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The application of plant growth promoting rhizobacteria (PGPR) in low input and organic cultivation of graminaceous crops; potential and problems*

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ABSTRACT

Although the uses of plant growth stimulating bacteria (PGPR) to improve the yield of graminaceous crops have been studied for over seventy years the utility of the technology remains uncertain. Increases in crop yield have often been inconsistent, reflecting a lack of understanding of the mechanisms by which PGPR exert their effects. Because PGPR are able to fix N_2, this was initially assumed to boost crops by supplementing soil N. However, it is now clear, that for most free living PGPR, other mechanisms affecting root development, and nutrient uptake can account for the observed increase in crop yields. Here we review the current state of our understanding of PGPR in graminaceous crop cultivation, identifying their potential contribution to more sustainable agricultural practices but also highlighting issues that need to be addressed before this technology can be appropriately assessed as a replacement for inorganic N addition.

INTRODUCTION

Despite an unprecedented increase in agricultural productivity during the twentieth century the world faces uncertainty over global food security. The most pressing issue is the predicted increase in global population, that is projected to rise from the current 6.8 billion and surpass 9 billion people by 2050. The burden of feeding these additional people will be felt most keenly by developing countries, whose populations are projected to rise from 5.6 billion in 2009 to 7.9 billion in over the same period (http://www.unfpa.org/public/).

Currently, the global population could be fed by the present level of agricultural output, and the global production of food is 145% greater today than it was in 1960 (Pretty 2008). However, it is unlikely that this growth in agricultural productivity can continue to keep pace with the rising population. In addition, increases in productivity over the last 50 years mask significant variations within developing regions that reflect political, economic and social challenges for the 1.2 billion people who currently live in poverty (Hazell and Wood 2008). For example, China has increased its overall food production fivefold and *per capita* production threefold since 1960. In contrast, Africa has observed a 10% decline in *per capita* food production over the same period (Pretty 2008). Moreover, most developing countries have environmental constraints that will impede the development of agricultural systems able to meet this challenge. These include lack of water, desertification and insufficient cultivable land. Potentially, such problems could be further exacerbated by climate change.

In addition, an increasingly urban global population poses additional challenges. For many people in rapidly developing economies an increased disposable income coincides with the adoption of a diet with a greater consumption of meat and processed cereal products. To meet this demand livestock will need to be raised intensively on a diet of cereals and oils (Pretty 2008). This in turn will place an increased pressure on the available agricultural land and how it is farmed. As a result, it has been argued that current models of low input agriculture relying on biological nitrogen fixation (BNF), and requiring large areas of land will be unlikely to provide the annual requirement of an extra 15 million tonnes of protein by 2050 to stave off widespread hunger (Jenkinson 2001; Smil 2001).

In order that its production practices are able to keep pace with the increasing demand for food, agriculture has relied on

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Table 1. A summary of the reported effects of PGPR on graminaceous crops under laboratory and field conditions.

PGPR	Crop	Effect of inoculation	Proposed mode of action	Reference
Isolates from rice rhizosphere	Rice (*Oryzia sativa* L.)	Isolate inoculation resulted in a significant increase in germination rates, plant height, root length, and dry matter production in rice seedlings	Produce phytohormones and solubilise phosphate	Ashrafuzzaman et al. (2009)
Azospirillum lipoferum		Increased root length, root surface area and root volume	Phytohormone synthesis and siderophore production	Boyer et al. (2008)
Azospirillum		Grain yield and N content was improved	Increased N fixation	Pedraza et al. (2009)
Pseudomonas spp.		Increased IAA levels	Phytohormone synthesis	Karnwal (2009)
Azospirillum; Enterobacter; Aeromonas veronii		Increase in root area, plant biomass and N fixation	Increased N fixation and phytohormone synthesis	Mehnaz et al. (2001)
Pseudomonas spp. *Azospirillum*		Increased shoot biomass and grain yield	Increased N fixation and phytohormone production	Mirza et al. (2006)
Herbaspirillum sp. strain B501 gfp1		Increased dry and fresh weight	Increased N fixation and phytohormone synthesis	Zakria et al. (2007)
Burkholderia vietnamiensis		Increased shoot and root weight and leaf surface	Mechanism is not addressed but hypothesize that increased N fixation and phytohormone production are involved	Van et al. (2000)
Isolates from wheat rhizosphere	Wheat (*Triticum aestivum* L.)	Improved yield and higher N content in grain and straw when used with recommended combination chemical fertilizers	Improved N use efficiency	Akhtar et al. (2009)
Azospirillum brasilense		Increased quantity of photosynthetic igments resulting in greener plants	Enhanced photosynthetic pigment production	Bashan et al. (2006)
Pseudomonas cepacia R55, R85, *P. aeruginosa* R80, *P. fluorescens* R92; *P. putida* R104		Increased root dry weight but results were very inconsistent	Interaction with AMF alters nutrient and water uptake but leads to inconsistent results	Germida and Walley (1996)
Pseudomonas fluorescens		Significant increase in yield	Regulate production of ethylene and longate roots by hydrolyzing 1-aminocyclopropane-1-carboxylic acid	Naveed et al. (2008a)
Pseudomonas spp. *Burkholderia caryophylli*		Significant root elongation, root height, and grain and straw yields	Increased ACC-deaminase activity, chitinase activity, phytohormone production and P solubilization	Shaharoona et al. (2007)
Pseudomonas fluorescens	Rye *(Secale cereale)*	Significant increase in foliar dry mass	Siderophore production and suppression of fungal pathogens	Kurek and Jaroszuk-Scisel (2003)
Bacillus licheniformis RCO2; *Rhodobacter capsulatus* RCO4;	Barley (*Hordeum vulgare* L.)	Increased root and shoot weight and Increased uptake of Fe, N, Mn and Zn	Increased N fixation and production of phytohormones	Cakmakci et al. (2007)
Comercially available Plant Growth Activator (PGA)	Maize *(Zea mays* L.*)*	Greater plant height	More efficient uptake of N and P	Adesemoye et al. (2008)
Azospirillum brasilense Az39; *Bradyrhizobium japonicum* E109		Seed germination and nodule formation were promoted	Production of phytohormones	Cassan et al. (2009)
Azospirillum ipoferum CRT1		Root growth was enhanced	No explanation given	El Zemrany et al. (2006)
Rhizobium spp.; *Sinorhizobium* spp.		Increased shoot and root dry biomass	Production of phytohormones and siderophores	Hossain and Martensson (2008)
Bacillus megaterium; B. subtilis; Pseudomonas corrugata		Increase in grain yield	Increase in fixed nitrogen, production of phytohormones, phosphate solubilization, siderophorestion of antibiotics and siderophores	Kumar et al. (2007)
Pseudomonas spp.		Increased grain yield and nutrient uptake	Hydrolyses ACC	Naveed et al. (2008b)

the application of huge amounts of inorganic N fertiliser to soil. This has removed the requirement for a fertility enhancing cycle in crop rotations and has enabled significant intensification of production of graminaceous crops from a given area of arable land. The exploitation of inorganic N fertiliser has contributed to an annual 4% increase in aggregate global cereal grain production in the forty years since 1960. During which period fertiliser consumption increased from $10.8 Mt_N \cdot yr^{-1}$ to $85.6 Mt_N \cdot yr^{-1}$ (Crews and Peoples 2004). The significance of inorganic N fertiliser and the Haber Bosch process that generates has been contextualised by Smil (2001) who asserted that by 2050 over half of the human population will owe its existence to synthetic N fertilisers.

Whilst the application of inorganic N has had significant benefits for agricultural food productivity and global food security in the short term, there are increasing concerns around the sustainability of this technology to provide a long term solution to ensuring that food production keeps pace with the burgeoning population. The management of agricultural soil is fundamental to ensuring a sustainable agricultural system, however, it is becoming clear that intensive agricultural systems lead to the degradation of agricultural soils as a result of, amongst other factors, the loss of organic matter, compaction and increased salinity, leaching of inorganic nitrate, along with, associated costs such as fuel requirements and the loss of water resources (Kibblewhite et al. 2008; Peoples et al. 1995; Smil 2001).

Consequently, there is increasing interest in developing agricultural management systems that embrace the principles of sustainability. Whilst such concepts are not novel, there is an increasing urgency in developing and implementing them due to increasing alarm that current conventional agricultural management systems cannot continue linearly increasing their reliance on fertilizer consumption, pesticide application, the expansion of agricultural land and machine usage indefinitely, without detriment to the environmental (Kitzes et al. 2008).

Here we review the potential contribution of PGPR that are indigenous or inoculated into soils, may make to the sustainable cultivation of graminaceous crops.

Mechanisms of action of PGPR

In many of the early studies on the exploitation of PGPR in graminaceous crop production, the mechanism of action of the bacteria was presumed to be due to the increased input of fixed nitrogen into the soil, as many PGPR are capable of fixing atmospheric nitrogen (Table 1). However, subsequent work has revealed that there are a variety of other mechanisms through which plant growth can be facilitated including: hormone production, enhanced nutrient acquisition, pathogen suppression and N_2-fixation, often working in parallel to produce the observed response. These effects have been extensively studied and reviewed. Here we summarise the key findings suggesting that PGPR frequently exert their effect through multiple mechanisms working simultaneously.

Biological nitrogen fixation (BNF) can occur in bulk or rhizospheric soil. Fixed nitrogen can then be acquired through root uptake and contribute to the nitrogen budget of the crop (Figure 1). The earliest large scale experiments, exploiting PGPR potential to enhance crop productivity used N_2 fixing bacteria, with the implicit assumption that it was this activity that was producing the enhanced crop yields. For example, large scale field trials in the 1950s used N_2 fixing bacteria, principally *Azotobacter chroococcum* as an inoculum on several million hectares of graminaceous crops including wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Cooper 1959). However, due to inconsistent results the trials were abandoned in the 1960s (Andrews et al. 2003). Other bacterial taxa, including *Azospirillum* spp. and *Agrobacterium radiobacter*, were also extensively studied and trialled as potential substitutes for N fertiliser. One study in Russia to test the potential of a strain of *Agrobacterium radiobacter*, isolated from the rhizosphere of rice (*Oryza sativa* L.), on winter wheat and spring barely appeared to give significant increases (5-30%) in yield in two out of three years. At the same time it was estimated that the contribution of N_2 fixation to total N assimilation was between 23-32% (Bairamov et al. 2001). However, the lack of consistency in the results from one year to the next reflected that of the earlier studies (Andrews et al. 2003). More significantly, in this example, *A. radiobacter*, now reclassified as *Rhizobium radiobacter* (Young et al. 2001), was a taxa that had never demonstrated the ability to fix N_2. Subsequent studies on this strain demonstrated unequivocally that, as with all members of this taxon, *R. radiobacter* was not capable of fixing atmospheric N_2, nor did it form a physical association with the roots of barley. The plant growth promoting substances it produced were most probably responsible for the increase in yields of graminaceous crops (Humphry et al. 2007).

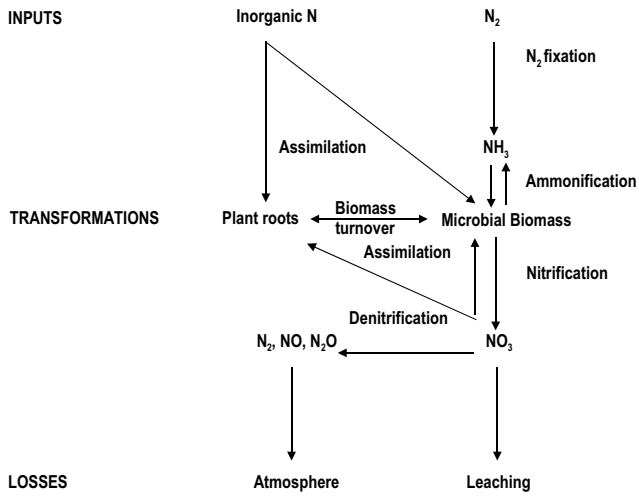

Figure 1. The input, transformation and loss of nitrogen in agricultural soil as a result of inorganic fertiliser application or biological nitrogen fixation.

N_2 fixing activity has been confirmed in PGPR in many other cases. *Azospirillum* species have, for example, been implicated in the enhancement of rice (Pedraza et al. 2009), maize (Montanez et al. 2009) and wheat (Sala et al. 2007) yields, through BNF mechanisms. As many of the PGPR exhibit N_2 fixing abilities, it will always remain a temptation to invoke this activity to explain some of the increased yields observed when such bacteria are used as inoculants on graminaceous crops (Andrews et al. 2003). However, it is apparent by careful analyses of the literature that their mechanisms of action in enhancing crop yields are often due to a range of other activities which, ironically, can reduce soil N rather than supplement it. What is clear is that none of the PGPR effects, studied to date, can match N fertiliser application as a consistent replacement for soil N deficiency (Andrews et al. 2003).

The early large scale studies of PGPR using *Azotobacter* (in the 1930s-1950s) and *Azospirillum* spp. (between 1976-late 1980s), demonstrated that, in field trials, it was possible to observe significant increases in yields with a number of graminaceous crops (Andrews et al. 2003 and references therein). However, consistent results remained elusive and as a result the technology was never adopted because the original hypothesis, that the increased crop yields were due to BNF by the PGPR increasing the soil N budget, could not be substantiated.

There are a number of studies in which the inoculation of PGPR, in tandem with the addition of inorganic N fertiliser, results in an increase in crop yields comparable or greater than that observed when conventional quantities of inorganic N are applied. A study on wheat demonstrated maximum increases in yields of grain and straw were observed in treatments where PGPR were used in combination with recommended dosages of inorganic fertiliser (Akhtar et al. 2009). A further study indicated that PGPR which demonstrated ACC-deaminase activity, such as *Pseudomonas fluorescens* and *Pseudomonas putida*, could improve wheat yield and reduce the dependence on inorganic N by 25%, whilst giving an increase in wheat grain yield of 96% (Naveed et al. 2008b).

Other workers have demonstrated positive responses on wheat yields with reductions in the requirement for inorganic fertiliser with strains of *Pseudomonas fluorescens* and *Azospirillum brasilense* (Sala et al. 2007; Shaharoona et al. 2008). Such work, whilst of interest needs to be rigorously followed up for several seasons. The mechanism of crop yield enhancement and the reduction on the requirement for inorganic N may reflect short term enhancement of N uptake from the pool already present in the soil complementing that provided by the fertiliser. Over time, as the residual N in the soil is depleted such applications of PGPR, with reduced levels of inorganic N addition, may result in deficits for crop growth. A consequence could be a reduction or inconsistent response of yield to this protocol, typical of those that bedevilled earlier attempts to develop PGPR as a tool for enhancing sustainability in agriculture.

Frequently, the mechanisms underlying the observed crop growth enhancement are not understood and as a result are attributed to a specific activity of the organism involved. In the case of free-living diazotrophs the additional provision of N to the plant is assumed to be significant in observed increases in yields, however, such organisms do not seem able to directly release fixed N to the plant and this occurs only through the turnover of the microbial biomass (Richardson et al. 2009). In tandem with the N_2 fixation, many PGPR also produce phytohormones that have a significant effect on the crop root biomass and surface area, as seen in studies on rice (Mirza et al. 2006) and maize (Kumar et al. 2007). As a consequence the increases in grain yield may reflect the indirect enhancement of plant nutrition through the increased root surface area, as opposed to a direct effect of increased fixed N being available to the plant from the diazotrophic bacteria. The effect of phytohormones on crop root growth probably explains the increased N use efficiency in rice (Van et al. 2000), and wheat (Akhtar et al. 2009) inoculated with PGPR.

A number of studies have proposed that the addition of PGPR to crops can enhance yields by increasing uptake of nutrients in addition to N, including, phosphorus, potassium and iron. The uptake of nutrients by plants represents a three way interaction between the plant root, the physical and chemical environment of the soil and the rhizospheric microbial community. As with increased N use efficiency the production of phytohormones by PGPR may increase the surface area of roots enabling greater uptake of key nutrients (Cakmaki et al. 2007). *Gluconacetobacter diazotrophicus* has been shown to solubilise Zn an essential micronutrient, a deficiency of which is in common sugar cane plants in which this bacterium is an endophyte (Saravanan et al. 2008); or they may mobilise key nutrients by the production of siderophores (Fischer et al. 2007). Similarly, studies on rice, wheat and maize have all demonstrated that bacteria with P solubilising activity can have a positive effect on plant growth (Adesemoye et al. 2008; Bashan et al. 2006). However, the mechanisms remain ambiguous and whether these organisms mobilise sufficient P to make a substantive contribution to plant nutrition has not been resolved and phytohormones may once again play a role in the positive increase in crop yields. Certainly, field studies have failed to consistently demonstrate such a response and few studies attempt to address the significance of P solubilisation by demonstrating a negation of the response when higher concentrations are applied (Richardson et al. 2009).

Large areas of agricultural land have been degraded by poor irrigation practice, resulting in damage such as salinization which affects 20% of total irrigated areas. Moreover, climate change appears to be a contributing factor to increased variability in rainfall (Hazell and Wood 2008). As a result, the impact of environmental stresses, such as drought and salinity, on crop yields is significant (Kibblewhite et al. 2008). There is some evidence that the inoculation of crops with PGPR enhances the tolerance of crops to such environmental stress. *Pseudomonas* spp. inoculated on legumes were shown to ameliorate the effects of drought stress on the growth and yield

of the crop (Arshad et al. 2008). However, effective inoculation of crops cultivated in soils subject to environmental stress requires that the bacteria deployed can tolerate these conditions and remain effective in promoting plant growth. Paul and Nair (2008) demonstrated that *Pseudomonas fluorescens* MSP-393, used as biocontrol agent of soil pathogens, remained capable of effectively colonising plant roots even in high salinity soils. However, development of PGPR inocula for soils subjected to one or several environmental stresses need to validate that they remain effective under such conditions.

In summary, the effects of PGPR as inoculants of graminaceous crops have been extensively studied, in both field and pot based trials. Many of the PGPR identified have N_2 fixing ability but it is also apparent (Table 1) that alternative mechanisms of action, that account for the enhanced plant growth can be observed. Unfortunately, in many of the studies reporting a positive response of a particular crop to PGPR addition, an explicit link is forged, often without robust data sets, to a particular bacterial attribute such as N_2 fixation or production of phytohormones. However, it is clear that the issues surrounding the lack of reproducibility of a crop's response to PGPR inoculation require a far more systematic approach before the technology can be effectively deployed in the field.

The experimental design of such studies need, among other things, to robustly test the mechanism(s) by which the crop responds to PGPR inoculation; its reproducibility from one growing season to the next; to measure the plant response consistently, such that meaningful comparisons between studies can be made (Vessey 2003); and appraise of the persistence of the inoculated PGPR in the soil (Strigul and Kravchenko 2006).

Applying PGPR inoculants to soil

There are well established technologies, developed in legume cultivation, to add bacterial inoculants either as a liquid to coat the seeds or directly to the soil, typically using a carrier, such as peat or other material such as perlite, composted cork or bagasse (Albareda et al. 2008). Peat carriers have been the most widely used on a commercial scale as they have a number of advantages, including, a long shelf life and increased bacterial viability compared to liquid inoculants added directly to the seed. However, they have frequently resulted in inconsistent effects on crop yield, due to either the quality of the inoculant being low (Brockwell and Bottomley 1995) or the bacteria being unable to survive in the soils to which they are added, as a result of adverse environmental conditions inhibiting bacterial survival, competition from the native bacterial flora (Catroux et al. 2001) or a combination of these two factors.

The use of PGPR on graminaceous crops is a different issue to their use on legumes, the mechanisms of action may occur in the rhizosphere (phytohormone production, pathogen suppression, enhanced nutrient uptake) or be associated with the colonization of the plant roots (phytohormones, BNF). In the first case, the aim of the inoculation process is to engineer the rhizosphere to accommodate the bacteria. The competitiveness of the introduced bacteria will reflect how well it adapts to soil conditions and competes with the indigenous flora. Studies utilizing genetically engineered *Pseudomonas putida* strains in the wheat rhizosphere, inoculated by broth culture application to the seed coat, have shown a rapid decrease in the numbers of introduced bacteria by five orders of magnitude between sowing and harvesting (Viebahn et al. 2003). The experiment was conducted over two growing seasons, in the first some perturbation of the indigenous microbial flora was observed but not in the second. Moreover, the effect of the genetically modified PGPR on increased plant growth was no greater than that observed after a conventional crop rotation event. A recent study on the impact of inoculation of rice seeds with *Azospirillum brasilense* on the diversity of bacteria in the phyllosphere showed no significant impact (Pedraza et al. 2009). In another study, *A. lipoferum*, was shown to significantly shift the rhizosphere population of field grown maize up to 35 days after sowing (Baudoin et al. 2009).

The influence of the plant genotype on the microbial community of the rhizosphere has been understood for almost forty years, following studies using several wheat lines (Neal et al. 1970). This reflects the differential rhizodeposition of different plant species and varieties. Ryan et al. (2008) have recently reviewed data from a number of studies indicating the differential population of *Pseudomonas fluorescens* found in the rhizospheres of both different wheat varieties (Mazzola et al. 2004) and plant hosts (Bergsma-Vlami et al. 2005).

The application and fate of inoculants on field grown crops needs to be carefully validated to ensure that they can produce some demonstrable benefit to yields. Recently, attempts have been made to mathematically model PGPR inoculation into the rhizosphere (Strigul and Kravchenko 2006). Such approaches are welcome as they enable the impacts of the different abiotic and biotic factors on PGPR survival to be considered. Strigul and Kravchenko (2006) demonstrated, through mathematical simulations, that the most significant factor affecting PGPR survival was the competition for limiting resources with indigenous flora, followed by the compatibility between the rhizodeposition of compounds by the plant host and the ability of the inoculated bacteria to utilise them. Such work is useful in framing ongoing studies in the use of PGPR, enabling a prediction of the success of a PGPR inoculation in a particular soil with a specific variety of crop to be made.

Future work

Engineering the rhizosphere of crops to improve productivity and plant health has been studied through a number of mechanisms, including manipulating the plants to: modify their

rhizosphere to promote nutrient availability, suppress pathogens, or encourage PGPR bacterial growth (Ryan et al. 2008). Similarly, the inoculation of soil with a PGPR leading to enhancement of crop yields implies that the bacteria have become established in the rhizosphere of the plant, and are exerting a stimulatory effect via one or several mechanisms described above. As a result there is an implicit assumption that the rhizosphere has been manipulated or engineered by the inoculation process. Such a response can be demonstrated in the field, for example, *Azospirillum lipoferum* inoculated onto the seed of field grown maize produced a statistically significant shift in the composition of the indigenous rhizobial community (Baudoin et al. 2009). However, several studies including a field based study on wheat have indicated that such inoculation effects are transient as a result of a rapid decline in inoculant numbers after the bacteria are added (Viebahn et al. 2003). Advances in our understanding of the ecological effects of inoculation will also be significant in enabling more effective modelling of the inoculation. Recent studies indicate invading bacteria might release anti-competitor toxins or parasitic phage to overcome the barrier presented by the resident flora in the rhizosphere (Brown et al. 2006). More explicit manipulation has been demonstrated by engineering PGPR strains to enhance their ability to suppress pathogens or inhibit the production of stress hormones by the plant (Ryan et al. 2008). It is unlikely that genetically engineered strains offer a realistic mechanism to exploit PGPR effectively in the short and medium term, as they would have to satisfy stringent regulatory criteria, demonstrate a reproducible positive impact on crop yield and in some areas significant public antipathy to such technology.

The effective utilisation of PGPR in the future will demand that there is a much more rational approach to the choice and delivery of the particular bacterium into the field. This will depend on a range of variables that require consideration (Trivedi et al. 2005). The development of 'bespoke' inocula that are adapted to specific soil and crop varieties is essential if the full benefit of PGPR increase in crop yields is to be realised (Cummings and Andrews 2003). However, a consequence of such parochial inoculants is that the cost of development and production may outweigh the benefits in terms of increased yields, and reduce the size of the potential market for such products such that they are not economically viable.

CONCLUSIONS

At present the potential contribution of PGPR to the sustainable cultivation of graminaceous crops remains ambiguous. The technology has had a long and chequered history, whilst the production of inoculants is relatively cheap, until they can be proven to produce a return for the additional cost it is unlikely to be widely taken up by farmers. Inoculant technology has developed significantly in recent years, in terms of scale and quality, particularly for legumes. The mechanisms by which PGPR seem to exert their most significant effect on crop growth is by enhanced nutrient uptake. However, they do not offer significant reproducible gains in graminaceous crop yield year on year. More systematic approaches to research questions should be adopted to determine how PGPR can be most effectively deployed to improve agricultural productivity.

PGPR represent a less significant threat to the environment than the use of inorganic N or pesticide application, in the longer term, the consequence of inoculation of soils with PGPR on microbial soil diversity is unknown. Most studies indicate such bacteria rapidly decline in competition with the indigenous flora. Genetically engineered strains are possible but remain an expensive and potentially more controversial approach to the technology. However, until it has been demonstrated to be a robust and reproducible method of crop yield enhancement this approach does not appear to be viable.

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