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QS – systems communication of Gram-positive bacterial cells

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Abstract In Gram-positive bacteria, cell-to-cell communication, also called *quorum sensing* (QS) mainly is dependent on extracellular signaling oligopeptide pheromones, which stimulate a response either indirectly, by activating a two-component phosphorelay, or directly, by binding to cytoplasmic effectors. The oligopeptide pheromones production and secretion are initiated in response to specific environmental stimuli or stresses. These pheromones are biosynthesized through different pathways and some have unusual functional chemistry as a result of post-translational modifications. In the cells of *Bacillus subtilis* and *Streptococcus pneumoniae* this system controls the acquisition of the state of competence, while in *Staphylococcus aureus* it regulates virulence. The review aims at giving an updated overview of these peptide-dependant communication pathways.

Systemy komunikacji *QS* w komórkach bakterii Gram dodatnich

Słowa kluczowe *quorum sensing,* bakterie Gram dodatnie, cząsteczki sygnałowe, ekspresja genów, kompetencja *Streptococcus pneumoniae* i *Bacillus subtilis,* wirulencja *Staphylococcus aureus*

Streszczenie U bakterii Gram dodatnich komunikacja od komórki do komórki, zwana także *guorum sensing* (QS) jest przede wszystkim zależna od zewnątrzkomórkowych sygnałowych oligopeptydowych feromonów, które stymulują odpowiedź także pośrednio poprzez aktywowanie dwuskładnikowych fosforanów lub bezpośrednio przez wiązanie efektorów cytoplazmatycznych. Wytwarzanie i wydzielanie feromonów oligopeptydowych jest inicjowane w odpowiedzi na specyficzne czynniki środowiskowe lub stres. Feromony są syntetyzowane różnymi drogami a niektóre mają jeszcze dodatkową funkcję chemiczną jako wynik modyfikacji potranslacyjnej. W komórkach *Bacillus subtilis* i *Streptococcus pneumoniae* ten system kontroluje nabycie stanu kompetencji, natomiast u *Staphylococcus aureus* reguluje wirulencję. Celem pracy był przegląd aktualnych danych dotyczących peptydozależnych dróg komunikacji.

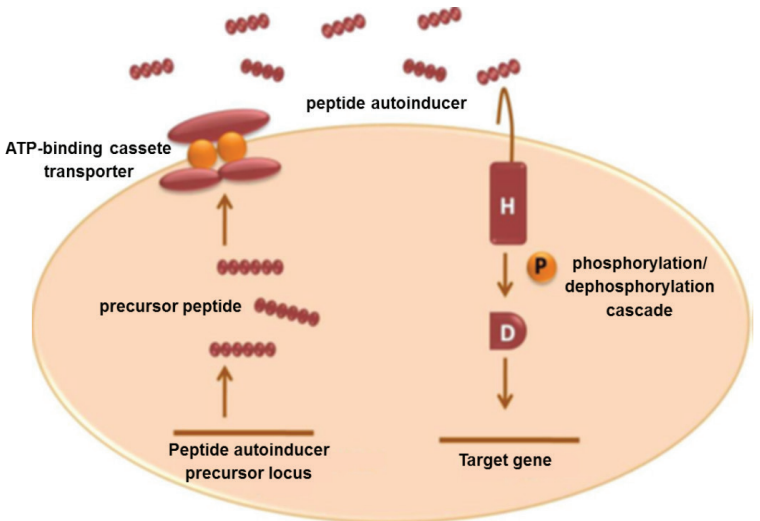
Introduction

Bacterial cell-cell communication systems also called *quorum sensing* (QS) are based on the secretion of signal molecules. Within Gram-positive bacteria, the expression of target genes

is controlled at the population level via signaling peptides, also known as pheromones (Cook, Federle, 2014; Monnet et al., 2016). Differences in the *QS* systems between Gram-positive and Gram-negative bacteria are seen in the mechanisms of synthesis of signal peptides and in the way they are transmitted from the sensor proteins to the cell effectors. Also, different molecules act as the signaling molecules in both groups of bacteria, and the differences in these chemicals being used in the sensing of quorum result primarily from differences in the construction of structures of the cell wall of bacteria that produce them. Gram-positive system usually uses secreted oligopeptides and two-component systems, which consist of membrane-bound sensor kinase receptors and cytoplasmic transcription factors that direct fluctuations in gene expression. Gram-negative bacteria often use several autoinducers, but new studies reveal unusual signaling molecules, novel regulatory components and heterogeneity in *QS* responses (Papenfort, Bassler, 2016).

QS – system communication

AI molecules produced by Gram-negative bacteria diffuse passively into and out of cells, whereas AIs synthesized by Gram-positive bacteria are actively transported (Miller, Bassler, 2001). Gram-positive bacteria communicate with each other using the two-component system of detection and respond to the presence of autoinducer (Li, Tian, 2012). Inside the bacterial cell, the oligopeptides are generated and then transported to the outside environment via the ABC transport protein (ATP-binding cassette transporter) (Siepka, Gładkowski, 2012). The mechanism of signal transmission occurs on a basis of cascade of phosphorylation and dephosphorylation (Kleerebezem et al., 1997). The signal oligopeptides released outside after reaching a threshold concentration are detected by transmembrane protein kinase that acts as a receptor protein.



H – transmembrane protein kinase, P – phosphate group, D – regulatory protein

Figure 1. The general scheme of the *quorum sensing* system in Gram-positive bacteria

Source: Vijayalakshmi (2013).

The interaction of protein kinase with the ligand leads to its autophosphorylation thereby initiating the cascade of reactions that result in a phosphorylation of regulatory protein. The phosphorylated form of regulatory protein is able to recognize and bind to the suitable promoters of target genes involved in *QS*, thereby initiating their expression (Li, Tian, 2012). A schematic model showing the system of *quorum sensing* in Gram-positive bacteria has been presented in Figure 1.

In different species of Gram-positive bacteria *QS* system has a different biological role. The examples can be here the cells of *Bacillus subtilis* and *Streptococcus pneumoniae*, in which this system controls the acquisition of the state of competence, while in *Staphylococcus aureus* it regulates virulence and in *Enterococcus faecilis* process of conjugation (Vijayalakshmi, 2013). In recent years, it has been noticed a large increase in studies discovering new pheromone pathways among Gram-positive bacteria that improve our understanding of peptide signaling described for model organisms like *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* (Cook, Federle, 2014).

QS in acquiring competence of *Streptococcus pneumoniae*

Streptococcus pneumoniae was the first species of bacteria in which in 1928 the genetic transformation was described (Lorenz, Wackernagel, 1994). The transformation process occurs only in the competent cells, which have the ability to collect the DNA from the surrounding environment. The bacterium *S. pneumoniae* reaches the state of competence spontaneously (in a natural way). The acquisition of competence depends on many complex physiological processes, many of which are under control of *QS* (Lee, Morrison, 1999).

In the *S. pneumoniae* system the signaling molecule is oligopeptide CSP (competence stimulating peptide) (Vijayalakshmi, 2013). It is built of 17 amino acids and is formed from the precursor peptide, called ComC. The oligopeptide CSP is transported out of the bacterial cell by the transporter protein, ABC. The protein of ComD kinase is a sensor detecting the accumulated extracellularly autoinducer CSP. At a high concentration of CSP in the environment of bacterial growth, ComD undergoes autophosphorylation and via the phosphorylation and dephosphorylation reactions pathway it transmits the signal to the final acceptor, which is a regulatory protein, ComE. The phosphorylated protein ComE activates the transcription of the *comX* gene encoding alternative sigma factor ComX required for the expression of structural genes involved in the acquisition of competence (Vijayalakshmi, 2013).

QS in acquiring competence of *Bacillus subtilis*

The process of acquiring the status of competence in bacteria of the genus *Bacillus subtilis* has been understood in details (Wolska, 2012). It is known that cells of these bacteria synthesize two types of signal molecules, one of which, ComX controls the process of acquiring the competence and the second, CFS regulates sporulation (Solomon et al., 1996). This allows the *B. subtilis* cells for precise response to external factors and appropriate adaptation to changing environmental conditions (Solomon et al., 1996).

When the population of cells reaches the high density in its growth environment, a ComP kinase, acting as a sensor for the autoinducer ComX, receives a cumulated signal and passes it through a cascade of reactions of phosphorylation and dephosphorylation to form a regulatory ComA protein (Vijayalakshmi, 2013). The phosphorylated ComA protein activates the expression

of the *comS* gene and the protein ComS inhibits proteolytic degradation of the second regulatory protein, ComK (thereby increasing the intercellular level of this protein), which is an activator of transcription that regulates expression of structural genes involved in the development of competence of *B. subtilis* (Vijayalakshmi, 2013).

The second of the above-mentioned signaling molecules – CFS, responsible for the stimulation of sporulation processes in adverse conditions, for example in reduced access to food sources, is imported into the cell by the bacterial ABC transporter called Opp (Jaworski et al., 2005). At high extracellular concentrations of CFS autoinducer, it comes to the inhibition of ComS protein, which in consequence inhibit the expression of competence genes and leads to activation of the metabolic pathways associated with cell sporulation. Conversely, in the case of low intercellular concentrations of CFS, it binds with RapC phosphatase and inhibits its activity, increasing this way the level of phosphorylated ComA protein and directs the cells of *B. subtilis* on the way of acquiring the status of competence (Vijayalakshmi, 2013).

QS in virulence of *Staphylococcus aureus*

S. aureus is a bacterium responsible for infections of skin and soft tissues, bacteraemia, endocarditis, sepsis, and toxic shock syndrome (Cheung et al., 2011). Treatment of *S. aureus* is complicated due to the devolvement of multidrug-resistant *S. aureus* strains, known as methicillin-resistant *S. aureus* (MRSA) and these virulence factors are a crucial part in the pathogenesis of that bacterial infections (Lowy, 2003). Virulence factors involve a wide range of various enzymes and exotoxins that enable the avoidance of the immune system and tissue adhesion damage to the host cell or enterotoxin release the toxic shock syndrome. Finally, α-hemolysin, which causes the destruction of membrane structures and can cause pneumonia (Gordon, Lowy 2008). The expression of different virulence factors is determined by external influences and is regulated by the cell-density-dependent *QS* accessory gene regulator (*agr*) system of *S. aureus* (Painter et al., 2014). The *agr* locus includes five genes *agrA*, *agrB*, *agrC*, *agrD*, and *hld* that are organized in one operon. The *agr* operon and *hld* are controlled by different promoters, named P2 and P3. Each of these proteins takes over different functions in the *QS* system, the last one is converted into the autoinducing peptide (AIP) that is used as cellular signaling molecule and with the *agr* system regulates the expression of virulence factors. However, many hospital-isolated strains of *S. aureus*, as the most frequently isolated pathogens in intensive care units, are *agr* defective and deficiency the main QS-controlled virulence regulatory system. Paulander et al. (2013) showed that *agr*-negative strains have an advantage over *agr*-positive strains what may be an explanation of so frequently isolated *agr*-defective *S. aureus* strains in hospitals. As peptide signaling systems remain to be discovered, there is a rising need to understand the details of these communication mechanisms. This information will deliver understanding how Gram-positives coordinate cellular events and help to board these pathways for infection treatments. The global emergence of antibiotic-resistance amongst infectious bacteria requires detection not only of new antimicrobials but also alternative therapeutic strategies to control pathogen (Jimenez, Federle, 2014; Aggarwal et al. 2015). One of those strategies is to control the virulence gene expression, mainly through the manipulation of *QS* systems in a process of *quorum sensing* inhibition (QSI) also discussed as "*quorum quenching*". Due to the importance of peptide systems in pathogenesis, there is emerging interest in *quorum-quenching* methods for therapeutic intervention (Cook, Federle, 2014). The quenching strategies that have successfully blocked signal biosynthesis are also covered. As new peptide systems are discovered and characterized, and if found to influence

pathogenic attributes of host-microbe interactions, then *QS*Is may become an increasingly important source of novel therapeutics against bacterial infections, while providing treatments that are less likely to impose the evolutionary constraints associated with the development of antibiotic resistance.

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