Beneficial microorganisms for sustainable agriculture

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Global agriculture has to double food production by 2050 in order to feed the world's growing population and at the same time reduce its reliance on inorganic fertilisers and pesticides. To achieve this goal, there is an urgent need to harness the multiple beneficial interactions that occur between plants and microorganisms. The beneficial influences of microorganisms on plant growth include nitrogen fixation, acquisition and uptake of major nutrients, promotion of shoot and root growth, disease control or suppression and improved soil structure. Some of the commonly promoted and used beneficial microorganisms in agriculture worldwide include *Rhizobia*, Mycorrhizae, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Trichoderma*, *Streptomyces* species and many more. Unravelling the biota black box using modern molecular methods is helping to find new suites of beneficial microorganisms that can help improve agricultural production worldwide.

N_2 fixing bacteria

Symbiotic associations between legume plants and root-nodulating bacteria belonging to the genera *Rhizobium*, *Bradyrhizobium*, *Ensifer* and *Mesorhizobium* produce around 80% of the nitrogen in Australian grains, with a value estimated at A$3 billion each year[1]. Nitrogen fixation is one of the essential beneficial biological processes for the economic and environmental sustainability of agriculture worldwide. Globally, annual inputs of fixed nitrogen from crop legume–rhizobia symbioses are estimated as 2.95 million tonnes for pulses and 18.5 million tonnes for oilseed legumes[1]. In spite of the in-depth knowledge about the biochemical and molecular steps involved in legume-rhizobium symbiosis, the holy grail of N_2 fixation by non-legumes, especially cereal food crops, is yet to be realised. Finding efficient rhizobia for the wide variety of legumes that are cultivated around the world and developing efficient management of symbioses in the field to realise the ~25 kg of N_2 fixation for each tonne of legume dry matter are the major challenges for the future.

"Diazotrophy", the ability to fix atmospheric nitrogen catalysed by the enzyme nitrogenase, is distributed among diverse groups of bacteria and archaea[2]. Free-living N_2 fixing bacteria (for example, *Azospirillum* spp., *Azotobacter* spp., *Acetobacter diazotrophicus*, *Herbaspirillum* spp., *Bacillus* spp., *Azoarcus* sp.) are found in the rhizosphere and rhizoplane environments of cereal crops. Recent evidence not only identified new genera of N_2 fixing bacteria and archaea in natural and managed ecosystems but also indicated significant edaphic and environmental groupings in genetic diversity and functionality[3,4]. Non-rhizobial N_2 fixing bacteria can grow as endophytes in a number of grasses, for example, in a recent study in South Australia *Pseudomonas* species were the most dominant group of *nif*H carrying bacteria found in the rhizosphere of perennial native grasses[5]. Evidence suggests the *nif*H gene is present in a number of non-*Frankia* actinobacteria (for example, *Agromyces, Microbacterium, Corynebacterium and Micromonospora*).

Thus the challenge is to identify (i) functionally significant N_2 fixing genera/species specific to biomes and crops, and (ii) key edaphic and environmental drivers regulating the genetic diversity and free living N_2 fixation in order to maximise benefits from these beneficial microbes both for sustainable primary production and climate change adaptation.

Mycorrhizae and phosphate solubilising microorganisms

The symbiotic association of plants and mycorrhizal fungi (arbuscular mycorrhizal fungi, AMF) has long been recognised for the benefits it provides with nutrient transport and uptake; however, there is considerable uncertainty about the functional benefits in intensive agricultural systems[5,6]. Even though AM symbiosis is widespread, the symbiotic functions of AMF species differ according to specific AMF isolates, host plants and soil properties. AMF associations are generally considered diffuse and non-specific because multiple species colonisation linking together two or more plants is not uncommon[7]. Identification of specific phylotypes of AMF and their relationship with soil properties is a crucial step to fully exploit the benefits from AMF[6,7]. New knowledge on the microbial interactions in the mycosphere has the potential to enhance our ability to manipulate plant-mycorrhizal associations. Restoration of AMF symbiosis is one of the key beneficial biological processes for the rehabilitation of contaminated soils and mining sites[6].

A diverse array of bacteria (for example, *Pseudomonas, Bacillus*, actinomycetes) and fungi (for example, *Aspergillus*, *Penicillium* species) are capable of solubilising and mineralising plant unavailable forms of phosphorus in soils, and the benefits from their use as inoculants are increasingly being recognised, especially in P-limited environments[8,9].

Plant growth-promoting rhizobacteria (PGPR)

Bacteria colonising roots that elicit shoot and root growth, referred generally as PGPR, are recommended (marketed) for improving plant growth and disease control both in agriculture and horticulture[10]. PGPR can promote root and shoot growth either by producing plant hormones or secondary metabolites, controlling diseases, induction of systemic resistance or through changing physicochemical interactions with plants. Bacterial inoculants applied as bio-fertilisers are also being explored to alleviate stress from abiotic factors, for example, drought and salinity[11].

Biological control organisms

Bacteria, fungi and actinobacteria can act as biocontrol agents against root diseases[12]. A number of bacterial and fungal inoculant formulations are available commercially to control diseases in agricultural and horticultural crops (http://www.oardc.ohio-state.edu/apsbcc/). Challenges associated with the introduction of biocontrol organisms against soil-borne plant pathogens include their poor survival, variable root colonisation and lack of adaptability to the natural environment. The success of biocontrol inoculants depends upon the ability to: (1) maintain the adequate populations needed to provide effective biological control; (2) lengthen the period during which a threshold population density is sustained in the rhizosphere; and (3) increase the magnitude of disease control provided by introduced rhizobacteria. Actinobacterial endophytes can colonise plants without disrupting the "normal" endophytic populations, can produce antifungal antibiotics and plant growth hormones, and can also induce systemic disease resistance in plants[13]. Biocontrol inoculants are generally tested for their antibiosis potential due to antagonism, hyper-parasitism, competition and predation by indigenous organisms; however, organisms that induce a systemic resistance to diseases and pests have the greatest potential to succeed under field conditions[10].

Some soils can suppress the severity of disease even in the presence of a pathogen, host plant and favourable climatic conditions for the disease. There are a number of examples, both in Australia (Figure 1) and worldwide, where agricultural soils have become suppressive to soil-borne pathogens[14-16]. Culture-based and culture-independent analyses have indicated the involvement of a diverse range of microorganisms involved in reducing pathogen inoculum and infection, plant growth promotion and induction systemic resistance (Table 1). Due to the difficulties associated with introducing inoculants, *in situ* enhancement of beneficial microorganisms involved in natural disease suppression could be the more effective and reliable control measure. It can also provide environmental benefits by reducing agrochemical dependency for disease control.

Probiotics for plants

Probiotics for human health are not new but the concept of managing plant health through the manipulation of probiotic organisms associated with plants has gained interest only recently[17]. Plant-specific stimulation of specific microbial groups in their rhizosphere suggests that plants may have evolved to strategically stimulate and support particular microbial groups capable of producing antibiotics as a defence against diseases caused by soil-borne pathogens[16]. Diseases caused by soil-borne fungal pathogens result in more than $150 million of annual production losses in cereal crops in Australia.

Soil bacteria belonging to the genus *Pseudomonas* are ubiquitous in most soils and have been linked to wide-ranging processes including plant growth promotion and inhibition, disease control, nutrient cycling, nitrogen fixation and bioremediation.

Figure 1. Natural biological suppression can reduce the impact of rhizoctonia disease in cereal crops. Inset: Damaged hyphae of *Rhizoctonia solani* AG8 in a suppressive soil.
Footnote: Enhanced biological disease suppression mediated by a variety of bacteria, actinomycetes and fungi has been identified under conservation management practices in South Australia. Unravelling the composition of such microbial communities has the potential to identify a new suite of beneficial microbes.

Their ability to respond quickly to changes in physical, chemical, carbon and nutritional conditions in soil has been linked to their functional significance in agricultural ecosystems. Pseudomonads have been studied for their biocontrol potential against fungi and oomycete pathogens for more than two decades and a number of promising candidates have been identified[18]. Antibiosis is the most commonly suggested trait responsible for their activity against plant pathogens and a number of antimicrobial compounds have been identified, for example, 2,4-diacetylphloroglucinol (2,4-DAPG), phenazines (PHZ), pyrrolnitrin (PRN), pyoluteorin (PLT), hydrogen cyanide (HCN) and biosurfactant antibiotics[17]. Conventional biochemistry-based characterisation of the chemicals is now complemented with molecular techniques (e.g. metabolomics and transcriptomics) to unravel the mechanisms of production, interactions with pathogens and plants, genotypic and phenotypic diversity of organisms capable of producing similar compounds and determine their activity in natural soil environments.

Finding new beneficial inoculants

Traditional cultivation and isolation methods have mainly targeted a small group of copiotrophic microorganisms as candidates for inoculants. Culture-independent investigations have revealed the presence of significant populations of new bacterial divisions (for example, *Verrucomicrobia, Acidobacteria*) in the rhizosphere which were not identified in the isolations using nutrient-rich media. Pyrosequencing targeting the surface protein encoding *cpn*60 could help generate a list of genera and their relative abundances *in situ*, which can complement the isolation efforts using semi-selective culture media and specialised cultivation methods[19,20].

Table 1. List of beneficial organisms isolated from suppressive soil with demonstrated functions in the suppression of diseases caused by soilborne necrotrophic pathogens[15].

Organisms	Functions
Bacillus spp.	***Reduced pathogen inoculum***
Microbacteria spp.	Competition
Pseudomonas brassicacearum	Parasitism
Pantoea agglomerans	Predation
Exiguobacterium acetylicum	***Reduced infection***
Pseudomonas fluorescens	General antibiosis
Streptoverticillium sp.	Disrupt growth of pathogen from source to root
Streptomyces spp.	Prevent access to infection sites
Trichoderma spp.	Metabolise/disrupt plant-microbe signalling
Penicillium griseofulvum	General antibiosis
Mycophagous amoebae	Induced systemic resistance
Fungal feeding nematodes	***Plant growth promotion***
	Production of secondary roots
	Improved nutrient availability

Conclusion

It is essential to enhance the activities of microbes that benefit plant nutrition, control diseases and assist plants to cope with a variety of abiotic stresses to sustain and improve global food production in future climate scenarios while maintaining environmental health. A diverse range of beneficial microorganisms have been found but their reliable use in field environments is yet to be fully realised. New knowledge on soil microbial diversity can lead to the discovery of new generation inoculants as well as improve survival and performance of beneficial microbes *in situ* following their introduction into foreign environments.

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Biography

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