Antonie van Leeuwenhoek (2014) 105:289–305
DOI 10.1007/s10482-013-0082-3

REVIEW PAPER

Bacterial quorum sensing: circuits and applications

Neera Garg · Geetanjali Manchanda · Aditya Kumar

Received: 19 August 2013 / Accepted: 16 November 2013 / Published online: 27 November 2013
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Abstract Bacterial quorum sensing (QS) systems are cell density—dependent regulatory networks that coordinate bacterial behavioural changes from single cellular organisms at low cell densities to multicellular types when their population density reaches a threshold level. At this stage, bacteria produce and perceive small diffusible signal molecules, termed autoinducers in order to mediate gene expression. This often results in phenotypic shifts, like planktonic to biofilm or non-virulent to virulent. In this way, they regulate varied physiological processes by adjusting gene expression in concert with their population size. In this review we give a synopsis of QS mediated cell–cell communication in bacteria. The first part focuses on QS circuits of some Gram-negative and Gram-positive bacteria. Thereafter, attention is drawn on the recent applications of QS in development of synthetic biology modules, for studying the principles of pattern formation, engineering bi-directional communication system and building artificial communication networks. Further, the role of QS in solving the problem of biofouling is also discussed.

N. Garg (✉) · G. Manchanda
Department of Botany, Panjab University,
Chandigarh 160014, India
e-mail: garg_neera@yahoo.com

A. Kumar
Government Medical College and Hospital,
Chandigarh, India

Keywords Autoinducers · Bacteria · Biofouling · Quorum sensing · Synthetic biology

Introduction

Bacteria are small unicellular prokaryotic organisms that neither have membrane bound nucleus nor membrane enclosed organelles, yet, they have the ability to communicate with one another using different chemical languages. Bacteria release, detect and respond to the accumulation of self generated chemical signaling molecules called autoinducers (AIs), whose concentration correlates to the abundance of secreting microorganisms in the vicinity. When the signal concentration reaches a threshold, the communicating microorganisms undertake a coordinated change in their gene-expression profiles (Turovskiy et al. 2007; Jayaraman and Wood 2008; Popat et al. 2008). The regulated gene expression enables them to act in unison as enormous multicellular organisms. This chemical communication, called as 'quorum sensing' (QS) is defined as a density dependent mechanism by which bacteria coordinate expression of specific target genes in response to a critical concentration of signal molecules (Pappas et al. 2004; Novick and Geisinger 2008; Antunes and Ferreira 2009; Ng and Bassler 2009; Williams and Camara 2009; Antunes et al. 2010; Ng et al. 2011). With this mechanism, they initiate complex activities such as

control of secondary metabolism, root nodulation, bioluminescence, protein secretion, motility, virulence factor production, plasmid transfer, and biofilm maturation in diverse bacteria (Fuqua and Greenberg 2002; Bassler and Losick 2006; Williams 2007). QS plays a critical role in both pathogenic and symbiotic bacteria-host interactions. QS is beneficial in pathogens, because virulence factors are released and coordinated attack on the host made only when the bacterial population reaches a high density. With this coordinated activity, bacteria besiege the host defenses and enhance their survival prospects. Similarly for symbiotic bacteria, QS allows bacteria to synchronize important cellular responses with the host (e.g. bioluminescence and root nodulation), that facilitates their mutual existence (Thoendel and Horswill 2010). Moreover, it has been proposed that AI molecules allow cells to assess whether the production of exofactors will be directly beneficial to the cell that produces them, in response to the rate of diffusion in the surrounding environment. This idea, termed as diffusion sensing suggests that QS need not be a social behavior but could rather be a nonsocial trait (Redfield 2002; Hense et al. 2007; Venturi and Subramoni 2009; West et al. 2012). Gram negative and Gram positive bacteria have different QS systems, with LuxIR circuits in Gram-negative bacteria and oligopeptide two-component circuits in Gram-positive bacteria. Gram-negative bacteria synthesize primarily *N*-acylhomoserine lactones (AHLs), whereas Gram-positive bacteria most commonly use modified peptides as signal molecules. Both Gram-positive and Gram-negative bacteria produce AI-2 family molecules which are derived from the precursor 4,5-dihydroxy-2,3-pentanedione (DPD) produced by the LuxS synthase (Ryan and Dow 2008).

Some functions controlled by LuxIR-type QS include the production of antibiotics in *Erwinia carotovora*, virulence gene expression and biofilm formation in *Pseudomonas aeruginosa* and expression of factors necessary for symbiosis in *Sinorhizobium meliloti* (de Kievit and Iglewski 2000). Notable examples of behaviors controlled by oligopeptide two-component circuits in Gram-positive bacteria include regulation of the expression of virulence and other accessory genes in staphylococci and regulation of sporulation and genetic competence in *Bacillus subtilis* regulated with a series of linear peptides (Thoendel et al. 2011).

QS circuits

Gram negative proteobacteria (α, β and γ subdivisions) use acyl-HSL communication system to control specific genes in response to population density. QS in these bacteria is an RI-sensory system consisting of I-genes (homologs of *luxI*), encoding a signal synthase protein and R-genes (homologs of *luxR*), encoding a LuxR-like protein, which interacts with its specific signal molecule. AHL is the most common class of AI used by Gram-negative bacteria (Wei et al. 2006). LuxI-type AHL synthases catalyze the formation of a specific AHL from the substrates *S*-adenosyl-L-methionine (SAM), an intermediate of the methione/homecysteine pathway and acyl-acyl carrier protein (acyl-ACP), generated as an intermediate in fatty-acid biosynthesis (Marketon et al. 2002; Dessaux et al. 2011). The amphipathic character of AHL molecules, due to the presence of hydrophobic side chain and the hydrophilic HSL ring, allows the AHLs to traverse the phospholipid bilayer of cell membrane and navigate the aqueous intracellular and extracellular environments (Fuqua et al. 2001). As the cell population density increases, AHL autoinducer concentration also increases. When the concentration of AHL eventually reaches a sufficiently high concentration at a given threshold cell number or bacterial quorum, several DNA-binding transcription factors called R proteins, such as LuxR or LasR recognize and bind specific AHL autoinducers. The LuxR/AHL complex activates or represses multiple target genes, including those coding for the AHL synthase (usually a member of the LuxI protein family), giving rise to a positive auto induction circuit (Whitehead et al. 2001; Zhang et al. 2002; Urbanowski et al. 2004; Bjarnsholt and Givskov 2007).

QS was first described in a study carried out in the early 1970s on bioluminescence phenomenon found in *Vibrio fischeri*, a marine bacterium associated with Hawaiian squid (Nealson et al. 1970). Bioluminescence in *V. fischeri* is generated by the *lux* operon *luxICDABEG*, which encodes bacterial luciferase enzyme as well as the enzymes for production of luciferase substrate, and is regulated by QS channels (Pérez and Hagen 2010; Pérez-Montaño et al. 2011). QS system of *V. fischeri* employs *N*-3-oxo-hexanoyl homoserine lactone (3-oxo-C6-HSL) as a QS signaling molecule. This bioluminescence gene cluster of *V. fischeri* consists of eight lux genes (*luxA-E*, *luxG*, *luxI*

and *luxR*) which are arranged in two bi-directionally transcribed operons. LuxI catalyzes acylation and lactonization reactions between the substrates SAM and hexanoyl-ACP to synthesize 3-oxo-C6-HSL, 5′-methylthioadenosine and apo-ACP (Miyashiro and Ruby 2012). Following synthesis, 3-oxo-C6-HSL diffuses freely in and out of the cell, and its concentration increases as the cell density of the population increases. When 3-oxo-C6-HSL accumulates to sufficiently high concentration, it is bound by a ~25 kDa cytoplasmic receptor LuxR (Hanzelka and Greenberg 1995; Fuqua and Greenberg 2002). The LuxR/3-oxo-C6-HSL complex acts as transcriptional activator and binds to a 20 bp sequence within the *luxR–luxI* intergenic region referred to as the '*lux* box' (Stevens et al. 1994), 42.5 bp upstream of the *luxI* promoter start site and activates expression of *luxICDABE* operon. The *luxA* and *luxB* genes encode the α and β subunits of the heterodimeric luciferase enzyme, which catalyses the oxidation of reduced flavomononucleotide (FMNH2), long chain aliphatic aldehyde and oxygen. Products of the reaction are oxidized flavomononucleotide (FMN), aliphatic acid and water. Simultaneous liberation of excess free energy, evident as blue-green light, results in the phenotype (Lupp et al. 2003; Nijvipakul et al. 2008).

Since the discovery of AHL-mediated QS in *V. fischeri*, parallel systems employing homologues of the LuxI and LuxR regulatory proteins have been identified in a number of Gram-negative bacteria (Lerat and Moran 2004). Rhizobia, as an example, use QS regulation of gene expression to coordinate their behaviour in ways that enhance and spread their symbiotic capacity (Downie 2010). Quorum communication via AHLs in rhizobia affects various aspects of the legume symbiosis, including motility, exopolysaccharide synthesis (important for infection, attachment and biofilm formation), symbiosome development, biofilm formation, symbiotic plasmid transfer, root nodulation efficiency, and nitrogen fixing efficiency (Hoang et al. 2004; Laus et al. 2005; Glenn et al. 2007; Downie and Gonzalez 2008; Edwards et al. 2009; Frederix et al. 2011; Janczarek 2011; Nievas et al. 2012). *Rhizobium* species produce a wide variety of AHLs. Several AHL synthesis and regulator genes have been described and vary from four in *Rhizobium leguminosarum* bv. viciae (*rhiR/rhiI*, *raiR/raiI*, *traR/traI*, and *cinR/cinI*) to two in *Rhizobium etli* CNPAF512 (*cinR/cinI* and *raiR/raiI*) and one (*traR/traI*) in *R. etli* CFN42. *N*-Octanoyl homoserine lactone (C8-HSL) and its 3-oxo and 3-hydroxyl derivatives (3-oxo-C8-HSL and 3-OH-C8-HSL, respectively) have been found in most of these strains (González and Marketon 2003). The control of production of exoenzyme virulence factors and a carbapenem antibiotic in the plant pathogen *E. carotovora* subspecies *carotovora* is also achieved via the 3-oxo-C6-HSL signalling molecule (Jones et al. 1993; Pirhonen et al. 1993). The 3-oxo-C6-HSL signal is synthesized by the CarI protein and the cognate LuxR homologue required for carbapenem production is called CarR (Jones et al. 1993; McGowan et al. 1996). The CarR/3-oxo-C6-HSL complex binds to DNA and activates expression of the *car* biosynthetic genes to bring about production of the carbapenem antibiotic at high cell densities (Welch et al. 2000).

Another complex QS system is seen in *P. aeruginosa*. It has adapted different systems (AHL synthesis and regulator genes, signaling molecules) to regulate gene expression: *lasI/lasR*, *rhlI/rhlR*; 3-oxo-C12-AHL, C4-AHL in *P. aeruginosa*; *ahlI/ahlR*; 3-oxo-C6-AHL in *P. syringae*; *phzI/phzR*, *csaI/csaR*; C6-AHL in *P. aureofaciens*; *phzI/phzR*; C6-AHL in *P. chlororaphis*; *ppuI/ppuR*; 3-oxo-C12-AHL in *P. putida*; *mpuI/mpuR*; long acyl-chain-AHL in *P. fluorescens*; *hdtS*; 3-OH-C14:1-AHL in *P. fluorescens* (Smith and Iglewski 2003; Wagner et al. 2003; Juhas et al. 2005; Chin et al. 2001, 2005; Bertani and Venturi 2004; Steidle et al. 2002; Venturi 2006).The *P. aeruginosa* QS systems control the expression of extracellular virulence factors as well as biofilm formation. secrete virulence factors such as secondary metabolites (pyocyanin, hydrogen cyanide, pyoverdin, etc.), toxins (exotoxin A), exoenzymes [elastase, proteases (delivered by a type II secretion system; T2SS), type IV pili (Tfp), flagella and a type III secretion system (T3SS) that can inject a set of eukaryote specific effectors across the plasma membrane of target cells (McKnight et al. 2000; Sadikot et al. 2005; Winstanley and Fothergill 2009; Coggan and Wolfgang 2012). In addition to AHLs, *P. aeruginosa* produces a third signalling molecule called PQS (*Pseudomonas* quinolone signal; 2-heptyl-3-hydroxy-4(1H)-quinolone) (Diggle et al. 2007). Like the AHLs, PQS regulates the production of virulence determinants including elastase, rhamnolipids, the galactophilic lectin, LecA, and pyocyanin (a blue-green phenazine pigment) and influences biofilm

development (Diggle et al. 2003; Deziel et al. 2005; Hodgkinson et al. 2010).

While AHL signaling has been found exclusively in Gram-negative bacterial species, many Gram-positive species have been shown to utilize peptides as AIs (autoinducing polypeptides; AIPs) for QS. These small peptides, which are usually the products of cleaved/modified oligopeptides, interact with two component systems—a membrane-bound histidine kinase receptor and a cognate cytoplasmic response regulator; and ultimately regulate the gene transcription (Taga and Bassler 2003; Waters and Bassler 2005). At threshold concentrations, the peptides are recognized by sensor kinases that initiate phosphotransfer to a response regulator. The peptides involved in Gram-positive QS are often specific for their cognate receptors (Reading and Sperandio 2006). One major difference between LuxIR-based and oligopeptide-based QS systems is the location of the cognate receptors; whereas the LuxR-type receptors are cytoplasmic, the sensors for oligopeptide AIs in Gram-positive bacteria are membrane-bound. The membrane-bound receptors transduce information via a series of phosphorylation events. A typical two-component system consists of a membrane-bound histidine kinase receptor and a cognate cytoplasmic response regulator, which functions as a transcriptional regulator (Mascher et al. 2006; Myszka and Czaczyk 2012). As in AHL QS systems, the concentration of secreted oligopeptide AI increases as the cell density increases. At threshold concentrations, the peptides are recognized by sensor kinases that initiate phospho-transfer to a response regulator. Peptide binding to the membrane-bound histidine kinase receptor stimulates its intrinsic autophosphorylation activity, resulting in ATP driven phosphorylation of a conserved histidine residue in the cytoplasm. The phosphate group is subsequently transferred to the conserved aspartate residue of a cognate response regulator. Phosphorylated response regulators are active and they function as DNA binding transcription factors to modulate expression of target genes. In many cases, the genes encoding the oligopeptide AI precursor, the histidine kinase receptor, and the response regulator form an operon, and its expression is auto-induced by QS.

Staphylococcus aureus, a commensal of humans and other mammals, is an opportunistic pathogen capable of causing nosocomial infections worldwide and is the etiologic agent of a wide range of diseases, from relatively benign skin infections to potentially fatal systemic disorders. The virulence of this organism is due to the secretion of diverse arsenal of invasive virulence factors, including hemolysins, superantigens, and tissue-degrading enzymes, which all contribute to pathogenesis. QS of this organism is one of the most studied systems on Gram-positive organisms. Staphylococci use a key two-component system, encoded in the QS *agr* (accessory gene regulator) locus to coordinate population density with the expression of a large set of accessory protein genes, many of which are involved in pathogenesis (Dunman et al. 2001; Novick and Geisinger 2008). *agr* system regulates over 70 genes, 23 of which are known virulence factors (George and Muir 2007). The *agr* locus consists of two divergent transcribed operons, RNAII and RNAIII controlled by the P2 and P3 promoters. P2 drives transcription of a four-gene operon (*agrBDCA*) (Novick et al. 1995; Roux et al. 2009), whose products are involved in the production (agrBD) and sensing (agrCA-AgrC is the signal transducer; AgrA the response regulator) of the AIP.

Bacillus subtilis, under stressful environmental conditions undergoes stochastic switching, in which it goes from the vegetative state to a competent state. In this physiological state, it has an increased ability to bind and take up high molecular weight exogenous DNA (transformation) to increase its chances of survival. It expresses a set of proteins involved in the uptake and integration of extracellular DNA. The expression of these proteins in the competent state is tightly controlled and initially regulated by a QS system. The ComX–ComP–ComA signalling pathway is a major quorum response pathway in *B. subtilis* and regulates the development of genetic competence. This QS system in *B. subtilis* is composed of the ComX pheromone and the two-component regulators ComP and ComA (Lazazzera and Grossman 1998; Dubnau and Lovett 2002).

Applications

Synthetic biology

Synthetic biology aims at the synthesis of complex, biologically based systems, which display functions non-existent in nature, through engineering of genetic

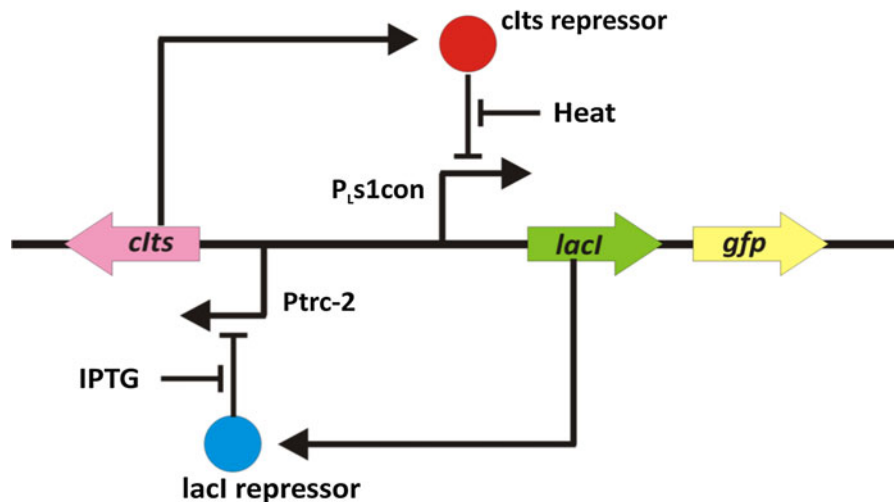

Fig. 1 Toggle switch-transcriptional gene circuits. As constructed by Gardner et al. (2000), the P_Ls1con promoter was used to drive transcription of *lacI*, the product of which represses a second promoter, Ptrc2 (a *lac* promoter variant). Conversely, Ptrc2 drives expression of a gene (*cI-ts*) encoding the temperature-sensitive CIts repressor protein, which inhibits the P_L promoter. Activity of circuit is monitored through a third gene expressing GFP, that is under the control of one of the two repressors. "Switch" functionality is conferred to the system with exogenous addition of the chemical inducer isopropyl-β-D-thiogalactopyranoside (IPTG) or with a transient increase in temperature

elements and the integration of basic elements into complex functional circuits (Endy 2005). Cell–cell communication represents a valuable mechanism to engineer novel signaling constructs in bacteria. The genetic elements of *V. fischeri* QS have been successfully used to engineer several cell–cell communication systems. This system, as discussed in the previous sections consists of sender cells producing AHL molecules, which, after diffusing through the bacterial membranes, reach receiver cells where they bind the LuxR transcription activator and stimulate bioluminescence. Gardner et al. (2000), with their construction and characterization of a transcriptional toggle switch, pioneered work in this field (Fig. 1). They constructed a synthetic, bistable gene-regulatory network in *Escherichia coli*, which composed of two repressors and two constitutive promoters. Each promoter is inhibited by a repressor that is transcribed by the opposing promoter. The toggle flipped between stable states using transient chemical or thermal induction. As a practical device, the toggle switch forms a synthetic, addressable cellular memory unit and has implications for biotechnology, biocomputing and gene therapy.

Insertion of QS in *E. coli* was the key to engineer a "pulse generating network" (Basu et al. 2004). Their system included sender cells that could be induced to synthesize AHL, which diffused to nearby pulse-generating receiver cells. Receiver cells responded to this long-lasting increase in the level of AHL by transiently activating, and then repressing, the expression of a GFP. The receiver circuit contained a feed-forward motif that in response to a stimulus exhibited an initial excitation followed by subsequent delayed inhibition. The circuit differentiated between various rates of increase in stimulus levels, enabling a spatiotemporal behavior. A step increase in concentration of the signaling molecule resulted in a pulse response with an amplitude that depended on the concentration of signal. The engineered bacteria could sense the time derivative of the signal concentration. As a consequence, receiver cells near the sender cells responded to the communication signal, whereas receiver cells that were further away ignored this signal. Basu et al. (2005) showed a synthetic multicellular system in which genetically engineered 'receiver' cells were programmed to form ring-like patterns by expressing reporter proteins of different colors in concomitance of diverse AHL concentrations (Fig. 2). The band-detect multicellular system programs *E. coli* receiver cells to fluoresce only at intermediate distances from sender cells.

Significant results were achieved in *E. coli*, by You et al. (2004). They built and characterized a 'population control' circuit that autonomously regulates the

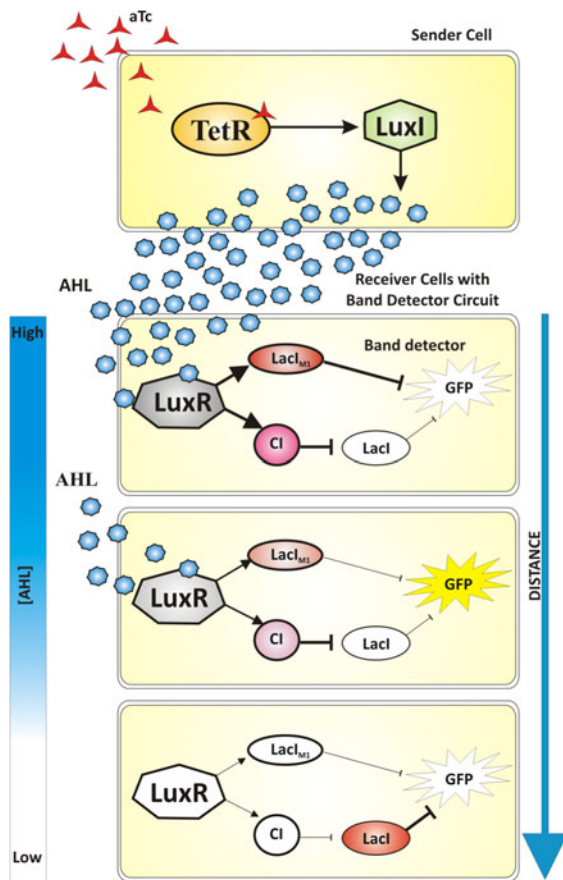

Fig. 2 Band detect network design and activity as a function of distance from a sender cell. Only receivers at intermediate distances from senders express the output protein. aTc initiates production of LuxI in the sender cells by binding the tetracycline repressor (TetR) and disabling it from repressing the tetracycline promoter. LuxI catalyses the synthesis of AHL, which diffuses through the cell membrane and forms a chemical gradient around the senders. In receivers, that are at close proximity to senders, AHL concentration is high. AHL binds to its cognate R protein, LuxR, that activates the expression of lambda repressor (CI) and Lac repressor (LacI, a product of a codon-modified *lacI*), which represses expression of GFP. At medium AHL concentrations, the cells produce moderate levels of CI and LacI, and while CI levels are sufficiently high to repress LacI expression the weaker LacI repressor does not inhibit expression of GFP. At low AHL levels, LacI and CI are not expressed and expression of a wild-type LacI, results in GFP repression

density of an *E. coli* population. They coupled QS with the synthesis of toxic proteins and obtained in this way a cell population control mechanism. The cell density is broadcasted and detected by elements from a bacterial QS system, which in turn regulate the death rate. At sufficiently high concentrations, AHL binds and activates the LuxR transcriptional regulator, which in turn induces the expression of a killer gene (E) under the control of a *luxI* promoter (p_{luxI}). One possible application of this population-based gene expression system is in the creation of cost-effective long-term fermentation processes for the self-regulated production and subsequent harvesting of exogenous cytotoxic proteins. Rasmussen et al. (2005) constructed a collection of screening systems, QS inhibitor (QSI) selectors, which enabled them to identify a number of novel QSIs among natural and synthetic compound libraries. An extension of these works on cell–cell communication is the generation of artificial ecosystems, where one cell produces molecules that the other needs. Bulter et al. (2004), developed artificial QS system to establish a gene-metabolic network where bacterial cells exchange acetate molecules that are produced in proportion to cell growth. This circuit uses a threshold concentration of acetate to induce gene expression by acetate kinase and part of the nitrogen-regulation two-component system. Anderson et al. (2006) engineered the interaction between bacteria and cancer cells. They constructed a synthetic gene network that enabled *E. coli* cells to invade cancer-derived cells only when tumor-indicative conditions were present. They characterized invasin (*inv*) gene from *Yersinia pseudotuberculosis* as an output module that enables *E. coli* to invade cancer-derived cells, including HeLa, HepG2, and U2OS lines. They constructed different plasmids containing the *inv* gene under QS control of the *lux* promoter, the hypoxia-responsive *fdhF* promoter, and the arabinose-inducible *araBAD* promoter. *E. coli* harboring these plasmids invaded cancer-derived cells in a density-dependent fashion, under anaerobic growth conditions, and upon arabinose induction, respectively. Thus, the *inv* expression was activated only at high cell density or during hypoxic growth conditions, which are the environmental conditions associated with the accumulation of bacteria at a tumor site and the high respiratory rate of cancerous cells. This approach could be used to engineer bacteria to sense the microenvironment of a tumor and respond by invading cancerous cells and releasing a cytotoxic agent.

In another system, Kobayashi et al. (2004) interfaced a toggle switch with a QS signaling pathway (Fig. 3). They constructed an engineered gene network containing three different plasmids: (i) a sensor

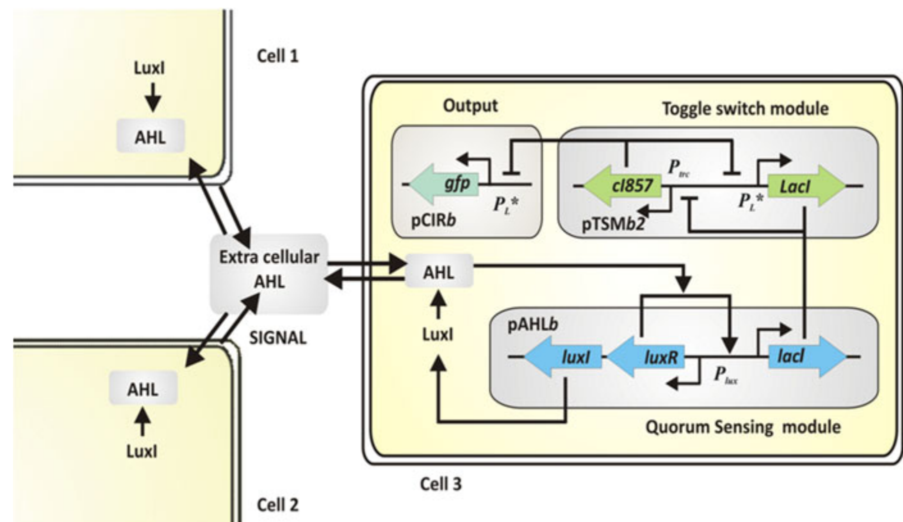

Fig. 3 Toggle switch with a quorum-sensing signaling pathway

plasmid (pAHL*b*; to enable the *E. coli* population to measure its own density through AHL), where the *luxI* gene from *V. fischeri* expressed polycistronically with the *luxR* gene and *lacI* expressed from the *P* promoter; (ii) the toggle switch plasmid (pTSM*b2*), for the expression of LacR and CI; and an output plasmid (pCIR*b*), with the reporter gene *gfp*. The signal molecule AHL diffuses between the culture and cells, and its extracellular concentration correlates with the cell density in cultures of cells that carry the *luxI* gene. AHL regulates the transcription of *lacI* in the sensor plasmid by formation of the AHL–LuxR dimer. As a result, *lacI* expression from the pAHL*b* plasmid is increased, when the cell density increases. The expression of LacR in the toggle switch plasmid is negatively regulated by CI, whereas the expression of CI is negatively regulated by the total LacR expressed from the sensor plasmid and the toggle switch plasmid. Because of the modular design of their system, density dependent synthesis of any protein can be achieved simply by replacing the *gfp* gene on the high-copy number reporter plasmid with a gene of interest.

Brenner et al. (2007) established a coordinated behavior between two bacterial populations (consortium) communicating via AHL. Two colocalized populations of *E. coli* conversed bidirectionally by exchanging AHLs. The consortium generates the gene-expression response if and only if both populations are present at sufficient cell densities. In this case, a particular gene is expressed only once both populations have reached a given density, which implies an AND gate with population concentrations as inputs. The microbial consensus consortium operates in diverse growth modes, including in a biofilm, where it sustains its response for several days. An engineered biofilm consortium might eliminate unwanted infection or even destroy harmful cells in the body. Furthermore, a synthetic predator–prey ecosystem was engineered in *E. coli* cells by using two different signals (Balagadde et al. 2008). Here, the predator cells emit a chemical that kills the prey by inducing the synthesis of a toxic protein; the prey, on the contrary, rescue predator cells by sending them the substance necessary to repress the production of the corresponding killer protein. Depending on the growth/death rate of the two groups, one of them will dominate or their concentrations will give rise to oscillations. Danino et al. (2010) described an engineered gene network with intercellular coupling that is capable of generating synchronized oscillations in a growing population of cells. The synchronized oscillator design is based on elements of the QS machineries in *V. fischeri* and *B. thurigensis*. They placed the *luxI* (from *V. fischeri*), *aiiA* (from *B. thurigensis*) and *yemGFP* genes under the control of three identical copies of the *luxI* promoter. By introducing AiiA, which degrades AHL, under the control of LuxR, cells were produced that responded to a quorum by destroying the QS signal. With the concept of QS in mind, Saeidi et al. (2011) genetically engineered nonpathogenic *E. coli* to first detect *P. aeruginosa* using its QS molecules and then releasing a protein toxin to kill the pathogen. They described the design and construction of a synthetic genetic system, in a

nonpathogenic chassis, *E. coli* which comprised QS, killing, and lysing devices, which enables *E. coli* to sense and kill a pathogenic *P. aeruginosa* strain through the production and release of pyocin. The sensing device was designed based on the Type I QS mechanism of *P. aeruginosa*. The *tetR* promoter, which is constitutively on, produces a transcriptional factor, LasR, that binds to AHL 3OC12HSL. The *luxR* promoter, to which LasR-3OC12HSL activator complex reportedly binds, was adopted as the inducible promoter in their sensing device (Gray et al. 1994). Next, the formation of the LasR-3OC12HSL complex, which binds to the luxR promoter, activates the killing and lysing devices, leading to the production of pyocin S5 and lysis E7 proteins within the *E. coli* chassis. Upon reaching a threshold concentration, the lysis E7 protein perforates membrane of the *E. coli* host and releases the accumulated pyocin S5. Pyocin S5, which is a soluble protein, then diffuses toward the target pathogen and damages its cellular integrity, thereby killing it.

Preventing biofouling

Natural and artificial substrata in the marine environment are colonized by micro- and macro-organisms in a process known as biofouling. Biofouling poses one of the most serious problems for aquaculture development, marine industries and navies around the world (Yebra et al. 2004). In the marine environment, natural and artificial surfaces immersed in seawater are colonized by biofoulers including micro-foulers such as marine bacteria, algae, and protozoa, and macro-foulers such as barnacles, bryozoans, and tubeworms (Callow and Callow 2002; Dobretsov et al. 2006). Although biofouling is primarily caused by marine invertebrates and plants, bacterial biofilms are believed to be the first colonizers of submerged surfaces, to which other marine organisms may attach (Rice et al. 1999). The process of biofouling occurs by both physical (reversible adsorption) and biochemical (irreversible adhesion) reactions. The physical reactions are governed by factors such as Brownian motion, van der Waals forces (Katsikogianni and Missirlis 2004), electrostatic interaction and water flow, and lead to formation of the conditioning biofilm composed of organic materials such as protein, polysaccharide, and proteoglycan, on the substrate surface. This step is short (1 min), and provides a stickier surface for microorganisms such as bacteria and microscopic eukaryotes (e.g. diatoms, fungi, and other heterotrophic eukaryotes) to adhere to the surface. Biofilms are three-dimensionally structured communities of microbes whose function depends on complex interactions that occur both within and between species and can be either competitive or cooperative (Stoodley et al. 2002; Li et al. 2012). After the formation and development of biofilm, larvae or spores of macrofoulers (invertebrate larvae and algal spores) (Wright et al. 2004) attach to the surface. Two or three weeks later, these finally evolve into a complex biological community. The biochemical reactions include secretion of extracellular polymeric substances (EPS; mainly composed of glucose- and fructose-based polysaccharide fibrils) (Decho 2000), which bacteria use to adhere temporarily to the surface. The mass of cells in biofilms accounts for only 2–5 % of the total weight with the remainder contributed by the EPS matrix. Diatoms are the most important contributors during biofilm formation in the marine environment (Callow 2000). Because most of the diatoms lack flagella, they are unable to actively approach a given surface, and land passively on the substratum. After the diatoms land on the surface, they actively form the initial reversible attachment called primary adhesion through secretion of EPS. In secondary adhesion, the diatoms reorient themselves and move along the surface into better positions based on their preferences, through diatom gliding. Diatom EPS (Wetherbee et al. 1998) is a multicomponent, mucilaginous, organic bioadhesive complex found exterior to the plasma membrane. In common with the EPS of many bacteria, the major matrix components are acidic polysaccharides that are frequently carboxylated or sulphated (Chiovitti et al. 2003; Molino and Wetherbee 2008; Zargiel et al. 2011). Proteoglycans are also implicated in both adhesion and gliding motility (Wetherbee et al. 1998). motile, quadriflagellate, naked spores (zoospores) of *Ulva* [formerly *Enteromorpha* (Hayden et al. 2003)], are extremely important in biofouling because of their abundance in seawater and adaptability to different environments. The spores settle through a process involving sensing of a surface, and temporary adhesion (Callow et al. 1997; Callow 2000). Barnacle cyprids use antennulae for crawling, attachment and sensory functions to investigate the surface. When an appropriate surface is found, cyprid larvae attaches via o-quinone cross-linking that resembles the dihydroxyphenylalanine (DOPA)-containing adhesive

proteins of *Mytilus* spp. (Wiegemann 2005). This cement embeds the antennular attachment organs and hardens because of protein polymerization. After stable settlement, cyprids metamorphose into juvenile barnacles, and finally become adults (Wiegemann 2005; Kristensen et al. 2008).

Biofoulers that accumulate on the ship hulls increase drag and surface corrosion, thereby severely diminishing ships' maneuverability and carrying capacity (Chambers et al. 2006). In addition, biofouling causes huge material and economic costs in maintenance of mariculture, naval vessels, and seawater pipelines (Yebra et al. 2004). In order to control biofouling, broad-spectrum metal biocides, such as tributyl tin (TBT), copper or organic compounds (e.g. sea-nine, isothiazolone) have been added to marine paints as antifouling compounds (Thomas et al. 2001; Bhadury and Wright 2004). Although very effective, all these chemicals are toxic to a wide range of non-target organisms and pollute the aquatic environment (Alzieu 2000; Konstantinou and Albanis 2004). For example, gastropod imposex, mussel larvae mortality, and oyster shell malformation have all been recorded as ecotoxicological effects of TBT even at extremely low concentrations (in the ng l^{-1} range) (Alzieu 2000). This led the International Maritime Organization (IMO) and Marine Environmental Protection Committee to prohibit the use of toxic organotin tributyltin-based paint product as anti-biofouling agents, effective from 17 September 2008 (IMO 2001; Qian et al. 2010). Alternative biocide-based anti fouling (AF) paints, containing compounds such as Irgarol 1051, diuron, Sea-Nine 211, chlorothalonil, dichlofluanid, and zinc pyrithione are the most frequently used booster biocides worldwide, and some of these have also been found to accumulate in coastal waters at levels that are deleterious for marine organisms (Omae 2003; Konstantinou and Albanis 2004; Bellas 2006; Thomas and Brooks 2010). Accordingly, in the absence of effective alternative to TBT, there is an urgent need for the development of 'environmental friendly' nontoxic antifoulants.

Marine invertebrate larvae utilize biofilms as indicators of substratum suitability for eventual settlement (Railkin 2004). Since the establishment of microbial biofilms is a prerequisite not only for subsequent microbial colonisation but also macrofoulers, such as invertebrate larvae and algal spores, disruption of bacterial biofilms can result in the diminution of macrofouling of submerged surfaces (Burgess et al. 2003; Patel et al. 2003; Dobretsov et al. 2007). Since QS controls bacterial biofilm differentiation and maturation (Sauer et al. 2002; Xavier and Bassler 2003), interruption of QS by inhibition of QS signal generation (Rasmussen and Givskov 2006); degradation of QS signals (Dong et al. 2002; Rajamani et al. 2011; Oh et al. 2012); inhibition of DNA transcription (Zhang and Dong 2004); competition with/suppression of QS receptors (Rasmussen and Givskov 2006) can be ideal treatments for controlling biofilm formation that would eventually lead to biofouling (de Nys et al. 2006; Dobretsov et al. 2007, 2009; Qian et al. 2010).

In the marine environment, many organisms including bacteria, cyanobacteria, algae, sponges, fungi and tunicates (Dobretsov et al. 2011) are endowed with effective defense mechanisms to disrupt bacterial biofilms and thus prevent or control microbial colonization. One of the well-documented strategies is the disruption of bacterial QS by the production of inhibitor compounds (Hentzer et al. 2002; Ni et al. 2009; Ng et al. 2012). The first QS inhibitor was isolated from the red macro-alga *Delisea pulchra* (Givskov et al. 1996). A broad range of secondary metabolites, brominated furanones [(5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanones] produced at the surface of this alga inhibit the growth of Gram-negative bacteria as well as the settlement of invertebrate larvae (Rasmussen et al. 2000; Kjelleberg et al. 2001; Fusetani 2011; Natrah et al. 2011). The structures of these halogenated furanones are similar to AHL, except that furanones have a furan-ring instead of a homoserine lactone ring. The halogenated furanones most probably bind to *LuxR* family proteins without activating them (Manefield et al. 1999; Rasmussen et al. 2000). It has been reported that these furanone-type QS inhibitors have an ability to inhibit some AHL-regulated phenotypes, such as swarming motility, flagella-driven movement, extracellular biosurfactant production, and eventually control microbial attachment and biofilm formation by several bacterial species (Hentzer et al. 2002). Manefield et al. (2000) reported that furanones repressed AHL-dependent expression of *V. fischeri* bioluminescence. Hentzer et al. (2002) applied furanones to *P. aeruginosa* biofilms and observed that furanones penetrated microcolonies and blocked cell signalling and QS in most of the biofilm cells. The compound did not affect initial attachment to

the abiotic substratum, however, it affected the architecture of the biofilm and enhanced the process of bacterial detachment from the substratum. A non-halogenated, commercially available 2(5H)-furanone has been found to inhibit the AHL molecules with varying chain lengths and significantly reduce the biofilm mass of *Aeromonas hydrophila* isolated from a biologically fouled RO membranes on polystyrene surface, which suggested that 2(5H)-furanone could be used as potential QS inhibitor compound that reduce the biofouling (Ponnusamy et al. 2010). Another seaweed belonging to the same family, *Bonnemaisonia hamifera*, also showed antifouling activities (Nylund and Pavia 2005). The seaweed inhibited growth of nine different bacterial strains from five different groups. Macroalga, *Ahnfeltiopsis flabelliformis* produces non-lactone QS inhibitors, such as floridoside, betonicine, and isethionic acid that interfere with bacterial AHL signals by an unknown mechanism (Kim et al. 2007). Brown algae from the family Laminariaceae were reported to produce hypobromous acid, which deactivates 3-oxo-acyl-HSL molecules (Borchardt et al. 2001). Green alga *Chlamydomonas reinhardtii* has been reported to produce a QS inhibitor (lumichrome) that mimics the activity of AHL signal molecules (Teplitski et al. 2004; Rajamani et al. 2008). Jha et al. (2013) tested thirty marine macroalgae for QS inhibition activity by using *Chromobacterium violaceum* CV026 as the reporter strain, and reported that *Asparagopsis taxiformis* showed antibacterial, as well as antiquorum sensing activities. Lyngbyoic acid obtained from a marine cyanobacterium is known to inhibit QS (Kwan et al. 2011). Some of the gorgonian corals from the Caribbean reef showed antibacterial and QS inhibitory effects (Hunt et al. 2012).

Dobretsov et al. (2011) screened 78 different natural products from chemical libraries containing compounds from both marine organisms and terrestrial plants. More than half of the natural products had activity against bacterial QS; 24 % inhibited the QS of *Chromobacterium violaceum* CV017 without any toxicity and 21 % inhibited QS of *C. violaceum* CV017, with some antibiotic activity at higher concentrations. Amongst the natural products isolated and identified by them from fungi, sponges and brown algae, hymenialdisin, dulcitol, kojic acid, midpacamide and tenuazonic acid were responsible for the most marked QS inhibition. Golberg et al. (2013) collected one hundred and twenty bacterial isolates from healthy coral species and screened them for their ability to inhibit QS. Approximately 12, 11, and 24 % of the isolates exhibited anti-QS activity against *Escherichia coli* pSB1075, *Chromobacterium violaceum* CV026, and *Agrobacterium tumefaciens* KYC55 indicator strains, respectively. Their study concluded that coral-associated bacteria are capable of producing compounds which inhibit QS and prevent the formation of biofilm.

Formation of biofilm can be inhibited by enzymatic degradation of the signals involved in biofilm formation (Rajamani et al. 2011; Oh et al. 2012; Dobretsov et al. 2013). Some bacteria can completely degrade AHLs through the combined action of several enzymes (Huang et al. 2003; Uroz et al. 2003, 2005). The AHL-degrading enzymes, are broadly classified into two groups—AHL lactonases, that hydrolyse the lactone ring in AHLs and AHL-acylases that that cleave the *N*-acyl bond of AHLs, found in several different types of bacteria including a *Ralstonia* strain XJ12B, pseudomonads, and a *Streptomyces* species (Dong and Zhang 2005; Chen et al. 2009; Feng et al. 2013). AHL-lactonases mostly fit into two families: Zn-dependent metallo-*b*-lactamases (e.g. the autoinducer inactivating enzyme (AiiA), originally identified and purified from a Gram-positive Bacillus strain (Dong et al. 2000)) and metallo-dependent phosphotriesterases (PTE, e.g. QsdA in *Rhodococcus erythropolis*). Purified AiiA protein cleaves the homoserine lactone ring in C4 to C12-HSLs, with or without substitution at carbon three position. PTE protein can degrade AHLs which have an acyl chain of C6–C14 in length (Uroz et al. 2008). The other family of AHL degradation enzymes is AHL acylase. This was first described in *Variovorax paradoxus* (Leadbetter and Greenberg 2000; Leadbetter 2001). The organism was isolated from soil based upon its ability to utilize 3-oxo-C-HSL as both an energy and nitrogen source. AHL is initially cleaved into a fatty acid and homoserine lactone moiety by an uncharacterized acylase in first reaction step, and subsequently the fatty acid is subjected to beta-oxidation as an energy material, while the homoserine lactone is degraded into ammonium chloride and carbon dioxide. A novel AHL-acylase was identified and isolated from the marine nitrogen-fixing filamentous cyanobacterium *Anabaena* sp. PCC 7120 (Romero et al. 2008). This enzyme, named 'autoinducer inhibitor from cyanobacteria' (AiiC), could be used by the

cyanobacterium to control the response of its own QS signals, but can potentially be used to interfere with signalling within mixed microbial communities. Further research revealed the presence of similar genes in other cyanobacteria, such as *Nostoc punctiforme*, *N. violaceus* and *Synechocystis* sp., which indicate that these enzymes may be widespread among cyanobacteria (Romero et al. 2008). Several naturally occurring bacteria producing AHL-degrading enzymes have been identified as promising agents in reducing biofouling of membranes in wastewater treatment facilities (Oh et al. 2012). Decho et al. (2009) examined the production of AHLs by marine microbial mats (stromatolites) composed mostly of cyanobacteria and sulphate-reducing bacteria and reported that microbial mats produce a wide range of AHLs (from C4- to C14-HSL) under natural conditions. Marine organisms not only respond to bacterial QS signals, but also interfere and block them, presumably to control the growth of alien bacteria, which would lead to biofilm formation and epibiosis (Skindersoe et al. 2008; Dobretsov et al. 2009). Investigations conducted on the Great Barrier Reef showed that 23 % of extracts of 284 marine organisms, including corals, sponges, and algae inhibited bacterial AHL-mediated QS signaling (Skindersoe et al. 2008).

Conclusion and future prospects

Bacteria exist in multifaceted communities and exploit elaborate systems of intercellular communication involving signals, signal sensors, and signal transduction mechanisms to facilitate their adaptation to changing environment. AHL and AIP-dependent gene regulation has received increasing recognition as an important form of cell–cell communication in bacteria. Although this review described a few signaling systems, in detail, it is clear that many more remain to be discovered. Biotechnological research is now focused on the development of molecules that are structurally related to AIs. Such molecules have potential use as antimicrobial drugs aimed at bacteria that use QS to control virulence. Study of bacterial QS systems promises to give biologists new insights into novel mechanisms of intra- and intercellular signal transmission, intra- and inter-species communication and the evolution of multicellular organisms. The majority of synthetic biology is currently practiced in microbes, however, many of the most pressing problems, and in particular those of human health, are inherent problems with mammalian systems. Therefore, a more concerted effort towards advancing mammalian synthetic biology is critical to next-generation therapeutic solutions, including engineering synthetic gene networks for stem cell generation and differentiation. Strategies that disrupt QS systems in pathogenic bacteria or in microorganisms causing fouling, could be a promising alternative for antibiotics or biocides in controlling these undesirable microbial behaviors.

Acknowledgments Authors thankfully acknowledge the financial support provided by Department of Science and Technology, India.

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