# Current perspectives and applications in plant probiotics





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**Abstract.** **As agriculture and food security face unprecedented challenges, emerging agricultural innovations and existing practices require ongoing examination in the context of sustainability. In this review, we focus on the use of probiotic microorganisms for improved plant production. As plants are enormously diverse, emphasis is placed on the fundamental sites of plant-microbe interactions regarding benefits and challenges encountered when altering the microbiome of these locations. The soil, the external plant epidermis, and internal plant tissue are considered in discussion regarding the type of plant probiotic application. Plant probiotics range from broader soil beneficial microorganisms (such as *Trichoderma* spp.) through to specialised epiphytes and endophytes (such as root nodule bacteria). As each site of interaction affects plant growth differently, potential outcomes from the introduction of these exogenous microorganisms are discussed with regard to plant productivity. Finally, recommendations regarding regulation and future use of plant probiotics are points of consideration throughout this review.**

## Introduction

Microbial communities (or microbiomes) are associated with all biotic systems, and the balance and function of a system can be altered by the metabolic activity and interaction of microorganisms within it. When the microbiome of a system is disturbed, it can result in changes in homeostasis in an organism or shifts in productivity in a system[1]. Depending on the change, this can lead to a deleterious or beneficial effect[2]. Probiotics is a term used when exogenous microorganisms are introduced, or endogenous microorganism populations are manipulated to elicit a beneficial change (for the purpose of this review we will conform to this nomenclature)[3]. The study of probiotics is an emerging field in mammals. For example, an imbalance of the human gut microbiome has been shown to result in disrupted homeostasis (for reviews see [4] and [5]). However, other higher organisms, including plants, are more diverse in function and physiology therefore conclusions regarding the effects of probiotics are often species related[6].

Species belonging to the kingdom Plantae are enormously diverse and occupy most terrestrial surfaces on every continent on Earth. Given this diversity, it is difficult to generalise plant-physiology. For simplicity, sites where interactions between microorganisms and plant tissue occur are summarised in Figure 1. The site of infection and colonisation can occur internal to the epidermis (endophyte) and on the surface of the epidermis (epiphyte). All interactions between host (plant) and symbiont (microorganism) vary and the relationship is defined by the effect the symbiont has on the host, as illustrated in Figure 2.

Plant roots penetrate various layers of soil substrata in search of nutrients and water. During their exploration of soil, plant roots encounter millions of different microorganisms and have developed advanced genetic and metabolic mechanisms to both recruit and defend against microorganisms. Colonisation of roots involves a complex molecular communication between microorganism and roots. Attracted by root exudates, microorganisms migrate towards roots via chemotaxis and may colonise the root surface (rhizoplane), or in the soil aggregates that form around

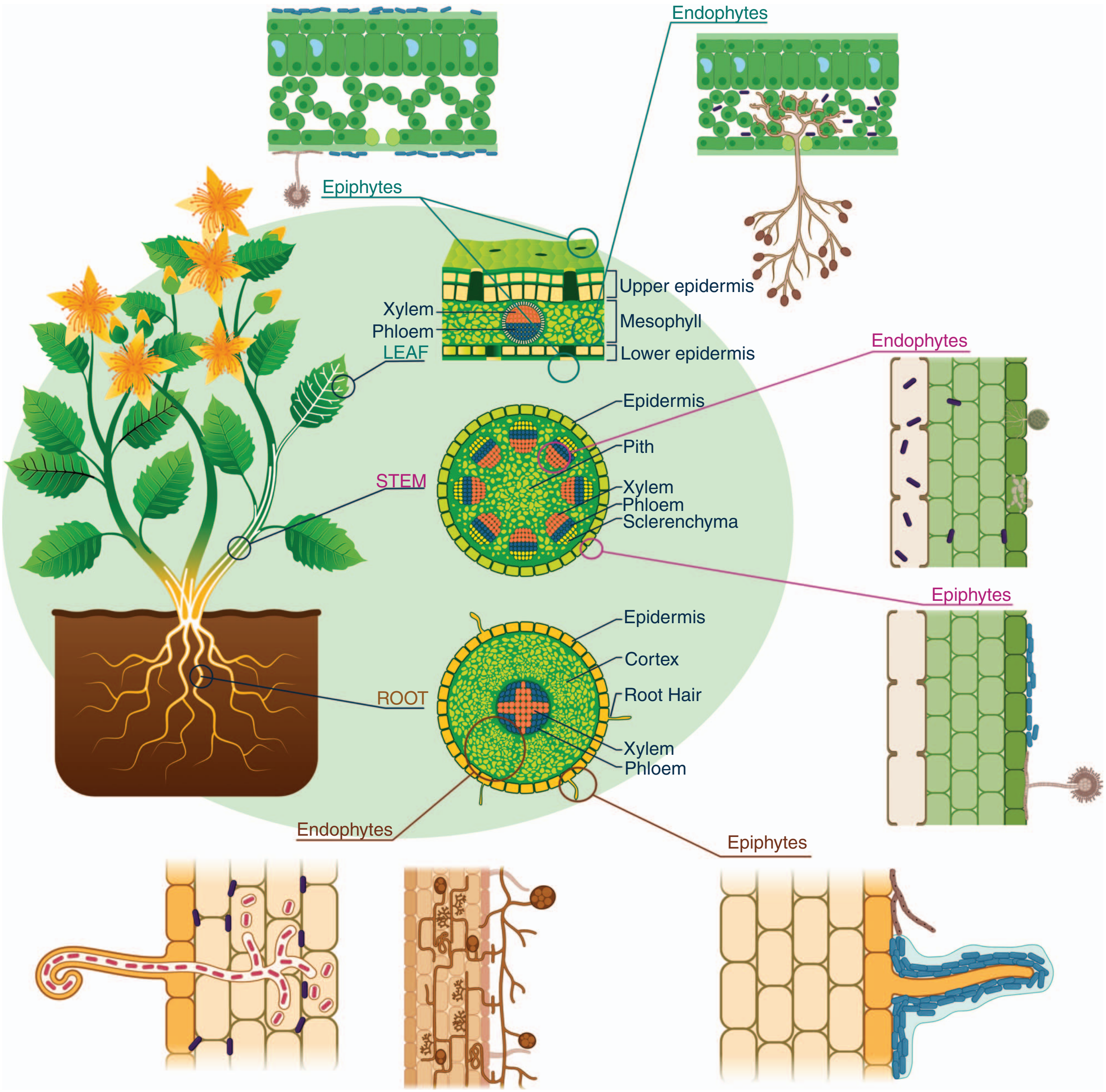


Figure 1. Diagram showing possible locations of interaction between epiphytes and endophytes on major types of plant tissue. Blue rods, bacterial epiphytes; dark purple rods, bacterial endophytes; red rods, root nodule bacteria; fungi shown in brown and grey. Not to scale.

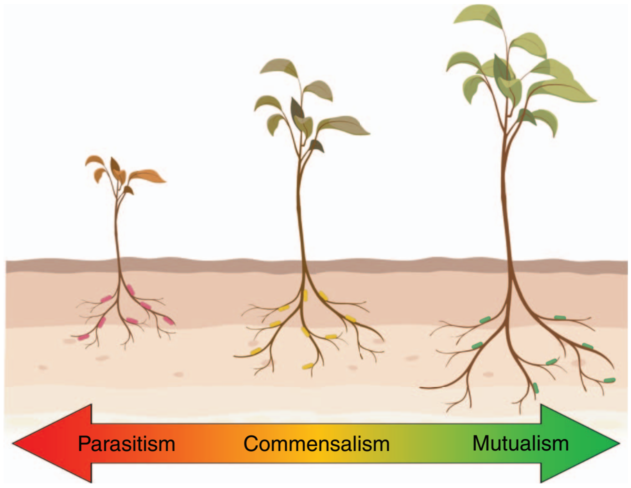


Figure 2. Overview of the types of interaction that occur between host and symbiont in plant-microbe interactions.

roots (rhizosphere), or both[7]. Most beneficial interactions between host and symbiont begin at the rhizosphere and should be considered the first point of manipulation for plant probiotics.

In this review, we will explore the microbiome of plants and the effect of changes in the plant status with the use of single or multiple species of microorganisms. Consideration will be given to discussions regarding host range and the use of promiscuous over narrow host range microorganisms as a point of critical consideration. The current applications in the use of plant probiotics will be described in the context of beneficial agricultural outputs under both biotic and abiotic stress conditions.

## Soil probiotics: biofertilisers

The introduction of beneficial microorganisms to soil (biofertilisers) can result in improved plant growth. However, the mechanisms underlying improved plant health are different from the direct interaction between plant and host. Indirectly, microorganisms improve soil nutritional status and health through various mechanisms including: (1) increased phosphate availability through the solubilisation of occluded soil phosphates; (2) fixation of atmospheric nitrogen into bioavailable forms by free-living diazotrophs; (3) increasing the organic content in soil by cell turnover; (4) production of biofilms resulting in increased water retention; and (5) pathogen suppression (see reviews [8] and [9]). These microorganisms promote plant growth by indirect interaction with plants, and are, arguably, better characterised as soil probiotics. Research into increasing soil health through the introduction of microorganisms, or by a mixture of microorganisms and carrier, is apparent with over 713 patent filings regarding biofertilisers within the last ten years (source: Google Patents).

The beneficial effects of biofertilisers on crop yields has been documented extensively. A two-year study by Zhang *et al.*[10] is presented as a case study. The authors used a controlled fertilisation regime of composted cattle manure or composted cattle manure supplemented with a single fungal species, *Trichoderma rossicum,* and monitored soil chemistry, plant biomass and microbiota fluctuations. At the end of the trial, the authors reported a significant increase in plant biomass on land treated with composted cattle manure supplemented with *T. rossicum*. Interestingly, improved soil chemistry and fungal diversity were correlated with treatments, but bacterial diversity was not. However, DNA for metabarcoding were extracted from the bulk soil and changes in the rhizosphere were not monitored. Therefore, it is unknown if the rhizosphere microbiome had altered between treatments and elicited an effect on plant growth.

While biofertilisers offer an attractive method of soil amendment, Hart *et al.*[11] offer a cautious approach to the use of biofertilisers, in particular arbuscular mycorrhizal fungi (AMF). The authors contend that the use of aggressive generalists in biofertilisers may result in the loss of local AMF communities with unknown future ecological consequences. An additional point of consideration presented is the lack of regulation of biofertilisers compared to more traditional fertilisers. As the use of soil probiotics increases, consideration must be given to the greater biological implications – both positive and potentially harmful.

## Plant probiotics: plant epiphytes – the generalists

Soil microorganism populations are more diverse than those found in the rhizosphere of plants, but soil microorganisms are much less abundant than the rhizosphere population[7]. For this reason, it is necessary to consider the ability of microorganisms to colonise plant roots for plant growth promoting properties. Within the rhizosphere, microorganisms play a crucial role in phosphate availability, they are also a source of nitrogen via diazotrophic nitrogen fixation and present a barrier (much like oral microflora in humans) to incoming pathogens (reviewed in [12]). The rhizosphere is an environment rich in organic acids, plant photosynthates and complex molecular signals. These plant compounds present selection pressure and may present a target for the development of plant probiotics intended for the rhizosphere. The current literature regarding plant growth promoting rhizosphere microorganisms is abundant. However, there are several key mechanisms that may elicit a positive plant growth phenotype.

The use of epiphytic microorganisms to alleviate abiotic stress is an emerging field of research especially considering arable land has become increasingly impacted by climate change[13]. Other mechanisms of PGP in the rhizosphere include the solubilisation and mobilisation of occluded phosphates. Microorganisms can mine phosphate from soil and increase the amount of labile phosphorous available to plants. Research in this area of plant probiotics is extensive and will not be covered in this review, but for further reading see [8,12–15].

Salinisation of soil results in decreased agricultural outputs. Mukhtar *et al*.[16] explored the possibility of utilising halotolerant rhizosphere microorganisms on salt stressed maize. They isolated rhizosphere microorganisms from plant halophytes *Salsola stocksii* and *Atriplex amnicola* and screened them for potential PGP characteristics. The selected isolates were inoculated onto maize by seed coat and planted in saline soil. The results indicated a significant increase in root and shoot biomass of plants containing halophilic microorganisms. This study presents an example of plant probiotics by utilising microorganisms that are adapted to a stressed environment. For further reading regarding salt stress see [12].

A novel approach in the use of plant probiotics is presented in several papers discussing the bioremediation of heavy metals by rhizosphere microorganisms. By introducing organisms that can colonise root tissue and incorporate or metabolise heavy metals, reductions in heavy metal accumulation in plant tissue have been observed across multiple plant species[8,17–19]. Like the microorganisms isolated from saline environments, these plant probiotic heavy metal remediating species could potentially be sourced from contaminated land for use in agriculture.

## Plant probiotics: plant endophytes – the specialists

Soil microorganisms and plant epiphytes confer PGP through a diverse array of mechanisms as previously discussed and generally these microorganisms can confer this benefit across multiple hosts. These are broad host range plant probiotic microorganisms. Endophytic microorganisms, in contrast, are much more selective and have a narrower host range. The most extensively studied plant endophytes are represented by the legume and root nodule bacteria interaction (RNB). For over a century, RNB have been used with their concomitant host to elicit a beneficial effect on plant growth by utilising the diazotrophic ability of the symbiont to increase plant nitrogen. However, this has presented a unique set of challenges due to genetic plasticity of RNB.

The inoculation of RNB onto a crop leads to an intimate symbiosis, but long-term exploitation of this symbiosis has led to unexpected consequences. Symbiotic genes are often located on plasmids or on symbiosis islands, and these genetic elements are susceptible to horizontal gene transfer. Transfer of symbiotic genes between similar species occurs at varying rates and, over time, can give rise to a population of native species that can outcompete inoculants and are ineffective nodule symbionts. This has been observed in several legume species including *Biserrula pelecinus*[20] and *Lotus japonicum*[21]. The rate at which horizontal gene transfer occurs between RNB may be greater than reported in the literature.

## Conclusion

Plant probiotics is an area of research that is anticipated to gain much traction in the coming years. With agriculture productivity facing increased strain from urbanisation, climate change and land use, the augmented use of plant probiotics offers mechanisms to alleviate saline stress, heavy metal contamination, reduce plant stress responses, and increase agronomic outputs. However, all alterations to the microbiome of plants result in some changes occurring. Some changes are macroscopic, such as increased biomass, and others occur on microscopic levels that may accumulate unnoticed. The challenge facing agronomists, ecologists and biologists rests in harmonising the balance between existing plant and soil microbiomes with the introduced plant probiotics. By careful monitoring of not just agricultural outputs, but also the perturbations within the communities of microorganisms that share soil and plant tissue, plant probiotic treatments can offer a useful and powerful tool for plant growth promotion.

## Conflicts of interest

The authors declare no conflicts of interest.

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## Biographies

**Dr Robert Walker** is plant and soil microbiologist at the University of Melbourne. His research interests include phosphorous and iron pathway regulation in plant growth promoting bacteria. He also works with fungal pathogens to understand plant defence priming using metabolomic and transcriptomic tools in Industrial Hemp with industry collaboration from Nutrifield.

**Carl Otto-Pille** is a Masters of Science student at the University of Melbourne. His research focuses on isolating bacteria from agricultural mixed-use land by trapping with the model plant, *Brachypodium distachyon*. These isolates are then screened for phosphate solubilising ability and their genome sequences will be available soon.

**Sneha Gupta** recently submitted her PhD thesis at the University of Melbourne. Sneha is a plant biologist who works with *Trichoderma harzianum* to elucidate biochemical interactions during salinity stress using advanced mass spectrometry imaging and analysis.

**Martino Schillaci** is a PhD candidate at the University of Melbourne. Martino studies the interaction between *Azospirillum brasilense* and the model plant *Brachypodium distachyon* under sub-optimal growth conditions. During his PhD, Martino studied plant phenotype using advanced imaging platforms at the Forschungszentrum Jülich (GER) and analysed the metabolome of plants using GC-MS and LC-MS at the University of Melbourne. Martino is due to submit his PhD thesis in 2020.

**Professor Ute Roessner** leads the Plant Biochemistry research group at the University of Melbourne. Professor Roessner developed novel GC-MS methods to analyse metabolites in plants. Together with the application of sophisticated data mining, the field of metabolomics was born and is today an important tool in biological sciences, systems biology and biomarker discovery. In 2003 she moved to Australia from Germany where she established a GC-MS and LC-MS based metabolomics platform as part of the Australian Centre for Plant Functional Genomics. Professor Roessner is currently in the position as Head of School, School of BioSciences, University of Melbourne.