Heterologous Expression of Quorum Sensing Inhibitory Genes in Diverse Organisms

Prasun Kumar, Shikha Koul, Sanjay K.S. Patel, Jung-Kul Lee, and Vipin C. Kalia

Abbreviations

AHL	Acyl homoserine lactone
C4-HSL	N-butanoyl-L-HSL
C6-HSL	N-hexanoyl HSL
C8-HSL	N-octanoyl HSL
C10-HSL	N-decanoyl HSL
C12-HSL	N-dodecanoyl HSL
C14-HSL	N-tetradecanoyl-HSL
3OC6-HSL	3-oxo-N-hexanoyl-HSL
3OC8-HSL	3-oxo-N-octanoyl-HSL
3OC10-HSL	3-oxo-N-decanoyl-HSL
3OC12-HSL	3-oxo-N-dodecanoyl- HSL
HSL	Homoserine lactone
QS	Quorum sensing
QSS	Quorum sensing systems
QQ	Quorum quenching

P. Kumar
Microbial Biotechnology and Genomics, CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi 110007, India

S. Koul • V.C. Kalia (✉)
Microbial Biotechnology and Genomics, CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi 110007, India

Academy of Scientific and Innovative Research (AcSIR), 2, Rafi Marg, Anusandhan Bhawan, New Delhi 110001, India
e-mail: vckalia@igib.res.in; vc_kalia@yahoo.co.in

S.K.S. Patel • J.-K. Lee
Department of Chemical Engineering, A1414, Konkuk University, 1 Hwayang-Dong, Gwangjin-Gu, Seoul 143-701, South Korea

Introduction

The discovery of antibiotics was a wonderful solution to provide relief to human beings from infectious diseases. However, indiscriminate usage of antibiotics turned out to be counterproductive. It was observed that patients were not getting cured in spite of the systematic use of antibiotics. In fact, microbes had developed resistance to antibiotics. This perturbation has been in operation even with antibiotics subsequently developed during the next 6–7 decades (D'Costa et al. 2006). Pharmaceutical companies are no longer interested in investing money into this business (Spellberg et al. 2004; Courvalin 2008). It obliged scientists to look for alternative drugs and new drug targets. It was realised that more than 80 % of the infectious diseases are caused by microbial pathogens, through specialised structures – biofilms. It enables bacteria to survive the lethal effect of drugs, as they "become" up to 1,000 times more resistant to antibiotics (Kalia 2013; Gui et al. 2014; Kalia et al. 2014a, b). These biofilms are developed by bacteria in a population density-dependent process called quorum sensing (QS) (Dong and Zhang 2005). Most Gram-negative bacteria operate through a QS system termed as LuxR/I-type, where acylated homoserine lactones (AHLs) acts as signals. QS signals consist of the lactone ring with varying acyl chains (Yang et al. 2012; Shang et al. 2014). QS regulates the expression of virulence factors, antibiotic production, nitrogen fixation, sporulation,

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conjugation, swarming, etc. (Borlee et al. 2008; Kalia and Purohit 2011; Kalia 2013; Wang et al. 2013; Zhang et al. 2013; Kalia et al. 2014a, b). These properties allow such bacteria to dominate the community structure. It is thus no surprise that the competing organisms have also developed mechanisms to interfere with the QSS and degrade these signals – a phenomenon termed as quorum quenching (QQ) (Kalia and Purohit 2011; Annapoorani et al. 2012; Bakkiyaraj et al. 2013; Kalia 2013; Agarwala et al. 2014).

Signal Degradation

QS signals – AHLs – can be degraded through: (1) chemical, (2) metabolic and (3) enzymatic routes. In the chemical degradation route, alkaline pH leads to the opening of the lactone ring which thus inactivates the AHL signals produced by plant pathogen – *Erwinia* species (Byers et al. 2002; Yates et al. 2002). This process can be reversed at acidic pH, where the cyclisation of the lactone ring leads to the reformation of an active signal molecule. In addition to chemical inactivation, bacteria, such as *Variovorax paradoxus* and *Pseudomonas aeruginosa* PAI-A can metabolise AHLs to use them as an energy source (Leadbetter and Greenberg 2000; Huang et al. 2003). In addition to these mechanisms, QS signals can be degraded enzymatically through AHL-lactonase, AHL-acylase, oxidoreductases and lactonase-like enzymes (paraoxonases) (Tables 1 and 2), which hydrolyze either the lactone ring or the amide bond of the AHL (Dong et al. 2000; Lee et al. 2002; Lin et al. 2003).

Diversity of Organisms Possessing AHL-Lactonase

The distribution of AHL-lactonase has been reported among diverse taxa: *Actinobacteria*, *Bacteroidetes*, and *Firmicutes*. It is interesting to learn that members of *Acidobacteria*, *Planctomycetes*, *Sphingobacteria*, and *Spirochaetales* possess only AHL-lactonase. However, most of bacteria belonging to these taxa do not show the presence of AHL-acylase (Kalia et al. 2011). AHL-lactonase belonging to the superfamily – metallohydrolase – has been reported to be produced by *Bacillus*, *Arthrobacter*, *Acidobacteria*, *Agrobacterium*, *Klebsiella*, *Streptomyces*, *Rhodococcus*, *Pseudomonas*, *Comamonas*, *Shewanella*, etc. (Kalia et al. 2011; Chen et al. 2013). The activity of the enzyme, AHL-lactonase, is influenced by a wide range of metal ions and chelating reagents. The variation in enhancing the activity has been observed with certain metal ions such as Mg^{2+} and Zn^{2+} at high concentration of 10 mM. However, this activity was observed to decline dramatically at lower metal ion concentration of 1 mM (Chen et al. 2010). In other cases, the enhancement in enzyme activity was similar due to the presence of metal ions such as Na^{+}, K^{+}, Ca^{2+}, Fe^{3+} and Mn^{2+} in the range of 1–10 mM. In contrast, quite a few ions at concentrations ranging between 0.2 and 2 mM did not have any effect on the activity of this enzyme: Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+} and Cd^{2+} (Wang et al. 2004). There was complete inhibition in AHL-lactonase activity in the presence of heavy metals (1–10 mM): Cu^{2+}, Cr^{3+}, Hg^{2+} and Ag^{+} (Chen et al. 2010). Sodium do-decyl sulphate inhibited the enzyme activity whereas reagents such as ethylenediaminetetraacetic acid helped to enhance AHL-lactonase activity at 10 mM (Chen et al. 2010). In previous studies, reagents like 2,2′-bipyridine and *o*-phenanthroline were found to have no impact on this enzyme (Wang et al. 2004). It is important to know the determinants that regulate the broad substrate specificity of AHL-lactonases. In case of *Bacillus thuringiensis* AiiA, F107 residue was found to have an important role in the selective interaction preferably for longer acyl-chain substrates (Liu et al. 2013). Identification of such residues may help in the development of highly selective QSIs as potential therapeutics.

Hydrolysis of the lactone ring inactivates the QS signal and thus influences the QS-mediated characteristics (Dong et al. 2000, 2002, 2004; Lee et al. 2002; Park et al. 2003; Ulrich 2004; Dong and Zhang 2005; Liu et al. 2005; Thomas et al. 2005; Bai et al. 2008; Riaz et al. 2008;

Table 1 Effect of heterologous expression of acyl homoserine lactone (AHL)-lactonase in diverse organisms on their quorum sensing systems

Source organism and gene	Host	QS signals and characteristic affected	References
Bacillus amyloliquefaciens PEBA20, *aii*A	*Escherichia coli* BL21(DE3)	Interferes with QS-mediated functions in *P. carotovorum* subsp. *carotovorum*	Yin et al. (2010)
Bacillus anthracis (Ames), *aii*A	*Burkholderia thailandensis*	In activated C6HSL, C8HSL and C10HSL	Ulrich (2004)
*Bacillus cereus, aii*A			
Bacillus subtilis subsp. *endophyticus* BS1, *aii*A	*E. coli* BL21(DE3) pLysS	Attenuated the soft rot symptoms caused by *Erwinia carotovora* var. carotovora	Pan et al. (2008)
Bacillus thuringiensis subsp. *morrisoni* and subsp. *kyushuensis, aii*A	*E. coli* BL21(DE3)	3OC6HSL, C6HSL and C8HSL Attenuates the pathogenicity caused by *E. carotovora*	Lee et al. (2002)
Bacillus sp. A24, *aii*A	*Pseudomonas aeruginosa* PAO1	3OC12HSL; reduces virulence gene expression and swarming motility in *P. aeruginosa* PAO1	Reimmann et al. (2002)
	Pseudomonas fluorescens P3	C4HSL and C6HSL; reduction in protease production and diseases caused by plant pathogens – *A. tumefaciens* and *Erwinia carotovora*	Molina et al. (2003)
	Serratia plymuthica HRO-C48	Abolished AHL production; reduced pyrrolnitrin and chitinase production	Müller et al. (2009)
	Lysobacter enzymogenes OH11A	Strong reduction of *Pectobacterium carotovorum* virulence on Chinese cabbage	Qian et al. (2010)
	S. plymuthica strain G3	Modification of the adhesion and biofilm forming abilities	Liu et al. (2011)
Bacillus sp. 240B1, *aii*A	*E. carotovora* SCG1 (E7-R3)	Reduced release of AI signals; decrease extracellular level of pectolytic enzymes and polygalacturonase; attenuation in pathogenicity on wide range of host plants	Dong et al. (2000)
	Transgenic tobacco and potato plants	Resistant to infection by the plant pathogen – *E. carotovora*	Dong et al. (2001)
Bacillus sp., *aii*A	*B. thuringiensis* BMB171	Delayed sporulation	Zhou et al. (2006)
Bacillus sp. BC6, *aiiA*	*E. coli* BL21(DE3)pLysS	Biofilm inhibition of *V. cholerae*	Augustine et al. (2010)
Bacillus sp. B546, *aii*A	*Pichia pastoris*	Resistance against *Aeromonas hydrophila*	Chen et al. (2010)
Agrobacterium tumefaciens; *att*M	*Azospirillum lipoferum* strain B518	Abolished pectinase activity, increased siderophore synthesis and reduced indole acetic acid production	Boyer et al. (2008)
Ochrobactrum sp. T63, *aid*H	*E. coli* BL21(DE3)	Interferes with QS-mediated functions in *P. fluorescens* 2P24 and *P. carotovorum*	Mei et al. (2010)
Pseudoalteromonas byunsanensis 1A01261, *qsd*H	*E. coli* BL21(DE3)	Attenuates the pathogenicity caused by *E. carotovora*	Huang et al. (2012)

(continued)

Table 1 (continued)

Source organism and gene	Host	QS signals and characteristic affected	References
Rhodococcus erythropolis W2	*E. coli* DH5α	C6-14 HSL with or without substitution at C3. Quench violacein synthesis in reporter strain	Uroz et al. (2008)
	P. aeruginosa 1855-344	Reduction in symptom severity of *P. carotovorum* PCC797	
	Agrobacterium tumefaciens 15955	Prevented accumulation of HSLs and conjugal transfer of Ti plasmid	
Chryseobacterium sp. StRB126, *aid*C	*E. coli* DH5α	C6HSL and C10HSL	Wang et al. (2012)
Metagenome-derived clones; *bpi*B01, *bpi*B04, *bpi*B07	*P. aeruginosa* PAO1	Inhibit motility and biofilm formation in *P. aeruginosa*	Schipper et al. (2009)

Uroz et al. 2009; Chan et al. 2010; Han et al. 2010; Deng et al. 2011; Yin et al. 2012). Among the different organisms known to produce lactonase, *Bacillus* has a broad substrate specificity with a preference for signals with (s)-configuration (Fuqua et al. 2001; Thomas et al. 2005). *Bacillus* spp. could degrade AHL signal HAI-1 of *V. harveyi* (Dong et al. 2002; Bai et al. 2008). Many homologues of this AHL-lactonase have been identified (Ulrich 2004) (Table 1). In a recent effort to look for diversity of AHL-lactonases, screening of 800 different bacteria revealed 42 strains of different *Bacillus* spp. to have *aiiA* gene (Huma et al. 2011). Other *Bacillus* species reported to express AHL-lactonase activity belonged to *B. amyloliquefaciens*, *B. subtilis*, *B. mycoides* and *B. microestinctum* (Dong et al. 2002; Pan et al. 2008; Han et al. 2010; Yin et al. 2010). Comparative genomics (*in silico*) study has also proved helpful in providing insights into genetic variability of genes for AHL-lactonase: *B. weihenstephanensis* KBAB4, *B. licheniformis* ATCC 14580, *Geobacillus* sp. WCH70, *Dorea longicatena* DSM 13814, *Pelotomaculum thermopropionicum* SI, *Moorella thermoacetica* ATCC 39073, *Clostridium beijerinckii* NCIMB 8052, *Lysinibacillus sphaericus* C3-41, *Bacillus* sp. B14905, *Staphylococcus saprophyticus* subsp. *saprophyticus* ATCC 15305, *Bacillus cereus* W, *Bacillus subtilis*, *B. thuringiensis*, *B. thuringiensis* serovar *israelensis* ATCC 35646, *B. thuringiensis* str. Al Hakam, *Clostridium kluyveri* DSM 555, *Staphylococcus aureus* RF122, *S. aureus* subsp. *aureus* MSSA476, *S. aureus* Mu50, *S. aureus* MW2, *Thermosinus carboxydivorans* Nor1, *Caldicellulosiruptor saccharolyticus* DSM 8903 and *Clostridium scindens* ATCC 35704 (Huma et al. 2011; Kalia et al. 2011).

Eukaryotic organisms are also known to exhibit activities similar to AHL-lactonases. *Phialocephala*, *Ascomycetes* and *Meliniomyces* are fungi which have lactonase activities for QS signals C6-HSL and 3OC6-HSL (Uroz and Heinon 2008). Human epithelial cells show activities for enzymes paraoxonases – PON1, PON2 and PON3 (Ng et al. 2005; Dong et al. 2007). These enzymes inactivate QS signals 3OC12-HSL produced by *P. aeruginosa* infecting human respiratory system (Chun et al. 2004; Hastings 2004). Human paraoxonases (PON2) hydrolyze and inactivate QS signals – 3OC6-HSL of pathogenic bacteria. PON lactonases are specific to six-member ring lactones than 5-member ring analogs (Draganov et al. 2005).

Diversity of Organisms Possessing AHL-acylase

The enzyme AHL-acylase, present in Gram-positive and Gram-negative bacteria, acts by cleaving the side chain of the signal molecule – AHL. The activity of AHL-acylase depends on acyl-chain lengths (Table 1). AHL-acylase reported from *Ralstonia eutropha* although quite specific, however has preference for long-chain AHLs more than 8 carbons (Lin et al. 2003).

Table 2 Effect of heterologous expression of acyl homoserine lactone (AHL)-acylase in diverse organisms on their quorum sensing systems

Source organism and gene	Host	QS signals and characteristic affected	References
Anabaena sp. PCC7120, *aii*C	*Escherichia coli*	Specificity for broad acyl-chain length HSLs	Romero et al. (2008)
Brucella melitensis, *aii*D	*B. melitensis*	Clumping phenotype and produce exopolysaccharide	Godefroid et al. (2010)
Ochrobactrum A44, *aii*O	*E. coli* BL21(DE3)	Inactivates C4 to C14 HSLs with or without 3-oxo or 3-hydroxy substituents	Czajkowski et al. (2011)
Pseudomonas aeruginosa PAO1, *qui*P (PA1032)	*E. coli* *P. aeruginosa*	Long-chain AHLs Decreased accumulation of the 3OC12HSL	Huang et al. (2006)
P. aeruginosa PAO1, *pvdQ* (PA2385)	*P. aeruginosa* *E. coli* DH10B	Dismantle the biofilm formation C7HSL, C8HSL, 3OC10HSL and 3OC12HSL	Sio et al. (2006)
P. aeruginosa, *pvdQ* (PA2385)	*P. aeruginosa* PAO1	Less virulent in *Caenorhabditis elegans* infection model; modulate its own pathogenicity	Papaioannou et al. (2009)
P. aeruginosa, *pa0305*	*P. aeruginosa* *E. coli*	Reduction in 3OC12HSL accumulation and the expression of virulence factors	Wahjudi et al. (2011)
Pseudomonas syringae, *hac*A, *hac*B and *psyr*3871	*E. coli*	AHLs with differing substrate specificities	Shepherd and Lindow (2009)
P. aeruginosa, PAI-A		Degrade long-chain AHLs but not short chain AHLs	Huang et al. (2003)
P. aeruginosa, *pvdQ*	*E. coli*	Rapid inactivation of long-chain AHLs	Huang et al. (2003)
	P. aeruginosa PAO1	Did not accumulate 3OC12HSL	
Ralstonia sp. XJ12B, *aiiD*	*E. coli*	Inactivated 3OC8HSL, 3OC10HSL and 3OC12HSL	Lin et al. (2003)
	P. aeruginosa PAO1	Influenced AHL accumulation, extracellular secretion and swarming motility, attenuated elastase and pyocyanin production, paralysed nematodes	
Ralstonia solanacearum GMI1000, *aac*	*E. coli* DH10B *Chromobacterium violaceum* CV026	C6HSL, C7HSL, C8HSL Inhibited violacein and chitinase activity	Chen et al. (2009)
Shewanella sp. MIB015, *aac*	*E. coli* *Vibrio anguillarum*	Long-chain AHLs Reduced AHL production and biofilm formation	Morohoshi et al. (2008)
*Streptomyces sp.*M664, *ahlM* gene	*Streptomyces lividans*	Long acyl chains; degrades penicillin G; decreased the production of virulence factors, including elastase, total protease and LasA	Park et al. (2005)
Metagenome-derived clone, *bpi*B09	*P. aeruginosa* PAO1	Reduced pyocyanin production, decreased motility and poor biofilm formation	Bijtenhoorn et al. (2011)

AHL-acylase from *Streptomyces* sp. M664 can be exploited for degrading AHLs with less than 8 carbons (Park et al. 2005). *P. aeruginosa* PAO1 can also degrade long-chain AHLs (Lamont and Martin 2003; Zhang and Dong 2004; Huang et al. 2006; Sio et al. 2006). The diversity of organisms showing AHL-acylase is reflected by their presence in *Ralstonia solanacearum* GMI1000 (Chen et al. 2009) and *Shewanella* sp. (Morohoshi et al. 2005). *Comamonas* can degrade acyl-chain lengths between 4 and 16 carbons with varying substitutions (Uroz et al. 2003, 2007). Homology to the acylase was seen in two diverse organisms such

as nitrogen-fixing cyanobacterium *Anabaena* (*Nostoc*) sp. PCC7120 and QuiP of *P. aeruginosa* PAO1 (Romero et al. 2008). In spite of a wide diversity of taxa having organisms possessing AHL-lactonase or AHL-acylase, however, cyanobacterial members have been reported to possess only AHL-acylase (Kalia et al. 2011).

Organisms Possessing Multiple AHL-Degrading Enzymes

Rhodococcus erythropolis is unique with a wide range of QQ abilities (Table 1). *R. erythropolis* W2 is one of those strains which possess activities for AHL-lactonase, AHL-acylase and oxidoreductase (Uroz et al. 2005; Park et al. 2006). In silico studies have also reported the presence of organisms with multiple AHL hydrolytic enzymes: (1) *Deinococcus radiodurans*, (2) *Hyphomonas neptunium* and (3) *Photorhabdus luminescens* (Kalia et al. 2011).

Expression of Prokaryotic Genes for AHL-Lactonase in Different Hosts

Attempts to enhance the activity of AHL-lactonase have been made by expressing the genes in different organisms (Table 1). Most of the studies have been targeted to express *aii*A of the *Bacillus* species (Kumar et al. 2013; Tinh et al. 2013). These heterologous expressions have proved effective in manipulating a wide range of QS-mediated characteristics in different organisms.

Expression of *Bacillus aiiA*

In *Escherichia*

Gene *aiiA* from *Bacillus subtilis* subsp. *endophyticus* BS1 expressed in *Escherichia coli* BL21 (DE3) pLysS proved effective in attenuating the soft rot symptoms caused by plant pathogen *Erwinia carotovora* (Pan et al. 2008). Using the same host, the expression of genes *aii*A from (1) *B. thuringiensis* and (2) *Bacillus amyloliquefaciens* interfered with the pathogenicity caused by *E. carotovora* by inhibiting the activities of the QS signals – 3OC6-HSL, C6-HSL and C8-HSL (Lee et al. 2002; Yin et al. 2010), whereas (3) *Bacillus* sp. BC6 could inhibit biofilm formation abilities of *Vibrio cholerae* (Augustine et al. 2010). The expression of *aii*A from *B. thuringiensis* subsp. *morrisoni* was weak in comparison to that of *B. thuringiensis* subsp. *kyushuensis*, as observed by their AHL degradation capacity. However, when *E. coli* BL21 (DE3) was used as a host, these differences were no longer evident with different QS signals: 3OC6-HSL, C6-HSL and C8-HSL (Lee et al. 2002).

In *Pseudomonas*

Expression of *aiiA* gene from *Bacillus* sp. A24 has been tested in a wide range of host organisms. There was a direct inhibitory impact on QS-mediated functions of *P. aeruginosa* PAO1, which include accumulation of QS signals (3OC12-HSL to approximately 0.10 μM), and expression of properties like swarming, motility and secretion of virulence factors (Reimmann et al. 2002). Similarly, soft rot disease of potatoes and eggplants caused by *Pectobacterium carotovorum* and crown gall disease of tomatoes caused by *Agrobacterium tumefaciens* could be prevented by expressing *aiiA* in *Pseudomonas fluorescens* (Dong et al. 2000; Molina et al. 2003). Vascular wilt of tomato plants caused by *Fusarium oxysporum* could be controlled when co-inoculated with *Pseudomonas chlororaphis*. However, this advantage was lost in the presence of AHL-lactonase producing *Bacillus* sp. A24 (Molina et al. 2003).

In *Burkholderia*

AHL-lactonase gene *aiiA* from *Bacillus anthracis* and *B. cereus* was effective in degrading AHL signal molecules when expressed in *Burkholderia thailandensis*. AHL signal molecules, such as C6- to C10-HSL, were significantly reduced from 2.4 to 300 pmol to undetectable levels. Subsequently, this gene was shown to be instrumental in retarding the growth rate such that generation time of *B. thailandensis* increased from 48 min in the wild-type to 243 min in the genetically

modified strain. In addition, this genetically engineered strain also affected the swarming and twitching motility of this pathogen. It has been envisaged that this genetic modification can prove effective in developing vaccine against Gram-negative pathogenic bacteria (Ulrich 2004).

In *Erwinia*

E. carotovora SCG1 (E7-R3) expressing *aiiA* gene of *Bacillus* sp. 240 B1 could effectively inhibit the release of AI signals into the milieu. The activities of extracellular pectolytic enzymes such as pectate lyase, pectin lyase and polygalacturonase were 3–10-fold lower than the wild type. This genetic change in *E. carotovora* was pivotal for reducing its ability to cause disease on eggplant, potato, celery and leafy vegetables such as cabbage, Chinese cabbage, carrot and cauliflower (Dong et al. 2000).

In *Serratia and Lysobacter*

Endophytic bacteria, such as *Serratia plymuthica*, are closely associated with plant rhizosphere and phyllosphere. It has been proposed to be a potential biocontrol agent against fungal diseases. The influence of *Bacillus* A24 *aiiA* gene on QSS of *S. plymuthica* G3 was recorded in terms of the modified adhesion and biofilm-forming abilities. QS signals were completely degraded, which diminished its antifungal activity but augmented its indole acetic acid biosynthesis (Liu et al. 2011). Similar impact on AHL signal concentration and consequent depletion in production of chitinase and pyrrolnitrin were evident in *S. plymuthica* HRO-C48 expressing *aiiA* gene (Müller et al. 2009). *aiiA* gene of *Bacillus* has been expressed in *Lysobacter enzymogenes*. Here it affected pathogenicity caused by *E. carotovora* on Chinese cabbage (Qian et al. 2010).

In Eukaryotes

Aeromonas spp. are known to cause infections in fishes through biofilm formation and efforts are being made to control it (Chu et al. 2013; Mahanty et al. 2013). A unique approach of overproducing AHL-lactonase using eukaryotic high yielding expression system led to many advantages. Expression of *aii*A in *Pichia pastoris* made the fish less susceptible to infection by the bacterial pathogen *Aeromonas hydrophila* (Chen et al. 2010; Chu et al. 2013). The secreted lactonase was found to be stable and active on a wide pH of 6.5–8.9, having thermal stability at 70 °C, and most importantly was protease resistant. This gave an advantage of having a QSI that can work strongly and efficiently on field trials, without the need to clone genes using biocontrol agents (Chen et al. 2010). *Bacillus* spp. with AHL-lactonase could protect *Macrobrachium rosenbergii*, a giant freshwater prawn, from infection caused by *Vibrio harveyi* infection (Nhan et al. 2010). Tobacco and potato plants modified by the introduction of *aii*A gene were resistant to *E. carotovora* infection (Dong et al. 2001).

In *Bacillus* spp.

In general, *aiiA* from *Bacillus* spp. shows higher expression in a heterologous host such as *E. coli.* However, certain mutants of the *B. thuringiensis* BMB171 were found to perform better, but the mutant strains were slow in sporulation process. This property was linked to modifications in its membrane channel. This scenario can be envisaged to allow sustainable commercial production of AHL-lactonase (Zhou et al. 2006).

Expression of Homologues of *aiiA* in Different Hosts

In Gram-positive bacterium *R. erythropolis* W2 a unique AHL-lactonase encoded by gene *qsd*A belonging to phosphotriesterases was found. Like other classes of lactonases, it also has broad substrate specificity for acyl-chain length upto C14. All the clones (*E. coli*) harbouring *qsd*A alleles efficiently inactivated AHL signals. *P. fluorescens* 1855–344 expressing $qsdA_{W2}$ also conferred resistance to infection against *P. carotovorum* PCC797. It completely abolished the QS-mediated Ti-plasmid conjugal transfer ability of *A. tumefaciens* expressing *qsd*A (Uroz et al. 2008). Similarly, another lactonase variant *aid*H was found in Gram-negative bacterium *Ochrobactrum* sp. T63. The encoded protein is a metal-dependent (Mn^{2+}) hydrolase belonging

to α/β hydrolase family. Cloning of *aid*H into *P. fluorescens* 2P24 and *P. carotovorum* Z3-3 curtailed biofilm formation and abolished pathogenicity in the tested plants (Mei et al. 2010). The absence of AHL-type substrate specificity makes AidH a unique AHL-lactonase to be used against QS-mediated harmful phenotypes. Similarly, expression of *qsd*H gene from *Pseudoalteromonas byunsanensis* in *E. coli* BL21 (DE3) was effective in inhibiting the QS-regulated functions and pathogenicity caused by *E. carotovora* (Huang et al. 2012). Heterologous expression of *att*M (a homologue of *aii*A) from *A. tumefaciens* was able to reduce the QS-mediated pectinase enzyme activity of *Azospirillum lipoferum*. It also influenced the siderophore and indole acetic acid production (Boyer et al. 2008). An *attM* paralogous gene (*aiiB*) was also found in *A. tumefaciens*, having potent activity against AHLs that can be exploited (Carlier et al. 2003).

Recent developments in metagenomic approaches to look for AHL-lactonase from uncultured bacteria are expected to add to the limited diversity of this enzyme (Williamson et al. 2005). Metagenome of a bacterial community from soil is another genetic resource of great potential to be exploited. Screening of various metagenomic clones allowed the identification of genes such as *qlc*A, *bpi*B01 and *bpi*B04, which had very low or no similarities to any known AHL-lactonases. Expression of *bpi*B01 in *P. aeruginosa* PAO1 inhibited its biofilm-forming ability (Riaz et al. 2008; Schipper et al. 2009).

Hetreologous Expression of Eukaryotic Lactonase

Expression of genes for three mouse paraoxonases (PONs) in mammalian cells led to the degradation of AHL in a manner which was quite similar to that of lactonases (Yang et al. 2005). Expression of human paraoxonase in *Drosophila melanogaster* allowed the organism to survive the onslaught of the lethal action of *P. aeruginosa* infection (Stoltz et al. 2008).

Heterologous Expression of AHL-Acylase Gene from *Ralstonia*

Cloning and expression of gene *aiiD* from *Ralstonia* sp. into *E. coli* was very effective in abolishing the 3 QS signals: 3OC8-HSL, 3OC10-HSL and 3OC12-HSL, while its overexpression within *Brucella melitensis* affected the QS-mediated process and clumping phenotypes (Godefroid et al. 2010) (Table 2). This inactivation was quite rapid as it was observed within 3 h of incubation. Wild-type *P. aeruginosa* PAO1 possessing plasmid pUCM9-PAO1 showed accumulation of QS signal in normal concentrations. In contrast, introduction of pUCaiiD encoding AHL-acylase gene into *P. aeruginosa* PAO1 affected a wide range of QS regulated expressions. The pathogenic bacterium could not accumulate AHL signals: C4-HSL and 3OC12-HSL. The genetically engineered *P. aeruginosa* was not able to produce elastase and pyocyanin in normal quantities, and its ability to swarm was significantly restricted. The pathogen could no longer paralyse the nematode, *Caenorhabditis elegans*, which had more than 80 % survival rate (Lin et al. 2003). Subsequently, an aculeacin A acylase was reported from *Ralstonia solanacearum* GMI1000. The *aac* gene had 83 % identity with acylase gene *aiiD*, but no significant resemblance with lactonase gene *aiiA* sequence (Chen et al. 2009). This enzyme is active against AHLs having side chains more than 6 carbons. Expression of *aac* gene in *C. violaceum* CV026 could inhibit AHL-mediated activities such as production of violacein and chitinase enzyme (Chen et al. 2009).

Over Expression of AHL-Acylase Gene from *Pseudomonas*

P. aeruginosa genes coding for AHL-acylase have the potential to be exploited for developing novel therapies against the infections caused by this pathogen. Over expression of gene PA2385 also known as *qsc112* and *pvdQ* (Whiteley et al. 1999; Lamont and Martin 2003) has differential behaviour against AHL signals.

It could not hydrolyse QS signals: C10-HSL, C12-HSL and C14-HSL (Sio et al. 2006). This gene was first cloned into the *P. aeruginosa-E. coli* shuttle vector pME6032 and electroporated in *P. aeruginosa* PAO1. Introduction of gene PA2385 absolutely extripated 3OC12-HSL, but there was no impact on the level of C4-HSL. Over expression of PA2385 abolished activities of enzymes, LasB, and also minimised the expression of *lecA* gene. It, however, did not affect production of pyoverdin (Sio et al. 2006).

A novel AHL-acylase coded by *aiiC* (*all3924*) was reported from filamentous nitrogen fixing cyanobacterium, *Anabaena* sp. PCC7120. It showed homology to *quiP* of *P. aeruginosa* PAO1. This gene was also cloned into shuttle vector pME6032 and expressed in *E. coli*. AiiC has broad specificity with respect to the acyl-chain length, but did not degrade short chain AHLs (Romero et al. 2008). Although *P. aeruginosa* PAO1 has been established to possess *pvdQ* and *qui*P, however, two more genes, pa1893 and pa0305 belonging to the Ntn hydrolase superfamily, have been predicted to encode for penicillin acylase (Wahjudi et al. 2011). Biosensor assays showed their ability to degrade C6 to C14-HSLs. Pa0305 was found to be 56 times more efficient in degrading 3OC12-HSL than C8-HSL. The enzyme was effective in killing *C. elegans* as well (Table 2).

Gene *ahlM* from *Streptomyces* sp. M664 was able to express and degrade AHLs in *Streptomyces lividans* (Park et al. 2005). AhlM enzyme had higher deacylation activity towards long acyl-chain AHLs. It could also degrade penicillin by the same mechanism. It had high activity, even at low concentration of 2 μg/ml. It could significantly affect the production level of virulence factors such as elastase, protease and LasA of *P. aeruginosa* (Park et al. 2005).

Horizontal Gene Transfer

Screening for AHL-degrading proteins has revealed the presence of *aiiA* gene in a large number of organisms. These organisms belong to taxonomically diverse genera. AHL-lactonases from different sources may share homology among themselves. Such genetic similarity can be explained on the basis of horizontal gene transfer (HGT). The occurrence of HGT among bacterial kingdom and its impact of genetic and phenotypic changes has been well reported (Lal et al. 2008). The unique AHL-lactonase of *R. erythropolis* W2 seems to be a case of HGT (Uroz et al. 2008).

In silico studies have also highlighted the occurrence of HGT involving AHL-degrading enzymes (Kalia et al. 2011). Genetic variability in AHL-lactonase coding gene *aii*A was reported on the basis of their phylogenetic relationships and the restriction digestion patterns (Huma et al. 2011; Kalia et al. 2011). Taxonomically diverse organisms were found to show discrepancies in their phylogenetic relationships for *aii*A. Genes for AHL-lactonase from *Firmicutes*, *Moorella thermoacetica* ATCC 39073, and β-*Proteobacteria*, *Burkholderia graminis* C4D1M, were very close to each other with a bootstrap value, BV of 1000. Similar correlations with high BVs were also recorded among: (i) *Actinobacteria* such as *Mycobacterium* sp. MCS and *Rubrobacter xylanophilus* DSM9941 on one hand and members of α-*Proteobacteria* (*Granulibacter bethesdensis* CGDNIH1, *Acidiphilium cryptum* JF-5 and *A. tumefaciens*) on the other and (ii) also between *D. radiodurans* R1 (*Deinococcus-Thermus*) and *Xylella fastidiosa* (γ-*Proteobacteria*).

Taxonomic discrepancies in the phylogenetic tree of the gene for AHL-acylase were reported between different groups: (1) *D. radiodurans* R1 and *Ralstonia* spp. and (2) δ-*Proteobacteria*, *Plesiocystis pacifica* SIR-1, and *Acidobacteria*, *Solibacter usitatus.* This emphasises that in nature, organisms live in symbiotic relationships and share these genes through HGTs for evolving better survival strategies (Kalia et al. 2011).

Conclusion

Heterologous expression of QS signal degrading enzymes can be exploited for producing them on a commercial scale. The immediate application is

to use them as potential drugs against biofilm-forming bacteria. Their usage in a range of biotechnological applications has been discussed in a subsequent chapter.

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