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MINIREVIEW

Communication Between Microorganisms as a Basis for Production of Virulence Factors

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Abstract

Quorum sensing (QS), or cell-to-cell communication in bacteria, is achieved through the production and subsequent response to the accumulation of extracellular signal molecules called autoinductors. The main role of QS is regulation of production of virulence factors in bacteria. Bacterial pathogenicity is often manifested by the expression of various cell-associated and secreted virulence factors, such as exoenzymes, toxins and biofilm. In bacteria, the expression of virulence factors is controlled coordinately by the global regulatory QS systems, which includes the AI-1/LuxIR-, AI-2/LuxS-, AI-3/QsC-, AIP/Agr-based systems. The regulation of production of virulence factors is extremely complex and many components influence it.

Key words: autoinductor, pathogenesis, quorum sensing, virulence factors

Introduction

One of the fundamental adaptation abilities of microorganisms is sensitivity to changes in the environment and to their behaviour by managing for survival purposes. While the environment of microorganisms changes, the reaction is often programmed by changes in the expression of genes, resulting from the improvement of survival. Pathogenesis may be considered as bacterial adaptation towards survival and development in a new niche in a host organism. During pathogenesis, numerous virulence factors are expressed and used in different ways to support the survival of bacteria in the host organism.

Many different factors may influence the evolution and course of pathogenesis, that is temperature, pH, chemical compounds from the host. Moreover, numerous natural microflora should be added to host factors, which may also detect signals deriving from other bacterial species. During this process, known as quorum sensing (QS), bacteria produce signal called autoinductor (AI), which is secreted to environment. Every AI, reaching critical concentration causes growth of the bacterial population and induces changes in the expression of genes resulting from switching life cycle and bacterial metabolism. Some bacteria use QS to detect one another when the community they belong to reaches population numerous enough to activate a "profitable" virulent way of life, *i.e.* biofilm formation. AI-based systems, which do not react strictly with QS model, are also used by bacteria, *i.e.* pathogenic bacteria utilize signal molecules to detect the presence of a host's microflora, indicating this way that the pathogen is localized in the specific niche as colonization. In these cases the detected signal is not transferred by bacteria – "invader", but it rather informs of the high population density. Hence, bacteria that colonize a host do not have to produce or secrete a signal, only to detect it.

There are known four common systems of bacterial cell-to-cell communication. Two of them, where autoinductor-1 (AI-1) and autoinductor-3 (AI-3) are used, exist in Gram-negative bacteria while Gram-positive have their own system based on an autoinducing polypeptide (AIP). The fourth system, which utilizes autoinductor-2 (AI-2), is present in both Gram-positive and Gram-negative bacteria and it is a very common signaling system. All the systems begin producing and secreting the autoinductor to the environment by either pathogen or natural microflora. Detection of these chemically different autoinductors and changes in genes' expression is specific to all of the systems.

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QS signal molecules, although largely considered as effectors of QS-dependent gene expression, are also emerging as multifunctional molecules that influence life, development and death in single and mixed microbial populations and impact significantly the outcome of host-pathogen interactions (Williams and Cámara, 2009). QS is a key regulatory system, which itself is affected by many other regulators (Girand and Bloemberg, 2008).

AI-1/LuxIR-based system

The first finding of this system is associated with explanation of the generation of luminescence by *Vibrio fischeri*. That is why LuxI/LuxR-based system is still a model QS-system, on which the others base. It includes LuxI, which produces *N*-acyl homoserine lactone (AHL) called AI-1 and LuxR, transcription factor responsible for gene expression control in the presence of AI.

LuxI and its homologues produce AI by fatty acid chain transfer from acylated acyl carrier protein (ACP) to *S*-adenosylonationine (SAM), releasing AHL and methylthioadenosine (Schaefer *et al*., 1996). AHL produced by LuxI homologues contain different fatty acids remains, what favours recognition of specific ACP by synthases and results from species- and genus-specific signal.

After LuxI synthesis, AI-1 diffuses through cell barriers and is released to environment. That is why, every single bacterium simultaneously produces AI-1 in growing population, and its concentration in the environment grows along with population growth. At high cell density, AI-1 concentration is high enough to diffuse inside the cell, where it bonds LuxR. After AI-1 binding, LuxR activates transcription of *luxCDABEGH*-operon by binding Lux-boxes localized in promoter (Devine *et al*., 1989). The product of this operon, luciferase, catalyzes chemical reaction, which gives luminescence.

Presence of LuxI/LuxR-system homologues has been proved in many Gram-negative bacteria, able to produce specific AHL. Among some bacterial species like *Pseudomonas aeruginosa*, *Serratia marcescens*, opportunistic pathogens, mechanisms of communication control pathways responsible for expression of different virulence factors. Both species, able to form biofilm on biomaterials, cause chronic infections in hospitalized patients. The nature of biofilms makes these infections more resistant to antibiotic treatment than planktonic bacteria cells. *P. aeruginosa* rods contain two AHL-based QS systems: LuxI/LuxR for biofilm formation control and production of extracellular enzymes, as well as transcription by different QS system, RhII/RhIR enriching by additional control level through AHL-signaling system (De Kievit and Iglewski, 2000). Both systems play a role in virulence of *lasI* and *rhII* strains, as mutants with one or two mutations. These strains colonize lungs of infant mouse more weakly than wild type (Pearson *et al*., 2000). LasI synthesize oxo-C12-homoserine lactone (HSL), which activates LasR, while RhII produces C4-HSL, activating RhIR (Schuster and Greenberg, 2006). Ueda and Wood (2009) discern a mechanism by which QS controls biofilm formation by screening 5850 transposon mutants *P. aeruginosa* PA14 for altered biofilm formation. This screen identified the PA3885 mutant, which had 147-fold more biofilm than the wild-type strain. Loss of PA3885 decreased swimming, abolished swarming, and increased attachment, although this did not affect production of rhamnolipids. The PA3885 mutant also had a wrinkly colony phenotype, formed pronounced pellicles, had substantially more aggregation, and had 28-fold more exopolysaccharide production. Expression of PA3885 in *trans* reduced biofilm formation and abolished aggregation. Whole transcriptome analysis showed that loss of PA3885 activated expression of the *pel* locus, an operon that encodes for the synthesis of extracellular matrix polysaccharide. Genetic screening identified that loss of PelABDEG and PA1120 protein (which contains a GGDEF-motif) suppressed the phenotypes of the PA3885 mutant, suggesting that the function of the PA3885 protein is to regulate 3.5-cyclic diguanylic acid (c-di-GMP) concentrations as a phosphatase since c-di-GMP enhances biofilm formation by activating PelD, and c-di-GMP inhibits swarming. Loss of PA3885 protein increased cellular c-di-GMP concentrations; hence, PA3885 protein is a negative regulator of c-di-GMP production. Purified PA3885 protein has phosphatase activity against phosphotyrosine peptides and is translocated to the periplasm. Las-mediated QS positively regulates expression of the PA3885 gene. The PA3885 protein responds to AHL signals and likely dephosphorylates PA1120, which leads to reduced c-di-GMP production. This inhibits matrix exopolysaccharide formation, which leads to reduced biofilm formation (Ueda and Wood, 2009).

P. aeruginosa forms a biofilm in lung tissue in *cystis fibrosis* (CF) patients and oxo-C12-HSL particles can be detected in sputum. *In vitro* studies indicate that oxo-C12-HSL is conducive to IL-8 production by bronchial epithelial cells, through induction of the transcription factors NF-kB and AP-2. *In vivo* studies using the adult mouse acute lung refection model indicate that *P. aeruginosa* Δ lasI/rhlI mutant, defective for the production of oxo-C12-HSL and C4-HSL, is attenuated for disease in mice. Oxo-C12-HSL, but not C4-HSL, induces a potent inflammatory response *in vivo.* Expression analysis indicated increased expression of the chemokines MIP-2 (macrophage inflamma-

tory protein), MCP-1 (monocyte chemotactic protein), MIP1-beta, IP-10, CTCA 3) T cell activation gene 3, IL-1 alpha and IL-6 (Pacheco and Sperandio, 2009). Activated T cells exposed to oxo-C12-HSL showed an increased production of IFN comparable to that induced by the control IL-12, but not IL-4, suggestion that oxo-C12-HSL induces a Th1 inflammatory response. These data suggest that oxo-C12-HSL stimulates inflammation in most tissues. Oxo-C12-HSL induces apoptosis in bone marrow derived macrophages and neutrophiles (Tateda *et al.*, 2003). Although there is an increasing evidence that oxo-C12-HSL manipulates host cell signaling, the mechanism of this regulation remains unknown.

Culture positivity for *lasR* mutant *P. aeruginosa* may serve as marker of early CF adaptive change of prognostic significance. Furthermore, as LasR inactivation alters susceptibility to antibiotics, infection with *las* mutant *P. aeruginosa* may impact response to therapy (Hoffman *et al*., 2009).

In *P. aeruginosa*, the expression of virulence genes is coordinately controlled by the global regulatory QS systems, which includes the *las* and *rhl* systems, as well as the *Pseudomonas* quinolone signal (PQS) system (Liang *et al.*, 2008). Phenazine compounds are among the virulence factors under the control of both the *rhl* and PQS systems. Gene PA0964, designated *pmpR* (*pqsR*-mediated PQS regulator), has been identified as novel regulator of the PQS system. It belongs to a large group of widespread conserved hypothetical proteins with unknown function, the YebC protein family (Pfam family DUF28). It negatively regulates the QS response regulator *pgsR* of the PQS system by binding at its promoter region. Alongside *phzA1* expression and phenazine and pyocyanin production, a set of virulence factors genes controlled by both *rhl* and the PQS were shown to modulated by PmpR. Swarming motility and biofilm formation are also significantly affected. The results added another layer of regulation in the rather complex QS systems in *P. aeruginosa* and demonstrated a clear functional clue for the YebC family proteins.

Nakamura *et al.* (2008) investigated extracellular lipopolysaccharide (LPS) and DNA in the supernatants of culture solutions from PAO1, the wild-type *P. aeruginosa*, and those of QS mutants. As compared to that of *las* QS mutants, the amount of LPS and DNA released was significantly higher in PAO1 and in *las* QS mutants complemented with *N*-(3-oxododecanoyl) homoserine lactone. QS is among the regulators involved in the release of extracellular LPS and DNA.

S. marcescens also has a homologous system LuxI/LuxR. This system in the presence of AHL, produced by *Serratia* spp. turns on the control of many pathways connected with *S. marcescens* strains pathogenesis, in comparison with wild type strain. Synthase SmaI AHL-deficient strain proves decrease in swarming motion, weaker adhesion (probably due to biofilm formation defect) and lower production level of extracellular casein, chitinase and hemolysin (Coulthurst *et al*., 2006). These defects are repaired by AHL addition, what indicates that these properties are caused directly or indirectly by cell-to-cell communication.

Studies suggest that QS systems are used to control the density of own population, but it does not always happen. *Escherichia coli* and *Salmonella enterica* serovar Typhimurium do not have LuxI-equivalents, hence they do not produce AI-1, but encode LuxR-homologues, called SdiA. When overproduced, they exhibit negative interaction on genes that influence cell adhesion of enterohemorrhagic *E. coli* strains (EHEC) (Kanamaru *el al*., 2000). Positive interaction is observed in case of different genes localized on *S.* Typhimurium virulence plasmid, including *rck* gene, producing protein involved in host immune response evasion (Ahmer *et al*., 1998). Although exact role of SdiA in pathogenesis is unclear, protein allows EHEC and *S.* Typhimurium strains to change genes' expression in response to the presence of AI-1, produced by other bacteria (Michael *et al*., 2001). It may have a particular significance for these pathogens, because of colonization gastrointestinal tract, where AI-1 may be present as a product of host's natural microflora.

AI-2/LuxS-based system

Not only AI-1-based system is available for cell-to-cell communication. Numerous Gram-negative and Gram-positive bacteria species have a system that detects extracellular signal known as autoinductor-2 (AI-2). This signal differs from the above described AHL-system and is synthesized by SAM metabolism. LuxS changes ribose – homocysteine into homocysteine and 4.5-dihydroxy-2.3-pentanedione (DPD) component, which in the presence of water, forms different furanone-derivatives.

AI-2, like AI-1, is released by bacteria and accumulated in the cells' environment. Two mechanisms of AI-2 detection, both different from recognition of AI-1, have been described. First of them, described in *V. harveyi,* is specific to borate diester forming AI-2. This mechanism detects AI-2 in periplasm by binding signal molecules to proteins binding specific autoinductor, LuxP. AI-2/LuxP-complex reacts subsequently with LuxQ-kinase sensor, initiating phosphate-transfer cascade by deactivation of negative response of LuxO regulator in luciferase production and luminescence.

Among pathogenic *V. cholerae* strains LuxO inhibits the production of transcription regulator HapR at low cell density, which further inhibits expression of some virulence factors (Zhu *et al*., 2002). These

bacteria have reversed "intuition" in virulence factors expression at low population density. To identify AI-regulated target genes in *V. cholerae* El Tor, strain responsible for the current cholera pandemic, luciferase expression was assayed in an AI^- strain carrying a random *lux* transcriptional reporter library in the presence and absence of exogenously added AI. Twenty three genes were identified and shown to require the QS transcription factor, HapR, for their regulation. Several of the QS-dependent target genes, annotated as encoding hypothetical proteins, in fact encode HD-GYP proteins, phosphodiesterases that degrade the intracellular second messenger cyclic dimeric GMP (c-di-GMP), which is important for controlling biofilm formation. Indeed, overexpression of a representative QS-activated HD-GYP protein in *V. cholerae* El Tor reduced the intracellular concentration of c-di-GMP, which in turn decreased exopolysaccharide production and biofilm formation. The *V. cholerae* classical biotype which caused previous cholera pandemics and is $HapR^-$, controls c-di-GMP levels and biofilm formation by the VieA signaling pathway. The VieA pathway is dispensable for biofilm formation in *V. cholerae* El Tor but that restoring HapR in *V. cholerae* classical biotype reestablishes QS-dependent repression of exopolysaccharides production (Hammer and Bassler, 2009).

AI-2 is used by *E. coli* and *S.* Typhimurium rods in different ways. These bacteria import AI-2 by the mechanism similar to capturing ribose by *rbs*-system. Distinctive LuxP/LuxQ-system, Lsr system regulated by LuxS, induces cellular response by transport of the AI-2 to cell cytoplasm. This process starts with recognition of the signal by periplasmic protein, LsrB, which binds to R-THMF form of AI-2. One bond of the transporter, Lsr ABC, contains LsrA and LsrC and allows AI-2 insertion to the cell, where it is phosphorylated by LsrK. Phosphorylated AI-2 form reacts with transcription repressor LsrR what extinguishes operon *Isr* (Taga *et al*., 2001). Although not demonstrated directly, this mechanism or a similar one, possibly results from decrease of the regulation of additional operons in the presence of phosphorylated form of AI-2.

LuxS/AI-2 systems have been detected among numbers of Gram-positive and Gram-negative bacteria species, what indicates that AI-2 system allows common communication among species (Xavier and Bassler, 2003). Considering a complicated nature of most natural bacteria communities, for example, in the mammalian gastrointestinal tract, this signal may be used for regulation of genes required to survive in the presence of other bacteria.

Pathogenic bacteria may use the same signals for activating mammalian cells, which let them gain an advantage in the same environment. The role of AI-2 in pathogenesis of bacteria other than *Vibrio* spp. is unclear. Studies using *luxS*-mutants have demonstrated the growth of the expression level of pili and type III secretion mechanism in EHEC strains only when autoinductor is present (Sperandio *et al*., 1999, 2001). It has been noticed that direct influence of the phosphorylated AI-2 or bonded AI-2 on these genes never affects the response of the regulator.

Many regulatory systems control virulence-associated traits in *Staphylococcus epidermidis*. *LuxS* mutant *S. epidermidis* shows increased biofilm formation *in vitro* and enhanced virulence in rat model of biofilm-associated infection. On the contrary, inactivation of *luxS* in various *S. aureus* strains has been reported not to affect virulence-associated traits. Externally added AI-2 almost completely restored gene expression patterns of the wild-type strain in the *luxS* mutant strain *S. epidermidis*. *S. epidermidis* regulates virulence-associated factors in addition to metabolism in an AI-2-dependent manner. There was dramatic AI-2-dependent alternation of pro-inflammatory phenol-soluble modulin (PSM) expression (Li *et al*., 2008). PSMs have been recently recognized as key pro-inflammatory and immune evasion factors in *S. epidermidis* and *S. aureus.*

AI-3/QsC-system

AI-3, different from AI-2-system, was first described as a component of used growth medium, which activates expression of genes responsible for EHEC adhesion to eukaryotic cells, the result of which is rearrangement of actin proteins (Sperandio *et al*., 2003). Structure and synthesis of that signal is unclear. Like AI-1, it represents family of particles.

Results indicate that LuxS influences some pathways, because AI-3 production is less efficient in *luxS* mutants. Other studies suggest that lack of AI-3 production in the same mutants is a result of change in a cell metabolism. When oxaloacetate was used instead of SAM as methionine precursor and L-aspartate was added to growth medium to reduce requirements of oxaloacetate, AI-3 production was reversed, but no result on AI-2 production was observed (Walters *et al*., 2006). These studies also indicate that numerous commensal bacteria, *i.e.* nonpathogenic *E. coli* and *Enterobacter cloacae* and pathogenic species like *Shigella* spp., *Salmonella* spp. and *Klebsiella* spp. produce AI-3. It suggests that AI-3 may represent other interspecies signal, which has not been detected in Gram-positive bacteria. The role of AI-3 in commensal communities is still vague.

AI-3 detection is possible using 2-complements system composed of QseC-kinase sensor and QseB-regulator response. In the presence of periplasmic AI-3, QseC is autophosphorylated first and transfers this phosphate on QseB afterwards, which activates

genes responsible for pili biosynthesis and motility by decrease in regulation of genes regulator responsible for host's cilium flhDC (Clarke *et al*., 2006). AI-3 presence is also connected with the process of adhesion and effacement (attaching and effacing, AE) of lesion by EHEC – phenomenon connected with increased regulation of 5 different loci of enterocyte effacement (LEE) operons localized on the EHEC chromosome (Sperandio *et al*., 2003). Cascade responsible for these genes' regulation is still unclear, probably requires QseA, LysR – family regulator that influences cell-to-cell communication and directly increases LEE genes regulation (Sperandio *et al.*, 2002). On the basis of these observations, it is suggested that intestinal pathogens may utilize AI-3 produced by host's microflora and pili overexpression as well as motility regulation is necessary to penetrate intestinal mucous membrane rich in epithelial cells. Proteins encoded by LEE genes are crucial to contact with epithelial cells and make pathogens able to adhere and finally colonize eukaryotic cells.

QseBC cascade is also responsible for adrenaline and noradrenaline signals, existing in gastrointestinal tract (Clarke *et al*., 2006). It suggests that QseC is responsible simultaneously for bacteria and host signals. Adrenergic receptors on eukaryotic cells may react to AI-3 in like they do to adrenaline and noradrenaline. Intestinal bacteria may have an advantage in this kind of communication for intestinal epithelial cells preparation for colonization.

AIP/Agr-system

This system of cell-to-cell communication occurs in Gram-positive bacteria. It is based on the prototype Agr system, first described in *S. aureus* strains. Gram-positive bacterial systems utilize polypeptide signals. They are bi-functional: they influence an organism which produces them as autoinductors and other organisms as inhibitors. That signal, called AIP, is encoded by *agrD* genes. After translation, propeptide AgrD is directed to membrane by N-terminal sequence of the signal. Near membrane, AgrB, membrane-related endopeptidase cuts C-terminal of the propeptide. N-terminal part of the propeptide, including signal sequence, is excluded by peptidase SpsB signal. Eventually, C-terminal part of the transformed polypeptide is covalently bonded with centrally placed cysteine to a ring form thiolactone with free N-end. Both structures are required for appropriate function of AIP. After reaching environment, AIP is recognized by AgrC-signal receptor. This protein contains transmembrane rest ended with N-sequence responsible for recognizing specific AIP and histidine kinase N-rest, which in the presence of specific AIP, phosphorylates the response of AgrA regulator. Phosphorylated AgrA activates transcription of selected genes by direct bond of repeated regions localized in promoter. Uniqueness of AIP/Agr system is the fact that AIP produced by one *Staphylococcus* spp. strain may interfere into Agr system of another one. This double role as activator and inhibitor is connected with interaction between AIP and AgrC. Cyclic structure of AIP is required for interactions with AgrC, but the N-terminal part is responsible for AgrC activation. Removal of this ending (tarl) results from a universal inhibitor, which bonds AgrC, but is not capable to activate Agr system (Lyon *et al*., 2000).

Agr system is connected with pathogenesis of numerous Gram-positive bacteria. Agr system is basic QS element among staphylococci. During the switch from late logarithmic to stationary phase, decrease in the level of expression of a few surface membrane proteins and increase of numerous virulence factors are observed. About 150 genes are under its control. It is found in about 14 species/subspecies of staphylococci. Its role, as a QS mediator, depends on the type of infection, time and environment which host provides. Agr expression is dynamic. Agr is exposed only in dynamically growing cell populations. Its activity is observed in two phases: phase I – first three hours after infection, important for abscess growth, phase II – 48-72 hours afterwards. Agr inactivation is connected with increase in resistance to antibiotics and small colony variants (SCV). SCV growth in the presence of AIP of isogenic strain restores or partially restores Agr activity. *S. aureus* strains, deprived of Agr system, have a decreased ability to cause *osteomyelitis* on both mice and rabbit models (Blevins *et al*., 2003; Gillaspy *et al*., 1995). AIP genes regulation by AgrA results from production and secretion of numerous *S. aureus* toxins, for instance, hemolysin alpha-, beta- and delta-, serine protease and toxic shock syndrome toxin (TSST-1).

The *S. aureus* genome encodes a number of transcriptional factors, the Sar (staphylococcal accessory regulator) family of proteins, including SarA, SarR, SarS, SarT, SarV, and Rot. SarA is transcriptional activator for the Agr system, as well as a transcriptional regulator that activates or represses a number of staphylococcal genes. SarA, for example, is a repressor of *spa* (staphylococcal protein A) and an upregulator for the fibronectin-binding protein A. SarA is also required for biofilm formation. SarR binds to the *sarA* promoter region to downregulate transcription from its P1promoter and thus reduces SarA protein expression. SarS is a positive regulator of *spa* transcription. SarT has been shown to positively regulate *sarS* transcription and negatively regulate expression of *hla* (which encodes alpha-hemolysin) and *sarU*. SarU is proposed to be a positive regulator of *agr* expression.

SarV is thought to be an important regulator in the autolytic pathway of *S. aureus*. SarX is a negative regulator of Agr. AgrA has been shown to be an activator of microcapsule synthesis, nuclease expression, and *norA* transcription but represses the expression of alpha-toxin, coagulase, protease, protein A, and certain genes involved in autolysis (Hsieh *et al*., 2008).

Hemolysin secretion and eukaryotic cells damage by protease facilitate staphylococci to adhere, while TSST-1 is a superantigen that bonds nonspecific receptors of the T-cells and eventually yields strong immune response caused by systemic cytokine production. Furthermore, high toxins regulation and high AIP concentration are responsible for decreased level of proteins exposed to the cell surface, like fibronectin binding proteins and protein A, two proteins connected with staphylococci adhesion. That drop of cell adhesion ability may impact negatively on the architecture of populations related to biofilm. Bacteria cells are released from their populations and as planktonic populations migrate to other structures of the host body. If they find an available place, they adhere again and multiply, causing secondary infections.

QS regulation in *S. epidermidis* has two major characteristics that reflect the adaptation to stationary growth phase: 1) up-regulation of virulence, resistance, stress and other factors that are needed for survival under suboptimal conditions and 2) down-regulation processes, such as translation and cell division that are typical of exponentially growing cells (Yao *et al.*, 2006). Biofilm formation is a key virulence determinant during chronic staphylococcal infections, particularly in infection by *S. epidermidis.* The low activity of *agr* is a characteristic feature of the main part of *S. epidermidis* biofilms, whereas exposed regions in a biofilm express *agr* at a higher level. *Agr* is differentially expressed during the course of infection.

Enterococcus faecalis is another Gram-positive bacterium that uses the AIP signal. These streptococci utilize a 2-component system corresponding to the Agr system in *Staphylococcus* spp. When AIP is detected, the cells produce and secrete two extracellular proteases: gelatinase (GeIE) and serine protease (SprE) (Qin *et al*., 2000). The role of these two proteins in pathogenesis is still unclear, but gelatinase facilitates cells migration into the host organism (Zeng *et al*., 2005). *E. faecalis* translocation through epithelial cells of the colon may allow the infection of vascular and lymphatic system, migration to other places, causing secondary infections.

AIP-based system is not the only one in *E. faecalis* QS. Cytolysin production by the enterococci is repressed at the level of transcription by two proteins: membrane connected sensor, CylR1 and DNA connected proteins, CylR2 (Haas *et al*., 2002). When cytolysin concentration outside the cell is low, CylR2 represses genes encoding subunits of cytolysins, $cylL_L$ and $cylL_S$, by *cylL* promoter bond. CylR1 monitors cytolysin concentration outside the cell and while the threshold concentration is reached, CylR1 changes the ability to bond DNA by CylR2. It results from CylR2 secretion by *cylL* promoter and increase in the cytolysin production. Cytolysin is regulated in a manner dependent on population, by means of its detection rather than AIP detection.

Streptococcus mutans uses the QS signaling system, which is dependent on competence stimulating peptide (CSP), to regulate diverse physiological activities including bacteriocin production, genetic transformation, and biofilm formation. The expression of QS-associated genes was increased 3.4-5.3-fold by CSP in biofilms. Cell viability of *S. mutant* grown in biofilms is affected by the CSP-dependent QS system (Zhang *et al*., 2009).

Summary

It is becoming more understandable that bacterial cell-to-cell communication plays an important role in interactions between bacteria and host. This is true for both natural symbiotic microflora and pathogenic "invading" bacteria. A great number of bacteria employ QS for regulation of various phenotypes as a part of their pathogenic or symbiotic lifestyles. In the case of a host's microflora, signaling particles are utilized to control density of the population for a better response coordination of numerous population, frequently occurring in the created, complex biofilm. Some pathogens use also signaling particles to coordinate a reaction dependent on population in case of a huge number of bacteria needed for colonization. They also use signaling particles produced by other populations, like natural microflora as indicator of their presence in host organism. Using that kind of strategy, the "passing" bacteria activate pathogenic genes only when they are in the environment where pathogenesis is successful. It secures bacteria from pointless using of additional part of energy required for pathogenesis.

A higher relatedness between the bacteria infecting a host (lower strain diversity) will lead to more prudent exploitation of the host, and hence lower virulence. A higher relatedness will favor higher levels of cooperation that in turn allows the host to be exploited more efficiently, and hence a higher virulence (Rumbaugh *et al*., 2009). QS molecule signals may modulate the physiology of other microbes in as-yet-undiscovered interspecies interactions (Shank and Kolter, 2009). QS signal molecules, although largely considered as effectors of QS-dependent gene expression are also emerging as multifunctional molecules that influence life, development and death in single

and mixed microbial populations and impact significantly the outcome of host-pathogen interactions (Williams and Cámara, 2009).

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