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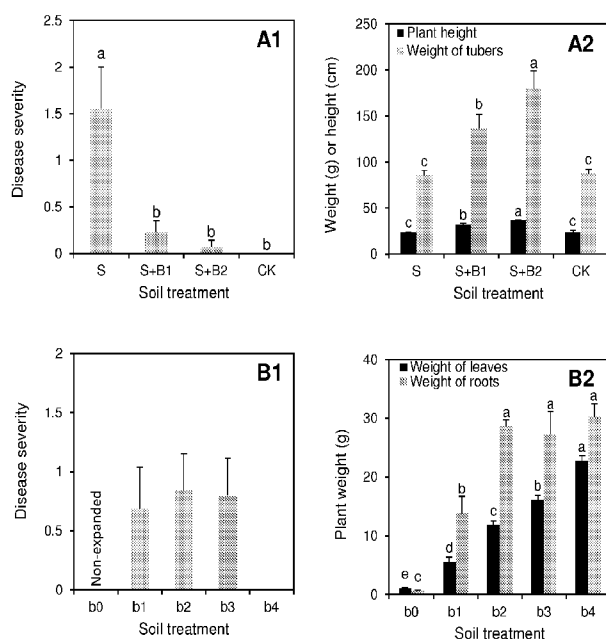


Figure 1

(57) Abstract: A novel *Bacillus amyloliquefaciens* strain BAC03, is isolated and characterized. This BAC03 strain provides protection against soil plant pathogen and enhances plant growth. Methods of using the strain are also provided.

NOVEL *BACILLUS AMYLOLIQUEFACIENS* STRAIN BAC03 AND METHODS OF USING SAME

RELATED APPLICATIONS

[0001] This application claims priority from United States Provisional Application No. 61/640,204, filed on April, 30 2012, which is hereby incorporated by reference in its entirety.

Background

[0002] In agricultural production, poor quality and bad cosmetic appearance due to soilborne diseases are responsible for significant economic crop loss. For example, *Streptomyces* species, like *Streptomyces scabies*, cause scabby diseases in potatoes, radishes, and other crops. Most of the current plant disease control methods have proven ineffective. Moreover, many synthetic fungicides are not permitted especially for organic production, and chemical fungicides do not prevent all diseases and often release toxic chemicals into the soil or food chain. The use of many agrochemicals are also banned or restricted due to environmental and health risks.

Summary

[0003] The use of microbial biopesticides (biocontrol) offers alternatives to chemical control of plant pests and diseases. A novel strain of *Bacillus amyloliquefaciens* strain BAC03 is isolated and described. The strain is one which has the identifying characteristics of the *B. amyloliquefaciens* BAC03 strain a representative sample of which has been deposited with the American Type Culture Collection (ATCC) as Deposit No. PTA-12890 (hereinafter "ATCC No. PTA-12890). The sequence of LCI (SEQ ID NO:1), an antimicrobial protein produced by this strain, has 99% homology to reported LCI DNA sequences. The bacterial strain promotes plant growth and enhances disease resistance. The strain and mutants thereof are useful as a biological control agent. Use in reducing adverse impact of a pathogen on a plant or plant part is provided. Plants, plant parts or soils are treated by applying an effective amount of BAC03, a supernatant of the strain or cell suspension or other forms of BAC03.

Sequence Listing

[0004] This application hereby incorporates by reference the material of the electronic Sequence Listing filed concurrently herewith.

Brief Description of the Drawings

[0005] FIGS. 1A-B. Effect of *Bacillus amyloliquefaciens* BAC03 on disease severity and plant growth in potato and radish. Panels A1 and A2: Potato was planted in potting mix treated with 200 ml of *S. scabies* at final concentrations 10^6 CFU/cm³ (treatment S), BAC03 at 10^5 CFU/cm³ (treatment B1), BAC03 at 10^6 CFU/cm³ (treatment B2), and tryptic soy broth as a control (CK). Panels B1 and B2: Radish was planted in potting mix treated with 30 ml of BAC03 at final concentration of 10^5 CFU/cm³ each time in the following treatments: b0 (non-treated), b1 [one time of application after planting (TAP)], b2 (2 TAPs), b3 (3 TAPs), and b4 (1 time before and 3 TAPs), with weekly intervals between applications. All pots were infested with *Streptomyces scabies* at 10^7 CFU/cm³ potting mix. Fresh plant weight was measured in the end of experiment.

[0006] FIGS. 2. Detection of *S. scabies* population in potting mix using quantitative real-time polymerase chain reaction assay. Radish was planted in potting mix treated with BAC03 at final concentrations of 10^5 CFU/cm³ in the following treatments: b0 (non-treated), b1 [one time after planting (TAP)], b2 (2 TAPs), b3 (3 TAPs), and b4 (1 time before and 3 TAPs). All pots were infested with *Streptomyces scabies* at 10^7 CFU/cm³ of potting mix. Soil-less potting mix (0.1 g) was collected from each treated pot for DNA extraction and PCR analyses. The quantity of *S. scabies* DNA was estimated based on external standards. The means of groups annotated with the same letters were not significantly different, tested by Fisher's Least Significant Difference method ($P > 0.05$).

[0007] FIGS. 3A-B (A) Antimicrobial activity of *Bacillus amyloliquefaciens* BAC03 ammonium sulfate precipitation fraction (AS) against *Streptomyces scabies*; and (B) Test for bactericidal versus bacteriostatic activity of BAC03 AS. *Streptomyces scabies* was recovered after incubation with AS for 0, 1, 3, and 5 days, followed by rinsing off AS through a 0.22 μ m membrane.

[0008] FIG. 4 Scanning electron microscopy (SEM) of mycelial morphology of *Streptomyces scabies* grown on yeast malt extract medium plates. Ten microliters of sodium phosphate buffer (untreated), or AS (treated) was directly dropped onto a *S. scabies* 2-day-old colony. Five days later, the untreated colonies produced white spores, but the treated culture did not show any sporulation. The culture specimen were processed following the method of Fan *et al.* (2011) and coated with metallic osmium in preparation for SEM.

[0009] FIG. 5. Test for active fraction of ammonium sulfate precipitate (AS) from *Bacillus amyloliquefaciens* BAC03 by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After fractionation on SDS-PAGE, the gel was overlaid with

yeast malt extract medium containing *Streptomyces scabies* (10^7 CFU/ml), and the inhibition zone was observed after 2 days of incubation at 28°C. Growth was detected by staining with thiazolyl blue tetrazolium bromide (MTT). The atomic mass of the active compound(s) was estimated by comparison to the position of the markers (in kDa).

[0010] FIGS. 6A and 6B. Effect of BAC03 to induce systemic resistance in potato against common scab. Figure 6A shows disease severity of four treatment groups, when exposed to BAC03, with or without exposure to *S. scabies*, and in control plants. Figure 6B shows biomass measurements.

[0011] FIGS. 7A and 7B. Effect of biomaterials on potato common scab in the field in location 1. Figure 7A shows disease severity and Figure 7B shows plant weight.

[0012] FIGS. 8A and B. Effect of biomaterials on potato common scab in the field in location 2. Figure 8A shows disease severity and Figure 8B shows plant weight.

Detailed Description of the Embodiments

[0013] In the following detailed description, embodiments are described in sufficient detail to enable those skilled in the art to practice them, and it is to be understood that other embodiments may be utilized and that changes (e.g. chemical and procedural) may be made without departing from the spirit and scope of the present subject matter. The following detailed description is, therefore, not to be taken in a limiting sense, and the scope of embodiments of the present invention is defined only by the appended claims.

[0014] *Bacillus amyloliquefaciens* is an aerobic bacterium that is a gram-positive motile, rod-shaped bacterium. See, for example Kang et al., US20110274673. Disclosed is a novel bacterial strain, *Bacillus amyloliquefaciens* (strain BAC03), having strong antimicrobial activity against a variety of bacterial and fungal pathogens. An embodiment is to a strain having all the identifying, biological and genetic characteristics of strain BAC03. The bacterial strain is an alternative to chemical control strategies since they are environmentally friendly and safe to humans. The bacteria produce antimicrobial substances that serve as antibiotics used to inhibit other microorganisms, such as plant pathogens. This strain has been isolated from a disease-suppressive soil and sequenced. The bacteria have been shown to have antimicrobial activity. The antimicrobial portion which is able to kill or inhibit a range of microorganisms, especially soilborne plant pathogens, was isolated and identified as having 99% homology to reported nucleotide sequences encoding an LCI protein, an antimicrobial substance, and disclosed here as SEQ ID NO: 1. As used here, the term “the bacteria”, “the strain”, “the bacterial strain”, depending on the context may be used

interchangeably to refer to the BAC03 strain, the antimicrobial portion of BAC03 (viz., LCI) or both.

[0015] The bacterial strain also stimulates plant growth by increasing biomass and inducing the plant to defend against soil borne pathogens. The strain has been demonstrated to have antimicrobial activity on plant pathogens including bacterial and fungal pathogens. Examples include, without limitation, agronomically important crop, vegetable and nut pathogens such as *Streptomyces scabies* (potato common scab), *Agrobacterium tumefaciens*, *Clavibacter michiganensi*, *Phytophthora cambivora*, *Phytophthora capsici*, *Rhizactonia solani* and *Sclerotinia sclerotiorum*. Still further examples of pathogens include those listed in Table 2 below. In some cases of pathogen inhibition, the strain possesses a greater antimicrobial effect (inhibition zone) than a known biocontrol product containing *Bacillus subtilis* sold under the trade name SERENADE™ and produced by AgraQuest. Furthermore, BAC03 promotes plant growth (e.g. increasing plant biomass) in plants such as potato, radish, tomato, turnip, beet, cucumber, pepper and carrot.

[0016] The term plant is used broadly herein to include any plant at any stage of development, or to part of a plant, including a plant cutting, a plant cell, a plant cell culture, a plant organ, a plant seed, and a plantlet. A plant cell is the structural and physiological unit of the plant, having a protoplast and a cell wall. A plant cell can be in the form of an isolated single cell or aggregate of cells such as a friable callus, a cultured cell, or can be part of a higher organized unit, for example, a plant tissue, plant organ, or plant. Thus, a plant cell can be a protoplast, a gamete producing cell, or a cell or collection of cells that can regenerate into a whole plant. As such, a seed, which includes multiple plant cells and is capable of regenerating into a whole plant, is considered a plant cell for purposes of this disclosure. A plant tissue or plant organ can be a seed, protoplast, callus, or any other groups of plant cells that is organized into a structural or functional unit. Particularly useful parts of a plant include harvestable parts and parts useful for propagation of progeny plants. A harvestable part of a plant can be any useful part of a plant, for example, flowers, pollen, seedlings, tubers, leaves, stems, fruit, seeds, roots, and the like. A part of a plant useful for propagation includes, for example, seeds, fruits, cuttings, seedlings, tubers, rootstocks, and the like. Tissue culture is capable of regenerating plants. For example, the regenerable cells in such tissue cultures will be embryos, protoplasts, meristematic cells, callus, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks, stalks or the like. Still further, plants may be regenerated from the tissue cultures.

[0017] The disclosed bacterial strain may be used with any plant species, whether monocotyledonous or dicotyledonous. Examples of monocotyledonous plants include, but are not limited to, asparagus, field and sweet corn, barley, wheat, rice, sorghum, onion, pearl millet, rye and oats. Examples of dicotyledonous plants include, but are not limited to tomato, potato tobacco, cotton, rapeseed, field beans, soybeans, peppers, lettuce, peas, alfalfa, clover, cole crops or *Brassica oleracea* (e.g., cabbage, broccoli, cauliflower, Brussels sprouts), radish, carrot, beets, eggplant, spinach, cucumber, squash, melons, cantaloupe, sunflowers and various ornamentals.

[0018] A culture of the microbial strain has been deposited with the ATCC, 10801 University Blvd., Manassas, Va. 20110-2209 USA on May 10, 2012 and, as noted above, has been designated as ATCC No. PTA-12890.

[0019] The strain or the antimicrobial protein when referred to as biologically pure or isolated refers to the strain or protein separated from materials with which it is normally associated in nature. A representative sample of a biologically pure or isolated culture of BAC03 strain refers to the strain deposited as ATCC No. PTA-12890. A monoculture of a strain, or a strain associated with other strains, or with compounds or materials that it is not normally found in nature, are examples of a biologically pure strain. The strain encompasses its various forms, including by way of example without limitation, cell suspensions, spores, vegetative cells or whole culture of the strain which may be used to provide the antimicrobial properties of BAC03. They may be prepared, for example, from a culture obtained by cultivating the strain by a conventional method. The whole culture obtained may be prepared into whole culture powder, for example, by freeze-drying the whole culture as it is. The vegetative cells may be prepared as a cell precipitate, for example, by centrifuging whole culture after the cultivation to remove contaminants, further centrifuging the resulting supernatant, and then washing the cells precipitated. In addition, the spores may be prepared as freeze-dried spore powder, for example, by suspending the cell precipitate obtained above in distilled water and freeze-drying the resulting suspension.

[0020] The strain may be a mutant (also referred to as a variant) of BAC03, a representative sample of which has been deposited as ATCC PTA-12890 that has all the identifying characteristics of the strain and retains antimicrobial properties to the pathogens described.

[0021] Such mutants may be those having a genome that hybridizes under conditions of high stringency to the genome of the strain a representative sample of which has been

deposited as ATCC PTA-12890. In another embodiment the mutants may be those having *recA*, *recN*, *cheA* and *gyrA* sequences which hybridize under stringent conditions to the *recA* (SEQ ID NO. 2), *recN* (SEQ ID NO. 3), *cheA* (SEQ ID NO. 4) and *gyrA* sequences (SEQ ID NO. 5) of BAC03, a representative sample of which has been deposited as ATCC PTA-12890.

[0022] In hybridization techniques, all or part of a known nucleotide sequence is used as a probe that selectively hybridizes to other corresponding nucleotide sequences present in a population of cloned genomic DNA fragments or cDNA fragments (e.g., genomic or cDNA libraries) from a chosen organism. The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ³²P, or any other detectable marker. Thus, for example, probes for hybridization can be made by labeling synthetic oligonucleotides based on the DNA sequences disclosed. Methods for probe preparation for hybridization and for cDNA or genomic libraries construction are generally known in the art and are disclosed (see e.g. Sambrook et al., 1989) Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.).

[0023] For example, the nucleic acid sequences disclosed herein, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding sequences. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique among the sequences to be screened and are preferably at least about 10 nucleotides in length, and most preferably at least about 20 nucleotides in length. Such sequences may alternatively be used to amplify corresponding sequences from a chosen plant by polymerase chain reaction (PCR). This technique may be used to isolate sequences from a desired plant or as a diagnostic assay to determine the presence of sequences in a plant.

[0024] Hybridization techniques include hybridization screening of DNA libraries plated as either plaques or colonies (Sambrook et al., 1989). Hybridization of such sequences may be carried out under stringent conditions. By “stringent conditions” or “stringent hybridization condition” is intended conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization or washing conditions or both, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to

allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.

[0025] Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 50°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 0.1% SDS at 37°C, and a wash in 0.1X SSC at about 60 to 65°C.

[0026] In general, sequences that correspond to and which hybridize to the nucleotide sequence disclosed herein will be at least about 50% to about 99% homologous; about 70% homologous, and even about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% homologous or more with the disclosed sequence. That is, the sequence similarity between probe and target may range, sharing at least about 50%, about 70%, and even about 85% or more sequence similarity.

[0027] Specificity is typically the function of post-hybridization washes, the factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl, *Anal. Biochem.*, 138:267-284 (1984): $T_m = 81.5^\circ\text{C} + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\% \text{ form}) - 500/L$; where M is the molarity of monovalent cations, % GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m , hybridization or wash conditions or both can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq 90\%$ identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the

thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at about 1 to about 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at about 6 to about 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at about 11 to about 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m , those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than about 45°C (aqueous solution) or about 32°C (formamide solution) it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids may for example be found in Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology “Hybridization with Nucleic Acid Probes” Part I, Chapter 2 (Elsevier, New York); and Ausubel et al., eds. (1995) Current Protocols in Molecular Biology, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.) and Haymes et al. (1985) In: Nucleic Acid Hybridization, a Practical Approach, IRL Press, Washington, D.C. Thus, mutant strains that have the antimicrobial activity described herein and which hybridize under stringent conditions to the sequences disclosed are encompassed by the various embodiments described herein.

[0028] A mutant may also be a strain having a genomic sequence having at least about 85% to about 95% identity to the genome of BAC03 as represented in ATCC No. PTA-12890. In another embodiment the mutant may be a strain having a *recA*, *recN*, *cheA* and *gyrA* sequences which have at least about 85% to about 95% identity to the *recA* (SEQ ID NO. 2), *recN* (SEQ ID NO. 3), *cheA* (SEQ ID NO. 4) and *gyrA* (SEQ ID NO. 5) sequences of BAC03, a representative sample of which has been deposited as ATCC No. PTA-12890. Any such mutant is one which retains the antimicrobial properties described herein. In other embodiments, a mutant may be one which produces the antimicrobial protein, an LCI protein.

[0029] The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides: (a) “reference sequence”, (b) “comparison window”, (c) “sequence identity” and (d) “percentage of sequence identity.”

[0030] (a) As used herein, “reference sequence” is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length promoter sequence, or the complete promoter sequence.

[0031] (b) As used herein, “comparison window” makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or deletions (e.g., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least about 20 to about 100 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to accurately reflect the similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

[0032] Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm.

[0033] Optimal alignment of sequences for comparison can use any means to analyze sequence identity (homology) known in the art, e.g., by the progressive alignment method of termed “PILEUP” (Morrison, (1997) *Mol. Biol. Evol.* 14:428-441, as an example of the use of PILEUP); by the local homology algorithm of Smith & Waterman (*Adv. Appl. Math.* 2: 482 (1981)); by the homology alignment algorithm of Needleman & Wunsch (*J. Mol. Biol.* 48:443-453 (1970)); by the search for similarity method of Pearson (*Proc. Natl. Acad. Sci. USA* 85: 2444 (1988)); by computerized implementations of these algorithms (e.g., GAP, BEST FIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.); ClustalW (CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, Calif., described by, e.g., Higgins(1988), *Gene* 73: 237-244; Corpet (1988), *Nucleic Acids Res.* 16:10881-10890; Huang, *Computer Applications in the Biosciences* 8:155-165 (1992); and Pearson (1994), *Methods in Mol. Biol.* 24:307-331); Pfam (Sonnhammer (1998), *Nucleic Acids Res.* 26:322-325); TreeAlign (Hein (1994), *Methods Mol. Biol.* 25:349-364); MEG-ALIGN, and SAM sequence alignment computer programs; or, by manual visual inspection.

[0034] Another example of algorithm that is suitable for determining sequence similarity is the BLAST algorithm, which is described in Altschul et al, (1990) *J. Mol. Biol.* 215: 403-

410. The BLAST programs (Basic Local Alignment Search Tool) of Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403-410) searches under default parameters for identity to sequences contained in the BLAST "GENEMBL" database. A sequence can be analyzed for identity to all publicly available DNA sequences contained in the GENEMBL database using the BLASTN algorithm under the default parameters.

[0035] Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/; see also Zhang (1997), *Genome Res.* 7:649-656 for the "PowerBLAST" variation. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold. These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff (1992), *Proc. Natl. Acad. Sci. USA* 89:10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The term BLAST refers to the BLAST algorithm which performs a statistical analysis of the similarity between two sequences; see, e.g., Karlin (1993), *Proc. Natl. Acad. Sci. USA* 90:5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0036] In an embodiment, GAP (Global Alignment Program) can be used. GAP uses the algorithm of Needleman and Wunsch (*J. Mol. Biol.* 48:443-453, 1970) to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number

of gaps. Default gap creation penalty values and gap extension penalty values in the commonly used Version 10 of the Wisconsin Package™ (Accelrys, Inc., San Diego, CA) for protein sequences are 8 and 2, respectively. For nucleotide sequences the default gap creation penalty is 50 while the default gap extension penalty is 3. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. A general purpose scoring system is the *BLOSUM62* matrix (Henikoff and Henikoff (1993), *Proteins* 17: 49-61), which is currently the default choice for *BLAST* programs. *BLOSUM62* uses a combination of three matrices to cover all contingencies. Altschul, J. Mol. Biol. 36: 290-300 (1993), herein incorporated by reference in its entirety and is the scoring matrix used in Version 10 of the Wisconsin Package™ (Accelrys, Inc., San Diego, CA) (see Henikoff & Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

[0037] (c) As used herein, “sequence identity” or “identity” in the context of two nucleic acid sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window.

[0038] (d) As used herein, “percentage of sequence identity” means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may include additions or deletions (e.g., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

[0039] Identity to the sequences disclosed would mean a polynucleotide sequence having at least about 65% to about 99% sequence identity, at least 70% sequence identity, at least 75% sequence identity, at least 80% identity, at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity.

[0040] When referring to “application” of the disclosed strain is intended to refer to exposing the plant, plant part, or soil with or without the plant or plant part to the strain in any convenient manner, such that adverse impact of a pathogen is reduced. The adverse impact of a pathogen on a plant or plant part may be measured by any of a variety of options

available to one skilled in the art, including by way of example without limitation, determination of improved plant growth, growth rate, biomass, increased survival rates, plant length, weight, leaf area, flower fertility, pollen fertility, seed weight or yield, seed germination, tuber weight or any combination of these, as compared to a plant exposed to the pathogen but which has not had the disclosed strain applied.

[0041] The strain may be applied to plants and soil in any convenient manner. The strain may be applied as a stand-alone living organism, since its spores can tolerate extreme environmental conditions. It may be provided as a suspension in whole broth culture (liquid culture containing cells and media) or supernatant of whole broth culture (liquid broth with cells removed by centrifugation, filtration or other of the many available methods) or precipitants having the antimicrobial LCI protein or an extraction of the protein or granular formulas. The application method is not limited by manner of producing or combining the strain with other components and one skilled in the art appreciates many variables exist. Where desired, the strain or supernatant or cell suspension or the like may also be combined with other compositions of interest, and provided in a convenient form, such as a formula, liquid, granule, suspension, microencapsulation or powder, for example. Examples of liquid compositions may include water, a saline solution or other aqueous solution. Further, inactive additives, plant health enhancing compositions, stabilizers or other components desired may be added.

[0042] The strain or any compositions combined with the strain may be applied in any advantageous manner, and may, for example, be applied prior to pathogen infection, at the time of infection, after infection, or as a seed treatment, and in multiple treatments. Application can also include, for example, applying to the soil in contact with the plant or plant part. In some embodiments, the concentration of the strain may need to be determined such that it does not affect seedling germination. For plants whose seedling emergence is not affected, the strain may be applied in a convenient strength to the seedling or in the soil. An effective amount of the strain is an amount sufficient to reduce adverse impact of a pathogen compared to a plant to which the strain has not been applied. The concentration of the strain may be dictated by economical considerations. For example, concentrations of 10^7 CFU cm^{-3} would be effective, yet costs may indicate a range of up to 10^6 CFU cm^{-3} . When the antimicrobial substance is used, economic considerations may also impact a concentration. By way of example without limitation, when tested *in vitro*, an inhibition rate of about 15%

was observed when the concentration of purified antimicrobial substance was 15µg/ml and an inhibition of almost 100% was observed with a concentration of 75µg/ml.

[0043] Disclosed is also a kit for the control, prevention or treatment of plant disease or inducing or promoting plant growth, where the kit includes the bacterial strain or protein composition and instructional material for applying the bacterial composition to control, prevent or treat a plant pathogen infection, or induce or promote plant growth as described herein. The skilled artisan will appreciate that the instructions for applying the disclosed bacterial composition can be any form of instruction means. Such instructions include, but are not limited to, written instruction material (e.g., a label, a booklet, a brochure, a pamphlet), oral instructional material (such as on an audio cassette or CD) or video instructions (such as on a video tape or DVD).

[0044] Some additional non-limiting exemplary embodiments are provided below.

1. A biologically pure or isolated culture of *Bacillus amyloliquefaciens* BAC03.
2. A biologically pure or isolated culture of *Bacillus amyloliquefaciens* BAC03 deposited as ATCC NO. PTA-12890.
3. A biologically pure or isolated bacterial culture having the physiological and morphological characteristics of the culture deposited as ATCC NO. PTA-12890.
4. A biologically pure or isolated culture of *Bacillus amyloliquefaciens* BAC03 and mutants or variants thereof.
5. A biologically pure or isolated culture of *Bacillus amyloliquefaciens* BAC03 strain deposited as ATCC No. PTA-12890 and having antagonistic or antimicrobial activity against plant soil pathogens.
6. A biologically pure or isolated bacterial culture having a gene sequence possessing at least 85% sequence identity to SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5.
7. An isolated protein from *Bacillus amyloliquefaciens* BAC03 having antimicrobial activity.
8. An isolated polynucleotide having SEQ ID NO:1 and having antimicrobial or plant growth promoting activity.
9. A biologically pure or isolated culture of *Bacillus amyloliquefaciens* BAC03 having antagonist activity against soil pathogens.
10. A biologically pure or isolated culture of *Bacillus amyloliquefaciens* BAC03 having antagonist activity against *Streptomyces* species.

11. A biologically pure or isolated culture of *Bacillus amyloliquefaciens* BAC03 having antagonist activity against common scab.
12. A biologically pure or isolated culture of *Bacillus amyloliquefaciens* BAC03 having plant growth promoting activity.
13. A composition comprising any of the embodiments 1-12.
14. A composition of embodiment 13 further comprising one or more other compositions.
15. A composition comprising any of the embodiments of 1-14 in a concentration up to 10^7 CFU.
16. A composition comprising spores, cells, whole culture or cell suspension of any of the embodiments 1-15.
17. A composition comprising an ammonium sulfate precipitated fraction of a supernatant of a whole cell culture of *Bacillus amyloliquefaciens* BAC03.
18. A kit for increasing plant growth, comprising a bacterial culture of any of embodiments 1-17, and instructions for use of the culture for promoting plant growth.
19. A kit for reducing disease severity of a plant pathogen, comprising a bacterial culture of any of embodiments 1-17, and instructions for use of the culture for reducing disease severity of a plant pathogen.
20. A method of reducing adverse impact of a plant pathogen on a plant or plant part, the method comprising applying to the plant, plant part or a soil an effective amount of any of embodiments 1-17.
21. The method of embodiment 20, wherein applying to the plant or plant part is more than once.
22. The method of embodiment 20, wherein the plant part is a seed.
23. A method of reducing adverse impact of a plant pathogen on a plant or plant part, the method comprising applying to the plant, plant part or a soil an effective amount of a composition comprising:
 - a. a polynucleotide having SEQ ID NO:1;
 - b. a BAC03 strain deposited as ATCC No. PTA-12890;
 - c. a strain having at least 80 % identity to SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5.
24. A method of producing a composition for reducing adverse impact of a soil plant pathogen on a plant or plant part, the method comprising producing a composition

comprising biologically pure or isolated culture of a *Bacillus amyloliquefaciens* strain BAC03 deposited as ATCC No. PTA-12890.

25. The method of embodiment 24, wherein the strain comprises spores, cells or whole culture of the strain.
26. The method of any of embodiments 20, 23 or 24, wherein the plant pathogen is selected from *Streptomyces scabies*, *Streptomyces acidiscabies*, *Streptomyces stelliscabiei*, *Streptomyces aureofaciens*, *Streptomyces* sp., *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Clavibacter michiganensi*, *Cladosporium cucumerinum*, *Fusarium solani*, *Cryphonectria parasitica*, *Phytophthora capsici*, *Penicillium citrinum*, *Phytophthora cambivora*, *Phytophthora cinnamomi*, *Botrytis cinerea*, *Rhizactonia solani* and *Sclerotinia sclerotiorum*.
27. A method for promoting plant growth, comprising:
applying to a plant a plant growth promoting bacteria comprising a biologically pure or isolated culture of *Bacillus amyloliquefaciens* BAC03 deposited as ATCC NO. PTA-12890 and mutants or variants thereof.
28. The method of embodiment 27, wherein the plant growth promoting bacteria have a gene sequence possessing at least 80% sequence identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5.
29. The method of embodiment 26, wherein the applying is effected by a method selected from applying:
 - a) to soil before or contemporaneously with sowing plant seeds in the soil;
 - b) to seeds before or contemporaneously with sowing plant seeds in the soil; and,
 - c) to seeds after plant seeds have been sown in the soil, or to growing plants or to the roots, stems, leaves or other parts thereof.

[0045] The various embodiments will be further described by reference to the following examples, which are offered to further illustrate various embodiments of the present invention. It should be understood, however, that many variations and modifications may be made while remaining within the scope of the present invention.

EXAMPLE 1

Materials and Methods

Collection of Microorganisms And Identification of BAC03

[0046] A bacterial strain, designated BAC03, was isolated from a Michigan potato field that is suppressive to potato common scab. *Bacillus subtilis* QST713 was obtained from the commercial biocontrol product SERENADE™ (AgraQuest Inc. Davis, CA, USA) as a positive control. *Bacillus amyloliquefaciens* FZB42 was obtained from the *Bacillus* Genetic Stock Center (Columbus, OH, USA). All other microorganisms used in this study are listed in Table 1.

[0047] BAC03 was identified based on sequence analysis of selected genes. BAC03 cultured in 200 µl tryptic soy broth (TSB, EMB Chemical Inc., Gibbstown, NJ, USA) for 24 hours was used for DNA extraction. The FastDNA Spin Kit (MP Biomedicals, Solon, OH, USA) was used following the manufacturer's instructions. Polymerase chain reaction (PCR) was conducted to amplify fragments of the 16S rDNA, *recA* and *recN* (DNA repair and recombination proteins), *cheA* (a histidine kinase), and *gyrA* (gyrase subunit A) genes with primers listed in Table 1. Primers for gene *cheA* and *gyrA* were designed based on the consensus sequence of these two genes retrieved from several *B. amyloliquefaciens* strains in National Center for Biotechnology Information (NCBI) database, and validated with DNAMAN software (Lynnon Corporation, Quebec, Canada). Each PCR reaction had a total volume of 25 µl, containing 5 U Taq DNA polymerase, 1X Taq polymerase PCR buffer (Promega, Madison, WI, USA), 200 mM dNTP mixture, 0.2 mM of each primer, and 1 µl (2 to 25 ng) of template DNA. Thermocycler (Bio-Rad Scientific Inc, Hercules, CA, USA) settings were as follows: an initial denaturation for 5 min at 94°C, followed by 36 cycles of 40 s at 94°C, 40 s at 58°C, and 1.5 min at 72°C; and extension for 7 min at 72°C. After PCR product visualization by electrophoresis on 1.2% (w/v) agarose gel stained with GelGreen (Biotium, Inc., Hayward, CA, USA), the PCR products were purified with a PCR purification kit (Denville Scientific Inc, Metuchen, NJ, USA), and sequenced at the Michigan State University Genomic Technology Support Facility (East Lansing, MI, USA). The sequences were analyzed using the BLAST algorithm against the GenBank database.

Antimicrobial Activity of BAC03

[0048] In vitro assay. Antimicrobial activity of BAC03 was determined against *Streptomyces* spp. using an agar-disk diffusion assay as described by Kimura *et al.* 1998. One hundred microliter spore suspension (10^5 CFU/ml, determined by dilution plating) of various *Streptomyces* spp. isolates were spread onto yeast malt extract agar (YME, EMB Chemical Inc., Gibbstown, NJ, USA) plates. Fifteen microliters of a 10^6 CFU/ml BAC03 or *B. subtilis* QST713, which were cultured for 24 hours in TSB, were placed as a drop on a

sterile filter paper disk (5 mm diam., Whatman #1, Piscataway, NJ, USA), which was placed on the agar medium with 2 disks per plate, at 2 cm distance from each other. After the plates were incubated at 28°C in darkness for 3 days, the diameter of the inhibition zone (if any) was measured with a ruler. Each isolate was replicated four times. The experiment was conducted twice.

[0049] To determine the potential antimicrobial activities of BAC03 against various microorganisms (fungi and oomycetes), a dual culture method was used. Briefly, one mycelial plug (5 mm diam.) were cut from freshly growing cultures using a sterile cork borer (5 mm diam), and transferred to approximately the center of a potato dextrose agar (PDA, EMB Chemical Inc., Gibbstown, NJ, USA) plate. Two paper discs (5 mm diam.) were placed on either side of the plug at 2 cm distance from the plug. An aliquot of 15 µl of BAC03 or QST713 was added to each filter paper disc using a pipette. The plates were incubated at 22°C for 3 to 4 days. The inhibition zone was measured as described above.

[0050] For other bacteria, the same technique was used as described for testing *Streptomyces* spp. All the microorganisms and media used are listed in Table 1. The experiment was conducted twice with 4 replicates for each isolate.

[0051] In plant assay. A pot assay was conducted in a greenhouse. *Streptomyces scabies* (Deposited as ATCC No. 49173) was cultured in oatmeal broth at 28°C for 4 to 5 days in an incubator shaker at 180 rpm, and the concentration of liquid culture was determined by plating on YME. Potato tuber pieces with one eye (cv. 'Snowden') at least were surface disinfested with 1% NaClO for 5 min, and then rinsed with sterile distilled water three times. After air-drying, the tuber was incubated in potting mix (ASB Greenworld Inc., New Brunswick, VA, USA) in a growth chamber at 25°C until seedling emergence. The potato seedlings were transferred to a 3.78 L plastic pot with potting mix, infested with *S. scabies* by pouring the inoculum to the top of the potting mix at a final concentration of 10^6 colony forming unit (CFU)/cm³. Liquid culture of BAC03 was added to the potting mix as a drench to give a final concentration of 10^5 (treatment B1) and 10^6 (treatment B2) CFU/cm³. Treatment with BAC03 was done two times; once when seedlings were transplanted and a second 20 days later. A positive control using *S. scabies* inoculum only (S), and a negative control of non-infested potting mix mixed with tryptic soy broth at a level similar to the amount added with B2 (CK) were used. Six weeks after transplanting, the height of the plant from the soil line to the apical meristem of potato was measured with a ruler. Plants were harvested 10 weeks after transplanting. Tubers were examined for lesions and given a

severity rating using the 0 to 5 scale where 0 = no disease, and 5 = the whole tuber is covered by scabby lesions. Tuber yield was determined by measuring the weight of potato tubers from each pot.

[0052] Radish (cv. “Cherry Belle”, Burpee Inc. Warminster, PA, USA) seeds were pre-germinated in a Petri dish (VWR International, LLC, Radnor, PA, USA) with moist filter paper overnight at 25 °C. After germination, seedlings were sown in a 1 L pot containing autoclaved potting mix infested with *S. scabies* (10^7 CFU/cm³), in a growth chamber (23°C and 14 h light). BAC03 was applied (10^5 CFU/cm³ for each time) as the following treatments: b0: non-treated; b1: 1 time after planting (TAP); b2: 2 TAPs; b3: 3 TAPs; and b4: 1 time before planting and 3 TAP. The intervals between BAC03 applications were one week. There were 4 replicates for each treatment. The disease severity was determined by giving a severity rating of lesions with 0 to 5 scale, where 0 = no disease, and 5 = the whole tuber is covered by scabby lesions. And the weight of leaves and roots were determined at harvest, 6 weeks after planting.

Detection of *S. Scabies* Population in BAC03-Potting Mix Using Quantitative Polymerase Chain Reaction (qPCR) Assay

[0053] After radish plants were pulled out for disease rating, the remnant potting mix from each pot was air-dried and mixed for 5 min by hand. Two samples (0.1 g each) of the potting mix were collected from each pot (eight samples/treatment). Total genomic DNA was extracted with the FastDNA Spin kit for soil (MP Biomedicals). DNA was analyzed using qPCR to quantify *S. scabies* population in potting mix by amplifying the genes *txtA* and *txtC* (Table 2), which were an indicator of quantity of pathogenic *S. scabies* (Loria *et al.* 2006). Reactions were prepared in triplicate in 96-well optical plates using the following master mix: 10 µl of ABI SYBR Green PCR master mix (2 X) (Applied Biosystems, Carlsbad, CA, USA), 2 µl of 1/10 diluted potting mix DNA, 0.5 µl of each primer (10 µM), and 7 µl of sterile ddH₂O. External standards for quantification consisted of six concentrations of serially diluted *S. scabies* genomic DNA (10, 1, 0.1, 0.01, 0.001, 0.0001 ng/µl), and were included in triplicate in each PCR run to calculate quantities of *S. scabies* in the unknown potting mix samples. The PCR was carried out in a StepOnePlus Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) using the conditions described by the manufacturer with 45 amplification cycles. To confirm single products, the melting curve and gel electrophoresis were used. The response of potato plant to foliar spray application of BAC03. Twenty ml of BAC03 culture broth (10^6 CFU/ml) was sprayed onto potato leaves when the plants reached 15 cm in height. Applications were sprayed weekly for 5 weeks.

The potting mix in the pots was covered with a plastic film to avoid contamination from foliar spray. *S. scabies* (10^6 CFU/cm³ potting mix) was slowly poured onto the surface of potting mix evenly 5 days after transplanting, and repeated two weeks later. A negative control of inoculation with *S. scabies* only, and 5 replicates were used for each treatment. Potato tubers were harvested after 3 months and the lesion severity was rated as above.

Effects of BAC03 on Seedling Emergence

[0054] Cut potato tubers, with at least one eye per piece, were surface disinfested with 1% NaClO for 5 min, and rinsed with sterile water three times. After air-drying overnight, the potato tubers were sown in 3.78 L pots containing 2.5 L of potting mix. The seed tubers were placed on the potting soil surface, with 4 tuber pieces per pot, followed by covering with the potting mix (5 cm depth). BAC03 cultured in TSB for 24 hours was mixed into the potting mix at potting to make the final concentrations of 10^4 , 10^5 , and 10^6 CFU/cm³ potting mix. Each treatment had five replicates. For radish (cv. Cherry Belle), 40 seeds were sown at a 2 cm depth in eight 1 L pots with five seeds each. BAC03 liquid culture was drenched into the potting mix to concentrations of 10^4 , 10^5 , and 10^6 CFU/cm³ potting mix. Potting mix with TSB only (same volume with 10^6 CFU/cm³ treatment) was prepared as a negative control. The total number of emerged seedlings was recorded when the seeds in control pots all were at approximately 2 cm high.

Isolation and Characterization of Potential Antimicrobial Substances

[0055] Precipitation. Two extraction methods were used to separate different types of secondary metabolites. BAC03 was grown in 200 ml of TSB at 28°C on a shaker incubator at 180 rpm for 48 h. The culture was centrifuged at 15,000 g for 20 min at 4°C and the supernatant was collected for all extractions.

[0056] For proteinaceous secondary metabolites, BAC03 culture supernatant was extracted using ammonium sulfate precipitation as described by Sutyak *et al.* (2008) with a little modification as follows. Eighty percent ammonium sulfate was added to the supernatant, and incubated at 4°C overnight. The mixture was centrifuged at 15,000 g for 30 min at 4°C, and the supernatant was discarded. The precipitate was dissolved in 10 mM sodium phosphate buffer (pH 6.0), and applied to a Sephadex G-50 column (Pure Biotech LLC, Middlesex, NJ, USA) and centrifuged at 2000 g for 3 min. The eluted fluid was passed through a 0.22 µm filter membrane (Millipore, Billerica, MA, USA), and the antimicrobial activity of this substance against *S. scabies* was tested by the agar diffusion assay as

described above. This extract was designated as ammonium sulfate precipitated material (AS) for the rest of the study.

[0057] Extraction of lipopeptides was carried out according to the combination of acid precipitation and solvent extraction described by Vater *et al.* (2002) with slight modifications. The pH of the culture filtrate was adjusted to 2.0 by adding 6 N HCl followed by precipitation at 4°C overnight. The pellet derived from centrifugation (15,000 g for 30 min at 4°C) following precipitation was dissolved in 100% methanol. The mixture was passed through a 0.22 µm filter membrane (Millipore, Billerica, MA, USA). The antimicrobial activity of the extract against *S. scabies* was tested with the agar diffusion assay described above. This material was designated acid precipitate material (AP) for the rest of the study.

[0058] Based on the results of the agar diffusion assay, the material from the ammonium sulfate precipitation produced larger inhibition zones (> 2 cm) and thus further study concentrated on this material. The lipopeptide fraction showed low activity (< 0.5 cm) and was not examined further. The concentration of protein in the AS was determined using a Bradford Protein assay kit (Thermo Fisher Scientific Inc., Rockford, IL, USA) with a Nanodrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) based on a standard curve constructed for colorimetric absorbance at 595nm.

[0059] To test the quantitative effect of the AS fraction on *S. scabies*, a spore suspension of *S. scabies* (10⁵ CFU/ml) was mixed with BAC03 AS at concentrations equivalent to 0, 15, 30, 45, 60, 75, and 90 µg of protein/ml as determined by the Bradford assay described above. The mixture was spread on YME plates and incubated at 28°C in the dark. Any inhibitory effect was evaluated by counting *S. scabies* colonies on the plate after 3-day incubation in comparison with a control of plating spore suspension only.

[0060] To determine whether the extract was bactericidal or bacteriostatic, 10⁵ CFU/ml of a *S. scabies* spore suspension was incubated with the AS fraction at a final concentration of 30, 60, and 90 µg/ml. After 0, 1, 3 and 5-day incubation at 28°C in the dark, the mixture was filtered through a 0.22 µm membrane, and the *S. scabies* collected from the filter surface was spread on YME to determine the concentration of viable *S. scabies*.

Effects of Temperature, pH, Enzymes, and Chemical Solvents On Antimicrobial Activity of Liquid Culture and AS of BAC03

[0061] Both BAC03 liquid culture and its derived AS fraction were examined for their reaction to various factors, including temperatures, pH levels, enzymes, and chemicals. To test the effect of temperature, the antimicrobial activity was assessed after being incubated at

temperatures of 40, 60, 80, and 100°C for 30 min, or 121°C (autoclaving) for 15 min. To test the effect of pH levels, the pH was adjusted from 1.0 to 14.0 by whole pH units using sterile 1 N HCl or 1 N NaOH. Material was incubated at these pH levels overnight at 4°C. Prior to assessing activity, the pH was readjusted to pH 7.0. Several enzymes were tested for their effect on activity by incubating the BAC03 culture or the AS fraction with 10 mg/ml of the following enzymes for 2 h at the optimal temperature for each enzyme according to the manufacturer's instruction. Enzymes were trypsin (MP Biomedicals, Solon, OH, USA, 25°C, pH 7.6); proteinase K (Sigma-Aldrich, Inc. St Louis, MO, 37°C, pH 7.5); pepsin (Sigma-Aldrich, Inc., 37°C, pH 2.0); α -chymotrypsin (Sigma-Aldrich, Inc., 25°C, pH 7.8); and catalase (MP Biomedicals, 25°C, pH 7.0). Effect of various chemical solvents (Table 3) was tested by incubating 5 h at 25°C with 10% organic solvents. Antimicrobial activities of the treated culture and the AS fraction were tested against *S. scabiei* using the disc diffusion assay as described above.

Scanning Electron Microscope Analysis

[0062] To examine the effect of the AS fraction on *S. scabiei*, scanning electron microscopy (SEM) was used to observe the morphology of mycelia on media. Ten microliters of the AS fraction was dropped onto a *S. scabiei* 2-day-old colony. Sodium phosphate buffer was used as a control. The plates were incubated at 28°C in the dark. Five days later when the control colonies became white with sporulation, the culture was coated with metallic osmium and processed according to the methods of Fan *et al.* (2011) by the Center for Advanced Mycology at Michigan State University, and examined with a scanning electron microscope (JEOL JSM 6400 (Japan Electron Optics Laboratories) at high voltage (10 kV).

Molecular Mass, Chemical Structure of the AS Fraction

[0063] The AS fraction was obtained by dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE). Following electrophoresis, the gel was vertically cut into two parts. One part of the gel, containing the sample and molecular weight standards, was stained with Coomassie Blue protein stain. The other part, containing the same sample, was tested for antimicrobial activity using the method of Naclerio *et al.* (1993). Briefly, the gel was treated with 20% isopropanol-10 mM Tris-HCl (pH 7.5) for 3 h, rinsed for 1.5 h in 10 mM Tris-HCl (pH 7.5), and washed twice with distilled water for a total of 1 h. The gel was placed in a Petri dish with 1% water agar, and overlaid with 0.8% YME mixed with a bacterial suspension of *S. scabiei* (10^7 CFU/ml). The plate was incubated at 28°C for 48 h,

then dyed with thiazolyl blue tetrazolium bromide (MTT, Sigma-Aldrich Co. LLC, St. Louis, MO, USA) to stain living organisms, and observed for any unstained zone(s). Molecular weight of the AS fraction was measured according to the standard molecular marker that was also run on SDS-PAGE gel.

[0064] The active band associated with the antimicrobial activity above was excised, eluted from the gel, and digested according to Shevchenko *et al.* (1996). Liquid chromatography-mass spectrometry (LC/MS/MS) was conducted at the Michigan State University Proteomics Facility Center. The resulting MS/MS spectra were converted to peak lists using BioWorks Browser v3.3.1 (Thermo Fisher Scientific Inc, Pittsburgh, PA, USA) and searched against all gram-positive bacterial protein sequences downloaded from NCBI. The Mascot output was analyzed using Scaffold v3.08 to probabilistically validate protein identifications using the ProteinProphet2 computer algorithm. Assignments validated above the Scaffold 95% confidence filter were considered valid.

PCR Detection

[0065] Based on the identified protein, the corresponding predicted nucleotide sequence was amplified with specific primers (Table 1 and reported by Hu *et al.* (2010)) using PCR. DNA extraction, PCR amplification and sequence determination were conducted as described above.

Table 1. Primers for polymerase chain reactions used in this study to amplify portions of bacterial genes.

Gene	Primer	Sequence (5'-3')	SEQ ID NO:
16s rDNA	63-F	CAGGCCTAACACATGCAAGTC	7
	1387-R	GGGCGGWGTGTACAAGGC	8
<i>recA</i>	recA-F	TGAGTGATCGTCAGGCAGCCTTAG	9
	recA-R	TTCTTCATAAGAATACCACGAACCGC	10
<i>recN</i>	recN-F	CTTTTGCGATCAGAAGGTGCGTATCCG	11
	recN-R	GCCATTATAGAGGAAGTACG ATTTC	12
<i>cheA</i>	cheA-F	GTTTGAAGCGCTTGATCATCTAGAA	13
	cheA-R	GGTTCATTGAAGAATCCAGTCTTTC	14
<i>gyrA</i>	gyrA-F	CGTACGCAATGAATGATTAGGCATG	15
	gyrA-R	ATTATGAAAGACGTCTGCAGGGCCGTT	16
<i>lci</i>	lci-F	CGCGGATCCATGAAATTCAAAAAG	17
	lci-R	GCGTCGACTTATTTATCTACACTTTCAT	18

<i>txtA</i>	txtA-F	TGCTCAACTCCGTGATCCAGTA	19
	txtA-R	GGGACACCTCGCGCAGTA	20
<i>txtC</i>	txtC-F	ACCATCTCGCTGTCCTTGGT	21
	txtC-R	CGTGGACGACGGAGAACTTC	22

Statistical Analysis

[0066] Data were analyzed using SAS software (Version 9.2, SAS Inc., Cary, NC, USA). Procedure GLM was used for analysis of variance, and Fisher's least significance difference (LSD) multiple comparisons were performed for mean separation. Procedure REG was used for linear regression.

Identification of BAC03

[0067] The 16s rDNA sequence of BAC03 had a similarity higher than 99% with both *B. subtilis* and *B. amyloliquefaciens*. However, based on fragment sequences of *recA* (SEQ ID NO. 2), *recN* (SEQ ID NO. 3), *cheA* (SEQ ID NO. 4) and *gyrA* sequences (SEQ ID NO. 5), the results showed the highest similarity between strain BAC03 and *B. amyloliquefaciens*.

Antimicrobial activity of BAC03

[0068] In vitro assay. Growth inhibition of microorganisms were evaluated on agar media by *Bacillus amyloliquefaciens* strains BAC03 and *B. subtilis* QST713, with 15 µl bacterial suspension (10⁶ CFU/ml) applied on a sterile filter paper disc.

Table 2. BAC03 Antimicrobial Activity.

Test microorganism	^x Source, location	Inhibition zone diameter (cm) ^y		^z Media
		BAC03	QST713	
<i>Streptomyces scabies</i> 49173	ATCC, USA	2.53*	1.70	YME
<i>Streptomyces. scabies</i> 1231	NRRL, USA	1.08*	0.62	YME
<i>Streptomyces acidiscabies</i> 49003	ATCC, USA	0.86*	0.23	YME
<i>Streptomyces stelliscabiei</i> Her21	Hao, MI	1.45	1.45	YME
<i>Streptomyces aureofaciens</i> 5404	NRRL, USA	0.78	0.58	YME
<i>Streptomyces</i> sp. DS3024	Hao, MI	1.16	0.90	YME
<i>Agrobacterium tumefaciens</i>	Day, USA	2.18*	1.80	LB
<i>Pseudomonas syringae</i>	Kirk, MI	1.48	1.30	LB
<i>Clavibacter michiganensis</i> ssp. <i>michiganensis</i>	Kirk, MI	2.40*	1.90	523
<i>Cladosporium cucumerinum</i>	Hammerschmidt, MI	0.80	0.70	PDA
<i>Fusarium solani</i>	Hao, MI	0.60	0.50	PDA
<i>Cryphonectria parasitica</i>	Fulbright, MI	1.50*	1.20	PDA
<i>Phytophthora capsici</i>	Hao, MI	1.05*	0.70	PDA
<i>Penicillium citrinum</i>	Hao, MI	0.25	0.15	PDA

<i>Phytophthora cambivora</i>	Fulbright, MI	1.15*	0.70	PDA
<i>Phytophthora cinnamomi</i>	Fulbright, MI	1.30	1.30	PDA
<i>Botrytis cinerea</i>	Hao, MI	2.00*	0.85	PDA
<i>Rhizoctonia solani</i>	Hao, MI	1.60*	0.85	PDA
<i>Sclerotinia sclerotiorum</i>	Hao, MI	1.80*	0.50	PDA

^x ATCC: American Type Culture Collection (<http://www.atcc.org/>), Manassas, VA, USA.
 NRRL: USDA-ARS Agriculture Collection (<http://nrml.ncaur.usda.gov/>), Peoria, IL, USA.
 Kirk, Hao, Fulbright, and Hammerschmidt are from collections of professors at Michigan State University.

^y Each number is the average of 8 measurements.

^z LB: Lysogeny broth medium; PDA: potato dextrose agar; 523: bacterial screening medium (SigmaAldrich, Inc. St Louis, MO, USA).

* indicates significant difference between the average inhibition zone size using strain BAC03 and QST713 by Student *t*-test ($\alpha = 0.05$).

[0069] Table 2 shows that BAC03 displays antagonistic activity against a broad spectrum of bacteria, fungi, and oomycetes. BAC03 showed significantly greater inhibition zones ($P < 0.05$) than QST713 on 13 out of 21 test organisms.

[0070] In the greenhouse, the severity of potato common scab was significantly reduced ($P < 0.05$, Fig. 1 A1), and potato growth (plant height and tuber weight) was significantly increased ($P < 0.05$, Fig. 1 A2) by BAC03 application compared to pathogen control; these effects were enhanced by increasing the concentration of BAC03 applied (Fig. 1A).

[0071] For radish, high concentrations of *S. scabies* inhibited root expansion, which interfered with the disease evaluation as indicative lesions were not visible (Fig. 1 B1). However, application of BAC03 reduced this inhibition, as radish root expansion was observed. The greatest response was obtained when BAC03 was applied before radish seeding; this resulted in no disease symptoms. One, two, or three times of applications after seeding also reduced the disease severity with the rating scale below 1 (Fig. 1 B1). The biomass, including weights of leaves and roots, of radish was significantly increased ($P < 0.05$, Fig. 1 B2), when BAC03 concentration increased. QPCR analysis showed that *S. scabies* population in potting mix inoculated with BAC03 before radish seeding was significantly ($P < 0.05$) lower than that in all other treatments (Fig. 2).

Effect of BAC03 on Disease Suppression with Foliar Spray

[0072] Foliar spray with BAC03 did not affect potato growth or disease severity; sprayed plants had disease severity average ratings of 0.76 ± 0.16 , and tuber weight of 48.04 ± 2.09 g/pot; non-treated plants had disease severity of 0.75 ± 0.17 and tuber weight of 47.82 ± 1.55 g/pot.

Effects of BAC03 on Plant Emergence

[0073] Emergence of potato and radish reacted differently to BAC03 treatment. BAC03 did not affect the amount of emergence of potato seedlings, regardless of concentrations. However for radish, only 20 ± 5.8 % plants emerged during the course of experiment when treated with BAC03 culture at 10^4 CFU/cm³ potting mix, compared to 100% radish emerged for the control. No radish emergence was visible following treatments with BAC03 at 10^5 and 10^6 CFU/cm³ potting mix.

Characterization of an Antimicrobial Substance

[0074] Based on the agar diffusion assay, the AS fraction was picked for further study. The AS fraction significantly reduced the *S. scabiei* population when evaluated by the co-culturing method, and the antimicrobial effect was positively correlated with the AS concentration (Fig. 3A). The inhibition was almost 100% when the AS concentration was 75 µg/ml. In an assay for bactericidal effect, a reduction in the number of *S. scabiei* colonies following the removal of the AS fraction was observed after co-incubation for different times, and the antimicrobial effect was enhanced with increasing exposure time to the AS fraction (Fig. 3B).

[0075] The antimicrobial activity was not affected by catalase, or any of the organic solvents tested (Table 3), but activity was completely removed by treatments with proteinase K, trypsin, pepsin, or chymotrypsin. The activity was relatively heat stable, and maintained the same level when treated by temperature up to 60°C, but activity was totally lost following autoclaving. The culture broth and AS fraction were also active over a wide pH range, but the effect was reduced in extremely acid (pH<3) or alkaline (pH>12) conditions (Table 3). Overall, the culture broth was more tolerant to adverse conditions than extracted AS fraction

Table 3. Effects of temperatures, pH levels, enzyme treatment, and chemical solvents on the activity of *Bacillus amyloliquefaciens* BAC03 as liquid culture in tryptic soy broth of the bacterium or as the ammonium sulfate precipitate fraction from BAC03 liquid culture.

Residual activity of BAC03 (% $\pm$ standard deviation) ^x			
	Treatment ^z	Liquid culture ^w	AS
None		100	100
Chemicals	Acetone	100	100
	Ethyl acetate	100	100
	Ethyl alcohol	100	100
	Chloroform	100	100
	Methyl alcohol	100	100
Enzymes	Proteinase K	5 ± 3	0
	Trypsin	7 ± 2	0
	Pepsin	15 ± 4	0
	Chymotrypsin	8 ± 4	0

	Catalase	100	100
Temperature	40 (30 min)	100	100
	60 (30 min)	100	100
	80 (30 min)	89 ± 3	61 ± 4
	100 (30 min)	26 ± 6	10 ± 3
	121 (15 min)	0	0
pH	1	76 ± 3	55 ± 6
	3	100	100
	5	100	100
	10	100	100
	12	100	100
	14	0	0

^zThe culture broth and ammonium sulfate precipitate (AS) were exposed to various treatments, including: chemical solvent (10%) for 5 h at 25°C temperatures of 40, 60, 80, and 100°C for 30 min, and 121°C (autoclaving) for 15 min pH levels from 1.0 to 14.0 (every single unit) using sterile 1 N HCl or 1 N NaOH, and was readjusted to pH 7.0 after incubation overnight at 4°C. A subset of levels is shown as pH 1, 3, 5, 10, 12, 14 in the table enzymes (10 mg/ml) for 2 h, including trypsin (25°C, pH 7.6), proteinase K (37°C, pH 7.5), pepsin (37°C, pH 2.0), α-chymotrypsin (25°C, pH 7.8), and catalase (25°C, pH 7.0).

^x Residual activity was measured by an agar disc diffusion assay, and calculated as [(diameter of inhibition zone on treated plate)/ (diameter of inhibition zone of control)]*100%

^w Culture broth: strain BAC03 was cultured in TSB for 24 hours (10⁶ CFU/ml) in a shaker with 180 rpm. AS: crude antimicrobial substance, which was extracted by ammonium sulfate precipitation from the culture broth supernatant, and filtered through Sephadex G-50 column and 0.22 µm filter membrane.

[0076] The AS fraction affected *S. scabiei* sporulation. When treated with the AS fraction, the *S. scabiei* colonies showed no color change throughout the 7 day incubation period, compared to non-treated colonies, which changed to white or whitish grey. The morphology of the mycelia observed by SEM displayed spiral spore chain formation on non-treated plates but no such structure formed on AS-treated cultures during the time period examined (Fig. 4).

Molecular Mass and Chemical Structure Determination Of AS And PCR Testing

[0077] The AS fractionated on SDS-PAGE displayed a clear band with a molecular mass of approximately 10 kDa after MTT staining, indicating no bacterial growth at an area (Fig. 5). LC/MS/MS results suggested that the most probable compound was the 10 kDa (molecular weight) LCI protein. The sequence similarity of the amplified putative LCI (SEQ ID NO. 1) was 99% between strains BAC03 and *B. amyloliquefaciens* FZB42 (accession number: CP000560.1) or C-31 (accession number: FJ904931.1). The DNA sequence and putative translated protein sequence were compared to those of reported *B. amyloliquefaciens* strains. All of the strains had a putative protein of 94 amino acids. The LCI protein sequence for BAC03 was 100% identical with strain C31 (Hu et al. 2010), but had one amino acid different from FZB42.

[0078] A reduction in scab severity was observed in both potato and radish, where the population of *S. scabies* was significantly suppressed by BAC03, and suppression was increased as the time of application was increased.

EXAMPLE 2

[0079] Plant growth promotion assay. In a growth chamber (23°C, 14h/8h light/dark cycle) the growth promoting effect of BAC03 (10^5 CFU/cm³) was evaluated on radish, pepper, beet, carrot, turnip, cucumber and tomato. Three seeds were planted per pot with four pots per treatment. Media only was used as a control. Weight and length of plant shoots and roots were measured after matured. See Table 4 for results.

[0080] The experiment is repeated with measurement including shoot length, fresh and dry shoot weight, fresh and dry root weight and compared to other strains of biocontrol product.

Table 4. Effect of BAC on growth of seven plant types. Two applications of BAC03 (10^5 CFU/cm³) were drenched into the soil after plant germination and two weeks later. The same volume of media was used for control.

	Measurement objective	BAC03	Control
Radish	Aboveground weight (g)	34.6 ± 4.1*	6.1 ± 1.9
	Weight of tubers (g)	46.0 ± 4.9*	8.6 ± 3.1
Tomato	Height (cm)	38.2 ± 4.1*	30.7 ± 2.2
	Aboveground weight (g)	29.1 ± 9.2*	15.2 ± 2.3
Turnip	Weight of tubers (g)	19.9 ± 9.7*	7.5 ± 5.4
Beet	Weight of tubers (g)	18.4 ± 3.4*	8.0 ± 2.2
Cucumber	Aboveground weight (g)	29.5 ± 2.8*	19.3 ± 3.1
Pepper	Aboveground weight (g)	26.5 ± 11.2	21.0 ± 3.5
Carrot	Weight of tubers (g)	13.5 ± 3*	7.7 ± 1.8

* indicates significant difference between the treated and control.

EXAMPLE 3

[0081] The ability of BAC03 to induce systemic resistance is determined by a split root system. In a greenhouse, potato roots of a plant were split into two parts, A and B, and each part placed in a separate pot when the root system was well developed. Four groups were created including: Group I, roots inoculated with BAC3 and *S. scabies* in root part A and with *S. scabies* in root part B; Group II, root inoculated with BAC03 in root part A and *S. scabies* in root part B; Group III, roots inoculated with *S. scabies* in root part A and non-

treated in root part B; and Group IV, roots not treated in both root parts A and B. See the results in Figure 6A, measuring disease severity, and Figure 6B measuring potato tuber weight. Where BAC03 was applied to one root part, the separated plant part had reduced disease severity and increased tuber weight. In the absence of exposure to BAC03, both plant parts did not show a decrease in disease severity nor enhanced growth. Thus it can be seen BAC03 was able to induce systemic resistance in plants.

EXAMPLE 4

[0082] Field trials were conducted by applying BAC03 liquid culture at two locations, where soil was naturally infested with high population of *Streptomyces scabies*, along with other biomaterial applications. To prepare liquid culture, strain BAC03 was cultured in the YME liquid medium (yeast extract 4.0 g, malt extract 10.0 g, dextrose 4.0 g, and agar 20.0 g per liter) for 24 h at 180 rpm. The cell concentration (10^6 CFU/ml) was determined by plating the diluted cultures on YME agar medium.

[0083] The field trial was conducted in a randomized block design with plot size of 2 feet by 25 feet in four replicates within each location. A common scab susceptible cultivar (cv. 'Snowden') was used in the field experiment. In the field, chestnut tissues (shells and pellicles) were applied at a rate of 15 tons/acre and oregano essential oil at a rate of 13.5 quarts/acre and pressed horseradish root applied at one ton per acre. Each of these three treatments was applied two weeks before planting. The BAC03 spore suspension (10^6 CFU/ml) and the tryptic soy broth are applied at 360 quarts/acre four times after planting at two week intervals. A non-treated control is also provided. Four replications were set for each treatment.

[0084] Potatoes were harvested and the tubers scored for common scab disease. Fifty tubers were arbitrarily selected from each plot for disease evaluation. Disease lesions on potato tubers were scored: 0 = no symptoms, 1 = 1 to 10% surface area with superficial or raised lesions, 2 = 11 to 25% surface area with superficial or raised lesions, 3 = 26 to 50% surface area with superficial or raised lesions, 4 = > 50% surface area with superficial or raised lesions or < 25% pitted lesion area, and 5 = >50% surface area with superficial or raised lesions or >25% pitted area. The disease severity index was calculated as $\Sigma(\text{score} * \text{number of tubers with that score}) / \text{total number of potato tubers evaluated}$. For yield assessment, all the potatoes in the middle area with 10 feet long in each plot were gathered and weighted. Yield and disease severity are assessed at harvest. Measurement of disease

severity and weight (evaluated at harvest) is shown for location 1 in Figure 7A and 7B and for location 2 in Figure 8A and 8B.

EXAMPLE 5

[0085] The experiment of Example 3 may be repeated using radish, pepper or tomato. Briefly, the plant roots may be split and placed into two separate pots infested with a pathogen. To one of the two pots may be applied the *Bacillus* solution and disease suppression may be evaluated. Assessing of disease suppression may be done by evaluating plant defense-related enzymes such as peroxidase (POX), polyphenol oxidase (PPO) or phenylalanine ammonia lyase (PAL). The plant growth promoting effect of the bacteria may also be evaluated by assaying for the production of indoleacetic acid (IAA), NH₃, or HCN, and presence of phosphate solubilization and ACC-deaminase. These secreted substances or reactions are correlated with the plant growth promotion.

[0086] All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the various embodiments described herein.

[0087] Although specific embodiments have been illustrated and described herein, it will be appreciated by those of ordinary skill in the art that any procedure that is calculated to achieve the same purpose may be substituted for the specific embodiments shown. For example, although the process has been discussed using potato plants, any other plant such as tomato, radish or pepper, for example, may be used. This application is intended to cover any adaptations or variations of the present subject matter. Therefore, it is manifestly intended that embodiments of this invention be limited only by the claims and the equivalents thereof.

WHAT IS CLAIMED IS:

1. A biologically pure or isolated culture deposited as ATCC NO. PTA-12890.
2. A composition comprising the culture of claim 1.
3. A composition for controlling plant disease severity of soil plant pathogens comprising *Bacillus amyloliquefaciens* BAC03 strain.
4. The composition of claim 2 or comprising spores, cells or whole culture of the strain.
5. The composition of claim 2 or comprising a substance isolated from a whole cell culture of the strain, an ammonium sulfate precipitated fraction of a whole cell culture of the strain, and spores of the strain.
6. The composition of claim 5 wherein the substance comprises an LCI protein.
7. The composition of claim 5 wherein the substance comprises sequence possessing at least 85% sequence identity to SEQ ID NO:1.
8. The composition claim 2 or 3 further comprising one or more other compositions.
9. The composition of claim 2 or wherein the strain is present in a concentration of up to 10^7 CFU cm^{-3} .
10. The composition of claim 3 wherein the plant disease is common scab.
11. The composition of claim 2 or having plant growth promoting activity.
12. The composition of claim 2 or having antagonistic or antimicrobial activity against plant soil pathogens.
13. The composition of claim 12 wherein the plant pathogen is selected from *Streptomyces scabies*, *Streptomyces acidiscabies*, *Streptomyces stelliscabiei*, *Streptomyces aureofaciens*, *Streptomyces* sp., *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Clavibacter michiganensi*, *Cladosporium cucumerinum*, *Fusarium solani*, *Cryphonectria parasitica*, *Phytophthora capsici*, *Penicillium citrinum*, *Phytophthora cambivora*, *Phytophthora cinnamomi*, *Botrytis cinerea*, *Rhizactonia solani* and *Sclerotinia sclerotiorum*.

14. A method of reducing disease severity of a plant pathogen, the method comprising applying to soil or a plant or plant part an effective amount of the *B. amyloliquefaciens* strain BAC03 deposited as ATCC NO. PTA-12890, or a substance selected from a whole cell culture of the strain, an ammonium sulfate precipitated fraction of a whole cell culture of the strain, and spores of the strain.
15. The method of claim 14 wherein the plant part is a root, stem or leaf.
16. The method of claim 14 wherein the plant part is a seed.
17. The method of claim 14 wherein applying is to the soil, plant, plant part and is more than once.
18. The method of claim 14 wherein applying is to soil before or contemporaneously with sowing plant seeds in soil.
19. The method of claim 14 wherein apply is to seeds after plant seeds have been sown in the soil or to growing plants.
20. The method of claim 14 wherein the plant pathogen is selected from *Streptomyces scabies*, *Streptomyces acidiscabies*, *Streptomyces stelliscabiei*, *Streptomyces aureofaciens*, *Streptomyces* sp., *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Clavibacter michiganensi*, *Cladosporium cucumerinum*, *Fusarium solani*, *Cryphonectria parasitica*, *Phytophthora capsici*, *Penicillium citrinum*, *Phytophthora cambivora*, *Phytophthora cinnamomi*, *Botrytis cinerea*, *Rhizactonia solani* and *Sclerotinia sclerotiorum*.

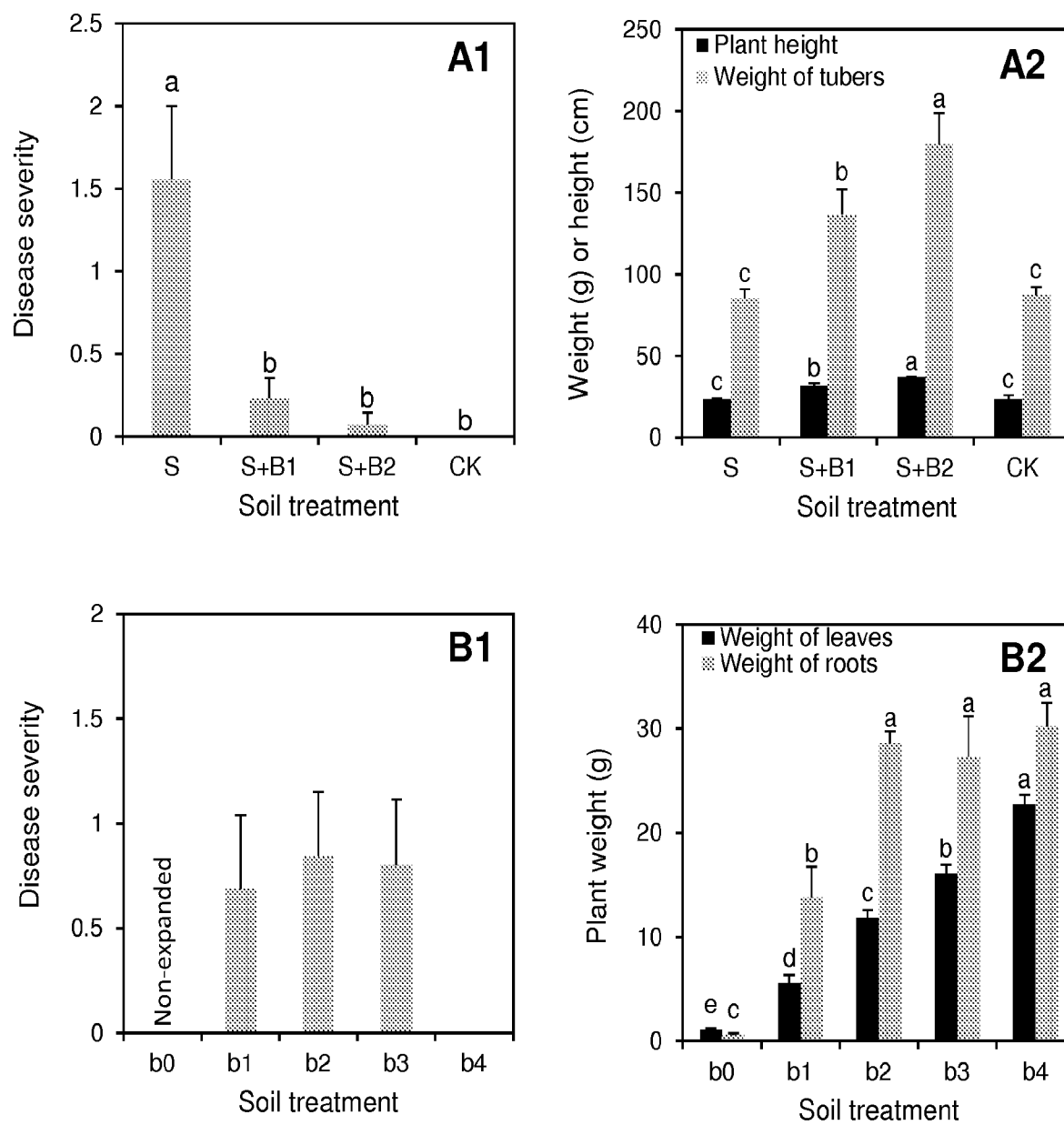


Figure 1

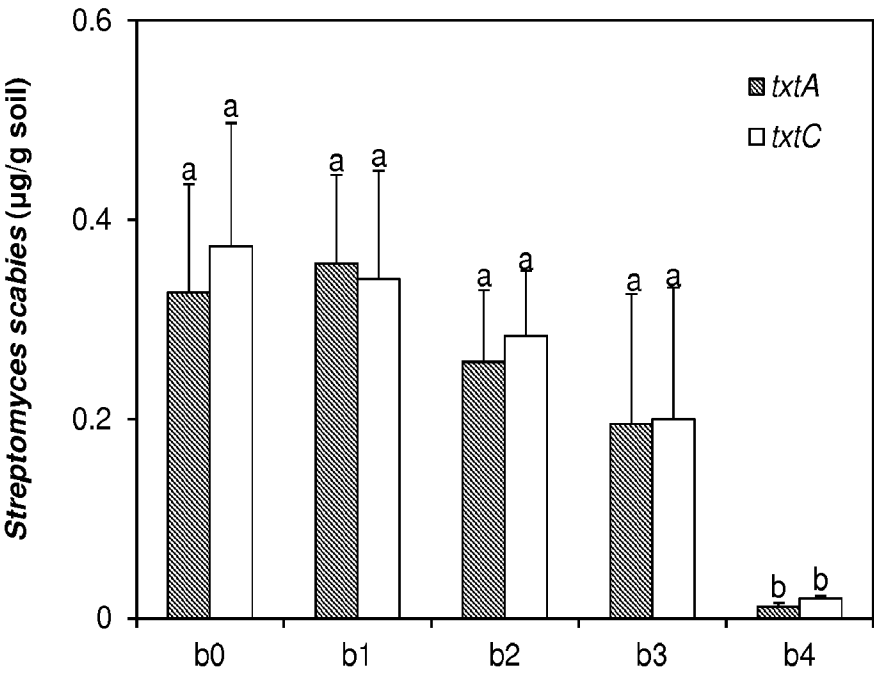


Figure 2

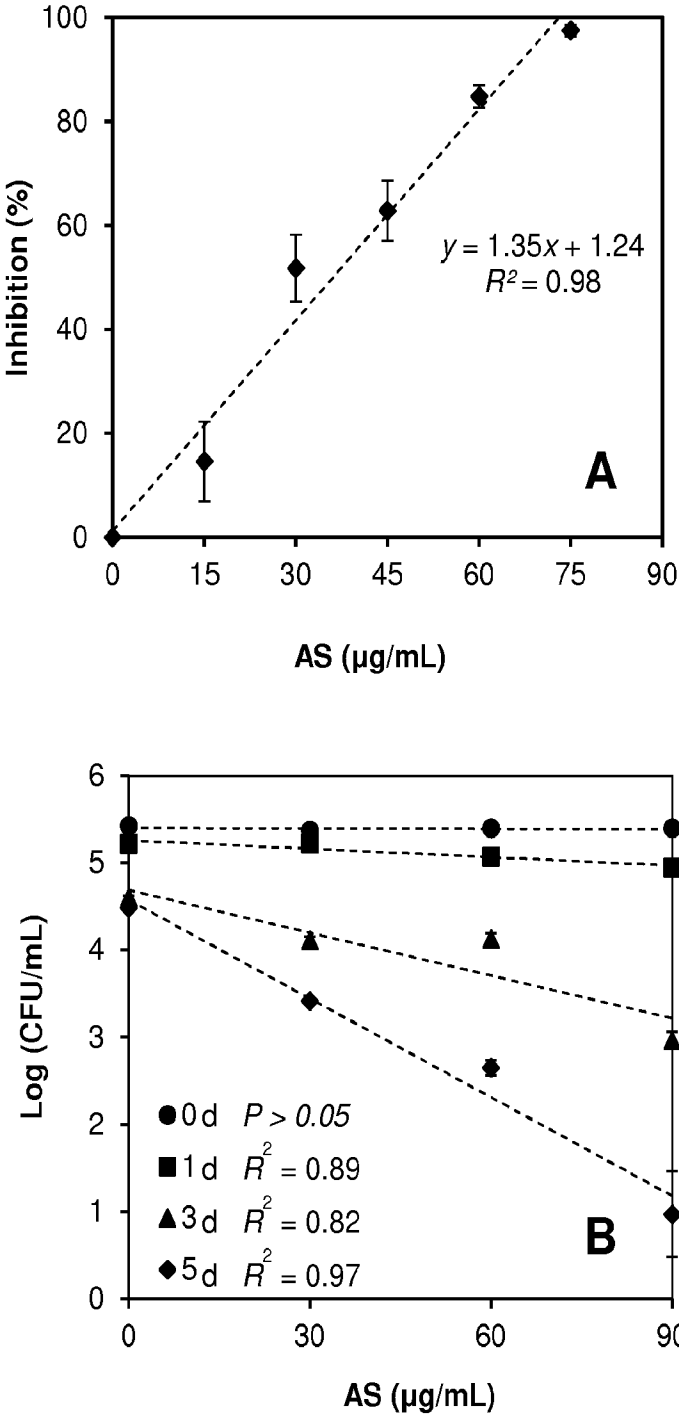
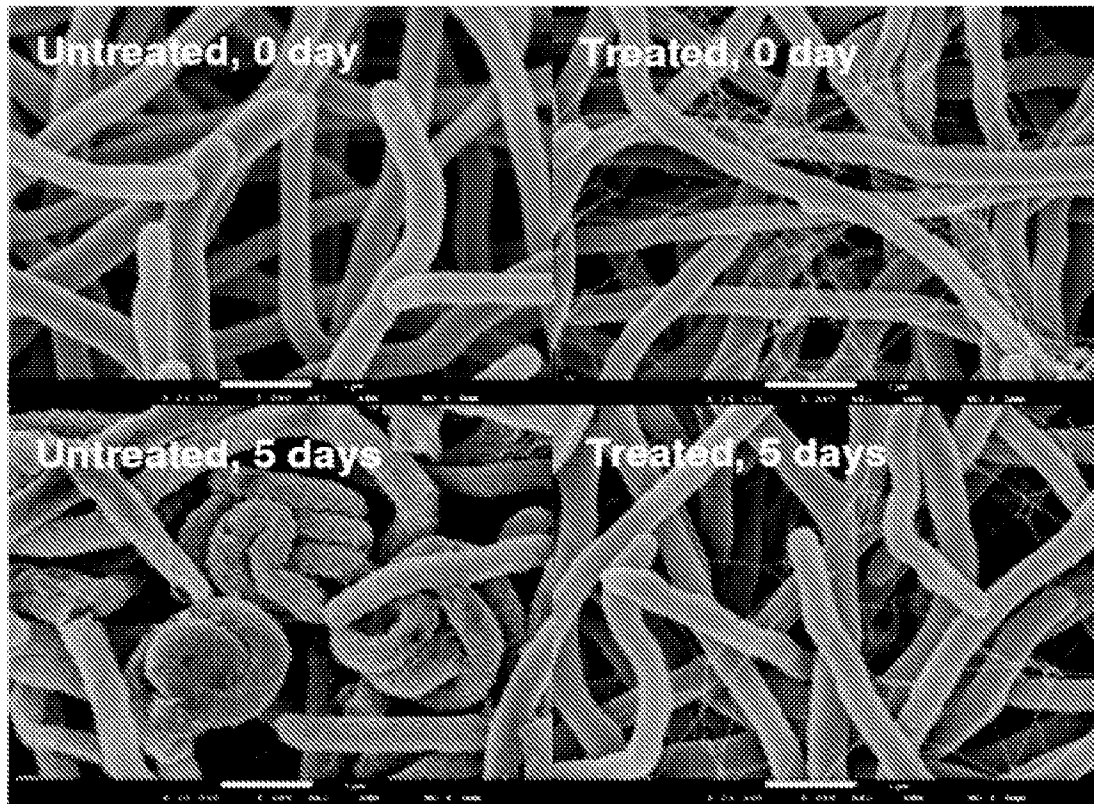
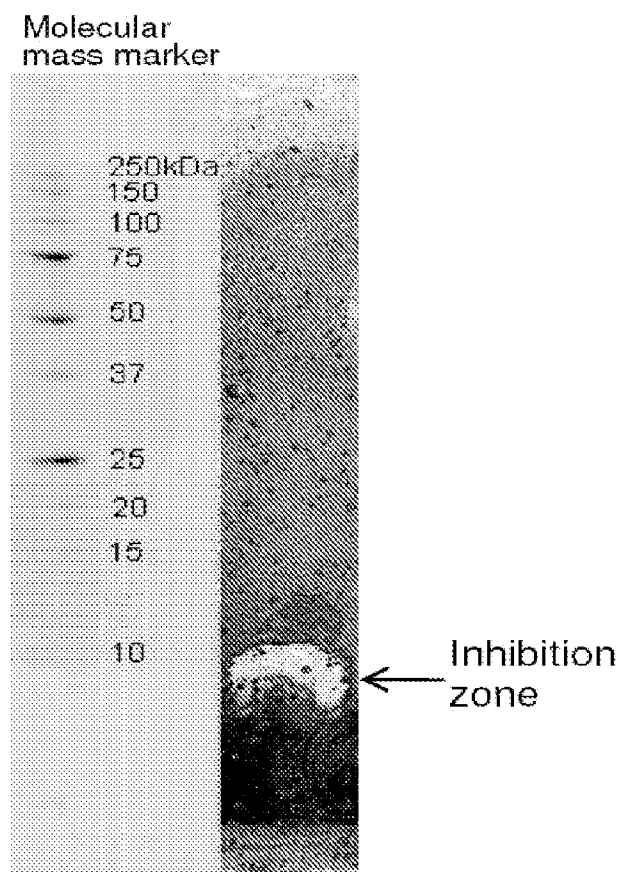
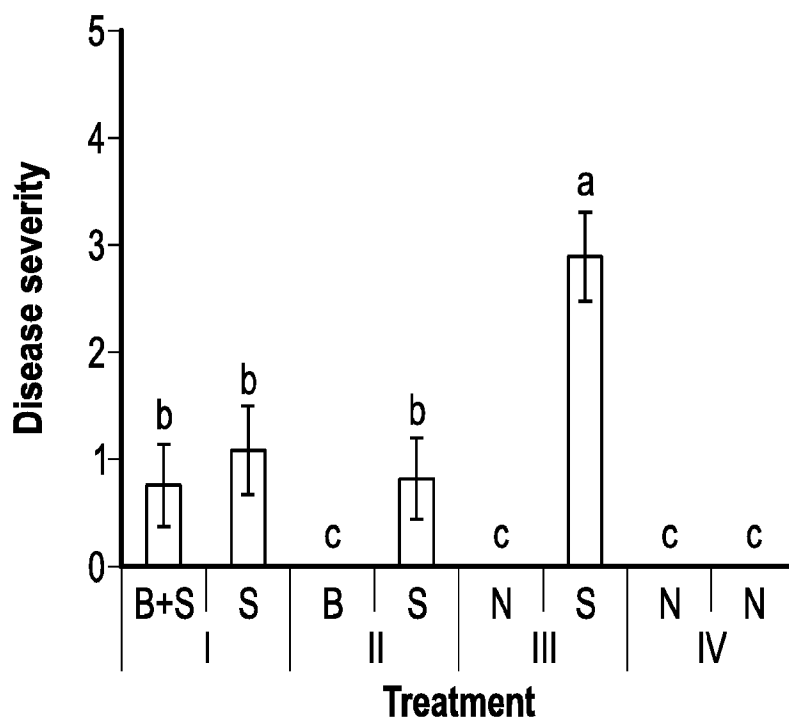
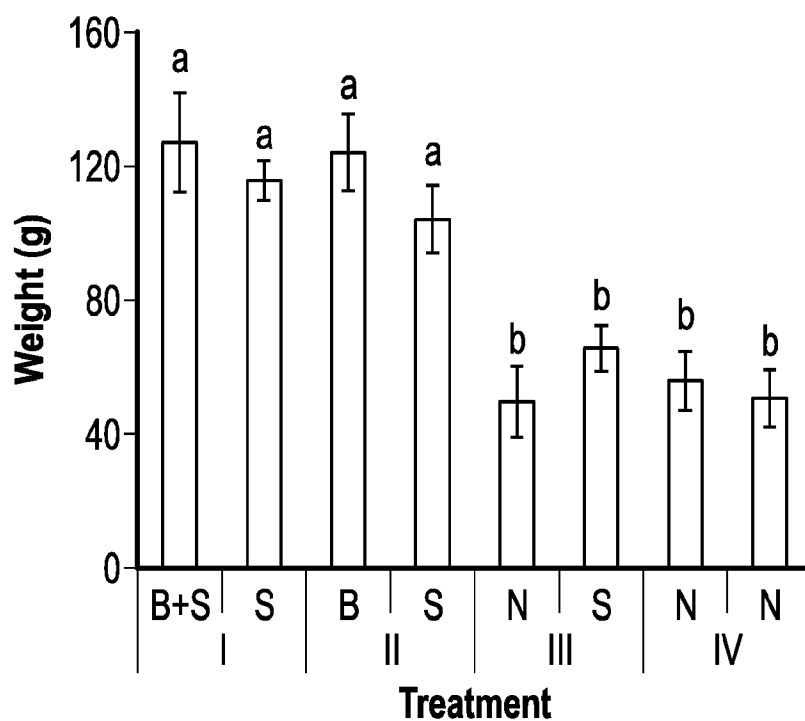


Figure 3

**Figure 4****Figure 5**

Effect of BAC03 on PCS using split-root method**FIG. 6A****Effect of BAC03 on potato tuber weight****FIG. 6B**

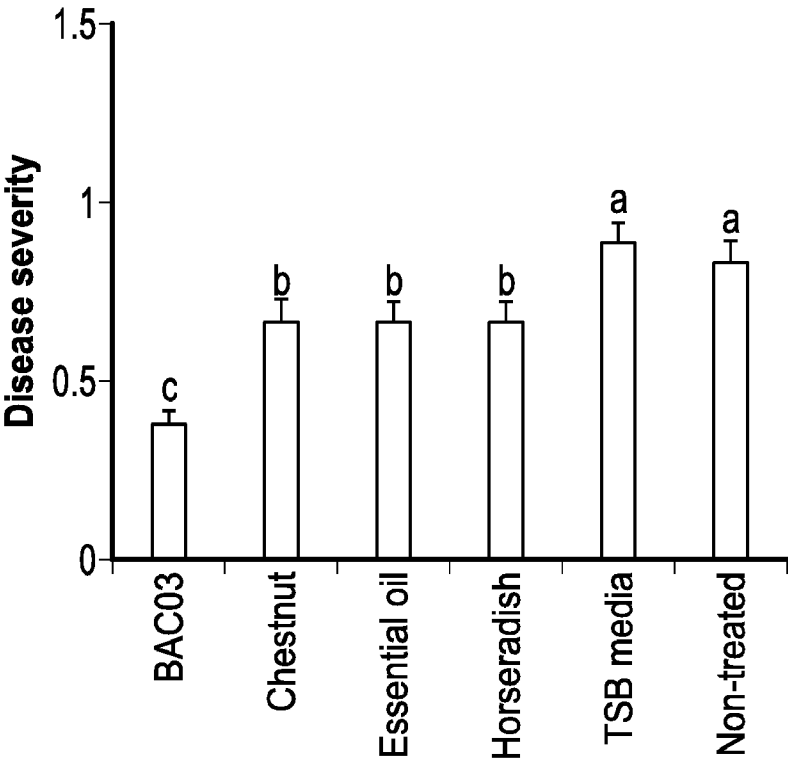


FIG. 7A

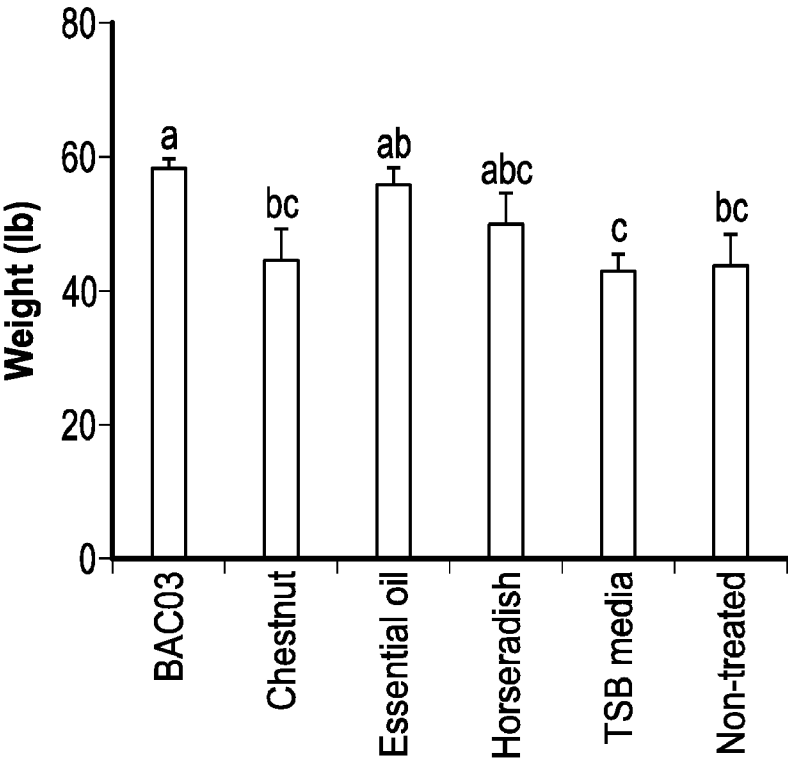


FIG. 7B

Figure 8A

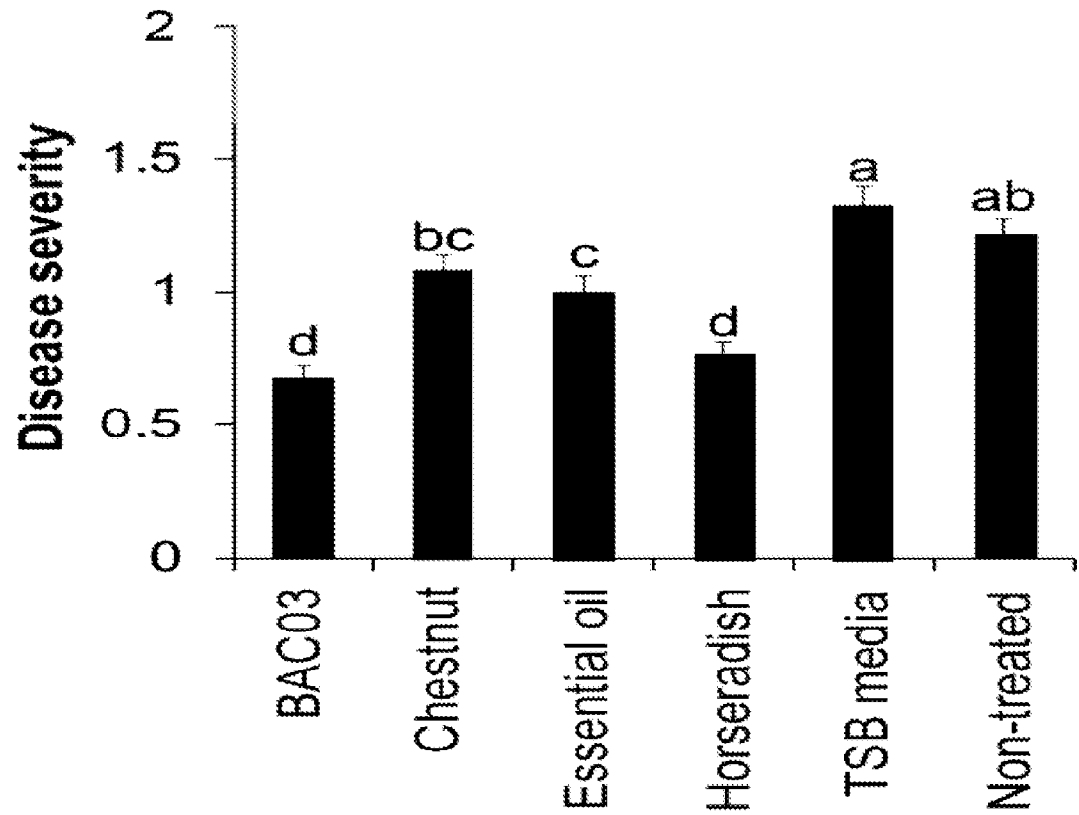
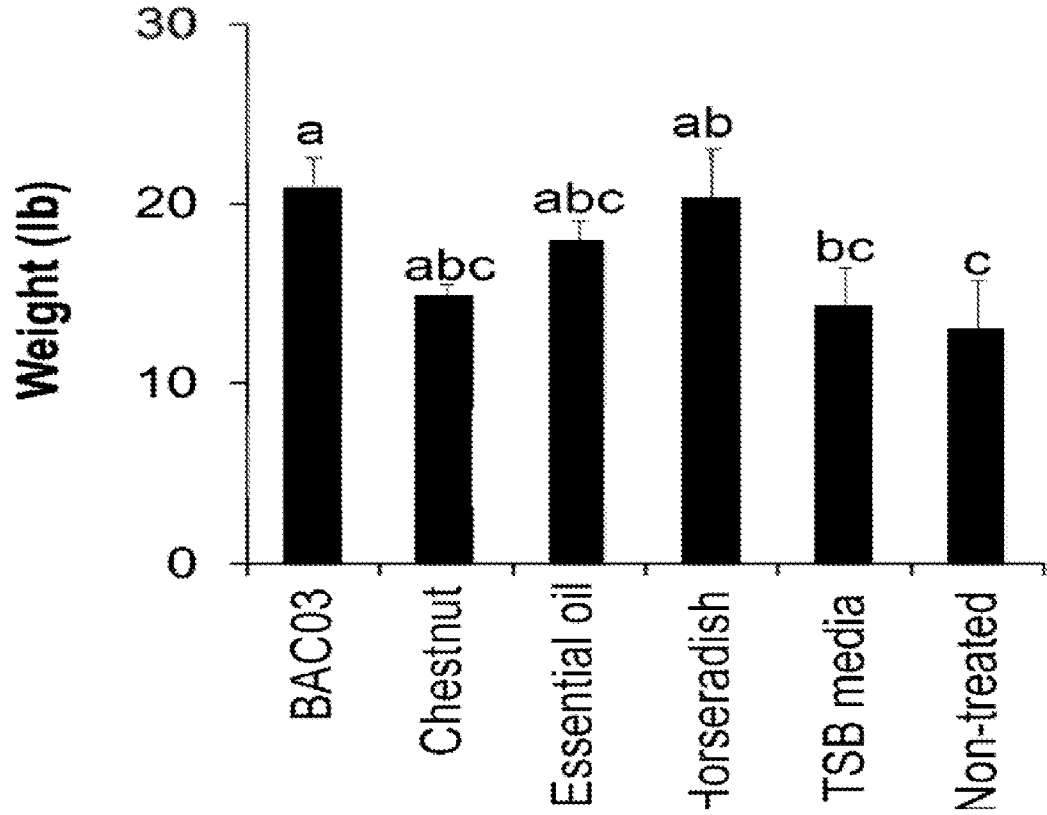


Figure 8B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/032246

A. CLASSIFICATION OF SUBJECT MATTER INV. C12N1/20 C12R1/07 A01N63/00 A01N63/02 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) C12R A01N C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MENG Q ET AL: "An inhibitory effect of a novel Bacillus sp strain against potato common scab", PHYTOPATHOLOGY, vol. 101, no. 6, Suppl. S, June 2011 (2011-06), pages S119-S120, XP009169718, & AMERICAN PHYTOPATHOLOGICAL SOCIETY (APS)/INTERNATIONAL PLANT PROTECTION CONGRESS(IPPS) JOINT MEETING; HONOLULU, HI, USA; AUGUST 06 -10, 2011 ISSN: 0031-949X the whole document ----- -/-	1-20
☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
24 May 2013		11/06/2013
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Giebeler, Katharina

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/032246

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAN QIAO HU ET AL: "Characterization of an antimicrobial material from a newly isolated <i>Bacillus amyloliquefaciens</i> from mangrove for biocontrol of <i>Capsicum</i> bacterial wilt", BIOLOGICAL CONTROL, vol. 54, no. 3, 27 June 2010 (2010-06-27), pages 359-365, XP055063584, ISSN: 1049-9644, DOI: 10.1016/j.biocontrol.2010.06.015 cited in the application the whole document	1-20
X	DEQUAN LI ET AL: "Screening of high-yielding biocontrol bacterium Bs-916 mutant by ion implantation", APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, SPRINGER, BERLIN, DE, vol. 75, no. 6, 5 June 2007 (2007-06-05), pages 1401-1408, XP019513765, ISSN: 1432-0614, DOI: 10.1007/S00253-007-0951-7 the whole document -& DATABASE UniProt [Online] 3 October 2012 (2012-10-03), "SubName: Full=Uncharacterized protein;", XP002697480, retrieved from EBI accession no. UNIPROT:J0DN49 Database accession no. J0DN49 sequence	1-20
X	CHEN XIAO-HUA ET AL: "More than anticipated - production of antibiotics and other secondary metabolites by <i>Bacillus amyloliquefaciens</i> FZB42", JOURNAL OF MOLECULAR MICROBIOLOGY AND BIOTECHNOLOGY,, vol. 16, no. 1-2, 1 January 2009 (2009-01-01), pages 14-24, XP009127778, DOI: 10.1159/000142891 the whole document	1-20

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2013/032246

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ARGUELLES-ARIAS ANTHONY ET AL: "Bacillus amyloliquefaciens GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens", MICROBIAL CELL FACTORIES, BIOMED CENTRAL, LONDON, NL, vol. 8, no. 1, 26 November 2009 (2009-11-26), page 63, XP021063197, ISSN: 1475-2859, DOI: 10.1186/1475-2859-8-63 the whole document	1-20
X	R. BORRISS ET AL: "Relationship of Bacillus amyloliquefaciens clades associated with strains DSM 7T and FZB42T: a proposal for Bacillus amyloliquefaciens subsp. amyloliquefaciens subsp. nov. and Bacillus amyloliquefaciens subsp. plantarum subsp. nov. based on complete genome sequence comparisons", INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, vol. 61, no. 8, 1 August 2011 (2011-08-01), pages 1786-1801, XP055043359, ISSN: 1466-5026, DOI: 10.1099/ij.s.0.023267-0 the whole document	1-20
X,P	Q.X. MENG ET AL: "Characterizing a novel strain of Bacillus amyloliquefaciens BAC03 for potential biological control application", JOURNAL OF APPLIED MICROBIOLOGY, vol. 113, no. 5, 1 November 2012 (2012-11-01), pages 1165-1175, XP055063435, ISSN: 1364-5072, DOI: 10.1111/j.1365-2672.2012.05420.x the whole document	1-20
A,P	QINGXIAO MENG ET AL: "Culture-Based Assessment of Microbial Communities in Soil Suppressive to Potato Common Scab", PLANT DISEASE, vol. 96, no. 5, 1 May 2012 (2012-05-01), pages 712-717, XP055063434, ISSN: 0191-2917, DOI: 10.1094/PDIS-05-11-0441 the whole document	1-20