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(54) **PLANT MICROBIOME AND METHODS FOR PROFILING PLANT MICROBIOME**

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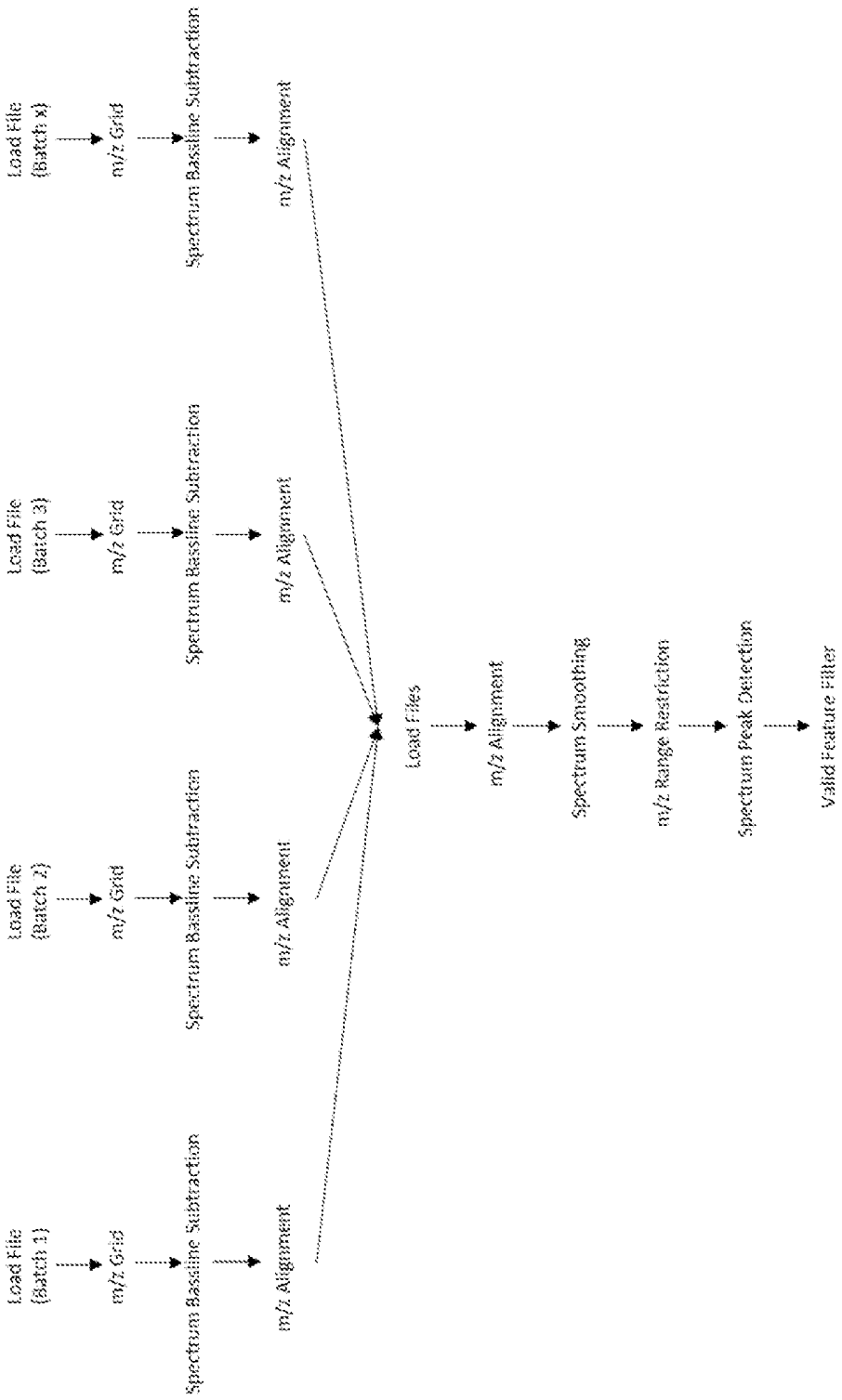
CPC ***C12Q 1/04*** (2013.01); *C12R 2001/01* (2021.05)

(57) **ABSTRACT**

The present invention provides a method for profiling plant endophyte microbiomes, wherein the method provides for identification of endophyte strains that are phylogenetically related to a desired endophyte strain. More particularly, the present invention relates to method for identifying, characterising and/or comparing endophyte strains and to novel endophyte strains selected and/or isolated by the method. The present invention also relates to methods for transferring endophyte strains between plants.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Spectrum Baseline Subtraction
m/z Alignment
Load File (Batch 2)
m/z Grid
Spectrum Baseline Subtraction
m/z Alignment
Load File (Batch 3)
m/z Grid
Spectrum Baseline Subtraction
m/z Alignment
Load File (Batch x)
m/z Grid
Spectrum Baseline Subtraction
m/z Alignment
Load Files
m/z Alignment
Spectrum Smoothing
m/z Range Restriction
Spectrum Peak Detection
Valid Feature Filter

FIG 1

m/z Grid
m/z Grid Method: Adaptive Grid
Scan Count: 1- 10

Spectrum Bassline Subtraction
Method: Quantile
Quantile: 10 %-20 %
m/z Window: 10-100 Da
Advanced
Export Background Function: false

m/z Alignment
Alignment: Reference Spectrum
Spectrum Index: 1-5
m/z Window: 5-1000 Da
Max. m/z Shift: 1-200 Da

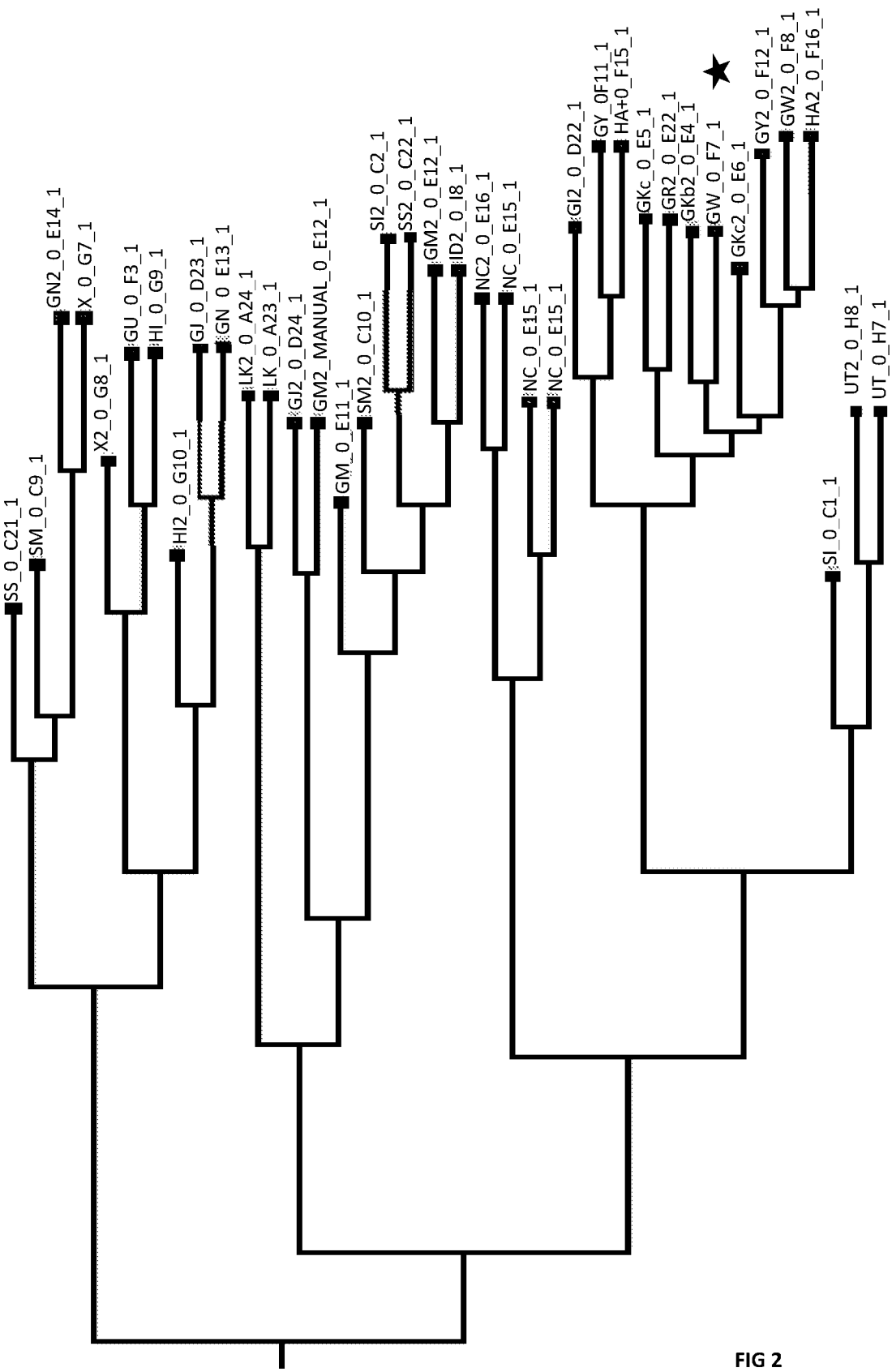
Spectrum Smoothing
Algorithm: Moving Average
m/z Window: 4 - 30 Points

m/z Range Restriction
m/z Minimum: 2000 Da
m/z Maximum: 20000 Da

Spectrum Peak Detection
Use Smoothing: false
Peak Detection: Resolution-based
Detection: Standard
Center Computation: Local Maximum
Boundary Determination: Maximum Curvature
Peak Filtering
Present in at least: 2 Experiments

Valid Feature Filter
Data Type: Auto Detect
Feature Type: Auto Detect
Validity Threshold: 0- 40 %Intensity
Present in at Least: 2 Experiments

FIG 1 (ctd)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X2_0_G8_1
GU_0_F3_1
HI_0_G9_1
HI2_0_G10_1
GJ_0_D23_1
GN_0_E13_1
LK2_0_A24_1
LK_0_A23_1
GJ2_0_D24_1
GM2_MANUAL_0_E12_1
GM_0_E11_1
SM2_0_C10_1
SI2_0_C2_1
SS2_0_C22_1
GM2_0_E12_1
ID2_0_I8_1
NC2_0_E16_1
NC_0_E15_1
NC_0_E15_1
NC_0_E15_1
GI2_0_D22_1
GY_0F11_1
HA+0_F15_1
GKc_0_E5_1
GR2_0_E22_1
GKb2_0_E4_1
GW_0_F7_1
GKc2_0_E6_1
GY2_0_F12_1
GW2_0_F8_1
HA2_0_F16_1
SI_0_C1_1
UT2_0_H8_1
UT_0_H7_1

FIG 2

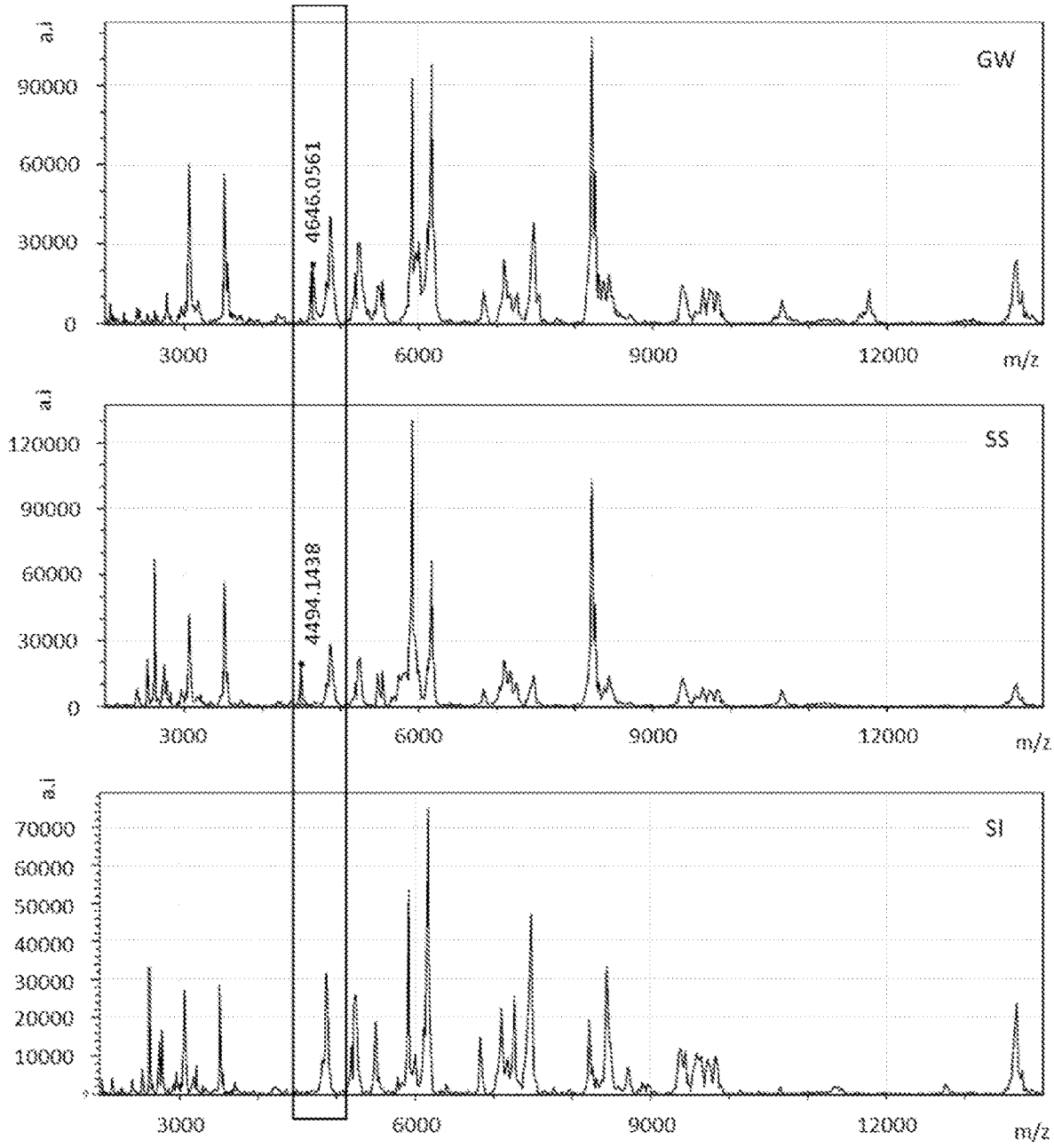
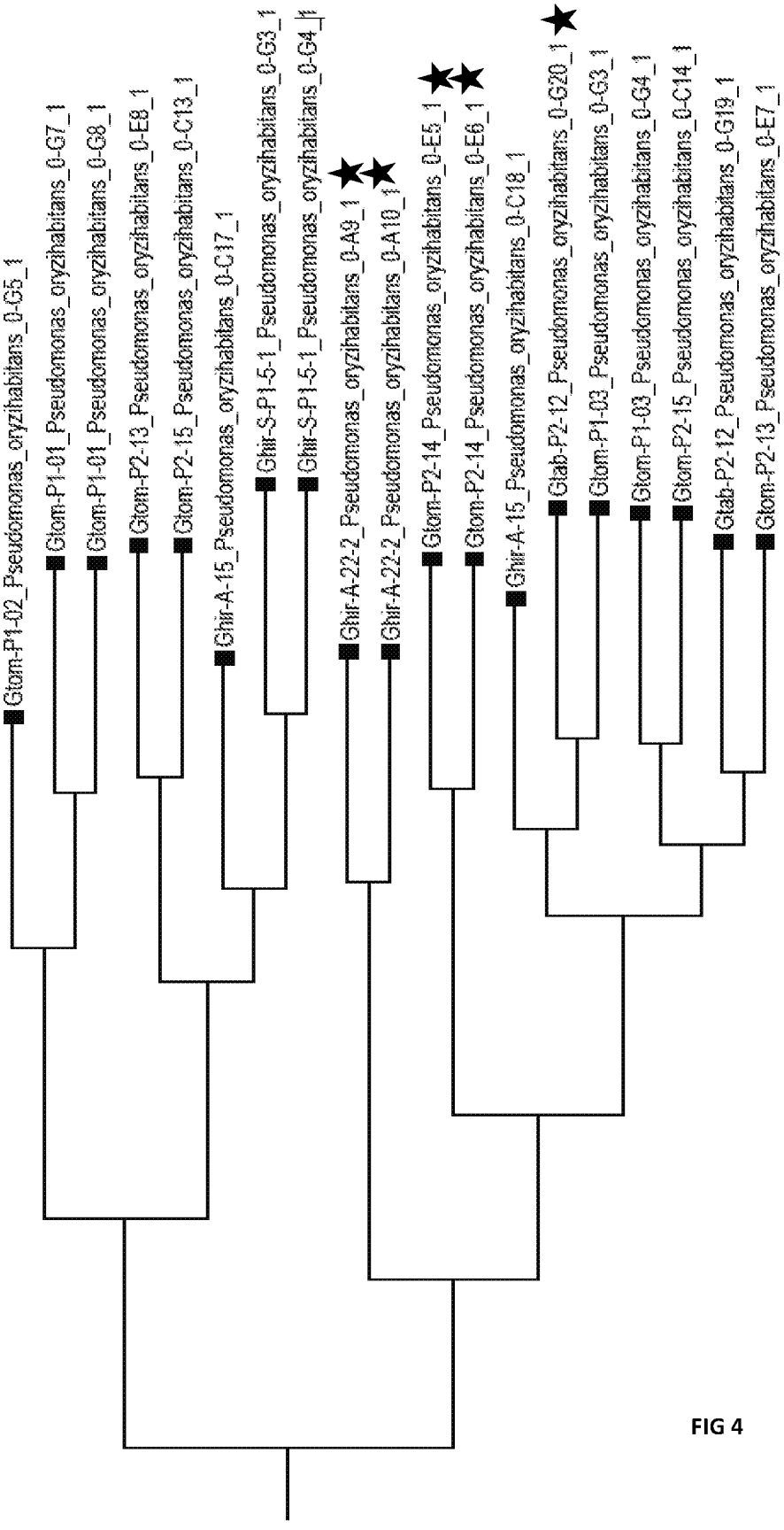

a.i
90000
60000
30000
0
4646.0561
GW
3000
6000
9000
12000
m/z
a.i
120000
90000
60000
30000
0
4494.1438
SS
3000
6000
9000
12000
m/z
a.i
70000
60000
50000
40000
30000
20000
10000
0
SI
3000
6000
9000
12000
m/z

FIG 3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Gtom-P1-01_Pseudomonas_oryzihabitans_0-G8_1
Gtom-P2-13_Pseudomonas_oryzihabitans_0-E8_1
Gtom-P2-15_Pseudomonas_oryzihabitans_0-C13_1
Ghir-A-15_Pseudomonas_oryzihabitans_0-C17_1
Ghir-S-P1-5-1_Pseudomonas_oryzihabitans_0-G3_1
Ghir-S-P1-5-1_Pseudomonas_oryzihabitans_0-G4_1
Ghir-A-22-2_Pseudomonas_oryzihabitans_0-A9_1
Ghir-A-22-2_Pseudomonas_oryzihabitans_0-A10_1
Gtom-P2-14_Pseudomonas_oryzihabitans_0-E5_1
Gtom-P2-14_Pseudomonas_oryzihabitans_0-E6_1
Ghir-A-15_Pseudomonas_oryzihabitans_0-C18_1
Gtab-P2-12_Pseudomonas_oryzihabitans_0-G20_1
Gtom-P1-03_Pseudomonas_oryzihabitans_0-G3_1
Gtom-P1-03_Pseudomonas_oryzihabitans_0-G4_1
Gtom-P2-15_Pseudomonas_oryzihabitans_0-C14_1
Gtab-P2-12_Pseudomonas_oryzihabitans_0-G19_1
Gtom-P2-13_Pseudomonas_oryzihabitans_0-E7_1

FIG 4

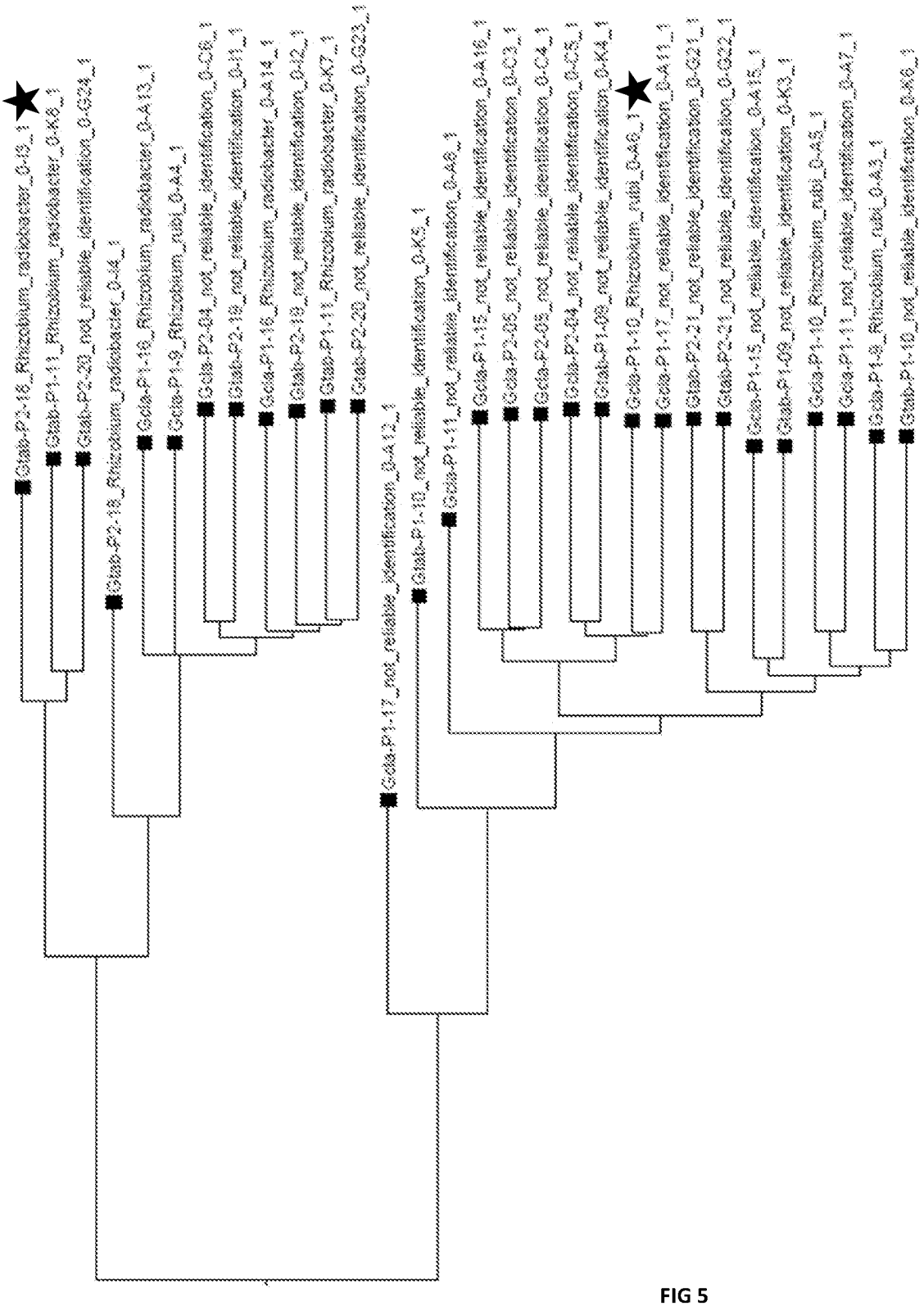

Gtab-P2-16_Rhizobium_radiobacter_0-I5_1
Gtab-P1-11_Rhizobium_radiobacter_0-K8_1
Gtab-P2-20_not_reliable_identification_0-G24_1
Gtab-P2-18_Rhizobium_radiobacter_0-I4_1
Gcla-P1-16_Rhizobium_radiobacter_0-A13_1
Gcla-P1-9_Rhizobium_rubi_0-A4_1
Gcla-P2-04_not_reliable_identification_0-C6_1
Gtab-P2-19_not_reliable_identification_0-I1_1
Gcla-P1-16_Rhizobium_radiobacter_0-A14_1
Gtab-P2-19_not_reliable_identification_0-I2_1
Gtab-P1-11_Rhizobium_radiobacter_0-K7_1
Gtab-P2-20_not_reliable_identification_0-G23_1
Gcla-P1-17_not_reliable_identification_0-A12_1
Gtab-P1-10_not_reliable_identification_0-K5_1
Gcla-P1-11_not_reliable_identification_0-A9_1
Gcla-P1-15_not_reliable_identification_0-A16_1
Gcla-P2-05_not_reliable_identification_0-C3_1
Gcla-P2-05_not_reliable_identification_0-C4_1
Gcla-P2-04_not_reliable_identification_0-C5_1
Gtab-P1-09_not_reliable_identification_0-K4_1
Gcla-P1-10_Rhizobium_rubi_0-A6_1
Gcla-P1-17_not_reliable_identification_0-A11_1
Gtab-P2-21_not_reliable_identification_0-G21_1
Gtab-P2-21_not_reliable_identification_0-G22_1
Gcla-P1-15_not_reliable_identification_0-A15_1
Gtab-P1-09_not_reliable_identification_0-K3_1
Gcla-P1-10_Rhizobium_rubi_0-A5_1
Gcla-P1-11_not_reliable_identification_0-A7_1
Gcla-P1-9_Rhizobium_rubi_0-A3_1
Gtab-P1-10_not_reliable_identification_0-K6_1

FIG 5

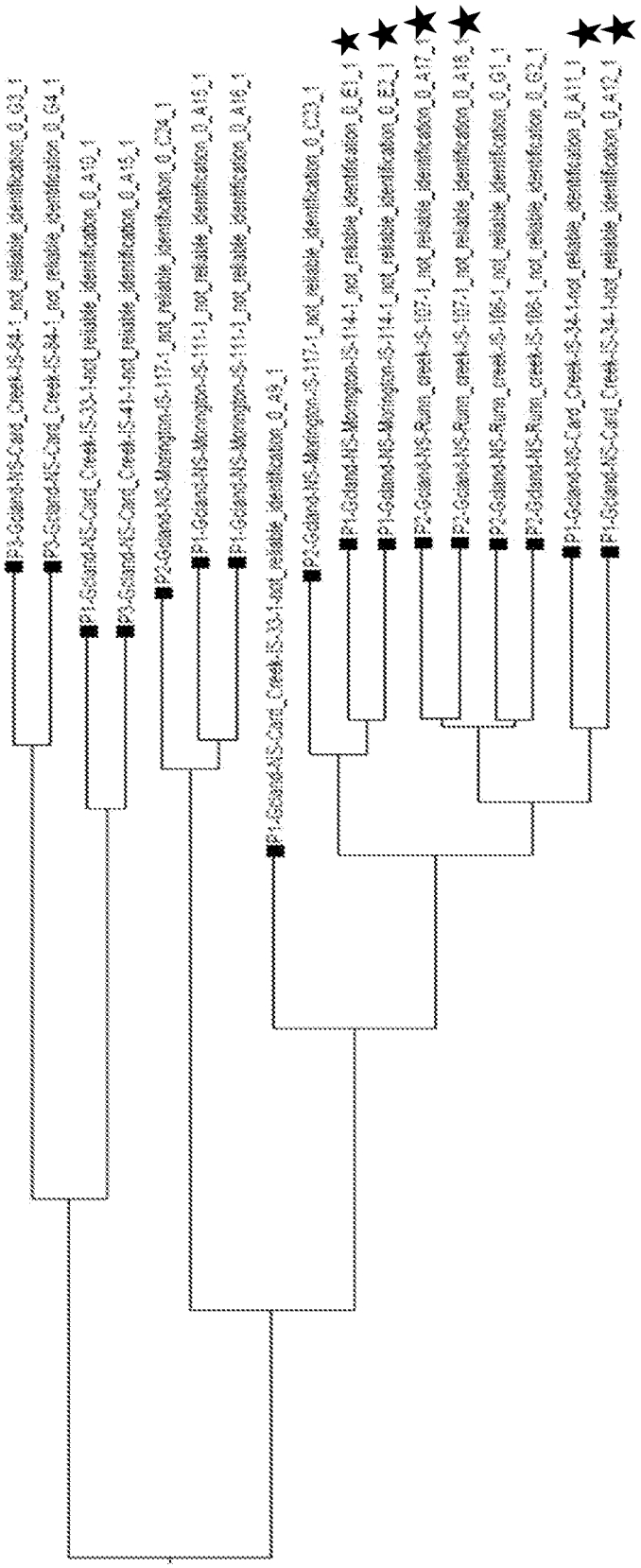

P1-Grand-NS-Card_Creek-IS-84-1_not_reliable_identification_0_G3_1
P2-Grand-NS-Card_Creek-IS-84-1_not_reliable_identification_0_G4_1
P1-Grand-NS-Card_Creek-IS-33-1-not_reliable_identification_0_A10_1
P3-Grand-NS-Card_Creek-IS-44-1-not_reliable_identification_0_A15_1
P2-Grand-NS-Muningan-IS-117-1_not_reliable_identification_0_C24_1
P1-Grand-NS-Muningan-IS-111-1_not_reliable_identification_0_A12_1
P1-Grand-NS-Muningan-IS-111-1_not_reliable_identification_0_A16_1
P2-Grand-NS-Muningan-IS-117-1_not_reliable_identification_0_C23_1
P1-Grand-NS-Muningan-IS-114-1_not_reliable_identification_0_E1_1
P1-Grand-NS-Muningan-IS-114-1_not_reliable_identification_0_E2_1
P2-Grand-NS-Rum_creek-IS-107-1_not_reliable_identification_0_A17_1
P2-Grand-NS-Rum_creek-IS-107-1_not_reliable_identification_0_A18_1
P2-Grand-NS-Rum_creek-IS-108-1_not_reliable_identification_0_G1_1
P2-Grand-NS-Rum_creek-IS-108-1_not_reliable_identification_0_G2_1
P1-Grand-NS-Card_Creek-IS-34-1-not_reliable_identification_0_A11_1
P1-Grand-NS-Card_Creek-IS-34-1-not_reliable_identification_0_A12_1

FIG 6

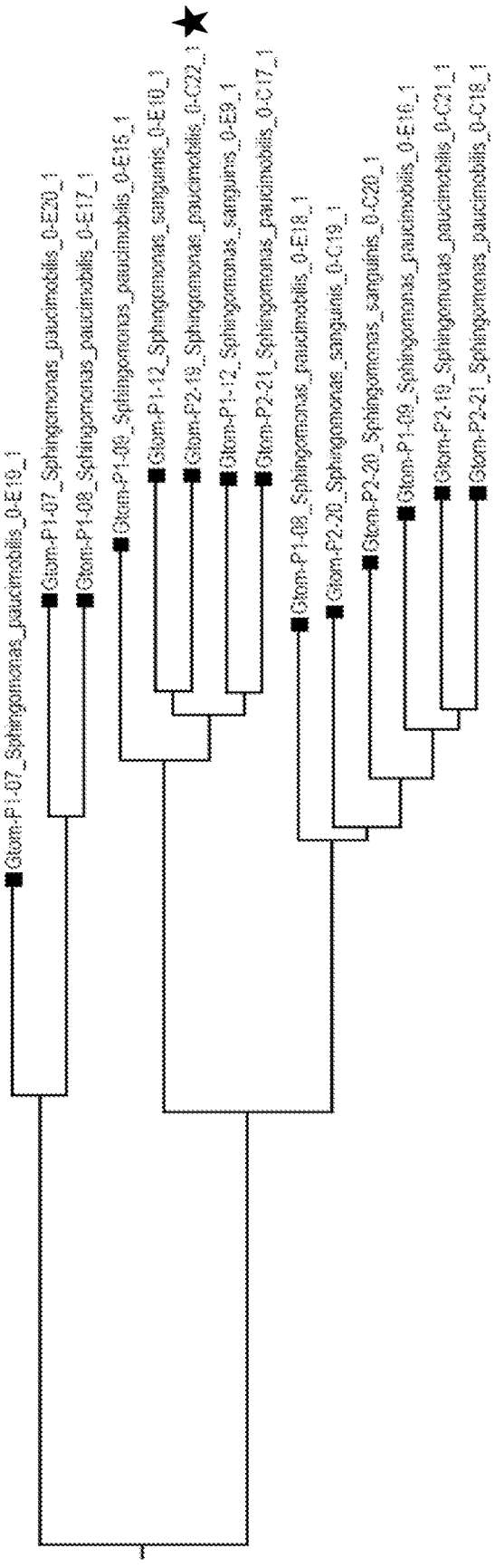

Gtom-P1-07_Sphingomonas_paucimobilis_0-E19_1
Gtom-P1-07_Sphingomonas_paucimobilis_0-E20_1
Gtom-P1-08_Sphingomonas_paucimobilis_0-E17_1
Gtom-P1-08_Sphingomonas_paucimobilis_0-E16_1
Gtom-P1-12_Sphingomonas_sanguinis_0-E10_1
Gtom-P2-19_Sphingomonas_paucimobilis_0-C22_1
Gtom-P1-12_Sphingomonas_sanguinis_0-E9_1
Gtom-P2-21_Sphingomonas_paucimobilis_0-C17_1
Gtom-P1-08_Sphingomonas_paucimobilis_0-E18_1
Gtom-P2-20_Sphingomonas_sanguinis_0-C19_1
Gtom-P2-20_Sphingomonas_sanguinis_0-C20_1
Gtom-P1-09_Sphingomonas_paucimobilis_0-E15_1
Gtom-P2-19_Sphingomonas_paucimobilis_0-C21_1
Gtom-P2-21_Sphingomonas_paucimobilis_0-C18_1

FIG 7

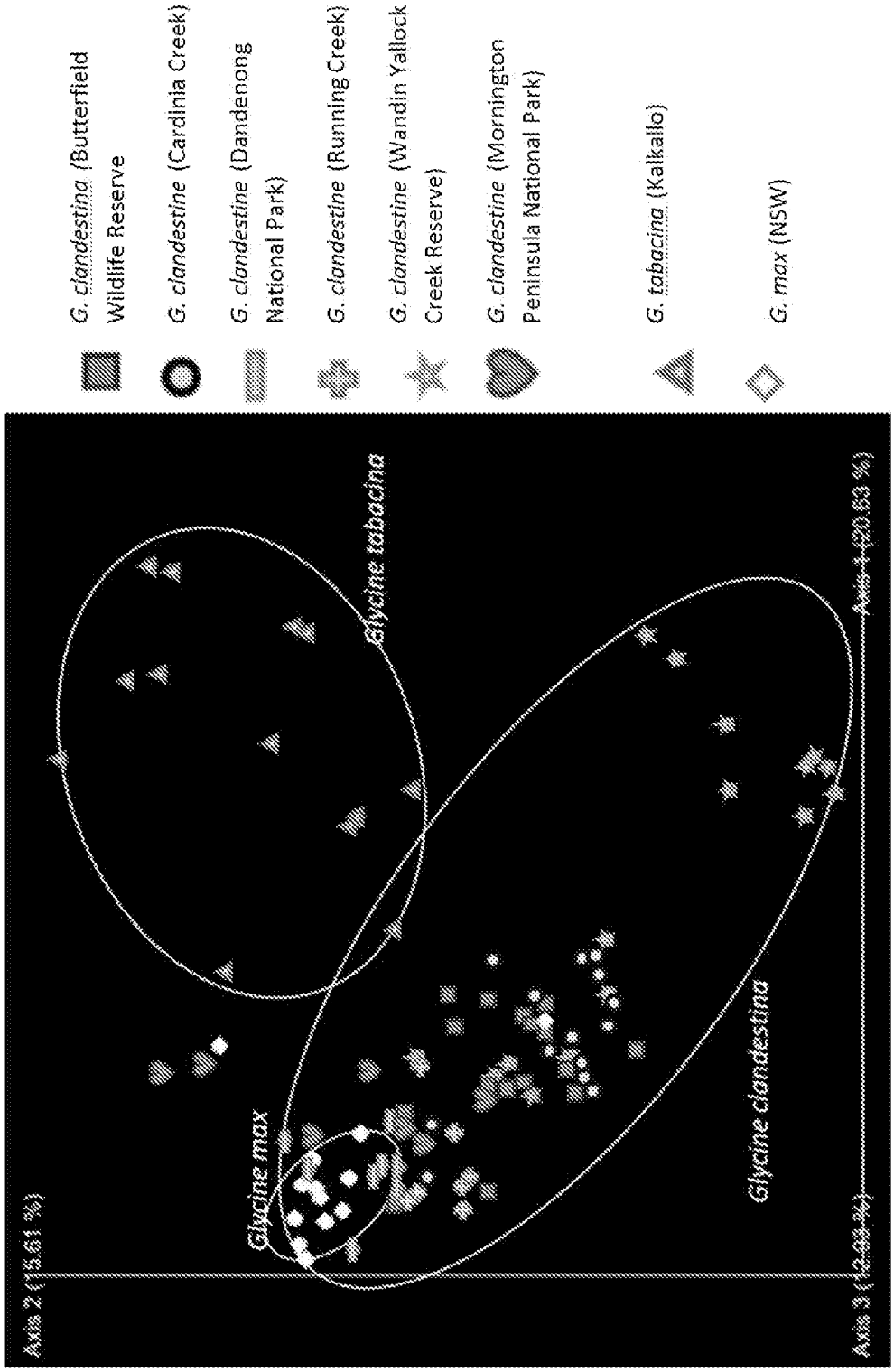
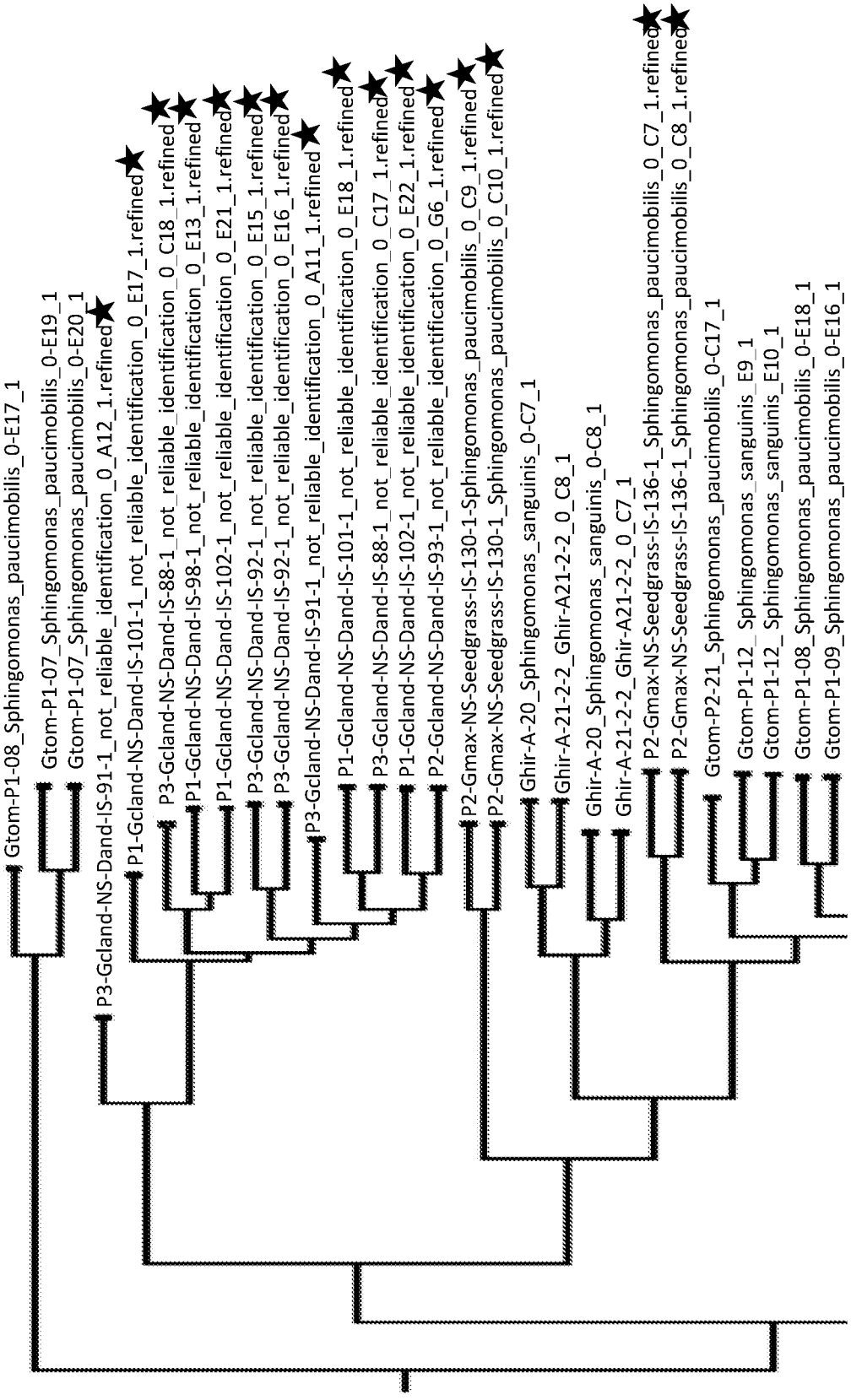

G. clandestina (Butterfield Wildlife Reserve
G. clandestine (Cardinia Creek)
G. clandestine (Dandenong National Park)
G. clandestine (Running Creek)
G. clandestine (Wandin Yallock Creek Reserve)
G. clandestine (Mornington Peninsula National Park)
G. tabacina (Kalkallo)
G. max (NSW)
Glycine max
Glycine tabacina
Glycine clandestina
Axis 2 (15.61 %)
Axis 3 (12.03 %)
Axis 1 (20.63 %)

FIG 8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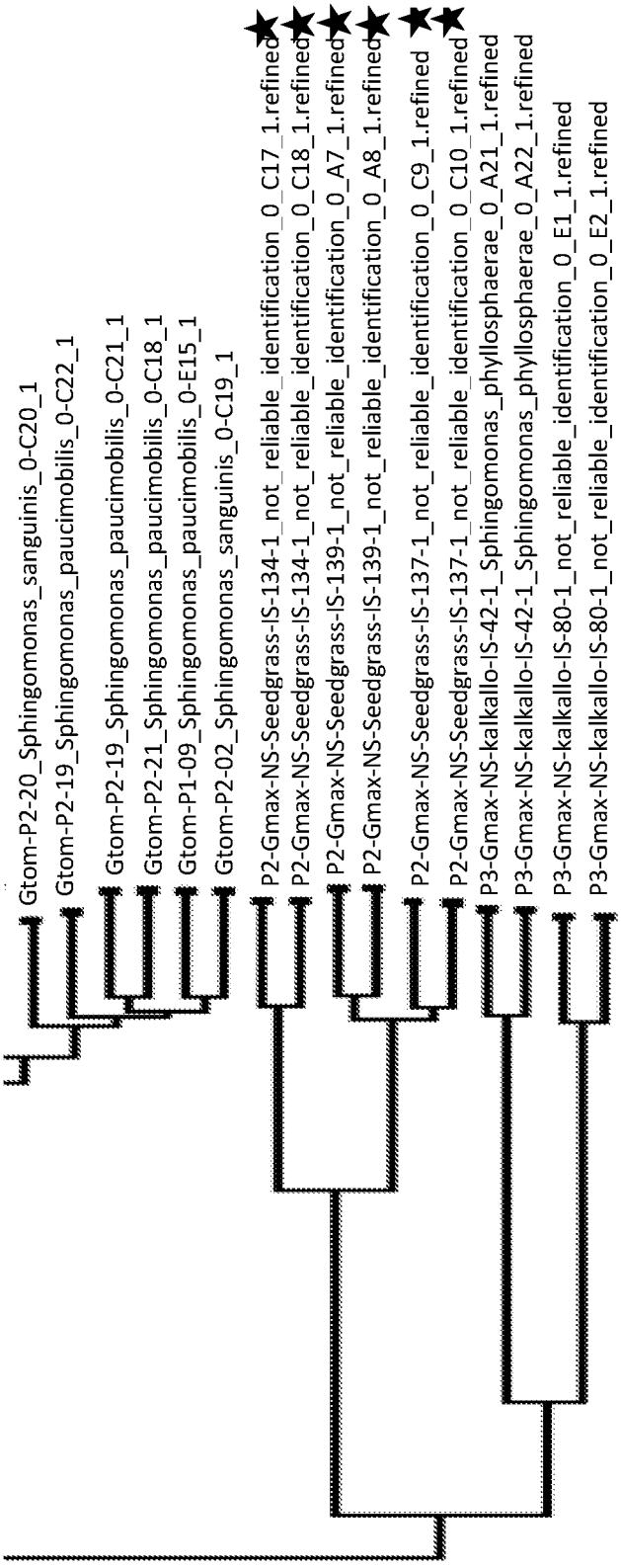
Gtom-P1-07_Sphingomonas_paucimobilis_0-E19_1
Gtom-P1-07_Sphingomonas_paucimobilis_0-E20_1
P3-Gcland-NS-Dand-IS-91-1_not_reliable_identification_0_A12_1.refined
P1-Gcland-NS-Dand-IS-101-1_not_reliable_identification_0_E17_1.refined
P3-Gcland-NS-Dand-IS-88-1_not_reliable_identification_0_C18_1.refined
P1-Gcland-NS-Dand-IS-98-1_not_reliable_identification_0_E13_1.refined
P1-Gcland-NS-Dand-IS-102-1_not_reliable_identification_0_E21_1.refined
P3-Gcland-NS-Dand-IS-92-1_not_reliable_identification_0_E15_1.refined
P3-Gcland-NS-Dand-IS-92-1_not_reliable_identification_0_E16_1.refined
P3-Gcland-NS-Dand-IS-91-1_not_reliable_identification_0_A11_1.refined
P1-Gcland-NS-Dand-IS-101-1_not_reliable_identification_0_E18_1.refined
P3-Gcland-NS-Dand-IS-88-1_not_reliable_identification_0_C17_1.refined
P1-Gcland-NS-Dand-IS-102-1_not_reliable_identification_0_E22_1.refined
P2-Gcland-NS-Dand-IS-93-1_not_reliable_identification_0_G6_1.refined
P2-Gmax-NS-Seedgrass-IS-130-1-Sphingomonas_paucimobilis_0_C9_1.refined
P2-Gmax-NS-Seedgrass-IS-130-1_Sphingomonas_paucimobilis_0_C10_1.refined
Ghir-A-20_Sphingomonas_sanguinis_0-C7_1
Ghir-A-21-2-2_Ghir-A21-2-2_0_C8_1
Ghir-A-20_Sphingomonas_sanguinis_0-C8_1
Ghir-A-21-2-2_Ghir-A21-2-2_0_C7_1
P2-Gmax-NS-Seedgrass-IS-136-1_Sphingomonas_paucimobilis_0_C7_1.refined
P2-Gmax-NS-Seedgrass-IS-136-1_Sphingomonas_paucimobilis_0_C8_1.refined
Gtom-P2-21_Sphingomonas_paucimobilis_0-C17_1
Gtom-P1-12_ Sphingomonas_sanguinis_E9_1
Gtom-P1-12_ Sphingomonas_sanguinis_E10_1
Gtom-P1-08_Sphingomonas_paucimobilis_0-E18_1
Gtom-P1-09_Sphingomonas_paucimobilis_0-E16_1

FIG 9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Gtom-P2-19_Sphingomonas_paucimobilis_0-C21_1
Gtom-P2-21_Sphingomonas_paucimobilis_0-C18_1
Gtom-P1-09_Sphingomonas_paucimobilis_0-E15_1
Gtom-P2-02_Sphingomonas_sanguinis_0-C19_1
P2-Gmax-NS-Seedgrass-IS-134-1_not_reliable_identification_0_C17_1.refined
P2-Gmax-NS-Seedgrass-IS-134-1_not_reliable_identification_0_C18_1.refined
P2-Gmax-NS-Seedgrass-IS-139-1_not_reliable_identification_0_A7_1.refined
P2-Gmax-NS-Seedgrass-IS-139-1_not_reliable_identification_0_A8_1.refined
P2-Gmax-NS-Seedgrass-IS-137-1_not_reliable_identification_0_C9_1.refined
P2-Gmax-NS-Seedgrass-IS-137-1_not_reliable_identification_0_C10_1.refined
P3-Gmax-NS-kalkallo-IS-42-1_Sphingomonas_phyllosphaerae_0_A21_1.refined
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P3-Gmax-NS-kalkallo-IS-80-1_not_reliable_identification_0_E1_1.refined
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FIG 9 (cont)

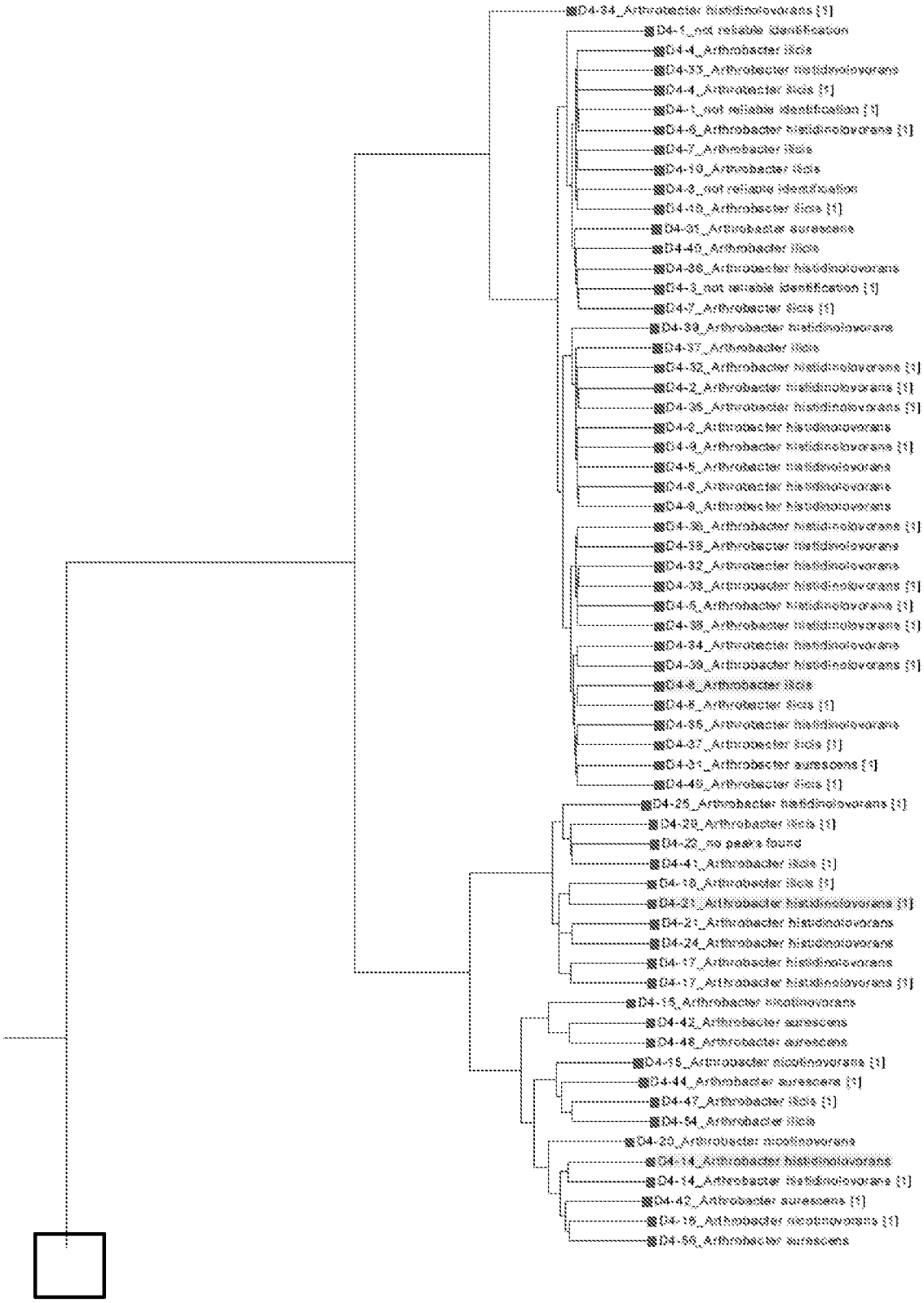
D4-34_Arthrobacter histidinolovorans [1]
D4-1_not reliable identification
D4-4_Arthrobacter ilicis
D4-33_Arthrobacter histidinolovorans
D4-4_Arthrobacter ilicis [1]
D4-1_not reliable identification [1]
D4-6_Arthrobacter histidinolovorans [1]
D4-7_Arthrobacter ilicis
D4-10_Arthrobacter ilicis
D4-3_not reliable identification
D4-10_Arthrobacter ilicis [1]
D4-31_Arthrobacter aurescens
D4-45_Arthrobacter ilicis
D4-36_Arthrobacter histidinolovorans
D4-3_not reliable identification [1]
D4-7_Arthrobacter ilicis [1]
D4-30_Arthrobacter histidinolovorans
D4-37_Arthrobacter ilicis
D4-32_Arthrobacter histidinolovorans [1]
D4-2_Arthrobacter histidinolovorans [1]
D4-35_Arthrobacter histidinolovorans [1]
D4-9_Arthrobacter histidinolovorans
D4-9_Arthrobacter histidinolovorans [1]
D4-5_Arthrobacter histidinolovorans
D4-8_Arthrobacter histidinolovorans
D4-9_Arthrobacter histidinolovorans
D4-30_Arthrobacter histidinolovorans [1]
D4-38_Arthrobacter histidinolovorans
D4-32_Arthrobacter histidinolovorans
D4-32_Arthrobacter histidinolovorans [1]
D4-5_Arthrobacter histidinolovorans [1]
D4-38_Arthrobacter histidinolovorans [1]
D4-34_Arthrobacter histidinolovorans
D4-39_Arthrobacter histidinolovorans [1]
D4-8_Arthrobacter ilicis
D4-5_Arthrobacter ilicis [1]
D4-35_Arthrobacter histidinolovorans
D4-37_Arthrobacter ilicis [1]
D4-31_Arthrobacter aurescens [1]
D4-45_Arthrobacter ilicis [1]
D4-25_Arthrobacter histidinolovorans [1]
D4-29_Arthrobacter ilicis [1]
D4-22_no peaks found
D4-41_Arthrobacter ilicis [1]
D4-19_Arthrobacter ilicis [1]
D4-21_Arthrobacter histidinolovorans [1]
D4-21_Arthrobacter histidinolovorans
D4-24_Arthrobacter histidinolovorans
D4-17_Arthrobacter histidinolovorans
D4-17_Arthrobacter histidinolovorans [1]
D4-15_Arthrobacter nicotinovorans
D4-42_Arthrobacter aurescens
D4-48_Arthrobacter aurescens
D4-15_Arthrobacter nicotinovorans [1]
D4-44_Arthrobacter aurescens [1]
D4-47_Arthrobacter ilicis [1]
D4-54_Arthrobacter ilicis
D4-20_Arthrobacter nicotinovorans
D4-14_Arthrobacter histidinolovorans
D4-14_Arthrobacter histidinolovorans [1]
D4-42_Arthrobacter aurescens [1]
D4-18_Arthrobacter nicotinovorans [1]
D4-56_Arthrobacter aurescens

FIG 10

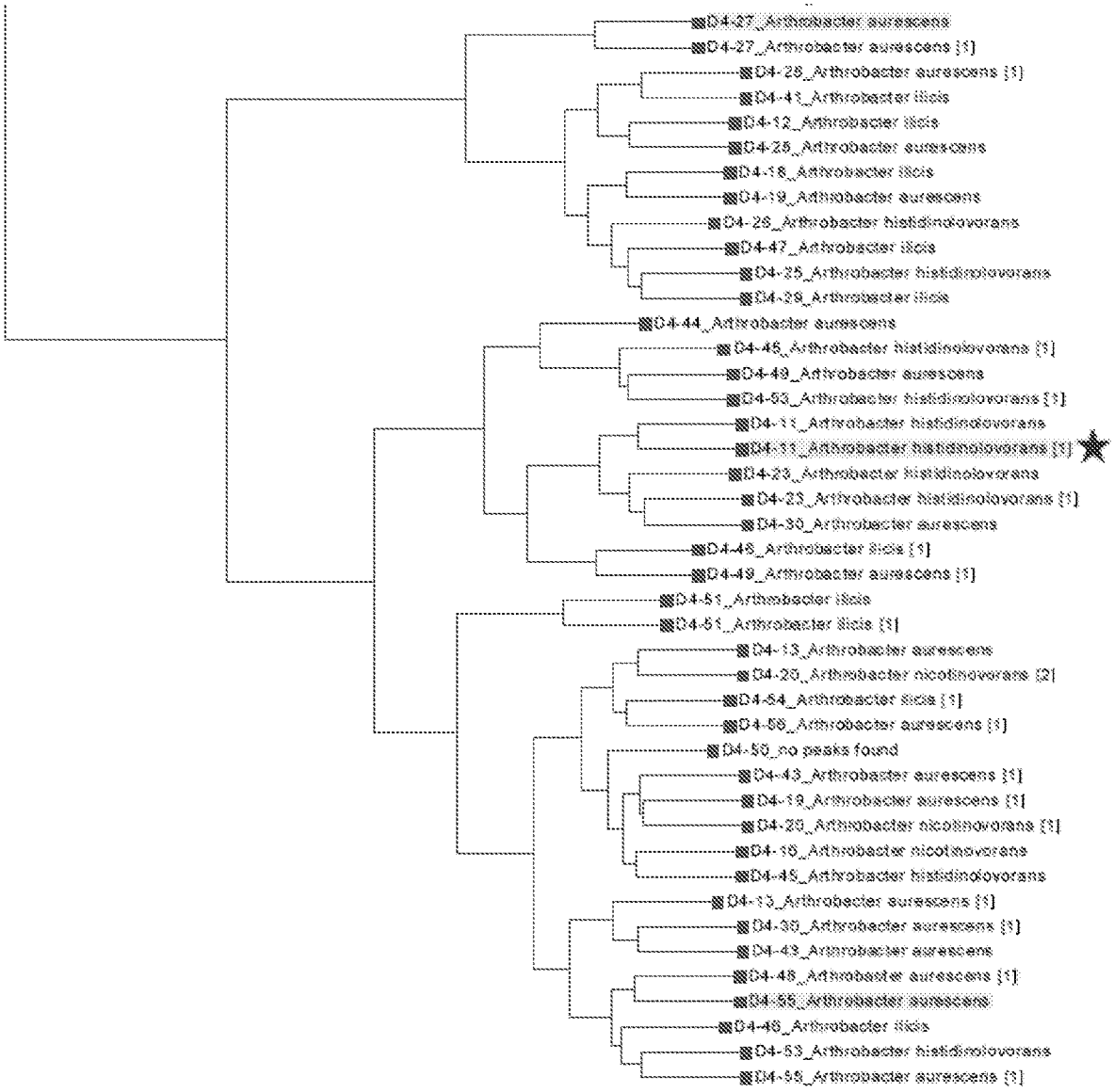
D4-27_Arthrobacter aurescens
D4-27_Arthrobacter aurescens [1]
D4-28_Arthrobacter aurescens [1]
D4-41_Arthrobacter ilicis
D4-12_Arthrobacter ilicis
D4-25_Arthrobacter aurescens
D4-16_Arthrobacter ilicis
D4-19_Arthrobacter aurescens
D4-26_Arthrobacter histidinolovorans
D4-47_Arthrobacter ilicis
D4-35_Arthrobacter histidinolovorans
D4-29_Arthrobacter ilicis
D4-44_Arthrobacter aurescens
D4-45_Arthrobacter histidinolovorans [1]
D4-49_Arthrobacter aurescens
D4-53_Arthrobacter histidinolovorans [1]
D4-11_Arthrobacter histidinolovorans
D4-11_Arthrobacter histidinolovorans [1]
D4-23_Arthrobacter histidinolovorans
D4-23_Arthrobacter histidinolovorans [1]
D4-30_Arthrobacter aurescens
D4-48_Arthrobacter ilicis [1]
D4-49_Arthrobacter aurescens [1]
D4-51_Arthrobacter ilicis
D4-51_Arthrobacter ilicis [1]
D4-13_Arthrobacter aurescens
D4-20_Arthrobacter nicotinovorans [2]
D4-54_Arthrobacter ilicis [1]
D4-56_Arthrobacter aurescens [1]
D4-50_no peaks found
D4-43_Arthrobacter aurescens [1]
D4-14_Arthrobacter aurescens [1]
D4-22_Arthrobacter nicotinovorans [1]
D4-15_Arthrobacter nicotinovorans
D4-45_Arthrobacter histidinolovorans
D4-13_Arthrobacter aurescens [1]
D4-30_Arthrobacter aurescens [1]
D4-43_Arthrobacter aurescens
D4-48_Arthrobacter aurescens [1]
D4-55_Arthrobacter aurescens
D4-46_Arthrobacter ilicis
D4-53_Arthrobacter histidinolovorans
D4-55_Arthrobacter aurescens [1]

FIG 10 (Cont.)

PLANT MICROBIOME AND METHODS FOR PROFILING PLANT MICROBIOME

FIELD OF THE INVENTION

[0001] The present invention relates to method for identifying, characterising and/or comparing endophyte strains and to novel endophyte strains selected and/or isolated by the method. The present invention also relates to methods for transferring endophyte strains between plants.

BACKGROUND OF THE INVENTION

[0002] Microbes represent an invaluable source of novel genes and compounds that have the potential to be utilised in a range of industrial sectors. Scientific literature gives numerous accounts of microbes being the primary source of antibiotics, immune-suppressants, anticancer agents and cholesterol-lowering drugs, in addition to their use in environmental decontamination and in the production of food and cosmetics.

[0003] A relatively unexplored group of microbes known as endophytes, which reside e.g. in the tissues of living plants, offer a particularly diverse source of novel compounds and genes that may provide important benefits to society, and in particular, agriculture.

[0004] Endophytes may be fungal or bacterial. Endophytes often form mutualistic relationships with their hosts, with the endophyte conferring increased fitness to the host, often through the production of defence compounds. At the same time, the host plant offers the benefits of a protected environment and nutriment to the endophyte.

[0005] Important forage grasses perennial ryegrass (*Lolium perenne*) are commonly found in association with fungal and bacterial endophytes. However, there remains a general lack of information and knowledge of the endophytes of these grasses as well as of methods for the identification and characterisation of novel endophytes and their deployment in plant improvement programs.

[0006] *Glycine* (soybean) is a genus in the bean family Fabaceae. The best known species is the cultivated soybean (*Glycine max*). Again, there remains a general lack of information and knowledge of the endophytes of these plants as well as of methods for the identification and characterisation of novel endophytes and their deployment in plant improvement programs.

[0007] Knowledge of the endophytes of perennial ryegrass may allow certain beneficial traits to be exploited in enhanced pastures, or lead to other agricultural advances, e.g. to the benefit of sustainable agriculture and the environment.

[0008] Identification of phylogenetically related microbes typically involves isolation of microbes from e.g. plant material and subsequent processing by genetic sequencing to allow for comparison of microbes. Microbes can then be clustered based on genetic similarities.

[0009] Further methods for identification and characterization of for microbes, in particular endophytes, are generally based on morphological characterisation and molecular taxonomy analyses. Morphological characterisation includes analyses of macroscopic and microscopic structures of microbes grown on culture media. Molecular taxonomy analysis is mainly based on gene sequence analysis of spacer regions in nuclear ribosomal DNA (nrDNA), particularly in phylogenomics. However, traditional methods of phylogenomics based on nrDNA sequences may not reflect the divergence of closely related species.

[0010] There exists a need to overcome, or at least alleviate, one or more of the difficulties or deficiencies associated with the prior art.

SUMMARY OF THE INVENTION

[0011] In one aspect, the present invention provides a method for profiling endophyte strains from a microbiome, said method including the steps of:

[0012] providing a microbiome;

[0013] obtaining protein profile spectra from one or more endophytes of the microbiome;

[0014] processing the protein profile spectra;

[0015] clustering the endophyte strains based on the processed protein profile spectra; and

[0016] selecting and/or isolating endophyte strain(s) having desired genetic and/or metabolic characteristics, or being phylogenetically related to a desired endophyte strain.

[0017] As used herein, the term 'profiling' endophyte strain(s) means identifying, characterising and/or comparing endophyte strain(s). For example, this may include selecting and/or isolating endophyte strain(s) having desired genetic and/or metabolic characteristics, or endophyte strain(s) that are phylogenetically related to a desired endophyte strain.

[0018] As used herein the term 'isolated' means that an endophyte is removed from its original environment (e.g. the natural environment if it is naturally occurring). For example, a naturally occurring endophyte present in a living plant is not isolated, but the same endophyte separated from some or all of the coexisting materials in the natural system, is isolated.

[0019] As used herein the term 'endophyte' is meant a bacterial or fungal strain that is closely associated with a plant. By 'associated with' in this context is meant that the bacteria or fungus lives on, in or in close proximity to a plant. For example, it may be endophytic, for example living within the internal tissues of a plant, or epiphytic, for example growing externally on a plant.

[0020] In a preferred embodiment the microbiome may be isolated from a plant material. The plant material may be of any suitable type. For example, the plant material may be from a grass, tree, flower, herb, shrub or bush, vine or legume, or a product thereof. The method according to the present invention is particularly applicable to grasses and legumes.

[0021] In a preferred embodiment the plant material may be from a perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinaceae*), corn (*Zea mays*), *Glycine* species, wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), or any combination thereof.

[0022] In a further preferred embodiment the *Glycine* species includes *Glycine tomentella, Glycine tabacina, Glycine latifolia, Glycine hirticaulis, Glycine microphylla, Glycine clandestine.*

[0023] In another preferred embodiment the *Glycine* species includes *Glycine max.*

[0024] In a preferred embodiment the plant material includes seeds, leaves, stems, petioles, roots, buds, flowers or any combination thereof.

[0025] In a preferred embodiment, the step of providing the microbiome includes the steps of:

[0026] providing plant material;

[0027] washing the plant material in an aqueous solution;

[0028] submerging the plant material in an aqueous solution;

[0029] macerating the plant material; and

[0030] applying the macerated plant material to a growth medium for growth of the microbiota to provide the isolated microbiome.

[0031] In a preferred embodiment, the microbiota grown on the growth medium may be subjected to a re-streaking so as to obtain an isolated endophyte colony.

[0032] In a preferred embodiment, the method may include obtaining protein profile spectra from one or more isolated endophyte colonies.

[0033] In a preferred embodiment, when the plant material includes a seed, the step of providing the microbiome may include the preliminary steps of:

[0034] harvesting the plant material;

[0035] sterilising the plant material;

[0036] germinating the plant material;

[0037] growing the germinated plant material.

[0038] In a further preferred embodiment, the aqueous solution may be a buffer solution. In a further preferred embodiment the buffer solution may be a phosphate buffered saline (PBS) solution.

[0039] In a preferred embodiment, the protein profile spectra may be obtained by mass spectrometry. In a further preferred embodiment, the mass spectrometry technique used to obtain the protein profile spectra may be matrix assisted laser desorption/ionisation (MALDI) mass spectrometry.

[0040] In a preferred embodiment, the protein profile spectra are processed by a data deconvolution workflow. In a further preferred embodiment the data deconvolution workflow includes performing the steps of:

[0041] a m/z scan to create a m/z grid;

[0042] a spectrum baseline subtraction; and

[0043] a m/z alignment;

wherein the data deconvolution workflow provides the processed protein spectra.

[0044] In a preferred embodiment, processed protein profile spectra may be obtained for one or more isolated endophyte colonies.

[0045] As used herein 'm/z' means a measurement of the mass to charge ratio resulting from a mass analysis experiment wherein one or more electrons are taken from molecules to create charged ions. The number of electrons removed is the charge number (for positive ions), wherein m/z represents mass (m) divided by charge number (z).

[0046] In a further preferred embodiment the m/z grid may be produced according to an adaptive grid method. In a more preferred embodiment the adaptive grid method scan count is between approximately 1 and 10.

[0047] In a further preferred embodiment the spectrum baseline subtraction may be performed according to a quantile normalization method. In a more preferred embodiment the quantile normalization method may be limited to between approximately 10% and 20%. In a more preferred embodiment the spectrum baseline subtraction may be performed wherein the m/z window is between approximately 10 and 100 Da.

[0048] In a preferred embodiment the m/z alignment may be performed with reference to a reference spectrum. In a further preferred embodiment the reference spectrum may be from *Escherichia coli* ATCC 25922. In a further preferred embodiment the m/z alignment may be performed with a spectrum index between approximately 1 and 5. In a further preferred embodiment the m/z alignment may be performed with a m/z window between approximately 5 and 1000 Da. In a further preferred embodiment the m/z alignment may be performed wherein the maximum m/z shift is between approximately 1 and 200 Da.

[0049] In a preferred embodiment, the method includes combining the protein profile spectra, for example protein profile spectra from one or more isolated endophyte colonies, and performing one or more of the steps of:

[0050] a m/z alignment;

[0051] a spectrum smoothing;

[0052] a m/z range restriction;

[0053] a spectrum peak detection; and

[0054] a valid peak filtration to remove peaks which do not meet a defined threshold.

[0055] In a preferred embodiment, the processed protein profiles may be converted into a matrix for analysis.

[0056] In a further preferred embodiment the m/z ratio alignment may be performed with reference to a reference spectrum. In a further preferred embodiment the m/z ratio alignment may be performed with reference to a reference spectrum from *Escherichia coli* ATCC 25922.

[0057] In a further preferred embodiment the m/z ratio alignment may be performed with a spectrum index between approximately 1 and 5. In a further embodiment spectrum smoothing may be performed according to a moving average algorithm. In a further preferred embodiment spectrum smoothing may be performed with a m/z window of approximately 4 to 30 points.

[0058] In a further preferred embodiment the m/z range may be between approximately 2000 and 20000 Da.

[0059] In a further preferred embodiment the spectrum peak detection may be performed by a resolution-based method. In a further preferred embodiment the boundary determination may be performed at maximum curvature peak filtering. In a further preferred embodiment the spectrum peak detection may be present in at least two experiments. In a further preferred embodiment the valid feature filter may have a threshold between approximately 0-40% intensity. In a further preferred embodiment the feature filter may be present in at least 2 experiments.

[0060] In a further embodiment the processed protein profile spectra may be used to perform hierarchal clustering. In a further embodiment the hierarchal clustering may be used to compare endophyte strains and, in particular, identify related endophyte strains.

[0061] In a preferred embodiment the hierarchal clustering provides a phenogram wherein endophyte strains are clustered based on similar protein profiles. In a further embodiment the hierarchical clustering provides a clade of endophytes having properties selected from:

[0062] i. related bioactivity;

[0063] ii. related geographic ranges; or

[0064] iii. belonging to plant lines having the same phenotype.

[0065] In another preferred embodiment the hierarchical clustering provides a clade of endophytes having properties selected from:

[0066] i. related bioactivity;

[0067] ii. related geographic ranges;

[0068] iii. belonging to plant lines having the same phenotype; or

[0069] iv. similar protein profiles.

[0070] In a further embodiment the related bioactivity may be selected from bioprotection and biofertilizer activity. In a preferred embodiment the bioprotection and biofertilizer properties may be the same or similar to those of *Xanthomonas* sp. bacterial strain GW.

[0071] By "bioactivity" or "bioactive properties" is meant the capacity of a compound to elicit pharmacological or toxicological effects in plants, humans or animals. In particular, plants may contain secondary compounds and metabolites with bioactive properties that are produced by endophytes.

[0072] For example, the bioactivity may be selected from bioprotection and biofertilizer activity. In a preferred embodiment the bioprotection and biofertilizer properties may be the same or similar to those of *Xanthomonas* sp. bacterial strain GW.

[0073] As used herein the term 'bioprotection and/or biofertilizer' means that the endophyte possesses genetic and/or metabolic characteristics that result in a beneficial phenotype in a plant harbouring, or otherwise associated with, the endophyte. Such beneficial properties include improved resistance to pests and/or diseases, improved tolerance to water and/or nutrient stress, enhanced biotic stress tolerance, enhanced drought tolerance, enhanced water use efficiency, reduced toxicity and enhanced vigour in the plant with which the endophyte is associated, relative to an organism not harbouring the endophyte or harbouring a control endophyte such as standard toxic (ST) endophyte.

[0074] The pests and/or diseases may include, but not limited to, fungal and bacterial pathogens. In a particularly preferred embodiment, the endophyte may result in the production of the bioprotectant compound in the organism with which it is associated.

[0075] As used herein, the term 'bioprotectant compound' means a compound that provides bioprotection to the plant or aids the defence of the plant with which it is associated against pests and/or diseases, such as fungal and/or bacterial pathogens. A bioprotectant compound may also be known as a 'biocidal compound'. In a particularly preferred embodiment, the endophyte produces a bioprotectant compound and provides bioprotection to the organism against fungal and/or bacterial pathogens. The terms bioprotectant, bioprotective and bioprotection (or any other variations) may be used interchangeably herein.

[0076] As used herein, a 'bioprotectant property' provides bioprotection to the plant or aids the defence of the plant against pests and/or diseases, such as fungal and/or bacterial pathogens. A bioprotectant compound may also be known as a 'biocidal compound'. In a particularly preferred embodiment, the endophyte produces a bioprotectant compound and provides bioprotection to the plant with which it is associated against fungal and/or bacterial pathogens. The terms bioprotectant, bioprotective and bioprotection (or any other variations) may be used interchangeably herein.

[0077] As used herein, a 'biofertilizer' improves the availability of nutrients to the plant with which the endophyte is associated, including but not limited to improved tolerance to nutrient stress.

[0078] The nutrient stress may be lack of or low amounts of a nutrient such as phosphate and/or nitrogen. The endophyte is capable of growing in conditions such as low nitrogen and/or low phosphate and enable these nutrients to be available to the plant with which the endophyte is associated.

[0079] In a further embodiment there is provided a method for clustering endophytes of the plant phenotype is for drought tolerance or drought resistance.

[0080] In a further embodiment the hierarchical clustering provides identification of a clade of endophytes of a group selected from:

[0081] i. multiple host species, wherein said host species is from multiple geographic locations;

[0082] ii. multiple host species, wherein said host species is from a single geographic location;

[0083] iii. one host species, wherein said host species is from multiple geographic locations; or

[0084] iv. one host species, wherein said host species is from a single geographic location.

[0085] In another aspect, the present invention provides a substantially purified or isolated endophyte strain, preferably selected and/or isolated using a method according to the present invention, as described herein.

[0086] In another aspect of the present invention there is provided a method for profiling a plant microbiome said method including the steps of:

[0087] providing plant material from a first plant species and plant material from a related second plant species;

[0088] characterising the microbiome of the first and second plant species by analysing the plant material; and

[0089] assessing the microbiome of the first and second plant species to identify endophyte strains found in both the first and second plant species or endophyte strains found in the second plant species but not in the first plant species.

[0090] In a preferred embodiment, the method of profiling a plant microbiome as described herein and the method of profiling endophyte strains from a microbiome as described herein may be performed sequentially.

[0091] In a preferred embodiment the first plant species may be selected from perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinaceae*), corn (*Zea mays*), *Glycine* species, wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*).

[0092] In a further preferred embodiment the first plant species may be selected from *Glycine tomentella, Glycine tabacina, Glycine latifolia, Glycine hirticaulis, Glycine microphylla, Glycine clandestine* and *Glycine Max.*

[0093] In a further embodiment the second plant species may be a crop wild relative (CWR) of the first plant species.

[0094] As used herein 'crop wild relative (CWR)' is meant plants that have not been domesticated and are genetically related to crop plants. These CWR may be used as a source of endophytes or alleles for crop plants that have been lost through their domestication. Accordingly, in a preferred embodiment, the present invention may include screening microbiomes of CWR, such as *Glycine* CWR, to identify endophytes that could be substituted for similar endophytes of the crop plant, or introduced into the crop plant, for example the crop plant *Glycine max* (soybean).

[0095] In a further embodiment providing the plant material from the first and second plant species includes the steps of:

[0096] sterilising the plant material; and

[0097] germinating the plant material,

wherein the germinated plant material provides seedlings for characterisation.

[0098] In a further preferred embodiment, the step of providing the plant material may include the steps of:

[0099] washing the plant material in an aqueous solution;

[0100] submerging the plant material in an aqueous solution;

[0101] macerating the plant material; and

[0102] applying the macerated plant material to a growth medium for growth of the microbiota to provide an isolated microbiome.

[0103] In a further preferred embodiment, when the plant material includes a seed, the step of providing the plant material may include the preliminary steps of:

[0104] harvesting the plant material;

[0105] sterilising the plant material;

[0106] germinating the plant material; and/or

[0107] growing the germinated plant material.

[0108] In a preferred embodiment the plant material may be from a grass, tree, flower, herb, shrub or bush, vine or legume, or a product thereof. The method according to the present invention is particularly applicable to grasses and legumes. In a further preferred embodiment the plant material may be selected from seeds, leaves, stems, petioles, roots, buds, flowers or any combination thereof.

[0109] In a further preferred embodiment, the aqueous solution may be a buffer solution. In a further preferred embodiment the buffer solution may be a phosphate buffered saline (PBS)

Solution

[0110] In a further embodiment characterising the microbiome of the first and second plant species includes the steps of:

[0111] extracting nucleic acid from the plant material; and

[0112] analysing the extracted nucleic acid to profile the plant material microbiome.

[0113] The steps of extracting nucleic acid and analysing the extracted nucleic acid as used herein may be performed by any suitable technique. In a particularly preferred embodiment analysis of the extracted nucleic acid may be performed by Unweighted UNIFRAC Distance Principal Components Analysis utilising operational taxomic units (OTU) phylogeny.

[0114] In a preferred embodiment, the microbiota grown on the growth medium may be subjected to a re-streaking so as to obtain an isolated endophyte colony.

[0115] In a preferred embodiment, the method may include obtaining protein profile spectra from one or more isolated endophyte colonies.

[0116] In a further preferred embodiment, the step of assessing the microbiome of the first and second plant species may include statistical analysis to determine microbiome operational taxomic units (OTU) present within each plant species.

[0117] As used herein 'operational taxomic units (OTU)' may include clusters of related organisms having similar physiological, bioactivity, genetic or peptide properties.

[0118] In a further preferred embodiment the microbiome OTU within the first plant species may be compared with that of the second plant species to determine microbiota which are either:

[0119] shared between the plant species, or

[0120] unique to the second plant species, relative to the first plant species.

[0121] In a preferred embodiment the identified microbiome OTU may be selected from the group including *Stenotrophomonas* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Holomonas* sp., *Enterobactereaceae* sp., *Pantoea* sp., *Burkholderiaceae* sp., *Ralstonia* sp., *Massilia* sp., *Herbaspirillum* sp., *Delftia* sp., *Curvibacter* sp., *Aquabacterium* sp., *Sphingomonas* sp., *Novosphingobium* sp., *Bradyrhizobium* sp., *Ochrobactrum* sp., *Methylobacterium* sp., *Lactobacillus* sp., *Staphylococcus* sp., *Bacillus* sp. and *Curtobacterium* sp.

[0122] In a further aspect of the present invention, there is provided a method for enhancing the bioactivity of a plant species, said method including:

[0123] identifying endophyte strains found in the first and second plant species; and

[0124] transferring one or more endophyte strains from the second plant species to the first plant species to enhance bioactivity of the first plant species,

wherein the transferred endophyte enhances bioactivity of the first plant species.

[0125] In a preferred embodiment, the step of identifying endophyte strains found in the first and second plant species may be performed by a method of profiling plant microbiome, as hereinbefore described.

[0126] In a further aspect of the present invention, there is provided a method for enhancing the bioactivity of a plant species, said method including:

[0127] identifying endophyte strains found in the second plant species but not in the first plant species, and

[0128] transferring one of said more endophyte strains from the second plant species to the first plant species,

wherein the transferred endophyte enhances bioactivity of the first plant species.

[0129] In a preferred embodiment, the step of identifying endophyte strains found in the second plant species but not in the first plant species may be performed by a method of profiling plant microbiome, as hereinbefore described.

[0130] By "enhancing the bioactivity of a first plant species" is meant that the first plant species has a beneficial phenotype relative to the same plant species not harbouring the transferred endophyte or harbouring a control endophyte such as standard toxic (ST) endophyte. Such beneficial properties include improved resistance to pests and/or diseases, improved tolerance to water and/or nutrient stress, enhanced biotic stress tolerance, enhanced drought tolerance, enhanced water use efficiency, reduced toxicity and enhanced vigour in the plant with which the endophyte is associated, relative to a plant not harbouring the endophyte or harbouring a control endophyte such as standard toxic (ST) endophyte.

[0131] In a preferred embodiment the microbiome may be isolated from plant material of the first and second plant species. The plant material may be of any suitable type. In

a preferred embodiment the plant material includes seeds, leaves, stems, petioles, roots, buds, flowers or any combination thereof.

[0132] By a "related second plant species" is meant a plant species sharing substantial genetic identity with the first plant species, for example greater than approximately 80% genetic identity, preferably greater than 90% genetic identity, more preferably greater than approximately 95% genetic identity, even more preferably greater than approximately 98% genetic identity.

[0133] In a preferred embodiment, the protein profile spectra may be obtained by mass spectrometry. In a further preferred embodiment, the mass spectrometry technique used to obtain the protein profile spectra may be matrix assisted laser desorption/ionisation (MALDI) mass spectrometry, as hereinbefore described.

[0134] The step of transferring one or more of said endophyte strains from the second plant species to the first plant species may be performed by any suitable technique. Preferably, the first plant species is infected with the endophyte by a method selected from the group consisting of inoculation, breeding, crossing, hybridization and combinations thereof.

[0135] In a further aspect, the present invention provides a plant, plant part or plant product with enhanced bioactivity produced by a method according to the present invention.

[0136] In another aspect, the present invention provides a substantially purified or isolated endophyte strain, preferably selected and/or isolated using a method according to the present invention, as described herein.

[0137] In a preferred embodiment, according to any aspect of the present invention, the endophyte strain may be a strain of *Xanthomonas* sp. which provides bioprotection and/or biofertilizer phenotypes to plants into which it is inoculated. In a preferred embodiment, the *Xanthomonas* sp. strain may be GW as described herein and as deposited with The National Measurement Institute of 1/153 Bertie Street, Port Melbourne, VIC 3207, Australia on 17 May 2019 with accession number V19/009902.

[0138] In another preferred embodiment the endophyte strain may be a strain of *Xanthomonas* sp. which provides enhanced bioactivity to plants into which it is inoculated.

[0139] In another preferred embodiment, according to any aspect of the present invention, the endophyte strain may be a strain of *Arthrobacter* sp. which provides bioprotection and/or biofertilizer phenotypes to plants into which it is inoculated. In a preferred embodiment, the *Arthrobacter* sp. strain may be a D4-11 strain as described herein and as deposited with The National Measurement Institute of 1/153 Bertie Street, Port Melbourne, VIC 3207, Australia on 9 Jul. 2019 with accession number V19/013680.

[0140] In another preferred embodiment the endophyte strain may be a strain of *Arthrobacter* sp. which provides enhanced bioactivity to plants into which it is inoculated.

[0141] In another preferred embodiment, according to any aspect of the present invention, the endophyte strain may be a strain of *Papiliotrema* sp. which provides bioprotection and/or biofertilizer phenotypes to plants into which it is inoculated. In a preferred embodiment, the *Papiliotrema* sp. strain may be a strain selected from the group consisting of P2-Gland-NS-Runn Creek IS-107-1, P1-Geland-NS-Mornington-IS-114-1, and P2-Geland-NS-Card Creek IS-34-1 strain as described herein and as deposited with The National Measurement Institute of 1/153 Bertie Street, Port Melbourne, VIC 3207, Australia on 9 Jul. 2019 with accession numbers V19/013679, V19/013678 and V19/013677, respectively.

[0142] In another preferred embodiment the endophyte strain may be a strain of *Papiliotrema* sp which provides enhanced bioactivity to plants into which it is inoculated.

[0143] In another preferred embodiment, according to any aspect of the present invention, the endophyte strain may be a strain of *Sphingomonas paucimobilis* which provides bioprotection and/or biofertilizer phenotypes to plants into which it is inoculated. In a preferred embodiment, the *Sphingomonas paucimobilis* strain may be Gtom-P2-19 as described herein and as deposited with The National Measurement Institute of 1/153 Bertie Street, Port Melbourne, VIC 3207, Australia on 9 Jul. 2019 with accession number V19/013676.

[0144] In another preferred embodiment the endophyte strain may be a strain of *Sphingomonas paucimobilis* which provides enhanced bioactivity to plants into which it is inoculated. In another preferred embodiment, according to any aspect of the present invention, the endophyte strain may be a strain of *Argobacterium lanymoorei* which provides bioprotection and/or biofertilizer phenotypes to plants into which it is inoculated. In a preferred embodiment, the *Argobacterium lanymoorei* strain may be a strain selected from Gcla-P1-10, and Gtab-P2-18 as described herein and as deposited with The National Measurement Institute of 1/153 Bertie Street, Port Melbourne, VIC 3207, Australia on 9 Jul. 2019 with accession numbers V19/013675 and V19/013671, respectively.

[0145] In another preferred embodiment the endophyte strain may be a strain of *Argobacterium lanymoorei* which provides enhanced bioactivity to plants into which it is inoculated.

[0146] In another preferred embodiment, according to any aspect of the present invention, the endophyte strain may be a strain of *Pseudomonas oryzihabitans* which provides bioprotection and/or biofertilizer phenotypes to plants into which it is inoculated. In a preferred embodiment, the *Pseudomonas oryzihabitans* strain may be a strain selected from Ghir-A-22-2, Gtab-P2-12 and Gtom-P2-14 as described herein and as deposited with The National Measurement Institute of 1/153 Bertie Street, Port Melbourne, VIC 3207, Australia on 9 Jul. 2019 with accession numbers V19/013672, V19/013674 and V19/013673, respectively.

[0147] In another preferred embodiment the endophyte strain may be a strain of *Pseudomonas oryzihabitans* which provides enhanced bioactivity to plants into which it is inoculated.

[0148] In this specification, the term 'comprises' and its variants are not intended to exclude the presence of other integers, components or steps.

[0149] In this specification, reference to any prior art in the specification is not and should not be taken as an acknowledgement or any form of suggestion that this prior art forms part of the common general knowledge in Australia or any other jurisdiction or that this prior art could reasonably expected to be combined by a person skilled in the art.

[0150] The present invention will now be more fully described with reference to the accompanying Examples and drawings. It should be understood, however, that the description following is illustrative only and should not be taken in any way as a restriction on the generality of the invention described above.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0151] In the Figures:

[0152] FIG. **1** shows the data deconvolution workflow and associated parameters (Refiner, Genedata) used to process raw MALDI spectra from multiple novel bacterial strains across multiple Batches, in order to align and smooth spectra, reduce background noise, and identify all valid protein peaks and their relative intensities.

[0153] FIG. **2** shows a region of the Hierarchical Clustering tree generated from protein spectra from novel bioactive bacterial strain GW and other *Xanthomonas* strains. The novel *Xanthomonas* sp. bacterial strain GW_0_F7_1 (V19/009902, star) is a representative strain of this clade (bioactive strain)

[0154] FIG. **3** shows the protein spectra of the three *Xanthomonas* sp. novel bacterial strains GW, SS and SI. A. Full chromatogram (2000-14000 Daltons). Proteins unique to either the novel bacteria strain GW (4646.0561) or SS (4494.1438) are evident within the boxed area.

[0155] FIG. **4** shows a region of the Hierarchical Clustering tree generated from protein spectra from novel *Pseudomonas oryzihabitans* bacterial strains isolated from three *Glycine* species, *Glycine tomentella* (Gtom designation), *Glycine hirticaulis* (Ghir designation) and *Glycine tabacina* (Gtab designation) from two locations, Kakadu National Park (NT), La Trobe University Wildlife Sanctuary (Victoria). The novel *Pseudomonas oryzihabitans* bacterial strains Ghir-A-22-2 (V19/013672, star) and Gtom-P2-14 (V19/013673, star) and Gtab-P2-12 (V19/013674, star) are representative strains of this clade (broad host range, broad geographic range).

[0156] FIG. **5** shows a region of the Hierarchical Clustering tree generated from protein spectra from novel *Agrobacterium lartymoorei* bacterial strains isolated from two *Glycine* species, *Glycine clandestina* (Gcla designation) and *Glycine tabacina* (Gtab designation) from one location, the La Trobe University Wildlife Sanctuary (Victoria). The novel *Agrobacterium lartymoorei* bacterial strains Gtab-P2-18 (V19/013671, star) and Gcla-P1-10 (V19/013675, star) are representative strains of this clade (broad host range, narrow geographic range).

[0157] FIG. **6** shows a region of the Hierarchical Clustering tree generated from protein spectra from fungal strains (*Papiliotrema* sp.) isolated from only *Glycine clandestina* from three locations, Mornington Peninsula National Park (Mornington designation), Cardinia Creek Parklands (Card_Creek designation) and Kinglake National Park: Running Creek (Runn_creek designation) (Victoria). The novel (*Papiliotrema* sp.) fungal strains P1-Gcland-NS-Card_Creek-IS-34-1 (V19/013677, star), P1-Gcland-NS-Mornington-IS-114-1 (V19/013678, star) and P2-Gcland-NS-Runn_creek-IS-107-1 (V19/013679, star) are representative strains of this clade (narrow host range, broad geographic range).

[0158] FIG. **7** shows a region of the Hierarchical Clustering tree generated from protein spectra from *Sphingomonas* bacterial strains isolated from only *Glycine tomentella* from one location, Kakadu National Park (NT). The novel *Sphingomonas paucimobilis* bacterial strain Gtom-P2-19 (V19/013676, star) is a representative strain of this clade (narrow host range, narrow geographic range).

[0159] FIG. **8**—Seed microbiome profiles of *Glycine clandestina, G. tabacina* and *G. max*, collected from various locations across the greater Melbourne region in Victoria, Australia.

[0160] FIG. **9**—*Sphingomonas* sp. clade from a Hierarchical Clustering analysis of MALDI protein spectra from strains isolated from *G. clandestina* (Gcland designation, light grey star) and *G. max* (Gmax designation, dark grey star), demonstrating a microbe that could potentially be transferred from one species to another.

[0161] FIG. **10** shows a region of the hierarchical tree generated from protein spectra from *Arthrobacter* bacterial strains isolated from drought tolerant wheat lines. The novel *Arthrobacter* sp. bacterial strain D4-11 (V19/013680, star) is a representative strain of this clade (associated with a plant phenotype—drought tolerance).

DETAILED DESCRIPTION OF THE EMBODIMENTS

Using Matrix Assisted Laser Desorption/Ionisation (MALDI) Mass Spectrometry for Plant Microbiome Profiling

[0162] The invention comprises methods for profiling plant bacterial and fungal microbiomes using MALDI. The bacterial and fungal microbiome is isolated from plants, and these microbes are identified and compared using MALDI. The spectra from bacterial and fungal strains are processed using novel filtering settings to produce validated spectra that are used for hierarchical clustering. Strains occupying the same clades of the tree shown to be phylogenetically related.

[0163] Applying this method allows the rapid identification of phylogenetically related microbes without the need to further process the microbes beyond their isolation, as whole colonies can be used for the MALDI spectra generation and comparison. The more time consuming and costly DNA sequencing can thus be targeted to microbes of interest.

[0164] This method can be used to specifically identify bacterial and fungal strains among a collection of isolated microbes that (i) are closely related to strains with bioactivity (e.g. bioprotection and biofertilizer properties); (ii) are closely related to strains with broad/narrow host ranges from broad/narrow geographic ranges; (iii) are closely related to strains from plant lines with a specific phenotype (e.g. drought tolerance).

Example 1—Microbe Isolation

[0165] Plant material (seeds, leaves, stems, petioles, roots, buds, flowers) was harvested from perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinaceae*), corn (*Zea mays*), *Glycine* species, wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*). Seed was surface sterilised, germinated and allowed to grow for up to 14 days prior to microbe isolation. All other plant material was washed in sterile distilled water or phosphate buffered saline (PBS) up to 5 times prior to microbe isolation. The plant material was submerged in sufficient PBS to completely cover the tissues, and ground using a Qiagen TissueLyser II, for 1 minute at 30 Hertz. A 10 μl aliquot of the macerate was added to 90 μl of PBS. Subsequent 1 in 10 dilutions of the 10^{-1} suspension were used to create additional 10^{-2} to 10^{-4} suspensions. Once the suspensions were well mixed 50 μl aliquots of each

suspension were plated onto Reasoners 2 Agar (R2A) for growth of bacteria and fungi. Dilutions that provided a good separation of microbial colonies were subsequently used for isolation of individual colonies through re-streaking of single colonies from the dilution plates onto single R2A plates to establish a pure colony.

Example 2—Spectra Acquisition, Processing and Analysis

Spectra Acquisition

[0166] MALDI spectra were acquired for all novel bacterial and fungal strains to determine the relatedness of each strain. The analysis acquired and compared spectra of protein profiles from each novel bacterial and fungal strain using the Bruker MALDI Biotyper system. Single bacterial and fungal colonies of each strain were generated through streaking from glycerol stocks onto R2A plates and allowing colony growth for 48 hours. Single bacterial and fungal colonies were applied to a Bruker MALDI Biotyper target plate using the Extended Direct Transfer (EDT) method. In the EDT method novel bacterial and fungal strains were inoculated on to two consecutive wells on the target plate (primary spot and secondary spot), treated with 70% formic acid (for up to 30 mins) and covered with HCCA (α-cyano-4-hydroxycinnamic acid) matrix solution [10 mg HCAA in 1 mL of solvent solution: 50% volume μL ACN (acetonitrile), 47.5% volume μL water, and 2.5% volume μL TFA (trifluoroacetic acid)]. The plate was dried at room temperature. *Escherichia coli* strain ATCC 25922 was included as a quality control. The target plate was analysed in a Bruker MALDI-TOF ultrafleXtreme according to manufacturer's instructions. Protein spectra were calibrated with the *Escherichia coli* ATCC 25922 quality control strain, and an internal standard. Automated analysis of the raw spectral data was performed by the MALDI BioTyper automation 2.0 software (Bruker Daltonics) using default settings. Protein spectra were compared to MALDI BioTyper library (3,746 spectra—Jun. 9, 2010) for preliminary identification and taxonomical assignment.

Spectra Processing

[0167] The raw protein spectra from each novel bacterial and fungal strain were processed through a data deconvolution workflow in the software Refiner, GeneData. The raw spectra from each plate (i.e. Batch) were processed separately, first by aligning spectra to create a m/z grid (m/z× sample), followed by spectrum baseline subtraction to reduce background noise across the grid, and finally aligning m/z across key reference spectra from the grid (e.g. *E. coli* ATCC 25922) (FIG. **1**). Batches were then merged and processed further, first by again aligning m/z across key reference spectra (e.g. *E. coli* ATCC 25922) from the grid, followed by spectrum smoothing to reduce intensity jitter of putative peaks, then restricting m/z from a defined range, then detecting spectrum peaks using a resolution-based method, and finally filtering valid peaks by removing those that did not meet specific thresholds. Parameters for the data deconvolution workflow are defined in FIG. **1**. The resultant processed data of valid peaks and intensities was converted into a matrix for statistical analysis.

Spectra Analysis

[0168] The matrix was analysed in the software Analyst, Genedata. A Hierarchical Clustering analysis was conducted to compare protein spectra between novel bacterial and fungal strains. The analysis utilised the Positive Correlation (1-r) distance algorithm, with complete linkage, and only included values present in 50% of samples. A Hierarchical Clustering tree was generated whereby novel bacterial and fungal strains clustered based on similar protein profiles.

Example 3—Use of MALDI Spectra to Identify Closely Related Bacterial Strains to Bioactive Strains

[0169] A method has been developed that uses MALDI to compare protein spectra from bioactive bacterial strains with other bacterial strains isolated from the same host plant, to identify closely related strains that may possess equivalent or superior bioactivity.

[0170] A total of 600 bacterial strains were isolated from seed, leaves and roots of perennial ryegrass (*Lolium perenne*). The novel *Xanthomonas* sp. bacterial strain GW isolated from seed was demonstrated to have bioprotectant and biofertilizer activity. The protein profiles of all bacterial strains were acquired, processed and analysed to determine their phylogenetic relatedness, including strains that were closely related to the novel bioactive *Xanthomonas* sp. bacterial strain GW.

[0171] The novel bioactive *Xanthomonas* sp. bacterial strain GW clustered with 17 additional strains in the phenogram. Some of these strains were isolated from seeds, while others were isolated from leaves and roots from mature plants. These strains included SS, SI, SM, GN, X, GU, HI, GJ, GM, LK, NC, NG, GY, HA, GKc, GKb, UT. The GW containing clade of the Hierarchical Clustering tree was referred to as a *Xanthomonas* clade (FIG. **2**). An assessment of the protein spectra of *Xanthomonas* sp. strains (GW, SS and SI) indicated that the profiles were highly similar, differing with respect to proteins, 3953.8004 Daltons (unique to strain GW), 4497.4911 Daltons (Unique to strain SS) and 12741.7201 Daltons (Unique to strains SS and SI) (FIG. **3**).

[0172] The 17 additional strains are candidates for further characterisation to determine if they possess equivalent or superior bioactivity.

Example 4—Use of MALDI Spectra to Identify Bacterial Strains Common within a Plant Genera (Host Range) and Across Diverse Geographic Locations (Geographic Range)

[0173] A method has been developed that uses MALDI to compare protein spectra from bacterial and fungal strains isolated from within a plant genera and from a diverse geographic locations, to identify closely related bacterial and fungal strains that (i) are specific to multiple host species from diverse locations (i.e. broad host range, broad geographic range), (ii) are specific to multiple host species from a single location (i.e. broad host range, narrow geographic range), (iii) are specific to one host species from diverse locations (i.e. narrow host range, broad geographic range) or (iv) are specific to one host species from a single location (i.e. narrow host range, narrow geographic range).

[0174] A total of 331 bacterial and fungal strains were isolated from seeds of soybean (*Glycine max*) and six native *Glycine* species (*Glycine* tomentella, *Glycine* tabacina, *Glycine* latifolia, *Glycine* hirticaulis, *Glycine* microphylla, *Glycine clandestina*). The protein profiles of all bacterial and

fungal strains were acquired, processed and analysed to determine their phylogenetic relatedness.

Broad Host Range, Broad Geographic Range

[0175] A clade was identified that contained closely related *Pseudomonas oryzihabitans* bacterial strains, that were isolated from three *Glycine* species, *Glycine* tomentella, *Glycine hirticaulis* and *Glycine tabacina* (FIG. **4**). The bacterial strains were isolated across a range of locations throughout Australia—Kakadu National Park (Northern Territory, NT), La Trobe University Wildlife Sanctuary (Victoria). These bacterial strains all had spectra with identical proteins, differing only in the intensity of those proteins. This clade represents closely related bacterial strains with a broad host range and from a broad geographic range.

Broad Host Range, Narrow Geographic Range

[0176] A clade was identified that contained closely related *Agrobacterium larrymoorei* bacterial strains that were isolated from two *Glycine* species, *Glycine clandestina* and *Glycine tabacina* (FIG. **5**). The bacterial strains were isolated from one location in Victoria—La Trobe University Wildlife Sanctuary. These bacterial strains all had spectra with identical proteins, differing only in the intensity of those proteins. This clade represents closely related bacterial strains with a broad host range and from a narrow geographic range.

Narrow Host Range, Broad Geographic Range

[0177] A clade was also identified that contained closely related fungal strains (*Papiliotrema* sp.), that were isolated from only *Glycine clandestina* (FIG. **6**). The fungal strains were isolated across a range of locations throughout Victoria—Mornington Peninsula National Park, Cardinia Creek Parklands and Kinglake National Park: Running Creek. These fungal strains had spectra with identical proteins, differing only in the intensity of those proteins. This clade represents closely related fungal strains with a narrow host range and from a broad geographic range.

Narrow Host Range, Narrow Geographic Range

[0178] A clade was also identified that contained closely related *Sphingomonas* bacterial strains that were isolated from only *Glycine tomentella* (FIG. **7**). The bacterial strains were isolated from one location, Kakadu National Park (NT). These bacterial strains all had spectra with identical proteins, differing only in the intensity of those proteins. This clade represents closely related bacterial strains with a narrow host range and from a narrow geographic range.

Example 5—Use of MALDI to Identify Bacterial Strains Exclusive to a Plant Phenotype

[0179] A method has been developed that uses MALDI to compare protein spectra from bacterial strains isolated plant lines exhibiting different phenotypes, to identify closely related bacterial strains that are exclusive to a specific phenotype.

[0180] A total of 580 bacterial strains were isolated from seeds of 11 wheat (*Triticum aestivum*) lines that were phenotyped as either drought tolerant or drought resistant. The protein profiles of all bacterial strains were acquired, processed and analysed to determine their phylogenetic relatedness.

[0181] A clade was identified that contained closely related *Arthrobacter* bacterial strains that were exclusive to the drought tolerant lines (FIG. **8**). These bacterial strains all had spectra with identical proteins, differing only in the intensity of those proteins. This clade represents closely related bacterial strains that are exclusive to a plant phenotype.

[0182] Finally, it is to be understood that various alterations, modifications and/or additions may be made without departing from the spirit of the present invention as outlined herein.

Example 6—Profiling the *Glycine* Seed Microbiome

[0183] The seed microbiome of *Glycine* CWR was compared to commercial *G. max* to determine similarities across the species. The core microbiome of *G. clandestina* was identified and compared to *G. max* to identify operational taxonomic units (OTUs) present in (Scenario I) both *Glycine* CWRs and *G. max*, in an effort to identify strains that could be transferred from one species to another, which could potentially offer enhanced bioactivity, or (Scenario II) bacteria that were only found in *Glycine* CWRs that could be introduced into *G. max*, which could potentially offer novel bioactivity. The process was validated by isolating strains from *Glycine* CWRs and *G. max*, which were taxonomically identified and compared using MALDI-TOF MS to determine if there were strains that clustered according to Scenario I or Scenario II. Candidate microbes were identified that fulfilled Scenario I, and the putative identities of microbes that fulfil criteria were identified within the core microbiome of *G. clandestina*.

[0184] Seed from *Glycine* max, *Glycine* clandestina, *Glycine* hirticaulis, *Glycine* tomentella, *Glycine tabacina*, and *Glycine microphylla* was collected across Victoria and the Northern Territory, and stored at room temperature (22-24° C.). The seed were washed 10 times with excess amount of sterile distilled water in sterile conditions. All the *Glycine* CWR seeds were then scarified using sterile scalpel blade to initiate the process of water absorption. Seeds of both *Glycine max* and CWR were germinated in large (12 cm diameter) sterile petri dishes containing three layers of sterile Whatman™ paper (two on bottom and one layer on top of seeds). Under aseptic conditions, 10-20 seeds were placed into each dish followed by the addition of 5-7 ml of sterile distilled water. The petri dishes were sealed with Parafilm™ and incubated first for 2-3 days in dark at room temperature. Once the seed germinated the top layer of filter paper was removed aseptically and petri dishes were then resealed with Parafilm™ allowed further to grow for 10-12 days. Once the seedlings were of sufficient size, a total of 16 seedlings were harvested per *Glycine* species and per location. DNA extraction of seedlings was performed in 96-well plates using the QIAGEN MagAttract 96 DNA Plant Core Kit according to manufacturers' instructions with minor modifications for use with a Biomek FX liquid handling station. The bacterial microbiome was profiled targeting the V4 region (515F and 806R) of the 16S rRNA gene according to the Illumina 16S Metagenomic Sequencing Library Preparation protocol, with minor modifications to include the use of PNA PCR blockers to reduce amplification of 16S

rRNA genes sequences derived from the plant chloroplast genome and mitochondrial genome (Wagner et al., 2016). Paired-end sequencing was performed on HiSeq3000 using a 2×150 bp v3 chemistry cartridge. Sequence data was trimmed and merged using PandaSEQ (removal of low quality reads, 8 bp overlap of read 1 and read 2, removal of primers, final merged read length of 253 bp) (Massela et al., 2012). QIIME2 (release 2019.4) was used for dereplication for taxonomy assignment, removal of organelle OTUs, and statistical analysis (multivariate statistics for qualitative and quantitative OTU analysis; presence/absence searches for core microbiome analysis).

sp., *Curvibacter* sp., *Aquabacterium* sp., *Sphingomonas* sp., *Novosphingobium* sp., *Bradyrhizobium* sp., *Ochrobactrum* sp., *Methylobacterium* sp., *Lactobacillus* sp., *Staphylococcus* sp., *Bacillus* sp. and *Curtobacterium* sp. (Table 1). Of these, a total of 14 OTUs were also present in *G. max*, with these OTUs representing microbes that could potentially be transferred between the two species. In addition, there were 8 OTUs that were absent from *G. max*, with these OTUs representing microbes that could potentially be introduced into *G. max*. These included *Stenotrophomonas* sp., *Acinetobacter* sp., *Holomonas* sp., *Pantoea* sp., *Curvibacter* sp., *Aquabacterium* sp., *Novosphingobium* sp. and *Bradyrhizobium* sp.,

TABLE 1

Core seed microbiome of *G. clandestina*, and assessment of their presence in *G. max*. OTUs in bold are present in both *G. clandestina* and *G. max* and represent microbes that could potentially be transferred between species, while OTUs in underline are only from *G. clandestina* and represent microbes that could potentially be introduced into *G. max*.

	Glycine clandestina						
Bacterial genera	Butterfield Wildlife Reserve	Cardinia Creek	Dandenong Ranges National Park	Mornington Peninsula National Park	Running Creek Road	Wandin Yallock Creek Reserve	*Glycine max* New South Wales
Curtobacterium sp.	**0.081%**	**0.053%**	**20.837%**	**1.288%**	**0.900%**	**0.006%**	**0.023%**
Bacillus sp.	**5.259%**	**2.229%**	**0.315%**	**0.058%**	**2.061%**	**0.146%**	**0.862%**
Staphylococcus sp.	**1.507%**	**0.198%**	**0.014%**	**0.024%**	**1.242%**	**0.021%**	**0.020%**
Lactobacillus sp.	**0.662%**	**0.234%**	**0.002%**	**1.257%**	**1.185%**	**0.011%**	**0.001%**
Methylobacterium sp.	**0.272%**	**0.006%**	**5.367%**	**0.247%**	**0.700%**	**0.000%**	**0.280%**
Ochrobactrum sp.	**0.253%**	**0.177%**	**0.024%**	**0.549%**	**0.155%**	**0.001%**	**0.019%**
Bradyrhizobium sp.	0.051%	0.006%	0.023%	0.584%	0.088%	0.001%	0.000%
Novosphingobium sp.	0.027%	0.002%	0.024%	0.522%	0.018%	0.000%	0.000%
Sphingomonas sp.	**2.309%**	**44.282%**	**42.417%**	**1.514%**	**2.075%**	**1.791%**	**2.132%**
Aquabacterium sp.	0.152%	0.503%	0.054%	2.208%	0.450%	0.004%	0.000%
Curvibacter sp.	0.172%	0.309%	0.014%	1.167%	0.872%	0.004%	0.000%
Delftia sp.	**3.926%**	**1.781%**	**0.627%**	**18.702%**	**9.746%**	**0.018%**	**0.224%**
Herbaspirillum sp.	**0.737%**	**0.332%**	**0.155%**	**1.851%**	**0.587%**	**0.004%**	**0.045%**
Massilia sp.	**9.919%**	**0.090%**	**0.867%**	**4.367%**	**5.578%**	**0.001%**	**1.387%**
Ralstonia sp.	**0.845%**	**2.128%**	**0.247%**	**3.509%**	**0.812%**	**0.005%**	**0.043%**
Burkholderiaceae	**0.445%**	**0.670%**	**0.195%**	**5.778%**	**5.947%**	**0.002%**	**0.443%**
Pantoea sp.	4.810%	3.705%	0.670%	24.130%	15.848%	0.058%	0.000%
Enterobacteriaceae	**5.621%**	**0.780%**	**0.537%**	**6.228%**	**2.276%**	**0.010%**	**0.093%**
Halomonas sp.	0.002%	0.080%	0.007%	0.199%	0.063%	0.000%	0.000%
Acinetobacter sp.	0.191%	0.480%	0.034%	1.854%	2.764%	0.010%	0.000%
Pseudomonas sp.	**52.354%**	**35.410%**	**1.906%**	**18.715%**	**13.147%**	**97.855%**	**43.240%**
Stenotrophomonas sp.	0.201%	0.001%	0.030%	0.594%	0.320%	0.000%	0.000%

[0185] Seed microbiomes were assessed from *G. clandestina* accessions collected from 6 locations across the greater Melbourne region of Victoria and compared to *G. tabacina* (one accession—greater Melbourne region) and *G. max* (one accession—NSVV). The comparison utilised Unweighted UNIFRAC Distance Principal Components Analysis, which is a qualitative assessment that utilises OTU phylogeny (FIG. **8**). The microbiomes of *G. clandestina* and *G. max* were similar with replicates clustering together, particularly replicates from *G. max* and *G. clandestina* (Dandenong National Park), indicating the phylogeny of OTUs within the microbiomes were similar. The seed microbiome of *G. tabacina* was different from *G. max* and *G. clandestina* forming a distinct cluster. An assessment of the core microbiome of *G. clandestina* identified 22 OTUs present in seed accessions from the six locations, including *Stenotrophomonas* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Holomonas* sp., *Enterobactereaceae* sp., *Pantoea* sp., *Burkholderiaceae* sp., *Ralstonia* sp., *Massilia* sp., *Herbaspirillum* sp., *Delftia*

Example 7—Isolation and Characterisation of the *Glycine* Microbiome

[0186] Seed from the 6 *Glycine* spp. from Victorian and the Northern Territory were washed and germinated as per Example 6. Seed was harvested by removing aerial tissue and root tissue, and discarding the seed coat. The plant tissues were submerged in Phosphate Buffered Saline (PBS) to cover the plant tissue, and ground using a sterile micropestle or Qiagen TissueLyser II, for 1 minute at 30 Hertz. A 10 µl aliquot of the macerate was added to 90 µl of PBS. Subsequent 1 in 10 dilutions of the 10^{-1} suspension were used to create additional 10^{-2} to 10^{-4} suspensions. Once the suspensions were well mixed 20 µl aliquots of each suspension were plated onto Reasoners 2 Agar (R2A) for growth of bacteria. Dilutions that provided a good separation of bacterial colonies were subsequently used for isolation of individual bacterial colonies through re-streaking of single bacterial colonies from the dilution plates onto single R2A plates to establish a pure bacterial colony. Around 400 bacterial strains were obtained from sterile seedlings.

[0187] MALDI spectra were acquired for all novel bacterial and fungal strains to determine the relatedness of each strain. The analysis acquired and compared spectra of protein profiles from each novel bacterial and fungal strain using the Bruker MALDI Biotyper system. Single bacterial and fungal colonies of each strain were generated through streaking from glycerol stocks onto R2A plates and allowing colony growth for 48 hours. Single bacterial and fungal colonies were applied to a Bruker MALDI Biotyper target plate using the Extended Direct Transfer (EDT) method. In the EDT method novel bacterial and fungal strains were inoculated on to two consecutive wells on the target plate (primary spot and secondary spot), treated with 70% formic acid (for up to 30 mins) and covered with HCCA (α-cyano-4-hydroxycinnamic acid) matrix solution [10 mg HCAA in 1 mL of solvent solution: 50% volume μL ACN (acetonitrile), 47.5% volume μL water, and 2.5% volume μL TFA (trifluoroacetic acid)]. The plate was dried at room temperature. *Escherichia coli* strain ATCC 25922 was included as a quality control. The target plate was analysed in a Bruker MALDI-TOF ultrafleXtreme according to manufacturer's instructions. Protein spectra were calibrated with the *Escherichia coli* ATCC 25922 quality control strain, and an internal standard. Automated analysis of the raw spectral data was performed by the MALDI BioTyper automation 2.0 software (Bruker Daltonics) using default settings. Protein spectra were compared to MALDI BioTyper library (3,746 spectra—Jun. 9, 2010) for preliminary identification and taxonomical assignment. The raw protein spectra from each novel bacterial and fungal strain were processed through a data deconvolution workflow in the software Refiner, GeneData. The raw spectra from each plate (i.e. Batch) were processed separately, first by aligning spectra to create a m/z grid (m/z×sample), followed by spectrum baseline subtraction to reduce background noise across the grid, and finally aligning m/z across key reference spectra from the grid (e.g. *E. coli* ATCC 25922). Batches were then merged and processed further, first by again aligning m/z across key reference spectra (e.g. *E. coli* ATCC 25922) from the grid, followed by spectrum smoothing to reduce intensity jitter of putative peaks, then restricting m/z from a defined range, then detecting spectrum peaks using a resolution-based method, and finally filtering valid peaks by removing those that did not meet specific thresholds. The resultant processed data of valid peaks and intensities was converted into a matrix for statistical analysis. The matrix was analysed in the software Analyst, Genedata. A Hierarchical Clustering analysis was conducted to compare protein spectra between novel bacterial and fungal strains. The analysis utilised the Positive Correlation (1-r) distance algorithm, with complete linkage, and only included values present in 50% of samples. A Hierarchical Clustering tree was generated whereby novel bacterial and fungal strains clustered based on similar protein profiles.

[0188] The Hierarchical Clustering tree was assessed for clades that contained strains (i) common to both *G. clandestine* and *G. max* that could be transferred to both species, and (ii) unique to *G. clandestine* that could be introduced to *G. max*. A clade was identified containing *Sphingomonas* sp. strains isolated from both *G. clandestine* and *G. max*, which is a bacterial species identified in the core microbiome of *G. clandestine* and *G. max* (FIG. **9**).

Example 8—Use of MALDI to Identify Bacterial Strains Exclusive to a Plant Phenotype

[0189] A method has been developed that uses MALDI to compare protein spectra from bacterial strains isolated plant lines exhibiting different phenotypes, to identify closely related bacterial strains that are exclusive to a specific phenotype.

[0190] A total of 580 bacterial strains were isolated from seeds of 11 wheat (*Triticum aestivum*) lines that were phenotyped as either drought tolerant or drought resistant. The protein profiles of all bacterial strains were acquired, processed and analysed to determine their phylogenetic relatedness.

[0191] A clade was identified that contained closely related *Arthrobacter* bacterial strains that were exclusive to the drought tolerant lines (FIG. **10**). These bacterial strains all had spectra with identical proteins, differing only in the intensity of those proteins. This clade represents closely related bacterial strains that are exclusive to a plant phenotype.

[0192] Finally, it is to be understood that various alterations, modifications and/or additions may be made without departing from the spirit of the present invention as outlined herein.

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

INTERNATIONAL FORM

TO:
Professor German Carlos Spangenberg
AgriBio, Centre for AgriBioscience,
5 Ring Road, La Trobe University,
Bundoora, Victoria 3083, Australia

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:
***Xanthomonas sp.* (GW)**	**V19/009902**

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 **17th May 2019** (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: **21st May 2019**

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

Appendix 3
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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO:
Professor German Carlos Spangenberg
AgriBio, Centre for AgriBioscience
5 Ring Road, La Trobe University,
Bundoora, Victoria, Australia 3083

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

I DEPOSITOR	II IDENTIFICATION OF THE MICROORGANISM
Name: **Professor German Carlos Spangenberg** Address: **AgriBio, Centre for AgriBioscience** **5 Ring Road, La Trobe University,** **Bundoora, Victoria, Australia 3083**	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **V19/009902** Date of the deposit or of the transfer [1]: **17th May 2019**
III VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on **17th May 2019** [2] On that date, the said microorganism was ☒ [3] viable ☐ [3] no longer viable	

1. Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2. In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

3. Mark with a cross the applicable box.

Appendix 3
page 22

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED [4]	
Deposit was grown on R2A (agar) at 21^{0}C for 24-48 hours	
V. INTERNATIONAL DEPOSITORY 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 authorized official(s) **Dean Clarke** Date: **21st May 2019**

4 Fill in if the information has been requested and if the results of the test were negative

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

INTERNATIONAL FORM

TO:
Attention German Spangenberg
AgriBio, Centre for AgriBioscience
5 Ring Road, La Trobe University,
Bundoora, Victoria, 3083, Australia

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:
***Argobacterium larrymoorei* (Gtab-P2-18)**	**V19/013671**

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 July 2019** (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: **12th July 2019**

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

Appendix 3
page 21

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

INTERNATIONAL FORM

TO:

Attention: German Spangenberg
AgriBio, Centre for AgriBioscience
5 Ring Road, La Trobe University,
Bundoora, Victoria, 3083, Australia

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

I DEPOSITOR	II IDENTIFICATION OF THE MICROORGANISM
Name: **German Spangenberg** Address: AgriBio, Centre for AgriBioscience 5 Ring Road, La Trobe University, Bundoora, Victoria, 3083, Australia	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **V19/013671** Date of the deposit or of the transfer [1]: **9th July 2019**
III VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on [2] **9th July 2019** On that date, the said microorganism was ☒ [3] viable ☐ [3] no longer viable	

1. Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2. In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

3. Mark with a cross the applicable box.

Appendix 3
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IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED [4]	
Deposit was grown on Reasoner's 2 Agar (R2A) at 21°C for 3 days.	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: NATIONAL MEASUREMENT INSTITUTE (FORMERLY AGAL) Address: 1/153 BERTIE STREET, PORT MELBOURNE VIC AUSTRALIA 3207 Phone: +61 3 9644 4837 Facsimile: +61 3 9644 4863	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) **Dean Clarke** Date: **12th July 2019**

4 Fill in if the information has been requested and if the results of the test were negative

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

INTERNATIONAL FORM

TO:
Attention German Spangenberg
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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:
***Pseudomonas oryzihabitans* (Ghir-A-22-2)**	**V19/013672**

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 Depositary Authority accepts the microorganism identified under I above, which was received by it on **9th July 2019** (date of the original deposit) [1]

IV RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary 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: **12th July 2019**

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
FOR THE PURPOSES OF PATENT PROCEDURE

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TO:
Attention: German Spangenberg
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VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
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I DEPOSITOR	II IDENTIFICATION OF THE MICROORGANISM
Name: **German Spangenberg** Address: AgriBio, Centre for AgriBioscience 5 Ring Road, La Trobe University, Bundoora, Victoria, 3083, Australia	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **V19/013672** Date of the deposit or of the transfer [1]: **9th July 2019**
III VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on [2] **9th July 2019** On that date, the said microorganism was ☒ [3] viable ☐ [3] no longer viable	

2. Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2. In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

3. Mark with a cross the applicable box.

Appendix 3
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IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED [4]	
Deposit was grown on Reasoner's 2 Agar (R2A) at 21°C for 3 days.	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: NATIONAL MEASUREMENT INSTITUTE (FORMERLY AGAL) Address: 1/153 BERTIE STREET, PORT MELBOURNE VIC AUSTRALIA 3207 Phone: +61 3 9644 4837 Facsimile: +61 3 9644 4863	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) **Dean Clarke** Date: **12th July 2019**

4 Fill in if the information has been requested and if the results of the test were negative

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

INTERNATIONAL FORM

TO:
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RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
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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:
***Pseudomonas oryzihabitans* (Gtom-P2-14)**	**V19/013673**

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 July 2019** (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: **12th July 2019**

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
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VIABILITY STATEMENT
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INTERNATIONAL DEPOSITARY AUTHORITY
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I DEPOSITOR	II IDENTIFICATION OF THE MICROORGANISM
Name: **German Spangenberg** Address: AgriBio, Centre for AgriBioscience 5 Ring Road, La Trobe University, Bundoora, Victoria, 3083, Australia	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **V19/013673** Date of the deposit or of the transfer [1]: **9th July 2019**
III VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on [2] **9th July 2019** On that date, the said microorganism was . ☒ [3] viable ☐ [3] no longer viable	

3. Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2. In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

3. Mark with a cross the applicable box.

Appendix 3
page 22

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED [4]	
Deposit was grown on Reasoner's 2 Agar (R2A) at 21°C for 3 days.	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: NATIONAL MEASUREMENT INSTITUTE (FORMERLY AGAL) Address: 1/153 BERTIE STREET, PORT MELBOURNE VIC AUSTRALIA 3207 Phone: +61 3 9644 4837 Facsimile: +61 3 9644 4863	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) **Dean Clarke** Date: **12th July 2019**

4 Fill in if the information has been requested and if the results of the test were negative

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

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I IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY:
***Pseudomonas oryzihabitans* (Gtab-P2-12)**	**V19/013674**

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 July 2019** (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: **12th July 2019**

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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I DEPOSITOR	II IDENTIFICATION OF THE MICROORGANISM
Name: **German Spangenberg** Address: AgriBio, Centre for AgriBioscience 5 Ring Road, La Trobe University, Bundoora, Victoria, 3083, Australia	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **V19/013674** Date of the deposit or of the transfer [1]: **9th July 2019**
III VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on [2] **9th July 2019** On that date, the said microorganism was ☒ [3] viable ☐ [3] no longer viable	

4. Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2. In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

3. Mark with a cross the applicable box.

Appendix 3
page 22

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED [4]	
Deposit was grown on Reasoner's 2 Agar (R2A) at 21°C for 3 days.	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: NATIONAL MEASUREMENT INSTITUTE (FORMERLY AGAL) Address: 1/153 BERTIE STREET, PORT MELBOURNE VIC AUSTRALIA 3207 Phone: +61 3 9644 4837 Facsimile: +61 3 9644 4863	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) **Dean Clarke** Date: **12th July 2019**

4 Fill in if the information has been requested and if the results of the test were negative

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

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Bundoora, Victoria, 3083, Australia

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
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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:
***Argobacterium larrymoorei* (Gcla-P1-10)**	**V19/013675**

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 July 2019** (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: **12th July 2019**

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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VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

I DEPOSITOR	II IDENTIFICATION OF THE MICROORGANISM
Name: **German Spangenberg** Address: AgriBio, Centre for AgriBioscience 5 Ring Road, La Trobe University, Bundoora, Victoria, 3083, Australia	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **V19/013675** Date of the deposit or of the transfer [1]: **9th July 2019**
III VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on [2] **9th July 2019** On that date, the said microorganism was ☒ [3] viable ☐ [3] no longer viable	

5. Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2. In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

3. Mark with a cross the applicable box.

Appendix 3
page 22

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED [4]	
Deposit was grown on Reasoner's 2 Agar (R2A) at 21°C for 3 days.	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: NATIONAL MEASUREMENT INSTITUTE (FORMERLY AGAL) Address: 1/153 BERTIE STREET, PORT MELBOURNE VIC AUSTRALIA 3207 Phone: +61 3 9644 4837 Facsimile: +61 3 9644 4863	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) **Dean Clarke** Date: **12th July 2019**

4 Fill in if the information has been requested and if the results of the test were negative

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

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TO:
Attention German Spangenberg
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RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
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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:
***Sphingomonas paucimobilis* (Gtom-P2-19)**	**V19/013676**

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 July 2019** (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: **12th July 2019**

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
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VIABILITY STATEMENT
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INTERNATIONAL DEPOSITARY AUTHORITY
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I DEPOSITOR	II IDENTIFICATION OF THE MICROORGANISM
Name: **German Spangenberg** Address: AgriBio, Centre for AgriBioscience 5 Ring Road, La Trobe University, Bundoora, Victoria, 3083, Australia	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **V19/013676** Date of the deposit or of the transfer [1]: **9th July 2019**
III VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on [2] **9th July 2019** On that date, the said microorganism was ☒ [3] viable ☐ [3] no longer viable	

6. Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2. In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

3. Mark with a cross the applicable box.

Appendix 3
page 22

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED [4]	
Deposit was grown on Reasoner's 2 Agar (R2A) at 21°C for 3 days.	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: NATIONAL MEASUREMENT INSTITUTE (FORMERLY AGAL) Address: 1/153 BERTIE STREET, PORT MELBOURNE VIC AUSTRALIA 3207 Phone: +61 3 9644 4837 Facsimile: +61 3 9644 4863	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) **Dean Clarke** Date: **12th July 2019**

4 Fill in if the information has been requested and if the results of the test were negative

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
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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:
***Papiliotrema sp.* (P2-Gcland-NS-Card Creek IS-34-1)**	**V19/013677**

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 July 2019** (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: **12th July 2019**

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
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TO:
Attention: German Spangenberg
AgriBio, Centre for AgriBioscience
5 Ring Road, La Trobe University,
Bundoora, Victoria, 3083, Australia

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

I DEPOSITOR	II IDENTIFICATION OF THE MICROORGANISM
Name: **German Spangenberg** Address: AgriBio, Centre for AgriBioscience 5 Ring Road, La Trobe University, Bundoora, Victoria, 3083, Australia	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **V19/013677** Date of the deposit or of the transfer [1]: **9th July 2019**
III VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on [2] **9th July 2019** On that date, the said microorganism was ☒ [3] viable ☐ [3] no longer viable	

7. Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2. In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

3. Mark with a cross the applicable box.

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IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED [4]	
Deposit was grown on Reasoner's 2 Agar (R2A) at 21°C for 3 days.	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: NATIONAL MEASUREMENT INSTITUTE (FORMERLY AGAL) Address: 1/153 BERTIE STREET, PORT MELBOURNE VIC AUSTRALIA 3207 Phone: +61 3 9644 4837 Facsimile: +61 3 9644 4863	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) **Dean Clarke** Date: **12th July 2019**

4 Fill in if the information has been requested and if the results of the test were negative

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

INTERNATIONAL FORM

TO:
Attention German Spangenberg
AgriBio, Centre for AgriBioscience
5 Ring Road, La Trobe University,
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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:
***Papiliotrema sp.* (Pl-Gcland-NS-Mornington-IS-114-1**	**V19/013678**

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 July 2019** (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: **15th July 2019**

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

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The viability of the microorganism identified under II above was tested on [2] **9th July 2019** On that date, the said microorganism was ☒ [3] viable ☐ [3] no longer viable	

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IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED [4]	
Deposit was grown on Reasoner's 2 Agar (R2A) at 21°C for 6 days.	
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Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY:
***Papiliotrema sp.* (P2-Gcland-NS-Runn Creek IS-107-1)**	**V19/013679**

II SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

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III VIABILITY STATEMENT	
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Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY:
***Arthrobacter sp.* (D4-11)**	**V19/013680**

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1-59. (canceled)

60. A method for profiling endophyte strains from a microbiome, said method including the steps of:

providing a microbiome, preferably wherein the microbiome is isolated from a plant material selected from seeds, stems, leaves, petioles, roots, buds, flowers or any combination thereof, preferably isolated from a plant selected from perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinaceae*), corn (*Zea mays*), *Glycine* species including *Glycine* max, *Glycine* tomentella, *Glycine* tabacina, *Glycine* latifolia, *Glycine* hirticaulis, *Glycine microphylla*, and *Glycine clandestine*, wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) and preferably wherein the microbiome includes bacteria and/or fungi;

obtaining protein profile spectra from one or more endophytes of the microbiome;

processing the protein profile spectra;

clustering the endophyte strains based on the processed protein profile spectra; and

selecting and/or isolating endophyte strain(s) having desired genetic and/or metabolic characteristics, or being phylogenetically related to a desired endophyte strain.

61. The method according to claim **60**, wherein the step of providing the microbiome includes the steps of:

providing plant material;

washing the plant material in an aqueous solution;

submerging the plant material in a aqueous solution;

macerating the plant material; and

applying the macerated plant material to a growth medium for growth of the microbiota to provide the isolated microbiome, preferably wherein the microbiota grown on the growth medium are subjected to a re-streaking so as to obtain an isolated endophyte colony.

62. The method according to claim **61**, wherein the plant material includes a seed, and wherein the step of providing the microbiome includes the preliminary step of:

harvesting the plant material;

sterilising the plant material;

germinating the plant material; and

growing the germinated plant material.

63. The method according to claim **60**, wherein the protein profile spectra are obtained by mass spectrometry, preferably matrix assisted laser desorption/ionisation (MALDI) mass spectrometry.

64. The method according to claim **60**, wherein the protein profile spectra are processed by a data deconvolution workflow, preferably wherein the data deconvolution workflow includes performing the steps of:

a m/z scan to create a m/z grid;

a spectrum baseline subtraction; and

a m/z alignment;

wherein the data deconvolution workflow provides the processed protein spectra.

65. The method according to claim **64**, wherein the m/z grid is produced according to an adaptive grid method, the spectrum baseline subtraction is performed according to a quantile normalization method and wherein the m/z alignment is performed with reference to a reference spectrum, preferably wherein the reference spectrum is of *Escherichia coli* ATCC 25922.

66. The method according to claim **60**, wherein protein profile spectra are obtained from one or more isolated endophyte colonies and wherein processing the protein profiles includes combining the protein profile spectra of each isolated endophyte colony.

67. The method according to claim **66** further including subsequently performing the steps of:

m/z alignment;

spectrum smoothing;

m/z range restriction;

spectrum peak detection; and

valid peak filtration to removing peaks which do not meet a defined threshold.

68. The method according to claim **67**, wherein the processed protein profiles are converted into a matrix for analysis, wherein the m/z ratio alignment is performed with reference to a reference spectrum, preferably wherein the reference spectrum is of *Escherichia coli* ATCC 25922, wherein the spectrum smoothing is performed according to a moving average algorithm, wherein the m/z range is restricted to between 2000 Da and 20000 Da, wherein the spectrum peak detection is performed by a resolution-based method, wherein the valid feature filter has a threshold between approximately 0-40% intensity, and wherein processed protein profile spectra are used to perform hierarchal clustering.

69. The method according to claim **68**, wherein said hierarchical clustering provides a clade of endophytes having properties selected from:

i. related bioactivity;

ii. related geographic ranges;

iii. belonging to plant lines having the same phenotype; or

iv. including similar protein profiles; and

wherein the related bioactivity is preferably selected from bioprotection and biofertilizer activity; and

wherein the plant phenotype is preferably for drought tolerance or drought resistance.

70. The method according to claim **60**, wherein prior to profiling endophyte strains from a microbiome, plant microbiome profiling is performed, said plant microbiome profiling including the steps of:

providing plant material from a first plant species and plant material from a related second plant species;

characterising the microbiome of the first and second plant species by analysing the plant material; and

assessing the microbiome of the first and second plant species to identify endophyte strains found in both the first and second plant species, or endophyte strains found in the second plant species but not in the first plant species.

71. A method for profiling a plant microbiome said method including the steps of:

providing plant material from a first plant species and material from a related second plant species, preferably wherein said first plant species is selected from perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinaceae*), corn (*Zea mays*), *Glycine* species including *Glycine* tomentella, *Glycine* tabacina, *Glycine* latifolia, *Glycine* hirticaulis, *Glycine microphylla, Glycine clandestine* and *Glycine Max*, wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), and preferably wherein the second plant species is a crop wild relative (CRW) of the first plant species;

characterising the microbiome of the first and second plant species by analysing the plant material; and

assessing the microbiome of the first and second plant species to identify endophyte strains found in both the first and second plant species or endophyte strains found in the second plant species but not in the first plant species.

72. The method according to claim **71**, wherein providing the plant material from the first and second plant species includes the steps of:

sterilising the plant material; and

germinating the plant material,

wherein the germinated plant material provides seedlings for characterisation; and

wherein the plant material is preferably selected from seeds, leaves, stems, petioles, roots, buds, flowers or any combination thereof.

73. The method according to claim **71**, wherein characterising the microbiome of the first and second plant species includes the steps of:

extracting nucleic acid from the plant material; and

analysing the extracted nucleic acid to profile the plant material microbiome.

74. The method according to claim **71**, wherein assessing the microbiome of the first and second plant species includes statistical analysis to determine microbiome operational taxomic units (OTU) present within each plant species.

75. The method according to claim **74**, wherein the microbiome OTU within the first plant species are compared to that of the second plant species to provides a means for determining microbiota which are either:

shared between the plant species, or

unique to the second plant species, relative to the first plant species.

76. The method according claim **74**, wherein the identified microbiome OTU are selected from the group including *Stenotrophomonas* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Holomonas* sp., *Enterobactereaceae* sp., *Pantoea* sp., *Burkholderiaceae* sp., *Ralstonia* sp., *Massilia* sp., *Herbaspirillum* sp., *Delftia* sp., *Curvibacter* sp., *Aquabacterium* sp., *Sphingomonas* sp., *Novosphingobium* sp., *Bradyrhizobium* sp., *Ochrobactrum* sp., *Methylobacterium* sp., *Lactobacillus* sp., *Staphylococcus* sp., *Bacillus* sp. and *Curtobacterium* sp.

77. A method of enhancing the bioactivity of a plant species, said method including:

identifying endophyte strains found in the first and second plant species according to the method of claim **71**; and

transferring one or more endophyte strains from the second plant species to the first plant species to enhance bioactivity of the first plant species;

wherein the transferred endophyte enhances bioactivity of the first plant species; or

said method including identifying endophyte strains found in the second plant species but not in the first plant species, according to the method of claim **71**, and

transferring one of said more endophyte strains from the second plant species to the first plant species,

wherein the transferred endophyte enhances bioactivity of the first plant species.

78. A substantially purified or isolated endophyte strain selected and/or isolated by the method according to claim **60**, preferably wherein said endophyte is a strain of *Xanthomonas* sp., more preferably wherein the *Xanthomonas* sp. strain is GW as described herein and as deposited with The National Measurement Institute on 17 May 2019 with accession number V19/009902; preferably wherein said endophyte is a strain of *Arthrobacter* sp., more preferably wherein the *Arthrobacter* sp strain is D4-11 as described herein and as deposited with The National Measurement Institute on 9 Jul. 2019 with accession number V19/013680; preferably wherein said endophyte is a strain of *Papiliotrema* sp., more preferably wherein the *Papiliotrema* sp strain is selected from the group consisting of P2-Gland-NS-Runn creek IS-107-1, P1-Geland-NS-Mornington-IS-114-1, and P2-Geland-NS-Card Creek IS-34-1, as described herein and as deposited with The National Measurement Institute on 9 Jul. 2019 with accession numbers V19/013679, V19/013678 and V19/013677, respectively; preferably wherein said endophyte is a strain of *Sphingomonas paucimobilis*, more preferably wherein the *Sphingomonas paucimobilis* strain is Gtom-P2-19 as described herein and as deposited with The National Measurement Institute on 9 Jul. 2019 with accession number V19/013676; preferably wherein said endophyte is a strain of *Argobacterium larrymoorei*, more preferably wherein said *Argobacterium larrymoorei* strain is selected from Gcla-P1-10, and Gtab-P2-18, as described herein and as deposited with The National Measurement Institute on 9 Jul. 2019 with accession numbers V19/013675 and V19/013671, respectively; preferably wherein said endophyte is a strain of *Pseudomonas oryzihabitans*, more preferably wherein the *Pseudomonas oryzihabitans* strain is selected from Ghir-A-22-2, Gtab-P2-12 and Gtom-P2-14, as described herein and as deposited with The National Measurement Institute on 9 Jul. 2019 with accession numbers V19/013672, V19/013674 and V19/013673, respectively.

79. A plant, plant part or plant product with enhanced bioactivity produced by the method according to claim **60**.

* * * * *