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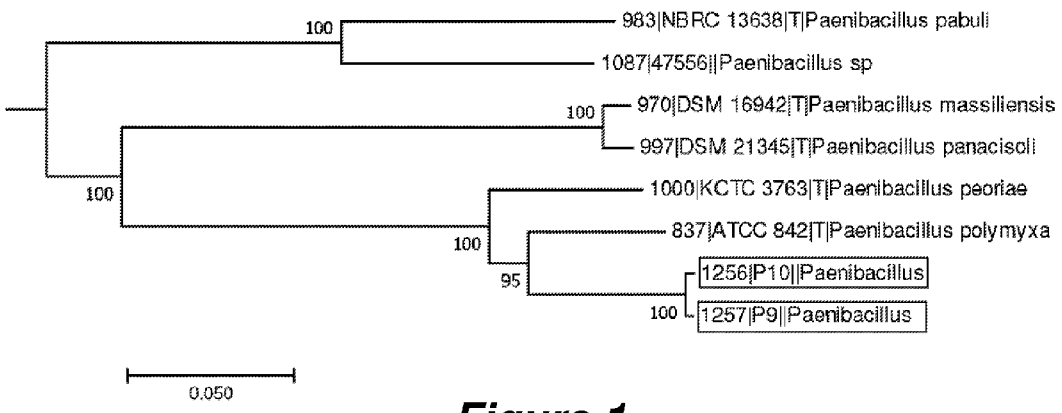

Figure 1

(57) Abstract: The present disclosure relates to bacterial inoculants, and methods for their use, to control a fungal root disease in a plant and promote plant growth in water limited conditions. In particular strains of *Paenibacillus* and *Streptomyces* are disclosed.

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BACTERIAL INOCULANTS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to Australian Provisional Application No. 2017901523, filed April 27, 2017, and U.S. Provisional Application No. 62/568,763, filed October 5, 2017, the disclosures of which are incorporated by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in conjunction with this specification and is hereby incorporated by reference in its entirety. Said copy is named 33524-40357_WO.txt, and is 26715 bytes in size.

FIELD

The present invention relates to bacterial inoculants, and methods for their use, to control a fungal root disease in a plant and promote plant growth in water limited conditions.

BACKGROUND

Root diseases are a major constraint in cropping systems worldwide. Root diseases are difficult to control with fungicides as they are below ground and management practices often provide only partial reductions in disease.

Two major genera of fungal root rot pathogens are *Rhizoctonia* and *Pythium* which infect multiple crop types in broad acre and horticulture crops. These pathogens infect roots of plants, reducing germination and establishment of emerging seedlings and causing loss of root hairs and breakdown of roots in established plants and thereby reducing the plants access to water and nutrients resulting in reduced growth and yield. In broad acre cereal cropping systems these pathogens are ubiquitous and the increase in minimal or no--till tillage practices has increased the impact of these diseases. Their broad host range means there are few non-host crops to use in rotations to reduce pathogen inoculum and there are no resistant cereal cultivars available.

In dryland cereal cropping systems, *Rhizoctonia* root rot, caused by *Rhizoctonia*

solani anastomosis group AG8 is the main fungal root disease, especially in low rainfall zones, causing an estimated yield loss of Aus $77 mil per annum in Australia. *R. oryzae* is also an important root pathogen in cereals. *Pythium* damping off and root rot is caused by a number of *Pythium* species, with *P. irregulare* and *P. ultimum* being the main species infecting cereals with the prevalence and severity of disease increasing in higher rainfall zones causing an estimated yield loss of Aus $11 mil per annum in Australia.

The incidence and severity of *Rhizoctonia* and *Pythium* diseases on crop plants are known to be influenced by soil and plant associated microbes, with numerous reports of bacteria and fungi able to reduce disease under controlled conditions in pots. However, further improved microbial inoculants to control fungal root diseases in valuable crops, such as wheat or canola, are desirable.

BRIEF DESCRIPTION OF THE DRAWINGS

The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

FIG. 1 depicts a phylogenetic tree visualizing the phylogenetic relationship between strain 9.4E (P9), strain 10.6D (P10), and other Paenibacillus strains.

FIG. 2 depicts a phylogenetic tree visualizing the phylogenetic relationship between strain HCA1273 (S12), and other Streptomyces strains.

FIG. 3 depicts a phylogenetic tree visualizing the phylogenetic relationship between strain BD141 (S14), and other Streptomyces strains.

DESCRIPTION

Nucleotide and amino acid sequences are referred to herein by a sequence identifier number (SEQ ID NO:). A summary of the sequence identifiers is provided below:

Sequence Identifier	Description
SEQ ID NO: 1	27f primer nucleotide sequence
SEQ ID NO: 2	1465r primer nucleotide sequence
SEQ ID NO: 3	*Paenibacillus* sp. 10.6D 16S rRNA gene nucleotide sequence
SEQ ID NO: 4	*Paenibacillus* sp. 9.4E 16S rRNA gene nucleotide sequence

SEQ ID NO: 5	*Streptomyces* sp. HCA1273 16S rRNA gene nucleotide sequence
SEQ ID NO: 6	*Streptomyces* sp. BD141 16S rRNA gene nucleotide sequence
SEQ ID NO: 7	*Paenibacillus* sp. 9.4E atpD gene nucleotide sequence
SEQ ID NO: 8	*Paenibacillus* sp. 9.4E recA gene nucleotide sequence
SEQ ID NO: 9	*Paenibacillus* sp. 9.4E trpB gene nucleotide sequence
SEQ ID NO: 10	*Paenibacillus* sp. 9.4E gyrB gene nucleotide sequence
SEQ ID NO: 11	*Paenibacillus* sp. 10.6D recA gene nucleotide sequence
SEQ ID NO: 12	*Paenibacillus* sp. 10.6D atpD gene nucleotide sequence
SEQ ID NO: 13	*Paenibacillus* sp. 10.6D trp gene nucleotide sequence
SEQ ID NO: 14	*Paenibacillus* sp. 10.6D gyrB gene nucleotide sequence
SEQ ID NO: 15	*Paenibacillus* sp. 10.6D full genome sequence
SEQ ID NO: 16	*Streptomyces* sp. HCA1273 full genome sequence
SEQ ID NO: 17	*Paenibacillus* sp. 9.4E full genome sequence
SEQ ID NO: 18	*Streptomyces* sp. BD141 full genome sequence

In this work over 2,000 microbial strains were screened in a multi--tiered screening system to identify strains which can reduce disease when applied to seeds under field cropping conditions. Strains were sequentially screened in a high--throughput plant-- pathogen tube system, then pot bioassay systems, characterised, and selected strains assessed in field trials. Field trials were carried out in cereal growers' paddocks with naturally occurring pathogen inoculum.

In a first aspect, the present invention provides a bacterial inoculant for controlling a fungal root disease on a plant.

A "bacterial inoculant" as referred to herein should be understood as any isolated microorganism which may be inoculated onto a plant in order to control a fungal root disease.

An "isolated" bacterial microorganism should be understood to be any bacterial microorganism which has been removed from its native environment and grown or cultured in vitro. In some embodiments, an isolated bacterial microorganism may be substantially purified and thus grown or cultured substantially in the absence of other microorganisms. Alternatively, in some embodiments, the isolated microorganism may be co--cultured with one or more additional microorganisms.

As referred to herein, terms such as "inoculating", "inoculated", "inoculation" and the like should be understood to include any method or process wherein a plant (including without limitation a plant seed, leaf, root) is brought into contact with a bacterial inoculant by human ingenuity such that the bacterial inoculant exists on or in the plant in a manner not found in nature prior to the application of the bacterial inoculant. In some embodiments inoculation may comprise the bacterial inoculant being applied to a wheat seed or canola plant seed. In some embodiments inoculation may comprise the bacterial inoculant being applied to soil in which a wheat or canola plant is growing or in which a wheat or canola seed will be planted. In some embodiments, inoculation may comprise the bacterial inoculant being applied to root and/or shoot tissue of a wheat or canola plant. In some embodiments inoculation may be the mechanical or manual application, artificial inoculation or disposition of a bacterial inoculant onto or into a plant or plant growth medium. A plant growth medium is any composition or environment in which a plant may be grown. In some embodiments, the plant growth medium is soil.

As described later, in some embodiments, the bacterial inoculants contemplated by the present invention are from a specific genus or species, comprise a defining 16S rRNA gene nucleotide sequence, and/or comprise a defined bacterial strain.

As also set out above, the present invention contemplates control of a fungal disease of a plant. In some embodiments, the fungal disease is a root disease of a monocot or dicot plant. In some embodiments, the monocot is a cereal plant. In some embodiments the cereal plant is member of the plant family *Poaceae* or *Gramineae*, for example: wheat, rice, corn, barley, millet, sorghum, oat, rye, or related grain producing plant. In some embodiments, the dicot is a member of the plant family *Fabaceae* or *Leguminosae,* for example: soybeans, peas, beans, lentils, peanuts, alfalfa, clover, or related plants. In some embodiments, the dicot is a member of the plant family *Brassicaceae* or *Cruciferae,* for example: canola, rapeseed, cabbage, cauliflower, kale, radish, mustard, turnip, or related plants.

A "wheat plant", as referred to herein, should be understood to include plants of the genus Triticum. In some embodiments, the term "wheat" should be understood to include one or more of diploid wheat, tetraploid wheat and/or hexaploid wheat. In

some embodiments, the wheat plant may be a cultivated species of wheat including, for example, *Triticum aestivum, Triticum durum, Triticum monococcum* or *Triticum spelta*. In some embodiments, the term "wheat" refers to wheat of the species *Triticum aestivum.*

A "canola plant", as referred to herein, should be understood to include plants of the genus *Brassica,* particularly *B. napus, B. rapa, B. campestris, B. oleracea*, *B. montana,* and hybrids thereof. In some embodiments, the term "canola" should be understood to include one or more of rape, rapeseed, oilseed rape, Argentine canola, and colza. In some embodiments, the canola plant may be a cultivated species of canola. In some embodiments, the canola plant may be a species canola of including, for example, *B. napus* subsp. *oleifera, B. napus* subsp. *napus, B. napus* subsp. *napus f. annua*, *B. napus* subsp. *napus f. napus*, *Brassica campestris* subsp. *napus,* or *Brassica rapa* subsp. *oleifera*. In some embodiments, the term "canola" refers to canola of the species *Brassica napus* L and subspecies thereof.

As also set out above, the present invention contemplates bacterial inoculants for the control of a fungal disease in a plant. In some embodiments, the fungal disease is a root disease of a monocot or dicot plant. In some embodiments, the present invention contemplates bacterial inoculants for the control of a fungal root disease in a wheat plant or a canola plant.

In some embodiments, "control" of a fungal root disease in a plant may be understood as enhancement of one or more growth parameters in an inoculated plant relative to an uninoculated plant of the same taxon in the presence of the fungal root disease. In some embodiments, the plant is a wheat plant or a canola plant.

In some embodiments, enhancement of a growth parameter will include an increase in the measured value of the growth parameter. For example, an increase in one or more of:

a length and/or mass of a shoot; a length and/or mass of a root; a number and/or mass of seed;

a concentration and/or amount of a nutrient; or a germination rate.

In some embodiments, an "increase" in a growth parameter may include, for example, a 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 2--fold, 5--fold, 10--fold, 20--fold, 50--fold, 100--fold increase in the growth parameter in an inoculated plant relative to a plant of the same taxon that has not been inoculated. In some embodiments, the plant is grown in the presence of a fungal root disease. In some embodiments, the plant is grown in water limited conditions. In some embodiments, the plant is a wheat plant or a canola plant.

In some embodiments, however, "enhancement" of the growth parameter may include a decrease in the measured value of the growth parameter. For example, a decrease in the concentration and/or amount of a pathogen, disease symptom and/or toxin in the plant, and/or a decrease in the time of germination of a wheat or canola plant seed, may be considered "enhancement" of such growth parameters.

In some embodiments, a "decrease" in a growth parameter may include, for example, a 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100% decrease in the growth parameter in an inoculated plant relative to a plant of the same taxon that has not been inoculated. In some embodiments, the plant is grown in the presence of a fungal root disease. In some embodiments, the plant is grown in water limited conditions. In some embodiments, the plant is a wheat plant or a canola plant.

In some embodiments, enhancement of a growth parameter may comprise enhancement within a particular time period. For example, in some embodiments, enhancement of the growth parameter may comprise enhancement over a time period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90 or 100 days.

As set out above, the present invention contemplates a bacterial inoculant for controlling a fungal root disease on a wheat or canola plant. A "fungal root disease" as referred to herein should be understood as any disease of a plant which infects or damages the roots of the plant and which is caused by a fungus or fungal--like pathogen. A "fungal--like" pathogen should be understood to specifically include

Oomycete pathogens such as pathogens of the genus *Pythium*. In some embodiments, the fungal root disease is a disease of a wheat or canola plant.

In some embodiments, the fungal root disease is caused by a pathogen of the genus *Rhizoctonia*. In some embodiments, the pathogen is of the species *Rhizoctonia solani*. In some embodiments, the pathogen is *Rhizoctonia solani* AG8. In some embodiments, the pathogen is of the species *Rhizoctonia oryzae*.

In some embodiments, the bacterial inoculant of the first aspect of the invention includes a microorganism of the genus *Paenibacillus* that is able to at least control a fungal root disease caused by a pathogen of the genus *Rhizoctonia.* In some embodiments, the microorganism of the genus *Paenibacillus* is able to at least control a pathogen of the genus *Rhizoctonia* on or in a wheat or canola plant.

In some embodiments bacterial inoculant of the genus *Paenibacillus* includes a microorganism having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 3. In some embodiments the microorganism comprises a 16S rRNA gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 3.

In some embodiments bacterial inoculant of the genus *Paenibacillus* includes a microorganism having an atpD gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 7. In some embodiments the microorganism comprises an atpD gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 7.

In some embodiments bacterial inoculant of the genus *Paenibacillus* includes a microorganism having a recA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 8. In some embodiments the microorganism comprises a

recA gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 8.

In some embodiments bacterial inoculant of the genus *Paenibacillus* includes a microorganism having a trpB gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 9. In some embodiments the microorganism comprises a trpB gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 9.

In some embodiments bacterial inoculant of the genus *Paenibacillus* includes a microorganism having a gyrB gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 10. In some embodiments the microorganism comprises a gyrB gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 10.

In some embodiments bacterial inoculant of the genus *Paenibacillus* includes a microorganism having a recA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 11. In some embodiments the microorganism comprises a recA gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 11.

In some embodiments bacterial inoculant of the genus *Paenibacillus* includes a microorganism having an atpD gene nucleotide sequence which is at least 98%

identical to SEQ ID NO: 12. In some embodiments the microorganism comprises an atpD gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 12.

In some embodiments bacterial inoculant of the genus *Paenibacillus* includes a microorganism having a trpB gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 13. In some embodiments the microorganism comprises a trpB gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 13.

In some embodiments bacterial inoculant of the genus *Paenibacillus* includes a microorganism having a GyrB gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 14. In some embodiments the microorganism comprises a gyrB gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 14.

When comparing nucleic acid sequences to calculate a percentage identity (in relation to any of the SEQ ID NOS herein, the compared nucleic acid sequences should be compared over a comparison window of, for example, at least 100 nucleotide residues, at least 300 nucleotide residues, at least 600 nucleotide residues, at least 1000 nucleotide residues, at least 1100 nucleotide residues, at least 1200 nucleotide residues, at least 1300 nucleotide residues or at least 1400 nucleotide residues. In some embodiments, the comparison window may comprise the region in each of the compared nucleotide sequences between and including the binding sites of the 27f primer (SEQ ID NO: 1) and the 1465r primer (SEQ ID NO: 2) on the compared nucleotide sequences. The comparison window may comprise

additions or deletions (i.e., gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms such as the BLAST family of programs as, for example, disclosed by Altschul et al. (Nucl. Acids Res. 25: 3389--3402, 1997). A detailed discussion of sequence analysis can be found in Unit 19. 3 of Ausubel et al. (Current Protocols in Molecular Biology, John Wiley & Sons Inc., Chapter 15,1998).

A number of particularly useful actinobacterial microorganisms of the present invention have been deposited with the National Measurement Institute ('NMI'), 1/153 Bertie Street, Port Melbourne, Victoria, 3207, Australia, in accordance with the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

Accordingly, in some embodiments, the bacterial inoculant includes microorganism *Paenibacillus* sp. 10.6D as deposited on 9 March 2017 with the National Measurement Institute under NMI accession number V17/004922; or a mutant or derivative of said deposited microorganism that retains the ability to control a fungal root disease in a wheat or canola plant. In some embodiments, the mutant or derivative retains the ability to control a fungal root disease in a wheat or canola plant, where the root disease is caused by a pathogen of the genus *Rhizoctonia*.

A "mutant or derivative" of the subject deposited microorganisms referred to herein should be understood to encompass, for example, any spontaneous or induced mutant, conjugation progeny or genetically modified form of a deposited strains which retains the ability to enhance one or more growth parameters of a plant. In some embodiments, a mutant or derivative retains the ability to enhance one or more growth parameters of a wheat or canola plant in the presence of the fungal root disease or under water limited conditions. Mutagenisation techniques that may be used to generate derivatives or mutants include, for example, chemical mutagenesis (e.g., EMS mutagenesis), ionising radiation--induced mutagenesis (e.g., X--ray mutagenesis, γ--ray mutagenesis and UV mutagenesis), genetic insertion mutagenesis methods (e.g., transposon mutagenesis) and the like.

In some embodiments, the bacterial inoculant of the first aspect of the invention includes a microorganism of the genus *Streptomyces* that is able to at least control a fungal root disease caused by a pathogen of the genus *Rhizoctonia.* In some embodiments, the microorganism of the genus *Streptomyces* is able to at least control a pathogen of the genus *Rhizoctonia* on or in a wheat or canola plant.

In some embodiments bacterial inoculant of the genus *Streptomyces* includes a microorganism having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 5. In some embodiments the microorganism comprises a 16S rRNA gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 5.

In some embodiments, the bacterial inoculant includes microorganism *Streptomyces* sp. HCA1273 as deposited on 9 March 2017 with the National Measurement Institute under NMI accession number V17/004924; or a mutant or derivative of said deposited microorganism that retains the ability to control a fungal root disease in a wheat or canola plant. In some embodiments, the mutant or derivative retains the ability to control a fungal root disease in a wheat or canola plant, where the root disease is caused by a pathogen of the genus *Rhizoctonia*.

In some embodiments, the fungal root disease is caused by a pathogen of the genus *Pythium*. In some embodiments, the pathogen is of the species *Pythium irregulare*. In some embodiments, the pathogen is of the species *Pythium ultimum*.

In some embodiments, the bacterial inoculant of the first aspect of the invention includes a microorganism of the genus *Paenibacillus* that is able to at least control a fungal root disease caused by a pathogen of the genus *Pythium.* In some embodiments, the microorganism of the genus *Paenibacillus* is able to at least control a pathogen of the genus *Pythium* in or on a wheat or canola plant.

In some embodiments bacterial inoculant of the genus *Paenibacillus* includes a

microorganism having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 4. In some embodiments the microorganism comprises a 16S rRNA gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 4.

In some embodiments, the bacterial inoculant includes microorganism *Paenibacillus* sp. 9.4E as deposited on 9 March 2017 with the National Measurement Institute under NMI accession number V17/004921; or a mutant or derivative of said deposited microorganism that retains the ability to control a fungal root disease in a wheat or canola plant. In some embodiments, the mutant or derivative retains the ability to control a fungal root disease in a wheat or canola plant, where the root disease is caused by a pathogen of the genus *Pythium*.

In some embodiments, the bacterial inoculant of the first aspect of the invention includes a microorganism of the genus *Streptomyces* that is able to at least control a fungal root disease caused by a pathogen of the genus *Pythium* on a wheat or canola plant. In some embodiments, the microorganism of the genus *Streptomyces* is able to at least control a pathogen of the genus *Pythium* on a wheat or canola plant.

In some embodiments bacterial inoculant of the genus *Streptomyces* includes a microorganism having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 6. In some embodiments the microorganism comprises a 16S rRNA gene nucleotide sequence which is at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to SEQ ID NO: 6.
In some embodiments, bacterial inoculants and methods disclosed herein include a microorganism having a gene, e.g., a 16S rRNA gene, having a nucleotide sequence at least 97%, 98%, 99% or 100% identical to the same gene nucleotide sequence found in one of the genomic sequences found in

Table 1. In some embodiments the microorganism comprises a gene having a nucleotide sequence of at least 97%, at least 97.1%, at least 97.2% at least 97.3%, at least 97.4%, at least 97.5%, at least 97.6%, at least 97.7%, at least 97.8%, at least 97.9%, at least 98%, at least 98.1%, at least 98.2% at least 98.3%, at least 98.4%, at least 98.5%, at least 98.6%, at least 98.7%, at least 98.8%, at least 98.9%, at least 99%, at least 99.1%, at least 99.2% at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% at least 99.9% or 100% sequence identity to the same gene nucleotide sequence found in one of the genomic sequences found in Table 1.

In some embodiments, the bacterial inoculant includes microorganism *Streptomyces* sp. BD141 as deposited on 9 March 2017 with the National Measurement Institute under NMI accession number V17/004923; or a mutant or derivative of said deposited microorganism that retains the ability to control a fungal root disease in a wheat or canola plant. In some embodiments, the mutant or derivative retains the ability to control a fungal root disease in a wheat or canola plant, where the root disease is caused by a pathogen of the genus *Pythium*.

In a second aspect, the present invention provides an inoculant composition comprising one or more bacterial inoculants as hereinbefore described.

In some embodiments, the inoculant composition further comprises a carrier or additive. The carrier or additives used will depend on the nature of the inoculant composition. For example, the inoculant composition may be in the form of a liquid composition, a solid composition (such as a powder, pellet or granular composition) a seed dressing or the like. In some embodiments, the inoculant composition comprises a seed dressing.

A range of useful carriers or additives would be readily apparent to those of skill in the art and may include, for example: one or more gums (including xanthan gum), clay or peat based carriers, one or more nutrients including carbon or nitrogen sources, one or more antifungal or antibacterial agents, one or more seed coating agents, one or more wetting agents and the like.

The inoculant compositions of the present invention may be adapted to be applied

to a plant, for example a wheat or canola plant, in any suitable way. For example, the inoculant composition could be adapted to be applied as a seed coating, applied as a solid or liquid composition to the foliage or roots of a plant, or applied as a solid or liquid composition to soil before, during or after sowing of a plant, for example a wheat or canola plant.

In a third aspect, the present invention provides a method for controlling a fungal root disease on a plant, the method comprising inoculating a plant with a bacterial inoculant or inoculant composition as hereinbefore described.

In some embodiments, the plant is a wheat plant or a canola plant.

In some embodiments, the root disease is caused by a pathogen of the genus *Rhizoctonia*.

In some embodiments, the root disease is caused by a pathogen of the genus *Pythium*.

In some embodiments, the bacterial inoculant or inoculant composition are inoculated onto a seed. In some embodiments, the bacterial inoculant or inoculant composition are inoculated onto a wheat seed or canola seed.

In a fourth aspect, the present invention provides a method for improving growth of a plant under water limited conditions, the method comprising inoculating a plant with a bacterial inoculant or inoculant composition as hereinbefore described.

In some embodiments, the method provides for improving growth of a monocot or dicot plant under water limited conditions. In some embodiments, the monocot is a cereal plant. In some embodiments the cereal plant is member of the plant family *Poaceae* or *Gramineae*, for example: wheat, rice, corn, barley, millet, sorghum, oat, rye, or related grain producing plant. In some embodiments, the dicot is a member of the plant family *Fabaceae* or *Leguminosae,* for example: soybeans, peas, beans, lentils, peanuts, alfalfa, clover, or related plants. In some embodiments, the dicot is a member of the plant family *Brassicaceae* or *Cruciferae,* for example: canola, rapeseed, cabbage, cauliflower, kale, radish, mustard, turnip,

or related plants.

Water limited conditions, include but are not limited to, drought conditions and dryland (non-irrigated) environments. In some embodiments, water limited conditions are growth conditions where the amount of water available to the plants is less than the amount necessary to support optimal plant growth. In some embodiments, the water limited condition comprises less than 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, less than 100% of the amount necessary to support optimal plant growth. In some embodiments, the amount of water necessary to support optimal plant growth is measured in average or above average yield. In some embodiments, the water limited conditions are the amount of water that result in a reduction in average yield of un-inoculated plants by at least 5%, at least 10%, between 5-15%, about 15%, at least 20%, about 20%, between 20-25%, or at least 25%. In some embodiments, the water limited conditions are a non-irrigated field. In some embodiments, the water limited condition comprises a 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50% reduction in rainfall relative to the 1 year, 2, 3, 4, 5 year, 6, 7, 8, 9, 10 year historical average rainfall for the geography. In some embodiments, the water limited conditions are controlled by human endeavor in greenhouse or laboratory assays. A non-limiting example of a laboratory assay conducted in water limited conditions is growth of a plant in an aqueous solution comprising polyethylene glycol (PEG), for example 7.5% PEG 6000.

In a fifth aspect, the present invention provides a bacterial inoculant as described herein with respect to any of the examples.

In a sixth aspect, the present invention provides an inoculant composition as described herein with respect to any of the examples.

In a seventh aspect, the present invention provides a method for controlling a fungal root disease on a wheat or canola plant as described herein with respect to any of the examples.

In an eighth aspect, the present invention provides a method for a method for improving growth of a wheat or canola plant under water limited as described herein with respect to any of the examples.

The present invention is further described with reference to the following non-limiting examples:

EXAMPLE 1 – BACTERIAL INOCULANTS FOR CONTROL OF FUNGAL ROOT DISEASE IN WHEAT OR CANOLA

A number of microbial strains were screened in a series of in planta bioassays, characterised and assessed in field trials using naturally occurring pathogen inoculum. Four strains were identified as being of interest, as shown in Table 1.

Table 1 - Strains identified as beneficial inoculants in grain cropping systems

Strain Identifier	Alternative name	Full Genome Sequence (SEQ ID NO:)	Genus	Function--crop
10.6D	P10	SEQ ID NO: 15	*Paenibacillus*	Rhizoctonia control--wheat or canola
HCA1273	S12	SEQ ID NO: 16	*Streptomyces*	Rhizoctonia control--wheat or canola
9.4E	P9	SEQ ID NO: 17	*Paenibacillus*	Pythium control--wheat
BD141	S14	SEQ ID NO: 18	*Streptomyces*	Pythium control--wheat

EXAMPLE 2 – IDENTIFICATION

DNA of each strain was extracted. Two sections of 16S rRNA were amplified by PCR using primers 27f (agagtttgat cctggctcag, SEQ ID NO: 1) and 1492r (tacggytacc ttgttacgac tt, SEQ ID NO: 2). PCR products were sequenced by Sanger sequencing, two replicate extractions and forward and reverse directions of PCR fragments were sequenced. Sequences were identified using Ezbiocloud (www.ezbiocloud.net). Sequences were aligned with 20 closest matches using ClustalW in Mega7 and phylogeny inferred using Maximum Likelihood Tree and Nearest Neighbour Joining Trees. Results are shown in Tables 2 and 3.

Table 2 -- Identification of 10.6D, 9.4E, HCA1273, BD141

Strain	Closest matches with type strains	Pairwise similarity %
10.6D	*Paenibacillus peoriae DSM8320^T*	99.63
	Paenibacillus kribbensis AM49^T	98.90
9.4E	*Paenibacillus peoriae DSM8320^T*	98.99
	Paenibacillus kribbensis AM49^T	98.52
HCA1273	*Streptomyces prasinosporus NRRLB12431^T*	98.66
	Streptomyces scopiformis NBRC14215^T	98.36
BD141	*Streptomyces cyaneofuscatus NRRLB2570^T*	99.86
	Streptomyces griseus subsp. griseus KCTC9080	99.86

Table 3 -- 16S rRNA sequences of 10.6D, 94.E, HCA1273, BD141, 5' to 3' orientation

Strain	16S rRNA sequence
10.6D	GGATGGGCCTGCGGCGCATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGA
	CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC
	CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCT
	GACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGC
	CAGGGAAGAACGTCTTGTAGAGTAACTGCTACAAGAGTGACGGTACCTGAGAAG
	AAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCG
	TTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCTCTTTAAGTCTGGTGT
	TTAATCCCGAGGCTCAACTTCGGGTCGCACTGGAAACTGGGGAGCTTGAGTGCAG
	AAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGG
	AACACCAGTGGCGAAGGCGACTCTCTGGGCTGTAACTGACGCTGAGGCGCGAAA
	GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGA
	ATGCTAGGTGTTAGGGGTTTCGATACCCTTGGTGCCGAAGTTAACACATTAAGCAT
	TCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGACC
	CGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA
	GGTCTTGACATCCCTCTGACCGGTCTAGAGATAGACCTTTCCTTCGGGACAGAGG
	AGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTC
	CCGCAACGAGCGCAACCCTTATGCTTAGTTGCCAGCAGGTCAAGCTGGGCACTCT
	AAGCAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA
	TGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCGGTACAACGGGAAGC
	GAAATCGCGAGGTGGAGCCAATCCTAGAAAAGCCGGTCTCAGTTCGGATTGTAG

Strain	16S rRNA sequence
	GCTGCAACTCGCCTACATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCC
	GCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTT
	ACAACACCCGAAGTCGGTGG
	(SEQ ID NO: 3)
9.4E	GGATGGGCCTGCGGCGCATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGA
	CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC
	CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCT
	GACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGC
	CAGGGAAGAACGTCTTGTAGAGTAACTGCTACAAGAGTGACGGTACCTGAGAAG
	AAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCG
	TTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCTCTTTAAGTCTGGTGT
	TTAATCCCGAGGCTCAACTTCGGGTCGCACTGGAAACTGGGGAGCTTGAGTGCAG
	AAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGG
	AACACCAGTGGCGAAGGCGACTCTCTGGGCTGTAACTGACGCTGAGGCGCGAAA
	GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGA
	ATGCTAGGTGTTAGGGGTTTCGATACCCTTGGTGCCGAAGTTAACACATTAAGCAT
	TCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGACC
	CGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA
	GGTCTTGACATCCCTCTGACCGGTCTAGAGATAGACCTTTCCTTCGGGACAGAGG
	AGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTC
	CCGCAACGAGCGCAACCCTTATGCTTAGTTGCCAGCAGGTCAAGCTGGGCACTCT
	AAGCAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA
	TGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCGGTACAACGGGAAGC
	GAAATCGCGAGGTGGAGCCAATCCTAGAAAAGCCGGTCTCAGTTCGGATTGTAG
	GCTGCAACTCGCCTACATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCC
	GCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTT
	ACAACACCCGAAGTCGGTGG
	(SEQ ID NO: 4)
HCA1273	GATGAAGCCCTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAA
	TCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATATGAG

Strain	16S rRNA sequence
	TCTCCACCGCATGGTGGGGGCTGTAAAGCTCCGGCGGTGCAGGATGAGCCCGCG
	GCCTATCAGCTTGTTGGTGAGGTAACGGCTCACCAAGGCGACGACGGGTAGCCG
	GCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACG
	GGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGC
	CGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGC
	GAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGC
	GGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGT
	AGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGT
	CGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGG
	TGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCC
	GATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTG
	GTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCCACGTCGTCC
	GTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTA
	AAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAAT
	TCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTGGA
	GACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG
	TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTG
	CCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCG
	GAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACA
	CGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGTGAGGTGGAGCGAATCTC
	AAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGA
	GTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGT
	ACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAAC
	CCC
	(SEQ ID NO: 5)
BD141	AGTCGAACGATGAAGCCTTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACAC
	GTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCG
	GATAACACTCTGTCCCGCATGGGACGGGGTTAAAAGCTCCGGCGGTGAAGGATG
	AGCCCGCGGCCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACG
	GGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAG
	ACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATG

Strain	16S rRNA sequence
	CAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGG
	GAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCA
	GCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAA
	GAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGG
	GTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCGGAATTCCTG
	GTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGA
	TCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATT
	AGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTTGGCGACATTC
	CACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCC
	GCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCAGCGGAGCATG
	TGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATATACCGGAA
	AGCATCAGAGATGGTGCCCCCCTTGTGGTCGGTATACAGGTGGTGCATGGCTGTC
	GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGT
	TCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCACAGGAGACTGCCGGG
	GTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTG
	GGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATGCCGCGAGGCGG
	AGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCA
	TGAAGTCGGAGTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCC
	CGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCG
	GTGGCCCAACCCCTTGTGGGAGGGAG

(SEQ ID NO: 6)

Additional gene sequences for strain 9.4E are depicted in Table 22, below.

Table 22—additional gene sequences, strain 9.4E

Gene	Strain 9.4E Sequence

atpD ATACGGGAGCTTGATGCGAGTGGATCAACCGCCGGGTAAATACCCATTTCGGAAA
TTTTACGTTCCAGATTCGTTGTAGCATCCAAATGGGCAAACGTCGTAGCAGGAGCC
GGGTCAGTGTAGTCATCCGCAGGCACATAGATCGCCTGAATGGAAGTAACAGAAC
CTTTTTTAGTAGAAGTAATCCGTTCTTGCAATTGACCCATCTCAGTAGCCAGCGTA
GGCTGGTAACCTACTGCTGAAGGCATACGTCCCAACAGAGCCGAAACCTCAGAAC
CCGCTTGAGTAAAGCGGAAAATGTTATCAATAAAGAGCAACACGTCACGGCCTTC
TTGGTCACGGAAGTATTCCGCCATCGTCAGACCTGTGAGAGCTACACGCAAACGT
GCACCTGGAGGCTCGTTCATTTGTCCAAACACCATCGCTGTTTTGTTGATAACGCC
GGAATCTCTCATTTCATGATACAAGTCATTTCCTTCACGTGTGCGTTCACCTACACC
CGCAAATACAGAAATACCACCATGCTCCTGAGCGATATTGTTAATCAATTCTTGAA
TGGTTACGGTTTTACCTACACCAGCACCACCAAACAATCCGACTTTACCACCTTTGG
CATAAGGAGCTAGCAAGTCGATAACTTTAATACCTGTTTCGAGCATCTC
(SEQ ID NO: 7)

recA CCCTGACCAAGGCGCTCACCTTCGTAGGAATACCAGGCTCCGCTCTTGTCGACAAT
GTCATGCTCCGTACCGATATCGATCAAGCTACCTTCTTTGGAAATACCCTCACCGTA
CATAATATCCACCTCAGCCTGACGGAAAGGAGGGGCAACTTTGTTCTTCACGACTT
TAATACGTGTGCGGTTACCCACAATGTCGTTACCCATTTTCAAACTTTCAATACGAC
GAACATCCAAACGTACCGTAGAGTAAAACTTCAAGGCACGTCCACCTGGTGTTGT
TTCAGGGTTACCGAACATAACACCTACTTTTTCACGTAGCTGGTTAATAAAAATAG
CAATGGTTTTCGACTTGTTAATGGCTCCAGAAAGCTTACGCAATGCCTGGGACATC
AAACGTGCTTGAAGACCAACGTGGGAATCTCCCATTTCGCCTTCAATCTCTGCCTT
GGGCACAAGTGCCGCTACGGAGTCAACAACTACAATGTCTACTGCTCCACTACGT
ACAAGGGCTTCGGCAATCTCAAGCGCCTGCTCTCCTGTATCTGGTTGCGATAGTAA
CAACTCATCAATATTGACACCCAGCTTGCTTGCATACGACGGATCAAGCGCATGCT
CGGCGTCGATAAAGGCGGCTTGTCCGCCTGTTTTTTGCACCTCTGCGATAGCGTGA
AGAGCTACTGTCGTTTTACCGGATGATTCCGGTCCGTATATTTCAATAACCCGGCC
(SEQ ID NO: 8)

trpB GTCATTGCTGAAACAGGCGCAGGACAGCATGGTGTCGCGACAGCGACTGTGGCT
GCGTTGCTCGGATTGGAATGCAAGGTGTTTATGGGCGAAGAGGATACTGTGCGC
CAGCAGCTGAACGTCTTTCGGATGCAGCTTTTGGGTGCAGAGGTCATTCCGGTGA
CATCAGGTACACGTACACTTAAGGATGCCGGGAATGAAGCTTTGCGTTACTGGGT
CAGCCATGTCCATGATACGTTCTATATTTTGGGTTCAGCTGTCGGCCCGCACCCGT
ATCCGATGATGGTGCGGGACTTCCAACGTGTGATTGGTGATGAAACACGTCGCCA
GATCCTAGAGAAGGAAGGCAGACTTCCGGATGTCATCGTGGCAGCGATCGGTGG
CGGAAGCAATGCCATCGGCATGTTTTATCCTTTTATTGAGGATCAGGGTGTCGCAT
TGATTGGCGTGGAGGCCGCTGGAAAAGGTGTCGAAACGGAATTCCATGCAGCTA
CGATGACCAAGGGAACACAAGGGGTCTTCCAAGGCTCTATGAGTTATCTGCTTCA
GGATGAGTATGGACAAGTGCAACCTGCGCATTCCATCTCGGCTGGATTAGATTAT
CCAGGTGTTGGACCGGAGCATTCATACCTGAAAGA
(SEQ ID NO: 9)

gyrB AGCTTTGTCTTGGTCTGACCCTCAAACTGTGGTTCTGGAATTTTGACGGAGATAAT
CGCCGTCAATCCTTCACGCACATCGTCACCGGTCAAGTTGGCGTTGTTGTCCTTAA
TCAAGCCATTTTTACGTGCATAATCGTTAATAATCCGGGTTAATGCACTCTTGAAAC
CTGATTCGTGAGTTCCGCCCTCATGGGTGTTGATGTTGTTGGCAAAAGAATAAATA
TTCTCGGTATAGCTGTCGTTATATTGCAATGCCACTTCGACTTGAATCATATCACGC
GAGCCTTCGACATAAATCGGCTGTTCATGCAGCGCTTCTCTTTTTTGATTCAAAAAT
TGCACATATTCACTGATTCCGCCCTCGTAGTGAAATGTATCGCTGGCGCCCGTCCG
TTCATCAGTCAAGCTGATTGCAATACCTTTGTTCAGGAAAGCCAACTCACGAATCC
GTGTCTGGAGCGTATCATAGTCATATACGGTCGTTTCTGTAAAGATTTGATCGTCA
GGATAAAAAGTCGTTTGGGTACCCGTCTCGTCTGTGTCACCGATGACTCTGACATC
ATACTGCGGAGCACCACGATGATATTCCTGCTCATACAGATGTCCGTCCCGTTTAA
CATGCACGATCATTTTGCTGGAGAGGGCATTTACTACGGATACACCAACCCCGTGC
AGACCACCGGATACCTTGTACCCTCCGCCTCCAAATTTACCACCTGCGTGAAGCAC
GGTCATAACGACTTCCAGCGCAGATTTTTTCATTTTGGCGTGTTCACTTACTGGAAT
ACCGCGACCGTTATCTGTAACGGTAATGCTATTGTCTTCGTGAACGACAACTTGAA
TGCTGTCACAGTAACCCGCCAGCGCTTCGTCAATGCTGTTGTCCACAACTTCCCAG
ACCAAATGATGGAGACCTTTGGCGCTCGTGGAGCCAATATACATCCCGGGACGTT
TCCGAACCGCTTCCAGGCCCTCAAGGACCTGAATCTCGCCCGCATCATAAGACGGT
TGATTCATAGACATGCCTTTCACCTACTTCTATAGATTCTATGGTTAAGCATTGGCA
ACAAACTGGTTTGCCCGCTTTTTGAGTGTTGTTGAGGAGATGGGGGAATAATACA
CCGTATTCTGAGTCACTACGATGGACTTGGCTTCCTCTTCGCCAATCATCTCGACAT
GCTTCTGCTGTTGGGCGTGGTTCACGTATTGCTTGGAGATTTTAGAGGATTTTTCA
ATCGAGATATCGAAAATAGCCACAAGCTCTGAAGAGCGGATGATTTTTTCTCCACC
CAGATGAAT
(SEQ ID NO: 10)

Additional gene sequences for strain 10.6D are depicted in Table 23, below.

Table 23-- additional gene sequences, strain 10.6D

Gene	Strain 10.6D sequence

recA GCATTCTCCCGTCCCTGACCAAGGCGCTCACCTTCGTAGGAATACCAGGCTCCGCT
CTTGTCGACAATGTCATGCTCCGTACCGATATCGATCAAGCTACCTTCTTTGGAAAT
ACCCTCACCGTACATAATATCCACCTCAGCCTGACGGAAAGGAGGGGCAACTTTG
TTCTTCACGACTTTAATACGTGTGCGGTTACCCACAATGTCGTTACCCATTTTCAAA
CTTTCAATACGACGAACATCCAAACGTACCGTAGAGTAAAACTTCAAGGCACGTCC
ACCTGGTGTTGTTTCAGGGTTACCGAACATAACACCTACTTTTTCACGTAGCTGGTT
AATAAAAATAGCAATGGTTTTCGACTTATTAATGGCTCCAGAAAGCTTACGCAATG
CCTGAGACATCAAACGTGCTTGAAGACCAACGTGGGAATCTCCCATTTCGCCTTCA
ATCTCTGCCTTGGGCACAAGTGCCGCTACGGAGTCAACAACTACAATGTCTACTGC
TCCACTACGTACAAGGGCTTCGGCAATCTCAAGCGCCTGCTCTCCTGTATCTGGTT
GCGATAGTAACAACTCATCAATATTGACACCCAGCTTGCTTGCATACGACGGATCA
AGCGCATGCTCGGCGTCGATAAAGGCGGCTTGTCCGCCTGTTTTTTGCACCTCTGC
GATAGCGTGAAGAGCTACTGTCGTTTTACCGGATGATTCCGGTCCGTATATTTCAA
TAACCCGGCC
(SEQ ID NO: 11)

atpD ATACGGGAGCTTGATGCGAGTGGATCAACCGCCGGGTAAATACCCATTTCGGAAA
TTTTACGTTCCAGATTCGTTGTAGCATCCAAATGGGCAAACGTCGTAGCAGGAGCC
GGGTCAGTGTAGTCATCCGCAGGCACATAGATCGCCTGAATGGAAGTAACAGAAC
CTTTTTTAGTAGAAGTAATCCGTTCTTGCAATTGACCCATCTCAGTAGCCAGCGTA
GGCTGGTAACCTACTGCTGAAGGCATACGTCCCAACAGGGCTGAAACCTCAGAAC
CCGCTTGAGTAAAGCGGAAAATGTTATCAATAAAGAGCAACACGTCACGGCCTTC
TTGGTCACGGAAGTATTCCGCCATCGTCAGACCTGTGAGAGCTACACGCAAACGT
GCACCCGGAGGCTCGTTCATTTGTCCGAACACCATCGCTGTTTTGTTGATAACGCC
GGAATCTCTCATTTCATGATACAAGTCATTTCCTTCACGTGTGCGTTCACCTACACC
CGCAAATACAGAAATACCACCATGCTCCTGAGCGATATTGTTAATCAATTCTTGAA
TGGTTACGGTTTTACCTACACCAGCACCACCAAACAATCCGACTTTACCACCTTTGG
CATAAGGAGCTAGCAAGTCGATAACTTTAATACCTGTTTCGAGCATCTCTGCTTGA
GTTGTCAGCTCATCGAAAGAAGGAGCTTGACGGTGAATCGG
(SEQ ID NO: 12)

trpB GTCATTGCTGAAACAGGCGCAGGACAGCATGGTGTCGCGACAGCGACTGTGGCT
GCGTTGCTCGGATTGGAATGCAAGGTGTTTATGGGCGAAGAGGATACTGTGCGC
CAGCAGCTGAACGTCTTTCGGATGCAGCTTTTGGGTGCAGAGGTCATTCCGGTGA
CATCAGGTACACGTACACTTAAGGATGCAGGGAATGAAGCTTTGCGTTACTGGGT
CAGCCATGTCCATGATACGTTCTATATTTTGGGTTCAGCTGTCGGCCCACATCCGT
ATCCGATGATGGTGCGGGACTTCCAACGCGTGATTGGTGATGAAACACGTCGCCA
GATCCTAGAGAAGGAAGGCAGACTTCCGGATGTCATCGTGGCAGCGATCGGTGG
CGGAAGCAATGCCATCGGAATGTTTTATCCTTTTATTGAGGATCAGGGTGTCGCAT
TGATTGGCGTGGAGGCCGCTGGAAAAGGTGTCGAAACGGAATTCCATGCAGCTA
CGATGACCAAGGGAACACAAGGGGTCTTCCAAGGCTCTATGAGTTATCTGCTTCA
GGATGAGTACGGACAAGTGCAACCTGCGCATTCCATCTCGGCTGGATTAGATTAT
CCAGGTGTTGGACCGGAGCATTCATACCTGAAAGA
(SEQ ID NO: 13)

GyrB AAGTTGGCGTTGTTGTCCTTAATCAAGCCATTTTTACGTGCATAATCGTTAATAATC
CGGGTTAATGCACTCTTGAAACCTGATTCGTGAGTTCCGCCCTCATGGGTGTTGAT
GTTGTTGGCAAAAGAATAAATATTCTCGGTATAGCTGTCGTTATATTGCAATGCCA
CTTCGACTTGAATCATATCACGCGAGCCTTCGACATAAATCGGCTGTTCATGCAGC
GCTTCTCTTTTTTGATTCAAAAATTGCACATATTCACTGATTCCGCCCTCGTAGTGA
AATGTATCGCTGGCGCCCGTCCGTTCATCAGTCAAGCTGATTGCAATACCTTTGTT
CAGGAAAGCCAACTCACGAATCCGTGTCTGGAGCGTATCATAGTCATATACGGTC
GTTTCTGTAAAGATTTGATCGTCAGGATAAAAAGTCGTTTGGGTACCCGTCTCGTC
TGTGTCACCGATGACTCTGACATCATACTGCGGAGCACCACGATGATATTCCTGCT
CATACAGATGTCCGTCCCGTTTAACATGCACGATCATTTTGCTGGAGAGGGCATTT
ACTACGGATACACCAACCCCGTGCAGACCACCGGATACCTTGTACCCTCCGCCTCC
AAATTTACCACCTGCGTGAAGCACGGTCATAACGACTTCCAGCGCAGATTTTTTCA
TTTTGGCGTGTTCACTTACTGGAATACCGCGACCGTTATCTGTAACGGTAATGCTA
TTGTCTTCGTGAACGACAACTTGAATGCTGTCACAGTAACCCGCCAGCGCTTCGTC
AATGCTGTTGTCCACAACTTCCCAGACCAAATGATGGAGACCTTTGGCGCTCGTGG
AGCCAATATACATCCCGGGACGTTTCCGAACCGCTTCCAGGCCCTCAAGGACCTG
AATCTCGCCCGCATCATAAGACGGTTGATTCATAGACATGCCTTTCACCTACTTCTA
TAGATTCTATGGTTAAGCATTGGCAACAAACTGGTTTGCCCGCTTTTTGAGTGTTG
TTGAGGAGATGGGGGAATAATACACCGTATTCTGAGTCACTACGATGGACTTGGC
TTCCTCTTCGCCAATCATCTCGACATGCTTCTGCTGTTGGGCGTGGTTCACGTATTG
CTTGGAGATTTTAGAGGATTTTTCAATCGAGATATCGAAAATAGCCACAAGCTCTG
AAGAGCGGATGATTTTTTCTCCACCCAGATG
(SEQ ID NO: 14)

Phylogenetic trees were generated as described above, using 16S, atpD, gyrB, recA, and trpB genes.

FIG. 1 depicts a phylogenetic tree visualizing the phylogenetic relationship between strain 9.4E, strain 10.6D, and other *Paenibacillus* strains.

FIG. 2 depicts a phylogenetic tree visualizing the phylogenetic relationship between strain HCA1273, and other *Streptomyces* strains.

FIG. 3 depicts a phylogenetic tree visualizing the phylogenetic relationship between strain BD141, and other *Streptomyces* strains.

A comparison of the 16S, atpD, gyrB, recA, and trpB genes between P9 and P10 strains confirm that the isolates are distinct isolates but are closely related. For example, while sequences for 16S and gyrB are completely similar, recA

sequences have 2 SNPs between P9 and P10, trpB sequences have 6 SNPs between P9 and P10, and atpD sequences have 4 SNPs between P9 and P10.

EXAMPLE 3 – SCREENING FOR *RHIZOCTONIA* CONTROL

All bioassays were conducted in a controlled envi9ronment room at 15°C, 12 hr day/night cycle. Wheat cv. Yitpi was used for all assays. For each assay there were three control treatments, (1) no--pathogen control (2) pathogen only control and (3) positive control of current best biocontrol strain, either *Trichoderma* strain TB or *Streptomyces* strain EN16. Bioassays were conducted in soils collected from fields with continuing *Rhizoctonia* problems. Soils were from Netherton SA (grey siliceous sand) or Waikerie SA (Red calcareous sand).

Primary tube *Rhizoctonia* bioassay

This assay consisted of 50 ml tube with 60 g Netherton soil at 8% moisture content with two *Rhizoctonia solani* infested millet seeds added and incubated 2 weeks at 15ºC. Two pregerminated wheat seeds were planted and microbial inoculum added as suspension (150 ul) directly onto the seeds and incubated for 2 wks. Plants were assessed by shoot height and number of roots reaching the bottom of the tube. Two replicates were used per treatment. Results are shown in Table 4.

Table 4 -- Primary Rhizoctonia assay results of 10.6D and HCA1273, n=2.

Strain	Treatment	Mean shoot height (cm)	Mean number of roots to bottom of tube
10.6D	Control	12.75	9
	Disease control	7.5	0
	10.6D	9.5	0.5
HCA1273	Control	8.75	4
	Disease control	6.75	0
	HCA1273	8.75	0.5

Secondary *Rhizoctonia* pot bioassay

To confirm efficacy, strains were assessed in a pot bioassay containing 300 g Waikerie soil at 8% moisture. Six *Rhizoctonia solani* infested millet seeds were added to the soil and incubated 2 weeks at 15°C. For seed inoculation, microbes were harvested from agar plates, diluted to absorbance of 0.5 at 550nm in 3 ml dilute sticker solution (0.005% Na Alginate, 0.03% xantham gum) and 1.5 g wheat seed added and soaked for 1 hr. The microbial suspension was drained and 7 seeds planted and later thinned to 5 after germination. Inoculum concentration was determined by dilution plate counts. Plants were grown for 4 wks. Plants were assessed for root disease as Percentage of Roots Infected (%RI) and on a Root Disease Score (RDS) on a 0-5 scale (0=no disease, 5=max disease). The length of seminal and nodal roots were measured and dry weights of roots and shoots obtained after drying at 60°C for 4 days. There were 4 replicates in a randomised complete block design. Data was analysed as ANOVA, RCBD. Results are shown in Tables 5 and 6.

Table 5 -- Results of secondary assay for strain 10.6D. Inoculum suspension 10.6D, 2.0 x 10^6 cfu/ml.

Treatment	Shoot DW mg/pot	Root DW mg/pot	%RI	Total Root Length (cm)	Length seminal roots (cm)	Length nodal roots (cm)	Root Disease Score (0-5)
No pathogen control	381*	364*	18*	59*	45*	13.5	0.8*
Pathogen only control	316	211	82	35	28	7.1	3.1
TB	311	270	73	40	29	11.8	2.1*
10.6D	313	301*	72	43	30	13.1	2.4*
Fprob	0.006	0.020	<0.001	0.003	<0.001	0.351	<0.001
LSD(0.05)	39.8	87.6	12.6	9.9	5.6	ns	0.70

**=significantly different from pathogen only control at P=0.05*

Table 6 -- Results of secondary assay for strain HCA1273. Inoculum suspension 6.0 x 10^8 cfu/ml. Treatment

	Shoot DW mg/pot	Root DW mg/pot	%RI	Total Root Length (cm)	Length seminal roots (cm)	Length nodal roots (cm)	Root Disease Score (0-5)
No pathogen control	165	155*	64*	41*	39*	2.2	0.2*
Pathogen only control	169	107	86	24	23	1.7	2.6
EN16	166	117	83	28	25	2.4	2.2
HCA 1273	161	136	80	30	28	3.0	1.5*
Fprob	0.846	0.022	0.001	0.004	0.002	0.163	0.003
LSD(0.05)	ns	30.8	9.3	8.1	7.0	ns	0.98

**=significantly different from pathogen only control at P=0.05*

Tertiary Rhizoctonia pot bioassay

A tertiary assay was conducted on selected strains based on results of the secondary assay. The tertiary pot bioassay was conducted the same as for the

secondary *Rhizoctonia* pot assay except that microbes were inoculated at 3 rates to indicate the most appropriate inoculation level. Seed cfu levels were measured on seeds at the highest inoculation rate by extracting cells from 5 seeds in 1 ml phosphate buffered saline (PBS) after shaking for 30 minutes, serially diluting the suspension and plating onto agar. Seed cfu levels at the lower rates were estimated from the highest rate. Results are shown in Tables 7 and 8.

Table 7 - Results of tertiary assay for strain 10.6D.

Treatment	Inoculum (cfu/seed)	Shoot DW mg/pot	Root DW mg/pot	%RI	Total Root Length (cm)	Length seminal roots (cm)	Length nodal roots (cm)	Root Disease Score (0-5)
No pathogen control		330*	268*	11*	61*	45*	15.8	0.6*
Pathogen only control		135	63	100	11	6	4.8	4.2
TB	2.0×10^4	191*	80	100	13	9	3.7	4.1
10.6D	3.3×10^4	210*	100	93	22*	16*	6.1	3.1*
10.6D	6.7×10^4	146	72	93	14	9	5.2	4.6
10.6D	1.3×10^5	157	93	90	22*	14*	8.2	3.4*
Fprob		0.006	0.020	<0.001	0.003	<0.001	0.351	<0.001
LSD(0.05)		39.8	87.6	12.6	9.9	5.6	ns	0.70

**=significantly different from pathogen only control at P=0.05*

Table 8 - Results of tertiary assay for strain HCA1273.

Treatment	Inoculum (cfu/seed)	Shoot DW mg/pot	Root DW mg/pot	%RI	Total Root Length (cm)	Length seminal roots (cm)	Length nodal roots (cm)	Root Disease Score (0-5)
No pathogen control		258.2	155.5	69	35.1	16	1.7	0.5
Pathogen only control		173.5	82.5	89	17.7	30.15	5	3.2

Treatment	Inoculum (cfu/seed)	Shoot DW mg/pot	Root DW mg/pot	%RI	Total Root Length (cm)	Length seminal roots (cm)	Length nodal roots (cm)	Root Disease Score (0-5)
EN16	1.5×10^6	205.2	108.2	85	19	17.75	1.25	2.3
HCA1273	5.9×10^4	198.2	114.2	78.5	28.8	23.9	4.9	1.85
HCA1273	3.0×10^5	192	99	84	23.4	21.88	1.5	2.58
HCA1273	3.0×10^6	185	106.2	82	21.3	19.75	1.55	2.35
Fprob		0.144	0.453	0.387	0.660	0.564	0.750	0.200
LSD(0.05)		ns	ns	ns	ns	ns	ns	ns

**=significantly different from pathogen only control at P=0.05*

Rhizoctonia field trials: Microplots

All Rhizoctonia field trials were carried out in fields used for commercial cereal production in South Australia with a continuing *Rhizoctonia* problem, with natural levels of *Rhizoctonia solani* AG8 DNA >100 pg/g soil (as measured by SARDI Root Disease Testing Service).

Selected strains were first assessed in the field in 1m long single row microplots in 2012 and 2013. Wheat cv. Grenade seeds were coated with microbes as a concentrated suspension in a sticker solution (0.3% xanthan gum, 0.05% Na alginate). Seeds were hand planted at 4 cm spacing using a seeding template. Microplot trials were a split plot design, with each treated row paired with an untreated row in a randomised complete block design, 6 replicates. *Rhizoctonia* root rot is a patchy disease, so a split--plot design with paired treated and untreated rows was used to measure disease in the same disease space. Plants (10) were harvested at 8 wks and assessed for root disease score (0-5 scale) caused by *Rhizoctonia* on seminal and nodal roots and for dry weights of roots and shoots. Each strain was assessed at 2 sites. In 2013, Chemical seed treatments (Vibrance, Syngenta; EverGol Prime, Bayer) and *Streptomyces* strain EN16 were included for comparison. Results are shown in Tables 9 and 10.

Table 9 - Combined results for 2012 microplot trials at Karoonda (SA, Mallee) and Port Julia (SA, Yorke Peninsula) at 8 wks, n=12. Seed inoculation, 10.6D $4.3x10^5$ cfu/seed.

Shoot DW (mg/plant)	Treated	272
	Un-treated	244
	% change from untreated[1]	11
Root DW (mg/plant)	Treated	55
	Un-treated	52
	% change from untreated	6
Nodal Root Disease Score (0-5)	Treated	1.5**
	Un-treated	2
	% change from untreated	-23
Seminal Root Disease Score (0-5)	Treated	0.9*
	Un-treated	1.2
	% change from untreated	-23

**significantly different from un--treated control at P=0.05*

***significantly different from un--treated control at P=0.01*

[1]*percent change of treated rows from untreated rows*

Table 10 - Combined results for 2013 microplot trials at Wynarka (SA, Mallee) and Lamaroo (SA, Mallee) at 8 wks, n=12. Seed inoculation, HCA1273 2.4x10^5 cfu/seed; EN16 2.4x10^4 cfu/seed.

		HCA1273	EN16	EverGol Prime	Vibrance
Shoot DW (mg/plant)	Treated	880*	876	829	854
	Un-treated	725	793	793	747
	% change from untreated[1]	21	10	5	14
Root DW (mg/plant)	Treated	120*	123	126	124*
	Un-treated	101	105	114	105
	% change from untreated	18	17	11	18
Nodal Root Disease Score (0-5)	Treated	2.7*	2.3*	2.1	2.2
	Un-treated	3.1	2.8	2.3	2.4
	% change from untreated	-23	-18	-8	-6
Nodal Root Disease Score (0-5)	Treated	1.4*	1.2*	1.3	1.3
	Un-treated	1.9	1.8	1.4	1.3
	% change from untreated	-27	-34	-8	0

**significantly different from un--treated control at P=0.05*

[1]percent change of treated rows from untreated rows

<u>*Rhizoctonia* field trials: 20m 3+3 row plots</u>

Strains selected from microplot trials and characterisation were assessed as seed coatings in larger field trials in 2013 and 2014 with 20m plots, six replicates. Three rows of each plot were treated and three rows untreated in a split--plot randomised complete block design to allow comparison in the same disease space due to the patchy nature of *Rhizoctonia* root rot. Wheat cv. Grenade seeds were coated with microbes as a concentrated suspension in a sticker solution (0.3% xanthan gum, 0.05% Na alginate). Seeds were planted with a plot scale seeder and herbicide and fertilisers applied as per local best practice. Plants (21) from each split--plot were assessed at 8 wks (2013) or 11 wks (2014) and assessed for root disease score (0--5 scale) caused by *Rhizoctonia* on seminal and nodal roots and for dry weights of roots and shoots. Seeds were harvested at

the end of season with a plot scale header. In 2014, *Streptomyces* strain EN16 and an in--furrow chemical treatment, Uniform (Syngenta) were included as controls. Results are shown in Table 11.

Table 11 - Results for 2013 and 2014 3+3 row 20 m plots field trials at Wynarka (SA, Mallee) and Lameroo (SA, Mallee) in 2013 and at Lameroo (SA, Mallee) in 2014 at 8 wks and yield, n=6. Percent change (% change) = [(Microbe treated/untreated)x100]-100

		2013 Lameroo 10.6D (9.6×10^4)	2013 Wynarka 10.6D (1.1×10^5)	2014 Lameroo			Uniform
				10.6D 4.8×10^4	HCA1273 4.2×10^4	EN16 4.5×10^5	
Shoot DW (mg/plant)	Treated	455	242	719	847	765	730
	Un-Treated	431	213	751	746	652	747
	% change from untreated[1]	6	13	-4	14	17	-2
Root DW (mg/plant)	Treated	52	39	94	105	100	104
	Un-Treated	51	35	102	97	92	96
	% change from untreated	2	12	-8	8	9	9
Nodal Root Disease Score (0-5)	Treated	2.1	2.4	2.1*	2.5*	1.9*	1.7*
	Un-Treated	1.8	2.7	2.6	3.2	2.4	2.2
	% change from untreated	14	-9	-20	-23	-21	-21
Seminal Root Disease Score (0-5)	Treated	1.0*	1.2	2.1*	1.9*	2	1.4*
	Un-Treated	1.4	1.3	2.5	2.8	2.4	2.2
	% change from untreated	-27	-12	-17	-32	-15	-35
Yield (t/ha)	Treated	2.86	2.23	2.68*	2.6	2.63	2.56*
	Un-Treated	2.75	2.14	2.57	2.52	2.56	2.49
	% change from untreated	4.1	3.8	4.2	2.8	2.5	3

**significantly different from un--treated control at P=0.05*

[1] percent change of treated rows from untreated rows

EXAMPLE 4 – SCREENING FOR PYTHIUM CONTROL ON WHEAT

Primary Pythium tube bioassay

The primary Pythium tube assay was set up as for the *Rhizoctonia* tube assay with 60g washed sand at 11% moisture with 3g/L Miracle Gro soluble fertiliser. *Pythium irregulare* strain 89 was added as one 11 mm agar plug, with no pre--incubation prior to seeding with two pre--germinated wheat cv. Yitpi seeds. For seed inoculation, microbes were harvested from agar plates, diluted to absorbance of 0.8 at 550nm in

a dilute sticker solution (0.005% Na alginate, 0.03% xanthan gum) and 150 ul added directly to seeds. Plants were assessed by shoot height and number of roots reaching the bottom of the tube. Two replicates per treatment. Results are shown in Table 12.

Table 12 -- Primary Pythium assay results of 9.4E, and BD141, n=2

Assay-strain	Treatment	Mean shoot height (cm)	Mean number of roots to bottom of tube
9.4E	Control	20.5	6
	Disease control	13.5	1
	9.4E	17.5	3
BD141	Control	8.0	6
	Disease control	5.3	0.5
	BD141	7.75	2

Secondary *Pythium* pot bioassay

Washed sand (200 g/pot) at 11% moisture with 1.5g/L Miracle Grow fertiliser was used. Pathogen was added as 3x8mm agar plugs *of Pythium irregulare* strain 89. Wheat cv. Yitpi seeds (2.2) were inoculated with a microbial suspension diluted to absorbance of 0.8 at 550nm in 3 ml dilute sticker solution (0.005% Na Alginate, 0.03% xanthan gum) and soaked for 1 hr prior to planting. Microbial suspension was drained and 7 seeds sown and thinned to 5 after 14 days. Plants were grown for 4 weeks and assessed for root disease on a 0--5 scale and for dry weight of shoots and roots. Results are shown in Tables 13 and 14.

Table 13 -- Results of secondary assay for strain 9.4E.

Treatment	Inoculum (cfu/seed)	No. plants emerged (7 d)	Shoot DW mg/plant	Root DW mg/plant	Root Disease Score (0-5)
No pathogen control		5.8*	35*	26*	0.4*
Pathogen only control		3.8	27	21	2.7
EN27	3.5×10^6	3.5	25	19	2.0
9.4E	1.7×10^5	3.3	24	19	1.4*
Fprob		0.004	0.002	0.003	<0.001
LSD(0.05)		2.01	4.9	3.9	0.95

**significantly different from pathogen only control at P=0.05*

Table 14 -- Results of secondary assay for strain BD141.

Treatment	Inoculum (cfu/seed)	No. plants emerged (6 d)	Shoot DW mg/plant	Root DW mg/plant	Root Disease Score (0-5)
No pathogen control		6.8*	35	26	0.2*
Pathogen only control		2.3	32	17	2.3
EN27	3.5×10^6	3.5	29	19	1.8
BD141	5.3×10^5	4.0*	35	22	1.3*
Fprob		0.020	0.581	0.795	0.006
LSD(0.05)		1.43	ns	12.6	0.74

**significantly different from pathogen only control at P=0.05*

Tertiary Pythium tub bioassay

An emergence assay in 100 ml tubs with 140 g Waikerie sand at 13% moisture was used to assess pre and post emergence damping off control. Twenty wheat cv. Yitpi seeds were planted, and covered with 1 g *Pythium irregulare* strain 89 sand--polenta inoculum. Plants were grown for 14d at 15°C, 12 hr day/night cycle, 4 replicates in randomised complete block design. EN27 was included as a positive control. The number of plants emerged was counted at 7, 11, and 14 days after planting. A chemical control (Dividend, difanconazole and metalaxyl) for *Pythium* was included in the assay with 9.4E assay. Results are shown in Tables 15 and 16.

Table 15 -- Results of secondary assay for strain 9.4E.

Treatment	Inoculum (cfu/seed)	No. plants Emerged (7 d)	No. plants Emerged (11 d)
No pathogen control		12.8*	18.8*
Pathogen only control		2.5	7.3
Dividend		8.0*	14.5*
EN27	2.3×10^6	4.8	10.5*
9.4E	8.9×10^4	4.5	8.0
9.4E	1.8×10^5	4.0	7.0
9.4E	3.5×10^5	7.5*	12.3*
Fprob		<0.001	<0.001
LSD(0.05)		3.1	3.1

**significantly different from pathogen only control at P=0.05*

Table 16 -- Results of secondary assay for strain BD141.

Treatment	Inoculum (cfu/seed)	No. plants Emerged (7 d)	No. plants Emerged (11 d)	No. plants Emerged (14 d)
No pathogen control		6.5	18.3*	18.3*
Pathogen only control		0.5	8.5	10.0
EN27	7.1×10^5	3.0	13.3*	14.5*
BD141	1.9×10^5	3.0	12.8*	13.5*
BD141	3.8×10^5	2.3	11.8*	12.3
BD141	7.7×10^5	2.5	13.0*	14.5*
Fprob		0.096	<0.001	<0.001
LSD(0.05)		ns	3.2	3.0

**significantly different from pathogen only control at P=0.05*

Pythium field trials

All *Pythium* field trials were carried out in fields used for commercial cereal production in South Australia with a continuing Pythium problem, with natural levels of Pythium group F DNA >100 pg/g soil (as measured by SARDI Root Disease Testing Service).

Seed coated microbes were assessed at two Pythium infested sited in 2015 and 2016 (Table 17). Plant establishment was increased in both years with microbial

inoculation compared to controls, but this was only significantly different at the Conmurra sites. Significant yield responses of 4.6 to 6.3 % increase were evident in 2015 at Turretfield with non-significant increases at Conmurra. Yield responses in 2016 were probably masked by nearly double the rainfall compared to 2015. Significant reductions in root disease were evident at Turretfield in 2015 and Conmurra in 2016.

Table 17. Results from control of Pythium root rot on wheat field trials. Data is the mean plants/m at 3-4 wks, shoot dry weight (DW) mg per plant and seminal root disease score (DS, 0-5) at eight weeks and final grain yield, six replicates. Control treatment contains no fungicide or microbial inoculation. Microbes were applied as seed coatings. % increase in yield is relative to untreated Control.

Site Year Treatment	Establishment plants/m	Shoot DW mg/plant	Seminal root DS (0-5)	Yield t/ha	% increase in yield
Turretfield 2015					
Control	36	892	3.1	2.51	0
Apron	38	872	2.7	2.49	-1.00
Paenibacillus 9.4E	37	960	2.4*	2.63*	4.63
Streptomyces BD141	39	870	2.8	2.67*	6.27
Streptomyces EN27	37	953	2.6*	2.51	0.06
Conmurra 2015					
Control	24	655	2.6	4.13	0
Apron	27	628	2.6	4.24	2.48
Paenibacillus 9.4E	30*	603	2.6	4.31	4.39
Streptomyces BD141	29*	579	2.6	4.01	-2.95
Streptomyces EN27	28*	638	2.7	3.97	-3.89
Turretfield 2016					
Control	37	2161	2.9	4.93	
Apron	40	2476	2.8	5.08	3.0
Paenibacillus 9.4E	39	2081	2.7	4.94	0.1
Streptomyces BD141	37	2359	2.6	5.17	4.9
Paenibacillus 10.6D	39	2181	2.9	4.86	-1.4
Streptomyces HCA1273	39	2200	2.6	4.75	-3.7
Streptomyces EN27	40	2220	2.7	4.77	-3.3
Conmurra 2016					
Control	22	688	1.8	4.16	0
Apron	29*	582	1.5	4.17	0.4
Paenibacillus 9.4E	32*	641	1.3*	4.07	-2.1
Streptomyces BD141	30*	616	1.5*	4.12	-0.9
Paenibacillus 10.6D	30*	641	1.4*	4.04	-2.8
Streptomyces HCA1273	31*	647	1.3*	4.20	1.1
Streptomyces EN27	22*	688	1.8	4.16	0

**Treatment significantly different from untreated control at P=0.05 by Fisher's LSD*

EXAMPLE 5 – MICROBIAL INOCULUM SURVIVAL ON WHEAT SEEDS

Microbial survival on seeds was assessed on 20 g seed lots (wheat cv. Yitpi) after inoculation and at 1, 2 and 7 days. Concentrated microbial suspensions were made in a sticker solution (0.3% xanthan gum, 0.05% Na alginate) at various concentrations depending on results of tertiary assays and 626 ul added to each 20 g seed lot and mixed until even coverage of seeds. To assess seed colony forming units (cfu), 5 seeds were placed in 1.5 ml tubes, 1 ml phosphate buffered saline added, vortexed 15 sec and shaken for 15 min on orbital shaker at maximum speed. The suspension was sampled, serially diluted, plated onto agar media and cfu/seed calculated. There were two replicates for strains 10.6D and HCA1273, three replicates for 9.4E and BD141 at each time point. Percent survival was calculated based on initial population at t=0. Results are shown in Table 18.

Table 18 -- Log10 cfu/seed and percent survival on seeds at t=0, 1, 2, and 7 days after inoculation. Values mean of two (10.6D, HCA1273) or three (9.4E, BD141) replicates.

Strain	*t=0 Log10 (cfu/seed)*	*t=1d Log10 (cfu/seed)*	*t=2d Log10 (cfu/seed)*	*t=7d Log10 (cfu/seed)*	*% survival t=1d*	*% survival t=2d*	*% survival t=7d*
10.6D	*4.50*	*4.56*	*4.30*	*4.32*	*116*	*63*	*65*
HCA1273	*3.54*	*3.64*	*3.42*	*3.71*	*124*	*75*	*148*
9.4E	*5.46*	*5.66*	*5.61*	*5.70*	*161*	*142*	*173*
BD141	*5.75*	*5.80*	*5.90*	*5.91*	*112*	*141*	*145*

EXAMPLE 6 – IN VITRO INHIBITION OF FUNGAL PATHOGENS

Strains identified for *Rhizoctonia* control were assessed for in vitro inhibition of four fungal pathogens, *R. solani* AG8 strain W19, *Pythium irregulare* strain 89 isolated from lucerne roots, *Gaeumannomyces graminis* var. *tritici* (Ggt) strain C3 isolated from wheat roots and *Fusarium pseudograminearum* strain B4a isolated from wheat crowns. Fungi were grown on PDA/4 for between 2 and 7 d depending on strain prior to use. Test fungal pathogens were added to the centre of 9 cm agar plates as 8 mm agar and test strains added as 2x 20 µl spots (10^7 cfu/ml) on opposite sides of the plate 30 mm from the centre. Inhibition zones were recorded at

2d *for P. irregulare*, 4 d for R. solani and 7 d for Ggt and *F. pseudograminearum*. There were three replicate plates for each pathogen-test strain combination in a randomised complete block design. Results are shown in Table 19.

Table 19 -- In vitro inhibition of root pathogens *Rhizoctonia solani* AG8, *Fusarium pseudograminearum*, *Pythium irregulare* and *Gaeumannomyces graminis tritici* (Ggt) by strains isolated for Rhizoctonia control. Response of fungal pathogen to test strains are given as: -- no sign of inhibition; + hyphal avoidance but no clear zone of inhibition; ++ inhibition zone 1-2 mm; +++ inhibition zone >3mm.

Strain	*Rhizoctonia solani*	*Fusarium pseudograminearum*	*Pythium irregulare*	*Ggt*
10.6D	+++	+++	+++	++
9.4E	+++	+++	+++	++
BD141	+	-	-	++
HCA1273	+++	+++	-	+++

EXAMPLE 7 -- SEED DRESSING COMPATIBILITY

Compatibility of strains with a subset of common seed dressings was assessed by adding the seed dressings at 8 times the recommended application rate per seed to a 5 mm antibiotic disk and applying the disk to a lawn of bacteria (10^5 cfu/plate) on an agar plate. Zones of inhibition were measured after 3 days. Results are shown in Table 20. Where a zone of inhibition of greater than 4 mm was observed, this was indicative of an inhibitory effect on the growth of the inoculant.

Table 20 - Inhibition of strains identified for *Rhizoctonia* control by common seed dressings. Data shows size of inhibition zones in mm surrounding the seed dressing soaked disk in the bacterial lawn, n=3.

Seed dressing (manufacturer)	*10.6D*	*HCA 1273*	*EN16*	*EN27*
Vitaflo C (Chemtura)	*0*	*0*	*0*	*0*
Rancona dimension (Chemtura)	*2-8*	*2*	*7*	*12*
Proleaf T (Chemtura)	*0*	*0*	*0*	*0*
Rancona C (Chemtura)	*0-4*	*2*	*7*	*10*
Raxil T (Bayer)	*0-5*	*1*	*8*	*>10*
Lamardor FS400 (Bayer)	*1-4*	*4*	*6*	*12*
Jockey Stayer (Bayer)	*0-5*	*0*	*5*	*10*
Vibrance (Syngenta)	*0*	*0*	*3*	*4*
Dividend M (Syngenta)	*0*	*1*	*5*	*5*

EXAMPLE 8 - ENHANCED CANOLA GERMINATION AND GROWTH UNDER WATER LIMITED CONDITIONS.

Seed coated microbes were assessed in field trials in a low rainfall zones in Parilla (Murray Mallee) in Australia. *Paenibacillus* 9.4E and *Streptomyces* BD141 had increased establishment growth (plants per meter), and significantly increased the number of secondary roots per plant (Table 21).

Table 21. Results from field trials assessing microbial inoculants for increasing establishment, growth and yield of canola in the low rainfall zone. Data is the mean plants/m at 6 wks (Parilla), shoot dry weight (DW) g per plant and number of secondary roots at 13 wks and final grain yield, six replicates. Control treatment contains no fungicide or microbial inoculation. Microbes were applied as seed coatings.

Site Year Treatment	Establishment Plants/m	Shoot DW g/plant	Secondary roots per plant
Control	8	4.12	10.8
Paenibacillus 9.4E	10*	3.80	11.9
Streptomyces BD141	11*	5.06	14.6*

**Treatment significantly different from untreated control at P*=0.05 by Fisher's LSD

EXAMPLE 9 - CANOLA GROWTH UNDER PYTHIUM ROOT ROT STRESS

Strains were assessed as seed coatings on canola at three sites with Pythium infested soil. There was no impact on grain yield at the two Spalding sites in 2015 or 2016, however *Streptomyces* BD141 and *Paenibacillus* 9.4E significantly increased the percentage of roots with root hairs at the 2016 Spalding site (Table 22). *Paenibacillus* 9.4E significantly increased grain yield at Turretfield in 2016 by 11.4%.

Table 22. Results from control of Pythium root rot on canola field trials. Data presented is the mean plants/m at 3-4 wks, shoot dry weight (DW) mg per plant, root disease score (DS, 0-10), % of root system with root hairs at eight weeks and final grain yield, six replicates. Control treatment contains no fungicide or microbial inoculation. Microbes were applied as seed coatings. % increase in yield is relative to untreated Control.

Site Year Treatment	Establishment plants/m	Shoot DW mg/plant	Root DS (0-10)	% Root hairs	Yield t/ha	% increase in yield
Spalding 2015						
Control	14	1184	2.2	64	2.00	0.0
Apron	15	1310	2.1	66	1.94	-2.8
Paenibacillus 9.4E	15	1344	2.3	64	1.96	-1.8
Streptomyces BD141	15	1177	2.1	66	2.01	0.5
*Streptomyces*EN27	15	1158	2.1	68	1.97	-1.5
Spalding 2016						
Control	14	878	0.4	80	2.91	0
Apron	15	899	0.3	82	2.90	-0.5
Paenibacillus 9.4E	15	890	0.2	86*	2.83	-3.0
Streptomyces BD141	13	905	0.3	86*	2.86	-1.9
Streptomyces EN27	15	1061	0.2	87	2.85	-2.2
Turretfield 2016						
Control	15	1210	<0.1	41	2.37	0.0
Apron	16	1368	<0.1	45	2.47	4.2
Paenibacillus 9.4E	17	1162	<0.1	44	2.65*	11.4
Streptomyces BD141	18	1236	<0.1	40	2.48	4.4
Streptomyces EN27	18*	1275	<0.1	38	2.68*	12.8

**Treatment significantly different from untreated control at P=0.05 by Fisher's LSD*

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features,

compositions and compounds referred to, or indicated in this specification, individually or collectively, and any and all combinations of any two or more of the steps or features.

Also, it must be noted that, as used herein, the singular forms "a", "an" and "the" include plural aspects unless the context already dictates otherwise. Thus, for example, reference to "a microorganism" includes a single microorganism as well as two or more microorganisms; "a wheat plant" includes a single wheat plant as well as two or more wheat plants; and so forth.

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Reference is made to standard textbooks of molecular biology that contain methods for carrying out basic techniques encompassed by the present invention, including DNA restriction and ligation for the generation of the various genetic constructs described herein. See, for example, Maniatis et al, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory Press, New York, 1982) and Sambrook et al. (2000, supra).

Additional Sheet for Biological Material

Identification of deposits:

1) The Name and Address of depositary institution for the deposits are:

National Measurement Institute
1/153 Bertie Street
Port Melbourne
Victoria, Australia, 3207

Date of deposits	Accession Numbers	Identification Reference
9 March 2017	V17/004921	*Paenibacillus* sp. 9.4e
9 March 2017	V17/004922	*Paenibacillus* sp. 10.6D
9 March 2017	V17/004923	*Streptomyces* sp. BD141
9 March 2017	V17/004924	*Streptomyces* sp. HCA1273

2) **Depositor:**

All above mentioned depositions were made by:

Professor Chris Franco
Flinders University
Room 4.19
Level 4 Health Sciences Building
Registry Road, Bedford Park, SA
Australia

Copies of the above mentioned deposit receipts follow:

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO:
Professor Chris Franco
Flinders University, Room 4.19,
Level 4 Health Sciences Building,
Registry Road, Bedford Park SA,
Australia 5042

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY:
***Paenibacillus* sp. 9.4e**	**V17/004921**

II SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☒ a scientific description

☒ a proposed taxonomic designation

(Mark with a cross where applicable)

III RECEIPT AND ACCEPTANCE

This International Depository Authority accepts the microorganism identified under I above, which was received by it on **9th March 2017** (date of the original deposit) [1]

IV RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depository Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V INTERNATIONAL DEPOSITARY AUTHORITY

Name: NATIONAL MEASUREMENT INSTITUTE Address: 1/153 BERTIE STREET PORT MELBOURNE VICTORIA, AUSTRALIA, 3207 Phone: +61 3 9644 4888 Facsimile: +61 3 9644 4999	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s) **Dean Clarke** Date: **17th March 2017**

1 Where Rule 6.4(d) applies, such date is the date on which the status of International Depositary Authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
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INTERNATIONAL FORM

TO:
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INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY:
***Paenibacillus* sp. 10.6D**	**V17/004922**

II SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:
☒ a scientific description
☒ a proposed taxonomic designation
(Mark with a cross where applicable)

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I IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY:
***Streptomyces* sp. BD141**	**V17/004923**

II SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:
☒ a scientific description
☒ a proposed taxonomic designation
(Mark with a cross where applicable)

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I IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY:
***Streptomyces* sp. HCA1273**	**V17/004924**

II SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:
☒ a scientific description
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1 Where Rule 6.4(d) applies, such date is the date on which the status of International Depositary Authority was acquired.

What is claimed is:

1. A bacterial inoculant for controlling a fungal root disease on a wheat or canola plant.

2. A bacterial inoculant according to claim 1 wherein the fungal root disease is caused by a pathogen of the genus *Rhizoctonia*.

3. A bacterial inoculant according to claim 2 wherein the bacterial inoculant includes a microorganism of the genus *Paenibacillus.*

4. A bacterial inoculant according to claim 2 or 3 wherein the bacterial inoculant includes a microorganism having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 3.

5. A bacterial inoculant according to any one of claims 2, 3, or 4 wherein the bacterial inoculant includes microorganism *Paenibacillus* sp. 10.6D as deposited under NMI accession number V17/004922; or a mutant or derivative of said deposited microorganism that retains the ability to control a fungal root disease in a wheat or canola plant.

6. A bacterial inoculant according to claim 2 wherein the bacterial inoculant includes a microorganism of the genus *Streptomyces*.

7. A bacterial inoculant according to claim 2 or 6 wherein the bacterial inoculant includes a microorganism having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 5.

8. A bacterial inoculant according to any one of claims 2, 6 or 7 wherein the bacterial inoculant includes microorganism *Streptomyces* sp. HCA1273 as deposited under NMI accession number V17/004924; or a mutant or derivative of said deposited microorganism that retains the ability to control a fungal root disease in a wheat or canola plant.

9. A bacterial inoculant according to claim 1 wherein the fungal root disease is caused by a pathogen of the genus *Pythium*.

10. A bacterial inoculant according to claim 9 wherein the bacterial inoculant includes a microorganism of the genus *Paenibacillus*.

11. A bacterial inoculant according to claim 9 or 10 wherein the bacterial inoculant includes a microorganism having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 4.

12. A bacterial inoculant according to any one of claims 9, 10 or 11 wherein the bacterial inoculant includes microorganism *Paenibacillus* sp. 9.4E as deposited under NMI accession number V17/004921; or a mutant or derivative of said deposited microorganism that retains the ability to control a fungal root disease in a wheat or canola plant.

13. A bacterial inoculant according to claim 9 wherein the bacterial inoculant includes a microorganism of the genus *Streptomyces*.

14. A bacterial inoculant according to claim 9 or 13 wherein the bacterial inoculant includes a microorganism having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 6.

15. A bacterial inoculant according to any one of claims 9, 13 or 14 wherein the bacterial inoculant includes microorganism *Streptomyces* sp. BD141 as deposited under NMI accession number V17/004923; or a mutant or derivative of said deposited microorganism that retains the ability to control a fungal root disease in a wheat or canola plant.

16. An inoculant composition comprising a bacterial inoculant according to any one of claims 1 to 15.

17. An inoculant composition according to claim 16 wherein the inoculant composition comprises a seed dressing.

18. A method for controlling a fungal root disease on a wheat or canola plant, the method comprising inoculating a wheat or canola plant with a bacterial inoculant or inoculant composition according to any one of claims 1 to 17.

19. A method according to claim 18 wherein the fungal root disease is caused by a pathogen of the genus *Rhizoctonia*.

20. A method according to claim 18 wherein the fungal root disease is caused by a pathogen of the genus *Pythium*.

21. A method according to any one of claims 18, 19 or 20 wherein the bacterial inoculant or inoculant composition are inoculated onto a wheat or canola seed.

22. A bacterial inoculant according to claim 1 as herein before described with respect to any of the examples.

23. An inoculant composition according to claim 16 as hereinbefore described with respect to any of the examples.

24. A method according to claim 18 as herein before described with respect to any of the examples.

25. A bacterial inoculant for improving growth of a canola plant under water limited conditions, wherein the bacterial inoculant includes a microorganism of the genus *Paenibacillus* having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 4.

26. A bacterial inoculant for improving growth of a canola plant under water limited conditions, wherein the bacterial inoculant includes a microorganism of the genus *Paenibacillus* sp. 9.4E as deposited under NMI accession number V17/004921; or a mutant or derivative of said deposited microorganism that retains the ability to improve growth in a canola plant under water limited conditions.

27. A bacterial inoculant for improving growth of a canola plant under water limited conditions, wherein the bacterial inoculant includes a microorganism of the genus *Streptomyces* having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 6.

28. A bacterial inoculant for improving growth of a canola plant under water limited conditions, wherein the bacterial inoculant includes a microorganism of the genus *Streptomyces* sp. BD141 as deposited under NMI accession number V17/004923; or a mutant or derivative of said deposited microorganism that retains the ability to improve growth in a canola plant under water limited conditions.

29. An inoculant composition comprising a bacterial inoculant according to any one of claims 25 to 28.

30. An inoculant composition according to claim 29 wherein the inoculant composition comprises a seed dressing.

31. A method for improving growth of a canola plant under water limited conditions, the method comprising inoculating a canola plant with a bacterial inoculant or inoculant composition comprising a microorganism of the genus *Paenibacillus* having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 4.

32. A method for improving growth of a canola plant under water limited conditions, the method comprising inoculating a canola plant with a bacterial inoculant or inoculant composition comprising a microorganism of the genus

Paenibacillus sp. 9.4E as deposited under NMI accession number V17/004921; or a mutant or derivative of said deposited microorganism that retains the ability to improve growth in a canola plant under water limited conditions.

33. A method for improving growth of a canola plant under water limited conditions, the method comprising inoculating a canola plant with a bacterial inoculant or inoculant composition comprising a microorganism of the genus *Streptomyces* having a 16S rRNA gene nucleotide sequence which is at least 98% identical to SEQ ID NO: 6.

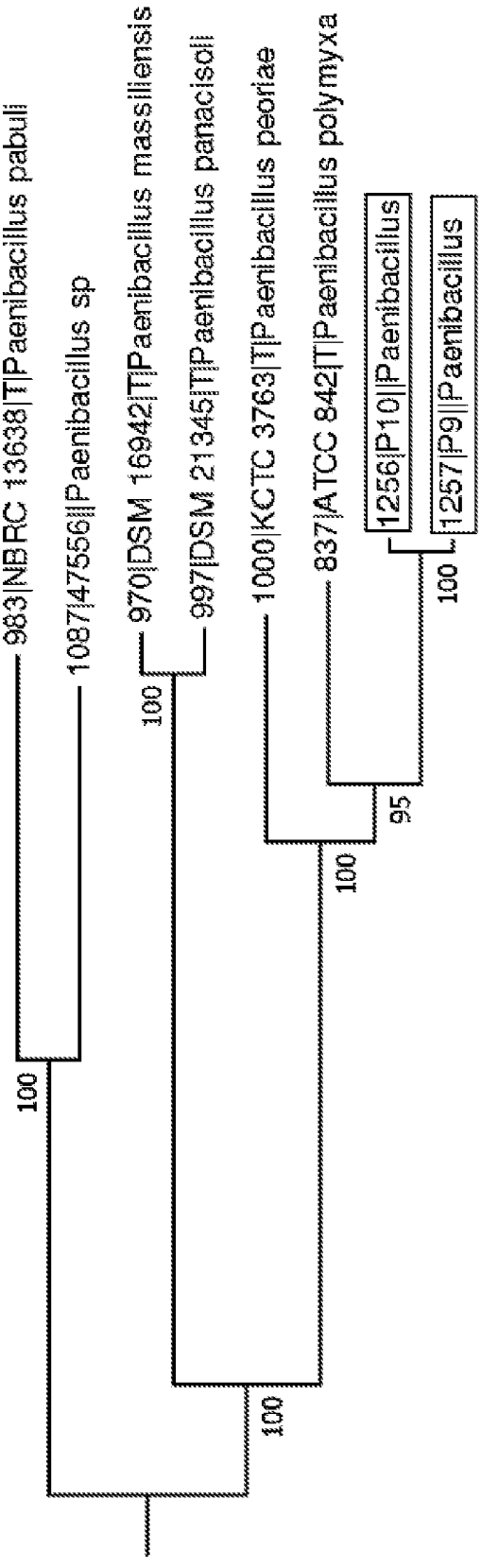
34. A method for improving growth of a canola plant under water limited conditions, the method comprising inoculating a canola plant with a bacterial inoculant or inoculant composition comprising a microorganism of the genus *Streptomyces* sp. BD141 as deposited under NMI accession number V17/004923; or a mutant or derivative of said deposited microorganism that retains the ability to improve growth in a canola plant under water limited conditions.

35. A method according to any one of claims 31 to 34 wherein the bacterial inoculant or inoculant composition are inoculated onto a canola seed.

36. A bacterial inoculant for improving growth of a canola plant under water limited conditions as hereinbefore described with respect to any of the examples.

37. An inoculant composition for improving growth of a canola plant under water limited conditions as hereinbefore described with respect to any of the examples.

38. A method for improving growth of a canola plant under water limited conditions as hereinbefore described with respect to any of the examples.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837|ATCC 842|T|Paenibacillus polymyxa
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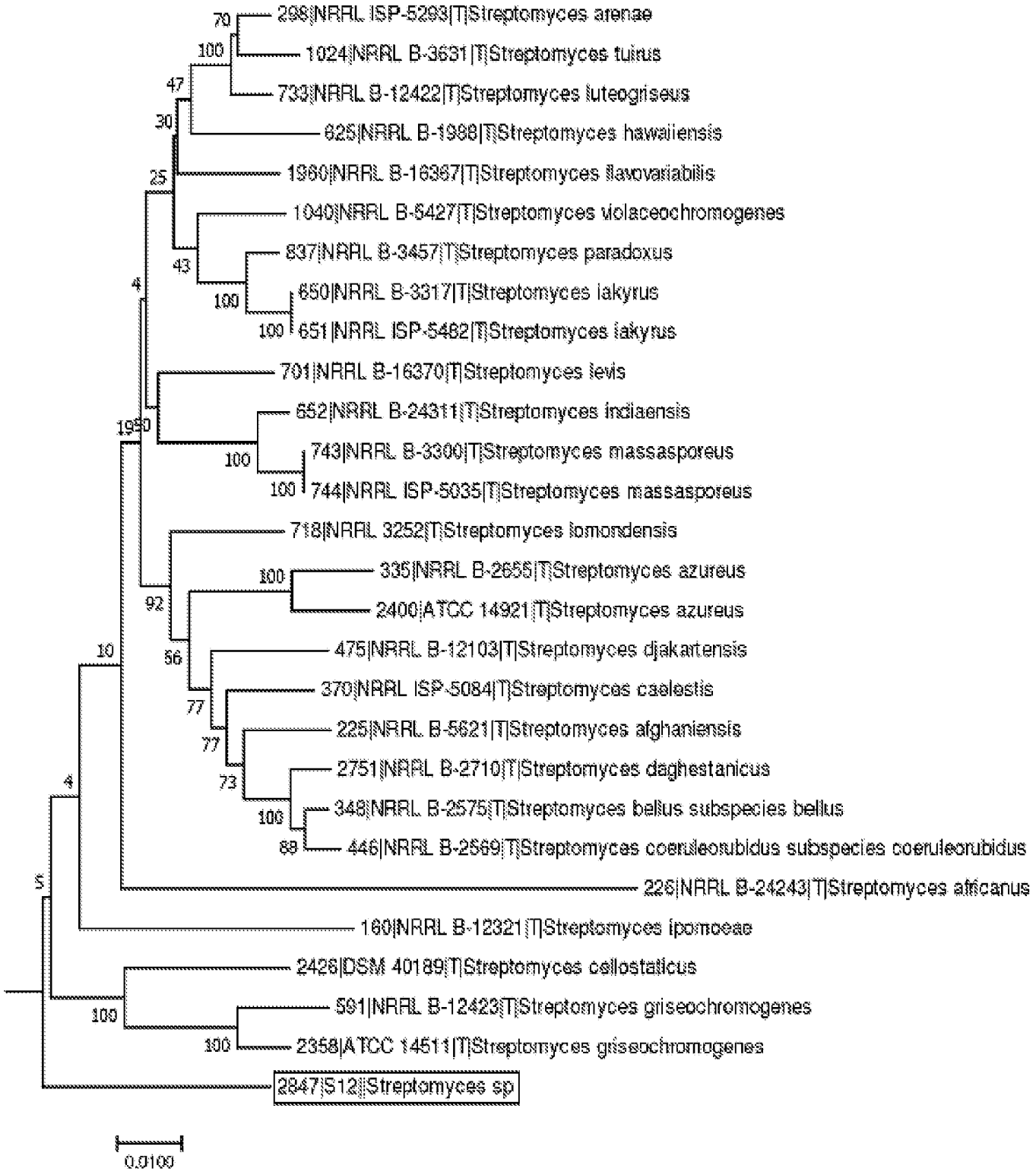
Figure 1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625|NRRL B-1998|T|Streptomyces hawaiiensis
1360|NRRL B-16367|T|Streptomyces flavovariabilis
1040|NRRL B-5427|T|Streptomyces violaceochromogenes
837|NRRL B-3457|T|Streptomyces paradoxus
650|NRRL B-3317|T|Streptomyces lakyrus
651|NRRL ISP-5462|T|Streptomyces lakyrus
701|NRRL B-16370|T|Streptomyces levis
652|NRRL B-24311|T|Streptomyces indiaensis
743|NRRL B-3300|T|Streptomyces massasporeus
744|NRRL ISP-5035|T|Streptomyces massasporeus
718|NRRL 3252|T|Streptomyces lomondensis
335|NRRL B-2655|T|Streptomyces azureus
2400|ATCC 14921|T|Streptomyces azureus
475|NRRL B-12103|T|Streptomyces djakartensis
370|NRRL ISP-5084|T|Streptomyces caelestis
225|NRRL B-5621|T|Streptomyces afghaniensis
2751|NRRL B-2710|T|Streptomyces daghestanicus
348|NRRL B-2575|T|Streptomyces bellus subspecies bellus
446|NRRL B-2569|T|Streptomyces coeruleorubidus subspecies coeruleorubidus
226|NRRL B-24243|T|Streptomyces africanus
160|NRRL B-12321|T|Streptomyces ipomoeae
2426|DSM 40189|T|Streptomyces cellostaticus
591|NRRL B-12423|T|Streptomyces griseochromogenes
2358|ATCC 14511|T|Streptomyces griseochromogenes
2847|S12|Streptomyces sp
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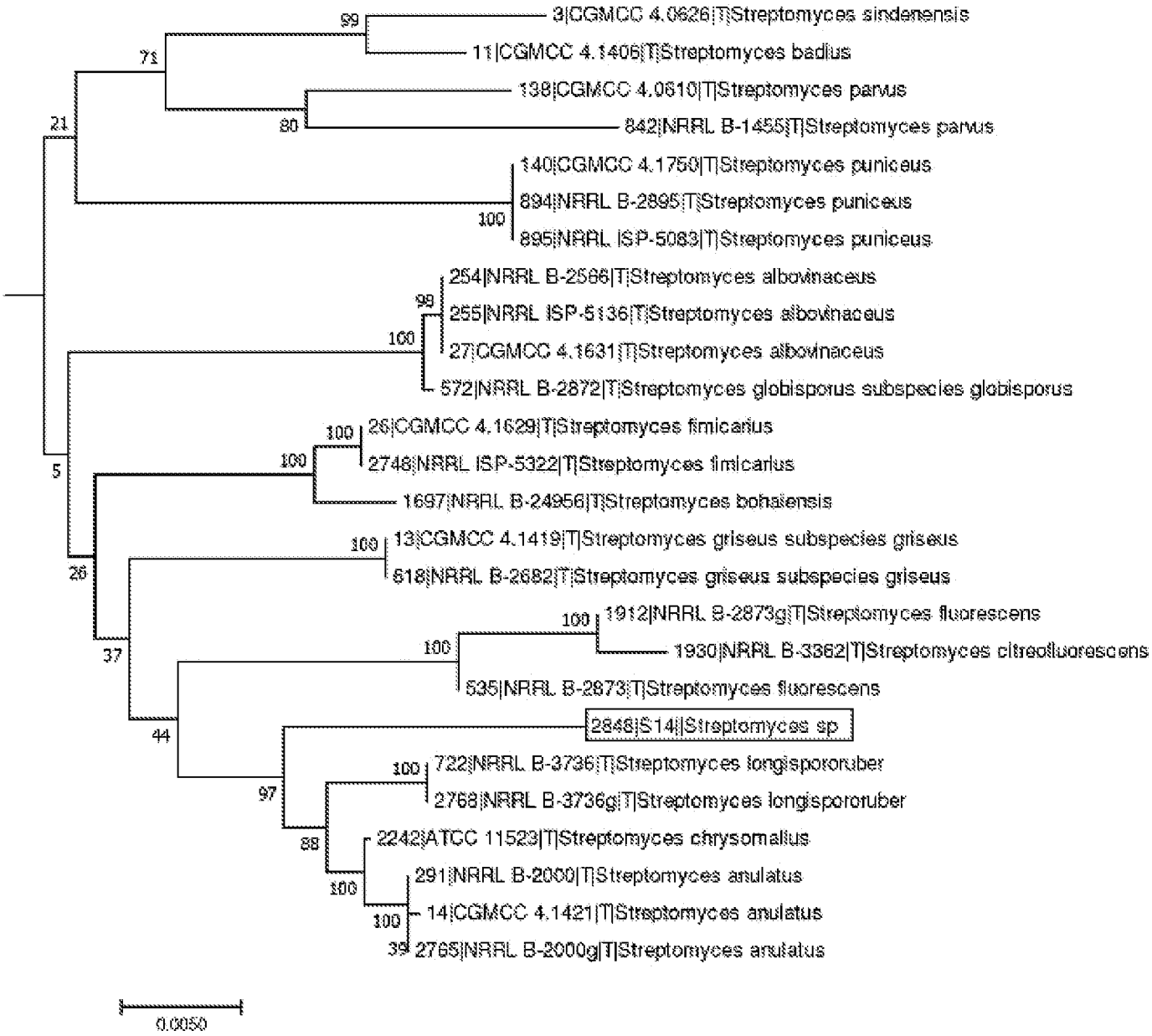
Figure 2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140|CGMCC 4.1750|T|Streptomyces puniceus
894|NRRL B-2895|T|Streptomyces puniceus
895|NRRL ISP-5083|T|Streptomyces puniceus
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27|CGMCC 4.1631|T|Streptomyces albovinaceus
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26|CGMCC 4.1629|T|Streptomyces fimicarius
2748|NRRL ISP-5322|T|Streptomyces fimicarius
1697|NRRL B-24956|T|Streptomyces bohaiensis
13|CGMCC 4.1419|T|Streptomyces griseus subspecies griseus
618|NRRL B-2682|T|Streptomyces griseus subspecies griseus
1912|NRRL B-2873g|T|Streptomyces fluorescens
1930|NRRL B-3362|T|Streptomyces citreofluorescens
535|NRRL B-2873|T|Streptomyces fluorescens
2848|S14|Streptomyces sp
722|NRRL B-3736|T|Streptomyces longispororuber
2768|NRRL B-3736g|T|Streptomyces longispororuber
2242|ATCC 11523|T|Streptomyces chrysomallus
291|NRRL B-2000|T|Streptomyces anulatus
14|CGMCC 4.1421|T|Streptomyces anulatus
2765|NRRL B-2000g|T|Streptomyces anulatus
0.0050

Figure 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2018/050387

A. CLASSIFICATION OF SUBJECT MATTER

A01N 63/00 (2006.01) C12N 1/20 (2006.01) C12R 1/01 (2006.01)

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)

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)

CAPLUS, BIOSIS, MEDLINE, CABA, PATENW: Keywords: A01N63/00, bacteria, Paenibacillus, Streptomyces, inoculate, co-inoculate, anti-fungal, actinobacteria, fungus, Rhizoctonia, Pythium, P. Irrregulare, P. Ultimum, root, disease, rot, wheat, tricitum, canola, brassica, napus, rapa, rapeseed, campestris, oleracea, cereal, grain and similar terms.

GENOMEQUEST: SEQ ID NOs: 3-14

GOOGLE, PUBMED, ESP@CE: Applicant/Inventor names searched.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 July 2018	12 July 2018
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Anita Cochrane AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262832118

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2018/050387**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/200987 A1 (INDIGO AGRICULTURE, INC.) 15 December 2016 Paragraphs [00072], [00141], [00148], [00164], [00223]; SEQ ID NO: 9 & 11	1-2, 6, 9, 13-14, 16-21, 27, 29-30, 33 & 35
X	FATIMA Z et al, "Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat", African Journal of Biotechnology, 2009, 8: 219-225 Abstract, pages 220, 222	1-2, 16-19 & 21
X	ORAKÇI GE et al, "Selection of antagonistic actinomycete isolates as biocontrol agents against root-rot fungi", Fresenius Environmental Bulletin, 2010, 19: 417-424 & GenBank Accession Number GQ475299, 5 October 2009 Abstract, pages 418, 421; Sequence comprises regions with 94.8% identity to SEQ ID NO: 5 and 99% identity to SEQ ID NO: 6	1-2, 6, 9, 13-14, 16-21, 27, 29-30 & 33
X	AL-ASKAR AA, "Microbiological studies on the *in vitro* inhibitory effect of *Streptomyces collinus albescens* against some phytopathogenic fungi", African Journal of Microbiology Research, 2012, 6: 3277-3283 & GenBank Accession Number AB184101, 20 May 2008 Abstract; Sequence comprises regions with 97.5% identity to SEQ ID NO: 5 and 95.8% identity to SEQ ID NO: 6	1-2, 6 & 16
X	US 6602500 B1 (KHARBANDA et al) 05 August 2003 Columns 2, 9, 28, Claims	1-3, 9-10 & 6-21
X	GenBank Accession Number KY643705, 27 February 2017 Sequence shares 99.57% identity with SEQ ID NO: 6	1-2, 6, 9, 13-14, 16, 27 & 29
X	GenBank Accession Number KF951483, 5 January 2014 Sequence shares 99.35% identity with SEQ ID NO: 6	1-2, 6, 9, 13-14, 16-17, 27 & 29
X	GenBank Accession Number KJ152029, 6 May 2015 Sequence shares 98.75% with SEQ ID NO:5	1-2, 6-7, 9, 13 & 16
X	GenBank Accession Number KJ162248, 8 April 2014 Sequence shares 98.32% with SEQ ID NO: 5	1-2, 6-7, 9, 13 & 16
P,X	BARNETT S et al, "Selection of microbes for control of Rhizoctonia root rot on wheat using a high throughput pathosystem", Biological Control, 6 July 2017, 113: 45-57 Abstract, Tables 6a & 6b	1-3, 6, 9-10, 13 & 16-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2018/050387

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:

because they relate to subject matter not required to be searched by this Authority, namely:

the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including

2. ☒ Claims Nos.: **22-24 & 36-38**

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See Supplemental Box

3. ☐ Claims Nos:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

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Supplemental Box

Continuation of **Box II**
Claims 22-24 and 36-38 do not comply with Rule 6.2(a) because they rely on references to the description.

Continuation of: **Box III**
This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-3, 6, 9-10 and 16-21 (in part) and 4-5 (in full) are directed to a bacterial inoculant for controlling fungal root disease in wheat or canola. The feature of having a 16S rRNA gene nucleotide sequence with at least 98% to SEQ ID NO: 3 or deposited under NMI accession number V17/004922 is specific to this group of claims.

- Claims 1-2, 6, 9, 13 and 16-21 (in part) and 7-8 (in full) are directed to a bacterial inoculant for controlling fungal root disease in wheat or canola. The feature of having a 16S rRNA gene nucleotide sequence with at least 98% to SEQ ID NO: 5 or deposited under NMI accession number V17/004924 is specific to this group of claims.

- Claims 1-3, 6, 9-10, 16-21, 29-30 and 35 (in part) and 11-12, 25-26, 31-32 (in full) are directed to a bacterial inoculant for controlling fungal root disease in wheat or canola. The feature of having a 16S rRNA gene nucleotide sequence with at least 98% to SEQ ID NO: 4 or deposited under NMI accession number V17/004921 is specific to this group of claims.

- Claims 1-2, 6, 9, 13, 16-21, 29-30 and 35 (in part) and 14-15, 27-28, 33-34 (in full) are directed to a bacterial inoculant for controlling fungal root disease in wheat or canola. The feature of having a 16S rRNA gene nucleotide sequence with at least 98% to SEQ ID NO: 3 or deposited under NMI accession number V17/004923 is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is bacteria capable of controlling fungal root disease in wheat or canola

However this feature does not make a contribution over the prior art because it is disclosed in:

FATIMA Z et al, African Journal of Biotechnology, 2009, 8: 219-225

ORAKÇI GE et al, Fresenius Environmental Bulletin, 2010, 19: 417-424

AL-ASKAR AA, African Journal of Microbiology Research, 2012, 6: 3277-3283

US 6602500 B1 (KHARBANDA et al) 05 August 2003

Each of these documents discloses anti-fungal activity of bacteria from wheat or canola.

Therefore in the light of this document this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori*.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2018/050387**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2016/200987 A1	15 December 2016	WO 2016200987 A1	15 Dec 2016
		AU 2016274683 A1	01 Feb 2018
		CA 2988764 A1	15 Dec 2016
		EP 3302068 A1	11 Apr 2018
US 6602500 B1	05 August 2003	US 6602500 B1	05 Aug 2003
		AU 3805799 A	06 Dec 1999
		AU 758577 B2	27 Mar 2003
		CA 2238289 A1	20 Nov 1999
		EP 1079692 A1	07 Mar 2001
		WO 9959412 A1	25 Nov 1999

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.