

# Transition of solitary to biofilm community life style in bacteria: a survival strategy with division of labour

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**ABSTRACT** Multicellularity is associated with higher eukaryotes having an organized division of labour and a coordinated action of different organs composed of multiple cell types. This division of different cell types and organizations to form a multicellular structure by developmental programming is a key to the multitasking of complex traits that enable higher eukaryotes to cope with fluctuating environmental conditions. Microbes such as bacteria, on the other hand, are unicellular and have flourished in diverse environmental conditions for a much longer time than eukaryotes in evolutionary history. In this review, we will focus on different strategies and functions exhibited by microbes that enable them to adapt to changes in lifestyle associated with transitioning from a unicellular solitary state to a complex community architecture known as biofilm. We will also discuss various environmental stimuli and signaling processes which bacteria utilize to coordinate their social traits and enable themselves to form complex multicellular-like biofilm structures, and the division of labour operative within such communities driving their diverse social traits. We will also discuss here recent studies from our laboratory using a plant-associated bacterial pathogen as a model organism to elucidate the mechanism of bacterial cell-cell communication and the transition of a bacterial community to a multicellular-like structure driven by the complex regulation of traits influenced by cell density, as well as environmental sensing such as chemotaxis and nutrient availability. These studies are shedding important insights into bacterial developmental transitions and will help us to understand community cooperation and conflict using bacterial cell-cell communication as a model system.

**KEY WORDS:** *quorum sensing, biofilm, adhesion, extracellular polysaccharide, heterogeneity, cheating, bet-hedging, fitness*

## Introduction

Bacteria have been generally considered as unicellular, and therefore solitary organisms that are associated with given environments such as soil, plants, animals and water. They have served as excellent model systems to study fundamental biological processes such as replication, transcription, translation and basic physiology. Most bacterial studies are done in broth cultures under laboratory conditions of presumably homogenous cultures with uniformly dispersed cells. The concept that bacteria can modulate their behaviour at high cell density came with the study of bioluminescence in the Gram-negative marine bacterium known as *Vibrio fischeri*, which forms a symbiotic association with some marine animals, such as *Euprymna scolopes* (Nealson *et al.*, 1970; Nealson 1977). The bacteria inhabit the light organs of *Euprymna* and emit light

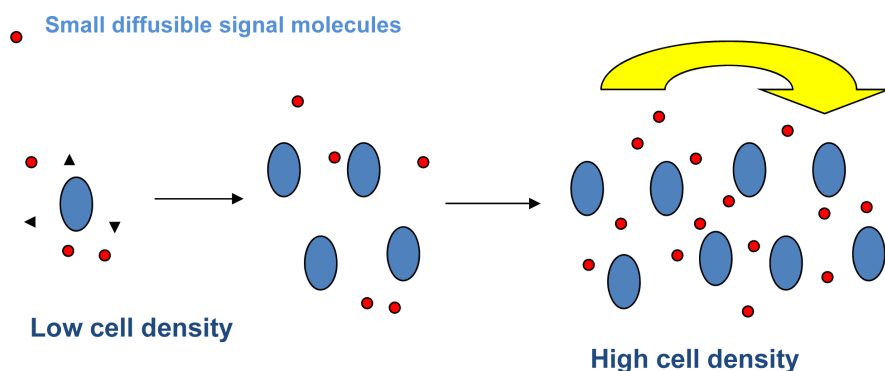
due to the activity of luciferase enzymes. Researchers observed that production of light by *V. fischeri* was density dependent as the bacterium at low cell density in broth culture was unable to produce bioluminescence. However, when the culture density increased to very high concentrations in broth culture (similar to the cell density attained in symbiotic association), the bacterial cells exhibited bioluminescence. The phenomenon of a coordinated response at a particular high cell density was coined as “quorum sensing (QS)” (Engebrecht *et al.*, 1983; Fuqua *et al.*, 1994). With further studies of bacterial behaviour and understanding the mechanism of quorum sensing in the last decade, the QS mediated communication system that is utilized by the unicellular organism to perform and

*Abbreviations used in this paper:* AHL, acyl-homoserine lactone; DSE, diffusible signal factor; rpf, regulation of pathogenicity factor; QS, quorum sensing.

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**Fig. 1. Quorum sensing.** In prokaryotes, quorum sensing is called density dependent cell-cell signaling. Bacteria (indicated in blue ovals) produce cell-cell signaling diffusible molecules (red dots), in a density dependent fashion which diffuse out (black triangles) of the cells. The quorum sensing signal concentration builds up with the increase in cell density. At a high cell density, the concentration of quorum signal molecules reaches a threshold level which synchronizes (yellow arrow) the bacterial cells to regulate gene expression in unison and to perform social tasks such as motility, biofilm formation, antibiotic production, production of extracellular enzymes etc. These community social behaviours enable bacteria to perform multiple tasks similar to multicellular organisms.



coordinate complex tasks similar to multicellular organisms has been elucidated (Miller and Bassler, 2001; Ng and Bassler, 2009). This also opened the field of sociomicrobiology (the connection between quorum sensing and biofilm formation) and researchers started looking at microbial communities in natural habitats or under laboratory conditions which mimic the natural environment (Parsek and Greenberg, 2005; Turovskiy *et al.*, 2007). The study of microbes on different surfaces in nature revealed that bacteria often formed highly organized structures known as biofilms. Microbial biofilms consist of either single or multiple layers of bacterial cells that adhere to various surfaces and form robust structures that provide protection from different environmental stresses such as antimicrobial compounds, low nutrient availability and changes in the temperature and pH of the surrounding environment (O'Toole *et al.*, 2000; Palková, 2004; Flemming *et al.*, 2007; Nadell *et al.*, 2009; López *et al.*, 2010). In this review, we will discuss the mechanism of formation of biofilms in bacteria and how they utilize these multicellular-like structures to perform complex social tasks. We will also discuss the mechanisms of the reverse process of biofilm dispersal which is also highly dynamic and reversible in nature. Finally, we will elucidate the complexity of the QS response and biofilm formation that often involves the emergence of cheaters and the interplay of coordinated and heterogeneous social responses that generates phenotypic diversity in an otherwise genetically identical bacterial population or community.

### Bacterial quorum sensing enables social communication among individual members within populations of solitary cells

Quorum sensing is a process by which bacteria communicate with each other via production and sensing of multiple types of secreted signaling molecules (Fig. 1). Several plant and animal-associated bacteria, including those inhabiting diverse environments exhibit quorum sensing (Fuqua *et al.*, 1994; Parsek and Greenberg, 2005; Turovskiy *et al.*, 2007; He and Zhang, 2008; Ng and Bassler, 2009). Diverse classes of quorum sensing signaling molecules have now been characterized that are involved in both intra-species as well as inter-species communication (Fuqua *et al.*, 2001; Turovskiy *et al.*, 2007; He and Zhang, 2008; Ng and Bassler, 2009). The most common and well studied quorum sensing system is that conferred by acyl-homoserine lactone (AHL) mediated signaling in several Gram-negative bacteria such as *Vibrio fischeri*, *Pseudomonas aeruginosa*, *Agrobacterium tumefaciens* and *Erwinia carotovora* (Fuqua

*et al.*, 2001; Miller and Bassler, 2001; Ng and Bassler, 2009). The AHL mediated QS process has been studied as a model for the mechanism of production and perception of QS signals. In general, this involves an AHL synthase such as AhlI or LuxI that is involved in the production of QS signal. At low cell density, only a basal level of expression of the signal synthase is operative, leading to low levels of AHL signal accumulation in or near the cell. As cell density increases, signal production increases and this is either diffused or transported rapidly out of the cell. When the concentration builds up above a threshold level, the signal molecule (ligand) binds to the transcriptional regulator such as AhlR or LuxR (receptor), that thereby gets activated and binds to DNA and typically acts as a regulator of gene expression (Fig. 2) (Fuqua *et al.*, 2001; Miller and Bassler, 2001; Ng and Bassler, 2009). In addition to AHL-mediated quorum sensing signaling, bacteria also exhibit QS mediated by other diverse signaling molecules such as furanosyl borate diester, fatty acid derivatives (3-Hydroxylpalmitic acid methyl ester, diffusible signal factor) and cyclic peptide (thiolactone) (in Gram-positive bacteria) (Parsek and Greenberg, 2005; Turovskiy *et al.*, 2007; He and Zhang, 2008; Ng and Bassler, 2009). In several bacteria, it has been shown that QS-mediated coordinated responses occur via synchronized regulation of gene expression leading to harmonious production and secretion of various extracellular products, often known as 'public goods', that are beneficial to the population as a whole (Greenberg, 1998; Palková, 2004; Darch *et al.*, 2012; Pai *et al.*, 2012). Several traits have been shown to be regulated by QS such as: (i) the production of extracellular polysaccharides, adhesions or attachment proteins that often play a role in biofilm formation, (ii) extracellular cell-wall hydrolyzing enzymes, (iii) iron chelating compounds known as siderophores, (v) virulence factors that are utilized for host colonization and infection, and (v) functions required for directional motility (Fuqua *et al.*, 2001; Parsek and Greenberg, 2005; Williams *et al.*, 2007; Ng and Bassler, 2009; Long *et al.*, 2009; Darch *et al.*, 2012; Pai *et al.*, 2012).

Our laboratory uses the *Xanthomonas* group of phytopathogens as model organisms to study quorum sensing, cooperation and social behaviour in bacterial community. This review will focus on *Xanthomonas* QS biology and will elaborate on how *Xanthomonas* coordinates the expression of multiple social traits via coordination of cell-cell signaling and environmental sensing to achieve multicellular-like social tasks. The *Xanthomonas* group are phytopathogens that cause disease in several economically important crop plants such as rice, tomato, cabbage, citrus etc (Niño-Liu *et al.*, 2006; Büttner and Bonas, 2010; Mansfield *et al.*, 2012).

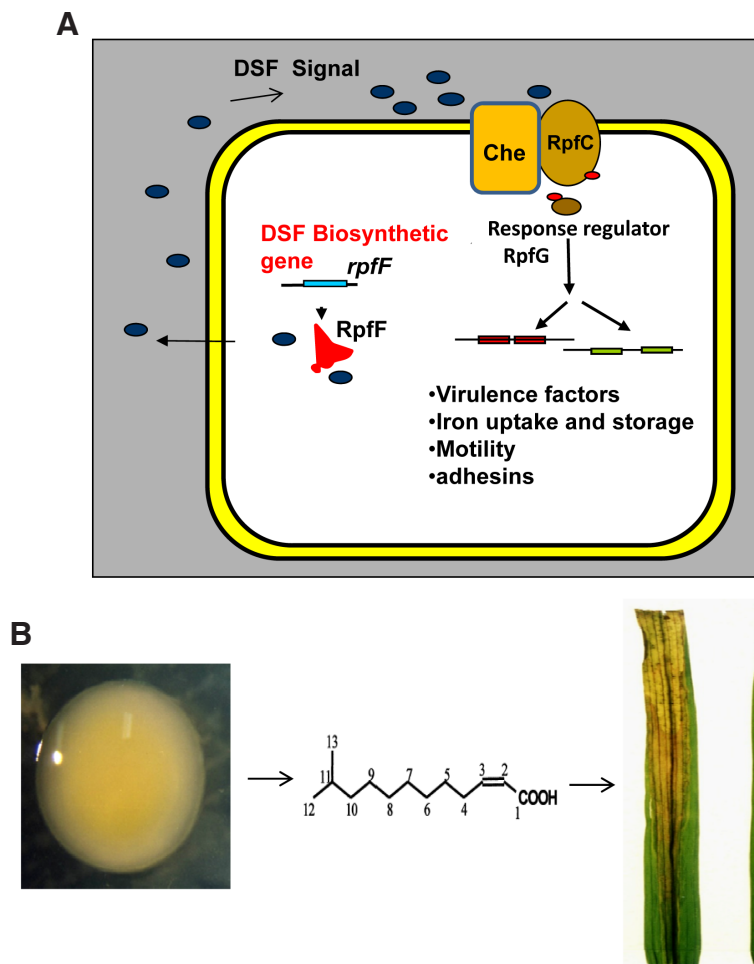
Among Xanthomonads, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) are important rice pathogens (Niño-Liu *et al.*, 2006; Mansfield *et al.*, 2012). In the early 2000s, while working on isolation of virulence-deficient mutants of *Xanthomonas* by genetic screening, at the Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India, we isolated a genetic mutant defective in a gene in a gene cluster known as “regulation of pathogenicity factor (*rpf*)” (Chatterjee and Sonti, 2002). Our group, along with scientists at the John Innes Centre in Norwich, UK independently showed that the *rpfF* gene is involved in the production of a diffusible signal factor that is involved in the virulence of *Xanthomonas* (Fig. 2) (Barber *et al.*, 1997; Chatterjee and Sonti, 2002). Further characterization of the signaling molecule and characterization of various mutants revealed that *Xanthomonas* produces an unusual fatty acid QS signal molecule (*cis*-11-methyl-2-dodecenoic acid) known as “diffusible signal factor (DSF)” (He and Zhang, 2008; Deng *et al.*, 2011; Ryan and Dow, 2011). With the appreciation of the novelty of the DSF family of signaling molecule, it has now increasingly become evident that several groups of bacteria, such as members of the genus *Xanthomonas*, *Burkholderia*, *Xylella*, *Stenotrophomonas* all communicate using the DSF family of signaling molecules (He and Zhang, 2008; Chatterjee *et al.*, 2008a,b; Deng *et al.*, 2011; Ryan and Dow, 2011). In the rice pathogen *Xanthomonas oryzae*, DSF is involved in the positive regulation of biofilm formation and adhesin production (required for attachment), and in the negative regulation of motility and produc-

tion of cell wall hydrolyzing enzymes (Rai *et al.*, 2012; Rai *et al.*, 2015). Characterization of the DSF-mediated signal transduction process in the *Xanthomonas* group of phytopathogens revealed that the regulation of virulence-associated functions by DSF mediated signaling is a complex process involving multiple sensors and response regulators that act in parallel and with complex regulatory interactions with each other (Chatterjee *et al.*, 2008a,b; Rai *et al.*, 2015). In *Xanthomonas oryzae*, DSF not only promotes the transition of a solitary to a community lifestyle or biofilm, but it is also involved in the regulation of iron uptake, chemotaxis, and motility in a density-dependent fashion (Chatterjee and Sonti, 2002; Rai *et al.*, 2012; Rai *et al.*, 2015). Particularly when in a biofilm, the availability of scarce nutrients such as iron is limiting, the pathogen has to acquire and store iron from diverse environmental or host iron sources. QS coordinates the expression of multiple iron sensing regulators to achieve iron homeostasis in a cell density-dependent fashion that enables optimum growth and survival of the cells at high cell density and within biofilms that experience nutrient scarcity (Pandey *et al.*, 2016; Pandey *et al.*, 2017; Pandey *et al.*, 2018).

### Biofilms coordinated by quorum sensing (QS) represent a microbial multicellular transition from a solitary lifestyle

QS regulates biofilm formation in many bacteria. Biofilm provides a stable, safe structure for the survival of bacteria wherein they can perform multiple tasks such as nutrient acquisition, defense against

host antimicrobial compounds, and stress tolerance (Costerton *et al.*, 1994; O’Toole *et al.*, 2000; Parsek and Greenberg, 2005; Danhorn and Fuqua, 2007; López *et al.*, 2010). *Xanthomonas* species form multi-layer biofilms only at high cell density both under laboratory conditions as well as inside the host plant (Fig. 3) (Rai *et al.*, 2012; Rai *et al.*, 2015; Pandey *et al.*, 2016). Us-

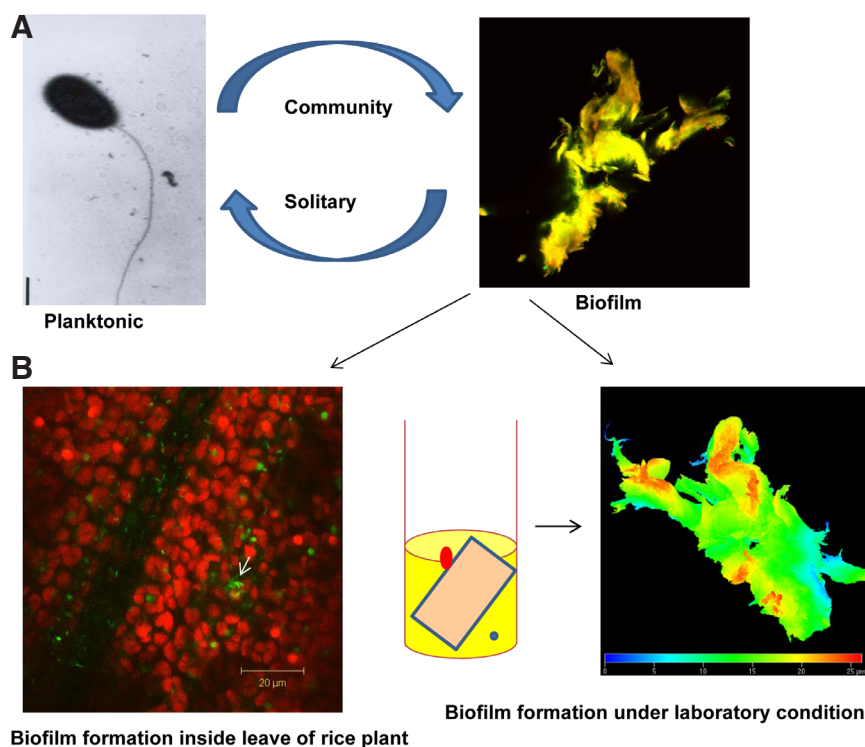


**Fig. 2. A model depicting the basic mechanism of quorum sensing signal transduction. (A)** Diffusible signaling molecule (DSF) is made by the enzymatic action of QS signal synthase. The QS signal concentration increases inside the cell and diffuses out into the extracellular space. As the cell number increases the production of QS signal increases and the concentration of QS signal builds up above a threshold limit which is then detected by either membrane bound or cytoplasmic receptor. Binding of the QS signal to the sensor leads to conformational change and induces auto phosphorylation of sensor kinase. The sensor kinase interacts with response regulator by phosphate-transfer and the response regulator may bind to target promoters to induce gene expression. In case of *Xanthomonas* QS system, the RpfF (DSF synthase) makes DSF signaling molecule which binds with the sensor RpfC and other sensors which activates the response regulator RpfG. RpfG is a cyclic Di-GMP hydrolyzing protein which degrades cyclic Di-GMP, regulates gene expression and modulates social behaviour. **(B)** Representative picture of a typical *Xanthomonas* colony on a laboratory medium. The bacteria produce extracellular polysaccharide and DSF signaling molecule in a density dependent fashion. Production of signal and regulation of virulence associated function are important for causing disease on rice plant (Shown from left to right are the leaves of a rice plant that are infected with the wild type and the QS-deficient mutant strains of *Xanthomonas oryzae* pv. *oryzae* respectively).

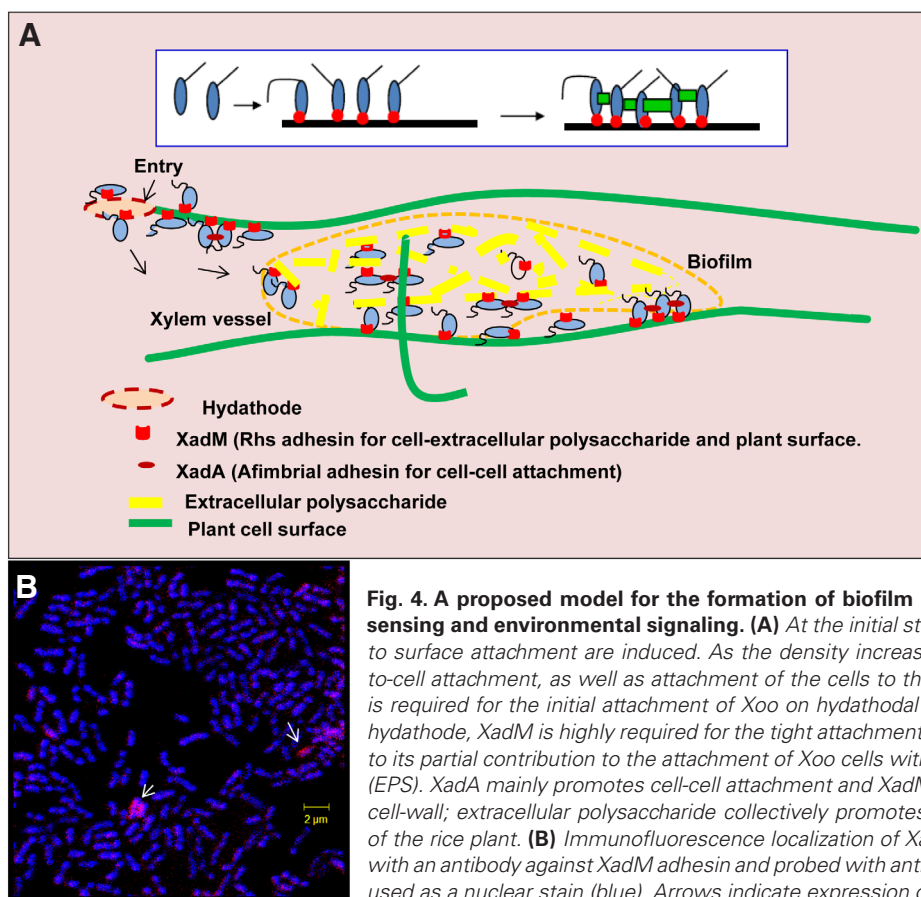
ing both confocal laser scanning microscopy in conjunction with probes for the study of biofilms revealed that the formation of biofilms in *Xanthomonas* is a stage-specific process that requires both cell-cell attachment as well as cell to surface attachment (Fig. 3; Fig. 4) (Das et al., 2009; Darsonval et al., 2009; Gottig et al., 2009; Rai et al., 2012; Rai et al., 2015). Since the infection process in the host involves both active directional motility to enter the host via specific portals followed by the migration and colonization the interior host tissue, such as xylem vessels, synthesis of biofilm-forming factors is coordinated in a density-dependent fashion (Rai et al., 2012; Verma et al., 2018). At low cell densities when DSF levels are low, the lack of a QS signal promotes chemotaxis or directional motility, enabling *X. oryzae* to enter the rice xylem vessel through small openings on the leaf surface known as hydathodes. Once inside the hydathodes, the pathogen spreads and forms microcolonies inside the xylem vessels, and different sets of attachment proteins known as “adhesins” (that are induced by both QS and environmental condition-dependent manner) are produced. The *in vitro* environmental conditions for such production mimic conditions inside the host (Fig. 3; Fig. 4) (Pradhan et al., 2012; Rai et al., 2012). It is logical to think that if all attachment proteins are made simultaneously, it will interfere with the systemic spread, since the colonization process involves

entry, migration, spread and disease progression (Fig. 4). We have characterized several adhesins (attachment proteins) in *Xanthomonas* that are required for virulence, biofilm formation and attachment such as XadA, YapH, and XadM. Study of the dynamics of biofilm formation, and the expression patterns of these virulence factors both inside the host plant and under host mimicking *in vitro* conditions revealed that the adhesins such as XadM are expressed in a density-dependent fashion within the bacterial community (Fig. 4) (Ray et al., 2002; Das et al., 2009; Pradhan et al., 2012; Pandey et al., 2016). In addition to various adhesins, *Xanthomonas oryzae* also produces extracellular polysaccharide (EPS) that is composed of xanthan (a complex polysaccharide) and glucan carbohydrate. EPS provides protection to the bacteria against harmful plant defense molecules, as well as acts as an extracellular matrix (Flemming et al., 2007; Kakkar et al., 2015). Attachment and biofilm formation studies revealed that (i) the production of EPS is also regulated by DSF-mediated QS, and (ii) EPS, together with surface-exposed adhesions such as XadM is involved in the process of attachment of bacterial cells to various surfaces (Pradhan et al., 2012; Kakkar et al., 2015; Rai et al., 2015). EPS also plays a role in the suppression of plant defense responses that protect bacterial cells during host colonization (Kakkar et al., 2015).

Interestingly, one of the components of EPS is a cyclic glucan that is both secreted and cell associated. Recent studies indicated that glucan is involved in iron homeostasis as it sequesters iron from the environment and also suppress harmful plant defense responses when bacteria are colonizing the host plant xylem vessels (Kakkar et al., 2015; Javvadi et al., 2018). In *Xanthomonas oryzae*, DSF-mediated signaling negatively regulates chemotaxis, thus contributing to biofilm stability (Rai et al., 2012; Rai et al., 2015). In *Xyloella fastidiosa* (an important insect transmitted plant pathogen and a close relative of *Xanthomonas*), it has been shown that mutants that exhibit hyper-motility are deficient in biofilm formation (Chatterjee et al., 2008a,b). Recent study to understand the role of the chemotaxis system in *Xanthomonas oryzae* revealed that chemotaxis mutants that are deficient in directional motility, form biofilms even better than the wild type strain (Verma et al., 2018). Study of the regulation of biofilm formation by DSF-mediated signaling (influenced by chemotaxis-specific nutrient availability) revealed that high cell density triggers biofilm formation, and there is a fine tuning of motility and chemotaxis that coordinates the transition of solitary to biofilm lifestyle in *Xanthomonas* (Rai et al., 2015; Pandey et al., 2016). Biofilms also enable bacteria to survive under nutrient-limiting conditions, wherein many essential nutrients such as iron, sugars are limited in amount to support microbial growth at high cell density (Cassat and Skaar, 2013). In addition to cell-cell signaling mediated by DSF, the regulation of iron uptake and metabolism also cross talks with QS signaling in *Xanthomonas* (Chatterjee



**Fig. 3. Quorum sensing coordinates the formation of biofilm.** (A) Transition of bacteria from a solitary (planktonic) lifestyle to a multicellular aggregate is triggered by quorum sensing and changes in environmental condition. (B) A typical biofilm formed by *Xanthomonas oryzae* consists of multicellular aggregates visualized by confocal laser scanning microscopy. Biofilm formation can be induced under laboratory condition under high cell density on liquid-air-surface interface, wherein multiple cells aggregate by cell to cell attachment and form a complex multicellular-like structure. Under natural conditions *Xanthomonas* form biofilm inside the leaves of rice plants during colonization and disease establishment. White arrow indicates a bacterial biofilm formed inside the infected leaf of a rice plant.



**Fig. 4. A proposed model for the formation of biofilm in *Xanthomonas oryzae* coordinated by quorum sensing and environmental signaling. (A)** At the initial stage, at low cell density, adhesins which promote cell to surface attachment are induced. As the density increases, adhesins such as XadM and YapH promote cell-to-cell attachment, as well as attachment of the cells to the xylem vessel entry points. XadM along with XadA is required for the initial attachment of Xoo on hydathodal entry points present on the leaf surface. Inside the hydathode, XadM is highly required for the tight attachment of the bacterial cells to the plant cell-wall, in addition to its partial contribution to the attachment of Xoo cells with each other by secreted extracellular polysaccharide (EPS). XadA mainly promotes cell-cell attachment and XadM is mainly involved in attachment of Xoo to the host cell-wall; extracellular polysaccharide collectively promotes stable biofilm formation inside the infected leaves of the rice plant. **(B)** Immunofluorescence localization of XadM in Xoo cells within a biofilm. Cells were stained with an antibody against XadM adhesin and probed with anti-rabbit FITC conjugate secondary antibody. DAPI was used as a nuclear stain (blue). Arrows indicate expression of XadM on the surface of bacterial cells in a biofilm.

and Sonti, 2002; Rai *et al.*, 2015). We have isolated a novel iron binding transcription factor named XibR (*Xanthomonas* iron binding Regulator) in *Xanthomonas campestris* pv. *campestris* that phenocopies many of the traits exhibited by QS regulatory mutants. These results indicate that bacteria employ complex sensing and signal transduction machinery to perform social task, such as biofilm formation that involves multiple regulators and sensors including those involved in sensing cell density and environmental conditions (Pandey *et al.*, 2016).

#### Division of labour in bacterial social behaviour: non genetic phenotypic heterogeneity in QS response

It has been argued that multicellular-like behaviour induced by bacterial QS in high cell densities and the associated uniform response is beneficial to the cells. However, such a system can also exhibit a division of labour (Costa *et al.*, 2006; Diggie *et al.*, 2007; Sandoz *et al.*, 2007; Xavier and Foster, 2007; Nadell *et al.*, 2009). Evolutionary theory predicts that maintaining phenotypic heterogeneity in social traits can lead to bet-hedging survival strategies that can force in a division of labour within the bacterial community (Gardner *et al.*, 2007; Davidson and Surette, 2008; Jacob and Schultz, 2010). In bacterial biofilms, the cells attach to form a multicellular-like structure that must be dynamic in nature in terms of phenotypic plasticity. Furthermore, the cells need to be free to migrate in search of new environments that might provide the needed nutrients (Costa *et al.*, 2006). For that

reason, phenotypic heterogeneity has been reported in diverse social processes such as chemotaxis-driven motility (Spudich and Koshland, 1976), persistence in the presence of antibiotics (Balaban *et al.*, 2004), bi-stability of gene expression (Novick and Weiner, 1957) and induction of natural competence in *Bacillus* (Süel *et al.*, 2006), that are often associated with biofilms. We have used *Xanthomonas* and *Pseudomonas* as model systems to address heterogeneity in QS response in microbial populations. Using single cell studies of QS dynamics via fluorescence activated cell sorting, live cell imaging, and competition experiments (with wild type and various QS mutants), we showed that bacteria exhibit reversible phenotypic heterogeneity in their QS response. Specifically, QS responding populations maintain a proportion of QS responding and non-responding cells in an approximately 80:20 ratio (Anetzberger *et al.*, 2009; Pradhan and Chatterjee, 2014). Using competition experiments coupled with imaging of cells that formed aggregates from previously solitary cells revealed that the non-responders in a biofilm move away from the biofilm and therefore may contribute to biofilm dispersal and systemic spread (Pradhan and Chatterjee, 2014). In our recent study of *Xanthomonas*-plant interaction, we have used whole cell dual-biosensors that can track both bacterial localization and quorum sensing response *in vivo* and have studied the dynamics of heterogeneity in the QS response inside the host plant (Samal and Chatterje, 2019). Our study indicates that division of labour consisting of QS-responsive and non-responsive populations gives stability to the bacterial community towards the initial successful

disease establishment, and the reversal of biofilm to planktonic cells contributes to the systemic spread of the disease within the host (Samal and Chatterjee, 2019). We proposed that this division of labour provides survival fitness to such a host-associated complex bacterial community under adverse conditions during its parasitic life cycle within the host.

## Concluding remarks

Bacteria are increasingly being used as model systems to study social life. Quorum sensing acts as a signal for the transition from a solitary (planktonic) to a biofilm (multicellular) organization and vice versa within a bacterial community. It therefore can be used as a model system to study the evolutionary transition from unicellular life form to complex multicellular architectures. Since bacterial systems are amenable to genetic manipulation, and exhibit short generation times and is thus subject to rapid evolutionary changes, it can serve as good system to ask many questions such as: Do bacteria as a community have long or short term memory or program that enables phase-specific morphological and/or physiological adaptation? What triggers heterogeneity in performing social tasks? How are multiple signaling pathways coordinated at a cellular level with environmental conditions to mediate appropriate outcomes for the community? What other phenotypic or genetic switches are involved in QS and environmental sensing to coordinate the transition from a planktonic to biofilm lifestyle and vice versa? In the near future, more detailed studies of the social life of bacteria should lead us to understand the evolutionary driving forces responsible for unicellular to multicellular transition in other forms of life.

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## References

- ANETZBERGER, C., PIRCH, T. and JUNG, K. (2009). Heterogeneity in quorum sensing-regulated bioluminescence of *Vibrio harveyi*. *Mol Microbiol* 73: 267–277.
- BALABAN, N.Q., MERRIN, J., CHAIT, R., KOWALIK, L. and LEIBLER, S. (2004). Bacterial persistence as a phenotypic switch. *Science* 305: 1622–1625.
- BARBER, C.E., TANG, J.L., FENG, J.X., PAN, M.Q., WILSON, T.J.G., SLATER, H., DOW, J.M., WILLIAMS, P. and DANIELS, M.J. (1997). A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. *Mol Microbiol* 24: 555–566.
- BÜTTNER, D. and BONAS, U. (2010). Regulation and secretion of *Xanthomonas* virulence factors. *FEMS (Fed Eur Microbiol Soc) Microbiol Rev* 34:107–133.
- CASSAT, J.E. and SKAAR, E.P. (2013). Iron in infection and immunity. *Cell Host Microbe* 13:509–519.
- CHATTERJEE, S. and SONTI, R.V. (2002). *rpfF* mutants of *Xanthomonas oryzae* pv. *oryzae* are deficient for virulence and growth under low iron conditions. *Mol Plant-Microbe Interact* 15: 463–471.
- CHATTERJEE, S., WISTROM, C. and LINDOW, S.E. (2008a). A cell-cell signaling sensor is required for virulence and insect transmission of *X. fastidiosa*. *Proc Natl Acad Sci USA* 105: 670–675.
- CHATTERJEE, S., ALMEIDA, R.P.P. and LINDOW, S.E. (2008b). Living in two worlds: The plant and insect lifestyles of *X. fastidiosa*. *Ann Rev Phytopathol* 46: 243–271.
- COSTA, E., PEREZ, J. and KREFT J.U. (2006). Why is metabolic labour divided in nitrification? *Trends Microbiol* 14: 213–219.
- COSTERTON, J.W., LEWADOWSKI, Z., DEBEER, D., CALDWELL, D., KORVER, D. and JAMES, G. (1994). Biofilms, the customized microniche. *J Bacteriol* 176: 2137–2142.
- DANHORN, T. and FUQUA, C. (2007). Biofilm formation by plant-associated bacteria. *Annu Rev Microbiol* 61:401–422.
- DARSONVAL, A., DARRASSE, A., DURAND, K., BUREAU, C., CESBRON, S. and JACQUES, M.A. (2009). Adhesion and fitness in the bean phyllosphere and transmission to seed of *Xanthomonas fuscans* subsp. *fuscans*. *Mol Plant-Microbe Interact* 22: 747–757.
- DARCH, S.E., WEST, S.A., WINZER, K. and DIGGLE, S.P. (2012). Density-dependent fitness benefits in quorum-sensing bacterial populations. *Proc Natl Acad Sci USA* 109: 8259–8263.
- DAS, A., RANGARAJ, N. and SONTI, R.V. (2009). Multiple adhesin-like functions of *Xanthomonas oryzae* pv. *oryzae* are involved in promoting leaf attachment, entry, and virulence on rice. *Mol Plant Microbe Interact* 22: 73–85.
- DAVIDSON, C.J. and SURETTE, M.G. (2008). Individuality in bacteria. *Ann Rev Microbiol* 42: 253–268.
- DENG, Y., WU, J., TAO, F. and ZHANG, L.H. (2011). Listening to a new language: DSF-based quorum sensing in gram-negative bacteria. *Chem Rev* 111: 160–173.
- DIGGLE, S., GRIFFIN, A., CAMPBELL, G. and WEST, S. (2007). Cooperation and conflict in quorum-sensing bacterial populations. *Nature* 450: 411–414.
- ENGBRECHT, J., NEALSON, K. and SILVERMAN, M. (1983). Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* 32: 773–781.
- FLEMMING, H.-C., NEU, T.R. and WOZNIK, D.J. (2007). The EPS matrix: the “House of biofilm cells”. *J Bacteriol* 189: 7945–7947.
- FUQUA, W.C., WINANS, S.C. and GREENBERG, E.P. (1994). Quorum sensing in bacteria: The LuxR-LuxI family of cell density-responsive transcriptional regulators. *J Bacteriol* 176:269–275.
- FUQUA, C., PARSEK, M.R. and GREENBERG, E.P. (2001). Regulation of gene expression by cell-cell communication: Acyl-homoserine lactone quorum sensing. *Annu Rev Genet* 35: 4439–4468.
- GARDNER, A., WEST, S.A. and GRIFFIN, A.S. (2007). Is Bacterial Persistence a Social Trait? *PLoS ONE* 2: e752. doi:10.1371/journal.pone.0000752.
- GOTTIG, N., GARAVAGLIA, B.S., GAROFALO, C.G., ORELLANO, E.G. and OTTADO, J. (2009). A filamentous hemagglutinin-like protein of *Xanthomonas axonopodis* pv. *citri*, the pathogen responsible for citrus canker, is involved in bacterial virulence. *PLoS ONE* 4: e4358.
- GREENBERG, E.P. (1998). The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 280: 295–298.
- HE Y.W. and ZHANG L.H. (2008). Quorum sensing and virulence regulation in *Xanthomonas campestris*. *FEMS Microbiol Rev* 32: 842–857.
- JACOB, E.B. and SCHULTZ, D. (2010). Bacteria determine fate by playing dice with controlled odds. *Proc Natl Acad Sci USA* 107: 13197–13198.
- JAVVADI, S., PANDEY S.S., MISHRA, A., PRADHAN, B.B. and CHATTERJEE, S. (2018). Bacterial cyclic  $\beta$ -(1,2)-glucans sequester iron to protect against iron-induced toxicity. *EMBO Rep* 19: 172–186.
- LONG, T., TU, L.C., WANG, Y., MEHTA, P., ONG, N.P., BASSLER, B.L. and WINGREEN, N.S. (2009). Quantifying the integration of quorum-sensing signals with single-cell resolution. *PLoS Biol* 7: e1000068.
- KAKKAR, A., NIZAMPATNAM, N.R., KONDRÉDDY A., PRADHAN, B.B. and CHATTERJEE, S. (2015). *Xanthomonas campestris* cell-cell signaling molecule DSF (diffusible signal factor) elicits innate immunity in plants and is suppressed by the exopolysaccharide xanthan. *J Exp Bot* 66: 6697–6714.
- LÓPEZ, D., VLAMAKIS, H. and KOLTER, R. (2010). Biofilms. *Cold Spring Harb Perspect Biol* 2: a000398.
- MANSFIELD, J., GENIN, S., MAGORI, S., CITOVSKY, V., SRIARIYANUM, M., RONALD, P., DOW, M., VERDIER, V., BEER, S.V., MACHADO, M.A., TOTH, I., SALMOND, G. and FOSTER, G.D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol Plant Pathol* 13: 614–629.
- MILLER, M.B. and BASSLER, B.L. (2001). “Quorum sensing in bacteria”. *Annu Rev of Microbiol* 55: 165–199.
- NADELL, C.D., XAVIER, J.B. and FOSTER, K.R. (2009). The sociobiology of biofilms. *FEMS Microbiol Rev* 33: 206–224.

- NEALSON, K.H., PLATT, T. and HASTINGS, J.W. (1970). Cellular control of the synthesis and activity of the bacterial luminescent system. *J Bacteriol* 104: 313–322.
- NEALSON, K.H. (1977). Autoinduction of bacterial luciferase. Occurrence, mechanism and significance. *Arch.Microbiol* 112: 73–79.
- NG, W.L. and BASSLER, B.L. (2009). Bacterial quorum-sensing network architectures. *Ann Rev Genet* 43:197–222.
- NIÑO-LIU, D.O., RONALD, P.C. and BOGDANOVA, A.J. (2006). *Xanthomonas oryzae* pathovars: model pathogens of a model crop. *Mol Plant Pathol* 7: 303-324.
- NOVICK, A. and WEINER, M. (1957). Enzyme induction as an all-or-none phenomenon. *Proc Natl Acad Sci USA* 43: 553-566.
- O'TOOLE, G., KAPLAN, H.B. and KOLTER, R. (2000). Biofilm formation as microbial development. *Annu Rev Microbiol* 54: 49-79.
- PAI, A., TANOUCI, Y. and YOU, L. (2012). Optimally and robustness in quorum sensing (QS)-mediated regulation of a costly public good enzyme. *Proc Natl Acad Sci USA* 109: 19810-19815.
- PALKOVÁ, Z. (2004). Multicellular microorganisms: laboratory versus nature. *EMBO Rep* 5: 470-476.
- PANDEY, S.S., PATNANA, P.K., LOMADA, S.K., TOMAR, A. and CHATTERJEE, S. (2016). Co-regulation of Iron Metabolism and Virulence Associated Functions by Iron and XibR, a Novel Iron Binding Transcription Factor, in the Plant Pathogen *Xanthomonas*. *PLoS Pathog* 12: e1006019. DOI: 10.1371/journal.ppat.1006019.
- PANDEY, S.S., PATNANA, P.K., RAI, R. and CHATTERJEE, S. (2017). Xanthoferrin, the  $\alpha$ -hydroxy carboxylate type siderophore of *Xanthomonas campestris* pv. *campestris* is required for optimum virulence and growth inside cabbage. *Mol Plant Pathol* 18: 949-962. DOI: 10.1111/mpp.12451.
- PANDEY, S.S., PATNANA, P.K., PADHI, Y. and CHATTERJEE, S. (2018). Low-iron conditions induces the hypersensitive reaction and pathogenicity *hrr* genes expression in *Xanthomonas* and is involved in modulation of hypersensitive response and virulence. *Env Microbiol Rep* 10: 522-531.
- PARSEK, M.R. and GREENBERG, E.P. (2005). Sociomicrobiology: The connections between quorum sensing and biofilms. *Trends Microbiol* 13: 27–33.
- PRADHAN, B.B., RANJAN, M. and CHATTERJEE, S. (2012). XadM, a novel adhesin of *Xanthomonas oryzae* pv. *oryzae*, exhibits similarity to Rh family proteins and is required for optimum attachment, biofilm formation and virulence. *Mol Plant-Microbe Interact* 25: 1157-1170.
- PRADHAN, B.B. and CHATTERJEE, S. (2014). Reversible non-genetic phenotypic heterogeneity in bacterial quorum sensing. *Mol Microbiol* 92: 557-569.
- RAI, R., PRADHAN, B.B., RANJAN, M. and CHATTERJEE, S. (2012). Atypical regulation of virulence associated functions by a Diffusible Signal Factor in *Xanthomonas oryzae* pv. *oryzae*. *Mol Plant-Microbe Interact* 25: 789-801.
- RAI, R., JAVVADI, S. and CHATTERJEE, S. (2015). Cell–cell signaling promotes ferric iron uptake in *Xanthomonas oryzae* pv. *oryzicola* that contribute to its virulence and growth inside rice. *Mol Microbiol* 96: 708-727.
- RAY, S.K., RAJESWARI, R., SHARMA, Y. and SONTI, R.V. (2002). A high molecular weight outer membrane protein of *Xanthomonas oryzae* pv. *Oryzae* exhibits similarity to non-fimbrial adhesins of animal pathogenic bacteria and is required for optimum virulence. *Mol Microbiol* 46: 637-647.
- RYAN, R.P. and DOW, J.M. (2011). Communication with a growing family: diffusible signal factor (DSF) signaling in bacteria. *Trends in Microbiol* 19: 145-152.
- SAMAL, B. and CHATTERJEE, S. (2019). New insight into bacterial social communication in natural host: Evidence for interplay of heterogeneous and unison quorum response. *PLoS Genet* 15: E1008395.
- SANDOZ, K.M., MITZIMBERG, S.M. and SCHUSTER, M. (2007). Social cheating in *Pseudomonas aeruginosa* quorum sensing. *Proc Natl Acad Sci USA* 104: 15876-15881.
- SPUDICH J.L. and KOSHLAND, D.E.JR. (1976). Non-genetic individuality: chance in the single cell. *Nature* 262: 467–471.
- SÚEL, G.M., GARCIA-OJALVO, J., LIBERMAN, L.M. and ELOWITZ, M.B. (2006). An excitable gene regulatory circuit induces transient cellular differentiation. *Nature* 440: 545–550.
- TUROVSKIY, Y., KASHTANOV, D., PASKHOVER, B. and CHIKINDAS, M.L. (2007). Quorum sensing: fact, fiction, and everything in between. *Adv Appl Microbiol* 62:191-234.
- VERMA, R.K., SAMAL, B. and CHATTERJEE, S. (2018). *Xanthomonas oryzae* pv. *oryzae* chemotaxis components and chemoreceptor Mcp2 is involved in sensing constituent of xylem sap and contribute to regulation of virulence associated functions and entry into rice. *Mol Plant Pathol* 19: 2397-2415.
- WILLIAMS, P., WINZER, K., CHAN, W.C. and 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.
- XAVIER, J.B. and FOSTER, K.R (2007). Cooperation and conflict in microbial biofilms. *Proc Natl Acad Sci USA* 104: 876–881.

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