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(54) **Title**: MICROBIAL INOCULANTS AND FERTILIZER COMPOSITIONS COMPRISING THE SAME

(57) **Abstract**: Provided herein are microbial inoculants for use in increasing plant growth, plant productivity and/or soil quality, comprising strains of one or more bacterial species selected from *Lactobacillus parafarraginis*, *Lactobacillus buchneri*, *Lactobacillus rapi* and *Lactobacillus zeae*. Optionally the microbial inoculants also comprise a strain of *Acetobacter fabarum* and/or a strain of *Candida ethanolica*. Also provided are fertilizer compositions comprising said microbial inoculants.

Microbial inoculants and fertilizer compositions comprising the same

Field of the Art

[001] The present disclosure relates generally to microbial inoculants, particularly for use as fertilizers, comprising one or more microbial species or strains as described herein, and to fertilizer compositions comprising such organisms. The disclosure also relates to methods of promoting plant growth, increasing availability of nutrients in the soil and remediating degraded soils and pastures using microbial inoculants and fertilizer compositions of the present disclosure.

Background

[002] The use of fertilizers to enhance plant and crop production and overcome poor soil quality is widespread. Most commonly employed commercially available fertilizers are inorganic chemical fertilizers. Such chemical fertilizers can be expensive to produce, can be hazardous to use and are often associated with environmentally damaging consequences, such as nitrate contamination in run off and ground water. Environmental sustainability can be promoted by limiting the use of chemical fertilizers.

[003] Fertilizer compositions comprising microorganisms (so-called "biofertilizers") are increasingly considered as alternatives to conventional chemical fertilizers. The ability of specific bacterial species to promote plant growth has long been recognised. For example, nitrogen-fixing bacteria such as *Rhizobium* species provide plants with essential nitrogenous compounds. Species of *Azotobacter* and *Azospirillum* have also been shown to promote plant growth and increase crop yield, promoting the accumulation of nutrients in plants. However bacteria of these genera are often unable to compete effectively with native soil and plant flora, thereby requiring the application of impractically large volumes of inoculum. Various *Bacillus* and *Pseudomonas* species have also found application in microbial-based fertilizers.

[004] To date, biofertilizers have typically met with limited success, often not proving to

be efficacious under real farming conditions. There remains a need for improved microbial-based fertilizers that are effective in providing nutrients for plant growth and are environmentally safe and nonhazardous.

Summary of the Disclosure

[005] A first aspect of the present disclosure provides a microbial inoculant for use in increasing plant growth, plant productivity and/or soil quality, comprising strains of one or more bacterial species selected from *Lactobacillus parafarraginis*, *Lactobacillus buchneri*, *Lactobacillus rapi* and *Lactobacillus zeae*.

[006] In a particular embodiments the inoculant comprises two of said *Lactobacillus* species, three of said *Lactobacillus* species or all of said *Lactobacillus* species. The inoculant may represent a symbiotic combination of two or more or three or more of said *Lactobacillus* species.

[007] The *Lactobacillus parafarraginis* strain may be *Lactobacillus parafarraginis* Lp18. In a particular embodiment the *Lactobacillus parafarraginis* strain is *Lactobacillus parafarraginis* Lp18 deposited with National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022945.

[008] The *Lactobacillus buchneri* strain may be *Lactobacillus buchneri* Lb23. In a particular embodiment the *Lactobacillus buchneri* strain is *Lactobacillus buchneri* Lb23 deposited with National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022946.

[009] The *Lactobacillus rapi* strain may be *Lactobacillus rapi* Lr24. In a particular embodiment the *Lactobacillus rapi* strain is *Lactobacillus rapi* Lr24 deposited with National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022947.

[0010] The *Lactobacillus zeae* strain may be *Lactobacillus zeae* Lz26. In a particular embodiment the *Lactobacillus zeae* strain is *Lactobacillus zeae* Lz26 deposited with

National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022948.

[0011] An inoculant of the first aspect may further comprise a strain of *Acetobacter fabarum*. The *Acetobacter fabarum* strain may be *Acetobacter fabarum* Afl5. In a particular embodiment the *Acetobacter fabarum* strain is *Acetobacter fabarum* Afl5 deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022943.

[0012] An inoculant of the first aspect may further comprise a yeast. The yeast may be a strain of *Candida ethanolica*. The *Candida ethanolica* strain may be *Candida ethanolica* Ce31. In a particular embodiment the *Candida ethanolica* strain is *Candida ethanolica* Ce31 deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022944.

[0013] One or more of the strains in the inoculant may be encapsulated. Where multiple strains are encapsulated, the strains may be individually encapsulated or combined in a single encapsulation.

[0014] A second aspect of the present disclosure provides a microbial inoculant comprising at least one *Lactobacillus* species, at least one *Acetobacter* species and at least one *Candida* species.

[0015] In a particular embodiment the at least one *Lactobacillus* species is selected from *Lactobacillus parafarraginis*, *Lactobacillus buchneri*, *Lactobacillus rapi* and *Lactobacillus zeae*. In a further particular embodiment, the microbial inoculant comprises at least one strain of each of said *Lactobacillus* species. In a further particular embodiment, the *Lactobacillus parafarraginis* is strain Lp18 (deposited under Accession Number V11/022945), *Lactobacillus buchneri* is strain Lb23 (deposited under Accession Number V11/022946), *Lactobacillus rapi* is strain Lr24 (deposited under Accession Number V11/022947) and *Lactobacillus zeae* is strain Lz26 (deposited under Accession Number V11/022948).

[0016] In a particular embodiment the at least one *Acetobacter* species is *Acetobacter fabarum*. In a further particular embodiment the *Acetobacter fabarum* is Afl5 (deposited under Accession Number V11/022943).

[0017] In a particular embodiment the at least one *Candida* species is *Candida ethanolica*. In a further particular embodiment the *Candida ethanolica* is Ce31 (deposited under Accession Number V11/022944).

[0018] A third aspect of the present disclosure provides a microbial inoculant comprising at least one bacterial strain selected from *Lactobacillus parafarraginis* Lp18, *Lactobacillus buchneri* Lb23, *Lactobacillus rapi* Lr24 and *Lactobacillus zeae* Lz26.

[0019] An inoculant of the third aspect optionally further comprises *Acetobacter fabarum* Afl5 and/or *Candida ethanolica* Ce31.

[0020] An inoculant of the first, second or third aspect may be used as a fertilizer.

[0021] A fourth aspect of the present disclosure provides a fertilizer composition comprising a microbial inoculant of the first, second or third aspect. The fertilizer composition may optionally comprise one or more additional components such as organic material, humic substances, penetrants, macronutrients, micronutrients and other soil and/or plant additives.

[0022] A fifth aspect of the present disclosure provides a method for increasing plant growth and/or productivity, the method comprising applying to the plant, plant seeds or to the soil in which the plant or plant seeds are grown an effective amount of a microbial inoculant of the first, second or third aspect or a fertilizer composition of the fourth aspect.

[0023] A sixth aspect of the present disclosure provides a method for improving soil quality, the method comprising applying to soil or to the plants or plant seeds in said soil an effective amount of a microbial inoculant of the first, second or third aspect or a fertilizer composition of the fourth aspect.

[0024] In accordance with the above aspects the plant may be, for example, a pasture plant, crop plant (including fruit and vegetable plants) or ornamental plant. The crop may be, for example, any human or animal food crop or crop for use as fuel or for pharmaceutical production. The food crop may be, for example, a fruit, vegetable, nut, seed or grain.

[0025] A seventh aspect of the present disclosure provides a method for remediating degraded soil or pasture, the method comprising applying to the soil or pasture an effective amount of a microbial inoculant of the first, second or third aspect or a fertilizer composition of the fourth aspect.

Brief Description of the Drawings

[0026] Aspects and embodiments of the present disclosure are described herein, by way of non-limiting example only, with reference to the following drawings.

[0027] **Figure 1**. Root development in tick bean plants, treated as described in Example 5. **A**, control group; **B**, T40 treatment group; **C**, SGL40 treatment group; **D**, T25%GL40 treatment group; **E**, GL40 treatment group.

[0028] **Figure 2**. Average rate of change of growth (height) of tomato plants over a 20 day treatment period in three different soils (**A-C**), treated as described in Example 6. Squares represent IMP Bio treated seedlings, diamonds represent FlowPhos treated seedlings, triangles represent IMP Bio plus FlowPhos treated seedlings, crosses ('x') represent untreated (water only) seedlings.

[0029] **Figure 3**. Comparison of plant height, foliage size and root development in tomato seedlings, treated as described in Example 6. GreatLand = IMP Bio treated seedling.

[0030] **Figure 4**. Comparison of vegetative growth (and density of growth) of strawberry plants, treated as described in Example 8. **A**, conventional fertilizer treated plants after 3 months. **B**, IMP Bio treated plants after 3 months.

Detailed Description

[0031] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, typical methods and materials are described.

[0032] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0033] In the context of this specification, the term "about," is understood to refer to a range of numbers that a person of skill in the art would consider equivalent to the recited value in the context of achieving the same function or result.

[0034] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0035] The term "plant productivity" as used herein refers to any aspect of growth or development of a plant, that is a reason for which the plant is grown. Thus, for purposes of the present disclosure, improved or increased plant productivity refers broadly to improvements in biomass or yield of leaves, stems, grain, fruit, vegetables, flowers, or other plant parts harvested or used for various purposes, and improvements in growth of plant parts, including stems, leaves and roots. For example, when referring to food crops, such as grains, fruits or vegetables, plant productivity may refer to the yield of grain, fruit, vegetables or seeds harvested from a particular crop. For crops such as pasture, plant productivity may refer to growth rate, plant density or the extent of groundcover. "Plant growth" refers to the growth of any plant part, including stems, leaves and roots. Growth may refer to the rate of growth of any one of these plant parts.

[0036] The term "yield" refers to the amount of produced biological material and may be used interchangeably with "biomass". For crop plants, "yield" may also mean the amount of harvested material per unit of production or per area (e.g. hectare). Yield may be defined in terms of quantity or quality. The harvested material may vary from crop to crop, for example, it may be seeds, above-ground biomass, below-ground biomass (e.g. potatoes), roots, fruits, or any other part of the plant which is of economic value. "Yield" also encompasses yield stability of the plants. "Yield" also encompasses yield potential, which is the maximum obtainable yield under optimal growth conditions. Yield may be dependent on a number of yield components, which may be monitored by certain parameters. These parameters are well known to persons skilled in the art and vary from crop to crop. For example, breeders are well aware of the specific yield components and the corresponding parameters for the crop they are aiming to improve. For example, key yield parameters for potato include tuber weight, number of tubers, and number of stems per plant.

[0037] By "improving soil quality" is meant increasing the amount and/or availability of nutrients required by, or beneficial to plants, for growth. By way of example only, such nutrients include nitrogen, phosphorous, potassium, copper, zinc, boron and molybdenum. Also encompassed by the term "improving soil quality" is reducing or minimising the amount of an element that may be detrimental to plant growth or development such as, for example iron and manganese. Thus, improving soil quality by use of microbial inoculants and fertilizer compositions of the present disclosure thereby assists and promotes the growth of plants in the soil.

[0038] The term "remediating" as used herein in relation to degraded pasture or soil refers to the improvement in plant nutrient content in the soil to facilitate improved plant growth and/or yield. Degraded pasture includes overgrazed pasture.

[0039] As used herein, the term "effective amount" refers to an amount of microbial inoculant or fertilizer composition applied to a given area of soil or vegetation that is sufficient to effect one or more beneficial or desired outcomes, for example, in terms of plant growth rates, crop yields, or nutrient availability in the soil. An "effective amount" can be provided in one or more administrations. The exact amount required will vary depending on factors such as the identity and number of individual strains employed, the

plant species being treated, the nature and condition of the soil to be treated, the exact nature of the microbial inoculant or fertilizer composition to be applied, the form in which the inoculant or fertilizer is applied and the means by which it is applied, and the stage of the plant growing season during which application takes place. Thus, it is not possible to specify an exact "effective amount". However, for any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

[0040] The term "crop" as used herein refers to any plant grown to be harvested or used for any economic purpose, including for example human foods, livestock fodder, fuel or pharmaceutical production (e.g. poppies).

[0041] The term "optionally" is used herein to mean that the subsequently described feature may or may not be present or that the subsequently described event or circumstance may or may not occur. Hence the specification will be understood to include and encompass embodiments in which the feature is present and embodiments in which the feature is not present, and embodiments in which the event or circumstance occurs as well as embodiments in which it does not.

[0042] In accordance with the present disclosure, novel microbial inoculants and microbial fertilizer compositions are presented which find application in increasing plant productivity and improving soil quality. In particular embodiments the microbial species present in the microbial inoculant or fertilizer composition provide a symbiotic combination of organisms.

[0043] In the broadest embodiments, a microbial inoculant of the present disclosure comprises strains of one or more bacterial *Lactobacillus* species. The *Lactobacillus* species may be selected from *Lactobacillus parafarraginis*, *Lactobacillus buchneri*, *Lactobacillus rapi* and *Lactobacillus zeae*. The inoculant may further comprise at least one *Acetobacter* species and at least one *Candida* species.

[0044] The *Lactobacillus parafarraginis* strain may be *Lactobacillus parafarraginis* Lp18. In a particular embodiment the *Lactobacillus parafarraginis* strain is *Lactobacillus parafarraginis* Lp18 deposited with National Measurement Institute, Australia on 27

October 2011 under Accession Number V11/022945. The *Lactobacillus buchneri* strain may be *Lactobacillus buchneri* Lb23. In a particular embodiment the *Lactobacillus buchneri* strain is *Lactobacillus buchneri* Lb23 deposited with National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022946. The *Lactobacillus rapi* strain may be *Lactobacillus rapi* Lr24. In a particular embodiment the *Lactobacillus rapi* strain is *Lactobacillus rapi* Lr24 deposited with National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022947. The *Lactobacillus zeae* strain may be *Lactobacillus zeae* Lz26. In a particular embodiment the *Lactobacillus zeae* strain is *Lactobacillus zeae* Lz26 deposited with National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022948.

[0045] The inoculant may further comprise a strain of *Acetobacter fabarum*. The *Acetobacter fabarum* strain may be *Acetobacter fabarum* Afl5. In a particular embodiment the *Acetobacter fabarum* strain is *Acetobacter fabarum* Afl5 deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022943.

[0046] The inoculant may further comprise a yeast. The yeast may be a strain of *Candida ethanolica*. The *Candida ethanolica* strain may be *Candida ethanolica* Ce31. In a particular embodiment the *Candida ethanolica* strain is *Candida ethanolica* Ce31 deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022944.

[0047] The concentrations of each microbial strain to be added to microbial inoculants and fertilizer compositions as disclosed herein will depend on a variety of factors including the identity and number of individual strains employed, the plant species being treated, the nature and condition of the soil to be treated, the exact nature of the microbial inoculant or fertilizer composition to be applied, the form in which the inoculant or fertilizer is applied and the means by which it is applied, and the stage of the plant growing season during which application takes place. For any given case, appropriate concentrations may be determined by one of ordinary skill in the art using only routine experimentation. By way of example only, the concentration of each strain present in the inoculant or fertilizer composition may be from about 1×10^2 cfu/ml to about 1×10^{10} cfu/ml, and may be about 1×10^3 cfu/ml, about 2.5×10^3 cfu/ml, about 5×10^3 cfu/ml, 1×10^4 cfu/ml, about 2.5×10^4

cfu/ml, about 5 x 10^4 cfu/ml, 1 x 10^5 cfu/ml, about 2.5 x 10^5 cfu/ml, about 5 x 10^5 cfu/ml, 1 x 10^6 cfu/ml, about 2.5 x 10^6 cfu/ml, about 5 x 10^6 cfu/ml, 1 x 10^7 cfu/ml, about 2.5 x 10^7 cfu/ml, about 5 x 10^7 cfu/ml, 1 x 10^8 cfu/ml, about 2.5 x 10^8 cfu/ml, about 5 x 10^8 cfu/ml, 1 x 10^9 cfu/ml, about 2.5 x 10^9 cfu/ml, or about 5 x 10^9 cfu/ml. In particular exemplary embodiments the final concentration of the Lactobacillus strains is about 2.5 x 10^5 cfu/ml, the final concentration of *Acetobacter fabarum* may be about 1 x 10^6 cfu/ml and the final concentration of *Candida ethanolica* may be about 1 x 10^5 cfu/ml.

[0048] Also contemplated by the present disclosure are variants of the microbial strains described herein. As used herein, the term "variant" refers to both naturally occurring and specifically developed variants or mutants of the microbial strains disclosed and exemplified herein. Variants may or may not have the same identifying biological characteristics of the specific strains exemplified herein, provided they share similar advantageous properties in terms of promoting plant growth and providing nutrients for plant growth in the soil. Illustrative examples of suitable methods for preparing variants of the microbial strains exemplified herein include, but are not limited to, gene integration techniques such as those mediated by insertional elements or transposons or by homologous recombination, other recombinant DNA techniques for modifying, inserting, deleting, activating or silencing genes, intraspecific protoplast fusion, mutagenesis by irradiation with ultraviolet light or X-rays, or by treatment with a chemical mutagen such as nitrosoguanidine, methylmethane sulfonate, nitrogen mustard and the like, and bacteriophage-mediated transduction. Suitable and applicable methods are well known in the art and are described, for example, in J. H. Miller, *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1972); J. H. Miller, *A Short Course in Bacterial Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1992); and J. Sambrook, D. Russell, *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001), *inter alia*.

[0049] Also encompassed by the term "variant" as used herein are microbial strains phylogenetically closely related to strains disclosed herein and strains possessing substantial sequence identity with the strains disclosed herein at one or more phylogenetically informative markers such as rRNA genes, elongation and initiation factor genes, RNA polymerase subunit genes, DNA gyrase genes, heat shock protein genes and

recA genes. For example, the 16S rRNA genes of a "variant" strain as contemplated herein may share about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with a strain disclosed herein.

[0050] Microbial inoculants and fertilizer compositions of the present disclosure may optionally further comprise one or more additional microbial organisms, for example, additional agronomically beneficial microorganisms. Such agronomically beneficial microorganisms may act in synergy or concert with, or otherwise cooperate with the organisms of the present disclosure in the inoculant or fertilizer. Examples of agronomically beneficial microorganisms include *Bacillus* sp., *Pseudomonas* sp., *Rhizobium* sp., *Azospirillum* sp., *Azotobacter* sp., phototrophic and cellulose degrading bacteria, *Clostridium* sp., *Trichoderma* sp. and the like. Those skilled in the art will appreciate that this list is merely exemplary only, and is not limited by reference to the specific examples here provided.

[0051] In the soil environment, inoculated bacteria can find survival difficult among naturally occurring competitor and predator organisms. To aid in survival of microorganisms present in microbial inoculants and fertilizer compositions of the present disclosure upon application in the environment, one or more of the strains may be encapsulated in, for example, a suitable polymeric matrix. In one example, encapsulation may comprise alginate beads such as has been described by Young *et al*, 2006, Encapsulation of plant growth-promoting bacteria in alginate beads enriched with humic acid, *Biotechnology and Bioengineering* 95:76-83, the disclosure of which is incorporated herein by reference in its entirety. Those skilled in the art will appreciate that any suitable encapsulation material or matrix may be used. Encapsulation may be achieved using methods and techniques known to those skilled in the art. Encapsulated microorganisms can include nutrients or other components of the inoculant or fertilizer composition in addition to the microorganisms.

[0052] Those skilled in the art will appreciate that any plant may benefit from the application of microbial inoculants and fertilizer compositions of the present disclosure to soil, seeds and/or vegetation. Particular embodiments are employed to aid the growth, development, yield or productivity of crops and pastures or other plants of economic value, including ornamentals and plants grown for oils or biofuel. The crop plant may be, for

example, a food crop (for humans or other animals) such as any fruit, vegetable, nut, seed or grain producing plant. Exemplary crop plants include, but are not limited to, tubers and other below-ground vegetables (such as potatoes, beetroots, radishes, carrots, onions, etc.), ground-growing or vine vegetables (such as pumpkin and other members of the squash family, beans, peas, asparagus, etc.), leaf vegetables (such as lettuces, chard, spinach, alfalfa, etc.), other vegetables (such as tomatoes, brassica including broccoli, avocadoes, etc.), fruits (such as berries, olives, stone fruits including nectarines and peaches, tropical fruits including mangoes and bananas, apples, pears, mandarins, oranges, mandarins, kiwi fruit, coconut, etc.), cereals (such as rice, maize, wheat, barley, millet, oats, rye etc.), nuts (such as macadamia nuts, peanuts, brazil nuts, hazel nuts, walnuts, almonds, etc.), and other economically valuable crops and plants (such as sugar cane, soybeans, sunflower, canola, sorghum, pastures, turf grass, etc).

[0053] Microbial inoculants and fertilizer compositions of the present disclosure may be applied directly to plants, plant parts (such as foliage) or seeds, or alternatively may be applied to soil in which the plants are growing or to be grown or in which seeds have been or are to be sown. Application may be by any suitable means and may be on any suitable scale. For example, application may comprise pouring, spreading or spraying, including broad scale or bulk spreading or spraying, soaking of seeds before planting, and/or drenching of seeds after planting or seedlings. Those skilled in the art will appreciate that multiple means of application may be used in combination (for example soaking of seeds prior to planting followed by drenching of planted seeds and/or application to seedlings or mature plants). Seeds, seedlings or mature plants may be treated as many times as appropriate. The number of applications required can readily be determined by those skilled in the art depending on, for example, the plant in question, the stage of development of the plant at which treatment is initiated, the state of health of the plant, the growth, environmental and/or climatic conditions in which the plant is grown and the purpose for which the plant is grown. For example, in the case of flowering crops such as tomatoes, it may be desirable to apply the microbial inoculant or fertilizer composition once or more than once during the flowering period.

[0054] Thus, in accordance with the present disclosure, microbial inoculants and fertilizer products as disclosed herein may be prepared in any suitable form depending on the means by which the inoculant or fertilizer composition is to be applied to the soil or to plant seeds

or vegetation. Suitable forms can include, for example, slurries, liquids, and solid forms. Solid forms include powders, granules, larger particulate forms and pellets. Solid form fertilizer particles can be encapsulated in water soluble coatings (for example dyed or undyed gelatin spheres or capsules), extended release coatings, or by micro-encapsulation to a free flowing powder using one or more of, for example, gelatin, polyvinyl alcohol, ethylcellulose, cellulose acetate phthalate, or styrene maleic anhydride. Liquids may include aqueous solutions and aqueous suspensions, and emulsifiable concentrates.

[0055] In order to achieve effective dispersion, adhesion and/or conservation or stability within the environment of inoculants and fertilizer compositions disclosed herein, it may be advantageous to formulate the inoculants and compositions with suitable carrier components that aid dispersion, adhesion and conservation/stability. Suitable carriers will be known to those skilled in the art and include, for example, chitosan, vermiculite, compost, talc, milk powder, gels and the like.

[0056] Additional components may be incorporated into inoculants and fertilizer compositions of the present disclosure, such as humic substances, trace elements, organic material, penetrants, macronutrients, micronutrients and other soil and/or plant additives.

[0057] Humus or humic substances that may be incorporated may include, but are not limited to, humic acid derived from, for example oxidised lignite or leonardite, fulvic acid and humates such as potassium humate.

[0058] Organic material added may include, but is not limited to, biosolids, animal manure, compost or composted organic byproducts, activated sludge or processed animal or vegetable byproducts (including blood meal, feather meal, cottonseed meal, ocean kelp meal, seaweed extract, fish emulsions and fish meal).

[0059] Penetrants include, but are not limited to, non-ionic wetting agents, detergent based surfactants, silicones, and/or organo-silicones. Suitable penetrants will be known to those skilled in the art, non-limiting examples including polymeric polyoxyalkylenes, allinol, nonoxynol, octoxynol, oxycastrol, TRITON, TWEEN, Sylgard 309, Silwet L-77, and Herbex (silicone/surfactant blend).

[0060] Exemplary trace elements for inclusion in microbial inoculants and fertilizer compositions are provided in Example 1. However those skilled in the art will recognise that suitable trace elements are not limited thereto, and that any trace elements (natural or synthetic) amy be employed.

[0061] Optional further soil and/or plant additives that can be added to inoculants and fertilizer compositions of the present disclosure include, for example, water trapping agents such as zeolites, enzymes, plant growth hormones such as gibberellins, and pest control agents such as acaracides, insecticides, fungicides and nematocides.

[0062] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[0063] The present disclosure will now be described with reference to the following specific examples, which should not be construed as in any way limiting the scope of the invention.

Examples

[0064] The following examples are illustrative of the invention and should not be construed as limiting in any way the general nature of the disclosure of the description throughout this specification.

Example 1- Microbial strains

[0065] The following microbial strains were used in the production of a biofertilizer.

[0066] *Lactobacillus parafarraginis* Lp18 was isolated from an environmental source. Partial 16S rRNA sequencing indicated 100% similarity to *Lactobacillus parafarraginis* AB 262735 which has a risk group of 1 (TRBA). When cultured on MRS media for 3 days at 34°C, anaerobically, Lp18 produces cream, round, slight sheen, convex, colony diameter

1-2mm (facultative anaerobe). Its microscopic appearance is Gram positive, non-motile, short rods rectangular, mainly diploid. *Lactobacillus parafarraginis* Lp18 was deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022945.

[0067] *Lactobacillus buchneri* Lb23 was isolated from an environmental source. Partial 16S rRNA sequencing indicated 99% similarity to *Lactobacillus buchneri* AB 429368 which has a risk group of 1 (TRBA). When cultured on MRS media for 4 days at 34°C, anaerobically, Lb23 produces cream, shiny, convex, colony diameter 1-2mm (facultative anaerobe). Its microscopic appearance is Gram positive, non-motile, rods in chains. *Lactobacillus buchneri* Lb23 was deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022946.

[0068] *Lactobacillus rapi* Lr24 was isolated from an environmental source. Partial 16S rRNA sequencing indicated 99% similarity to *Lactobacillus rapi* AB 366389 which has a risk group of 1 (DSMZ). When cultured on MRS media for 4 days at 34°C, anaerobically, Lr24 produces cream, round, shiny colonies with a diameter of 0.5mm (facultative anaerobe). Its microscopic appearance is Gram positive, non-motile, short rods single or diploid. *Lactobacillus rapi* Lr24 was deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022947.

[0069] *Lactobacillus zeae* Lz26 was isolated from an environmental source. Partial 16S rRNA sequencing indicated 99% similarity to *Lactobacillus zeae* AB 008213.1 which has a risk group of 1 (TRBA). When cultured on MRS media for 48 hours at 34°C, anaerobically, Lz26 produces white, round, shiny, convex, colonies with a diameter of 1mm (facultative anaerobe). Its microscopic appearance is Gram positive, non-motile, short rods almost coccoid, diploid and some chains. *Lactobacillus zeae* Lz26 was deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022948.

[0070] *Acetobacter fabarum* Af15 was isolated from an environmental source. Partial 16S rRNA sequencing indicated 100% similarity to *Acetobacter fabarum* AM 905849 which has a risk group of 1 (DSMZ). When cultured on Malt extract media for 3 days at 34°C, AF15 produces opaque, round, shiny, convex, colony diameter 1mm (aerobic). Its

microscopic appearance is Gram negative, rods single or diploid. *Acetobacter fabarum* Af15 was deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022943.

[0071] *Candida ethanolica* Ce31 was isolated from an environmental source. Partial 16S rRNA sequencing indicated 89% similarity to *Candida ethanolica* AB534618. When cultured on Malt extract media for 2 days at 34°C, Ce31 produces cream, flat, dull, roundish, colony diameter 2-3mm (aerobic). Its microscopic appearance is budding, ovoid yeast. *Candida ethanolica* Ce31 was deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022944.

Maintenance of cultures

[0072] 30% glycerol stocks were made of each isolate and maintained at -80°C for long-term culture storage. Short-term storage of the cultures are maintained at 4°C on agar slopes (3 month storage) and on agar plates which are subcultured monthly. To maintain the isolates original traits, a fresh plate is made from the -80°C stock following three plate subcultures.

Inoculum and growth media

[0073] The *Lactobacillus* strains were grown with or without air (*L. rapi* prefers anaerobic) either in MRS broth (Difco) or on MRS agar plates depending on application. The cultures were routinely grown for 2 days at a mesophilic temperature of 30-34°C. The *Acetobacter* and *Ethanolica* strains are grown aerobically either in Malt extract broth (Oxoid) or on Malt extract agar plates depending on application. The cultures are routinely grown for 2 days at a mesophilic temperature of 30-34°C.

Fermenter 'seed' preparation

[0074] For individual strains, using a sterile nichrome wire a single colony is removed from a fresh culture plate and transferred to a universal bottle containing 15mL of sterile media. The bottle is securely placed in a shaking incubator set at 30^0C, 140rpm for 48hrs (*L.rapi* is not shaken). After incubation a cloudy bacterial growth should be visible. 'Seed' inoculation bottles are stored at 4°C until required (maximum 1 week).

[0075] Typically a 5% bacterial inoculation is required for a fermenter run. The stored 15ml culture seed is added to a Schott bottle containing a volume of sterile media which is 5% of the total fermenter working volume. The culture is incubated and shaken in the same way as the 15ml seed. Large scale automatic fermenters are used to grow pure cultures of each isolate. There is an automatic feed of alkali, antifoam and glucose. Typically the temperature is maintained at 30-34°C, pH 5.5 but the oxygen and agitation varies depending on the microorganism.

Sample analysis

[0076] After each large scale culturing of an isolate a sample is aseptically withdrawn and a viability count undertaken using 10 fold serial dilutions, performed in a laminar flow hood. A wet slide is also prepared and purity observed using a phase contrast microscope to double check for contaminants that may be present but unable to grow on the culture media. After 48 hours the viability plates are checked for a pure culture (same colony morphology) and the colonies counted to produce a colony forming unit per ml (cfu/ml) value. A Grams stain is also performed.

Example 2- Pasture trials

[0077] Field trials on pasture were conducted using a biofertlizer as disclosed herein, in comparison to untreated pasture and pasture treated with conventional inorganic fertilizers.

[0078] The biofertilizer (hereinafter "IMP Bio") comprised the six microbial strains listed in Example 1, at final concentrations of 2.5 x 10^5 cfu/ml for each of the *Lactobacillus* strains, 1.0 x 10^5 cfu/ml for *Candida ethanolica* and 1.0 x 10^6 cfu/ml for *Acetobacter fabarum*. The strains were grown as described in Example 1 and mixed with 2% trace elements, 0.3% humate (Soluble Humate, LawrieCo), 3% molasses and 0.1-0.2% phosphoric acid. Phosphoric acid was added to the point where pH was in the range 3.8 to 4.0. The trace elements component typically comprised the following (per 1,000L):

Table 1 Trace elements component of biofertilizer

Material	Volume (kg)
Water	200 kg
Potassium Sulphate	15.25 kg
Copper Complex[1]	25.6 kg
Magnesium Citrate[2]	175.0 kg
Chromium Citrate[3]	10.0 kg
Calcium Sokolate[4]	52.0 kg
Citric Acid	11.15 kg
Ferrous Sulphate	4.0 kg
Cobalt Sulphate	750 g
Nickel Sulphate	250 g
Manganese Sulphate	4.0 kg
Urea	31.0 kg
Zinc Sulphate	4.0 kg
Borax	4.5 kg
M A P	13.25 kg
Sodium Molybdate	2.5 kg
Acetic Acid	10.8 kg
Sugar	50.0 kg

[0079] Conventional inorganic fertilizers used as comparators were Spray Gro Liquid Urea, DAP (diammonium phosphate), and 14:16:11 commercial NPK mix.

[0080] Sites for the pasture trials were selected based on rainfall levels, soil type, pasture composition and past fertilizing practices. The following locations in Tasmania were used: Nabageena (high rainfall; rye grass, cocksfoot, Yorkshire fog and other grasses), Cuprona (high rainfall; rye grass), West Moorville/Upper Burnie (high rainfall; rye grass), Connorville (dryland pasture; degraded) and Connorville (irrigated pasture; rye grass).

[0081] At each location, multiple 4 x 10m strips of pasture were prepared by mowing to a height of 45mm (and removal of clipped plant material prior to fertilizing). At West Moorville/Upper Burnie and Nabageena, IMP Bio was applied to replicate plots at 20L/ha, 30L/ha or 50L/ha, and 14:16:11 NPK mix was applied to replicate plots at 250kg/ha. At West Moorville, DAP was also applied to replicate plots at 125kg/ha. At Cuprona, IMP

Bio was applied to replicate plots at 20L/ha, 30L/ha or 50L/ha, and Spray Gro Liquid Urea was applied at 50L/ha. At Connorville, IMP Bio was applied to replicate plots at 20L/ha, 30L/ha or 50L/ha, and DAP was applied to replicate plots at 125kg/ha. IMP Bio and SprayGro Urea were applied as large droplets through 2m backpack boom sprays in a single pass. 14:16:11 NPK mix and DAP were applied by hand distribution. At each location, replicate control (unfertilized) plots were set aside.

[0082] Plant yield and leaf nutrient content were analysed 6-8 weeks after treatment.

[0083] Results for plant yield are shown in Table 2 below. These results indicate that the IMP Bio fertilizer produced yields at least similar to, and in some cases superior to, conventional inorganic fertilizers.

Table 2. Yield (kg/ha/day)

Area	**Control**	**Conventional fertilizer**	**IMP Bio (30L/ha)**	**IMP Bio (half strength)**
Cuprona	65	79 (Spray Gro)	75	-
Nabageena	60	78 (14:16:11)	73	-
Connorville (dryland)	35	34 (DAP)	32	31
Connorville (irrigated)	44	56 (DAP)	51	44
West Moorville	67	90 (DAP)	87	-

[0084] Plant material nutrient analysis was conducted, as shown in Table 3 below. Key elements required by, or beneficial to, the pasture for growth (such as nitrogen, phosphorous, postassium, calcium, copper, zinc, boron, molybdenum) were present in plant material from the IMP Bio treated plots at levels equivalent to or higher than those plots treated with the comparator conventional inorganic fertilizer, despite these nutrients not being added in the IMP Bio fertilizer.

Table 3

Nutrient	Control	Conventional fertilizer	IMP Bio (30L/ha)
Connorville (irrigated)			
Nitrogen (%)	1.95	2.23	1.94
Phosphorus (%)	0.28	0.38	0.26
Potassium (%)	2.61	2.69	2.53
Sulphur (%)	0.26	0.27	0.24
Carbon (%)	43.5	43.7	43.8
Calcium (%)	0.33	0.30	0.41
Magnesium (%)	0.28	0.25	0.28
Sodium (%)	0.24	0.45	0.30
Copper (ppm)	4.2	4.5	5.3
Zinc (ppm)	19	21	24
Manganese (ppm)	372	339	309
Iron (ppm)	113	90	109
Boron (ppm)	4.9	4.7	6.0
Molybdenum (ppm)	0.7	0.7	0.7
Cobalt (ppm)	0.2	0.1	0.2
Silicon (ppm)	201	169	193
Nitrogen : Sulphur ratio	7.4	8.4	8.1
Nitrogen : Phopshorus ratio	7.0	5.9	7.4
Nitrogen : Potassium ratio	0.7	0.8	0.8
Carbon : Nitrogen ratio	22.3	19.6	22.6
Crude protein (%N x 6.25)	12.2	13.9	12.1
West Moorville			
Nitrogen (%)	1.56	1.57	1.64
Phosphorus (%)	0.34	0.33	0.31
Potassium (%)	2.26	2.43	2.11
Sulphur (%)	0.25	0.24	0.24
Carbon (%)	44.0	44.1	43.9
Calcium (%)	0.78	0.59	0.57
Magnesium (%)	0.24	0.20	0.18

Sodium (%)	0.18	0.14	0.15
Copper (ppm)	4.8	4.3	4.7
Zinc (ppm)	18	18	19
Manganese (ppm)	37	33	35
Iron (ppm)	172	114	120
Boron (ppm)	10	7.7	8.3
Molybdenum (ppm)	1.3	1.0	1.1
Cobalt (ppm)	0.1	<0.1	<0.1
Silicon (ppm)	316	268	244
Nitrogen : Sulphur ratio	6.2	6.5	7.0
Nitrogen : Phopshorus ratio	4.5	4.7	5.3
Nitrogen : Potassium ratio	0.7	0.6	0.8
Carbon : Nitrogen ratio	28.2	28.1	26.8
Crude protein (%N x 6.25)	9.8	9.8	10.2
Cuprona			
Nitrogen (%)	3.68	3.60	3.68
Phosphorus (%)	0.39	0.38	0.39
Potassium (%)	3.43	3.43	2.90
Sulphur (%)	0.38	0.41	0.41
Carbon (%)	43.6	44.2	43.6
Calcium (%)	0.51	0.49	0.58
Magnesium (%)	0.24	0.27	0.26
Sodium (%)	0.26	0.36	0.44
Copper (ppm)	8.7	9.1	8.4
Zinc (ppm)	24	25	22
Manganese (ppm)	106	118	74
Iron (ppm)	104	110	103
Boron (ppm)	6.2	4.2	4.4
Molybdenum (ppm)	0.3	0.3	0.9
Cobalt (ppm)	<0.1	<0.1	<0.1
Silicon (ppm)	267	284	214
Nitrogen : Sulphur ratio	9.8	8.8	9.1
Nitrogen : Phopshorus ratio	9.5	9.5	9.3

Nitrogen : Potassium ratio	1.1	1.1	1.3
Carbon : Nitrogen ratio	11.8	12.3	11.9
Crude protein (%N x 6.25)	23.0	22.5	23.0

Example 3- Soil quality

[0085] To determine the effect of a biofertilizer as disclosed herein on soil quality, 2 x 150g of soil from a farm in Tasmania were each weighed into 2 x clean 150ml Schott bottle. 10mls of a 1:10 dilution of IMP Bio fertilizer (see Examples 1 and 2) was dripped over the top of the soil in one bottle and the lid replaced and incubated at 34°C for one week. The second bottle had no biofertilizer added was incubated 34°C. The soil from both bottles was analysed by Environmental Analytical Laboratories (EAL, Southern Cross University Lismore, NSW) using standard soil testing methods.

[0086] The results for the one week treatment of soil with IMP Bio are summarised in Table 4. Soil tests on the untreated incubated sample are not shown as these were substantially the same as the initial untreated soil test. It is clear from the soil tests on the two treated samples that there is a marked difference in the soil after incubation with IMP Bio. The second sample analysed, shows a general trend of increasing the levels of available cations (calcium, magnesium, potassium, sodium and all trace elements – zinc, manganese, iron and copper) and ammonium nitrogen, while the total levels under the acid extractions were slightly lower across all nutrients. Organic matter increased by 1% (14.6% to 15.5%) between the samples dates. The overall decrease in total nutrients does not appear to be significant.

[0087] There was a greater than three-fold increase in ammonium nitrogen, although no increase in nitrates. This indicates an increase in mineralisation of nitrogen from the organic nitrogen pool, and may be linked to the transformation of organic material, the level of which in this soil is particularly high. This could also indicate nitrogen fixation.

Table 4

Nutrient/soil characteristic	Prior to IMP Bio treatment	After IMP Bio treatment
Calcium (mg/kg)	1006	1431
Magnesium (mg/kg)	181	279
Potassium (mg/kg)	218	317
Phosphorus (mg/kg)	2.2	3.3
Nitrate nitrogen (mg/kg)	13.3	14.2
Ammonium nitrogen (mg/kg)	21.2	72.6
Sulfur (mg/kg)	28.5	33.8
Zinc (mg/kg)	3.0	3.6
Manganese (mg/kg)	19	48
Iron (mg/kg)	335	369
Copper (mg/kg)	3.4	3.5
Boron (mg/kg)	0.82	0.98
Silicon (mg/kg)	24	28
Total carbon (%)	8.33	8.85
Total nitrogen (%)	0.52	0.53
Carbon : Nitrogen ratio	16.1	16.6
Organic matter (%)	14.6	15.5
pH	6.41	6.36
Conductivity (dS/m)	0.132	0.202

Example 4 - Potato trials

[0088] A field trial was conducted in which Bondi variety potatoes were treated with the IMP Bio biofertilizer (see Example 2) at planting. The trial was conducted at Waterhouse, Tasmania. IMP Bio was applied in furrows to rows 30m long at a rate of 50L/ha, either alone, or together with the conventional chemical fertilizer 5-10-16 at either 650kg/ha (delivering 32kg/ha nitrogen, 63kg/ha phosphorus and 100kg/ha potassium) or 1250kg/ha (delivering 63kg/ha nitrogen, 125kg/ha phosphorus and 200kg/ha potassium). In a fourth replicate, 5-10-16 was applied at 1250kg/ha together with the fungicide Amistar. Four

plots of 4m length were dug from each treatment and tubers assessed for size and yield. The results are shown in Table 5.

Table 5. Potato yield

Treatment	Total yield >45g (t/ha)	Seed (t/ha)	Yield >350g (t/ha)	Yield <45g (t/ha)	Stems/plant
IMP Bio	42.4	38.1	4.3	2.9	3.7
IMP Bio + 5-10-16 (650kg/ha)	39.2	35.4	3.8	1.7	3.3
IMP Bio + 5-10-16 (1250kg/ha)	35.6	30.0	5.6	1.6	3.3
5-10-16 + Amistar	39.3	33.4	5.9	1.8	3.2

[0089] There was an increase in stem numbers per plant in the IMP Bio treatment, which is desirable (higher stem numbers typically correlating with higher tuber numbers). The reduction in large (>350g) tubers observed with IMP Bio treatment is also significant as larger tubers have lower commercial value than seed sized tubers (45-350g). Additionally, the 14% increase (5 tonnes/ha) in seed weight in the IMP Bio compared to the 5-10-16 + Amistar treatment is also of significant economic value. The IMP Bio treated potato plants were also observed to be approximately three weeks more developed (in terms of maturity) than those treated with 5-10-16.

Example 5 – Tick bean trials

[0090] A greenhouse experiment was conducted to establish the effect of IMP Bio biofertilizer (see Example 2) on tick bean plant growth, compared to the commercial fertilizer Baileys TriStar (8.3%N, 0% P, 16% K, 14% S, 1% Fe, 2% Mg).

[0091] The treatment groups and regimes employed for seedlings post-germination were as follows:

Control: 300 µl water
"T40": 300 µl TriStar at 40L/ha
"SGL40": 300 µl IMP Bio at 40L/ha
"T25%GL40": 300 µl TriStar 25% plus IMP Bio at 40L/ha

"GL40": 300 µl IMP Bio at 40L/ha

[0092] Seeds in the T40, SGL40 and T25%GL40 groups were soaked for 1 hour in 100ml of a 1:10 dilution of IMP Bio solution prior to planting. Control and GL40 seeds remained dry prior to planting. Three replicates of each treatment group (and two replicates of the control group) were used. Seeds were planted 5mm deep in the middle of each pot and the pots placed in a temperature-controlled greenhouse at 16-18°C under hydroponic lights. After germination, all seedlings were treated every two weeks (for a total of four weeks) using the treatments described above. Seedlings were watered once a day.

[0093] At the conclusion of the experiment it was observed that the tallest plants, and the plants with the strongest main stem were those of the T25%GL40 treatment group. Overall, the best growth was observed in the T25%GL40 and SGL40 groups (data not shown). However the most noticeable differences observed were in root development (see Figure 1). Roots of the control plants were the least dense and the shortest (Figure 1A). Roots of the T40 plants had good root density and length (Figure 1B), however development was not as extensive as in the plants treated with IMP Bio. In the SGL40 plants the root system shown good desnity and length (Figure 1C). Root nodules were present as were black nodule-like growths. In the T25%GL40 plants the root system was more dense and longer than in other treatment groups (Figure 1D). Root nodules were present but black nodule-like growth was not seen. In the GL40 plants the root system was similarly dense, long and well developed (Figure 1E). Root nodules were present as were black nodule-like growths.

Example 6 - Tomato trials

[0094] A greenhouse experiment was conducted to investigate the effect of IMP Bio biofertilizer (see Example 2) on the growth rate of tomato plants over a 20 day period. Tomato seedlings were provided by Cedenco. Water only was used as a control, and the commercial fertilizer FlowPhos (Yara Nipro) used as a comparator. Seedlings were potted into 50 mm pots in one of three different soils obtained from different locations (Cedenco) and drenched once with either: (i) 10 ml water; (ii) 10 ml of IMP Bio (100 ml in 900 ml water); (iii) 10 ml of FlowPhos (7.5 ml in 900 ml water); or (iv) 10 ml of FlowPhos plus IMP Bio (7.5 ml FlowPhos and 100 ml IMP Bio made to a total volume of 1000ml with

water). Three replicates of the control (water) group and eight replicates of each of the treatment groups. Plants were watered twice a day with 30 ml water. Plant height was measured every third day over the 20 day period of the experiment.

[0095] The average rate of change of growth (height) of tomato seedlings over the 20 day period for all treatment groups, in each of the three soils, is shown in Figure 2. As can be seen, the IMP Bio treated plants were the only plants that consistently showed increases in growth over the course of the experiment, resulting in taller plants. Figure 3 shows an exemplary comparison of difference in plant height, foliage and root system development in control plants, FlowPhos treated plants and IMP Bio (GreatLand) treated plants, in which the advantages of IMP Bio treatment are clearly evident.

[0096] A field trial was then conducted at Timmering, Victoria in which tomato plants were treated with IMP Bio by foliar application during flowering, either at a rate of 80L/ha or 40L/ha during early flowering followed by 40L/ha during mid flowering. Yield of tomato fruit was determined and compared to the yield from the same number of untreated plants. For the plants that received 80L/ha IMP Bio, total fruit yield was 149.87 tonnes/ha, compared to 128.87 tonnes/ha for the untreated plants. For the plants that received two applications of 40L/ha IMP Bio, total fruit yield was 130.15 tonnes/ha, compared to 103.05 tonnes/ha for the untreated plants.

Example 7 - Macadamia trials

[0097] A field trial was conducted in which macadamia trees in a 100 ha farm in Lismore, NSW were treated with the IMP Bio biofertilizer (see Example 2) by spraying at the rate of 40L/ha, every 2-3 months for a period of 12 months. IMP Bio was applied in conjunction with chemical fertilizer (Easy N Fertilizer), the same fertilizer used for at least the previous four years. The yield of macadamia nuts following the 12 month treatment was approximately 70 tonnes, compared to an average yield of 35 tonnes per year over the previous four years. The benefits offered by the IMP Bio biofertilizer allowed for a significant reduction in the application of chemical fertilizer.

[0098] Leaf and soil analysis was also conducted at four sites across the farm after 45 days of IMP Bio use. Significant increases were observed in levels of zinc, manganese, iron

and boron in macadamia leaves, and in ammonium nitrogen, nitrate nitrogen, phosphorus, potassium, calcium, copper and boron in the soil.

Example 8 - Strawberry trials

[0099] A field trial was conducted in Beerwah, Qld to establish the effect of IMP Bio biofertilizer (see Example 2) on strawberry plant growth and fruit yield over an 8 ha plot. The IMP Bio was applied at a rate of 40L/ha to the soil pre-planting, again at the same rate at planting, and weekly during the vegetative growth and flowering stage (weeks 2-4), during the fruiting stage (weeks 5-8) and during the picking stage (weeks 9-16). In comparison to conventional fertilizer (NitroPhoska(blue) applied preplanting at 1000 kg/ha), plant growth rate was significantly increased and plants showed increased vegetative growth and leaf area (Figure 4). Fruit yield was also significantly increased (38,000 kg as compared to 20,000 kg).

Example 9 – Other trials

[00100] Preliminary trials have also been conducted on sugar cane, lettuce, raspberries, roses, wheat, basil and turf grass (golf course green). In each case IMP Bio biofertilizer (see Example 2) was observed to result in increased rate of growth of plants compared to untreated plants (data not shown).

Claims

1. A microbial inoculant for use in increasing plant growth, plant productivity and/or soil quality, comprising strains of one or more bacterial species selected from *Lactobacillus parafarraginis*, *Lactobacillus buchneri*, *Lactobacillus rapi* and *Lactobacillus zeae*.

2. A microbial inoculant according to claim 1 wherein the inoculant comprises two of said *Lactobacillus* species.

3. A microbial inoculant according to claim 1 wherein the inoculant comprises three of said *Lactobacillus* species.

4. A microbial inoculant according to claim 1 wherein the inoculant comprises all of said *Lactobacillus* species.

5. A microbial inoculant according to any one of claims 1 to 4 wherein the inoculant is a symbiotic combination of two or more, or three or more, of said *Lactobacillus* species.

6. A microbial inoculant according to any one of claims 1 to 5 wherein the *Lactobacillus parafarraginis* strain is *Lactobacillus parafarraginis* Lp18.

7. A microbial inoculant according to claim 6 wherein the *Lactobacillus parafarraginis* strain is *Lactobacillus parafarraginis* Lp18 deposited with National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022945.

8. A microbial inoculant according to any one of claims 1 to 7 wherein the *Lactobacillus buchneri* strain is *Lactobacillus buchneri* Lb23.

9. A microbial inoculant according to claim 8 wherein the *Lactobacillus buchneri* strain is *Lactobacillus buchneri* Lb23 deposited with National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022946.

10. A microbial inoculant according to any one of claims 1 to 9 wherein the *Lactobacillus rapi* strain is *Lactobacillus rapi* Lr24.

11. A microbial inoculant according to claim 10 wherein the *Lactobacillus rapi* strain is *Lactobacillus rapi* Lr24 deposited with National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022947.

12. A microbial inoculant according to any one of claims 1 to 11 wherein the *Lactobacillus zeae* strain is *Lactobacillus zeae* Lz26.

13. A microbial inoculant according to claim 12 wherein the *Lactobacillus zeae* strain is *Lactobacillus zeae* Lz26 deposited with National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022948.

14. A microbial inoculant according to any one of claims 1 to 13 further comprising a strain of *Acetobacter fabarum*.

15. A microbial inoculant according to claim 14 wherein the *Acetobacter fabarum* strain is *Acetobacter fabarum* Afl5.

16. A microbial inoculant according to claim 15 wherein the *Acetobacter fabarum* strain is *Acetobacter fabarum* Afl5 deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022943.

17. A microbial inoculant according to any one of claims 1 to 16 further comprising a yeast.

18. A microbial inoculant according to claim 17 wherein the yeast is a strain of *Candida ethanolica*.

19. A microbial inoculant according to claim 18 wherein the *Candida ethanolica* strain is *Candida ethanolica* Ce31.

20. A microbial inoculant according to claim 19 wherein the *Candida ethanolica* strain is *Candida ethanolica* Ce31 deposited with the National Measurement Institute, Australia on 27 October 2011 under Accession Number V11/022944.

21. A microbial inoculant according to any one of claims 1 to 20 wherein one or more of the strains in the inoculant is encapsulated.

22. A microbial inoculant comprising at least one bacterial strain selected from *Lactobacillus parafarraginis* Lp18, *Lactobacillus buchneri* Lb23, *Lactobacillus rapi* Lr24 and *Lactobacillus zeae* Lz26.

23. An inoculant according to claim 22 further comprising *Acetobacter fabarum* Af15 and/or *Candida ethanolica* Ce31.

24. An inoculant according to any one of claims 1 to 23 for use as a fertilizer.

25. A fertilizer composition comprising a microbial inoculant according to any one of claims 1 to 24.

26. A fertilizer composition according to claim 25 further comprising one or more additional components such as organic material, humic substances, penetrants, macronutrients, micronutrients and other soil and/or plant additives.

27. A fertilizer composition according to claim 25 or 26 for use in fertilizing pasture plants, crop plants or ornamental plants.

28. A fertilizer composition according to claim 27 wherein the crop is a human or animal food crop or crop for use as fuel or for pharmaceutical production.

29. A fertilizer composition according to claim 28 wherein the food crop is a fruit, vegetable, nut, seed or grain.

30. A method for increasing plant growth and/or productivity, the method comprising applying to the plant, plant seeds or to the soil in which the plant or plant seeds are grown

an effective amount of a microbial inoculant according to any one of claims 1 to 24 or a fertilizer composition according to any one of claims 25 to 29.

31. A method for improving soil quality, the method comprising applying to soil or to the plants or plant seeds in said soil an effective amount of a microbial inoculant according to any one of claims 1 to 24 or a fertilizer composition according to any one of claims 25 to 29.

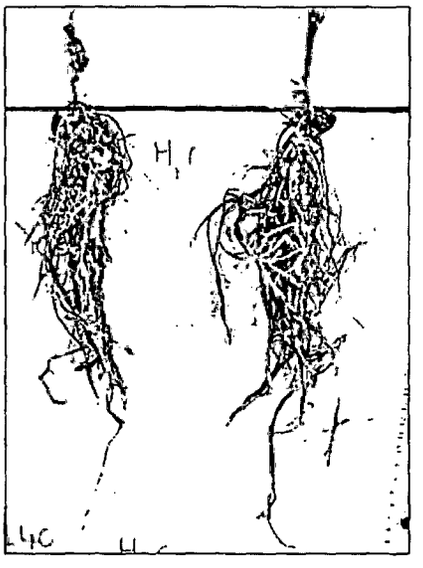
32. A method according to claim 30 or 31 wherein the plant is a pasture plant, crop plant or ornamental plant.

33. A method according to claim 32 wherein the crop is a human or animal food crop or crop for use as fuel or for pharmaceutical production.

34. A method for remediating degraded soil or pasture, the method comprising applying to the soil or pasture an effective amount of a microbial inoculant according to any one of claims 1 to 24 or a fertilizer composition according to any one of claims 25 to 29.

Figure 1

A



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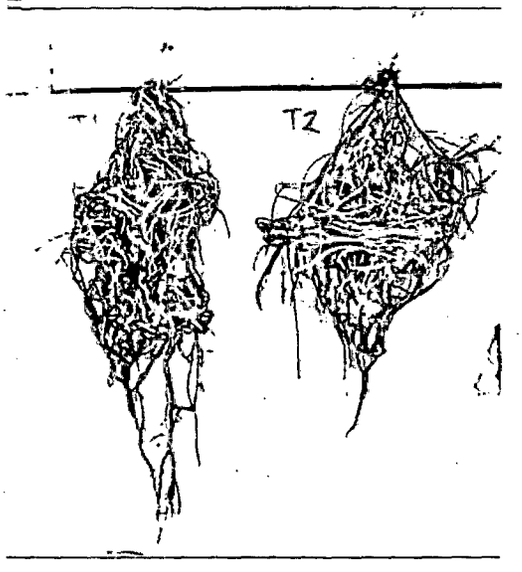

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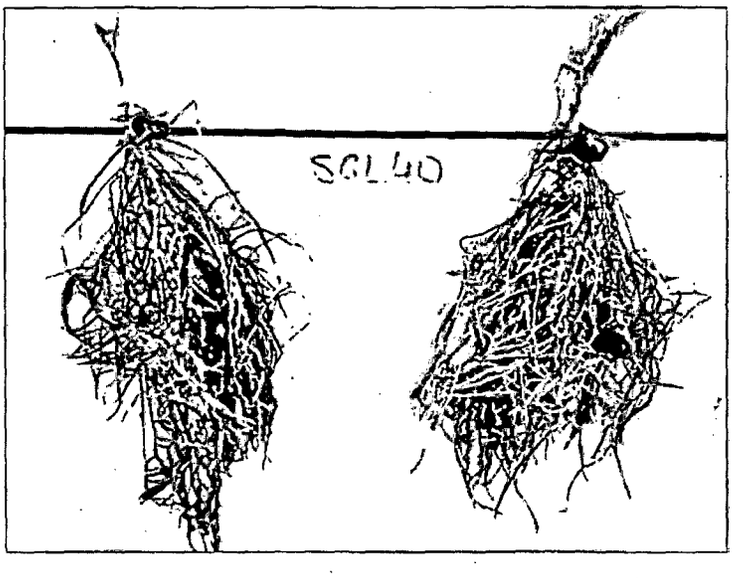

Figure 1 (cont'd)

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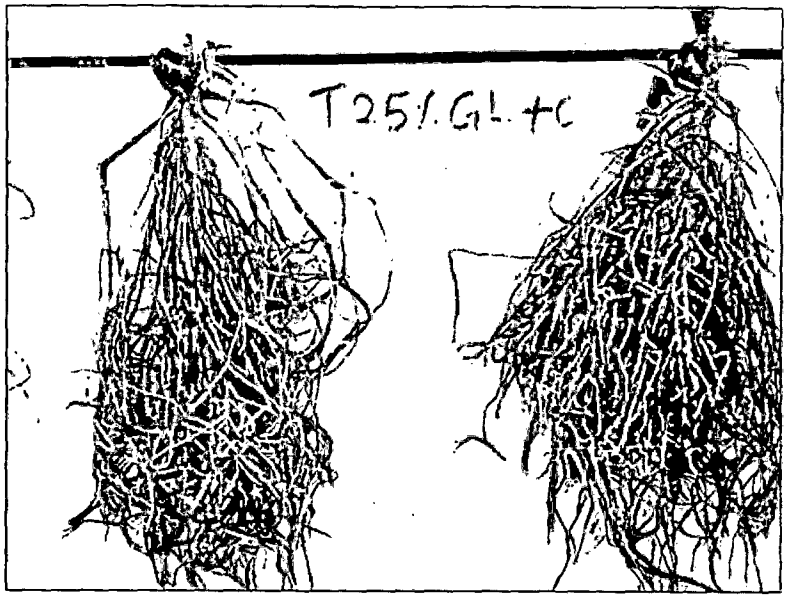

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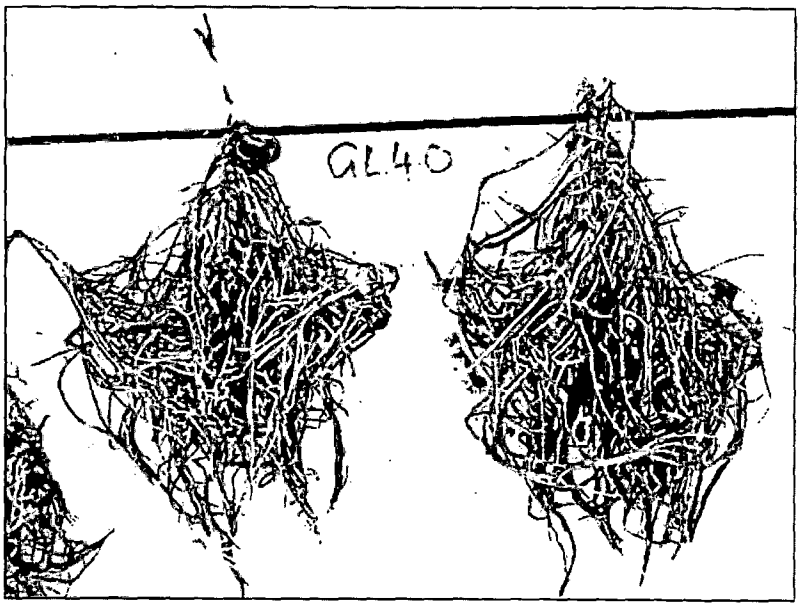

Figure 2

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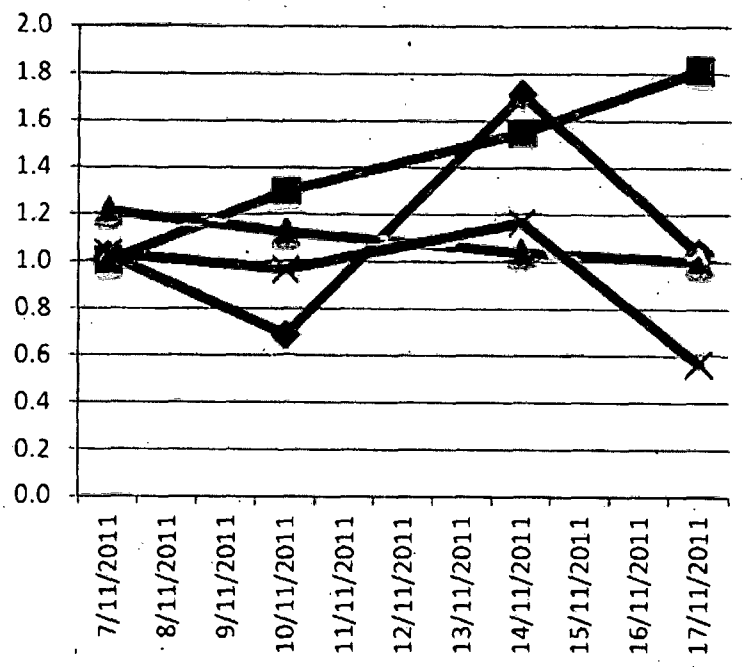
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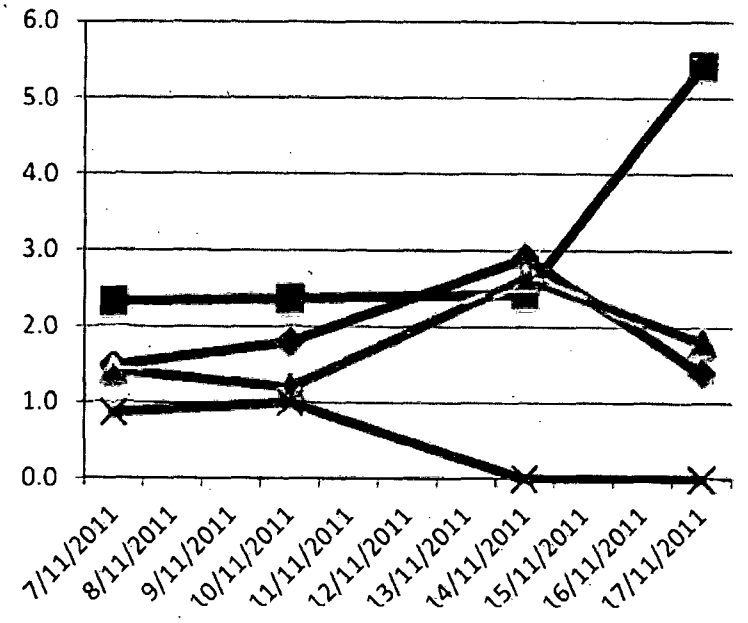
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Figure 2 (cont'd)

C

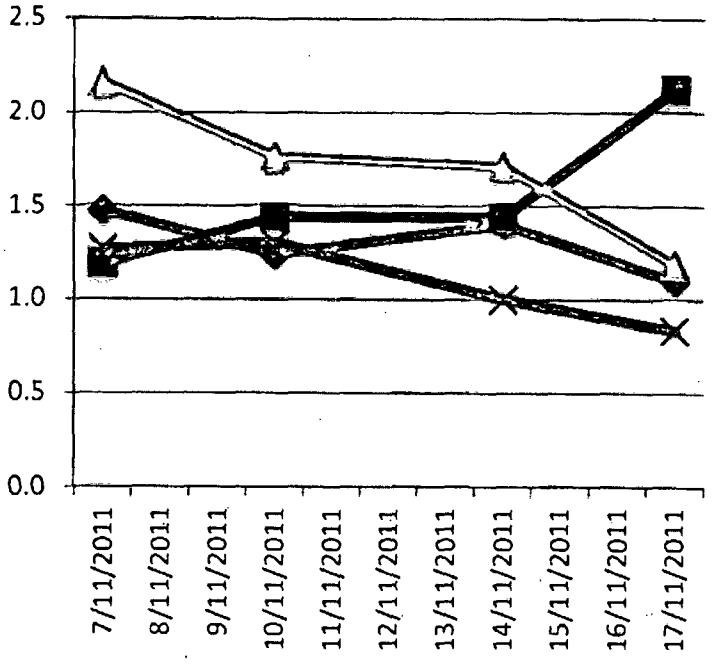

Figure 3

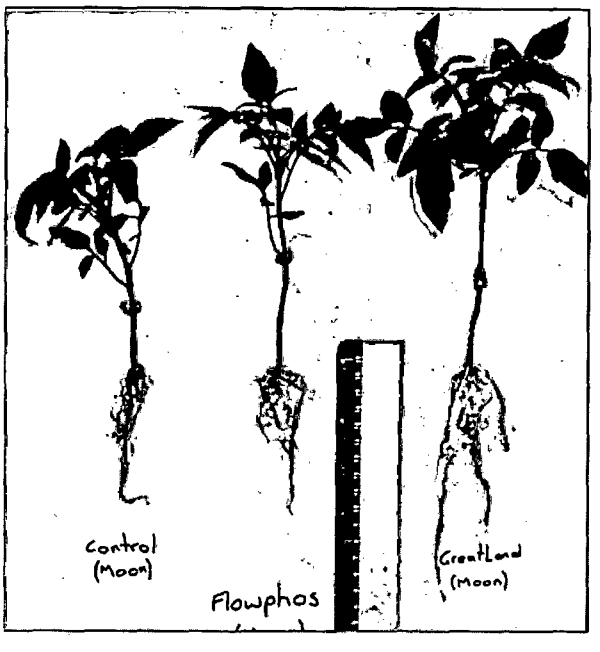

Figure 4

A

B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2012/001355

A. CLASSIFICATION OF SUBJECT MATTER

C05F 11/08 (2006.01) C09K 17/00 (2006.01) C09K 101/00 (2006.01) C12N 1/20 (2006.01) C12N 11/02 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases: EPODOC, WPI, MEDLINE, BIOSIS, HCAPLUS, AGRICOLA. Keywords: *Lactobacillus parafarraginis, Lactobacillus buchneri, Lactobacillus rapi, Lactobacillus zeae*, fertilizer, soil quality, bioremediation & like terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

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

* Special categories of cited documents:

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 December 2012	04 December 2012

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INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. PCT/AU2012/001355
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KR 100664747 B1 (JEONG et al.) 04 January 2007 Abstract; Working Examples 1-3	1, 21, 24-30 and 32-33
X	WO 2011012680 A2 (BARRY CALLEBAUT AG) 03 February 2011 Claim 1; Tables 1, 3; Example 1	1-2, 5 and 24-29
X	TABACCO, E., et al., 'Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as influenced by different lactic acid bacteria inocula', Journal of Dairy Science, 2011 (Mar), vol. 94, pages 1409-1419 page 1410, 'Crop and Ensiling'	1 and 24-29
X	WATANABE, K., et al., 'Lactobacillus kisonensis sp. nov., Lactobacillus otakiensis sp. nov., Lactobacillus rapi sp. nov. and Lactobacillus sunkii sp. nov., heterofermentative species isolated from sunki, a traditional Japanese pickle', International Journal of Systematic and Evolutionary Microbiology, 2009, vol. 59, pages 754-760 Abstract; page 755, left hand column, second paragraph; page 759, 'Description of Lactobacillus rapi sp. Nov.'	1 and 24-29
X	ASHARA, T., et al., 'Protective effect of Lactobacillus casei strain Shirota against lethal infection with multi-drug resistant Salmonella enterica serovar Typhimurium DT104 in mice', Journal of Applied Microbiology, 2011 (Jan), vol. 110, pages 163-173 page 164, 'Lactobacilli'	1 and 24-29
A	US 6277167 B1 (ITO et al.) 21 August 2001	

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2012/001355**

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

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
KR 100664747 B1	04 Jan 2007	KR 100664747 B1	04 Jan 2007
WO 2011012680 A2	03 Feb 2011	AU 2010277607 A1	09 Feb 2012
		EP 2459699 A2	06 Jun 2012
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		KR 20000017379 A	25 Mar 2000
		NZ 335602 A	29 Sep 2000
		US 6277167 B1	21 Aug 2001

End of Annex

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