

SIGNALING IN BACTERIA

Abstract

The chapter delves into the intricate mechanisms governing communication among bacterial populations. It investigates quorum sensing, elucidating its molecular intricacies and its impact on collective behaviors like biofilm formation and virulence factor expression. Additionally, the chapter explores two-component signal transduction systems, uncovering their role in bacterial adaptation via environmental sensing and response regulation. Emerging signaling molecules like c-di-GMP and small RNAs are discussed, showcasing their contribution to complex regulatory networks. The chapter also emphasizes the significance of bacterial signaling in pathogenesis, exemplified by pathogens like *Vibrio cholerae*. Overall, the chapter underscores the pivotal role of bacterial signaling in shaping microbial behaviors, ecological interactions, and potential practical applications.

Keywords: Quorum sensing; Two-component systems; Molecular mechanisms; Biofilm formation; Virulence factors; c-di-GMP; Ecological interaction.

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I. CHAPTER OVERVIEW

This chapter is divided into 5 major sections each dealing with a unique dimension of the signaling widely spread throughout prokaryotes.

- 1. Quorum sensing: coordinating collective behaviors:** This section explores the concept of quorum sensing as a pivotal mechanism through which bacteria coordinate their behaviors based on population density. It covers the diverse types of quorum sensing systems, the molecular processes behind quorum sensing molecule synthesis and detection, and the regulatory networks they activate. The role of quorum sensing in biofilm formation, virulence factor expression, and symbiotic interactions is explored, shedding light on its significance in various ecological contexts and potential practical applications.
- 2. Two-component signal transduction systems: environmental adaptation:** The section provides a comprehensive exploration of these vital communication pathways within bacteria. It starts by introducing the fundamental components of these systems, highlighting their prevalence and variations across bacterial species. The sensory capabilities of sensor kinases are detailed, explaining how these domains detect environmental cues such as nutrients, temperature, and pH shifts. The subsequent discussion on response regulators delves into the intricate processes of phosphorylation, signal transduction, and diverse response mechanisms that these regulators employ to influence gene expression and trigger specific outputs. The integration of sensing and response is examined, showcasing how crosstalk and cross-phosphorylation contribute to intricate regulatory networks. This section also emphasizes the role of two-component systems in bacterial adaptation, emphasizing their rapid response to environmental changes and their impact on the evolutionary fitness of bacteria. Furthermore, stress responses and nutrient sensing are explored, revealing the involvement of two-component systems in various stress-related pathways and metabolic regulation. Collectively, this section provides a comprehensive understanding of how two-component systems contribute to bacteria's ability to adapt and thrive in dynamic environments.
- 3. Emerging players in bacterial signaling:** In this section, we explore the diverse array of emerging signaling molecules beyond traditional quorum sensing paradigms. We delve into the discovery of molecules like Autoinducer-2 (AI-2) and their role in mediating interspecies communication. Additionally, we investigate the role of cyclic di-GMP (c-di-GMP), a second messenger molecule, in regulating biofilm formation and other bacterial behaviors. The significance of small RNAs in post-transcriptional gene regulation is discussed, along with their involvement in stress responses and adaptation. The section also emphasizes the intricate cross-talk and integration of these signaling pathways, highlighting the complexity of bacterial regulatory networks. Finally, the potential applications of this expanding knowledge, including synthetic biology and therapeutic interventions, are contemplated.
- 4. Signaling and pathogenesis: *Vibrio cholerae* case study:** Here, we explore the role of bacterial signaling in the context of pathogenesis, focusing on the case study of *Vibrio cholerae*, a well-known pathogenic bacterium responsible for cholera outbreaks. The section begins by introducing *Vibrio cholerae* as a model pathogen, emphasizing its significance in both historical and modern contexts. It then delves into the intricate

relationship between quorum sensing and virulence in *Vibrio cholerae*, discussing specific quorum sensing systems (such as HapR and CqsA/CqsS) and their regulation of toxin production and infection-related factors. Furthermore, the section explores the role of two-component systems in pathogenicity of *Vibrio cholerae*, highlighting key players like TcpPH and ToxR-Regulated Systems. The implications of these signaling pathways for the development of innovative treatments are also examined, including the potential for targeting quorum sensing and modulating two-component systems as strategies to mitigate bacterial infections. The section concludes by addressing the challenges and prospects of translating these insights into practical therapeutic applications

5. Practical applications and future perspectives: The practical implications of understanding bacterial signaling pathways are far-reaching. Biotechnology stands to benefit from manipulating bacterial communication for various purposes. Controlled biofilm formation could optimize industrial processes requiring surface attachment, while exploiting quorum sensing can enhance bioremediation strategies by guiding microbes to degrade pollutants efficiently. Additionally, modulating signaling pathways offers potential for producing valuable compounds through microbial factories. From a therapeutic perspective, disrupting bacterial communication pathways holds promise as an alternative to traditional antibiotics. Inhibiting quorum sensing in pathogens could render them less virulent and easier to treat. Similarly, targeting two-component systems might provide new avenues for tackling antibiotic-resistant bacteria, potentially synergizing with existing antibiotics for improved outcomes. Looking ahead, the exploration of bacterial signaling is still in its infancy, presenting exciting avenues for future research. Investigating inter-species communication and its ecological implications could unveil new dimensions of microbial interactions. Furthermore, manipulating bacterial signaling within the human microbiome might lead to innovative health interventions. In the realm of synthetic biology, designing custom signaling pathways could revolutionize biotechnological applications. In conclusion, delving into the practical applications of bacterial signaling not only unveils innovative biotechnological and therapeutic prospects but also opens doors to uncharted territories in microbial communication research.

II. INTRODUCTION

Bacterial signaling is a unique process by which bacteria communicate and coordinate their behavior by the secretion, perception and accumulation of signaling molecules[1,2]. This phenomenon was termed as ‘Quorum Sensing’ by Clay Fuqua, E.P.Greenberg and Steve Winson in 1994 [2]. Quorum sensing is a molecular approach of signaling that mediates cell-to-cell communication[2]. Bacteria have a well-organized social life. QS network provides bacteria with a method to collect information from its surrounding and make appropriate decisions using the stimuli from the environment [2]. Using bacterial quorum sensing, both intra-species and inter-species gene regulation is possible and the bacteria can control a variety of traits and several physiological and biochemical functions [3]. The QS helps in regulation of a range of social behavior such as those which promote the fitness of other bacteria or have a positive impact in presence of other competing bacteria by secreting substances that can alter the environment or by secreting toxins that can negatively affect other bacterial strains or species, and horizontal gene transfer[1]. Besides, the signaling plays a vital role in the interaction between the bacteria and the set of mobile elements it contains, for instance, the plasmids, prophages, pathogenicity islands, restriction and modification

systems, transposons and others [1,4]. The intercellular signaling is also the backbone of host-pathogen interaction [1]. Bacteria accomplish this by secreting out diffusible signal molecule that can be sensed by the producer bacteria itself[1]. QS is ubiquitously present in the bacterial kingdom and has undergone several independent evolutionary cycles. These multiple independent evolutions have become the reason for the diverse kinds of signaling molecules, the mode of sensing, the conditions accompanying the synthesis and the detection of signal molecules and the type of mediated response [1]. Plant or animal hosts and the parasites of bacteria can monitor the bacterial signaling and manipulate it either by causing the disruption of signal molecules or by producing molecules that can either activate or inhibit the QS receptors. This is an effective defense strategy[1]. In contrast, the symbiotic and pathogenic bacteria need the QS to maintain their relationships with the hosts[5].

III. DISCOVERY

The genetic circuit of QS was first discovered in *Vibrio fischeri*(*Allivibriofischeri*), a gram-negative, marine and symbiotic bacterium frequently found populating the light organ of *Euprymna scolopes* (bobtail squid). The Lux operon produces bioluminescence due to expression of Luciferase enzyme in high cell density[6]. Because the QS regulated trait is easy to detect, *Vibrioshave* become a model organism to study QS[7]. When the moonlight penetrates the sea waters at night, organisms cast shadows when seen from below and so any opaque organism can easily be spotted by the predators[8]. To prevent this, the *E. scolope* recruits the bacteria *V. fischeri* inside its light organ. As some of the bacteria enter the organ, they begin to multiply at a rapid rate and soon, large number of bacteria populate the light organ and produce bioluminescence[8]. This helps the squid avoid casting shadows and hence being seen by predators. But light production is certainly costly for bacteria in terms of energy requirements and that is why quorum sensing is useful. QS helps the bacteria in detecting the cell density and make decision for investing energy is energy-intensive process. If the cell density is low in the light organ, then the bioluminescence is not produced. The bacteria produce bioluminescence only their numbers are high enough to produce required light. This helps the squid avoid predators and also the bacteria benefit by getting a nutrient rich environment[8]. The LuxI protein (product of *luxI* gene) is responsible for the production of a 3OC6-homoserine lactone (HSL) which acts as the autoinducer. When the signal concentration reaches threshold at HCD, the HSL binds to the product of *luxR* gene (LuxR protein). The HSL-LuxR structure binds to the promoter of the *luxCDABE* operon and activates its transcription which further leads to increase in transcription of *luxI* gene and ultimately bioluminescence is produced [8].

IV. QUORUM SIGNALING IN VIBRIO HARVEYI

This bacterium is capable of synthesizing and responding to at least three different autoinducers as shown in Table 1. LuxO is a response regulator protein which is phosphorylated at low-cell density (LCD) as there are insufficient number of signal molecules to bind the receptors and hence they act as kinase and phosphorylate the phosphotransfer protein, LuxU which transfers the phosphate to the response regulator, LuxO[7]. The LuxO~P can activate the transcription of genes which produces 5 small RNA known as quorum regulatory RNAs (qrr 1-5)[7,9]. It can destabilize the *LuxR* transcript and hence the master regulator, LuxR is not produced. Simultaneously, the qrr can activate the translation of AphaA (transcription factor) and hence AphaA is produced. At high cell density, when the autoinducers bind to their sensors, the phosphorelay is reversed and the AI/Sensor

act as phosphatase which via LuxU can dephosphorylate the LuxO. This stops the transcription of genes as well as the production of qrr (sRNAs) and this stimulates the *LuxR* transcript to code of the LuxR protein while the AphaA production comes to halt [7,9].

Table 1: Different Autoinducers Found in *Vibrio harveyi*

| Producer of Autoinducer | LuxM | CqsA | LuxS |
|-------------------------|--|-------------------------------------|---|
| Type of autoinducer | HAI-1 (3-hydroxybutanoyl homoserine lactone) | CAI-1 [(S)-3-hydroxytridecan-4-one] | AI-2 [(2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuranborate] |
| Sensor/Receptor | LuxN | CqsS | LuxPQ |
| Communication mediated | Intra-species | Intra-genera | Inter-species |

LuxR is a member of the TetR superfamily and has the ability to control the expression of genes in the QS regulon. It can activate as well as repress the expression of genes [9]. Different classes of QS target genes according to their affinity for LuxR are shown in Table 2.

Table 2: Classes of QS genes

| Target genes | Class I | Class II | Class III |
|--------------------------------|-----------------|-----------------------|---------------|
| Affinity of promoters to LuxR | Lowest affinity | Intermediate affinity | High affinity |
| Concentration of LuxR requires | high | intermediate | lowest |

V. ROLE OF QS IN THE SURVIVAL OF BACTERIA

Quorum signaling helps bacteria in detecting the population size of bacteria by sensing concentration of signal molecules in their environment and thereby controlling investment of energy in processes regulating costly phenotypic traits which is possible only in high cell-density [10]. The synthesis and the release of QS signals leads to the regulation of the production of diverse kinds of extracellular factors from cells which controls behaviors like swarming and motility, virulence, conjugation, competence, scavenging for nutrients, bacteriocin production, pathogenesis, synthesis of antibiotics, suppression of host immune system, formation of biofilms [3,10]. These behaviors can only be achieved by cooperation and coordination of the all members of bacterial community [10]. At lower cell-density, QS signals are produced by cell at basal rate. As the cell-density approaches threshold, the concentration of signal molecule increases in the environment and leads to the expression of QS-dependent genes and the traits regulated by QS are switched on [10]. Several QS-systems employ an autoregulatory positive feedback loop that promotes the production of

further signals [11]. Many QS-regulated phenotypes are examples of so-called ‘public good’ which are the products secreted by individual for the benefit of all cells of the population[11].

VI. METHODS OF SIGNAL TRANSDUCTION

Unlike complex multicellular organisms that have receptor organs to sense environmental stimuli, prokaryotes display a simplified signal transduction mechanism to sense environmental alterations and regulate their cellular responses accordingly. The changing environmental conditions may sometimes not be optimal and the survival of bacteria in such unstable environmental conditions requires quick, adaptive responses under control of regulatory protein. Studies focusing on bacterial communication, have reported two pathways for signal transduction – one component systems (OCS) and two component systems (TCS)[12].

Most researchers consider the two-component system as the primary signal transduction pathway using which the prokaryotes respond to their environment. It typically comprises of two proteins – first, a receptor histidine kinase that senses the environmental cue using its extracellular input domain and second, the cytosolic cognate response regulator for which the output domain is phosphorylated and initiates a cellular response[13]. This mostly controls the gene expression at transcriptional level through the DNA-binding HTH (helix-turn-helix) domain of response regulator. Even though this system provides efficient mode of signaling, it is less frequently utilized as compared to the one-component pathway which dominates the signal transduction in prokaryotes[12]. OCS is an evolutionarily primitive pathway and has more simple makeup as the input domain and the output domain are fused into a single protein molecule. Furthermore, greater diversity is witnessed with respect to the input and output domains in one-component than the two-component systems. Consequently, it is assumed that the OCS are progenitors of the TCS[14]. The regulatory properties of several one-component systems have been experimentally determined. More than 20 families or classes of one-component pathway involved in regulating different processes have been determined. These families can be described on the basis of conserved sequence of amino acids or the primary structure of protein in their DNA-binding domain and are defined by different conserved motifs. The two-component system were the first major signal transduction to be identified in the prokaryotes. Genomics tools have helped in better identification and characterization of the OCS and TCS in bacterial genomes as well as in complex eukaryotic organisms. Unlike ONCs, majority of the TCS have been found to be membrane associated rather than being cytoplasmic. In bacteria, the signaling systems have been shown to promote virulence. This system is composed of two protein units – one of which acts as sensor and the other acts as the regulator but there may be additional components in few others[15]. The histidine-kinase makes up the input or the sensor domain consists of a transmitter unit within. The response regulator contains the receiver and the regulator or the output domain[16]. The input domain communicates via the phosphorylation[15]. Quorum sensing that occurs in response to high cell density also utilizes the two-component system especially in the gram-positive bacteria. The OCS and the TCS are related mainly due to the shared repositories of the sensor and the receptor protein domains.

VII. QUORUM SENSING SYSTEMS

Several families of QS regulators have been elucidated in gram-positive as well as gram-negative bacteria. Each bacteria reveals the presence of several such families of regulator molecules working simultaneously in the cell. The enormous diversity of the signaling molecules and further their intraspecific signaling variants (known as pherotypes) imply towards the existence of selection forces that contributes to the creation and maintenance of diversity. According to Aframian and Elder, the wide diversity is probably driven either from the interaction between different variants or because of the different chemical nature of the signal molecules within a family that affect their properties in a given set of environmental condition [1]. At the molecular level, the evolution of a novel signal QS signaling variant is a co-evolutionary process in which both the signaling genes and the receptor genes undergo mutations.

- 1. AHLs (N-Acyl homoserine lactones):** These signal molecules mediate QS in gram-negative bacteria. The AHL-dependent QS is mediated by homologs of two proteins, LuxI and LuxR [10]. LuxI is the synthase which produces the signal molecule, AHL. LuxR is the AHL signal receptor [10]. It is a cytoplasmic transcriptional regulator that controls expression of genes responsible for group behavior [10]. The lipophilic AHL molecules can easily cross the cell membranes without the use of transporters [17]. Due to the solubility of AHLs in lipids, they can rapidly enter mammalian cells and induce apoptosis in the host cells [18]. Each organism produces different AHL molecules, which usually differ in the length of fatty acid chains generally varying from C4-C18. Sometimes, they may also contain unsaturation or substitutions in chain [10]. Each AHL system is considered to be species-specific. As each organism recognizes the AHL produced by them. The AHL system require few components, namely LuxR, AHL synthases and the relevant promoters.
- 2. AIP (Autoinducing Peptides):** These are also known as peptide pheromone network [6]. These are small peptides that mediate QS in gram-positive bacteria [10]. These are 5-17 amino acids long linear or cyclic peptides synthesized as precursors from the ribosomes [10]. AIPs are synthesized by ribosomes and exhibit enormous structural variety and are frequently subjected to post-translational changes during secretion, aided by an ABC (ATP-binding cassette) transporter located on the membrane and they become activated and stabilized. When population density rises, AIPs accumulate in the environment [17]. The peptides act by binding to sensor kinase signal receptor in the bacterial membrane which consequently phosphorylates conserved histidine residue when a particular threshold level is reached [17]. The phosphoryl group is then transferred by the active receptor kinase to a conserved aspartate residue of the intracellular cytoplasmic response regulator which is then activated can control transcription of target genes such as AIP genes, receptor kinase and response regulator genes, and ABC transporter genes [10,17].
- 3. AI-2 (Autoinducer 2):** It is a furanosyl borate diester [11,19]. It is a product of the LuxS enzyme [3]. The *luxS* gene can be found in more than half of all sequenced bacterial genomes. LuxS enzyme synthesizes the precursor of AI-2, DPD (4,5-dihydroxy-2,3-pentanedione) as a by-product of AMC (activated methyl cycle). It is highly active and can spontaneously cyclize and rearrange to form DPD derivatives [20]. These DPD derivatives are interconvertible and the different forms are seen to exist in equilibrium

and are sensed as autoinducers by different bacteria [20]. In *E. coli*, AI-2 is imported into the cell by LsrACDB. It is then phosphorylated by the LsrK kinase and the phosphorylated AI-2 (AI-2~P) binds to the repressor, LsrR, ultimately relieving the repression of the bidirectional *lsr* promoter [21]. This causes transcription of the *lsr* operon and overexpression of LsrACDB, LsrK, LsrR, and LsrFG, resulting in rapid uptake of AI-2 and reduction of AI-2 from the extracellular media. LsrFG eventually metabolizes the AI-2 molecule [21]. This is present in both gram-positive and gram-negative bacteria and thus AI-2 has been proposed as a method of inter-species communication [11]. It has been accepted as a universal language by which bacteria communicate [20]. This has been noted in the interaction of *Escherichia coli* and *Vibrio harveyi*. The AI-2 produced by *E. coli* can induce the QS of *V. harveyi* to produce bioluminescence and the AI-2 of the *V. harveyi* can be sensed by *E. coli* to induce its Lsr system [3,20].

- 4. Indole:** Indole is widely spread in prokaryotic and eukaryotic communities and can serve as language for inter-species and inter-kingdom communication [22]. Certain bacteria have enzyme tryptophanase (TnaA), which has the ability to convert the substrate L-tryptophan into indole [3]. This signaling molecule is known to regulate cell division, stability of plasmid, antibiotic tolerance and spore formation in gram-positive bacteria [22].
- 5. QS System in gram-negative Bacteria:** *Vibrio cholerae* is a curved, rod-shaped, motile, gram-negative bacteria that lives in aquatic environment. It is the causative agent of cholera [23]. Cholera is a highly contagious bacterial diarrheal disease and is a major public health disease affecting millions of people. Since 1817, there has been 7 pandemics involving cholera, starting from 1961. The incubation period for cholera can last from 12 hours to 5 days. *V. cholerae* must quickly become adapted to the human digestive system after ingestion and for this purpose, the bacteria exploits a signal transduction network that controls the gene expression in response to various environmental stimuli throughout the gastrointestinal tract [23]. *Vibrio cholerae* depends on QS to regulate crucial cellular processes for the survival and adaptability both inside and outside of its hosts. The *V. cholerae* QS system has served as a model for understanding how pathogens use QS for precise regulation of virulence factor production. According to the present understanding, when *V. cholerae* cells first enter the human small intestine, the population of cells is relatively low, and hence the QS response is not triggered. The master QS regulator HapR, which represses *V. cholerae* virulence genes, is not expressed at this stage of infection. As a result, virulence factors such as Toxin-Coregulated Pili (TCP) and Cholera Toxin (CTX) are produced. The production of these substances allows the pathogen to colonize inside the small intestine of host and cause the characteristic watery diarrhea. Once *V. cholerae* has colonized the host, population density rises and the QS response is activated. As a result, HapR is generated and production of virulence factor is suppressed. Some of the genes activated by QS at high cell density (HCD) are considered to enable the *V. cholerae* population from exiting the host. *V. cholerae* mutants stuck in HCD state do not create TCP or CTX and are not capable of colonizing hosts [24].

Vibrio cholerae has two parallel QS systems to control virulence including AI-2/LuxQ and CAI1/CqsA [10,24]. The first system is made of CAI-1 ((S)-3-hydroxytridecan-4-one), the signal molecule which is synthesized by the enzyme, CqsA synthase and the CqsS, its cognate receptor which is a transmembrane

histidine kinase (HK)[24,25]. The second system is composed of AI-2(2S, 4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate), synthesized by the LuxS synthase enzyme, as the signal molecule and its cognate receptor LuxPQ, another transmembrane HK. CqsS and LuxPQ are two-component proteins that have both the kinase and phosphatase activities[25]. The kinase activity of the receptors, CqsS and LuxPQ is inversely proportional to the concentration of signal molecules CAI-1 and AI-2 respectively. This means that the total receptor kinase activity is inversely proportional to the cell density[25].

At low cell density (LCD), when the HK receptors, LuxQ and CqsA are not bound to their ligand signal molecules, they cause phosphorylation of transcription factor LuxO that induces transcription of *qrr* sRNA which represses master regulator HapR[10,25]. At LCD, *hapR* gene is repressed and the expression of virulence factor is stimulated [10]. The repression of HapR activates expression of genes of *ctx* and *tcp* operon. The two membrane proteins, ToxR and ToxS control the expression of both the *tcp* and *ctx* operons[26]. ToxR can bind to the promoter of the *ctx* operon, which contains genes coding for the two outer membrane proteins OmpU and OmpT, and the gene encoding ToxT, a downstream transcription regulator of ToxR regulon which when expressed can directly affect the gene expression of *ctx* and *tcp* operon[26]. However, two other membrane proteins, TcpP and TcpH, are also required to work along with ToxR and ToxS to activate *toxT* transcription[26]. Virulence factors like CT which causes severe diarrhea and TCP which promotes self-aggregation of bacteria and colonization are produced[10]. Both the CT and TCP are under control of QS and exhibited at LCD[10]. When the cell-density becomes high, HapR upregulates the production of flagellum and represses genes involved in biofilm and virulence[10]. This lets bacteria be transferred to the diarrheal fluids and be excreted outside and allows further transmission[10].

- 6. QS system in gram-positive bacteria:** QS has been well-studied in infections caused by *Staphylococcus aureus* which is known to be a leading cause of both acute- and chronic-infections [10]. The QS is mediated by AIP, a post-transcriptionally modified oligopeptide which is synthesized from expression of *agrD*, and exported by AgrB, a membrane protein [10,19,27]. The mature AIP binds to AgrC, histidine kinase transmembrane receptor [10]. It further causes the phosphorylation of AgrA, the cognate response regulator [10,27]. The phosphorylation of AgrA leads to production of two transcripts, RNAI (which regulates Agr system) I and RNAIII (which regulates the expression of several virulence genes) through the activation of promoters P2 and P3 respectively [10]. Low levels of *agr* expression, promotes colonization and high levels of *agr* expression which leads to production of enzymes which can damage tissues and allows their dispersal to other tissues via bloodstream [10].

VIII. c-di-GMP

c-di-GMP is a secondary messenger molecule belonging to the class of cyclic dinucleotides (CDN). It has been known since the late 1980s and now has been recognized as being nearly ubiquitous that controls various aspects of bacterial growth and behavior. c-di-GMP was the first CDN to be discovered in 1987 by Moshe Benziman. He reported “an unusual cyclic nucleotide activator”, a compound that was able to stimulate cellulose synthase from *Komagataeibacter xylinus*, and this compound was later recognized as bis-(3'-

5')-cyclic diguanylic acid (c-di-GMP). It is the most intensely studied and the best understood member of this protein family.

The c-di-GMP monomer has two GMP moieties that are fused by a 5'-3' macrocyclic ring and it displays two-fold symmetry. The c-di-GMP is a monomer in solution, and the intercalated dimers develop through the sequential binding of two monomers to particular effector proteins. The cellular levels of c-di-GMP are controlled by both internal and external signals. This is accomplished through the activity of two antagonistic enzyme families: diguanylate cyclases (DGCs) and c-di-GMP-specific phosphodiesterases (PDEs) with enzymes responsible for c-di-AMP metabolism [28]. Changes in the expression or activity of c-di-GMP-metabolizing enzymes (CMEs) in response to quorum sensing (QS) signals influence cellular levels of c-di-GMP. The enzymes of the DGC and PDE families are found in all major bacterial phyla, making them two of the biggest known signaling protein families in prokaryotes.

DGCs catalyze the formation of c-di-GMP by the cooperative action of their two catalytic GGDEF domains, which are arranged antiparallel with one GTP molecule attached to each protomer [28]. Diguanylate cyclases (DGCs) use a characteristic glycine-glycine-aspartic acid-phenylalanine (GGDEF) motif to synthesize c-di-GMP from two GTP molecules. c-di-GMP molecule is degraded by hydrolases with conserved glutamic acid-alanine-leucine (EAL) or histidine-aspartic acid/glycine-tyrosine-proline (HD-GYP) motifs. The HD-GYP motif converts c-di-GMP directly into two GMP molecules, and the EAL-domain proteins linearize c-di-GMP to pGpG, which are then cleaved into two GMP molecules [29].

The effect of this molecule on bacterial physiology and behavior is dependent on its sensors, which includes mRNA riboswitches and target proteins such transcriptional regulators, enzymes, metabolic switches, and adaptor protein. c-di-GMP signaling has been known to modulate virulence in a variety of mammalian pathogens. Low c-di-GMP levels are linked with motility and highly pathogenic state, whereas high c-di-GMP levels favor biofilm formation and persistent infections. Recent studies have revealed that c-di-GMP adversely regulates the type III secretion systems (T3SSs) of different pathogenic bacteria [30].

IX. SMALL RNAs: POST-TRANSCRIPTIONAL REGULATION

Studies on short RNA (sRNA) regulation of QS were first published in 2006 and 2007. The QS-circuits of the gram-negative family Vibrionaceae and the Agr QS circuit of the gram-positive Staphylococci are completely dependent on sRNA. These are auxiliary regulators that directly or indirectly affect the QS signaling [31]. The small regulatory RNA act by base-pairing with the target mRNA targets to repress or activate the gene expression. In *P. aeruginosa*, the sRNAs PrrF1, PrrF2, and PhrS control the PQS (*Pseudomonas* Quinolone Signal) system in response to limiting levels of iron and oxygen respectively. PhrS stimulates translation of an ORF at the 5'-end of the pqsR mRNA and this initiates pqsR translation by changing the secondary structure surrounding the pqrR RBS. PrrF1 and PrrF2 suppress the expression of genes coding for enzymes that degrade anthranilate (the precursor of PQS), thus allowing increased PQS formation under low iron conditions. RsmY and RsmZ are two sRNAs involved in the QS control of *P. aeruginosa*. These are activated by the GacS/GacA, two-component system in response to limiting levels of magnesium along with additional unknown signals. RsmY and RsmZ inhibit the transcription factor RsmA, which in turn

inhibits the synthesis of 3OC12HSL and C4HSL which are lipid-soluble AHL unique to this bacterium[32,33].sRNAs are categorized according to their genomic location and also the mechanism of regulation. They are not expressed constitutively and instead respond to environmental variates to alter the expression of the target gene. These particular situations may include stationary phase transition, thermal shock, oxidative stress, and a variety of other stimuli. While some sRNAs are conserved across the bacterial groups, say for instance the structural "housekeeping" sRNAs like 6S, tmRNA, and RNaseP, they are frequently specific to particular order or species. The sRNAs have better properties as compared to the usual transcription factors, as they act in a fast, reversible manner and can bind to multiple targets while consuming less energy as energy is not required in initiating the translation. These features allow them to control a wide spectrum of biological activities [34]. sRNA is known to upregulate and downregulate several genes involved in the virulence of a bacteria. In *S. aureus*, RNAlII (sRNA) coordinates the transition from colonization to infection stage by directly or indirectly controlling the expression of large set of genes. In *Salmonella typhimurium*, a pathogenicity encoded island sRNA, IsrM is seen which does not have contribution in the bacterial growth but it is seen to be upregulated in ileum of small intestine during infections. A special feature of sRNAs is their ability to alter host-pathogen connections after being delivered into host cells via outer membrane vesicles. The sRNA content in extracellular vesicles has been characterized and linkages between sRNAs and host immunological response have also been found in *P.aeruginosa*, *Helicobacter pylori*, and *Vibrio fischeri*[34]. sRNA is also seen to mediate antimicrobial responses and resistance. When multi-drug resistant bacterial cell cultures were exposed to antibiotic doses, expression of several sRNAs were seen. A lot of sRNAs are involved in the regulation of fitness and adaptability[34].

X. METHODS OF DECODING BACTERIAL COMMUNICATION

Systems Biology approaches are used to unravel the regulatory networks and to understand the complex microbial relationship. Systems biology provides an effective experimental technique and offers a holistic approach for the characterization of microbial communities. In this approach, complete microbial communities are regarded as metaorganisms, and each of the DNA, RNA, proteins, and metabolites are investigated along with in situ environmental variables. Each level of biological information provides a different level of characterization of the metaorganisms. The metagenome talks about the potential of microbial communities by revealing the genes that could possibly be expressed by the metaorganism. The metatranscriptome, including mRNA and non-coding RNAs, provides some information about the regulatory networks and gene expression at the time of sampling. The metaproteome together with the metatranscriptome provides information on the functionality of the microbial communities. Moreover, the metaproteome also gives access to regulatory networks and when combined with the metabolome, gives significant information on the microbial activities[35].

XI. APPLICATIONS

- 1. Synthetic Biology:** The field of synthetic biology aims to synthesize biological models with programmed predictive behaviors. It also aims to incorporate engineering design principles. Various studies in synthetic biology makes use of QS circuits to program cell behavior. Researches on QS and synthetic biology have complemented each other as the

research on QS expands the toolkit of synthetic biology and reciprocally, the research in synthetic biology provides new tool for investigating quorum sensing[21].

- QS-based genetic circuits confer special properties to cell that can be utilized in several different purposes in engineering[2]. Bacteria use QS and behave like multicellular systems. In the past decade, several research groups have demonstrated artificial intra-species and inter-species communication with the help of synthetic circuits that incorporate several components of the bacterial QS system. These engineered QS-based circuits have tremendous biotechnological application especially with respect to production of biochemicals. It is also helpful in tissue engineering and mixed species fermentation[36].

The field of metabolic engineering has seen advancement by incorporating QS-network into production strain. QS has been used to synchronize gene expression across the bacterial population to decrease variability between cells in order to increase the yield of engineered traits [6].

- Redesigned QS circuit can promote communication and aid in development of synthetic microbial consortium [2].
 - Engineered QS system can intervene between interkingdom signaling as in interaction of eukaryotic host (for example, plants and mammalian cells) and bacterial pathogen [2]. It has been discovered that QS signals can also modulate behavior of viruses. QS-mediated interaction is also seen between bacteria and abiotic artificial electrodes [2].
 - QS incorporated bio-hybrid devices offer innovative approaches to program the cell behavior and biological functions [2]. Examples include logic gates, genetic oscillators and toggle switches that use AHL-mediated signaling [3]. Combining these devices with their autoinducers and synthetic QS circuits can help in dynamic control of bacterial populations which may include control of the population size, control of the physiological processes and the metabolic control in order to synthesize desired products [3].
- 2. Whole-cell Biosensors:** These are engineered cells that can detect and signal the presence of a particular condition. They also have advantage of being biocompatible as well as renewable as compared to their chemical and electrical parts[2]. There are biosensors that can be used to detect new QS inhibitors (made from *V. harvey* strain BB170) [2]. The cells of *Pseudomonas aeruginosa* were returned into biosensor by fusing the QS promoters with fluorescence genes to report the genetic expression of four different QS networks[2]. QS-based biosensors also find application in detecting new pathogens, infection markers and also pollutants [2]. Most of them can act on recognition of AHLs produce by gram negative bacteria [36].
 - 3. Therapeutics:** Due to the recent advancements in the discipline of synthetic biology, bacteria can be programmed with specific therapeutic functions. In-vitro tests have confirmed the ability of bacteria to detect infections caused by the *Pseudomonas aeruginosa* and *Enterococcus faecalis* by exploiting the species specificity of the innate quorum signals [2]. The scope of this is not just limited to diagnostics but also effective

inhibition and elimination of the bacteria as well as biofilms by the release of bacteriostatic or bactericidal compounds[2]. By manipulating QS-systems, cells that are seen to preferentially grow in certain environments, like *Salmonellain* the tumor tissues, have been made to express genes coding for cytotoxic compounds and unwanted expression in healthy body tissues is avoided. In this way, cells have been converted into anti-cancer therapies[2]. It is known that QS systems aid in forming persister cells, virulence factors and extracellular matrices which can lead to formation of biofilms [37]. Therefore, inhibiting the QS can be an effective strategy to mitigate infections caused by biofilm formations. For this reason, quorum sensing inhibitors (QSIs) which block the QS and quorum quenching (QQ) enzymes which degrade the signals can be utilized [3,37]. This can be done in three ways – by blocking the production of AHL, inactivation of AHL molecule and interference of the receptor [38]. The inactivation of AHL by enzymes can be done by the use of lactonases, oxidoreductases or the amidases [39].

- 4. Restoration of Degraded Environment:** Modulating QS of biofilm forming bacteria is a promising strategy to can help in the bioremediation of persistentorganic pollutants (POPs). The POPs are non-biodegradable and they tend to stay in the environment and can also be a major health concern as it may bioaccumulate in the tissues of animals and humans [40]. Occurrence of numerous severe diseases have been correlated with the presence of POPs. Hence, their removal is essential for protecting the health and environment. With the help of quorum sensing, bacteria can form biofilms that can help in biodegradation of organic pollutants and can speed up the process of wastewater treatment. The biofilms can also trap and immobilize toxic metals within itself [40].
- 5. Biosynthesis of Desired Compounds:** QS systems can be exploited for metabolic engineering that can remodel the natural regulatory mechanism for deriving maximum benefit[2]. The advantage of engineered QS circuits is that it allows production of required compounds without external induction or input and can avoid the state of metabolic burden and can signal metabolic burden if it exists during the initial stages as a result of genetic modifications [2].Such a system was created in *Escherichia coli* which was modified to be a self-induction system for production of bisabolene. Another semi-autonomous circuit was also established for the production of isopropanol[2]. Another method involved repressing genes of the metabolic pathway for the synthesis of desired products. In *Saccharomyces cerevisiae*, p-hydroxybenzoic acid production was stimulated by silencing the genes that coded for competing products. In *Pantoeastewartia*, production of D-glucaric acid was increased by switching off shikimate kinase and phosphofructokinase-I. This led to increase in production of glucaric acid, myo-inositol and shikimic acid [2].
- 6. Engineering Microbial Consortium:** Microbial consortium is more stable as compared to an individual microbe and can perform several functions due to interactions and division of labor between different sub-populations of bacteria. They show versatility, cooperation and communication between species and are robust enough to face environmental challenges [2]. From the perspective of a synthetic biology, engineering microbial consortium can be more useful as it will reduce the complexity involved in engineering bacteria by distributingtasks between sub-populations to avoid metabolic burden [2]. This compartmentalization of functionleads to specialization and increase in the modularity which allow easy and precise control of different parts of the system[2]. The bidirectional communication in microbial co-cultures allows population density-

dependent control and regulation of gene expression which could be used for production of enzymes, toxins. It also paved way for the development of synthetic biofilms [2]. Synthetic biology can also provide tools that has ability to detect and sometimes every modify the natural microbial consortium such as the gastrointestinal tract, skin microbiome and plant microbiome[21].Despite the potential benefits, engineering microbial consortia comes several difficulties, the most prominent of which is crosstalk between quorum systems. There can be signal crosstalk that involves binding of a receptor with AI of another quorum system. An example of this is seen in infection of cystic fibrosis, where *Burkholderiacepacia* and *Pseudomonas aeruginosa*, are known to co-infect patients and the QS regulator of each pathogen responds to the autoinducer of each other resulting in the coordination of virulence gene expression [6]. Promoter crosstalk is seen when activated receptors can bind to a different promoter. In certain cases, a combination of signal and promoter crosstalk is also evident where a receptor activated by a different AI which binds to another promoter [2]. This crosstalk can be avoided by developing QS system with complete orthogonality (inability to interact despite similarities) can be developed. Another possibility is to exploit this crosstalk and create unique QS circuits with novel applications [2]. Synthesizing microbial consortium with artificial genetic circuits based on QS-devices is an appealing therapeutic alternative to deal with biofilms of infectious pathogen especially *Pseudomonas aeruginosa*.

XII. CONCLUSION

The exploration of bacterial signaling unveils a complex tapestry of communication mechanisms that orchestrate microbial behaviors, interactions, and adaptations. From the foundational role of quorum sensing in coordinating collective actions to the nuanced functioning of two-component systems in environmental responsiveness, the intricate world of bacterial signaling is both fascinating and pivotal. The discovery of emerging signaling molecules like c-di-GMP and small RNAs adds depth to our understanding of the regulatory networks that shape bacterial behaviors. Moreover, the study of bacterial signaling's implications in pathogenesis, exemplified by the case of *Vibrio cholerae*, highlights its potential for innovative therapeutic strategies against bacterial infections. As we navigate practical applications, including biotechnological advancements and therapeutic interventions, the horizon of bacterial signaling research continues to expand. This chapter underscores the far-reaching impact of signaling mechanisms on microbial communities, ecological dynamics, and the broader spectrum of scientific inquiry. As new discoveries await and uncharted pathways beckon, exploring bacterial signaling remains an exciting avenue for unlocking the mysteries of microbial life and harnessing its potential for diverse applications.

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