Quorum-Sensing Systems in *Bacillus*

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Introduction

Since centuries bacteria were thought to be unicellular organisms. The discovery of bacterial communication through small molecules has asserted that bacteria can efficiently coordinate intraspecies as well as interspecies. The bacteria become more benefitted and suitable of behaving like a multicellular organism to adopt new modes of growth in limited nutrient supply. Under adverse conditions, single bacterial cell has less chance to survive in isolation; consequently bacterial language has been developed during evolution to communicate with its neighbours through self-generated signals (Bassler and Losick 2006). These signalling small molecules are called as pheromones or autoinducers. These autoinducers sense a critical bacterial density in population (Kievit and Iglewski 2000; Williams et al. 2007).

Communication in bacterial population is also observed in symbiotic bacteria which use these pheromones while interacting with their host. Bacteria are associated with the lives of *Homo sapiens* as well as live stocks concerning that they cause deadly diseases, can be in symbiotic relationship or are employed in various food processing industries (Steidle et al. 2001). *Bacillus* genera belong to the Firmicutes phylum of bacterial kingdom and include various significant organisms, namely, *B. anthracis*, the aetiological agent of anthrax; *B. cereus* that causes diarrhoeal symptoms; and *B. thuringiensis*, the insect pathogen. Numerous pathogens are known to cause upper respiratory and urinary tract infections, given their ability to communicate intraspecies and thus form multicellular organization called biofilms. Enhanced antibiotic resistance is also observed when these pathogenic microorganisms form clinically more interesting recalcitrant biofilms (Davey and O'toole 2000; Donlan and Costerton 2002). Furthermore, *B. subtilis* is known to colonize the *Arabidopsis thaliana* roots through matrix-enclosed multicellular communities in which matrix production is triggered by plant exopolysaccharides and reported to be beneficial for plant growth (Beauregard et al. 2013).

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V.C. Kalia (ed.), *Quorum Sensing vs Quorum Quenching: A Battle with No End in Sight*,
DOI 10.1007/978-81-322-1982-8_15,

Quorum-Sensing-Mediated Biofilm Formation

Bacillus subtilis is the most extensively studied organism in *Bacillus* genera. *B. subtilis* is a gram-positive, endospore-forming, rod-shaped and aerophilic bacteria (Kumar et al. 2013). It secretes diffusible oligopeptides for communication with the neighbouring cells; the process referred as quorum sensing (QS) (Kalia and Purohit 2011; Kalia 2013). Neighbouring bacteria perceive and broadcast the signals in its vicinity consequently causing behavioural modifications in the bacterial population (Parsek and Greenberg 2005; Bassler and Losick 2006; Mehta et al. 2009; Boyle et al. 2013; Vlamakis et al. 2013). Biofilms are the cocoons made up of extracellular polymeric substances (EPS) in which bacteria thrive under adverse environmental conditions (Flemming et al. 2007). The biofilm matrix consists of exopolysaccharides, proteins, enzymes and extracellular DNA along with pili and flagella. Biofilm provides adhesion that facilitates the initial step of colonization and imparts protection against the innate host defence. Following adhesion, biofilm also maintains the moisture content and absorption of nutrients for the better survival throughout the infectious phase or under environmental stress (Flemming and Wingender 2010; Li and Tian 2012; Kostakioti et al. 2013). Biofilm formation precisely describes the transition between unicellular bacteria to the partial multicellular organism (Aguilar et al. 2007; Shank et al. 2011). It is an ingenious plan of nature to sweep over the nutritional stress using coordinated biological pathways (Lemon et al. 2008; Shank et al. 2011). Bacteria within the biofilm display heterogeneity at phenotypic and genotypic level, and the social behaviour is governed through paracrine signalling (Stewart and Franklin 2008; López et al. 2009; Monds and O'Toole 2009; Kalia et al. 2011; Kalia 2013).

Competence and sporulation stimulating factor (CSF) is the master regulator of QS-mediated biofilm formation, competence and sporulation (Waters and Bassler 2005). The CSF is conserved in *Bacillus* spp.; however, the protein sequence shows the polymorphism. CSF is secreted in its precursor form (40 amino acids) encoded by *phrC* gene. The N-terminal of pre-CSF contains the guiding sequence for secretion. Membrane-associated serine proteases cleave the C-terminal of precursor CSF and release mature CSF (Miller and Bassler 2001; López et al. 2009). Mature CSF is a pentapeptide secreted into extracellular milieu, which regulates the competence factors ComA, ComK, ComS and ComX expression. The competence factor ComA activates the surfactin operon (*srfA-D*). Additionally, the ComK interacts with ComP and switches on the *srfA-D* operon, which ensures the cell to be competent and able to produce the exopolysaccharides. Surfactin-producing cells are capable to show the competency as ComS is synthesized, while the ComK shows the alternative cascade regulation; only a fraction of cells producing surfactin displays the competency (Shank and Kolter 2011). The pathways of competency and biofilm formation are similar in *B. subtilis* and in other organisms, for example, *Streptococcus pneumoniae*. Although the mechanism of action of QS molecules is well established, how the QS molecules are synthesized in the cell is relatively less understood (Turovskiy et al. 2007; orthington et al. 2012).

Quorum-Sensing-Mediated Cannibalism

Bacillus undergoes sporulation process in high stress condition such as nutritional imbalance or heat stress (Fig. 1). Sporulation is highly energy-driven and time-investing process in which vegetative cell differentiates into a dormant structure. Among different subpopulations under starvation conditions, a discrete set of bacterial cells secretes two toxin peptides, namely, Sdp (sporulation-delaying factor) and Skf (sporulation-killing factor) whose function is to delay sporulation and kill the siblings, respectively (Lamsa et al. 2012). The Skf peptide toxin kills their neighbouring sensitive cells

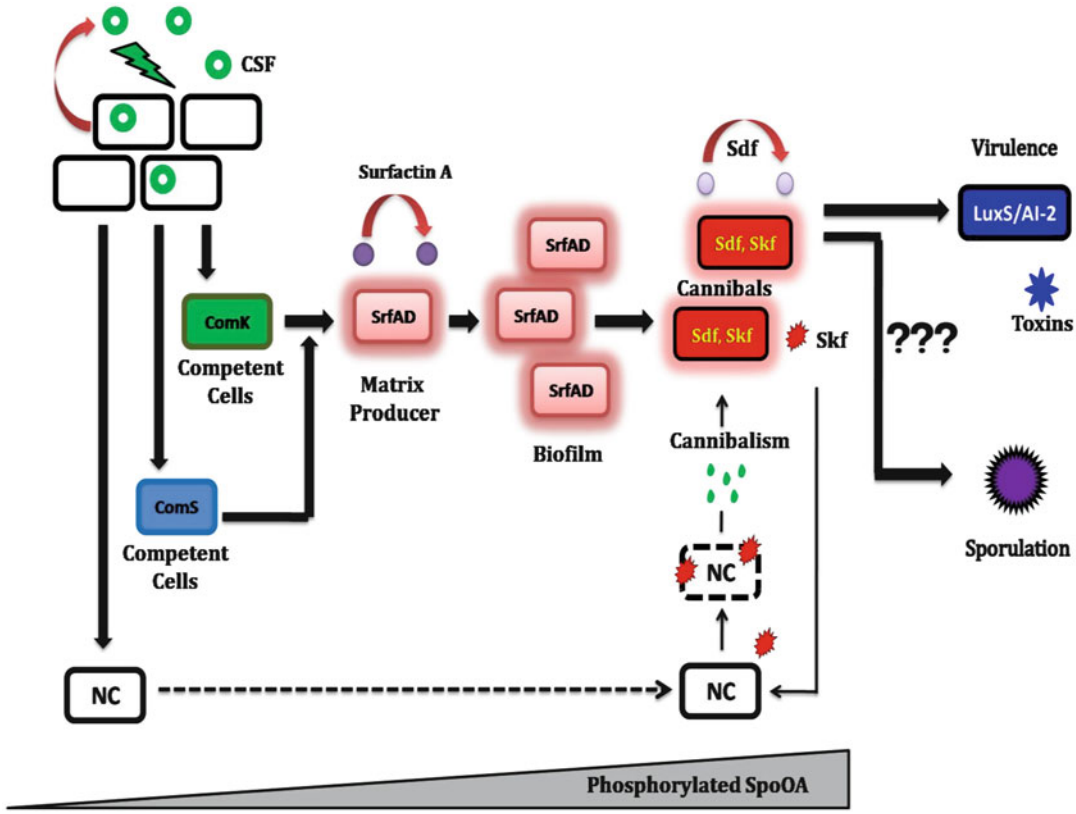

Fig. 1 Quorum-sensing systems in *Bacillus*: Heterogeneous bacterial population undergoes competence with the trigger of the colony and sporulation stimulating factor (CSF) during low phosphorylated Spo0A levels. The CSF acts differently on the cells, giving rise to distinct subpopulations of non-competent cells (NC), ComS and ComK expressing cells. The ComK majorly regulates the expression of surfactin-encoding operon (*srfA-D*), which triggers onset of matrix production by ComK and ComS expressing competent cells. Under persistent nutritional limitations, the matrix producers generate quorum-sensing (QS) signal through releasing sporulation-killing factor (Skf) which causes the killing of neighbouring non-competent cells, hence releasing nutrients to be used for prolonged survival and matrix production, the process known as cannibalism. Additionally, the matrix producers generate other signals known as a sporulation-delaying factor (Sdf), which results in enhanced survival in vegetative phase of the bacterial life cycle. At early stationary phase, cells generate QS molecules, i.e. autoinducer AI-2 signals, and regulate various virulence factors like toxin synthesis and secretion, S-layer formation, etc. At higher levels of phosphorylated Spo0A signals, sporulation is triggered simultaneously in the whole bacterial population. The QS regulators behind this highly synchronized onset of sporulation process are still unknown

(non-competent cells) and utilizes the nutrients released as food to overcome the nutrient limitations. The procedure of eating their siblings is termed as cannibalism and is described to be transitory in nature (Schultz et al. 2009; Shank and Kolter 2011). The sporulation master regulator Spo0A governs the expression of Sdp and Skf proteins. The low level of phosphorylated Spo0A regulates the *sdpABC, srf* operon consequently causing matrix production (López and Kolter 2009). The same subpopulation forms the biofilm, out of which some cells display cannibalistic behaviour. Both biofilm formation and cannibalism are reported to be triggered by the QS signal molecule, surfactin. Higher level of phosphorylated Spo0A promotes the cell into the committed sporulation phase of the bacterial life cycle (Fujita and Losick 2005).

Cannibalism is analogous to programmed cell death, in which cells not required for development of bacterial community are removed (López et al. 2009; Li and Tian 2012). In *B. subtilis*

paracrine signalling through QS molecules regulates the three major life events in the life cycle, e.g. biofilm formation, cannibalism and sporulation. Similarly the other *Bacillus* spp. like *Lactococcus lactis* secrete the cannibalism toxin nisin that functions as the antimicrobial peptide (Williams et al. 2007). The nisin treatment with *B. subtilis* kills the cells, those that are unable to produce the cannibalism toxin, consequently giving rise to stronger biofilm that are competing for survival in limited nutrients. It has been reported that *B. subtilis* also kills the neighbouring bacteria through the Skf cannibalism toxin (Turovskiy et al. 2007). Further investigations are needed to see the role of QS molecules and cannibalism toxins in multispecies biofilm formation.

Quorum-Sensing-Mediated Virulence

QS governs the major phenomenon in the bacterial alternating life stages. Interestingly, the virulence of pathogenic members of *Bacillus* is also reported to be governed by the societal communication (Fig. 1). The pathogenic clade of *Bacillus* species includes *B. anthracis*, *B. cereus* and *B. thuringiensis. B. thuringiensis* is an insect pathogen, which is a parasite of the economically significant crop, cotton. The paracrine signalling peptide PlcR (34 kDa or 48aa) is secreted by the *B. thuringiensis* and imported by neighbouring bacterial cells through oligopeptide permease. The PlcR interacts with PapR and binds to the DNA, and this ternary interaction is known to cause pleiotropic effects, including secretion of toxins (Kievit and Iglewski 2000). The PlcR regulator is also secreted by another pathogen *B. cereus* causing diarrhoeal and nausea symptoms upon food poisoning. PlcR is known to express at the onset of stationary phase that regulates the synthesis of various toxins like enterotoxins, cytotoxins and hemolysins. Also, the deletion of *plcR* causes abolished virulence in animal model systems (Grenha et al. 2012). Thus, PlcR plays central role in controlling the virulence in *B. thuringiensis* and *B. cereus* through QS (Atkinson and Williams 2009; Hong et al. 2012; Rutherford and Bassler 2012).

Anthrax is a zoonotic disease prevalent in developing countries, and recurrent outbreaks of the disease are reported across the world. *B. anthracis* is the aetiological agent of anthrax and secretes the tripartite toxin, namely, lethal factor, oedema factor and protective antigen encoded by *lef, cya* and *pag* genes, respectively. The toxin genes are present on the pathogenic islands of extrachromosomal plasmid pXO1. Small molecular weight protein AI-2 secreted by *B. anthracis* is known to modulate the pathogenicity. Jones and co-workers have investigated the role of *luxS* in the secretion of toxins from *B. anthracis*. Using microarray, they demonstrated that *B. anthracis* LuxS regulates the AI-2-dependent toxin secretion, bacterial growth and S-layer protein expression. The concentration of autoinducer AI-2 is found to be directly proportional to the bacterial cell density and toxin secretion (Kievit and Iglewski 2000; Turovskiy et al. 2007; Jones et al. 2010).

Conclusion

Bacillus exhibits the alternative life phase, i.e. vegetative cells and spore. Furthermore, it displays temporary multicellular organism behaviour that facilitates bacteria to survive in different environmental conditions. Under nutritional stress, bacteria show matrix production and cannibalism. However, persistent environmental stress or nutritional imbalance leads to the sporulating fate of vegetative cells in the biofilms. *Bacillus* is also reported to express the toxins at critical bacterial cell density. Sporulation is a highly energy-driven process in which the active vegetative cells undergo cellular differentiation process and form a dormant structure which can be viable over years. The decision to enter sporulation is reported to be critical for bacterial cell. Therefore, to avoid the sporulation, bacterium secretes proteins that are toxic to neighbouring cells, and this process of feeding on the siblings is known as cannibalism. The bacteria utilize the degraded cell components very efficiently

for delayed survival under starving conditions. Consequently, cannibalistic behaviour assists the bacterium to maintain the vegetative phase of its life cycle. Despite that, prolonged starvation leads its entry into the sporulation phase.

These major life events in *Bacillus* are temporally interdependent and are founded on precise intercellular communications in the bacterial population through its own molecular language, i.e. QS. The QS-mediated biofilm formation and consequent cannibalism behaviour are well elucidated. However, the QS modulators involved in sporulation are still not known.

Acknowledgements The authors wish to thank the director of CSIR-Institute of Genomics and Integrative Biology (IGIB), CSIR-GENCODE (BSC0123), Government of India, for providing the necessary funds and facilities. Authors are also thankful to the Academy of Scientific and Innovative Research (AcSIR), New Delhi. LKS is thankful to UGC and ND is thankful to CSIR for granting Senior Research Fellowships. We highly acknowledge Dr. V. C. Kalia and Mr. Prasun Kumar from CSIR-IGIB, Delhi, India, for their critical comments in the manuscript.

References

Aguilar C, Vlamakis H, Losick R, Kolter R (2007) Thinking about *Bacillus subtilis* as a multicellular organism. Curr Opin Microbiol 10:638–643. doi:10.1016/j.mib.2007. 09.006

Atkinson S, Williams P (2009) Quorum sensing and social networking in the microbial world. J R Soc Interface 6:959–978. doi:10.1098/rsif.2009.0203

Bassler BL, Losick R (2006) Bacterially speaking. Cell 125:237–246. doi:10.1016/j.cell.2006.04.001

Beauregard PB, Chai Y, Vlamakis H, Losick R, Kolter R (2013) *Bacillus subtilis* biofilm induction by plant polysaccharides. Proc Natl Acad Sci 110:E1621–E1630. doi:10.1073/pnas.1218984110

Boyle KE, Heilmann S, Ditmarsch DV, Xavier JB (2013) Exploiting social evolution in biofilms. Curr Opin Microbiol 16:207–212. doi:10.1016/j.mib.2013.01.003

Davey ME, O'toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64:847–867

Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15:167–193. doi:10.1128/CMR.15.2.167-193.2002

Flemming HC, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633. doi:10.1038/nrmicro2415

Flemming HC, Neu TR, Wozniak DJ (2007) The EPS matrix: "The house of biofilm cells". J Bacteriol 189:7945–7947. doi:10.1128/JB.00858-07

Fujita M, Losick R (2005) Evidence that entry into sporulation in *Bacillus subtilis* is governed by a gradual increase in the level and activity of the master regulator Spo0A. Genes Dev 19:2236–2244. doi:10.1101/gad.1335705

Grenha R, Slamti L, Nicaise M, Refes Y, Lereclus D, Nessler S (2012) Structural basis for the activation mechanism of PlcR virulence regulator by the quorum-sensing signal peptide PapR. Proc Natl Acad Sci 110:1047–1052. doi:10.1073/pnas.1213770110

Hong KW, Koh CL, Sam CK, Yin WF, Chan KG (2012) Quorum quenching revisited-from signal decays to signalling confusion. Sensors 12:4661–4696. doi:10.3390/s120404661

Jones MB, Peterson SN, Benn R, Braisted JC, Jarrahi B, Shatzkes K, Ren D, Wood TK, Blaser MJ (2010) Role of luxS in *Bacillus anthracis* growth and virulence factor expression. Virulence 1:72–83. doi:10.4161/viru.1.2.10752

Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31:224–245. doi:10.1016/j.biotechadv.2012.10.004

Kalia VC, Purohit HJ (2011) Quenching the quorum sensing system: potential antibacterial drug targets. Crit Rev Microbiol 37:121–140. doi:10.3109/1040841X.2010.532479

Kalia VC, Raju SC, Purohit HJ (2011) Genomic analysis reveals versatile organisms for quorum quenching enzymes: acyl-homoserine lactone-acylase and lactonase. Open Microbiol J 5:1–13. doi:10.2174/1874285801105010001

Kievit TR, Iglewski BH (2000) Bacterial quorum sensing in pathogenic relationships. Infect Immun 68:4839–4849. doi:10.1128/IAI.68.9.4839-4849.2000

Kostakioti M, Hadjifrangiskou M, Hultgren SJ (2013) Bacterial biofilms: development, dispersal and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harb Perspect Med 3:a010306. doi:10.1101/cshperspect.a010306

Kumar P, Patel SKS, Lee JK, Kalia VC (2013) Extending the limits of *Bacillus* for novel biotechnological applications. Biotechnol Adv 31:1543–1561. doi:10.1016/j.biotech adv.2013.08.007

Lamsa A, Liu WT, Dorrestein PC, Pogliano K (2012) The *Bacillus subtilis* cannibalism toxin SDP collapses the proton motive force and induces autolysis. Mol Microbiol 84:486–500. doi:10.1111/j.1365-2958.2012.08038

Lemon KP, Earl AM, Vlamakis HC, Aguilar C, Kolter R (2008) Biofilm development with an emphasis on *Bacillus subtilis*. Curr Top Microbiol Immunol 322:1–16

Li YH, Tian X (2012) Quorum sensing and bacterial social interactions in biofilms. Sensors 12:2519–2538. doi:10.3390/s120302519

López D, Kolter R (2009) Extracellular signals that define distinct and coexisting cell fates in *Bacillus subtilis*. FEMS Microbiol Rev 34:134–149. doi:10.1111/j.1574-6976.2009.00199.x

López D, Vlamakis H, Losick R, Kolter R (2009) Cannibalism enhances biofilm development in *Bacillus subtilis*. Mol Microbiol 74:609–618. doi:10.1111/j.1365-2958.2009.06882.x

Mehta P, Goyal S, Long T, Bassler BL, Wingreen NS (2009) Information processing and signal integration in bacterial quorum sensing. Mol Syst Biol 5:325. doi:10.1038/msb. 2009.79

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199. doi:10.1146/annurev.micro.55.1.165

Monds RD, O'Toole GA (2009) The developmental model of microbial biofilms: ten years of a paradigm up for review. Trends Microbiol 17:73–87. doi:10.1016/j.tim.2008.11.001

Parsek MR, Greenberg EP (2005) Sociomicrobiology: the connection between quorum sensing and biofilms. Trends Microbiol 13:27–33. doi:10.1016/j.tim.2004.11.007

Rutherford ST, Bassler BL (2012) Bacterial quorum sensing: Its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med 2:a012427. doi:10.1101/cshperspect.a012427

Schultz D, Wolynes PG, Ben Jacob E, Onuchic JN (2009) Deciding fate in adverse times: sporulation and competence in *Bacillus subtilis*. Proc Natl Acad Sci 106:21027–21034. doi:10.1073/pnas.0912185106

Shank EA, Kolter R (2011) Extracellular signaling and multicellularity in *Bacillus subtilis*. Curr Opin Microbiol 14:741–747. doi:10.1016/j.mib.2011.09.016

Shank EA, Klepac-Ceraj V, Collado-Torres L, Powers GE, Losick R, Kolter R (2011) Interspecies interactions that result in *Bacillus subtilis* forming biofilms are mediated mainly by members of its own genus. Proc Natl Acad Sci 108:E1236–E1243. doi:10.1073/pnas.1103630108

Steidle A, Sigl K, Schuhegger R, Ihring A, Schmid M, Gantner S, Stoffels M, Riedel K, Givskov M, Hartmann A, Langebartels C, Eberl L (2001) Visualization of N-acylhomoserine lactone-mediated cell-cell communication between bacteria colonizing the tomato rhizosphere. Appl Environ Microbiol 67:5761–5770. doi:10.1128/AEM.67.12.5761-5770.2001

Stewart PS, Franklin MJ (2008) Physiological heterogeneity in biofilms. Nat Rev Microbiol 6:199–210. doi:10.1038/nrmicro1838

Turovskiy Y, Kashtanov D, Paskhover B, Chikindas ML (2007) Quorum sensing: fact, fiction and everything in between. Adv Appl Microbiol 62:191–234. doi:10.1016/S0065-2164(07)62007-3

Vlamakis H, Chai Y, Beauregard P, Losick R, Kolter R (2013) Sticking together: building a biofilm the *Bacillus subtilis* way. Nat Rev Microbiol 11:157–168. doi:10.1038/nrmicro2960

Waters CM, Bassler BL (2005) Quorum sensing: cell-to cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346. doi:10.1146/annurev.cellbio.21.012704.131001

Williams P, Winzer K, Chan WC, Cámara M (2007) Look who's talking: communication and quorum sensing in the bacterial world. Philos Trans R Soc Lond B Biol Sci 362:1119–1134. doi:10.1098/rstb.2007.2039

Worthington RJ, Richards JJ, Melander C (2012) Small molecule control of bacterial biofilms. Org Biomol Chem 10:7457–7474. doi:10.1039/c2ob25835h