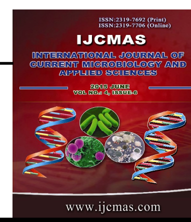
*Int.J.Curr.Microbiol.App.Sci* (2015) 4(6): 884-896

*International Journal of Current Microbiology and Applied Sciences*
*ISSN: 2319-7706* **Volume 4 Number 6 (2015) pp. 884-896**
**http://www.ijcmas.com**



**Review Article**

# Modulation of the Extracellular Polymeric Substances (EPS) Production by Quorum Sensing (QS) in Bacteria

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**Keywords**

Quorum sensing, Extracellular Polymeric Substances, Biofilms

**A B S T R A C T**

Microbial communities are capable of networking to adapt and survive under diverse ecosystems on the planet. While growing either as single species or in consortium they synthesize and release macromolecules known as extracellular polymeric substances (EPS). EPS are complex polymers comprised of a variety of high molecular weight biomolecules. These are produced by both prokaryotic and eukaryotic microorganisms irrespective of their origin, and have significant role in biofilm formation, providing structural support, mass transfer, adsorption of organic/inorganic compounds and different metals. In bacterial populations certain traits are expressed and coordinated in cell density-dependent manner and this mechanism is known as Quorum Sensing (QS). The QS help in cell to cell communication and signaling among the crowded bacterial community to assess their local population by sensing the release of diffusible signal molecule secreted by them. Various physiological activities are controlled by this quorum sensing circuitry. Quorum sensing is the most studied regulatory mechanism that has been found to control the EPS production and biofilm formation. Several bacteria which have been intensively studied and have revealed information where quorum sensing regulated modulation had a significant impact on the EPS production and biofilm formation are reviewed.

## Introduction

Microorganisms are ubiquitous in their presence on the planet but the communication network they use to adapt, grow and survive is still under the exploration among the biologist world. The prokaryotic and eukaryotic microorganisms grow as single species or in consortium under diverse ecosystems synthesize and release macromolecules known as extracellular polymeric substances (EPS). These 80% exopolysaccharides released in the ecosystem are having their origin from the microbial sources (Flemming and Wingender, 2001).They are present on the outer surface of bacterial cell. Exopolysaccharides produced by diverse

microbial groups multiplying under biofilms have been reviewed extensively by many researchers (Sutherland, 2001a,b; Guezennec, 2002; DePhilippis *et al*., 2001; Allison, 2003). EPS from different sources are composed of complex biopolymers having high molecular weight organic biomolecules such as polysaccharides, phospholipids, proteins, nucleic acids along with some low molecular weight non-polymeric components (Wingender and Flemming, 1999). The EPSs generated from microbial origin are diverse in their physico-chemical properties, composition, number of monosaccharides units and non-carbohydrate components. These are classified into two types as Homopolysaccharides and Hetero-polysaccharides. The homopolysaccharides have monosaccharides repeated units, of 10 or less in a linear chain with range of $1\times10^5$ to $3 \times 10^5$ Da as an average molecular weight and nature type is neutral. The repeating units are generally pentoses (as D-arabinose, D-Ribose and D-Xylose), hexoses (D-Glucose, D-Galactose, D-Mannose, D-Allose, L-Rhamnose, L-Fucose), amino sugars (D-Glucosamine and D-Galactosamine) or uronic acids (D-Glucuronic acids and D-Galacturonic acids) moieties. The 1,4-β or 1,3-β linkages between monosaccharides in the backbones of biopolymer are rigid but 1,2-α or 1,6-α linkages are the flexible ones. Heteropolysaccharides are composed of monosaccharides and non carbohydrate substituents which are polyanionic, constituted of either uronic acids (glucuronic acid, galacturonic acid and mannuronic acid) or ketal-linked pyruvate and inorganic residues like phosphate, acetate, pyruvate, succinate or rarely sulphate. The physical properties of polysaccharides are attributable to the arrangement of monosaccharides and the assembly of the single polymeric chains (Sutherland, 1994; Pal and Paul, 2008; Poli *et al*., 2011). EPS also contain lipid and protein derivatives such as lipopolysaccharides, glycoproteins and lipoproteins (Marvasi *et al*., 2010; Sheng *et al*., 2010; Simoes *et al*., 2010; More *et al*., 2014). Owing to the structural complexity and diversity of the EPS, their production is regulated at different stages via various mechanisms (Waters and Bassler, 2005; Ruiz *et al*., 2008). Quorum sensing is such one regulatory mechanism for EPS production and biofilm formation in bacterial community (Miller and Ruiz *et al*., 2008).

## Quorum sensing: A bacterial twitter

The coordinating, networking in aggregation and processing capacity of bacteria in populations for survivorship and development is called as Quorum Sensing (QS) and it regulates the expression of some characters in a density dependant manner (Loh *et al*., 2002; Von Bodman *et al*., 2003). It is the mechanism of cell to cell communication and signaling among the crowded bacterial community which regulates the expression of many traits as whole (Fuqua, 1994; Fuqua, 2001). Bacteria use quorum sensing to assess their local population by sensing the release of diffusible signal molecule secreted by them, this support them in recognition, which increases with their population density, resulting in a order of synchronized gene expression.

The regulation of phenotypic and physiological traits by QS confront the traditional perception of bacteria being an self-governing agent by allowing them to function in aggregated groups and sustain in specific environmental niches. This also reinforces the concept that individual bacteria are benefitted from their mutual cooperative group behavior on and actions

to survive and strive in nature. The phenotypes regulated by QS are enormously varied in their behavior and performance , with many having a significant impact upon healthcare, agriculture, and the environment. Quorum sensing is mediated by small diffusible signaling molecules termed autoinducers (Nealson, 1977; Galloway, 2010). These autoinducers are synthesized intracellularly by the bacteria and then secreted in their local outer environment throughout the growth phase, accumulate and reaches a critical value (Galloway, 2010). At this point the population is considered quorate, which in turn triggers a signal transduction pathway resulting in switching on the gene expression and the initiation of 'co-operative' behaviors among the members of the community that mutually benefit the whole (Fuqua *et al*., 1994; Bassler and Losick, 2006; Boyer and Wisniewski 2009; Atkinson and Williams, 2009; Galloway, 2010; McInnis and Blackwell, 2011).The different species of the bacteria varies in the chemical nature of signal transferring molecule, its receptors, signal transduction mechanism and expression of phenotypic characters (Waters and Bassler, 2005; Galloway, 2011).

## Molecular mechanisms of Quorum sensing (QS) in bacteria

The QS phenomenon was first time reported in *Vibrio fischeri* and *V. harveyi*, these marine bacteria were found to be luminescent at high cell density (Nealson *et al*., 1970). The phenomenon of QS involves the release and exchange of signal molecules among members *via* autoinducers (AIs) and cognate receptor as shown in the Fig. 1. The AIs diffuse out of the cell or are actively transported and AI concentration can be positively correlated with the bacteria population present in such environment, thus AI level serving as an effective indicator of cell density. The minute level of the signal goes higher with the increasing population to threshold level, where it activates the receptor proteins. The binding of the AIs to their target (intracellular or membrane-bound) receptors activates the transcription of genes required for QS phenotypes as well as those associated with biosynthesis of AI. The bacterial population reaching the "quorum", which is sufficient density in environment will promote ample of gene transcription for the expression of the QS regulated phenotype. As the production of the AI increases, the signal boosts the sensitivity of the signalling process (i.e., autoinduction) and facilitates population-wide harmonization of the QS-regulated phenotype, which is essential for quorum sensing.

The primary requirement of QS is therefore cell growth in close propinquity, as biofilm or aggregated manner in an enclosed, limited environment. In any of the above condition the signal concentration will reach the thresh hold value to make happen the QS regulated trait (Nealson *et al*., 1970). There are various quorum-sensing systems reported in literature in the diverse bacterial world, but the two most widely discussed are the acyl-homoserine lactone (acyl-HSL) systems of Gram-negative species and the peptide-based signaling systems of Gram positive species (Fuqua *et al*., 2001; Bassler, 2002 and Sturme *et al*., 2002). N-acylated-L-homoserine lactones (AHLs) are the most common class of autoinducer used by Gram-negative bacteria (Whitehead *et al*., 2001; Thoendel *et al*., 2011). They are formed by LuxI-type synthase enzymes and bind to cytoplasmic LuxR-type receptors to exert a regulatory output whereas cyclic peptides also called Quormones are the major class of autoinducer in Gram-positive bacteria. These are advocated by either membrane-

associated histidine kinases or cytoplasmic receptors (Thoendel *et al*., 2011).

**Modulation of extracellular polymeric substances via quorum sensing**

The different physiological activities regulated by this quorum sensing are antibiotic production, sporulation, biofilm development, conjugation and even in some bacteria bioluminescence is reported (Fuqua *et al*., 1994; Dunlop, 1999; DeKievit and Iglewski, 1999; Lazazzera, 2000). These vastly coordinated group behavior can have intense implications on the survival and pathogenicity of a bacterial population present in the particular environmental niche. These characters of living in aggregation make these to manage the rising stress responses and develop mechanisms under high cell density which is advantageous for these organisms to coordinate the release of toxins and antigenic factors and thus overcome the host immune system with the release of virulence factors (Senadheera and Cvitkovitch, 2008).

Quorum sensing controls production of bacterial extracellular polymeric substances (EPS) which in turn have important role in biofilm formation, providing structural support to biofilm (resistant to shear), adsorption of different metal/ organic/inorganic compounds and mass transfer by biofilm (Flemming and Leis, 2003; Neyens *et al*., 2004; Czaczyk. and Myszka, 2007).

It had been reported that QS controls the secretion of extra polymeric substances in biofilm formation and its key constituent is the putative exopolysaccharides in the extracellular matrix of the floating biofilms (Fuqua *et al*., 2001; Marketon *et al*., 2003). This EPS worked as a barrier to protect the microorganisms from microbicides and make available an enclosed space for biofilm formation (Richard and Melander, 2009). The different studies were carried out and lot of study the mechanism to inhibit QS for the control of EPS release and thus biofilm formation. In bio film reactor addition enhancement of EPS for production of biofilm formation of *Pseudomonas aeruginosa* with N-(3-oxooxtanoyl)- L-homoserine lactone (C -oxo-HSL, one of the common QS signal in environment) addition was reported as an engineering tool in waste water treatment systems (Xia *et al*., 2012). Zhou *et al*. in 2014 reported the effect of pH, temperature and salinity on EPS of *P. aeruginosa* biofilm and concluded that C8-oxo-HSL addition should be carried out at pH 5-7 and biofilm reached the highest concentration at 20°C. In another study carried out by Johnson and Boese-Marrazzo (1980); Koch *et al*., (1991), the rhl quorum sensing system of *P. aeruginosa* was reported to regulate the production of rhamnolipid (lipopolysaccharide) which is essential for the maintenance of architecture in structured biofilms because of its hemolytic and biosurfactant properties (Davey *et al*., 2003). Many research groups reported that rhamnolipid production is impaired in rhl mutant strains (Pearson *et al*., 1997; Davey *et al*., 2003) as *rhl*A mutant formed biofilms that were unstructured as compared with parent strain. But Davies *et al*., (1998) reported that the rhl mutant of *P. aeruginosa* formed biofilms similar in architecture to the parent, which is contradictory because the rhl quorum-sensing system of *P. aeruginosa* controls rhamnolipid production. However, Wagner *et al*., 2003; Schuster *et al*., 2003; Hentzer *et al*., 2003 found that the rhamnolipid gene expression also responds to the las signaling system. Based upon the above results it is hypothised that sufficient activation of rhamnolipid synthesis in *rhl*1 mutant via the las system is responsible for the

development of structured biofilms. Because the las system is required to activate the rhl system, so *las*I mutant produces negligible amounts of rhamnolipid (Parsek and Greenberg, 2005). These results strengthen and underline the effect of co-culturing conditions which enable us to promote that quorum sensing could be explored to develop more reliable and advanced biofilm technology.

Quinones *et al*., (2005) reported that quorum sensing in *Pseudomonas syringae* strain B728a is directly governed by the production of alginate, a major component of EPS *i.e* release of 3-oxo-hexanoyl-homoserine lactone AHL. This signal molecule is released in a cell-density-dependent approach under the expression of AHL synthase gene, *ahlI* and the AHL regulator gene, *ahlR* (Dumenyo *et al*., 1998; Quiñones *et al*., 2004).

As *ahlI* catalyzes the production of 3-oxo-C6-HSL and *ahlR* forms a stable complex and activates transcription of *ahlI* that leads to elevated amounts of AHL production with higher concentration. This *ahlI-ahlR* quorum-sensing system also is amenable to modulation by additional regulatory protein (Quiñones *et al*., 2004).The AHL deficient mutants were found to be feeble in alginate production and *ahlI –ahlR*-double mutants produced 30% less alginate than the parental strain. This study resulted that *ahlI-ahlR* quorum-sensing system are positive regulators of EPS and higher levels of alginate production.

In another study nitrogen-fixing gram-negative soil bacterium *Sinorhizobium meliloti,* symbiotic with *Medicago sativa* "Alfalfa" common leguminous plant was reported to produce two exopolysaccharide polymers, succinoglycan and EPS II, which promotes symbiosis and function in nodule invasion (Becker *et al*., 2002;Fraysse *et al*., 2003;Skorupska *et al*., 2006). Succinoglycan is a polymer of repeating octasaccharide subunits composed of one galactose and seven glucose residues with acetyl, succinyl, and pyruvyl modifications in a ratio of 1:1:1 (Aman *et al*., 1981; Reinhold *et al*., 1994). *S. meliloti* possesses a quorum-sensing system composed of two transcriptional regulators, SinR and ExpR, and the SinR controlled autoinducer synthase SinI, responsible for the biosynthesis of AHLs (Marketon *et al*., 2002a, b). These AHLs, in conjunction with the ExpR regulator, control a variety of downstream genes (Hoang *et al*., 2004). EPS II is encoded by the *exp* genes (Becker *et al*., 1997), and its synthesis stops in the absence of one or more of the quorum-sensing regulatory components (Pellock *et al*., 2002; Marketon *et al*., 2003).

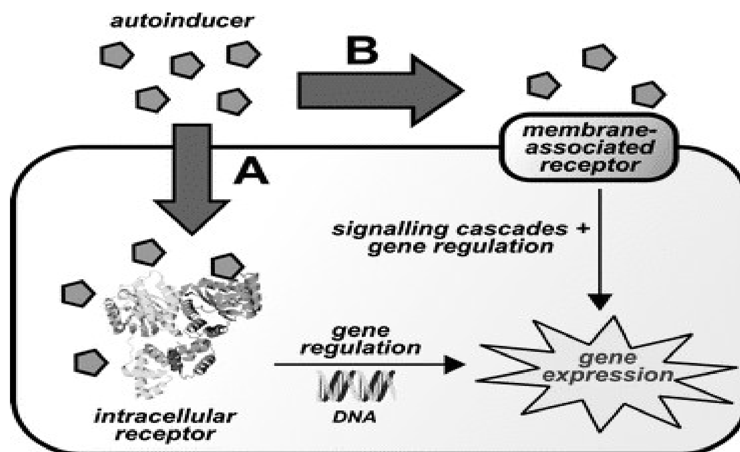
The findings of Glenn *et al*., (2007) showed that the production of succinoglycan is encoded by *exo* genes and is represses without a functional ExpR/Sin system. The ExpR/Sin quorum-sensing system is a switch on or off for succinoglycan production like EPS II and modulates through the *ExpR* regulator in combination with the *sin* AHLs. In the absence of the ExpR regulator, production of succinoglycan remains high and unaltered by the *sin* AHLs, signifying that in this situation, biosynthesis of succinoglycan becomes free of quorum-sensing control. The advocacy for the observed result is that ExpR acts as a repressor of succinoglycan biosynthesis in the absence of the *sin* AHLs but in the absence of ExpR, repression does not occur.

Another gram-negative bacterium *Pantoea stewartii* ssp.*stewartii*, a phytopathogen in maize causes Stewart's wilt disease by production of stewartan exopolysaccharide in vascular occlusion while colonizing and growing in the xylem tissue forms biofilm (Braun, 1982; Koutsoudis *et al*., 2006).

**Table.1** Extracellular Polymeric Substances regulated by Quorum sensing in bacteria

| EPS producing Bacteria | QS System Responsible for Auto inducer Induction/ | Autoinducer/AHl Present | EPS Composition/Functional Groups Regulated | References |
|---|---|---|---|---|
| *Pseudomonas areuginosa* | *las*I | 3oxododecenoyl homoserine lactone | Glucose Rich Matrix Polysaccharide | Kolter and Sakuragi,2007 |
| *Pseudomonas areuginosa* | *Rhl* | *N*-butyrylhomoserine lactone | Rhamnolipid | Pearson *et al.*, 1995;1997 |
| *Pseudomonas areuginosa* | *Las* | N-(3-oxooxtanoyl)-L-homoserine lactone | Proteins and Polysaccharide | Xia *et al.*, 2012; Zhou *et al.*, 2014 |
| *Pseudomonas Syringae* | *ahl*I-*AhlI*R | 3-oxo-hexanoyl-homoserine lactone | Alginate | Quinones *et al.*, 2005 |
| *Sinorhizobium meliloti* | sinR/Sinl | $C_{12}$-Homoserine lactone, 3-oxo $C_{14}$ homoserine lactone $C_{16}$homoserine lactone, $C_{18}$homoserine lactone | EPS II Production | Glenn *et al.*, 2007; *Bahlawane et al.*, 2008; Mc Intosh *et al.*, 2008 |
| *Sinorhizobium meliloti* | ExpR | Palmitoleyl homoserine lactone, 3oxododecenoyl homoserine lactone, dodecenoyl homoserine lactone | Succinoglycan (Repeating octasaccharide subunits consisting of one galactose, seven glucose, and one each of succinyl, acetyl, and pyruvyl modifications)<br><br>EPSII(Repeating disaccharide subunits consisting of alternating galactose and glucose, along with acetyl and pyruvyl modifications) | Pellock *et al.*, 2002; Marketon *et al.*, 2003; Hoang *et al.*, 2004; Gao *et al.*, 2005; Glenn *et al.*, 2007; Bahlawane *et al.*, 2008; McIntosh *et al.*, 2008 |
| *Pantoeastewartiissp. stewartii* | EsaI/EsaIR | 3oxo $C_6$homoserine lactone,3oxo$C_8$homoserine lactone | Stewartan EPS (Anionic polymer of heptasaccharide repeat units of gactose,glucose and glucuronic acid) | Von Bodman and Farrand, 1995; Von Bodman *et al.*, 1998 |
| *Xanthomonascampestris* | *rpf*C-*rpf*G | Diffusible factor Diffusible signaling factor (*cis*-11-methyl-2dodecenoic Acid) | Xanthan | Tang *et al.*, 1991; Barber *et al.*, 1997; Slater *et al.*, 2000 |

**Fig.1** Diagrammatic expression of the Quorum sensing (QS) mechanism in bacteria
A. Intracellular receptors based QS system in Gram-negative bacteria. B. Membrane based receptors type QS system in Gram-positive bacteria. (Praneenararat *et al*., 2012)




Stewartan EPS is an anionic polymer of heptasaccharide repeat units of galactose, glucose and glucuronic acid in a 3:3:1 ratio (Nimtz *et al*., 1996a; Yang *et al.*, 1996). In *P. stewartii*, the biosynthesis of stewartan EPS is encoded by the cps gene cluster (Coplin and Majerczak, 1990). The mutation in cpsA-M gene is the primary reason of wilting, resulting in loss of systemic movement (Coplin and Majerczak, 1990), lay down the stewartan EPS as a principal virulence factor. The cps genes of *P. stewartii* are regulated by the Rcs (regulator of capsule synthesis) two-component signal transduction system. The plasma-membrane sensory protein Rcs C detects environmental signals, regulates osmolarity and outcome in phosphorylation and activation of the RcsB response regulator. An accessory protein, RcsA, is also needed for full induction of the cps genes, presumably by forming a more effective RcsA/RcsB activation complex (Gottesman, 1995). EPS synthesis in *P. stewartii* strain DC283 is regulated in part by the EsaI/EsaR QS system. The AHL synthase, EsaI, catalyzes the production of 3-oxoC6HLand minor amounts of 3-oxoC8HL (Von Bodman and Farrand, 1995). The EsaI gene is constitutive expressed and not subject to EsaR-mediated autoregulation. The mutation in EsaI gene eliminating AHL production, EPS synthesis and virulence, whereas mutations in the EsaR gene lead to constitutive, growth independent hypermucoidy (Von Bodman and Farrand, 1995). In comparison, the wild-type strain produces EPS in a population density-dependent manner at significant levels detected primarily at population densities $>10^8$ cells/ml (Von Bodman *et al*., 1998). This result that EsaR mutants are fully induced for EPS production at low cell density signifies that EsaR acts as a negative regulator of the cps genes in the absence of AHL (Von Bodman *et al*., 1998). Another well known phytopathogen *Xanthomonas campestris* responsible for virulence in citrus plants have been extensively studied and it was reported that *Xcc* system regulates the quorum-sensing and extracellular enzyme and EPS production. The modular proteins *rpf*C and *rpf*G form a two component quorum sensing system (Slater *et al*., 2000;Barber *et al*., 1997) regulated by *rpf*F gene. Quorum-sensing in this phytopathogen is mediated by two different autoinducers: DF ‘diffusible factor’ and DSF ‘diffusible signalling factor’

(Wang *et al*., 2004). The DF controls production of xanthomondin pigments and extracellular polysaccharides (EPS) and DSF coordinate the synthesis of extracellular enzymes such as proteases, pectinases and cellulases leads to the control of production of the extracellular polysaccharide xanthan gum (Tang *et al*., 1991; Barber *et al*., 1997; Slater *et al*., 2000).

From the above review of results it has been concluded that production of extracellular polymeric substances by modulation of quorum sensing systems is widely studied and still a lot of research work is needed to emphasize the effects associated with it in production of biofilm for waste water treatment plants and control of biofilm in infectious diseases and to regulate the damages of phytopathogens in plants. It's like both sides of the coin but more attention on QS regulation is needed to understand fundamentals of our present QS mechanisms. Genetic studies and *in vitro* experiments are needed to know how gene mediates repression and to test the most logical regulatory scenarios, which are still inconclusive. The more extensive research analysis has to be made on QS system using modern biotechnological tools towards better understanding about this microbial language to learn about their diversity and ecology.

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