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Review

Scoping the potential uses of beneficial microorganisms for increasing productivity in cotton cropping systems

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ABSTRACT

There is a growing body of evidence that demonstrates the potential of various microbes to enhance plant productivity and yield in cropping systems. Realizing the potential of beneficial microbes requires an understanding of the role of microbes in growth promotion, particularly in terms of fertilization and disease control, the underlying mechanisms and the challenges in application and commercialization of plant growth-promoting (PGP) microbes. This review focuses specifically on the use of PGP microbes in the cotton industry and summarizes the commercial bioinoculant products currently available for cotton; highlighting factors that must be considered for future development of PGP microbial products for the cotton industry. Given the paucity of information on beneficial microbes for cotton production systems in comparison to those for other cropping systems (e.g. legumes and grains), a snapshot of the current research is critical in light of the increased interest in cotton inoculants, mainly in developing countries such as India, and the overall increased interest in PGP applications as part of promoting sustainable agriculture.

1. Introduction

Agricultural industries such as the cotton industry rely heavily on the use of chemical fertilizers, herbicides and pesticides. One of the aims of agricultural biotechnology is to develop microbial inoculants to enhance plant growth and suppress plant disease, with a key goal of reducing reliance on chemical fertilizers and pesticides (Adesemoye et al., 2009). Many factors need to be taken into consideration during the development of such inoculants commercially (Berg, 2009) including selection of appropriate plant growth-promoting (PGP) microbes based on target host plant, soil type, indigenous microbial communities, environmental conditions, inoculant density, suitability of carriers and compatibility with integrated crop management.

Plant growth and productivity is heavily influenced by the interactions between plant-roots and the surrounding soil, including the microbial populations within the soil. The plant rhizosphere harbours microorganisms that may have positive, negative or no visible effect on plant growth. Although most rhizospheric microbes appear to be benign, deleterious microorganisms include pathogens and microbes producing toxins that inhibit root growth or those that remove essential substances from the soil. By contrast the main mechanisms for plant growth promotion include suppression of disease (biocontrol); enhancement of nutrient availability (biofertilization); and production of plant hormones (phytostimulation) (reviewed by Martinez-Viveros et al., 2010; Bhattacharyya and Jha, 2012). Studies of PGP microbes indicate that multifunctionality is a hallmark of the most beneficial (Vassilev et al., 2006; Avis et al., 2008).

The indigenous rhizospheric microbial population of agricultural soils is greatly influenced by agricultural practices (e.g. soil cultivation, season, stubble retention, burning etc.), crop plant species, cultivar and genotype, as well as soil type (Berg and Smalla, 2009; Reeve et al., 2010). Plant exudates may cause changes to soil characteristics such as pH and carbon availability, impacting the diversity and activity of microbial populations (Haichar et al., 2008). Bioaugmentation, the addition of microbes to agricultural soils, thus becomes a valuable influence on soil microbial processes.

In light of this, the question under consideration is the potential for successful application of biofertilization, biocontrol and phytostimulation in cotton production systems. This review summarizes the types of PGP microbes and the mechanisms by which they enhance plant growth, with particular attention to those tested on cotton, and discusses the factors essential to the practical application and commercialization of microbial inoculants for cotton. In addition, currently available commercial PGP and biocontrol products for cotton production systems are evaluated.

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2. Plant growth promotion in cotton: biocontrol, biofertilization and phytostimulation

2.1. Mechanisms of disease suppression

Globally, crop growth protection and health is continuously challenged by emerging, re-emerging and endemic plant pathogens (Miller et al., 2009). Chemical pesticide and fungicide use has led to environmental concerns and pathogen resistance, forcing constant development of new agents (Fernando et al., 2006). Rhizospheric microbes that suppress plant pathogens could be used as biocontrol agents, and may be considered as alternative to chemical pesticides. There are a number of mechanisms for plant pathogen suppression including direct inhibition of pathogen growth through production of antibiotics, toxins, hydrogen cyanide (HCN) and hydrolytic enzymes (chitinases, proteases, lipases) that degrade virulence factors or pathogen cell-wall components (reviewed in Whipps, 2001; Compant et al., 2005).

Antibiotics are a normal part of the self-protective arsenals of bacteria, such as *Pseudomonas* species (e.g. *Pseudomonas fluorescens* strains) (Haas and Defago, 2005) and *Bacillus* species (e.g. *Bacillus subtilis*) (Kim et al., 2003), as well as fungal species such as *Trichoderma*, *Gliocladium, Ampelomyces* and *Chaetomium* (Kaewchai et al., 2009) and therefore these organisms have great potential for soil conditioning. Multifunctional organisms such as *Trichoderma harzianum* Rifai 1295-22 appear to enhance plant growth by solubilising phosphate (P) and micronutrients required by plants, such as iron and manganese, and also suppresses plant pathogens (Altomare et al., 1999). HCN production suppresses microbial growth and may inhibit pathogens such as root-knot, bacterial canker and black rot in tomato and tobacco (Voisard et al., 1989; Siddiqui et al., 2006; Lanteigne et al., 2012). However HCN might be harmful to plants by inhibiting energy metabolism and reducing root growth (Siddiqui et al., 2006). Many different bacterial genera produce HCN, including *Alcaligenes*, *Aeromonas*, *Bacillus*, *Rhizobium* and *Pseudomonas* spp. (Ahmad et al., 2008).

Pathogen suppression can also occur competitively through indirect inhibition. Selected bacteria and fungi produce siderophores as iron chelating agents especially during iron deficiency (Sharma and Johri, 2003), including *Bradyrhizobium*, *Pseudomonas*, *Rhizobium*, *Streptomyces*, *Serratia*, and *Azospirillum* (Martinez-Viveros et al., 2010). Their ability to deplete iron from their surroundings makes it unavailable to pathogenic fungi, creating a competitive advantage (O'Sullivan and O'Gara, 1992; Loper and Henkels, 1999). Inoculation with siderophore-producing bacteria grown under iron limiting conditions has a positive effect on plant growth (Carrillo-Castaneda et al., 2002); however the potential role for a combination of several PGP mechanisms and not siderophore production alone cannot be discounted.

Other mechanisms involved in disease suppression include activation of the plant's own defence system, known as induced systemic resistance (ISR). Volatile compounds released by PGP bacteria and fungi can trigger ISR, resulting in enhanced expression of defence-related genes in the host (Ryu et al., 2005; Hossain et al., 2007; Naznin et al., 2014).

2.2. Microbes that suppress disease in cotton

Cotton pathogens present a high economic burden to growers (Pereg, 2013). Seedling disease complexes are caused by several fungal and bacterial pathogens including *Pythium ultimum, Rhizoctonia solani, Fusarium* spp., *Verticillium* spp., *Thielaviopsis basicola* and *Xanthomonas camprestris* pv. *malvacerum* (*Xcm*). Management strategies to prevent disease include selection of suitable varieties and planting times, crop rotation with non-host species, optimised seed bed preparation and irrigation schedules, agrochemicals and improved farm-hygiene practices. Unfortunately, quite often fungicides are not effective against soil-borne pathogens and management strategies that control disease caused by one pathogen not only may not be effective in controlling others but might actually increase damage by other pathogens (Pereg, 2013). Disease-resistant cotton varieties with increased resistance to *Fusarium* and *Verticillium* spp. have been selected (Kappelman, 1980; Gore et al., 2009). While pathogen-specific resistance can be incredibly valuable, this is too restrictive in the face of the number of cotton pathogens, and commercial transgenic varieties with resistance to multiple soil-borne diseases are currently unavailable. Despite attempts to develop such resistant variants, cotton seedling disease remains an ongoing issue for producers. Consequently the studies that have identified PGP microbes with potential as biocontrol agents against common cotton pathogens (see Table 1) provide an important alternative.

Table 1
Biocontrol agents identified to control common cotton pathogens.

Biocontrol agent	Pathogen/s controlled (geographic region)	References
Trichoderma virens	*Pythium ultimum (USA)*	Howell, 1982; Howell and Stipanovic, 1983; Howell, 2002
	Rhizoctonia solani (USA)	Howell et al., 2000
	Fusarium oxysporum	Zhang et al., 1996a
	Verticillium dahliae	Hanson, 2000
Pseudomonas fluoroscens	*Pythium ultimum*	Howell and Stipanovic, 1980; Loper, 1988; Hagedorn and Nelson, 1990; Howie and Suslow, 1991; Loper, 1988
	Rhizoctonia solani	Howell and Stipanovic, 1979
	verticillium dahlia	Mansoori et al., 2013; Erdogan and Benlioglu, 2010
	Xanthomonas camprestris (Xcm) (India)	Habish, 1968; Mondel et al., 2000, 2001
Streptomyces lydicus	*Pythium ultimum (USA)*	Yuan and Crawford, 1995
Burkholderia cepacia	*Rhizoctonia solani (USA)*	Zaki et al., 1998
Trichoderma harzianum	*Rhizoctonia solani (Israel)*	Elad et al., 1980
	Fusarium oxysporum	Sivan and Chet, 1986
Cladorrhium foecundissimum	*Rhizoctonia solani (Argentina)*	Gasoni and Stegman de Gurfinkel, 2009
Bacillus subtilis	*Fusarium oxysporum*	Zhang et al., 1996a
	Verticillium dahliae	Mansoori et al., 2013

A number of organisms can cause damping-off in cotton, resulting in substantial losses to growers. *P. ultimum* soil infestation is one such organism, but research has demonstrated that several rhizospheric microbes have an antagonistic effect against *P. ultimum* infection in cotton, such as *Entobacter cloacae* and *Erwinia herbicola* (Nelson, 1988). The fungus *Trichoderma (Gliocladium) virens* improves the survival of cotton seedlings, possibly due to the production of the antibiotic compound gliovirin (Howell, 1982; Howell and Stipanovic, 1983). Several *Trichoderma* spp. control the disease by competing for metabolites released from the germinating seeds (Howell, 2002). *P. fluorescens* increases seedling survival and cotton stand in *P. ultimum* infested soil, possibly through antibiosis and antagonistic siderophore production (Howell and Stipanovic, 1980; Loper, 1988; Hagedorn and Nelson, 1990; Howie and Suslow, 1991). *Streptomyces lydicus* can destroy germinating oospores and damage the cell walls of fungal hyphae, making it a potential biocontrol agent against *Pythium* seed and root rot in cotton and other crops (Yuan and Crawford, 1995).

Similarly *R. solani* also plays a critical role in the pronounced losses due to cotton damping-off. Seed treatment with a *P. fluorescens* strain from the rhizosphere of cotton seedlings, or pyrrolnitrin, an antibiotic produced by *P. fluorescens*, greatly increased seedling survival in *R. solani* infested soils. Pyrrolnitrin

also inhibits growth of other pathogenic fungi including *T. basicola* and *Verticillium dahliae* (Howell and Stipanovic, 1979). In field trials, a soil drench of *Burkholderia cepacia* improved plant stand in *R. solani* infested soils, possibly due to the production of growth-inhibiting antifungal compounds (Zaki et al., 1998). *Trichoderma* spp. including *T. harzianum* and *Trichoderma virens* have been identified as biocontrol agents against *R. solani* (Elad et al., 1980). Interestingly, *T. virens* controls *R. solani* through induction of the plant's defence response, whereas its control of another pathogen, *P. ultimum*, is through antibiotic production (Howell et al., 2000). The endophytic fungus *Cladorrhinum foecundissimum* colonises cotton seedling roots and reduces disease incidence amongst plants transplanted into *R. solani* infested soils (Gasoni and Stegman de Gurfinkel, 2009).

Numerous *Fusarium* spp. have been found to be associated with cotton seedling roots, however only some species are pathogenic, causing *Fusarium* wilt (Zhang et al., 1996b). *T. harzianum* controls *Fusarium* wilt in both naturally and artificially *Fusarium oxysporum* infested soils and persists in the soil through consecutive plantings, reducing disease incidence at each planting (Sivan and Chet, 1986). Growth chamber and greenhouse experiments have demonstrated that both *T. virens* and *B. subtilis* reduce seedling colonisation and supress the incidence and severity of wilt (Zhang et al., 1996a). Cotton-associated bacteria including *Aureobacterium sapardae, Bacillus pumilus, Pseudomonas putida* and *Burkholderia solanacearum* also reduce disease severity in *F. oxysporum* infected cotton (Chen et al., 1995).

Although the pathogenic fungus *Verticillium dahlia* causes *Verticillium* wilt, one of the most important cotton diseases, *P. fluorescens* and *Bacillus* spp. strains reduce its incidence when applied to cotton seeds before planting in *V. dahlia* inoculated soil (Mansoori et al., 2013). Further, treatment with *Pseudomonas* spp., *T. virens* or *Enterobacter* sp. HA02 decrease wilt incidence and improve cotton growth parameters (Hanson, 2000; Erdogan and Benlioglu, 2010; Li et al., 2012). Similarly, mycorrhizal fungi of *Glomus* spp. including *G. etunicatum* can diminish the symptoms of *Verticillium* cotton wilt under controlled conditions (Kobra et al., 2009).

Xcm, a cause of bacterial cotton blight, is also suppressed by *P. fluorescens* (Habish, 1968), potentially through production of growth-inhibiting antimicrobial compounds (Mondel et al., 2000, 2001).

2.3. Mechanisms of biofertilization

Biofertilizers are microorganisms that enhance nutrient availability to plants, contributing to plant nutrition either by facilitating nutrient uptake or by increasing primary nutrient availability in the rhizosphere. They might also be used to increase crop yield when applied complementary to, or as replacement for, chemical fertilizers.

Nitrogen (N) is an essential plant nutrient that is often limited in agricultural soils due to high losses by emission or leaching. N fixation can be carried out by non-symbiotic bacteria such as *Azospirillum, Burkholderia, Gluconacetobacter* and *Pseudomonas* species (Dobbelaere et al., 2003), and may be used in biofertilization of non-leguminous crops such as rice (Mirza et al., 2006; Muthukumarasamy et al., 2007), sugarcane (Suman et al., 2005, 2008), wheat (Egamberdiyeva and Hoflich, 2002) and maize (Estrada et al., 2005). The *Azotobacter* strain Azo-8 was also found to be effective as bio-inoculant for wheat grown under dryland conditions in combination with urea and manure (Singh et al., 2013).

Although soils generally contain substantial total phosphorus, available phosphorus is often quickly depleted from the rhizosphere (Richardson et al., 2009). Microorganisms play an important role in the soil phosphorus cycle and, thus, in mediating phosphorus availability to plants, enhancing the capacity of plants to acquire phosphorus from the soil by directly solubilising and mineralising inorganic phosphorus or by facilitating the mobility of organic phosphorus through microbial turnover and/or increasing the root system (Richardson and Simpson, 2011). Myriad soil microbes that solubilise inorganic phosphorus have been isolated, including bacteria such as *Actinomycetes*, *Pseudomonas, Rhizobium* and *Bacillus* spp. (Richardson et al., 2009; Richardson and Simpson, 2011; Bhattacharyya and Jha, 2012). In addition, some fungal members of the *Penicillium* genus excrete organic acids that facilitate the conversion of immobilised soil phosphorus into soluble forms available to plants (Wakelin et al., 2004).

The rate of root growth and the plasticity of root architecture along with the 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 phosphorus in non-mycorrhizal plants (Fohse et al., 1991). Mycorrhizal fungi colonise the root cortex and extend externally, connecting the roots with surrounding soil and increasing efficiency of phosphorus acquisition by mycorrhizal plants (Barea et al., 2008). Mycorrhizal symbiosis may potentiate plant growth through enhancement of plant establishment, protection against stress, improved soil structure and increased nutrient uptake, particularly phosphorus and essential micronutrients, such as Zn, Cu (and also other nutrients such as Mg, Ca and K, depending on soil pH) (Clark and Zeto, 2000; Richardson et al., 2009).

2.4. Microbial fertilization in cotton production

Over the past decade the number of field and laboratory studies on PGP microbial inoculants for cotton has grown (Table 2), with several studies focusing on co-inoculation with multiple organisms. Various N-fixing, P-solubilising and indole-3-acetic acid (IAA)-producing bacteria from *Azotobacter, Azospirillum, Acetobacter* and *Pseudomonas* genera have been used as inoculants under irrigation. Multiple strains increased boll number and weight, and could promote this increased yield under reduced levels of chemical fertilization (Narula et al., 2005). Gomathy et al. (2008) found that using a mix of *Azospirillum, Methylobacterium* and P-solubilising *Bacillus* spp. in combination with NPK fertilization significantly increased cotton growth and yield in field trials under drip irrigation. Co-inoculation of fields with *Azospirillum* sp., P-solubilising bacteria and methylotrops significantly enhances root and shoot growth, fibre yield, and, to some extent, fibre quality when used in combination with fertilizers (Dhale et al., 2010, 2011), as well as increased yield under reduced levels of chemical fertilizers (Nalayini et al., 2010). Similarly, treatment of cottonseeds with a mixture of *Pseudomonas aeruginosa* Z5 and *Bacillus fusiformis* S10 isolated from cotton in Pakistan improved yield of cotton under reduced fertilizer conditions (Yasmin et al., 2013).

Several biofertilizers have been tested individually. The strain and the type of formulation of *P. fluorescens* was shown to impact the ability of the bacterium to promote plant growth. Strain Q18 was more effective than strain CKK-3, and utilising bentonite as a mineral carrier promoted greater seedling height and root length than talc or organic carriers such as peat and rice bran (Ardakani et al., 2010). In addition, the potassium-mobilizing bacterium *Bacillus edaphicus* enhanced the root and shoot growth of seedlings in pot trials of cotton grown in potassium-deficient soil and increased the N and P concentration in plants through root proliferation (Sheng, 2005).

Table 2
PGP microbial inoculants beneficial to cotton in field and laboratory trials conducted over the last decade.

Microbial inoculant	Experimental system	Effects	Reference
Azotobacter, Azospirillum, Acetobacter and *Pseudomonas* spp.	Irrigated field cotton	Increased boll number and weight; reduced chemical fertilization	Narula et al., 2005
Coinoculation of *Azospirillum, Methylobacterium*, P-solubilising *Bacillus* spp.	Field inoculation under drip irrigation	Increased growth and yield when combined with chemical fertilizer	Gomathy et al., 2008
Coinoculation of *Azospirillum*, methylotrops, P-solubilising bacteria	Applied on top of seeds, cotton fields under irrigation	Enhanced root and shoot growth, fibre yield and quality when combined with chemical fertilizer	Dhale et al., 2010, 2011
Coinoculation of *Azospirillum*, methylotrops, P-solubilising bacteria	Field trials in winter irrigated cotton	Increased cotton yield with reduced application of chemical fertilizer	Nalayini et al., 2010
Pseudomonas aeruginosa Z5 + *Bacillus fusiformis* S10	Applied as seed coating and tested in field trials	Improved growth and yield with reduced application of chemical fertilizer	Yasmin et al., 2013
Pseudomonas fluorescens	Greenhouse trials using different formulations for application	Promoted plant growth, type of formulation important	Ardakani et al., 2010
Bacillus edaphicus	Greenhouse pot trials	Increased root and shoot growth	Sheng, 2005
Raoultella planticola	Pot trials, saline soils	Enhanced seed germination, increased plant height and weight	Wu et al., 2012
Azotobacter chroomcoccum + mycorrhizal fungi	Seed treatment, field trials	Improvement in plant height, boll number and boll weight. Synergistic effect of coinoculation	Paul et al., 2011

2.5. Mechanisms of phytostimulation

One of the most important mechanisms of plant growth promotion is the production of plant hormones, or phytostimulation, by some rhizospheric microorganisms. PGP microbes enhance plant growth by producing growth hormones, such as auxins, gibberellins and cytokinins in the proximity of the roots, or by controlling the levels of ethylene produced by plants. The size and depth of root systems influence the capacity of plants to efficiently capture nutrients from soil and *vice versa*: root growth and morphology may change in response to nutrient availability (Wijesinghe et al., 2001). Having both shallow and deep roots allows the plant to reach both mineralized nitrogen available in topsoils, for example, as well as leached nitrogen in the depth (Gastal and Lemaire, 2002; Ho et al., 2005). Consequently, using phytostimulation for enhancing plant root development could play a significant role in improving nutrient uptake, especially if applied in combination with biofertilization.

IAA, the main plant auxin, stimulates root growth and shapes architecture (e.g., lateral root initiation, root vascular tissue development, root hair positioning) (Aloni et al., 2006). Many different rhizobacteria, including pathogenic, beneficial, associative and free living, are able to produce IAA (Tsavkelova et al., 2006). Examples include *Azospirillum*, *Aeromonas*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas* and *Rhizobium* (Spaepen et al., 2006; Martinez-Viveros et al., 2010). Cytokinins stimulate plant cell division and control root development by inhibiting primary root elongation and lateral root formation and promoting root hair formation (Werner et al., 2003; Riefler et al., 2006). They are produced by some PGP rhizobacteria, such as *Arthrobacter*, *Azospirillum*, *Pseudomonas* and *Paenibacilus* species, but their involvement in plant growth promotion is not well understood (Richardson et al., 2009). Similarly, gibberellins promote the development of stem tissue, root elongation and lateral root extension (Barlow et al., 1991; Yaxley et al., 2001), and are produced by species of PGP rhizobacteria, such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Herbaspirillum*, *Gluconobacter* and *Rhizobium* (MacMillan, 2002; Bottini et al., 2004).

Ethylene is an important plant hormone essential for plant growth and development, although it may have different effects on plant growth depending on its concentrations in plant roots (Pierik et al., 2006). 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 (Broekaert et al., 2006). Certain PGP bacteria, such as *Azospirillum brasilense*, can produce small amounts of ethylene, which may promote root hair development (Ribaudo et al., 2006). Ethylene is produced in plants from the substrate 1-aminocyclopropane-1-carboxylate (ACC), which is released by plants into the rhizosphere in times of stress, and reabsorbed by the roots to be converted to ethylene. However, ethylene accumulation in the roots causes reduced root growth, exacerbating plant stress (Babalola, 2010). Rhizospheric PGP fungi and bacteria (e.g. *P. putida*) that can degrade ACC reduce the adsorption of ethylene by the roots and allow the plant to re-establish a healthy root and cope with environmental stress (Glick, 2005). Plant growth promotion by ACC degrading microbes seems to be particularly important under stress such as cold, drought, saline soils or flooded soils contaminated by heavy metals (Grichko and Glick, 2001; Mayak et al., 2004). Microbes able to degrade ACC include *Achromobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Pseudomonas* and *Rhizobium* strains (Martinez-Viveros et al., 2010).

2.6. Phytostimulation in cotton

Table 2 also summarises recent field and laboratory studies on phytostimulation for cotton, conducted in the last decade. Many inoculants (see section 2.4), such as *Azospirillum* and *Pseudomonas* spp. have multiple beneficial traits. Differentiating between plant growth promotion due to phytostimulation versus biofertilization can be accomplished by examining whether mutant strains deficient in plant hormone production are still able to promote plant growth. For example, *Azospirillum brasilense* mutants with reduced levels of IAA production are affected in their ability to promote wheat growth (Dobbelaere et al., 1999; Spaepen et al., 2008). IAA and ACC deaminase production by the rhizobacterium *Raoultella planticola* as well as enhanced uptake of N, P and other nutrients are the mechanisms suggested for the increased germination rate, height and weight in cotton seedlings observed under salinity stress (Wu et al., 2012). The IAA producing bacterium *Azotobacter chroomcoccum*, particularly when co-inoculated with arbuscular mycorrhizal fungi, improved seed germination, seedling development, plant height, boll number and boll weight when applied as a seed treatment (Paul et al., 2011).

Soil aggregation is also important for allowing root penetration and soil aeration, as well as infiltration and retention of water,

leading to improved plant growth (Miller and Jastrow, 2000). Examples of microbes that could contribute to the formation of soil aggregates in cotton growing soils are arbuscular mycorrhiza (Rillig, 2004) and exopolysaccharide-producing bacteria such as *Azospirillum*, which can attach to soil particles depending on soil type and overall conditions (Bashan, 1999).

3. Considerations for use and commercialisation of PGP microbes for cotton production

Although many microbes have been demonstrated to stimulate plant growth and yield in the laboratory, the results have been poorly repeatable in field trials (Bhattacharjee et al., 2008; Martinez-Viveros et al., 2010), creating a barrier to commercialization and widespread use (Richardson et al., 2009). Further progress in this area depends on a clear understanding of the factors that influence the efficacy of microbial inoculants in the field, including plant species, soil type, local microbial communities, environmental conditions, inoculant carrier and other management practices such as fertilization, cultivation, irrigation and pest control. Together, plant species and soil type shape microbial communities in the rhizosphere (Garbeva et al., 2004; Berg and Smalla, 2009), and this must be taken into consideration when introducing microbial inoculants. Plant root exudates affect the surrounding soil, and can impact the ability of different microbial species to colonise and thrive in the rhizosphere (Rovira, 1969). Soil type and farm management practices also have a great influence on rhizospheric microbe populations (Reeve et al., 2010), with nutrient availability such as N and P, different pH, moisture content varying widely across soil types, with divergent capacities to support colonisation and growth of microbes. Indeed, Neumann et al. (2011) demonstrated that soil factors had a much greater influence on the growth of alfalfa than inoculation with PGP microbes. The composition of indigenous microbial communities within soils will also impact the ability of introduced microbes to effectively colonise the rhizosphere in sufficient numbers to effect plant growth (van Veen et al., 1997). Competition with the resident flora could rapidly deplete the population of introduced microbes, and may account in part for the inconsistencies observed between greenhouse studies and field trials (Martinez-Viveros et al., 2010).

The majority of cotton production takes place in arid or semi-arid soils, which poses additional challenges for the design and use of bio-inoculants (Bashan, 1998). Low rainfall and high temperatures characterize arid or semi-arid regions and soils in these regions are often nutrient poor, prone to salinity and often contain high amounts of insoluble P, with only approximately 2–4% available for plants (Richardson et al., 2009). These factors must be taken into consideration when selecting bio-inoculants, especially for dryland cotton, as introduced microbes must have the ability to colonise and promote plant growth under these environmental conditions. Delivery methods and the nature of the carrier also need to be optimized to assure rapid colonization of target plants, as the harsh conditions in these regions can quickly diminish the population of introduced microbes (Bashan, 1998), with technological advances targeting efficient delivery of bio-inoculants in crop production systems (Carr et al., 2014).

Indigenous strains of rhizobacteria, isolated from the intended plant and better adapted to the local environment, may have more competitive power and be more effective as bio-inoculants (Khalid et al., 2004). The importance of increasing the fitness of the biocontrol agent in the field was highlighted 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®. The local strain showed the most effective control of damping-off caused by *R. solani*, especially when combined with chemical fungicides (Zaki et al., 1998).

It is recognized 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 (Martinez-Viveros et al., 2010). 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 (Khalid et al., 2004); therefore, it would be beneficial to utilise those biofertilizers 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 (Vassilev et al., 2006). Alternatively, the use of compatible inoculant mixes could serve the same purpose. There is evidence from trials in cotton that co-inoculation with multiple PGP microbes can increase plant yield compared to single inoculums (Paul et al., 2011; Yasmin et al., 2013). In addition, the use of multiple biocontrol agents can overcome some of the variability observed in field trials and broaden the environmental conditions under which a biocontrol agent can be used (Guetsky et al., 2001). Given the aforementioned cotton growth conditions, it would be beneficial to utilize PGP microbes that have been implicated in stress protection, perhaps in conjunction with other biological agents. Studies have already identified microbes that improve cotton growth and yield under conditions of potassium-limitation (Sheng, 2005) and saline stress (Wu et al., 2012), and further research into these microbes and their possible inclusion in a bio-inoculant seems warranted.

Microbial inoculants have numerous advantages when compared with chemical fertilizers, 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 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; and they can be used on their own or in combination with conventional pest management (Berg, 2009). When used together with chemical fertilisers, it would be necessary to define the most effective ratio between inoculum size and the concentration of fertilisers. Management strategies combining pesticides or herbicides application and bio-inoculants must test for resistance of the bio-inoculant to the agrochemicals and for optimal methods of co-application.

In addition to the ecological considerations outlined above, there are also economic and manufacturing factors that need to be taken into account with regards to the commercialization of microbial inoculants. The mass production of microbes can be technically challenging and expensive; products need to be formulated to have long shelf life (transport and storage), which may be problematic in particular with gram-negative bacteria that do not form spores. Further, registration procedure can be expensive and time consuming, and application must be both simple and compatible with agronomic practices and equipment (Berg, 2009; Kaewchai et al., 2009; Figueiredo et al., 2010). A study into the adoption of biological inputs in cotton production in India showed that some of the factors influencing the usage of bio-inoculants included concerns about timely availability and reduced shelf life of bio-inoculants, and cumbersome application methods (Sundaravardarajan et al., 2006). The success of the biocontrol agent Kodiak® in cotton production may be largely attributed to its integration with standard chemical fungicides, allowing for ease of application and long–term activity (Brannen and Kenney, 1997).

3.1. Commercial PGP and biocontrol products for use in cotton production

The increased research focus on PGP microbes has led to the commercialization of a number of products for use in the agricultural industry. This section examines the commercial products marketed for use on cotton specifically or on all agricultural soils/crops including cotton.

In 1992, *B. subtilis* GB03 was registered as a commercial biocontrol product for cotton pathogens in the USA, named Kodiak® (Gustafson Inc. USA). The development of the biocontrol agent used in Kodiak® originated in Australia (1970s to late 1980s) with *B. subtilis* (isolate A-13), which was well documented as a biocontrol and growth promoting agent in wheat and peanut, leading to the cotton-adapted strain GB03 used in Kodiak® (Brannen and Kenney, 1997). Kodiak® works as a biocontrol against *Rhizoctonia* and *Fusarium* spp.. Mahaffee and Backman (1993) found that cotton seed-factors, including surface pH, cultivar, and presence of fungicide coating, influenced the colonization of cotton and its rhizosphere by this biocontrol agent; thus, such factors have to be considered when developing an inoculant product for cotton.

Current products in the USA include Ascend™ PA, a biofertilizer containing the mycorrhizal fungi *Glomus intraradicies*, and the information provided suggests that it increases growth in cotton by 300% (BioScientific, Inc., Arizona, USA, www.BioSci.com). PIX PLUS® combines *Bacillus cereus* with mepiquat chloride, and is marketed to increase boll number and size, increasing yield by up to 82lb/acre on average (Arysta LifeScience, USA, www.arysta-na.com). Deny® (Stine Microbial Products, USA) and Intercept® (Soil Technologies Corp., USA) are two biocontrol products marketed for use on cotton and a variety of other crops, which contain *Burkholderia cepacia*, and are used for the control of *Rhizoctonia, Pythium* and *Fusarium* spp.. SoilGard® (Certis Inc., USA) is marketed for the control of *Pythium, Rhizoctonia* and *Fusarium* spp., through the active agent *Trichoderma virens*. Contans® WG (Prophyta Biologischer Pflanzenschutz GmbH, Germany) is a *Coniothyrium minitans*-containing biocontrol agent active against *Sclerotinia sclerotiorum* and *S. minor* in all susceptible crop species including cotton. Afla-Guard® (Syngenta Crop Protection Inc., USA) contains *Aspergillus flavus* NRRL 21882, which acts to control aflatoxin-producing fungal pathogens in a wide range of crop species including cotton.

In Australia current products include BioAg Soil and Seed® (BioAg, AU, www.bioag.com.au) for improvement of soil fertility, promotion of rapid seed germination and early root development. This formulation can be applied via irrigation or used as a seed inoculant. Table 3 summarises currently available commercial bioinoculants for use in cotton production systems.

In addition to commercial products currently in use there are also a number of other microorganisms registered with the U.S. Environmental Protection Agency as biopesticides (http://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:46:0:NO:::). The Arizona Cotton Research and Protection Council (USA) has registered *A. flavus* AF36 as a biopesticide to control the growth of aflatoxin-producing *A. flavus* on cotton.

4. Summary and conclusions

Plant growth promotion is a complex phenomenon rarely attributable to a single mechanism as most PGP microbes influence plant growth through multiple mechanisms, and in some cases their PGP effect may only occur through interactions with other microbes. Any microbial agent added to the rhizosphere has 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. Fig. 1 presents the steps from PGP isolation to commercialization.

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 the number of successful cases increasing. The direct benefits of such research are both financial, from reductions in the use of chemical fertilizers and pesticides, and productive through improved crop yield, while indirect benefits include reduced toxin accumulation in agricultural soils and reduced environmental pollution with agricultural runoff. Success is often associated with a combination of inoculants possessing complementary beneficial traits, e.g. biofertilizers 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 prove the most effective; such microbes suit the environmental conditions in the cropping system for which they are intended. Nevertheless, indigenous microbes would still have to out-compete other microbes for resources and, in the case of biocontrol agent, suppress pathogens.

The Australian cotton industry is one industry that could greatly benefit from research into isolation of crop-specific beneficial microbes. In general, it can be said that similar groups of beneficial microbes seem to be involved in promoting the growth of different plants, with examples including bacteria from the *Bacillus*, *Azospirillum*, *Pseudomonas* groups and mycorrhizal fungi. Nevertheless, there is sufficient evidence to suggest that particular microbial species, or even strains, benefit specific plants under defined conditions; thus there is a need to carry out region specific research to produce inoculants specific to the crop, agronomic practices, soil type and other environmental conditions.

In addition to isolating microbial agents for augmentation, further research should be directed into cropping practices that enhance both existing and introduced beneficial microbes, such as

Table 3
Commercial biocontrol and biofertilizer products currently marketed for use in cotton production.

Commercial product	PGP microbe	Use	Company
Kodiak	*Bacillus subtillus* GB03	Control of *Fusarium* and *Rhizoctonia* spp.	Gustafson Inc, USA
Ascend/BuRIZE	*Glomus intraradicies*	Increases cotton growth	Bioscientific Inc., USA
PIX PLUS	*Bacillus cereus*	Increase boll number and size	Arysta LifeScience, USA
Deny	*Burkholderia cepacia*	Control of *Rhizoctonia*, *Fusarium* and *Pythium* spp.	Stine Microbial Products, USA
Intercept	*Burkholderia cepacia*	Control of *Rhizoctonia*, *Fusarium* and *Pythium* spp.	Soil Technologies Corp., USA
SoilGard	*Trichoderma virens*	Control of *Rhizoctonia*, *Fusarium* and *Pythium* spp.	Certis Inc., USA
Contans WG	*Coniothyrium minitans*	Control of *Sclerotinia* spp.	Prophyta Biologischer Pflanzenschutz GmbH, Germany
Afla-Guard	*Aspergillus flavus* NRRL 21882	Control of aflatoxin-producing fungi	Syngenta Crop Protection Inc, USA
BioAg Soil and Seed	Unspecified	Improve soil fertility and promote rapid seed germination and early root development	BioAg, Australia

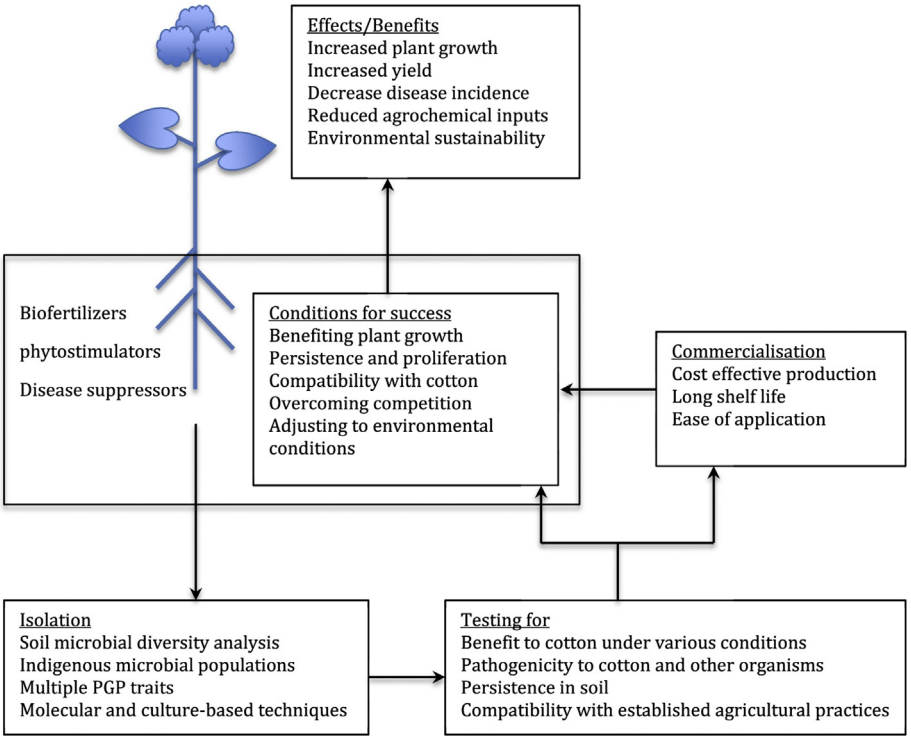

Fig. 1. Cotton growth-promoting rhizospheric microbes: from isolation to application of bio-inoculants.

controlling the amount of chemical inputs, as over-application of chemicals may suppress the activity of beneficials and increase the activity of detrimental microorganisms. Consequently there is great scope for collaborations to develop technology to screen for and identify microbes with beneficial traits; assess the benefit to the plant; test strains for commercialization; design the best formulations for inoculant delivery; detect and assess the performance of inoculants in the soil; analyze soil microbial communities and the effects of the soil inoculation on soil health; and study the general effects of cropping practices on specific microbial communities.

Molecular techniques, such as proteomics and transcriptomics, add a new dimension to the understanding of the overall responses of plants and pathogens during disease cycles (Nittler et al., 2005; Coumans et al., 2009, 2010, 2011). Such information can be useful in the development of disease control measures, including biocontrol. Genome analysis can indicate the presence of virulence genes and transcriptome analysis can determine the expression of such genes, allowing for the screening of virulence suppressive factors. In searching for new PGP traits, it is possible to screen the genomic library of certain beneficial microbial species, e.g. *P. fluorescens*, for sequences that may be involved in plant growth promotion (Berg, 2009).

Soil microbial diversity analysis (e.g. DNA-microarrays and pyrosequencing) under different crop management strategies can supply information about the presence of pathogens and/or PGP microbes in the rhizosphere, while methods such as qRT-PCR and RNA sequencing can supply information on the active growth of different microbes, and whether specific functional genes are being expressed. Such modern techniques can be used in promoting crop production practices that enhance PGP activity in the soil, reducing nutrient removal (e.g. denitrification) and suppressing pathogen virulence. Natural disease suppressive soils, where disease suppression is due to biological factors, can give clues as to the structure of microbial communities associated with disease suppression. Such soils have also a good potential to be a source of biocontrol agents.

While microbial communities and their functions can be studied using molecular techniques, culturing techniques need to be employed in the isolation of PGP microbes. Such methods vary and are dependent on the mechanism sought after and the biology of the microorganism. The development of biocontrol agents requires vigorous screening. There is no defined screening for biocontrol agent as it depends on the crop, the affected part of the plant, the target pathogen, and the cropping system. Observation of zones of pathogen growth inhibition led to the identification of many useful bacterial biocontrol agents, although this method does not identify biocontrol agents with other modes of action such as induced systemic resistance or competition (McSpadden et al., 2002). As previously mentioned, indigenous and suppressive soils could be good sources of PGP microbes; however, current techniques for initial screening of pathogen suppressive microbes are very labor intensive and new, more direct ways of isolating beneficial microbes from soils are required.

Pathogen suppressive parasites may be isolated from buried propagules of the pathogen retrieved from the soil. Microbes controlling pathogen populations by competition may be those that are fast colonizers of sterilized soil and can exclude growth of other organisms as well as looking for microbes that colonize the same niches as the pathogen.

In conclusion, the search for new biocontrol microbes is ongoing and gaining importance, as issues of pathogenic resistance grow in the face of increased need for crops commensurate with a growing world population. It is recognized 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. Consequently there is a pressing need for cross-disciplinary collaborations and a

better and more comprehensive understanding of soil–plant–microbe interaction.

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