

RALSTONIA AHL-ACYLASE GENE

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] This invention pertains to the field of molecular biology. In particular, the invention relates to an N-acyl homoserine lactone acylase gene from *Ralstonia* sp. XJ12B.

Description of the Background Art

[0002] N-acyl homoserine lactones (AHLs), also known as autoinducers, are widely used quorum sensing signal molecules in many Gram-negative bacteria. These compounds regulate certain classes of target genes in bacteria, such as virulence genes or biofilm differentiation genes. Generally, quorum sensing molecules are highly conserved and share an identical homoserine lactone moiety. The length and structure of their acyl side chains are different, however. Although the target genes regulated by AHLs in different bacteria species are varied, basic mechanisms of AHL biosynthesis and gene regulation are conserved among different bacterial species.

[0003] The general feature of AHL-mediated gene regulation is that it is cell population dependent (quorum sensing). Bacteria secrete AHLs into the environment; extracellular concentration of AHLs increases as bacterial cell populations grow. When AHL accumulates to a threshold extracellular concentration, the expression of certain sets of target genes are triggered in the bacteria.

[0004] Bacteria using these signals release, detect and respond to the accumulation of AHL signal molecules for synchronizing expression of a particular sets of genes and coordinating cellular activities within the bacterial cell population. AHLs are involved in regulation of a range of biological functions, including bioluminescence in *Vibrio* species (13, 4), Ti plasmid

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conjugal transfer in *Agrobacterium tumefaciens* (31), induction of virulence genes in *Burkholderia cepacia*, *Erwinia carotovora*, *Erw. chrysanthemi*, *Erw. stewartii*, *Pseudomonas aeruginosa*, and *Xenorhabdus nematophilus* (3, 6, 12, 17, 19, 22, 23, 24, 26), regulation of antibiotic production in *P. aureofaciens* and *Erw. carotovora* (6, 26), swarming motility in *Serratia liquifaciens* (14) and biofilm formation in *P. fluorescens* and *P. aeruginosa* (1, 8). In many other bacterial species the relevant biological functions controlled by AHLs remain to be investigated (2, 5, 11).

[0005] A number of plant, animal and human bacterial pathogens use AHL quorum-sensing signals to regulate expression of pathogenic genes and aid in the formation of biofilms. Therefore, AHL quorum-sensing signal molecules are group of molecular targets for genetic and chemical manipulations since disruption of these signaling mechanisms can prevent or reduce the ability of these bacteria to infect plant and animal tissues or to form biofilms.

[0006] The gene encoding an AHL-inactivation enzyme (AiiA) from a Gram-positive bacterium (*Bacillus* strain 240B1) has been cloned (9). AiiA (also known as AHL-lactonase) inactivates AHL activity by hydrolyzing the lactone bond of AHLs (10).

Expression of *aiiA* in transformed *Erw. carotovora* (a pathogenic strain which causes soft rot disease in many plants) significantly reduces the release of AHL, decreases extracellular pectrolytic enzyme activities, and attenuates pathogenicity on potato, eggplant, Chinese cabbage, carrot, celery, cauliflower, and tobacco (9). Transgenic plants expressing AHL-lactonase showed a significantly enhanced resistance to *Erw. carotovora* infection and delayed development of soft rot symptoms (10). AHL-inactivation mechanisms appear to be widely distributed. For example, a bacterial isolate of *Variovorax paradoxus* was reported to use AHL molecules as its energy and nitrogen sources, indicating the possible presence of AHL-degrading enzymes (18).

[0007] Further methods to counteract AHL-mediated plant, animal and human disease and plant pathogen virulence by interfering with bacterial intercellular communication would be highly desirable.

SUMMARY OF THE INVENTION

[0008] Accordingly, in this study, the cloning and characterization of a gene encoding an AHL-acylase from a bacterial isolate *Ralstonia* sp. JX12B is reported.

[0008a] In a first aspect of the present invention, there is provided a nucleic acid according to SEQ ID NO: 1.

[0008b] In a second aspect of the present invention, there is provided a nucleic acid which comprises nucleotides 1234-3618 of SEQ ID NO: 1.

[0008c] In a third aspect of the present invention, there is provided a peptide having a sequence encoded by a nucleic acid consisting of nucleotides 1234-3618 of SEQ ID NO: 1.

[0008d] In a fourth aspect of the present invention, there is provided a peptide having a sequence according to SEQ ID NO: 2.

[0008e] In a fifth aspect of the present invention, there is provided a peptide having a sequence comprising amino acids 36-217 of SEQ ID NO:2.

[0008f] In a sixth aspect of the present invention, there is provided a peptide having a sequence comprising amino acids 233-794 of SEQ ID NO:2.

[0009] An embodiment, may be a composition of matter which comprises a nucleic acid according to SEQ ID NO:

1. Another embodiment, may be a composition of matter which comprises a nucleic acid selected from the group consisting of nucleotides 1234-3618 of SEQ ID NO: 1, a fragment thereof and a substantially homologous variant thereof.

[00010] Yet a further embodiment, may be a nucleic acid according to claim 2 which comprises nucleotides 1234-3618 of SEQ ID NO: 1.

[00011] Yet a further embodiment, may be a composition of matter which comprises a peptidic sequence selected from the group consisting of a peptidic sequence according to SEQ ID NO: 2, a fragment thereof and a substantially homologous variant thereof.

[00012] Yet a further embodiment, may be a composition of matter which comprises a peptidic sequence encoded by a nucleic acid selected from the group consisting of nucleotides 1234-3618 of SEQ ID NO: 1, a fragment thereof and a substantially homologous variant thereof.

[00013] Yet a further embodiment, may be a composition of matter which comprises a peptidic sequence selected from the group consisting of SEQ ID NO: 2, a fragment thereof, a subunit thereof and a substantially homologous variant thereof, such as a peptidic sequence according to SEQ ID NO: 2, a peptidic sequence comprising amino acids 36-217 233-794 of SEQ ID NO: 2 or a peptidic sequence comprising amino acids 233-794 of SEQ ID NO: 2.

[00014] Yet a further embodiment, may be a composition of matter as described above which inactivates AHL.

[00014a] In a tenth aspect of the present invention, there is provided an in vitro method of modulating AHL signaling activity which comprises contacting said AHL with a peptide as defined above.

[00015] Yet a further embodiment, may relate to a method of modulating AHL signaling activity which comprises contacting said AHL with a composition of matter as described above.

[00015a] In an eleventh aspect of the present invention, there is provided a transgenic plant comprising a nucleic acid as defined above.

[00015b] In a twelfth aspect of the present invention, there is provided a transgenic non-human animal comprising a nucleic acid as defined above.

[00016] Yet a further embodiment, may be a transgenic plant or non-human mammal harboring a nucleic acid as described above.

[00016a] In a thirteenth aspect of the present invention, there is provided use of a nucleic acid as defined above and its peptide product for the manufacture of a medicament for controlling a bacterial disease in a mammal wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.

[00017] Yet a further embodiment, may relate to a method of controlling a bacterial disease in a mammal which comprises administering to said mammal a composition of matter as described above, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.

[00017a] In a fourteenth aspect of the present invention, there is provided use of a peptide as defined above for the manufacture of a medicament for controlling a bacterial disease in a mammal, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.

[00018] Yet a further embodiment, may relate to a method of controlling a bacterial disease in a plant which comprises administering to said plant a composition of matter as described above, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.

[00018a] In a fifteenth aspect of the present invention, there is provided a method of controlling a bacterial disease in a plant which comprises administering to said plant a peptide as defined above, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.

[00018b] In a sixteenth aspect of the present invention, there is provided a method of controlling a bacterial disease in a plant which comprises transforming the plant with a nucleic acid as defined above.

[00018c] In a seventeenth aspect of the present invention, there is provided a method of controlling a bacterial disease in a plant using a bacteria transformed with a nucleic acid as defined above.

BRIEF DESCRIPTION OF THE DRAWINGS

[00019] Figure 1 is a photograph showing AHL inactivation bioassay results for bacterial cultures and bacterial proteins from the indicated bacterial clones. Figure 1A shows the results of a bioassay with bacterial cultures of *E. coli* DH5 α strains 13H10 (slice 1), 2B10 (slice 2), MUB3 (slice 3), MUC6 (slice 4), GST-QsbA (slice 5) and GST (slice 6), which contain plasmid clones or constructs p13H10, p2B10, pMUB3, pMUC6, pGST-QsbA, and pGST, respectively. Figure 1B shows results for bioassay of the indicated bacterial proteins GST-QsbA and GST.

[00020] Figure 2 is a graph showing the temperature and pH optimum profiles of AHL acylase.

[00021] Figure 3 is a graph showing the time course of OOHL inactivation by the purified AHL-acylase.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[00022] A bacterial isolate of *Ralstonia* sp. XJ12B from a biofilm sample in a water treatment system was found to enzymatically inactivate AHLs, bacterial quorum-sensing molecules, in a bioassay using *Agrobacterium tumefaciens* strain Nt1 (*traR*; *tra::lacZ749*) as an indicator for AHL activity. The gene encoding the protein exhibiting this enzyme activity for AHL inactivation (*qsba*) was cloned from a bacterial strain isolated from the biofilm sample and found to encode a peptide of 794 amino acids.

[00023] Bacterial cultures and bacterial proteins were assayed for the ability to inactivate AHL using *Agrobacterium tumefaciens* indicator cells. A *tumefaciens* was cultured at 28°C in MM medium as described in Zhang et al. (31). The bacteria or protein to be assayed is first mixed with an AHL substrate, for example N- β -oxooctanoyl-L-homoserine lactone (OOHL), and the reaction (inactivation of the AHL) is allowed to proceed. If AHL inactivation activity is present in the sample (i.e. the AHL has been cleaved and inactivated), then the inactivated AHL products fail to trigger the expression of *lacZ* reporter gene which is under the control of a *TraR*-dependent promoter. The strain *A. Tumefaciens* NT1 hosting the *lacZ* reporter system therefore does not turn blue in the presence of substrate 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-gal). See Example 2 for details of the bioassay. Any AHL may be used in the assay, as desired. Of course, any suitable assay for cleavage of AHL, including traditional *in vitro* enzyme assays may be used to detect the AHL inactivation activity. Those of skill in the art are able to modify or devise assays to detect and/or quantitate AHL inactivation.

[00024] *Escherichia coli* strain DH5a was used as a host for DNA manipulation. Both *Ralstonia* sp. and *E. coli* were cultured in LB medium (tryptone, 10 g/L, yeast extract, 5 g/L, and NaCl, 10 g/L, pH 7.0) at 37°C. Appropriate antibiotics were added when necessary at the following concentrations: ampicillin, 100 μ g/ml; tetracycline, 10 μ g/ml; and kanamycin, 20 μ g/ml.

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[00025] The gene encoding the protein responsible for the detected AHL inactivation was isolated using a cosmid library of 1600 clones with the genomic DNA of *Ralstonia* sp. strain XJ12B, constructed in *E. coli*. *E. coli* transfectants were screened for the ability to inactivate AHL. One clone, p13H10, was found to inactivate AHL. Cosmid DNA from p13H10 was digested, fused into a cloning vector, ligated and transformed into *E. coli*. The *E. coli* clones again were assayed for AHL inactivating activity. One clone, containing a 4 kb insert, had AHL inactivation activity.

[00026] Plasmids were subsequently purified for sequencing. The 4 kb fragment from clone p2B10 was completely sequenced according to known methods using ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems). See Table I, below. The sequence contained an open reading frame of 2385 nucleotides which was the AHL inactivation gene, *qsba*, encoding a predicted polypeptide of 794 amino acids (85,373 Daltons).

Table I. *QsbA* Gene (*Ralstonia* sp.) Nucleotide Sequence (SEQ ID NO: 1).

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gtttgggaaagtgggnagcgcgctgtgcag  cggcccgccctcagccgcgagctcggg  cgcaccgaatgcgcgcgctggggcgccc  90
ggcggctggcgggtgtggcgccggtacagg  cgcgggaaggcggacatgtcgtgataacc  cactgttcggcgattgcgctcaggtcagc  180
gtgctgacttccagcaggtggcaggcgcg  tccacgcgcagccggtgcagcaattgcag  ggcgaggtgccagggtcttggtagaatgc  270
cgcagcagcgtgcgctcgtgtgtagggcg  gcggcgccagcttggccaggtcgaacggc  tcgtgcaggtgctgctgcaggtagcgccc  360
gcccgcagttaccacgctggtgcggtggcg  ggcttgcgtgcgcagccagatggcggtgg  tcaccgcgcgacgggtggtcgcagcaggg  450
tgccgaggggtgcgtgccagcgggtgtcg  gccagcgcgccgaccagcgctgcgtgagc  gccacgcgctgctccatcgcgcgccgct  540
accagcttgcgctgctgacgatggcctgc  tccgccaccaccttcagctgcgggtagtt  ccgtgcagccagccggcgatcagccagtc  630
accgtcaagcgccggcgccggcgagcgcg  ccggccagcagccacgcggtgaaggac  gaggccaccaagcctgcggcgctccagtc  720
gcgcggatggtggcgcggttccactccag  cagggccaggcgctgctccagcgtgctgat  gtggtcgaaatgcaggggcgggagcacc  810
cgctgcgcccagcgcgctgcgcccggcg  cagcgctgagcagcgccaggggtctc  ggcggcgcgctgcagcggcgcggtcgcg  900
cgcgaccagcgccacccgaacacccggct  ggcgcatcgccagcgttgcggcgatgcat  ggagcgagcgcatggccacgcgaggg  990
gtcgcgacggtgcgcccagcgtggagagg  ggctcgggaaaggtcagcaggtcgatgtc  ggcatccgcaaagtataggggagggcg  1080
gaggcctcctgcgtggcggtgattgaccca  actctgggggaataacctcttccctcg  cgggccccagtcgacgatacggcggtg  1170
gcgctcgcgccgcgcgaagactagagcg  acacaagacaaagcagcaacaggagaca  cgcATGATGcAGGgATTCGCGCTGCGCGC  1260
ACGCTCGCATGCGCGCTGCGCGCTGCGC  GCGCGCTGCGCGCTGCGCGCTGCGCGC  TGGGGGTGCGCTGCGCGACCGCGCTGTCC  1350
GCCGAGATCGCGCGCACCGGCTTCGGCAT  CCGCACATCGCGCGCAACGACTACGCCAG  CTCGCTATGGCATGGCTATGCCATCGCG  1440
CAGGACACCTGTGCTGCTGGCGACCGAC  GTGGTCACCGTCAACGGCGAGCGCTCGA  ACCTTCGGGCCCCGAGGGCACCCTGACGG  1530
TCGTTCAAGCGCATCCCCACCTGCAGTCG  GACGCTTCTTCAAGGGCATCTTCGACGAG  GACGGCTGCGCGCGGTTATGCGCGAGAT  1620
TCGCGCGAGGCGCGCGAGTGTGCGCGCG  TACATCGCGCTTCAACCGCTATCTCAAG  GACACGCGCGCGCGCAACTTCCGCGCGCG  1710
TGCGCGAATGCGCGCTGGGTGCGCTGCG  ACGCTGGGCGACATGATGCGCATGGGCG  GAGAAGGCGATCCAGGCGAGCGCGCGCG  1800
ATGCTGGCGGGCATGCTGCGCGCGCAGCG  CCGGGCGCGACGCGCGGTGGCGGAGCGG  ATTCCGCGCGAGGCGCTGACACCGTGGCG  1890
CTGGACCGCGAACTGCAGCTGCGCGACAT  CCGATCGGCTCCACGGCTGGGCTTTCGG  GCTGACCGCACCGCGCAACCGCGCGCGG  1980
CTGCTCGGCAATCCGCACTTCCCGTGGAG  ACCACCAACCGCTTCTACCGAGTCCACCT  ACGGTGCCCGGCAAGCTCGAGCTGATGG  2070
GCCCTCGATCGCGCGCTTCCCGTGGTGAG  ATCGGCTTCAACAAGGACGTGGCGTGGAC  CACACCGTCTCCACCGCGCGCGCTTACC  2160
TTGTTGGAATGAAGCTGGCGGAAGCGGAC  CCGACCACTTACCTGGTGCAGCGGCGCG  CACAAGATGACCAACCGCACGGTTCGCT  2250
GACGTGAAGCTGCGCGAGCGCGCGCTCGA  CGCGCGACGCAACCTTCTACGACACCAT  TACGGCGCGGTGCTGTCGATCGCGAGCG  2340
GGCATGCGCGTGGACCGCAGAGGCGCTAC  GCGCTGCGCGAGCGCAACCGCAACACAG  CGCTCGGTGCGACGCTGGCTGCATATCG  2430
CAGGCGCGGAGCGTGGCGCGCATCCGCGAG  GCCATCGGCAACCTGGGCTTCTTGGGTC  AACACCATCGCGACCGCGCAACCGCGCG  2520
GCCCTGTTGCGCGAGCTGTCGACACGCGG  GACGTGCGCGCGCGGAGCTCCAGCGCTGT  GCCCGTTCGCGCTGGCGCGCAACTCTTC  2610
AAGGACGCGCGGCTGGTGTCTCGACGGC  TCGCGCGGCGACCTGCAACTGGCAGTTC  CCGGCTTCGCGGTACCGCGGCTGGTGGG  2700
CCGCGCGCATGCGCGGTGCTCGACGGGAC  GACTACGTCCCGCAACAGTACACGCTTC  TGGCTGACCAACCGCGCGCAAAAGTGA  2790
GGCTTCTGCGCGGTGATGGGCTCGGTCGAC  GTACCGCAGCGGCTGCGCACGCGCATCG  CTGATCGAGATCGGCGCGCGCTGGCGCG  2880
ACCGACGGATGCGCGGCAACCGCATCGAT  CTGCGGAACCTGCGAGCGATGATCTTCA  AATGCCAACCTGGCGGAGCAACTGGTGT  2970
GGCGACCTGCTCGCGCATGCAAGGCCACG  CCGGCCCGGATGCGGAGCTGCGCGAGCG  TGGCGCGCGCTCGGCGAGTGAACCGCAC  3060
AGCAACGCGCGACCGCGCGCGCGCACCTG  TTCCGCGAGTCTTGGATGCGCGCAAGGAC  ATCGCGCAGGTGCACGCGCTCGAGTTCG  3150
CGGCGCGACCGGCTCCACGCGCGCGCGCG  CTGCGCATGACGCGCGAGCTAGCGACG  GCGGTGTTCAAGCGCGTGAAGGAAGCGG  3240
GGGCGGTGCGCAAGCGCGGCTTCGCGCTG  GATGCGCGGCTGCGCGAGCTACAGGCGCG  CACGCACCGGACGGCTCCATCGCGCTGC  3330
GGCGCGGAGGAATACGAAGGCGTGTCAAC  AAGCTGCAAAACCTGCGGATCGGCGCGA  GGGCTGCGCGGTGATTTTCGCGACCGAG  3420
ATCCAGACCGTGCAGCTTCGACGACGAGG  CCGGTGCGCGAGCGCATCTTCACTACGG  GAATCGAGCGACCGCGCTCGCGCGACGG  3510
TTCGACCGATGCGGTGCTGCTGCGGCAAG  CACTGGAACCGGCTGCGCTTCTCCGAAG  GCCATCGCGCGCGATCGGCGCTGAAGTG  3600
ATCGGTTGTGCGAGTGAAGgggtgcgggtg  cctggaaaaacgccccgttgcggggcg  ttttttgcagtggtgaatggctcaatcgt  3690
gttggaaaccgcatccggacatgactgtat  tgtgactctgcctgtgtccgtgt 3743

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The predicted open reading frame of the *qsbA* gene is shown in upper case letters with the start codon and stop codon in bold. A putative ribosome binding site (AGGAGA) is underlined.

[00027] Sequence analysis of this peptide indicated that *QsbA* did not have any significant homology with the known AHL-lactonase quorum-sensing molecule inactivator encoded by the *aiiA* gene from *Bacillus* sp. 240B1, however the deduced peptide sequence was typical of the primary structure of aculeacin A acylases (AACs) and penicillin G acylases, with signal peptide- α subunit-spacer- β subunit organization (16, 30). The *Ralstonia* sequence shares substantial identity with AACs from *Deinococcus radiodurans* strain R1, *Actinoplanes utahensis* and a putative acylase from *Pseudomonas aeruginosa*, all of which catalyze deacylation of their substrates. These AAC genes are translated as single precursor polypeptide and then processed to the active

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form, which has two subunits. Aculeacin A is an echinocandin-type antifungal antibiotic with a long fatty side chain.

Aculeacin A acylases purified from *A. utahensis* catalyze the hydrolysis of the amide bond on the palmitoyl side chain of aculeacin A (29). The primary structure of the protein, as well as enzyme activity analysis with different substrates, discussed below, therefore indicates that *qsbA* encodes an AHL-acylase which cleaves the amide linkage between the acyl side chain and the homoserine lactone moiety of AHLs.

[00028] The presumed α and β subunits of QsbA are located at amino acid positions 36-217 and 233-794, respectively, of SEQ ID NO: 2, with a 15 amino acid spacer between them, as determined by alignment with the peptide sequences from *D. radiodurans* strain R1, *A. utahensis* and *P. Aeruginosa*. See Table II.

Table II. Aligned Amino Acid Sequences of QsbA from *Ralstonia* sp. XJ12B (SEQ ID NO: 2), *D. radiodurans* strain R1 acylase (SEQ ID NO: 3), *A. utahensis* acylase (SEQ ID NO: 4) and *P. aeruginosa* acylase (SEQ ID NO: 5).

R.sp	MMQGF---ALRGTLAMAALALAGCA-----SSTDGRWGSLSDTGLSAEIRRTGFGIPHIRANDYASLGGMAYAYAQDN 72
D.rad	MSR-----SPFSSVSLPARLLGSLI-----LGPLMLGGAASAQTYQVQIQTARHGIPIQASDLGGIGYGVGYSYAQDN 70
A.uta	MTSSY---MRLKAAAIAGFVIVATAA-----VPSPAS-GREHDGGYAALIRRASYGVPHITADDFGSLGFGVGYVQAEDN 71
P.aer	MSRPFPRPLCRETTSNMGMRVTLTGLAGMLLGSMMPVQADMPRPTGLAADIRWTAYGVPHIRAKDERGLGYGIGYAYARDN 80
	* : : : . * : : : * * * * * : : * * *
	1
R.sp	LCLLADQVTVNGERSKTFGPEGTVTVSFKPIPNLQSDAFFKGFDEDDGLRAGYAQMSPEARELLRGGYIAGFNRYLKDTP 152
D.rad	LCLLADQVMTVRGERSKFLGAEKTVVGFQPVNNLSDVFFKTVIEFGRQLQAGYRDQ-PQILALMRGYVAGVNNRYLRDTP 149
A.uta	ICVIAESVVTANGERSRWFGATGPDDADVTTSSQTQIDDRVAERLLEGPRDGVRAPCDDVDRDQMRGFVAGVNNHFLRRTG 151
P.aer	ACLLAEEIVTARGERARYFGSEGKSSAELD---NLPSDIFYAWLNQPEALQAFWQAQTPAVRQLLEGYAAGFNRLFLREAD 157
	* : : : : * * : : * . . . :
R.sp	PANFP-AACRNAAWVRPLTLGDMMRMGEEKAIQASAGAMLGIVAAQPPGRTPVAEREITPPQAVDTVALDRELQLRDMPI 231
D.rad	PEQWP-SACRNADWVRPLTELDMRLGEEKAIQASAGAMVSAITSARPPQ---AGASTAAPRPDLAAFNQYRFNDLPI 224
A.uta	VHRLTDPACRGKAWVRPLSEIDLWRTSWDSMVRAGSGALLDGIVAATPPT---AAGPASAPEAPDAAAIAAALDGTSGAI 228
P.aer	GKTTS---CLGQPWLRAIATDLDLRLRLRLVGGVGFADALVAAAPPG---AEKVALSGEQAFQVAEQRRQRFRLER 230
	. * . * : : : * : * : : * * : : * * *
	1
R.sp	GSNGWAFGADATANRRGVLLGNPHFPWTTNRFYQVHLTVPGKLDVMGASIAAFPVVVSIGFNKDVAVHTVSTGRRFTLF 311
D.rad	GSNGWAFGSEATTNGRGLLGNPHFPWETSNRFYQLHLTLPGQFDVMGASLGGMPVVNIGFNQDVAVHTVSTDKRFTLA 284
A.uta	GSNAYGLGAQATVNGSGMVLNPHFPWQGAERFYRMHLKVPGRYDVEGAALIGDPIIEIGHNRTVAWSHTVSTARFVNH 288
P.aer	GSNAIavgSERSADGKMILLNPHFPWNGAMRFYQHLTIPGRLDVMGASLPGLPVVNIGFSRHLAWHTVDTSSHFTLY 290
	***. . : : : * : * * * * * : * : * * * * * : * : * * * * * : *

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R.sp      ELKLAEGDPTTYLVDGTPHKMTTRTVAFDVKLPDGRLERRTHTFYDTIYGVLSPSGGMPWTTQKAYALRDANRNNTRS 391
D.rad     ALTIVPGDPLSYVDKQQRRRLQRRTAIVIEVKTANG-PRLHTRTVYFTPEGELVNLPAAGLTWTPQYAFALRDANRNNTRM 383
A.uta     RLSLVPGDPTSYVVDGRPERMRARTVTVTQTGSG-----PVSRTFHDTRYGFVAVVP-GTFDWTPTATAYAITDVNAGNNRA 383
P.aer     RLALDPKDPFRYLVDGRSLPLEEKSAIEVRGADGKLSRVEHKVYQSIYGLPVVWP-GKLDWNRSEAYALRDANLENTRV 390
          * * * * * : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
          * * * * * : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :

R.sp      VDSWLHIGQARDVAGIRQAIG-NLGIPVWNTIATDRNGRALFADVSTTPDVPAEELQRCAPSPLAGKLFKDAGLVLLDGS 470
D.rad     LATWLGFAGAKSVRDIRASLN-VQGIPIWNTIAADRAGSALYADISSSPNVSAQQQACTPPPLA-PLFPAAGLAVLDGS 461
A.uta     FDGWLRMGQAKDVRALKAVLDRHQFLPWVNVIAADARGEALYGDHSSVVPRTGALAAACIPAPFQ-PLYASSGQAVLDGS 461
P.aer     LQQWYSINQASDVADLRRRVEALQGIPIWNTLADEQGNALYMNQSVVPYLPKPELIPACAIPLQV-----AEGLPALQGG 464
          . * : * . * : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
          . * : * . * : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :

R.sp      RGTCTNWQVDPASVPVGLVAPARMPVLERDDYVANSNDSSWLTPAQLTGFSPVMGSDVPQRLRTRIGLIEIGRRLAGT 550
D.rad     HSACDWTDPASRVPGRLRAPDKMPVLIRODFVANSNNSAWLANPAAPQTGLDPLVGEVNAPQSPRTRMGLLEIGRRLSGT 541
A.uta     RSDCALGADPDAAVPGILGPASLPVRFRDDYVNTNSDSHWLASPAAPLEGFPRLGNERTPRSLRTRGLDQIQRLAGT 541
P.aer     DSRCAWSRDPAAQAAGITPAAQLPVLLRRDFVQNSNDSAWLTNPASPLQGFSPVLSQE-KPIGPRARYALSRLQCKQP-- 543
          . * * * : . * : . : * * * * * : : : : : : : : : : : : : : : : : : : : : : : : : : : : :

R.sp      DGLPGNRIDLPLNLOAMIFSNANLAGQLVLGDLAACKATPAPDAD-----VRDGCALGQWNRTSNADA-RAAHLFREF 630
D.rad     DGLPGRTFDIPTLQATLLRESNLTGEMYAADAALKQS--AGGAE-----LQPACNALAAWDRSSQES-RGAALWREF 619
A.uta     DGLPGKGFTTARLWQVMFNGRMHGAELVRDDLVALCRRQPTATASNGAIVDLTAACIALSRFDERADLDS-RGAHLFTEF 621
P.aer     -----LEAKTLEEMVTANHVFSADQVLPDLRLCRDN-QGEKS-----LARACAALAQWDRGANLDSGSGFVYFQRF 613
          : * : : : : * * : : : : : : : : : : : : : : : : : : : : : : : : : : : :

R.sp      WMRAKDIAQVHAVEFDPADPVHTPRGLR-MNDATVRTAVFKALKEAVGAVRKAGFALDAPLGTVQAAHAPDGSIALHGGE 702
D.rad     WRRARAI PNVAVPFDPADPVNTPRGLN-TADPAQTALLGALREAAALTAAGIPFDAPLGEVQGVVRGGDFISLPGGA 691
A.uta     LAGG-----IRFADTFEVTDPVRTPAPFWNTTDFRVRTALADACNGSPASPSTR-----SVGDIHTDSRGERRIPINGGR 690
P.aer     MQRFAELDGAWKEPFAQRLDTPQGIA-LDRPQVATQVROALADAAAEVEKSGIPDGARWGDLOVSTRQERIAIPGGD 686
          * : * : * : : . . * : * : : . : : * : : * : : * : : * : : * : : * : : * : : * : : * : : * : :

R.sp      EYEGVLNKLQTLPIGPKGLPVYFG--TSYIQVTFDDQGPVADAILTYGESTDHPHAFDQMRAYS GKHNRLPFSEAA 780
D.rad     EFEGVLDKIDFNPLAPGGYRGVVGNGASSYIQTVGFTDSGVQAEAVLTYSQSSNPESPYFSQTRLEFSRSEWVKLPFTQPE 771
A.uta     GEAGTFNVITNPLVPGVGYQVVGHG-TSFMMAVELGPHGPGSRQILTYAQSTNPNSPWYADQTVLYSRKGDWDTIKYTEAQ 769
P.aer     GHFGVYNAIQS--VRKGDHLEVVG--TSYIQVTFPEEGPKARGLLAFSQQSSDPERSPHYRDQTELFSTRQWQTLFPFSRQ 763
          * . : : : . . : : : * : * . : : : : : : : : * * * : * . * : : :

R.sp      IAADPALKVMRLSQ--- 794
D.rad     IEADPTRTVQLSE--- 785
A.uta     IAADPNLRVYRVAQRGR 786
P.aer     IDADPQLQRLSIRE--- 777
          * * * : :

```

* = identical residues, : = conserved substitutions; . = semi-conserved substitutions; ! = post-translational processing sites for signal peptide and subunits; - = spacers.

[00029] The coding region of the *gsbA* gene was amplified by PCR. The amplified PCR products were digested, fused in-frame to the coding sequence of the glutathione S-transferase (GST) gene and expressed in *E. coli*. Protein extracts from the recombinant *E. coli* cells were assayed for the ability to inactivate AHL. Protein from *E. coli* expressing GST alone

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served as a control. The results demonstrated that GST-QsbA fusion protein effectively eliminated AHL activity. See Figure 1B.

[00030] The substrate specificity of QsbA was determined by assaying total soluble protein extracted from the recombinant *E. coli* (pGST-QsbA) for inactivation of AHLs using substrates with acyl chains of differing lengths. QsbA was able to completely inactivate 3-oxo group acyl-HSLs having acyl chains of 8, 10 and 12 carbons. QsbA also strongly inactivated methylene group acyl-HSLs having acyl chains of 8 and 10 carbons. QsbA also inactivated the butyl and hexyl esters of N- β -octanoyl-L-homoserine, whereas the AHL-lactonase encoded by *aiiA* was unable to inactivate them. The substrate specificity data indicate that QsbA is an AHL-acylase.

[00031] QsbA and *qsbA* provide new tools for down regulation of AHL-mediated biological activities, such as the expression of virulence genes and biofilm differentiation in pathogenic bacteria, both *in vitro* and *in vivo*. The *qsbA* gene, which encodes the AHL inactivation enzyme (QsbA), or a functional fragment, subunit or substantially homologous variant thereof, may be introduced into a plant genome to produce a genetically modified plant with the ability to quench pathogen quorum-sensing signaling. Transgenic plants expressing an enzyme that inactivates AHLs can exhibit a significantly enhanced resistance to infection by bacterial pathogens, even when bacteria are present in high concentrations.

[00032] Methods of genetic manipulation and transformation of plant cells are well known in the art, as are methods of regenerating fertile, viable transformed plants. In general, any method of cloning the coding region of *qsbA* or a functional fragment or substantially homologous variant thereof into a suitable expression vector may be used. It is convenient to ligate the *qsbA* coding region into a vector, followed by ligation into a plant transformation vector, however those of skill are well aware of alternative methods to achieve the same results. Any suitable plant transformation vector may be used.

The vector contains the *qsbA* gene, or a functional fragment, subunit or substantially homologous variant thereof, so long as expression of the gene results in a QsbA protein or functional fragment, subunit or variant thereof which inactivates AHL.

[00033] A functional promoter preferably controls expression. Many suitable promoters are known in the art, such that a convenient promoter may easily be selected by a skilled artisan depending on the expression system being used. Such selection of a suitable promoter to achieve the desired level of translational expression is considered routine in the art. For example, it is advantageous to optimize *qsbA* expression by modification of codon usage and coupling to a strong promoter such as the double 35S promoter.

[00034] A suitable marker gene, such as kanamycin resistance, green fluorescent protein or any other convenient marker is advantageously used. Variations of the commonly used and well known methods for transforming plants with a gene, are well within the skill of the ordinary artisan in genetic manipulation of plants. Expression constructs may contain a signal sequence to direct secretion of the expressed QsbA protein, or may lack such a sequence, as desired. The plant transformation vectors containing the *qsbA* gene and a marker gene may be used to transform plant cells using *Agrobacterium*-mediated transformation. *Agrobacterium*-mediated transformation is conveniently used to transform plants with the *qsbA* gene, however any suitable method known in the art may be used, depending on the plant being transformed. For example, certain monocotyledonous plants are more efficiently transformed using other methods such as microprojectile bombardment, vacuum filtration or any other method known in the art to introduce and integrate DNA plasmids or fragments into the plant genome. Those of skill in the art are familiar with means to transform gymnosperms, monocots and dicots. All of these methods known in the art are contemplated for use with this invention.

[00035] After selection for transformants carrying the *qsbA* gene, transgenic plants may be regenerated according to known

methods in the art. Plants selected for a marker gene, for example kanamycin resistance, may be assayed, for example by PCR and DNA gel blot to determine how many copies of the *qsbA* gene are present in the plant tissue. Any suitable method known in the art is contemplated for use with the gene of this invention. QsbA enzyme activity may be detected in transgenic plants transformed with the *qsbA* gene by the bioassay method described in Example 2 or by any convenient method.

[00036] By "functional fragment, subunit or substantially homologous variant thereof," when referring to a *qsbA* nucleotide sequence, it is meant any fragment, subunit, variant or homologous sequence of *qsbA* (nucleotides 1234-3618 of SEQ ID NO: 1) which encodes a protein or peptide sequence capable of inactivating N-acyl homoserine lactones. "Substantially homologous variants" of a nucleotide sequence generally are those the complement of which hybridizes with *qsbA* under stringent or highly stringent conditions, for example temperatures of about 30°C to about 50°C, for example 30°C, 35°C, 37°C, 40°C, 45°C or 50°C, and/or salt concentrations of about 200 mM to about 1000 mM NaCl or the equivalent ionic strength, for example 200 mM, 250 mM, 300 mM, 400 mM, 500 mM, 750 mM or 1000 mM. The stringency conditions are dependent on the length of the nucleic acid and the base composition of the nucleic acid and can be determined by techniques well known in the art. Those of skill in the art are familiar with these conditions and ranges which are useful. Generally, a substantially homologous nucleotide sequence is at least about 75% homologous to SEQ ID NO: 1 or a fragment or subunit thereof, preferably at least about 85% homologous, and most preferably 90%, 95% or 99% homologous or more.

[00037] Those of skill in the art are familiar with the degeneracy of the genetic code, and thus are aware that nucleic acid sequences may be less than 100% homologous and yet encode the same protein or peptide sequence. Such variation in any of the sequences, fragments, subunits or substantially homologous variants also are contemplated as part of this invention.

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[00038] Peptide and protein sequences which are encompassed by this invention include any sequences encoded by the *qsba* gene, or any fragment, subunit or substantially homologous variant thereof. Such sequences therefore include any functional protein or peptide which retains the ability to inactivate AHL, including protein and peptide fragments of the complete QsbA protein, such as, for example, the sequences of amino acids 36-217 and 233-794 encoding by SEQ ID NO: 1 and substantially homologous variants thereof. A substantially homologous variant of the QsbA protein includes sequences which are at least about 50% homologous, preferably at least about 60% homologous, and most preferably 70%, 80% or 90% homologous or more. Therefore, a protein which is a substantially homologous variant of QsbA is about 50% to about 99.9% homologous with QsbA. Both conservative and non-conservative amino acid substitutions are contemplated, as well as sequences containing non-traditional or modified amino acids such as those known in the art.

[00039] The term "fragment" is intended to indicate any portion of the nucleotide of SEQ ID NO: 1 or protein/peptide sequence of SEQ ID NO: 2 which is greater than about 300 nucleotide bases or about 100 amino acids, up to one nucleotide or amino acid less than the entire sequence. The term "subunit" is intended to encompass any functional unit of the QbsA protein, such as, for example, amino acids 36-217 or 233-794 of SEQ ID NO: 2.

[00040] A protein or peptide sequence which is considered to inactivate N-acyl homoserine lactones is one which is capable of inactivating at least 55 pmoles N-acyl homoserine lactone (OOHL) per μ g protein per minute at 30°C.

[00041] It has been previously demonstrated that quenching bacterial quorum sensing by inactivation of N-acyl homoserine lactone with AHL-lactonase stops bacterial infection (9, 10). The gene and protein described here, which is likely an AHL-acylase, represent a new and effective tool for inactivation of AHL signals and thus control bacterial infection. Similarly,

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the gene and protein described here targets AHL quorum-sensing signals that regulate expression of pathogenic genes of many bacterial pathogens at a threshold concentration. This tool is applicable to all plant, animal or human diseases where the expression of pathogenic genes of bacterial pathogens is activated by AHL signals, such as, for example, plant pathogens *Erw. carotovora*, *Erw. Chrysanthemi*, *Erw. Stewartii*; human pathogens *P. aeruginosa*, *B. cepacia*; and animal pathogens *X. nematophilus*, *P. fluorescens* (1, 3, 6, 12, 17, 19, 22, 23, 24, 26).

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[00042] The following examples are provided to illustrate the invention described herein and should not be construed to limit the appended claims.

EXAMPLES

Example 1. Bacterial Isolation.

[00043] A bacterial biofilm sample was collected from a water treatment system and screened to isolate AHL inactivation bacterial strains. The bacterial mixture was suspended in sterilized water with shaking for 1 hour before spreading onto YEB medium (yeast extract, 5 g/l; casein hydrolysate, 10 g/l; NaCl, 5 g/l; sucrose, 5 g/l; MgSO₄·7H₂O, 0.5 g/l and agar, 15 g/l) plates. Individual colonies were restreaked on new plates to ensure purity of the isolates. Bacterial isolates were cultured in LB medium (tryptone, 10 g/L; yeast extract, 5 g/L, and NaCl, 10 g/L; pH7.0) in 1.5-ml Eppendorf™ tubes or 96-well plates at 28°C, with shaking, overnight, and assayed for AHL inactivation activity.

Example 2. AHL Inactivation Bioassay.

[00044] The bacterial culture to be assayed was mixed with an equal volume of fresh medium containing 20 μ M N- β -oxooctanoyl-L-homoserine lactone (OOHL), or another AHL, when specified, to form a reaction mixture. The reaction mixture was incubated at 28°C for 4 to 5 hours, followed by 30 minute sterilization under UV light. Plates containing 20 ml MM agar medium (K_2HPO_4 , 10.5 g/L; KH_2PO_4 , 4.5 g/L; $MgSO_4 \cdot 7H_2O$, 0.2 g/L; $FeSO_4$, 4.5 mg/L; $CaCl_2$, 10 mg/L; $MnCl_2$, 2 mg/L; $(NH_4)_2SO_4$, 2.0 g/L; mannitol, 2.0 g/L; pH 7.0) supplemented with 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-Gal, 40 μ g/ml) were prepared. The solidified medium was cut, still inside the plate, into separated slices (approximately 1 cm in width). See Figure 1. Five microliters of sterilized reaction mixture was loaded at the top of an MM agar strip, and then AHL indicator cells (*Agrobacterium tumefaciens* strain NT1 (*traR*; *tra::lacZ749*) (25) 0.7 μ l cell suspension with an optical density at 600 nm of 0.4) were spotted at progressively further distances from the loaded samples. Plates were incubated at 28°C for 24 hours. A positive result for AHL inactivation is shown by the absence of blue colonies on the slice. A negative result for AHL inactivation is shown by the presence of blue colonies on the slice. For assay of protein for enzyme activity, total soluble bacterial protein was incubated with 20 μ M of OOHL (or other AHL) at 37°C for 1 hour as the reaction mixture.

Example 3. Identification and Cloning the *qsba* Gene.

[00045] Two bacterial isolates from the biofilm sample with distinct phenotypes, XJ12B and XJ12A, were found to possess the ability to inactivate AHL, with XJ12B showing stronger enzyme activity. The XJ12B isolate was cultured, centrifuged and sonicated. The strongest enzymatic activity was associated with the cell debris fraction rather than the soluble protein and supernatant fractions. These results indicated that the AHL inactivation activity is membrane associated. Sequencing of 16S rRNA was performed to identify the XJ12A and XJ12B isolates.

The 16S rRNA sequences of these isolates showed 97% and 96% identity, respectively, with that of *Ralstonia eutropha*.

[00046] To identify the gene encoding for AHL inactivation, a cosmid library of 1600 clones was constructed in *E. coli* with the genomic DNA of *Ralstonia sp.* strain XJ12B. Genomic DNA from the isolated *Ralstonia sp.* strain XJ12B was partially digested with Sau3A. The resulting DNA fragments were ligated to the dephosphorylated BamHI site of cosmid vector pLAFR3 (28). The ligated DNA was packed with Gigapack III XL Packaging Extract (Stratagene) and transfected into *E. coli* DH5alpha. These *E. coli* transfectants were screened for AHL inactivation activity according to the methods described in Example 2 using OOHl as the substrate. Only a single clone (p13H10) was identified as showing AHL inactivation activity (see Figure 1A, slice 1). To subclone the gene encoding the detected activity, cosmid DNA from the positive clone p13H10 was partially digested with Sau3A and fused into BamHI digested cloning vector pGEM-3Zf (+). The plasmids were ligated and transformed into *E. coli*, and the *E. coli* were assayed for the ability to inactivate AHL as described in Example 2. Clone p2B10, which contains a 4 kb insert, had AHL inactivation activity (see Figure 1A, slice 2). The TGS™ Template Generation System F-700 (Finnzymes OY) was used to mutate p2B10 plasmid DNA by randomly inserting the artificial Mu transposon, following the manufacturer's instructions. Plasmid clone p2B10, which contains the 4 kb insert containing the *qsba* gene, was used as a template. Fifteen mutant clones were produced, and none was able to inactivate AHL. Bacterial cultures of *E. coli* DH5α containing pMUG3 and pMUC6 are shown as examples in Figure 1A, slices 3 and 4, respectively. Plasmids were subsequently purified for sequencing using primers supplied in the kit.

Example 4. Sequencing and Sequence analysis of the *qsba* Gene.

[00047] Sequencing was performed according to known methods using ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems). The 4 kb

fragment from clone p2B10 was completely sequenced and is shown in Table I. The sequence contains an open reading frame of 2385 nucleotides with an ATG start codon and a TGA stop codon (SEQ ID NO: 1, nucleotides 1259-3643). Based on the MU transposon mutagenesis data described in Example 3, this open reading frame is the coding region of the AHL inactivation gene, designated as *qsbA*. A putative ribosome binding site (AGGAGA) is located 6 base pairs upstream of the first ATG start codon (underlined in Table I).

[00048] The deduced peptide sequence shows the typical polypeptide primary structure of aculeacin A acylases (AACs) and penicillin G acylases, with signal peptide- α subunit-spacer- β subunit organization (16, 30). There are four additional potential start codons located 3, 36, 189 and 384 downstream from the first ATG. The longest open reading frame encodes 794 amino acids, with a predicted molecular weight of 85373 Daltons. The deduced peptide has 78 strongly basic and strong acid amino acid residues and a predicted isoelectric point of 7.48. The first 20 amino acid residues of the assumed open reading frame appear to be a signal peptide.

[00049] The peptide sequence of *qsbA* deduced from the open reading frame shares 40-52% identity with AACs from *Deinococcus radiodurans* strain R1, *Actinoplanes utahensis* and a putative acylase from *Pseudomonas aeruginosa*. The AACs' catalyze deacylation of their substrates. These AAC genes are translated as single precursor polypeptide and then processed to the active form of two subunits. By alignment with the peptide sequences from *D. radiodurans* strain R1, *A. utahensis* and *P. aeruginosa*, Table II, the presumed α and β subunits are located at amino acid positions 36-217 and 233-794, respectively, with a 15 amino acid spacer between them. *QsbA* shares less than 28% homology with penicillin G acylase (20) and cephalosporin acylase (21). See Table II. The amino acid sequence alignment in Table II was analyzed by the Clustal W program available from the European Bioinformatics Institute website (<http://www.ebi.ac.uk>).

Example 5. Expression of the *QsbA* Gene.

[00050] The coding region of the *qsbA* gene was amplified by PCR using a forward primer 5'-CGTGGATCCATGATGCAGGATTCGCCGCTGCGC-3' (SEQ ID NO: 6) and a reverse primer 5'-CGCGAATTCACCGGCAGCCCTCATGCGACAAC-3' (SEQ ID NO: 7) containing BamHI and EcoRI restriction sites, respectively. The amplified PCR products were digested using the above restriction enzymes and fused in-frame to the coding sequence of the glutathione S-transferase (GST) gene under the control of the isopropyl β -D-thiogalactopyranoside (IPTG) inducible lac promoter in vector pGEX-2T (Amersham Pharmacia) to generate construct pGST-QsbA. pGST-QsbA was transformed into *E. coli* and expressed.

[00051] Total soluble protein was extracted from the recombinant *E. coli* cells harboring the GST-QsbA-encoding fusion construct according to methods known in the art, based on the methods described in Dong et al. (9), and assayed for AHL inactivation. The total soluble protein from *E. coli* containing GST vector only was used as a control. For the bioassay, 50 μ l of the soluble protein preparation (20 μ g/ μ l) was added to the same volume of 40 μ M AHL, e.g., OOHl. After a 1 hour incubation at 37°C, the reaction mixture was assayed as described in Example 2. Representative data, shown in Figure 1B, slice 1, indicate that the soluble GST-QsbA fusion protein effectively eliminated OOHl activity.

Example 6. Characterization of the Substrate Spectrum of GST-QsbA Fusion Protein Expressed in *E. coli*.

[00052] To determinate the substrate spectrum of *QsbA*, total soluble protein extracted from the recombinant *E. coli* (pGST-QsbA) was assayed for inactivation of AHLs with acyl chains of differing lengths according to the methods of Example 2. The following AHLs were synthesized according to known methods as described by Zhang et al. (31): (1) N-octanoyl-L-homoserine lactone (C8HSL, OOHl); (2) N-decanoyl-L-homoserine lactone (C10HSL, DHL); (3) N- β -oxohexanoyl-L-homoserine lactone (3-oxo-

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C6HSL, OHHL); (4) N- β -oxohexanoyl-L-homoserine lactone (3-oxo-C12HSL, OdDHL); (5) N- β -oxohexanoyl-L-homoserine lactone (3-oxo-C8HSL, OOHHL). The butyl and hexyl esters of N- β -oxohexanoyl-L-homoserine were prepared by esterification of N- β -oxohexanoyl-L-homoserine lactone with 1-butanol and 1-hexanol respectively, in the presence of small amount of Dowex 50H+ resin (Aldrich). The reaction was conducted at 60°C for 2 hours and the products were purified by silica column chromatography.

[00053] QsbA completely inactivated OOHHL, N- β -oxodecanoyl-L-homoserine (ODHL) and N- β -oxododecanoyl-L-homoserine (OdDHL), which have acyl chains of 8, 10 and 12 carbons, respectively, at the concentrations tested (data not shown). QsbA also strongly inactivated N- β -octanoyl-L-homoserine (OHL) and N- β -decanoyl-L-homoserine (DHL), which have acyl chains of 8 and 10 carbons, respectively (data not shown). However, under the same reaction conditions, QsbA had less inactivating activity for N- β -oxohexanoyl-L-homoserine (OHHL), which has an acyl chain of 6 carbons (data not shown). The total soluble protein extract from control *E. coli* (pGST) did not show any activity against AHLs (data not shown).

[00054] QsbA also completely inactivated the butyl and hexyl esters of N- β -octanoyl-L-homoserine (data not shown). These two esters of N- β -octanoyl-L-homoserine showed comparable induction activity with OOHHL when assayed with the AHL reporter strain *A. tumefaciens* NT1 (*traR*; *tra::lacZ749*) (data not shown). AHL-lactonase (encoded by *aiaA*) did not inactivate these substrates. These substrate specificity data are consistent with identification of QsbA as an AHL-acylase.

Example 7. Purification of AHL-acylase encoded by the *qsbA* gene

[00055] The GST-[AHL-acylase] fusion protein was purified using a glutathione Sepharose 4B affinity column following the manufacturer's instructions (Pharmacia). AHL-acylase was cleaved by digestion with thrombin (Sigma). Protein concentration was determined by measuring OD₂₈₀.

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[00056] The purified AHL-acylase was incubated with OOHL for 20 minutes and the relative enzyme activity was measured by determining the residual OOHL in the reaction mixture, which contained 8 μ M OOHL and about 0.6 μ g AHL-acylase in a total reaction volume of 50 ml 1x PBS buffer. The reactions were stopped by addition of 1% SDS before loading on the assay plate. Determination of the OOHL activity was carried out in quadruplicate. AHL-acylase degraded OOHL in a range of temperatures from 22-42°C at pH 7.0. See Figure 2. The optimal temperature for enzyme activity was found to be approximately 28°C. Reaction temperature higher than 42°C decreased enzyme activity sharply. The optimal pH for enzyme activity also was determined. The AHL-acylase has a relatively narrow optimal pH range from pH 6.5 to 7.5. See Figure 2. The time course of OOHL inactivation by the purified AHL-acylase was determined at 30°C. See Figure 3. After 10 minutes, more than 82% OOHL had been degraded; the reaction rate was estimated to be about 55 pmols per μ g AHL-acylase per minute.

CLAIMS:

1. A nucleic acid according to SEQ ID NO: 1.
2. A nucleic acid which comprises nucleotides 1234-3618 of SEQ ID NO: 1.
3. A peptide having a sequence encoded by a nucleic acid consisting of nucleotides 1234-3618 of SEQ ID NO: 1.
4. A peptide having a sequence according to SEQ ID NO: 2.
5. A peptide having a sequence comprising amino acids 36-217 of SEQ ID NO: 2.
6. A peptide having a sequence comprising amino acids 233-749 of SEQ ID NO: 2.
7. A peptide according to claim 4 which inactivates AHL.
8. An *in vitro* method of modulating AHL signaling activity which comprises contacting said AHL with a peptide according to any one of claims 3 to 7.

9. A transgenic plant comprising a nucleic acid of claim 2.
10. A transgenic non-human animal comprising a nucleic acid of claim 2.
11. A method of controlling a bacterial disease in a plant which comprises administering to said plant a peptide according to any one of claims 3 -7, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.
12. A method of controlling a bacterial disease in a plant which comprises transforming the plant with a nucleic acid according to claim 2.
13. A method of controlling a bacterial disease in a plant using a bacteria transformed with a nucleic acid according to claim 2.
14. A nucleic acid according to claim 2 or a peptide according to any one of claims 3-7 for use in therapy.
15. Use of a nucleic acid according to claim 2 and its peptide product for the manufacture of a medicament for controlling a bacterial disease in a mammal wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.
16. Use of a peptide according to any one of claims 3-7 for the manufacture of a medicament for controlling a bacterial disease in a mammal, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.
17. The use of claim 15 or 16 wherein said mammal is a human.

SEQUENCE LISTING

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<222> (16)..(16)

<223> unknown

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cgccggaagg cggacatgtc gtgataaccg cactgttcgg cgattgccgt caggctcagc	180
gtgctgactt ccagcaggtg gcaggcgcg tccacgcgca gccggtgcag caattgcagc	240

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ctgctcggca atccgcactt cccgtggacg accaccaacc gcttctacca ggtccacctg	2040
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atcggcttca acaaggacgt ggcgtggacg cacaccgtct ccaccggccg ccgcttcacc	2160
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tgtgactctg cctgtgtccg tgt

3743

<210> 2

<211> 794

<212> PRT

<213> Ralstonia sp.

<400> 2

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 20 25 30

Leu Ser Asp Thr Gly Leu Ser Ala Glu Ile Arg Arg Thr Gly Phe Gly
 35 40 45

Ile Pro His Ile Arg Ala Asn Asp Tyr Ala Ser Leu Gly Tyr Gly Met
 50 55 60

Ala Tyr Ala Tyr Ala Gln Asp Asn Leu Cys Leu Leu Ala Asp Gln Val
 65 70 75 80

Val Thr Val Asn Gly Glu Arg Ser Lys Thr Phe Gly Pro Glu Gly Thr
 85 90 95

Val Thr Val Ser Phe Lys Pro Ile Pro Asn Leu Gln Ser Asp Ala Phe
 100 105 110

Phe Lys Gly Ile Phe Asp Glu Asp Gly Leu Arg Ala Gly Tyr Ala Gln
 115 120 125

Met Ser Pro Glu Ala Arg Glu Leu Leu Arg Gly Tyr Ile Ala Gly Phe
 130 135 140

Asn Arg Tyr Leu Lys Asp Thr Pro Pro Ala Asn Phe Pro Ala Ala Cys
 145 150 155 160

Arg Asn Ala Ala Trp Val Arg Pro Leu Thr Leu Gly Asp Met Met Arg
 165 170 175

Met Gly Glu Glu Lys Ala Ile Gln Ala Ser Ala Gly Ala Met Leu Ala
 180 185 190

Gly Ile Val Ala Ala Gln Pro Pro Gly Arg Thr Pro Val Ala Glu Arg
 195 200 205

Glu Ile Pro Pro Gln Ala Val Asp Thr Val Ala Leu Asp Arg Glu Leu
 210 215 220

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Gln Leu Arg Asp Met Pro Ile Gly Ser Asn Gly Trp Ala Phe Gly Ala
 225 230 235 240
 Asp Ala Thr Ala Asn Arg Arg Gly Val Leu Leu Gly Asn Pro His Phe
 245 250 255
 Pro Trp Thr Thr Thr Asn Arg Phe Tyr Gln Val His Leu Thr Val Pro
 260 265 270
 Gly Lys Leu Asp Val Met Gly Ala Ser Ile Ala Ala Phe Pro Val Val
 275 280 285
 Ser Ile Gly Phe Asn Lys Asp Val Ala Trp Thr His Thr Val Ser Thr
 290 295 300
 Gly Arg Arg Phe Thr Leu Phe Glu Leu Lys Leu Ala Glu Gly Asp Pro
 305 310 315 320
 Thr Thr Tyr Leu Val Asp Gly Thr Pro His Lys Met Thr Thr Arg Thr
 325 330 335
 Val Ala Phe Asp Val Lys Leu Pro Asp Gly Arg Leu Glu Arg Arg Thr
 340 345 350
 His Thr Phe Tyr Asp Thr Ile Tyr Gly Pro Val Leu Ser Met Pro Ser
 355 360 365
 Gly Gly Met Pro Trp Thr Thr Gln Lys Ala Tyr Ala Leu Arg Asp Ala
 370 375 380
 Asn Arg Asn Asn Thr Arg Ser Val Asp Ser Trp Leu His Ile Gly Gln
 385 390 395 400
 Ala Arg Asp Val Ala Gly Ile Arg Gln Ala Ile Gly Asn Leu Gly Ile
 405 410 415
 Pro Trp Val Asn Thr Ile Ala Thr Asp Arg Asn Gly Arg Ala Leu Phe
 420 425 430
 Ala Asp Val Ser Thr Thr Pro Asp Val Pro Ala Ala Glu Leu Gln Arg
 435 440 445
 Cys Ala Pro Ser Pro Leu Ala Gly Lys Leu Phe Lys Asp Ala Gly Leu
 450 455 460
 Val Leu Leu Asp Gly Ser Arg Gly Thr Cys Asn Trp Gln Val Asp Pro
 465 470 475 480
 Ala Ser Pro Val Pro Gly Leu Val Ala Pro Ala Arg Met Pro Val Leu
 485 490 495
 Glu Arg Asp Asp Tyr Val Ala Asn Ser Asn Asp Ser Ser Trp Leu Thr
 500 505 510
 Asn Pro Ala Gln Lys Leu Thr Gly Phe Ser Pro Val Met Gly Ser Val
 515 520 525

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Asp Val Pro Gln Arg Leu Arg Thr Arg Ile Gly Leu Ile Glu Ile Gly
 530 535 540

Arg Arg Leu Ala Gly Thr Asp Gly Leu Pro Gly Asn Arg Ile Asp Leu
 545 550 555 560

Pro Asn Leu Gln Ala Met Ile Phe Ser Asn Ala Asn Leu Ala Gly Gln
 565 570 575

Leu Val Leu Gly Asp Leu Leu Ala Ala Cys Lys Ala Thr Pro Ala Pro
 580 585 590

Asp Ala Asp Val Arg Asp Gly Cys Ala Ala Leu Gly Gln Trp Asn Arg
 595 600 605

Thr Ser Asn Ala Asp Ala Arg Ala Ala His Leu Phe Arg Glu Phe Trp
 610 615 620

Met Arg Ala Lys Asp Ile Ala Gln Val His Ala Val Glu Phe Asp Pro
 625 630 635 640

Ala Asp Pro Val His Thr Pro Arg Gly Leu Arg Met Asn Asp Ala Thr
 645 650 655

Val Arg Thr Ala Val Phe Lys Ala Leu Lys Glu Ala Val Gly Ala Val
 660 665 670

Arg Lys Ala Gly Phe Ala Leu Asp Ala Pro Leu Gly Thr Val Gln Ala
 675 680 685

Ala His Ala Pro Asp Gly Ser Ile Ala Leu His Gly Gly Glu Glu Tyr
 690 695 700

Glu Gly Val Leu Asn Lys Leu Gln Thr Leu Pro Ile Gly Pro Lys Gly
 705 710 715 720

Leu Pro Val Tyr Phe Gly Thr Ser Tyr Ile Gln Thr Val Thr Phe Asp
 725 730 735

Asp Gln Gly Pro Val Ala Asp Ala Ile Leu Thr Tyr Gly Glu Ser Thr
 740 745 750

Asp His Ala Ser Pro His Ala Phe Asp Gln Met Arg Ala Tyr Ser Gly
 755 760 765

Lys His Trp Asn Arg Leu Pro Phe Ser Glu Ala Ala Ile Ala Ala Asp
 770 775 780

Pro Ala Leu Lys Val Met Arg Leu Ser Gln
 785 790

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<210> 3

<211> 785

<212> PRT

<213> D. radiodurans

<400> 3

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 Leu Gly Ser Leu Leu Leu Gly Pro Leu Met Leu Gly Gly Ala Ala Ser
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 Ala Gln Thr Tyr Gln Val Gln Ile Gln Arg Thr Ala His Gly Ile Pro
 35 40 45
 His Ile Gln Ala Ser Asp Leu Gly Gly Ile Gly Tyr Gly Val Gly Tyr
 50 55 60
 Ser Tyr Ala Gln Asp Asn Leu Cys Leu Leu Ala Asp Gln Val Met Thr
 65 70 75 80
 Val Arg Gly Glu Arg Ser Lys Phe Leu Gly Ala Glu Gly Lys Thr Val
 85 90 95
 Val Gly Phe Gln Pro Val Asn Asn Leu Asp Ser Asp Val Phe Phe Lys
 100 105 110
 Thr Val Ile Glu Pro Gly Arg Leu Gln Ala Gly Tyr Arg Asp Gln Pro
 115 120 125
 Gln Ile Leu Ala Leu Met Arg Gly Tyr Val Ala Gly Val Asn Arg Tyr
 130 135 140
 Leu Arg Asp Thr Pro Pro Glu Gln Trp Pro Ser Ala Cys Arg Asn Ala
 145 150 155 160
 Asp Trp Val Arg Pro Leu Thr Glu Leu Asp Val Met Arg Leu Gly Glu
 165 170 175
 Glu Lys Ala Ile Gln Ala Ser Ala Gly Ala Met Val Ser Ala Ile Thr
 180 185 190
 Ser Ala Arg Pro Pro Gln Ala Gly Ala Ser Thr Ala Ala Pro Arg Pro
 195 200 205
 Asp Leu Ala Ala Phe Asn Arg Gln Tyr Arg Phe Asn Asp Leu Pro Ile
 210 215 220
 Gly Ser Asn Gly Trp Ala Phe Gly Ser Glu Ala Thr Thr Asn Gly Arg
 225 230 235 240

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Gly Leu Leu Leu Gly Asn Pro His Phe Pro Trp Glu Thr Ser Asn Arg
 245 250 255
 Phe Tyr Gln Leu His Leu Thr Leu Pro Gly Gln Phe Asp Val Met Gly
 260 265 270
 Ala Ser Leu Gly Gly Met Pro Val Val Asn Ile Gly Phe Asn Gln Asp
 275 280 285
 Val Ala Trp Thr His Thr Val Ser Thr Asp Lys Arg Phe Thr Leu Ala
 290 295 300
 Ala Leu Thr Leu Val Pro Gly Asp Pro Leu Ser Tyr Val Lys Asp Gly
 305 310 315 320
 Gln Gln Arg Arg Leu Gln Arg Arg Thr Ala Val Ile Glu Val Lys Thr
 325 330 335
 Ala Asn Gly Pro Arg Leu His Thr Arg Thr Val Tyr Phe Thr Pro Glu
 340 345 350
 Gly Pro Leu Val Asn Leu Pro Ala Ala Gly Leu Thr Trp Thr Pro Gln
 355 360 365
 Tyr Ala Phe Ala Leu Arg Asp Ala Asn Arg Asn Asn Thr Arg Met Leu
 370 375 380
 Ala Thr Trp Leu Gly Phe Ala Gly Ala Lys Ser Val Arg Asp Ile Arg
 385 390 395 400
 Ala Ser Leu Asn Val Gln Gly Ile Pro Trp Val Asn Thr Ile Ala Ala
 405 410 415
 Asp Arg Ala Gly Ser Ala Leu Tyr Ala Asp Ile Ser Ser Ser Pro Asn
 420 425 430
 Val Ser Ala Ala Gln Gln Gln Ala Cys Thr Pro Pro Pro Leu Ala Pro
 435 440 445
 Leu Phe Pro Ala Ala Gly Leu Ala Val Leu Asp Gly Ser His Ser Ala
 450 455 460
 Cys Asp Trp Lys Thr Asp Pro Ala Ser Arg Val Pro Gly Leu Arg Ala
 465 470 475 480
 Pro Asp Lys Met Pro Val Leu Ile Arg Gln Asp Phe Val Ala Asn Ser
 485 490 495
 Asn Asn Ser Ala Trp Leu Ala Asn Pro Ala Ala Pro Gln Thr Gly Leu
 500 505 510
 Asp Pro Leu Val Gly Glu Val Asn Ala Pro Gln Ser Pro Arg Thr Arg
 515 520 525
 Met Gly Leu Leu Glu Ile Gly Arg Arg Leu Ser Gly Thr Asp Gly Leu
 530 535 540

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Pro Gly Arg Thr Phe Asp Ile Pro Thr Leu Gln Ala Thr Leu Leu Arg
 545 550 555 560
 Glu Ser Asn Leu Thr Gly Glu Met Tyr Ala Ala Asp Ala Ala Lys Leu
 565 570 575
 Cys Gln Ser Ala Gly Gly Ala Glu Leu Gln Pro Ala Cys Asn Ala Leu
 580 585 590
 Ala Ala Trp Asp Arg Arg Ser Ser Gln Glu Ser Arg Gly Ala Ala Leu
 595 600 605
 Trp Arg Glu Phe Trp Arg Arg Ala Arg Ala Ile Pro Asn Val Tyr Ala
 610 615 620
 Val Pro Phe Asp Pro Ala Asp Pro Val Asn Thr Pro Arg Gly Leu Asn
 625 630 635 640
 Thr Ala Asp Pro Ala Ala Gln Thr Ala Leu Leu Gly Ala Leu Arg Glu
 645 650 655
 Ala Ala Ala Ala Leu Thr Ala Ala Gly Ile Pro Phe Asp Ala Pro Leu
 660 665 670
 Gly Glu Val Gln Gly Val Val Arg Gly Gly Asp Phe Ile Ser Leu Pro
 675 680 685
 Gly Gly Ala Glu Phe Glu Gly Val Leu Asp Lys Ile Asp Phe Asn Pro
 690 695 700
 Leu Ala Pro Gly Gly Tyr Arg Gly Val Val Gly Asn Ala Ser Ser Tyr
 705 710 715 720
 Ile Gln Thr Val Gly Phe Thr Asp Ser Gly Val Gln Ala Glu Ala Val
 725 730 735
 Leu Thr Tyr Ser Gln Ser Ser Asn Pro Glu Ser Pro Tyr Phe Ser Asp
 740 745 750
 Gln Thr Arg Leu Phe Ser Arg Ser Glu Trp Val Lys Leu Pro Phe Thr
 755 760 765
 Gln Pro Glu Ile Glu Ala Asp Pro Thr Arg Thr Val Val Gln Leu Ser
 770 775 780
 Glu
 785

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<210> 4

<211> 786

<212> PRT

<213> A. utahensis

<400> 4

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Met Thr Ser Ser Tyr Met Arg Leu Lys Ala Ala Ala Ile Ala Phe Gly
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20      25      30
His Asp Gly Gly Tyr Ala Ala Leu Ile Arg Arg Ala Ser Tyr Gly Val
35      40      45
Pro His Ile Thr Ala Asp Asp Phe Gly Ser Leu Gly Phe Gly Val Gly
50      55      60
Tyr Val Gln Ala Glu Asp Asn Ile Cys Val Ile Ala Glu Ser Val Val
65      70      75      80
Thr Ala Asn Gly Glu Arg Ser Arg Trp Phe Gly Ala Thr Gly Pro Asp
85      90      95
Asp Ala Asp Val Arg Thr Thr Ser Ser Thr Gln Ala Ile Asp Asp Arg
100     105     110
Val Ala Glu Arg Leu Leu Glu Gly Pro Arg Asp Gly Val Arg Ala Pro
115     120     125
Cys Asp Asp Val Arg Asp Gln Met Arg Gly Phe Val Ala Gly Tyr Asn
130     135     140
His Phe Leu Arg Arg Thr Gly Val His Arg Leu Thr Asp Pro Ala Cys
145     150     155     160
Arg Gly Lys Ala Trp Val Arg Pro Leu Ser Glu Ile Asp Leu Trp Arg
165     170     175
Thr Ser Trp Asp Ser Met Val Arg Ala Gly Ser Gly Ala Leu Leu Asp
180     185     190
Gly Ile Val Ala Ala Thr Pro Pro Thr Ala Ala Gly Pro Ala Ser Ala
195     200     205
Pro Glu Ala Pro Asp Ala Ala Ala Ile Ala Ala Ala Leu Asp Gly Thr
210     215     220
Ser Ala Gly Ile Gly Ser Asn Ala Tyr Gly Leu Gly Ala Gln Ala Thr
225     230     235     240

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Val Asn Gly Ser Gly Met Val Leu Ala Asn Pro His Phe Pro Trp Gln
 245 250 255
 Gly Ala Glu Arg Phe Tyr Arg Met His Leu Lys Val Pro Gly Arg Tyr
 260 265 270
 Asp Val Glu Gly Ala Ala Leu Ile Gly Asp Pro Ile Ile Glu Ile Gly
 275 280 285
 His Asn Arg Thr Val Ala Trp Ser His Thr Val Ser Thr Ala Arg Arg
 290 295 300
 Phe Val Trp His Arg Leu Ser Leu Val Pro Gly Asp Pro Thr Ser Tyr
 305 310 315 320
 Tyr Val Asp Gly Arg Pro Glu Arg Met Arg Ala Arg Thr Val Thr Val
 325 330 335
 Gln Thr Gly Ser Gly Pro Val Ser Arg Thr Phe His Asp Thr Arg Tyr
 340 345 350
 Gly Pro Val Ala Val Val Pro Gly Thr Phe Asp Trp Thr Pro Ala Thr
 355 360 365
 Ala Tyr Ala Ile Thr Asp Val Asn Ala Gly Asn Asn Arg Ala Phe Asp
 370 375 380
 Gly Trp Leu Arg Met Gly Gln Ala Lys Asp Val Arg Ala Leu Lys Ala
 385 390 395 400
 Val Leu Asp Arg His Gln Phe Leu Pro Trp Val Asn Val Ile Ala Ala
 405 410 415
 Asp Ala Arg Gly Glu Ala Leu Tyr Gly Asp His Ser Val Val Pro Arg
 420 425 430
 Val Thr Gly Ala Leu Ala Ala Ala Cys Ile Pro Ala Pro Phe Gln Pro
 435 440 445
 Leu Tyr Ala Ser Ser Gly Gln Ala Val Leu Asp Gly Ser Arg Ser Asp
 450 455 460
 Cys Ala Leu Gly Ala Asp Pro Asp Ala Ala Val Pro Gly Ile Leu Gly
 465 470 475 480
 Pro Ala Ser Leu Pro Val Arg Phe Arg Asp Asp Tyr Val Thr Asn Ser
 485 490 495
 Asn Asp Ser His Trp Leu Ala Ser Pro Ala Ala Pro Leu Glu Gly Phe
 500 505 510
 Pro Arg Ile Leu Gly Asn Glu Arg Thr Pro Arg Ser Leu Arg Thr Arg
 515 520 525
 Leu Gly Leu Asp Gln Ile Gln Gln Arg Leu Ala Gly Thr Asp Gly Leu
 530 535 540

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Pro Gly Lys Gly Phe Thr Thr Ala Arg Leu Trp Gln Val Met Phe Gly
 545 550 555 560
 Asn Arg Met His Gly Ala Glu Leu Val Arg Asp Asp Leu Val Ala Leu
 565 570 575
 Cys Arg Arg Gln Pro Thr Ala Thr Ala Ser Asn Gly Ala Ile Val Asp
 580 585 590
 Leu Thr Ala Ala Cys Thr Ala Leu Ser Arg Phe Asp Glu Arg Ala Asp
 595 600 605
 Leu Asp Ser Arg Gly Ala His Leu Phe Thr Glu Phe Leu Ala Gly Gly
 610 615 620
 Ile Arg Phe Ala Asp Thr Phe Glu Val Thr Asp Pro Val Arg Thr Pro
 625 630 635 640
 Ala Pro Phe Trp Asn Thr Thr Asp Pro Arg Val Arg Thr Ala Leu Ala
 645 650 655
 Asp Ala Cys Asn Gly Ser Pro Ala Ser Pro Ser Thr Arg Ser Val Gly
 660 665 670
 Asp Ile His Thr Asp Ser Arg Gly Glu Arg Arg Ile Pro Ile His Gly
 675 680 685
 Gly Arg Gly Glu Ala Gly Thr Phe Asn Val Ile Thr Asn Pro Leu Val
 690 695 700
 Pro Gly Val Gly Tyr Pro Gln Val Val His Gly Thr Ser Phe Val Met
 705 710 715 720
 Ala Val Glu Leu Gly Pro His Gly Pro Ser Gly Arg Gln Ile Leu Thr
 725 730 735
 Tyr Ala Gln Ser Thr Asn Pro Asn Ser Pro Trp Tyr Ala Asp Gln Thr
 740 745 750
 Val Leu Tyr Ser Arg Lys Gly Trp Asp Thr Ile Lys Tyr Thr Glu Ala
 755 760 765
 Gln Ile Ala Ala Asp Pro Asn Leu Arg Val Tyr Arg Val Ala Gln Arg
 770 775 780
 Gly Arg
 785

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<210> 5

<211> 777

<212> PRT

<213> *P. aeruginosa*

<400> 5

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Met Ser Arg Pro Phe Arg Pro Pro Leu Cys Arg Glu Thr Thr Ser Met
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Gly Met Arg Thr Val Leu Thr Gly Leu Ala Gly Met Leu Leu Gly Ser
20          25          30
Met Met Pro Val Gln Ala Asp Met Pro Arg Pro Thr Gly Leu Ala Ala
35          40          45
Asp Ile Arg Trp Thr Ala Tyr Gly Val Pro His Ile Arg Ala Lys Asp
50          55          60
Glu Arg Gly Leu Gly Tyr Gly Ile Gly Tyr Ala Tyr Ala Arg Asp Asn
65          70          75          80
Ala Cys Leu Leu Ala Glu Glu Ile Val Thr Ala Arg Gly Glu Arg Ala
85          90          95
Arg Tyr Phe Gly Ser Glu Gly Lys Ser Ser Ala Glu Leu Asp Asn Leu
100         105         110
Pro Ser Asp Ile Phe Tyr Ala Trp Leu Asn Gln Pro Glu Ala Leu Gln
115         120         125
Ala Phe Trp Gln Ala Gln Thr Pro Ala Val Arg Gln Leu Leu Glu Gly
130         135         140
Tyr Ala Ala Gly Phe Asn Arg Phe Leu Arg Glu Ala Asp Gly Lys Thr
145         150         155         160
Thr Ser Cys Leu Gly Gln Pro Trp Leu Arg Ala Ile Ala Thr Asp Asp
165         170         175
Leu Leu Arg Leu Thr Arg Arg Leu Leu Val Glu Gly Gly Val Gly Gln
180         185         190
Phe Ala Asp Ala Leu Val Ala Ala Ala Pro Pro Gly Ala Glu Lys Val
195         200         205
Ala Leu Ser Gly Glu Gln Ala Phe Gln Val Ala Glu Gln Arg Arg Gln
210         215         220
Arg Phe Arg Leu Glu Arg Gly Ser Asn Ala Ile Ala Val Gly Ser Glu
225         230         235         240

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Arg Ser Ala Asp Gly Lys Gly Met Leu Leu Ala Asn Pro His Phè Pro
 245 250 255
 Trp Asn Gly Ala Met Arg Phe Tyr Gln Met His Leu Thr Ile Pro Gly
 260 265 270
 Arg Leu Asp Val Met Gly Ala Ser Leu Pro Gly Leu Pro Val Val Asn
 275 280 285
 Ile Gly Phe Ser Arg His Leu Ala Trp Thr His Thr Val Asp Thr Ser
 290 295 300
 Ser His Phe Thr Leu Tyr Arg Leu Ala Leu Asp Pro Lys Asp Pro Arg
 305 310 315 320
 Arg Tyr Leu Val Asp Gly Arg Ser Leu Pro Leu Glu Glu Lys Ser Val
 325 330 335
 Ala Ile Glu Val Arg Gly Ala Asp Gly Lys Leu Ser Arg Val Glu His
 340 345 350
 Lys Val Tyr Gln Ser Ile Tyr Gly Pro Leu Val Val Trp Pro Gly Lys
 355 360 365
 Leu Asp Trp Asn Arg Ser Glu Ala Tyr Ala Leu Arg Asp Ala Asn Leu
 370 375 380
 Glu Asn Thr Arg Val Leu Gln Gln Trp Tyr Ser Ile Asn Gln Ala Ser
 385 390 395 400
 Asp Val Ala Asp Leu Arg Arg Arg Val Glu Ala Leu Gln Gly Ile Pro
 405 410 415
 Trp Val Asn Thr Leu Ala Ala Asp Glu Gln Gly Asn Ala Leu Tyr Met
 420 425 430
 Asn Gln Ser Val Val Pro Tyr Leu Lys Pro Glu Leu Ile Pro Ala Cys
 435 440 445
 Ala Ile Pro Gln Leu Val Ala Glu Gly Leu Pro Ala Leu Gln Gly Gln
 450 455 460
 Asp Ser Arg Cys Ala Trp Ser Arg Asp Pro Ala Ala Ala Gln Ala Gly
 465 470 475 480
 Ile Thr Pro Ala Ala Gln Leu Pro Val Leu Leu Arg Arg Asp Phe Val
 485 490 495
 Gln Asn Ser Asn Asp Ser Ala Trp Leu Thr Asn Pro Ala Ser Pro Leu
 500 505 510
 Gln Gly Phe Ser Pro Leu Val Ser Gln Glu Lys Pro Ile Gly Pro Arg
 515 520 525
 Ala Arg Tyr Ala Leu Ser Arg Leu Gln Gly Lys Gln Pro Leu Glu Ala
 530 535 540

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Lys Thr Leu Glu Glu Met Val Thr Ala Asn His Val Phe Ser Ala Asp
 545 550 555 560
 Gln Val Leu Pro Asp Leu Leu Arg Leu Cys Arg Asp Asn Gln Gly Glu
 565 570 575
 Lys Ser Leu Ala Arg Ala Cys Ala Ala Leu Ala Gln Trp Asp Arg Gly
 580 585 590
 Ala Asn Leu Asp Ser Gly Ser Gly Phe Val Tyr Phe Gln Arg Phe Met
 595 600 605
 Gln Arg Phe Ala Glu Leu Asp Gly Ala Trp Lys Glu Pro Phe Asp Ala
 610 615 620
 Gln Arg Pro Leu Asp Thr Pro Gln Gly Ile Ala Leu Asp Arg Pro Gln
 625 630 635 640
 Val Ala Thr Gln Val Arg Gln Ala Leu Ala Asp Ala Ala Ala Glu Val
 645 650 655
 Glu Lys Ser Gly Ile Pro Asp Gly Ala Arg Trp Gly Asp Leu Gln Val
 660 665 670
 Ser Thr Arg Gly Gln Glu Arg Ile Ala Ile Pro Gly Gly Asp Gly His
 675 680 685
 Phe Gly Val Tyr Asn Ala Ile Gln Ser Val Arg Lys Gly Asp His Leu
 690 695 700
 Glu Val Val Gly Gly Thr Ser Tyr Ile Gln Leu Val Thr Phe Pro Glu
 705 710 715 720
 Glu Gly Pro Lys Ala Arg Gly Leu Leu Ala Phe Ser Gln Ser Ser Asp
 725 730 735
 Pro Arg Ser Pro His Tyr Arg Asp Gln Thr Glu Leu Phe Ser Arg Gln
 740 745 750
 Gln Trp Gln Thr Leu Pro Phe Ser Asp Arg Gln Ile Asp Ala Asp Pro
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32