Biofilm dispersion and quorum sensing

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Abstract

Biofilm development and quorum sensing are closely interconnected processes. Biofilm formation is a cooperative group behaviour that involves bacterial populations living embedded in a self produced extracellular matrix. Quorum sensing (QS) is a cell-cell communication mechanism that synchronizes gene expression in response to population cell density. Intuitively, it would appear that QS might coordinate the switch to a biofilm lifestyle when the population density reaches a threshold level. However, compelling evidence obtained in different bacterial species coincides in that activation of QS occurs in the formed biofilm and activates the maturation and disassembly of the biofilm in a coordinate manner. The aim of this review is to illustrate, using four bacterial pathogens as examples, the emergent concept that QS activates the biofilm dispersion process.

Introduction

Bacteria are elementary, unicellular organisms able to grow, divide, sense and adapt to environmental signals autonomously. Despite their self-sufficiency, bacteria coordinate efforts with neighbours to accomplish cooperative activities such as bioluminescence production, biofilm development, and exoenzyme secretion. Coordination occurs through a mechanism of cell-to-cell communication called quorum sensing (QS) (Reviewed in [1-3]). QS confers bacteria the capacity to recognize the population density by measuring the accumulation of a specific signalling molecule that members of the community secrete. Only when the population density is high, the accumulation of the signal in the extracellular environment is sufficient to activate the response. Structurally, QS signal molecules have a low molecular weight and belong to a wide range of chemical classes including acyl homoserine lactones (AHLs), furanosyl borate diesters (AI2), *cis*-unsaturated fatty acids (DSF family signals) and peptides.

One of the most common processes that bacteria accomplish in a cooperative manner is biofilm development. Biofilms are communities of microorganisms that grow attached to a surface or interphase and embedded in a self produced extracellular matrix [4]. Inside the biofilm, bacteria grow protected from environmental stresses, such as desiccation, attack by the immune system, protozoa ingestion, and antimicrobials. Our understanding of how bacteria build the biofilm comprises three sequential stages: irreversible adhesion to the surface, followed by bacterial division and production of the extracellular matrix and finally, disassembly of the matrix and dispersion of bacteria [5]. When thinking about the relationship between biofilm development and QS, the first question that comes to mind is at which step bacterial density reaches the threshold level that allows QS signalling to participate in biofilm regulation. Intuitively, the initial adhesion step seems inappropriate for the accumulation of quorum signals because it

involves bacteria that are swimming freely in the media. It is later, when the attached bacteria divide and form microcolonies that the population density increases and quorum signals can reach sufficient levels to activate the maturation and disassembly of the biofilm in a coordinate manner. In support of this view, recent evidences indicate that many bacterial species use QS to coordinate the disassembly of the biofilm community. Biofilm dispersion is essential to allow bacteria to escape and colonize new niches when nutrients and other resources become limited and waste products accumulate. There are different strategies to accomplish biofilm dispersion: ending the synthesis of the biofilm matrix compounds, degrading the matrix and also, disrupting noncovalent interactions between matrix components (Table 1) [6]. Because QS regulatory networks are usually very intricate and may include several genes whose products affect biofilm development at different stages, it is not always easy to understand how the activation of QS finally triggers biofilm dispersion. In this review, we summarize the regulatory connections between QS signalling and biofilm development in four bacterial pathogens (*Pseudomonas aeruginosa*, *Vibrio cholerae*, *Xanthomonas campestris* and *Staphylococcus aureus*) to illustrate QS mediated biofilm dispersion.

Pseudomonas aeruginosa

Biofilm formation has been extensively studied in the Gram negative bacterium *P. aeruginosa* because of its implication in causing severe chronic infections in patients with cystic fibrosis (CF) [7]. As regards QS, *P. aeruginosa* harbors two complete AHL circuits, LasI/LasR and RhlI/RhlR, being the LasI/R circuit hierarchically positioned upstream the RhlI/R circuit (Fig. 1). These two QS systems are composed of a LuxI type synthase, responsible of AHL synthesis, and a LuxR type receptor. At high cell

density (HCD), AHLs accumulate and specifically interact with LuxR type transcription factors. AHL binding stabilizes the LuxR type proteins, allowing them to fold, bind DNA, and regulate transcription of target genes. In many cases, AHL bound LuxR type proteins also activate transcription of *luxI*, providing a signal amplification mechanism via a feed forward autoinduction loop. In addition, *P. aeruginosa* has two orphan LuxR homologues, VqsR and QscR, and it also presents the *Pseudomonas* quinolone signal (PQS), which are interconnected with the LasI/LasR and RhlI/RhlR signalling circuitries [3,8].

The first evidence of the relationship between *P. aeruginosa* QS and biofilm formation was shown in 1998 by Davies et al. [9]. Results showed that the LasI/LasR system, although not involved in the initial attachment and growth stages, was required for the subsequent biofilm differentiation process. From then on, several *in vitro* studies have addressed the role of QS in *P. aeruginosa* biofilm differentiation but results have been discrepant. The reasons behind this disagreement seem to be related to differences in the biofilm model used and/or culture conditions [10]. In those cases in which biofilm development has been proved to depend on QS, this dependency has been linked to different factors involved at determined stages of biofilm development. For example, QS induced extracellular DNA (eDNA) release plays a part in offering structural stability to the biofilm [11]. QS control of swarming motility has been linked to an early step of biofilm formation, since swarming dictates initial coverage of the substratum [10]. With respect to exopolysaccharide production, different groups have obtained contradictory results. Initially, it was shown that LasI/LasR system activated transcription of the *pel* genes [12] whose products are responsible for the production of a glucose-rich exopolysaccharide (PEL) that builds the biofilm matrix. On the contrary, Ueda and Wood reported that Las mediated QS inhibits the production of this

exopolysaccharide [13]. These authors demonstrated that LasI/LasR positively regulates the expression of the tyrosine phosphatase TpbA. TpbA not only inhibits the expression of *pel* genes but also leads to decreased levels of c-di-GMP, probably through regulation of the activity of the diguanylate cyclase TpbB. Such low levels of c-di-GMP result in a decrease in PEL production, since binding of c-di-GMP to the c-di-GMP receptor PelD is needed for PEL synthesis (Fig. 2). Another element controlled by QS, specifically by both AHL and PQS signaling, that plays an important role in *P. aeruginosa* biofilm development is rhamnolipids production [14]. These biosurfactants were first shown to influence a late stage of biofilm development, maintaining the channels between the mushroom shaped structures of the biofilm, once they are formed [15]. These channels allow fluids to flow throughout the biofilm, resulting in the distribution of nutrients and oxygen and removal of waste products. Although the expression of the rhamnolipids synthesis operon *rhlAB* occurs primarily in the stalks of the mushroom like structures [16], rhamnolipids play a role in mushroom cap formation by promoting bacterial twitching motility [17]. A notable demonstration that secretion of the right amount of rhamnolipids is critical for proper biofilm development was shown by Boles et al. [18]. In this study, spontaneous *P. aeruginosa* variants that exhibited accelerated biofilm detachment were analysed. Results revealed that increased biofilm detachment was due to the overproduction of rhamnolipids. Furthermore, exogenous addition of purified *P. aeruginosa* rhamnolipids to wild type *Pseudomonas* biofilms or even to biofilms produced by other microorganisms (*Bordetella bronchiseptica* and *Candida albicans*) caused bacterial detachment [19,20]. In summary, QS promotes biofilm dispersion in *P. aeruginosa* at least by reducing the synthesis of one of the major exopolysaccharides of the biofilm matrix (PEL) and inducing the synthesis of surfactant molecules (rhamnolipids) (Fig. 2) (Table 1). The

finding that QS promotes the release of eDNA, which is a component of the biofilm matrix, might seem contradictory with the concept of QS induced biofilm dispersion. However, since this eDNA comes from the lysis of bacteria, cell death promoted by QS might also be considered as part of the dispersion mechanism. Interestingly, in addition to promoting bacterial release, rhamnolipids appear to provide protection from the innate immune defense by causing necrotic cell death of polymorphonuclear leukocytes [21]. This activity would confer some local protection to the biofilm and also to the bacteria released during the dispersion process.

Vibrio cholerae

V. cholerae, the causative agent of the cholera disease, has two QS pathways that function in parallel. At low cell density (LCD), the levels of the two autoinducers, CAI-1 ((S)-3-hyroxytridecan-4-one), synthesized by CqsA, and AI-2, synthesized by LuxS, are low and their membrane bound two-component receptors, CqsS and LuxPQ act as kinases. As a result, the phosphotransfer protein LuxU is phosphorylated and then the phosphate is transferred to the response regulator LuxO. Phosphorylated LuxO activates the transcription of four small RNAs (*qrr*1-4) that via base pairing prevent ribosome binding to *hapR* mRNA, encoding the QS master regulator, leading to its degradation. Also, the *qrr*1-4 small RNAs promote c-di-GMP synthesis and biofilm development by base pairing with the *vca0939* mRNA which encodes a GGDEF domain protein. This pairing relieves an inhibitory structure that occludes the ribosome binding site of *vca0939* mRNA and thus, activates its translation [22]. At HCD, CAI-1 and AI-2 accumulate, and their receptors bound to AIs act as phosphatases. Unphosphorylated LuxO cannot activate the transcription of *qrr*1-4 and *hapR* mRNA is translated (Fig. 1) [3]. Several lines of evidence indicate that activation of HapR at HCD is the key to

biofilm dispersion (Fig. 2). Firstly, HapR activates transcription of the *hap* gene encoding haemagglutinin protease (HA/P) [23], leading to detachment of cells from biofilms that had been formed at LCD (Table 1). Second, HapR represses transcription of the *vps* exopolysaccharide (VPS) biosynthesis operons by binding to the promoter of the *vpsT* transcription factor, which is a positive activator of *vps* transcription [24]. Third, HapR controls the transcription of several genes encoding proteins that synthesize (GGDEF domain proteins) and degrade c-di-GMP (EAL and HD-GYP domain proteins) resulting in a reduction in cellular c-di-GMP levels [24,25]. This decrease in c-di-GMP has consequences on the activity of two c-di-GMP receptors, VpsT itself and VpsR. On one hand, VpsT activity is repressed, since only upon c-di-GMP binding it oligomerizes and gains the capacity to bind to and activate *vps* transcription (Fig. 2) [26]. On the other, VpsR is no longer able to activate the transcription of *vpsT* [27] (Table 1). Interestingly, two recent publications in *Vibrio vulnificus*, a close relative of *V. cholerae*, have shown that activation of SmcR, the HapR homologue, promotes biofilm dispersion at HCD by downregulating expression of VpsT and a GGDEF protein and upregulating the synthesis, amongst others of the VvpE protease and the capsule exopolysaccharide (CPS) (Fig. 2) [28,29]. At LCD, expression of CPS is repressed but when QS signaling is activated in the mature biofilm, synthesis or exogenous addition of CPS restricts the growth of the biofilm, limiting its size (Table 1). Although exopolysaccharides are very often essential components of the biofilm matrix, there are several examples showing that they can also have antibiofilm properties [30,31]. Their mode of action remains poorly characterized but it appears that they would act as surfactant molecules that modify the physical characteristics of bacterial cells. Thus, similarly to the situation in *P. aeruginosa*, QS in

Vibrio represses the synthesis of biofilm matrix compounds and induces the synthesis of molecules with surfactant properties.

Xanthomonas campestris

In the last few years, DSF (diffusible signal factor) family signals have been unveiled as a novel QS system that is widespread in Gram negative bacterial pathogens. These *cis*-unsaturated fatty acids have been shown to regulate a range of biological functions including cell growth, biofilm development and virulence [32,33]. DSF was first identified and characterized as *cis*-11-methyl-2-dodecenoic acid in *Xanthomonas campestris* pv *campestris* (*X. camprestis*), the causal agent of black rot of cruciferous plants [34]. In *X. campestris*, biosynthesis of DSF is dependent on *rpfF* and *rpfB*, which encode a crotonase enzyme and a putative long chain fatty acyl CoA ligase, respectively, and are located in the *rpf* gene cluster (*rpfA-I*) [35]. In addition, the *rpfC* gene encodes a hybrid two-component regulator that functions as a DSF sensor and regulates DSF biosynthesis. At LCD, RpfC remains unphosphorylated and maintains a conformation that promotes the formation of a complex with RpfF, limiting DSF production. At HCD, DSF molecules accumulate, triggering the autophosphorylation of RpfC and thus the release of RpfF, resulting in increased DSF production (Fig. 1) [36,37]. Moreover, RpfC constitutes a two component regulatory system with RpfG, a protein that contains a typical receiver domain and a HD-GYP domain, which is responsible of degrading c-di-GMP to two molecules of GMP. Phosphorylation of RpfG activates its phospodiesterase activity and results in reduced c-di-GMP levels (Fig. 1) [38].

Assessment of biofilm formation in *X. campestris* has been carried out by visualization of bacterial aggregation in liquid medium [39,40]. Max Dow and colleagues

demonstrated that the DSF mediated QS system controls *X. campestris* biofilm dispersal (Fig. 2). Mutants in *rpfF*, *rpfC* or *rpfG* formed cell aggregates in L medium, whereas the wild type grew planktonically under the same conditions. In these aggregates, bacteria were held together in a matrix of extracellular material. Addition of DSF triggered dispersion of the *rpfF* mutant strain aggregates, but not those of the rest of the mutants, indicating that the DSF mediated dispersal acted through the RpfC/RpfG two-component signalling system. The molecule responsible for biofilm dispersion, acting downstream DSF, was identified as endo-β-1,4-mannanase, which is an extracellular enzyme encoded by the *manA* gene, that could disperse the cell aggregates produced by all *rpf* mutants. However, ManA was not the only factor responsible for DSF inducible biofilm dispersal, because it had no detectable activity against soluble xanthan, an exopolysaccharide needed for the integrity of the *Xanthomonas* biofilm, and also because DSF was still able to disperse the aggregates of a double *rpfF*/*manA* mutant [41]. In this respect, Tao et al. identified that RpfC/RpfG can also induce biofilm dispersion by repressing transcription of *xagABC* operon, encoding a putative glycosyltransferase system required for the synthesis of an exopolysaccharide essential for biofilm formation (Fig. 2) [40]. This work also implicated the cyclic-AMP receptor-like protein Clp as an element responsible for linking DSF signaling (and alteration in c-di-GMP) to the expression of *manA* and the repression of the *xagABC* operon. Several lines of evidence suggest that Clp plays a role in the regulation of biofilm dynamics in response to alterations in the c-di-GMP level. Mutation of *clp* leads to the downregulation of expression of *manA*, which is implicated in biofilm dispersal and, conversely, in the upregulation of *xag* gene expression, which is implicated in biofilm formation. The binding of Clp to promoters of both *manA* and *xag* genes is inhibited by c-di-GMP (Fig. 2) [42]. In addition, a very recent transcriptome analysis has shown that

the Rpf/DSF dependent regulon is very complex and comprises over 480 genes encoding for putative candidates that might participate in the DSF induced biofilm dispersal process [43]. Altogether, the DSF mediated QS acts as a regulatory mechanism in modulation of *X. campestris* biofilm dispersal, at least by means of positively regulating ManA and negatively controlling *xagABC* expression (Table 1).

Intriguingly, it has been shown that one bacterial species may produce more than one DSF family signal and that DSF signals are implicated not only in intraspecies signalling but also in interspecies and interkingdom communication [32,44]. In this respect, Davies et al. demonstrated that *P. aeruginosa* encodes *dspI* (PA0745), a *rpfF* homologue, which is required for synthesis of a DSF like molecule, cis-2-decanoic acid. Furthermore, cis-2-decanoic acid induces the dispersion not only of established *P. aeruginosa* biofilms but also of those formed by a variety of Gram negative and positive bacteria and even the yeast *Candida albicans* [45]. This study and others suggest that these cis-unsaturated fatty acid signals might constitute a broadly used mechanism for the induction of biofilm dispersal.

Staphylococcus aureus

QS regulation of *S. aureus* biofilm development has been assumed to depend on the Agr system [46,47]. Following the classical QS signalling in Gram positive bacteria, the Agr system consists of a membrane bound protein (AgrB) that modifies and exports the QS peptide (AgrD) and a bacterial two-component signal transduction system, composed of the sensor histidine kinase (AgrC) and its cognate response regulator (AgrA). When modified AgrD accumulates in the extracellular media, note that in contrast with other QS systems, the bacterial membrane is impermeable to the peptide, it binds to the membrane bound AgrC which autophosphorylates at a conserved histidine residue.

Then, AgrC transfers the phosphate to AgrA and phosphorylated AgrA activates its own transcription as well as transcription of other targets including the regulatory RNA, RNAIII (Fig. 1) [1,48]. Early on in the analysis of Agr function on biofilm development, it became apparent that *agr* mutants displayed an increased capacity to produce a biofilm [46]. Because the Agr system upregulates extracellular proteases production, it was initially assumed that decreased accumulation of proteases in the *agr* mutant was responsible for the enhanced biofilm phenotype (Table 1). This explanation was also supported by the fact that mutants in genes encoding for extracellular proteases displayed improved biofilm formation [47]. However, the influence of the Agr system in biofilm development is more complex than regulation of protease production (Fig. 2). This system also regulates the synthesis of biofilm matrix compounds. *S. aureus* can produce two types of biofilm matrices, one utilizing the exopolysaccharide PIA/PNAG and the other based on surface proteins. Experimental evidence suggests that the Agr system does not regulate the synthesis of PIA/PNAG. In contrast, it downregulates the expression of surface adhesins such as fibronectin binding proteins (FnBPs) and protein A [49], which under specific environmental conditions are capable of inducing a proteinaceous biofilm matrix [50-53] (Table 1). More recently, an additional role for the Agr system in biofilm dispersion has been identified. The group of M. Otto demonstrated first in *S. epidermidis* and then in *S. aureus* that a specific class of secreted peptides (phenol soluble modulins, PSMs) with surfactant like properties mediates the main impact of Agr in biofilm dispersion [54,55] (Table 1). PSM operons transcription is under strict control by AgrA and consequently *agr* mutants lack PSM production. Analysis of biofilm tridimensional structure using confocal laser scanning microscopy revealed that PSMs were not only necessary for biofilm dispersion but also impacted the biofilm volume, thickness, roughness, and channel formation. In these

studies, the nature of the biofilm matrix produced by the strains under study was not determined, and therefore, additional studies would be necessary to determine whether PSMs show similar effects when the biofilm matrix is built with exopolysaccharide or proteins. Interestingly, under certain growth conditions PSMs can polymerize into aggregates that exhibit biochemical and biophysical characteristics of amyloid-like fibers [56]. The PSMs derive amyloid like fibers contribute to biofilm development in these particular conditions and mutants deficient in PSMs are unable to produce a biofilm. These results indicate that PSMs can play a dual function in biofilm development depending on their aggregation state. As monomers, they have surfactant properties that promote biofilm disassembly, but when they polymerize in fibers they favor biofilm development. The environmental conditions that control the switch between the monomeric and polymeric state are still undetermined.

In addition to the Agr system, recent studies indicate that *S. aureus* possesses a functional *luxS* gene and has the ability to produce AI-2 [57,58]. Mutation of *luxS* results in increased biofilm formation compared with the wild type strain under static and flow conditions. Quantitative RT-PCR analysis showed that AI-2 activated the expression of IcaR, the main negative regulator of PIA/PNAG exopolysaccharide synthesis (Fig. 2) (Table 1) [59]. Because the potential AI-2 receptor has not been found, the regulatory pathway that connects AI-2 signal with IcaR expression remains unknown.

Concluding remarks

There is an enormous interest to better understand bacterial biofilm development, because the biology supporting this process is anticipated to be instrumental for the development of new treatments. Early studies mainly focused on the initial steps of

biofilm development identified surface adhesins responsible for the interaction with both biotic and abiotic surfaces. Then, the efforts were aimed at understanding the regulation of the synthesis of biofilm matrix compounds and we learned that most bacteria use cyclic nucleotides to induce the synthesis of biofilm matrix exopolysaccharides. More recent studies are showing that many bacteria use QS to activate, in a coordinate manner, the dispersion of the biofilm structure. The biological rational behind this last strategy is that disassembly of the matrix would be a titanic task for individual bacteria. An important consideration of this scenario is that antimicrobials directed against QS systems would have the unintended consequence of impairing biofilm disassembly whereas molecules that mimicry QS signals would induce dispersion of the biofilm. Another interesting lesson learnt from these studies is that most bacteria use surfactant molecules to promote biofilm detachment. Because very often the same surfactant molecule is able to induce biofilm dispersion in different bacterial species, it appears that a combination of surfactant molecules with antimicrobials might be a promising alternative for the eradication of bacterial biofilms.

Figures

Figure 1. Connection between QS signaling and biofilm matrix compounds occurring at HCD. Schematic representation of QS regulatory cascades that end in the activation of a master regulator that governs the synthesis of biofilm matrix compounds in the selected four bacterial pathogens. In *P. aeruginosa*, LasI, RhlI, and PqsABCDH synthesize the QS signal molecules 3OC12-HSL, C4-HSL, and PQS, respectively. The transcription factors LasR, RhlR, and PqsR detect their respective signal molecules, leading to a feed forward autoinduction loop and also to the regulation of transcription of target genes. The three circuitries are interconnected as indicated by arrows and T-bars, which represent positive and negative regulation, respectively. QS induces eDNA release on one hand and on the other it inhibits the production of PEL exopolysaccharide. In *V. cholerae*, LuxS and CqsA synthesize AI-2 and CAI-1 respectively. These signal molecules are detected by their corresponding receptors, the two-component histidine kinases LuxPQ and CqsS. Signal binding promotes their phosphatase activity, resulting in unphosphorylated LuxO, cessation of Qrr1-4 transcription and induction of HapR expression. HapR, the HCD master transcriptional regulator, represses transcription of the *vps* exopolysaccharide biosynthesis operons. In *X. campestris*, RpfF synthesizes DSF, which is sensed by the membrane-bound histidine kinase protein, RpfC. Ligand binding triggers the autophosphorylation of RpfC, which provokes the release of RpfF, leading to increased DSF production. RpfC transfers the phosphate to RpfG, which activates its PDE activity and decreases the c-di-GMP pool. The RpfC/RpfG two-component system represses the expression of *xagABC*, which encodes putative glycosyl transferases required for exopolysaccharide synthesis, and induces the production of xanthan. In *S. aureus*, the QS peptide is synthesized as a longer precursor by *agrD*, and is processed and secreted via AgrB. The extracellular signal is detected by

the membrane-located histidine kinase AgrC and signal transduction occurs by phosphorelay to the AgrA response regulator. AgrA inhibits the expression of the biofilm matrix proteins, FnBPs and Protein A. LuxS synthesizes AI-2, which inhibits PIA/PNAG exopolysaccharide synthesis through an unknown QS cascade.

HCD, high cell density; eDNA, extracellular DNA; DSF, diffusible signal factor; PDE, phosphodiesterase; c-di-GMP, cyclic di-GMP; FnBPs, Fibronectin binding proteins.

Figure 2. Biofilm dispersion mechanisms activated at HCD by QS in bacteria. Schematic representation of biofilm mushroom-like pillars indicating the mechanisms of biofilm dispersion activated by QS signal accumulation in each bacterial species. In *P. aeruginosa*, QS positively regulates the expression of the periplasmic tyrosine phosphatase TpbA. TpbA dephosphorylates the membrane-anchored GGDEF protein TpbB deactivating its DGC activity and thus reducing c-di-GMP levels in the cell. As a result, the c-di-GMP receptor PelD is not longer bound to c-di-GMP and PEL polysaccharide production is decreased. QS also promotes the synthesis of rhamnolipids whose overproduction results in biofilm detachment. In *Vibrio spp.*, QS signal accumulation provokes a cessation in *qrr*1-4 small RNAs transcription. In *V. cholerae*, *qrr*1-4 cannot longer base pair with the *vca0939* mRNA, which encodes a GGDEF domain protein, and thus its translation is inhibited and c-di-GMP levels decrease. On the other hand, the expression of the HCD master transcriptional regulators HapR and SmcR of *V. cholerae* and *V. vulnificus* increases. HapR and SmcR downregulate expression of VpsT, a positive regulator of *vps* transcription. HapR and SmcR also control the transcription of c-di-GMP metabolizing enzymes resulting in a reduction of c-di-GMP. This causes a decrease in VPS polysaccharide production since the c-di-GMP receptor VpsT needs c-di-GMP binding to activate *vps* transcription. In addition,

HapR and SmcR activate the production of HA/P and VvpE proteases, respectively. SmcR also upregulates the synthesis of CPS, which restricts the growth of the biofilm. In *X. campestris*, accumulation of DSF leads to a decrease in c-di-GMP levels. Clp, which encodes a c-di-GMP responsive transcriptional regulator becomes able to bind to *manA* and *xag* promoters resulting in an increased production of ManA that has biofilm dispersing activity and suppression of *xagABC* expression, leading to a reduction in exopolysaccharide synthesis. In *S. aureus*, QS peptide accumulation causes the phosphorylation of the AgrA response regulator that directly activates expression of PSMs and proteases and represses the synthesis of the biofilm matrix proteins, FnBPs and Protein A. On the other hand, LuxS inhibits PIA/PNAG exopolysaccharide synthesis via induction of expression of IcaR.

DGC, diguanylate cyclase; HA/P, haemagglutinin protease; CPS, capsule exopolysaccharide; ManA, endo-β-1,4-mannanase; PSMs, phenol soluble modulins; FnBPs, Fibronectin binding proteins.

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Table 1. Summary of main biofilm dispersion mechanisms regulated by QS

		Biofilm dispersion strategies					
	QS system	**Inhibition of matrix compounds synthesis**		**Matrix degradation**		**Surfactants**	
P. aeruginosa	LasI/R RhlI/R PQS	Pel	[13]			Rhamnolipids	[18]
X. campestris	DSF	XagABC	[40]	ManA (endo-β-1,4-mannanase)	[39]		
V. cholerae/V. vulnificus	CAI1 AI2	Vps	[22,24,25]	Haemagglutinin protease VvpE	[23] [28]	Capsule	[29]
S. aureus	Agr AI2	FnbAB Protein A PIA/PNAG	[49] [59]	Proteases	[47]	PSMs	[55]

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An important study that demonstrates for the first time posttranscriptional activation of a GGDEF protein. Qrr sRNAs base-pair with and activate translation of Vca0939 mRNA. Also, this is the first description of a *V. cholerae* QS controlled process that is independent of HapR.

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**of outstanding interest

This study shows that PSMs are key contributors to *S. aureus* biofilm maturation processes, specifically to the formation of the characteristic channel-containing biofilm structure, biofilm detachment, control of biofilm expansion, and dissemination from biofilms *in vivo*.

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Highlights Solano et al. 2014

1. Biofilm development and quorum sensing are social bacterial behaviours
2. Quorum sensing regulates genes involved in biofilm development
3. Quorum sensing promotes biofilm dispersion
4. Quorum sensing upregulates the synthesis of surfactant molecules

Figure 1

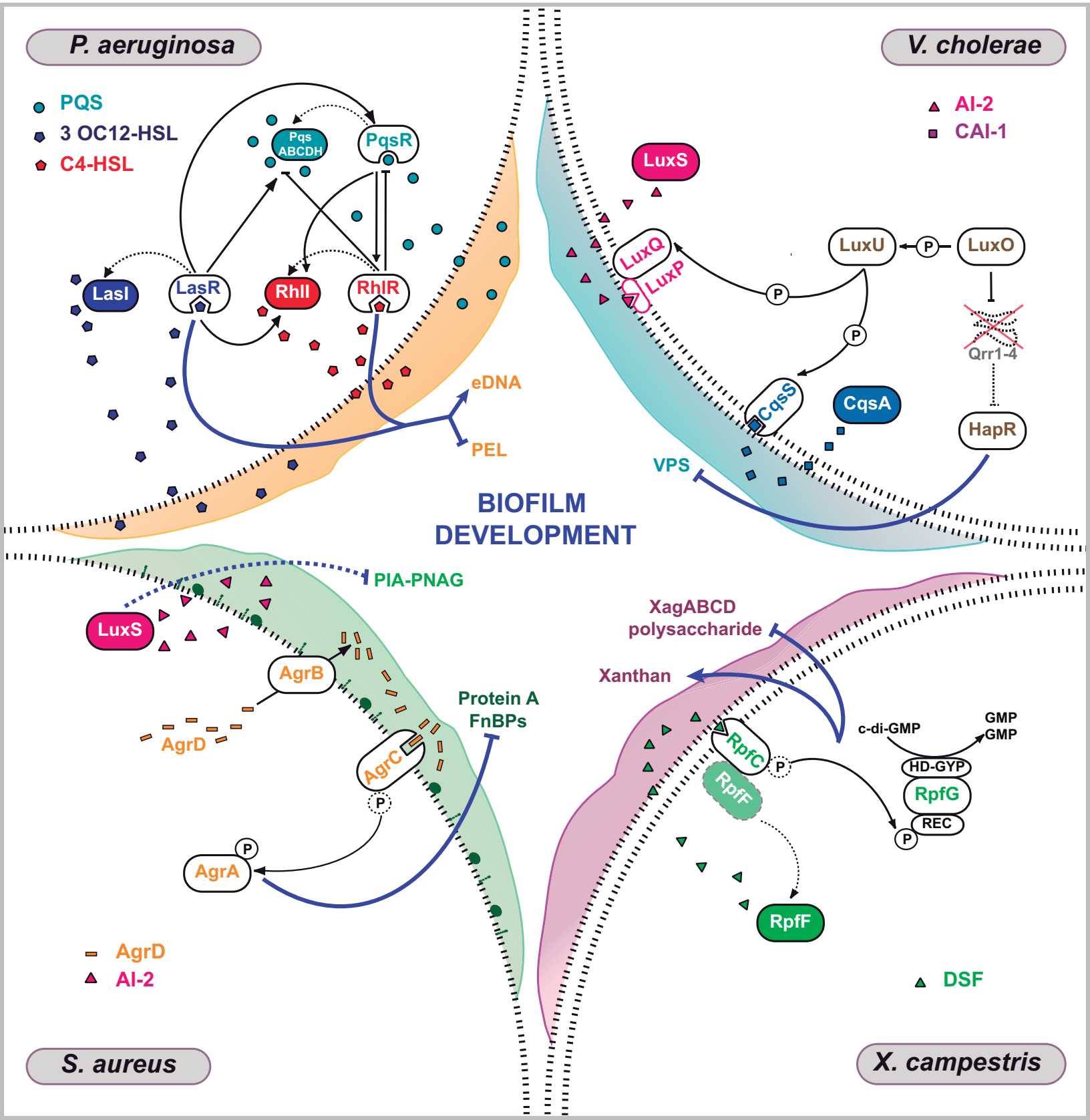
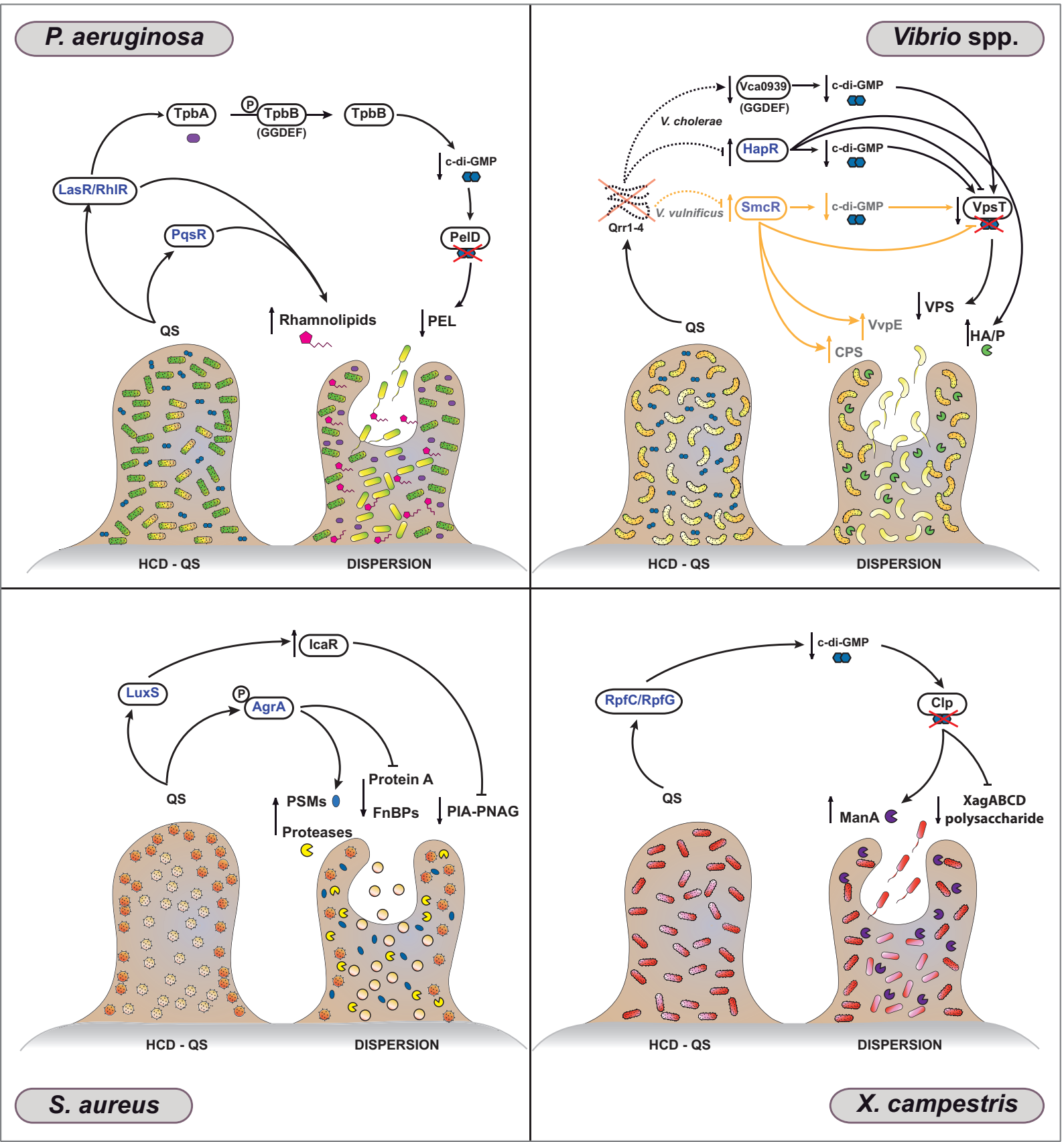

P. aeruginosa
PQS
3 OC12-HSL
C4-HSL
Pqs ABCDH
PqsR
LasI
LasR
RhlI
RhlR
eDNA
PEL
V. cholerae
AI-2
CAI-1
LuxS
LuxQ
LuxP
LuxU
P
LuxO
Qrr1-4
CqsS
CqsA
HapR
VPS
BIOFILM DEVELOPMENT
PIA-PNAG
LuxS
AgrB
AgrD
AgrC
P
AgrA
Protein A
FnBPs
AgrD
AI-2
S. aureus
XagABCD polysaccharide
Xanthan
RpfC
RpfF
c-di-GMP
GMP
GMP
HD-GYP
RpfG
REC
P
RpfF
DSF
X. campestris

Figure 2


P. aeruginosa
TpbA
P
TpbB
(GGDEF)
TpbB
c-di-GMP
LasR/RhlR
PqsR
PelD
QS
Rhamnolipids
PEL
HCD - QS
DISPERSION
Vibrio spp.
Vca0939
(GGDEF)
c-di-GMP
V. cholerae
HapR
c-di-GMP
V. vulnificus
SmcR
c-di-GMP
VpsT
Qrr1-4
VPS
VvpE
HA/P
CPS
QS
HCD - QS
DISPERSION
S. aureus
IcaR
LuxS
P
AgrA
QS
Protein A
PSMs
FnBPs
PIA-PNAG
Proteases
HCD - QS
DISPERSION
X. campestris
c-di-GMP
RpfC/RpfG
Clp
QS
ManA
XagABCD
polysaccharide
HCD - QS
DISPERSION