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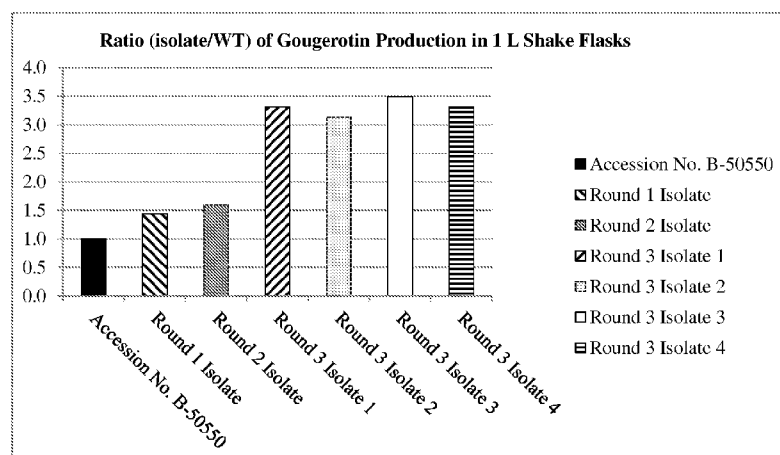
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(54) **Title:** *STREPTOMYCES MICROFLAVUS* STRAINS AND METHODS OF THEIR USE FOR THE TREATMENT AND PREVENTION OF CITRUS GREENING DISEASE

FIG. 1

(57) **Abstract:** The present invention relates to the use of strains of *Streptomyces microflavus* for preventing and treating bacterial diseases such as citrus greening in a plant. The invention also relates to a method of preventing or treating a bacterial disease in a plant comprising applying a fermentation product comprising a gougerotin-producing *Streptomyces* strain to the plant, to a part of the plant and/or to a locus of the plant.

STREPTOMYCES MICROFLAVUS STRAINS AND METHODS OF THEIR USE FOR THE TREATMENT AND PREVENTION OF CITRUS GREENING DISEASE

FIELD OF INVENTION

[0001] The present invention relates to the field of bacterial strains and their ability to control plant diseases.

BACKGROUND OF INVENTION

[0002] Problems with insect-vectored bacterial diseases in plants such as commercial crops are well known and documented. There is often a serious problem with yield loss due to the lack of effective disease prevention or control measures, particularly for new strains of infectious diseases.

[0003] Huanglongbing, HLB, or citrus greening disease was first reported in southern China in 1919 (Reinking, O.A. (1919) Philippine Agricultural 8:109-135), but it has been suggested to have originated in Africa. The disease is now found in approximately 40 different Asian, African, North and South American countries and has recently become a serious threat in Florida, California, Louisiana, Texas and Brazil, all of which are major citrus-producing locations. Citrus greening disease is caused by the phloem-limited fastidious prokaryotic α -proteobacterium *Candidatus Liberibacter* spp., *Ca. L. africanus*, *Ca. L. asiaticus*, and *Ca. L. americanus*. Two psyllids, *Diaphorina citri* and *Trioza erytreae*, are known to vector the disease (Manjunath, et al., (2002) Zhiwu Baohu Xuehui Huikan 44:279-302.) Citrus trees that become infected with the devastating citrus greening disease go into decline, producing misshaped, off-flavor fruit, and then die within a few years. The \$1.4 billion annual Florida citrus industry is severely threatened by this vector-disease pathosystem. Further, the disease threatens to wipe out the \$1.3 million annual citrus industry in California.

[0004] Citrus greening reduces the quantity and quality of citrus fruits, eventually rendering infected trees useless. In areas of the world affected by citrus greening the average productive lifespan of citrus trees has dropped from 50 or more years to 15 or less. The trees in the orchards usually die 3-5 years after becoming infected and require removal and replanting. An infected tree produces fruit that is unsuitable for sale as fresh fruit or for juice.

[0005] Citrus plants infected by the citrus greening bacteria may not show symptoms for years following infection. Initial symptoms frequently include the appearance of yellow shoots on a tree. As the bacteria move within the tree, the entire canopy progressively develops a yellow color. The most characteristic symptoms of citrus greening are a blotchy leaf mottle and vein yellowing that develop on leaves attached to shoots, providing the overall yellow

appearance. Fruit from diseased trees are small, often misshapen, and typically some green color remains on ripened fruit. This symptom is the origin of the common name “greening.” Yields are almost minimal, and any developed fruit is rendered worthless due to small size, poor color, and bad taste.

[0006] Presently, there is no cure for this disease and trees are routinely destroyed once severely infected. Moreover, there are no known relevant cultivars that are resistant to citrus greening disease. Since 2005, it is estimated that about 650,000 trees have been destroyed in Brazil and a similar number in Florida to slow the disease. Therefore, there is a need to develop an effective treatment method for the reduction of the incidence of insect-vectored bacterial infections such as citrus greening that stunt citrus plant development or kill plants.

SUMMARY OF INVENTION

[0007] The present invention provides the *Streptomyces microflavus* strain NRRL B-50550 or a mutant (strain) derived therefrom.

[0008] The present invention also provides a composition containing *Streptomyces microflavus* strain NRRL B-50550 or a mutant strain derived therefrom. In one aspect, the composition is a fermentation product of the *Streptomyces microflavus* strain NRRL B-50550 or a mutant strain derived therefrom.

[0009] The present invention also provides a fermentation broth obtained by cultivating a gougerotin-producing *Streptomyces* strain, wherein the fermentation broth contains at least about 1 g/L gougerotin.

[00010] The present invention also provides a method of preventing or treating a bacterial disease in a plant, wherein the method comprises applying a bactericidal *Streptomyces microflavus* strain NRRL B-50550 and/or a mutant thereof having all the identifying characteristics of the respective strain, and/or at least one metabolite produced by the respective strain to the plant, to a part of the plant and/or to a locus of the plant. In one embodiment, a fermentation product of the strain or a fermentation product of a mutant derived therefrom is applied to the plant and/or to a locus of the plant.

[00011] The present invention also provides a method of preventing or treating a bacterial disease in a plant, wherein the method comprises applying to the plant, to a part of the plant and/or to a locus of the plant a composition comprising a *Streptomyces microflavus* strain NRRL B-50550 or a mutant strain derived therefrom in an effective amount to control the bacterial disease.

[00012] In some embodiments, the gougerotin-producing *Streptomyces* strain is a mutant strain that produces increased amounts of gougerotin compared to amounts of gougerotin

produced by a wild-type strain of the same species. The mutant strain may be *Streptomyces microflavus* strain M with Accession No. 091013-02.

[00013] The present invention is also directed to a method of preventing or treating a bacterial disease in a plant comprising applying a fermentation product comprising a gougerotin-producing *Streptomyces* strain to the plant, to a part of the plant and/or to a locus of the plant. In certain aspects, the *Streptomyces* strain is a *Streptomyces microflavus* strain or a *Streptomyces puniceus* strain. In other aspects, the fermentation product contains at least about 1 g/L, at least about 2 g/L, at least about 3 g/L, at least about 4 g/L, at least about 5 g/L, at least about 6 g/L, at least about 7 g/L or at least about 8 g/L gougerotin.

[00014] In some embodiments, the bacterial disease is an insect-vectored bacterial disease. The insect vector may be a psyllid such as *Diaphorina citri* and *Trioza erytreae*, and the bacterial disease may arise from infection of the plant by any one of *Candidatus Liberibacter* spp., *Candidatus Liberibacter africanus*, *Candidatus Liberibacter asiaticus*, and *Candidatus Liberibacter americanus*. In certain aspects the insect-vectored bacterial disease is citrus greening.

[00015] In other embodiments, the bacterial disease is caused by any one of the following bacterium: *Acidovorax avenae*, *Acidovorax konjaci*, *Burkholderia glumae*, *Burkholderia* spp., *Candidatus Liberibacter* spp., *Candidatus Liberibacter africanus*, *Candidatus Liberibacter americanus*, *Candidatus Liberibacter asiaticus*, *Candidatus Phytoplasma* spp., *Clavibacter michiganensis* subsp. *Michiganensis*, *Clavibacter xyli* subsp. *cynodontis*, *Clavibacter xyli* subsp. *xyli*, *Corynebacterium*, *Curtobacterium flaccumfaciens*, *Erwinia* spp., *Janibacter melonis*, *Pectobacterium carotovorum* subsp. *Atrosepticum*, *Pectobacterium carotovorum* subsp. *Carotovorum*, *Pseudomonas syringae* pv. *actinidae*, *Pseudomonas syringae* pv. *glycinea*, *Pseudomonas syringae* pv. *lachrymans*, *Pseudomonas syringae* pv. *tomato*, *Pseudomonas syringae*, *Ralstonia solanacearum*, *Serratia marcescens*, *Spiroplasma citri*, *Spiroplasma kunkelii*, *Spiroplasma phoenecium*, *Streptomyces* spp., *Xanthomonas albilineans*, *Xanthomonas axonopodis* pv. *citri*, *Xanthomonas axonopodis* pv. *glycines*, *Xanthomonas axonopodis*, *Xanthomonas campestris* pv. *musacearum*, *Xanthomonas campestris* pv. *pruni*, *Xanthomonas campestris* pv. *vesicatoria*, *Xanthomonas campestris*, *Xanthomonas fragariae*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas* spp., *Xanthomonas translucens*, and *Xylella fastidiosa*. In some embodiments, the bacterial disease comprises Huanglongbing (HLB).

BRIEF DESCRIPTION OF THE DRAWINGS

[00016] **FIG. 1** shows increased gougerotin production observed in mutant strains created from the parent strain *Streptomyces microflavus* NRRL No. B-50550.

[00017] FIG. 2 shows gougerotin production in NRRL No. B-50550 mutant strains cultured in bioreactors.

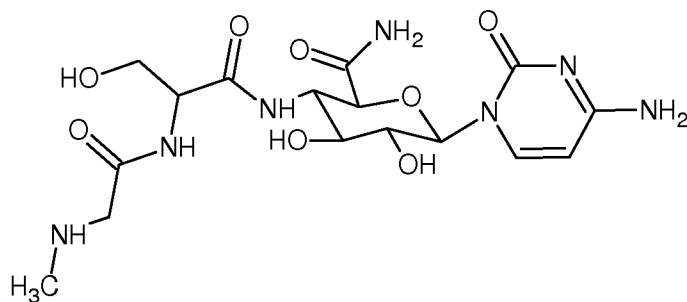
DETAILED DESCRIPTION OF INVENTION

[00018] All publications, patents and patent applications, including any drawings and appendices, herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[00019] The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed inventions, or that any publication specifically or implicitly referenced is prior art.

[00020] The present invention provides the *Streptomyces microflavus* strain NRRL B-50550 or mutant thereof having all the identifying characteristics of the respective strain, such as *Streptomyces microflavus* strain M. *Streptomyces microflavus* strain NRRL B-50550 and *Streptomyces microflavus* strain M are described in U.S. Patent Application No. 14/052,094, the contents of which are hereby incorporated by reference. It has been found that the strain NRRL B-50550 has a variety of advantageous properties. Not only does the strain NRRL B-50550 (or its fermentation product) have acaricidal activity as such but, for example, also shows a high UV stability, a good translaminar activity, good ovicidal activity, long residual activity, drench activity as well as activity against a broad range of mites and thus meets the requirements for an effective acaricide. In addition, the strain NRRL B-50550 (or its fermentation product) possesses both insecticidal activity and activity against various fungal phytopathogens such as leaf rust and powdery mildew. This unique combination of activities makes the strain NRRL B-50550 a highly versatile candidate and renders the strain suitable to be broadly employed in methods of treating plants to control a plant disease and/or a plant pest. Such a broad range of activities and possible applications in agriculture has not yet been reported for known *Streptomyces* strains. In relation to a possible agricultural use, *Streptomyces* strains have been predominantly described in publications from the late 1960's and early 1970's. See, for example, the British Patent No. GB 1 507 193 that describes the *Streptomyces rimofaciens* strain No. B-98891, deposited as ATCC 31120, which produces the antibiotic B-98891. According to GB 1 507 193, filed March 1975, the antibiotic B-98891 is the active ingredient that provides antifungal activity of the *Streptomyces rimofaciens* strain No. B-98891 against powdery mildew. U.S. Patent No. 3,849,398, filed August 2, 1972, describes that the strain *Streptomyces toyocaensis* var. *aspiculamyceticus* produces the antibiotic aspiculamycin which is also known as gougerotin (see,

Toru Ikeuchi et al., 25 J. ANTIBIOTICS 548 (Sept. 1972). According to U.S. Patent No. 3,849,398, gougerotin has parasiticidal action against parasites on animals, such as pin worm and the like, whereas gougerotin is said to show a weak antibacterial activity against gram-positive, gram-negative bacteria and tubercule bacillus. Similarly, Japanese Patent Application No. JP 53109998 (A), published 1978, reports the strain *Streptomyces toyocaensis* (LA-681) and its ability to produce gougerotin for use as miticide. However, it is to be noted that no miticidal product based on such *Streptomyces* strains is commercially available. Thus, the *Streptomyces microflavus* strain NRRL B-50550 with its broad efficacy against acari (based on gougerotin production), fungi and insects and its favorable properties in terms of mode of action (e.g., translaminar activity and residual activity) represents a significant and unexpected advancement in terms of biological and advantageous properties which as such have not been reported for known *Streptomyces* strains. Additionally, Applicant has solved the problem of producing a fermentation broth containing high concentrations of gougerotin, making feasible the ultimate use of the fermentation broth as a commercial pesticide or as a source of gougerotin for use as a commercial pesticide. Thus, this invention encompasses fermentation broths containing gougerotin at concentrations of at least about 0.5 g/L. In addition, this invention encompasses fermentation broths containing gougerotin at concentrations of at least about 1 g/L, at least about 2 g/L, at least about 3 g/L, at least about 4 g/L, at least about 5 g/L at least about 6 g/L, at least about 7 g/L or at least about 8 g/L. In other embodiments the fermentation broth contains gougerotin in a concentration ranging from about 2 g/L to about 15 g/L, including in a concentration of about 3 g/L, of about 4 g/L, of about of about 5 g/L, of about 6 g/L, of about 7 g/L, of about 8 g/L, of about 9 g/L, of about of 10 g/L, of about 11 g/L, of about 12 g/L, of about 13 g/L, and of about 14 g/L. In some embodiments the fermentation broths are from *Streptomyces* species. In specific embodiments, the fermentation broths are from *Streptomyces microflavus*. In still other specific embodiments, the fermentation broths are from *Streptomyces microflavus* NRRL-50550 or mutants derived therefrom. See structure of gougerotin below.



[00021] It should be understood that any numerical range recited herein is intended to include all sub-ranges subsumed therein. For example, a range of 1 to 10 is intended to include

all sub-ranges between and including the recited minimum value of 1 and the recited maximum value of 10, that is, having a minimum value equal to or greater than 1 and a maximum value of equal to or less than 10.

[00022] As used herein, unless otherwise expressly specified, all numbers such as those expressing values, ranges, amounts or percentages may be read as if prefaced by the word “about”, even if the term does not expressly appear. Any numerical range recited herein is intended to include all sub-ranges subsumed therein. Plural encompasses singular and vice versa; e.g., the singular forms “a,” “an,” and “the” include plural referents unless expressly and unequivocally limited to one referent.

[00023] The phrase “effective amount” as used herein is intended to refer to an amount of an ingredient used such that a noticeable reduction in effects caused by a bacterial disease is observed in plants treated using the method of the present invention, compared to plants that did not receive treatment.

[00024] As used herein, the term “control” when used in reference to a plant disease means to kill or inhibit the growth of the causative agent of the disease (e.g., bacterium). In certain embodiments, the methods and compositions of the present invention may be used to control at least 10% of a bacterial disease, at least 20% of a bacterial disease, at least 30% of a bacterial disease, at least 40% of a bacterial disease, at least 50% of a bacterial disease, at least 60% of a bacterial disease, at least 70% of a bacterial disease, at least 80% of a bacterial disease, at least 85% of a bacterial disease, at least 90% of a bacterial disease, or at least 95% of a bacterial disease.

[00025] The microorganisms and particular strains described herein, unless specifically noted otherwise, are all separated from nature (i.e., isolated) and grown under artificial conditions, such as in shake flask cultures or through scaled-up manufacturing processes, such as in bioreactors, as described herein.

[00026] In one embodiment, a mutant strain of the *Streptomyces microflavus* strain NRRL B-50550 is provided. The term “mutant” refers to a genetic variant derived from *Streptomyces microflavus* strain NRRL B-50550. In one embodiment, the mutant has one or more or all the identifying (functional) characteristics of *Streptomyces microflavus* strain NRRL B-50550. In a particular instance, the mutant or a fermentation product thereof controls (as an identifying functional characteristic) mites at least as well as the parent *Streptomyces microflavus* NRRL B-50550 strain. In addition, the mutant or a fermentation product thereof may have one, two, three, four or all five of the following characteristics: translaminar activity in relation to the miticidal activity, residual activity in relation to the miticidal activity, ovicidal activity, insecticide activity, in particular against Diabrotica species, or activity against fungal

phytopathogens, in particular against powdery mildew and rust disease. In another particular instance, the mutant or a fermentation product thereof controls (as an identifying functional characteristic) bacteria at least as well as the parent *Streptomyces microflavus* NRRL B-50550 strain.

[00027] Such mutants may be genetic variants having a genomic sequence that has greater than about 85%, greater than about 90%, greater than about 95%, greater than about 98%, or greater than about 99% sequence identity to *Streptomyces microflavus* strain NRRL B-50550. Mutants may be obtained by treating *Streptomyces microflavus* strain NRRL B-50550 cells with chemicals or irradiation or by selecting spontaneous mutants from a population of NRRL B-50550 cells (such as phage resistant or antibiotic resistant mutants) or by other means well known to those practiced in the art.

[00028] Suitable chemicals for mutagenesis of *Streptomyces microflavus* include hydroxylamine hydrochloride, methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), 4-nitroquinoline 1-oxide (NQO), mitomycin C or N-methyl-N'-nitro-N-nitrosoguanidine (NTG), to mention only a few (*cf.*, for example, Stonesifer & Baltz, Proc. Natl. Acad. Sci. USA Vol. 82, pp. 1180-1183, February 1985). The mutagenesis of *Streptomyces* strains by, for example, NTG, using spore solutions of the respective *Streptomyces* strain is well known to the person skilled in the art. *See*, for example Delic et al., Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, Volume 9, Issue 2, February 1970, pages 167–182, or Chen et al., J Antibiot (Tokyo), 2001 Nov; 54(11), pages 967-972.). In more detail, *Streptomyces microflavus* can be subjected to mutation by NTG using the protocol described in Kieser, T., et al., 2000, *supra*. Practical *Streptomyces* Genetics, Ch. 5, John Innes Centre, Norwich Research Park, England (2000), pp. 99-107. Mutagenesis of spores of *Streptomyces microflavus* by ultraviolet light (UV) can be carried out using standard protocols. For example, a spore suspension of the *Streptomyces* strain (freshly prepared or frozen in 20% glycerol) can be suspended in a medium that does not absorb UV light at a wave length of 254 nm (for example, water or 20% glycerol are suitable). The spore suspension is then placed in a glass Petri dish and irradiated with a low pressure mercury vapor lamp that emits most of its energy at 254 nm with constant agitation for an appropriate time at 30° C (the most appropriate time of irradiation can be determined by first plotting a dose-survival curve). Slants or plates of non-selective medium can, for example, then be inoculated with the dense irradiated spore suspension and the so obtained mutant strains can be assessed for their properties as explained in the following. *See* Kieser, T., *et al.*, 2000, *supra*.

[00029] The mutant strain can be any variant strain that has one or more or all the identifying characteristics of *Streptomyces microflavus* strain NRRL B-50550 and in particular miticidal activity that is comparable or better than that of *Streptomyces microflavus* NRRL B-

50550, such as *Streptomyces microflavus* strain M. The miticidal activity can, for example, be determined against two-spotted spider mites (“TSSM”), meaning culture stocks of the mutant strain of *Streptomyces microflavus* NRRL B-50550 can be grown in 1 L shake flasks in Media 1 or Media 2 of Example 5 at 20-30° C for 3-5 days, and the diluted fermentation product can then be applied on top and bottom of lima bean leaves of two plants, after which treatment, plants can be infested on the same day with 50-100 TSSM and left in the greenhouse for five days.

[00030] A “variant” is a strain having all the identifying characteristics of the NRRL or ATCC Accession Numbers as indicated in this text and can be identified as having a genome that hybridizes under conditions of high stringency to the genome of the NRRL or ATCC Accession Numbers.

[00031] “Hybridization” refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. Hybridization reactions can be performed under conditions of different “stringency”. In general, a low stringency hybridization reaction is carried out at about 40° C in 10 X SSC or a solution of equivalent ionic strength/temperature. A moderate stringency hybridization is typically performed at about 50° C in 6 X SSC, and a high stringency hybridization reaction is generally performed at about 60° C in 1 X SSC.

[00032] A variant of the indicated NRRL or ATCC Accession Number may also be defined as a strain having a genomic sequence that is greater than 85%, more preferably greater than 90% or more preferably greater than 95% sequence identity to the genome of the indicated NRRL or ATCC Accession Number. A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example, those described in Current Protocols in Molecular Biology (F. M. Ausubel et al., eds., 1987) Supplement 30, section 7. 7. 18, Table 7. 7. 1.

[00033] In another aspect of the invention, the *Streptomyces microflavus* strain NRRL B-50550 or a mutant strain thereof may have drench activity. The term “drench activity” is used herein in its regular meaning in the art to mean pesticidal activity that travels from soil or other growth media upward through the plant via the xylem. In one aspect of the invention, drench

activity can still be observed (is present) after several days (e.g., about 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 days).

[00034] In another aspect of the invention, the *Streptomyces microflavus* strain NRRL B-50550 or a mutant strain thereof may have also insecticide activity. The target insect may be a Diabrotica. The Diabrotica may be Banded cucumber beetle (*Diabrotica balteata*), Western spotted cucumber beetle (*Diabrotica undecimpunctata undecimpunctata*), or a corn rootworm such as Northern corn rootworm (*Diabrotica barberi*), Southern corn rootworm (*Diabrotica undecimpunctata howardi*), Western cucumber beetle (*Diabrotica undecimpunctata tenella*), Western corn rootworm (*Diabrotica virgifera virgifera*), Mexican corn rootworm (*Diabrotica virgifera zae*) and combinations of such Diabrotica. The insecticidal activity of a mutant strain of *Streptomyces microflavus* NRRL B-50550 alone or in comparison to *Streptomyces microflavus* NRRL B-50550 can be determined against corn rootworm, using the method as described in Example 5.

[00035] In certain aspects, the disclosed strains and compositions prevent the feeding, development, and/or reproduction of insect vectors that carry bacterial disease. In non-limiting examples, the strains and compositions of the present invention have insecticidal activity when applied to psyllids such as *Diaphorina citri* and *Trioza erytreae*.

[00036] The present invention also encompasses methods of treating a plant to control plant pests and diseases by administering to a plant or a plant part, such as a leaf, stem, flowers, fruit, root, or seed or by applying to a locus on which plant or plant parts grow, such as soil, one or more of a gougerotin-containing fermentation broth of *Streptomyces*, a *Streptomyces microflavus* strain, the *Streptomyces microflavus* strain NRRL B-50550 or a mutant strain thereof or cell-free preparations thereof or metabolites thereof.

[00037] As used herein, the term “plant” refers to any living organism belonging to the kingdom Plantae (i.e., any genus/species in the Plant Kingdom). This includes familiar organisms such as but not limited to trees, herbs, bushes, grasses, vines, ferns, mosses and green algae. The term refers to both monocotyledonous plants, also called monocots, and dicotyledonous plants, also called dicots. The plant is in some embodiments of economic importance. In some embodiments the plant is a human-grown plant, for instance a cultivated plant, which may be an agricultural, a silvicultural or a horticultural plant. Examples of particular plants include but are not limited to corn, potatoes, roses, apple trees, sunflowers, wheat, rice, bananas, tomatoes, opo, pumpkins, squash, beans (e.g., lima beans), lettuces, cabbages, oak trees, guzmania, geraniums, hibiscus, clematis, poinsettias, sugarcane, taros, duck weeds, pine trees, Kentucky blue grasses, zoysia, coconut trees, brassica leafy vegetables (e.g., broccoli, broccoli raab, Brussels sprouts, cabbages, Chinese cabbages (Bok Choy and Napa),

cauliflowers, cavalos, collards, kales, kohlrabi, mustard greens, rape greens, and other brassica leafy vegetable crops), bulb vegetables (e.g., garlic, leeks, onions (dry bulb, green, and Welch), shallots, and other bulb vegetable crops), citrus fruits (e.g., grapefruits, lemons, limes, oranges, tangerines, citrus hybrids, pummelo, and other citrus fruit crops), cucurbit vegetables (e.g., cucumbers, citron melons, edible gourds, gherkins, muskmelons (including hybrids and/or cultivars of cucumis melons), watermelons, cantaloupes, and other cucurbit vegetable crops), fruiting vegetables (including eggplants, ground cherries, pepinos, peppers, tomatoes, tomatillos, and other fruiting vegetable crops), grapes, leafy vegetables (e.g., romaines), root/tuber and corm vegetables (e.g., potatoes), lentils, alfalfa sprouts, clover and tree nuts (almonds, pecans, pistachios, and walnuts), berries (e.g., tomatoes, barberries, currants, elderberries, gooseberries, honeysuckles, mayapples, nannyberries, Oregon-grapes, see-buckthorns, hackberries, bearberries, lingonberries, strawberries, sea grapes, blackberries, cloudberries, loganberries, raspberries, salmonberries, thimbleberries, and wineberries), cereal crops (e.g., corn, rice, wheat, barley, sorghum, millets, oats, ryes, triticales, buckwheats, fonio, and quinoa), pome fruits (e.g., apples, pears), stone fruits (e.g., coffees, jujubes, mangos, olives, coconuts, oil palms, pistachios, almonds, apricots, cherries, damsons, nectarines, peaches and plums), vines (e.g., table grapes, wine grapes), fiber crops (e.g., hems, cottons), ornamentals, to name a few. The plant may, in some embodiments, be a household/domestic plant, a greenhouse plant, an agricultural plant, or a horticultural plant. As already indicated above, in some embodiments the plant may be a hardwood such as one of acacia, eucalyptus, hornbeam, beech, mahogany, walnut, oak, ash, willow, hickory, birch, chestnut, poplar, alder, maple, sycamore, ginkgo, a palm tree and sweet gum. In some embodiments the plant may be a conifer such as a cypress, a Douglas fir, a fir, a sequoia, a hemlock, a cedar, a juniper, a larch, a pine, a redwood, spruce and yew. In some embodiments the plant may be a fruit bearing woody plant such as apple, plum, pear, banana, orange, kiwi, lemon, cherry, grapevine, papaya, peanut, and fig. In some embodiments the plant may be a woody plant such as cotton, bamboo and a rubber plant. The plant may in some embodiments be an agricultural, a silvicultural and/or an ornamental plant, i.e., a plant which is commonly used in gardening, e.g., in parks, gardens and on balconies. Examples are turf, geranium, pelargonium, petunia, begonia, and fuchsia, to name just a few among the vast number of ornamentals. The term “plant” is also intended to include any plant propagules.

[00038] The term “plant” generally includes a plant that has been modified by one or more of breeding, mutagenesis and genetic engineering. Genetic engineering refers to the use of recombinant DNA techniques. Recombinant DNA techniques allow modifications which cannot readily be obtained by cross breeding under natural circumstances, mutations or natural

recombination. In some embodiments a plant obtained by genetic engineering may be a transgenic plant.

[00039] As used herein, the term “plant part” refers to any part of a plant including but not limited to the shoot, root, stem, seeds, stipules, leaves, petals, flowers, ovules, bracts, branches, petioles, internodes, bark, wood, tubers, pubescence, tillers, rhizomes, fronds, blades, pollen, stamen, microspores, fruit and seed. The two main parts of plants grown in typical media employed in the art, such as soil, are often referred to as the “above-ground” part, also often referred to as the “shoots”, and the “below-ground” part, also often referred to as the “roots”.

[00040] In a method according to the invention a composition containing *Streptomyces microflavus* NRRL B-50550 or a mutant strain thereof can be applied to any plant or any part of any plant grown in any type of media used to grow plants (e.g., soil, vermiculite, shredded cardboard, and water) or applied to plants or the parts of plants grown aurally, such as orchids or staghorn ferns. The composition may for instance be applied by spraying, painting, atomizing, vaporizing, scattering, dusting, watering, squirting, sprinkling, pouring or fumigating. As already indicated above, application may be carried out at any desired location where the plant of interest is positioned, such as agricultural, horticultural, forest, plantation, orchard, nursery, organically grown crops, turfgrass and urban environments. In certain embodiments, the composition is applied as a foliar and/or soil drench to treat citrus greening in a plant.

[00041] Compositions of the present invention can be obtained by culturing *Streptomyces microflavus* NRRL B-50550 or mutants derived from it using conventional large-scale microbial fermentation processes, such as submerged fermentation, solid state fermentation or liquid surface culture, including the methods described, for example, in U.S. Patent No. 3,849,398; British Patent No. GB 1 507 193; Toshiko Kanzaki et al., Journal of Antibiotics, Ser. A, Vol. 15, No.2, Jun. 1961, pages 93 to 97; or Toru Ikeuchi et al., Journal of Antibiotics, (Sept. 1972), pages 548 to 550. Fermentation is configured to obtain high levels of live biomass, particularly spores, and desirable secondary metabolites in the fermentation vessels. Specific fermentation methods that are suitable for the strain of the present invention to achieve high levels of sporulation, cfu (colony forming units), and secondary metabolites are described in the Examples section.

[00042] The bacterial cells, spores and metabolites in culture broth resulting from fermentation (the “whole broth” or “fermentation broth”) may be used directly or concentrated by conventional industrial methods, such as centrifugation, filtration, and evaporation, or processed into dry powder and granules by spray drying, drum drying and freeze drying, for example.

[00043] The terms “whole broth” and “fermentation broth,” as used herein, refer to the culture broth resulting from fermentation (including the production of a culture broth that contains gougerotin in a concentration of at least about 1 g/L) before any downstream treatment. The whole broth encompasses the microorganism (e.g., *Streptomyces microflavus* NRRL B-50550 or a mutant strain thereof) and its component parts, unused raw substrates, and metabolites produced by the microorganism during fermentation. The term “broth concentrate,” as used herein, refers to whole broth (fermentation broth) that has been concentrated by conventional industrial methods, as described above, but remains in liquid form. The term “fermentation solid,” as used herein, refers to dried fermentation broth. The term “fermentation product,” as used herein, refers to whole broth, broth concentrate and/or fermentation solids. Compositions of the present invention include fermentation products. In some embodiments, the concentrated fermentation broth is washed, for example, via a diafiltration process, to remove residual fermentation broth and metabolites.

[00044] In one embodiment, the fermentation broth contains at least about 1×10^5 colony forming units (CFU) of the microorganism (e.g., *Streptomyces microflavus* NRRL B-50550 or a mutant strain thereof)/mL broth. In another embodiment, the fermentation broth contains at least about 1×10^6 colony forming units (CFU) of the microorganism (e.g., *Streptomyces microflavus* NRRL B-50550 or a mutant strain thereof)/mL broth. In yet another embodiment, the fermentation broth contains at least about 1×10^7 colony forming units (CFU) of the microorganism (e.g., *Streptomyces microflavus* NRRL B-50550 or a mutant strain thereof)/mL broth. In another embodiment, the fermentation broth contains at least about 1×10^8 colony forming units (CFU) of the microorganism (e.g., *Streptomyces microflavus* NRRL B-50550 or a mutant strain thereof)/mL broth. In another embodiment, the fermentation broth contains at least about 1×10^9 colony forming units (CFU) of the microorganism (e.g., *Streptomyces microflavus* NRRL B-50550 or a mutant strain thereof)/mL broth. In another embodiment, the fermentation broth contains at least about 1×10^{10} colony forming units (CFU) of the microorganism (e.g., *Streptomyces microflavus* NRRL B-50550 or a mutant strain thereof)/mL broth. In another embodiment, the fermentation broth contains at least about 1×10^{11} colony forming units (CFU) of the microorganism (e.g., *Streptomyces microflavus* NRRL B-50550 or a mutant strain thereof)/mL broth. One of skill in the art will understand that the concentrations described above relate to CFU measured shortly after completion of fermentation but that CFU levels will decline over time, depending on storage conditions. CFU levels of unformulated fermentation products of the microorganisms described herein are stable when the products are maintained in cold storage (e.g., about 4° C) but decline at room temperature.

[00045] In another embodiment, the fermentation product of the present invention has potency of at least 40%, at least 50%, or at least 60%, wherein the potency is measured as follows. Dilute the fermentation product in a water surfactant solution (using the amount of surfactant recommended on the product label) to obtain a solution that is 5% whole broth (or whole broth equivalent, as described below, if dealing with a fermentation solid derived from whole broth). Apply the diluted solution to the top and bottom surfaces of a leaf (such as the leaf of a lima bean) until both surfaces are wet, but do not apply to run-off. Allow plants to dry and then infest with 10-20 two-spotted spider mites (*Tetranychus urticae* Koch). Four days after treatment, inspect the treated leaves and count live and dead adult females and deutonymphs on the leaves. Use the Sun-Shepard formula to calculate potency (i.e., corrected mortality). $\text{Corrected \%} = 100 (\% \text{ reduction in the treated plot} \pm \% \text{ change in untreated population}) / (100 \pm \% \text{ change in untreated population})$. In this application, potency calculated by the above-described method will be referred to as “Spider Mite Potency.”

[00046] In one embodiment, the fermentation broth or broth concentrate can be formulated into liquid suspension, liquid concentrate, emulsion concentrate, or wettable powder with the addition of stabilization agents, preservatives, adjuvants, and/or colorants.

[00047] In another embodiment, the fermentation broth or broth concentrate can be dried with or without the addition of carriers, inerts, or additives using conventional drying processes or methods such as spray drying, freeze drying, tray drying, fluidized-bed drying, drum drying, or evaporation.

[00048] In some embodiments, the fermentation broth, broth concentrate or fermentation solid is treated in order to kill the microorganism, resulting in a fermentation product that consists of the killed microbe, its metabolites and residual fermentation media. Suitable treatments to accomplish this are known to those of skill in the art and include heat treatments.

[00049] In embodiments in which the fermentation broth or broth concentrate is freeze dried, one gallon of fermentation broth yields about 0.2 lb to about 1 lb freeze dried powder. In a particular instance, one gallon of fermentation broth yields about 0.4 lb to about 0.6 lb freeze dried powder. In another instance, one gallon of fermentation broth yields about 0.5 lb freeze dried powder.

[00050] In a further embodiment, the resulting dry products may be further processed, such as by milling or granulation, with or without the addition of inerts or additives to achieve specific particle sizes or physical formats or physical properties desirable for agricultural applications.

[00051] In addition to the use of whole broth or broth concentrate, cell-free preparations of fermentation broth of the novel variants and strains of *Streptomyces* of the present invention can be obtained by any means known in the art, such as extraction, centrifugation and/or filtration of fermentation broth. Those of skill in the art will appreciate that so-called cell-free preparations may not be devoid of cells but rather are largely cell-free or essentially cell-free, depending on the technique used (e.g., speed of centrifugation) to remove the cells. The resulting cell-free preparation may be dried and/or formulated with components that aid in its application. Concentration methods and drying techniques described above for fermentation broth are also applicable to cell-free preparations.

[00052] Compositions of the present invention may include formulation ingredients added to compositions comprising cells, cell-free preparations or metabolites to improve efficacy, stability, and physical properties, usability and/or to facilitate processing, packaging and end-use application. Such formulation ingredients may include carriers, inerts, stabilization agents, preservatives, nutrients, or physical property modifying agents, which may be added individually or in combination. In some embodiments, the carriers may include liquid materials such as water, oil, and other organic or inorganic solvents and solid materials such as minerals, polymers, or polymer complexes derived biologically or by chemical synthesis. In some embodiments, the ingredient is a binder, adjuvant, or adhesive that facilitates adherence of the composition to a plant part, such as leaves, seeds, or roots. *See*, for example, Taylor, A.G., et al., "Concepts and Technologies of Selected Seed Treatments," *Annu. Rev. Phytopathol.* 28: 321-339 (1990). The stabilization agents may include anti-caking agents, anti-oxidation agents, desiccants, protectants or preservatives. The nutrients may include carbon, nitrogen, and phosphorus sources such as sugars, polysaccharides, oil, proteins, amino acids, fatty acids and phosphates. The physical property modifiers may include bulking agents, wetting agents, thickeners, pH modifiers, rheology modifiers, dispersants, adjuvants, surfactants, antifreeze agents or colorants. In some embodiments, the composition comprising cells, cell-free preparation or metabolites produced by fermentation can be used directly with or without water as the diluent without any other formulation preparation. In a particular embodiment, a wetting agent is added to a fermentation solid, such as a freeze-dried powder. A wetting agent increases the spreading and penetrating properties of the active ingredient (once diluted) when it is applied to surfaces. Exemplary wetting agents are known to those of skill in the art and include sulfocinates and derivatives, such as MULTIWET™ MO-70 (Croda Inc., Edison, NJ); trisiloxanes such as BREAKTHRU® (Evonik, Germany); nonionic compounds, such as ATLOX™ 4894 (Croda Inc., Edison, NJ); alkyl polyglucosides, such as TERWET® 3001 (Huntsman International LLC, The Woodlands, Texas); C12-C14 secondary alcohol ethoxylate,

such as TERGITOL™ 15-s-15 (The Dow Chemical Company, Midland, Michigan); phosphate esters, such as RHODAFAC™ BG-510 (Rhodia, Inc.); and alkyl ether carboxylates, such as EMULSOGEN® LS (Clariant Corporation, North Carolina).

[00053] In some embodiments, the formulation inerts are added after concentrating fermentation broth and during and/or after drying.

[00054] The present invention encompasses fermentation broths containing gougerotin at a concentration of at least about 1 g/L. In some embodiments such whole broth cultures come from gougerotin-producing strains of *Streptomyces*. In a particular embodiment, such gougerotin-producing strain is *Streptomyces microflavus*, *Streptomyces puniceus*, or *Streptomyces gramineus*. In yet another particular embodiment, such gougerotin-producing strain is *Streptomyces microflavus* NRRL B-50550 or mutants thereof. In yet another particular embodiment, such gougerotin-producing strain is *Streptomyces gramineus* CGMCC 4.506, deposited at China General Microbiological Culture Collection Center CGMCC. Fermentation broths containing at least about 1 g/L gougerotin may be obtained in several ways, such as fermentation optimization and/or mutagenesis of a parent gougerotin-producing strain in order to attain a mutant strain that produces higher levels of gougerotin than the parent strain.

[00055] Thus, the present invention also encompasses a method of producing a fermentation broth of a gougerotin-producing *Streptomyces* strain, wherein the fermentation broth contains at least about 1 g/L gougerotin. The method comprises cultivating the *Streptomyces* strain in a culture medium that contains a digestible carbon source and a digestible nitrogen source under aerobic conditions, wherein the culture medium contains a precursor to gougerotin, such as cytosine; a nucleobase; and/or an amino acid at a concentration effective to achieve a gougerotin concentration of at least 1 g/L.

[00056] In some embodiments, the *Streptomyces* strain is cultivated in the culture medium until the culture medium contains gougerotin in a concentration of at least about 0.5 g/L, of at least about 1 g/L, of at least about 2 g/L, of at least about 3 g/L, of at least about 4 g/L, of at least about 5 g/L, of at least about 6 g/L, of about at least 7 g/L or of at least about 8 g/L gougerotin.

[00057] In other embodiments, the *Streptomyces* strain is cultivated in the culture medium until the culture medium contains gougerotin in a concentration ranging from about 0.5 g/L to about 25 g/L gougerotin, meaning the fermentation broth contains gougerotin in a concentration ranging typically ranging from about 0.5 g/L to about 15 g/L gougerotin after completion of the fermentation.

[00058] In this context it is noted that the amino acid that is added at a concentration effective to achieve a gougerotin concentration of at least 1 g/L is provided to the culture medium

as a separate individual component in a defined concentration and not part of a composition such as a yeast extract or a protein hydrolysate (for example, casein hydrolysate, soy flour hydrolysate, soy peptone, soy acid hydrolysate, to name only a few) in which amino acids may be present in a mixture with other compounds such as oligopeptides and partially hydrolyzed proteins. Thus, by “a concentration effective to achieve a gougerotin concentration of at least 1 g/L” in the fermentation broth is meant a concentration of an amino acid in the culture medium that is specifically chosen to provide such a gougerotin concentration. In some embodiments, the concentration effective to achieve the desired gougerotin concentration is a concentration of the amino acid in the culture medium of at least about 1 g/L. This “effective concentration” may thus be higher than 2 g/L and may, for example, range from about 2 g/L to about 15 g/L. The concentration may be about 3 g/L, about 4 g/L, about 5 g/L, about 6 g/L, about 7 g/L, about 8 g/L, about 9 g/L, about 10 g/L, about 11 g/L, about 12 g/L, about 13 g/L, or about 14 g/L.

[00059] The amino acid may be any amino acid which provides for a concentration of gougerotin of at least about 0.5 g/L or a higher concentration such as at least about 1 g/L, at least about 2 g/L, at least about 3 g/L, at least about 4 g/L, at least about 5 g/L, or at least about 6 g/L. In some embodiments the amino acid is glycine, L-glutamic acid, L-glutamine, L-aspartic acid, or a mixture thereof. In some embodiments the culture medium contains glycine at a concentration of about 5 g/L to about 15 g/L, whereas in other embodiments the culture medium contains glutamic acid in an initial concentration of about 5 g/L to about 15 g/L. It is also possible that the culture medium contains both glycine and L-glutamic acid (or L-glutamine) in a concentration of about 5 g/L to about 15 g/L.

[00060] Any carbon source that is digestible (and thus available) for *Streptomyces* strains can be used in the method of producing a fermentation broth (or fermentation method) as described here. Examples of suitable carbon sources include glucose, fructose, mannose, galactose, sucrose, maltose, lactose, molasses, starch (as an example for a polysaccharide), dextrin, maltodextrin (as an example of an oligosaccharide) or glycerin, to name only a few. The total initial concentration of the carbon source (or sources) may be any concentration that provides a suitable growth of *Streptomyces* and production of the desired concentration of gougerotin and may be determined experimentally (determining the final concentration of gougerotin in the fermentation broth dependent from the concentration of the used carbon source(s)). The total initial carbon source concentration may, for example, be in the range of about 10 g/L to about 150 g/L, for example, about 10 g/L, about 20 g/L, about 30 g/L, about 40 g/L, about 50 g/L, about 60 g/L, about 70 g/L, about 80 g/L, about 90 g/L, about 100 g/L, about 110 g/L or about 120 g/L. In some embodiments, the carbon source might be a mixture of two or more carbon sources, for example, a mixture of glucose with a polysaccharide such as starch, a

mixture of glucose and an oligosaccharide such as dextrin or maltodextrin or a mixture of glucose, starch and dextrin. In some embodiments the culture medium contains as carbon source a mixture of glucose and an oligosaccharide. The oligosaccharide may be maltodextrin or dextrin. In such embodiments, the initial maltodextrin concentration in the culture medium may be about 50 g/L to about 100 g/L or about 60 g/L to about 80 g/L. The initial glucose concentration in the culture medium may be about 20 g/L to about 80 g/L, for example, about 30 g/L, about 40 g/L, about 50 g/L, about 60 g/L or about 70 g/L. In other embodiments in which glucose is used as carbon source with maltodextrin or dextrin, the glucose concentration may be about 20 g/L to 60 g/L or about 30 g/L to about 50 g/L.

[00061] Any nitrogen source that is digestible can be used in the fermentation process described here. The nitrogen source can be a single source or a mixture of sources. In illustrative embodiments the nitrogen source is (at least partially) selected from the group consisting of soy peptone, soy acid hydrolysate, soy flour hydrolysate, casein hydrolysate, yeast extract, and mixtures thereof. The total initial concentration of the nitrogen source(s) may be any concentration that provides a suitable growth of *Streptomyces* and production of the desired concentration of gougerotin and may be determined experimentally. Suitable total concentrations in the culture medium may, for example, be in the range of about 10 g/L to about 60 g/L, for example, about 20 g/L, about 30 g/L, about 40 g/L, about 50 g/L. In illustrative embodiments, the nitrogen source may be a mixture of casein hydrolysate and soy flour hydrate or a mixture of yeast extract and soy acid hydrolysate, wherein for example the yeast extract is used in the culture medium in a concentration (or amount) of 10 g/L and the soy acid hydrolysate is used in a concentration/amount of 20 g/L.

[00062] The culture medium can further contain a calcium source such as calcium chloride, or calcium carbonate. If present, the culture medium may contain a calcium source such as calcium carbonate in an initial concentration of about 1 g/L to 3 g/L.

[00063] In this context, it is noted that concentrations of all ingredients of the culture medium are given as concentration at the beginning of the fermentation (initial concentrations) unless indicated otherwise. The concentrations are based on the post inoculation volume that is used for the fermentation. The initial concentrations as given here can either be maintained during the fermentation by continuous nutrient feeding or, alternatively, the ingredients (carbon source, nitrogen source, amino acid) can be added only at the beginning of the fermentation. However, the pH of the culture medium/fermentation broth is typically continuously monitored and controlled by addition of a suitable acid (such as sulfuric acid or citric acid) and/or of a suitable base (such as sodium hydroxide or ammonia solution or potassium hydroxide). An appropriate pH can be determined empirically. In typical embodiments the pH of the culture

medium/fermentation broth is in range of 6.5 to 7.5, for example, 6.8 to 7.0. Also process parameters such as temperature and aeration rate are usually controlled over the course of fermentation process. Since the cultivation of the *Streptomyces* strain is carried out under aerobic conditions, the fermentation broth is typically aerated with air, oxygen enriched air or if necessary, pure oxygen. The temperature is usually chosen to be within a range of 20° C to 30° C, however higher temperatures are also contemplated herein. Standard fermentation reagents such as antifoam agents may also be added continuously. The production of the fermentation broth can be carried out using conventional large-scale microbial fermentation processes, such as submerged fermentation, solid state fermentation or liquid surface culture, including the methods described, for example, in U.S. Patent No. 3,849,398; British Patent No. GB 1 507 193; Toshiko Kanzaki et al., Journal of Antibiotics, Ser. A, Vol. 15, No.2, Jun. 1961, pages 93 to 97; or Toru Ikeuchi et al., Journal of Antibiotics, (Sept. 1972), pages 548 to 550.

[00064] Any gougerotin-producing *Streptomyces* strain can be used for producing the gougerotin-containing fermentation broth disclosed herein. In illustrative embodiments the *Streptomyces* strain is a *Streptomyces microflavus* strain, *Streptomyces puniceus* strain or a *Streptomyces graminearus* strain. The *Streptomyces microflavus* strain may, for example, be *Streptomyces microflavus* strain NRRL B-50550 or a mutant strain derived therefrom. In addition, parent bacterial strains, such as various *Streptomyces* (including, but not limited to, *Streptomyces microflavus*, *Streptomyces puniceus* and *Streptomyces graminearus*) and *Bacilli*, capable of producing gougerotin, even at low levels, may be mutagenized for enhanced gougerotin production. Example 3 describes one way to produce such mutants and resulting fermentation broths containing at least 1 g/L gougerotin.

[00065] Selection of specific carbon and nitrogen sources and other nutrients during fermentation may be used to optimize the production of gougerotin. Suitable carbon sources for enhancing gougerotin production are starch, maltodextrin, dextrin, sugars and glucose. In a specific embodiment a combination of glucose and an oligosaccharide is used as the carbon source and/or procures. Suitable nitrogen sources for enhancing gougerotin production are soy protein hydrolysate, casein hydrolysate, soy peptone, yeast extract, and other nitrogen sources that are less nutrient rich. Other suitable nitrogen sources include amino acids and/or precursors to gougerotin such as glycine, glutamic acid, including L-glutamic acid, aspartic acid, including L-aspartic acid and cytosine. Cytosine may be added as part of a media component that has a high concentration of cytosine, such as a yeast extract having high nucleobase content. Examples of fermentation media capable of producing a fermentation broth having an increased level of gougerotin are provided in Examples 1-2.

[00066] In some embodiments the compositions of the present invention are used to treat a wide variety of agricultural and/or horticultural crops, including those grown for seed, produce, landscaping and those grown for seed production. Representative plants that can be treated using the compositions of the present invention include but are not limited to the following: brassica, bulb vegetables, cereal grains, citrus, cotton, cucurbits, fruiting vegetables, leafy vegetables, legumes, oil seed crops, peanut, pome fruit, root vegetables, tuber vegetables, corm vegetables, stone fruit, tobacco, strawberry and other berries, and various ornamentals.

[00067] The compositions of the present invention may be administered as a foliar spray, as a soil treatment, and/or as a seed treatment/dressing. When used as a foliar treatment, in one embodiment, about 1/16 to about 5 gallons of whole broth are applied per acre. When used as a soil treatment, in one embodiment, about 1 to about 15 gallons or about 1 to about 5 gallons of whole broth are applied per acre or about 0.1 mg to about 14 mg, or about 0.2 mg to about 10 mg, or about 0.2 mg to about 8 mg fermentation product, such as a freeze dried product, depending on the size of the seeds to be treated and the concentration of colony forming units in the fermentation product. When used for seed treatment about 1/32 to about 1/4 gallons of whole broth are applied per acre. For seed treatment, the end-use formulation contains at least 1×10^8 colony forming units per gram.

[00068] In some embodiments, application of the compositions of the present invention to plants, plant parts or plant loci is preceded by identification of a locus in need of treatment.

[00069] A fermentation product, such as a whole broth culture or a fermentation solid, including a freeze-dried powder, of the microorganism (e.g., *Streptomyces microflavus* NRRL B-50550 or a mutant strain thereof)/mL is diluted and applied to plants foliarly. Application rates are provided in gallons or pounds per acre and can be adjusted proportionally to smaller applications. For larger applications, the fermentation product is diluted in 100 gallons of water before application. In one embodiment, about 0.5 gallons to about 15 gallons, about 1 gallon to about 12 gallons or about 1.25 gallons to about 10 gallons whole broth culture (diluted in water and, optionally, with a surfactant) are applied to plants foliarly per acre. In another embodiment, about 0.2 lbs to about 8 pounds of freeze-dried powder, about 0.4 lbs to about 7 pounds, or about 0.4 lbs to about 6 lbs (diluted in water and, optionally, a surfactant) are applied to plants foliarly per acre. In a particular instance, the fermentation product has Spider Mite Potency of at least about 40%, at least about 50% or at least about 60%. In another instance, the fermentation product is about 0.5% to about 12% gougerotin, about 1% to about 10% gougerotin, or about 2% to about 9% gougerotin, where all percentages are weight by weight.

[00070] In a particular embodiment, 1.25 pounds of fermentation product, such as freeze-dried powder, (diluted in water and, optionally, with a surfactant) are applied to plants foliarly per acre. In these embodiments, the end-use formulation is based on a starting fermentation broth containing at least about 1×10^6 colony forming units per mL, at least about 1×10^7 colony forming units per mL, at least about 1×10^8 colony forming units per mL, at least about 1×10^9 colony forming units per mL, or at least about 1×10^{10} colony forming units per mL. In another example, this fermentation product contains at least about 1% by weight gougerotin, at least about 2% by weight gougerotin, at least about 3% by weight gougerotin, at least about 4% by weight gougerotin, at least about 5% by weight gougerotin, at least about 6% by weight gougerotin, at least about 7% by weight gougerotin, or at least about 8% by weight gougerotin.

[00071] In certain embodiments, the method of the present invention further comprises applying at least one further compound selected from the group consisting of bactericides, antibiotics, fungicides, micronutrients, micronutrient containing compounds, and lipochito-oligosaccharide compounds (LCO). In certain aspects, the at least one further compound is selected from the group consisting of isotianil, fosetyl-Al, penflufen, strobilurins (e.g., azoxystrobin and trifloxystrobin), copper-containing compounds, propineb, mancozeb, lipochito-oligosaccharide compounds (LCO), kasugamycin, streptomycin, and oxytetracycline.

[00072] In the context of the present invention, micronutrients and micronutrient-containing compounds relate to compounds selected from the group consisting of active ingredients containing at least one metal ion selected from the group consisting of zinc, manganese, molybdenum, iron and copper or the micronutrient boron. More preferably these micronutrients and micronutrient-containing compounds are selected from the group consisting of the zinc containing compounds Propineb, Polyoxin Z (zinc salt), Zineb, Ziram, zinc thiodazole, zinc naphthenate and Mancozeb (also containing manganese), the manganese containing compounds Maneb, Metiram and Mancopper (also containing copper), the iron containing compound Ferbam, copper (Cu) and the copper containing compounds Bordeaux mixture, Burgundy mixture, Cheshunt mixture, copper oxychloride, copper sulfate, basic copper sulfate (e.g., tribasic copper sulfate), copper oxide, copper octanoate, copper hydroxide, oxine-copper, copper ammonium acetate, copper naphthenate, chelated copper (e.g., as amino acid chelates), mancopper, acypetacs-copper, copper acetate, basic copper carbonate, copper oleate, copper silicate, copper zinc chromate, cufraneb, cuprobam, saisentong, and thiodiazole-copper, and combinations thereof. More preferably the micronutrients and micronutrient-containing compounds are selected from the group consisting of copper (Cu), copper-hydroxide, copper-sulfate, copper- oxychloride, Propineb and Mancozeb. Even more preferably the micronutrients

and micronutrient-containing compounds are selected from the group consisting of copper-hydroxide, copper-sulfate, and Propineb.

[00073] In the meaning of the invention, a lipochito-oligosaccharide (LCO) compound is a compound having the general LCO structure, i.e., an oligomer backbone of β -1,4-linked N-acetyl-D-glucosamine residues with a N-linked fatty acyl chain at the non-reducing end, as described in U.S. Patent Nos. 5,549,718; 5,646,018; 5,175,149; and 5,321,011. This basic structure may contain modifications or substitutions found in naturally occurring LCO's, such as those described in Spaink, Critical Reviews in Plant Sciences 54: 257-288, 2000; D'Haeze and Holsters, Glycobiology 12: 79R- 105R, 2002. Naturally occurring LCO's are defined as compounds which can be found in nature. This basic structure may also contain modifications or substitutions which have not been found so far in naturally occurring LCO's. Examples of such analogs for which the conjugated amide bond is mimicked by a benzamide bond or which contain a function of benzylamine type are the following compounds of formula (I) which are described in WO 2005/063784 and WO 2008/071672, the content of which is incorporated herein by reference. The LCO's compounds may be isolated directly from a particular culture of Rhizobiaceae bacterial strains, synthesized chemically, or obtained chemo-enzymatically. Via the latter method, the oligosaccharide skeleton may be formed by culturing of recombinant bacterial strains, such as *Escherichia coli*, in a fermenter and the lipid chain may then be attached chemically. LCO's used in embodiments of the invention may be recovered from natural Rhizobiaceae bacterial strains that produce LCO's, such as strains of Azorhizobium, Bradyrhizobium (including *B. japonicum*), Mesorhizobium, Rhizobium (including *R. leguminosarum*), Sinorhizobium (including *S. meliloti*), or from bacterial strains genetically engineered to produce LCO's. These methods are known in the art and have been described, for example, in U.S. Patent Nos. 5,549,718, and 5,646,018, which are incorporated herein by reference. Hungria and Stacey (Soil Biol. Biochem. 29: 819-830, 1997) list specific LCO structures that are produced by different rhizobial species. LCO's may be utilized in various forms of purity and may be used alone or with rhizobia. Methods to provide only LCO's include simply removing the rhizobial cells from a mixture of LCOs and rhizobia, or continuing to isolate and purify the LCO molecules through LCO solvent phase separation followed by HPLC chromatography as described by Lerouge et al. (U.S. Patent No. 5,549,718). Purification can be enhanced by repeated HPLC, and the purified LCO molecules can be freeze-dried for long-term storage. This method is acceptable for the production of LCO's from all genera and species of the Rhizobiaceae. Commercial products containing LCO's are available, such as OPTIMIZE® (EMD Crop Bioscience). LCO compounds, which can be identical or not to naturally occurring LCO's, may also be obtained by chemical synthesis and/or through genetic engineering.

Synthesis of precursor oligosaccharide molecules for the construction of LCO by genetically engineered organisms is disclosed in Samain et al., Carbohydrate Research 302: 35-42, 1997. Preparation of numerous LCO compounds wherein the oligosaccharide skeleton is obtained by culturing recombinant bacterial strains, such as recombinant *Escherichia coli* cells harboring heterologous gene from rhizobia, and wherein the lipid chain is chemically attached is disclosed in WO 2005/063784 and WO 2008/07167, the content of which is incorporated herein by reference. Examples of lipochito-oligosaccharide compounds include, but are not limited to LCO compounds specifically disclosed in WO 2010/125065.

[00074] In one embodiment, the at least one further compound is selected from the group consisting of: Antibiotics such as kasugamycin, streptomycin, oxytetracycline, validamycin, gentamycin, aureofungin, blasticidin-S, cycloheximide, griseofulvin, moroxydine, natamycin, polyoxins, polyoxorim and combinations thereof.

[00075] In certain aspects, the compositions of the present invention are applied in an effective and non-phytotoxic amount wherein the expression “effective and non-phytotoxic amount” means an amount of the ingredients and the active compositions according to the invention which is sufficient for controlling or destroying pathogenic bacterial organisms present or liable to appear on the plants, by notably avoiding the development of resistant strains to the active ingredients and in each case does not entail any appreciable symptom of phytotoxicity for the crops. Such an amount can vary within a wide range depending on the bacterial pathogen to be combated or controlled, the type of crop, the climatic conditions and the compounds included in the bactericide composition according to the invention.

[00076] The bactericidal *Streptomyces microflavus* strain NRRL B-50550 and/or mutant thereof having all the identifying characteristics of the respective strain, and/or at least one metabolite produced by the respective strain and/or at least one further compound may be applied simultaneously (i.e., in combination) or sequentially to the plant, to the part of the plant and/or to the locus of the plant. In this context, the term “combination” means various combinations of at least two of the abovementioned active ingredients which may be presented, for example, as ready mixes, tank mixes (which is understood as meaning spray slurries prepared from the formulations of the individual active ingredients by combining and diluting prior to the application) or combinations of these (for example, a binary ready mix of two of the abovementioned active compounds is made into a tank mix by using a formulation of the third individual substance). According to the invention, the individual active ingredients may also be applied sequentially, i.e., one after the other, at a reasonable interval of a few hours or days, in the case of the treatment of seed for example also by applying a plurality of layers which contain different active ingredients.

[00077] In certain embodiments, the present invention is directed to use of a bactericidal *Streptomyces microflavus* strain NRRL B-50550 and/or a mutant thereof having all the identifying characteristics of the respective strain, and/or at least one metabolite produced by the respective strain for preventing, controlling, or treating a bacterial disease in a plant.

DEPOSIT INFORMATION

[00078] A sample of the *Streptomyces microflavus* strain of the invention has been deposited with the Agricultural Research Service Culture Collection located at the National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604, U.S.A., under the Budapest Treaty and has been assigned the following depository designation: NRRL B-50550.

[00079] A sample of a mutant of *Streptomyces microflavus* strain NRRL B-50550 (designated herein as *Streptomyces microflavus* strain M and also known as AQ6121.002) has been deposited with the International Depositary Authority of Canada located at 1015 Arlington Street Winnipeg, Manitoba Canada R3E 3R2 on October 9, 2013 and has been assigned Accession No. 091013-02.

[00080] The following examples are given for purely illustrative and non-limiting purposes of the present invention.

EXAMPLES

Example 1 – Fermentation Product Containing Increased Levels of Gougerotin

[00081] Fermentation was conducted to optimize gougerotin production and bactericidal activity of NRRL B-50550. A primary seed culture was prepared using a media composed of 10.0 g/L starch, 15.0 g/L glucose, 10.0 g/L yeast extract, 10.0 g/L casein hydrolysate (or 10.0 g/L soy peptone) and 2.0 g/L CaCO₃ in 2 L shake flasks at 20-30° C. When there was abundant mycelial growth in the shake flasks, after about 1-2 days, the contents were transferred to fresh media (same as above, with 0.1% antifoam) and grown in a 400 L fermentor at 20-30° C. When there was abundant mycelial growth, after about 20-30 hours, the contents were transferred to a 3000 L fermentor and grown for 160-200 hours at 20-30° C in media composed of 80.0 g/L (8.0%) Maltodextrin, 30.0 g/L (3.0%) glucose, 15.0 g/L (1.5%) yeast extract, 20.0 g/L (2.0%) soy acid hydrolysate, 10.0 g/L (1.0%) glycine and 2.0 g/L (0.2%) calcium carbonate and 2.0 ml/L antifoam. Gougerotin levels are shown in **Table 1**.

Table 1. Yield and *Normalized* Gougerotin Productivity

	Harvest Titer (mg/g)	Harvest Weight (kg)	Total Gougerotin (kg)	Target Volume (L)	Normalized Volumetric Titer (g/L)
First 3000 L Fermentation	1.7	3397	5.78	3000	1.9
Second 3000 L Fermentation	1.8	3511	6.33	3000	2.1

[00082] Using the first 3000 L fermentation as an example, the yield of gougerotin in the fermentor is calculated as follows. $3397 \text{ kg} \times 1.7 \text{ mg/g Fermentation broth} = 5774.90 \text{ g gougerotin} = 5.78 \text{ kg}$. The initial weight in the fermentor was 3496 kg (3256 kg Medium + 240 kg Seed), which resulted in a final volume more than the target volume 3000 L. Since the target volume 3000 L is the basis for calculating the amount of all ingredients in the production medium, the normalized volumetric productivity is: $5774.9 \text{ g}/3000 \text{ L} = 1.9 \text{ g/L}$. This gougerotin concentration was similar to the 1.8 g/L achieved in a 20 L fermentation conducted using the same media as described above, with the final fermentation step and media containing glycine (as amino acid).

Example 2– Fermentation Product Containing Increased Levels of Gougerotin

[00083] Fermentation was conducted to optimize gougerotin production and bactericidal activity of NRRL No. B-50550. A primary seed culture was prepared using a media composed of 10.0 g/L starch, 15.0 g/L glucose, 10.0 g/L yeast extract, 10.0 g/L casein hydrolysate (or 10.0 g/L soy peptone) and 2.0 g/L CaCO_3 in 1 L shake flasks at 20-30° C. When there was abundant mycelial growth in the shake flasks, after about 1-2 days, the contents were transferred to fresh media (same as above, with 0.1% antifoam) and grown in 1 L shake flasks at 20-30° C. When there was abundant mycelial growth, after about 20-30 hours, the contents were transferred to a 20 L fermentor and grown for 160-200 hours at 20-30° C in media composed of 60.0 g/L (8.0%) starch, 30.0 g/L (3.0%) dextrose, 15.0 g/L (1.5%) yeast extract, 20.0 g/L (2.0%) soy acid hydrolysate, 12.0 g/L (1.0%) L-glutamic acid and 2.0 g/L (0.2%) calcium carbonate and 2.0 mL/L antifoam.

[00084] This gougerotin concentration using L-glutamic acid as amino acid in this fermentation was 1.1 g/L.

Example 3 - Gougerotin-Overproducing Mutants

[00085] With the goal of increasing gougerotin production and bioactivity, mutants were created from the parent strain *Streptomyces microflavus* NRRL No. B-50550 through an antibiotic-resistant mutant screening program in which libraries of mutants resistant to individual antibiotics (gentamicin, rifampicin, streptomycin, paromomycin or tobramycin) were produced. See, Okamoto-Hosoya, Y., et al., The Journal of Antibiotics 43(12) Dec 2000. The parent strain was subjected to mutagenesis using N-methyl-N'-nitro-N-nitrosoguanidine ("NTG") and then resulting antibiotic resistant mutants selected and screened. A detailed description of creation and screening of mutant libraries from which gougerotin-overproducing strains were selected for further development is described below.

[00086] Spore suspensions of *Streptomyces microflavus* B-50550 were prepared from soy flour maltose (SFM) agar plates containing B-50550 grown for approximately 14 days or to sporulation and stored at -80° C in 20% glycerol. NTG, dissolved in suitable buffer, was added to the spore suspensions in an amount suitable to obtain 50% kill (0.5 mg/mL at pH 8.5 slowly shaken for 1 hour at 37° C). NTG-treated spore suspensions were then plated onto GYM (glucose 4 g/L, yeast extract 4 g/L, malt extract 10 g/L, and agar 12 g/L) supplemented with the concentrations of antibiotics shown in **Table 2**.

Table 2. Antibiotic Concentrations Used for Mutant Screening

ANTIBIOTIC	1x	2x	5x	10x	20x
Streptomycin SO ₄	10 mg/L	20 mg/L	50 mg/L	100 mg/L	200 mg/L
Rifampicin (Fresh)	3.5 mg/L	7 mg/L	17.5 mg/L	35 mg/L	70 mg/L
Paromomycin SO ₄	1 mg/L	2 mg/L	5 mg/L	10 mg/L	20 mg/L
Tobramycin SO ₄	4.5 mg/L	9 mg/L	22.5 mg/L	45 mg/L	90 mg/L
Gentamycin SO ₄	5.5 mg/L	11 mg/L	27.5 mg/L	55 mg/L	110 mg/L

See Kieser, T., et al., Practical *Streptomyces* Genetics, Ch. 5, John Ines Centre Norwich Research Park, England (2000), pp. 99-107. Approximately 350 individual antibiotic-resistant colonies were isolated, purified, and screened as described below.

[00087] Each isolate removed from GYM antibiotic plates was re-plated onto SFM agar plates. Agar plugs containing antibiotic-resistant bacteria were used to inoculate 24-well blocks containing 2.5 mL of seed media. Bacteria in these inoculated blocks were grown for 3 days and the resulting culture broth used to inoculate 24-well blocks containing production media. Bacteria in production blocks were grown for seven days at 28° C. Each well in the seed blocks contained Trypticase Soy Broth (TSB) (Per liter of DI H₂O: 17 g Bacto Tryptone (Pancreatic Digest of Casein), 3 g Bacto Soytone (Pancreatic Digest of Soybean Meal), 2.5 g Dextrose, 5 g NaCl, 2.5 g Dipotassium Phosphate) and in the production blocks contained the following media: Proflo 20 g/L, malt extract 20 g/L, KH₂PO₄ monobasic 6 g/L, K₂HPO₄ dibasic 4.8 g/L.

[00088] The whole broth from each well of the production block was tested for gougerotin production as follows using analytical HPLC chromatography. 2.4 mL water was added to each well of the production block. Blocks were vortexed and centrifuged. 0.8 mL supernatant was transferred to an extraction block containing 4 mL of water per well. 3.2 mL water was added to the cell pellet in each well of the production block and the block vortexed and centrifuged again. This 3.2 mL of wash water was then added to the appropriate well of each extraction block. The aqueous extracts in the extraction block were then assayed for gougerotin content using analytical HPLC chromatography. Specifically, a sample was injected onto a Cogent Diamond hydride column (100A, 4 µm, 150 x 4.6 mm) fitted with a Diamond Hydride guard column. The column was eluted with a 30 minute Acetonitrile/NH₄O Ac gradient (see below). The flow rate was 1 mL/min. Gougerotin was detected at 254 nm. Gougerotin elutes as a single peak with an approximate retention time of 19 minutes. Top over-producing mutants were confirmed by re-growing in both 24 well blocks and 250 mL flasks to confirm gougerotin levels. Once confirmed some isolates were then subjected to at least one more round of mutagenesis and antibiotic-resistance screening. Each subsequent round of mutagenesis coupled with antibiotic-screening was performed using the remaining antibiotics to which an isolate derived in the previous round had not developed resistance. Small (1.2x) increases in gougerotin production were found after a single round of screening, and subsequent rounds lead to greater increases from isolates generated from the same original low level overproducer as shown in **FIG. 1**.

[00089] Selected mutants with higher gougerotin production and ability to sporulate on SFM agar plates were grown in 1 L baffled shake flasks and subsequently scaled up to 5 L Sartorius B-plus bioreactors and/or 20 L bioreactors containing Medium 2. Gougerotin levels produced by the mutants in the bioreactors are shown in **FIG. 2**.

Example 4 - Field Activity Against Citrus Mite

[00090] Field trials were conducted to determine efficacy of NRRL B-50550 against citrus rust mites (*Phyllocoptruta oleivora*) on Valencia oranges. Shake flasks containing Medium 1 (2.0 % starch, 1.0% dextrose, 0.5% yeast extract, 0.5% casein hydrolysate and 0.1% CaCO₃) were inoculated with frozen cultures of NRRL B-50550 and grown 1-2 days at 20-30° C. This was repeated. The resulting fermentation product was used to seed a 20 L bioreactor containing the following media: 6.0% starch, 3.0% dextrose, 1.5% yeast extract, 2.0% soy acid hydrolysate, 0.6% glycine, and 0.2% calcium carbonate. This medium was fermented at between 28° C for 8 days. The resulting whole broth was used to create a freeze dried powder (“FDP”) used in the following trials. The freeze dried powder was diluted in water and applied at 100 gal/acre at the rates shown in **Table 3**. The miticide ENVIDOR® (spirodiclofen, Bayer CropScience, Germany) was used as positive control. In treatments 1-3, the BREAK-THRU FIRST CHOICE® adjuvant (polyether-polymethylsiloxane-copolymer, see above) was added at 0.66% v/v. The fermentation product applied at a rate of 0.625 lb/A showed a better miticidal activity than ENVIDOR® spirodiclofen applied at a rate of 16-fl oz/A.

Table 3. Activity of NRRL B-50550 Against Citrus Mites

	Treatment	Rate	No. Mites/cm² Fruit
1.	NRRL B-50550 FDP	0.625 lb/A	0.29
2.	NRRL B-50550 FDP	1.25 lb/A	1.43
3.	NRRL B-50550 FDP	2.5 lb/A	0.78
4.	NRRL B-50550 + 435 Oil	2.5 lb/A + 5 gal/A	0.76
5.	ENVIDOR® 2SC (spirodiclofen)	16 fl oz/A	0.41
6.	Untreated Check	- -	13.09

Example 5 – Corn Rootworm Activity

[00091] Tests were conducted to determine efficacy of NRRL B-50550 against corn rootworm. NRRL B-50550 whole broth was prepared in Medium 1 or Medium 2. Medium 1 was composed of 2.0 % starch, 1.0% dextrose, 0.5% yeast extract, 0.5% casein hydrolysate and 0.1% CaCO₃. Medium 2 was composed of 2% ProFlo cotton seed meal, 2% malt extract, 0.6% KH₂PO₄ and 0.48% K₂HPO₄. NRRL B-50550 whole broth was diluted and fed to larvae of western spotted cucumber beetle (*Diabrotica undecimpunctata*) in a diet-based assay conducted in a microtiter plate. Activity was assessed and rated on a scale of 1 to 4. For insects and eggs, 1 indicates 100% mortality, 1.5 indicates 90% to 95% mortality, 2.0 represents 75% to 90%

mortality; 2.5 represents 40% to 55% mortality; 3.0 represents 20% to 35% mortality and 4.0 represents 0% to 10% mortality. The termiticide/insecticide TERMIDOR® SC (5-amino-1-(2,6-dichloro-4(trifluoromethyl)phenyl)-4-((1R,S)-(trifluoromethyl)sulfinyl)-1-H-pyrazole-3-carbonitrile, commonly known as fipronil, was used as positive control. Results are shown in **Table 4**. NRRL B-50550 showed the same insecticidal activity as the insecticide TERMIDOR® SC, which contains the active ingredient fipronil.

Table 4. Activity of NRRL B-50550 Against Corn Rootworm

Treatment	Dosage	Rating
NRRL B-50550 Media 1	100%	1.0
NRRL B-50550 Media 1	25%	1.0
NRRL B-50550 Media 1	6.25%	1.0
NRRL B-50550 Media 1	1.56%	3.75
NRRL B-50550 Media 2	100%	1.0
NRRL B-50550 Media 2	25%	1.0
NRRL B-50550 Media 2	6.25%	1.0
NRRL B-50550 Media 2	1.56%	4.0
TERMIDOR® SC 8.3 mg/mL	100.0%	1.0
TERMIDOR® SC	25.0%	1.0
TERMIDOR® SC	1.56%	3.75
Untreated		4.0

Example 6 – Activity Against *Pseudomonas syringae* pv. *tomato*

[00092] Three separate preparations of NRRL B-50550 fermentation broth were generated according to the procedure outlined in Example 1. These preparations are designated NRRL B-50550 #1, NRRL B-50550 #2, and NRRL B-50550 #3 in **Table 5**. In addition, a gougerotin-overproducing NRRL B-50550 mutant strain designated “strain M” was isolated by the procedure described in Example 3 and analyzed. The fermentation product of this NRRL B-50550 mutant strain was prepared in the same manner as were the three NRRL B-50550 preparations.

[00093] The fermentation broths from NRRL B-50550 #1, NRRL B-50550 #2, NRRL B-50550 #3, and strain M were diluted and foliarly applied to tomato plants at dosages of 2.5%, 5%, and 10%. The tomato plants were subsequently inoculated with *Pseudomonas*

syringae pv. *tomato* and rated 7 days later for percent disease control relative to untreated control plants. The results of the analysis are presented in **Table 5**.

Table 5. Activity of NRRL B-50550 Against *Pseudomonas syringae* pv. *tomato*

Treatment	Dosage	% Disease Control
NRRL B-50550 #1	10%	80%
NRRL B-50550 #1	5%	77%
NRRL B-50550 #1	2.5%	50%
NRRL B-50550 #2	10%	88%
NRRL B-50550 #2	5%	50%
NRRL B-50550 #2	2.5%	58%
NRRL B-50550 #3	10%	88%
NRRL B-50550 #3	5%	67%
NRRL B-50550 #3	2.5%	58%
Strain M	10%	92%
Strain M	5%	68%
Strain M	2.5%	33%
Untreated	--	0%

Example 7 – Activity Against *Xanthomonas campestris* pv. *vesicatoria*

[00094] The fermentation products for NRRL B-50550 #1, NRRL B-50550 #2, NRRL B-50550 #3, and strain M were prepared as described in Example 1.

[00095] The fermentation products from NRRL B-50550 #1, NRRL B-50550 #2, NRRL B-50550 #3, and strain M were foliarly applied to pepper plants at dosages of 2.5%, 5%, and 10%. The pepper plants were subsequently inoculated with *Xanthomonas campestris* pv. *vesicatoria* and rated 14 days later for percent disease control relative to untreated control plants. The results of the analysis are presented in **Table 6**.

Table 6. Activity of NRRL B-50550 Against *Xanthomonas campestris* pv. *vesicatoria*

Treatment	Dosage	% Disease Control
NRRL B-50550 #1	10%	100% *
NRRL B-50550 #1	5%	17%
NRRL B-50550 #1	2.5%	38%
NRRL B-50550 #2	10%	83%
NRRL B-50550 #2	5%	17%
NRRL B-50550 #2	2.5%	33%
NRRL B-50550 #3	10%	77%
NRRL B-50550 #3	5%	60%
NRRL B-50550 #3	2.5%	37%
Strain M	10%	90%
Strain M	5%	80%
Strain M	2.5%	17%
Untreated	--	0%

*Phytotoxic injury was so severe that it was difficult to precisely determine percent disease control with this particular treatment.

Example 8 – Control of Huanglongbing (HLB) in Citrus

[00096] A wettable powder containing NRRL B-50550 is prepared and applied to citrus trees with 100% HLB symptoms to determine the efficacy of NRRL B-50550 in treating citrus greening.

[00097] The wettable powder is prepared from freeze-dried powder of NRRL B-50550, which is obtained with a procedure similar to that described in Example 1. Freeze-dried powder is formulated with inert ingredients (a wetting agent, stabilizer, carrier, flow aid and dispersant) to make the wettable powder. The formulated product comprises 75% by weight freeze-dried powder. Wettable powder is diluted in water and applied at 100 gal/acre at the rates shown in **Table 7**.

[00098] Citrus trees are evaluated for the severity of HLB at regular intervals after each application. It is expected that NRRL B-50550 will control citrus greening.

Table 7. NRRL B-50550 Treatments for Application on Citrus Trees with HLB

Treatment	Dosage (Rate per Grove Acre)	Application
NRRL B-50550 Wettable Powder	3-lb	ABC (spray)
NRRL B-50550 Wettable Powder	6-lb	ABC (spray)
NRRL B-50550 Wettable Powder	3-lb	ABC (drench)
NRRL B-50550 Wettable Powder	6-lb	ABC (drench)
Untreated	--	--

Note: The initial application (A) is followed up by subsequent applications (B and C) depending on the treatment outcome of A.

Example 8 – Effect of *Streptomyces microflavus* NRRL B-50550 Treatment on HLB-Affected Citrus

[00099] A wettable powder containing *Streptomyces microflavus* NRRL B-50550 was prepared as described in Example 7. The wettable powder was diluted in water and applied as a drench to the rooting zone of citrus trees (“drench”) or as a foliar spray (“spray”) at the dosages indicated in **Table 8**. Initial treatments (“A” and “B” in **Table 8**) were followed 57 days later by a second round of treatments (“C” and “D” in **Table 8**). HLB severity was very high throughout the grove of citrus trees at the initiation of this study.

Table 8. NRRL B-50550 Treatments for Application on Citrus Trees with HLB

Treatment	Dosage (Rate per Grove Acre)	Application
Untreated	--	--
NRRL B-50550 Wettable Powder	3-lb/a	AC (spray)
NRRL B-50550 Wettable Powder	6-lb/a	AC (spray)
NRRL B-50550 Wettable Powder	3-lb/a	BD (drench)
NRRL B-50550 Wettable Powder	6-lb/a	BD (drench)

[000100] HLB disease severity was rated 102 days after the second round of treatments. No difference in disease severity was observed between the treatment groups and the untreated control group in this study.

[000101] Tree canopy volumes were also determined the day of the first set of treatments (*i.e.*, at Day 0) and 102 days after the second set of treatments (*i.e.*, at Day 159). Ten trees were measured in each of four replicate plots per treatment. The percent change in canopy volume between the two sets of measurements was recorded for each individual tree, and the average percent change within each group is presented in **Table 9**.

Table 9. Percent Change in Canopy Volumes of Citrus Trees with HLB Treated with NRRL B-50550

Treatment	Percent Change in Canopy Volume
Untreated	68.3%
NRRL B-50550 at 3-lb/a (spray)	142.1%
NRRL B-50550 at 6-lb/a (spray)	111.1%
NRRL B-50550 at 3-lb/a (drench)	194.8%
NRRL B-50550 at 6-lb/a (drench)	191.4%

[000102] Generally, trees treated with *Streptomyces microflavus* NRRL B-50550 experienced an increase in canopy volume whereas untreated trees in the control group suffered a decrease in canopy volume.

[000103] This trial did not run long enough to directly assess the effect of *Streptomyces microflavus* NRRL B-50550 on fruit production. However, it is reasonable to expect based on similar studies that treatments resulting in increased tree health evidenced by greater size, root density, canopy volume, and/or stem diameter will contribute to tree productivity.

[000104] Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present invention, the preferred methods and materials are described herein. All publications, patents, and patent publications cited are incorporated by reference herein in their entirety for all purposes.

[000105] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

[000106] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in

general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

CLAIMS

We claim:

1. A method of preventing or treating a bacterial disease in a plant, wherein the method comprises applying a bactericidal *Streptomyces microflavus* strain NRRL B-50550 and/or a mutant thereof having all the identifying characteristics of the respective strain, and/or at least one metabolite produced by the respective strain to the plant, to a part of the plant and/or to a locus of the plant.
2. The method of Claim 1, wherein the *Streptomyces microflavus* strain NRRL B-50550 or bactericidal mutant strain is applied in a composition comprising the *Streptomyces microflavus* strain NRRL B-50550 or the mutant strain derived therefrom.
3. The method of Claim 2, wherein the composition is a fermentation product of the *Streptomyces microflavus* strain NRRL B-50550 or the mutant strain derived therefrom.
4. The method according to any one of Claims 1 to 3, further comprising simultaneously or sequentially applying at least one further compound selected from the group consisting of bactericides, antibiotics, fungicides, micronutrients, micronutrient containing compounds, and lipochito-oligosaccharide compounds (LCO).
5. The method of Claim 4, wherein the at least one further compound is selected from the group consisting of isotianil, fosetyl-Al, penflufen, strobilurins, copper-containing compounds, propineb, mancozeb, LCO, kasugamycin, streptomycin, and oxytetracycline.
6. The method according to any one of Claims 1 to 5, wherein the composition is applied in an effective amount to control the bacterial disease in the plant.
7. The method of Claim 6, wherein application of the effective amount of the composition controls at least 60% of the bacterial disease in the plant.
8. The method of Claim 7, wherein application of the effective amount of the composition controls at least 70% of the bacterial disease in the plant.
9. The method to any one of Claims 1 to 8, wherein the method comprises applying the composition to soil.
10. The method of Claim 9, wherein the composition is applied before, during or after the plant or plant part comes into contact with the soil.
11. The method of Claim 10, wherein the plant part is selected from the group consisting of a seed, root, corm, tuber, bulb and rhizome.
12. The method according to any one of Claims 1 to 8, wherein the composition is applied one or more times to roots of the plant during planting or transplanting, in-furrow and/or as a root dip.

13. The method according to any one of Claims 1 to 8, wherein the composition is applied one or more times to foliage, trunks and/or roots of the plant during plant growth.

14. The method according to any one of Claims 1 to 8, wherein the composition is applied to the plant by spraying, painting, or drenching.

15. The method of any one of Claims 1 to 14, wherein the bacterial disease to be prevented or treated is an insect-vector-borne bacterial disease.

16. The method of Claim 15, wherein the bacterial disease is carried by an insect vector selected from the group consisting of *Diaphorina citri* and *Trioza erytreae*.

17. The method of Claim 15, wherein the bacterial disease is caused by a bacterium selected from the group consisting of *Candidatus Liberibacter* spp., *Candidatus Liberibacter africanus*, *Candidatus Liberibacter asiaticus*, and *Candidatus Liberibacter americanus*.

18. The method of Claim 17, wherein the bacterial disease comprises Huanglongbing (HLB).

19. The method of any one of Claims 1 to 14, wherein the bacterial disease is caused by a bacterium selected from the group consisting of *Acidovorax avenae*, *Acidovorax konjaci*, *Burkholderia glumae*, *Burkholderia* spp., *Candidatus Liberibacter* spp., *Candidatus Liberibacter africanus*, *Candidatus Liberibacter americanus*, *Candidatus Liberibacter asiaticus*, *Candidatus Phytoplasma* spp., *Clavibacter michiganensis* subsp. *Michiganensis*, *Clavibacter xyli* subsp. *cynodontis*, *Clavibacter xyli* subsp. *xyli*, *Corynebacterium*, *Curtobacterium flaccumfaciens*, *Erwinia* spp., *Janibacter melonis*, *Pectobacterium carotovorum* subsp. *Atrosepticum*, *Pectobacterium carotovorum* subsp. *Carotovorum*, *Pseudomonas syringae* pv. *actinidae*, *Pseudomonas syringae* pv. *glycinea*, *Pseudomonas syringae* pv. *lachrymans*, *Pseudomonas syringae* pv. *tomato*, *Pseudomonas syringae*, *Ralstonia solanacearum*, *Serratia marcescens*, *Spiroplasma citri*, *Spiroplasma kunkelii*, *Spiroplasma phoenecium*, *Streptomyces* spp., *Xanthomonas albilineans*, *Xanthomonas axonopodis* pv. *citri*, *Xanthomonas axonopodis* pv. *glycines*, *Xanthomonas axonopodis*, *Xanthomonas campestris* pv. *musacearum*, *Xanthomonas campestris* pv. *pruni*, *Xanthomonas campestris* pv. *vesicatoria*, *Xanthomonas campestris*, *Xanthomonas fragariae*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas* spp., *Xanthomonas translucens*, and *Xylella fastidiosa*.

20. The method of any one of the preceding Claims, wherein the composition is applied at a rate of from about 0.625 pounds/acre to about 6 pounds/acre.

21. A method of preventing or treating a bacterial disease in a plant comprising applying a fermentation product comprising a gougierotin-producing *Streptomyces* strain to the plant, to a part of the plant and/or to a locus of the plant.

22. The method of Claim 21, wherein the gougerotin-producing *Streptomyces* strain is a mutant strain that produces increased amounts of gougerotin compared to amounts of gougerotin produced by a wild-type strain of the same species.

23. The method of Claim 22, wherein the mutant strain is *Streptomyces microflavus* strain M with Accession No. 091013-02.

24. The method according to Claim 21 or 22, wherein the *Streptomyces* strain is a *Streptomyces microflavus* strain or a *Streptomyces puniceus* strain.

25. The method according to any one of Claims 21 to 24, further comprising simultaneously or sequentially applying at least one further compound selected from the group consisting of bactericides, antibiotics, fungicides, micronutrients, micronutrient containing compounds, and LCO.

26. The method according to any one of Claims 21 to 25, wherein the bacterial disease is caused by a bacterium selected from the group consisting of *Acidovorax avenae*, *Acidovorax konjaci*, *Burkholderia glumae*, *Burkholderia* spp., *Candidatus Liberibacter* spp., *Candidatus Liberibacter africanus*, *Candidatus Liberibacter americanus*, *Candidatus Liberibacter asiaticus*, *Candidatus Phytoplasma* spp., *Clavibacter michiganensis* subsp. *Michiganensis*, *Clavibacter xyli* subsp. *cynodontis*, *Clavibacter xyli* subsp. *xyli*, *Corynebacterium*, *Curtobacterium flaccumfaciens*, *Erwinia* spp., *Janibacter melonis*, *Pectobacterium carotovorum* subsp. *Atrosepticum*, *Pectobacterium carotovorum* subsp. *Carotovorum*, *Pseudomonas syringae* pv. *actinidae*, *Pseudomonas syringae* pv. *glycinea*, *Pseudomonas syringae* pv. *lachrymans*, *Pseudomonas syringae* pv. *tomato*, *Pseudomonas syringae*, *Ralstonia solanacearum*, *Serratia marcescens*, *Spiroplasma citri*, *Spiroplasma kunkelii*, *Spiroplasma phoenecium*, *Streptomyces* spp., *Xanthomonas albilineans*, *Xanthomonas axonopodis* pv. *citri*, *Xanthomonas axonopodis* pv. *glycines*, *Xanthomonas axonopodis*, *Xanthomonas campestris* pv. *musacearum*, *Xanthomonas campestris* pv. *pruni*, *Xanthomonas campestris* pv. *vesicatoria*, *Xanthomonas campestris*, *Xanthomonas fragariae*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas* spp., *Xanthomonas translucens*, and *Xylella fastidiosa*.

27. The method according to any one of Claims 21 to 25, wherein the bacterial disease comprises HLB.

FIG. 1

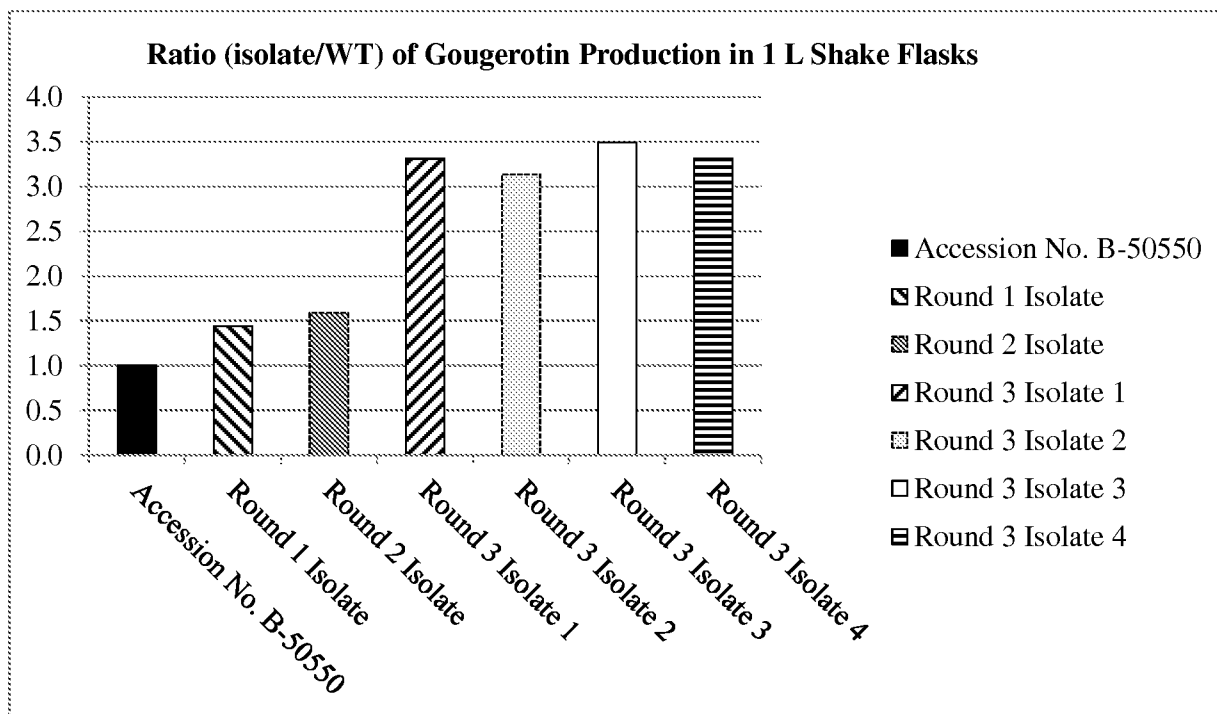
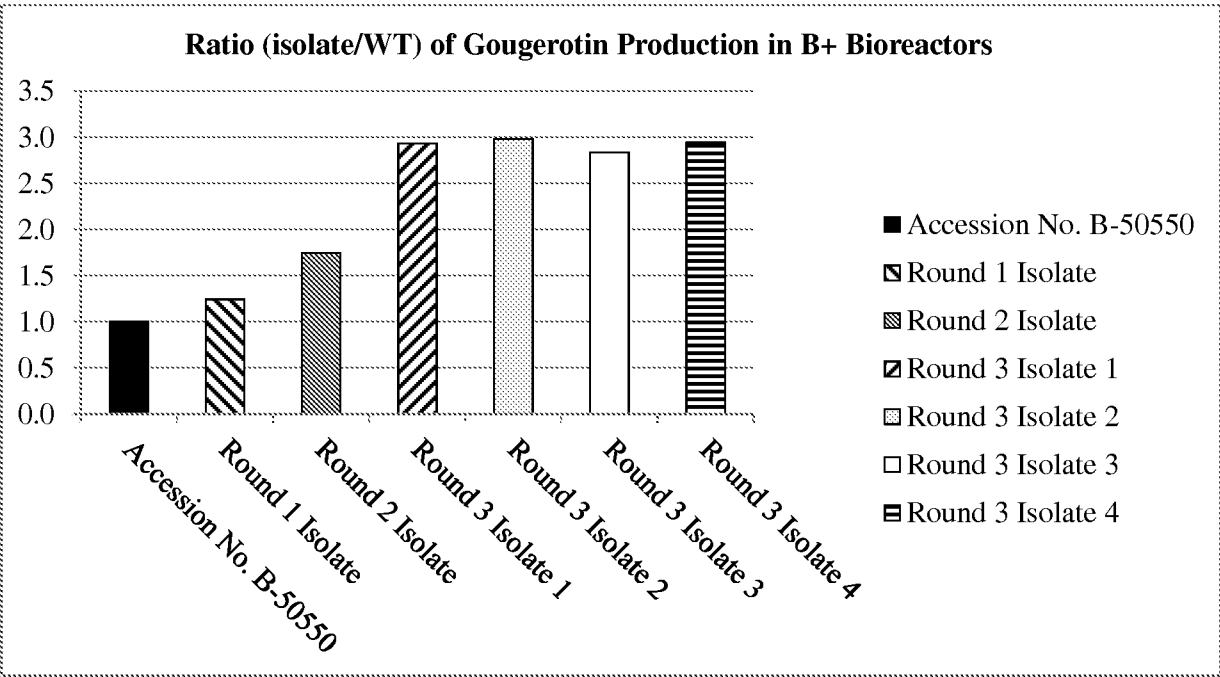


FIG. 2



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/058017

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01N63/00 A01P1/00
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)
A01N

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 02/26041 A2 (AGRAQUEST INC [US]; LEHMAN LORI JO [US]; YUAN CAIYAO [US]; ORJALA JIMM) 4 April 2002 (2002-04-04) the whole document -----	1-27
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☒ 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

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"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

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Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/058017

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International application No

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