes [18,20]. Upon attaining a certain concentration threshold level, binding of the AIP to its receptor activates the receptor kinase by receptor phosphorylation on a conserved H residue [16]. Subsequently, this phosphate group is transferred by the activated receptor kinase to a conserved aspartate (D) residue of the intracellular response regulator, thus activating the transcription regulators of targeted genes [21].

Several studies on the structure-activity relationship have reported the key structural characteristics of the AIPs, proving the significance of the AIP structure and its critical properties for AIP function and QS system activation [19,22]. Several types of AIPs have been reported in studies. QSPs are the most common peptides produced by Gram-positive bacteria. Besides acting as bacterial communication signals, it has an impact on the host as well [23]. QSPs exhibit species specificity, varying in length and adopting either cyclic or linear conformation after PTMs [24]. In addition to QSPs, competence stimulating peptides (CSPs) act as autoinducer molecules that control competence in bacteria via a TCS system formed by ComD, a H-kinase, and ComE, a response regulator. Due to ubiquity of cell-to-cell interactions within the bacterial communities, these QS systems play a vital role in regulation of robust cell behaviors.

3. Quorum sensing circuits in Gram-positive bacteria

3.1. Quorum sensing peptide based Agr system of Staphylococcus aureus

Cell-to-cell communication in *S. aureus* is based on the Agr signaling system [25]. The Agr QS mechanism is the key regulator of virulence phenotypes in *S. aureus* and several reports have shown that it influences the pathogenesis of bacteria [26]. It is a TCS QS system which makes use of an AIP consisting of about 8 amino acids, as a signaling molecule [17]. The accumulated AIP initiates the signal transduction events that activate the *agr* locus. The *agr* locus produces two mRNA transcripts such as RNA II and RNA III, which are under the transcriptional regulation of P2 and P3 promoters, respectively. These promoters are directionally opposite to one another. The RNA II transcript from the P2 promoter is encoded by the operon consisting of four genes: *agr*A, *agr*B, *agr*C and *agr*D, and is transcribed in the following order *agr*BDCA [8,27]. This forms the main Agr sensing system. AgrB encoded by the *agrB* gene acts as a transmembrane transporter and brings about the transport of the AIP such as AgrD, encoded by the *agrD* gene.

One of the key components of the Agr QS transduction system is a membrane-bound H-kinase receptor, AgrC which is encoded by the *agr*C gene [18,19]. AgrC is an obligate dimeric protein with a sensor module that is membrane-embedded and a cytoplasmic H-kinase module. The H-kinase module comprises a catalytic adenosine triphosphate (ATP) - binding domain and dimerization or a H-phosphotransferase domain [28]. AgrC is a transmembrane protein, consisting of N-terminal half which acts as the input or sensor domain, which interacts with AIP, and the C-terminal half which is a transmitter that gets autophosphorylated at a conserved histidine residue upon stimulation by the AIP [29]. The AIP binding to the N-terminal sensor domain of AgrC induces a conformational change in the AgrC C-terminal. This C-terminal is a cytoplasmic helix that forms a link between the sensor and kinase domains, enables autophosphorylation and activation of the AgrC kinase domains using ATP [30]. The activated AgrC then interacts with the intracellular response regulator, AgrA, which results in AgrA phosphorylation at conserved D residue, thus activating the response regulator [21]. Moreover, AgrB is also involved in processing of AgrD into an eight amino acid containing peptide to produce functional AIP and modification of AIP by formation of cyclic thiolactone, both of these functions being required for the transport of AIP outside the cell [26]. AgrD acts as the precursor of the AIP which gets processed by AgrB and SpsB. It first gets associated to the internal cell membrane

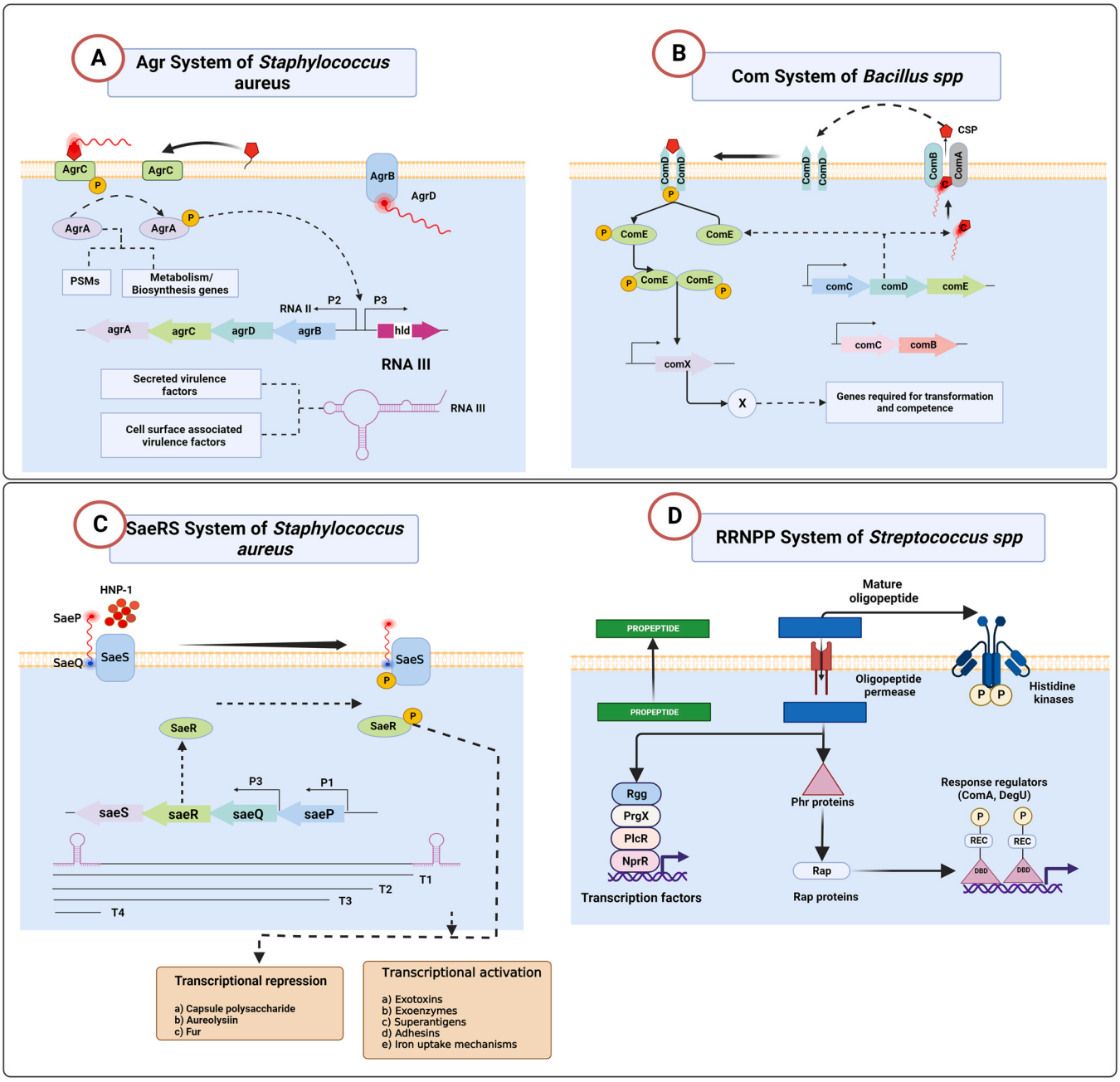

Fig. 1. QS regulatory systems in Gram-positive pathogenic bacteria: A) Agr System of *S. aureus*: *agr*BDCA regulatory operon consisting of gene coding for AgrD which is modified and exported via AgrB. The AgrD AIP then triggers autophosphorylation of the AgrC histidine kinase, which further phosphorylates and activates DNA-binding response regulator AgrA, which activates transcription of target genes. B) Com System of *Bacillus spp.* CSP based signaling where ComC binds to ComAB, a membrane protein complex, and is processed and exported as CSP. CSP then binds to the dimeric ComD membrane histidine kinase. Upon CSP binding, ComD is autophosphorylated and the phosphate group is transferred to the ComE response regulator. The phosphorylated ComE then dimerizes and activates transcription of target genes by binding to their promoters. C) SaeRS System of *S. aureus*: The signaling cascade in the SaeRS system starts by detection of environmental signals such as HNPs by SaeS membrane histidine kinase. This results in autophosphorylation of SaeS. The phosphate group is then transferred to the SaeR response regulator. The phosphorylated SaeR then binds the promoters of its target genes and activates their transcription. D) RRNPP System of *Streptococcus spp*: Immature pro-peptides are processed and secreted as oligopeptide signals. The mature oligopeptides then bind to and regulate histidine kinases or bind to oligopeptide permeases, which import the oligopeptides inside the bacterial cell. These oligopeptides further bind to and regulate the activity of transcription factors (TF), such as Rgg, PlcR and NprR, or PrgX. Alternatively, the oligopeptides bind to and regulate the *Bacillus* Rap proteins. The Rap proteins function as phosphatases or as transcriptional anti-activators targeting response regulator TFs such as ComA and DegU.

through its N-terminal leader sequence and is acted upon by AgrB which acts as the integral membrane endopeptidase and AIP transporter [31]. Agr is, thus, a master regulator of virulence, activated by AIP in *S. aureus* (Fig. 1**A**).

3.2. Competence stimulating peptide-based Com QS system in Streptococcus spp.

Another family of cell surface receptor containing QS signaling systems is the Com family, present in different *Streptococcus* species. These QS systems control bacterial virulence, competence and the AIP, which is referred to as CSP [32]. In this signaling system, ComC binds to ComAB, a transmembrane protein complex that then

processes and exports ComC as CSP molecule to the extracellular space. The processed CSP then binds to a dimeric membrane H-kinase, ComD. CSP binding then promotes ComD autophosphorylation at its H residue and transfers the phosphate group to the response regulator ComE. Phosphorylated ComE is then dimerized and this dimer activates transcription of genes present in *comX*, *comCDE*, and *comAB* operons by binding to their promoters [33]. ComAB are the secretory proteins while, ComX is a competence-specific operon [32]. The unphosphorylated form of ComE also binds to the promoters of these genes, repressing the transcription through their promoters. Synthesis of the ComX directs transcription of genes involved in genetic transformation and other functions [33]. In *Streptococcus mutans*, CSP is also involved in regulating the production of biofilms and bacteriocins, thereby playing a significant role in pathogenesis (Fig. 1**B**).

3.3. SaeRS quorum sensing system based on environmental stimuli

In addition to the Agr QS system, the SaeRS TCS system in *S. aureus* also regulates the expression of several virulence factors such as leukocidins, hemolysins, surface proteins, superantigens, and proteases [34]. Due to its significant impact on *S. aureus* virulence and pathogenesis the SaeRS TCS has been extensively researched. The SaeRS TCS comprises of a sensor H-kinase SaeS and a response regulator SaeR along with two auxiliary proteins SaeP and SaeQ. The *sae* operon contains four genes (*saeP*, *saeQ*, *saeR*, and *saeS*), regulated by two promoters including P1 and P3. The P3 promoter is located within the coding region of *saeQ* gene and only transcribes *saeR* and *saeS*, referred to as T3 transcript [34]. SaeRS TCS plays a major role in the sensing of and defense against neutrophils, with exposure to neutrophils acts as the key stimulus for the *saeRS* operon activation. The signaling cascade in the SaeRS system starts by detection of environmental signals by SaeS such as human neutrophil peptides (HNPs), leading to SaeS autophosphorylation at the conserved H residue [35]. The phosphate group is then transferred to the D residue of SaeR. The phosphorylated SaeR then binds the promoters of its target genes and activates their transcription [34]. *saeP* and *saeQ* are auxiliary genes upstream of *saeRS* [36] encoding a lipoprotein and a membrane protein respectively [37,38]. SaeP and SaeQ are dispensable for Sae system activation. However, they induce SaeS's phosphatase activity by forming a SaePQS ternary complex [37]. SaeRS acts synergistically with Agr QS system in *S. aureus* in some cases. The upstream transcripts i.e., SaeS and SaeR, along with the RNAIII of Agr QS, are induced during log phase of bacterial growth. The activation of these transcripts requires RNAIII, but is blocked by several environmental signals overriding RNAIII effects. SaeRS is involved in activation of exoprotein pathway, and coordinating the effects of environmental signals with the Agr QS system, it is a key mediator in the overall regulatory strategy that *S. aureus* senses and responds to its environment [36] (Fig. 1**C**).

3.4. RRNPP system

RRNPP is a special type of QS system found in Gram-positive bacteria that use oligopeptides for signal transduction where the receptor directly interacts with its corresponding signaling peptide. The protein family 'RRNPP' include the Rap phosphatases (aspartyl phosphate phosphatases, Rgg, NprR proteins (neutral protease regulator), PlcR protein (phospholipase C regulator) and PrgX protein (sex pheromone receptor) intracellular receptors [39]. The RNPP system of QS is primarily found in the Gram-positive bacteria such as *B. subtilis, E. faecalis*, and *B. cereus* [40–42]. The genes which encode for the RRNPP family proteins, along with their cognate pro-signaling peptides form transcriptional cassettes in the bacterial chromosome or plasmids [42]. These receptors remain inside the cell, but a fragment of pro-signaling peptide C-terminus is exported and processed by proteases to form the mature signaling peptide which is then internalized by an oligopeptide permease to bind the intracellular receptor protein [40,42].

In spite of several proteins playing a role in the RRNPP QS system, the underlying mechanism of downstream signal transduction is conserved among all the members. The signaling process begins with the production of the oligopeptide signals followed by their secretion. The next step is maturation of these peptide signals from precursor form to mature form in the extracellular environment. The mature peptides are internalized into the cytoplasm via oligopeptide permeases. The cognate signaling peptides are then recognised by the respective RRNPP receptors in the cytoplasm followed by modulation of the regulatory properties of the corresponding RRNPP regulators by allosteric structural changes induced by the signal peptides [43–46]. However, peptide sensing by receptors of the RRNPP family show varying downstream consequences in bacteria (Fig. 1**D**).

4. Computational tools for studying quorum sensing signaling in *Staphylococcus aureus*

The role of Agr QS in the pathogenesis and virulence of *S. aureus* is well explored and continues to be actively researched. The AIPs of *S. aureus* play a very crucial role in pathogenesis. Understanding their mechanism of action will aid in finding novel opportunities to combat *S. aureus* infections by designing drug candidates targeting these AIPs or other components of the Agr system [12]. Various experimentally validated drugs and approaches have been developed to target the Agr QS system which primarily depend on identification of the components of the Agr system including AIPs. However, there is a high cost and time associated with the identification as well as screening of QSPs in bacteria [11]. Therefore, there is a need to develop bioinformatics/computational tools and frameworks to aid the screening and identification of QSPs. These computational tools help in rapid, efficient and high-throughput identification of QSPs using only QSP sequence information [12]. Computational tools have a potential to relibly predict QSP and identify inhibitors targeting these QSPs. The following section describes the various computational and bioinformatics tools that can be used to study QS systems across different bacterial species including *S. aureus* (Fig. 2).

4.1. Databases for quorum sensing peptides and molecules

4.1.1. Quorumpeps®

QSPs, such as AIPs have wide variety of structures and are prone to modifications that can result in the alterations or their functionality. The Quorumpeps® database, developed by Wynendaele et al., (2013) provides a structured and detailed overview of the QSPs found in Gram-positive and Gram-negative bacteria. The database included information on the microbial origin of peptides and functionality (including mechanism of action, the result of their action and their receptor), peptide linkages and their physicochemical properties (PCPs), which depends on their 3D-structure [47]. Since these peptides play a very crucial role in the QS signaling and have a potential diagnostic and therapeutic properties [48], data from the Quorumpeps database, which contains information on modified QSPs, can be used to develop new bio-active compounds targeting the QS of various pathogenic bacteria, and thus treating the infections caused by them [47]. The MySQL backend is used to implement the Quorumpeps database, providing a simple search interface based on keywords to access the available data. Each search result provides the user with a complete and detailed overview of the QSP that matches the input query, allowing for in-depth information about the selected peptides to be obtained. Quorumpeps is a useful tool to justify peptide choices for evaluating different responses or studying

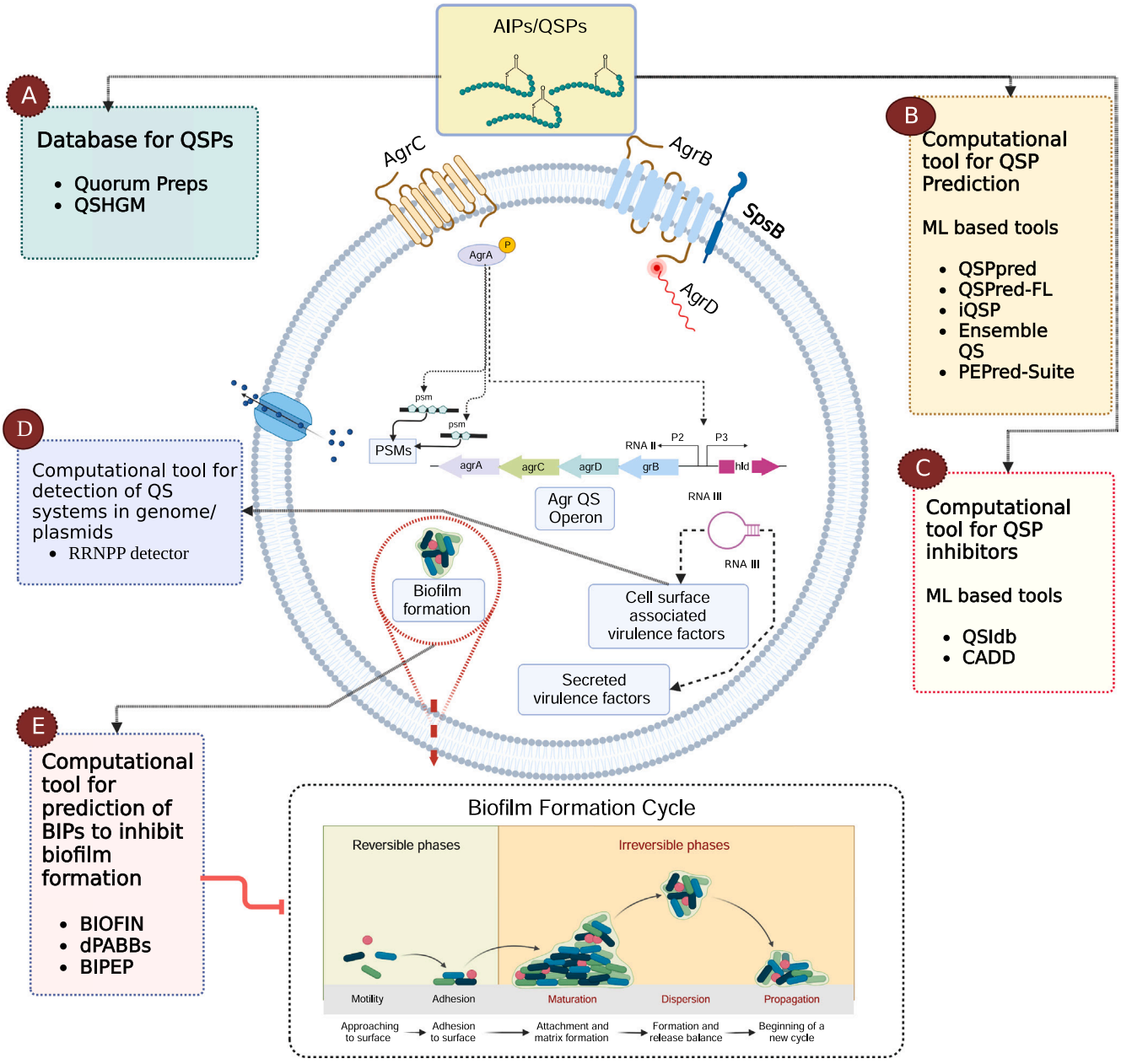

Fig. 2. Schematic representation of comprehensive computational approaches available for prediction and detection of QSPs and their inhibitors in Gram-positive bacteria. A) Databases for detailed structures of QSPs, B) Machine learning (ML) based tools for prediction of QSPs, C) Tools for identification of QS inhibitors, D) Tools for detection of QSPs in genomes/plasmids and E) Tools for detection of Biofilm inhibiting peptides (BIPs). The names of databases and tools applicable for particular approaches are mentioned in boxes (bullet points).

quantitative structure property relationships (QSPRs) of *S. aureus* AIPs, enabling the development of bio-active molecules that target these AIPs. Quorumpeps is freely available at http://quorumpeps.ugent.be [47].

4.1.2. Quorum sensing of human gut microbes (QSHGM)

Similar to Quorumpeps, QSHGM is a QS repository of human gut microbiota (HGM). With the help of QSHGM, it is possible to search diverse microbial interactions based on bacterial communication within the HGM. A QS communication network (QSCN) is further constructed and analyzed for about 818 HGMs, forming one of the key maps of the HGM, that will support future applications. These include novel manipulations in the synthetic microbiota and designing potential therapies to gut diseases targeting these QSPs [49]. The database was developed by following a pipeline which included QS training, QS expanding, and QS mining modules and combining basic local alignment search tool (BLAST) and amino acid sequence descriptors, extracting various QS relevant proteins. The database is available at http://www.qshgm.lbci.net. A similar approach could be used to create a database specific to different *Staphylococci* species, as most of these species are normal commensals of the human body and can act as opportunistic pathogens. *S. aureus*, one of the most notorious drug resistant pathogens, belongs to this group, and developing such a database will help in easy identification of *S. aureus* QSPs and designing therapeutic strategies targeting these QSPs.

4.2. Tools/Algorithms for prediction of quorum sensing peptides and molecules

4.2.1. QSPpred

Despite the significance of QSPs, Quorumpeps is currently the only available database of experimental QSPs. However, detailed bioinformatic analysis of these QSPs is still lacking. With this in mind, Rajput et al., (2015) developed a Support Vector Machine (SVM), a type of machine learning (ML) technique, based algorithm called QSPpred. This is the first web server for QSP prediction and design and developed using experimentally proven QSPs and basis of the sequence features of these peptides. Additionally, the distinctiveness and uniqueness of these peptides helps the algorithm for predicting the QSP status of unknown peptides. The prediction has a maximum accuracy of 93%, Mathew's correlation coefficient (MCC) of 0.86 and Receiver operating characteristic (ROC) of 0.98, suggesting a very high accuracy of QSPpred based peptide prediction [24].

QSPpred is freely available at http://crdd.osdd.net/servers/qsppred. Its web server provides three predictive models based on SVM: 1) QSPepPred, 2) QSPepDesign and 3) QSPepMap. These models utilize amino acid composition (AAC), binary characteristics, PCPs and the hybrids of QSPs for peptide prediction. QSPepPred helps in predicting either a FASTA or multiFASTA peptide input format as a QSP or non-QSP. On the other hand, QSPepDesign, enables designing all possible single amino acid position mutants of the given peptide and then predict the QS status whereas, QSPepMap helps to identify potential regions in protein, which may function as QSPs [24]. In addition, the web server also includes various analysis tools such as QSMotifScan which finds all known motifs that occur in a given amino acid sequence; MutGen, which allows mutation of sequences according to a specified mutation model and helps in generating customized mutations; PhysicoProp, which provides graphical representation of PCPs of peptide sequence such as percentage of AAC, hydrophobicity, preference for beta strands etc; and ProtFrag, which helps in generating fragment of input protein sequence in peptide of desirable length. Owing to its high accuracy in QSP prediction, the predictor "QSPpred" would accelerate research in QS field. Thus, QSPpred can be easily used to identify *S. aureus* AIPs that can be targeted and the motifs/domains/amino acids in these AIPs that can serve as primary targets.

4.2.2. QSPred-FL

QSPred-FL is a sequence-based feature descriptor for peptide representation developed by Wei et. al., (2018), that uses a representation learning strategy to effectively capture the sequence determinants and improve predictive performance. It is the first tool that can detect QSPs directly from proteins. QSPred-FL uses several peptide features, such as AAC, binary profile features (BPF), composition-transition-distribution (CTD), g-gap dipeptide composition (GDC), overlapping property features (OVP) and information theory features (IT). The tool also uses a two-step descriptor feature selection strategy to determine the optimal peptide feature subset that can be used for QSP prediction. The first step involves the minimum Redundancy and Maximum Relevance (mRMR) method [50,51] and the second step uses sequential forward search (SFS). Later, using the selected feature subset, the predictive model based on Random Forest (RF) ML algorithm, performs QSP prediction [52]. Studies demonstrate that QSPred-FL outperforms other currently available QSP predictors and is more effective and accurate in QSP prediction. In comparative studies performed by Wei *et. al*., (2018), QSPred-FL demonstrated better performance than the other previously available QSP predictors such as QSPpred. QSPred-FL is thus a powerful tool for the discovery of novel QSPs and providing insights into the QSP functional mechanisms [52]. Currently, it is publicly accessible at http://server.malab.cn/QSPred-FL.

4.2.3. iQSP

Although, the above-mentioned computational tools offer promising results in QSP prediction, additional improvement is still required in the performance and interpretability of QSP predictors. iQSP a sequence-based QSP structure predictor for rapid, efficient and high-throughput QSP identification, prediction and analysis was developed based on the sequence information of peptide [12]. iQSP was developed to overcome the drawbacks of QSPpred [24] and QSPpred-FL [52] and to improve the prediction and interpretability performances. Similar to QSPpred, iQSP is based on SVM ML algorithm, which cooperates with 18 features from PCPs such that peptide sequence is represented with PCPs. In this, sequence of amino acids of a peptide is used for characterization of the properties between QSPs and non-QSPs. It also contains a set of rules referred to as IR-QSP extracted using RF-ML, used for predicting and analysing QSPs. These IR-QSPs yield a prediction accuracy of greater than 80%. iQSP has an accuracy, MCC and ROC of 93.00%, 0.91% and 0.96%, respectively, which shows a significant improvement in QSP prediction when compared with QSPpred [12].

Thus, iQSP provides more reliable and accurate results with respect to unknown peptides. iQSP is considered a free QSP predictor that helps in classification of peptide sequence query as being either a QSP or a non-QSP. iQSP is available online at https://codes.bio/iqsp/. It is predicted that due to its high-performance accuracy, iQSP may become a powerful and cost-effective approach for predicting and analysing peptides on a large scale.

4.2.4. EnsembleQS

The above mentioned QSP prediction tools, such as QSPpred [24], QSPpred-FL and iQSP are based on only one ML algorithm, i.e., either SVM or RF. Previous studies have demonstrated that predictions based on a single ML algorithms can be complex and can provide either overestimated or underestimated predictions [53]. This demands for further improvements. In this view, EnsembleQS is a type of stacked generalization ensemble model with Gradient Boosting Machine (GBM)-based feature selection for prediction of QSPs with high accuracy. It predicts the QSPs using amino acid sequence information while outperforming the existing ML tools [54]. For the QSP prediction, the algorithm consists of two types of datasets: 1) Positive data obtained from QSPs from Quorumpeps [47] and existing literature and 2) Negative data obtained from Uniprot database. Along with the data it also consists of the feature descriptors that are extracted from the peptide sequences using the iFeature package [55]. In EnsembleQS, six such baseline expert ML algorithms are used as prediction classifiers. These included: SVM, RF, Decision Tree (DT), K-Nearest Neighbors (KNN), Bernoulli Naïve-Bayes (BNB) and Gaussian NB (GNB). The model also reduces the time taken to predict the QSPs and is easier to interpret and understand [54]. Due to these features of EnsembleQS, it outperforms baseline ML classifiers and demonstrates a robust performance with a 4% higher accuracy as compared to other ML approaches for QSP prediction [54]. This indicates the superiority of the model in QSP prediction thereby making it a novel computational framework for predicting QSPs providing future scope for proteomics research. EnsembleQS is publicly available at https://github.com/proteinexplorers/EnsembleQS.

4.2.5. PEPred-Suite

PEPred-Suite is a bioinformatics tool used for generic prediction of QSPs based on adaptive feature representation strategy that learns the most representative features for peptides, similar to QSPred-FL [52]. However, the difference between QSPred-FL and PEPred-Suite is that the latter includes more information by means of integrating other peptide features, including PCPs. More importantly, PEPred adaptively extracts the most discriminative peptide features using Area under the ROC curve (AUC)-based feature space optimization to

identify a specific type of peptide. Due to this, the tool helps in predicting different peptides reliably and thus exploring an effective way for peptide feature representation. PEPred-Suite contains RF ML-based model for the prediction of different QSPs along with seven other different types of peptides, which makes it a potential predictor. Comparative results based on many studies with cross-validation evaluations, as well as independent evaluations suggest that PEPred-Suite generally gives a better overall performance. This tool can thus help in the computational discovery of more candidate functional peptides, facilitating the better understanding of their functional mechanisms and accelerating the applications of disease therapy [56]. PEPred-Suite freely accessible at http://server.malab.cn/PEPred-Suite.

4.3. Databases/tools/algorithms for designing inhibitors targeting the peptides/molecules involved in bacterial quorum sensing

4.3.1. QSIdb

QSIdb is a specialized repository of quorum sensing interference (QSI) molecules (QSIMs) that disrupt and manipulate QS, resulting in dynamic control of bacterial populations and their virulence. It consists of 633 reported QSIMs and 73,073 expanded QSIMs, including both the agonists and the antagonists. The database contains a pipeline that uses algorithms such as SMILES-based similarity assessment and docking-based validations to mine potential QSIMs from existing compounds in PubChem database. The repository also includes a feature referred to as *pocketedit*, which is used to calculate the similarity index of active protein pockets in the identified QSIMs. This helps in the identification of crosstalk between QSIMs and developing potential QSIMs, and having broad-spectrum activity for multiple QS receptors [57]. QSIdb is a specialized database containing reported as well as potential QSIMs, and is conveniently available at the webserver http://qsidb.lbci.net/. QSIdb can help in understanding of QS-associated therapeutics, manipulation of QS-based genetic circuits, development of broad-spectrum QSIMs and expanding new ligands for other receptors [57]. For example, in case of *S. aureus* it can be used to identify QSIMs, that target the AgrC to treat *S. aureus* infections by blocking the Agr system. A single broad spectrum QSIM targeting the AgrC of *S. aureus* belonging to different *Agr* subgroups (I-IV) and thus having AgrC of different specificity can be developed.

4.3.2. Inhibitors targeting quorum sensing peptides involved in biofilm formation

Computational tools can also be used to inhibit the QSPs which help in the formation of bacterial biofilms. Biofilms are crucial in pathogenesis of a bacteria. Gram-positive bacteria use AIPs as signaling molecules for development of biofilms. However, designing bacterial biofilm inhibitory peptides (BIPs) is crucial and therefore, developing computational tools for the prediction of BIPs or inhibitors targeting the QSPs involved in biofilm formation is inevitable and important. Following are some of the computational tools commonly used for this purpose.

4.3.2.1. BIOFIN. It is a unique computational method aimed at the prediction of BIPs. The model uses the sequences of experimentally validated BIPs for extraction of peptide sequence-based features and identification of unique sequence motifs in the peptides. These individual sequence-based features are then utilized for construction of SVM-based BIP prediction models which along with sequence motifs information predicts the appropriate BIPs [58]. It is based on a database of BIPs known as Biofilm active-Anti Microbial Peptides (BaAMPs) [59]. BIOFIN for the prediction of BIPs is available freely as web server at http://metagenomics.iiserb.ac.in/biofin/. and http://metabiosys.iiserb.ac.in/biofin/.

4.3.2.2. dPABBs. The design Peptides Against Bacterial Biofilms (dPABBs). is a web server that facilitates in the prediction and designing of anti-biofilm peptides. It is the tool for predicting biofilm-active peptides and creating their mutants having improved activity and optimized PCPs [60]. dPABBs is freely accessible on: http://ab-openlab.csir.res.in/abp/antibiofilm/. It consists of six SVM and Weka-based ML models trained on 80 biofilm-active anti-microbial peptides (AMPs) obtained from the database BaAMPs and 88 QSPs that were retrieved from QSPpred [24] and were cross referenced with Quorumpeps [47]. Using this data, the tool aids in identification of anti-biofilm peptides based on their whole AAC, selected amino acid residue features and the positional preference of these amino acids [60].

4.3.2.3. BIPEP. Although, the tools discussed earlier have played crucial roles in prediction of BIPs, there is a need to improve the prediction accuracy in order to minimize the number of false positives and to access better BIP candidates for experimental validation. BIPEP is one such computational prediction tool that screens large number of peptide sequences for selection of potential candidate peptides which can act as QSP and biofilm inhibitors. In BIPEP, different peptide features are extracted from NMR spectra of amino acids and are used along with PCPs for identification of inhibitory peptides. In this, different peptide descriptors such as CTD along with the newly introduced NMR-based features are used for mapping peptide sequences to numeric feature vectors as the input for prediction methods. Various ML classification algorithms including SVM, RF, and KNN are then used to evaluate the performance of these identified inhibitors [61]. The developed user-friendly web-server is accessible at http://cbb1.ut.ac.ir/BIPClassifier/Index.

4.3.3. Multi-level computational approach for identification of potential inhibitors targeting quorum sensing peptides and their receptors

Computer-aided drug design (CADD), is a commonly used computational method in the search for QS inhibitors which target different aspects of QS such as the QSPs, receptors of these QSPs, transcriptional regulators, peptides involved in biofilm formation, etc. CADD can be used for understanding the target binding determinants and specific properties of the binding pocket of the peptides/receptors acting as a QS inhibition model target. Thus, it can be used to identify new promising molecules for blocking QS. Various computational methods are combined in this approach. Some of these approaches are: 1) Structure identification of the target molecule, 2) Protein–ligand docking studies to identify the molecule which specifically binds with maximum binding efficiency to the target molecule, 3) Virtual screening to determine the efficacy of interaction, 4) Molecular dynamics (MD) simulations for validating results of protein–ligand docking and evaluating the structural stability of the protein–ligand complex and 5) MM/PB(GB)SA mathematical calculations for determining the binding free energy (BFE) of each ligand. This approach results in identification of compounds with higher potential for blocking QS [62].

4.4. Tools for detecting quorum sensing systems in chromosomes, plasmids and phages of Gram-positive bacteria

4.4.1. RRNPP_detector

RRNPP_detector is a python-based software for detection of the RRNPP QS system signature in the chromosomes and mobile genetic elements (MGEs) of Gram-positive bacteria. It is not based on homology searches as that would limit the output of analysis to representatives of already known QS signals. Rather than relying on homology searches, RRNPP_detector relies on multiple signature criteria, that are common across distinct families of experimentally validated RRNPP QS systems. As this signature is generic while being

specific to the canonical mechanism of RRNPP QS, it enables the discovery of novel RRNPP QS systems. The detector reveals thousands of candidates RRNPP QS systems in complete bacterial chromosomes, plasmids and phage genomes.

RRNPP QS systems regulate genes adjacent to them in a density-dependent manner, post-processing genomic context analyses can couple the output of RRNPP_detector with various functional insights. RRNPP_detector is thus, a first step towards the large-scale *in silico* identification of peptide-based QS systems. However, the tool is dedicated only to the detection of RRNPP QS signals and its usage can be expanded towards the detection of peptide-based QS systems with two-component systems such as the Agr QS system of *S. aureus*.

4.5. Other bioinformatic approaches for studying quorum sensing

Instead of using ML based computational tools and algorithms, multiple commonly used bioinformatics tools can also be used stepwise to study bacterial QS. Many such approaches have been previously described by researchers. For instance, Hegde et al., (2019) used bioinformatics for identification of the proteins associated with QS and formation of biofilms in *Mycobacterium tuberculosis* infection. Firstly, *M. tuberculosis* proteins having a possible association with biofilm formation or QS were shortlisted using comparative genomics and literature mining. Furthermore, a functional relationship among these shortlisted proteins was established by carrying out interaction studies such as protein-protein interactions (PPIs) and regulatory interactions, as well as gene expression correlation studies using Cytoscape. This was followed by graph centrality and motif analyses to predict the importance of these proteins in *M. tuberculosis* biofilm formation. Finally, the analysis of conservation across other biofilm-forming bacteria performed using phylogenetic analysis suggests that most of these genes are conserved in mycobacteria. As the processes, such as QS, involve diverse pathways and protein interactions, system-wide studies like this provide a novel perspective towards understanding mycobacterial persistence and help in easy identification of the proteins associated with QS [63].

Similarly, Rajput et al. used bioinformatics tool for exploration of putative LuxR solos in archaea and their functional implications through multidimensional perspectives, using their distribution, similarity with bacteria, functional characterization followed by correlation between taxonomy and ecological niche. This involved, data collection, Multiple Sequence Alignment (MSL), domain analysis, motif analysis, Gene Ontology (GO), prediction of ligand binding followed by phylogenetic analyses and ecological niche studies, executed to observe the evolutionary relationship of LuxR-containing protein of Archaea with other families of LuxR and, to correlate the taxonomy and ecological distribution of Archaeal LuxR solos [64]. Hence, various bioinformatics approaches can be used to explore the extent and functionality of the Agr QS system in *S. aureus* and to identify the QSPs of *S. aureus* that take part in the Agr QS, in order to develop treatment approaches targeting these functionalities. This demonstrates the utility of computational and bioinformatics tools and algorithms in studying the QS signaling and identification of QS compounds as well as the inhibitors of QS. Table 1 provides a brief overview about the tools mentioned above.

5. *In Silico* approaches towards targeting the *Staphylococcus aureus* Agr quorum sensing system

Over the last decade, computational or *in silico* methods are frequently used in the discovery and optimization of new molecules having target affinity [65]. *In silico* methods have widely been used to identify compounds inhibiting the *S. aureus* Agr system, especially those that target the response regulator, AgrA, and the transmembrane histidine kinase, AgrC, of the system. One such method is molecular docking. It is a widely used tool for the process of computer aided structure-based rational drug design. Following sections describe molecular docking studies performed to identify compounds targeting Agr system components:

5.1. AgrA

As mentioned earlier, AgrA regulates the QS response in *S. aureus* by controlling the production of hemolysins as well as many other virulence factors. For this, AgrA acts as the transcription factor (TF) and binds to the bacterial DNA (virulent genes) via its C-terminal LytTR domain. This AgrA-LytTR domain recognizes and binds to a consensus nine-base-pair sequence (5′-ACAGTTAAG-3′) of the DNA through interactions which are base-specific and driven by three β-loops (L1–3) protruding from the edges of β-sheets. Although this domain is not found in human beings, it is common in several bacterial pathogens [66]. This makes it a possible target for antimicrobial treatments.

For instance, molecular docking analysis of savirin, an FDA approved AgrA inhibitor demonstrated the key interactions involved in savirin-AgrA binding. The ligand savirin interacts with Tyr229 and Arg218 of the AgrA-LytTR domain having −6.1 kcal/mol of docking score [67]. The major interactions are given in Table 2. Similarly, in a study conducted by Khayat et al., (2022) antidiabetic gliptins, were analysed using *in silico* tools to identify its AgrA binding activity. To verify the anti-QS activities of these gliptins, a validated molecular modelling simulation study against AgrA was performed. The molecular docking, using PyMol2.0.6 graphical visualization software and molecular operating environment (MOE) tools, demonstrated sitagliptin's (SIT), trelagliptin's (TRG) and omarigliptin's (OMR)

Table 1
Databases and tools for prediction of quorum sensing peptides and inhibitors in Gram-positive bacteria.

Name of Tool	Type of Tool	Reference link
Quorumpeps	Database for quorum sensing signaling peptides	https://quorumpeps.ugent.be/
QSHGM	Database for QSPs in HGM and QSCN	http://www.qshgm.lbci.net/
QSPpred	SVM ML technique-based algorithm for predicting QSP	http://crdd.osdd.net/servers/qsppred/
QSPred-FL	QSP Predictor	http://server.malab.cn/QSPred-FL
iQSP	Sequence based tool for prediction of QSP	https://codes.bio/iqsp/
EnsembleQS	GBM-based feature selection to predict structure of QSPs	https://github.com/proteinexplorers/EnsembleQS
PEPred-Suite	QSP Predictor	http://server.malab.cn/PEPred-Suite
QSIdb	Database for QS interference molecules	http://qsidb.lbci.net/
BIOFIN	BIP Predictor	http://metagenomics.iiserb.ac.in/biofin/ http://metabiosys.iiserb.ac.in/biofin/
dPABBs	BIP Predictor	http://ab-openlab.csir.res.in/abp/antibiofilm/
BIPEP	QSP Inhibitor Predictor	http://cbb1.ut.ac.ir/BIPClassifier/Index
CADD	QSP Inhibitor Predictor	
RRNPP_Detector	QS system Detector	https://github.com/TeamAIRE/RRNPP_detector

Table 2
Inhibitors of QS receptors in *S. aureus* bacteria identified by molecular docking.

Inhibitor	Receptor	Interaction residue	Nature of interaction	Docking score	Reference
Manool	AgrA	Ile238	H-Bond	-15.747	[68]
Sclareol		Arg233; Asn234		-23.294	
4-phenoxyphenol		Arg198; Asn201		− 22.191	
9 H-Xanthene-9-carboxylic acid		Asn201, Asn234		− 20.841	
Savirin	AgrA	Glu217; Arg218	H-bond	-6.100	[67]
		Tyr229; Arg233	π-H		
		His200; Asn201	π-H		
Sitagliptin (SIT)	AgrA	Arg233; Arg218	H- bond	-6.666	[28]
		Asn201; Val232; Tyr229; His200	π-H		
	AgrC	Asp374	H-Bond	-5.224	
Trelagliptin (TRG)	AgrA	Arg233	H-H	-6.964	[28]
		His169	π-π	-6.276	
	AgrC	Asp374; Arg377	H- bond	-4.950	
Omargliptin (OMR)	AgrA	His169	π-π	-4.477	[28]
	AgrC	Asp373; Asp374	H-Bond	-3.770	
Raloxifene	NorA	Phe16 and Asn340	H-Bond	-9.064	[69]
		Phe140	π-π		
Carvacrol	SarA	Asp120; Tyr162	π-π	-6.860	[70]
c-di-GMP		Ile106; Ile103; Thr104		-4.470	
Eugenol		Asn161; Asp120; Tyr162		-4.390	
Morin	SarA	Leu60; Asn61; Phe10; Ser14; Glu123	H-Bond	-4.430	[71]

ability to bind to AgrA and hinder the activity of the QS receptors suggesting that gliptins and other similar chemical groups can act as effective anti-virulence and anti-QS agents. The study also confirmed the anti-AgrA activity of the FDA approved AgrA inhibitory drug savirin [28].

A similar approach was also used to study the anti-AgrA activity of avellanin B, thymol, sclareol, manool, staquorsin, axitinib, dantrolene sodium and diflunisal which showed strong binding affinity with AgrA receptor. Yet another study, identified three chemical compounds, 4-phenoxyphenol, 9 H-xanthene-9-carboxylic acid and 2-(4-methylphenyl)− 1,3-thiazole-4-carboxylic acid, as potential AgrA inhibitors by performing docking studies using AutoDock Vina which indicated that these three compounds bind to the amino acid residues present in LytTR domain of AgrA. Thus, the molecular docking analysis suggests that the DNA binding domain i.e., LytTR domain of AgrA could serve a primary target for the development of antimicrobial drugs. A detailed information about the key interactions between the above-mentioned compounds, the amino acid residues involved in these interactions and the docking score are provided in Table 2**.**

5.2. AgrC

AgrC, a transmembrane histidine kinase, of *S. aureus* comprises an important catalytic ATP-binding domain that is conserved across variable bacterial TCSs. However, this ATP-binding domain is not present in the tyrosine and serine/threonine kinases of and other H-kinases of humans [72,73]. Thus, targeting this domain of AgrC confers selectivity and permits effective and safe management of *S. aureus* infection.

Similar to AgrA, Khayat et al., (2022) also performed docking studies on antidiabetic gliptins such as SIT, TRG and OMR along with a known AgrC inhibitor ADP-β-N to evaluate their AgrC-ATP domain binding activity. The study demonstrated the ability of these compounds to bind to key AgrC amino acid residues of Asp373/374, Asn339, Arg393, Met368, Ile375, Leu381, Phe382, Thr414, Ile415, Ile416, Phe420, and Phe421 and hinder the activity of AgrC suggesting that these gliptins and other related chemical moieties can serve as effective anti-QS agents [28].

Another study identified two novel compounds 3 R,4 R)− 1-(7a-Methyl-5-oxo-2,3,6,7-tetrahydropyrrolo[2,1-*b*][1,3]thiazole-3-carbonyl)− 4-phenylpyrrolidine-3-carboxylic acid and (3 R,4 R)− 1-(3-Pentanoyl-1,3-thiazolidine-4-carbonyl)− 4-phenylpyrrolidine-3-carboxylic acid as potent inhibitors of AgrC activity [74]. Thus, the molecular docking analysis suggests that the ATP binding domain of AgrC could act as a primary target for antimicrobial drug development. A detailed information about the key interactions between the above-mentioned compounds, the amino acid residues involved in these interactions and the docking score are provided in Table 2.

5.3. SarA

SarA a major Agr regulatory molecule controls the expression of many *S. aureus* virulence genes making it an important therapeutic target. In this view, computer-assisted drug design can be used to produce specific compounds with low molecular weight interacting with SarA and disrupt the QS mechanism. One such compound identified is 2-[(Methylamino)methyl] phenol also known as SarABI-12. Molecular docking studies suggest that, SarABI-12 binds SarA at residues Glu-89 and Arg-90 with a docking score of less than − 18 kcal/mol and down-regulates virulence gene expressions as well as biofilm formation [75]. Similarly, in another study 3–Hydroxybenzoic Acid (3-HBA) derived from *Illicium verum* inhibited SarA by interactions with the active site residues Arg210 and Glu129 with a docking score of − 4.1 kcal/mol [76]. Other compounds including carvacrol, eugenol, morin were proved as target for QS receptors [70,71]. Thereby, the molecular docking analysis implies that SarA could serve as a crucial target for the development of antimicrobial drugs. A detailed information about the key interactions between the above-mentioned compounds, the amino acid residues involved in these interactions and the docking score are provided in Table 2**.**

5.4. RAP/TRAP system or RNAIII

In silico-based studies can be used to find out analogues of substrates for bacterial virulence systems, leading to the discovery of competitive inhibitors. For instance, hamamelitannin (HAM), which targets the RNAIII-activating protein (RAP)/ (Target of RAP) TRAP QS system of *S. aureus* and thus the RNAIII molecule using the *in silico* method for analog development [77]. Based on this, Kiran et al., (2008) discovered RNAIII-inhibiting peptide (RIP) analogs that prevent formation of biofilm using ribosomal protein L2 (a TRAP ortholog), to develop a model of RIP, which was later used to screen 300,000 compounds from the available chemicals database (ACD).

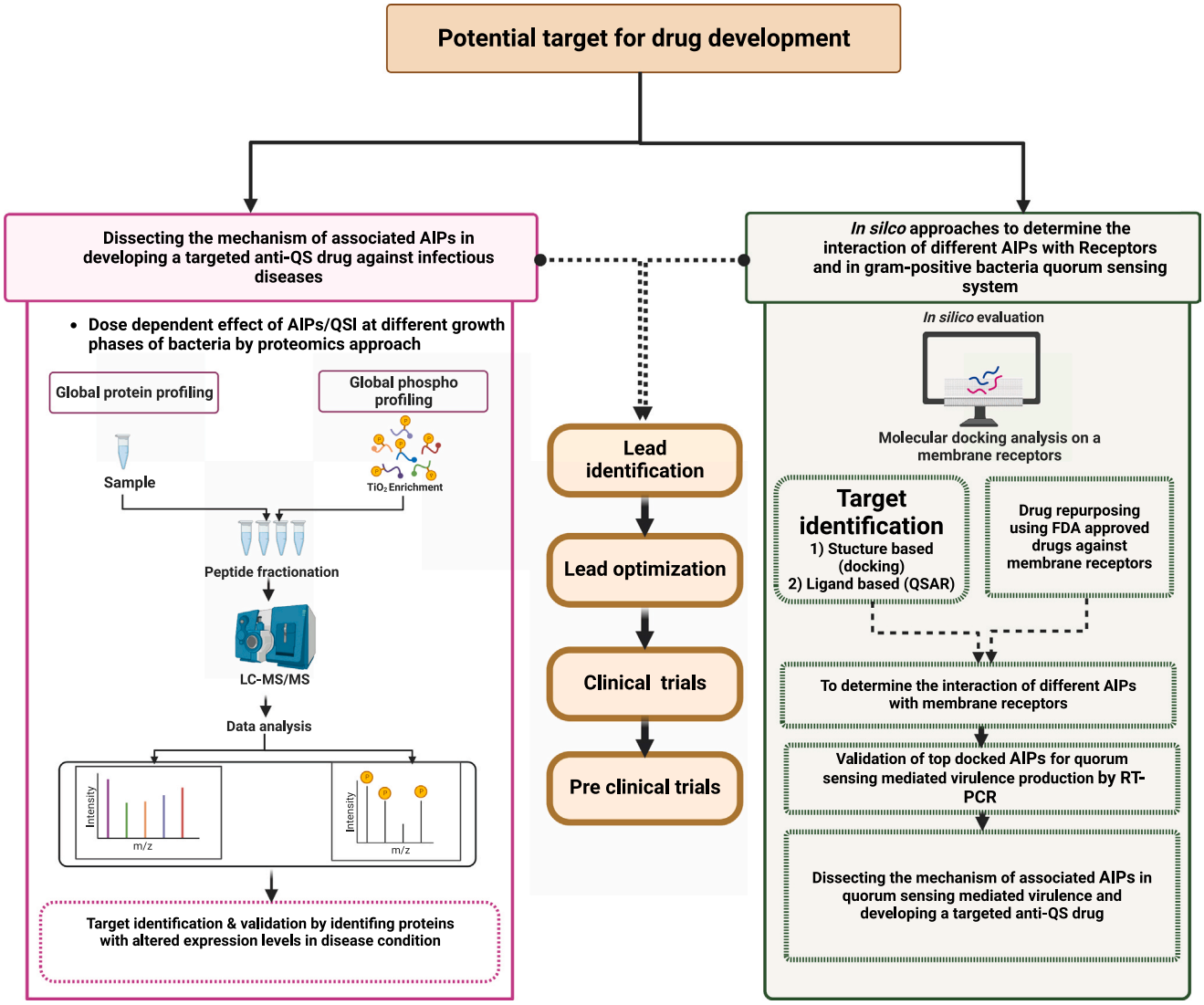

Fig. 3. Target identification and validation by using proteomics and *in silico*-based approaches for the drug development.

This screening was carried out with the Integrated Scientific Information System (ISIS) software from Elsevier MDL. In this, the search of the suitable ACD was carried out based on the approach of pharmacophore. For this, the queries were defined by a set of distance ranges between aromatic rings of amino acids such as Tyr, Phe, and Trp and the hydrogen bond donors or acceptors, based on the RIP model. Subsequently, the coordinates of the top hits were transformed to PDB format by program BABEL. Lastly, the structures of the top hits were imposed on the RIP model and were viewed either with program SwissPDBViewer on a PC or with program on an SGI Octane workstation [78]. This identified HAM as a non-peptide analogue of RIP that reduced surface attachment and virulence of *S. aureus* and increased the susceptibility of its biofilm to antibiotics [77].

5.5. Other targets

Besides the inhibitors for specific target systems, a study by Qu et al., (2020) demonstrates that a coumarin derivate DCH (3,3′-(3,4-dichlorobenzylidene)-bis-(4-hydroxycoumarin)), effectively combats MRSA and exhibits potent antibiofilm activity. Using molecular docking assays, the study demonstrated that the arginine repressor ArgR, an important regulator of the arginine catabolic pathway, is the target of DCH. These findings suggest that DCH is a promising lead compound in the development of novel drugs to fight MRSA biofilms. Molecular docking results revealed that DCH had a very similar binding pocket to arginine while binding to ArgR. The two important interactions involved DCH-ArgR are between the DCH benzene rings and two amino acids Gln25 and Asp46 of ArgR active site [79]. This is important as the ways by which *S. aureus* forms biofilms and protect themselves against antibiotics are not deeply understood and targeting other factors involved in virulence is important.

Previous studies have demonstrated that QS regulates and plays a crucial regulatory role in the drug efflux pumps. These efflux pumps are important contributors to *S. aureus* biofilm formation and thus can serve as potential targets for anti-microbial therapy [80]. NorA is one of the most popular efflux pumps in *S. aureus* which is studied and developing inhibitors of the NorA is a promising approach to potentially reverse multidrug resistance (MDR) as well as inhibit biofilm formation and virulence [81]. In one study performed by Tintino et al., (2021) 2,3,4-trifluoro-N-(5-chloro-1,8-naphthyridin-2-yl) benzenesulfonamide, a 1,8-naphthyridines derivative, was identified as a potential NorA inhibitor. Docking simulations were carried out for compound-efflux pump complexes to minimize the energy and stabilize the docked complex [82]. In another study, with molecular docking analysis, raloxifene was identified to have NorA binding ability with docking score of −9.064 kcal/mol. The key

amino acid residues involved in this interaction were Asn340, Phe16, Gln51, Thr336, and Phe140 and the major interactions were hydrogen bonds and Pi-Pi interactions. Other potential NorA inhibitors identified in this study were ezetimibe, propafenone, capsaicin, nefazodone, chlorprothixene, and pyrvinium [69,83]. Molecular docking and stimulation studies also highlighted the important interactions involved between the NorA protein and Reserpine, an FDA approved NorA inhibitor [84], the details of which are provide in the Table 2 and Supplementary table (S1). Therefore, *in silico* approaches can be easily used to identify potential inhibitors targeting different components of *S. aureus* Agr QS system as well as other molecules playing important role in its virulence.

6. Conclusion and future prospects

Currently, QS inhibition is a major focus of research, as our understanding of QS regulatory systems in pathogenesis is increasing. This review provides a comprehensive and reliable information on databases and tools that are potential predictors of QSPs and QS interference molecules. Researchers can explore the tools and databases summarised in this article for identifying QSPs and inhibitors and ligands for QS receptors and validate them by experimental procedures to understand the molecular mechanisms associated with QS and for development of target biomarkers. Thus, due to the potential applications of tools such as Quorumpeps, iQSP, QSIDB, several systematic approaches can be applied to predict other proteins and peptides involved in QS regulatory mechanism.

Quorum sensing in Gram-positive bacteria is operated at the post-transcriptional level, which requires an indispensable proteome analysis of QS regulated functions. However, the 'gold standard' for a comprehensive characterization of such complex regulatory systems should be the collaborative application of system-level approaches, i.e., a combination of proteomics, transcriptomics, and metabolomics technologies, intending to evaluate all genes, proteins, and metabolites that are affected by QS. As QS leads to biofilm formation, it involves diverse pathways and interactions between proteins. These system-wide studies will provide a novel perspective towards understanding bacterial persistence. The integrated omics analyses methods can be used to identify different components of QS systems such as the receptors or their autoinducers as well as downstream QS-regulated proteins thereby assessing the role of bacterial cell-to-cell communication in pathogenesis. Proteomic approaches can also be used to map the temporal changes in QS proteins controlled by both transcriptional and post-transcriptional mechanisms facilitating in understanding the role of bacterial QS in global gene regulation. It can further be exploited along with other *in silico* approaches including molecular docking, QSAR studies and molecular dynamics, to predict the target specificity of natural and synthetic QS inhibitors having a great potential as therapeutics for treating bacterial infections. Apart from this, proteomics can also be used to study the PPIs between QS components. These PPI studies will help in elucidating the bacterial cross-talk as well as in drug target identification and validation. Thus, proteomics is an indispensable tool to elucidate the regulatory mechanisms of the diverse language signals diffusing through different microbial communities.

Computational approaches along with emerging omics methods, offer a powerful tool for studying and guiding experimental designs related to QS in pathogenic bacteria. These approaches can generate hypothetical and simulation models, providing a quantitative understanding of how signal transduction occurs during QS. Such modelling offers promising prospects for future experimental validation and for drug repurposing aimed towards QS inhibition (Fig. 3). As the demand for more efficient and accurate, omics technologies grow, cutting edge methods such as metagenomics will make it possible to explore the evolution of the QS dynamics *invitro* and further advance our understanding of this complex regulatory systems.

CRediT authorship contribution statement

Shreya Kumar: Writing – original draft, Writing – review & editing, Visualization. **Rex Devasahayam Arokia Balaya:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization. **Saptami Kanekar:** Writing – original draft, review & editing, Visualization. **Rajesh Raju:** Writing – review & editing. **Thottethodi Subrahmanya Keshava Prasad**: Writing – review & editing. **Richard K. Kandasamy:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2023.02.051.

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