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Review article

Applications of free living plant growth-promoting rhizobacteria

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Abstract

Free-living plant growth-promoting rhizobacteria (PGPR) can be used in a variety of ways when plant growth enhancements are required. The most intensively researched use of PGPR has been in agriculture and horticulture. Several PGPR formulations are currently available as commercial products for agricultural production. Recently developing areas of PGPR usage include forest regeneration and phytoremediation of contaminated soils. As the mechanisms of plant growth promotion by these bacteria are unravelled, the possibility of more efficient plant-bacteria pairings for novel and practical uses will follow. The progress to date in using PGPR in a variety of applications with different plants is summarized and discussed here.

Introduction

New and novel solutions for plant growth enhancements are required to ease the burden imposed on our environment and other resources. Here we look at potential solutions to these issues by examining some of the research conducted regarding the biological applications of free-living plant growth promoting rhizobacteria (PGPR). The major applications of bacteria for improved plant growth include agriculture, horticulture, forestry and environmental restoration. This review presents an overview of information available on these various applications.

Indirect mechanisms used by PGPR include antibiotic protection against pathogenic bacteria, reduction of iron available to phytopathogens in the rhizosphere, synthesis of fungal cell wall-lysing enzymes, and competition with detrimental microorganisms for sites on plant roots. Direct mechanisms of plant growth by PGPR include the provision of bioavailable phosphorus for plant uptake, nitrogen fixation for plant use, sequestration of iron for plants by siderophores, production of plant hormones like auxins, cytokinins and gibberellins, and lowering of plant ethylene levels (Glick 1995; Glick et al. 1999).

Applications of PGPR in Agriculture

The most intensively studied application for free living PGPR is agriculture. Researchers in the former Soviet Union and India conducted widespread tests in the early to the mid part of the 20th century studying the effects of PGPR on different crops. Though results from different experiments were not harmonized and were often inconsistent, up to 50 to 70% yield increases were reported. Inconsistency of results was due to a lack of quality in experimental design and analysis of results (Brown 1974; Cooper 1959). Moreover, during this time an understanding of the detailed mechanisms of plant growth promotion by rhizobacteria was largely unknown. Nevertheless, these field experiments provided clues concerning the optimal conditions for bacterial colonization and growth promotion of target crops (Brown 1974).

The results of many studies of the effect of free-living rhizobacteria on various crop plants, conducted

over approximately the last twenty-five years, are summarized in Table 1. Plant growth benefits due to the addition of PGPR include increases in germination rates, root growth, yield (including grain), leaf area, chlorophyll content, magnesium content, nitrogen content, protein content, hydraulic activity, tolerance to drought, shoot and root weights, and delayed leaf senescence. Another major benefit of PGPR use is disease resistance conferred to the plant, sometimes known as 'biocontrol'.

The use of PGPR to increase crop yield has been limited due to the variability and inconsistency of results between laboratory, greenhouse and field studies (Mishustin and Naumova 1962). Soil is an unpredictable environment and an intended result is sometimes difficult to obtain (Bashan 1998). For example, in a study by Frommel et al. (1993), poor colonization of the PGPR on plant roots occurred at one site due to adverse conditions, including high *Verticillum* infection of the soil, low soil pH, high mean temperature and low rainfall during the growing season. These undesirable growing conditions most likely contributed to the low root colonization (Dobbelaere et al. 2001; Klein et al. 1990; Parke 1991; Suslow and Schroth 1982). Climatic variability also has a large impact on the effectiveness of PGPR (Okon and Labandera-Gonzalez 1994) but sometimes unfavourable growth conditions in the field are to be expected as a normal functioning of agriculture. Increased yields obtained with wheat inoculated by *Pseudomonas* species in the growth chamber have also been observed in the field (Weller and Cook 1986). Even though there is a possibility of great variability in field results, if a positive effect of a PGPR is seen on a specific crop in greenhouse studies, there is a strong likelihood that those benefits will carry through to field conditions.

There is a great deal of contradictory information on the effectiveness of PGPR on plants in soils under various conditions of fertilization, especially with *Azospirillum* species. In the past, the main mechanism of plant growth promotion by *Azospirillum* was thought to occur by providing the plant with fixed nitrogen. In fact, it has been reported that the plant growth promotion effect of *Azospirillum* only occurs in nitrogen-limited conditions (Dobbelaere et al. 2001; Fallik and Okon 1996). However, in other cases, much greater increases in plant growth promotion have been observed after the addition of fertilizers (Okon and Labandera-Gonzalez 1994). Another way in which *Azospirillum* species improve plant growth is through the production of indole acetic acid (IAA), a plant hormone (Dobbelaere et al. 1999; Okon and Labandera-Gonzalez 1994).

Different soil types can influence the effectiveness of PGPR (e.g., Kloepper et al. 1980). In a study with wheat and a pseudomonad, results suggested that the less fertile the soil, the greater the plant growth stimulation by the PGPR (De Freitas and Germida 1990). This is similar to observations in studies conducted with *Azospirillum* species, despite the fact that pseudomonads fix little or no nitrogen. On the other hand, growth promotion of maize with a strain of *Azospirillum lipoferum* has been reported to be independent of cultivar or soil type in the field (Fages 1994). When choosing an effective PGPR for a plant at a specific site, it is imperative to consider the nutrient level in the soil and how the intended PGPR would perform at that location.

Soil moisture content affects the colonization of the plant rhizosphere by the PGPR after inoculation (Burr et al. 1978). Studies in the Soviet Union suggested that optimum results were obtained when the soil moisture was 40% (Brown 1974; Cooper 1959). However, this may be a function of the type of bacterium utilized since high moisture content may decrease the oxygen content of the soil.

In some instances, specific strains of bacteria may promote bacterial growth only in certain crops. In one example, it was found that out of four *Pseudomonas* strains that promoted the growth of radish, only one was effective in promoting the growth of potato (Kloepper et al. 1980). It has also been observed that maximum increases in germination and yield often occur in crops inoculated with PGPR strains isolated from the plants native rhizosphere (Fages and Arsac 1991; Favilli et al. 1987).

During the initial stages of testing in the laboratory, PGPR survival in a microcosm of the field environment should be determined. This is to ensure that any manipulations conducted on the bacteria are not detrimental to their growth promotion effect and their competitiveness in the field (Tang et al. 1995). In the field, the number of PGPR cells applied to the plant is often vital for proper growth promotion (Boddey and Dobereiner 1988). Many researchers (Table 1) have applied up to 10^8 colony forming units (CFU) per seed (De Freitas and Germida 1991; Di Ciocco and Rodriguez-Caceres 1994; Fages 1994; Okon et al. 1988; Tran Van et al. 2000; Weller and Cook 1986) or up to 10^9 CFU/g of inoculant (Barrios et al. 1984; De Freitas and Germida 1990a, 1990b; Fallik and

Table 1. Examples of free-living plant growth promoting rhizobacteria tested on various crop types

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Azospirillum (local isolates from Argentina)	Wheat, Maize	Field	– in wheat cultivars over seven seasons, increases of yield from 15 to 30 %, and increases in yield of 50-60% when fertilized – over six seasons, increases of maize yield from 15 to 25% observed, and with fertilization, yield increased up to 40%	Okon and Labandera-Gonzalez 1994
Azospirillum brasilense	Guinea grass Pearl millet, *Digitaria decumbens*	Field	– greater dry matter yield compared to uninoculated controls – approximately 40 kg/ha per year of nitrogen estimated as saved due to inoculation	Smith et al. 1978
Azospirillum brasilense	Finger millet, Sorghum, Pearl millet	Field	– average of up to 15% yield increase for finger millet – for sorghum, average increase is 19% – in ten years of study, *Azospirillum* successful in significantly increasing yield in 60% of trials	Rao 1986
Azospirillum brasilense	Sorghum	Hydroponic system in greenhouse	– significant increases in dry matter, leaf area and grain yield after four weeks – leaf senescence delayed	Sarig et al. 1990
Azospirillum brasilense	Sorghum	Greenhouse	– increased total number and length of adventitious roots by 33 to 40% and increased hydraulic conductivity by 25-40%	Sarig et al. 1992
Azospirillum brasilense	Bean	Hydroponic growth chamber	– increased fresh root and shoot weights	Vedder-Weiss et al. 1999
Azospirillum brasilense	Sorghum, Wheat, Barley	Field (tested on over 200,000 ha)	– consistent positive effects with up to 26% increase of yield – best results seen in light sandy soils with intermediate amounts of fertilizers	Dobbelaere et al. 2001
Azospirillum brasilense Cd	Fountaingrass Sorghum Sudangrass	Field	– increases in yield of 11 to 24% at one location	Smith et al. 1984
Azospirillum brasilense Cd	Maize, Millet Sorghum, Wheat	Field	– summer crops show significant yield increases in 75% of trial plots, while winter crops only have significant yield increases in 5-12% of trials	Okon et al. 1988

Table 1. Continued.

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Azospirillum brasilense Cd *Azospirillum liporferum* Br-17	Maize	Field	– consistent increases of yield at intermediate soil fertility – replaces 35-40% of nitrogen fertilizers	Okon and Labandera-Gonzalez 1994
Azospirillum brasilense Cd	Maize	Field	– significant increase in the number of adventitious roots, root length, root and shoot dry weight in three different regions	Dobbelaere et al. 2001
Azospirillum brasilense Cd	Chickpeas, Faba beans	Greenhouse and field	– significant increases in root nodulation by native rhizobia and improved root and shoot development – when irrigated by saline water, inoculated plants are not as negatively affected compared to control – field results show a significant increase in shoot growth and crop yield	Hamaoui et al. 2001
Azospirillum brasilense Cd, 245	Oat, Maize, Sorghum	Greenhouse and field	– positive increases in maize and sorghum biomass in both field and greenhouse, but not statistically significant – significant increase of biomass of oat for one year's field test but not for the second year	Dobbelaere et al. 2001
Azospirillum brasilense Cd, Az-39 *Azospirillum lipoferum* Az-30	Millet	Field	– increased yield up to 30% and 21% in two years of plant growth	Di Ciocco and Rodriguez-Caceres 1994
Azospirillum brasilense Cd, Az-39	Wheat	Field	– plant yield increased, especially with Az-29	Caceres et al. 1996
Azospirillum brasilense NO40	Rice	Field	– increased yield by 15-20% in two locations	Omar et al. 1989
Azospirillum brasilense Sp 245 *Azospirillum irakense* KBCl	Winter wheat, Maize	Field	– plant growth promotion effect disappeared when plants over fertilized with nitrogen – in plots with low nitrogen, higher yields not obtained, likely due to adverse weather conditions at test plots	Dobbelaere et al. 2001
Azospirillum brasilense Sp-111	Wheat	Field	– yield increases of 1.3 to 2 fold over the five year study – variable results due to climactic conditions	Okon and Labandera-Gonzalez 1994

Table 1. Continued.

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Azospirillum brasilense Sp-245, Sp-107st	Wheat	Field	– significant increases of grain yield and plant nitrogen content – strain Sp-245 most effective on wheat	Boddey and Dobereiner 1988
Azospirillum lipoferum	Sunflower	Greenhouse	– strains originally selected from plant rhizosphere – positive growth responses with respect to germination	Fages and Arsac 1991
Azospirillum lipoferum CRT1	Maize	Field	– growth promotion effect occurred early, despite rapid decrease of bacterial density of introduced bacteria – plant height, primary root length and root fresh weight all enhanced by the addition of the bacteria	Jacoud et al. 1998
Azospirillum lipoferum CRT1	Maize	Field	– average grain yields and N content higher for the inoculated plants, but not statistically significant – larger root systems, and lower grain moisture measured	Dobbelaere et al. 2001
Azospirillum lipoferum CRT-1	Maize	Field	– positive response of yield to inoculation regardless of cultivar or soil type	Fages 1994
Azospirillum sp.	Maize	Field	– increased yield of 6.7 to 75.1%	Kapulnik et al. 1981
Azospirillum sp.	Wheat	Field	– changes in yield of − 9.6 to 14.8%	Reynders and Vlassak 1982
Azospirillum sp.	Wheat	Field	– changes in yield of − 15.8 to 31%	Baldani et al. 1987
Azospirillum sp.	Millet	Field	– changes in yield of − 12.1 to 31.7%	Kloepper et al. 1989
Azospirillum sp.	Mustard	Field	– increased yield of 16 to 128%	Kloepper et al. 1989
Azospirillum sp.	Rice	Field	– increased yield of 4.9 to 15.5%	Kloepper et al. 1989
Azospirillum sp.	Maize	Field	– significant increases in yield in light soils and at intermediate nitrogen fertilization – in fields with no nitrogen fertilization, a definite yield increase over uninoculated plants, but not statistically significant – in fields with high nitrogen fertilization, no growth enhancement effect	Okon and Labandera-Gonzalez 1994

Table 1. Continued.

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Azospirillum sp.	Maize	Field	– no effect of inoculation on plant yields when soils are heavy and high in nitrogen content – in light soils low in nitrogen fertilization, yield increase of 11-14%	Fallik and Okon 1996
Azospirillum sp.	Sorghum	Field	– increased yield of 20.5 to 30.5%	Kapulnik et al. 1997
Azospirillum sp.	Maize	Field	– significant increase in dry matter yield – increased magnesium percentage	Hernandez et al. 1997
Azospirillum sp.	Sorghum	Field	– increased yield of 12 to 18.5%	Sarig et al. 1998
Azospirillum sp.	Maize	Greenhouse	– increased activity of glutamate dehydrogenase and glutamine synthetase – increased N content in leaves and roots	Ribaudo et al. 2001
Azospirillum sp.	Wheat	Greenhouse	– higher biomass, grain yield, protein content and plant nitrogen content – concluded that nitrogen uptake is the mechanism of plant growth promotion in this case	Saubidet et al. 2002
Azotobacter chroococcum	Barley	Growth chamber assays	– increase of seed germination and seedling development – no change in the number of seeds germinated, but increase seen in the extension of the growing root – addition of nitrate decreases plant stimulation effect – some inconsistent results seen, and authors conclude that *Azotobacter* is not a reliable inoculant	Harper and Lynch 1979
Azotobacter sp. *Bacillus* sp. *Enterobacter* sp. *Xanthobacter* sp.	Rice	Field	– increases of total dry matter yield, grain yield, and nitrogen accumulation by 6 to 24% over two years of study – hypothesised that yield increases due to increase in root length, leaf area and chlorophyll content	Alam et al. 2001
Bacillus amyliquefaciens IN937 *Bacillus pumilis* INR7, SE34 *Bacillus subtilis* GB03 *Bacillus cereus* C4	Tomato, Pepper	Field	– statistically significant increases of plant growth in two growing years in stem diameter, stem area, leaf surface area, weights of roots and shoots and number of leaves – transplant vigour and fruit yield improved – pathogen numbers and disease not reduced in tomatoes or pepper with the exception of reduction of galling in pepper by root-knot nematode	Kokalis-Burelle et al. 2002

Table 1. Continued.

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Bacillus polymyxa	Wheat	Field	– increases in plant yield	Caceres et al. 1996
Bacillus polymyxa *Burkholderia* sp. *Pseudomonas* sp.	Sugar beet	Field	– significant increases in root yield (6.1 to 13.0 %), sugar yield (2.3 to 7.8 %) – yields further enhanced by N, P and NP applications	Cakmakci et al. 2001
Bacillus sp.	Sorghum	Field	– increased yield of 15.3 to 33%	Broadbent et al. 1977
Bacillus sp.	Wheat	Field	– changes in yield of 0 to 114%	Kloepper et al. 1977
Bacillus subtilis A-13	Peanut	Field	– yield increases up to 37%; only two of 24 test sites produced negative results – plant responses most positive when subjected to stress like limited water, poor nutrition, cold temperatures – plant disease reduced	Turner and Backman 1991
Bacillus subtilis B2	Onion	Growth chamber	– significant increases in shoot dry weight (12-94%), dry root weight (13-100%) and shoot height (12-40%) over controls – rhizosphere populations of inoculated bacteria decreased throughout study, but growth promotion effect still observed	Reddy and Rahe 1989
Beijerinckia mobilis *Clostridium* sp.	Beet, Barley, Wheat, Red radish, Cucumber	Lab experiments and greenhouse	– in combination with mineral fertilizers, increase of plant production by 1.5 to 2.5 times – positive increases seen with seed germination rate, mean plant length and plant weight – different cucumber cultivars demonstrated variability in seed germination response	Polyanskaya et al. 2000
Burkholderia vietnamiensis TVV75	Rice	Outdoor pot and field trials	– when plants inoculated and transplanted at day 24, increases of shoot weight (up to 33%), root weight (up to 55%) and leaf surface (up to 30%) observed – end grain yield increase of 13-22% – grain weight (a late-season yield component) significantly increased due to inoculation, but not due to the addition of nitrogen	Tran Van et al. 2000
Enterobacter cloacae CAL3	Tomato, Pepper, Mung bean	Greenhouse	– positive seedling growth response by all three plant species to PGPR treatment, especially tomato, where no exogenous mineral nutrients added – early stimulation effect on seedlings observed	Mayak et al. 2001

Table 1. Continued.

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Non-fluorescent pseudomonad isolated from onion rhizoshpere	Potato	Growth chamber	– plantlets showed significant increases in root dry weight of 44 to 201%, stem length 26 to 28%, lignin up to 43%, and enhanced stem hair formation 55 to 110%	Frommel et al. 1991
OTHER Species and Mixtures				
Pseudomonads spp. (fluorescent strains)	Winter wheat	Field	– biocontrol effects seen against take all (Gaeumannomyces graminis), 27% yield increase due to inoculation	De Freitas and Germida 1990
Pseudomonas cepacia *Pseudomonas fluorescens* *Pseudomonas putida*	Winter wheat	Potted plants in growth chamber	– strains demonstrate biocontrol against *Rhizoctonia solani* and *Leptosphaera maculans* – different strains stimulate different plant parts – growth of plants in less fertile soil stimulated	De Freitas and Germida 1990
Pseudomonas cepacia MR85, R85 *Pseudomonas putida* MR111, R105	Winter wheat	Field	– bacteria inoculated on plants able to overwinter on roots and survive; with levels reaching 10^4 to 10^8 CFU/g – wheat grain yields increased significantly at several sites with these strains, however overall results were not significant due to variability of results between some trials	De Freitas and Germida 1992b
Pseudomonas cepacia R55, R85 *Pseudomonas putida* R104	Winter wheat	Growth chamber	– antagonism demonstrated against *Rhizoctonia solani* – increase of dry weight of inoculated plants (62-78%) grown in *R. solani* infected soil – dry root weight increased by 92-128% and shoot dry weight increased by 28-48%	De Freitas and Germida 1991
Pseudomonas cepacia R85 *Pseudomonas fluorescens* R104, R105 *Pseudomonas putida* R111	Winter wheat	Potted plants in growth chamber	– two soil types tested under simulated fall conditions (5ºC) – response of wheat nutrient uptake to inoculation dependant on soil composition – grain yield enhanced 46-75% in more fertile soil	De Freitas and Germida 1992a
Pseudomonas chlororaphis 2E3, O6	Spring wheat	Field and laboratory	– increased emergence at two different sites by 8 to 6% – strong inhibition of *Fusarium culmorum* – no promotion effect of inoculated plants evident in soils free of *Fusarium* infection	Kropp et al. 1996
Pseudomonas corrugata *Azotobacter chroococcum*	*Amaranthus paniculatus* *Eleusine coracana*		– plant growth and nitrogen content increased – hypothesised that the growth promotion effect is due to the stimulation of native bacterial communities	Pandey et al. 1999

Table 1. Continued.

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Pseudomonas fluorescens *Pseudomonas putida* TL3, BK1	Potato	Field	– lack of growth promotion effect and bacterial survival in dry soils – in normal conditions statistically significant increases of yield of 14-33% in 5 of 9 plots	Burr et al. 1978
Pseudomonas fluorescens	Winter wheat	Field and growth chamber	– in growth chamber, seedling height promotion seen – in *Pythium*-contaminated sites, significant increases in stand, plant height, number of heads, and grain yield	Weller and Cook 1986
Pseudomonas fluorescens 63-28, R17-FP2, QP5, R15-A4	Tomato	Greenhouse with natural lighting	– in favourable light conditions, fruit yields increased by 5.6 to 9.4 % – in unfavourable light conditions, yields increased up to 18.2 %	Gagné et al. 1993
Pseudomonas fluorescens 63-49, 63-28, 15 *Pseudomonas corrugate* 13 *Serratia plymthica* R1GC4	Cucumber	Field	– strain 63-49 significantly increases fruit numbers by 12% and fruit weight by 18% – strains 13, 15, R1GC4 slightly increase yields – in *Pythium* infected soils, yield increased up to 18% with addition of strains 63-49 and 63-28	McCullaugh et al. 2001
Pseudomonas fluorescens Pf5 *Bacillus pumilus*	Highbush blueberry		– leaf area and stem diameter increases	de Silva et al. 2000
Pseudomonas putida *Pseudomonas putida biovar* B *Pseudomonas fluorescens* *Arthrobacter citreus* *Serratia liquefaciens*	Canola (*Brassica campestris* L. and *Brassica napus* L.)	Field and greenhouse	– in greenhouse, selected strains produce 57% increase in yield – in field conditions, select strains increase seedling emergence and vigour – yield increase from 6 to 13 % over a two year test period	Kloepper et al. 1988
Pseudomonas putida *Bacillus subtilis* *Enterobacter aerogenes* *Enterobacter agglomerans* *Bacillus cereus*	Cucumber	*In vitro* and greenhouse	– most strains increased root length in *Pythium*-infected plants *in vitro* – in greenhouse, increases of the weight of cucumber plants by 29%, fruit yield by 14% and fruit number by 50% by *B. subtilis*	Uthede et al. 1999
Pseudomonas putida GR12-2	Canola	Greenhouse	– non-nitrogen fixing mutants provide greater root elongation effects and greater phosphate uptake	Lifshitz et al. 1987

Table 1. Continued.

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Pseudomonas putida GR12-2	Canola, Lettuce, Tomato, Barley, Wheat, Oat	Growth chamber	– in dicot plants, root elongation stimulated by the bacteria, but in monocot plants, little to no growth promotion seen – difference due to sensitivity differences to ethylene, this bacterial strain contains gene to reduce ethylene synthesis (i.e. ACC deaminase)	Hall et al. 1996
Pseudomonas putida W4P63	Potato	Field	– increased yield of 10.2 to 11.7% – potato soft rot (*Erwinia carotovora*) suppressed	Xu and Gross, 1986
Pseudomonas sp. *Vario-vovax* sp. *Agrobacterium* sp. *Phyllobacterium* sp.	Canola	Growth chamber	– significant increase in root dry weight from 11 to 52% – most important promotion effect by *Phyllobacterium* sp.	Bertrand et al. 2001
Pseudomonas sp. PsJN	Potato	Greenhouse and field trials	– in greenhouse, increase of whole plant dry weight; result not influenced by soil sterility – in field, early emergence stimulated and significant tuber yield increases in 3 of 4 trials	Frommel et al. 1983
Pseudomonas spp. (fluorescent strains) A1, B10, TL3, BK1, E6	Potato	Greenhouse and field	– treated seed pieces produce larger root systems in greenhouse – significant yield increases occurred in all test fields, but different strains promote plant growth differently in different soil types – early plant responses correlated to increased yields	Kloepper et al. 1980
Pseudomonas spp. (fluorescent strains) A1, B2, B4, E6, RV3, SH5	Sugar beet	Greenhouse and field trials	– increases of seedling mass by all strains in greenhouse – effects of inoculation positive on yield in field trials, but results variable from site to site – study authors conclude that promotion effect due to antagonism of plant disease	Suslow and Schroth 1982
Pseudomonas spp.	Potato	Field	– changes in yield of − 10 to 37%	Howie and Echandi 1983
Pseudomonas spp.	Potato	Field	– changes in yield of − 9 to 20%	Geels et al. 1986
Pseudomonas spp.	Potato	Field	– changes in yield of − 14 to 33%	Kloepper et al. 1989
Pseudomonas spp.	Rice	Field	– increased yield of 3 to 160%	Kloepper et al. 1989

Table 1. Continued.

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Pseudomonas spp.	Lettuce, Cucumber, Tomato, Canola	Hydroponic growth chamber	– increases of root and shoot weights for all plants tested – most significant positive growth responses in lettuce, tomato and cucumber	Van Peer and Schippers 1998
Pseudomonas spp. 7NSK2	Maize, Barley, Wheat	Field	– increased yield of 15 to 25%	Iswandi et al. 1987
Pseudomonas syringae pv. *Phaseolicola*	Bean	Greenhouse	– poor establishment of test pathogen in plants inoculated with *P. syringae* – increased amounts of protein in inoculated plants	Alstrom 1995
Pseudomonas W34 *Bacillus cereus* S18	Lettuce, Tomato	Pot experiment	– significant reduction in galling and enhanced seedling biomass in soils infested with *Meloidogyne incognita* – *B. cereus* S18 causes up to a 9% yield increase compared to the control	Hoffmann-Hergarten et al. 1998
Serratia liqufaciens *Pseudomonas* sp. *Bacillus* sp.	Maize	Greenhouse	– increased of yield of 8 to 14 % – *S. liquefaciens and Pseudomonas* sp. gives the highest stimulation effect in different soils	Lalande et al. 1989
Serratia proteamaculans 102 *Serratia liquefaciens* 2-68	Soybean	Field	– treatment effects of both bacterial strains not significant over two study years	Pan et al. 2002
Xanhomonas maltophila	Sunflower	Lab and greenhouse	– increased germination rate	Fages and Arsac 1991
'Yield Increasing Bacteria' (YIB) (Species Unknown)	Rice, Wheat, Corn, Millet, Sweet Potato, Cotton, Beet, Rapeseed, Watermelon	Field (Total area of test crops is 3.46 million ha)	– major study conducted in China in various provinces – average yield increases of 10.0 to 22.5 % after application of YIB on crops	Mei et al. 1990

Okon 1996; Lalande et al. 2002; Paredes-Cardona 1988). While there may be a threshold number of bacteria that should be inoculated on a given plant, excessively large numbers of bacteria could be detrimental to the germination and growth of seeds or plants (Chanway 1997). However, growth promotion effects can still occur even with lower bacterial populations (Jacoud et al. 1998). It has been shown under controlled experimental conditions, that initial bacterial binding to seed, not necessarily the roots after germination, is most important for enhanced plant root elongation (Hong et al. 1991).

The over-wintering ability of PGPR is fundamental when considering uses in colder climates. There is evidence that *Pseudomonas* species are able to overwinter in sufficient quantities on the roots of winter wheat (De Freitas and Germida 1990b). It has also been argued that antifreeze protein activity of many bacterial species may contribute to their survival in colder climates (Sun et al. 1995; Xu et al. 1998). On the other hand, strains of *Azospirillum* often have low survival rates in soils that are colder (Lifshitz et al. 1986).

There are a few other points of interest that relate to agricultural uses of PGPR. For example, it has been shown that some PGPR strains are able to counteract irrigation problems by reducing the negative effect of irrigation of crops with highly saline water (Hamaoui et al. 1996). This may reflect the lowering of plant ethylene levels elevated by salt stress by means of 1-amino-cyclopropane-1-carboxylate (ACC) deaminase-containing PGPR (S. Mayak, T. Tirosh, B.R. Glick, unpublished results). Also, it has been observed that PGPR numbers decline rapidly in the rhizosphere after inoculation (Jacoud et al. 1998), although their effects last throughout the growing season. Several studies show that growth promotion effects are seen early in plant development, and these subsequently translate into higher yields (Glick et al. 1997; Hoffmann-Hergarten et al. 1998; Kloepper et al. 1988; Polyanskaya et al. 2000). Evidence of late-season grain weight increases have also been reported in studies with PGPR and rice (Tran Van et al. 2000).

Free living PGPRs can be administered to crops in some formulations that are available commercially (Table 2). The majority of these products are biocontrol agents which contribute indirectly to the growth promotion of crops (Chet and Chernin 2002; Glick et al. 1999). Commercial free living PGPR inoculants provide a possible alternative to the use of pesticides and fertilizers on various crops, though they are not used widely at present (Glick et al. 1999).

A broad array of methods and materials exist for the delivery of bacteria to crops in the field. Presently the non-free living symbiotic *Rhizobium* spp. are most commonly incorporated into peat as inoculant carriers. Peat carriers, although cheap and easily used, have many disadvantages. Peat is generally used as a non-sterile medium and holds a large contaminant load. Also, peat quality can be variable and peat itself is not necessarily readily available worldwide. Heat sterilization of peat can release substances toxic to the chosen bacteria. Some peat is known to inhibit plant growth, probably a reason for some of the negative effects of inoculation by PGPR seen in Table 1. Finally, bacteria in peat formulations are vulnerable to temperature fluctuations and have a limited shelf life (Bashan 1998).

Understanding the mechanisms of plant growth promotion is important when deciding what type of bacteria to use with a plant in a given situation. For example, *Pseudomonas putida* GR12-12 contains the gene for ACC deaminase, which inhibits ethylene synthesis, ethylene being a product of stress. This mechanism is most effective on plants that are more susceptible to the effects of ethylene, such as dicotyledonous plants (Hall et al. 1996) especially under such stress conditions as flooding (Grichko and Glick, 2001) drought (S. Mayak, T. Tirosh, B.R. Glick, unpublished results) and phytopathogens (Wang et al. 2000).

More recent knowledge of indirect mechanisms of plant growth promotion by soil bacteria may aid the agricultural production of certain legume crops. The hydrogen gas that is produced as a by-product of nitrogen fixation by rhizobia within legume nodules may be recaptured and recycled by those rhizobial strains that contain a hydrogen uptake system (Evans et al. 1987). It is clearly beneficial to the plant to obtain its nitrogen from a symbiotic diazotroph that has a hydrogen uptake system; however, this trait is not common in naturally occurring rhizobial strains (Evans et al. 1987). The lack of an endogenous hydrogen uptake system notwithstanding, many soils contain large numbers of free living microorganisms that can capture some of the hydrogen gas produced during nitrogen fixation and thereby indirectly promote the growth of the rhizobia-treated plants (Dong and Layzell 2001; McLearn and Dong 2002). In these instances, the organisms responsible for this plant growth promotion effect have not been characterized.

Table 2. Examples of commercial products using free-living plant growth promoting rhizobacteria (Chet and Chernin 2002, Glick et al. 1999)

Bacterial Content	Product	Intended Crop
Agrobacterium radiobacter	Diegall Galltrol-A Nogall Norbac 84 C	Fruit, nut, ornamental nursery stock and trees
Azospirillum brasilense	Azo-Green	Turf and forage crops
Azospirillum brasilense Cd *Azospirillum lipoferum* Br-17	Zea-Nit	Maize
Azospirillum lipoferum CRT1	AZOGREEN-m	Maize
Bacillus amyloliquefaciens GB99 *Bacillus subtilis* CB122	Quantum 4000 BioYield™	Broccoli, cabbage, cantaloupe, cauliflower, celery, cucumber, lettuce, ornamentals, peppers, tomato, and watermelon
Bacillus subtilis	Epic HiStick N/T Kodiak Rhizo-Plus Serenade Subtilex System 3	Barley, beans, cotton, legumes peanut, pea, rice, and soybean
Bulkholderia cepacia	Blue Circle Deny Intercept	Alfalfa, barley, beans, clover, cotton, maize, peas, sorghum, vegetables and wheat
Pseudomonas fluorescens	BlightBan A506 Conquer Victus	Almond, apple, cherry, mushroom, peach, pear, potato, strawberry and tomato
Pseudomonas syringae	Bio-save10	Citrus and pome fruit
Streptomyces griseovirdis K61	Mycostop	Field, ornamental and vegetable crops

In other cases, free living bacteria that promote the rhizobial-legume symbiosis have been identified and characterized (e.g., Andrade et al. 1998; Marek-Kozaczuk et al. 2000; Xu et al. 1994). In these cases, the free living bacteria are thought to act by decreasing the interference in the nodulation process by other soil microorganisms.

What is currently missing from the research of PGPR in agriculture is a lack of comparative studies between crop types and different species or strains of rhizobacteria. For example, when *Pseudomonas putida* GR12-2 is inoculated on various crops, there are dissimilarities in the plant stimulation between monocot and dicot plants (Hall et al. 1996). There are also significant differences in yield between summer versus winter crops following inoculation with *Azospirillum brasilense* Cd (Okon et al. 1988). Nevertheless, as noted by Okon et al. (1994), the positive effects of PGPR shown for several rhizobacterial types on many economically important crops is a valid phenomenon, and these results can act as a basis for the effective utilization of PGPR in a variety of applications.

Applications of PGPR in Forestry

Research on the use of PGPR in forestry is much less widespread than for agricultural applications. PGPR and their effect on angiosperms were the initial research focus through the 1980s, however from the 1990s to the present there has been more research of PGPR on gymnosperms (Chanway 1997). A wider scope of studies of both of these tree types and PGPR could benefit the commercial forestry sector, as well as reforestation efforts worldwide. Table 3 summarizes many of the studies that have been conducted with different PGPR and tree species.

There are different considerations that must be taken into account when evaluating the performance of the inoculation of PGPR on tree species, in contrast to agricultural crops. Fruit and grain yield increases are obviously not an imperative aspect of tree growth but biomass increases due to inoculation are quite important. Aspects such as seedling emergence and reduction in seedling transplant injury during the transfer from nursery to field are also significant (Shishido and Chanway 2000). While some tree types are very good at rapid and effective germination, without inoculation, many have difficulty in getting established to grow into an adult tree (Zaady and Perevoltsky 1995). Soil type may also be a major consideration when testing PGPR in a forest environment. Many forest soils are acidic, especially those of conifer forests, and some PGPR are sensitive to low pH conditions (Brown 1974).

Winter survival of PGPR is imperative, especially with trees intended for the colder regions of the world (e.g., Canada, Scandinavia, Russia) and also particularly since trees are perennial plants in contrast to most agricultural crops. Chanway et al. (2000) has shown that there is promise for many PGPR, such as strains of *Bacillus polymyxa* and *Pseudomonas fluorescens* to over-winter on the roots of field-planted trees. In that study, from one year to the next, there was a decrease of approximately two orders of magnitude in the inoculated bacterial populations; however, the benefits of inoculation were seen the next year (Chanway et al. 2000).

The medium in which a bacterial strain is prepared preceding inoculation may affect the root colonization pattern of the inoculated bacteria (Zaady et al. 1993). This study showed that malate-grown bacteria were better able to promote the growth of oak, than bacterial cells of the same strain grown in fructose-based media. It was found that malate-grown cells have a tendency to aggregate, while the fructose cells disperse through the soil substrate. Fructose-grown cells may be adequate for growth promotion of surface-rooted plants like maize, however, they were not sufficient for the growth promotion of trees such as oak with deep tap roots (Zaady et al. 1993).

Similar to the specificity observed in agricultural crops, a specific bacterial strain may promote growth only in certain tree species. (Enebak et al. 1998; Shishido and Chanway 2000). Sometimes even the tree ecovar is important. For example, while a strain of bacteria was effective at promoting growth in one type of pine species it was not effective with another pine species (Chanway 1995). Ecotypes, or trees of the same species from different regions or altitudes, also show differential responses to bacterial inoculation (Chanway 1995). For example in one case, *Hydrogenophaga pseudoflava* consistently promoted the growth of only one of two spruce ecovars (Chanway and Holl 1993). However, there are also some broad-range bacterial strains, such *Bacillus polymxa* L6, which consistently promote the growth of many pine varieties and other tree species (Chanway 1995; Holl and Chanway 1992). It is interesting to note that this strain of bacteria was originally isolated from the rhizosphere of perennial ryegrass, and not a tree species (Holl and Chanway 1992).

Table 3. Examples of free-living plant growth promoting rhizobacteria tested on various tree species.

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Agrobacterium radiobacter	Beech, Scotch pine	Greenhouse	– beech biomass increases of up to 235% – pine biomass increases of up to 15%	Leyval and Berthelin 1989
Arthrobacter citreus *Pseudomonas fluorescens* *Pseudomonas putida*	Black spruce, Jack pine, White spruce	Greenhouse	– height and biomass increases demonstrated	Beall and Tipping 1989
Arthrobacter oxydans *Pseudomonas aureofaciens*	Douglas Fir	Greenhouse and field	– height and biomass increases of up to 68% – also increased branch and root weights – some variability in response of fir ecotypes to inoculation	Chanway and Holl 1994
Arthrobacter sp.	Pine	Lab assay	– shoot length increases up to 69%	Pokojska-Burdziej et al. 1982
Azospirillum brasilense	River Oak	Greenhouse	– biomass increase of 90%	Rodriguez-Barrueco et al. 1991
Azospirillum brasilense Cd	Oak	Greenhouse	– root growth increases of up to 70% – these growth promotion observed only with cells cultured in malate and not fructose	Zaady et al. 1993, Zaady and Perevoltsky 1995
Azotobacter chroococcum	Oak, ash	Growth chamber	– biomass increases of 13 to 26%	Akhromeiko and Shestakova 1958
Azotobacter chroococcum	Quercus serrata	Potted plant experiment outdoors	– biomass increases of up to 38%	Pandey et al. 1986
Azotobacter chroococcum *Bacillus megaterium*	Eucalyptus	Potted plant experiment outdoors	– biomass increases of up to 44%	Mohammed and Prasad 1998
Bacillus licheniformis CECT 5105 *Bacillus pumilis* CECT 5106	Silver spruce	Greenhouse	– statistically significant increase in aerial plant growth – root system development not affected – increased plant nitrogen content	Probanza et al. 2002
Bacillus licheniformis Phylobacterium sp.	Mangrove	Greenhouse	– doubling of nitrogen incorporation into plant – increased leaf development	Bashan and Holguin 2002, Rojas et al. 2001

Table 3. Continued.

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Bacillus polymyxa	Douglas Fir, Lodgepole Pine, White spruce	Growth chamber and greenhouse	– for lodgepole pine, significant increases in root dry weight (1.35-fold) and the number and length of secondary roots (1.44-fold and 1.92-fold, respectively) – in pine, root growth, emergence, height and weight, root collar diameter increases present for plants grown in sterile versus non-sterile media – seedling emergence increases for white spruce	Chanway et al. 1991
Bacillus polymyxa	Western hemlock	Greenhouse	– increased seedling height and biomass up to 30%, due to plant height and weight increases of 1.19 and 1.30-fold respectively – degree of hemolock growth promotion different for biovars from different altitudes	Chanway 1995
Bacillus polymyxa *Pseudomonas fluorescens*	Loblolly pine, slash pine	Greenhouse	– significant increases in the speed of seedling emergence and total biomass – post-emergence damping off reduced in loblolly pine – two bacterial strains show reduction of biomass of both pine species – loblolly pine has increased root length with some strains	Enebak et al. 1998
Bacillus polymyxa *Pseudomonas fluorescens*	Hybrid spruce	Field	– increase of spruce seedling dry weight up to 57% above uninoculated spruce plants at five of nine test sites – all test strains increase dry weight of spruce at four of the nine sites – some plant growth inhibition detected due to inoculation at some sites	Chanway et al. 2000
Bacillus polymyxa *Staphylococcus hominis*	Hybrid spruce	Greenhouse (using field soil)	– significant growth increase up to 59%	O'Neill et al. 1992
Bacillus polymyxa L6	Lodgepole Pine	Greenhouse	– some seedlings co-inoculated with mycorrhizae – no effect with *Bacillus* alone – shoot and root biomass increases	Chanway and Holl 1991
Bacillus polymyxa L6	Lodgepole Pine	Growth chamber	– statistically significant increases in seedling biomass at 6 weeks of plant growth – mean shoot and root weights increased up to 35%	Holl and Chanway 1992

Table 3. Continued.

Bacteria	Plant	Conditions	Results of addition of bacteria to plant	Reference
Bacillus subtilis *Pseudomonas* spp.	Hybrid spruce	Greenhouse plants outplanted to field	– significant increases of plant biomass by inoculation with *Pseudomonas* strain of 10 to 234% at all sites with typical increases of 28 to 70% – reduction in seedling shoot injury after transplant – *Bacillus* strain ineffective	Shishido and Chanway 2000
Hydrogenophaga pseudoflava *Pseudomonas putida*	Hybrid spruce	Field	– *H. pseudoflava* increased seedling biomass and root branch numbers up to 49% in two spruce ecotypes tested – one ecotype shows root growth promotion – *P. putida* increases seedling biomass in two trials and has inhibitory effect in other trials	Chanway and Holl 1993
Pseudomonas spp.	Apple	Greenhouse and field	– growth increases of seedlings up to 65% and of root-stocks 179% – some biocontrol seen against pathogenic fungi	Caesar and Burr 1987

Sterility of the bacterial inoculant carrier can have an impact on the amount of plant growth promotion. Chanway et al. (1991) demonstrated that pine trees inoculated with PGPR in sterilized peat-vermiculite carrier material promoted growth to a greater extent compared to when they were inoculated with PGPR in non-sterilized material (Chanway et al. 1991). Also, many researchers tend to inoculate seedlings or older plants. This is in contrast to the agricultural use of PGPR, where there is a tendency to inoculate seeds, or the substrate surrounding the seed. This may have to do with differential practices in these two disciplines, since many trees in the forestry industry have their beginnings in a nursery and are long lived, while crop plants are usually started in the field and are relatively short lived.

There are currently very few research groups working in the area of forestry PGPR research. As a consequence, there is currently no field data for deciduous trees and still comparatively little field data for coniferous trees (Chanway 1997).

Applications of PGPR for Environmental Remediation

An extension of PGPR technology is the emerging use of the bacteria with plants for environmental applications. Recent studies in this area include many different uses: for growth promotion of soil stabilizing plants (Bashan et al. 1999); to counteract flooding stress of plants (Grichko and Glick 2001); to aid plant growth in acidic conditions (Belimov et al. 1998a); to counter high temperature stress (Bensalim et al. 1998); and the use of PGPR in phytoremediation technologies (Burd et al. 1998; Burd et al. 2000; Huang et al. 2000; Huang et al. 2003a, b). Besides environmental uses, some of the outcomes of these studies may also have an impact on agricultural or forestry applications.

Phytoremediation is the use of plants to extract, degrade or stabilize hazardous substances present in the environment. (Cunningham and Berti 1993; Cunningham et al. 1995; Cunningham and Ow 1996). Plants used for phytoremediation should be able to accumulate high amounts of the contaminant, and also be able to produce a large biomass. However, very often plants can be compromised by growing on contaminated sites due to the inherent toxicity, so adding PGPR can aid plant growth (Burd et al. 2000). Clearly, when plants used for phytoremediation are

able to grow well, the site detoxification will be greatly enhanced.

Table 4 shows examples of research that has been conducted using free-living rhizosphere bacteria with plants and contaminants, to study their potential for use in phytoremediation technologies. The bacteria listed in this Table include both bacteria with established plant growth promoting properties as well as bacteria with previously unknown credentials as plant growth promoters. Table 4 includes examples of rhizobacteria which are beneficial to plants by mobilization of soil contaminants as well as those which promote plant growth. There also exist rhizosphere bacteria that degrade contaminants but are not necessarily PGPR. In this case, plant roots serve only as a site for contaminant breakdown by the rhizobacteria (Anderson et al. 1993) and these examples have not been included in Table 4.

The plant properties that are improved by PGPR during phytoremediation include biomass, contaminant uptake, and plant nutrition and health. Grain yield was measured by Belimov et al. (1998b) as an indication of plant health and growth, however this attribute is obviously not important in plants used for phytoremediation.

Some plants like barley, tomato, canola (*Brassica campestris)* and Indian mustard (*Brassica juncea*) do not accumulate more contaminants (namely metal) per gram of plant material with the addition of PGPR, even though the total biomass increases (Belimov et al. 1998c; Burd et al. 2002; Burd et al. 1998; Nie et al. 2002). However, Hoflich and Metz (1997) and Whiting et al. (2001) have shown that bacterial inoculation of maize and *Thlaspi caerulescens* increases the uptake of heavy metals by these plants. In addition, de Souza et al. (1999) found increased selenium accumulation by *Brassica juncea* after inoculation. Upon closer examination, it appears that the very low level of contaminants used in some studies have probably influenced the amount of uptake so that increased contaminant accumulation in the presence of PGPR occurs only at low contaminant concentrations and not at the higher levels that inhibit plant growth.

When choosing a PGPR to increase metal uptake by plants, it is important to ensure that the bacteria used do not cause a reduction in metal uptake. With barley, the addition of *Azospirillum lipoferum* 137 significantly reduced the amount of radiolabelled cesium uptake per gram of plant dry weight (Belimov et al. 1998c).

Survival and PGPR success is essential for use in phytoremediation. It has been shown that high contaminant levels can have inhibitory effects on the growth of PGPR. For example, *Enterobacter cloacae* CAL2 growth was inhibited by 50% in 20 mM arsenate contaminated soils, but was only inhibited by 2% in 2 mM arsenate contamination (Nie et al. 2002). Also, some bacteria are sensitive to one contaminant, but not another. In a study by Belimov et al. (1998b), it was shown that *Flavobacterium* sp. is very sensitive to cadmium, but not to lead. In practice, it is essential that the bacteria used are at least somewhat resistant to the levels of contaminants endogenous to the environment to be cleaned up. Thus, preliminary selection of resistant strains, as performed by Burd et al. (1998) for nickel on *Kluyvera ascorbata,* is imperative for the practical use of these organisms.

Pairing of PGPR with transgenic plants may be a good way of increasing the efficacy of phytoremediation. In the presence of arsenate, fresh root and shoot weights of canola plants were greatly increased due to the concerted action of the PGPR *Enterobacter cloacae* CAL2 and the canola plants which express the stress tolerance gene ACC deaminase (Nie et al. 2002).

Plant exudates are essential for the association of bacteria with the rhizosphere of the plant as shown by Belimov and Dietz (2000) who demonstrated that the addition of an alternate carbon source to the soil caused an abolition of plant growth promotion in a contaminated site.

PGPRs in association with plants have an important role in the phytoremediation of soils but the research in this area has been limited. As seen in Table 4, there have been no field studies of this work and only controlled studies in greenhouses and/or growth chambers have been conducted. Also, only rudimentary inoculation procedures have been used. In addition, only a few known PGPR and plants types have been tested. Despite the lack of extensive data, PGPR inoculation technology has a great deal of potential in the area of phytoremediation.

Conclusions

PGPR present an alternative to the use of chemicals for plant growth enhancement in many different applications. Extensive research has demonstrated that PGPRs could have an important role in agriculture and horticulture in improving crop productivity. In

Table 4. Examples of free-living plant growth promoting rhizobacteria tested for phytoremediation technologies.

Bacteria	Plant	Contaminant	Conditions	Results of pairing of bacteria and plant	Reference
Agrobacterium radiobacter 10 *Arthrobacter mysorens* 7 *Azospirillum lipoferum* 137 *Flavobacterium* sp. L30	Barley	Cadmium, Lead	Pot experiments in greenhouse	– *Flavobacterium* sp. L30 very negatively sensitive to cadmium – *Flavobacterium* sp. L30 and *A.mysorens* 7 give increases of grain yield – enhanced lead accumulation by plants inoculated with *A. radiobacter* 10 and *A. mysorens* 7 – significant growth improvements seen in plants inoculated by all strains at higher cadmium concentrations	Belimov et al. 1998b
Agrobacterium radiobacter 10 *Arthrobacter mysorens* 7 *Azospirillum lipoferum* 137 *Flavobacterium* sp. L30	Barley	^{134}Cesium	Pot experiments in greenhouse	– *Flavobacterium* sp. L30 increases ^{134}Cs uptake by barley, but not significantly, due to increased plant biomass – *A. lipoferum* 137 significantly decreases the total accumulation of ^{134}Cs	Belimov et al. 1998c
Agrobacterium radiobacter 10 *Arthrobacter mysorens* 7 *Azospirillum lipoferum* 137 *Flavobacterium* sp. L30	Barley	Cadmium	Pot experiments in growth chamber	– increased absorption of essential nutrients from contaminated growth medium – slight stimulation of root length and biomass in contaminated growth medium – *A. lipoferum* 137 increased concentration of cadmium in roots, but no change in cadmium uptake by plants inoculated with other strains	Belimov and Dietz 2000
Agrobacterium sp. *Pseudomonas* sp. *Stenotrophomas* sp.	Maize, Rye, Pea, Lupin	Cadmium, Copper, Lead, Nickel, Zinc, Chromium	Pot experiments in growth chamber	– bacteria stimulates growth of maize and increases metal uptake by maize; this effect more pronounced on more weakly polluted soils compared to heavily-polluted soils – growth and metal uptake of lupin, pea and rye not affected by bacteria addition	Hoflich and Metz 1997
Azospirillum brasilense Cd *Enterobacter cloacae* CAL 2 *Pseudomonas putida* UW3	Tall fescue	Polycyclic aromatic hydrocarbons (PAHs)	Pot experiments in growth chamber	– accelerated and more complete PAH removal from the soil with inoculation – effectiveness of PAH removal further enhanced in combination with the use of land-farmed soil and inoculation with PAH-degrading bacteria	Huang et al. 2003a, In press

Table 4. Continued.

Bacteria	Plant	Contaminant	Conditions	Results of pairing of bacteria and plant	Reference
Azospirillum brasilense Cd *Enterobacter cloacae* CAL2 *Pseudomonas putida* UW3	Kentucky bluegrass, Tall fescue, Wild rye	PAHs	Pot experiments in growth chamber	– increased PAH removal from soil – germination of all three plant types increased dramatically in PAH-spikrd soil with inoculation – root biomass significantly increased in all plant types	Huang et al. 2003b, In press
Enterobacter cancerogenes Microbacterium saperdae Pseudomonas monteilii	*Thlaspi caerulescens* *Thlaspi arvense*	Zinc	Pot experiments in growth chamber	– T. *caerulescens* has two-fold increase of zinc concentration in roots after inoculation and four fold increase of zinc accumulation in shoots – *T. caerculescens* has higher shoot biomass with inoculation – *T. arvense* has no increased growth or metal accumulation with inoculation	Whiting et al. 2001
Enterobacter cloacae CAL2	Canola	Arsenate	Pot experiments in growth chamber	– slight inhibitory effect of CAL2 on germination of canola in presence of arsenate – partnered with transgenic plants, bacteria induced significantly higher root and shoot weights in plants – no increase of arsenate concentration by roots of plants	Nie et al. 2002
Kluyvera ascorbata SUD165	Canola, Tomato	Nickel	Pot experiments in growth chamber	– for tomato and canola, both roots and shoots protected from toxicity with inoculation – significant decease in ethylene production by plants – no increase or change in nickel uptake in plant material with inoculation	Burd et al. 1998
Kluyvera ascorbata SUD165/26, SUD165	Indian mustard, Canola, Tomato,	Nickel, Lead, Zinc	Pot experiments in growth chamber	– both strains decrease some plant growth inhibition by the metals, but not always significantly – SUD165/26 decreases plant growth inhibition best – no increase of metal uptake with either strain over noninoculated plants	Burd et al. 2000

addition, these organisms are also useful in forestry and environmental restoration purposes, though research in these areas is minimal. PGPR have been shown to cause very real and positive effects when matched correctly to the right plant and the right environmental situation.

What is needed for the future is a clear definition of what bacterial traits are useful and necessary for different environmental conditions and plants, so that optimal bacterial strains can either be selected or constructed. Also, it would be very useful to have a better understanding of how different bacterial strains work together for the synergistic promotion of plant growth. Additional studies need to be conducted on the effectiveness of different and novel inoculant delivery systems such as alginate encapsulation. In addition, a better understanding of the factors that facilitate the environmental persistence of the PGPR strains would be very useful. Finally inoculant strains should be labelled (e.g., with *lux* of *gfp* genes), so they can be readily detected in the environment after their release.

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