BACTERIAL STRAINS, GENES AND ENZYMES FOR CONTROL OF BACTERIAL DISEASES BY QUENCHING QUORUM-SENSING SIGNALS

FIELD OF THE INVENTION

The present invention relates to genes encoding regulators of bacterial metabolism, more particularly to genes encoding enzymes that quench quorum-sensing signals. The present invention further relates to methods of control of bacterial diseases comprising expression of genes encoding autoinducer inhibitors.

BACKGROUND OF THE INVENTION

N-acyl-homoserine lactones, known as autoinducers (AIs), are widely conserved signal molecules present in quorum-sensing systems of many Gram-negative bacteria. It has been found that AIs are involved in the regulation of a range of biological functions, including bioluminescence in *Vibrio* species (Eberhard et al., 1981; Cao and Meighen, 1989), Ti plasmid conjugal transfer in *Agrobacterium tumefaciens* (Zhang et al., 1993), induction of virulence genes in *Erwinia carotovora, Erw. chrysanthemi, Erw. stewartii, Pseudomonas aeruginosa, P. solanacerum,* and *Xenorhabdus nematophilus* (Jones et al., 1993; Passador et al., 1993; Pirhonen et al., 1993; Pearson et al., 1994; Beck von Bodman and Farrand, 1995; Flavier et al., 1998; Costa and Loper, 1997; Nasser et al., 1998;),

regulation of antibiotic production in *P. aureofaciens* and *Erw. carotovora* (Costa and Loper, 1997; Pierson et al., 1994), regulation of swarming motility in *Serratia liquifaciens* (Eberl et al., 1996), and biofilm formation in *P. fluorescens* and *P. aeruginosa* (Allison et al., 1998; Davies et al., 1998). Many more bacterial species are known to produce AIs, but the relevant biological functions have not yet been established (Bassler et al., 1997; Dumenyo et al., 1998; Cha et al., 1998). Biofilm formation is of particular significance to bacterial pathogenicity, as it makes bacteria more resistant to antibiotics and host defense responses, and causes microbial contamination in medical devices and in drinking water pipelines.

Different bacterial species may produce different AIs. All AI derivatives share identical homoserine lactone moieties, but differ in the length and structure of their acyl groups. Although the target genes regulated by AIs are extremely varied, the basic mechanism of AIs biosynthesis and gene regulation seems to be conserved in different bacteria. The general feature of gene regulation by AIs is cell density dependence, also known as quorum sensing. At low cell densities the AIs are at low concentrations, and at high cell densities the AIs can accumulate to a concentration sufficient for activation of related regulatory genes (Fuqua and Winans, 1996). The biological functions regulated by AIs are of considerable scientific, economic, and medical importance. New approaches for up or down regulation of bacterial quorum sensing systems would be of

significant value, not only in science, but also in practical applications.

It has been reported recently that a novel gene encoding autoinducer inactivation (*aiiA*) has been cloned from the Gram-positive bacterium *Bacillus* sp. strain 240B1 (Dong et al., 2000). Expression of the *aiiA* in transformed *Erw. carotovora* strain SCG1, a pathogen that causes soft rot disease in many plants, significantly reduces the release of AI, decreases extracellular pectrolytic enzyme activities, and attenuates pathogenicity on potato, eggplant, Chinese cabbage, carrot, celery, cauliflower, and tobacco. The results indicate the promising potential of using the AI-inactivation approach for prevention of diseases in which virulence is regulated by quorum sensing signals.

SUMMARY OF THE INVENTION

Bacterial strains and enzymes capable of efficient inactivation of N-acyl homoserine lactone autoinducers (AIs) are of considerable interest for biotechnology applications. With the present invention it is disclosed that all *Bacillus thuringiensis* strains and their closely related species tested were capable of enzymatic inactivation of AIs. One AI synthesis minus mutant of *Agrobacterium tumefaciens* strain A6, caused by Tn5 insertion mutagenesis, was also found capable of producing AI inactivation enzyme. The genes encoding for AI inactivation enzymes were cloned either by a functional cloning approach or by a PCR approach from the selected bacterial strains. A peptide sequence comparison indicates that all of these enzymes belong to the metallohydrolase family, with amino acid identity ranging from 35.4% - 94.0 % to the previously

reported AiiA enzyme. The *B. thuringiensis* strains effectively quench AI activity when co-cultured with AI producing pathogenic bacteria, and provide effective biocontrol of potato soft rot disease caused by *Erwinia carotovora*. The data suggest that quenching biosignals which regulate virulence is an useful strategy for disease control, and that *B. thuringiensis* strains which are known for insecticidal activity are also promising biocontrol agents for prevention of diseases in which virulence is regulated by AIs.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the time course of AI (OOHL) inactivation by the protein extract of *A. tumefaciens* strain M103. The total protein of M103 was extracted by sonication disruption of bacterial cells in 1/15 M phosphate buffer (pH 8.0). Equal volumes of M103 protein extract (1.46 mg/ml) and 5000 nM OOHL were mixed and incubated in a 1.5 ml Eppendorf centrifuge tube at 28°C. Same protein extract was denatured by boiling for 5 min and used as a control. The samples were taken after 1, 3, 6 hr after reaction and the reaction was stopped by boiling for 3 min. The samples were analyzed for AI activity.

Figure 2 shows the cloning of the AI inactivation region from the cosmid clones of mutant M103. Two cosmid clones were contained in cosmid vector pLAFR3 while the four sub-clones in plasmid vector pBluescript II SK(+). Symbols: +, positive in AI inactivation; -, negative in AI inactivation; E: *Eco*RI; P: *Pst*I.

Figure 3 shows (A) The potential ORFs in the 1.5 kb AI inactivation region predicted with a sequence analysis program; and (B) Deletion analysis to define

the ORF encoding AI inactivation enzyme (AiiB). PCR amplified fragments were cloned into vector pBluescript II SK(+) (pBM clones) or in vector pKK223-3 (pKM clones). The numbers under each clone indicate the start and stop positions of the PCR fragments corresponding to the nucleotide sequences of the 1.5 kb region. All constructs were confirmed by sequencing analysis. The start codon (GTG) and stop codon (TAA) of the *aiiB* ORF are shown under the clone pKM103-315. Solid arrows indicate the location and direction of *lac* and *tac* promoter in these clones, the ORFs were indicated with open arrows. Symbols: +, positive AI inactivation activity; -, negative AI inactivation activity.

Figure 4 shows (A) the nucleotide sequence (SEQ ID NO 1) and (B) predicted peptide sequence (SEQ ID NO 11) of the *aiiB* gene cloned from *A. tumefaciens* M103. The putative ribosome binding (SD) region and two *Pst*I restriction enzyme sites are underlined, and the putative transcription termination codon is indicated.

Figure 5 shows the protein sequence comparison of AiiB (SEQ ID NO 11) and AttM (SEQ ID NO 21), a putative protein encoded by the *attM* gene in the *att* region of *A. tumefaciens*, but its biological function has not been demonstrated experimentally (GenBank accession No. U59485). These two proteins exhibit a high degree of similarity (the center sequence represents the consensus sequence, four fragments identical to amino acids 8-43, 45-158, 160-186 and 188-263 of SEQ ID NO 11), but functional AiiB protein has an additional 7 amino acids in the N-terminus.

Figure 6 shows a protein sequence comparison of

AiiB (SEQ ID NO 11) and AiiA (SEQ ID NO 22), a putative metallohydrolase which inactivates AI cloned from *Bacillus sp.* 240B1. The two conserved zinc binding regions are underlined.

Figure 7 shows the functional cloning of the *aiiC* gene. (A) Enzymatic inactivation of AI by the suspension culture of Bt strain Cot1. Equal volume of cell suspension culture (OD_{600} = 1.1) and 40 µM OOHL were mixed and incubated at 28°C (▲). The boiled culture and OOHL at same concentrations were used as control (■). The samples were taken at times as indicated for AI activity assay. (B) Direct subcloning of AI-inactivation regions from the cosmid clones pLAFR3-aiiC of *B. thuringiensis* Cot1. The cosmid clone was digested by *Eco*RI and subcloned into the pGEM-7Z vector. The AI inactivation positive clone pGEM7-aiiC was identified by enzyme activity assay. The pGEM7-aiiC was further subcloned in the pBluescript II SK(+) vector after *Bam*HI digestion. The AI inactivation region of about 1.4 kb in size contained in clone pBS-aiiC was completely sequenced. Restriction enzymes: E: *Eco*RI; B: *Bam*HI.

Figure 8 shows (A) the nucleotide sequence (SEQ ID NO 2) and (B) predicted peptide sequence (SEQ ID NO 12) of the *aiiC* gene cloned from the Bt strain Cot1. The nucleotide sequence of the *aiiC* ORF is indicated by the uppercase letters and the untranslated regions are indicated by the lower case letters.

Figure 9 shows the nucleotide sequences and predicted protein sequences of the genes *aiiD* (SEQ ID NOS 3 & 13), *aiiE* (SEQ ID NOS 4 & 14), *aiiF* (SEQ ID NOS 5 & 15), *aiiG* (SEQ ID NOS 6 & 16), *aiiH* (SEQ ID NOS 7 &

17), *aiiI* (SEQ ID NOS 8 & 18), *aiiJ* (SEQ ID NOS 9 & 19) and *aiiK* (SEQ ID NOS 10 & 20) from Bt strains B1, B2, B17, B18, B20, B21, B22 and B25, respectively.

Figure 10 shows a phylogenetic tree analysis and amino acid identity of 11 cloned AI inactivation genes. The phylogenetic tree was produced by DNASTAR sequence analysis software (DNASTAR Inc.). The distance is shown below the tree. The amino acid identity of each sample to AiiA is shown at the right hand of the graph.

Figure 11 shows the effect of Bt strains on AI production by *Erwinia carotovora*. *Erw. carotovora* SCG1 was inoculated alone in 15 ml LB medium (◆) or co-inoculated respectively in 1:1 ratio with Bt strains Cot1 (Δ) and B1 (●), *E. coli* DH5α (▲) and *B. fusiformis* (■) in the same medium. The inoculum concentration (T_0) was 1 x 10^7 CFU/ml (colony forming unit per milliliter) for SCG1 and 1 x 10^6 CFU/ml for others. After incubation of 1, 2, 3, 4, 6 and 24 hours at 30°C, the bacterial suspensions were taken and the supernatants were used to bioassay the AI produced. The data were means of four repeats.

Figure 12 shows the effect of Bt strain Cot1 on control of potato soft rot disease caused by *Erw. carotovora* SCG1. Dip: Potato slices were dipped into suspensions of Bt strain Cot1 (C), *E. coil* DH5α (D) or *B. fusiformis* (Bf) at a level of about 5 x 10^8 CFU/ml or water (W) for about 20 sec and then dried in a sterile air flow for about 20 min. The slices which showed no moisture on the surface were inoculated with 2.5 µl of bacterial suspension containing SCG1 cells equivalent to 5 x 10^5 or 5 x 10^4 CFU. Mixture: the cell culture of SCG1 (2 x 10^8 or 2 x 10^7 CFU/ml) was mixed respectively

with equal volumes of Cot1 (C), *E. coil* DH5α (D), *B. fusiformis* (Bf) (5 x 10^8 CFU/ml) or water (W). Two point five microlitres of the mixture was inoculated onto the top of slices. The final cell numbers of SCG1 inoculated are 2.5 x 10^5 or 2.5 x 10^4 CFU, as marked in the second line below the graph. After 20 hours incubation at 28°C the maceration area was measured. The data were the means of 4 or 12 (12 for Cot1) repeats.

Figure 13 shows the influence of Bt strains Cot1 and B1 on the growth of *Erwinia carotovora* SCG1. *Erw. carotovora* SCG1 (■) was inoculated alone or coinoculated with Bt strain Cot1 (◆) and B1 (▲) respectively in a 1:1 ratio in 15-ml LB medium. Each strain was inoculated to a final concentration of about 1 x 10^7 CFU/ml for SCG1 and 1 x 10^6 CFU/ml for the others in the T_0 medium. After 2, 4, 6 and 24 hours culture at 30°C, the bacterial suspensions were taken and diluted accordingly for spreading on plates for colony counting. The experiment was repeated four times and mean data were presented. Top: SCG1, Cot1, and B1 were incubated and grown separately; Middle: SCG1 was co-incubated with Cot1; Bottom: SCG1 was co-incubated with B1.

Figure 14 shows changes in bacterial cell numbers (A) and development of soft rot symptom (B) on inoculated potato slices. Potato slices were dipped into Cot1 suspensions (5 x 10^8 CFU/ml) (▲) or water (◆) for about 20 sec and then dried in a Laminar Flow for about 20 min. The slices were then inoculated with 5 µl of *Erw. carotovora* SCG1 (2 x 10^9 CFU/ml). After incubation of 1, 2, 3 and 4 days at 28°C, the

inoculated slices were cut into small pieces and 10 ml of 0.1 M NaCl solution was added for resuspension of bacterial cells. The mixture was shaken for 30 min and the suspension was diluted accordingly and spread on to plates for colony counting. The colony numbers of SCG1 were shown as log10 CFU/slice (▲ SCG1 only; ■ dipped in Cot1) and the numbers of Cot1 (◆) as log10 CFU/mm^2. The experiment was repeated four times and mean data were presented.

DETAILED DESCRIPTION OF THE INVENTION

Ten genes encoding AI inactivation enzymes have been cloned from 9 Gram positive bacterial isolates and one Gram negative bacterium (*A. tumefaciens*). The genes showed different levels of homologies to the *aiiA* gene, which encodes a putative metallohydrolase with strong AI inactivation activity (Dong et al., 2000). Similar to AiiA, the zinc binding motif regions are highly conserved in the enzyme proteins encoded by these newly cloned AI inactivation genes. It is very likely that these ten enzymes are also members of the metallohydrolase family, and use the same molecular mechanism as the AiiA for inactivation of N-acyl homoserine lactone autoinducers. The present invention further enriches the gene pool of AI inactivation enzymes.

In *A. tumefaciens*, N-acyl homoserine lactone autoinducers, mainly OOHL, are involved in regulation of Ti plasmid conjugal transfer (Zhang et al., 1993). The production of OOHL in *A. tumefaciens* is induced by the conjugal opines secreted by crown gall tumours (Zhang and Kerr, 1991). The OOHL in turn induces the expression of *tra* genes. Tra proteins are responsible

for completing the process of Ti plasmid conjugal transfer. Only a few hours are required from opine induction to completion of Ti plasmid conjugal transfer, so the Ti plasmid conjugal transfer can therefore be regarded as only a transient event. One embodiment of the present invention, the *aiiB* gene for N-acyl homoserine lactone degradation, identified in *A. tumefaciens*, highlights the possibility that the bacterium has a sophisticated mechanism for control of AI signal turn over. It is plausible that AI is degraded in *Agrobacterium* after completion of the Ti plasmid conjugal transfer.

It has been noted that a majority of bacterial isolates capable of AI inactivation are Gram positive, belonging to *B. thuringenesis* and closely related species. So far, most of the characterised quorum-sensing signals in Gram-negative bacteria are N-acyl homoserine lactones (Fuqua et al., 1996), while Gram-positive bacteria produce oligopeptides as quorum-sensing signals (Dunny and Leonard, 1997).

Bacillus thuringiensis (Bt) has been used extensively as a microbial insecticide during the last 30 years. The microorganism is a gram-positive, spore-forming soil bacterium, and produces a crystalline parasporal body consisting of one or more crystal (Cry) proteins during sporulation, which shows biocidal activity against insect families such as lepidopteran, dipteran, and colepteran insects at larval stages (Lambert and Peferoen, 1992). Some Bt strains have also been reported to be active against other insect families, as well as mites, nematodes, flatworms, and protozoa (Feitelson et al., 1992). Different Bt

strains produce more than 28 different but related groups of insecticidal crystal proteins (http://www.biols.susx.ac.uk/ Home/Neil_ Crickmore/Bt/). Different groups of crystal proteins are usually active against a specific spectrum of insects, but do not affect other beneficial insects in agriculture. Currently, Bt-based formulations are the most widely used and most effective microbial insecticides in agriculture.

As a valuable biocontrol agent, Bt has several advantages including its specificity for target insects, its low development cost, and its environmental compatibility (Lambert and Peferoen, 1992). Bt is commonly found in natural soil, and normally multiplies by cell division, but forms spores when nutrients are depleted or when the environment becomes adverse. These spores are highly resistant to stress conditions such as heat and drought, enabling the bacterium to survive periods of stress. This sporulating Gram-positive micro-organism can be formulated readily into stable products, such as a dry powder, for insect or disease biocontrol. Bt also has been subjected to many safety tests, with no harmful effects for animals or human beings.

Bt has not been exploited for disease control because it usually does not produce effective antibiotics against bacteria and fungi. In the present invention, it has been found that all tested Bt strains are capable of inactivating AI, and that Bt strains provide effective biocontrol against *Erw. carotovora* infection, whereas *B. fusiformis* and *E. coli* strains which do not have AI inactivation genes were unable to

provide biocontrol against *Erw. carotovora*. Bt strains did not produce any antibiotics and were not inhibitory to the growth of pathogen. The data strongly suggest the important role of AI inactivation genes in disease biocontrol. Because the AI diffuses easily into bacterial cells, Bt, capable of eliminating AI constantly from its surroundings, is a promising biocontrol agent, not only for control of plant soft rot disease caused by *Erw. carotovora*, but also for control of other diseases in which the virulence genes are regulated by AIs.

Accordingly, an object of the present invention is to provide a method for increasing resistance in a plant or animal to a disease in which virulence is regulated by AIs [such as the diseases caused by *Pseudomonas aeruginosa*, *Erwinia stewartii*, *Erwinia chrysanthemi*, *Pseudomonas solanacerum*, and *Xanthomonas campestris* (Passador, *et al.*, 1993; Pirhonen, *et al.*, 1993; Pearson, *et al.*, 1994; Beck von Bodman and Farrand, 1995; Barber, *et al.*, 1997; Clough, *et al.*, 1997; Costa and Loper, 1997; Nasser, *et al.*, 1998), and especially plant soft rot disease caused by *Erw. carotovora*] comprising administering to the plant or animal an effective amount of a bacterium that is capable of producing an autoinducer inhibitor. In a preferred embodiment of this aspect of the invention, the bacterium administered is a *Bacillus* sp., more preferably a variety of *Bacillus thuringiensis*, most preferably a variety of *B. thuringiensis* selected from the group consisting of B1, B2, B17, B18, B20, B21, B22 and B25. In another preferred embodiment of this aspect of the invention, the animal to be treated is a

human.

It is another object of the present invention to provide isolated nucleic acid molecules encoding autoinducer inactivation proteins. These nucleic acid molecules encode autoinducer inactivation proteins that share the conserved amino acid motif ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}. Preferred embodiments of these nucleic acid molecules encode the proteins of SEQ ID NOS 11-20, and most preferred embodiments of these nucleic acid molecules have the sequences of SEQ ID NOS 1-10.

Another object of the present invention is to provide an expression vector that comprises at least one nucleic acid sequence encoding an autoinducer inactivation protein, wherein the encoded protein comprises the conserved amino acid motif ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}, wherein the expression vector propogates in a procaryotic or eucaryotic cell. Preferred embodiments of these expression vectors comprise at least one nucleic acid sequence encoding a protein having a sequence selected from the group consisting of SEQ ID NOS 11-20, and most preferred embodiments have the nucleic acid sequences of SEQ ID NOs 1-10.

Yet another object of the present invention is to provide a cell of a procaryote or eucaryote transformed or transfected with an expression vector of the present invention.

Yet another object of the present invention is to provide an isolated protein which has autoinducer

inactivation activity, where the protein comprises the conserved amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}. Preferred embodiments of the invention comprise proteins having the amino acid sequences of SEQ ID NOS 11-20.

Yet another object of the present invention is to provide a method for increasing disease resistance in a plant or animal, which method comprises introducing into a cell of such plant or animal at least one nucleic acid molecule that encodes an autoinducer inactivation protein in a manner that allows said cell to express said nucleic acid sequence, wherein said autoinducer inactivation protein comprises the conserved amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}. Preferred embodiments of this aspect of the invention comprise introducing at least one nucleic acid molecule encoding a protein having a sequence selected from the group consisting of SEQ ID NOS 11-20, and most preferred embodiments comprising introducing at least one nucleic acid sequence selected from the group consisting of SEQ ID NOS 1-10.

Yet another object of the present invention relates to a method of preventing or reducing bacterial damage to a plant or animal, which method comprises administering to a plant or animal in need of such prevention or reduction an effective amount of at least one autoinducer inactivation protein, wherein said protein comprises the conserved amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif

^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}. Preferred embodiments of this aspect of the invention comprise providing at least protein having the amino acid sequences of SEQ ID NOS 11-20.

Yet another object of the present invention relates to a method of preventing or reducing the formation of bacterial biofilms, which method comprises exposing biofilm-forming bacteria to at least one autoinducer inhibitor protein, wherein said protein comprises the conserved amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}. Preferred embodiments of this aspect of the invention comprise exposing the biofilm-forming bacteria to at least protein having the amino acid sequences of SEQ ID NOS 11-20.

It is possible to further enhance the efficiency of Aii-producing bacterial strains by using a genetic approach to modify such strains, for example by introducing genes encoding for additional, or more active, autoinducer inhibitors. It also is possible to optimise the enzyme activity of *aii* genes by an *in vitro* DNA evolution approach. Increasing the expression of Aii enzymes by coupling the *aii* gene to a strong promoter or increasing the copy number of the *aii* gene in Bt cells would be another useful way to improve the capacity of Bt strains to quenching AI signals. It is likely that genetically modified Bt strains which secrete AI inactivation enzyme or contain the enzyme in the outer membrane of the cell could have better efficiencies in quenching AI signals than their wild type parent strain. This is achievable by fusing an *aii* gene to a sequence encoding a secretion or a

membrane attachment signal peptide.

The sequence may be introduced into plant or animal cells by well-known methods. Methods for the transformation or transfection of eukaryotic cells with exogenous nucleic acid sequences include transfection, projectile bombardment, electroporation or infection by *Agrobacterium tumefaciens*. These methods are likewise familiar to the person skilled in the area of molecular biology and biotechnology and need not be explained here in detail.

As pathogenic bacteria cells are confined to the intercellular area of plant tissues, it is desirable to target the Aii protein into the intercellular spaces. Such may be accomplished by fusing a secretion signal peptide to the Aii protein (Sato, *et al.*, 1995; Firek, *et al.*, 1993; Conrad and Fiedler, 1998; Borisjuk, *et al.*, 1999). Alternatively, a plant membrane attachment motif can be incorporated into the peptide sequence of Aii for anchoring the Aii enzyme in the outer surface of plant cell membrane.

The present invention also contemplates usage of a bacterial autoinducer inactivation protein directly to treat or prevent bacterial damage. For example, the protein may be applied directly to plants in need of such treatment or prevention. In a preferred embodiment, the protein is applied in the form of a composition which comprises an effective amount of the protein and a suitable carrier. The composition may have a wide variety of forms, including solutions, powders, emulsions, dispersions, pastes, aerosols, *etc.*

The bacterial autoinducer inactivation protein may also be used to treat bacterial infections in animals,

including humans. In that application, an effective amount of the active ingredient is administered to an animal in need of such treatment.

For therapeutic treatment, the active ingredient may be formulated into a pharmaceutical composition, which may include, in addition to an effective amount of the active ingredient, pharmaceutically acceptable carriers, diluents, buffers, preservatives, surface active agents, and the like. Compositions may also include one or more other active ingredients if necessary or desirable.

The pharmaceutical compositions of the present invention may be administered in a number of ways as will be apparent to one of ordinary skill in the art. Administration may be done topically, orally, by inhalation, or parenterally, for example. Topical formulations may include ointments, lotions, creams, gels, drops, suppositories,sprays, liquids and powders. Oral formulations include powders, granules, suspensions or solution in water or non-aqueous media, capsules or tablets, for example. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be used as needed. Parenteral formulations may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. The dose regimen will depend on a number of factors which may readily be determined, such as severity and responsiveness of the condition to be treated.

Traditionally, microbial biocontrol has depended on production of antibiotics or antimicrobial compounds (Cronin et al., 1997; Liao and Sapers, 1999; Emmert and Handelsman, 1999). The present invention offers an

alternative strategy for biocontrol, based on quenching biosignals that are essential for virulence.

Example 1: Bacterial strains capable of inactivating autoinducers

To identify the genes responsible for inactivation of autoinducer signals, more than 400 field and plant bacterial isolates and about 100 stains of the laboratory bacterial culture collection were screened. The bacterial strains used to test the ability of inactivating autoinducer signals were isolated from soil and plant suspensions as described previously (Dong et al., 2000), or obtained from *Bacillus* Genetic Stock Centre (BGSC) and the American Type Culture Collection (ATCC). *Erwinia carotovora* SCG1 was isolated from Chinese cabbage leaves showing soft rot symptoms. It was confirmed by 16S DNA sequence and its characteristic production of autoinducer and induction of soft rot disease in potato and Chinese cabbage. These strains were grown at 28°C in Luria-Bertani (LB) medium with shaking when necessary. *Agrobacterium tumefaciens* strains were grown at 28°C in YEB, in BM minimal medium (basic minimal nutrient added with mannitol as sole carbon source), or on nutrient agar plates (Difco Laboratories). Mannitol at a final concentration of 0.2% was used as the sole carbon source in the minimal medium. *Escherichia coli* strains were grown at 37°C in LB or on LB agar plates. Antibiotics were added at the following concentrations, when required: rifampin at 50 µg/ml, streptomycin at 100 µg/ml, ampicilin at 100 µg/ml, kanamycin at 50 µg/ml, and tetracycline at 10µg/ml. X-gal (5-bromo-4-

chloro-3-indolyl-B-D-galactopyranoside) (Promega) was included in media at 50 µg/ml for detection of β-galactosidase enzyme activity.

More than 30 strains showed different levels of AI inactivation activity. To characterise the unknown isolates, the 16S rRNA sequences of these isolates were analysed by PCR amplification and subsequent sequencing. The sequence search showed the 16S rRNA sequences of those strains capable of inactivating AI are highly homologous to that of *Bacillus thuringiensis* (Bt).

To test whether other *Bacillus* strains also have the AI-inactivation ability, known strains of *B. thuringenesis*, *B. cereus*, *B. mycoides*, and *B. sphaericus* were selected for bioassay. For determination of the AI inactivation ability of bacterial strains and isolates, the autoinducer, N-β-oxo-hexanoyl-L-homoserine lactone (OHHL), or N-β-oxo-octanoyl-L-homoserine lactone (OOHL) was added to the over-night bacterial cultures which were diluted to OD_{600} = 1.1, or to the protein extracts, at a final concentration of 20 µM, and incubated at 28 °C for 30 min. The AI remaining in the supernatant was then determined as previously described (Zhang, 1993; Dong et al., 2000).

Table 1 shows the AI inactivation activities of the selected strains and some newly identified isolates. All the tested bacterial strains, except *B. sphaericus* and *B. fusiformis*, eliminated AI (at a concentration of 20 µM OHHL) with different levels of enzyme activities. These strains include 13 known *Bacillus* species (strains starting with a "B" in Table

1), 1 known *Agrobacterium* and 9 *Bacillus* species identified by 16S rDNA sequence analysis. Among them, 12 bacterial strains showed a high level of AI-inactivation activity (> 30 μM/h/OD_{600}); 8 showed a medium level of activity (25-30 μM/h/OD_{600}); and the *A. tumefaciens* strain M103 showed a low level of activity (4.5 μM/h/OD_{600}). Except for *A. tumefaciens*, all these AI-inactivation strains are Gram-positive and belong to *B. thuringenesis* or its close related species.

Table 1. Bacterial strains and their AI-inactivation activity

	Strains		Source	Enzyme activity ($\mu M/h/OD_{600}$)
28-32	*Bacillus thuringiensis*		This work	32.4 ± 1.1
258-3	*Bacillus thuringiensis*		This work	32.5 ± 1.2
69	*Bacillus thuringiensis*		This work	30.9 ± 2.3
60-1	*Bacillus thuringiensis*		This work	28.2 ± 5.1
250	*Bacillus thuringiensis*		This work	23.4 ± 3.9
262	*Bacillus thuringiensis*		This work	23.1 ± 1.5
B18	*Bacillus thuringiensis*		This work	27.4 ± 3.0
B20	*Bacillus thuringiensis*		This work	32.7 ± 2.4
B21	*Bacillus thuringiensis*		This work	33.1 ± 0.8
B22	*B. thuringiensis ssp. kurstaki**		This work	32.8 ± 1.3
B23	*B. thuringiensis ssp. Israelensis**		BGSC(4Q7)	26.7 ± 3.5
B1	*B. thuringiensis ssp. thuringiensis*		BGSC (4A3)	32.5 ± 0.3
B2	*B. thuringiensis ssp. kurstaki*		BGSC (4D1)	33.0 ± 0.6
B12	*B. thuringiensis ssp. Aizawai*		BGSC (4J4)	33.5 ± 0.9
B17	*B. thuringiensis ssp. Wuhanensis*		Mycogen(PSS2A1)	28.8 ± 4.1
B25	*Bacillus cereus*		This work	33.7 ± 0.8
B14579	*Bacillus cereus*		ATCC (14579)	31.7 ± 0.6
B6462	*Bacillus mycoides*		ATCC (6462)	29.8 ± 2.2
240B	*Bacillus sp.*		This work	33.0 ± 1.0
Cot	*Bacillus thuringiensis*		This work	25.1 ± 2.4
M103	*Agrobacterium tumefaciens*		This work	4.5
269	*Bacillus fusiformis*		This work	0
B29	*Bacillus sphaericus*		BGSC (12A4)	0

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* Plasmid minus

** Equal volume bacterial suspension (diluted to OD_{600} = 1.1 from overnight cultures) and OHHL (40 µM) were incubated at 28°C for 30 min and then OHHL remaining in the supernatant was determined as previously described (Zhang, 1993). The enzyme activity is shown as digested µM of OOHL per hour per OD_{600} of bacterial culture. Values represent mean ± standard deviation of 4 replicates. Strains starting with a "B" prefix are the known *Bacillus* species. Other *Bacillus* strains were identified by 16S rDNA sequence analysis.

The evidence suggests that the AI-inactivation gene is located in chromosomal DNA but not in a plasmid, because Bt ssp. *kurstaki* strain B2 and its plasmid minus derivative strain B22, both showed a similar level of enzyme activity. The second plasmid minus strain B23, belonging to *B. thuringenesis* ssp. *Israelensids*, was also capable of enzymatic inactivation of AI.

To investigate the genetic diversity of genes for AI-inactivation, the representative bacterial strains showing high, medium or low levels of AI-inactivation activity were chosen for further cloning experiments.

Example 2: Functional cloning of the *aiiB* gene from *Agrobacterium tumefaciens* strain M103

The suicide plasmid pSUP10 (Simon et al, 1983) in *E. coli* SM10 was used to introduce transposon Tn5 insertions into the genome of *A. tumefaciens* octopine strain A6 by the protocol described by Garfinkel and Nester (1980), except that the bacterial suspensions were spread onto BM minimal plates containing kanamycin (100 µg/ml). Total DNA of *A. tumefaciens* mutant strain M103 was partially digested with *Eco*RI, the 20-30 kb fragments were recovered from lower melting point agarose gel and purified. The purified fragments were ligated to the dephosphorized *Eco*RI site of the cosmid vector pLAFR3 (Staskawicz et al., 1987). The ligation mixture was packaged with GigapackTMIII XL Packaging Extract (Stratagene) and then transfected into *E. coli* DH5α. About 2000 individual colonies grown on the selective medium containing tetracycline were maintained as the genomic library of *A. tumefaciens*

mutant strain M103. The cosmid clones containing Tn5 were selected on the medium containing kanamycin and were further assayed for AI inactivation activity by using the bioassay method described above. Subcloning into the sequencing vector pGEM-7Zf(+) was carried out by routine techniques (Sambrook et al., 1989). Sequencing was performed on both strands by using the ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems).

Agrobacterium tumefaciens strain A6 produces N-acyl homoserine lactone autoinducers (AI) which are involved in regulation of Ti plasmid conjugal transfer (Zhang and Kerr, 1991). But its derivative M103 caused by Tn5 insertional mutagenesis is capable of inactivation of AI. (Table 1 and Fig. 1). It is likely that the gene encoding for AI degradation in strain A6 is regulated by a negative regulator, and the Tn5 insertion resulted in constitutive expression of the gene for AI inactivation.

Based on the assumption that the AI inactivation gene may be located downstream of the Tn5 insertion site, the cosmid clones containing Tn5 transposon were selected by the kanamycin resistance phenotype. Two cosmid clones resistant to kanamycin and showing AI inactivation activity were obtained from the cosmid library of M103. Restriction analysis and bioassay showed that a 5.2 kb *Eco*RI fragment conferred the AI inactivation activity. Further subcloning narrowed down the region to a 1.5 kb *Pst*I fragment (Fig. 2). Sequence analysis showed that several putative open reading frames (ORFs) starting with ATG or UTG were in the fragment. One of the ORFs showed 96.8% identity in

nucleotide sequence and 98% in amino acid sequence to the *attM* gene (U59485) of *A. tumefaciens* identified previously. However, AI inactivation activity was not detected when expressing the *attM* in *E. coli* via an expression vector pKK223-3. Deletion analysis of the 1.5 kb fragment showed that a 792bp ORF, its start codon a GTG rather than the normal ATG, encoding for AI inactivation (Fig. 3). The gene was named as *aiiB* (Fig. 4). In comparison with the AttM whose biological function has not been identified experimentally, the AiiB has 7 extra amino acids at the N terminus (Fig. 5). AiiB showed 35.4% identity at the amino acid level compared to the previously reported AiiA (Fig. 6).

Example 3: Functional cloning of the *aiiC* gene from *B. thuringinesis* strain Cot1

The suspension culture of strain Cot1 eliminated AI (20 μM) completely after 2 hr incubation, but bacterial cells killed by boiling for 5 min failed to inactivate AI (Fig. 7A), indicating an enzymatic inactivation mechanism. To identify the gene encoding for AI inactivation from Cot1, a cosmid library was constructed by *Eco*RI partial digestion of the genomic DNA of the bacterial isolate Cot1. Genomic DNA was extracted from bacterial isolate Cot1 and digested partially with *Eco*RI. The DNA fragments were ligated to the dephosphorylized *Eco*RI site of cosmid vector pLAFR3. Ligated DNA was packaged and transfected into *E. coli* DH5α. Cosmid clones with AI inactivation activity were identified by using the bioassay method described above. Subcloning into the sequencing vector pGEM-7Zf(+) or pBlueScript SK were carried out by

routine techniques.

One clone showing AI inactivating function was identified from the one thousand cosmid clones screened. Restriction analysis showed that this clone contains an insert of 24 kb. All five fragments generated by *Eco*RI complete digestion were subcloned into pGEM7 vector. The bioassay of these subclones showed that one clone, pGEM7-aiiC with an insert of 5 kb, conferred the AI inactivation activity. Further subcloning identified a 1.4 kb *Bam*HI fragment contained in the clone pBS-AiiC which was responsible for the AI inactivation function (Fig. 7B). The complete sequence of the clone pBS-AiiC showed that there is an ORF of 750 bp nucleotides (from 166 to 918) which encodes a protein of 250 amino acids (Fig. 8). Cloning of this ORF in the *E. coli* expression vector confirmed that it encoded a functional AI inactivation enzyme, designated as AiiC. At the peptide sequence level, the AiiC gene showed 91% and 33% identity, to the AiiA and the AiiB respectively. The *aiiC* gene has no significant similarity to other known sequences in the databases by FASTA and BLAST analysis at either nucleotide or peptide levels.

Example 4: The autoinducer inactivation genes in Bt belong to the same gene family

Among the tested bacterial isolates with AI inactivation activity, all except the *A. tumefaciens* strain M103, are Gram positive, and belong to *B. thuringiensis* (Bt) or closely related bacterial species. The *aiiA* and *aiiC* genes from the two *Bacillus* strains showed a high level of similarity. It is very

likely that the *aiiA* and *aiiC* genes are highly conserved among *B. thuringiensis* strains. DNA hybridisation (Southern blot) analysis was performed using an *aiiC* fragment as a probe. The genomic DNA was isolated from 18 selected bacterial strains, B1 (Bt ssp. *thuringiensis*), B2, B3 and B4 (Bt ssp. *kurstaki*), B22 (Bt ssp. *kurstaki* plasmid minus), B12 (Bt ssp. *Aizawai*), B16 and B17 (Bt ssp. *Wuhanensis*), B23 (Bt ssp. *Israelensis*), and other Bt strains B18, B20, B21, 240B1, 471W, and Cot1 as well as B25 and B26 (*B. cereus*), and B29 (*B. sphaericus*). Genomic DNA (20 µg) digested with *Eco*RI was separated by electrophoresis in 0.8% agarose gel and then the DNA was transferred onto Hybond-N+ membrane (Amersham Pharmacia, Biotech.) according to manufacture's instructions. The 1.4 kb *Bam*HI fragment containing the *aiiC* codon region was labelled with DIG for use as a probe for hybridisation. After hybridisation at 65°C, the membrane was washed twice in 2x SSC, 0.1% SDS at room temperature for 5 min, followed by washing twice in 0.1x SSC, 0.1x SDS at 65°C for 15 minutes. After washing, the membrane was detected with anti-DIG-AP conjugate, the NBT/BCIP solution was used as colour substrate according to manufacture's protocol (Boehringer Mannheim).

The result showed that one hybridising band was clearly detected from all tested strains, except for B29 (*B. sphaericus*). These results indicated that there is a single gene, with sequence similar to *aiiC*, present in all tested *B. thuringiensis* strains and its closely related species *B. cereus*. This is in agreement with the bioassay data (Table 1).

Example 5: Cloning of other AI inactivation genes from more bacterial isolates

Since the genes for AI inactivation are highly conserved, a PCR approach was used for the cloning of other AI inactivation genes from the selected *B. thuringiensis* isolates. Genomic DNA isolated from the bacterial isolates B1, B2, B17, B18, B20, B21, B22 and B25 was used as template. Primers were designed based on the conserved sequences of the 5' and 3' ends of the *aiiA* and *aiiC* gene. Standard PCR conditions were used to amplify AI-inactivation genes from the selected bacterial isolates. The primer sequences were: C5f: 5'- ATG GGA TCC ATG ACA GTA AAG AAG CTT TAT - 3'; C3r: 5'-GTC GAA TTC CTC AAC AAG ATA CTC CTA ATG -3'. The PCR reactions were performed for 35 cycles of 30 sec at 94°C, 30 sec at 55°C and 1 min at 72°C using a Perkin Elmer GenAmp PCR System 2400. Two separate PCR reactions were performed to make sure there was no error in the amplified sequences. The PCR products were purified by using QIAquick PCR Purfication Kit (QIAGEN) and the purified PCR fragment was ligated to pGEM-T vector (Promega). Clones having inactivating autoinducer activity were chosen for further study. Two such clones from each strain were sequenced. Nucleic acid sequence data and deduced amino acid sequences were analysed with the DNASTARTM sequence analysis software package (DNASTAR Inc.) and GCG sequence analysis software (Genetics Computer Group, Wisconsin). Database searches were performed using the BLASTA search algorithm.

Fig. 9 shows the nucleotide and deduced peptide sequences of 8 AI inactivation genes (named *aiiD* to

aiiK) cloned from Bt strains B1, B2, B17, B18, B20, B21, B22 and B25 respectively. These sequences all contain an ORF of 750 bp, which encodes a protein of ~~250 amino acids~~.

Example 6: The autoinducer inactivation genes are highly conserved among members of Bt and closely related *Bacillus* spp.

Except for the *aiiB* gene, all other genes were cloned from the Gram positive bacterial isolates. Sequence analysis indicates that the *aii* genes cloned from the Gram positive bacterial isolates are highly conserved, with high amino acid identities ranging from 90.4% to 94.0%, in comparison to that of AiiA (Fig. 10). The *aiiB* gene cloned from the Gram negative *A. tumefaciens* showed less similarity to other *aii* genes and clustered as a single group in the phytogenetic tree (Fig. 10). These results indicate that the autoinducer inactivation genes are highly conserved among members of Bt and closely related *Bacillus*.

In these Aii protein sequences, all except AiiB contain several invariant histidines with aspartate residues showing a pattern of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa ~D^{191}; the AiiB of *A. tumefaciens* contains the similar, but distinct motif ^{103}HXHXDH^{108}~72aa~H^{180}~21aa ~D^{202}. This pattern agrees with the metallohydrolase criterion (Vallee and Galdes, 1984). The motif HXHXDH in the *Arabidopsis* glyoxalase II was suggested to be involved in binding to zinc ion (Crowder et al., 1997). Site-directed mutagenesis has shown that all these residues except the first histidine (^{104}H in AiiA) in this motif are necessary for AiiA activity. These invariant

histidines and aspartate residues are also present in AiiB to AiiK, indicating they belong to the same group of autoinducer metallohydrolases.

Example 7: Effect of Bt strains on AI production by *Erwinia carotovora*

To test the effect of Bt strains on quenching AI production by pathogenic bacteria, *Erw. carotovora* SCG1 was co-cultured with Bt strains Cot1, B1, *E. coil* DH5α, and *B. fusiformis* respectively. AI was assayed as in Example 1. The AI produced by strain SCG1 was detected after 2 hours incubation, and a rapid increase was observed from 2 to 6 hours incubation (for cell numbers, see Fig. 14), whereas no AI was detected in the culture supernatant of SCG1 co-cultured with either Cot1 or B1 strain, which produce AI inactivation enzymes. In the co-culture supernatants of SCG1 with either *E. coil* DH5α or *B. fusiformis*, which do not contain *aii* genes, AI production levels were detected that were similar to those observed with SCG1 culture alone (Fig. 11). These results indicate that Bt strains effectively quench AI signals produced by the pathogen *Erw. carotovora* SCG1 when the two are cultured together.

Example 8: Effect of Bt strains on the pathogenesis of *Erwinia carotovora*

It is known that AI play a key role in regulation of the virulence determinates of several pathogenic bacterial species. Since Bt strains effectively quenched AI signals produced by the pathogen, it is likely this new function of Bt strains can be exploited

for disease control. To test this possibility, the effect of Bt strains for biocontrol against plant soft rot disease was investigated. Potato (*Solanum tuberosum* L. cv. Bintje) tubers were obtained from local stores. After rinsing in tap water and drying on paper towel, potato tubers were surface-sterilized with 70% ethanol, and then were sliced evenly to a 3mm thickness. For the dip treatment, the potato slices were dipped into the bacterial suspension of Cot1, or other bacterial strains, diluted to a concentration of 5×10^8 colony forming unit (CFU) per ml, for about 20 seconds. Sterilised water was used as a control. The slices were dried in a laminar flow cabinet for about 20 min to remove surface moisture before inoculation with 2.5 µl of *Erw. carotovora* SCG1 bacterial suspension containing approximately 2×10^8 or 2×10^7, CFU/ml onto the top of each slice. For the mixture treatment, equal volumes of each testing organism (5×10^8 CFU/ml), or sterile water were mixed with *Erw. carotovora* SCG1 bacterial suspension (2×10^8 or 2×10^7 CFU/ml). The mixture (2.5 µl) was inoculated to a cut surface of the potato slices. All the potato slices were incubated in a Petri dish at 28°C. Maceration area was measured during incubation. Each treatment was repeated 4 to 12 time (12 for Cot1), each repeat was inoculated 3 places on one slice. For the colonisation experiment, each treatment was repeated 4 times, each tuber slice was inoculated only once at the centre of slice. Potato tuber slices were either treated with Bt strain Cot1 or other controls first before inoculation of *Erw. carotovora* SCG1, or SCG1 bacteria were mixed with Cot1 or other controls before inoculation onto

potato slices.

Erw. carotovora SCG1 caused severe tissue maceration of potato slices 20 hr after inoculation, whereas on Bt strain Cot1 pre-treated potato slices the maceration symptom was significantly attenuated (Fig. 12). Co-inoculation of SCG1 with the Bt strain Cot1 also attenuated soft rot symptoms, especially at the lower concentration of inoculum. In contrast, control treatments, either pretreatment of potato slices with *E. coli* or *B. fusiformis* before inoculation of SCG1, or co-inoculation of SCG1 with *E. coli* and *B. fusiformis* respectively, showed severe tissue maceration symptoms (Fig. 12). These results suggest that Bt strains could be used as biocontrol agents against soft rot disease in plants.

Example 9: *In vitro* competition between Bt strain and *Erwinia carotovora* SCG1

The Bt strains Cot1 and B1 were tested for production antibiotics against *Erw. carotovora* SCG1. Competition experiments were conducted by co-inoculation of the Bt strain and *Erw. carotovora* in a 1:1 ratio. Each strain was inoculated at the level of about 1×10^7 CFU/ml for *Erw. carotovora* and 1×10^6 CFU/ml for other strains. The mixture was incubated at 30°C. At different time points the bacteria samples were taken for bioassay of AI production (the bioassay performed as in Example 1), and were diluted in suitable concentrations to spread on plates for colony counting. The experiment was repeated four times. For the colonisation experiment, the potato slices inoculated with *Erw. carotovora* were taken at times as

indicated, and plant tissues about 15 x 15 mm circling the inoculation site were cut. The cut tissues were cut into small piece and placed in 10 ml of 0.1M NaCl. After shaking for 30 min, the supernatant was diluted in suitable concentrations. Viable numbers of bacterial cells were counted.

On plates of both rich and minimum media, Bt strains did not show any inhibitory effect on the growth of SCG1. When strain SCG1 and Bt strain Cot1 or B1 were coinoculated, both Bt strains and SCG1 grew normally, showing the same growth trend over a 24 hr period (Fig. 13).

Example 10: Effect of Bt strain on colonisation of tuber slice by *Erwinia carotovora*

To investigate colonisation of *Erw. carotovora* SCG1 on potato slices after incubation, an expression vector containing the GFP gene was transformed into strain SCG1. The expression vector can be maintained in strain SCG1 stably without selection pressure. There was no difference in virulence between the SCG1 (GFP) and the wild-type SCG1. To investigate the effect of Bt bacteria on the survival and growth of SCG1 on plants, potato tuber slices were either dipped into bacterial suspensions of Cot1, then inoculated with SCG1(GFP), or simultaneously inoculated with SCG1(GFP) and Cot1. Changes in bacterial cell numbers and development of soft rotting symptoms of potato tissue were monitored daily for 4 days. Results showed that there were no big changes in cell numbers between SCG1(GFP) on the Cot1-treated slices and the SCG1(GFP) on the water-treated slices during 4-days incubation

(Fig. 14). The result indicates that Bt strain Cot1 did not significantly affect the growth of SCG1(GFP) on the potato tube slices, suggesting that attenuation of the virulence of *Erwinia* SCG1(GFP) by Bt strain Cot1 was not due to inhibition of SCG1(GFP) cell growth.

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CLAIMS

1. An isolated nucleic acid molecule encoding an autoinducer inactivation protein, wherein the encoded protein comprises an amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

2. An isolated nucleic acid molecule encoding an autoinducer inactivation protein, wherein the encoded protein comprises an amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22 but an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

3. An isolated nucleic acid molecule of claim 2, comprising a sequence selected from the group consisting of SEQ ID NOS 1-10.

4. A method for increasing in a susceptible plant or animal resistance to a disease in which virulence is regulated by autoinducers, comprising administering to the plant or animal an effective amount of a bacterium that is capable of producing an autoinducer inhibitor.

5. The method of claim 4, wherein the bacterium administered is a Bacillus sp.

6. The method of claim 5, wherein the Bacillus sp. is a variety of Bacillus thuringiensis.

7. The method of claim 6, wherein the Bacillus thuringiensus variety is selected from the group consisting of B1, B2, B17, B18, B20, B21, B22 and B25.

8. The method of any of claims 4-7, wherein administration is to an animal.

9. The method of claim 8, wherein the animal is a human.

10. An expression vector comprising at least one nucleic acid sequence encoding for an autoinducer inactivation protein, wherein the encoded protein comprises an amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191} and, and wherein the expression vector propogates in a procaryotic or eukaryotic cell, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

11. An expression vector, comprising at least one nucleic acid sequence encoding for an autoinducer inactivation protein, wherein the encoded protein comprises an amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, and wherein the expression vector propagates in a procaryotic or eukaryotic cell, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22 but an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

12. An expression vector according to claim 11, comprising a sequence selected from the group consisting of SEQ ID NOS 1-10.

13. A protein having autoinduction inactivation activity, where the protein comprises an amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191} and, with the proviso that the protein does not consist of the amino acid sequence of SEQ ID NO 22.

14. A protein having autoinduction inactivation activity, wherein the protein comprises an amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the protein does not consist of the amino acid sequence of SEQ ID NO 22 but an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

15. A method for increasing disease resistance in a plant or animal, which method comprises introducing into a cell of such plant or animal at least one nucleic acid sequence that encodes an autoinducer inactivation protein, in a manner that allows said cell to express said nucleic acid sequence, wherein said autoinducer inactivation protein comprises an amino acid sequence selected from the group consisting of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191} and^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D~^{202}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

16. A method according to claim 15, wherein the encoded protein comprises the amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

17. A method according to claim 15, wherein the at least one nucleic acid is selected from the group consisting of SEQ ID NOS 1-10.

18. A method of reducing bacterial damage to a plant or animal, which method comprises administering to a plant or animal in need of such reduction an effective amount of an autoinducer inactivation protein, wherein said protein comprises an amino acid sequence selected from the group consisting of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}and ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D~^{202}, with the proviso that the protein does not consist of the amino acid sequence of SEQ ID NO 22.

19. A method according to claim 18, wherein the protein comprises the amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the protein does not consist of the amino acid sequence of SEQ ID NO 22.

20. A method according to claim 18, wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

21. A method according to any of claims 18-20, wherein administration is to an animal.

22. A method according to claim 21, wherein the animal is a human.

23. A cell of a procaryote or eukaryote stably transformed with at least one nucleic acid molecule encoding an autoinducer inactivation protein, wherein the encoded protein comprises an amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

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24. A cell of a procaryote or eukaryote stably transformed with at least one nucleic acid molecule encoding an autoinducer inactivation protein, wherein the encoded protein comprises an amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22 but an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

25. A cell of claim 24, comprising a sequence selected from the group consisting of SEQ ID NOS 1-10.

26. A method of reducing the formation of bacterial biofilms, comprising exposing biofilm-forming bacteria to at least one autoinducer inhibitor protein, wherein said protein comprises an amino acid sequence selected from the group consisting of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191} and ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D~^{202}, with the proviso that the protein does not consist of the amino acid sequence of SEQ ID NO 22.

27. A method of claim 26, wherein the protein comprises the amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the protein does not consist of the amino acid sequence of SEQ ID NO 22.

28. A method of claim 27, wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

29. A cell of claim 28, comprising a sequence selected from the group consisting of SEQ ID NOS 1-10.

30. A method of reducing the formation of bacterial biofilms, comprising exposing biofilm-forming bacteria to at least one autoinducer inhibitor protein, wherein said protein comprises an amino acid sequence selected from the group consisting of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191} and ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}, with the proviso that the protein does not consist of the amino acid sequence of SEQ ID NO 22.

31. A method of claim 30, wherein the protein comprises the amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the protein does not consist of the amino acid sequence of SEQ ID NO 22.

32. A method of claim 31, wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.