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# Quorum Sensing in *Bacillus Cereus* in Relation to cysteine Metabolism and the Oxidative Stress response

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Chapter 21.5. Quorum sensing in *Bacillus cereus* in relation with cysteine metabolism and oxidative stress response.

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Running Title: Description of PlcRa/AIP and Lsr-like/AI-2 quorum-sensing systems in *Bacillus cereus*

**Keywords:** *Bacillus cereus*; quorum sensing; AIP; PlcRa; cysteine metabolism; CymR; AI-2; Lsr-like system; oxidative stress response; biofilm; global transcriptional analysis.

## **Abstract**

A number of peptide-mediated quorum sensing (QS) systems regulate important biological functions in bacteria of the *Bacillus cereus* group. Sporulation, virulence and biofilm formation are controlled by such systems in these bacteria. PapR, a secreted heptapeptide, activates PlcR, the main transcriptional regulator of virulence in *B. thuringiensis* and *B. cereus*. We recently found that PlcRa, a PlcR paralogue, also works in QS and is involved in oxidative stress response and cysteine metabolism during the early stationary phase of bacterial growth. Notably, we have reported that PlcRa activates the expression of several genes involved in the pathway of cysteine synthesis from methionine including the S-adenosylmethionine (SAM) recycling pathway. This transcriptional control of SAM recycling pathway by PlcRa impacts another QS, the autoinducer-2 (AI-2) system. AI-2 is a signal metabolic molecule produced by LuxS, a central metabolic enzyme involved in SAM recycling, found in many bacterial species and thus proposed to enable interspecies communication. We have reported that *B. cereus* synthesizes and recognizes AI-2 as an extracellular signal. Most particularly, we have shown that AI-2 inhibits biofilm formation in a concentration-dependent manner. In this review, we provide an overview of these two QS systems in *B. cereus* species (signal production, detection, transduction) with the description of AI-2 connection. We highlight how CymR

regulon -involved in the global regulation of gene expression in response to cysteine availability- together with PlcRa system is important for AI-2 production and present the role of PlcRa in oxidative stress response.

### **21.5.1. Introduction**

Quorum sensing (QS) allows bacteria to share information about cell density to adjust gene expression accordingly. This cell-cell communication relies on secreted signal molecules, called autoinducers (AI) or pheromones. This system enables bacteria to undergo energetically expensive processes as a collective only when the impact of these processes on their environment is more likely to be efficient (Dunny and Leonard, 1997).

Intercellular communication processes are crucial in coordinating growth, adaptation, pathogenicity, biofilm formation and sporulation in *B. cereus* group (Slamti et al., 2014). The *B. cereus* group includes well-known spore-forming pathogens of mammals (*B. anthracis* and *B. cereus*) and insects (*B. thuringiensis*). *B. cereus* is frequently associated with food-borne infections causing gastroenteritis (Stenfors Arnesen et al., 2008). *B. cereus* has also been described as the cause of nosocomial infections in immuno-compromised patients and is

involved in endocarditis, endophthalmitis or meningitis that have proved fatal (Stenfors Arnesen et al., 2008). The capacity of *B. cereus* to sporulate allows this bacterium to resist the usual cleaning procedures used in the food industry, resulting in the presence of *B. cereus* in many raw and processed foods, such as rice, spices, milk, vegetables, meats and various desserts (Abee et al., 2011).

The PlcR/PapR QS system controls the expression of genes encoding exported virulence factors, including degradative enzymes, enterotoxins and hemolysins in *B. cereus* and *B. thuringiensis* species (Agaisse et al., 1999). The transcription of these virulence genes in bacteria growing in a rich medium is activated at the onset of stationary phase or transition phase by the active quorum sensor PlcR. PlcR is activated by binding to PapR, a signaling peptide produced as a propeptide under the control of PlcR (Figure 21.5.1). PapR undergoes extracellular processing, to generate an active heptapeptide (Bouillaut et al., 2008), which is then re-imported into the bacterial cell via the oligopeptide permease system, OppABCDF. Within the cell, PapR interacts with PlcR and the resulting complex binds PlcR target sites on DNA (Slamti and Lereclus, 2002), resulting in the activation of the PlcR regulon, which contains 45 genes (Gohar et al., 2008). For a more detailed overview of PlcR/PapR system we recommend a recent review by Slamti (Slamti et al., 2014)

Determination of the structure of PlcR resulted in the identification of a new family of central regulatory quorum sensors (the RNPP family) found exclusively in Gram-positive bacteria with a low G+C content (Declerck et al., 2007). These quorum sensors include PlcRa and NprR from *B. cereus* (Huillet et al., 2012; Perchat et al., 2011), PrgX from *Enterococcus faecalis* (Shi et al., 2005) and RAP phosphatases from *Bacillus subtilis* (Parashar et al., 2011; Solomon et al., 1996). All these RNPP regulators are activated through a secreted signaling peptide that interacts with the TPR activation domain.

We recently characterized PlcRa, which activates the oxidative stress response and cysteine metabolism in transition state cells (Huillet et al., 2012). PlcRa displays about 29% sequence identity and about 50% similarity to PlcR. We found that the PlcRa protein is a 3D structural homolog of PlcR, suggesting that the TPR peptide-binding domain may be arranged similarly to form a pocket that is responsible for peptide binding. PlcRa is activated by the product of the *papRa* gene, a secreted signaling peptide (Figure 21.5.1) (Huillet et al., 2012). Biochemical and genetic analyses demonstrated the role of the heptapeptide PapRa<sub>7</sub> in PlcRa activity. Purified PlcRa specifically binds to a PlcRa-controlled promoter in the presence of PapRa heptapeptide (*abrB2* gene) (Huillet et al., 2012). The production, the maturation of

PapRa and the binding of PapRa<sub>7</sub> with the TPR activation domain remain to be established (Figure 21.5.1).

Figure 21.5.1 here.....

In Gram-negative bacteria, QS systems use mainly acylated homoserine lactones as signal molecules, while linear or cyclic peptides fulfill this function in Gram-positive bacteria. However, a conserved quorum-sensing system is found both in Gram-positive and Gram-negative bacteria (Pereira et al., 2013). The signal molecule of this system is AI-2 (autoinducer 2), a detoxification product of the activated methyl cycle or SAM recycling linked to cysteine biosynthesis from methionine (Figure 21.5.2). AI-2 is a derivative of (S) 4,5-dihydro-2,3-pentanedione (DPD). DPD itself is a by-product of the activated methyl cycle (AMC) which generates the methyl donor, S-adenosylmethionine (SAM), required for a number of metabolic reactions including methylations and polyamine biosynthesis. Release of methyl from SAM results in the production of S-adenosyl homocysteine (SAH), a toxic compound which conversion to DPD and homocysteine is catalysed by the Pfs and LuxS enzymes. DPD spontaneously converts to AI-2 while homocysteine is recycled to SAM by the MetE, Meth

and MetK enzymes. Therefore, AI-2 is both a product of the central metabolism and a signal molecule.

Figure 21.5.2 here.....

AI-2 is exported by a still unknown mechanisms. When its threshold concentration in the extracellular milieu is reached, the signal molecule is re-imported in the cell by a specific channel - the LsrACD transporter in *Bacillaceae* (Figure 21.5.3). Once in the cell, AI-2 is phosphorylated by the LrsK kinase, enabling its binding to, and the inactivation of, the LrsR transcriptional repressor. Genes regulated by LrsB can therefore be transcribed (Figures 21.5.3, 21.5.4).

Despite the presence of PfS and LuxS in most bacteria, not all of these bacteria use AI-2 as a signal molecule for quorum-sensing purposes, since some of them lack the AI-2 receptor (Rezzonico and Duffy, 2008). However, pathogens of the *B. cereus* group (*B. anthracis*, *B. cereus* and *B. thuringiensis*) possess the genetic equipment for AI-2 secretion, importation and processing. In addition, the LuxS-dependent production of AI-2 and its effect on growth has been demonstrated in *B. anthracis* (Jones and Blaser, 2003).

Figure 21.5.3 here.....

Figure 21.5.4 here.....

In this review, we provide an overview of PlcRa and Lsr-like QS systems in *B.cereus* with the AI-2 connection between the two systems (Figure 21.5.4) and discuss in the concluding remarks section the physiological effects of this QS interplay during bacterial growth.

### **21.5.2. AIP-mediated signalling in *B.cereus*: PlcRa system**

In this section, we first provide a brief overview of cysteine metabolism in *Bacillus* and present the major regulatory system that govern it -CymR regulon- in the Gram-positive model bacterium, *B.subtilis*. Then, we present transcriptomic changes in relation with CymR regulon induced by deletion of *plcRa* gene in *B.cereus*. Finally, we provide a brief overview of the oxidative stress defense mechanisms including CymR control in *B.subtilis*

and then present phenotypic changes in the *plcRa* mutant strain in the presence of hydrogen peroxide and diamide.

**CymR: master regulator of cysteine metabolism in *B. subtilis*.** The sulfur-containing amino acid, cysteine, plays a major role in cellular physiology and its synthesis is tightly controlled due to its reactivity. Cysteine is a biosynthetic precursor of methionine, thiamine, biotin, lipoic acid, coenzyme A and coenzyme M. Cysteine is also required for the biogenesis of [Fe-S] clusters and it is found in the catalytic site of several enzymes and helps protein folding and assembly by forming disulfide bonds.

Cysteine metabolism is well characterized in *B. subtilis* (Burguiere et al., 2004; Even et al., 2006; Hullo et al., 2007). In this bacterium, cysteine is synthesized either from homocysteine via the reverse transsulphuration pathway (Hullo et al., 2007) or from sulphide or thiosulphate via the thiolation pathway that directly incorporates these compounds into O-acetyl-L-serine (OAS) (Figure 21.5.5). Sulphide is obtained from the transport and reduction of inorganic sulphate or from organic sulphonates via their oxidation. Finally, methionine degradation is a biosynthesis pathway of cysteine formation and it proceeds via the formation of homocysteine using the S-adenosyl-methionine (SAM) recycling pathway followed by reverse transsulphuration,

involving the MccA cystathionine  $\beta$ -synthase, and the MccB cystathionine  $\gamma$ -lyase (Figure 21.5.5).

Figure 21.5.5 here.....

In *B. subtilis* (Even et al., 2006; Tanous et al., 2008) CymR for "cysteine metabolism repressor" is the master regulator of cysteine metabolism. CymR represses the transcription of a large set of genes involved in cystine uptake and cysteine biosynthesis. The CymR repressor belongs to the widespread and poorly characterized Rrf2 family of transcription factors (Tanous et al., 2008; Shepard et al., 2011). CymR displays a particular mechanism of control of its activity, with the formation of a regulatory complex with CysK, an OAS-thiol-lyase. In this complex, CymR is the DNA-binding protein, and CysK increases the stability of the interaction between CymR and DNA (Tanous et al., 2008).

In the signal transduction pathway controlling cysteine metabolism, CysK, via its substrate OAS, is the sensor of the cysteine pool in the cell for the regulatory complex, and transmits this information to the CymR repressor (Hullo et al., 2007). OAS is produced from acetyl-CoA and serine by CysE, the

serine acetyltransferase while the OAS-thiol-lyase, CysK, further condenses sulphide and OAS to form cysteine. Cysteine limits the OAS pool via its negative control of CysE synthesis and activity. In the presence of cysteine, the intracellular concentration of OAS is low, the CymR-CysK complex is formed, and transcriptional repression of the CymR regulon occurs (Figure 21.5.6.A). Under conditions of cysteine limitation, the pool of OAS increases until it reaches a concentration preventing the formation of the CymR-CysK complex or dissociating the complex *in vitro* (Figure 21.5.6.B). Thus, no repression of transcription of the CymR regulon occurs when cysteine is limiting (Even et al., 2006; Hullo et al., 2007; Tanous et al., 2008).

Figure 21.5.6.A here.....

Figure 21.5.6.B here.....

**PlcRa controls the expression of CymR regulon.** We characterized the regulatory role of PlcRa, by comparing the transcriptomes of the  $\Delta plcRa$  and wild-type *B. cereus* ATCC 14579 strains during early stationary phase in a complex rich medium, the Luria Bertani broth. Assessments were carried out one hour after the

onset of stationary phase ( $t_1$ ), when *plcRa* expression increases, and two hours after the onset of stationary phase ( $t_2$ ), when *plcRa* expression has reached a plateau (Huillet et al., 2012). Twenty of the 49 genes upregulated by PlcRa encode proteins involved in sulfur metabolism (Table 21.5.1). These genes were found to be differentially expressed two hours after the onset of stationary phase (Huillet et al., 2012). The sulfur metabolism genes of *B. cereus* remain poorly annotated, despite the availability of several *B. cereus* genomes. We therefore reconstructed the sulfur metabolism pathway, by searching for orthologs of *B. subtilis* genes in the *B. cereus* ATCC 14579 genome (Huillet et al., 2012) (Figure 21.5.5). The transport of sulfur sources (Burguiere et al., 2004) and the two major cysteine biosynthetic pathways in *B. subtilis* – the thiolation pathway, which requires sulfide, and the reverse transsulfuration pathway, which converts homocysteine to cysteine, with cystathionine formed as an intermediate (Even et al., 2006; Hullo et al., 2007) – are conserved in *B. cereus* ATCC 14579. We were able to identify all the enzymes and transporters required for these pathways other than those for the reduction of sulfite to sulfide (Huillet et al., 2012) (Table 21.5.1 & Figure 21.5.5).

Table 21.5.1 here.....

A total of 18 PlcRa-controlled genes are involved in cysteine metabolism: transporters (*tcyP*) and biosynthesis of cysteine from sulfate (*cysH* operon and *cysK* gene) or methionine (*yrrT-mtnN-mccAB* operon and *luxS* gene) (Table 21.5.1 & Figure 21.5.5).

BC4393, which shares 76 % identity with CymR from *B. subtilis* is probably the global regulator of cysteine metabolism in *B.*

*cereus*. The CymR DNA-binding motif (WTAW2N4ATWNRWNANAN2GGAATT) is present in the promoter region of 7 PlcRa-controlled genes or operons involved in cysteine metabolism (Huillet et al., 2012) (Figure 21.5.7). A total of 14 PlcRa-controlled genes belongs to the CymR regulon (Table 21.5.1).

Figure 21.5.7 here.....

No repression of transcription of the CymR regulon occurs when cysteine is limiting in *B.subtilis*. We hypothesized that PlcRa activates CymR-controlled genes when cysteine is limiting during the transition phase in *B.cereus*. Indeed, we found that the expression of the *PyrrT'*-lacZ fusion was induced at the onset of stationary phase in the wild-type strain, suggesting cysteine depletion in the growth medium leading to the induction of the

methionine-to-cysteine conversion pathway (Huillet et al., 2012). No such induction was observed in the *plcRa* mutant strain (Huillet et al., 2012). Here a proposed model for the global regulation of gene expression in response to cysteine availability in *B.cereus* (Figure 21.5.6AB). During the vegetative growth, in the presence of the preferred sulfur sources, sulfate or cysteine, CymR is active and represses the expression of CymR-controlled genes. Upon sulfate or cysteine limitation, as presumably observed at the onset of stationary phase, CymR repressor is inactive, repression is thus relieved (see previous paragraph or legend Figure 21.5.6AB for details). In this growth condition, PlcRa activator, active during this transition phase, activates CymR-controlled genes.

### **Role of the cysteine metabolism in oxidant protection in**

***Bacillus***. Many agents that restrict bacterial growth and developmental behavior induce the production and/or accumulation of reactive substances, such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and toxic electrophiles.

Increased levels of reactive species accompany stress, in many of its manifestations, and lead to macromolecular damage, an increased rate of mutagenesis, and cell death (Zuber,2009).

*Bacillus* respond to oxidative stress by the activation of

different cellular defence mechanisms (Zuber, 2009). These are composed of scavenging enzymes, as well as protection and repair systems presumably organized in highly sophisticated networks (Zuber, 2009; Mols and Abee, 2011). Cysteine alone, cysteine molecules and cysteine proteins are major actors in oxidant protection. In particular, cysteine is the direct precursor of the low-molecular mass antioxidant molecule, Bacillithiol, a key actor of the maintenance of the cytosolic redox balance and of the adaptation to reactive oxygen species in *Bacilli* (Newton et al., 2009; Helmann, 2011). In addition, S-cysteinylation is a general mechanism for thiol protection of *B. subtilis* proteins after oxidative stress (Hochgrafe et al., 2007). Consequently, the CymR regulon involved in cysteine biosynthesis is involved in oxidant protection. It has been shown that a *cymR* mutant is highly sensitive to H<sub>2</sub>O<sub>2</sub>, disulfide, paraquat, copper and tellurite induced stresses in *B. subtilis* (Soutourina et al., 2009). The CymR regulon is induced during disulfide or superoxide stresses in *B. subtilis* (Mostertz et al., 2004).

**PlcRa: role in the oxidative stress response.**

PlcRa is mainly involved in the activation of genes that function in oxidant protection through the control of the expression of CymR regulon (Huillet et al., 2012). PlcRa also activates the

expression of *ahpCF* operon encoding the alkyl hydroperoxyreductase (AhpR), a detoxification system composed of two enzymes that catalyses the breakdown of molecular oxygen to hydrogen peroxide ( $H_2O_2$ ) which is reduced by the second enzyme to water. PlcRa activates the expression of *dps2* gene encoding a Dps-like mini-ferritin protein (BC5044) which is homologous to the Dps2 protein of *B.anthraxis* that has recently been shown to play a major role in oxidative stress resistance (Huillet et al., 2012). These genes are induced during  $H_2O_2$  stress in *B. cereus* (Ceragioli et al., 2010).

We tested the effect of *plcRa* inactivation on the susceptibility of *B. cereus* to  $H_2O_2$  and diamide stresses. Diamide causes thiol oxidation and disulfide stress. The viability of the  $\Delta plcRa$  mutant and the parental strain was tested after the addition of  $H_2O_2$  (1 mM). A 7-fold reduction in survival was observed for the  $\Delta plcRa$  mutant compared to the wild-type strain. The complementation of the  $\Delta plcRa$  mutant restored a wild-type phenotype. Then, the viability of the  $\Delta plcRa$  mutant and the parental strain was tested after the addition of diamide (10 mM). A 4-fold reduction in survival was observed for the *plcRa* mutant compared to the wild-type strain while the introduction of *plcRa* in trans restored a wild-type phenotype (Huillet et al., 2012). These results showed that *plcRa* inactivation led to an increased sensitivity to  $H_2O_2$  and disulfide stresses. We also investigated

the effects of cystine addition during peroxide stress (Huillet et al., 2012). Cystine (1 mM) was added to the culture in mid-exponential growth phase (figure 21.5.8). Two hours later, at the end of exponential phase, H<sub>2</sub>O<sub>2</sub> (0.4 mM) was added to the medium. Both strains presented a growth arrest one hour after the H<sub>2</sub>O<sub>2</sub> addition which was characterized by a OD<sub>600</sub> measurements drop. In addition, in the presence of H<sub>2</sub>O<sub>2</sub>, OD<sub>600</sub> measures of the  $\Delta$ plcRa mutant strain were lower than the wild type strain. When cystine was added, the growth arrest for both strains was abolished. Thus, cystine significantly reduced the sensitivity of these cells to H<sub>2</sub>O<sub>2</sub> stress in our growth conditions. Moreover, these results strongly suggest that cystine transport is efficient in the  $\Delta$ plcRa mutant, as in the wild type and it might be due at least partly to the TcyABC system (BC0872-BC0873-BC0874) that is not controlled by PlcRa (figures 21.5.5, 21.5.6).

Figure 21.5.8 here.....

There is accumulating evidence suggesting that metabolic imbalances also induce bacterial processes that are normally associated with the oxidative stress response and/or with the general stress response. Altered transcriptional activities in *plcRa* mutant (117 genes differentially expressed with a more than

three-fold difference) probably resulted in metabolic changes which seem to have caused general and oxidative stresses (Huillet et al., 2012). Indeed, in the *plcRa* mutant, in LB medium without treatment, 10 genes that belong to the  $\sigma^B$  regulon, are strongly higher expressed (Huillet et al., 2012). This includes  $\sigma^B$  and its associated regulatory proteins RbsV, RbsW and RbsP. In *B. subtilis*, elevated activity of  $\sigma^B$  factor and induction of the  $\sigma^B$  general stress regulon confer increased oxidant resistance (Zuber et al., 2009). Upon exposure of *B. cereus* to mild and lethal  $H_2O_2$  concentrations the expression of numerous genes was affected, including genes involved in the  $\sigma^B$  common response to general stresses (Ceragioli et al., 2010). Oxidative and disulfide stresses regulation of prophage genes has been reported in *Lactobacillus plantarum*, *L. reuteri* and *B. subtilis* (Wilson et al., 2012). Notably in a *plcRa* mutant, the expression of 31 prophage genes is higher (Huillet et al., 2012).

### **21.5.3. AI-2 mediated signalling in *B. cereus*: Lsr-like system**

The production of AI-2 has been shown in *B. anthracis* to be LuxS-dependent, and this production is maximal at the end of the exponential phase of growth and decreased in stationary phase

(Jones and Blaser, 2003). In *B. cereus* too, production of AI-2 increased in exponential phase and decreased in stationary phase (Figure 21.5.8), suggesting that this quorum-sensing system is active during the transition between the exponential and the stationary phases of growth. In the same species, the effect of exogenously added AI-2 in a microtiter plate biofilm assay was assayed. AI-2 inhibited in a dose-dependent way the production of biofilm in these conditions. It also induced a decrease in biofilm biomass when this compound was added to mature biofilms (Figure 21.5.9) (Auger et al., 2006).

Figure 21.5.9 here.....

In Gram-positive as well as in Gram-negative bacteria, the AI-2 quorum-sensing system controls a number of phenotypes, including chemotaxis or toxin production in pathogens. However, the phenotype the most frequently targeted by this quorum-sensing system is biofilm formation (Pereira et al., 2013).

Interestingly, while AI-2 promotes biofilm formation in most species, the inverse effect is observed in *B. cereus*. This effect might be of importance in the formation of multispecies biofilm, where AI-2, working as an interspecies communication molecule, could modulate the balance of biofilm species.

While the *lsr* system appears to be fully functional in pathogenic bacteria of the *B. cereus* group, this is not a general rule in all bacteria of the Firmicutes phylum. In *Listeria monocytogenes* in *Clostridium difficile* or in *Lactobacillus reuteri*, for instance, the effect of *luxS* deletion is not a consequence of AI-2 absence, but is due to a perturbation of the bacterium metabolism (Challan Belval et al., 2006; Garmyn et al., 2009; Carter et al., 2005).

#### **21.5.4. Concluding remarks: interplay between PlcRa and Lsr-like QS during the transition between vegetative and sporulation phases.**

At the end of the vegetative growth phase, bacterial cells face a number of challenges, including a decrease in the nutrient content of their environment and accumulation of oxidative products. Under these conditions, spore-forming bacteria may initiate sporulation, producing spores that can survive in unfavorable environmental conditions (Piggot and Hilbert, 2004). Bacteria make use of various strategies to cope with environmental changes during the transition between the vegetative and sporulation phases (Phillips and Strauch, 2002). Alternatively to sporulation, bacteria can enter in another developmental state, the biofilm. The choice between sporulation and biofilm is likely to be a consequence of the speed at which

Spo0A, the sporulation master regulator, is activated by kinases. Low Spo0A~P concentrations, while unable to initiate the sporulation cascade of events, can promote biofilm formation by repressing or activating the transcription of other regulator genes (namely *abrB* and *sinI*) (Fujita and Losick, 2005).

In this review, we presented a new peptide-mediated QS system, PlcRa/PapRa involved in AI-2 production and in the control of cysteine biosynthesis during the transition between vegetative and sporulation phases. The two QS systems, PlcRa and Lsr-like, are interconnected through AI-2 production. In this review, we highlight that cysteine metabolism is an essential component of *B.cereus* physiology at the entry of stationary phase for three major reasons: (i) adaptation to sulfur poor condition (ii) oxidative stress response (iii) biofilm control. AI-2 is found in many bacterial species and thus proposed to enable interspecies communication. We identified a unique transcriptional control of AI-2 production via CymR and PlcRa regulators. This control is specific to *B.cereus* and *B.thuringiensis* species.

However, many specific and global questions surround Lsr-like and PlcRa/PapRa systems: PapRa signal production, AI-2 signal transduction; Lsr receptor characterization ; full phenotypic characterization of AI2/Lsr-like system; role of PlcRa/papRa in virulence; what is the benefit of AI-2 tight transcriptional

control for biofilm control? what is the benefit of QS-mediated control of cysteine metabolism during the infectious process of *B.cereus*?

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## **Figure Legends**

**Figure 21.5.1. Schematic representation of the PlcR-PapR and the PlcRa-PapRa QS systems in *B.cereus*: production of AIP (PapR and PapRa), activation of cognate quorum sensor (PlcR and PlcRa) and presentation of major gene targets.** The product of the *papR* gene is a 48 amino-acid peptide. PapR is secreted, probably via the Sec machinery, processed by the NprB protease and presumably other peptidases. PapR is found in the extracellular compartment and in the bacterial cytoplasm mainly as a seven amino-acid peptide. It is imported in the cell via the oligopeptide permease Opp. Once inside the cell PapR binds to PlcR allowing it to activate the expression of 45 genes composing the PlcR regulon including *plcR* and *papR* genes. PlcRa activates the expression of 18 genes involved in cysteine metabolism and it activates the expression of 5 genes involved in oxidative stress response. The product of the *papRa* gene is a 93 amino-acid peptide. As described for PapR, a typical Gram-positive N-terminal signal peptide has been identified for PapRa suggesting that PapRa is

also probably secreted via the Sec machinery. '?' Indicates that maturation and import of PapRa have not been reported yet. Black arrows represent a positive effect on gene transcription. Green lines indicate the circuit (production, export, processing, import, activation) followed by PapR and PapRa peptides.

**Figure 21.5.2. Biosynthesis of DPD.** The transfer of a methyl moiety from SAM (S-adenosyl methionine) to a substrate (R1) generates SAH (S-adenosyl homocysteine), which is converted to SRH (S-ribosyl homocysteine) by Pfs. LuxS catalyses the production of DPD (S-4,5-dihydroxy-2,3-pentanedione) and homocysteine. SAM is then regenerated by MetE, MetH and MetK. R2 stands for THF (N-methyltetrahydrofolate) or THPG (5-methyltetrahydropteroyl glutamate).

**Figure 21.5.3. Organisation of the *lsr* locus in *B. cereus*.** The *lsr* locus includes genes encoding an import channel (in green), the AI-2 receptor (in pink), the transcriptional repressor LsrR (in orange), the AI-2 kinase (in blue) and an isomerase involved in the degradation of AI-2 (in yellow). Putative promoters are figured by dark arrows.

**Figure 21.5.4. Schematic representation of two QS systems in *B. cereus* in relation with cysteine metabolism: LsrR/AI-2 & PlcRa/AIP circuits.** '?' Indicates that AI2 export remains uncharacterized. Black dashed arrow indicates a role of the

cysteine metabolism in oxidant protection. Black arrows represent a positive effect on gene transcription and the cross end of the arrows represent a negative effect on gene transcription. Orange lines indicate the circuit followed by AI2 and blue lines represent the circuit followed by AIP.

**Figure 21.5.5. Reconstruction of the sulfur metabolism pathway in *B. cereus*: transport and biosynthesis of sulfur-containing amino acids.** The putative proteins involved in the uptake and assimilation of inorganic (sulfate) and organic sulfur sources (sulfonates, cystine, methionine) are indicated by the corresponding genes. Cysteine is typically present in its oxidized form -cystine- in the extracellular environment. The BC numbers (ATCC14579 strain) for *B. cereus* genes are shown, with gene names according to the orthologs in *B. subtilis*. '?' indicates genes probably involved in the pathway or a step for which a gene is lacking or remains to be identified. All the PlcRa-regulated genes involved in sulfur metabolism are indicated by a downward black arrow and the putative functions of all the corresponding proteins are presented in Table 21.5.1. Presumed direct targets of CymR are indicated in bold typeface. OAS, O-acetyl-serine; SAM, S-adenosyl-methionine.

**Figure 21.5.6A-B. Schematic representation of the interplay between PlcRa and CymR regulators in *B.cereus*.** Cysteine is typically present in its oxidized form -cystine- in the extracellular environment. **(A)** In the presence of cystine, as indicated by "+", the intracellular concentration of O-acetylserine (OAS) is low, the CymR-CysK complex is formed, and transcriptional repression of the CymR regulon occurs. **(B)** Under conditions of cystine limitation, as indicated by "-", the pool of OAS increases until it reaches a concentration preventing the formation of the CymR-CysK complex. Thus, no repression of transcription of the CymR regulon occurs when cysteine is limiting. In this growth condition, PlcRa activates CymR-controlled genes '?' indicates that the exact mechanism of PlcRa positive control remains to be defined. Black arrows represent a positive effect on gene transcription and the cross end of the arrows represent a negative effect on gene transcription. Green arrows indicate cysteine biosynthesis pathways. Plain arrow, high uptake & biosynthesis; dashed arrow, low uptake & biosynthesis Int, intracellular; ext, extracellular.

**Figure 21.5.7. Identification of a motif common to the promoter regions of putative CymR targets in the *B. cereus* ATCC14579 strain.** An alignment of the promoter regions of the *ssuB*, *yrrT*, *tcyP*, *luxS*, *cysK*, *cysH* and BC1090 genes is presented. The

consensus sequence for the CymR-binding site was determined with the WebLogo tool (<http://weblogo.berkeley.edu/>).

**Figure 21.5.8. Cystine addition to a  $\Delta plcRa$  culture improved peroxide stress resistance.** Sensitivity to hydrogen peroxide ( $H_2O_2$ ) of a *B. cereus plcRa* mutant in the presence or absence of cystine in the growth culture was performed. We assessed the growth inhibition of wild-type and  $\Delta plcRa$  strains in early stationary phase. Growth curves of the wild-type strain (black circles) and the mutant (black triangles) in LB medium without (solid line) or with 1 mM cystine (dashed line). Hydrogen peroxide (0.4 mM) was added at an OD of 2 ( $\sim t-0.3$ ). White symbols indicate cultures treated with  $H_2O_2$ . The onset of stationary phase ( $t_0$ ) is indicated by a black arrow and hydrogen peroxide addition by a white arrow.

**Figure 21.5.9. Production and role of AI-2 in *B. cereus*** (A) AI-2 concentration in the culture supernatant of planktonic cultures was assayed by a luminescence assay in *Vibrio harveii*. Green circles represent the growth curve and pink squares show AI-2 concentration in arbitrary units (au). (B) Effect of increasing concentrations of exogenously added AI-2 on biofilm formation in a microtiter plate assay. Error bars show the standard deviation. Experiments were run in triplicates.

