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ORIGINAL ARTICLE

Multiplicity of Quorum Quenching Enzymes: A Potential Mechanism to Limit Quorum Sensing Bacterial Population

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Abstract Bacteria express certain of their characteristics especially, pathogenicity factors at high cell densities. The process is termed as quorum sensing (QS). QS operates via signal molecules such as acylhomoserine lactones (AHLs). Other bacteria inhibit QS through the inactivation of AHL signals by producing enzymes like AHL-lactonases and -acylases. Comparative genomic analysis has revealed the multiplicity of genes for AHL lactonases (up to 12 copies per genome) among *Bacillus* spp. and that of AHL-acylases (up to 5 copies per genome) among *Pseudomonas* spp. This genetic evolution can be envisaged to enable host to withstand the attacks from bacterial population, which regulates its functioning through QS.

Keywords Acylase · Bacteria · Lactonase · Pathogenicity · Quorum quenching · Quorum sensing

Introduction

Bacteria within a community depend upon each other for their survival. However, to maintain their identity and to ensure their existence they produce an arsenal of bioactive molecules [1–3]. The bacterial arsenal consists of antibiotics, toxins, hydrolytic enzymes, antipathogenic and antibacterial molecules [4–6]. *Vibrio* spp. fight their competitors through toxin loaded molecular guns—the Type III and VI secretion system (T3SS, T6SS) [7, 8]. *Pseudomonas* spp. possess the unique ability to produce antibiotics and other pathogenicity factors such as proteases, elastase, rhamnolipid, pyocyanin, superoxide dismutases for their defense against reactive oxygen species (ROS), to evade the immune response, and degrade surfactant proteins [9]. Most of these pathogenicity factors are produced through the phenomenon of quorum sensing (QS) [10, 11]. QS is a cell density dependent phenomenon, which operates through signal molecules. Above a threshold concentration, these signal molecules lead to the expression of selective genes, including those responsible for virulence [12, 13]. QS systems (QSS) such as LuxI/R and their homologs range from a single in *Vibrio* species to multiple in *Sinorhizobium* and *Pseudomonas* species. Each QSS is operated by its unique signal molecule, such as acyl-homoserine lactones (AHLs), oligopeptides, methyl esters, methyl dodecanoic acid, etc. AHLs consist of a lactone ring attached to a carbon side chain, which range from C4 to C18 in length. In this category of AHLs, the most prevalent ones are: *N*-butanoyl-homoserine lactone (C4HSL), *N*-hexanoyl-HSL (C6HSL), *N*-octanoyl-HSL (C8HSL) and *N*-dodecanoyl-HSL (C12HSL) [14, 15]. Multiplicity of QSS and variability in the signal molecules allow bacteria to use these in various combinations and permutations. This raises the complexity of the mechanisms responsible for controlling the pathogenicity of such bacteria [16–19].

To counter the bacterial population operating through QS mediated mechanism, rival bacteria produce enzymes—AHL-lactonases and -acylases for the AHL-degradation leading to disruption of QS, also called as Quorum Sensing inhibitors (QSIs) [14, 20, 21]. The AHL-lactonases hydrolyze the lactone ring of AHLs and are

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found in numerous *Bacillus* spp. [22–24]. Similarly, the AHL-acylases break the amide linkages of the AHL acyl chains and have been reported to be produced by bacteria such as *Ralstonia* spp. [25, 26]. Although, most bacteria possess one of these QSI enzymes, only a few of them have been reported to possess both AHL-lactonase and AHL-acylase, e.g., *Rhodococcus erythropolis, Deinococcus radiodurans, Photorhabdus luminescens*, *Hyphomonas neptunium* [14, 20, 27].

Based on the observations that there is a multiplicity of QSS and QSIs, we did a comparative genomic analysis to explore the possibility of the existence of multiplicity of genes governing the expression of AHL-lactonases and AHL-acylases. This information will further elucidate how this genetic material helps the host to withstand the attacks from bacterial population, which regulates its functioning through QS.

Materials and Methods

Conserved Domains of Acyl-Homoserine Lactone Degrading Enzymes

The detailed procedures used in this study are as described earlier [20]. Briefly, protein sequences for the enzymes AHL-lactonase from *Bacillus* sp. SB4 (Accession No. AAR85482.1) and AHL-acylase from *Ralstonia* sp. XJ12B (Accession No. AAO41113.1) were collected from the NCBI Protein database. The nucleotide sequences which corresponded to the protein sequences were downloaded from NCBI Genbank. The conserved domains of these enzymes for reference organisms (Tables 1, 2) are as described in our previous study [20]. We used BLASTP for the similarity searches and NCBI Conserved Domains [28, 29] for searching the conserved domains. The sequences showing multiple copies of AHL-lactonase and AHL-acylase have been analysed here.

Results

The presence of genes encoding for AHL-lactonase and -acylase has been observed in taxonomically diverse bacteria: gram-positive and -negative groups [20]. In the present study, the focus has been on identifying organisms with multiple copies of genes for AHL-lactonase and AHL-acylase.

Multiplicity of Genes Coding for AHL-Lactonase

Among all those organisms which show the presence of conserved domains for the enzyme—AHL-lactonase, the highest prevalence of the gene for this enzyme was recorded in Firmicutes (Gram-positive) and Proteobacteria (Gram-negative). Among Gram-positive bacteria, multiplicity of the gene for AHL-lactonase was recorded in (a) Firmicutes—(1) *Bacillus* spp., and (2) *Lysinibacillus*, and (b) *Deinococcus thermus—D. radiodurans* (Table 1). Interestingly, within *Bacillus* spp., (1) *B. cereus* strains were quite unique, where some showed a single copy of the gene for AHL-lactonase, whereas others possessed 2–5 copies of the same, (2) the strains of *B. subtilis* had 3 copies per genome, and (3) *B. thuringiensis* strains had 1–12 copies per genome. On the other hand, among Gram-negative bacteria, *Agrobacterium* sp. and *Bradyrhizobium* sp. had single and multiple copies of the gene for AHL-lactonase. Apart from these bacteria, *Thermoplasma* was observed to possess 3 copies of *AttM* related AHL-lactonase gene (Table 1).

Multiplicity of Genes Coding for AHL-Acylase

Multiple copies of gene coding for AHL-acylase, were found to be distributed among gram-negative bacteria representing different taxa: β-, γ-, and δ-Proteobacteria (Table 2). *Ralstonia* sp. XJ112B is known to possess active AHL-acylase and within the conserved domain of Ntn-hydrolase superfamily, represented by two partial domains. *Ralstonia* sp. in general have been observed to possess single copies of the gene coding for AHL-acylase, except *Ralstonia picketti* 12D, which possesses two copies (denoted by different gi numbers), within the sequenced genome. Among the members of γ-Proteobacteria, (1) *Azotobacter vinelandii*, (2) *Pseudomonas* sp., (3) *P. aeruginosa* strains PAO1, PA7, PACS2, UCBPP-PA14, 2197 and C379, (4) *P. entomophila*, (5) *P. fluorescens*, (6) *P. putida* strains F1, GB,W619, (7) *P. syringae* strains Phaseolicola 448A, syringae B728a, and tomato str. DC3000, (8) Marine proteobacterium exhibits multiple copies of this gene per genome.

Similarly, in gram-positive organisms multiplicity of the gene for AHL-acylase was recorded in (a) *Deinococcus—D. radiodurans* R1, (b) Actinobacteria—*Streptomyces* sp. Mg1, *Streptomyces griseus subsp. griseus* NBRC 13350 (c) Cyanobacteria—*Cyanothece* sp. PCC 7424, *Crocosphaera watsonii* WH 8501 (Table 2).

Discussion

Biological control of diseases caused by pathogenic bacteria such as *Pseudomonas* spp. in soil or rhizosphere has been reported with the help of rhizobacteria, e.g., *Bacillus* spp. [3, 30]. *Bacillus* spp. have been shown to control pathogens such as *Erwinia caratovora,* [31],

Table 1 Taxonomic distribution of organisms showing multiplicity of AHL lactonase

Organism	Enzyme	CD[a]	GI No:
Gram positive bacteria			
Firmicutes			
Bacillus cereus	AiiA	Full (F)	gi|22095307|gb|AAM92142.1|
	AiiA		gi|62945899|gb|AAY22194.1|
	AHL-lactonase		gi|19773609|gb|AAL98724.1|
	AHL-lactonase		gi|66576020|gb|AAY51613.1|
	AHL-lactonase		gi|115418822|emb|CAJ84442.1|
B. cereus G9842[b]	Metal-dependent hydrolase (MDH)	F	gi|168144103|ref|ZP_02587332.1|
B. cereus H3081.97[b]	AHL-lactonase	Partial (P)	gi|206975410|ref|ZP_03236323.1|
B. cereus ATCC 14579	AHL-hydrolase	F	gi|30021556|ref|NP_833187.1|
			gi|30021556|ref|NP_833187.1|
	MDH		gi|30022770|ref|NP_834401.1|
B. cereus B4264	AHL-hydrolase	F	gi|168133282|ref|ZP_02576511.1|
	MDH	P	gi|168137760|ref|ZP_02580989.1|
Bacillus sp. 240B1[b]	MDH	F, P	gi|7416989|gb|AAF62398.1|
Bacillus sp. 42[b]	AHL-lactonase	F	gi|146743333|gb|ABQ42909.1|
Bacillus sp. 91[b]	AHL-lactonase	F	gi|146743335|gb|ABQ42910.1|
Bacillus sp. A24	AiiA	F, P	gi|21541343|gb|AAM61772.1
Bacillus sp. COT1[b]	AHL-lactonase	F	gi|19773593|gb|AAL98716.1|
Bacillus sp. CSX-1[b]	AHL-hydrolase	F	gi|109809891|gb|ABG46349.1|
Bacillus sp. SB4[b]	AHL-lactonase	F	gi|40388447|gb|AAR85482.1|
Bacillus sp. B14905	HP BB14905_11105	F	gi|126653419|ref|ZP_01725520.1|
	YtnP		gi|126651311|ref|ZP_01723518.1|
B. subtilis	AHL-lactonase	F	gi|66576014|gb|AAY51610.1|
			gi|66576016|gb|AAY51611.1|
			gi|150256376|gb|ABR68029.1|
B. thuringiensis	AHL-lactonase	F	gi|19773603|gb|AAL98721.1
			gi|19773605|gb|AAL98722.1
			gi|19773595|gb|AAL98717.1|
			gi|19773599|gb|AAL98719.1|
			gi|19773601|gb|AAL98720.1|
B. thuringiensis	AHL-lactonase	F	gi|66576018|gb|AAY51612.1|
			gi|34500091|gb|AAQ73629.1|
			gi|34500091|gb|AAQ73629.1|
			gi|90421378|gb|ABD93926.1|
			gi|67107096|gb|AAY67830.1|
			gi|90421372|gb|ABD93923.1|
			gi|124389890|gb|ABN11118.1|
			gi|90421376|gb|ABD93925.1|
			gi|197253281|gb|ACH54082.1|
			gi|38564660|gb|AAR23790.1|
		F, P	gi|90421370|gb|ABD93922.1|
			gi|90421374|gb|ABD93924.1|
		P(2)	gi|146743337|gb|ABQ42911.1|

Table 1 continued

Organism	Enzyme	CD[a]	GI No:
B. thuringiensis	AiiA	F	gi|209944153|gb|ACI96332.1|
			gi|209944159|gb|ACI96335.1|
			gi|209944167|gb|ACI96339.1|
			gi|209944163|gb|ACI96337.1|
			gi|209944151|gb|ACI96331.1|
			gi|209944161|gb|ACI96336.1|
			gi|209944165|gb|ACI96338.1|
			gi|209944169|gb|ACI96340.1|
			gi|209944155|gb|ACI96333.1|
			gi|209944157|gb|ACI96334.1|
			gi|209944171|gb|ACI96341.1|
			gi|209944173|gb|ACI96342.1|
B. thuringiensis	AHL-lactonase	F	gi|194709246|pdb|3DHA|
			gi|85544298|pdb|2BR6|
			gi|125711135|gb|ABN51242.1|
B. thuringiensis serovar canadensis	AiiA-like protein	F	gi|22095279|gb|AAM92128.1|
B. thuringiensis serovar galleriae	AiiA-like protein		gi|22095283|gb|AAM92130.1|
B. thuringiensis serovar kyushuensis	AiiA-like protein		gi|22095289|gb|AAM92133.1|
B. thuringiensis serovar ostriniae	AiiA-like protein		gi|22095293|gb|AAM92135.1|
B. thuringiensis serovar pakistani	AiiA-like protein		gi|22095295|gb|AAM92136.1|
B. thuringiensis serovar toumanoffi	AiiA-like protein		gi|22095301|gb|AAM92139.1|
B. thuringiensis serovar japonensis	AHL-lactonase		gi|33187780|gb|AAP97743.1|
B. thuringiensis serovar kim	AHL-lactonase		gi|33187778|gb|AAP97742.1|
B. thuringiensis serovar jinhongiensis	AiiA-like enzyme		gi|28413984|gb|AAO40748.1|
B. thuringiensis serovar oswaldocruzi	AiiA-like enzyme		gi|28413776|gb|AAO40747.1|
B. thuringiensis serovar kurstaki	AHL-hydrolase		gi|75766091|pdb|2A7 M|
B. thuringiensis serovar alesti	AHL-hydrolase		gi|124014030|gb|ABM88266.1|
B. thuringiensis str. Al Hakam	MDH		gi|118479835|ref|YP_896986.1|
B. thuringiensis serovar israelensis ATCC 35646	AHL-hydrolase	F	gi|75761848|ref|ZP_00741777.1|
	MDH		gi|75761592|ref|ZP_00741546.1|
Lysinibacillus sphaericus C3-41	HP Bsph_3377	F	gi|169828841|ref|YP_001698999.1|
	YtnP		gi|169829648|ref|YP_001699806.1|
Deinococcus-Thermus			
Deinococcus radiodurans R1	Hypothetical protein (HP) DR_0172	F	gi|15805209|ref|NP_293896.1|
	HP DR_1823		gi|15806823|ref|NP_295546.1|
Gram negative bacteria			
Alphaproteobacteria			
Agrobacterium tumefaciens str. C58	MDH	F	gi|16119885|ref|NP_396590.1|
	MDH	P	gi|159186505|ref|NP_396071.2|
A. tumefaciens[b]	AttM/AiiB	P	gi|17223785|gb|AAL13075.1|
BradyRhizobium sp. ORS278	putative metallo-beta-lactamase family protein	F	gi|146341021|ref|YP_001206069.1|
	putative MDH		gi|146343732|ref|YP_001208780.1|
	putative signal peptide		gi|146339571|ref|YP_001204619.1|
BradyRhizobium japonicum[b]	AttM-like protein	F	gi|6655034|gb|AAF22881.1|

Table 1 continued

Organism	Enzyme	CD[a]	GI No:
B. japonicum USDA 110[b]	AttM/AiiB family protein	F	gi|27380160|ref|NP_771689.1|
Euryarchaeota			
Thermoplasma volcanium GSS1	MDH (AttM-related)	F	gi|13542116|ref|NP_111804.1|
	MDH		gi|13541013|ref|NP_110701.1|
	HP		gi|14324397|dbj|BAB59325.1|

[a] Reference organism used was *Bacillus* sp. SB4 (Accession No. AAR854821) for searching Conserved Domains (CDs) of Lactamase_B, superfamily

[b] This information has been retained in the Table to show variability in copy number in different species of a given Genus

Xanthomonas campesteris [32], *Phytophthora infestens* [33], *P. syringae* [34]. Heterologous expression of *Bacillus* AiiA lactonase in *Burkholderia thailandensis*, *P. aeruginosa* PAO1, *Vibrio harveyi*, and *E. carotovora* have been found to disrupt QS mediated properties [35, 36]. In fact, certain antibiotics become effective on biofilms, which have been exposed to AHL degrading enzymes, which implies that a combination of the two treatments can be complementary and effective in controlling the pathogenic organism(s) [30].

The presence of AHL lactonase throughout the gram positive genus—*Bacillus* shows the evolutionary stability of the a*iiA* gene. *B. thuringiensis* has been observed to exhibit a maximum of 12 copies of the complete AHL lactonase gene and *B. cereus* with 5 copies. In case of the gram negative group, taxa wide horizontal gene transfer (HGT) is suggested by the presence of the lactonase from *Agrobacterium tumifaciens* and *Bradyrhizobium* sp. (α-Proteobacteria), into *Thermoplasma volcanium* belonging to Euryarchaeota. Potential cases of HGT have been reported in the case of AHL-lactonase, where *Moorella* sp. (Firmicutes) and Burkholderia sp. (β-Proteobacteria) were found to show high phylogenetic similarity. Similarly, (1) *Mycobacterium* sp. and *Rubrobacter* sp. belonging to Actinobacteria were proposed to have shared this gene with the members of α-Proteobacteria (*Granulibacter* sp., *Acidiphilum* sp. and *A. tumefaciens*), and (2) *D. radiodurans* (Deinococcus-Thermus) and *Xylella fastidiosa* (γ-Proteobacteria) [20].

In the case of AHL-acylases, *Ralstonia* sp., contains an assortment of enzymes AHL-acylase, Penicillin amidase (PAM), Peptidase and Aculeacin A acylase. PAM in addition to the peptidase, can be seen to be distributed through gram negative as well as gram positive taxas. This is indicative of HGT in the case of AHL-Acylase as well. In fact, previous study has proposed HGT between members of (1) Acidobacteria and δ-Proteobacteria, and (2) *D. radiodurans* and *Ralstonia* spp. (β-Proteobacteria) [20]. A maximum of 5 copies of PAM is present in the Bacteriodetes, *Robiginitalea biformata* followed by the *Actinobacteria*, *Nocardioides* sp. JS614 having 4 copies. γ-Proteobacteria KT-71, contains 4 copies of peptidase and PAM. The major carrier of multiple gene copies has been found to be the *Pseudomonas* sp. in γ-Proteobacteria, majorly having peptidase.

AHL-lactonase and -acylase have been proved to inhibit bacterial infections, and prevent biofilm growth, etc. It may be professed that the hydrolysis of the lactone ring is an efficient method for Quorum Quenching (QQ) [19, 20]. In future, these genes may acquire novel functions beneficial for the survival of the host, as in the unique case of mammalian paraoxonases (PONs). It has been speculated that these AHL-lactonases have emerged within three super-families through convergent/parallel evolution [22, 37–39]. It is further stated that PONs have evolved from lactonases only after the introduction of the parathion, an organophosphate pesticide [38–40]. The evolutionary significance of lactonases has been found to be established in mammals, where they subsequently acquired the detoxifying functions to act as a tool of innate immunity [41]. The present classes of mammalian PONs: (1) PON1 (2) PON2 (3) PON3 have been shown to have originated from bacterial lactonases via horizontal gene transfer from an endosymbiotic bacteria. They still exhibit the presence of ancestral AHL lactonase relics in association with their detoxification characteristics [41]. PON2 however, seems to have gained new functions and has also reverted to the ancestral AHL lactonase activity [41].

In the face of increasing antibiotic resistance alternate therapy approaches need to be developed. QQ enzymes from a diverse range of organisms can be used for this purpose. The activity of AHL lactonase is lactone ring specific and not dependent upon the acyl chain length, in the case of AHL acylase [19, 20]. As elucidated in QSSs, such a multiplicity has been observed here for AHL-lactonase and AHL-acylase as well. In the virtual battle going on in the environment between QS and QSIs, in contrast to antibiotics, QSIs act at very low concentrations without killing the bacteria. This does not generate the pressure on bacteria to develop resistance against antibiotics. The

Table 2 Taxonomic distribution of organisms showing multiplicity of AHL-acylase

Organism	Enzyme	CD[a]	GI No:
Gram-negative			
Betaproteobacteria			
Ralstonia sp. XJ12B[a]	AHL-acylase	Partial (P(2))	gi|28376389|gb|AAO41113.1|
Ralstonia eutropha JMP134[b]	Penicillin amidase (PAM)	P	gi|73541530|ref|YP_296050.1|
R. eutropha H16[b]	PAM or acylase (PAC)	P(2)	gi|113867898|ref|YP_726387.1|
R. pickettii 12J[b]	Peptidase S45, PAM	P(2)	gi|187929893|ref|YP_001900380.1|
R. solanacearum GMI1000[b]	Aculeacin A acylase	P(2)	gi|17547266|ref|NP_520668.1|
R. metallidurans CH34[b]	Peptidase S45, PAM	P(2)	gi|94313782|ref|YP_586991.1|
R. pickettii 12D	Peptidase S45, PAM	P(2)	gi|153885673|ref|ZP_02006829.1|
	Aculeacin A acylase	P(2)	gi|153885674|ref|ZP_02006830.1|
Gammaproteobacteria			
Azotobacter vinelandii AvOP	Peptidase S45, PAM	P(2)	gi|67157312|ref|ZP_00418614.1|
		P	gi|67157375|ref|ZP_00418677.1|
Pseudomonas sp. SY-77-1	Glutaryl-7-amino-cephalosporanic-acid acylase	P(2)	gi|116242485|sp|P07662.2|
	Chain A	P(2)	gi|47168470|pdb|1OQZ|
	Chain B	P	gi|47168473|pdb|1OR0|B
Pseudomonas aeruginosa PAO1	AHL acylase PvdQ	P(2)	gi|15597581|ref|NP_251075.1|
	Hypo. protein (HP) PA1893	P	gi|15597090|ref|NP_250584.1|
P. aeruginosa PA7	AHL acylase	P(2)	gi|152987503|ref|YP_001348236.1|
	HP PSPA7_3392	P	gi|152987014|ref|YP_001348752.1|
	Putative PAM	P	gi|152985857|ref|YP_001349702.1|
P. aeruginosa PACS2	HP PaerPA_01002361	P	gi|107101327|ref|ZP_01365245.1|
	HP PaerPA_01002874	P(2)	gi|107101829|ref|ZP_01365747.1|
	HP PaerPA_01001501	P	gi|107100476|ref|ZP_01364394.1|
P. aeruginosa UCBPP-PA14	PAC	P(2)	gi|116050326|ref|YP_790857.1|
	HP PA14_40040	P	gi|116049847|ref|YP_791346.1|
	PAM	P	gi|116048960|ref|YP_792238.1|
P. aeruginosa 2192	PvdQ	P(2)	gi|194551803|ref|YP_002086830.1|
	HP PA2G_00931	P	gi|194551272|ref|YP_002086299.1|
P. aeruginosa C3719	HP PACG_00864	P	gi|194545971|ref|YP_002080999.1|
	HP PACG_00002	P	gi|194545153|ref|YP_002080181.1|
	PvdQ	P(2)	gi|194546375|ref|YP_002081403.1|
P. entomophila L48	PAM	P(2)	gi|104781218|ref|YP_607716.1|
	AHL-acylase	Full (F),P	gi|104784167|ref|YP_610665.1|
P. fluorescens Pf-5	PAM	P(2)	gi|70730269|ref|YP_260008.1|
		P	gi|70733204|ref|YP_262977.1|
P. fluorescens Pf0-1	Peptidase S45	P(2)	gi|77458788|ref|YP_348294.1|
	PAM, SepQ protein	F,P	gi|77457440|ref|YP_346945.1|
P. mendocina ymp[b]	Peptidase S45, PAM	P	gi|146309254|ref|YP_001189719.1|
P. putida F1	Peptidase S45, PAM	P(2)	gi|148548004|ref|YP_001268106.1|
		P	gi|148550271|ref|YP_001270373.1|
P. putida GB-1	Peptidase S45, PAM	P(2)	gi|167033881|ref|YP_001669112.1|
		P	gi|167036204|ref|YP_001671435.1|
P. putida W619	Peptidase S45, PAM	P(2)	gi|170721584|ref|YP_001749272.1|
		P	gi|170719488|ref|YP_001747176.1|
P. syringae pv. phaseolicola 1448A[b]	PAM	P(2)	gi|71736215|ref|YP_274165.1|
P. syringae pv. syringae str. B728a[b]	Peptidase S45, PAM	P(2)	gi|66045211|ref|YP_235052.1|

Table 2 continued

Organism	Enzyme	CD[a]	GI No:
P. syringae pv. tomato str. DC3000[b]	PAM	P(2)	gi|28869364|ref|NP_791983.1|
Shewanella benthica KT99	Aculeacin A acylase	P(2)	gi|163749206|ref|ZP_02156456.1|
		P(2)	gi|163749207|ref|ZP_02156457.1|
Gamma proteobacterium KT 71	Peptidase S45, PAM	F	gi|88703935|ref|ZP_01101650.1|
		P	gi|88706973|ref|ZP_01104671.1|
		P(2)	gi|88706945|ref|ZP_01104644.1|
Marine Gamma proteobacterium HTCC2080	Aculeacin A acylase	F, P(2)	gi|119505017|ref|ZP_01627093.1|
	PAM	P	gi|119504715|ref|ZP_01626793.1|
Deltaproteobacteria			
Plesiocystis pacifica SIR-1	PAM, PAC	F, P(2)	gi|149923822|ref|ZP_01912213.1|
		F	gi|149917617|ref|ZP_01906114.1|
Stigmatella aurantiaca DW4/3-1	Aculeacin A acylase	P(2)	gi|115376563|ref|ZP_01463795.1|
		P(2)	gi|115378259|ref|ZP_01465428.1|
Gram-positive			
Deinococcus			
Deinococcus radiodurans R1b	Aculeacin A acylase	P(2)	gi|15807918|ref|NP_285578.1|
Actinobacteria			
Nocardioides sp. JS614	Peptidase S45, Penicillin Amidase (PAM)	P(2)	gi|119717562|ref|YP_924527.1|
		P(2)	gi|119715569|ref|YP_922534.1|
Streptomyces sp. Mg1	PAM	F, P(2)	gi|197333590|ref|ZP_03175656.1|
	Putative acylase	F, P(2)	gi|197754175|ref|YP_002177538.1|
Streptomyces griseus subsp. griseus NBRC 13350	PAM	P(2)	gi|182440602|ref|YP_001828321.1|
	Putative acylase	P	gi|182438087|ref|YP_001825806.1|
S. sviceus ATCC 29083	PAM	F, P(2)	gi|197933677|ref|ZP_03197971.1|
	Putative acylase	P(2)	gi|197780832|ref|YP_002203628.1|
Cyanobacteria			
Cyanothece sp. PCC 7424	Glutaryl-7-amino-cephalosporanic-acid acylase	F,P	gi|186900662|ref|ZP_02973619.1|
	Peptidase S45, PAM	P(2)	gi|186902234|ref|ZP_02975180.1|
Crocosphaera watsonii WH 8501	AHL-acylase	P(2)	gi|67923096|ref|ZP_00516587.1|
		F	gi|67923095|ref|ZP_00516586.1|
Chloroflexi			
Chloroflexus aurantiacus J-10-fl	Peptidase S45, PAM	P	gi|163847876|ref|YP_001635920.1|
		P	gi|163849271|ref|YP_001637315.1|
Herpetosiphon aurantiacus ATCC 23779	peptidase S45, PAM	P	gi|159898624|ref|YP_001544871.1|
		P	gi|159901506|ref|YP_001547753.1|
Bacteroidetes			
Robiginitalea biformata HTCC2501	PAM	P(2)	gi|88805528|ref|ZP_01121047.1|
		P	gi|88804265|ref|ZP_01119785.1|
		P(2)	gi|88804870|ref|ZP_01120390.1|

[a] Reference organism used was *Ralstonia* sp. XJ12B (Accession No. AAO41113.1) for searching Conserved Domains (CDs) of Ntn_hydrolase superfamily

[b] This information has been retained in the Table to show variability in copy number in different species of a given Genus

implications of this diversity and multiplicity of AHL-lactonases and -acylases along with HGT and continuous gain of new favourable functions, as a weapon to interfere with QS opens a new paradigm of evolutionary benefits to the host and therein may lie our answer for antibiotic resistance.

Conclusion

We have been able to elucidate the presence of multiple copies of AHL-lactonase and -acylase in diverse organisms. These genes may acquire novel functions to increase the fitness of the host organisms against those organisms, which operate specific functions through QS. *Bacillus* sp. being designated as GRAS (Generally Regarded As Safe) by the FDA and an industrial workhorse, is also the predominant species that produces lactonase and has a great potential in this field.

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Compliance with ethical standards

Conflict of interest All authors declare no conflicts of interest in this paper.

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