Talking About Talking Microbes

2

Abstract

Bacterial quorum sensing mechanism is considered as the gene expression regulator in response to fluctuations in bacterial cell population density. This communication process is controlled by autoinducers. So bacteria can talk to each other using autoinducers. We introduce bacterial talking mechanism or communication process in this chapter. We briefly discuss quorum sensing process in cases of different bacteria such as LuxI/ LuxR type quorum sensing, LasI/LasR- RhlI/RhlR system, TraI/TraR system, ExpI/ExpR-CarI/CarR system, ComD/ComE system, ComP/ComA system, AgrC/AgrA system and LuxS family (interspecies communication). Here, we study the communication among the bacteria through chemical signalling only.

2.1 Bacterial Quorum Sensing Mechanism

Bacteria secrete molecules which are used for their communication with other surrounding bacteria (interspecies and intraspecies). This small secreted diffusible molecule is a key controller of the communication mechanism which is formally known as autoinducer or quorum sensing molecule (QSM) or chemical signalling molecule. Bacteria receive these chemical signals from other bacteria with the purpose of coordinating a collective behaviour. Bacteria emit and receive small chemical signal in order to extend in concentration as a function of bacterial cell number density. An important factor to be mentioned is that when bacteria continue to emit autoinducers in the environment, then the external concentration of the autoinducers is directly proportionate to the cell population density, making bacteria aware of the threshold concentration of the autoinducers as a result of which, gene expression starts altering [1–3]. Thus, we can say it as bacterial quorum sensing mechanism or chemical signalling mechanism. Bacterial communication systems regulate variety of physiological activity, which include biofilm formation, motility,

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Fig. 2.1 Quorum sensing: Bacteria emit autoinducers at low cell density, but they are not able to communicate with the surrounding bacteria. Bacteria emit and receive autoinducers at high cell density and the autoinducers concentration achieves a threshold. Quorum sensing begins at that point of time. This bacterial collective behaviour is a density dependent phenomenon

symbiosis, sporulation, virulence, conjugation, competence, antibiotic production. Quorum sensing was first observed in marine bacterium called *Vibrio fischeri*, which can be found as living microorganism as well as a symbiont in the light producing organ of an animal host (i.e. Hawaiian bobtail squid). *V. fischeri* is non-luminescent at low density, when the cell population grows up at a certain level and autoinducers concentration reaches a threshold, a coordination change is initiated. At that point of time, gene expression takes place and generates the enzyme luciferase, which leads to bioluminescence [2]. So, it is very much understandable that bacteria are talking to each other via small molecule as a collective behaviour which we call quorum sensing (Fig. 2.1).

Gram-negative bacteria use N-acyl homoserine lactones (HSL), fatty acid methyl esters, alkyl quinolones as autoinducers (chemical signalling molecules) and gram-positive bacteria use oligo peptides for conversation. Here we track some quorum sensing bacteria with their features in Table 2.1.

2.2 Quorum Sensing in Gram-Negative Bacteria

In the last few decades, several gram-negative bacteria are identified, which communicate using chemical signalling molecules or autoinducers (Fig. 2.2). Gram-negative bacterial communication contains at least two homologues regulatory proteins, known as LuxI and LuxR. Biosynthesis of autoinducers (specific acylated homoserine lactone) is controlled by LuxI link proteins and the autoinducers concentration elevates with rise of cell population density. Thereafter, LuxR link protein binds with the autoinducers (specific acylated homoserine lactone) and reaches the threshold concentration. Finally, target gene transcription is activated by

Table 2.1 List of gram-negative quorum sensing bacteria with chemical signalling molecules, regulatory proteins and phenotypes

Organism	Chemical signalling molecules	Regulatory proteins	Phenotypes
Agrobacterium tumefaciens	3-Oxo-C_8-HSL	TraI/TraR	Ti plasmid conjugation
Aeromonas hydrophila	C_4-HSL	AhyI/AhyR	Exoprotease production
Aeromonas salmonicida	C_4-HSL	AsaI/AsaR	Extracellular protease
Burkholderia cepacia	C_8-HSL	CepI/R	Protease, siderophores
Chromobacterium violaceum	C_6-HSL	CviI/CviR	Exoenzymes, antibiotics, cyanide, violacein
Erwinia chrysanthemi	3-Oxo-C_6-HSL C_6-HSL	ExpI/ExpR	Pectate lyases
Erwinia stewartii	3-Oxo-C_6-HSL	EsaI/EsaR	Exopolysaccharide, virulence factors
Enterobacter agglomerans	3-Oxo-C_6-HSL	EagI/EagR	–
Escherichia coli	–	–/SdiA	Cell division, attachment and effacing lesion formation
Erwinia carotovora subsp. *carotovora*	3-Oxo-C_6-HSL	ExpI/ExpR CarI/CarR	Exoenzymes Carbapenem antibiotics
Pseudomonas aeruginosa	3-Oxo-C_{12}-HSL C_4-HSL	LasI/LasR RhlI/RhlR	Biofilm formation, multiple extracellular enzymes, Xcp, RhlR secondary metabolites, RpoS
Pseudomonas aureofaciens	C_6-HSL	PhzI/PhzR	Phenazine antibiotics
Pseudomonas syringae	3-Oxo-C_6-HSL	AhlI/AhlR	Epiphytic fitness, cell aggregation
Pseudomonas chlororaphis	C_6-HSL	PhzI/PhzR	Phenazine-1-carboxamide biosynthesis
Pseudomonas putida	3-Oxo-C_{12}-HSL	PpuI/PpuR	Biofilm development
Pseudomonas fluorescens	Long acyl-chain-HSL	MpuI/MpuR	Mupirocin biosynthesis
Rhizobium leguminosarum	C_6-HSL	RhiI/RaiR	RhiABC rhizosphere-expressed genes, nodulation
Rhizobium etli	–	RaiI/RaiR	Restriction of number of nitrogen fixing nodules
Ralstonia solanacearum	C_8-HSL	SolI/SolR	–

(continued)

Table 2.1 (continued)

Organism	Chemical signalling molecules	Regulatory proteins	Phenotypes
Rhodobacter sphaeroides	7-*cis*-C_{14}-HSL	CerI/CerR	Dispersal from bacterial aggregates
Serratia liquefaciens	C_4-HSL	SwrI/SwrR	Extracellular protease, swarming
Salmonella typhimurium	–	–/SdiA	Resistance to competence killing
Vibrio fischeri	3-Oxo-C_6-HSL	LuxI/LuxR	Bioluminescence
Vibrio harveyi	3-Hydroxy-C_4-HSL	LuxLM/LuxN Lux-/LuxPQ	Bioluminescence
Vibrio anguillarum	3-Oxo-C_{10}-HSL	VanI/VanR	–
Yersinia enterocolitica	C_6-HSL	YenI/YenR	–
Yersinia pseudotuberculosis	C_8-HSL	YtbI/YtbR	Bacterial aggregation, motility

the LuxR-autoinducers complexes [4–6]. In general, this type of circuit is observed in different gram-negative bacteria with few exceptions (i.e. *M.xanthus*, *V. harveyi*) [2] (see more details in [1, 7–9]). We discuss some well understood quorum sensing circuits of gram-negative bacteria in this section.

2.2.1 Quorum Sensing Circuit of *Vibrio fischeri*

It has been observed that the *V. fischeri* has symbiotic relationship with the eukaryotic host. This bacterium lives in a nutrient rich environment and the cell density grows inside the light organ of the host [10–12]. In the signalling cascade, we observed two regulatory protein such as LuxI and LuxR. LuxI activates the production of *N*-(3-oxohexanoyl)- homoserine lactone (autoinducers of *V. fischeri*) and LuxR binds with *N*-(3-oxohexanoyl)- homoserine lactone. The interaction between LuxR and autoinducers exposes the LuxR DNA binding domain, which allows LuxR to combine with *luxICDABE* promoter and activate transcription of the *luxICDABE* operon [4, 13–17]. The LuxR-autoinducer complex behaves as a negative feedback loop (i.e. luxR expression), which decreases the positive feedback loop (i.e. *luxICDABE* expression) [4]. The concentration of autoinducers is same in intercellular as well as extracellular environment, because *N*-(3-oxohexanoyl)-homoserine lactone is easily diffusible across the cell membrane [18]. *V. fischeri* culture grows over the time and cell density reaches around 10^{11} cells/ml [19]. The autoinducers concentration reaches a threshold level (around 1–10 µg/ml) [20] and starts communication with other bacteria inside the host. So, the cell density is correlated with light production. Luciferase enzymes are needed for the production of light in these bacteria, which are encoded by *luxCDABE* (being as a part of

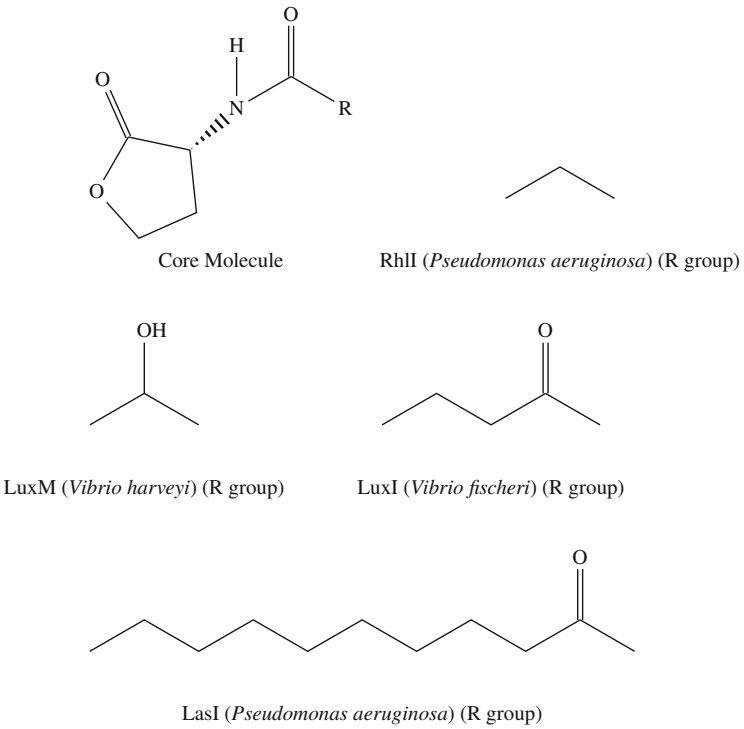


Fig. 2.2 Chemical structures: The core molecule and R groups of some Acyl-homoserine lactones (autoinducers)

luxICDABE operon) [4, 21] (Fig. 2.3). This light production feature is known as bioluminescence. Eukaryotic host utilizes this light for particular purposes such as attracting preys and staying away from predators [22]. For example, *Monocentris japonicus* uses this *V. fischeri* light to attract a mate and *Euprymna scolopes* uses this same lightning feature of *V. fischeri* for antipredation strategy [2].

2.2.2 Quorum Sensing Circuit of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a well known pathogenic bacteria, which has a hierarchical LuxI/R quorum sensing process. *P. aeruginosa* is responsible for the lung disease called cystic fibrosis and also regulate the biofilm formation [2]. Quorum sensing system of this bacteria has two signalling cascade such as LasI/LasR [23] and RhlI/RhlR [24] (both pairs are LuxI/LuxR homologues). LasI and RhlI produce autoinducers *N*-(3-oxododecanoyl)-homoserine lactone [25] and *N*-(butryl)-homoserine lactone [26], respectively, to regulate the quorum sensing circuit and control virulence genes. LasR binds with *N*-(3-oxododecanoyl)-homoserine lactone (autoinducer) and the complex (LasR-autoinducer) binds with

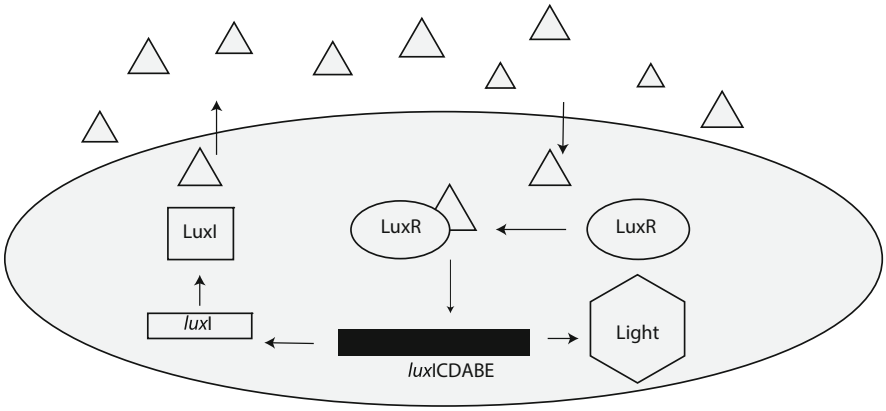


Fig. 2.3 Illustration of quorum sensing circuit of *Vibrio fischeri* (LuxI/LuxR): The oval shape shows a bacterial cell. This system consists of two regulatory genes (*luxI* and *luxR*) and five luciferase structural genes (*luxCDABE*). The triangles are autoinducers. LuxI (protein) produces autoinducers. The concentration of autoinducers increases, when the cell population density rises. When the concentration of autoinducers reaches a certain level LuxR (protein) binds with autoinducers. LuxR-autoinducers complex binds with promoter region of *luxICDABE* and active the transcription process of the operon *luxICDABE* and produce light

the promoter region before the genes encoding virulence factors (i.e. alkaline phosphatase, exotoxinA, protease and elastase are encoded by *aprA*, *toxA*, *lasA* and *lasB*, respectively) [1, 23, 27, 28]. The infection mechanism of the host begins and is controlled by these secreted virulence factors. A positive feedback loop is observed, when the complex (LasR-autoinducer) triggers *lasI* expression [29].

In other signalling cascade, *rhlR* expression is activated by the complex (LasR-autoinducer). RhlI produces *N*-(butryl)-homoserine lactone (autoinducer) and RhlR binds with the autoinducer [30]. Two genes expressions (*lasB* and *aprA*) are also controlled by the complex (RhlR-autoinducer). Moreover, RhlR-autoinducer complex triggers specific genes such as *rpoS*, *rhlAB* and *lecA* [1, 8, 9, 24, 30–37]. We can observe an autoregulatory loop in the system (activation of *rhlI*). Both the signalling mechanisms (RhlI/RhlR and LasI/LasR) work sequentially (Fig. 2.4).

Beside this above mention signalling cascades, *P. aeruginosa* uses 2-heptyl-3-hydroxy-4-quinolone (also known as *Pseudomonas* quinolone signal (PQS). PQS is considered as an additional link between Rhl and Las circuits and partially controls *lasB* gene expression [38].

2.2.3 Quorum Sensing Mechanism of *Agrobacterium tumefaciens*

The crown gall tumours are induced by the plant pathogenic bacteria *Agrobacterium tumefaciens*. Bacterium transfers oncogenic Ti plasmid to the host for the formation

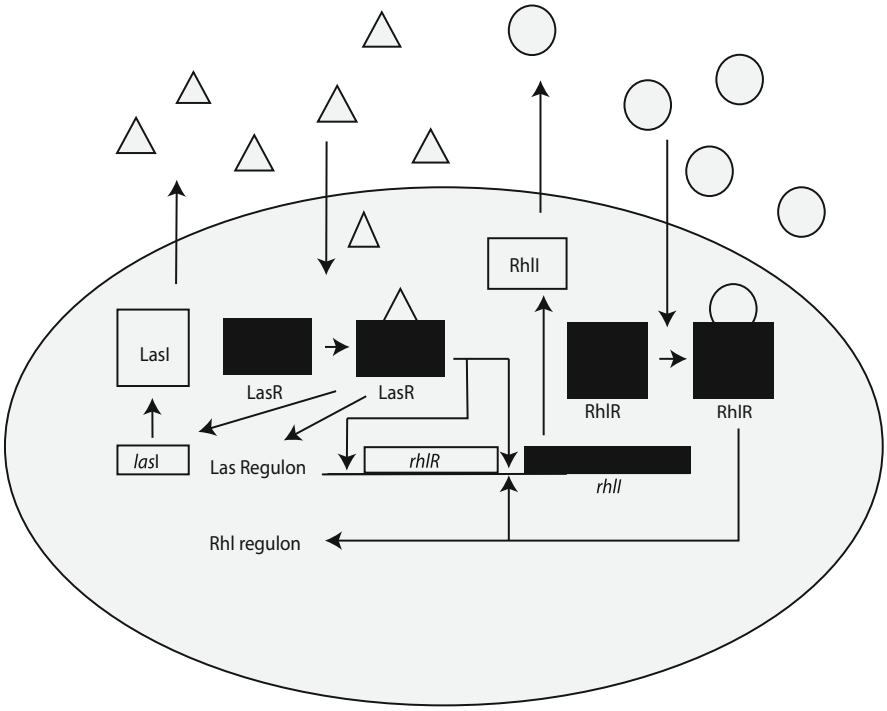


Fig. 2.4 Quorum sensing circuit of *Pseudomonas aeruginosa*: The oval shape shows the bacterial cell. The triangle and the circle represent two different autoinducers such as *N*-(3-oxododecanoyl)-homoserine lactone and *N*-(butryl)-homoserine lactone, respectively. There are two signalling cascades (LasI/LasR and RhlI/RhlR). LasI produces *N*-(3-oxododecanoyl)-homoserine lactone (autoinducer) that binds to LasR. The complex (LasR-autoinducer) activates different targeted genes (including virulence genes), induces transcription of *rhlR* as well as initiates the second signalling cascade. RhlI also produces *N*-(butryl)-homoserine lactone (autoinducer) and RhlR binds with autoinducer. The RhlR-autoinducer complex triggers set of targeted genes

of tumour [39, 40]. Opines secretion in the plant and biosynthesis is controlled by the genes on the Ti plasmid. The conjugation between cells needs autoinducer signal and opine signal. Opines control the communication mechanism and are considered as nutrient source for bacteria. Opine regulates the TraR expression. Two different class of opine such as nopaline type and octapine type regulate conjugal Ti plasmids. *A. tumefaciens* quorum sensing circuit is very much similar with *V. fischeri* at low cell population density. Bacterium uses *N*-(3-oxoctanoyl)-homoserine lactone (autoinducer) for their communication [41,42]. We can observe TraI/TraR signalling cascade in this communication process. TraI produces autoinducers and TraR binds with autoinducers and forms a (TraR-autoinducer) complex, which induces the *traI* expression. In this way, a positive autoinduction loop is created. The complex (TraR-autoinducer) regulates *tra* operon, *trb* operon and *traM* gene [2, 43–45]. *trb* operon encodes necessary genes and *tra* operon triggers Ti plasmid mobilization.

Moreover, the complex (TraR-autoinducer) induces TraM and down regulates the communication process. TraM is an additional level of regulation in this quorum sensing circuit.

2.2.4 Quorum Sensing Mechanism of *Erwinia carotovora*

We can find soft rot in potato because of plant pathogenic bacteria *Erwinia carotovora* [46]. The quorum sensing process of *E. carotovora* consists of two signalling cascade ExpI/ExpR and CarI/CarR. ExpI/ExpR homologues to LuxI/LuxR that regulates the cascade to mount a victorious infection [2]. Exoenzymes secretion is controlled by ExpI/ExpR at high cell density. The second signalling cascade is CarI/CarR, which has a similarity with LuxI/R. ExpI and CarI both produce the same autoinducer known as *N*-(3-oxohexanoyl)-homoserine lactone [47]. ExpR and CarR response to the same biochemical signal. CarI/CarR system generates antibiotics as well [48, 49].

2.3 Quorum Sensing in Gram-Positive Bacteria

Gram-positive bacteria regulate the cell-to-cell communication process using oligopeptides (autoinducers). We observe a precursor protein in this system, which is translated from peptide signal precursor locus and divided into peptides (autoinducers). Peptides are transported via ABC transporter, because it is not diffusible across cell membrane. The autoinducers concentration increases and reaches the threshold concentration. Gram-positive bacteria have two-component histidine sensor kinases for detection of autoinducer. Then, we notice a series of phosphoryl events, which is initiated by peptide ligand. This phosphorylation triggers response regulator (DNA binding transcription process). Finally, targeted genes transcription is activated by the phosphorylated response regulator [2,3,7,50–52]. Here, we are mainly discussing three gram-positive quorum sensing system (Figs. 2.5 and 2.6).

2.3.1 Quorum Sensing Process of *Streptococcus pneumoniae*

We observe genetic transformation in a gram-positive quorum sensing bacterium called *Streptococcus pneumoniae* [53]. This biochemical process needs that the bacterium becomes competent in order to get exogenous DNA molecules. This competent state is very complex phenomenon and partially controlled by cell-to-cell communication mechanism [54]. Competent state arises at the time of exponential growth. The *S. pneumoniae* loses the ability in later stage and departs from the competent state [53, 55, 56]. The competent state is developed by the signalling peptide known as competence stimulating peptide (CSP). ComC (41-amino acid precursor peptide) produces CPS (17-amino acid peptide) [57, 58]. This system

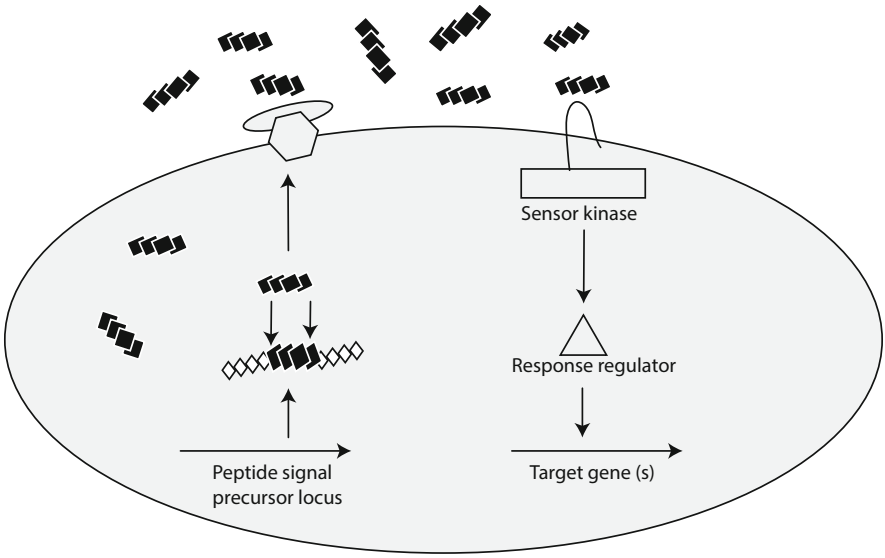


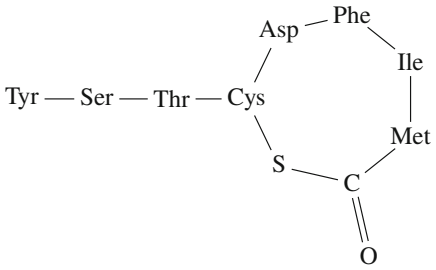
Fig. 2.5 In general, schematic diagram of a quorum sensing system of a gram-positive bacteria. This quorum sensing mechanism is mediated by peptides. The oval shape represents bacterial cell. Black diamonds are signalling peptides (autoinducers). Precursor protein (black and white diamonds) is translated from a peptide signal precursor and generates autoinducers. These autoinducers transport through ABC transporter. Peptides (autoinducers) detected by sensor kinase, at high cell density and phosphoryl group is transferred to response regulator by autophosphorylation. The targeted genes are activated by phosphorylated response regulator

has ABC transporter, ComAB. ComAB secretes processed CSP [59, 60]. ComD is the sensor kinase protein, which can detect CSP at high cell density [61]. Autophosphorylation of ComD is induced by high level of CSP and phosphoryl group is transferred to ComE (response regulator). Finally, *comX* gene transcription is triggered by phospho-ComE [62].

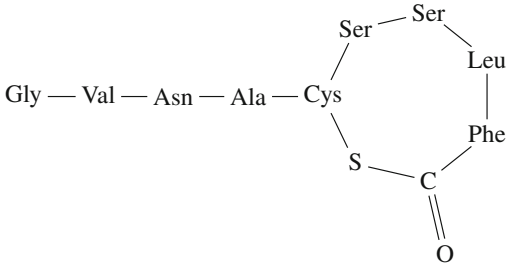
2.3.2 Quorum Sensing Process of *Bacillus subtilis*

The peptide quorum sensing system is also observed in another gram-positive bacteria known as *Bacillus subtilis*. We notice competent state and sporulation mechanism, which are controlled by the two peptide mediated communication process. *B. subtilis* reaches the competent state at the transition between logarithmic and stationary phase growth [51,63]. When the bacteria live in limited nutrients condition and the environmental condition have also deteriorated, then the sporulation process occurs in *B. subtilis* [64]. Quorum sensing mechanism is mediated by two peptides, ComX and CSF (competence and sporulation factor). These peptides are ejected and the concentration of peptides (autoinducers) increases as the cell density rises. 55-amino acid precursor peptide generates ComX and ComQ is needed for

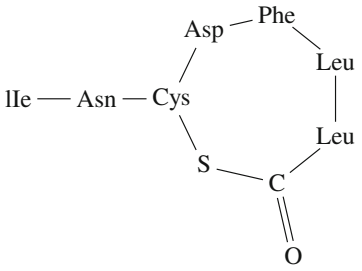
Fig. 2.6 Chemical structures: Oligopeptide autoinducers



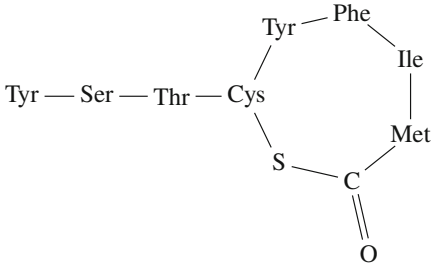
AIP-I (*Staphylococcus aureus*)



AIP-II (*Staphylococcus aureus*)



AIP-III (*Staphylococcus aureus*)



AIP-IV (*Staphylococcus aureus*)

production of ComX. ComP is a sensor kinase required for the detection of ComX. ComA is a response regulator of this signalling mechanism. The *comS* gene is activated by the phospho-ComA [65–68]. The degradation of ComK is inhibited by phospho-ComA. ComK is transcriptional activator associated with competence pathway.

B. subtilis also uses CFS (pentapeptide) to regulate the communication process. CSF is generated from the precursor peptide PhrC [66]. CSF is secreted via Opp (ABC type oligopeptide transporter). RapC (ComA-specific phosphatase) is inhibited by CSF (at low intracellular CSF concentration). *comS* gene expression is induced by CFS (at high intracellular CFS concentration) [66, 67, 69, 70]. So, competence is promoted at low intracellular CSF concentration, whereas sporulation is induced at high intracellular CSF concentration. RapB is inhibited by CSF, which dephosphorylates Spo0A (response regulator) and smooth the sporulation pathway [63, 70–72].

2.3.3 Quorum Sensing Mechanism of *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive pathogenic bacteria. This is a multitalented bacterium, which causes several diseases such as endocarditis, toxic shock syndrome and skin infection. The *S. aureus* quorum sensing system is regulated by autoinducing peptide (AIP) [73]. We can also notice variation in AIPs. The density dependent pathogenicity is regulated by RNAIII (RNA molecule). RNAIII is partially controlled by *agrBDCA* operon. *agrBDCA* is transcribed from *hld* gene. *hld* encodes the RNAIII transcript. Octapeptide is produced from AgrD (precursor peptide). This production process depends on AgrB-dependent mechanism [74–80]. We observe a thio-lactone ring in AIP and a two competent system AgrC/ArgA (sensor kinase/ response regulator) which is this communication system [80–82]. The concentration of RNAIII is increased by phospho-AgrA. RNAIII triggers the gene expression as well as virulence factors.

2.4 Cross-Species Cell-to-Cell Communication

Bacteria can talk with other bacterial species, which is formally known as interspecies or cross-species communication process. This notion arose with the finding of autoinducers-2 (AI-2) in *Vibrio harveyi*. *luxS* gene is needed for AI-2 production and LuxS synthesis the AI-2. Bacteria use AI-2 based quorum sensing mechanism for interspecies cell-to-cell communication [7, 83, 84]. For example, *V. harveyi* lives in a mixed population (with other bacterium) and communicates with each other using two different type of autoinducers (AI-1 and AI-2). Bacteria use AI-1 for intraspecies communication and AI-2 for interspecies communication [83, 84]. There are several number of gram-negative and gram-positive bacteria that contain luxS gene (required for interspecies communication), such as *B. subtilis*, *S. aureus*, *E. coli*, *V. cholerae*, *Y. pestis*, *S. paratyphi*, *H. influenzae*, *K. pneumoniae*, *M.*

tuberculosis and many more [7, 84]. LuxS generates DPD (4,5-dihydroxy-2,3-pentonedione). DPD is highly reactive and derives signalling molecules AI-2 [3].

So, we conclude that bacteria can talk to each other (intraspecies and interspecies) using different types of chemical signalling molecules for their own survival strategies. Gram-negative bacteria use acyl-homoserine lactones (autoinducers) and gram-positive bacteria use peptide for regulating the quorum sensing systems. We will see how bacteria can regulate other biochemical phenomena such as biofilm formation, virulence, swarming and many more (with mathematical modelling approach) in the next couple of chapters.

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