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Title:
Scoping the potential uses of beneficial microorganisms for increasing productivity of cropping systems

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A report for the CRDC on the international discoveries of microorganisms that benefit cropping systems as they relate to identification, augmentation and application, with examples given from different crops, in particular with relevance to cotton.

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1. Introduction

There is a growing body of research showing that beneficial microbes can enhance plant productivity as well as yield in cropping systems. To appreciate the potential uses of beneficial microbes for increasing yield, it is necessary to understand how the microbes promote the growth of the plant they associated with in terms of fertilization and disease control, what are the mechanisms employed, what are the challenges in PGP microbe isolation, application and commercialization as well as what might hinder their successful application. This report summarises this information giving ample of examples of identified plant growth promoting microbes (including commercial products) and how they may benefit the plant, with particular focus on cotton and cotton related systems (e.g. plant pathogen of other plants that are also known to affect cotton). The author wishes to point out that relative to some other cropping systems (e.g. legumes and grains) there has been only limited availability of published information on beneficial microbes related to cotton production. In the last decade there is increased interest and research related to cotton inoculants mainly in developing countries, such as India.

1.1 Plant beneficial microorganisms

The rhizosphere (roots and surrounding soil) of plants harbours microorganisms that may have positive, negative or no visible effect on the plant growth. Rhizospheric microbes may be found in the root interior (endophytic), attached to root surface or free living. Their interactions with the plant may be symbiotic, associative or casual in nature. Most microbes in the rhizosphere have no known or measurable effect on plant growth. Those that inflict negative effects include pathogens and microbes producing toxins that inhibit root growth or those that remove essential substances from the soil. Plant beneficial microorganisms, or plant growth-promoting (PGP) microbes,

include bacteria, fungi or viruses that enhance plant growth. The known mechanisms by which microbes promote plant growth vary and can be divided into four main groups:

- enhancing nutrient availability (biofertilisation);
- production and regulation of plant hormones (phytostimulation);
- disease suppression (biocontrol/biopesticides); and
- improving soil properties,

all have the potential to increase productivity in cropping systems. It is important to note that studies of PGP microbes indicate that the most beneficial strains are multifunctional (demonstrate more than one mechanisms of plant growth promotion) and that the PGP properties are distributed among many different microbial groups, most of which are indigenous members of the soil microbial community.

Factors including agricultural practices (e.g. soil cultivation, season, stubble retention, burning etc), the crop plant species, cultivar and genotype as well as soil amendments have great influence on the diversity and activity of the indigenous microbes in the soil. It became increasingly evident that the microbial community in the rhizosphere is greatly shaped by the plant cultivar. Plant exudates may cause changes to the soil characteristics, such as the pH and carbon availability for microbial growth. Another way to influence microbial process in the soil is by **bioaugmentation** – addition of microbes that promote plant growth. One of the aims of Agricultural Biotechnology is to develop microbial inoculants to enhance plant growth with reduction in the use of chemical fertilisers and pesticides. Plant beneficial microorganisms and their potential application in cropping systems will be discussed in this review, with special attention given to examples with relevance to cotton and semi arid production regions.

2. Microorganisms that enhance nutrient availability to plants

Microorganisms that enhance nutrient availability to plants are often referred to as **biofertilisers**.

Biofertilisers contain live microorganisms that may contribute to plant nutrition either by facilitating nutrient uptake or by increasing primary nutrient availability in the rhizosphere. Biofertilisers, such as nitrogen fixing and phosphate solubilising microbes, may be used to increase crop yield when applied as complementary to, or as replacement for, chemical fertilisers. They may be applied as seed coat, directly onto the plant surface or into the soil.

An area, which is less studied, is the role of saphrophytic microbes that decompose soil detritus and subsequently increase nutrient availability through mineralization and microbial turnover. The direct effect on plant growth by such microbes cannot be easily measured, but they are often the microbes responsible for growth promotion related to soil supplement with organic fertilisers.

2.1 Microbes involved in Nitrogen bioavailability

The main nitrogen sources for the growing plant are ammonium (NH_4^+) and nitrate (NO_3^-). The regulation of whole plant N uptake in crop production is poorly understood, however, there is evidence that the concentrations of both nitrate and ammonium in soil solutions as well as plant status are involved. In return, uptake of different forms of N has a significant effect on the pH of the soil and its chemical composition, thus influencing the uptake of other nutrients (e.g. P and micronutrients such as Zn, Mn and Fe) and the structure of the microbial community around the root, including potential inoculants.

Nitrogen (N) is one of the principal plant nutrients, however it is often limited in agricultural soils due to high losses by emission or leaching. Microorganisms play a significant role in the nitrogen cycle in soils. Nitrogen fixation is the only way to turn atmospheric nitrogen into available form (ammonia) for plants, but recycling of organic carbon is also an important source of available N (ammonia and nitrate) for plants.

Available nitrogen can be removed from the soil by the reaction of denitrification and by leaching, often responsible for the low N availability in agricultural soils.

Nitrogen fixation is limited to a group of bacteria and their close relatives, cyanobacteria that contain the enzyme **nitrogenase**. These bacteria have the ability to fix atmospheric nitrogen (N_2) into forms available for plants (ammonia). Therefore, nitrogen-fixing microbes isolated from the rhizosphere (soil surrounding root zone and root surface) and phylosphere of plants were tested for their potential to enhance the growth of plants in lab based and field experiments. One of the most widely known and extensively used in agriculture is the application of rhizobial strains to legumes. Rhizobia can form endophytic symbiosis with legume, in which the plants directly absorb N fixed by the bacteria. Note that this review does not include rhizobia, the symbiotic bacteria that colonise legumes, unless the *Rhizobium* strain was used to inoculate non-leguminous plants.

Benefits from the associations of nitrogen fixing bacteria with non-leguminous plants were studied mainly in cropping systems involving rice, maize, sugarcane and wheat production. Bhattacharjee et al. (2008) summarised benefits of the association of nitrogen fixing bacteria with non-leguminous plants. The following table (Table 1) is an extract from the extensive table presented in their review. Adesemoye and Kloepper (2009) also listed studies on nitrogen biofertilisation with improved crop yield, as summarised in Table 2.

Table 1. Nitrogen fixing bacteria and their influence on crop growth (adopted from Bhattacharjee et al. 2008).

Plant	Experimental system	Bacterial strains tested	Range of biomass/yield increase
Rice	Greenhouse or laboratory conditions	*Azoarcus; Burkholderia;* photosynthetic *Bradyrhizobium; Gluconacetobacter diazotrophicus; Herbaspirillum seropedicae; Serratia marcescens; Pantoea agglomerans*	Biomass increase (total plant, seed or shoot biomass) 16% to over 70%
Rice	Field	*Rhizobium leguminosarum; Bradyrhizobium vietnamiensis;* bacterial strain mix *(G. diazotrophicus, H. seropedicae, Azospirillum lipoferum and Bradyrhizobium vietnamiensis)*	Yield increase up to 24%
Maize	Greenhouse or laboratory conditions	*Burkholderia sp.; Azospirillum brasilense; R. trifolii; Sinorhizobium sp.; R. etli bv. Phaseoli; H. seropedicae; Pseudomonas sp.*	Increase of 12-90% yield
Maize	Field	*Burkholderia sp.; R. trifolii; Azospirillum brasilense; H. seropedicae;*	Yield increase up to 33%
Sugarcane	Greenhouse or laboratory conditions	*G. diazotrophicus; H. seropedicae; H. rubrisubalbicans; Enterobacter*	19-70% yield increase
Sugarcane	Field	*B. vietnamiensis; G. diazotrophicus; H. seropedeceae; Kleibsiella sp. GR9*	Up to 19.5% yield increase
Wheat	Greenhouse or laboratory conditions	*H. seropedicae; R. trifolii; Cellulomonas sp.* (root growth)	24-82% yield increase
Wheat	Field	*Cellulomonas sp.*	33% increase in root growth

Table 2. PGP microbes improving nitrogen cycling and plant utilisation (adopted from Adesemoye and Kloepper 2009).

Plant	Experimental system	Bacterial strains tested	PGP effects
Maise	Greenhouse	AMF* with or without: N fixer (*Azotobacter chroococcum*), P solubeliser (*bacillus megaterium*) K solubeliser (*b. mucilaginous*)	Microbial inoculants increased the growth and nutritional assimilation of nutrients (N, P and K) and improved soil properties
Cotton	Pot experiment with soil from non-fertilised field (Uzbekistan)	*Pseudomonas alcaligens* PsA15 and *Mycobacterium phlei* MbP18	Increase shoot and/or root N content
Wheat	Pot and field	Two strains of *Pseudomonas fluorescens* (ACC_{50} and ACC_{73})	Increased efficiency of N and P uptake in all tested NPK levels tested
Pomegranate	Field in India	AMF* with or without Bacteria: *Azotobacter chroococcum* and *Azospirillum brasilense*	Inoculation with mixed strains gave higher biomass production and higher increase in nutrient uptake as well as more consistent results

*AMF = mycorrhizal fungi

Some past observations suggest that rhizobacteria can supply crops with substantial quantity of N, however other studies claim the free living N fixers only provide 1-10% of the N quantities commonly practiced in modern agriculture (150-200 kg N /ha/year). Understanding the soil potential for N biofertilisation versus N removal and the influence of agricultural practices and level of chemical fertilisation on the indigenous N production in the rhizosphere could enhance the contribution of the rhizobacteria to the N pool and, if so, lead to reduce fertilisation requirements.

2.2 Microbial phosphate solubilisation

Phosphorous (P) is an essential plant nutrient. Nowadays many agricultural soils have a high total P due to long-term fertilisation. Although soils generally contain a large amount of total P, much of this P is in mineral forms and only a small proportion is immediately available for plant uptake (plant obtain P as orthophosphate anions from the soil). Moreover, available P is quickly depleted from the rhizosphere. Microorganisms play an important role in the soil P cycle and, thus, in mediating P availability to plants. Microbes can enhance the capacity of plants to acquire P from the soil through directly solubilising and mineralising P from inorganic and organic P, facilitating the mobility of organic P through microbial turnover and/or increase root system (either by hormonal stimulation of root growth or by extension of existing root system, e.g. by mycorrhizal association).

Bacteria and fungi able to mobilise orthophosphate from different forms of organic and inorganic P have been isolated from the rhizosphere of plants.

A wide range of soil microbes able to solubilise inorganic P have been isolated, e.g. the fungi *Aspergillus* and *Penicillium* and the bacteria *Actinomycetes*, *Pseudomonas* and *bacillus*. Some fungal members of the *Penicillium* genus excrete organic acids that facilitate the conversion of immobilised soil P into soluble forms available to plants.

Penicillium radicum isolated from Australian wheat roots is capable of solubilising P and significantly increase plant growth. Bio-Care Technology developed it into a commercial inoculum product for use on broad-acre farms. Field tests showed that increase in plant growth was due to several mechanisms, in particular enhancement of root branching, enabling more efficient nutrient uptake from the soil and improving early plant establishment. *P. radicum* was most effective on infertile soil with neutral to acidic pH. However, another species of *Penicillium*, *P. bilaiae*, was effective (crop yield increase by 10%) on alkaline soils. Inconsistent results in field trials have been reported and are mainly attributed to variability in the ability of the introduced microbe to compete with other microbes in the soil and poor

understanding of the actual mechanisms involved in growth promotion, where other factors, such as increased root formation, may play the main role in growth promotion.

Adesemoye and Kloepper (2009) listed the effect of inoculants on uptake of P, N and other elements, as summarised in Table 3.

Table 3. Influence of PGP microbes on uptake of nutrients and other elements (adopted from Adesemoye and Kloepper 2009).

Plant	Bacterial strains tested	PGP effects
Chick pea and barley	*Mesorhizobium mediterraneum*	Significant increase in K, Ca, Mg, P and N in both plants
Lettuce (under different levels of water stress)	*Pseudomonas mendocina* and AMF strains	Enhanced uptake of N, P, Fe, Ca and Mn
Eggplant	Fertilisation with rock P and K and inoculation with P-solubilising *Bacillus megaterium* and K-solubilising bacterium *B. mucilaginosus*	Increased uptake of P and K

Organic P may constitute 30-50% of the soil total P, mostly in the form of phytate, which may be mineralised by bacteria that produce the enzyme phytase. Rhizospheric bacteria that produce phytase and are extremely efficient in mineralising organic P include species belonging to *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Serratia* and *Staphylococcus* genera.

A *Pseudomonas* species that utilised phytate was isolated from different soils in Australia by Richardson and Hadobas (1997). This efficient strain released up to 80% of the P contained in phytate and increased the ability of pasture plants to acquire P from phytate upon inoculation. Phytate utilising *Burkholderia* species isolated from lupin significantly promoted the growth of the lupin.

2.3 Microbial facilitation of nutrient acquisition by plant roots

The rate of root growth and the plasticity of root architecture along with development of the rhizosphere, through either root growth or extension of root hair, are clearly important for effective exploration of soil and interception of nutrients. Root hair can constitute up to 70% of root volume and may absorb up to 80% of P in non-mycorrhizal plants. Mycorrhizal fungi colonise the root cortex and extend externally, connecting the roots with surrounding soil. This is mainly responsible for the increased efficiency of P acquisition by mycorrhizal plants (by reaching P from longer distance and by extended surface area for P absorption). Mycorrhizal symbiosis may enhance plant growth through enhancement of plant establishment, protection against stress, improved soil structure and increased nutrient uptake, particularly P and essential micronutrients, such as Zn, Cu (but also N and, depending on soil pH may enhance K, Ca and Mg uptake).

3. Microorganisms that influence the levels of plant hormones

Phytohormones, such as indole-3-acetic acid (IAA), cytokinins, gibberellins and ethylene, play significant roles in the process of plant growth. One of the most important mechanisms of plant growth promotion is the production of plant hormones by some microorganisms in the rhizosphere, including bacteria and fungi. PGP microbes can enhance plant growth by producing plant growth hormones that stimulate growth, such as auxins (e.g. IAA), gibberellins and cytokinins in the proximity of the roots or by controlling the levels of ethylene produced by plants. IAA production by bacteria in the rhizosphere may enhance root formation and thus promote water and nutrient uptake by the plant. The production of plant hormones that enhance plant/root growth is often referred to as phytostimulation.

Note that the size and depth of root systems influence the capacity of plants for efficient capture of N (and other nutrients) and *vice verse*: root growth and morphology may change in response to N availability. Having both shallow and deep roots would allow the plant to reach both mineralized N available

often in topsoils as well as leached N in the depth. Indeed wheat lines with vigorous vertical root growth and more extensive horizontal root development have shown superiority in N uptake. Therefore, using phytostimulation for enhancing plant root development could play a significant role in improving nutrient uptake, especially if applied in combination with biofertilisation.

3.1 Indole acetic acid (IAA) producing rhizobacteria

IAA, the main plant auxin, controls processes involved in plant development and growth. It has key role in stimulating root growth and shaping its architecture (e.g., lateral root initiation, root vascular tissue development, root hair positioning). Many different rhizobacteria, including pathogenic and beneficial, associative and free living bacteria, are able to produce IAA.

IAA producing bacteria isolated from the rhizosphere include *Azospirillum* (the best studied), *Aeromonas*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas* and *Rhizobium*. Inoculation with IAA producing bacterial strains was used to stimulate seed germination, to speed up root growth, modify root growth pattern, increase root biomass and increase root and shoot length. Interestingly, using combination of inoculants gave higher stimulation that inoculation with a single strain.

3.2 Production of other plant hormones

Cytokinins stimulate plant cell division and control root development, inhibit primary root elongation and lateral root formation but can promote root hair formation. It is produced by some PGP rhizobacteria, such as *Arthrobacter*, *Azospirillum*, *Pseudomonas* and *Paenibacilus* species, but its involvement in plant growth promotion is not well understood.

Gibberellins promote the development of stem tissue, root elongation and lateral root extension. It is produced by species of PGP rhizobacteria, such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Herbaspirillum*, *Gluconobacter* and rhizobia.

3.3 Microbial regulation of plant ethylene levels

Ethylene is an important plant hormone that is essential for plant growth and development, but it may have different effects on plant growth depending on its concentrations in plant roots. Ethylene is required for the induction of systemic resistance during interaction with associative microbes and higher concentrations are involved in plant defence in response to pathogen infection. Certain PGP bacteria, such *Azospirillum brasilense*, can produce small amounts of ethylene, which seemed to promote root hair development in tomato roots.

However, ethylene accumulation inside plants in the root zone causes poor stem and root growth and diminished ability to acquire water and nutrients, thus reduce crop performance. Ethylene is produced in plants from the substrate 1-aminocyclopropane-1-carboxylate (ACC). ACC is released by the plants into the rhizosphere in times of stress and reabsorbed by the roots, where it is converted to ethylene. Ethylene accumulation in the roots causes reduced root growth and further stress to the plant.

Rhizospheric PGP fungi and bacteria (e.g. *Pseudomonas putida*) that can degrade ACC (contain the enzyme ACC deaminase) reduce adsorption and ethylene production by the roots and allow the plant to re-establish a healthy root and cope with environmental stress. Plant growth promotion by ACC degrading microbes seem to be particularly important under stress, such as cold, drought, saline soils or flooded soils contaminated by heavy metals. Microbes able to degrade ACC isolate from different soils include *Achromobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Pseudomonas* and *Rhizobium* strains. Examples of the beneficial effects of ACC deaminase producing bacteria on plant growth are given in Table 4.

Table 4. Plant responses to inoculation with microbes producing ACC deaminase

Inoculant/s	Plant	Plant Response
Enterobacter cloacae and *Pseudomonas putida*	Inoculated tomato seeds	Increase in plant resistance to prolonged flooding
Bacillus species: *circulans*, *firmus* & *globisporus*	Cabbage	Stimulated root elongation
Achromobacter piechaudii	Inoculated tomato plants	Significant increase in plant biomass under water and saline stress conditions
Pseudomonas asplenii	Inoculated raps seeds	In soils with high copper content, increase in plant biomass (roots and air parts)

3.4 Production of Nitric oxide

Nitric oxide (NO) is an intermediate in the denitrification pathway (the complete pathway removes N nutrients by converting nitrate to N_2) and as such its production might contribute to nitrate loss from soils. However, NO is an important signalling molecule controlling root growth, stimulating seed germination and is involved in plant defence response against pathogens. It also influences other plant signalling pathways, such as that involving IAA. Therefore, NO production by rhizospheric bacteria, such as *Azospirillum brasilense* 245, is considered to be another mechanism of plant growth promotion and is under investigation with some positive results in tomato and wheat.

3.5 Mixed mechanisms

Many studies indicated that evaluation and ranking of P-solubilising and N-fixing microbes under laboratory conditions do not necessarily correspond to the efficiency of the PGP microbe for enhancing P or N uptake under field conditions. The production of plant growth hormones that improve root surface

area may improve the ability of the plant to absorb these and other nutrients from the rhizosphere. Therefore, it would be beneficial to utilise those biofertilisers that can undertake dual actions – solubilise/mineralise P and/or fix N as well as stimulate roots growth or mycorrhizal formation that enhance the adsorption of these nutrients from the rhizosphere. Alternatively, the use of compatible inoculant mixes could serve the same purpose. Table 5, adopted from Martinez-Viveros et al. (2010), lists beneficial microbes shown to posses multiple PGP traits.

Table 5. PGP microbes shown to posses more than one PGP trait (adopted from Martinez-Viveros et al. 2010).

Species	CP	IAA	ACCD	HCN	SID	Other mechanisms
Azotobacter chroococcum A4	+	+	ND	+	+	
Bacillus sp. PSB1	+	+	ND	+	+	
Bacillus sp. PSB10	+	+	ND	+	+	
Enterobacter BNM 0357	+	+	ND	ND	ND	Nitrogenase +
Enterobacter sp. NBRI K28	+	+	+	ND	+	
Pseudomonas sp. SF4c	_	+	_	ND	+	
Pseudomonas aeruginosa BFPB9	+	+	ND	+	+	Protease+ Cellulase+
P. fluorescens PSRB21	+	+	ND	ND	+	
P. mosselli FP13	+	+	ND	-	+	Protease+
P. fluorescens biotype G (N3)	ND	+	+	ND	ND	Chitinase+
P. plecoglossicida FP12	+	+	ND	-	+	Protease+
P. putida PSRB6	+	+	ND	ND	+	Chitinase+
Serratia marcescens	ND	+	ND	ND	+	

CP = P solubilisation; IAA = indol acetic acid production; ACCD = ACC deaminase activity; HCN = hydrogen cyanide production; SID = siderophore production; Nitrogenase = ability to fix nitrogen, Protease/cellulase/chitinase = secretion of enzymes that degrading enzymes.

4. Microbes that suppress plant disease

Plant pathogens have great impact on crop yield and crop quality. The ability of growers worldwide to protect plant growth and health is continuously challenged by emerging, re-emerging and endemic plant pathogens. The commonly control strategy is the use of chemical pesticides and fungicides, however this has led to environmental concerns and to pathogen resistance, forcing constant development of new pesticides. Rhizospheric biocontrol microbes that suppress pathogens are being considered as alternative to chemical pesticides. Organism that suppresses the growth of a pathogen (or a pest) is referred to as biological control agent (BAC).

There is a growing list of mechanisms involved in disease suppression. The best known mechanisms are microbial antagonism (opposition; suppression) mechanisms. Examples for microbial antagonism include:

1) Direct inhibition of pathogen growth via production of antibiotics, toxins, hydrogen cyanide (HCN) and hydrolytic enzymes (chitinases, proteases, lipases) that degrade virulence factors or pathogen cell-wall components. Bacteria that produced antibiotics include *Pseudomonas* species (e.g. *P. fluorescence* strains) and *Bacillus cereus*. Fungi include *Trichoderma* and *Gliocladium*. *Trichoderma harzianum* Rifai 1295-22 suppresses plant pathogens and can enhance plant growth, the latter is probably due to its ability to solubilise P and micronutrients required by plants, such as iron and manganese.

   HCN production suppresses the growth of microbes (fungi and bacteria). It may inhibit fungal pathogens such as root-knot and black rot in tomato and tobacco caused by nematodes and the fungus *Thielaviopsis basicola*. However HCN might also have harmful effects on plants due to inhibition of energy metabolism and reduced root growth. Many different bacterial genera can produce HCN, including species of *Alcaligenes*, *Aeromonas*, *Bacillus*, *Rhizobium* and *Pseudomonas*.

2) Indirect inhibition via competition for a niche (nutrients or plant infection sites). Indirect inhibition also includes competition for minerals, such as iron, through production of siderophores. Selected bacteria and fungi produce siderophores as iron (Fe) chelating agents especially during iron deficiency. The ability of these microbes to deplete iron from their surrounding enhances their competitive fitness both against pathogens and in plant colonisation. Inoculation with siderophore producing bacteria was reported to increase plant biomass. PGP effects may result from a combination of several mechanisms. Microbes producing siderophores include *Bradyrhizobium*, *Pseudomonas*, *Rhizobium*, *Streptomyces*, *Serratia*, and *Azospirillum*.

Mechanisms involved in disease suppression also include activation of the plant defence system so that it can better defend itself against the pathogen, i.e. activation of the plant's systemic resistance responses (this is often referred to as induced systemic resistance (ISR), which has similar effects to acquired systemic resistance (SAR)).

Biocontrol agents may be used in combination with reduced amounts of chemical treatment or on their own. In both cases they may lead to a substantial reduction in the amount of chemical used. To be successful with biocontrol application for disease management, the user requires understanding of the biology and ecology of the pathogen and the microbial biocontrol agent as well as the agricultural practices associated with the crop.

Table 6 (adopted from Martinez-Viveros et al. 2010) presents some examples of response of crop plants to inoculation with PGP rhizobacteria under different experimental conditions, with focus on broad acreage crops.

Table 6. Response of crop plants to inoculation with PGP rhizobacteria under different experimental conditions, with focus on broad acreage crops (table adopted from Martinez-Viveros et al. 2010).

Plant	PGPR Inoculant	PGPR Mechanisms involved	Plant Growth Parameter (measure unit)	Increased plant parameters (%)	Assay condition and limitation
Cotton (Gossypium sp.)	*Bacillus subtilis* FZB 24 ®	IAA production, phytase activity and antibiotics production	- Average yield (t ha^{-1}) - Bolls/plant mean number - Mean plant height (cm)	31 19 11	- Field experiment - Commercial strain (Yao et al. 2006)
Maize (zea mays L.)	*Azotobacter*	IAA production	- Straw yield (t ha^{-1}) - Fresh biomass (t ha^{-1}) - Plant height (cm) - Fresh cob weight (g) - Cob length (cm) - Grain rows cob^{-1} - 1000 grain weight (g)	17 12 7 13 6 3 7	- Field experiment - Commercial strain
Maize (zea mays L)	*Pseudomonas fluorescens* (MPp4, MBf21 and MBf15)	IAA production and antagonism against *Fusarium verticillioides*	- Shoot length (cm) - Longest root length (cm) - Shoot fresh weight (g) - Root fresh weight (g) - Plants showing disease symptoms (%) - Disease reduction (%)	30-32 47-63 24-32 76-88 10-30 60-87	- Microcosm and greenhouse experiments - Non-commercial strains - Not proven at field level
Oat	*Azospirillum* sp (Cho6 and Cho8) *Azotobacter* sp.(ChO5) *Pseudomonas* sp (ChO9)	IAA production and acetylene reducing activity	- Root length (mm) - Root area (cm^2) - Shoot dry weight (mg $plant^{-1}$) - Total N (mg $plant^{-1}$) - Proportion of plant N fixed from atmosphere (%Ndfa)	-12-23 8-500 6-93 -50-60 50-64	- In vitro - Non commercial strains - Not proven at field level.
Sorghum (Sorghum bicolour (L.) Moench)	*B. cereus* (KBE7-8), *B. cereus* (NAS4-3) and *Stenotrophomonas maltophilia* (KBS9-B)	Siderophore production, IAA production and P solubilisation	- Shoot height (mm) - Shoot fresh weight (g) - Shoot dry weight (g) - Chlorophyll (spad units) - Leaf width (mm) - Root length (mm) - Root dry weight (g)	104-182 1133-2255 180-260 68-78 103-326 214-279 1300-1525	

5. Microbes that improve soil properties

Beneficial microbes capable of biodegradation and uptake of chemicals are important in agricultural systems not only due to plant growth promotion, but also due to their role in the management of the harmful environmental side effects of fertilizer use, be it chemical or organic fertilizers, due to leaching and run-off. Nitrate leaching and runoff might lead to eutrophication of aquatic systems and death of marine life. P-rich soil particles might erode from fields and reach surface water; nutrient runoff might cause harmful algal and

vegetation blooms, increasing dissolved oxygen and killing fish; release of greenhouse gases, ozone layer depletion, global warming and acid rain might all result from extensive fertilizer use.

Soil nutrients are transferred to the root surface via the rhizosphere; in the case of association with mycorrhizal fungi, through the fungal network, which enhances the roots surface, to the root. This fungal network also contributes to aggregation of soil particles and improved soil moisture.

Other microbes that improve soil structure and contribute to water retention are rhizospheric bacteria, such as Azospirillum, that produces thick layer of polysaccharides on the cells surface, in particular in times of stress. The sticky polysaccharides attach to soil particles and promote aggregation.

6. Isolation, identification and detection of PGP microbes from soil and plants

Methods for isolation and identification of PGP microbes vary and are dependent on the mechanism sought after and the biology of the microorganism. The development of biocontrol agents requires vigorous screening. It is the initial and most important stage as the success of all the following steps depends on the ability of a screening procedure to identify an appropriate candidate. There is no single correct way to screen for biocontrol agent as it depends on the crop, the target pathogen, and the cropping system. Where to look for the biocontrol agent and how to screen depends on what the most logical strategy appears to be, e.g. if the pathogen is affecting the roots, it would be logical to look for the biocontrol agent in the rhizosphere, in particular from nearby naturally suppressive soils (where the pathogen does not inflict disease).

Observation of zones of pathogen growth inhibition on Petri dishes led to the identification of many useful bacterial biocontrol agents. Nevertheless, this method does not identify biocontrol agents with other modes of action such as induced systemic resistance or competition. In order to screen for parasitism, propagules of the pathogen can be buried and then retrieved with the hope of

isolating parasites. Competition is screened via e.g. looking for microbes that are fast colonisers of sterilised soil and can exclude growth of other organisms as well as looking for microbes that colonise the same niches as the pathogen. New biocontrol microbes are continuously sought after and it is most probable that continued production of new biocontrol agents will be required to diversify the potential applications of biocontrol and in order to replace commonly used biocontrol products in case resistance develops.

Identification of the PGP microbes involves general methods in microbiology i.e. classical and molecular taxonomy. *Classical taxonomy* refers to the classification of organisms by characteristics based on the morphology, physiology, biochemistry (e.g. fatty acid composition; enzymatic activity) and metabolic (including nutritional) properties, that is, the physical and chemical characteristics of that microbe. *Molecular taxonomy,* on the other hand, refers to the *genotype*. The genotype is the genetic composition of the organism as specified in the nucleotide or base order of the DNA. Molecular taxonomy is frequently used as a definitive test for the identification of a known or in some cases unknown organism. Nowadays, identification of a microbial species mostly involves modern molecular methods, in particular sequencing of the ribosomal RNA gene (16S subunit for bacteria and 18S for fungi).

Using molecular techniques one can detect microbes with specific PGP traits in a mixed population of microbes. One way to do it is by using carefully designed DNA probes that anneal (attach) to genes that encode known PGP traits. The DNA probes are often labeled with fluorescent markers, making them easy to detect. Examples include use of *nifH* gene probes to detect nitrogen-fixing bacteria and *accA* gene probes to detect ACC degrading microbes. It is also possible to identify new or known factors involved in microbial interactions with plants by screening the genomic library of certain beneficial microbial species, e.g. *Pseudomonas fluorescens*, for sequences that may be involved in plant growth promotion.

7. Microbial growth promotion and disease suppression in cotton

7.1 Discovery of PGP fungi for cotton

Species of the fungus ***Trichoderma*** control many crop diseases including root rot, *Fusarium* wilt and seedling diseases in cotton sorghum, tomato, sugarcane, tobacco and many other plants. *Trichoderma* is used to control a range of fungal plant pathogens including *Fusarium* spp., *Pythium*, *Rhizoctonia solani* and others. The antagonistic nature of *Trichoderma* and its ability to control pathogens in the soil are known for decades. In the 1970's Elad et al. (1979) found that *T. harzianum* was an effective biocontrol agent against *Sclerotium rolfsii* and *Rhizoctonia solani* in cotton fields (as well as in bean and tomato).

Different strains of the fungal antagonist ***Gliocladium virens*** were effective in suppressing the cotton seedling pathogens *Pythium ultimum* and *Rhizoctonia solani*. Depending on the type of antibiotic excreted by the *G. virens* strain, it was more or less effective in suppressing *P. ultimum* or *R. solani*, showing the importance of vigorous screening for selecting the most suitable biocontrol agent among strains (Howell et al., 1993). Combining *G. virens* with the bacterium *Bacillus subtilis* strain GB03 (see Table 8) gave promising results for suppression of *Fusarium* wilt of cotton roots (Zhang et al., 1996).

The **mycorrhizal** fungi *Glomus mosseae*, *Glomus versiforme* and *Sclerocystis sinuosa* decreased *verticillium* wilt disease incidence on cotton (*Gossypium hirsutum* and *G. barbadense*) when tested in sterilised soil in pots (Liu 1995).

7.2 Discovery of PGP bacteria for cotton

Interest in rhizobacteria and their biocontrol ability increased towards the end of the 1980's and researchers such Hagedron et al. (1989) reported on detection of a range of rhizospheric bacteria that suppress cotton seedling diseases caused by *Pythium ultimum* and *Rhizoctonia solani* in the US. Hagedron et al., (1990) evaluated some of the disease suppressive strains

and found that ***Pseudomonas fluorescence*** could repress cotton seedling diseases (caused by *Pythium ultimum* and *Rhizoctonia solani*) under glasshouse conditions. When tested under field conditions they found inconsistency in disease suppression, although results were more promising with direct application (in-furrow, both liquid and granular) than with seed coating, presumably due to poor survival of the biocontrol agent on the seeds (Hagedron et al., 1993).

The production of **Kodiak®** represented a successful biocontrol commercial product for suppression of soil-borne plant pathogen of cotton. In 1992, ***Bacillus subtilis*** **GB03** was registered as a commercial biocontrol product in the US, namely Kodiak® (see Table 7). Mahaffee and Backman (1993) found that cotton seed-factors, including surface pH, cultivar, and presence of fungicide coating, influenced the colonisation of cotton and its rhizosphere by this biocontrol agent. Thus, such factors have to be considered when developing an inoculant product for cotton.

The development of the biocontrol agent used in Kodiak® originated in Australia (1970s to late 1908s) with *Bacillus subtilis* (isolate A-13), which was well documented as biocontrol and growth promoting agent in wheat and peanut. This strain was used to develop a cotton-adopted strain GB03 used in Kodiak®. The success of Kodiak® in the cotton market could be largely attributed to its integration with standard chemical fungicides, which expended the activity spectrum and allowed for long-term activity. This is a classic example of integrated pest management (IPM) maximising the benefits of both components to provide optimum disease control (Brannen and Kenny, 1997).

Streptomyces lydicus (strain WYE108) was also investigated under lab conditions for its potential to suppress *P. ultimum* causing seedling disease of cotton and it was capable of quickly colonising cotton roots and secreting fungal cell-wall digesting enzymes. It was suggested as a good candidate for biocontrol of seedling diseases (Yuan and Crawford, 1995).

The importance of increasing the fitness of the biocontrol agent in the field was demonstrated in field tests in Arizona, where the effectiveness of ***Burkholderia cepacia***, locally isolated from cotton fields was compared with that of several commercial products, including Kodiak® and Deny® (see Table 7). The local strain showed the most effective control of damping-off caused by *R. solani*, especially when combined with chemical fungicides (Misaghi et al., 1998), again demonstrating the importance of IPM in maximising treatment effectiveness.

7.3 Commercial biocontrol products for plant pathogens focusing on cotton

In 2002 Gardener and Fravel listed biocontrol products commercially available in the US. An extract from that list related to cotton is given in the table below (Table 7).

Fravel (2005) further listed microorganisms registered with the U.S. Environmental Protection Agency as biopesticides in his review of commercialisation and implementation of biocontrol. Table 8 is an extract from this list summarising the information related to cotton.

Current products in the US include Ascend™ PA, containing VA-Mycorrhizal soil and root inoculants. The information provided suggests that it increases growth in cotton by 300% (BioScientific, Inc., Arizona, USA, www.BioSci.com).

In Australia current products include BioAg Soil and Seed® for improvement of soil fertility (www.bioag.com.au) to be applied to soil 48 hours before seeding or cultivation but could also be applied through irrigation (5-10 litre/ha for cotton).

Table 7. An extract from Gardener and Fravel (2002) – Biocontrol products commercially available in the US

Contans WG, Intercept WG	
Biocontrol Organism	*Coniothyrium minitans*
Target Pathogen/Disease	*Sclerotinia sclerotiorum* and *S. minor*
Crop	**All agricultural soils**
Formulation	Water dispersible granule
Application Method	Spray
Manufacturer/Distributor	Prophyta Biologischer Pflanzenschutz GmbH, Germany; www.prophyta.com
Deny	
Biocontrol Organism	*Burkholderia cepacia*type Wisconsin
Target Pathogen/Disease	*Rhizoctonia, Pythium, Fusarium*, and disease caused by lesion, spiral, lance, and sting nematodes
Crop	Alfalfa, barley, beans, clover, **cotton**, peas, grain sorghum, vegetable crops, and wheat
Formulation	Peat-based dried biomass from solid fermentation; aqueous suspension
Application Method	Applied to seeds with a sticking agent in planter box (aqueous suspension formulation is for use in drip irrigation or as a seedling drench)
Manufacturer/Distributor	Stine Microbial Products, KS; Helena Chemicals, Memphis, TN, www.helenachemical.com
Intercept	
Biocontrol Organism	*Burkholderia cepacia*
Target Pathogen/Disease	*Rhizoctonia solani, Fusarium* spp., *Pythium* sp.
Crop	Maize, vegetables, **cotton**
Manufacturer/Distributor	Soil Technologies Corp. USA; www.soiltechcorp.com
Kodiak (several formulations)	
Biocontrol Organism	*Bacillus subtilis* GB03
Target Pathogen/Disease	*Rhizoctonia solani, Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. that attack roots
Crop	**Cotton**, legumes
Formulation	Dry powder; usually applied with chemical fungicides
Application Method	Added to a slurry mix for seed treatment; hopper box treatment
Manufacturer/Distributor	Gustafson, Inc. USA; www.gustafson.com
Actigard	
Biocontrol Agent	Acibenzolar-S-methyl
Target Pathogen/Disease	Many
Crop	Tobacco, tomato, lettuce, spinach
Formulation	Water dispersible granule
Application Method	Drench
Manufacturer/Distributor	Syngenta Crop Protection, NC; www.syngentacropprotection-us.com/

Table 8. An extract from Flavel (2005) - Microorganisms registered with the U.S. Environmental Protection Agency as biopesticides

Biocontrol agent	Year registered	Target organism or disease	Crop or use	Product name	Manufacturer or distributer
Aspergillus flavous AF36 (non-pathogen)	2003	*Aspergillus flavous*	**Cotton**	*Aspergillus flavous* AF36	Arizona Cotton research and Protection Council USA
***Bacillus subtilis* GBO3**	1992	*Rhizoctonia*, *Fusarium*, *Aspergillus* and others	Crop seeds for **cotton**, peanuts, wheat, barley	**Kodiak**; Companion	Gustafson, USA; Growth Products, USA
Bacillus subtilis MBI600	1994	*Fusarium*, Rhizoctonia, *Alternaria* and *Aspergillus*	**Cotton**, wheat, corn and others	Subtilex; Histick N/T	Becker Underwood; Premier horticulture, USA
Gliocladium virens GL-21	1990	Soilborne pathogens	**Cotton** and others	Soilgard	Thermo Trilogy Corp. USA

7.4 Recent studies on PGP for use in cotton production

Developing countries

Recent interest in bio-inoculants (both biocontrol and biofertilisers) in developing countries, most probably driven by the high costs of chemical fertilisers and fungicides, led to testing of microbial agents for enhancing yield and soil health in cotton production. Table 9 lists field and lab studies on cotton inoculants conducted during the last decade.

Table 9. Effect of inoculants on cotton in field and laboratory: results from the last decade mostly from developing countries

Microbial inoculant	Reference	Experimental system	Effects
Phenolics compounds produced from *Pseudomonas fluorescence* CRb-26	Mondal et al. (2001) India	Leaf infiltration using syringe, under glass house conditions	Protected cotton leaves from bacterial blight caused by *Xanthomonas camprestris* pv. *malvacearum* (Xcm)
Nitrogen fixing *Azotobacter*	Narula et al. (2004) India	Effect on wheat in fields, grown under wheat and cotton rotation, applied with or without chemical Nitrogen fertiliser	Pronounced effect on growth of wheat. Inoculant persisted into next season, saving in nitrogen fertilisers
N fixing, P solubilising, IAA producing *Azotobacter*, *Azospirillum*, *Acetobacter* and *Pseudomonas*	Narula et al. (2005) India	Cotton (*Gossypium hirsutum*) under irrigation, in field, plant height and boll weight measured	Inoculants survived in the soil to different degrees, there was an increase in boll biomass and significant savings of chemical N fertilisation required.
P releasing *Bacillus edaphicus* NBT	Sheng (2005) China	Pot experiments on cotton and rape with K-deficient soil from non-fertilised filed	Increased root and shoot growth of cotton and rape; increased N and P content in above-ground plant components; increased soil P content by 26-30%
Mixes of *Azospirillum*, P- solubilising *Bacillus megaterium* strain and *Methylobacterium* sp.	Gomathy et al. (2008) India	Field inoculation under drip irrigation, with or without drip fertilisation with NPK	Combining NPK fertilisation and use of inoculant mixes enhanced the growth parameters measured (plant height, biomass and yield).
C*ladorrhinum foecundissimum* (S8 and A32) from grass roots	Gasoni and Stegman (2009) Argentina	Pot trials	Has potential as biocontrol agent against *R. solani* in cotton crops
Several AM *Glomus* fungal species	Kobra et al. (2009) Iran	Greenhouse	Reduced *verticillium* wilt symptoms

Microbial inoculant	Reference	Experimental system	Effects
Mixes of *Azospirillum* with P-solubilising bacteria	Dale et al. (2010, 2011) India	Mixes applied on top of seed in the field, which under irrigation	Combining fertilisation and use of inoculant mixes enhanced root, shoot and yield, as well as fibre quality to some extent
Pseudomonas fluorescence (two isolates Q18 and CKK-3) isolated from local cotton root zones	Ardakani et al. (2010) Iran	Different formulations tested in greenhouse experiments	Plant growth promotion was observed and some formulations gave better results than others
Comparing *Azotobacter chroococcum* with its heat resistant mutant	Mittal et al. (2010) India	Pot experiments under different temperatures	Thermo-tolerant strains showed better performance in increasing cotton biomass
Isolated local disease suppressing organisms - *Trichoderma harzianum and T. viride* strains were found to be superior	Gaur and Sharma (2010) India	Pots in glasshouse	50% reduction of dry root rot caused by the pathogen *Rhizoctonia bataticola* on cotton. Potential for combination with fungicides.
AM fungi in combination with N-fixing *Azotobacter*	Paul et al. (2011) India	Field studies, high clay and silt soil, basal application of NPK, seed treatment with inoculant	Significant improvement in plant height, no of flowers and bolls, boll weight and seed cotton yield. Synergistic effect of dual inoculation was observed

<u>Australian cotton</u>

Over a decade ago, NSW Agriculture researchers (S. Allen and S. Putcha) collected endophytic microbes from cotton roots with the hope to isolate microbes that are able to suppress cotton diseases, in particular fungal diseases. They have managed to collect strains that suppressed cotton major pathogens, such as *Verticillium dahliae*, *Thielaviopsis basicola*, *Rhizoctonia*

solani and *Pythium ultimum*. Following glasshouse and field-testing two promising biocontrol strains were isolated, a *Pseudomonas* and a *Bacillus*. The Bacillus strain was effective in suppressing Fusarium wilt and field tests showed that it was as effective as the commercial product Kodiak registered in the US (see Table 7 for Kodiak). This study was concluded in 2001. It showed promising potential for the use of biocontrol in the Australian cotton industry (Tingay et al. 2002).

During the last decade a large body of information on soil biology, biocontrol and biofertilisation have been generated that could be used in further developing and applying biological inoculants for the Australian cotton industry. Important message highlighted repeatedly in inoculation studies in cotton and other crop plants are the importance of the survival of the inoculant and the suitability of the environment to support the beneficial activity of the inoculant. It can thus be concluded that inoculants should be sought after from the local cropping system where they are going to be applied. Indeed, indigenous microbes often exceeded introduced microbes when used as inoculants.

8. Examples from Semi Arid environments

A review conducted by Okon and Labandera-Gonzalez (1994) reported some success worldwide with *Azospirillum* inoculation of crop fields, including in arid and semi arid areas or in the hot season. They report some cases of reduction of N fertilisation by up to 50% due to inoculation. Note that the mechanisms of plant growth promotion by *Azospirillum* vary with strains capable of N fixation, IAA production and/or disease suppression.

A bacterial strain of the genus *Bacillus* (strain RM-2) posses multiple plant beneficial traits: it solubilise P 10 times better than reported for *Pseudomonas fluorescens*, *Bacillus megaterium*, and *Azospirillum lipoferum*; it produces IAA and ACC deaminase, which are probably responsible for increased root development. Inoculation of cowpea with this strain in semi arid fields (semi

arid soils often contain high amounts of insoluble P with only approx 2-4% available for plants) significantly enhanced all growth parameter measured, including P and N content in the shoots, shoot fresh or dry weight, seed weight, grain weight, root length, leaf area and root fresh or dry weight (Minaxi et al, 2011).

Similar to most *Bacillus* and *Pseudomonas* strains (including strains from arid areas, such as *Pseudomonas aeruginosa* RM-3 from deserts of India), RM-2 also produced ammonia, which is suggested to be involved in suppression of pathogen by this strain, another mechanism of plant growth promotion.

9. Consideration for use and limitations of microbial inoculants

Several factors have to be taken into account when considering the practical application of innovative biocontrol microbes:

1) Mass production of microbes can be technically challenging and expensive,
2) Formulation into product with long shelf life (transport and storage) may be problematic in particular with gram-negative bacteria which do not form spores,
3) The registration procedure can be expensive and time consuming (one way to get around this is to register as general plant growth promoting product without claiming specific activity),
4) Plant associated microbes might prove to be human pathogens or harmful for other organisms and the environment,
5) Survival of the inoculants in the soil as well as their activity, are influenced by the soil conditions, including temperature, pH, moisture, plant exudates (host cultivar) and interactions with other microbes. This point also includes the knowledge required on the most effective dose of inoculant (e.g. Optimum concentration for *Azospirillum* inoculation is 1×10^7 cfu per seed or seedling),
6) Extensive field testing will be required in the production system for which the biocontrol product intended

10. Commercialisation and markets for microbial inoculants

A variety of microbial inoculants are already on the market globally with increasing popularity booth by organic and conventional growers due to enhanced effectiveness and sustainability achieved as a result of extensive and systematic research. Surveys performed at the USA predict yet growing interest in the bioinoculant products in future years. Bolckmans (2008) reports (in: Berg 2009) that in 2004, the global market for biocontrol was valued at about $US588 million, with 11.2% of sales in Oceania (including Australia).

Microbial inoculants have numerous advantages when compared with chemical fertilisers, fungicides and pesticides: through careful selection of suitable microbes there is a reduced risk of environmental damage and potentially human health; they are safer to apply; their activity is more targeted; they are effective in small quantities; they are able to multiply themselves given appropriate conditions (where their population size is controlled by the plant and indigenous microbes) and may survive to the next season; they decompose faster and more effectively; they can be used on their own or in combination with conventional pest management.

Critical stages of successful commercialisation of microbial inoculants include:

1. Formulation must be economical with appropriate shelf life and suitable for transport and application
2. Risk to human health and environment must be minimal
3. Having good market potential
4. Simplicity in production and application, guaranteed propagule viability and efficiency (if required- over long term)
5. Compatibility with agronomic practices and equipment

Bacteria belonging to the genera *Agrobacterium, Bacillus*, *Pseudomonas*, and *Streptomyces* and fungi belonging to the genera *Ampelomyces, Candida, Coniothyrium,* and *Trichoderma* were successfully commercialized and marketed as EPA-registered biopesticides in the United States. Microbes

(including those isolated from plant or soil) registered for biocontrol and active compounds at the United State can be found listed in the following address:

http://www.epa.gov/oppbppd1/biopesticides/ingredients/index_p-s.htm#s.

A few examples of biocontrol and other PGP microbial products include: *Agrobacterium radiobacter* K1026 (Nogall®), *Bacillus pumilus* QST 2808 (Sonata® TM), *B. pumilus* GB34 (YieldShield®), *B. subtilis* GBO3 (Kodiak®), *Pantoea agglomerans* C9-1 (BlightBan C9-1®), *P. agglomerans* E325 (Bloomtime®), *Pseudomonas aureofaciens* Tx-1(Spot-Less®T), *P. syringae* ESC-10 and ESC-11 (Bio-save®), *P. fluorescens* A506 (BlightBan®), *P. chlororaphis* MA 342 (Cedomon®), *Streptomyces griseoviridis* K61 (Mycostop®), and *S. lydicus* WYEC 108 (Actinovate®).

The U.S. market in consultation with the American Phytopatology Society in 2005 has registered products based on the *Bacillus* (BioYield, Companion, EcoGuard, HiStick N/T, Kodiak , Mepplus, Serenade, Sonata, Subtilex, Yield-Shield), *Burkholderia cepacia* (Deny and Intercept), and *Pseudomonas* (AtEze, Bio-save, BlightBan, Frostban, Spot-Less). However, to be able to make the application of biofertilisers PGP microbes a widely spread practice, there is a need to address the inconsistency of their effect in the field. PGP microbes should be used in the ecological niche they survive best, including association with a suitable plant cultivar, suitable climate and soil conditions. One way to address such requirements could be to source the PGP microbes from the cropping system and the geographical region in which they are intended, i.e., a more localized solutions using indigenous microbe.

Table 10, adopted from Berg (2009), lists representatives of microbial inoculant products available in the market containing microbes that employ a wide range of PGP mechanisms.

Table 10. Representative of microbial inoculants (adopted from Berg, 2009).

Microorganisms	Product Name	Plants, pathogens, or pathosystems	Company
Ampelomyces quisqualis M-10	AQ10 Biofungicide	Powdery mildew on apples, cucurbits, grapes, ornamentals, strawberries and tomatoes	Ecogen
Azospirillum spp.	Biopromoter	Paddy, millets, oilseeds, fruits, vegetables, sugarcane, banana	Manidharma Biotech
Bacillus subtilis FZB24	FZB24 li, TB, WG Rhizoplus	Potatoes, vegetables, ornamentals, strawberries, bulbs, turf and woods	AbiTep
Bacillus subtilis GB03	Kodiak	Growth promotion; *Rhizoctonia* and *Fusarium* spp.	(Gustafson); Bayer Cropscience
Bacillus pumilus GB34	YiedShield	Soil-borne fugal pathogens	(Gustafson); Bayer Cropscience
Bacillus subtilis QS716	Serenade	Tobacco, Tomato, lettuce, spinach	AgraQuest
Bacillus subtilis GB03, other *B. subtilis*, *B. lichenformis*, and *B. megaterium*	Companion	*Rhizoctonia*, *Pythium*, *Fusarium*, and *Phytophthora*	Growth Products
Bradyrhizobium japonicum	Soil implant+	Soy bean	Nitragin
Coniothyrium minitans	Contans WG, Interecept WG	*Sclerotinia sclerotiorum*, *S. minor*	Prophyta Biologischer Pflanzenschutz
Delftia acidovorans	BioBoost	Canola	Brett-Young Seeds Limited
Paecilomyces lilacinus	Bioact WG	Nematodes	Prophyta Biologischer Pflanzenschutz
Phlebiopsis gigantea	Rotex	*Heterobasidium annosum*	E-nema Biologischer p Pflanzenschutz
Pseudomonas chlororaphis	Cedomon	Leaf stripe, net blotch, *Fusarium* sp., sot blotch, leaf spot, etc. on barley and oats	BioAgri AB
Pseudomonas fluorescens A506	BlightBan A506	Frost damage, *Erwinia* amylovora, and russet –inducing bacteria on almond, apple, peach, pear, etc.	NuFarm
Pseudomonas trivialis 3Re-27	Salavida	Lettuce	Sourcon Padena
Pseudomonas spp.	Proradix	*Rhizoctonia solani*	Sourcon Padena
Serratia plymuthcia HRO-C48	RhizoStar	Strawberries, oilseed rape	Prophyta Biologischer Pflanzenschultz
Streptomyces griseoviridis K61	Mycostop	*Phomopsis* spp., *Botrytis* spp., *Phythophora* spp.	Kemira Agro Oy
Trichoderma harzianum T22	RootShield, Plantshield T22, Planter box	*Pythium* spp., *Rhizoctonia solani*, *Fusarium* spp,	Bioworks

Kaewchai et al. (2009) summarise a long list of fungal commercial products based on their use as biofertilisers for improved nutrition or as mycofungicides (fungal-based fungicides) for disease suppression. Note that most biofertilising fungal products contain either *Trichoderma* species or endomycorrhizal fungi (AM) or ectomycorrhizal fungi (ECM). The lists are presented in the following tables: Table 11 lists mycofungicides and Table 12 lists fungal biofertilisers, both tables adopted from Kaewchai et al. (2009).

Table 11. Some of the biocontrol products available in the global market (adopted from Kaewchai et al. 2009).

Products	Fungus	Target Pathogen*	Formulation	Producer
AQ 10 Biofungicide	*Ampelomyces quisqualis* M-10	Powdery mildew	Water-dispersible granule	Ecogen, Inc. Langhorne, PA www.algonet.se/-binab/index2.html
Bineb (several products)	*Trichoderma harzianum* and *T. Polysporum*	Fungi causing wilt, root rot	Wettable powder; Granules pellet	
Bioderma	*Trichoderma viride*	*Sclerotinia*, *Rhizoctonia*	Wettable powder	Biotech International Ltd., India www.biotech-int.com
Bioderma-H	*Trichoderma harzianum*	A long list including: *Phytophthora*, *Fusarium*, *Pythium* spp.,	Wettable powder	Biotech International Ltd., India
Biofox C	*Fusarium oxysporum* (non-pathogenic)	*Fusarium oxysporum Fusarium miniliforme*		SIAPA, Italy
Biofungus	*Trichoderma* spp.	*Sclerotinia, Phytophthora, Rhizoctonia solani, Pythium* spp., *Fusarium, Verticillium*	Granule Wettable powder	Grondortsmetting en deCuester n.v., Belgium
Cotans WG	*Coniothyrium minitans*	*Sclerotinia* spp.	Granules	Prophyta, Germany www.prophyta.de
Fungi-Killer	*Trichoderma harzianum*	*Phytophthora, Fusarium*	Powder	Bangkok Organic Compost Ltd. Thailand
Fusaclean	*Fusarium oxysporum* Fo47 (non-pathogenic)	*Fusarium oxysporum*	Spores, microgranule	Natural Plant Protection, France
Ketocin	*Chaetomium cupreum*	*Fusarium oxysporum* f_sp. *lycopersici*	Powder	Neoworld Ltd, Thailand

Products	Fungus	Target Pathogen*	Formulation	Producer
Ketomium	*Chaetomium globosum* *Chaetomium cupreum*	A long list including: *Phytophthora* *Fusarium oxysporum* f. sp.	Pellets Powder	Guangxi Guilin Green Harvest Fertilizer Factory, China. Nova Science, Thailand.
Koni	*Coniothyrium minitans*	*Sclerotinia* spp.		BIOVED Ltd., Hungary www.bioved.hu
Novacide	*Chaetomium cupreum*	*Fusarium oxysporum* f_sp. *lycopersici*	Powder	Nova Science, Thailand.
Polygandron Polyversum	*Pythium oligandrum*	*Pythium ultimum*	Granule or powder	Plant Protection Institute, Slovak Republic
Prestop Primastop	*Gliocladium catenulatum* strain JI446	*Pythium* spp. *Rhizoctonia solani,* *Botrytis* spp. *Didymella* spp.	Wettable powder	Kemira Agro Oy, Finland.
Promote	*Trichoderma Harzianum* *T. viride*	*Pythium* *Rhizoctonia* *Fusarium*		JH biotech Inc.,Ventura, CA, USA www.jhbiotech.com
RootShield PlantShield	*Trichoderma Harzianum* Strain T-22	*Pythium, Rhizoctonia, Fusarium, Sclerotina*	Granules and Wettable powder	Bioworks, Inc. NY.USA www.bioworksbiocontrol.com
Sentinel ^{R}	*Trichoderma* spp Strain LC52	*Botrytis cinera*	Wettable powder	Agrimms Technologies Ltd www.vinevax.com
SoilGard (GlioGard)	*Gliocladium Virens* GL21	Several plant diseases Damping-off and root pathogens	Granules Alginate prill	Thermo Triology, USA
Trichodex	*Trichoderma Harzianum* T-39	Fungal diseases e.g. *Botrytis cinerea* *Colletotrichum,* *Monilinia laxa,* *Plasmospara viticola* *Rhizopus stolonifer*	Wettable powder	Makhteshim-Agan, DeCeuster, Belgiium
Vinevax^{TM}	*Trichoderma* Spp.	Wood-infecting fungal pathogens of vineyard, orchard, ornamental trees, and vines	Wettable powder	Agrimms Technologies Ltd, www.vinevax.com

*Mechanisms of disease suppression: ***Gliocladium*** and ***Trichoderma*** species produce a range of antibiotics and use other mechanisms to suppress disease, such as production of pathogen cell-wall degrading enzymes, competition for nutrients and plant colonisation sites (pathogen displacement). ***Chaetominum*** sp. can degrade the cell wall of plant fungal pathogens and ***Periconia byssoides*** can degrade cell walls, inhibit growth and spore germination of fungal pathogens. ***Trichoderma*** is also capable of activating the plant induced resistance system for protection against pathogens and non-pathogenic strains of *Fusarium*, *Pythium* and *Rhizoctonia* could induce plant resistance to pathogenic strains.

Table 12. Some fungal biofertilisers available globally (adopted from Kaewchai et al. 2009)

Products	Fungi*	Companies
AgBio-Endos AgBio-Ectos	ECM AM	AgBio Inc, Westminter, USA Agbio-inc.com
AM120	AM	Reforestation Techonologies International, USA www.reforest.com
Bioorganic Plus	*Trichoderma Harzianum* *T. hamatum*	NovaScience Co. Ltd, Thailand
Bio Vam	AM Tri	T&J Enterprises, USA www.tandjenterprises.com
BuRize	AM Fungi	Bio Scientific Inc, Arizona, USA www.biosci.com
Diehard™ mycorrhizal inoculant	AM Tri	Horticultural Alliance, Inc, FL, USA www.horticulturalalliance.com
Endo mycorrhizal inoculants (BEI), Inoculants micronized (BEIM), Mycorrhizal root dip	AM	Bio-Organics, Oregon, USA www.bio-organics.com
MycoApply® Endo MycoApply® Endo/Ecto MycoApply® Maxx	ECM AM	Mycorrhizal application Inc, Oregon, USA www.mycorrhizae.com
Plant Success ™ Mycogrow ™	ECM AM	Fungi perfecti, LCC, WA, USA www.fungi.com
Mycomax	AM (*Glomous intraradices*)	JHBiotech Inc. California, USA www.jhbiotech.com
Myke; Myke® Pro Mycorise®	AM	Premier Tech Biotechnologies, Canada www.premiertech.com
PLantmate®	Tri	Agrimms Technologies Ltd, www.vinevax.com
Promote®	ECM	JHBiotech Inc.California, USA www.jhbiotech.com
Rhizanova	AM	Becker- Underwood Inc., USA www.beckerunderwood.com
Rootgrow, Rootgrow Professional	AM	PlantWorks Ltd., United Kingdom www.plantworksuk.co.uk
SoilMoist™	ECM AM	JMR chemical, Inc. Ohio, USA www.soilmoist.com
Superzyme	Tri	JH Biotech, Inc,. Ventura, CA. USA www.jhbiotech.com
Tricho®	Tri	Agrimms Technologies Ltd, www.vinevax.com

***Note:** Tri = Trichoderma species; AM = mycorrhizal or endomycorrhizal fungi; ECM = ectomycorrhizal fungi.

11. Summary and conclusions

Plant growth promotion is a complex phenomenon that often cannot be attributed to a single mechanism, as most PGP microbes are capable of multiple mechanisms and in some cases their PGP effect may only occur through interactions with different microbes. It is important to note that any microbial agent added to the rhizosphere would have to interact not only with the plant but also with any other organism sharing the same ecological niche. To be successful the inoculant has to maintain a critical population mass in the soil and have the right conditions to exert its beneficial activity.

Despite the challenges, a growing variety of microorganisms with properties that can be exploited in plant growth promotion are being discovered and tested under field conditions, with increasing number of cases reporting success. Reductions in the uses of chemical fertilisers and pesticides as well as improved yield are the main direct benefits reported from use of inoculants. Indirect benefits are reduced build up of toxins in agricultural soils and reduced environmental pollution with agricultural runoff. Success is often associated with using a combination of inoculants with complementary beneficial traits, e.g. biofertilisers that increase nutrient availability in the proximity of the roots together with a mycorrhizal fungus that enhances the root system and assists the plant to absorb the nutrients. It is not surprising that often indigenous microbes are proving to be the most effective, as such microbes would suit the environmental conditions in the cropping system they are intended for. Nevertheless, indigenous microbes would still have to out compete other microbes for resources and, in the case of biocontrol agent, suppress pathogens.

The Australian GRDC has been investing in a Soil Biology research since 1992. Between 2002 and 2008 they have invested approx AUD 8,000,000 on the Soil Biology initiative, which they report was successful in generating economical, environmental and social benefits. Economical benefits were attributed to investment areas related to inoculants, disease control and nutrient balance. Environmental benefits were attributed to a reduced use of

fertilisers and reduced energy input. The GRDC announced their next generation soil biology plan to run between 2009 and 2014. One of the main focuses of the plan is the Beneficial Microbes Program, which aims to isolate, test and commercialise microbial bio-inoculants for the grain industry. One of their rationales for such an investment is the readiness of the Australian farmers to reduce on-farm chemical inputs and replace them with biological alternatives in order to reduce costs, increase sustainability and maintain healthy soils and ecosystems (GRDC Five years Business plan, 2009-2014).

The cotton industry, via the CRDC, would benefit from maintaining its program on soil biology and, from reasons outlined above, extend research into isolation of cotton specific beneficial microbes, from different cotton growing regions in Australia. Other than isolating microbial agents for augmentation, further research should be directed into cropping practices that enhance existing and introduced beneficial microbes, such as controlling the amount of chemical inputs, which might otherwise suppress the activity of beneficials and increase the activity of detrimental organisms.

There is a great scope for collaborations between the different Research and Development Corporations in Australian agriculture and worldwide with regard to the development of technology for e.g. screening for and identification of microbes with beneficial traits; assessment of the benefit to the plant; testing strains for commercialisation; designing best formulations for inoculant delivery; detecting and assessing the performance of inoculants in the soil; analysing soil microbial communities and the effects of the soil inoculation on soil health; and the studying of general effects of cropping practices on specific microbial communities. In general, it can be said that similar groups of beneficial microbes seem to be involved in promoting the growth of different plants. Examples for this are bacteria from the *Bacillus*, *Azospirillum*, *Pseudomonas* groups and mycorrhizal fungi. Nevertheless, there is enough evidence to suggest that particular microbial species, or even strains, benefit specific plants under defined conditions. Therefore, beside collaborative efforts, regional efforts need to be maintained to produce inoculants specific to the crop, agronomic practices, soil type and other environmental conditions.

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