Signaling Molecules of Quorum Sensing in Bacteria

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Abstract

Bacteria use quorum sensing (QS) systems to change and coordinate the behavior between cells, thus present certain physiological characteristics. This system accomplishes certain physiological functions and regulatory mechanisms that can’t be accomplished by a single bacterium. The whole process of QS depends on the production, release, detection and response level of extracellular signaling molecules. In this review, we focus on introducing QS in gram-positive bacteria and gram-negative bacteria with different signal molecules.

Keywords

Quorum Sensing; Signaling Molecular; Gram-Positive Bacteria; Gram-Negative Bacteria

Introduction

Quorum Sensing (QS) is a phenomenon of intercellular communication discovered mainly in bacteria. One or several small signaling chemical molecules secreted by bacteria spread from cell to cell, facilitating communication among individual bacteria, thus coordinating group behavior. Bacteria can sense changes in the cell population density of their own or other bacteria in surrounding environment through signaling molecules. With the increase of bacterial population density and a critical concentration of signaling molecules in the environment, Bacteria change and coordinate the behavior between cells through activating the expression of specific genes, for achieving certain physiological functions and regulatory mechanisms that a single bacteria cannot complete.

Gram-positive bacteria and gram-negative bacteria have different QS systems. The main differences are the diverse QS signal molecules. General signaling molecules in gram-negative bacteria include n-Acyl-Homo-Serine Lactones (AHLs), quinolones, Diffusion Signaling Factor (DSF), (S)-3-hydroxytetradecanoe-4-ketone (CAI-1) and soon. Modified oligopeptide (AIP) is the most usual signaling molecule in gram-positive bacteria. Besides, there is another kind of non-species-specific Auto Inducer 2 (AI-2), used for intraspecific and interspecific communication both in gram-negative and gram-positive bacteria (Table 1).

QS in gram-negative bacteria

There are various kinds of autoinducers in gram-negative bacteria. For example, AHLs, one of the most common type of...
autoinducer in gram-negative bacteria, has a core n-acylated high serine lactone ring, containing modified 4-18 carbonyl chains. The length of the acyl chain affects stability, which may have an impact on signal transmission [1-3]. Hundreds of bacteria now contain the LuxI synthetase that produce AHL. The LuxI synthetase produces AHL by catalyzing the acyl side chain of carrier protein binding to S-Adenosine Methionine (SAM) [4]. Four common features were investigated in this kind of QS systems. First, the autoinducer was able to spread freely through the bacterial membrane. Second: the autoinducer is bound to specific receptors located in intracellular membranes or cytoplasm. Third: QS usually alters dozens to hundreds of genes that support various biological processes. Forth: in the process of autoinduction, QS driven by autoinducer stimulates the synthesis of autoinducer and establishes a feedforward ring for promoting the synchronous gene expression in the population [5].

DSF and CAI-1 are atypical autoinducers. DSF family contains cis-2-unsaturated fatty acids with different chain lengths and branched chains. Cis-11-methyl-2-dodecanoic acid was first found in Xanthomonas campestris [6]. Then, much more different signal molecules were reported in different bacteria, such as rice bacterial leaf blight pathogen X. oryzae pv. Oryzae (Xoo) [BDSF (cis-2-dodecylene acid), CDSF twelve carbon (cis-11-methyl-2,5-diene acid)] [7], Burkholderia bacterias (cis-2-dodecylene acid) [8], Pseudomonas aeruginosa (cis-2-decanoic acid) [9], Xylella fastidiosa (cis-2-14 carbon olefine acid) and Xanthomonas oryzae (cis-11 methyl dodecarbonate 2,5-dienoic acid). These signal molecules are associated with bacterial virulence, biofilm formation, and antibiotic resistance.

RpfF, an enzyme of crotonic acid superfamily, is involved in the synthesis of DSF in all bacteria species [10]. It has the activity of desaturase and thioesterase and takes use of fatty acyl ACP derived from the biosynthesis of fatty acids as substrates. DSF family signaling factors are produced by 3-hydroxy-aliphatic acyl ACP through continuous coordinated action of desaturase and thioesterase [11].

Besides, 3-hydroxypalmitate-methyl (3-OH PAME) and (R)-methyl-3-hydroxynutmeg ((R)-3-oh) MAME) are synthesized by the PhcB protein in Ralstonia solanacearum, related to virulence and biofilm formation [12,13]. HSL is synthesized by plant-related bacteria, for example, Isopentyl-HSL is synthesized by Bradyrhizobium japonicum and Aeromonas spp., while cinnamyI-HSL [14,15], is synthesized by Bradyrhizobium BTaI.

Here, we will detailed introduce several gram-negative bacteria and outline their QS mechanisms.

**P. aeruginosa**

*P. aeruginosa* is a common gram-negative bacterium that causes human acute and chronic infections. *P. aeruginosa* has four QS systems: LuxI, LuxR, LasI/LasR and RhlI/RhlR, all of which contains the signaling factors, AHL (N-acylhomoserine lactone). In addition, 2-hept-3-hydroxy-4-quinolone (PQS) are found both in the PqsR- controlled quinolone signaling system (PQS system) and IQS system acted under phosphate restriction conditions [16].

In the first circuit, 3-oxododecanoyl-homoserine lactone (3OC12HSL) was synthesized by LasI. At HCD, AI was detected by LasR in cytoplasmic. The complex of LasR-3OC12HSL activates the transcription of target genes, including encoding elastase, protease, and virulence factors such as exotoxin A. The target of LasR-3OC12HSL are LasI and RhlI. LasI establishes an autoinducer feedforward loop [17] and RhlI synthesizes the second AI-butylated high serine lactone (C4HSL), which combine with RhlR at a high concentration [18]. RhlR-C4-HSL can activate target genes, including encoding elastase, protease, and iron carriers [19]. *P. aeruginosa* also take use of the quinolone signaling system to control virulence factor gene expression. PQS are produced by PqsA, PqsB, PqsC, PqsD and PqsH, and detected by the regulator PqsR.

Table 1: Several Signaling Molecules in Quorum Sensing of Bacteria.

<table>
<thead>
<tr>
<th>Signaling molecules</th>
<th>Types</th>
<th>Species</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHL</td>
<td>Gram-negative bacteria</td>
<td>Vibrio fischeri</td>
<td>Bioluminescence</td>
</tr>
<tr>
<td>DSF</td>
<td>Gram-negative bacteria</td>
<td>Xanthomonas campestris</td>
<td>Black rot of cruciferous plants</td>
</tr>
<tr>
<td>CAI-1</td>
<td>Gram-negative bacteria</td>
<td>Vibrio cholerae</td>
<td>Produce enterotoxin</td>
</tr>
<tr>
<td>AI-2</td>
<td>Gram-negative bacteria</td>
<td>Vibrio harveyi</td>
<td>Produce fluorescence</td>
</tr>
<tr>
<td>AIP</td>
<td>Gram-positive bacteria</td>
<td>Streptococcus suis</td>
<td>Septicemia, meningitis and pneumonia</td>
</tr>
<tr>
<td></td>
<td>Gram-positive bacteria</td>
<td>Staphylococcus aureus</td>
<td>Pneumonia, bacteremia and sepsis</td>
</tr>
</tbody>
</table>
The expression of PqsH and PqsR was activated by LasR-3OC12HSL, while PqsABCD and PqsR are inhibited by RhlR-C4HSL. PqsR-PQS induces the synthesis of PQS and activated the expression of rhl and rhlR (Figure 1). Therefore, PQS system is closely related to LasI/LasR and RhlI/RhlR QS system, which also affects the generation of virulence factors [20-22].

**Vibrio Cholerae**

Cholera is a diarrheal disease characterized by watery diarrhea, can lead to dehydration or death if not treated properly. *V. cholerae*, the pathogenic factor of cholera, is controlled by QS to produce an enterotoxin called cholera toxin [23].

(S)-3-hydroxysterane-4-ketone was found in *V. cholerae*, as an autoinducer in cholera, called Cholera Autoinducer 1 (CAI-1) [24]. In *V. cholerae*, CAI-1 autoinducer synthase (CqsA) acts on SAM and decanoyl-CoA to produce amino-CAI-1, which is then converted to CAI-1 spontaneously [25,26]. CqsA enzymes were found in all *Vibrio* genera, and they can produce modified CAI-1 with different acyl chain length [27] (Figure 2).

**QS in gram-positive bacteria**

The QS of gram-positive bacteria is different from that of gram-negative bacteria. The signaling factors are mainly modified oligopeptide (autoinducing peptide AIP). Oligopeptides can be secreted and accumulated extracellularly like AHLs, but with the assistance of ABC transporters or other channel proteins. QS systems with oligopeptides as signal molecules are usually two-component signal systems. Signal molecules outside the cell are recognized and combined by the binary signal system located on the bacteria when they have reached the threshold, and finally regulate the transcriptional expression of target genes through a complex signal transduction process.

Diverse functions of gram-positive bacterial QS systems have been reported, such as virulence factor production in the *Bacillus cereus*, biofilm formation in *Bacillus subtilis*, and plasmid transfer in enterococcus faecalis [28]. Here, we take *Staphylococcus aureus* and *Bacillus cereus* as examples.

**S. aureus**

*S. aureus* is found in normal human skin, which causes minor skin infections if epithelial tissue is damaged. These infections can lead to pneumonia, bacteremia and sepsis [29,30]. The likelihood of infected disease may depend on the expression of a range of adhesion molecules, toxins and compounds which affect the immune system. QS regulates the expression of genes encoding these virulence factors.

Precursor Autoinducing Peptide (pro-AIP) is encoded by the agrD gene, which is processed into AIP by cell membrane protein AgrB and secreted to extracellular cells. AIP was detected by two-component signal transduction pathway. AgrC is a membrane-bound histidine kinase, and AgrA is a reaction regulator. Phosphorylated AgrA activates P2 and P3 promoters of the agr operon, and regulates RNA II and RNA III RNA, respectively. Virulence factor are produced post-transcriptional activation of RNA III [31].

**B. cereus**

*B. cereus* is closely related to human health. It usually causes intestinal and parenteral infections, especially food poisoning. The production and secretion of hemolysin, phospholipase, and toxins cause acute diarrheal disease [32].

QS in *B. cereus* requires the transcription factor PlcR. PapR protein was encode by 70 base pairs downstream of PlcR, which has 48 amino acids and contains amino terminal signal peptide [33]. After leaving the cell, PapR pro-AIP is processed by extracellular...
neutral protease B (NprB) to form mature heptapeptide AIP. Mature AIP is transported back to cells by Opp, binds to the transcription factor PlcR and activates it. The PlcR-AIP complex regulates the production of virulence factors and activates the expression of PapR [34] (Figure 3).

**Gram-positive and gram-negative bacteria share QS system**

The LuxS/AI-2 system is a common signal system in both gram-positive and gram-negative bacteria. Its signal molecule is difuryl borate, called Autoinducer 2 (AI-2), which can be recognized by different species of bacteria.

AI-2 is produced by decomposing S-adenosine Homocysteine (SAH) into adenine, homocysteine, and 4,5-dihydroxy-2,3-pentanedione (DPD) through a series of reactions of homocysteine nucleosidase (Pfs) and s-adenosine homocysteine nucleosidase (LuxS). DPD has high reactivity and can be spontaneously cycled into various furanones. Specific bacterial species detect different forms of DPD as active AI-2 signal. LuxD is a DPD synthetase that exists in more than 500 bacteria species [35], making AI-2 by far the most common bacterial autoinducer.

So far, it was known that there are two main forms of AI-2 in living organisms. The first active form of AI-2 was found in *V. harveyi*, was automatically condensed into diester furanyl borate through 4s-4,5-dihydroxy-2,3-pentanedione (4S-DPD) and boric acid molecules, causing the bacteria to spontaneously produce fluorescence. The second active form of AI-2, tetrahydroxy tetrahydrofuran (2R, 4S) without boron, found in *Salmonella typhimurium*, is produced by directly condensing and cyclizing of DPD and H₂O.

Current studies have reported that the LuxS/AI-2 system can regulate the luminescence of *V. harveyi*, the biofilm formation of *E. coli*, the toxicity of *V. cholera* and the pathogenicity of *P. aeruginosa*, etc.

**Conclusions and Future Perspectives**

QS is a widely existing regulatory mechanism in bacteria, regulates the formation of buds, fusion and transference of Ti plasmid, luminescence of bacteria [36], pigment [37], antibiotics [38], biofilm [39], virulence factor [40], bacterial movement and so on. Some plant diseases are caused by the regulation of the expression of pathogenic genes and virulence factors by the QS system in plant pathogens. Therefore, it is effective to target the QS signal molecules of pathogenic bacteria and weaken the pathogenicity of pathogens by interfering and destructing the quorum sensing system of pathogen. In all, it is of great theoretical and practical significance to study the regulation mechanism of bacterial QS deeply.

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