PLANT GROWTH-PROMOTING BACTERIA SUITABLE FOR THE PHYTOSTABILIZATION OF MINE TAILINGS

by

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ABSTRACT

Mining activities and their resulting wastes, mine tailings, have created a sizable problem globally. Semiarid lands have been particularly impacted due to intense mining activities in these areas. Growing concerns regarding human health risks and environmental consequences associated with these tailings has created a need for efficient and effective remediation strategies. Phytostabilization, the establishment of a vegetative cover on mine tailings to reduce erosion and dispersion of material, is emerging as a cost-effective remediation technology. However, due to elevated levels of metal contaminants, acidic pH values and poor substrate quality many tailings sites are inhospitable to plant growth. The addition of compost amendments can mitigate the toxic effects of tailings material and facilitate plant growth; however, in many instances the necessary compost amendments may be cost prohibitive. The use of specialized bacterial isolates, known as plant growth-promoting bacteria (PGPB), to enhance plant growth is a developing technology that has a broad range of applications. The use of PGPB to enhance one or more aspect of plant establishment and growth has been demonstrated to be effective in hundreds of previous studies conducted primarily under agricultural settings. To date, very few studies have utilized PGPB in attempts to enhance plant growth in mine tailings. The current study is an investigation into the potential for utilizing PGPB to enhance plant growth during the phytostabilization of semiarid mine tailings. During this investigation a large collection of bacterial isolates was screened for common plant growth-promoting mechanisms such as siderophore and indole-3-acetic acid production, phosphate solubilization and ACC-deaminase activity. Isolates

possessing beneficial qualities were utilized in a series of greenhouse screening studies to evaluate their abilities to enhance the growth of native desert plants in various tailings materials. A number of isolates tested have demonstrated the ability to enhance plant growth in composted and non-composted tailings material. Optimization of this technology has now indicated that alginate-encapsulated inoculation of target plants is a beneficial and practical technology.

CHAPTER 1

INTRODUCTION

ENVIRONMENTAL APPLICATIONS OF PLANT GROWTH-PROMOTING BACTERIA

Outline

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Abstract

Plant growth-promoting bacteria (PGPB), have been widely used to enhance plant growth in agricultural settings. This review focuses on an emerging area of PGPB application, restoration and remediation of the environment. The first part of the review describes the successful use of PGPB to address a variety of environmental issues including; the phytoextraction and phytostabilization of metal contaminated substrates, phytodegradation of organic pollutants, revegetation of desertified lands, restoration of mangrove ecosystems, and enhancing wastewater treatment. The second part of the review discusses research needs in the area of environmental applications highlighting the need for information regarding plant-microbe-substrate interactions that occur during these phytoremediation efforts. Examples include; understanding the specific interactions between PGPB and plants, developing studies that more accurately mimic intended field applications, understanding PGPB behavior under field applications, and designing long-term studies that can translate from the laboratory- to the field-scale.

1.0 Background

Anthropogenic release of organic and inorganic pollutants as well as exploitation and development of pristine lands have resulted in the decline of ecosystem health worldwide. As the incidence and severity of contaminated and desertified sites continues to rise, demands for cost-effective and efficient remediation strategies have increased (Berti and Cunningham, 2000). Recently, phytoremediation, an emerging technology that uses plants to extract, mitigate, or stabilize both organic and inorganic contaminants, has been explored as a solution to remediate and/or restore contaminated or damaged ecosystems (McCutcheon and Schnoor, 2003; Cunningham et al., 1995). Phytoremediation is an attractive alternative to other remediation strategies as it can be considerably less expensive, can be minimally invasive to contaminated substrates, places an aesthetically pleasing vegetative cover on contaminated or desertified sites, and promotes the development of healthy soils and microbial communities, which are vital to ecosystem function (Cunningham et al., 1995; Mendez and Maier 2008a; 2008b; Mendez et al., 2008).

In some cases phytoremediation applications are limited due to the inability of plants to survive or perform desired functions under unfavorable conditions including; low nutrient availability, poor soil structure, severely impacted heterotrophic microbial communities, extreme pH values, and high contaminant concentrations. There is increasing evidence that these conditions can be mitigated to some extent by application of plant growth promoting microorganisms. Plant growth-promoting bacteria (PGPB; Bashan and Holguin 1998) have been used since the mid 1970s to directly or indirectly

enhance plant growth in agricultural applications (Döbereiner et al. 1976; Kloepper et al., 1980; 1987). This success has inspired research to investigate whether PGPB can be used to overcome limitations to plant growth in remediation or restoration applications (Bashan, 1998; Bashan and de-Bashan 2005 Bashan et al., 1999; Bashan and Holguin, 2002; Grandlic et al., 2008; Lebeau et al., 2007).

The objectives of the current review are to (i) summarize recently developed approaches using PGPB for environmental applications including; phytoremediation of organic contaminants, phytoextraction of heavy metals, phytostabilization of eroded lands, re-vegetation of desertified and contaminated sites, re-forestation of mangrove forests, and enhancement of tertiary wastewater treatment, (ii) reveal aspects of these studies that warrant further research and improvement and, to (iii) identify potential future directions of this rapidly expanding field.

2.0 Plant Growth-Promoting Mechanisms

PGPB mechanisms are categorized as traditional (direct and non-direct) and non-traditional. Traditional mechanisms have been characterized and investigated for use in agricultural applications and include; the production of siderophores and phytohormones such as IAA, gibberellins and nitrous oxide, phosphate solubilization, ACC deaminase activity, nitrogen fixation, enhanced water and mineral uptake and biocontrol of numerous plant pathogens ranging from fungi, bacteria, virus and nematodes (Bashan and de-Bashan, 2005; Compant et al., 2005; Glick and Bashan 1997). Figure 1 depicts some well-characterized plant growth-promoting (PGP) mechanisms as they are thought to

occur in the rhizosphere. Non-traditional mechanisms are less well-understood, yet may prove to be important during environmental applications. Such mechanisms include increasing plant tolerance to acidic environments, organic or metal contaminants, drought conditions, and salinity. While such mechanisms have been observed for agricultural applications; their importance increases for environmental applications.

Traditional PGPB effects on plant growth involve the ability to provide plants with nutrients or growth factors which are generally growth-limiting under typical conditions. Growth factors include the bacterial production of cytokinins or phytohormones such as indole-3-acetic acid (IAA) which enhances cell division and root elongation (Garcia de Salamone et al., 2001; Ryu et al., 2003). IAA production has been established as an important plant growth-promoting (PGP) mechanism where an insertional mutant *Pseudomonas putida* isolate lacking the *ipdc* gene required for IAA biosynthesis lost much of its ability to enhance plant root elongation (Patten and Glick, 2002) and similarly in inoculation with *Azospirillum* species (Spaepen et al. 2007). Several PGPB have also demonstrated an ability to immobilize heavy metals in nutrient media and soils; a mechanism which could decrease levels of plant bioavailable heavy metals (Belimov et al., 1998; Glick 2003, 2004; Pishchik et al., 2002). The ability of many PGPB to aid the plant in nutrient acquisition may be especially beneficial when attempting to cultivate plants under nutrient-limiting conditions. Essential nutrients provided by bacterial isolates include nitrogen, phosphorous, and iron (Vazquez et al., 2000). Diazotrophic bacteria associated with mangrove plant rhizospheres have the ability to fix atmospheric nitrogen into ammonium that is in turn assimilated by the plant

(Bashan et al., 1998; Toledo et al., 1995). In addition, rhizobial species form synergistic relations with legumes in the form of nitrogen-fixing nodules on plant roots. PGPB can also aid in iron uptake through microbially-produced siderophores which are manufactured under iron-limiting conditions and can be subsequently taken up by plants (Bar-Ness et al., 1991; 1992). This mechanism is especially beneficial when establishing plants in alkaline soils where iron typically has extremely low bioavailability (Jing et al., 2007). PGPB can also provide the plant with phosphorous and other micronutrients such as K, Mn, and Zn through the production of organic acids (Bashan et al. 1990; Rodriguez et al., 2006; Saravanan et al., 2007).

A distinct PGP mechanism that has recently gained attention is known as ACC-deaminase activity (Glick et al., 1998; Arshad et al., 2007). Plants can induce the production of ethylene when residing under stressful conditions including high concentrations of heavy metals, the presence of salt stress and flooding (Burd et al., 1998; Grichko and Glick, 2001; Mayak et al., 2004). The compound ACC (1-aminocyclopropane-1-carboxylic acid) is a precursor to ethylene and is internally cleaved by the plant to produce ethylene which accumulates and retards or halts plant growth (Glick et al., 1998). Bacterial isolates with ACC-deaminase activities compete with plant enzymes by cleaving exogenous ACC into an α-ketobutyrate and ammonium molecule serving as a bacterial nitrogen source and thereby reducing ethylene concentrations in plant tissues. Some bacterial isolates have also been reported to utilize ACC as a carbon source giving them an additional edge in sequestering plant ethylene concentrations (Belimov et al., 2005). Belimov et al. (2005) reported a positive correlation between

ACC-deaminase activity and an isolates ability to enhance root elongation in the commonly used metal-accumulating Indian mustard (*Brassica juncea* L. Czern.). This PGP mechanism is proving to especially valuable under environmental applications of PGPB where it is thought to ameliorate the effects of inhospitable environmental conditions such as heavymetal contaminants and other environmental stresses (Arshad et al., 2007; Grichko and Glick; Belimov et al., 2005).

Non-traditional PGP mechanisms are still not fully understood, but may have broad applications during remediation efforts. For example; tolerance to salt and drought stress has been studied under agricultural conditions (Abd El-Samad et al, 2004; Creus et al., 2004; Mayak et al., 2004); however, these roles could also have valuable benefits during phytoremediation projects executed in arid and semiarid environments. The mitigation of flooding stress could also find uses in remediation applications of areas that receive frequent and heavy rain fall (Grichko and Glick, 2001). Bacterial strains that mitigate heavy metal toxicity may also have obvious roles during phytoremediation projects at heavy metal-contaminated sites such as mine tailings (Belimov et al., 2001; Burd et al., 1998; 2000). Some PGPB may also play roles in enhancing plant tolerance to acidic substrates during remediation efforts (Belimov et al., 1998). Screening procedures are occasionally used to identify new strains of potentially useful PGPB from large collections of bacterial isolates (Figure 2) (Cattelan et al., 1999; Grandlic et al., 2008). When utilized properly, such a screening can produce a set of beneficial isolates suitable for specific environmental applications.

Screening procedures typically begin with large collections of bacterial isolates (Figure 2). Careful planning should be placed into determining appropriate locations and the media used for obtaining isolates from environmental samples. A number of options regarding obtaining isolates are available including; plant tissue samples, rhizosphere samples, and soil samples, previously published PGPB, natural non-contaminated environments, and anthropogenically-impacted environments. The media used for isolation may ultimately play a large role in the taxonomy of the isolates collected as high-nutrient media tend to facilitate the growth of easily culturable, fast-growing strains and may occlude fastidious slow-growing isolates. After obtaining a collection of isolates, each individual is screened for various common plant growth-promoting mechanisms such as IAA and siderophore production, phosphate solubilization, and ACC-deaminase activity. Isolates can also be screened for tolerance to certain environmental factors including elevated levels of metal or organic pollutants, saline conditions, and extreme pH ranges. Isolates that display beneficial mechanisms at this point as selected based on defined criteria specific to each project and are typically utilized in a series of screening assays. A handful of PGPB that consistently and significantly enhance plant growth in screening assays are then finally chosen for optimization and eventual field-scale trials. Optimization may include investigating optimal inoculation methods and dosages.

3.0 Environmental Applications of PGPB

Revegetation is an economical approach to reduction of risks generated by contaminated and desertified sites. Such risks include the generation of particulate matter (PM_{10} and $PM_{2.5}$) which may be associated with allergens or site contaminants. Further, a vegetative cap reduces water erosion which is a major cause of the loss of top soil from desertified sites and contaminant spread from organic- or metal-containing sites. Finally, a vegetative cap can increase evapotranspiration within the site effectively preventing leaching of contaminants into the subsurface and groundwater supplies.

Revegetation of contaminated and desertified sites has common challenges. These sites suffer from various combinations of characteristics that make plant growth difficult. These can include: lack of soil structure, low nutrient content (carbon, nitrogen, phosphorus), extremes of pH, drought stress, salinity, elevated levels of organic or metal contaminants, and severely impacted neutrophilic heterotrophic microbial communities (Velázquez-Rodríguez et al. 2001; Mendez and Maier, 2007; 2008). Many of these limitations can be overcome with organic amendments such as compost or biosolids, topsoil, lime, or fertilizer. Such amendments are often one of the most costly components of the remediation process. Interest in PGPB arises because they have the potential to reduce or replace the need for traditional soil amendments while improving plant tolerance to drought stress and high salinity.

3.1 Phytodegradation

Phytodegradation, or rhizoremediation, is a remediation strategy that utilizes rhizosphere bacteria to degrade recalcitrant organic compounds. This effect can be manifested through shear numbers - the rhizosphere hosts bacterial populations that are several orders of magnitude higher than in the surrounding bulk soil which can result in more rapid removal of target contaminant compounds. In addition, some heterotrophic rhizosphere inhabitants are biochemically adapted to organic exudates from plant roots that may have structural similarities to organic contaminants of concern, e.g., aromatics, (Donnelly et al., 1994; Macková et al., 2007).

Several approaches to the use of PGPB in phytodegradation have been explored. For example, Singh et al. (2002) suggested PGPB could be used to enhance phytodegradation by stimulating the plant to produce phenolic compounds resulting in acclimation of the rhizosphere community and increased degradation of aromatic organic compounds. Huang et al. (2004) employed a multi-step approach to remediate a soil artificially contaminated with creosote. In the study, both polyaromatic hydrocarbon-(PAH) degrading bacteria and PGPB (*Pseudomonas putida*, *Azospirillum brasilense*, and *Enterobacter cloacae*) were added. PGPB used were thought to increase plant tolerance to elevated levels of organic and metal contaminants. Removal of PAH was more rapid in soils inoculated with PGPB than the uninoculated control. In addition, the authors observed an improved removal of larger PAH and attributed these results to the ability of the added PGPB to relieve plant stress via ACC-deaminase activity. A phytodegradative PGPB was genetically engineered to aid in removal of 2-chlorobenzoic acid and oil

contaminated soils (Radwan et al., 2005; Siciliano and Germida et al., 1997). In this case, a plasmid encoding toluene-degrading genes was transferred from *Burkholderia cepacia* to an endophytic PGPB strain of this species. The PGPB not only protected its host against toluene phytotoxicity but also lowered the amount of toluene that escaped to the atmosphere by evaporation (Barac et al. 2004; Glick 2004). The use of phytodegradation technologies has several advantages opposed to more invasive chemical or physical remediation methods to remove recalcitrant organics is beneficial for a number of reasons including; the preservation of soil structure and microbial communities, energy is derived from sunlight, potentially lower application costs, and it is more aesthetically attractive (Huang et al., 2004).

3.2 Phytoextraction of Metals

Phytoextraction is the use of hyperaccumulating plants to translocate metal pollutants from contaminated soil or water into above-ground shoot tissues (Cunningham et al. 1995; Pilon-Smits 2005). Accumulated metals in plant tissues can be disposed of or the metals can be recovered. About 400 plant species comprising 11 families are known hyperaccumulators and are widely used in phytoextraction studies. The majority of identified hyperacccumulators belong to the Brassicaceae family (Kumar et al., 1995; Gratão et al. 2005; Prasad and Freitas 2003).

The major drawback of hyperaccumulators is that many grow slowly, particularly in heavily contaminated soils, so that the phytoextraction process takes a long time. The benefits of PGPB application in phytoextraction come from: (i) enhanced mobility or

bioavailability of contaminants resulting in increased plant metal accumulation and (ii) direct enhancement of plant biomass production (Lebeau et al., 2007). Some bacterial species may be multi-functional in the phytoextraction process as they are thought to both enhance plant growth and metal solubilization (Rajkumar and Freitas, 2008; Saravanan et al., 2007; Sheng and Xia, 2006).

Examples of PGPB that aid in plant growth in the presence of metals include a *Variovorax* sp. with the ability to enhance root elongation in the presence of Cd (Belimov et al., 2005). Similar results have been reported using native rhizosphere bacteria to enhance arsenic accumulation in ferns (Jankong et al., 2007). Reichman (2007) revealed that inoculation with a plant growth-promoting rhizobium significantly increased dry plant biomass in arsenic-contaminated soils. This inoculation did not increase nitrogen uptake or decrease arsenic accumulation leading the authors to conclude that growth promotion was due to bacterial production of plant hormones. Finally, Wu et al., (2006a) used a bacterial consortium (*Azotobacter chroococcum*, *Bacillus megaterium*, and *B. mucilaginosus*) to enhance plant growth during the phytoextraction of Pb and Zn in a mine tailing. Although this inoculation did not significantly increase metal uptake into plant shoots, it did significantly affect plant biomass at certain low compost rates. The inoculation was less effective at higher compost rates.

A number of studies have reported significant increases in metal accumulation upon inoculation with bacterial isolates (Hoflich and Metz, 1997; Rajkumar and Freitas, 2008; Reed and Glick, 2005; Whiting et al., 2001). Although plant biomass may not be directly enhanced in these studies, the introduced strains of bacteria may improve overall

plant nutrition. de Souza et al. (1999a; 1999b) investigated the potential of utilizing phytoextraction of Se and Hg in an artificially constructed wetland. The authors reported that rhizobacteria had the ability to significantly increase volatilization and metal accumulation in plant tissues. It was later suggested that the reported increases in metal solubilization and subsequent plant uptake may be due to organic acid production by some PGPB (Saravanan et al., 2007).

3.3 Phytostabilization of Metal-Contaminated Sites

In some cases it is not practical or economical to remove contaminants from a site using phytoextraction or other technologies. Phytostabilization is an emerging alternative strategy that involves the establishment of a vegetative cap on a contaminated site. In contrast to phytoextraction, shoot accumulation of metals is not desirable for phytostabilization since the vegetative cap is considered permanent and exposure of surrounding wildlife and humans should be minimized. Several recent studies have demonstrated the benefits of employing PGPB to enhance plant growth during phytostabilization of metal-contaminated substrates (Grandlic et al., 2008; Petrisor et al., 2004; Vivas et al., 2006). This work suggests that PGPB can significantly increase a variety of quantifiable plant properties such as biomass, root and shoot length, and overall plant nutrition in the presence of heavy metals, but the mechanisms are not fully understood.

As for many other effective environmental applications of PGPB, the beneficial results observed in several phytostabilization studies have been attributed, at least in part,

to ACC-deaminase activities (Belimov et al., 2001; Burd et al., 1998; Reed et al., 2005). For example, rhizobacteria with ACC-deaminase activity stimulated root elongation and overall plant growth in the presence of cadmium-supplemented soils, yet did not result in increased metal uptake into shoot tissues (Belimov et al., 2001). Some authors have also suggested that successful PGPB have the ability to mitigate the toxicity of heavy metals and or reduce metal uptake into plant tissues (Belimov et al., 2004; Burd et al., 1998; Vivas et al., 2006). A number of these studies have shown reduced plant sensitivity to elevated levels of metal contaminants such as Cd, Cu and Ni along with increased plant growth (Burd et al., 1998; Reed et al., 2005). Vivas et al. (2006) reported an isolate from the genus *Brevibacillus* had the ability to enhance plant growth in the presence of increasing zinc concentrations. This isolate decreased zinc uptake into plant tissues, increased nitrogen and phosphorous content in plant tissues, and encouraged mycorrhizal colonization. The ability of the PGPB *Enterobacter cloacae* to facilitate the growth of both non-transformed and ACC deaminase-expressing canola plants was tested in the presence of arsenate. In both the presence and absence of the added PGPB, transgenic canola plants grew to a significantly greater extent than non-transformed canola plants (Nie et al.2002).

Although many bacterial species show promise for phytostabilization of metal contaminated sites, some isolates may alter the speciation and mobility of specific metal contaminants. Such results were observed in the study by Wu et al. (2006) where PGPB had a small effect on water and DTPA-extractable Cd, Cu, Pb and Zn, but significantly increased Cd uptake into shoot tissues. Petrisor et al. (2004) also reported an increase in

Cu, Cd, Mn and Zn concentrations in shoot tissues following PGPB inoculation. Reed and Glick (2005) noted that increases in shoot metal concentrations after inoculation are more likely to occur at low substrate metal concentrations that do not inhibit plant growth.

PGPB have recently been applied to enhance the phytostabilization of mine tailings. These substrates are challenging as they often contain elevated levels of heavy metals, lack structure and nutrients, can have extremely acidic pH ranges, and have severely impacted microbial communities (Mendez et al., 2007). Petrisor et al. (2004) used a mixed inoculation consisting of *Azotobacter chroococcum* and *Bacillus megaterium* in place of a chemical fertilizer to enhance the growth of native plant species in phosphogypsum (pH 4.5) and sulfidic (pH 2.0) tailings. Plant growth and microbial activity substantially increased after six months with repeated inoculations. Grandlic et al. (2008) inoculated seeds prior to planting and showed that PGPB significantly increased plant biomass in native desert plants grown in composted and non-composted sulfidic mine tailings after. The PGPB used in this study were obtained by screening a large collection of rhizosphere isolates for various PGP mechanisms and their abilities to survive acidic pH ranges and elevated heavy metal concentrations. The effectiveness of PGPB in this study varied with plant species and compost level as inoculations were effective in *Atriplex lentiformis* plants at 10% compost (w/w) and in non-composted *Buchloe dactyloides* plants demonstrating that the effectiveness of PGPB may vary with amendments and individual plant species. Finally, Rao and Tak (2001) inoculated surface-sterilized seeds with the PBPB *Bradyrhizobium* sp. and demonstrated

significantly increased nodulation, nitrogenase activity, plant height, root and shoot dry weight and nitrogen content of *Albizia lebbeck* trees grown in gypsum and limestone mine spoils. This inoculation also enhanced the metabolic activities in the spoils (as measured by dehydrogenase, acid phosphatase, alkaline phosphatase activities) and arbuscular mycorrhizal fungi spore counts and infection rates in tree rhizospheres.

3.4 Revegetation of Desertified Sites

Desertification is an increasingly important global issue that affects agriculture, forestry and human health. This process is especially problematic in areas of the southwestern United States, northwestern Mexico, the Sahel in Africa, northeastern China and in other arid or semi-arid areas across the globe. Natural succession and revegetation in these climates occurs slowly, if at all, and establishing vegetation on desertified sites can be difficult and time-intensive. The best-studied PGPB for restoration of desertified sites is *Azospirillum brasilense* a well-known PGPB that has been extensively used in agricultural applications. *A. brasilense* has multiple PGP mechanisms including; production of IAA and nitric oxide, N_2-fixation, and several small magnitude mechanisms all working in concert to enhance plant growth (Bashan et al., 2004). For example, this PGPB has been shown to acidify the plant rhizosphere which aids in solubilizing plant nutrients such as potassium, phosphate and iron that normally have low bioavailability in alkaline desert soils (Carrillo et al., 2002). It can also enhance enzymes in the phosphogluconate pathway of mesquite trees growing in poor soils (Leyva and Bashan 2008).

Azospirillum brasilense has been successfully inoculated onto cardon (*Pachycereus pringlei*), the world's largest cactus, resulting in soil stabilization and improved plant growth characteristics. Success was strain dependent and PGPB were recoverable for at least 300 days following inoculation (Puente and Bashan, 1993). This PGPB was also used to stabilize a highly eroded urban dirt road where three different inoculated cactus species had significantly higher survival rates after 3.5 years, 76% compared to 2% for uninoculated control plants. Plants in this study were inoculated three times per year for two years and inoculation increased plant volumes, improved soil nutrient status and eliminated soil erosion in the area (Bashan et al., 1999).

Studies with *A. brasilense* suggest that the PGPB is most effective in highly impacted sites. Carrillo-Garcia et al. (2000) reported no PGPB effect on the growth of the giant columnar cactus, *Pachycereus pringlei*, in healthy soils; however, root and above ground growth of inoculated treatments increased linearly with declining soil quality enhancing dry shoot mass by 60% and root length by 100% in the poorest quality soil. Similarly, Bacilio et al. (2006) showed that compost amendment made *A. brasilense* inoculation less effective. These studies stress the importance of determining soil quality to provide a preliminary understanding of the potential for success of PGPB treatment.

Other PGPB also show potential for enhancing restoration of desertified sites. Recent research on rocky sites devoid of soil has revealed that bacterial communities residing on the root surfaces of established cacti and trees play a role in weathering rock surfaces and solubilizing minerals for the plant (Puente et al., 2004a; 2004b). Although discussion of mycorrhizae alone is outside the scope of this review, it is well-known that

these fungi help in establishment and growth of plants in stressed environments (Requena et al., 1997). There have been several studies that show PGPB enhance mycorrhizal colonization and performance in desertified sites. In this case, the PGPB are termed mycorrhizal helper bacteria (Garbeye, 1994). For example, Valdenegro et al. (2001) showed that PGPB increased growth of plants infected with the arbuscular mycorrhizal (AM) fungus *Glomus deserticola* as well as a rhizobium strain by 40% in a semi-arid soil from southeastern Spain. In this greenhouse study, combinations of AM fungi, rhizobium, and other PGPB enhanced plant growth more than single inoculants alone (Requena et al., 1997). However, the authors note that the combinations that produced optimal growth differed, i.e., different PGPB worked better with different AM fungi. This group extended this work to a five-year field trial which confirmed that a combination of a native AM fungus and a rhizobial PGPB enhanced plant growth by 100%, and increased the aggregation and organic matter and nitrogen content of the soil (Requena et al., 2001).

3.5 Mangrove Forest Revegetation

Mangrove forests are vital coastal ecosystems that are diminishing due to coastal development and a growing aquaculture industry (Rönnbäck 1999). These ecosystems provide an important environmental services acting as breeding grounds and nursery areas for marine species and waterfowl (Holguin et al. 2001) and creating critical barriers that protect coastal cities and rice fields from tropical storms and tidal waves. Mangrove ecosystems in arid and semi-arid environments are particularly sensitive to stress and are

difficult to reforest following disruption (Toldeo et al. 2001). The importance of mangrove forests as ecosystems has driven interest in their restoration.

Several PGPB have been evaluated for their impact on restoration of mangroves. The cyanobacterium *Microcoleus chthonoplastes*, which was isolated from aerial roots of mangroves, increased root colonization and nitrogen accumulation (Bashan et al., 1998; Toledo et al., 1995). This PGPB as well as several others also obtained from mangrove ecosystems, have also stimulated growth of *Salicornia bigelovii* plants, a halophytic plant that co-inhabits coastal marshes in association with mangrove forests. PGPB inoculation of *S. bigelovii* increased the length of the plant life cycle and enhanced plant biomass by 70% (Bashan et al. 2000). Kathiresan and Selyam (2006) screened a collection of 48 mangrove isolates and reported that two, *Azotobacter vinelandii* and *Bacillus megaterium* demonstrated the potential to increase plant growth by greater than 100% making them excellent candidates for reforestation projects. Finally *Bacillus licheniformis* (a phosphorus solubilizer) and *Phyllobacterium* sp. (a nitrogen fixer) were shown to enhance mangrove growth (Rojas et al., 2001). These two isolates performed better together than as single inoculants.

3.6 PGPB to Improve Wastewater Treatment

A recent development in the use of PGPB is their application to tertiary treatment of wastewater. Microalgae are commonly used during tertiary treatment to remove nitrogen and phosphorous from domestic and municipal wastewater (de-Bashan and Bashan, 2004; Tam and Wong, 2000; Valderrama et al., 2002). Recent work has

investigated the use of the PGPB Azospirillum brasilense in combination with the microalgae *Chlorella vulgaris* and *C. sorokinian*. When jointly immobilized in alginate beads, *A. brasilense* and *C. vulgaris* resulted in a 93% and 75% removal of ammonium and phosphorous, respectively, after two days compared to 53% (ammonium) and no phosphorus removal by *C. vulgaris* cells alone (de-Bashan et al., 2002a). In this study, the effect of A. brasilense on C. vulgaris included increased pigment production, lipid content, diversification of fatty acids, increased cell numbers and cell size (de-Bashan et al., 2002b; 2004). *Pseudomonas diminuta* and *P. vesicularis*, isolated from algal cultures, were also shown to stimulate growth of *Chlorella* sp. (Mouget et al., 1995). The authors speculate that the PGPBs in this case utilize oxygen produced by photosynthesis to allow photosynthesis to continue at a steady rapid rate.

The effect of starvation periods on the viability and phosphorus uptake of two different species of microalgae, *C. vulgaris* and *C. sorokiniana*, jointly immobilized with *A. brasilense* cells were recently examined (Hernandez et al., 2006). Inoculation with *A. brasilense* enhanced growth and phosphorous absorption of both species in synthetic wastewater and *C. sorokiniana* in domestic wastewater following a three-day starvation period in a saline solution. Phosphorous removal was not increased following a starvation period and a return of cells to the same wastewater; however, if cells were returned to "fresh" wastewater phosphorous removal continued. The most effective removal of phosphorous, 72%, was achieved when pre-starved co-immobilized cells were used for treatment for one cycle and replaced with a fresh pre-starved culture.

4.0 Research Needs

Despite prior success in utilizing PGPB during various remediation efforts further research is necessary to develop these technologies into viable remediation strategies. This section of the review focuses on specific areas for which there are knowledge gaps. These include understanding the PGPB colonization of roots, the effect of nutrient status on PGPB behavior and understanding mechanisms as they occur *in situ*.

There is currently a large knowledge gap regarding plant-microbe-substrate dynamics that occur during remediation efforts. Information describing these applications over long time frames and under actual field conditions is scarce. Finally, optimizing current inoculation methodology may make these technologies more efficient and user-friendly.

4.1 PGPB Colonization of Roots

Colonization of roots is a prerequisite for successful inoculation of PGPB (Bashan and de-Bashan 2005; Lucy et al. 2004). A number of factors, both biotic and abiotic, may play key roles in an isolate's ability to colonize a specific host plant. Some PGPB may display desirable growth promotion mechanisms, yet remain ineffective due to their inability to colonize and/or compete in the rhizosphere of host plants (Benizri et al., 2001). Studies reporting poor colonization have attributed these results to adverse soil conditions (Frommel et al., 1993; Dobbleare et al., 2001). Soil moisture content following inoculation may also play a role in root colonization (Burr et al., 1978; Oliveira et al., 2004). Soil temperature has been suggested as an important abiotic colonization

factor as two strains of *Cellulomonas sp.* had greater colonization abilities at 16^{o}C as opposed to 26^{o}C (Egamberdiyeva and Höflich, 2002).

Jjemba and Alexander (1999) reported that an isolate's ability to survive in large numbers in the soil was a major determinant in colonization of the rhizosphere; however, in many cases root colonization at lower densities is sufficient for exerting the desired effect as it is in the case of *Azospirillum*-cereals interaction (Bashan and Holguin 1997). Motility and growth rate have been proposed as critical factors in the effectiveness of prospective PGPB as isolates lacking motility cannot reach active areas of the plant rhizosphere and cells with slow growth rates cannot replicate fast enough to compete or keep up with growing root tips (Benizri et al., 2001; Simons et al., 1996). Rhizosphere-competent isolates can reach plant roots through flagellular-assisted motility and chemotactic responses (Compant et al., 2005). Attachment to plant roots takes place in a two-step process (i) a quick reversible adsorption and (ii) a slow irreversible anchoring to the root surface involving attraction and attachment controlled by a number of molecular factors (Bashan and Holhuin, 1993; De Troch and Vanderleyden, 1996). Synthesis of the O-antigen of LPS, amino acids, vitamin B_1, and exudation of NADH dehydrogenase have all been suggested as biotic factors that aid introduced isolates in root colonization (Dekkers et al., 1998; Simons et al., 1996; 1997)

Native microorganisms often have a better chance to successfully colonize roots. Therefore, screening potential PGPB from the rhizospheres of plants intended for use in phytoremediation projects may yield better performing PGPB (Belimov et al., 1999; Puente et al. 2004a). Normally such screenings are based on traditional PGPB

mechanisms but such screening studies may benefit from the addition of (i) motility and colonization assays and (2) site specific (e.g., ability to survive low pH) assays when searching for PGPB suitable for a specific application. In fact, screening for native bacteria has been demonstrated to be a successful strategy in previous studies (Belimov et al., 1999; Grandlic et al., 2008; Puente et al. 2004b).

4.2 PGPB Behavior and Nutrient Status

There is evidence suggesting that the effectiveness of introduced PGPB may depend on the nutrient status or environmental characteristics of the substrate used for plant growth (De Freitas and Germida, 1990; Egamberdiyeva, 2007). For example, three PGPB had a greater effect on maize growth and uptake of nitrogen, phosphorous and potassium in nutrient-deficient soil than in nutrient rich soil (Egamberdiyeva, 2007). Further, it has been shown that *Azospirillum brasilense* only promotes plant growth under nitrogen-limiting conditions (Dobbelaere et al., 2001; Bashan et al. 2004). Thus, if plants have favorable growth conditions, introduced PGPB may be ineffective or even hinder some aspects of plant growth (Belimov et al., 2002). Belimov et al. (2002) reported that ACC-deaminase-positive PGPB introduced to phosphorous-sufficient rape increased root elongation, yet had no affect or even decreased root elongation in phosphorous deficient seedlings; furthermore, enhancement of root elongation was eliminated in the presence of high ammonium concentration. The study by Carrillo-Garcia et al. (2000) also demonstrated that the PGPB, *A. brasilense*, had the greatest effect on plant growth in soils with the poorest quality, while its effect was negligible in healthier substrates. Plant

response to some PGPB treatments has also been shown to vary with soil organic matter content as treatments were more effective in low organic matter soils (Cakmakci et al., 2006). Farwell et al. (2007) found that the PGPB, *Pseudomonas putida* UW4, enhanced shoot biomass of wild-type and transgenic canola plants under low flood stress; however, under high flood stress this bacterium actually decreased plant growth compared to non-inoculated controls. These initial reports warrant further research in the area of nutrient status and PGPB effectiveness and stress the importance of identifying the nutrient status of substrates prior to conducting field-scale applications.

4.3 Optimizing PGPB Technology for Field-Scale Applications

Developing a better understanding of the specific interactions that occur between PGPB, plant hosts and their physical environments may enable the selection of specific strains necessary to achieve a desired result during a particular application. Some PGPB isolates have been shown to perform better under certain conditions (Carrillo-Garcia et al., 2000; Hernandez et al., 2006; Zaady et al., 1994). In addition, although a specific bacterium displays a set of desirable PGP mechanisms *in vitro*, its actual mechanism of growth promotion *in situ* may be unknown. Desired results may be difficult to obtain under certain conditions (Bashan, 1998). Such results were observed previously where phosphate-solubilizing isolates enhanced plant growth without increasing phosphorous concentrations in plant tissues (de Freitas et al., 1997). Inoculating seeds with the well-known diazotrophic bacterium *A. brasilense* did not result in nitrogen fixation as no nitrogenase activity was detected in treatments (Carrillo-Garcia et al., 2000). Some

PGPB contain all the required genes for phosphate solubilization, and they do it in culture, yet, they failed to do so in the field (Rodriguez et al. 2006). Understanding these specific mechanisms of plant growth promotion at molecular and micro-scales may enable the exploitation of PGPB for specific environmental applications.

4.4 Appropriate Study Design

PGPB inoculation methods must be practical and optimized for the particular application. Multi-component inoculations, single inoculums composed of multiple PGPB strains, may be more effective than inoculating with a single microorganism (Bashan and Holguin, 1997; Bashan et al., 2004; Raupach and Kloepper, 1998). The concentration of introduced bacterial inoculants may also need optimization as variable results in germination have been reported when immersing seeds in bacterial suspensions ranging from $10^4 – 10^7$ CFU mL^{-1} with maximum germination occurring at 10^6 CFU mL^{-1} (Puente and Bashan, 1993). This concentration is also favorable for growth promotion by several PGPB (Bashan et al. 2000, Puente et al. 2004b). These results using modest PGPB concentrations contrast with those of Petrisor et al. (2004) who reported substantial increases in plant growth after inoculating with 2.5×10^{10} CFU mL^{-1}, the latter are impractical under field practices. Optimal concentrations may vary with specific strains or species and further investigations may focus on optimizing concentrations and inoculation methods for specific applications. PGPB should be inoculated under field conditions only after formulation with a carrier (Bashan 1998). Most PGPB carriers are based on peat and some are liquid or synthetic. Alginate (a polymer from marine algae)

has also been shown to be an effective carrier as it can deliver a high concentration of cells using a small volume and has long-term storage capabilities (Reed and Glick, 2005). Alginate-encapsulated cells also can behave as a time-release capsule releasing small concentrations of cells over a prolonged time period (Bashan et al., 2002). Such applications may be more beneficial than introducing a larger quantity of cells during a single inoculation event. Inoculating native desert plants using alginate-encapsulated PGPB has enhanced plant biomass production in an arid-climate mine tailings (de-Bashan et al., 2008; Grandlic et al., 2008).

4.5 Understanding PGPB Mechanisms In Situ

Previous studies have produced important data on the application of PGPB under different environmental conditions; however, the vast majority of studies have been conducted under environmental conditions that do not accurately mimic the intended use of their PGPB. Screening studies involving root elongation and germination assays are commonly performed under laboratory conditions using surface-sterilized seeds germinated under sterile conditions (Bashan et al., 2000b; Belimov et al., 2002; 2005; Donte-Correa et al., 2004). While these isolates no doubt enhance one aspect or another of plant growth under controlled conditions, the extrapolation of these functions to actual field applications is uncertain. The use of sterilized substrates is also common in many laboratory and greenhouse studies (Puente and Bashan, 1993; Jankong et al., 2007; Viva et al., 2006). Such studies provide valuable data regarding specific effects of an

individual isolate, yet do not demonstrate the microbe's effectiveness under actual field conditions.

The effects of some of these PGPB may become negligible once introduced to actual substrates where they may not have to ability to compete with resident microbial populations. Jankong et al. (2007) observed different results in field trails compared to greenhouse studies when prospective PGPB were used to enhance arsenic accumulation in plant shoots. Çakmakçi et al. (2006) reported the use of a PGPB collection under greenhouse and field-scale trails. These PGPB remained effective under field trials; however, their ability to enhance plant growth was greater under greenhouse conditions and was less effective than fertilizer amendments under field-scale applications. While some investigators have reported mixed results when translating from the bench scale to the field; a number of agricultural studies have found success in conducting field-scale trials (Raupach and Kloepper, 1998). Despite the success of these agricultural applications, a shortage of such studies remains under environmental applications. Recently, restoration of severely eroded desert field with three legumes trees and cardon cacti inoculated with several PGPB combined with AM fungi was carried out with high survivability and growth promotion of all plant species four years after inoculation (Bashan et al. unpublished). The current body of literature will benefit from the continuation of these initial screening studies into field applications as there is little information available describing the translation of laboratory or greenhouse-scale applications to successful field trials.

4.6 A Need for Long-Term Field Studies

The current body of literature also lacks studies describing the performance of these isolates under field conditions over long-term time scales. Many investigations have been conducted at the bench or greenhouse-scale where environmental factors such as temperature, irrigation, humidity and photoperiod can be carefully controlled (Bashan et al., 2000b; Grandlic et al., 2008; Petrisor et al., 2004; Vivas et al., 2006). These studies have supplied valuable information regarding the potential benefits of selected isolates in various substrates (Abd et al., 2004; Grandlic et al., 2008; Mayak et al., 2004). Although PGPB isolates may certainly prove to be effective under these conditions, valuable data can be provided by monitoring their performance in situations with naturally fluctuating environmental states. Screen-house studies are perhaps more suitable for investigating various environmental applications of PGPB as these houses more closely resemble natural field conditions, while offering protection from grazing animals.

Study length is also a factor which should be addressed during experimental design and should be the focus of future investigations. Short-term studies are common in the body of literature describing environmental applications of PGPB. Many of these studies highlight the effectiveness of these isolates under diverse applications; however, the shortage of literature tracking these isolates over periods greater than a few months leaves their long-term success in question. In addition, those isolates that appear to be ineffective during short-term studies may actually be quite beneficial over long-term timescales (Bashan et al., 1999; Puente et al. 2004a). There are also contradicting reports

conducted under agricultural settings which show that the effectiveness of PGPB are more pronounced during the early stages of plant growth (Cakmakci et al., 2006; Dobbelaere et al., 2002). In pot studies lasting 30 or 45 days, introduced PGPB demonstrated the ability to significantly increase plant growth; however, the nutrient contents, P, K, S, Ba, Mo, were decreased in shoot tissues of some treatments (Belimov et al., 2002). Such results suggest that although plant growth is increased, the nutrient status of the plant may become compromised over the long-term.

5.0 Conclusions

Plant growth-promoting bacteria have recently found many uses in environmental applications. Despite the success of described in recent PGPB studies much remains unknown regarding plant-microbe-substrate interactions that occur during these remediation efforts. Developing investigations with the goal of transferring technologies to field-scale applications may ultimately lead to the increased success and decreased costs of remediation efforts. Further research is required to optimize these applications to their full potential including:

- Understanding growth-promoting mechanisms as they occur under field conditions
- Investigating the colonization abilities of introduced PGPB
- Developing laboratory or greenhouse studies that better mimic intended field applications
- Understanding how the behavior of PGPB may vary with substrate nutrient status

- Conducting long-term field studies
- Optimizing inoculation methods to meet intended applications

Addressing these issues will produce valuable information required to develop PGPB-assisted remediation efforts into viable technologies.

6.0 References

Abd El-Samad Hamdia, M., M.A.K. Shaddad, and M.M. Doaa. 2004. Mechanisms of salt tolerance and interactive effects of *Azospirillum brasilense*. Plant Growth Regulation. **44:**165-174.

Andrade G., Linderman R.G. and Bethlenfalvay G.J. 1998. Bacterial associations with the mycorrhizosphere and hyphosphere of the arbuscular mycorrhizal fungus *Glomus mosseae*. Plant and Soil 202: 79-87

Arshad, M., M. Saleem, and S. Hussain. 2007. Perspectives of bacterial ACC deaminase in phytoremediation. TRENDS Biotech. **25:**356-362.

Bacilio M., Hernandez, J.-P., and Bashan, Y. 2006. Restoration of giant cardon cacti in barren desert soil amended with common compost and inoculated with *Azospirillum brasilense.* Biology and Fertility of Soils 43: 112-119.

Bacilio M., P. Vazquez, and Y. Bashan. 2003. Alleviation of noxious effects of cattle ranch composts on wheat seed germination by inoculation with *Azospirillum* spp. Biol. Fert. Soils. **38:**261-266.

Barac, T ., Taghavi, S ., Borremans, B ., Provoost, A ., Oeyen, L ., Colpaert, JV ., Vangronsveld, J ., van der Lelie, D. 2004. Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nat. Biotechnol. 22, 583-588.

Bar-Ness, E. Y. Chen, Y. Hadar, H. Marchner, and V. Römheld. 1991. Siderophores of *Pseudomonas putida* as an iron source for dicot and monocot plants. Plant Soil. **130:**231-241.

Bar-Ness, E., Y. Hadar, Y. Chen, A. Shanzer, and J. Libman. 1992. Iron uptake by plant from microbial siderophores. Plant Physiol. **99:**1329-1335.

Barriuso, J., M.T. Pereyra, J.A. Lucas Garcia, M. Megias, F.J. Gutierrez Manero, and B. Ramos. 2005. Screening for putative PGPR to improve establishment of the symbiosis *Lactarius deliciosus-Pinus* sp. Microbial Ecology. **50:**82-89.

Bashan, Y. 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol. Adv. **16:**729-770.

Bashan, Y., E.A. Davis, A. Carrillo, and R.G. Linderman. 2000. Assessment of VA mycorrhizal inoculum potential in relation to the establishment of cactus seedlings under mesquite nurse-trees in the Sonoran desert. Applied Soil Ecology **14**:165-176

Bashan, Y. and de-Bashan, L.E. 2005. Bacteria / Plant growth-promotion. In: Encyclopedia of soils in the environment. (Ed.) D. Hillel, Elsevier, Oxford, U.K. Vol. 1., pp. 103-115.

Bashan, Y., Harrison, S.K. and Whitmoyer, R.E. 1990. Enhanced growth of wheat and soybean plants inoculated with *Azospirillum brasilense* is not necessarily due to general enhancement of mineral uptake. Applied and Environmental Microbiology 56: 769-775.

Bashan, Y. and Holguin, G. 1993. Anchoring of *Azospirillum brasilense* to hydrophobic polystyrene and wheat roots. Journal of General Microbiology 139: 379-385.

Bashan, Y. and Holguin, G. 1997. Azospirillum/plant relationships: environmental and physiological advances (1990-1996). Canadian Journal of Microbiology 43: 103-121.

Bashan, Y., and Holguin, G. 1998. Proposal for the division of Plant Growth-Promoting Rhizobacteria into two classifications: biocontrol-PGPB (Plant Growth-Promoting Bacteria) and PGPB. Soil Biology and Biochemistry 30:1225-1228

Bashan, Y., A. Rojas, and M.E. Puente. 1999. Improved establishment and development of three cactus species inoculated with *Azospirillum brasilense* transplanted into disturbed urban soil. Can. J. Microbiol. **45:**441-451.

Bashan, Y., and G. Holguin. 2002. Plant growth-promoting bacteria: a potential tool for arid mangrove reforestation. Trees Struc. Func. **16:**159-166.

Bashan, Y., G. Holguin, and L.E. de-Bashan. 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Can. J. Microbiol. **50:**521-577.

Bashan, Y., J.-P. Hernandez, L.A. Leyva, and M. Bacilio. 2002. Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. Biol. Fertil. Soils. **35:**359-368.

Bashan, Y., M. Moreno, and E. Troyo. 2000b. Growth promotion of the oilseed halophyte *Salicornia bigelovii* in seawater inoculated with mangrove rhizosphere bacteria and *Azospirillum* . Biol. Fert. Soils. **32:** 265-272.

Bashan, Y., M.E. Puente, D.D. Myrold, G. Toledo. 1998. In vitro transfer of fixed nitrogen from diazotrophic filamentous cyanobacteria to black mangrove seedlings. FEMS Microbiol. Ecol. **26:**165-170.

Belimov, A.A., A.M. Kunakova, N.D. Vasilyeva, T.S. Kovatcheva, V.F. Dritchko, S.N. Kuzovatov, I.R. Trushkina, Yu.V. Alekseyev. 1998. Accumulation of radionucleotides by associative bacteria and the uptake of 134Cs by the inoculated barley plants in: Malik, K.A., M.S. Mizra, J.K. Ladha (Eds.), Nitrogen fixation with non-legumes. Kluwer Academic Publishers, Dordrecht, pp 275-280.

Belimov, A.A., A.M. Kunakova, V.I. Safronova, V.V. Stepanok, L.V. Yudkin, Y.V. Alekseev, and A.P. Kozhemyakov. 2004. Employment of rhizobacteria for the inoculation of barley plants cultivated in soil contaminated with lead and cadmium. Microbiol. **73:**99-106.

Belimov, A.A., A.Y. Ivanchikov, L.V. Yudkin, O.F. Khamova, S.M. Postavskaya, P.V. Popolzukhin, A.A. Shmakova, G.Y. Kozlova. 1999. New strains of associative growth-stimulating bacteria dominating the rhizoplane of barley seedlings: characterization and introduction. Mikrobiologiya. **47:**337-342.

Belimov, A.A., N. Hontzeas, V.I. Safronova, S.V. Demchinskaya, G. Piluzza, S. Bullitta, and B.R. Glick. 2005. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). Soil Boil. Biochem. **37:**241-250.

Belimov, A.A., V.I. Safronova, T. Mimura. 2002. Response of spring rape to inoculation with plant growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase depends on nutrient status of the plant. Can. J. Microbiol. **48:**189-199.

Belimov, A.A., V.I. Safronova, T.A. Sergeyeva, T.N. Egorova, V.A. Matveyeva, V.E. Tsyganov, A.Y. Borisov, I.A. Tikhonovich, C. Kluge, A. Preisfeld, K.-J. Dietz, and V.V. Stepanok. 2001. Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. Can. J. Microbiol. **47:**642-652.

Benizri, E., E. Baudoin, and A. Guckert. 2001. Root colonization by inoculated plant growth-promoting rhizobacteria. Biocontrol Sci. Technol. **11:**557-574.

Berti W.W.R., and S.D. Cunningham. 2000. Phytostabilization of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals – using plants to clean up the environment. John Wiley & Sons, Inc. New York. pp 71–88.

Burd, G.I., D.G. Dixon, and B.R. Glick. 1998. A plant growth-promoting bacterium that decreases nickel toxicity in seedlings. Appl. Environ. Microbiol. **64:**3663-3668.

Burd, G.I., D.G. Dixon, and B.R. Glick. 2000. Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can. J. Microbiol. **46:**237-245.

Burr, T.J., M.N. Schroth and T. Suslow. 1978. Increased potato yields by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathol. **68:** 1377–1383.

Çakmakçi, R., F. Donmez, A. Aydin, and F. Sahin. 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biol. Biochem. **38:**1482-1487.

Carrillo-Garcia, A., Y. Bashan, E. Diaz Rivera, and G.J. Bethlenfalvay. 2000. Effects of resource island soils, competition, and inoculation with *Azospirillum* on survival and growth of *Pachycereus pringlei*, the giant cactus of the Sonoran Desert. Res. Ecol. **8:**65-73.

Carrillo, A.E., C.Y. Li, and Y. Bashan. 2002. Increased acidification in the rhizosphere of cactus seedlings induced by *Azospirillum brasilense.* Naturwissenschaften. **89:**428-432.

Cattelan, A.J., P.G. Hartel, J.J. Fuhrmann. 1999. Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci. Soc. Am. J. **63:**1670-1680.

Compant, S., B. Duffy, J. Nowak, C. Clément, and E. Ait. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action and future prospects. Appl. Environ. Microbiol. **71:**4951-4959.

Creus, C.M., R.J. Sueldo, and C.A. Barassi. 2004. Water relations and yield in *Azospirillum*-inoculated wheat exposed to drought in the field. Can. J. Bot. **82:**273-281.

Cunningham, S.D., W.R. Betri, J.W. Huang. 1995. Phytoremediation of contaminated soils. Trends Biotechnol. **13:**393-397.

de-Bashan, L.E., J.-P. Hernandez, T. Morey, and Y. Bashan. 2004. Microalgae growth-promoting bacteria as "helpers" for microalgae: a novel approach for removing ammonium and phosphorus from municipal wastewater. Water Res. **38:**466-474.

de-Bashan, L.E., Antoun, H., and Bashan Y. 2008. Involvement of indole-3-acetic-acid produced by the microalgae growth-promoting bacterium *Azospirillum* spp. in growth promotion of *Chlorella vulgaris*. **Journal of Phycology 44:** (In press)

de-Bashan, L.E., M. Moreno, J.-P. Hernandez, and Y. Bashan. 2002a. Removal of ammonium and phosphorous ions from synthetic wastewater by the microalgae *Chlorella*

vulgaris coimmobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillum brasilense*. Water Res. **36:**2941-2948.

de-Bashan, L.E., Y. Bashan. 2004. Recent advances in removing phosphorous from wastewater and its future use as fertilizer (1997-2003). Water Res. **38:**4222-4246.

de-Bashan, L.E., Y. Bashan, M. Moreno, V.K. Lebsky, and J.J. Bustillos. 2002. Increased pigement and lipid content, lipid variety, and cell and population size of the microalgae *Chlorella* spp. when co-immobilized in alginate beads with the microalgae-growth-promoting bacterium *Azospirillum brasilense*. Can. J. Microbiol. **48:**514-521.

de-Bashan, L.E., Magallon, P., Antoun, H., and Bashan Y. 2008. Participation of glutamate dehydrogenase and glutamine synthetase in ammonium assimilation by *Chlorella vulgaris* jointly immobilized with the microalgae growth-promoting bacterium *Azospirillum brasilense.* **Journal of Phycology 44:** (In press).

de-Bashan L.E., Trejo A., Huss V.A.R., Hernandez J.-P. and Bashan, Y. 2008. *Chlorella sorokiniana* UTEX 2805, a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater. **Bioresource Technology** 99: 4980–4989

de Freitas, J.R. and J.J Germida. 1990. Plant growth promoting rhizobacteria for winter wheat. Can. J. Microbiol. **36:**265–272.

de Freitas, J. R., M. R. Banerjee, J. J. Germida. 1997. Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus L.*). Biol. Fertil. Soils. **24:**358-364.

Dekkers, L. C., A. J. van der Bij, I. H. M. Mulders, C. C. Phoelich, R. A. R. Wentwoord, D. C. M. Glandorf, C. A. Wijffelman, and B. J. J. Lugtenberg. 1998. Role of the O-antigen of lipopolysaccheride, and possible roles of growth rate and of NADH:ubiquinone oxidoreductase (*nuo*) in competitive tomato root-tip colonization by *Pseudomonas fluorescens* WCS365. Mol. Plant-Microbe Interact. **11:**763–771.

de Souza, M.P., D. Chu, M. Zhao, A.M. Zayed, S.E. Ruzin, D. Schichnes, and N. Terry. 1999a. Rhizosphere bacteria enhance selenium accumulation and volatilization by Indian mustard. Plant Physiol. **119:**565-573.

de Souza, M.D., C.P. Huang, N. Chee, N. Terry. 1999b. Rhizosphere bacteria enhance the accumulation of selenium and mercury in wetland plants. Planta. **209:**259-263.

De Troch, P., and J. Vanderleyden. 1996. Surface properties and motility or *Rhizobium* and *Azospirillum* in relation to plant root attachment. Microb. Ecol. **32:**149-169.

Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, Y. Okon, and J. Vanderleyden. 2002. Effects of inoculation on wild type *Azospirillum brasilense* and *A. irakense* strains in development and nitrogen uptake of spring wheat and grain maize. Biol. Fertil. Soils. **36:**284-297.

Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, J. Vanderleyden, P. Dutto, C. Labandera-Gonzalez, C. Mellado, J.F. Aguirre, Y. Kapulnik, S. Brener, S. Burdman, D. Kadouri, S. Sarig and Y. Okon. 2001. Responses of agronomically important crops to inoculation with *Azospirillum*. Aust J. Plant Physiol. 28: 871–879.

Döbereiner, J., Marriel, I. E., and Nery, M. 1976. Ecological distribution of *Spirillum lipoferum* Beijerinck. Can. J. Microbiol. **22**: 1464-1473.

Donate-Correa, J., M. Leon-Barrios, and R. Perez-Galdona. 2004. Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. Plant and Soil. **266:**261-272

Donnelly, P.K., R.S. Hedge, and J.S. Fletcher. 1994. Growth of PCB-degrading bacteria on compounds from photosynthetic plants. Chemosphere. **28:**981-988.

Duponnois, R., M. Kisa, K. Assigbetse, Y. Prin, J. Thioulouse, M. Issartel, P. Moulin, and M. Lepage. 2006. Fluorescent pseudomonads occurring in *Macrotermes subhyalinus* mound structures decrease Cd toxicity and improve its accumulation in sorghum plants. Science Total Environ. **370:**391-400.

Egamberdiyeva, D. 2007. The effect of nutrient uptake of maize in two different soils. Appl. Soil Ecology. **36:**184-189.

Egamberdiyeva, D., and G. Höflich. 2002. Root colonization and growth promotion of winter wheat and pea by *Cellulomonas spp.* at different temperatures. Plant Growth Reg. **38:**219-224.

Garbaye J.1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytologist 128, 197-210

Gratão P. L.; Prasad M. N. V.; Cardoso P. F.; Lea P. J.; Azevedo R. A. 2005. Phytoremediation: green technology for the clean up of toxic metals in the environment. Braz. J. Plant Physiol. 17: 53-64.

Farwell, A.J., S. Vesely, V. Nero, H. Rodriguez, K. McCormack, S. Shah, D.G. Dixon, B.R. Glick. 2007. Tolerance of transgenic canola plants (*Brasica napus*) amended with plant growth-promoting bacteria to flooding stress at a metal-contaminated site. Environ. Poll. **147:**540-545.

Felker P., D. Medina, C. Soulier, G. Velicce, M. Velarde, and C. Gonzalez. 2005. A survey of environmental and biological factors (*Azospirillum* spp*, Agrobacterium rhizogenes, Pseudomonas aurantiaca*) for their influence in rooting cuttings of *Prosopis alba* clones. J Arid Environ. **61:**227-247.

Flores-Mireles, A.L., S.C. Winans, and G. Holguin. 2007. Molecular Characterization of Diazotrophic and Denitrifying Bacteria Associated with Mangrove Roots. Applied and Environmental Microbiology 73: 7308–7321.

Frey-Klett, P., J.C. Pierrat, and J. Garbaye. 1997. Location and survival of mycorrhiza helper *Pseudomonas fluorescens* during establishment of ectomycorrhizal symbiosis between *Laccaria biocolor* and douglas fir. Appl. Environ. Microbiol. **63:**139-144.

Frommel. M.I., J. Nowak, and G. Lazarovitis. 1991. Growth enhancement and developmental modifications of in vitro grown potato *Solanum tuberosum* ssp. *tuberosum*. Plant Physiol. **96:** 928–936.

Garcia de Salamone, I.E., R.K. Hynes, and L.M. Nelson. 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can. J. Microbiol. **47:**404-411.

Glick, B.R. 2003. Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol. Adv. **21:** 383-393.

Glick, B.R. 2004. Teamwork in phytoremediation. Nature Biotechnol. **22:** 526-527.

Glick, B.R., and Bashan, Y. 1997. Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnological Advances 15: 353-378.

Glick, B.R., D.M. Penrose, and J. Li. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J. Theo. Biol. **190:**63-68.

Gonzalez-Acosta, B., Y. Bashan, N.Y. Hernandez-Saavedra, F. Ascencio, and G. De la Cruz-Agüero. 2006. Seasonal seawater temperature as the major determinant for populations of culturable bacteria in the sediments of an intact mangrove in an arid region. FEMS Microbiol. Ecol. **55:**311-321.

Gonzalez L.E., and Y. Bashan. 2000. Increased growth of the microalgae *Chlorella vulgaris* when coimmobilized and cocultured in alginate beads with the plant growth-promoting bacterium *Azospirillum brasilense.* Appl. Environ. Microbiol. **66:**1527-1531.

Grandlic, C.J., M.O. Mendez, J. Chorover, B. Machado, and R.M. Maier. 2008. Plant growth-promoting bacteria for phytostabilization of mine tailings. Environ. Sci. Tech. *In Press.*

Grichko, V.P., and B.R. Glick. 2001. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol. Biochem. **39:**11-17.

Hernandez, J.-P., L.E. de-Bashan, and Y. Bashan. 2006. Starvation enhances phosphorous removal from wastewater by the microalga *Chlorella* spp. co-immobilized with *A. brasilense*. Enzyme Microbial Tech. **38:**190-198.

Herrera, M. A., C. P. Salamanca, and J. M. Barea. 1993. Inoculation of woody legumes with selected arbuscular mycorrhizal fungi and rhizobia to recover desertified Mediterranean ecosystems. Applied and Environmental Microbiology 59:129-133.

Hoflich, G. R. and Metz. 1997. Interactions of plant-microorganism associations in heavy metal containing soils from sewage farms. Bodenkultur. **48:**239–247.

Holguin, G. and Bashan, Y. 1996. Nitrogen-fixation by *Azospirillum brasilense* Cd is promoted when co-cultured with a mangrove rhizosphere bacterium *(Staphylococcus* sp.) **Soil Biology and Biochemistry 28**:1651-1660.

Holguin, G., P. Gonzalez-Zamorano, L.E. de-Bashan, R. Mendoza, E. Amador, and Y. Bashan. 2006. Mangrove health in an arid environment encroached by urban development – a case study. Sci. Total Environ. **363:**260-274.

Holguin, G., Guzman, M.A. and Bashan, Y. 1992. Two new nitrogen-fixing bacteria from the rhizosphere of mangrove trees, isolation, identification and in vitro interaction with rhizosphere *Staphylococcus* sp. **FEMS Microbiology Ecology 101**: 207-216.

Holguin, G. Vazquez, P., and Bashan, Y. 2001. The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. Biology and Fertility of Soils **33**: 265-278.

Huang, X.-D., Y. El-Alawi, D.M. Penrose, B.R. Glick, and B.M. Greenberg. 2004. A multi-process system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ. Poll. **130:**465-476.

Jankong, P., P. Visoottiviseth, S. Khokiattiwong. 2007. Enhanced phytoremediation of arsenic contaminated land. Chemosphere. **68:**1906-1912.

Jjemba, P.K., and M. Alexander. 1999. Possible determinants of rhizosphere competence of bacteria. Soil Biol. Biochem. **31:**623-632.

Kathiresan, K., and M. Masilamani Selvam. 2006. Evaluation of beneficial bacteria from mangrove soil. Botanica Marina. **49:**86-88.

Khan, A.G. 2005. Role of soil microbes in the rhizospheres of plants growing on trace metals contaminated soils in phytoremediation. J Trace Elem. Med. Biol. **18:**355-364.

Kloepper, J.W., J. Leong, M. Teintze, M.N. Schroth. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature. **286:**885-886.

Kloepper, J.W., R. Lifshitz, and R. Zablotowicz. 1987. Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol. **7:**39-44.

Kumar, P.B.A., V. Dushenkov, H. Motto, and I. Raskin. 1995. Phytoextraction: the use of plants to remove heavy metals from soils. Environ. Sci. Tech. **29:**1232-1238.

Lebeau, T., A. Braud, and K. Jezequel. 2007. Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. Environ. Poll. dio:10.1016/j.envpol.2007.09.015. better put the pages as it was published last year

Lebsky, V.K., L.E. Gonzalez-Bashan, and Y. Bashan. 2001. Ultrastructure of interaction in alginate beads between the microalga *Chlorella vulgaris* with its natural associative bacterium *Phyllobacterium myrsinacearum* and with the plant growth-promoting bacterium *Azospirillum brasilense.* Can. J. Microbiol. **47:**1-8.

Leyva L.A. and Bashan, Y. 2008. Activity of two catabolic enzymes of the phosphogluconate pathway in mesquite roots inoculated with *Azospirillum brasilense* Cd **Plant Physiology and Biochemistry** (In press)

Lodewyckx, C., S. Taghavi, M. Mergeay, J. Vangronsveld, H. Clijsters, and D. Van Der Lelie. 2001. The effect of recombinant heavy metal-resistant endophytic bacteria on heavy metal uptake by their host plant. Int. J. Phytoremediat. **3:** 173-187.

Lucas Garcia, J., J. Domenech, C. Santamaria, M. Camacho, A. Daza, F.J. Gutierrez Manero. 2004. Growth of forest plants (pine and holm-oak) inoculated with rhizobacteria: relationship with microbial community structure and biological activity of its rhizosphere. Environ. Experimental Botany. **52:**239-251.

Lucy, M. Reed E.and Glick B. R. 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie van Leeuwenhoek J. G. **86:** 1–25.

Macková, M., B. Vrchotová, K. Francová, M. Sylvestre, M. Tomaniová, P. Lovecká, K. Demnerová, and T. Macek. 2007. Biotransformation of PCBs by plants and bacteria – consequences of plant-microbe interactions. European J. Soil Biology. **43:**233-341

Mayak, S., T. Tirosh, and B.R. Glick. 2004. Plant growth-promoting bacteria that confer resistance in tomato to salt stress. Plant Physiol. Biochem. 42:565-572.

McCutcheon, S.C. and Schnoor, J.L. 2003. Phytoremediation: Transformation and control of contaminants. Wiley-Interscience, 1024 p.

Mendez, M.O., E.P. Glenn, and R.M. Maier. 2007. Phytostabilization potential of quailbush for mine tailings: growth, metal accumulation, and microbial community changes. J. Environ. Qual. **36:**245-253.

Mendez MO and Maier RM. 2008a. Phytoremediation of mine tailings in temperate and arid environments. Rev. Environ. Sci. Biotechnol. 7:47-59.

Mendez, M.O., and R.M. Maier. 2008b. Phytostabilization of mine tailings in arid and semiarid environments – an emerging remediation technology. Environ. Health Perspect. **116:**278-283.

Nagendran, R., A. Selvam, K. Joseph, and C. Chiemchaisri. 2006. Phytoremediation and rehabilitation of municipal solid waste landfills and dumpsites: a brief review. Waste Management. **26:**1357-1369.

Nie, L., S. Shah, A. Rashid, G.I. Burd, D.G. Dixon, and B. Glick. 2002. Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth-promoting bacterium *Enterobacter cloacae* CAL2. Plant Physiol. Biochem. **40:** 355-361.

Oliveira, A.L.M., E.L. Canuto, E.E. Silva, V.M. Reis, and J.I. Baldani. 2004. Survival of endophytic diazotrophic bacteria in soil under different moisture levels. Brazilian J. Microbiol. **35:**295-299.

Patten, C.L., and B.R. Glick. 2002. Role of *Psuedomonas putida* indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. **68:**3795-3801.

Petrisor, I., S. Dobrota, K. Komnitsas, I. Lazar, J.M. Kuperberg, and M. Serban. 2004. Artificial inoculation – perspectives in tailings phytostabilization. Int. J. Phytoremediation. **6:**1-15.

Pilon-Smits E. 2005. Phytoremediation. Annu. Rev. Plant Biol. 56:15-39**.**

Pishchik, V.N., N.I. Vorobeyv, I.I. Chernyaeva, S.V. Timofeeva, A.P. Kozhemyakov, Y.V. Alexeev, S.M. Lukin. 2002. Experimental and mathematical simulation of plant growth promoting rhizobacteria and plant interactions under cadmium stress. Plant and soil. **243:**173-186.

Prasad MNV and Freitas H. 2003. Metal hyperaccumulation in plants-biodiversity prospecting for phytoremediation technology. Electronic Journal of Biotechnology 6:275-321.

Primavera J.H., R.S. Sadaba, M.J.H.L. Lebata, and J.P. Altamirano. 2004. Handbook of mangroves in the Philippines-Panay. Iloilo, Philippines. Southeast Asian Fisheries Development Center Aquaculture Department; 106 pp.

Puente, M.-E., C.Y. Li, and Y. Bashan. 2004a. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings. Plant Biol. **6:**643-650.

Puente, M.-E., and Y. Bashan. 1993. Effect of inoculation with *A. brasilense* strains on the germination and seedlings growth of the giant columnar Cardon cactus (*Pachycereus pringlei*). Symbiosis. **15:**49-60.

Puente, M.-E., Y. Bashan, C.Y. Li, and V.K. Lebsky. 2004b. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. root colonization and weathering of igneous rocks. Plant Biol. **6:**629-642.

Puente, M.-E., G. Holguin, B.R. Glick, and Y. Bashan. 1999. Root surface colonization of black mangrove seedlings by *Azospirillum halopraeferens* and *Azospirillum brasilense* in seawater. FEMS Microb. Ecol. **29:**283-292.

Radwan, S.S., N. Dashti, and I.M. El-Nemr. 2005. Enhancing the growth of *Vivia faba* plants by microbial inoculation to improve their potential for oily desert areas. Int. J. Phytoremediation. **7:**19-32.

Rai, U.N., K. Pandey, S. Sinha, A. Singh, R. Saxena, and D.K. Gupta. 2004. Revegetating fly ash landfills with *Prosopis juliflora* L.: impact of different amendments and *Rhizobium* inoculation. Environment International. **30:**293-300.

Rajkumar, M., and H. Freitas. 2007. Effects of inoculation of plant growth-promoting bacteria on Ni uptake by Indian mustard. Bioresour. Technol. doi:10.1016/j.biortech.2007.07.046.

Rajkumar, M., and H. Freitas. 2008. Influence of metal-resistant plant growth-promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. Chemosphere. **71:**834-842.

Rao, A.V., and R. Tak. 2001. Effect of rhizobial inoculation on *Albizia lebbeck* and its rhizosphere activity in mine spoils. Arid Land Res. Management. **15:**157-162.

Raupach, G.S., and J.W. Kloepper. 1998. Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology. **88:**1158-1164.

Reed, M.L.E., B.G. Warner, and B.R. Glick. 2005. Plant growth-promoting bacteria facilitate the growth of the common reed *Phargmites australis* in the presence of copper or polycyclic aromatic hydrocarbons. Current Microbiol. **51:**425-429.

Reed, M.L.E., and B.R. Glick. 2005. Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can. J. Microbiol. **51:**1061-1069.

Reichman, S.M. 2007. The potential use of legume-rhizobium symbiosis for the remediation of arsenic contaminated sites. Soil Biol. Biochem. **39:**2587-2593.

Requena, N., E. Perez-Solis, C. Azcon-Aguilar, P. Jefferies, and J.-M. Barea. 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Appl. Environ. Microbiol. **67:**495-498.

Requena, N., I. Jimenez, M. Toro, and J.M. Barea. 1997. Interactions between plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and *Rhizobium* spp. in the rhizosphere of *Anthyllis cytisoides*, a model legume for revegetation in Mediterranean semi-arid ecosystems. New Phytol. **136:**667-677.

Rodriguez, H., Fraga, R., Gonzalez, T., and Bashan, Y. 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant and Soil 287: 15-21

Rojas, A., G. Holguin, B.R. Glick, and Y. Bashan. 2001. Synergism between *Phyllobacterium* sp. (N-fixer) and *Bacillus licheniformis* (P-solubilizer), both from a semiarid mangrove rhizosphere. FEMS Microb. Ecol. **35:**181-187.

Rönnbäck P. 1999. The ecological basis for economic value of seafood production supported by mangrove ecosystems. Ecol Econ. 29:235–252.

Ryu, C.-M., M.A. Farag, C.-H. Hu, M.S. Reddy, H.-X. Wei, P.W. Pare, and J.W. Kloepper. 2003. Bacterial volatiles promote growth in Arabidopsis. Proc. Natl. Acad. Sci. USA. **100:**4927-4932.

Saravanan, V.S., M. Madhaiyan, and M. Thangaraju. 2007. Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. Chemosphere. **66:**1794-1798.

Sheng, X.-F., and J.-J. Xia. 2006. Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. Chemosphere. **64:**1036-1042.

Shishido M., Petersen D. J., Massicotte H. B., Chanway, C. P. 1996. Pine and spruce seedling growth and mycorrhizal infection after inoculation with plant growth promoting Pseudomonas strains. FEMS Microbiology Ecology 21 , 109–119

Simons, M., A. J. van der Bij, L. A. de Weger, C. A. Wijffelman, and B. J. Lugtenberg. 1996. Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. Mol. Plant-Microbe Interact. **9:**600–607.

Simons, M., H. P. Permentier, L. A. de Weger, C. A. Wijffelman, and B. J. J. Lugtenberg. 1997. Amino acid synthesis is necessary for tomato root colonization by *Pseudomonas fluorescens* strain WCS365. Mol. Plant-Microbe Interact. **10:**102–106.

Singh, U.P. B.K. Sarma, D.P. Singh, and A. Bahadur. 2002. Plant growth-promoting rhizobacteria mediated induction of phenolics in pea (*Pisum sativum*). Curr. Micorbiol. **44:**396-400. .

Spaepen, S., Vanderleyden, J. & Remans, R. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol. Rev. 31, 425-448.

Strangmann, A., Bashan, Y. and Giani, L. 2008. Methane in pristine and impaired mangrove soils and its possible effect on establishment of mangrove seedlings. Biology and Fertility of Soils 44: 511–519

Tam, N.F.Y., and Y.S. Wong. 2000. Effect of immobilized microalgal bead concentration on wastewater nutrient removal. Environ. Pollut. **107:**145-151.

Toledo, G., Y. Bashan, A. Soeldner. 1995. In vitro colonization and increase in nitrogen fixation of seedling roots of black mangrove inoculated by a filamentous cyanobacteria. Can. J. Microbiol. **41:**1012-1020.

Toledo, G., Rojas, A., and Bashan, Y. 2001. Monitoring of black mangrove restoration with nursery-reared seedlings on an arid coastal lagoon. **Hydrobiologia 444**: 101-109

Velázquez-Rodríguez AS, Flores-Román D and Acevedo-Sandoval OA. 2001. Aggregate formation in tepetate by effect of plant species. Agrociencia 35: 311-320.

Valdenegro, M., J.M. Barea, and R. Azcón. 2001. Influence of arbuscular-mycorrhizal fungi, *Rhizobium meliloti* strains and PGPR inoculation on the growth of *Medicago*

arborea used as model legume for re-vegetation and biological reactivation in a semi-arid Mediterranean area. Plant Growth Reg. **34:**233-24

Valderrama, L.T., C.M. Del Campo, C.M. Rodriguez, L.E. de-Bashan, and Y. Bashan. 2002. Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga *Chlorella vulgaris* and the macrophyte *Lenna minuscula.* Water Res. **36:**4185-4192.

Van Loon LC and Glick BR 2004. Increased plant fitness by rhizobacteria. In; Molecular ecotoxicology of plants. Ecological Studies, Vol. 170. (Ed.) H. Sandermann. Springer-Verlag, Berlin, pp. 177-205.

Vazquez, P., G. Holguin, M.E. Puente, A. Lopez-Cortez, and Y. Bashan. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol. Fertil. Soils. **30:**460-468.

Vivas, A., B. Biro, J.M. Ruiz-Lozano, J.M. Barea, and R. Azcon. 2006. Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zu-toxicity. Chemosphere. **62:**1523-1533.

Whiting, S.N., M.P. De Souza. and N. Terry. 2001. Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*. Environ. Sci. Technol. **35:**3144–3150.

Wu, S.C., K.C. Cheung, Y.M. Luo, and H.M. Wong. 2006a. Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by *Brassica juncea*. Environmental Pollution. **140:** 124-135.

Wu, S.C., Y.M. Luo, K.C. Cheung, and M.H. Wong. 2006b. Influence of bacteria on Pb and Zn speciation, mobility and bioavailability in soil: a laboratory study. Environ. Poll. **144:**765-773.

Yabur, R., Y. Bashan, G. Hernandez-Carmona. 2007. Alginate from the macroalgae *Sargassum sinicola* as a novel source for microbial immobilization material in wastewater treatment and plant growth promotion. J. Appl. Phycol. **19:**43-53.

Zaady, E., Y. Okon, and A. Perevolotsky. 1994. Growth response of Mediterranean herbaceous swards to inoculation with *Azospirillum brasilense*. Journal of Range Management. **47:**12-15.

Zhuang, X., J. Chen, H. Shim, and Z. Bai. 2007. New advances in plant growth-promoting rhizobacteria for bioremediation. Environ. Inter. **33:**406-413.

7.0 Figure Legends

Figure 1. Plant rhizosphere displaying selected plant growth-promotion mechanisms. PGPB associated residing in the rhizosphere can play a number of beneficial roles in assisting plant growth; (a) diazotrophic bacteria, free-living N-fixers, fix atmospheric N_2 producing bioavailable NH_4, (b) siderophore-producing bacteria can sequester Fe^{3+} in environments where Fe is limiting and has an extremely low bioavailability, (c) some PGPB can directly enhance plant growth through the production of phytohormones such as indole-3-acetic acid (IAA) and gibberellins which can increase root growth and plant cell division, (d) P-solubilizing bacteria can dissolve mineral-phase P increasing its bioavailability through the production of organic acids, (e) PGPB that posses the enzyme ACC-deaminase can lower plant ethylene levels and reduce the stress response of plants residing under adverse conditions.

Figure 2. Stepwise process for obtaining and utilizing PGPB in environmental applications. The utilization of PGPB in environmental applications can be achieved through a six-step procedure where each step narrows a large collection of isolates down to a few PGPB that are appropriate for field-scale application. (1) Investigations may begin with obtaining a large collection of potentially hundreds of bacterial isolates. (2) Each of these isolates are then screened for various PGP mechanisms that are relevant to the intended application. (3) The large collection can then be narrowed to a sub-set of isolates that display desirable PGP mechanisms. (4) Isolates in this sub-set are then screened on a small-scale under conditions that mimic the intended application. (5) A small collection of isolates that consistently perform well in small-scale screenings can then be optimized for field-scale application. (6) Finally, field-scale trials are conducted using one or two isolates that have the greatest ability to enhance the intended remediation application.

Figure 1 Plant rhizosphere displaying selected plant growth-promotion mechanisms

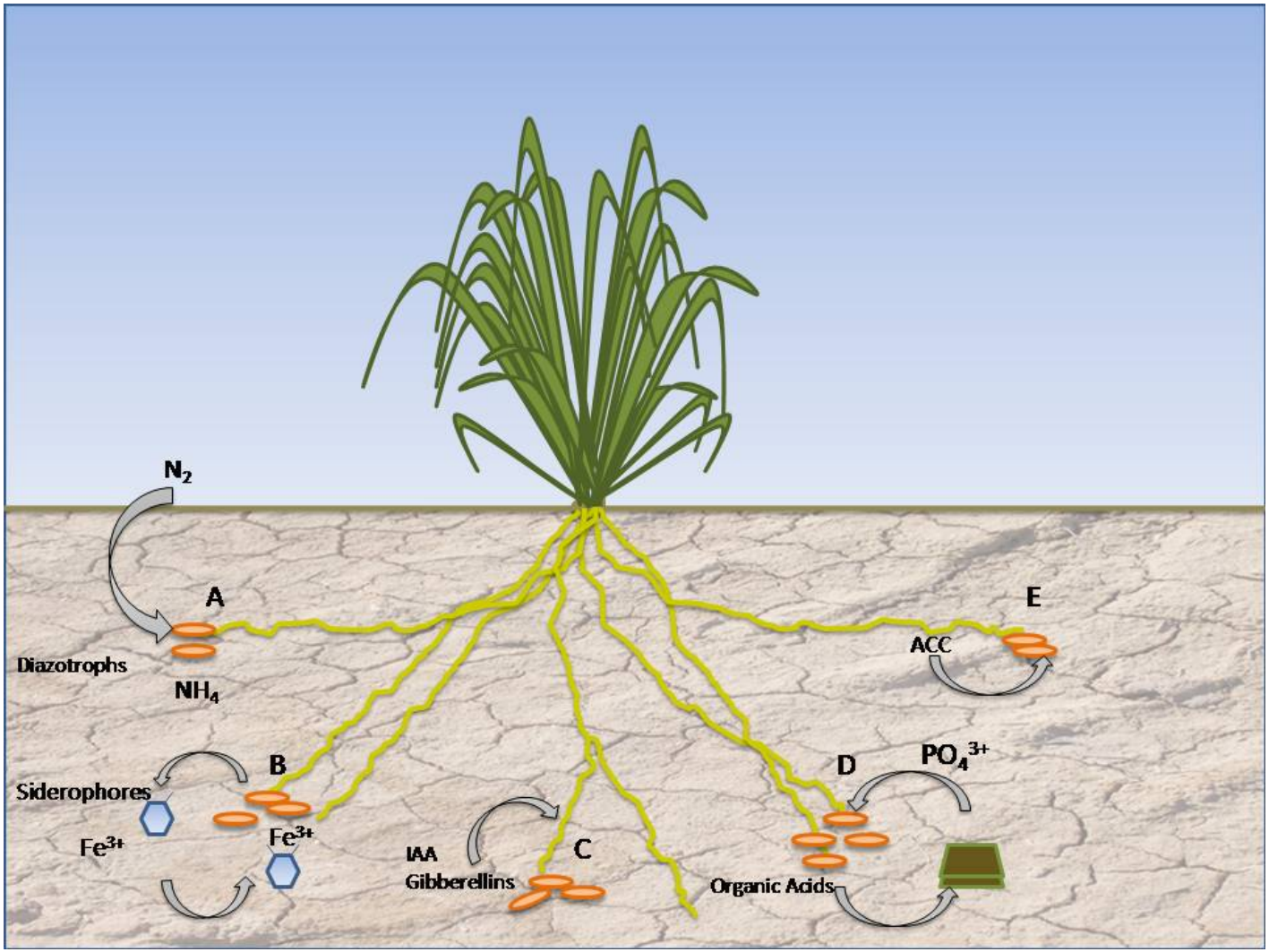
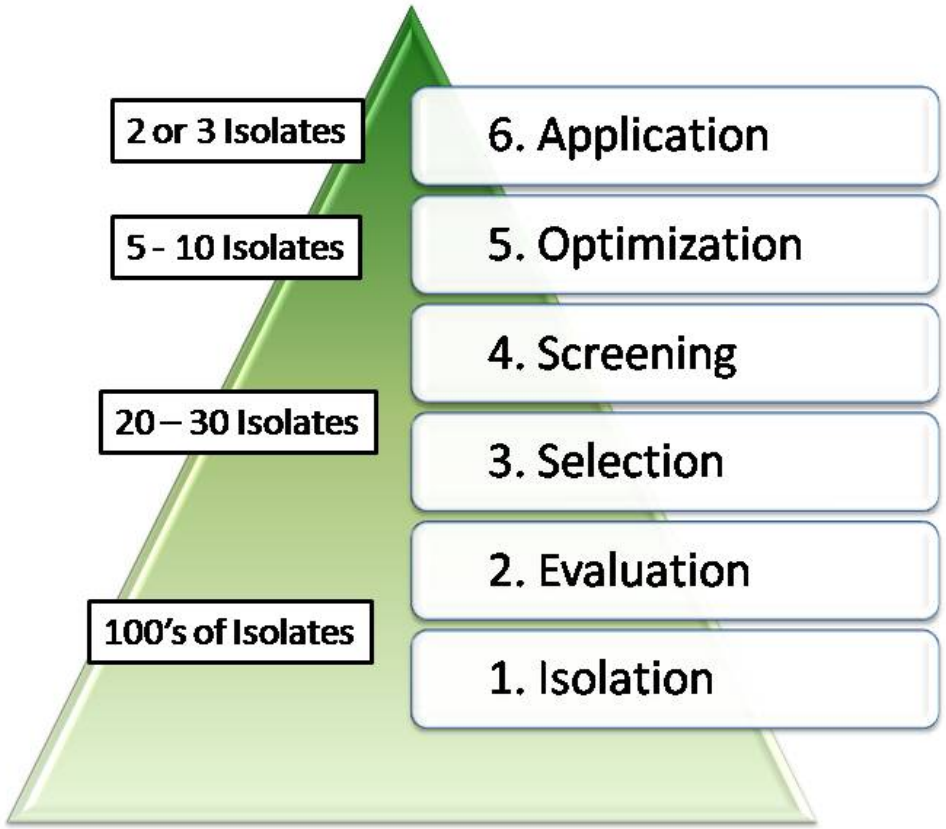


Figure 2 Stepwise process for obtaining and utilizing PGPB in environmental applications



CHAPTER 2

PLANT GROWTH-PROMOTING BACTERIA FOR THE PHYTOSTABILIZATION OF MINE TAILINGS

Abstract

Eolian dispersion of mine tailings in arid and semi-arid environments is an emerging global issue for which economical remediation alternatives are needed. Phytostabilization, the revegetation of these sites with native plants, is one such alternative. Revegetation often requires addition of bulky amendments such as compost which greatly increases cost. We report the use of plant growth-promoting bacteria (PGPB) to enhance the revegetation of mine tailings and minimize the need for compost amendment. Twenty promising PGPB isolates were used as seed inoculants in a series of greenhouse studies to examine revegetation of an extremely acidic, high metal content tailings sample previously shown to require 15% compost amendment for normal plant growth. Several isolates significantly enhanced growth of two native species, quailbush and buffalo grass, in tailings. In this study, PGPB/compost outcomes were plant specific; for quailbush, PGPB were most effective in combination with 10% compost addition while for buffalo grass, PGPB enhanced growth in the complete absence of compost. Results indicate that selected PGPB can improve plant establishment and reduce the need for compost amendment. Further, PGPB activities necessary for aiding plant growth in mine tailings likely include tolerance to acidic pH and metals.

1.0 Introduction

Mining activities have created a global problem in the form of mine tailings, with heavily impacted regions located in arid and semi-arid environments in northern Mexico and the western United States, the Pacific coast of South America (Chile and Peru), southwestern Spain, western India, South Africa, and Australia (Custer, 2003). Mine tailings are generated during mineral ore processing and are the primary component of mining waste (Mendez and Maier, 2007). Acidic pH, high metal content, low nutrients, impacted microbial community, and lack of soil structure leave these sites barren of vegetative cover and highly susceptible to both eolian dispersion and water erosion. The Klondyke mine tailings (Klondyke, AZ), constitute a state Superfund site that contains approximately 70,000 m^3 of acidic tailings with elevated levels of As, Cd, Pb, and Zn (Tummala and Humble, 1998). The latter two metals are considered most problematic for plant and microbial growth because they are present at solid phase concentrations up to several thousand mg kg^{-1}. Erosion of these tailings is of concern because they are adjacent to Aravaipa Creek, an important riparian system in southern Arizona, and elevated Pb and Cd have been detected in fish downstream of the tailings site (King and Martinez, 1998).

Efforts are currently underway to investigate the potential for remediation of Klondyke using phytostabilization. The goal of this strategy is to stabilize tailings piles by establishing a vegetative cover that does not hyperaccumulate metals. The desired outcomes include a significant decrease in eolian and waterborne tailings dispersion and a

visually attractive plant cover with above ground tissues meeting acceptable metal levels to protect surrounding wildlife. Prior greenhouse research has indicated that optimal growth of a model desert native plant, quailbush, in Klondyke tailings requires addition of 15% compost (w/w) (Mendez et al., 2007). Such a compost requirement is feasible but adds significantly to the cost and logistics of remediation. Thus, it is desirable to investigate possibilities for reducing the amount of compost necessary for plant establishment.

Previous studies have demonstrated the benefits of utilizing plant growth-promoting bacteria (PGPB) for establishment of vegetation on desertified or contaminated sites (Bashan et al., 1999; Burd et al., 1998; Dell 'Amico et al., 2005) and a limited number of studies have explored the use of PGPB in mine tailings (Petrisor et al., 2004; Wu et al., 2006). PGPB have been shown to enhance plant growth through a variety of mechanisms including: the production of growth hormones such as indoleacetic acid (IAA), siderophore production to aid in nutrient acquisition and suppression of plant pathogens, phosphate solubilization activity, and the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase which is thought to be an important mechanism for reducing plant ethylene concentrations under stressed conditions (Compant et al., 2005; Glick et al., 1998; Holguin and Glick, 2003; Patten and Glick, 2002; Penrose and Glick, 2001; Press et al., 2001; Rodriguez and Fraga, 1999; Singh and Kapoor, 1999; Vessey, 2003).

Here we report the use of PGPB to reduce the amount of compost necessary to establish two native plants, quailbush and buffalo grass, in the Klondyke tailings. A collection of 131 bacterial isolates was obtained from the rhizospheres of quailbush plants grown in composted Klondyke tailings, bulk mine tailings, and our laboratory collection. The objectives of our research were: (1) to evaluate this collection of bacterial isolates for their potential plant growth-promoting (PGP) activities, the ability to grow in acidic conditions, and their tolerance to metal stress, and (2) to evaluate a subset of 20 isolates from this collection that displayed desirable PGP mechanisms for the ability to enhance the growth of quailbush and buffalo grass in Klondyke tailings.

2.0 Experimental Section

Bacterial Isolates Ninety three isolates were obtained from the rhizosphere of quailbush plants grown in Klondyke tailings (Mendez et al., 2007), 21 from bulk tailings samples collected from the Klondyke site (Mendez et al., 2007), 5 from the Boston Mill mine tailings site on the San Pedro River (Rosario et al., 2007), and 12 isolates from our laboratory culture collection. Samples were plated in triplicate on R2A (EM Science, Gibbstown, NJ) amended with 200 mg L^{-1} cyclohexamide to inhibit fungal growth and incubated for five days at 23^{o}C. Colonies were selected and isolated based on morphological differences. All 131 bacterial isolates were maintained on R2A at 22^{o}C, gram-stained, and screened as described below.

Native Plants Two plants native to the desert southwest ecosystem surrounding Klondyke were used in this study, the shrub *Atriplex lentiformis* (Torr.) S. Wats.,

commonly known as quailbush and the grass *Buchloe dactyloides* (Nutt.) Engelm., commonly known as buffalo grass. Seeds were obtained from Carter Seeds, Vista, CA (quailbush) and Western Native Seed, Coaldale, CO (buffalo grass).

Mine Tailing and Soil Control A single composite Klondyke tailings sample was oven-dried at 105°C, sieved through a 2-mm mesh screen, and analyzed for texture (Burt, 2004). Tailings mineralogy was derived from powder X-ray diffraction and X-ray absorption spectroscopy analysis. Total organic carbon (TOC) and total nitrogen (TN) were measured in the solid phase using a Shimadzu high temperature combustion TOC/TN analyzer (nitrogen includes both organic and inorganic forms). The pH, electrical conductivity (EC), dissolved organic carbon (DOC) and dissolved nitrogen (DN, organic plus inorganic) were measured in a saturated paste extract (1:1) in deionized water. Samples were prepared by microwave acid digestion using EPA Method 3051 (US Environmental Protection Agency, 2004) for total element (As, Cd, Cu, Fe, K, Mn, Na, Pb, Zn) analysis by ICP–MS. Vinton Loamy Sand, previously characterized (Jordan et al., 2004), was employed as an uncontaminated control soil.

PGPB Assays Isolates were tested for the ability to solubilize phosphate on dicalcium phosphate medium (DCP) (Goldstein, 1987). Isolates producing a zone of clearing on DCP plates after 14 days at 23^{o}C were considered positive for phosphate solubilization activity.

Qualitative Siderophore Assay Siderophore production was assayed for on iron-deficient chromazurol S (CAS) plates (Schwyn and Neilands, 1987). Colonies were transferred

from R2A onto the CAS medium and allowed to incubate at 23^{o}C for 14 days. Colonies that produced orange halos on CAS plates were considered positive for siderophore production.

Quantitative Siderophore Assay Siderophore production was quantified from isolated colonies from an R2A plate as described previously (Schwyn and Neilands, 1987). Siderophore quantification was conducted in triplicate and mean siderophores production values are reported in μg 10^8 CFU.

Indoleacetic Acid Assay Isolates were assayed for the ability to produce IAA (Patten and Glick, 2002), except R2B was used as the growth medium. Isolates were transferred from R2A plates to 5 mL of R2B and incubated on a rotary shaker (200 rpm) at 23^{o}C for 48 h. Turbid R2B (50 μL) culture were then transferred to 5 mL of fresh R2B supplemented with 0, 100, or 500 mg L^{-1} of tryptophan and incubated for 48 h. A 1.5 mL aliquot was removed and centrifuged at (22,000 x *g*) for 10 min. Cell supernatant (1 mL) was removed and mixed with 4.0 mL of Salkowski's Reagent (150 mL concentrated H_2SO_4, 250 mL distilled deionized H2O, and 7.5 mL 0.5 M $FeCl_3$-$6H_2O$), incubated for 20 min at room temperature, and then IAA was quantified spectrophotometrically at 535 nm. A standard curve was created using R2B containing known concentrations of IAA (Fisher Scientific). IAA production was measured in triplicate at 500 mg L^{-1} tryptophan and mean values were reported as μg per 10^7 CFU.

ACC Deaminase Activity Isolates were screened for their ability to obtain nitrogen from ACC a potential mechanism for decreasing plant ethylene levels (Glick and Penrose,

1998). Isolates were grown on a rotary shaker (200 rpm) at 22°C in 5.0 mL R2B until turbid and then 50 µL were transferred to 5.0 mL of DF medium, supplemented with 30 mM NH_4^+ as $(NH_4)_2SO_4$, pH 7.2. Cultures reaching visual turbidity in this medium were transferred to 5.0 mL of fresh DF medium containing 3.0 mM ACC as a sole source of N. Isolates producing visible turbidity in DF-ACC medium were assumed to have the ability to use ACC as a sole source of N. Each isolate was transferred four additional times to fresh DF-ACC medium for confirmation.

Tolerance of Acidic pH To determine tolerance to acidic pH, isolates were initially grown in 5.0 mL R2B (pH 7.2) and then transferred to 5.0 mL R2B (pH 5.0). Cultures were monitored and isolates displaying visible turbidity within 8 days were considered positive for growth. All cultures positive for growth at pH 5.0 were transferred (50 µL) to 5.0 mL R2B (pH 4.0) and again monitored for 8 days.

Metal Tolerance Screening Isolates were screened for the ability to grow on modified R2A (g L^{-1}; 1.0, glucose; 0.3, proteose peptone No. 3; 0.3, yeast extract; 0.3, starch; 0.3, sodium pyruvate; 0.1, K_2HPO_4; 0.1, $Na_5O_{10}P_3$; 0.1, $MgSO_4 \cdot 7H_2O$; 15 noble agar, adjusted to pH 6.5 using $C_8H_5O_4K$) amended with either 0, 0.25, 0.5, 1.0 or 1.5 mM $Pb(NO_3)_2$ or $ZnSO_4$. Nitrate and sulfate concentrations were normalized to 10 mM in all Pb and Zn plates using KNO_3 and $MgSO_4 \cdot 7H_2O$, respectively. Isolates that grew on these plates after 5 days were considered to show tolerance to the respective metal concentration.

Isolate Identification Selected isolate colonies were inoculated into 5 mL of R2B and incubated on a shaker (180 rpm) at 23°C until turbid. A 1 mL aliquot of bacterial culture was centrifuged at 14,000 x *g*, decanted, resuspended in 1 mL of sterile distilled water, and cells were lysed using two freeze-thaw cycles followed by 15 min in a boiling water bath. The 16S rRNA gene was amplified from 3 μL cell lysate as previously described (Ikner et al., 2007). Products were purified using the QIAquick® PCR purification kit (QIAGEN Inc., Valencia, CA) and then submitted to the University of Arizona Research Labs Genomic Analysis and Technology Core for sequencing with an ABI3730xl DNA Analyzer (Applied Biosystems, Foster City, CA). Nucleotide sequences were used to identify bacterial isolates by BLAST analysis and then were submitted to the GenBank database under accession numbers DQ507202 to DQ507211 and DQ898296 to DQ898301.

PGPB Inoculation and Growth of Quailbush in Vinton Soil and in Klondyke Tailings A series of three greenhouse experiments were performed to evaluate the selected PGPB. The first experiment examined growth of quailbush in Vinton soil to assess whether any of the PGPB were pathogenic or otherwise inhibitory for growth. Vinton soil was wetted to field capacity 48 h prior to planting and then placed into 50 cm^3 pots. Quailbush seeds were surface sterilized by placing approximately 200 seeds into a sterile 50 mL centrifuge tube containing 30 mL of a 2% bleach solution for 10 min followed by a 3 min rinse in a 0.01% sodium thiosulfate solution to neutralize the bleach. The seeds were then rinsed three times in 30 mL of sterile water. PGPB cultures were prepared 48 h prior to inoculation by transferring single colonies from R2A plates to 5.0 mL of R2B

and incubating on a rotary shaker (200 rpm) at 23°C. Immediately prior to inoculation, the cultures were centrifuged at 12,100 x *g* for 10 min, culture supernatant was removed and cells were resuspended in sterile PBS (g L^{-1}): 8.0 NaCl, 0.2 KCl, 1.44 Na_2HPO_4, 0.24 KH_2PO_4, adjusted to pH 7.4. Sterilized seeds were aseptically transferred to each individual isolate suspension and allowed to incubate for 10 min with a 5 sec vortexing period every minute. Inoculated seeds were planted 0.5 cm deep with a sterile tweezers at depth of approximately 0.5 cm and the pots were placed into a controlled greenhouse environment at 30°C receiving irrigation for three min three times daily. Non-inoculated, sterile seed control treatments were created by subjecting seeds to the same sterilization and planting procedures minus the inoculation (referred to as the sterile control). Non-sterilized seeds, soaked in sterile water were used as a second control (referred to as the nonsterile control). Each treatment consisted of 5 seeds per pot and 10 replicate pots. After 30 d, seedlings were counted to determine germination and then were thinned to one plant per pot. Dry plant biomass was determined 75 days after planting. At this time each plant was carefully harvested (roots and shoots were separated) and rinsed gently under running water to remove all tailings and compost material. Plant roots and shoots were then placed in individual aluminum foil packets, dried for 48 h in a 60°C oven, and weighed.

The second greenhouse experiment was performed to evaluate the impact of the PGPB on growth of quailbush in Klondyke tailings amended with either 0% or 10% compost (w/w) (obtained from the University of Arizona Controlled Environment Agricultural Center, Tucson, AZ). Preparation and inoculation was as described for the

first experiment. After 30 days of growth, germination was evaluated and seedlings were thinned to one plant per pot. Dry plant biomass was determined for each plant after 75 days of growth.

The third greenhouse experiment was performed to further evaluate promising PGPB from the first two studies in larger pots and a second plant, *B. dactyloides*, was tested in addition to *A. lentiformis*. Each plant was grown in tailings amended with either 0% or 10% compost (w/w). This study was performed using larger (1625 cm^3) pots and a 90 d growth period after which dry plant biomass was determined for each plant. Each treatment had 5 replicate pots with 10 seeds per pot. For *A. lentiformis*, pots were thinned to one plant per pot after 4 wk. The *B. dactyloides* treatments were not thinned.

In each study, plants received 0.6 cm irrigation pot^{-1} three times daily. Daily temperature fluctuations were held constant with high humidity at 20^oC during the evening and approximately 35^oC during the day, simulating typical growth conditions during a Sonoran Desert monsoon season (mid July – late August). No supplemental lighting was used.

Statistical Analysis Statistical analysis was conducted using SAS Version 9.1 (SAS Institute Inc. Cary, NC). Significant treatment effects for plant root, shoot and total biomass and root-to-shoot data were evaluated using a one-way ANOVA ($p < 0.05$). For each treatment, significant differences between means were determined by the Duncan's Multiple Range Test ($p < 0.05$).

3.0 Results and Discussion

Bacterial Isolation and Screening This study reports the screening, identification, and testing of PGPB for use in phytostabilization of mine tailings found in arid and semi-arid environments with the goal of minimizing compost amendment. PGPB have been widely studied for agricultural applications (Cattelan et al., 1999; Gupta et al., 1999; Mayak et al., 2004), and some studies have demonstrated that PGPB can aid in revegetation of desertified regions (Bashan et al., 1999). However, little is known about phytostabilization of mine tailings; previous studies performed have investigated the use of soil-isolated PGPB for the phytostabilization of mildly toxic mine tailings at high compost amendment rates (Petrisor et al., 2004; Wu et al., 2006).

PGPB useful for establishment of plants in mine tailings may require specific traits beyond those normally associated with PGPB effective for agriculture. For example, Burd et al. (1998) reported that a heavy-metal resistant, siderophore producing, ACC-deaminase positive strain of *Kluyvera ascorbata* increased plant growth in a Ni-contaminated soil. The authors proposed that this inoculant acted by reducing Ni toxicity and alleviating Ni-induced stress via ACC-deaminase activity. Based on this consideration, we hypothesized that microbial populations residing in bulk mine tailings or those associated with plants grown in tailings are subject to selective pressures that may make them more suitable for use in revegetation of tailings than isolates not exposed to these conditions. For example, desirable traits, in addition to traditional PGP mechanisms, might include the ability to grow at acidic pH and the ability to tolerate elevated metal concentrations. Thus, our initial collection of 131 isolates was a diverse

assortment of bacteria obtained primarily from bulk mine tailings and from the rhizosphere of quailbush plants grown in composted tailings. Of these isolates, 86 were Gram positive and 45 were Gram negative (Table 2). A majority (62%) produced siderophores while smaller proportions of the collection had the ability to solubilize calcium phosphate (40%), both abilities important for providing plants with essential nutrients (Rodriguez and Farga, 1999). Nearly 21% of the isolates possessed ACC-deaminase activity while 57% of the isolates produced IAA. A large proportion of the isolates (80%) were able to grow at pH 5, and 16% of isolates grew at pH 4, demonstrating their potential to survive in the acidic tailings environment. In general, PGP mechanisms were distributed evenly among the Gram-positive and Gram-negative isolates in the collection.

A further quantitative analysis was performed for two of the above PGP activities; siderophore and IAA production (Table 3). Results show that 27% of isolates produced siderophores at a concentration of 10 mg L^{-1} or greater, while 15% produced at 100 µg mL^{-1} or greater. These levels of siderophore production are comparable to production by soil rhizobacteria (56 to 140 mg L^{-1}) (Alexander and Zuberer, 1991). In terms of IAA production, while no isolates produced IAA without the supplementation of tryptophan, 20% of isolates produced IAA in concentrations from 10 to 25 µg mL^{-1} medium when supplemented with 100 µg mL^{-1} tryptophan and 76% of isolates produced IAA when supplemented with 500 µg mL^{-1} tryptophan (Table 2). Thakuria et al. (2004) report comparable levels of IAA production, ranging from 2.0 to 21.6 mg L^{-1}, in culture supernatants from soil rhizosphere bacteria; however, these isolates were supplied with

substantially higher amounts of L-tryptophan. In summary, the collection of 131 isolates from bulk and planted tailings exhibited a variety of PGP activities and a large proportion of the collection grew at a moderately acidic pH of 5.

Following screening of the collection, a sub-set of 20 isolates was subjectively selected based on the PGPB assay results (Table 1). Selection criteria included isolates exhibiting only one PGPB activity (e.g., K6-19, K6-11B, and MTR-18) and isolates with different mixtures of PGPB activities (e.g., *B. vietnamensis*, MTR-21A, and MTR-61). One isolate, SP-1, did not have any of the measured PGPB activities and was chosen as a control. Many of the genera represented by this sub-set have been previously reported to enhance plant growth. For example, MTR-1, MTR-61, M22, and K6-19 represent bacterial genera previously identified as exhibiting biological control against plant pathogens (Compant et al., 2005).

Mine Tailings Analysis Selected physical-chemical analyses showed that the tailings have a pH of 4.54 ± 0.02, TOC and TN of 360 ± 68 and 67 ± 12 g kg^{-1}, respectively, DOC and DN of 38 ± 6.9 and 20 ± 1.2 g kg^{-1}, respectively, and EC of 3.0 ± 0.12 dS m^{-1}. The tailings texture is 51.9% sand, 26.4% silt, and 21.7% clay with major mineral constituents of quartz, orthoclase feldspar, and jarosite, and minor contributions from plumbojarosite and goslarite, which are the principal Pb and Zn bearing constituents, respectively. The major metals in the tailings are (mg kg^{-1} ± relative standard deviation) As, 91 ± 10%; Cd, 2.4 ± 0.7%; Cu, 653 ± 1%; Fe, 26,560 ± 1%; Mn, 2,811 ± 1%; Pb, 4,620 ± 1%; Zn, 1,400 ± 1%.

Greenhouse Studies An initial greenhouse study, conducted in the Vinton soil, showed that none of the 20 isolates inhibited growth of quailbush, e.g., there was no difference between the inoculated treatments and control plants ($p < 0.05$) (data not shown).

A second greenhouse study was performed to screen each of the 20 selected isolates for the ability to enhance growth of quailbush in either 0% or 10% compost-amended tailings (w/w). These amendment levels were chosen because a previous study showed that while 15% compost addition restored growth to levels comparable to that observed in an off-site soil control, the 0 and 10% treatments did not (Mendez et al., 2007). We therefore wanted to test whether PGPB could enhance plant growth at these sub-optimal compost treatment levels.

This 75 d experiment revealed that the average total biomass production in the 0% compost control treatments (0.071 ± 0.066 g) was similar to the biomass produced in the 10% compost control treatments (0.077 ± 0.027 g), although there was less variability in growth among plants in the 10% treatment. One noticeable difference between two compost levels in this experiment was that the root:shoot biomass ratio was greater for the 0% than for the 10% compost treatment. This suggests that in the absence of compost (and the nutrients provided therein) the plants must put most of their energy into establishing a root system with large surface area to aid in nutrient uptake.

For the inoculated 0% compost treatments, only one isolate, SP-1 (most closely related to *Microbacterium)*, significantly ($p < 0.05$) increased total plant biomass in the non-composted tailings by approximately 4-fold to 0.35 ± 0.28 g although again there

was great variability in individual plant growth (Fig. 1). Although *Microbacterium* isolates have previously been shown to enhance growth in field-grown potato plants (Sessitsch et al., 2004), SP-1 did not display any PGPB activities nor did it show tolerance to either metals or to low pH (Table 1) so its PGPB activity is not apparent. Further, SP-1 did not stimulate growth in the third longer-term study suggesting it may not have good survival potential for tailings.

The average quailbush survival rate in the 0% compost treatment was 4.8 ± 1.5 out of 10. Two isolates, K6-11B and MTR-71, enhanced plant survival to 8 (Fig. 1). This suggests that some PGPB may play a role in facilitating plant survival while not necessarily enhancing plant biomass and could be used as part of a PGPB mixture to enhance overall survival and growth. It should also be noted that four isolates (MTR-18, MTR-21A, MTR-61, and MTR-70) in the 0% compost treatment resulted in less than three surviving plants and were not included in Fig. 1.

In the 10% compost treatment, plant survival was improved to 7.9 ± 1.6 which was significantly higher than survival in the 0% treatment ($p < 0.0001$). In terms of plant growth, plants inoculated with 18 out of the 21 isolates had an average total dry biomass (0.12 ± 0.031 g) that was 1.5-fold greater than the control plants (0.077 ± 0.027 g). Four of these isolates (MTR-21A, MTR-1, MTR-18, and MTR-11) had significantly ($p < 0.5$) higher average dry plant biomass (0.016 ± 0.018 g) than the control plant treatments, with the increase ranging from 2 to 2.5-fold (Fig. 2). Three of these isolates, representing the genera *Clavibacter* (MTR-21A), *Microbacterium* (MTR-18), and *Streptomyces* (MTR-1),

have been reported to improve plant growth in previous studies of PGPB for agricultural applications (Bertrand et al., 2001; Sturz et al., 2001).

One intriguing observation is that MTR-18 and MTR-21A, the isolates that resulted in the greatest plant biomass increase in the 10% compost treatment, were not effective in the 0% compost treatment. These results suggest that some isolates may display PGP activities under different environmental conditions, e.g., plant type, compost rate. For example, neither MTR-18 nor MTR-21A showed tolerance to Pb and Zn (Table 1), which may have resulted in their ineffectiveness at the 0% compost treatment in which there was no compost material present to buffer metal toxicity. Thus, a minimum amount of compost amendment may be necessary to allow growth of some PGP isolates such as MTR-18 and MTR-21A.

A third 90 d greenhouse study was performed with selected isolates that enhanced quailbush growth in the previous experiment. The goals of this study were to confirm the previous results, scale up the size of the pots used for the study to allow for a longer growth period, and to extend the study to a second plant, buffalo grass. Results with quailbush showed that the addition of compost alone doubled the biomass in control plants from 1.2 ± 0.5 g (0% compost, data not shown) to 2.1 ± 0.4 g (10% compost). This is in contrast to the previous study where there was no difference between the control plants in the 0% and 10% treatments. Also, there was up to a 30-fold increase in total biomass produced in the controls from the 75 and 90 d experiments. These data suggest that the length of the study is an important consideration.

There was no significant effect of the inoculated isolates on quailbush growth in the 0% compost treatment (data not shown). However, for the 10% compost treatment, plants inoculated with MTR 21A had significantly ($p < 0.05$) higher biomass (3.2 ± 1.1 g) than the uninoculated control (2.1 ± 0.4 g) (Fig. 3). Isolate MTR 45B also increased the average biomass to 3.3 ± 2.5 but due to high variability in growth among replicates, this increase was not significantly different from the control. These results indicate that even over the longer term, inoculation has promise to aid in plant growth and establishment. However, it should be noted that only one of the four isolates, MTR 21A, that produced significantly better growth in the short-term study was also effective in the longer-term study. Tolerance to low pH seemed to be an important factor in these longer studies; both MTR-21A and MTR-45B were able to grow at pH 4 (only five of the 21 isolates tested had this ability) (Table 1).

Results with buffalo grass showed that the addition of compost alone resulted in a 5-fold increase in total biomass from 0.71 ± 0.35 g to 3.7 ± 2.0 g. While several isolates inoculated into the 10% treatment increased biomass production by up to 1.5-fold, the increase was not statistically significant (data not shown). However, four of the six isolates tested, MTR-1, MTR-11, MTR45B, and a mix of MTR-44 and K4-10C (Arthrobacter Mix) doubled the growth of buffalo grass in the 0% compost treatment ($p < 0.5$) (Fig. 4). All of these isolates except K4-10C, which was part of the mixed inoculum, showed tolerance to both Pb and Zn and all of these isolates showed growth at pH 5 (Table 1).

In summary, the establishment of a successful vegetative cover on mine tailings found in arid and semi-arid environments is challenging and normally requires amendment with organic matter. The goal of this study was to determine whether the requirement for organic matter could be reduced by the use of PGPB to improve the economics of this remediation approach. The results of this study demonstrate that PGPB can successfully enhance growth of native plants in mine tailings from arid environments and at least partially replace the requirement for compost amendment. The impact of PGPB seems to be plant specific, e.g., buffalo grass responded more strongly to the PGPB at the 0% compost treatment while quailbush responded more strongly in the 10% compost treatment. This study also suggests that very short-term (small pot) trials may not accurately identify successful PGPB, possibly due to poor persistence of the PGPB in the rhizosphere over a longer time scale. Finally, this study strongly indicates the importance of identifying PGPB that can persist in the tailings rhizosphere, e.g., exhibit metal and pH tolerance, in addition to providing plants with key PGP mechanisms. There is great likelihood that the performance of PGPBs could be enhanced through the use of PGPB mixtures and a better understanding of exactly what types of tolerance and PGP mechanisms are most important for optimal plant establishment.

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5.0 References

Alexander, D.B., and D.A. Zuberer. 1991. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol. Fertil. Soils. **12:**39-45.

Bashan, Y., A. Rojas, and M.E. 1999. Puente. Improved establishment and development of three cactus specie inoculated with *Azospirillum brasilense* transplanted into disturbed urban desert soil. Can. J. Microbiol. **45:**441-451.

Bertrand, H., R. Nalin, R. Bally, and J.-C. Cleyet-Marel. 2001. Isolation and identification of the most efficient plant growth-promoting bacteria associated with canola (*Brassica napus)*. Biol. Fertil. Soils. **33:**152-156.

Burd, G.I., D.G. Dixon, and B. Glick. 1998. A plant growth promoting bacterium that decreases nickel toxicity in seedlings. Appl. Environ. Microbiol. **64:**3663-3668.

Burt, R. 2004. Ed. Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report No. 42. Version 4.0, NRCS.

Cattelan, A.J., P.G. Hartel, and J.J. Fuhrmann. 1999. Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Soc. J. America. **63:**1670-1680.

Compant, S., B. Duffy, J. Nowak, C. Clément, and E.A. Barka. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. **71:**4951-4959.

Custer, K. 2003. Cleaning Up Western Watersheds; Mineral Policy Center; EarthWorks: Washington DC.

Dell' Amico, E., L. Cavalca, and V. Andreoni. 2005. Analysis of rhizobacterial communities in perennial *Graminaceae* from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. Microbial Ecol. **52:**153-162.

Glick, B., D.M. Penrose, and J. Li. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J. Theor. Biol. **190:**63-68.

Goldstein, A. H. 1987. Bacterial solubilization of mineral phosphates: historical perspective and future prospects. Am. J. Altern. Agric. **1:**51-57.

Gupta, A., V. Rai, N. Bagdwal, R. Goel. 2005. In situ characterization of mercury-resistant growth-promoting fluorescent pseudomonads. Microbiol. Res. **160:**385-388.

Holguin, G., and Glick, B.R. 2003. Transformation of *Azospirillum brasilense* Cd with an ACC deaminase genes from *Enterobacter cloacae* UW4 fused to the Tet^R gene promoter improves its fitness and plant growth promoting ability. Microbial Ecol. **46:**122-133.

Ikner, L.A., R.S. Toomey, G. Nolan, J.W. Neilson, B.M. Pryor, and R.M. Maier. 2007. Cultural microbial diversity and the impact of tourism in Kartchner Caverns, Arizona. Microbial Ecol. **53:**30-42.

Jordan, F.L., S.K. Sandrin, R.J. Frye, M.L. Brusseau, and R.M. Maier. 2004. The influence of system complexity on bacterial transport in porous media. J. Contam. Hydrol. **74:**19-38.

King, A.K., and M. Martinez. 1998. *Metals* in Fish Collected from Aravaipa Creek; U.S. Fish and Wildlife Service; Arizona Ecological Services Field Office: Phoenix, AZ.

Mayak, S., T. Tirosh, and B. Glick. 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Phys. Biochem. **42:**565-572.

Mendez, M.O. E.P. Glenn, and R.M. Maier. 2007. Phytostabilization potential of quailbush for mine tailings: growth, metal accumulation, and microbial community changes. J. Environ. Qual. **36:**245-253.

Mendez, M.O., and R.M. Maier. 2007. Phytoremediation of mine tailings in temperate and arid environments. Rev. Environ. Sci. Bio/Tech. DOI: 10.1007/s11157-007-9125-4.

Patten, C.L., and B.R. Glick. 2002. Role of *Pseudomonas putida* indoleacetic acid development of the host plant root system. Appl. Environ. Microbiol. **68:**3795-3801.

Penrose, D.M., and B.R. Glick. 2001. Levels of ACC and related compounds in exudate and extracts of canola seeds treated with ACC deaminase-containing plant growth-promoting bacteria. Can. J. Microbiol. **47:**368-372.

Petrisor, I.G., S. Dobrota, K. Komnitsas, I. Lazar, J.M. Kuperberg, and M. Serban. 2004. Artificial inoculation – perspectives in tailings phytostabilization. Int. J. Phytostabilization. **6:**1-15.

Press, C.M., J.E. Loper, and J.W. Kloepper. 2001. Role of iron in rhizobacteria-mediated induced systemic resistance of cucumber. Phytopathology. **6:**593-598.

Rodriguez, H., and R. Fraga. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. **17:**319-339.

Rosario, K., S.L. Iverson, D.A. Henderson, S. Chartrand, C. McKeon, E.P. Glenn, and R.M. Maier. 2007. Bacterial community changes during plant establishment at the San Pedro River mine tailings site. J. Environ. Qual. **36:**1249-1259.

Schwyn, B., and J.B. Neilands. 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. **160:**47-56.

Sessitsch, A., B. Reiter, and G. Berg. 2004. Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. Can. J. Microbiol. **50:**239-249.

Singh, S., and K.K. Kapoor. 1999. Inoculation with phosphate-solubilizing microorganisms and a vesicular-arbuscular mycorrhizal fungus improves dry matter yield and nutrient uptake by wheat grown in a sandy soil. Biol. Fertil. Soils. **28:**139-144.

Sturz, A.V., B.G. Matheson, W. Arsenault, J. Kimpinski, and B.R. Christie. 2001. Weeds as a source of plant growth promoting rhizobacteria in agricultural soils. Can. J. Microbiol. **47:**1013-1024.

Thakuria, D., N.C. Talukdar, C. Goswami, S. Hazarika, R.C. Boro, and M.R. Khan. 2004. Characterization and screening of bacteria from the rhizosphere of rice grown in acidic soils of Assam. Current Science. **86:**978-985.

Tummala, P.S.L., and W. Humble. 1998. Public Health Assessment; Klondyke Mine Tailings; Arizona Department of Health Services.

US Environmental Protection Agency. 2004. Test Methods for Evaluating Solid Waste; EPA SW-846: Washington, DC.

Vessey, J.K. 2003. Plant growth-promoting rhizobacteria as biofertilizers. Plant Soil. **255:**571-586.

Wu, S.C., K.C. Cheung, Y.M. Luo, and H.M. Wong. 2006. Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by *Brassica juncea*. Environ. Poll. **140:**124-135.

6.0 Figure Legends

Figure 1. The effect of PGPB inoculation on the total average dry plant mass of quailbush grown in Klondyke tailings in the absence of compost for 75 d (mean + 1 SD for total plant biomass). Lower and upper bars represent the average dry root biomass and average dry shoot biomass for each treatment, respectively. PGPB isolate IDs correspond to those given in Table 1. The number at the bottom of each bar represents the surviving plants out of ten replicates. Surface-sterilized (Sterile Control) and non-sterilized (Non-Sterile Control) *A. lentiformis* seeds served as controls. Four isolates, MTR-18, MTR-21A, MTR-61, and MTR-70, are not included because there were less than three surviving plants. Means with different letters are significantly different in total dry plant biomass at $p < 0.05$.

Figure 2. The effect of PGPB inoculation on the total average dry plant mass of quailbush grown in Klondyke tailings in the presence of 10% compost for 75 d (w/w) (mean + 1 SD for total plant biomass). Lower and upper bars represent the average dry root biomass and average dry shoot biomass for each treatment, respectively. PGPB isolate IDs correspond to those given in Table 1. Surface-sterilized (Sterile Control) and non-sterilized (Non-Sterile Control) *A. lentiformis* seeds served as controls. The number at the bottom of each bar represents the surviving plants out of ten replicates. Means with different letters are significantly different at $p < 0.05$.

Figure 3. The effect of PGPB inoculation on the total average dry plant mass of quailbush grown in Klondyke tailings in the presence of 10% compost for 90 d (w/w) (mean + 1 SD for total plant biomass). Lower and upper bars represent the average dry root biomass and average dry shoot biomass respectively, for each treatment. PGPB isolate IDs correspond to those given in Table 1. Surface-sterilized (Sterile Control) *A. lentiformis* seeds served as the control. The number at the bottom of each bar represents the surviving plants out of five replicates (following thinning). Means with different letters are significantly different at $p < 0.05$.

Figure 4. The effect of PGPB inoculation on the total average dry plant mass of buffalo grass grown in Klondyke tailings in absence of compost for 90 d (w/w) (mean + 1 SD for total plant biomass). Lower and upper bars represent the average dry root biomass and average dry shoot biomass respectively, for each treatment. PGPB isolate IDs correspond to those given in Table 1. Surface-sterilized (Sterile Control) *A. lentiformis* seeds served as the control. The number at the bottom of each bar represents the surviving plants out of fifty seeds. Means with different letters are significantly different at $p < 0.05$.

Table 1. Selected sub-set of bacterial isolates displaying desirable plant growth-promotion mechanisms. Identities and the results for each individual isolate's bioassay or tolerance assay results are listed. Isolates represented in this table were used in a series of greenhouse studies to evaluate each individual's potential to enhance plant growth in composted and non-composted Klondyke mine tailings.

Table 2. Qualitative assay screening results. Percentage of either Gram-positive, Gram-negative or the total collection of isolates that was positive for the listed plant growth-promotion mechanism or tolerant to the listed environmental factor.

Table 3. Quantitative assay screening results. Percentage of isolates that produced at or above the specified quantity of siderophores or IAA. The three different quantities listed for the IAA assay represent three different medium tryptophan concentrations.

Figure 1 *Atriplex lentiformis* biomass resulting from PGPB-treated seeds in non-composted Klondyke tailings

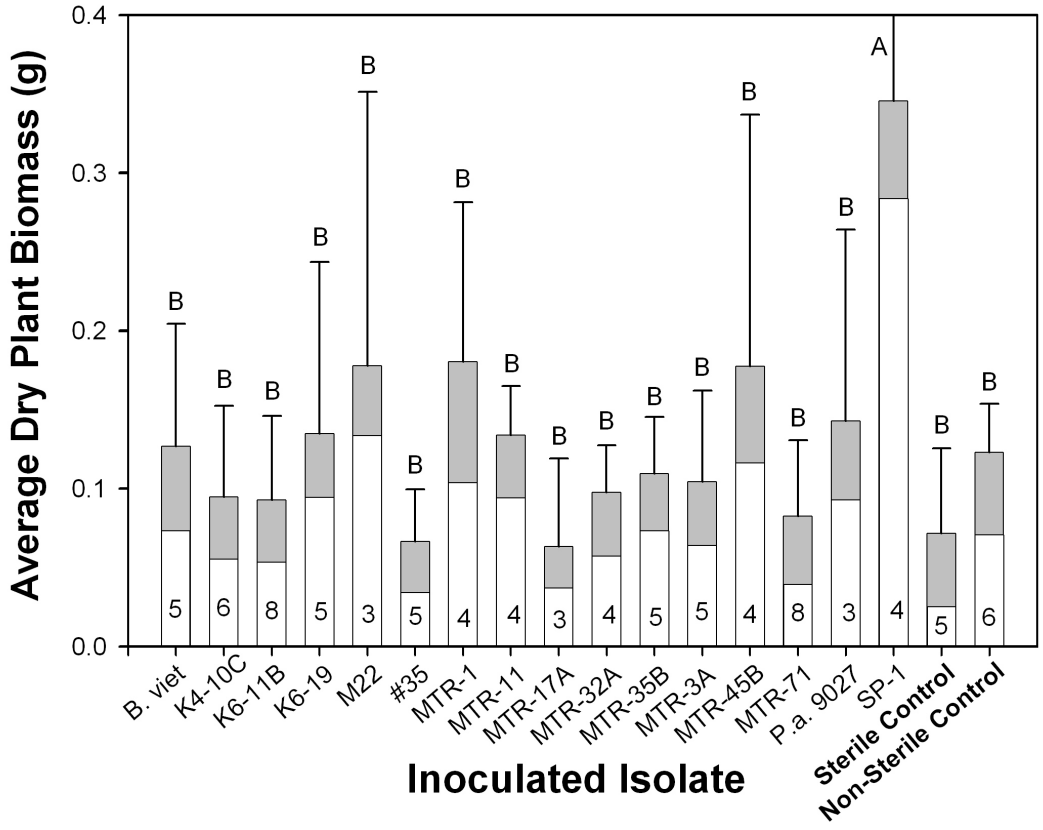


Figure 2 *Atriplex lentiformis* biomass from PGPB-treated seeds in Klondyke tailings receiving 10% compost (w/w)

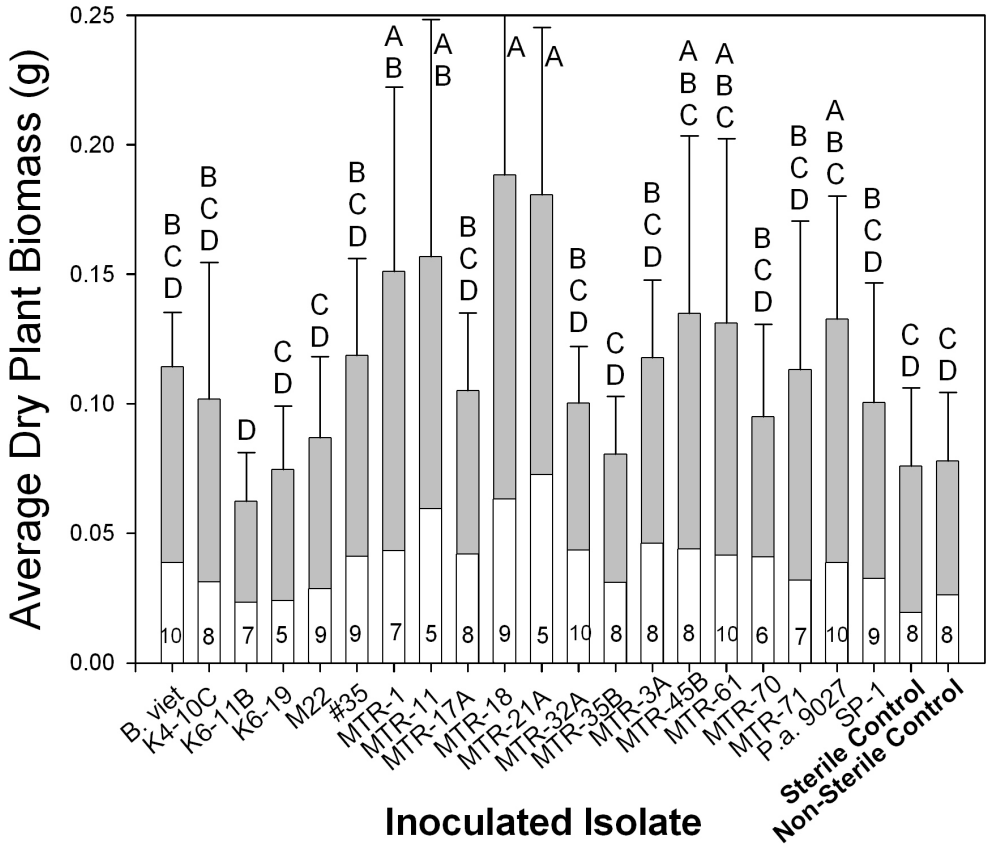


Figure 3 *Atriplex lentiformis* biomass in Klondyke tailings receiving 10% compost (w/w)

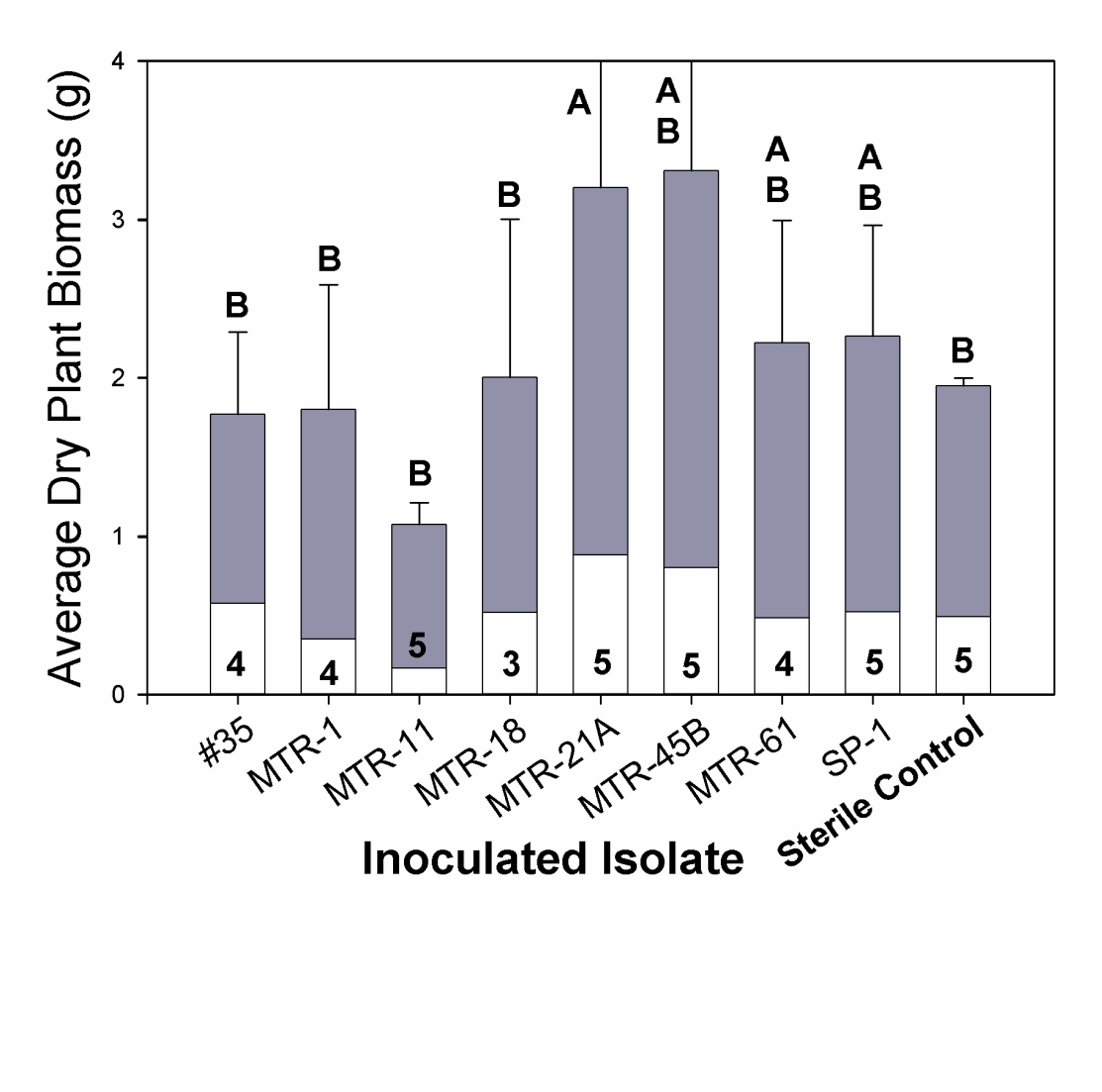


Figure 4 *Buchloe dactyloides* biomass in non-composted Klondyke tailings after 90 days

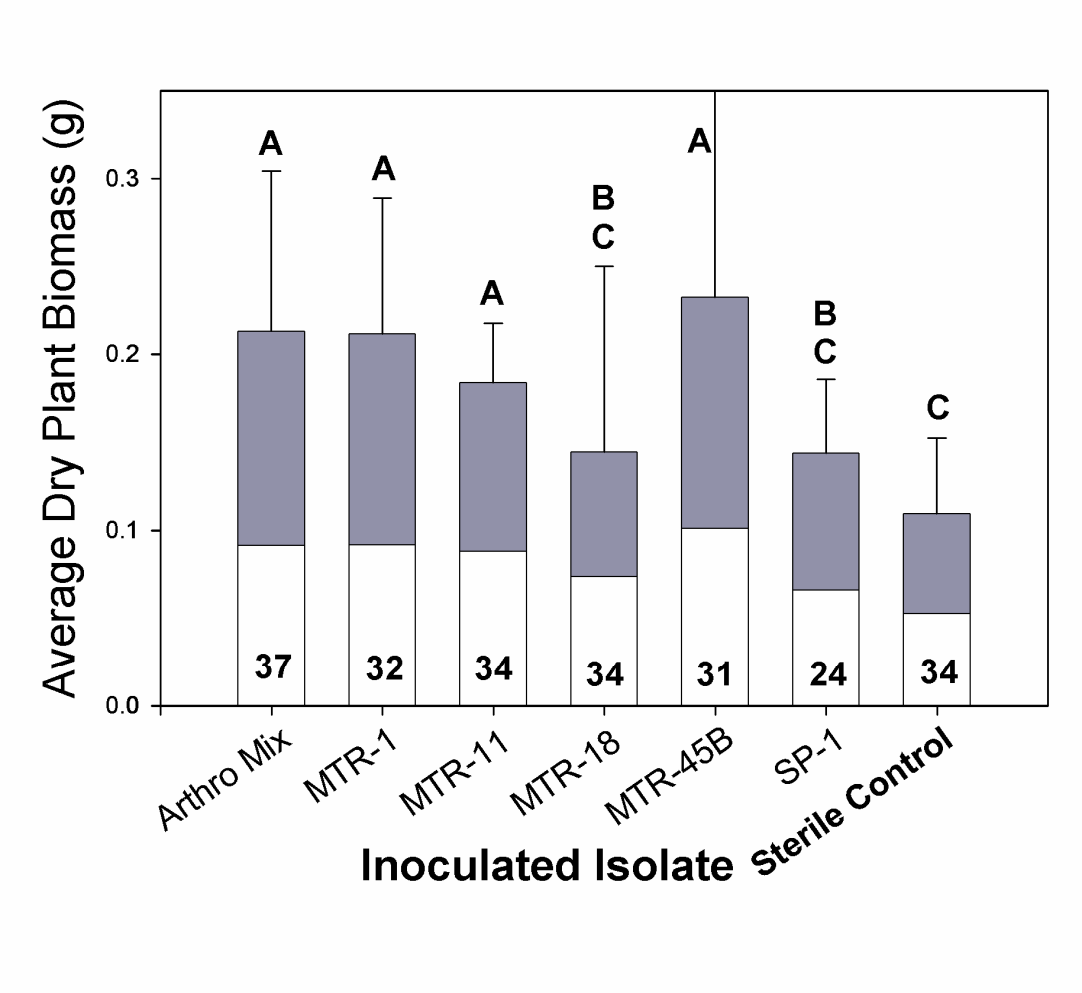


Table 1 Assessment of Potential Plant Growth-Promoting Qualities Displayed in Selected Bacterial Isolates

Isolate ID (Accession Number)	Nearest Neighbor (Accession Number)	Growth pH 5	Growth pH 4	Pb Assay[e]	Zn Assay[e]	CAS Assay	DCP Assay	ACC Deaminase	IAA Production[f]	Siderophore Production[g]
MTR-1 (DQ507205)	*Streptomyces sp.*[a] (AF389344)	+	-	0.25	1.5	-	-	+	55 ± 42	24 ± 10
MTR-3A (DQ898296)	*Rhizobium sp.*[a] (AM084043)	+	-	0.25	1.5	+	-	-	22 ± 2	0
MTR-11 (DQ898297)	*Gordonia sp.*[a] (DQ448700)	+	-	1.0	1.5	+	-	-	0	0
MTR-17A (DQ507206)	*Agrobacterium sp.*[a] (DQ14504)	+	-	1.0	1.5	+	+	-	0	112 ± 33
MTR-18 (DQ507207)	*Microbacterium sp.*[a] (AY569297)	+	-	0	0	-	-	-	13 ± 2	0
MTR-21A (DQ507208)	*Clavibacter sp.*[a] (AM237375)	+	+	0	0.5	+	+	-	0.1 ± 0.2	0
MTR-32A (DQ507209)	*Rhizobium sp.*[a] (AY662032)	+	+	0	0	+	-	-	25 ± 11	30 ± 4
MTR-35B (DQ507210)	*Agrobacterium sp.*[a] (AY174112)	+	-	0.5	1.5	+	+	-	28 ± 2	4 ± 3
MTR-44[d] (EU034524)	*Arthrobacter sp*[a]. (DQ298782.1)	+	-	1.0	1.0	-	+	+	86.8	57.6
MTR-45B (DQ507211)	*Rhodanobacter sp.*[a] (AB244763)	+	+	0.25	0.5	+	-	-	2 ± 0.1	0
MTR-61 (DQ898298)	*Pseudomonas sp.*[a] (DQ241591)	+	-	0.5	0.25	+	+	+	3 ± 0.1	41 ± 9

MTR-70 (DQ898299)	*Rhodococcus sp.*[a] (DQ490431)	+	+	1.0	1.5	-	-	+	0.6 ± 0.1	0
MTR-71 (DQ898300)	*Sphingomonas sp.*[a] (AY162145)	-	-	0	0	+	-	-	0	0
M22 (AY864081)	*Ralstonia sp.*[c] (AY864081)	+	-	1.0	1.5	+	+	+	0	8 ± 3
#35 (DQ196472)	*Methylobacterium sp.*[c] (DQ196472)	+	-	1.0	1.5	-	-	+	0	0
K4-10C (DQ507202)	*Arthrobacter sp.*[b] (AJ785568)	+	-	0	0.25	+	-	-	14 ± 0.1	163 ± 14
K6-11B (DQ507203)	*Methylobacterium sp.*[b] (D32236)	+	-	0.5	0	-	-	-	0	0
K6-19 (DQ507204)	*Paenibacillus sp.*[c] (AB073188)	-	-	0	0	-	-	-	30 ± 3	0
SP-1 (DQ898301)	*Microbacterium sp.*[b] (AF408999)	-	-	0	0	-	-	-	0	0
P.a 9027	*P. aeruginosa* 9027[c] (ATCC 9027)	+	-	1.0	1.5	+	+	-	0	21 ± 6
B. viet (AF312031)	*Burkholderia*[c] (AF312031)	+	+	1.5	1.5	+	+	+	0.4 ± 0.1	5 ± 2

a. Obtained from *A. lentifromis* rhizosphere

b. Obtained from bulk mine tailings

c. Obtained from laboratory culture collection

d. Not included in initial screening studies

e. Highest concentration (mM) of metal resistance

f. Reported as µg IAA 10^{-7} CFU

g. Reported as µg Siderophore 10^{-8} CFU

Table 2 Qualitative Assay Screening Results[a]

Assay	Gram Negative[b]	Gram Positive[b]	Total[c]
Siderophore Production	62[d]	60[e]	62[f]
Phosphate Solubilization	30	44	40
IAA Production	65	50	57
ACC-Deaminase Activity	17	23	21
Growth (pH 5)	71	85	80
Growth (pH 4)	15	17	16

[a]Qualitative screening tests were performed on the 131 isolates collection

[b]86 of 131 isolates were Gram positive, 45 of 131 isolates were Gram negative, isolates

[c]Total refers to the entire collection

[d,e,f]Percentage of [d]Gram positive, [e]Gram negative and the [f]total collection of isolates that produced a positive result upon screening

Table 3 Quantitative Assay Screening Results

Assay	Siderophore or IAA Produced (mg L^{-1})				
	10	25	50	75	100
	-----Percent of Isolates Producing[a]-----				
Siderophore Production	27	25	19	16	15
IAA Production (0 mg L^{-1} tryptophan added)	0	0	0	0	0
IAA Production (100 mg L^{-1} tryptophan added)	19	1	0	0	0
IAA Production (500 ml L^{-1} tryptophan added)	37	21	13	4	1

[a] Number given signifies the percentage of isolates with the ability to produce at or above the indicated level.

CHAPTER 3

PLANT SPECIES AND PLANT GROWTH-PROMOTING BACTERIA SUITABLE FOR THE RESTORATION OF A SEMIARID LOW-METAL, ACIDIC MINE TAILINGS SITE (NACOZARI, SONORA, MX)

1.0 Abstract

Mine tailings contamination poses a health risk in arid and semiarid environments globally. Phytoremediation is now emerging as a viable and cost-effective remediation technology to reduce the mobility of tailings material. Two native desert plant species, *Atriplex lentiformis* and *Buchloe dactyloides*, were investigated for their potential use during the phytostabilization of a "low-metal, low pH" tailings located in Nacozari, Soñora, Mexico. Growth of both plant species was extremely limited in non-composted tailings, but significantly increased from 0.7 ± 0.6 and 0.9 ± 0.6 g to 5.9 ± 1.6 and 12.7 ± 1.7 g average dry pot biomass with the addition of 5% compost material (w/w), representing a 84 – 140 fold increase. For *A. lentiformis*, there was a slight increase in biomass between the 5% and 10% composted treatments (w/w), while there was no difference in average dry pot biomass between the 10 and 20% composted (w/w) treatments; however, for *B. dactyloides* there was a difference in the biomass between the 5% and 10% composted (w/w) treatments, but a significant decrease in average dry pot biomass between the 10% to 20% composted (w/w) treatments, suggesting that some plant species may grow optimally at lower compost rates. For *B. dactyloides*, root-to-shoot ratios decreased substantially between the 5% and 10% treatments, suggesting that lower compost rates may encourage plants to allocate more biomass to the root tissues

further enhancing phytostabilization. Finally, three alginate-encapsulated bacterial inoculants, *Azospirilum brasilense* Cd, *Arthrobacter spp.* mixture, and *Rhodanobacter sp.,* significantly enhanced average dry plant biomass in *A. lentiformis* compared to non-inoculated controls at 5% compost (w/w), demonstrating that alginate-encapsulated PGPB could be used to enhance the growth of native plant species in these tailings.

2.0 Introduction

Mining activities have created a sizable problem in the form of mine tailings contamination on a global scale (Navarro et al., 2006; 2008). In particular, arid and semiarid lands including northern Mexico, western United States, South Africa, Australia, and the Pacific Coast of South America have been heavily impacted (Munshower, 1994; Tordoff et al., 2000). Tailings piles range in size from less than an acre to square kilometers and can vary in toxicity depending on pH and heavy metal content. These sites also pose serious concerns regarding respiratory health from eolian dispersion of particulate matter (Coelho et al., 2007). Phytostabilization, the establishment of a vegetative cover on tailings piles to stabilize tailings materials without translocation of heavy metal contaminants into plant shoot tissues, has emerged as a viable remediation solution to reduce the erosion and contaminant mobility from these tailings areas (Cunningham, 1995; Mendez et al., 2007; Mendez and Maier, 2008a; 2008b). However, there are generally a number of limitations to employing phytostabilization as an effective remediation technology including; elevated levels of metal contaminants, extremely acidic pH, lack of plant-available nutrients, lack of soil structure, and severely impacted heterotrophic microbial communities (Mendez et al., 2007; Tordoff et al., 2000). Often, these limitations can be overcome with the addition of organic compost material, liming, or by covering with topsoil (Munshower, 1994). For example, Mendez et al., (2007) demonstrated that a compost rate of 15% (w/w) was necessary to achieve plant growth that was significantly similar to off-site controls in an extremely acidic, heavy metal-contaminated mine tailing. Although potentially less

costly than some alternative remediation strategies, phytostabilization may still be cost-prohibitive for some regions where the transport and application of high compost levels onto large sites may be economically infeasible.

The use of plant growth-promoting bacteria (PGPB) to enhance one or more aspect of plant growth has been widely applied in agricultural settings (Bashan, 1998; Kloepper et al., 1987). PGPB are generally introduced to seeds prior to planting and aid the plant in acquisition of essential nutrients such as N, P, and Fe or directly promote growth through well-studied mechanisms such as IAA production and ACC-deaminase activity (Bar-Ness et al., 1992; Glick 1998; Patten and Glick, 2002; Vazquez et al., 2000). Recently, PGPB have been utilized to enhance the growth of plants under an assortment of environmental applications, including phytostabilization and the restoration of desertified lands (Bashan et al., 1999; Carrillo-Garcia et al., 2000; Grandlic et al., 2008; Petrisor et al., 2004). The bacterium *Azospirillum brasilense* has been widely applied to enhance plant growth under a variety of agricultural and environmental applications. Recently, Grandlic et al. (2008) reported a unique collection of PGPB that significantly enhanced *A. lentiformis* and *B. dactyloides* growth in semiarid mine tailings. Utilizing PGPB during these applications can facilitate the establishment of native plant species while potentially reducing the mass of compost amendments necessary to achieve optimal plant growth thus decreasing remediation costs. Recent research has also developed the use of alginate-encapsulated isolates making this PGPB technology increasingly more field-applicable (Bashan et al., 2002). Alginate-encapsulation has a

distinct advantage over traditional inoculation methods as it allows for prolonged storage of an easily transportable inoculum that requires minimal training for field use.

The Nacozari tailings site, located in Nacozari, Soñora, Mexico, poses a health risk to the surrounding community. The tailings volume and proximity (less than 10 m) to local residents have placed them near the top of the SEMARNAP priority list. This site's large size makes other remediation solutions such as capping and relocation economically unfeasible. To date, no information is available regarding the phytostabilization of low-metal, low-pH mine tailings located in semiarid environments. Furthermore, there are no data available regarding appropriate plant species and optimal compost rates that are necessary to achieve satisfactory plant growth in these tailings. The objectives of the current study were to (i) obtain a chemical and physical characterization of the Nacozari tailings (ii) identify optimal compost amendment rates for two plant species, *Atriplex lentiformis* and *Buchloe dactyloides* which are native to the Soñoran desert (iii) and to investigate whether alginate-encapsulated PGPB have the potential to increase *A. lentiformis* growth in the Nacozari tailings at a minimal compost rate.

3.0 Materials and Methods

Site Characteristics. The Nacozari mine tailings, located in Nacozari, Soñora, Mexico, cover approximately 52 hectares and are located in extreme proximity to residential areas with some residences less than 10 m from the pile. Currently, no vegetation is present on

the site. Tailings samples for physical/chemical analysis and plant growth screening were collected in June 2007 at GPS coordinates; (30^o 22' 02.47" N; 109^o 41' 40.50" W).

Substrate Characterization. Vinton soil, used as an off-site control, is a loamy sand previously characterized by Jordan et al. (2004). The Nacozari tailings material was characterized for particle size distribution, pH, electrical conductivity (EC), dissolved organic carbon and nitrogen, bulk mineralogy, and total and plant-available metals.

Extractions for Chemical Characterization. Triplicate aqueous saturated paste extractions at a mass ratio of 1:1 solid: solution, were performed using an end-over-end rotator for 1 or 24 hours at 10 rpm. The supernatant was separated by centrifugation and filtered before analysis. The solution pH and EC were measured directly. Dissolved organic carbon and nitrogen were measured using the Shimadzu Carbon Analyzer (TOC-VCSH), anions were measured using Dionex system with an AS-11 column, and trace metals analysis was performed using the ICP-MS (Perkin Elmer SCIEX Elan DRC II).

In order to access plant-available metals, triplicate DTPA and KCl extractions were performed as outlined by Bertch and Bloom, and Amacher, respectively (Amacher, 1996; Bertsch and Bloom, 1996). Briefly, 20 mL of DTPA extracting solution (0.005M DTPA, 0.1M TEA, and 0.01M $CaCl_2$, pH=7.3) were combined with 10 g of tailings and shaken for 2 hr. The KCl extraction was performed by combining 5 g of soil with 25 g of 1M KCl followed by shaking for 30 min. The supernatant for each was separated by centrifugation and filtration prior to acidification to pH< 3.0 for KCl and metals analysis by ICP-MS.

Solid state Carbon and Nitrogen

The total carbon, inorganic carbon and total nitrogen were measured using the Shimadzu Carbon Analyzer TOC-VCSH. Five measurements were performed on approximately 100 mg of solid tailings samples.

Particle Size Analysis

Particle size separation was performed in triplicate using the pipette sedimentation method outlined in Jackson and Burt (Burt, 2004; Jackson, 1985). The tailings were dispersed using 50 mL of sodium hexametaphosphate solution by shaking for 24 hours prior to removal of the sand fraction by sieving. The smaller size fractions were transferred into a 1 L graduated cylinder for sedimentation and the clay content was sampled after 3 hr. 38 min. in 23ºC water. After further settling, clays were decanted and the silt fraction was washed and then centrifuged in pH 9.5 Na_2CO_3. The clay-containing supernatant was added to the decanted clays and flocculated using NaCl. The clay suspension was then dialyzed (VWR, Spectrapor MWCO 12-14k, 25225-260). Some of the clay fraction was stored in suspension at room temperature for creating oriented clay slides for XRD analysis and the rest was freeze-dried for further analysis.

Bulk Mineralogy and Total Elemental Analysis

The bulk mineralogy of the tailings was analyzed by X-ray diffraction (XRD) at the Stanford Synchrotron Radiation Laboratory on Beamline 11-3, operating at ~12,700eV and a Mar detector operating with a 345 mm diameter with 100 µm pixels. Ring

potential at SSRL was 2GeV with a current varied from 100 to ~40mA during data collection. Data were collected in transmission mode with the sample in Scotch Magic Matte finish tape 150 mm from the detector. The sample was scanned over a 1mm section normal to the beam in 64 points (8x8 grid). Five or more scans were collected for each sample for subsequent addition to avoid detector saturation by the quartz peaks. The data were reduced using area diffraction machine with a mask covering the beamstop (Lande et al., 2008). The spectra were added, reduced, converted to Cu K-alpha scale, and analyzed using X'Pert software.

The clay composition of the tailings was determined by collecting XRD data for oriented clay spectrums (Jackson, 1985). Magnesium saturated clay slides were then saturated with glycerol and analyzed at room temperature. Potassium saturated slides were analyzed at room temperature and after 1hr. heating at 100°C, 300°C, or 550°C. The patterns were collected between 2 and 30 degrees 2-theta with a step size of 0.01 degrees 2-theta and a dwell time of 2 sec. per step. All oriented clay XRD patterns were collected at 45 kV and 40 mA on a Panalytical X'Pert PRO XRD in the Rasmussen Laboratory at the University of Arizona. Total elemental analysis was determined using the lithium metaborate/tetraborate fusion ICP Whole Rock method package Code 4B and a trace element ICP/MS package Code 4B2 at the Activation Laboratories (Ancaster, ON, Canada).

Native Plants

Two plants native to the Sonoran desert ecosystem surrounding Nacozari were used in this study, the shrub *Atriplex lentiformis* (Torr.) S. Wats., commonly known as quailbush and the grass *Buchloe dactyloides* (Nutt.) Engelm., commonly known as buffalo grass. Seeds were obtained from Carter Seeds, Vista, CA (quailbush) and Western Native Seed, Coaldale, CO (buffalo grass).

Evaluation of A. lentiformis and B. dactyloides Growth Nacozari Tailings

A series of greenhouse studies were performed to evaluate the effect of compost amendment on the growth of *A. lentiformis* and *B. dactyloides* in the Nacozari tailings in June/July 2007. Tailings/compost mixtures of 0, 5, 10 and 20% were prepared on a gravimetric basis, homogenized and distributed to 3 L pots. Five replicates were used for each individual plant type and compost rate. Substrate mixtures were wetted to field capacity 48 hr prior to planting seeds. Following this initial wetting period, 10 seeds of either *A. lentiformis* or *B. dactyloides* were planted in each pot. Plants were irrigated three times daily via mister distribution totaling 2.0 cm (362 mL) pot^{-1} day^{-1}. After 75 days, plants were harvested and thoroughly rinsed to remove all tailings and compost material. The roots and shoots were separated for each treatment and dried in a drying over at 65^{o}C for 72 hr. Biomass measurements of dried plant material were then collected.

Preparation of Alginate Encapsulated PGPB Isolates

Bacterial isolates that have demonstrated the ability to enhance plant growth in previous studies were selected as candidates to enhance *A. lentiformis* growth at 5% compost

(w/w) (Bashan et al., 1999; 2004; Grandlic et al., 2008). To prepare alginate-encapsulated cells, individual isolates were grown at 25°C on rotary shaker (200 rpm) in 3, 0.5 L volumes of 3XR2A; (g L^{-1}) 1.5 yeast extract, 1.5 glucose, 1.5 casein hydrolysate, 1.5 soluble starch, 1.5 proteose peptone No. 3, 0.9 K_2HPO_4, 0.9 sodium pyruvate, and 0.1 MgSO4·7H2O, adjusted to pH 7.4. After 48 – 72 hr (cell density of 10^9 CFUs mL^{-1}) cells were harvested and concentrated by centrifuging at 12,000 x *g* for 15 min. The supernatant was removed, discarded and the resulting cell pellets were re-suspended in 10 mL of 50 mM PIPES buffer (pH 7.4) for a cell density of approximately 10^{11} CFU mL^{-1}. The cell suspension was then thoroughly mixed with an equal volume of a sterile 3.5% alginate solution and forced through a sterile 30.5 gauge syringe into a chilled (4°C) 0.15 M $CaCl_2$ solution. *Arthrobacter* sp. mixture beads (MTR-44 and K4-10C) were produced by combining equal volumes of the two cultures after centrifugation prior to addition of the sodium alginate mixture. Alginate beads (approximately 300 – 500 µM) formed immediately upon contacting the $CaCl_2$ solution and were allowed to set at a minimal stirring speed (100 rpm) for at least 2 hr. Polymerized alginate beads were collected by filtration through a sterile Buchner funnel and rinsed 3 times with sterile 50 mM PIPES buffer. Alginate-encapsulated cells were aseptically transferred to 50 mL plastic centrifuge bottles and lyophilized until completely dried for 48 – 72 hr. Cell densities (CFU $gram^{-1}$ dry beads) were obtained by dissolving a determined mass of beads in 5 mL of 0.25 M sodium carbonate for 30 min, diluting in PBS, plating onto R2A agar and enumeration after 5 days.

Assessment of Bacterial Isolates Abilities to Enhance A. lentiformis Growth

To evaluate the potential for using selected PGPB in a field-scale application, a greenhouse study was conducted using *A. lentiformis* and alginate-encapsulated PGPB. *A. lentiformis* seeds were surface sterilized prior to inoculation; seeds were soaked in sterile distilled water for 10 min followed by a 1 min immersion in 95% ethanol, seeds were immediately rinsed with sterile distilled water and soaked for 10 min in 1% sodium hypochlorite solution. Following this soak, seeds were rinsed three times with sterile distilled water and soaked for three min in a 0.1% sodium thiosulfate solution and rinsed a final time. Lyophilized beads for each individual isolate were introduced to surface sterilized seeds at a rate of 2×10^7 CFU $seed^{-1}$. An appropriate volume of beads was added to inoculate 75 seeds per treatment. After inoculation the seed/bead mixture was aseptically homogenized. Seeds were transferred to Nacozari tailings amended with 5% compost material (w/w) at a rate of 10 seeds per pot. Five replicate pots were used per treatment. Plants were subjected to a similar watering regime as previously mentioned, harvested after 90 days, and evaluated for dry shoot, root, and total plant biomass as previously mentioned.

Statistical Analysis

Statistical analyses were conducted using SAS Version 9.1 (SAS Institute Inc. Cary, NC). Significant treatment effects for plant root, shoot and total biomass and root-to-shoot data were detected by employing a one-way ANOVA ($p < 0.05$). For each treatment, significant differences between means were determined by the Duncan's Multiple Range Test ($p < 0.05$).

4.0 Results and Discussion

Physical and Chemical Characterization of the Tailings. The Nacozari tailings can be classified as a "low-pH, low-metal" tailings material with a pH of 3.68 ± 0.02 and total metal concentrations of (mg Kg^{-1}) 17.7 ± 2.9, 513 ± 107, 32.4 ± 12.4, and 83.3 ± 15.3 for As, Cu, Pb and Zn, respectively (Table 1). The levels are lower than those typically observed at heavily contaminated sites (Grandlic et al., 2008; Mendez et al., 2007); however levels of plant-available Al and Cu are elevated in the tailings (Table 2). The most recognizable effect of Al toxicity in plants is an inhibition of root growth (Delhaize and Ryan, 1995). The bioavailability of Al in soils is controlled largely by soil pH, clay content and organic matter content (Adams, 1981; Ulrich, 1983). The low pH and low clay content of the Nacozari tailings likely contribute substantially to a high level of plant-available Al, 3700 ± 124 mg L^{-1}. Soil solution Al^{3+} concentrations of less than 200 mg L^{-1} were shown to affect plant growth in dozens of plant species, suggesting that Al-toxicity may contribute to the lack of natural re-vegetation in the Nacozari tailings (Andersson, 1988). Furthermore, Cronan (1995) has indicated soil solution ratios of Ca/Al are appropriate predictors for estimating the risk of adverse affects of Al toxicity in acidic soils, with a ratio of 1.0 leading to a 50% risk and ratios of 0.5 and 0.2 leading to a 75% and 100% risk, respectively. The solution Ca/Al ratio in the Nacozari tailings is 0.26, suggesting that Al toxicity is probable in the Nacozari tailings material. The elevated levels of plant-available Cu may also contribute to the lack of natural re-vegetation in the Nacozari tailings. Critical levels of 140 – 180 mg kg^{-1} were suggested

to be detrimental to most crop species while many crop plants cannot tolerate plant-available levels of greater than 70 mg kg^{-1} (Jiang et al., 2004; Yang et al., 2002).

Effect of Compost Rates on Plant Growth. The primary objective of the present study was to determine the minimal compost rates that are necessary to achieve plant growth in the Nacozari tailings. There are generally a number of limitations to plant growth in mine tailings including; a lack of soil structure and nutrients, severely impacted microbial communities, acidic pH values and elevated levels of heavy metals. The Nacozari tailings, although low in metal contamination, are acidic in nature and lack robust microbial communities, soil structure, and plant-available nutrients (Tables 1 and 2). Counts of heterotrophic bacteria in these tailings are extremely low, less than 100 CFU g^{-1} tailings, compared to healthy soils which are 6 or 7 orders of magnitude higher. Compost amendments facilitate plant growth by decreasing bulk densities, increasing water holding capacity, metal (e.g. Al^{3+}) complexing capacity, and providing some plant nutrients and heterotrophic microbes. For both *A. lentiformis* and *B. dactyloides* plant growth was extremely limited in the non-composted Nacozari tailings (Figure 1 and 2), which may be a result of Al toxicity. An addition of 5% compost material (w/w) resulted in a 84 – 140 fold increase in average pot biomass. A lack of compost also decreased *A. lentiformis* survival as only 13 plants survived after 75 days compared to 25 - 28 plants in remaining treatments. For this plant a maximum average pot biomass was achieved at 10% compost, with no differences between the 10% and 20% treatments; however, the 10% treatment produced plants that had greater root-to-shoot ratios (Table 2). For *B. dactyloides* average pot biomasses were essentially equal in the 5 and 10% compost

treatments, while average pot biomass decreased by greater than 40% in the 20% compost treatment (Figure 2). Although the 5 and 10% treatments had nearly equal pot biomasses, there were very different in root-to-shoot ratios as increasing the compost rate decreased this ratio from 0.50 ± 0.21 to 0.21 ± 0.07 (Table 3).

These results stress an important point in that not only is overall biomass production important in phytostabilization, it is also important to evaluate the distribution of biomass in plants in the form of root-to-shoot ratios. Developing a healthy, sizable root biomass is important to site stabilization. These results indicate that at different compost rates (10 and 20% for *A. lentiformis* and 5 and 10% for *B. dactyloides*) overall biomasses may not change but root biomass may decrease with increasing compost amendments. Furthermore, for some plant species, such as *B. dactyloides* high compost rates may actually be detrimental to overall plant biomass production as a 40% decrease in average pot biomass production was observed going from 10 to 20%. These results indicate that compost rates must be investigated prior to conducting field-scale applications. Our results have also indicated that both *B. dactyloides* and *A. lentiformis* are suitable candidates for the phytostabilization of the Nacozari tailings site.

Effect of PGPB on Plant Growth. An investigation was made into the potential for using alginate-encapsulated PGPG to enhance *A. lentiformis* growth at a minimal compost rate of 5% (w/w). *A. lentiformis* was selected as opposed to *B. dactyloides* as there were no differences in *B. dactyloides* biomass between the 5% and 10% composted treatments. Three of the PGPB applied to *A. lentiformis* seeds in this study demonstrated the potential to enhance plant growth at a minimal compost rate of 5% (w/w) (Figure 3).

Three treatments, the *Arthrobacter* Mixture, MTR-45B, and *Azospirillum* Cd, significantly enhanced total plant biomass in the Nacozari tailings receiving 5% compost by 19, 27, and 47%, respectively. The PGPB treatments *Arthrobacter* Mixture, MTR-21A, MTR-45B, and M22 enhanced the growth of *A. lentiformis* and *B. dactyloides* in composted and/or non-composted acidic mine tailings with high levels of both Pb and Zn (Grandlic et al., 2008). *Azospirillum brasilense* Cd has been used to enhance plant growth in agricultural and environmental applications in dozens of prior studies (Bashan, 1999; 2004; Carrillo-Garcia, 2000). The present study demonstrates that these isolates are again effective in yet another application. Furthermore, this study demonstrates that inoculating plants using the alginate-encapsulation method is a viable technology during phytostabilization. To the best of our knowledge, this is the first report of utilizing alginate-encapsulated PGPB isolates to enhance plant growth during the phytostabilization of mine tailings. As previously mentioned, this method has a number of advantages over traditional inoculation methods in that inoculums can be stored for extended periods of time at high densities and its application requires minimal training (Reed and Glick, 2005).

*5.0 Conclusi*ons

Investigations into potential native plant species and optimal compost rates for use during phytostabilization should be done prior to beginning large-scale remediation efforts. Similar research has been conducted in “acidic, high-metal” tailings and “neutral-pH, high-metal” tailings; however, no information is available regarding the potential for

using phytostabilization in “low-pH, low-metal” semiarid mine tailings (Grandlic et al., 2007; Mendez et al., 2007; Rosario et al., 2007). In the Nacozari tailings, two native desert plant species, *A. lentiformis* and *B. dactyloides*, have extremely stunted growth in non-composted tailings; however, grow optimally in tailings amended with 5 or 10% compost material (w/w) depending on plant species. Furthermore, employing alginate-encapsulated PGPB during phytoremediation efforts may significantly enhance plant biomass production at a minimal compost rate (5%). This method of inoculation is also beneficial as PGPB can be stored at high densities for extended periods of time with minimal preparation during their application (Reed and Glick, 2005).

6.0 References

Adams, F. 1981. Nutritional imbalances and constraints to plant growth on acid soils. J. Plant Nutr. **4:**81.

Amacher. 1996. Nickle, cadmium and lead. p. 754-756. *In* J.M. Bartels (ed.) Methods of soil analysis part 3: Chemical methods. Soil Science Society of America, Madison, WI.

Andersson, M. 1988. Toxicity and tolerance of aluminum in vascular plants. Water, Air and Soil Poll. **39:**439-462.

Bar-Ness, E., Y. Hadar, Y. Chen, A. Shanzer, and J. Libman. 1992. Iron uptake by plant from microbial siderophores. Plant Physiol. **99:**1329-1335.

Bashan, Y. 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol. Adv. **16:**729-770.

Bashan, Y., G. Holguin, and L.E. Bashan. 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Can. J. Microbiol. **50:**521-577.

Bashan, Y., A. Rojas, and M.E. Puente. 1999. Improved establishment and development of three cactus species inoculated with *Azospirillum brasilense* transplanted into disturbed urban soil. Can. J. Microbiol. **45:**441-451.

Bashan, Y., J.-P. Hernandez, L.A. Leyva, and M. Bacilio. 2002. Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. Biol. Fertil. Soils. **35:**359-368.

Bertsch and Bloom. 1996. Aluminum. p. 524-531. *In* J.M. Bartels (ed.) Methods of soil analysis part 3: Chemical methods. Soil Science Society of America, Madison, WI.

Burt, R. 2004. Soil physical and fabric-related analyses. p. 34-43. *In* R. Burt (ed.) Soil survey laboratory methods manual. 4.0th ed. Soil Survey Investigations Report, National Resources Conservation Service.

Carrillo-Garcia, A., Y. Bashan, E. Diaz Rivera, and G.J. Bethlenfalvay. 2000. Effects of resource island soils, competition, and inoculation with *Azospirillum* on survival and growth of *Pachycereus pringlei*, the giant cactus of the Sonoran Desert. Res. Ecol. **8:**65-73.

Coelho, P., S. Silva, J. Roma-Torres, C. Costa, A. Henriques, J. Teixeira, M. Gomes, and O. Mayan. 2007. Health impact of living near an abandoned mine – case study: Jales mines. International J. Hygiene and Environmental Health. **210:**399-402.

Cunningham, S.D., W.R. Berti, J.W.W. Huang. 1995. Phytoremediation of contaminated soils. Trends Biotechnol. **13:**393-397.

Delhaize, E., and P.R. Ryan. 1995. Al toxicity and tolerance in plants. Plant Physiol. **107:**315-321.

Glick, B.R., D.M. Penrose, and J. Li. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J. Theo. Biol. **1990:**63-68.

Grandlic, C.J., M.O. Mendez, J. Chorover, B. Machado, and R.M. Maier. 2008. Plant growth-promoting bacteria for phytostabilization of mine tailings. Environ. Sci. Tech. *In Press.*

Jackson, M.L. 1985. *Soil chemical analysis*. Parallel Press, Madison, Wisconsin.

Jiang, L.Y., X.E. Yang, Z.L. He. 2004. Growth response and phytoextraction of copper at different levels in soils by *Elsholtzia splendens*. Chemosphere. **55:**1179-1187.

Jordan, F. L.; Sandrin, S. K.; Frye, R. J.; Brusseau, M. L.; Maier, R. M. 2004. The influence of system complexity on bacterial transport in porous media. J. Contam. Hydrol. **74:**19-38.

Kloepper, J.W., R. Lifshitz, and R. Zablotowicz. 1987. Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol. **7:**39-44.

Lande, J., S.M. Webb and A. Mehta. Area diffraction machine. Http://groups.google.com.ezproxy2.library.arizona.edu/group/area-Diffraction-Machine.

Mendez, M.O., E.P. Glenn, and R.M. Maier. 2007. Phytostabilization potential of quailbush for mine tailings: growth, metal accumulation, and microbial community changes. J. Environ. Qual. **36:**245-253.

Mendez, M.O., and R.M. Maier. 2008a. Phytostabilization of mine tailings in arid and semiarid environments – an emerging remediation technology. Environ. Health Perspect. **116:**278-283.

Mendez, M.O., and R.M. Maier. 2008b. Phytoremediation of mine tailings in temperate and arid environment. Rev. Environ. Sci. Biotech. **7:**1569-1705.

Munshower, F.F. 1994. Practical handbook of disturbed land revegetation. Boca Ratton, FL. Lewis Publishing.

Navarro, M.C., C. Pérez-Sirvent, M.J. Martínez-Sánchez, J. Vidal, and J. Marimón. 2006. Lead, cadmium and arsenic bioavailability in the abandoned mine site of Cabezo Rajao (Murcia, SE Spain). Chemosphere. **63:**484-489.

Navarro, M.C., C. Pérez-Sirvent, M.J. Martínez-Sánchez, J. Vidal, P.J. Tovar, and J. Bech. 2008. Abandoned mine sites as a source of contamination by heavy metals: a case study in a semi-arid zone. Geochemical Exploration. **96:**183-193.

Patten, C.L., and B.R. Glick. 2002. Role of *Psuedomonas putida* indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. **68:**3795-3801.

Petrisor, I., S. Dobrota, K. Komnitsas, I. Lazar, J.M. Kuperberg, and M. Serban. 2004. Artificial inoculation – perspectives in tailings phytostabilization. Int. J. Phytoremediation. **6:**1-15.

Reed, M.L.E., and B.R. Glick. 2005. Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can. J. Microbiol. **51:**1061-1069.

Tordoff, G.M., A.J.M. Baker, A.J. Willis. 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere. **41:**219-228.

Ulrich, B. 1983. *In* A. Cammerer (ed.), Pollution Stresses on Forest Ecosystems, Transl. From LOLF-Mitteilun- gen 1982 Sonderheft. Translation - Environment Canada.

Vazquez, P., G. Holguin, M.E. Puente, A. Lopez-Cortez, and Y. Bashan. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol. Fertil. Soils. **30:**460-468.

Yang, X.E., X.X. Long, W.Z. Ni, Z.L. He, P.J. Stoffella, D.V. Calvert. 2002. Assessing copper thresholds for phytotoxicity and potential toxicity in selected crops. J. Environ. Sci. Health B **37:**625–635.

7.0 Figure Legends

Figure 1. The effect of compost amendments on the total average dry pot biomass of *A. lentiformis* grown in Nacozari tailings for 75 d (mean + 1 SD for total plant biomass). Lower and upper bars represent the average dry root biomass and average dry shoot biomass for each treatment, respectively. The number at the bottom of each bar represents the surviving plants out of fifty planted seeds. Means with different letters are significantly different in total dry plant biomass at $p < 0.05$.

Figure 2. The effect of compost amendments on the total average dry pot biomass of *B. dactyloides* grown in Nacozari tailings for 75 d (mean + 1 SD for total plant biomass). Lower and upper bars represent the average dry root biomass and average dry shoot biomass for each treatment, respectively. The number at the bottom of each bar represents the surviving plants out of fifty planted seeds. Means with different letters are significantly different in total dry plant biomass at $p < 0.05$.

Figure 3. The effect of PGPB treatments on the total average dry plant biomass of *A. lentiformis* grown in Nacozari tailings amended with 5% compost material after 75 d (mean + 1 SD for total plant biomass). The number at the bottom of each bar represents the surviving plants out of fifty planted seeds. Means with different letters are significantly different in total dry plant biomass at $p < 0.05$.

Table 1: Selected physical-chemical properties of the tailings

	pH[1]	***EC***[1]	***TOC***[2]	***TIC***[2]	***TN***[2]	***sand***[3]	***silt***[3]	***clay***[3]	***As***	***Cu***	***Pb***	***Zn***	**mineralogy**[4]
		uS/cm		mg/kg			percent		Total trace metal content (mg/kg)[6]				
N	3.68 ± 0.02	262 ± 2	145 ± 23	BDL[5]	18 ± 2	87 ± 3	5 ± 2	4 ± 2	17.7 ± 2.9	513 ± 107	32.4 ± 12.4	83.3 ± 15.3	Q, O, M

[1] Measured on a 1:1 solid to solution ratio 1 hour extraction performed in triplicate.
[2] Solid state measurements on Shimadzu TOC-VCSH performed in triplicate.
[3] Measured in triplicate using the pipette method (Jackson, 1985).
[4] Mineralogy: Q- quartz; O- orthoclase; M- mica; in order of relative abundance.
[5] Below Detection Limit, less than 0.02mg inorganic carbon.
[6]Determined using lithium metaborate/tetraborate fusion trace element ICP/MS package Code 4B2 (Activation Laboratories, Ancaster, ON, Canada)

Table 2: Nutrients and plant-available toxic metals present in the tailings (mg kg^{-1}).

	DOC[1]	*DN*[1]	NO_3^- [1]	PO_4^{3-} [1]	Mn^{2+} [2]	Zn^{2+} [2]	Pb^{2+} [2]	Cu^{2+} [2]	Ca^{2+} [3]	Al^{3+} [3]
N	3 ± 1	2.5 ± 0.3	0.0064 ± 0.0007	BDL[4]	97 ± 3	66 ± 3	5 ± 1	650 ± 30	970± 20	3700 ± 124

[1] Measured on a 1:1 solid to solution ratio 1 hour extraction.
[2] Plant-available contaminants were determined using a DTPA extraction (Bertsch and Bloom, 1996).
[3] Plant-available contaminants were determined using a 1M KCl extraction (Amacher, 1996).
[4] Below Detection Limit, less than 5 µmoles PO_4^3 L^{-1}.

Table 3: Root-to-shoot ratios[a] with increasing compost rates

	Vinton Soil	**0% Compost**	**5% Compost**	**10% Compost**	**20% Compost**
A. lentiformis	0.46 ± 0.07	0.39 ± 0.17	0.26 ± 0.06	0.26 ± 0.02	0.20 ± 0.06
B. dactyloides	0.84 ± 0.39	0.61 ± 0.13	0.50 ± 0.21	0.21 ± 0.07	0.21 ± 0.06

[a]Root-to-shoot ratios were calculated by dividing the dry root biomass by the dry shoot biomass for five replicates at each compost rate. The ratios on the table represent the average of five replicates.

Figure 1 *A. lentiformis* dry biomass

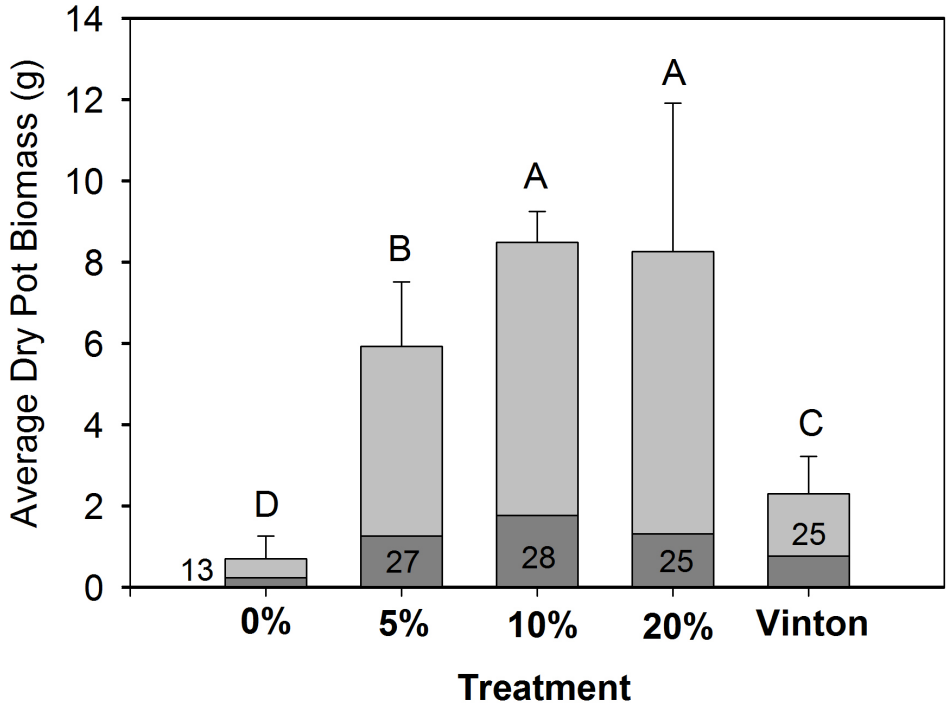


Figure 2 *B. dactyloides* dry biomass

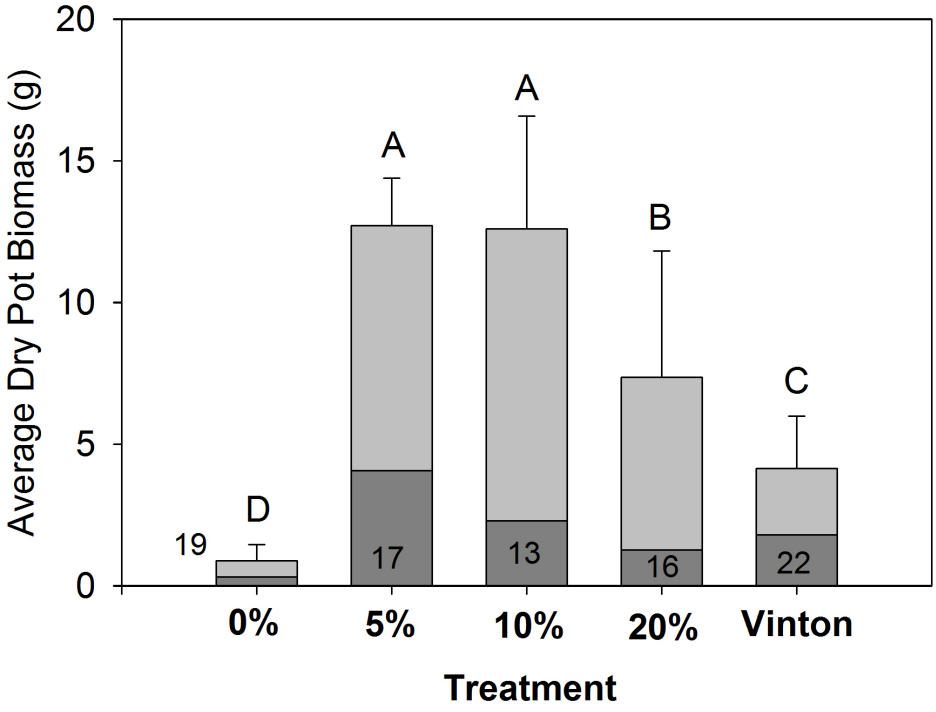
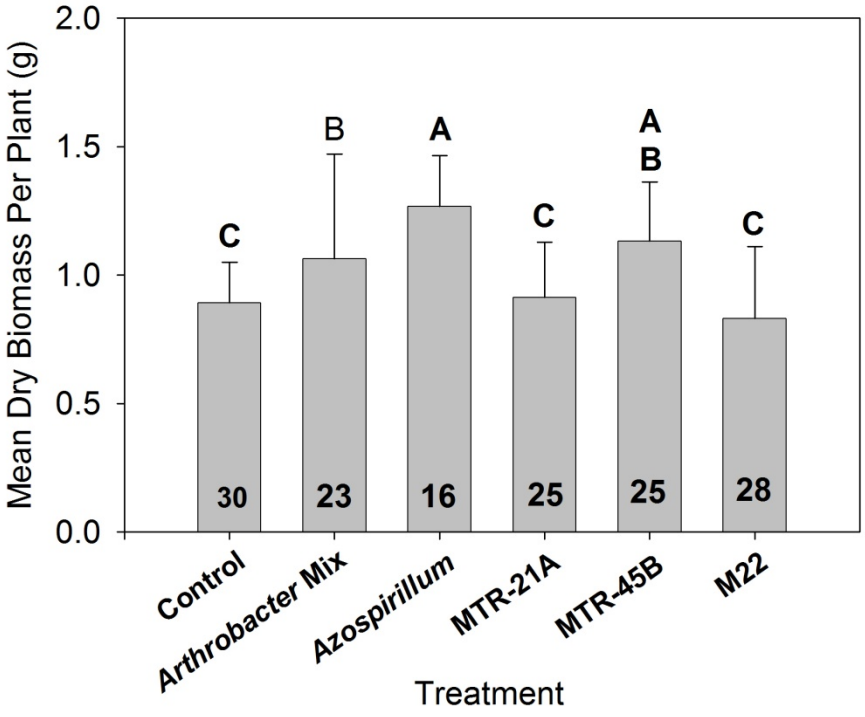


Figure 3 Effect of selected plant growth-promoting bacteria on *A. lentiformis* biomass



CHAPTER 4

OPTIMIZATION OF PLANT GROWTH-PROMOTING BACTERIA-ASSISTED PHYTOSTABILIZATION OF MINE TAILINGS

1.0 Abstract

Recent studies have indicated that plant growth-promoting bacteria (PGPB) can improve revegetation of mine tailings as measured by biomass production. The goals of the present study were first to evaluate how application of known PGPB affects their performance. Parameters investigated included surface sterilization of seeds (a common practice in phytoremediation trials) and comparison of two application methods; immersion and alginate encapsulation. Two native desert plant species, *Atriplex lentiformis* and *Buchloe dactyloides*, were used in this study. Results show that seed surface sterilization prior to inoculation was not necessary to achieve beneficial effects of introduced PGPB in the two plant species tested. In fact surface sterilization was harmful to *B. dactyloides* growth reducing the biomass of plants by 40 – 50% compared to non-sterilized controls. Both immersion and alginate-encapsulation generally enhanced plant growth although results are both plant and PGPB specific. For example, a mixture of two *Arthrobacter spp.* (*Arthro* Mix) enhanced the growth of both plant species by 70 – 430% regardless of the inoculation method used. These results demonstrate that alginate encapsulation, which allows for long-term storage and easier application to seeds, is an effective way to inoculate PGPB. The second goal of the study was to examine the effect of PGPB inoculation on microbial community development following plant establishment. Denaturing gradient gel electrophoresis (DGGE) analysis of bacterial

communities in the tailings following plant establishment show that community profiles from triplicate treatments inoculated with the same PGPB are more similar to each other than to other PGPB, tailings alone, or compost alone. This result was more pronounced when seeds were surface sterilized. These results suggest that introduced PGPB have the potential to direct the development of rhizosphere community structures.

2.0 Introduction

Mining tailings sites in arid and semi-arid environments remain barren of vegetation following deposition due to a combination of factors including metal toxicity, acidic pH, poor soil structure and nutrient levels and heavily impacted microbial communities (Moynahan et al., 2002). Phytostabilization, the establishment of a vegetative cap on mine tailings using plants that minimize metal accumulation into shoot tissues, is a remediation strategy of interest to ameliorate wind and water erosion of tailings in a cost-effective manner (Cunningham et al., 1995; Mendez et al., 2007; Mendez and Maier, 2008a; 2008b). Limitations to plant establishment can be overcome through the addition of compost material which acts immediately to decrease bulk density, increase pH, mitigate metal toxicity, increase water retention, and add necessary nutrients (Mendez et al., 2007). At heavily contaminated sites (high metal, low pH) high levels of compost material may be necessary to achieve desired plant growth which increases remediation costs. Recently, plant growth-promoting bacteria (PGPB) have been shown to enhance plant growth in acidic, high-metal tailings at lower than optimal compost rates (Grandlic et al., 2008). PGPB are beneficial strains of bacteria that are introduced to seeds prior to planting to enhance one or more aspect of plant growth through a number of potential mechanisms including the production IAA and siderophores, phosphate solubilization, ACC-deaminase activity, and mitigation of metal toxicity (Glick et al., 1998; Patten and Glick, 2002; Vazquez et al., 2000). PGPB have traditionally found wide use in agricultural applications and are now being explored for

environmental applications (Bashan, 1998; Bashan et al., 2004; Burd et al., 2000; Carrillo et al., 2004).

There are two primary methods of introducing PGPB to seeds prior to planting. The first, known as immersion, requires soaking seeds in PGPB suspensions prior to planting and the second method, alginate encapsulation, introduces PGPB to the seed surface in the form of alginate-encapsulated beads (Puente and Bashan, 1993; Bashan et al., 2002). PGPB that are inoculated using the immersion method must be prepared at the time of use; however, isolates encapsulated in alginate beads can be lyophilized and stored at high density for extended periods of time, making this method appealing for field-scale use (Reed and Glick, 2005). Alginate beads are also thought to offer protection to the PGPB while acting as a time-release capsule as they slowly break-down and release PGPB to the germinating plant (Bashan et al., 2002).

The vast majority of previous studies have prepared seeds for inoculation by surface sterilization prior to introducing desired strains of PGPB regardless of the inoculation method used (Bashan et al., 2000b; 2002; Belimov et al., 2002; 2005; Donte-Correa et al., 2004; Reed and Glick, 2005). This is thought to give the introduced isolate a competitive advantage in colonizing the surface of seed; however, it also adds an additional step to the procedure and may be potentially harmful to plant germination (Wilson, 1976).

Currently there is little information regarding the fate of PGPB following introduction to plants during phytoremediation applications. Even less is known regarding the development of rhizosphere microbial communities in mine tailings and

plant rhizospheres during phytostabilization. Yet there is evidence for dynamic and large changes in the rhizosphere community during phytostabilization. For example, enumeration of neutrophilic heterotrophic and iron- and sulfur-oxidizing autotrophic communities following plant growth in composted tailings (high metal, low pH) showed dramatic shifts in the numbers of these three communities (Mendez et al., 2007). A clone library analysis of these tailings further showed large differences in the diversity and community structure in tailings samples compared to an off-site control sample (Mendez et al., 2008). Significant community changes were also observed in a 18-month field trial in a high metal, neutral pH tailings using denaturing gradient gel electrophoresis (DGGE) to temporally monitor bacterial community profiles during plant establishment. In this case plant-associated community profiles significantly diverged from unplanted controls at 5 months following planting of transplants (Rosario et al., 2007). Interestingly, transplants were planted with and without compost and no compost effect was found for either biomass production or community profiles in these tailings.

The objectives of the current study were (i) to investigate whether surface sterilization of seeds prior to inoculation is necessary for PGPB to enhance plant growth in mine tailings, (ii) to compare two inoculation methods; immersion versus alginate-encapsulation, and (iii) to investigate how PGPB inoculation of seeds alters rhizosphere bacterial communities as measured by DGGE profile analysis. The mine tailings used in this study were obtained from the Klondyke mine tailings site, an Arizona State Superfund site located in the southeast corner of Arizona (Tummala and Humble, 1998). This site is similar to many found in the southwestern United States in that it is acidic in

nature and has elevated levels of metal contaminants, in particular lead and zinc. Previous research has indicated that a compost rate of 15% (w/w) is necessary to achieve plant growth in the Klondyke tailings that is statistically similar to offsite controls (Mendez et al., 2007). Additional research has indicated that some strains of PGPB have to ability to significantly increase the growth of native desert plant species (*Atriplex lentiformis* and *Buchloe dactyloides*) at compost rates of 0 and 10% in these tailings (Grandlic et al., 2008).

3.0 Materials and Methods

Site description. Tailings samples were collected and homogenized from the Klondyke mine tailings site located in Aravaipa Valley, Graham County, Arizona where a lead/zinc flotation mill was in operation from 1948 to 1952 (Wilson, 1959). Physical and chemical characteristics for the tailings used in this study have been previously described (Grandlic et al., 2008).

Native Plants. Two plants native to the desert southwest were evaluated for growth in tailings in this study, the shrub *Atriplex lentiformis* (Torr.) S. Wats., commonly known as quailbush and the grass *Buchloe dactyloides* (Nutt.) Engelm., commonly known as buffalo grass. *B. dactyloides* seed was obtained from Western Native Seed, Coaldale, CO. *A. lentiformis* seed was obtained from Carter Seeds, Vista, CA.

Bacterial isolates. Three PGPB were used in this study; MTR-21A (*Clavibacter sp.*), MTR-45B (*Rhodanobacter sp.*) and K4-10/MTR-44, a mixture of two *Arthrobacter sp.* (K4-10C and MTR-44) referred to as Arthro Mix. All three inoculants have been previously characterized and have demonstrated the ability to enhance dry plant biomass production of *A. lentiformis* and *B. dactyloides* in the Klondyke tailings (Grandlic et al., 2008). Briefly, MTR-21A has the ability to grow under an acidic pH of 4 and 5, is moderately tolerant to Zn, solubilizes phosphate, and produces small amounts of indole-3-aciticacid (IAA) and siderophores; MTR-45B has the ability to grow at pH 4 and 5, is tolerant to both Pb and Zn, and produces IAA and siderophores; K4-10C has the ability to grow at pH 5, is tolerant to Zn and produces both IAA and siderophores; MTR-44 has the ability to grow at pH 5, is tolerant to both Pb and Zn, solubilizes phosphate, possesses ACC-deaminase activity, and produces IAA and siderophores. All isolates used were maintained on R2A in a laboratory culture collection.

Experimental design and greenhouse conditions. A total of four treatments were tested. To evaluate the effects of surface sterilization on plant biomass production, seeds from both *A. lentiformis* and *B. dactyloides* were either surface-sterilized or remained non-sterilized (see descriptions below). To evaluate the effects of the two different inoculation methods on plant biomass production, both surface-sterilized and non-sterilized seeds were inoculated using either the immersion or the alginate-encapsulation methods. Following inoculation, seeds were transferred to 3 L pots filled with Klondyke tailings amended with 10% compost (w/w); tailings were pre-wetted to field capacity 48

h prior to planting. Fifteen seeds were planted in each pot and five replicates were used per treatment. Four controls were used; non-inoculated surface sterilized and non-inoculated non-sterilized seeds that were either subjected to the immersion method with no PGPB or inoculated with PGPB-free alginate beads.

Following planting, pots were placed in a greenhouse located at the University of Arizona's Controlled Environment Agriculture Center (Tucson, AZ) for 75 d (Dec. 2007 – Feb. 2008). Each pot was irrigated three times daily via a drip irrigation system distributing a total depth of 1.5 cm $pot^{-1}d^{-1}$. The greenhouse was maintained at high humidity with a constant temperature of 32°C. Fluorescent supplemental lighting (200 $\mu m\ m^{-2}\ s^{-1}$) was used to extend the daily photoperiod to 13 h d^{-1} as necessary.

Seed surface preparation. Surface sterilized seeds were prepared prior to inoculation as follows; seeds were soaked in sterile distilled water for 10 min followed by a 1 min immersion in 95% ethanol, seeds were immediately rinsed with sterile distilled water and soaked for 10 min in a 2% sodium hypochlorite solution. Following this soak, seeds were rinsed three times with sterile distilled water and soaked for three min in a 0.1% sodium thiosulfate solution and rinsed a final time. Non-sterilized seeds were prepared by soaking in sterile distilled water for 25 min. Following inoculation all seeds were transferred to Klondyke tailings receiving 10% compost (w/w).

Preparation and inoculation of PGPB using the immersion method. PGPB cultures were prepared 48 h prior to inoculation by transferring single colonies from R2A plates to

100 mL of R2B in a 250 mL Erlenmeyer flask and incubating on a rotary shaker (200 rpm) at 23°C. Immediately prior to inoculation, the cultures were centrifuged at 12,100 x *g* for 10 min, the culture supernatant was removed and cells were re-suspended in sterile PBS; (g L^{-1}) 8.0 NaCl, 0.2 KCl, 1.44 Na_2HPO_4, 0.24 KH_2PO_4, adjusted to pH 7.4. Isolate suspensions were adjusted to an absorbance = 1 at 600 nm using PBS which was equivalent to an approximate concentration of 10^9 CFU mL^{-1}. Counts were confirmed by enumerating all isolate suspensions used.

Approximately 100 surface sterilized seeds or non-sterilized seeds were aseptically transferred to each individual isolate suspension and allowed to incubate for 10 min with a 5 sec vortexing period every min. The suspension/seed mixtures were then subjected to a vacuum of 700 mm Hg for 5 min after which the vacuum was quickly removed forcing the bacterial suspension into micro-pore spaces on the seed surface originally occupied by air (Puente and Bashan, 1993). Seeds were then transferred to Klondyke tailings at a rate of 15 per pot.

Preparation and inoculation of alginate-encapsulated PGPB isolates. To prepare alginate-encapsulated cells, individual isolates were grown at 23°C on rotary shaker (200 rpm) in three 0.5 L volumes of 3X R2A; (g L^{-1}) 1.5 yeast extract, 1.5 glucose, 1.5 casein hydrolysate, 1.5 soluble starch, 1.5 Proteose peptone No. 3, 0.9 K_2HPO_4, 0.9 sodium pyruvate, and 0.1 MgSO4·7H2O, adjusted to pH 7.4. After 48 – 72 hr (cell density of 10^9 CFUs mL^{-1}) cells were harvested and concentrated by centrifuging at 12,000 x *g* for 15

min. The supernatant was removed, discarded and the resulting cell pellets were re-suspended in 10 mL of 50 mM PIPES buffer (pH 7.4) for a cell density of approximately 10^{11} CFU mL^{-1}. The cell suspension was then thoroughly mixed with an equal volume of a sterile 3.5% alginate solution and forced through a sterile 30.5 gauge syringe into a chilled ($4^{o}C$) 0.15 M $CaCl_2$ solution. *Arthrobacter spp*. mixture beads (MTR-44 and K4-10C) were produced by combining equal volumes of the two cultures after centrifugation prior to addition of the sodium alginate mixture. Alginate beads (approximately 300 – 500 µM) formed immediately upon contacting the $CaCl_2$ solution and were allowed to set at a minimal stirring speed (100 rpm) for at least 2 hr. Polymerized alginate beads were collected by filtration through a sterile Buchner funnel and rinsed 3 times with sterile 50 mM PIPES buffer. Alginate-encapsulated cells were aseptically transferred to 50 mL plastic centrifuge bottles and lyophilized until completely dried for 48 – 72 hr. Cell densities (CFUs $gram^{-1}$ dry beads) were obtained by dissolving 0.05 g of beads in 5 mL of 0.25M sodium carbonate for 30 min, diluting in PBS and plating onto R2A agar. Lyophilized beads for each individual inoculation were introduced to surface sterilized seeds or non-sterilized at a rate of 2 x 10^7 CFU $seed^{-1}$. An appropriate volume of beads was added to inoculate 75 seeds per treatment. After inoculation the seed/bead mixture was aseptically homogenized allowing the dried beads to adhere to the seeds, and seeds were aseptically transferred to Klondyke tailings amended with 10% compost material (w/w) at a rate of 15 seeds per pot.

Plant biomass and metal uptake analysis. Dry plant biomass was determined 75 d after planting. At this time each plant was carefully harvested (roots and shoots were separated) and rinsed gently under running water to remove all tailings and compost material. Plant roots and shoots were then placed in individual foil packets, dried for 96 h in a 65^{o}C oven, and weighed.

Microbial community profiling. Selected bacterial community profiles associated with plant rhizospheres and mine tailings were evaluated using DGGE analysis. Triplicate rhizosphere samples for each treatment were obtained at the end of the experiment by aseptically scraping approximately 2 g of substrate from plant root surfaces. Samples were transferred into sterile 1.8 mL Eppendorf tubes and stored at -20^{o}C. Genomic DNA extractions from samples were done using FastDNA SPIN Kits for Soil® (Bio 101, Inc., Vista, CA) following the instructions as recommended by the manufacturer. Genomic DNA extractions were stained using PicoGreen® and quantified using a fluoroimager (Invitrogen).

DNA extracts were amplified using a modified protocol originally described by Colores et al. (2000) using the universal bacterial primers 1070f and 1406r-GC (Ferris et al, 1996). Each 50 µL reaction contained 1X buffer (10nM Tris-HCl, 50 mM KCl, 2.0 mM MgCl2 – pH 8.3), 0.5 µM each primer, 400 mg L-1 bovine serum albumin, 0.2 mM each bNTP, 5% DMSO, 1 U of Hot Start Taq DNA polymerase, and 2 ng of DNA template (genomic DNA extraction). The amplification protocol used was 95^{o}C for 15 min followed by 30 cycles of 94^{o}C, 55^{o}C, 72^{o}C for 45 sec each and a final extension at

72°C for 10 min followed by 4°C. After amplification, PCR products were visualized on a 1.5% agarose gel using an Alpha Imager (Alpha Innotech, San Leandro, CA). PCR products were quantified by comparison to a known standard, Mass Ruler™ (Fermentas Inc., Glen Burnie, MD), using the "density" feature on Quantity One® software (Bio-Rad Laboratories, Inc., Hercules, CA).

PCR products were loaded at a rate of 500 ng per lane on to DGGE gels containing 7% acrylamide and a 40 – 80% urea-formamide denaturing gradient. Gels were run at 60°C and 60 V for 17 h. Following the run, gels were stained in 1 X SYBR Green (Molecular Probes, Eugene, OR) for 20 min followed by a 20 min rinse in 1 X TAE and imaged using an Alpha Imager. Microbial community banding profiles on DGGE gels were analyzed using the Quantity One® software package (Bio-Rad Laboratories, Inc., Hercules, CA). The profiles obtained from DGGE gels were analyzed via Kruskal's Non-Metric Multidimensional Scaling (KNMDS, Venables and Ripley, 2002) to evaluate similarities among profiles for the each treatment tested (Rosario et al., 2007). This non-parametric ordination method was used to visualize and interpret changes in the bacterial community based on binary distance where similar bacterial communities (i.e., profiles) cluster more closely than those with low similarity in multidimensional space. Differences between bacterial communities were evaluated with a permutation test ($\alpha = 0.05$). A stress factor was also calculated to reflect goodness-of-fit of the model ($sf < 0.1$ was considered a good fit). The configurations in multidimensional space were evaluated using three or four dimensions (D) to minimize

stress. Comparisons of the bacterial community could be performed only for samples within the same gel.

Statistical analysis. Statistical analysis was conducted using SAS Version 9.1 (SAS Institute Inc. Cary, NC). Significant treatment effects for plant root, shoot and total biomass and root-to-shoot data were detected by employing a one-way ANOVA ($p < 0.05$). For each treatment, significant differences between means were determined by the Duncan's Multiple Range Test ($p < 0.05$).

4.0 Results and Discussion

Effect of surface sterilization. In the absence of PGPB inoculation (control treatments), surface sterilization of seeds did not affect the average total dry pot biomass of *A. lentiformis* in either the immersion or the alginate treatments (Table 1, Figure 1). In contrast, for *B. dactyloides*, surface sterilization of seeds resulted in a significant (approximately 3-fold) decrease in biomass production for both immersion and alginate treatments (Table 1, Figure 2). Surface sterilization of *B. dactyloides* plants also appeared to affect plant survival as immersion-treated and alginate-treated controls decreased from 48 to 39 and 57 to 45 surviving plants when seeds were sterilized, respectively (Figure 2).

Surface sterilization of seeds in combination with PGPB inoculation did not impact any *A. lentiformis* treatment; there was no effect of seed sterilization for any of the PGPB tested (Table 1, Figure 1). However, for *B. dactyloides* (which exhibited decreased biomass production when seeds were surface sterilized in control treatments),

the seed sterilization effect was mitigated in some instances by the addition of PGPB. Specifically, the Artho Mix negated the impact of seed sterilization for both treatments, while the other two PGPB (MTR-21A and MTR-45B) mitigated the effect for immersion but not for alginate treatments (Figure 2). These results indicate that surface sterilization may be potentially harmful to some plant species stressing the importance of investigating the effects of seed preparation on plant growth prior to conducting large-scale studies. This is consistent with previous work including that of Wilson (1976) who observed that seed surface sterilization using sodium hypochlorite was detrimental to wheat seed germination, however, had no effect on the germination of sorghum and soybean. More recently, surface sterilization of seeds prior to introducing a PGPB was shown to have a negative effect on seed surface colonization (Miché and Balandreau, 2001).

Comparison of Inoculation Methods. For *A. lentiformis*, the maximum biomass produced across all treatments was approximately 9 g pot^{-1}. This level of biomass production was only achieved in the presence of PGPB and was 1.6 to 3-fold higher than uninoculated controls although these differences were not significant (Figure 1). All three PGPB generally enhanced plant biomass production regardless of inoculation method (Figure 1). These results suggest that alginate inoculation can be successfully used to apply PGPB to seeds prior to planting. This is important because alginate encapsulation is a method that allows the inoculum to be stored stably for long periods of

time. In fact, we have stored lyophilized alginate encapsulated PGPB for periods exceeding one year and recovered normal levels of culturable counts (data not shown).

Interestingly, control *A. lentiformis* treatments that contained PGPB-free alginate beads had lower average dry pot biomass than control treatments inoculated using the immersion method regardless of surface sterilization procedure suggesting that alginate itself is detrimental to *A. lentiformis* biomass production (Table 1). However, when alginate-encapsulated PGPB were introduced, total pot biomass increased to levels consistent with good PGPB performance for all three isolate treatments regardless of surface sterilization procedure (Figure 1). These results suggest that the potentially deleterious effects of alginate alone are overcome when it is used to encapsulate PGPB.

For *B. dactyloides*, the maximum biomass produced was approximately 8 g pot^{-1}. This level of production was achieved only with selected PGPB and was 1.5 to 5-fold (significant) higher than for uninoculated controls. The Arthro Mix was the only PGPB to result in consistently high biomass production for all treatments, although MTR-45B performed well in the non-sterile immersion treatment and MTR-21A performed well in the non-sterile alginate treatment (Table 1, Figure 2).

Taken together, these results suggest that surface sterilization of seeds prior to use in such phytostabilization trials is not necessary whether or not a PGPB is used. Further, alginate encapsulation of PGPB seems compatible with their use in phytostabilization although this must be qualified in the following way. This study shows that some PGPB, in this case the Arthro Mix, are more robust than others across different plants (*A. lentiformis* and *B. dactyloides*), seed preparation (sterile vs. non-sterile), and inoculation

methods (immersion vs. alginate encapsulation). Further, this work cautions that screening studies should be performed to demonstrate the efficacy of PGPB under the conditions that they will be applied.

Community Structure Analysis. The difference observed in PGPB performance across different treatments as well as our inability to detect any of the PGPB at the end of the 75 d experiment using isolate specific PCR primers with a sensitivity of 10^5 CFU g^{-1} dry substrate and by comparing DGGE community profiles with PGPB isolate profiles (data not shown) led us to hypothesize that the PGPB inoculants in some way influence the development of the rhizosphere. Therefore, rhizosphere samples were collected from *B. dactyloides* plants at 75 d and subjected to DGGE analysis to compare the bacterial community profiles. We chose to focus this work on two treatments, sterile immersion and non-sterile immersion, in one plant, *B. dactyloides*. We chose these samples because they exhibited the greatest biomass differences between the uninoculated controls and inoculated treatments (Figure 2). In addition, the bacterial community in the compost amendment was examined with each of the two treatments.

Analysis of DGGE profiles from triplicate biological replications (rhizosphere samples were taken from different pots for each replicate) indicated that community profiles are influenced by treatment. Bacterial community profiles taken from the sterile immersion treatment are shown in Figure 3, while community profiles taken from non-sterile immersion treatment are shown in Figure 4. The DGGE profiles were then compared using KNMDS to determine whether there were PGPB or compost effects on

the bacterial community. Each KNMDS graph illustrates the first two out of either three or four dimensions. In these graphs the relative distance between samples indicates the amount of similarity or difference among data points.

The KNMDS analysis of the DGGE profiles from the *B. dactyloides* sterile immersion treatment showed four significantly different ($p = 0.32$) clusters 75 d after planting (Figure 5). Results show that the rhizosphere bacterial communities on plants that received PGPB inoculation clustered differently from those did not. This suggests that PGPB have a long-term influence on the rhizosphere bacterial community that develops following plant establishment in mine tailings. Examination of the individual PGPB inoculants shows a separation into two clusters, one of rhizosphere communities from plants receiving the Arthro Mix and one that represents samples from five of the six plants receiving either MTR-21A or MTR-45B. It is of interest to note that the Arthro Mix, which clusters separately, is also the best-performing PGPB. This leads to the intriguing idea that the Arthro Mix may direct the development of a unique rhizosphere community that aids in establishment and growth of *B. dactyloides*. Figure 5 also shows that the compost community was significantly different from all uninoculated (control) and PGPB inoculated communities suggesting that there is little influence of the original compost bacterial community on the plant rhizosphere community at the end of the experiment.

The influence of PGPB on community development was also examined for the non-sterile immersion treatment. The results reveal three significantly different clusters ($p = 0.47$) (Figure 6). Similar to the sterile immersion treatment, the PGPB inoculated

rhizosphere communities were different from the uninoculated control and compost communities. Also similar to the sterile immersion treatment, the Artho Mix rhizosphere communities clustered separately from 5 out of the 6 MTR-21A and MTR-45B communities. The major difference between the sterile and non-sterile immersion treatments was in the relationship between the uninoculated controls and the compost. In the non-sterile immersion treatment these communities clustered together rather than separately indicating that in this case the initial compost community does influence the development of the uninoculated control rhizosphere communities. Although this needs further exploration, these results suggest that surface sterilization of seeds alone has an effect on community development.

This study provides an intriguing glimpse into the possibility that introduced PGPB can help direct the development of bacterial communities in the plant rhizosphere. de-Ridder-Duine et al. (2005) conducted a similar PCR-DGGE investigation of natural rhizosphere microbial community structures in wild sand sedge (*Carex arenaria*). The authors reported that rhizosphere microbial communities clustered more closely to bulk soil samples from their original location than to samples collected from the same plant species in different soil types, suggesting that the microbial communities in bulk soil influences community development. Other authors have also proposed similar conclusions (Buyer et al., 2002; Dalmastri et al., 1999; Buyer et al., 2002). Our investigation suggests that PGPB may have the potential to influence the direction of community formation within the first 75 d of *B. dactyloides* growth. Introduced PGPB may have a greater potential to do this in the harsh mine tailings environment than in a

healthy soil as tailings have severely impacted microbial communities (Mendez et al., 2007). While further investigation into this phenomenon is necessary, these results suggest there is potential for identifying bacterial isolates or consortia that direct the development of desired, healthy rhizosphere communities during phytostabilization applications.

5.0 Conclusions

The use of PGPB to enhance plant establishment in mine tailings and metal contaminated soils has been previously demonstrated. Simplifying this process by removing unnecessary and potentially harmful steps such as surface sterilization and developing user-friendly methods such as alginate inoculation will make this technology more applicable to field-scale use. Our results show that surface sterilization is not necessary to achieve desirable results when applying PGPB to enhance plant establishment in mine tailings. The present study also demonstrates that the introduction alginate-encapsulated PGPB is a viable method for enhancing plant growth in composted Klondyke tailings. Finally, investigation of the bacterial community associated with *B. dactyloides* rhizospheres suggests that introduced PGPB may have the potential to influence rhizosphere community development.

6.0 References

Bashan, Y. 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol. Adv. **16:**729-770.

Bashan, Y., G. Holguin, and L.E. de-Bashan. 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Can. J. Microbiol. **50:**521-577.

Bashan, Y., J.-P. Hernandez, L.A. Leyva, and M. Bacilio. 2002. Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. Biol. Fertil. Soils. **35:**359-368.

Bashan, Y., M. Moreno, and E. Troyo. 2000b. Growth promotion of the oilseed halophyte *Salicornia bigelovii* in seawater inoculated with mangrove rhizosphere bacteria and *Azospirillum* . Biol. Fert. Soils. **32:** 265-272.

Belimov, A.A., N. Hontzeas, V.I. Safronova, S.V. Demchinskaya, G. Piluzza, S. Bullitta, and B.R. Glick. 2005. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). Soil Boil. Biochem. **37:**241-250.

Belimov, A.A., V.I. Safronova, T. Mimura. 2002. Response of spring rape to inoculation with plant growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase depends on nutrient status of the plant. Can. J. Microbiol. **48:**189-199.

Berti, W.W.R., and S.D. Cunningham. 2000. Phytostabilization of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals – using plants to clean up the environment. John Wiley & Sons, Inc. New York. pp 71–88.

Burd, G.I., D.G. Dixon, and B.R. Glick. 2000. Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can. J. Microbiol. **46:**237-245.

Buyer, J.S., D.P. Roberts, and E. Russek-Cohen. 2002. Soil and plant effects on microbial community structure. Can. J. Microbiol. **48:**955–964.

Carrillo, A.E., C.Y. Li, and Y. Bashan. 2002. Increased acidification in the rhizosphere of cactus seedlings induced by *Azospirillum brasilense.* Naturwissenschaften. **89:**428-432.

Castro-Larragoitia J., U. Kramar, and H. Puchelt. 1997. 200 years of mining activities at La Paz, San Luis Potosi, Mexico – consequences for environment and geochemical exploration. J. Geochem. Explor. **58:**81–91.

Colores, G.M., R.E. Macur, D.M. Ward, and W.P. Inskeep. 2000. Molecular analysis of surfactant-driven microbial population shifts in hydrocarbon-contaminated soil. Appl. Environ. Microbiol. **66:**2959–2964.

Cunningham, S.D., W.R. Betri, J.W. Huang. 1995. Phytoremediation of contaminated soils. Trends Biotechnol. **13:**393-397.

Dalmastri, C., L. Chiarini, C. Cantale, A. Bevivino, S. Tabacchioni. 1999. Soil type and maize cultivar affect the genetic diversity of maize root-associated *Burkholderia cepacia* populations. Microbial Ecol. **38:**273–284.

De-Ridder-Duine, A.S., G.A. Kowalchuk, P.J.A. Klein Gunnewiek, W. Smant, and J.A. van Veen. 2005. Rhizosphere bacterial community composition in natural stands of *Carex arenaria* (sand sedge) is determined by bulk soil community composition. Soil Biol. Biochem. **37:**349-357.

Donate-Correa, J., M. Leon-Barrios, and R. Perez-Galdona. 2004. Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. Plant and Soil. **266:**261-272.

Ferris, M.J., G. Muyzer, and D.M. Ward. 1996. Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring mat community. Appl. Environ. Microbiol. **62:**340–346.

Glick, B.R., D.M. Penrose, and J. Li. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J. Theo. Biol. **190:**63-68.

Gonzalez, R.C., M.C.A Gonzalez-Chavez. 2006. Metal accumulation in wild plants surrounding mining wastes: soil and sediment remediation (SSR). Environ. Poll. **144:**84-92.

Grandlic, C.J., M.O. Mendez, J. Chorover, B. Machado, and R.M. Maier. 2008. Plant growth-promoting bacteria for phytostabilization of mine tailings. Environ. Sci. Tech. *In Press.*

Marschner, P., C.H. Yang, R. Lieberei, and D.E. Crowley. 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol. Biochem. **33:**1437–1445.

Mendez, M.O., E.P. Glenn, and R.M. Maier. 2007. Phytostabilization potential of quailbush for mine tailings: growth, metal accumulation, and microbial community changes. J. Environ. Qual. **36:**245-253.

Mendez, M.O. and Maier R.M. 2008a. Phytoremediation of mine tailings in temperate and arid environments. Rev. Environ. Sci. Biotechnol. 7:47-59.

Mendez, M.O., and R.M. Maier. 2008b. Phytostabilization of mine tailings in arid and semiarid environments – an emerging remediation technology. Environ. Health Perspect. **116:**278-283.

Miché, L., and J. Balandreau. 2001. Effects of rice seed surface sterilization with hypochlorite on inoculated *Burkholderia vietnamiensis*. Appl. Environ. Microbiol. **67:**3046-3052.

Moynahan O.S., C.A. Zabinski, and J.E. Gannon. 2002. Microbial community structure and carbon utilization diversity in a mine tailings revegetation study. Restor. Ecol. **10:**77-87.

Munshower, F.F. 1994. Practical handbook of disturbed land revegetation. Boca Ratton, FL:Lewis Publishing.

Patten, C.L., and B.R. Glick. 2002. Role of *Psuedomonas putida* indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. **68:**3795-3801.

Puente, M.E. and Y. Bashan. 1993. Effect of inoculation with *Azospirillum brasilense* strains on the germination and seedlings growth of the giant columnar cardon cactus (*Pachycereus pringlei*). Symbiosis. **15:**49–60.

Reed, M.L.E., and B.R. Glick. 2005. Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can. J. Microbiol. **51:**1061-1069.

Rosario, K., S.L. Iverson, D.A. Henderson, S. Chartrand, C. McKeon, E.P. Glenn, and R.M. Maier. 2007. Bacterial community changes during plant establishment at the San Pedro River mine tailings site. J. Environ. Quality. **36:**1249-1259.

Tordoff, G.M., A.J.M. Baker, A.J. Willis. 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere **41:**219-228.

Tummala, P. S. L., and W. Humble. 1998. Public health assessment; Klondyke mine tailings. Arizona Department of Health Services.

Vazquez, P., G. Holguin, M.E. Puente, A. Lopez-Cortez, and Y. Bashan. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol. Fertil. Soils. **30:**460-468.

Venables, W.N., and B.D. Ripley. 2002. Modern applied statistics with S. 4th ed. p. 385–389. Springer Statistics and Computing Series, New York.

Wilson, D.O. 1976. Evaluation of chemical seed coat sterilants. Plant Soil. **44:**703-707.

7.0 Figure Legends

Figure 1. Average dry pot biomass of surface-sterilized or non-sterilized *A. lentiformis* seeds inoculated with PGPB treatments (*Arthrobacter* Mixture, MTR-21A, and MTR-45B) 75 d after planting. Both surface-sterilized and non-sterilized seeds were inoculated using the immersion method or alginate-encapsulated PGPB. Error bars represent +/- 1 SD for total pot biomass. Lower and upper bars represent the average dry root biomass and average dry shoot biomass for each treatment, respectively. The number at the bottom of each bar represents the surviving plants out of 75 total planted seeds. Means with different letters are significantly different in total dry pot biomass at $p < 0.05$.

Figure 2. Average dry pot biomass of surface-sterilized or non-sterilized *B. dactyloides* seeds inoculated with PGPB treatments (*Arthrobacter* Mixture, MTR-21A, and MTR-45B) 75 d after planting. Both surface-sterilized and non-sterilized seeds were inoculated using the immersion method or alginate-encapsulated PGPB. Error bars represent +/- 1 SD for total pot biomass. Lower and upper bars represent the average dry root biomass and average dry shoot biomass for each treatment, respectively. The number at the bottom of each bar represents the surviving plants out of 75 total planted seeds. Means with different letters are significantly different in total dry pot biomass at $p < 0.05$.

Figure 3. DGGE analysis of triplicate *B. dactyloides* rhizosphere bacterial community profiles obtained from non-sterilized treatments inoculated using the immersion method. The identity of each inoculated PGPB is indicated at the top of the gel. Control samples are uninoculated treatments. Compost samples represent the community present in the compost prior to mixing with the tailings. These samples were examined to determine the similarity between the compost bacterial community and the rhizosphere communities at the end of the study.

Figure 4. DGGE analysis of *B. dactyloides* triplicate rhizosphere microbial community profiles obtained from surface-sterilized treatments inoculated using the immersion method. The identity of each inoculated PGPB is indicated at the top of the gel. Control samples are uninoculated treatments. Compost samples represent the community present in the compost prior to mixing with the tailings. These samples were examined to determine the similarity between the compost bacterial community and the rhizosphere communities at the end of the study.

Figure 5. Kruskal's Non-Metric Multidimensional Scaling (KNMDS) analysis of DGGE bacterial community profiles from the *B. dactyloides* sterilized immersion treatments. Labels correspond to the lanes in the DGGE gel in Figure 3. Circles delineate four clusters that are significantly different from each other ($p = 0.032$, stress factor = 0.041, D = 4 where D = dimension).

Figure 6. Kruskal's Non-Metric Multidimensional Scaling (KNMDS) analysis of DGGE bacterial community profiles from the *B. dactyloides* non-sterilized immersion treatments. Labels correspond to the lanes in the DGGE gel in Figure 4. Circles delineate four clusters that are significantly different from each other ($p = 0.047$, stress factor = 0.069, D = 3 where D = dimension).

Table 1. Effect of inoculation method on average dry pot biomass[a]

Treatment	Sterile Immersion	Non-Sterile Immersion	Sterile Alginate	Non-Sterile Alginate
		Atriplex lentiformis		
Control	5.6 ± 2.8 (1.0)	5.0 ± 4.0 (1.0)	2.9 ± 3.6 (1.0)	3.0 ± 2.9 (1.0)
Arthro Mix	9.3 ± 4.6 (1.7)	8.7 ± 2.7 (1.7)	6.9 ± 4.3 (2.4)	5.9 ± 3.6 (1.9)
MTR-21A	7.1 ± 2.2 (1.3)	5.3 ± 3.5 (1.1)	9.4 ± 2.4 (3.2)	6.3 ± 3.0 (2.1)
MTR-45B	7.5 ± 5.1 (1.3)	8.3 ± 3.6 (1.7)	8.1 ± 3.6 (2.8)	6.7 ± 1.3 (2.2)
		Buchloe dactyloides		
Control	1.5 ± 2.4 (1.0)	3.9 ± 4.0 (1.0)	2.1 ± 1.1 (1.0)	5.6 ± 2.9 (1.0)
Arthro Mix	8.0 ± 1.3 (5.3)	8.1 ± 1.9 (2.1)	8.4 ± 3.3 (4.0)	8.3 ± 1.8 (1.5)
MTR-21A	5.3 ± 4.4 (3.5)	4.6 ± 4.6 (1.2)	1.4 ± 2.0 (0.7)	7.3 ± 3.0 (1.3)
MTR-45B	5.8 ± 2.0 (3.9)	8.2 ± 3.7 (2.1)	4.0 ± 2.1 (1.9)	3.8 ± 1.8 (0.7)

[a] Biomass values are presented as the average and standard deviation of 5 replicates in g dry biomass pot^{-1}. The number in parentheses following biomass values is the fold increase or decrease in biomass in comparison to the control which is normalized to 1.

Figure 1 *A. lentiformis* Biomass

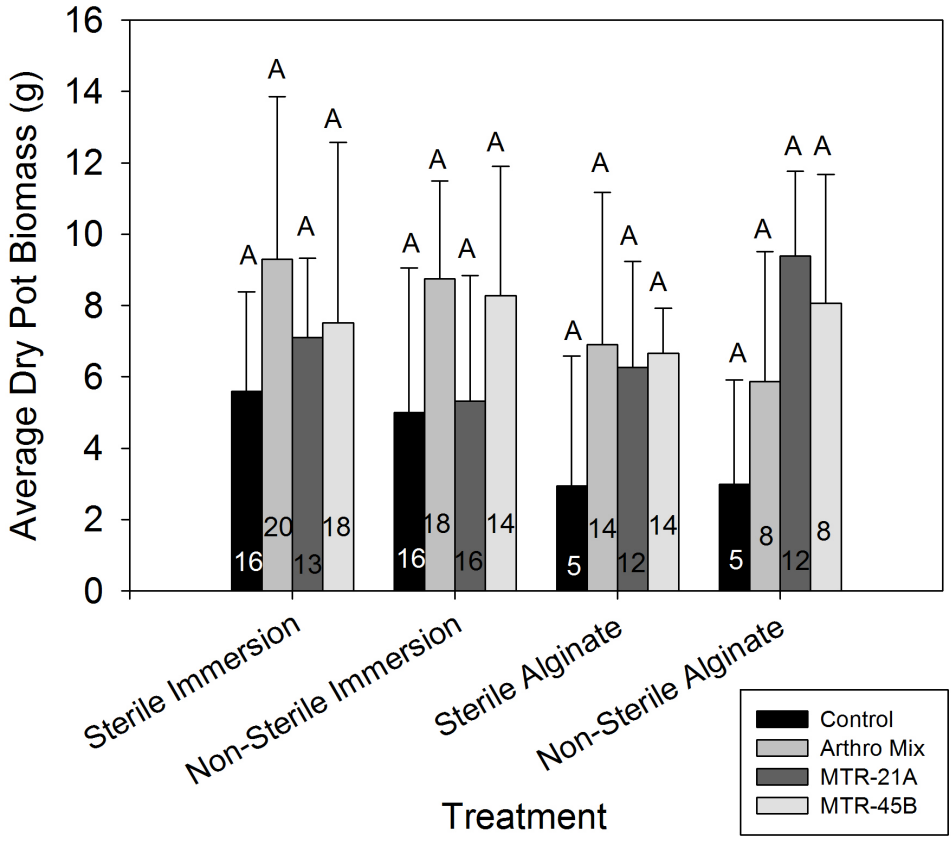


Figure 2 *B. dactyloides* Biomass

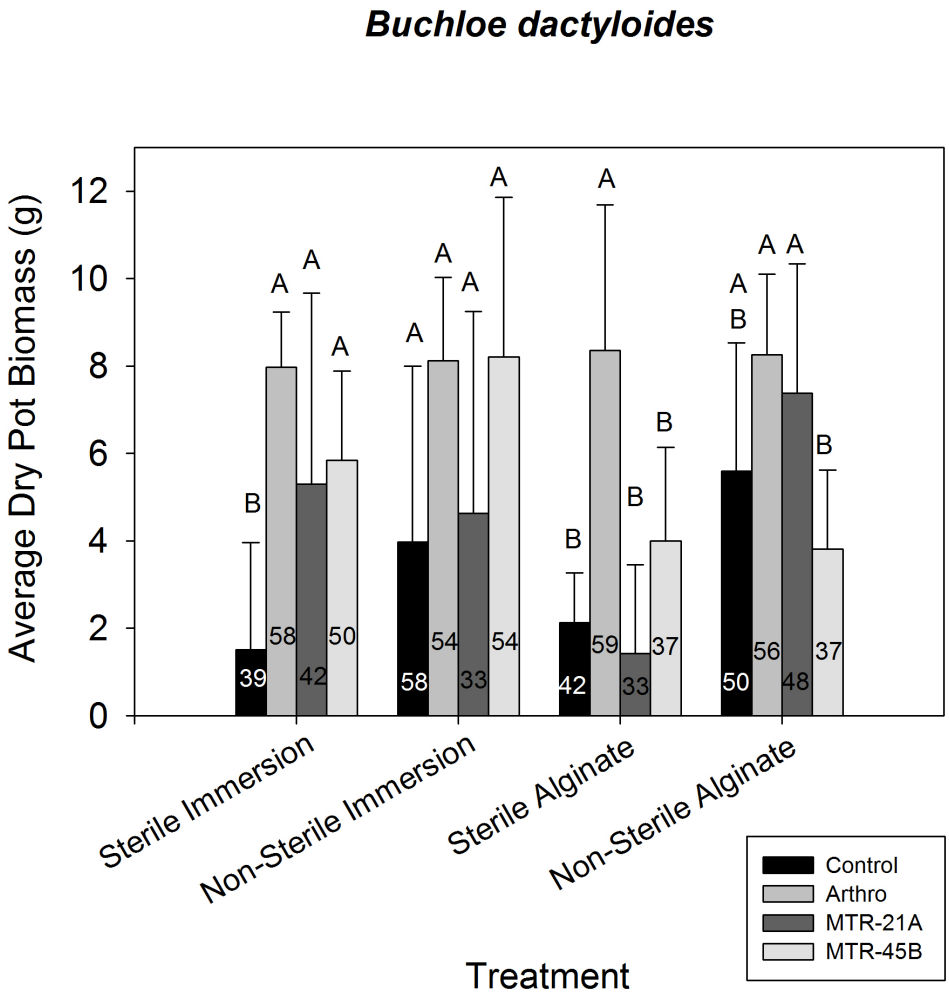


Figure 3 DGGE Image Displaying Microbial Community Profiles Obtained from *B. dactyloides* Non-Sterile Immersion Treatments

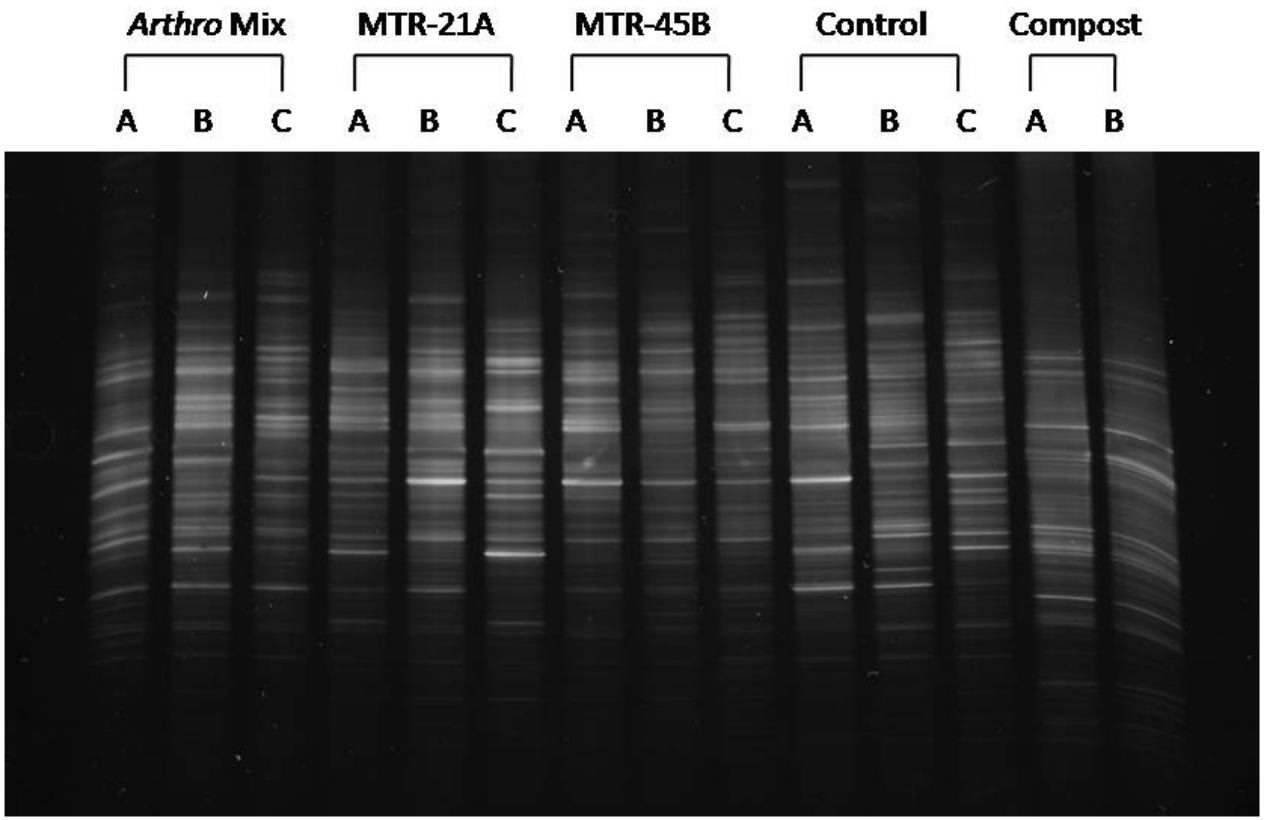


Figure 4 DGGE Image Displaying Microbial Community Profiles Obtained from *B. dactyloides* Sterilized Immersion Trea

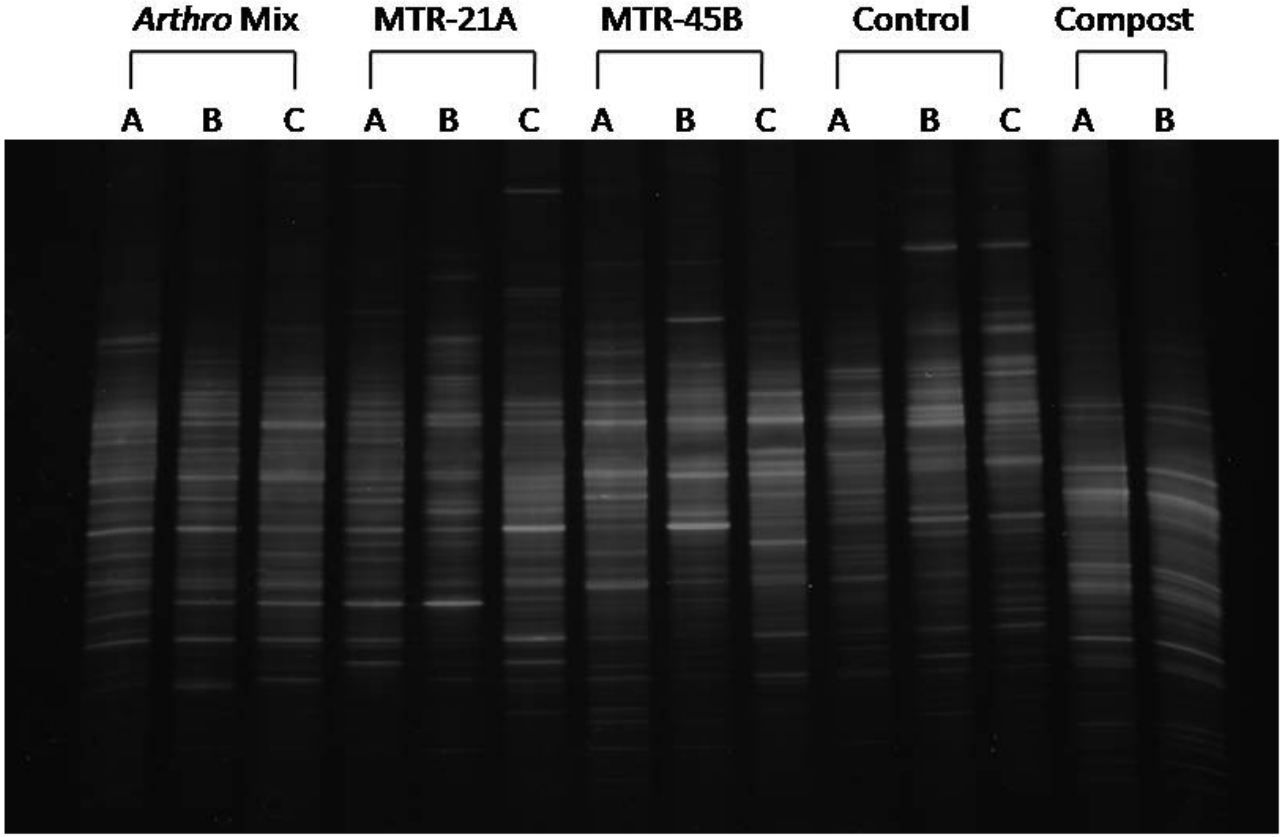


Figure 5 Kruskal's Non-Metric Multidimensional Scaling (KNMDS) analysis of DGGE bacterial community profiles from the *B. dactyloides* sterilized immersion treatments. Labels correspond to the lanes in the DGGE gel in Figure 3.

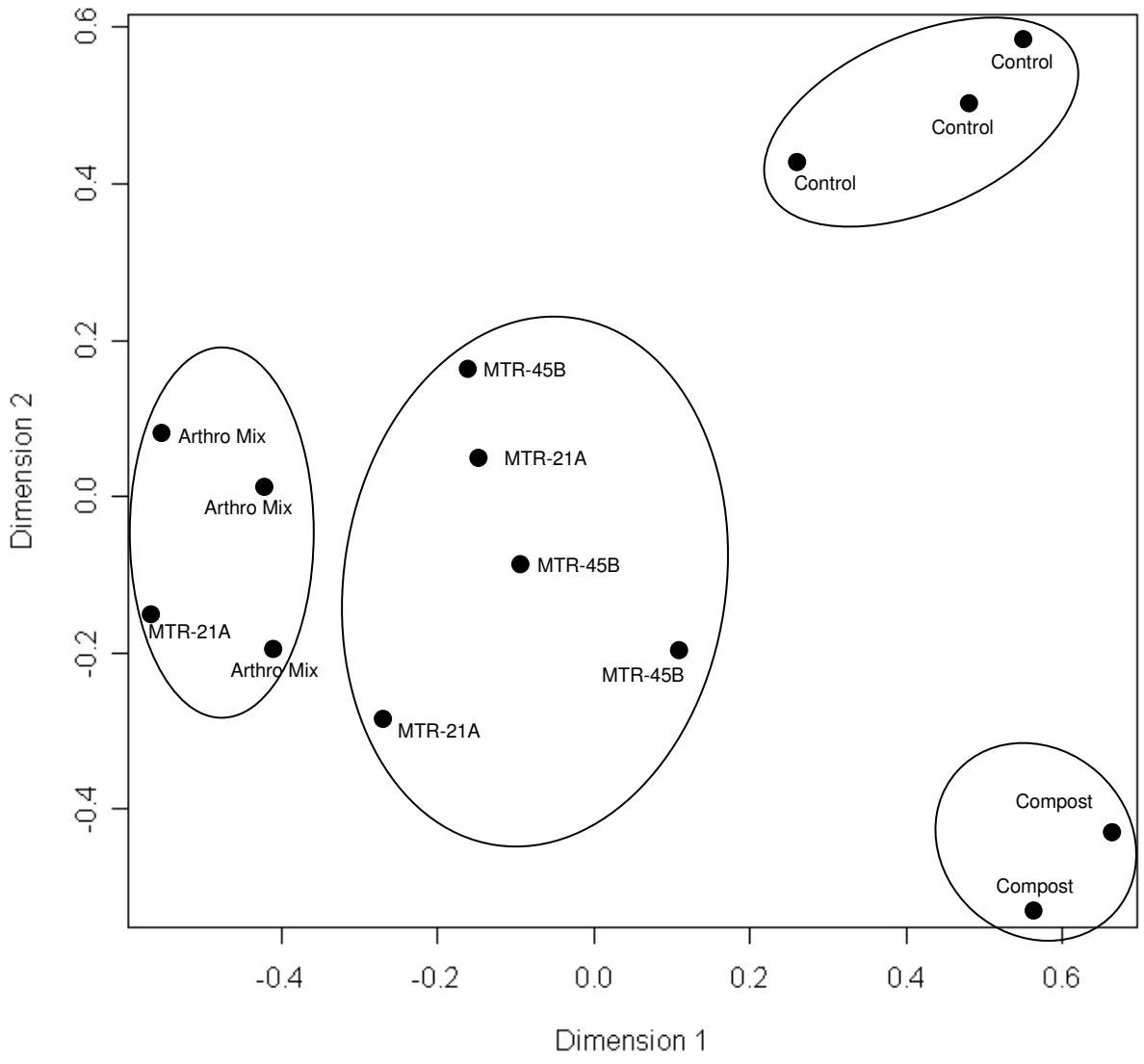
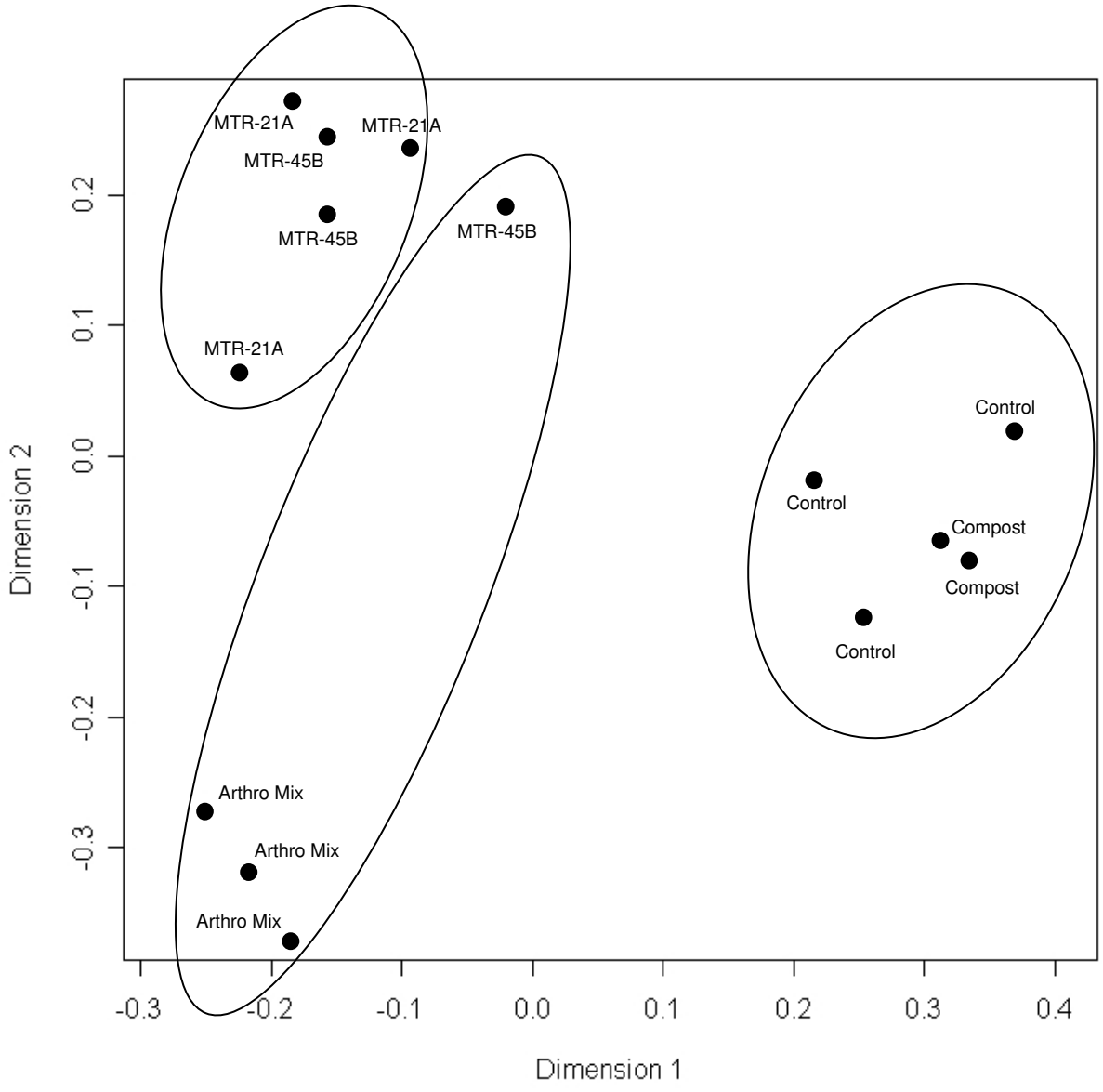


Figure 6 Kruskal's Non-Metric Multidimensional Scaling (KNMDS) analysis of DGGE bacterial community profiles from the *B. dactyloides* non-sterilized immersion treatments. Labels correspond to the lanes in the DGGE gel in Figure 4.



APPENDIX A

SCREENING FOR COMMON PLANT GROWTH-PROMOTING MECHANISMS

Prelude to Appendix

This appendix was created to familiarize the reader with many of the commonly studied plant growth-promoting (PGP) mechanisms. The initial edition of the following literature review was created in the fall of 2005 while attending a nutrient dynamics course taught by Dr. Tom Thompson in the Department of Soil, Water and Environmental Science. This edition has since been revised and improved. The objectives of this section are to: (1) familiarize the reader with many of the commonly studied (PGP) mechanisms, (2) discuss how these mechanisms can be adapted to enhance phytoremediation projects, and finally to (3) present data that was obtained by screening a library of bacterial isolates for PGP mechanisms.

1.0 Introduction

All plants have resident microbial communities associated with their rhizospheres. Many members of these communities can form symbiotic relationships with their host plants. These microorganisms are provided with nutrients and substrates in the form of root exudates and in turn supply the plant with an assortment of nutrients and growth factors thereby enhancing one or more aspect of plant growth and survival. The importance of both rhizosphere-associated bacteria and mycorrhizal fungi have been well-documented (Requena et al., 2001). Bacterial strains that are beneficial to plant growth have been widely studied and have been termed plant growth-promoting bacteria (PGPB) (Kloepper et al., 1987). In recent decades numerous studies have reported the benefits of inoculating plants with PGPB in order to achieve enhanced plant establishment, growth and survival. These studies have been conducted under a wide assortment of settings with varying objectives. Perhaps the most widely used and studied application of PGPB has been in agriculture with the goals of enhancing crop survival and productivity (Bashan, 1998). Application of PGPB to crops may also benefit agriculture by reducing the need for fertilizer application as many of these bacteria are known for their abilities to supply plants with critical nutrients such as phosphorous and nitrogen (Bashan, 1998; Rodriguez and Fraga, 1999). Developing the use of PGPB as biofertilizers is now becoming more critical than ever as global fertilizer stocks are now in high demand, while prices of nitrogen and phosphorous fertilizers have doubled or tripled between 2007 and 2008 (Hargrove, 2008).

PGPB also have now found additional uses in areas such as tree farming (Barriuso et al., 2005), reestablishing vegetation in desertified ecosystems (Bashan et al., 1999; Bashan and Holguin, 2002; Herrera et al., 1993; Vazquez et al. 2000), and the remediation/stabilization of contaminated sites (Burd et al., 1998). Extensive efforts have been made to utilize nitrogen-fixing and phosphate-solubilizing bacteria to re-establish damaged mangrove ecosystems (Bashan and Holguin, 2002; Vazquez et al., 2000). If successful, these efforts could aid in establishing vegetation along coastal regions and thus protect shorelines from erosion. Developing methods which utilize PGPB to aid in the reestablishment of desertified regions had been a topic that has recently gained much popularity (Carrillo et al., 2002; Requena et al., 2001). Requena et al. (2001) conducted a five-year field study in which mycorrhizal fungi and rhizobacteria were used as an inoculum to reestablish vegetation (*Anthyllis cytisoides*) in a desertified Mediterranean shrub land. Not only did this inoculation improve plant growth over the uninoculated control, but it also improved soil quality and structure by increasing aggregation, and total nitrogen and organic matter content. Another such example was reported by Carrillo et al. (2002) who utilized the plant growth-promoting bacterium *Azospirillum* sp. to reestablish native cardon cacti (*Pachycereus pringlei*) and develop a soil structure in the southern Sonoran desert. A similar study was conducted by Bashan et al. (1999) who used strains of *Azospirillum* to establish cacti near an eroded dirt road in hopes of developing a vegetation cover and establishing soil structure to reduce dust pollution. The authors revealed remarkable results as 76% of inoculated plants survived after 3.5 years, while only 2% of the uninoculated control plants remained. In addition, soil

compaction in the area was reduced to that seen in undisturbed, native soil, while soil organic matter increased from 30 mg/kg to 110 mg/kg. Such studies demonstrate the great potential in introducing bacterial and mycorrhizal inoculants to seeds/plants when attempting to reestablish desertified ecosystems.

Plant growth-promoting bacteria and mycorrhizae can be categorized into two major groups; microbes that promote growth by increasing nutrient availability or via the synthesis of growth-promoting substances, or microbes that promote growth by inhibiting pathogens from establishing in the rhizosphere, known as biocontrol-PGPB (Bashan and Holguin, 1998; Vessy, 2003). A wide distribution of microbes are well-known for their abilities to increase the availability of nutrients, the most important being phosphorous, nitrogen, and to a lesser extent iron and potassium. Major mechanisms for increasing plant-available phosphorous, nitrogen, and iron include phosphate solubilization, nitrogen fixation, and siderophore production, respectively. A number of bacteria are also well-known for synthesizing plant-specific growth factors (phytohormones) such as indoleacetic acid (IAA), cytokinins, and gibberellins (Garcia de Salamone et al., 2001; Patten and Glick, 2002; Ryu et al., 2002). IAA production has been reported in a wide distribution of bacterial species and is known for its effects on stimulating plant root development (Cattelan et al., 1999; Patten and Glick, 2002). The potential for microbial promotion of plant growth does seem vast as new reports of novel plant growth promoting microbes are published each year.

PGPB can currently be used effectively in many agricultural situations and to date much of the research involving the applications of these microbes pertains to such conditions (Bashan, 1998). Although much is currently known about the various types and mechanisms of PGPB, there remains much to be investigated and understood before these microbes can be utilized to their full potential. Obtaining a bacterium that displays the potential to promote plant growth does not necessarily imply that the microbe is a PGPB. In addition, some isolates my possess a desirable mechanism during *in vitro* assays, yet fail to enhance nutrient acquisition *in vivo*. Such a situation was emphasized by de Fritas et al. (1997) who reported that a phosphate-solubilizing bacterium did increase plant growth, yet did not increase phosphate concentrations in phosphate-deficient plants. Such a study suggests that there may be unknown PGP mechanisms at work and stresses the importance of conducting further research on plant growth promoting mechanisms. A better understanding of plant-microbe interactions would be particularly valuable for developing microbial inoculants for establishing and enhancing plant growth in desertified ecosystems or on contaminated sites. The objectives of this review are to familiarize the reader with some currently known plant growth-promoting mechanisms, to highlight what needs to be considered when developing a plant growth-promoting microbial inoculum, and to provide suggestions of future directions in utilizing plant growth promoting microbes to reestablish/stabilize desertified and contaminated sites.

2.0 Microbial Plant Growth-Promoting Mechanisms

2.1 Phosphate Solubilization

Microbial solubilization of mineral-phase phosphates has been reported in a variety of microbes including *Mycorrhizae*, *Aspergillus*, *Bacillus*, and *Pseudomonas*. de Fritas et al. (1997) stressed the importance of *Bacillus* sp. as being perhaps the most abundant phosphate solubilizing bacterium in soil. It has been repeatedly stressed that phosphate solubilization is a key plant growth-promoting mechanism (Bashan, 1998; Richardson, 2001; Rodriguez and Fraga, 1999; Singh and Kapoor, 1999). Previous studies have made it a priority to search for this mechanism when screening a large collection of bacterial isolates for potential PGPB (Cattelan et al., 1999; Chung et al., 2005; de Frietas et al., 1997; Johansen and Binnerup, 2002). Phosphate solubilization may also have an effect on additional plant growth-promoting mechanisms as available phosphorous in the cell controls many biological activities such nitrogen fixation which requires high levels of ATP (Requena et al., 1997). A number of mechanisms for increasing the availability of phosphorus to plants have been described (Rodriguez and Fraga, 1999). These mechanisms include; reducing the pH in the rhizosphere, producing organic acids/chelates, and excreting phosphatases to free phosphorous bound in organic matter. It has also been proposed that the mechanism used to solubilize phosphates may vary with chemical speciation (Chung et al., 2005; Illmer et al., 1995; Illmer and Schinner, 1995).

Organic acid production has been a commonly proposed phosphate solubilization mechanism (Chen et al., 2006; Kim et al., 1997; Rodriguez and Fraga, 1999; Vazquez et al., 2000). Rodriguez et al. (2000) demonstrated that gluconic acid production was important for the solubilization of P as transfromants introduced with a gene for the production of PPQ synthase, which is directly involved in the synthesis of gluconic acid, obtained the ability to grow on mineral phase phosphate. Vazquez et al. (2000) studied the solubilizing potential of fungi collected from mangrove roots. The mangrove ecosystem has a very low amount of bioavailable phosphate (18 µg/ml of orthophosphates compared to 73 µg/ml in seawater) due to the high pH of the environment (8.2). The authors identified a number of organic acids produced by phosphate-solubilizing fungi including acetic, propionic, isobutyric, isovaleric, valeric, lactic, fumaric, succinic, and additional unidentified compounds. This also was the first report of phosphate-solubilizing microbes collected from mangrove roots. Kim et al. (1997) identified a phosphate solubilizing isolate of *Rhanella aquatilis* that lowered the pH of hydroxyapatite-supplemented growth medium from 6.5 to 4.5 via the production of gluconic acid. This isolate was able release greater than 200 µg mL^{-1} phosphate *in vitro*. Previous research has also demonstrated that both bacteria and fungi have the ability to increase inorganic phosphate concentrations in culture without physically contacting the phosphates (Illmer and Schinner, 1995). The authors demonstrated that the soluble concentrations of inorganic phosphate increased without increasing concentrations in microbial cells, which contradicted the notion that solubilization occurs as microbes remove phosphates from the medium thus disturbing equilibrium. A further investigation

also revealed that solubilized phosphate did not increase linearly with added gluconic acid, leading the authors to conclude that organic acid production was not the sole factor in phosphate solubilization.

Solubilization via ammonium assimilation and subsequent proton excretion is one mechanism that is commonly used to explain the solubilization of phosphate by microbial isolates (de Freitas et al., 1997; Illmer et al., 1995; Illmer and Schinner, 1995). This mechanism was thought to be the most probable mechanism of solubilization used by *Penicillium simplicissimum* and *Pseudomonas* sp. in a study conducted by Illmer et al. (1995). A fungal species, *Aspergillus niger*, solubilized a highly insoluble form of phosphate, $AlPO_4$, while producing organic acids (citrate, oxalate, and gluconate), while the *Penicillium* and *Pseudomonas* displayed solubilization without producing detectable levels of organic acids. This led the authors to propose that the main mechanism of phosphate solubilization for these two microbes was via the production of H^+ during respiration and ammonium assimilation.

2.2 Nitrogen Fixation

Nitrogen-fixing bacteria (NFB) play critical roles in plant establishment and development as they supply plants with much needed nitrogen, which is often a limiting nutrient. NFB – plant relationships have been studied extensively, especially the symbiosis these bacteria form with legumes. Attention has also been given to the plant growth-promoting potentials of diazotrophs, free-living NFB (Dobbelaere et al., 2003). It has been noted that diazotrophs, such as *Acetobacter* and *Herbaspirillum*, may be

beneficial PGPB in non-legume crops (Kennedy et al., 1997). Other well-known NFB include *Azospirillum, Rhizobium*, *Bradyrhizobium*, *Burkholderia*, *Sinorhizobium*, *Mesorhizobium*, and *Azorhizobium* (Dobbelaere et al., 2003). Previous studies have screened large numbers of bacteria for NFB with the goals of developing these microbes as PGPB (Cattelan et al., 1999; Johansen and Binnerup, 2002). Bertrand et al. (2001) isolated a collection of Gram-negative NFB on a nitrogen-deficient medium and tested their abilities to enhance growth in canola plants (*Brassica napus*). These bacteria showed potential as PGPB by increasing the dry weights of plant roots by 11 to 52%. The plant growth-promoting abilities of N-fixing *Bacillus*, *Pseudomonas*, and *Rhodobacter* were demonstrated by Cakmack et al. (2005). Representatives from each genus significantly increased leaf, root and sugar weight in sugar beet plants compared to an untreated control after 60 days of growth. These bacteria also had a significantly greater effect on sugar beet growth compared to fertilizing the plants with 110 kg N/ha and 90 kg P_2O_5/ha. Genes involved in nitrogen fixation (Nif) were shown to be critical to an isolates, *Acetobacter diazotrophicus*, ability to enhance plant growth as Nif-mutants lost their ability to enhance plant growth under N-limiting conditions (Sevilla et al., 2001). The results of these studies demonstrate the potential for utilizing nitrogen-fixing bacteria to supply necessary nitrogen to both legumes and non-legume plants in nutrient-deficient environments.

2.3 Iron Acquisition

Many bacteria and fungi have the ability to acquire iron under extremely low iron conditions through to the production of siderophores. Iron availability is highly dependent on soil pH, and in general has a much lower bioavailability than is required by plants and microbes. Siderophores are molecules which have an extremely high affinity for iron, thereby binding insoluble iron and making it available to both plants and microbes. The ability to directly enhance plant growth through the production of siderophores is not completely understood. It has been noted that siderophore production may in fact enhance plant growth indirectly through pathogen suppression and increasing phosphate availability. Jayachandran et al. (1989) proposed a mechanism by which bacteria could solubilize insoluble phosphates by using siderophores to remove iron from iron-phosphates, thus freeing and supplying phosphate to the plant. Siderophore production had also been proposed as being more important in controlling pathogens than supplying a plant with iron. Many bacteria are known to have siderophores which have a greater affinity for iron than fungal siderophores. In addition, some species of bacteria, such as Bradyrhizobium, are known to use their own siderophores as well as those other microbes (Antoun et al., 1998). Both mechanisms can reduce the levels of available iron for other bacteria, creating a competitive exclusion.

2.4 Synthesis of Plant-Specific Growth Factors

Plant growth-promoting mechanisms such as phosphate solubilization, nitrogen fixation, and to a lesser extent, siderophore production are critical for directly supplying

the plant with necessary nutrients. Many microbes are also known for their abilities to effect plant growth through the production of plant hormones and signaling molecules such as auxins, cytokinins, and gibberellins (Garcia de Salamone et al., 2003; Patten and Glick, 2002; Ryu et al., 2003).

One plant growth-promoting mechanism that is thought to have high potential is bacterial ACC deaminase activity which works to reduce ethylene concentrations in plant roots (Glick et al., 1998; Glick, 1995). This mechanism is distributed among many genera of bacteria and is thought to play an important part in early plant development (Cattelan et al., 1999; Wenbo et al., 2003). Ethylene can be used to slow plant root development during periods of stress. The growth-promoting mechanism is accomplished via a competition between the plant enzyme ACC oxidase and the bacterial ACC deaminase (Glick, 1995). 1-aminocyclopropane-1-carboxylate (ACC) is synthesized by plants and can be broken down into ethylene by the plant derived ACC oxidase. Alternatively, ACC can be broken down by the bacterial derived ACC deaminase, which converts ACC into an ammonium and a molecule of α-ketobutyrate, thus supplying the microbe with a nitrogen source. In doing this the bacterium both reduces ACC and ethylene levels in the plant root, thereby preventing slower root growth. ACC-deaminase-positive strains of *Pseudomonas* and *Enterobacter* demonstrated an ability to significantly reduce ACC levels and increase root length in canola seedlings compared to an uninoculated control (Penrose et al., 2001). An ACC-deaminse-producing isolate facilitated the upregulation of genes involved in cell division and the downregulation of genes associated with stress-response in canola plants

(Hontzeas et al., 2004). This stress-reducing mechanism may prove to be beneficial when attempting to reestablish vegetation on contaminated sites.

Aside from directly supplying the plant with necessary nutrients a number of bacteria have been reported to have the abilities to synthesis plant-specific signaling molecules and growth factors. Indole-3-acetic acid (IAA) is thought to be one of the most important phytohormones produced by plants as it plays a critical role in cell elongation/division and root development (Patten and Glick, 2002). Bacterial synthesis of IAA has been reported in a number of genera including *Azospirillum, Burkholderia*, *Enterobacteria,* and *Pseudomonas* (Kuklinsky-Sobral et al., 2004; Patten and Glick, 2002). Antoun et al. (1998) reported that 58% of 266 *Rhizobium* and *Bradyrhizobium* isolates produced IAA. Patten and Glick (2002) demonstrated the benefits of IAA synthesis using wild-type and mutant strains of Pseudomonas putida as PGPB on canola plants. The wild-type strain produced an average of 35-50% longer roots compared to plants inoculated with an IAA-deficient mutant while no differences in shoot lengths were observed. Tryptophan-dependent synthesis of auxins (IAA) has been demonstrated as mutants lacking the necessary enzymes for this biosynthetic pathway had reduced abilities to enhance plant growth (Idris et al., 2007).

The regulatory effects of IAA may be concentration dependent as low concentrations (10^{-12} – 10^{-9} M) have a stimulatory effect while concentrations greater than 10^{-9} M can have an inhibitory effect on primary root development (Patten and Glick, 2002). Patten and Glick (2002) reported these effects by again using two different *P.*

putia strains. While the wild-type produced a greater number of roots in mung bean plants (*Vigna radiata*) these roots were smaller compared to those produced by plants inoculated with a mutant that produced low levels of IAA. The authors commented that the wild-type strain produced a large number of adventitious roots, while the mutant strain produced longer roots with a higher overall surface area. These results demonstrate the need for better understanding a bacterial inoculum prior to its application, as its effects on root development may vary with the levels of IAA produced. Choosing bacterial strains with specific IAA-producing abilities may help tailor an inoculum for a desired effect on plant development. Aside from IAA production, many PGPB such as *Azotobacter*, *Azospirillum*, *Rhizobium*, *Peanibacillus,* and *Pseudomonas* have been reported to produce a wide variety of additional phytohormones including cytokinins, gibberellins, and abscisic acid (Dobbelaere et al., 2003).

2.5 Biocontrol

Pathogen reduction is another area which has gained considerable attention in recent years. Certain species of bacteria have been known to increase plant growth and productivity by acting as pathogen-suppressing microbes (Dobbelaere et al., 2003). Pathogen prevention can arise from competitive exclusion, or through the production of specific biocontrol molecules such as hydrogen cyanide or anti-fungal enzymes (Antoun et al., 1998; Friedlander et al., 1993). Friedlander et al. (1993) identified strains of Pseudomonas cepacia that possessed the enzyme β – 1,3 glucanse, which has the ability to break-down fungal cell wall components.

2.6 Enhancing Mycorrhizal-Plant Interactions

An additional method of plant growth promotion that should be further explored is the ability of some bacteria to promote or establish mycorrhizal relationships (Barriuso et al., 2005; Frey-Klett et al., 1997; Requena et al., 2001). Frey-Klett et al. (1997) demonstrated the effectiveness of this technique by using a Pseudomonas strain to promote ectomycorrhizal fungi establishment on Douglas Fir roots. It has also been suggested that mycorrhizal-helping bacteria may beneficial in the reestablishment of desertified ecosystems where mycorrhizal abundance is low compared to native soils (Requena et al., 2001).

2.7 Additional Plant-Growth Promoting Mechanisms

Many additional plant growth-promoting mechanisms have been previously described. Many of these mechanisms have received little attention to date and their potentials are yet to be fully explored. Bacterial vitamin production is one such mechanism. It is thought that healthy plants typically have sufficient resources to synthesize the majority of their required vitamins, however, under stressful or nutrient-deficient conditions obtaining vitamins from a bacterial source may be important (Dobbelaere et al., 2003). Another mechanism which shows potential in plant growth promotion is carbon cycling. Many rhizosphere bacteria produce extracellular enzymes which degrade complex organics thus turning over carbon, nitrogen, and phosphorous. Johansen and Binnerup (2002) isolated 4,474 bacterial isolates from bulk soil and the rhizospheres of barley (*Hordeum vulgare L.*) and screened them for their abilities to

turnover nitrogen, phosphorous and carbon. The occurrence of these bacteria was up to 8.1 times greater in the rhizosphere than in bulk soil, the majority being fluorescent pseudomonads and cytophage-like bacteria.

Nitric oxide (NO) production by strains of *A. brasilense* has recently been suggested to play a significant role in enhancing the development of plant roots (Molina-Favero et al., 2008). Plants use NO to regulate IAA utilization pathways. Work conducted by Molina-Favero et al. (2008) has indicated that NO-production may potentially be more important than the production of IAA as mutant strains of *A. brasilense* lacking the ability to produce NO, yet retaining their abilities to produce IAA, lost their plant growth promoting potential.

3.0 Developing a Plant Growth-Promoting Inoculum

3.1 Factors to Consider

The group of plant growth-promoting microbes not only contains an enormous phylogenetic diversity, but also possesses a vast array of plant growth-promoting mechanisms. With this said, one has an abundance of information at their disposal when beginning to develop an inoculum for a specific purpose. However, a number of factors should be taken into consideration in addition to the types of microbes and plant growth-promoting mechanisms that one wishes to use. Such factors may include but are not limited to, the efficiency of a PGPB when used in the field, whether to use a single microbe or multiple microbes in an inoculum, the cell density of the inoculum, and persistence of the inoculum in the rhizosphere.

3.1.1. Inoculum Efficiency

The efficiency or effectiveness of a mechanism should receive some attention before investing a considerable amount of time and money into an inoculum. The study conducted by de Freitas et al. (1997) reinforces the need to investigate the function of an inoculum prior to using it on a large scale. These investigators used bacterial inocula composed of phosphate-solubilizing *Bacillus* or *Xanthomonas* with the goal of promoting growth in canola plants in a phosphate-deficient soil. None of these bacteria enhanced canola growth when inoculated with rock phosphates, while some even hindered growth by 16-30% compared to an non-inoculated control. After rock phosphate was removed, most of the bacterial isolates enhanced some aspect of plant growth (plant height, pod number, pod weight); however, none of these bacteria increased the concentration of phosphorous in the plants. This example stresses the point that bacteria that perform a function in the laboratory may not perform the function in the rhizosphere. In addition, these bacteria demonstrated the ability to promote growth without increasing phosphate, implying than an unknown mechanism (possibly phytohormone production) was occurring (de Freitas et al., 1997). Similar results have been reported in a study where phosphate-solubilizing and nitrogen-fixing bacteria significantly enhanced growth in inoculated plant compared to those fertilized with nitrogen and phosphorous, suggesting that additional plant-growth promoting mechanisms were present (Cakmack et al., 2005).

3.1.2 Persistence in the Rhizosphere

It may also be worth while to consider the persistence of the chosen inoculum in the field. Microbes that are going to provide a long-term effect must have the ability to compete with other microbes present in field conditions. For example, if one desires to use an inoculum to supply a plant with bioavailable phosphorous, the microbes involved should remain active in the rhizosphere as long as the plant is needed. The observed effects of some microbial inoculations may also be time-dependent. The most pronounced effects on plant growth may be observed early in plant development, or they may not appear until years after they have been introduced (Bashan et al., 1999; Cakmakc et al., 2005; Requena et al., 2001). Cakmakc et al. (2005) observed the greatest effects of their inoculum during early stages of sugar beet growth. The bacterial inoculum used in their study initially increased root and shoot mass, but differences between treated plants and the control decreased over the growing season. Frey-Klett et al. (1997) were unable to detect an inoculated *Pseudomonas* sp. after 19 weeks into a study, but reported the benefits of establishing a mycorrhizal-plant relationship was still present. Opposite outcomes have been observed where significant results became more pronounced over long time periods of 3.5 to 5 years (Bashan et al., 1999; Requena et al., 2001). It is possible that a decrease in differences between a treatment and the control may be a result of the inoculum decreasing in numbers with time, stressing the importance of using microbes that are able to persist under field conditions. On the other hand, if the goal is to establish vegetation on a site, the inoculum may only be necessary during the initial establishment of plant growth and become less important with time.

3.2. Collecting an Inoculum

Developing a plan for obtaining a collection of microbes is another important factor in creating an inoculum. Previous studies have demonstrated that collecting microbes from a wild-type plant in a native location is advantageous (Barriuso et al., 2005, Requena et al., 2001). Requena et al. (2001) demonstrated that using native mycorrhizae to establish vegetation on a desertified site was more beneficial than using non-native strains. Collecting microbes from the rhizosphere of the plant one wishes to use will indicate that the chosen microbes will likely have the ability to establish a relationship with this plant.

3.3. Single or Multiple Species in an Inoculum

One issue that must be addressed when developing an inoculum is whether to use a single or multiple microbial species. The majority of previous studies have analyzed the performances of single microbial species inoculated onto seeds or plant roots (Antoun et al., 1998; Bashan et al., 1999; Bertrand et al., 2001; Canbolat et al., 2005; Cakmakc et al., 2005; Carrillo et al., 2002; Cattelan et al., 1999; de Freitas et al., 1997; El-Komy, 2005; Friedlander et al., 1993; Ryu et al., 2003). These studies have provided key information regarding the individual effects and performances of single plant growth-promoting isolates. However, it would be beneficial to consider using multiple species of microbes in a single inoculum as a number of recent studies have indicated that doing so may in fact enhance overall inoculum performance (Frey-Klett et al., 1997; Halsall and Gibson, 1989; Holguin and Bashan, 1996; Requena et al., 2001). Holguin and Bashan

(1996) found the nitrogen-fixing capabilities of a co-culture of *Azospirillum* with *Staphylococcus* sp. to increase by nearly 40% compared to using the *Azospirillum* alone. The authors also reported increased plant growth when *Azospirillum* with other microorganisms including a phosphate-solubilizing bacterium (Bashan and Holguin, 1997). Staphylococcus cells were thought to enhance nitrogen fixation through the production signaling factors. Halsall and Gibson (1989) reported using a coculture of *Azospirillum* and *Cellulomas* sp. The two were thought to mutually benefit one another as the *Cellulomas* sp. provided Azospirillum with carbon from cellulose and the *Azospirillum* provided the *Cellulomas* with fixed nitrogen.

Using multiple microbial species as a single inoculum could prove to be beneficial when addressing complex issues such as establishing vegetation on low-nutrient contaminated or desertified sites. In these situations one would ideally want to find a microbe that demonstrates a potential to promote plant growth by providing essential nutrient to the plant in addition to be resistant to any site contaminants. However, it may be difficult to find a single microbe that possesses the ability to fix nitrogen, solubilize phosphate, and stimulate plant growth via growth factors, in addition to being resistant to site contaminants. Mechanisms such as metal resistance and nitrogen fixation are ATP-intensive and would utilize a large potion of the cells energy. It would therefore be beneficial to identify a group of microbes that could for instance complement one another by alleviating metal toxicity while fixing nitrogen. One drawback to this is that developing such an inoculum would be time intensive. Ideally one would initially want to screen a large collection of isolates for specific growth-

promoting mechanisms individually followed by screening the isolates for their abilities to complement one another in cocultures. Doing so would ensure that a collection of isolates with desirable qualities had been obtained in addition to ensuring that these microbes did not have antagonistic behaviors towards one another.

4.0 Potential Applications of Mycorrhizae

Although bacterial species have been widely used in agricultural plant growth-promoting research, the potential for using mycorrhizae to reestablish desertified or contaminated sites needs to be addressed. Mycorrhizal diversity is thought to be an essential component of desert ecosystems as it is critical in maintaining plant diversity and biomass (van der Heijden et al., 1998). In addition, mycorrhizae have demonstrated potential to alleviate stressful conditions and aid in the establishment of resource islands in the Sonoran Desert (Carilli-Garcia et al., 1999). In desertified or contaminated areas mycorrhizal numbers can be dramatically reduced making the task of establishing vegetation on these sites difficult (Bethlenfalvay et al., 1999). Requena et al. (2001) reported an increased survival of *Anthyllis cytisoides* plants when arbuscular mycorrhizal fungi (AMF) were introduced to a desertified site. The effects of these AMF were still apparent five years after the initiation of the study suggesting that beneficial AMF were still active.

The benefits of using mycorrhizal fungi to establish vegetation on damaged and desertified ecosystems has been reported in a number of previous studies (Azcon and Barea, 1997; Carrillo et al., 2002; Requena et al., 1996; Wright and Upadyaya, 1998).

Azcon and Barea (1997) used AMF to facilitate the establishment of lavender shrubs (*Lavandula* sp.) in a desertified semi-arid Mediterranean ecosystem. These inoculations increased plant phosphate, nitrogen, and potassium uptake and therefore helped to restore nutrient cycling to the damages ecosystem. The benefits of these AMF inoculations were further highlighted when the AMF-treated shrubs were compared to uninoculated fertilized plants as AMF-inoculated plants had higher levels of growth. The authors concluded that the AMF not only aided in nutrient uptake, but also helped the plant cope with stressful conditions. Not only are mycorrhizae important in plant nutrient acquisition, but they have also been shown to be important in developing soil structure due to their abilities to form glomalin (Bethlenfalvay et al., 1999). Glomalin acts as glue holding soil particles together which ultimately increases the development of soil aggregates. Wright and Upadyaya (1998) found a direct correlation between glomalin production in mycorrhizae and soil aggregate stability. Creating soil structure and aggregate stability may prove to an important mechanism for stabilizing contaminated sites.

Requena et al. (1996) made attempts to establish a woody legume (*A. cytisoides*) on a desertified site. The site had low nutrient levels and low organic matter content with frequent periods of drought. In this study native mycorrhizae, *Glomus coronatum*, was shown to be an important microbe in plant establishment. In addition, *G. coronatum* was most effective at increasing plant nitrogen and phosphorous when used in conjunction with a *Rhizobium* sp. Another mycorrhizal species, *Glomus intaradices*, was more effective at promoting plant growth with a completely different strain of *Rhizobium*

(Requena et al., 1996). This study highlights three important points. First, it demonstrates the benefits of using mycorrhizae when attempting to establish a desertified site. Secondly, it emphasizes the point that using an inoculum with multiple microbial species may offer more potential for enhancing plant growth than using an inoculum containing an individual species. Finally, it highlights the fact that many microbes may be fastidious by nature and may only perform optimally when used in conjunction with specific partners.

5.0 Future Directions: Using PGPB to Phytostabilize Contaminated Sites

It is well-established that many rhizosphere-associated bacteria and mycorrhizae have great potential to promote plant growth when used as inocula. These microbes play invaluable roles as they cycle nutrients such as nitrogen and phosphorous to plants. In addition, many PGPB have demonstrated abilities to synthesize growth-promoting phytohormones such as IAA or promote growth through special mechanisms such as ACC-deaminase production. Using the information that is currently available along with some additional research it may be possible to develop a microbial inoculum with the underlying goal of establishing a vegetative cover on and thereby phytostabilizing contaminated sites. Abandoned mine tailings are a prime example of such sites that would greatly benefit from the development of such an inoculum. Mine tailings are the remnants of mining activities and often contain high levels of toxic heavy metals which are harmful to both humans and surrounding ecosystems. Often the high levels of heavy metals on these sites leave them devoid of any vegetation and lacking soil structure,

making them highly susceptible to wind and water erosion. One cost-effective and desirable remedy for these sites is known as phytostabilization, which aims to establish a vegetative cover on the tailings thereby dramatically reducing wind and water erosion.

One potential strategy for the phytostabilization of mine tailings may involve paying more attention to establishing mycorrhizal-bacterial-plant relationships. As previously mentioned, a number of studies have eluded to the importance of using mycorrhizae as plant growth-promoting microbes when establishing vegetation in desertified ecosystems. To the best of the author's knowledge, very few studies have looked into using mycorrhizae as plant growth-promoting inocula for phytostabilizing mine tailings sites (Hetrick et al., 1994; Shetty et al., 1994). Both Hetrick et al. (1994) and Shetty et al. (1994) used a mycorrhizal inoculum on prairie grasses in attempts to establish a vegetative cover on lead-zinc mine tailings. Hetrick et al. (1994) reported promising results as both grasses used in their study benefited from mycorrhizal inoculation. Shetty et al. (1994) were unable to establish grasses in the tailings. Neither of these studies used a combination of mycorrhizal and bacterial species as an inoculum. As previously mentioned, studies that have used both bacteria and mycorrhizae in a single inoculum to establish vegetation have reported promising results (Frey-Klett et al., 1997; Requena et al., 1996). In addition, it had been shown that the establishment of mycorrhizal fungi on plant roots can be increased through the use of mycorrhizal helper bacteria (MHB) (Garbaye, 1994). Using an inoculum that contained both mycorrhizae and known PGPB would be ideal for establishing a vegetative cover on barren mine tailings.

The development of an ideal phytostabilizing inoculum should have a number of specific qualities including; the ability to provide plants with essential nutrients, the potential to enhance growth via microbial synthesis of phytohormones, the ability to persist in the rhizosphere, and the ability to reduce stress on the plant. Identifying bacteria that demonstrate plant growth-promoting abilities would be the first step towards identifying the ideal inoculum. These potential PGPB should have the ability to supply the plant with appropriate levels of phosphorous and nitrogen as mine tailings are generally low-nutrient environments. Utilizing bacteria with ACC-deaminase activities may also prove to be beneficial as this mechanism is thought be stress-reducing (Glick et al., 1998). Using bacteria that produce phytohormones such as IAA or cytokinins would provide plants with additional growth stimulation. It is also important that these selected bacteria have the ability to persist and perform their functions in the presence heavy metals. Dell'Amico et al. (2005) and Burd et al. (1998) both screened potential PGPB for resistances to metals, yet neither screened for various plant growth-promoting mechanisms in the presence of heavy metals. With this said, screening methods should include testing selected microbes for the ability to solubilize phosphates, fix nitrogen, break-down ACC, and synthesize phytohormones in the presence of heavy metals such as lead, zinc, and cadmium.

It is highly unlikely that any one microbe will possess all of these desired qualities; therefore, a selection of microbes that specialize in each desired mechanism may be utilized together to enhance performance. A second step towards developing the inoculum would involve screening each selected microbe as individual inoculants

introduced to target plants to ensure that any chosen microbe does not hinder plant development. The third step would involve testing the performance of this collection of microbes to ensure that no antagonistic behaviors or competitive exclusions are occurring and that the individual desired plant-growth promoting effects are still occurring within this group. Final steps should involve obtaining mycorrhizae from wild-type target plants and testing their abilities to work in combination with the bacterial inoculum to facilitate establishment of the plant in mine tailings.

Although there is currently much known regarding microbes and their abilities to facilitate plant growth, a great deal of investigation remains to be done. Studies such as the one conducted by de Freitas et al. (1997) demonstrate that there may be plant growth-promoting mechanisms or interactions that are currently unknown. Sevilla et al. (2001) also found that Nif-deficient *Acetobacter* mutants were lost their ability to enhance plant growth under N-limiting conditions; however, these isolates were still able to enhance plant growth under N-sufficient condition, suggesting that there were unknown PGP mechanisms present. Also, the majority of previous studies have used PGPB as single inoculants on target plants, while very few studies have used more than one microbe. Further investigations need to be made into the interactions between PGPB, mycorrhizae, and plants in order to better understand these complex relationships. With continued research, future results may provide a database of known PGPB along with techniques for creating site specific inocula.

6.0 References

Antoun, H., C. J. Beauchamp, N. Goussard, R. Chabot, and R. Lalande. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). Plant and Soil. **204:**57-67.

Azcon, R., and J. M. Barea. 1997. Mycorrhizal dependency of a representative plant species in Mediterranean shrublands (*Lavandula spica* L.) as a key factor to its use for revegetation strategies in desertification-threatened areas. **7:**83-92.

Barriuso, J., M. T. Pereyra, J. A. Lucas Garcia, M. Megias, F. J. Gutierrez Manero, and B. Ramos. 2005. Screening for putative PGPR to improve establishment of the symbiosis *Lactarius deliciosus-Pinus* sp. Microb. Ecol. **50:**82-89.

Bashan, Y. 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotech. Adv. **16:**729-770.

Bashan, Y., A. Rojas, and M. E. Puente. 1999. Improved establishment and development of three cactus specie inoculated with Azospirillum brasilense transplanted into disturbed urban desert soil. Can. J. Microbiol. **45:**441-451.

Bashan, Y., and G. Holguin. 1997. *Azospirillum*-plant relationships: environmental and physiological advances (1990–1996). Can. J. Microbiol. **43:**103–121.

Bashan, Y. and G. Holguin. 1998. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. Soil. Boil. Biochem. **30:**1225-1228.

Bashan, Y., and G. Holguin. 2002. Plant growth-promoting bacteria: a potential tool for arid mangrove reforestation. Trees. **16:**159-166.

Bertrand, H., R. Nalin, R. Bally, J.-C. Cleyet-Marel. 2001. Isolation and identification of the most efficient plant growth-promoting bacteria associated with canola (*Brassica napus).* Biol. Fertil. Soils. **33:**152-156.

Bethlenfalvay, G. J., I. C. Cantrell, K. L. Mihara, and R. P. Schreinre. 1999. Relationships between soil aggregation and mycorrhizae as influenced by soil biota and nitrogen nutrition. Biol. Fertil. Soils. **28:**356-363.

Burd, G. I., D. G. Dixon, and B. Glick. 1998. A plant growth promoting bacterium that decreases nickel toxicity in seedlings. Appl. Environ. Microbiol. **64:**3663-3668.

Cakmakc, R., F. Donmez, A. Aydm, F. Sahin. 2005. Growth promotion of plants by plant growth promoting rhizobacteria under greenhouse and two different field soil conditions. Soil. Biol. Biochem. In Press.

Canbolat, M., S. Bilen, R. Cakmakc, F. Sahin, and A. Aydin. 2005. Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol. Fertil. Soils. In Press.

Carilli-Garcia, A., J.-L. Leon de la Luz, Y. Bashan, G. J. Bethlenfalvay. 1999. Nurse plants, mycorrhizae, and plant establishment in a disturbed area of the Sonoran Desert. Restor. Ecol. **7:**321-335.

Carrillo, A. E., C. Y. Li, and Y. Bashan. 2002. Increased acidification in the rhizosphere of cactus seedlings induced by *Azospirillum brasilense*. Environ. Microbiol. **89:**428-432.

Cattelan, A. J., P. G. Hartel, and J. J. Fuhrmann. 1999. Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci. Soc. Am. J. **63:**1670-1680.

Chen, Y.P., P.D. Rekah, A.B. Arun, F.T. Shen, W.-A. Lai, and C.C. Young. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl. Soil Ecol. **34:**33-41.

Chung, H., M. Park, M. Madhaiyan, S. Seshadri, J. Song, H. Cho, and T. Sa. 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil. Biol. Biochem. **37:**1970-1974.

de Freitas, J. R., M. R. Banerjee, J. J. Germida. 1997. Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus L.*). Biol. Fertil. Soils. **24:**358-364.

Dell' Amico, E., L. Cavalca, and V. Andreoni. 2005. Analysis of rhizobacterial communities in perennial *Graminaceae* from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. **52:**153-162.

Dobbelaere, S., J. Vanderleyden, and Y. Okon. 2003. Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit. Rev. Plant Sci. **22:**107-149.

El-Komy, H.M.A. 2005. Coimmobilization of *A. lipoferum* and *B. megaterium* for plant nutrition. Food Technol. Biotech. **43:**19–27.

Favero-Molina, C., C.M. Creus, M. Simontacchi, S. Puntarulo, and L. Lamattina. 2008. Aerobic nitric oxide production by *Azospirillum brasilense* Sp245 and its influence on root architecture in tomato. Mol. Plant-Microbe Interactions. **21:**1001-1009.

Frey-Klett, P., J. C. Pierrat, and J. Garbaye. 1997. Location and survival ofmycorrhiza helper *Pseudomonas fluorescens* during establishment of ectomycorrhizal symbiosis between *Laccaria bicolor* and Douglas Fir. Appl. Environ. Microbiol. **63:**139-144.

Fridlender, M., J. Inbar, and I. Chet. 1993. Biological control of soilborne plant pathogens by a β – 1,3 glucanase-producing Pseudomonas cepacia. Soil Biol. Biochem. **25:**1211-1221.

Garbaye, J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol. **128:**197-210.

Garcia de Salamone, I. E., R. K. Hynes, and L. M. Nelson. 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can. J. Microbiol. **47:**404-411.

Glick, B. R. 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. **41:**109-117.

Glick, B., D. M. Penrose, and J. Li. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J. Theor. Biol. **190:**63-68.

Halsall, D. M., and A. H. Gibson. 1989. Nitrogenase activity of a range of diazotriphic bacteria on straw, straw breakdown products and related compounds. Soil. Biol. Biochem. **21:** 291-298.

Hargrove, T. 2008. World fertilizer prices soar as food and fuel economies merge. International Center for Soil Fertility and Agricultural Development. http://ifdc.org.

Herrera, M. A., C. P. Salamanca, and J. M. Barea. 1993. Inoculation of woody legumes with selected arbuscular mycorrhizal fungi and rhizobia to recover desertified Mediterranean ecosystems. **59:**129-133.

Hetrick, B. A. D., G. W. T. Wilson, and D. A. H. Figge. 1994. The influence of mycorrhizal symbiosis and fertilizer amendments on establishment of vegetation in heavy metal mine spoil. Enviorn. Poll. **86:**171-179.

Holguin, G., and Y. Bashan. 1996. Nitrogen-fixation by Azospirillum brasilense CD is promoted when co-cultured with a mangrove rhizosphere bacterium (Staphylococcus sp.). Soil Biol. Biochem. **28:**1651-1660.

Hontzeas, N., S.S. Saleh, and B. Glick. 2004. Changes in gene expression in canola roots induced by ACC-deaminse-containing plant growth-promoting bacteria. Mol. Plant-Microbe Interactions. **17:**865-871.

Idris E.E., D.J. Iglesias, M. Talon, and R. Borris. 2007. Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. Mol. Plant-Microbe Interactions. **20:**619-626.

Illmer, P., and F. Schinner. 1995. Solubilization of inorganic calcium phosphates – solubilization mechanisms. Soil Boil. Biochem. **27:**257-263.

Illmer, P., A. Barbato, and F. Schinner. 1995. Solubilization of hardly soluble AlPO4 with P-solubilizing microorganisms. Soil. Biol. Biochem. **27:**265-270.

Jayachandran, K., A. P. Schwab, and B. A. D. Hetrick. 1989. Mycorrhizal mediation of phosphorous availability: synthetic iron chelate effects on phosphorous availability. Soil Soc. Amer. J. **53:**1701-1706.

Johansen, J. E., and S. J. Binnerup. 2002. Contribution of Cytophage-like bacteria to the potential of turnover of carbon, nitrogen, and phosphorous by bacteria in the rhizosphere of barley (Hordeum vulgare L.). Microb. Ecol. **43:**289-306.

Kennedy, I. R., L. L. Pereg-Gerk, C. Wood., R. Deaker, K. Gilchrist, and S. Katupitiya. 1997. Biological nitrogen fixation in non-leguminous field crops: facilitating the evolution of an effective association between *Azospirillum* and wheat. Plant and Soil. **194:**65-79.

Kim, K.Y., D. Jordan, and H.B. Krishnan. 1997. *Rahnella aquatillis*, a bacterium isolated from soybean rhizosphere, can solubilize hydroxyapatite. FEMS Microbiol. Lett. **153:**273-277.

Kloepper, J. W., R. Lifshitz, and R. Zablotowicz. 1987. Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol. **7:**39-44.

Kuklinsky-Sobral, J., W. L. Aravjo, R. Mendes, I. O. Geraldi, A. A. Pizzirani-Kleiner, and J. L. Azevedo. 2004. Isolation and characterization of soy bean-associated bacteria and their potential for plant growth promotion. **6:**1244-1251.

Patten, C. L., and B. R. Glick. 2002. Role of Pseudomonas putida indoleacetic acid development of the host plant root system. Appl. Environ. Microbiol. **68:**3795-3801.

Penrose, D. M., and B. R. Glick. 2001. Levels of ACC and related compounds in exudate and extracts of canola seeds treated with ACC deaminase-containing plant growth-promoting bacteria. Can. J. Microbiol. **47:**368-372.

Requena, N., P. Jeffries, and J. Miguel Barea. 1996. Assessment of natural mycorrhizal potential in a desertified semiarid ecosystem. Appl. Enviorn. Microbiol. **62:**842-847.

Requena, N., I. Jimenez, M. Toro, and J. M. Barea. 1997. Interactions between plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and Rhizobium spp. in the rhizosphere of Anthyllis cytisoides, a model legume for revegetation in the Mediterranean semi-arid ecosystems. New Phytol. **136:**667-677.

Requena, N., E. Perez-Solis, C. Azcon-Aguilar, P. Jeffries, and J.-M. Barea. 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Appl. Environ. Microbiol. **67:**495-498.

Richardson, A. E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorous by plants. Aust. J. Plant Physiol. **28:**897-906.

Rodriguez, H., T. Gonzalez, and G. Selman. 2000. Expression of a mineral phosphate solubilizing gene from *Erwinia herbicola* in two rhizobacterial strains. Biotechnol. **84:**155-161.

Rodriguez, H., and R. Fraga. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. **17:**319-339.

Ryu, C.-M., M. A. Farag, C.-H. Hu, M. S. Reddy, H.-X. Wei, P. W. Pare, and J. W. Kloepper. 2003. Bacterial volatiles promote growth in Arabidopsis. Proc. Natl. Acad. Sci. USA. **100:**4927-4932.

Sevilla, M., R.H. Burris, N. Gunapala, and C. Kennedy. 2001. Comparison of benefit to sugarcane plant growth and 15N2 incorporation following inoculation of sterile plant with *Acetobacter diazotrophicus* wild-type and Nif- mutant strains. Mol. Plant-Microbe Interactions. **14:**358-366.

Shetty, K. G., B. A. D. Hetrick, D. A. H. Figge, and A. P. Schwab. 1994. Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. **86:**181-188.

Singh, S., and K. K. Kapoor. 1999. Inoculation with phosphate-solubilizing microorganisms and a vesicular-arbuscular mycorrhizal fungus improves dry matter yield and nutrient uptake by wheat grown in a sandy soil. Biol. Fertil. Soils. **28:**139-144.

Vazquez, P., G. Holguin, M. E. Puente, A. Lopez-Cortez, and Y. Bashan. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol. Fertil. Soils. **30:**460-468.

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature. **396:**69-72.

Vessy, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. **255:**571-586.

Wenbo, M., S. B. Sebestinaova, J. Sebestian, G. I. Burd, E. C. Guinel, and B. Glick. 2003. Prevalence of 1-aminocyclopropane-1-carboxylate deaminase in *Rhizobium* spp. Leeuwenhoek. **83:**285-291.

Wright, S. F., and A. Upadhyaya. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil. **198:**97-107.

APPENDIX B

PLANT GROWTH-PROMOTING BACTERIA; ISOLATION AND SCREENING RESULTS

The following tables have been included to give the reader an impression of the diversity and assortment of plant growth-promoting mechanisms that are typically present in a large collection of bacterial isolates. Table 1 is a collection of isolates there were obtained with the help of Dr. Monica O. Mendez. The majority of these isolates (those with identifications that begin with "MTR") were obtained from the rhizospheres of *Atriplex lentiformis* plants grown in composted and non-composted mine tailings. Isoaltes that have identifications beginning with K4 or K6 were collected from bulk mine tailings taken from the Klondyke site. The methods involved in obtaining, identifying and screening these isolates are described in the "Experimental" section of Chapter 2 of this document. Following screening, a subset of isolates was selected and used in a series of greenhouse-scale investigations that are described in Chapters 2, 3 and 4 of this document. The majority of these isolates have been cataloged and stored for future investigations.

Table 2 of this appendix displays a large collection of bacterial isolates that were obtained from wild, native plant sources. Isolates with identities beginning with AL came from *Atriplex lentiformis* rhizospheres, "GR" from alkali sacaton (*Sporobolus airoides*), PV from palo verde (*Parkinsonia microphylla*), and MQ from mesquite (*Prosopis velutina*). These isolates were not associated with mine tailings prior to conducting a series of assays for plant growth-promoting mechanisms. The methods

used for characterizing each of these isolates are the same as those listed in the "Experimental" section of Chapter 2. These isolates have also been cataloged, stored and are currently being used in a series of investigations.

Table 1 Bacterial Isolates Collected From Bulk Mine Tailings and "Impacted" *A. lentiformis* Rhizospheres

Isolate Identification	Gram Stain	Growth pH 5	Growth pH4	Growth on CAS	Growth on DCP	Siderophore Production (µM)	IAA Production (µg mL^{-1})	ACC Deaminase
K6-1 *Microbacterium paraoxydans*	Gram (+)	Yes	No	Yes	No	0	0	No
K6-2 *Curtobacterium flaccumfaciens*	Gram (+)	Yes	No	Yes	Yes	28.4	8.3	No
K6-7 *Amycolatopsis keratinophila*	Gram (+)	Yes	No	Yes	Yes	0	1.1	Yes
K6-8 *Amycolatopsis decaplanensis*	Gram (+)	Yes	No	No	Yes	0	6.5	No
K6-11B *Methylobacterium sp.*	Gram (-)	Yes	No	Yes	No	24	1.5	No
K6-13 *Arthrobacter aurescens*	Gram (+)	Yes	No	Yes	Yes	0	2.1 ug/ml (12.5 ug/ml)	No
K6-15 Unidentified	Gram (+)	Yes	No	Yes	Yes	0	1.1	No
K6-16 *Arthrobacter nitroguaiacolicus*	Gram (+)	Yes	No	Yes	Yes	0	4.0	No
K6-19 *Paenibacillus lautus*	Gram (+)	No	No	No	No	0	35	No
K6-20 *Amycolatopsis keratinophila*	Gram (+)	Yes	No	Yes	Yes	0	1.6	No
K6-23 *Arthrobacter nitroguaiacolicus*	Gram (+)	Yes	No	Yes	Yes	0	2.8	No
K6-24 *Amycolatopsis keratinophila*	Gram (+)	Yes	No	Yes	Yes	0	0.8	No
K6-25A *Arthrobacter nitroguajacolicus*	Gram (+)	Yes	No	Yes	Yes	0	2.9	Yes

K6-25B *Amycolatopsis keratinophilia*	Gram (+)	Yes	No	Yes	Yes	0	1.0	Yes
K6-25C *Arthrobacter nitroguajacolicus*	Gram (+)	Yes	No	No	Yes	0	3.2	No
K6-28 *Nordella oligomobilis*	Gram (-)	Yes	No	Yes	Yes	0	0	No
70 *Janthinobacterium agaricidamnosum*	Gram (-)	Yes	No	No	Yes	0	18.3	No
71 *Bacillus pumilis*	Gram (+)	Yes	No	No	Yes	0	0	Yes
M22 *Ralstonia detusculanense*	Gram (-)	Yes	No	Yes	Yes	400	0.8	Yes
50 *Ralstonia* sp. S23	Gram (-)	Yes	No	Yes	No	0	0.6	No
35 *Methylobacterium* sp. F18	Gram(-)	Yes	No	No	No	0	1.6	Yes
Pseudomonas aeruginosa L1	Gram (+)	Yes	No	Yes	Yes	105	0.9	No
Pseudomonas aeruginosa R4	Gram (+)	Yes	No	Yes	Yes	16.6	1.0	No
Burkholderia viet	Gram (-)	Yes	Yes	Yes	Yes	0	1.3	Yes
Rhodococcus	Gram (+)	Yes	No	Yes	Yes	38	0.7	No
MTN 11	Gram (-)	Yes	No	Yes	Yes	0	7.3	No
Pseudomonas putida ATCC 35546	Gram (+)	Yes	No	Yes	Yes	25.5	0	No
Pseudomonas aeruginosa 9027	Gram (+)	Yes	No	Yes	Yes	100-200	1.3	No
K4-1 *Paenibacillus cineris*	Gram (+)	Yes	No	No	Yes	0	2.6	No
K4-2 *Paenibacillus illinoisensis*	Gram (+)	Yes	No	Yes	Yes	0	17.8	No
K4-3 *Paenibacillus illinoisensis*	Gram (+)	No	No	Yes	Yes	0	21.4	No
K4-4 *Bacillus megaterium*	Gram (+)	Yes	No	Yes	Yes	0	8.8	No

K4-5 *Bacillus sp.*	Gram (+)	No	No	Yes	Yes	0	3.3	No
K4-6 *Bacillus pumilus*	Gram (+)	No	No	Yes	Yes	0	3.2	No
K4-7B	Gram (+)	Yes	No	Yes	Yes	0	0.9	No
K4-10A	Gram (+)	Yes	No	Yes	Yes	0	0	No
K4-10A1 *Bacillus pumilus*	Gram (+)	Yes	No	Yes	Yes	0	15.5	No
K4-10A3 *Bacillus pumilus*	Gram (+)	Yes	No	Yes	Yes	0	2.2	No
K4-10A4 *Bacillus pumilus*	Gram (+)	Yes	No	Yes	Yes	0	2.6	No
K4-10C *Arthrobacter globiformis*	Gram (+)	Yes	No	Yes	Yes	91.3	71.8	No
K4-11 *Sphingomonas sp.*	Gram (-)	Yes	No	No	Yes	0	0	No
MTR-1 *Streptomyces sp.*	Gram (+)	No	No	No	Yes	200	41.6	Yes
MTR-2	Gram (+)	Yes	No	No	Yes	200	5.2	No
MTR-3A *Rhizobium sp.*	Gram (-)	Yes	No	Yes	Yes	0	57	No
MTR-3B	Gram (-)	Yes	No	Yes	Yes	0	69	Yes
MTR-4	Gram (+)	Yes	No	No	Yes	0	1.9	Yes
MTR-5	Gram (+)	No	No	Yes	Yes	0	0.8	No
MTR-6	Gram (-)	No	No	No	Yes	0	110	No
MTR-7A	Gram (-)	No	No	Yes	Yes	0	21	No
MTR-7B	Gram (-)	Yes	No	Yes	No	0	26	No
MTR-8A *Streptomyces thermoviolaceus*	Gram (+)	No	No	Yes	Yes	149	6.3	Yes
MTR-9	Gram (-)	Yes	No	No	Yes	198	2.3	No
MTR-10	Gram (+)	Yes	No	No	Yes	26	0	Yes
MTR-11 *Gordonia alkalivorans*	Gram (+)	No	No	Yes	Yes	63	1.5	No
MTR-12	Gram (-)	No	No	No	Yes	0	26.4	No
MTR-13	Gram (+)	Yes	No	No	Yes	0	4.9	No
MTR-14A	Gram (+)	Yes	No	No	Yes	9.0	0	Yes
MTR-14B	Gram (-)	Yes	No	No	No	0	11.7	No
MTR-15	Gram (-)	No	No	No	Yes	0	3.2	Yes
MTR-16	Gram (-)	Yes	No	Yes	Yes	171	25.4	No

MTR-17A *Agrobacterium sp.*	Gram (-)	Yes	No	Yes	Yes	0	2	No
MTR-17B	Gram (-)	Yes	No	Yes	Yes	0	80	No
MTR-18 *Microbacterium sp.*	Gram (+)	Yes	No	No	No	0	104.4	No
MTR-19	Gram (+)	Yes	No	Yes	Yes	0	26	No
MTR-20	Gram (-)	No	NO	Yes	Yes	0	6.4	Yes
MTR-21A *Clavibacter michiganensis*	Gram (+)	Yes	Yes	Yes	Yes	100-200	7.2	No
MTR-21A1	Gram (+)	Yes	Yes	No	Yes	0	18.7	No
MTR-21B	Gram (-)	Yes	Yes	Yes	Yes	0	25	No
MTR-22	Gram (+)	Yes	No	Yes	Yes	0	53	No
MTR-23A *Rhizobium sp.*	Gram (+)	No	No	No	Yes	0	5	No
MTR-23B	Gram (-)	Yes	No	Yes	Yes	167	63.7	No
MTR-24	Gram (-)	Yes	No	Yes	Yes	157	33	No
MTR-25A	Gram (-)	Yes	No	Yes	Yes	0	17.2	No
MTR-25B	Gram (+)	Yes	No	No	No	0	54	No
MTR-25B1	Gram (-)	Yes	No	Yes	No	58	3.3	No
MTR-25C	Gram (+)	Yes	No	Yes	No	0	57	No
MTR-26	Gram (+)	Yes	No	No	Yes	66	6.3	No
MTR-27 *Arthrobacter oxydans*	Gram (+)	Yes	Yes	No	Yes	190	58	Yes
MTR-28	Gram (-)	Yes	No	Yes	Yes	5	1.3	No
MTR-29	Gram (+)	No	No	No	Yes	64	13	No
MTR-30	Gram (+)	No	No	No	Yes	0	3	Yes
MTR-31	Gram (-)	Yes	Yes	Yes	Yes	0	60	No
MTR-32A *Rhizobium sp.*	Gram (-)	Yes	Yes	Yes	Yes	196	67.3	No
MTR-32B	Gram (+)	No	No	Yes	Yes	0	6.2	No
MTR-33	Gram (+)	Yes	No	Yes	Yes	0	19.8	No
MTR-34	Gram (+)	Yes	Yes	Yes	Yes	0	8.3	No
MTR-35A	Gram (+)	Yes	No	Yes	Yes	198	54	No

MTR-35B *Agrobacterium sp.*	Gram (-)	Yes	No	Yes	Yes	196	51	No
MTR-36	Gram (-)	No	No	No	Yes	0	32	No
MTR-37	Gram (-)	Yes	Yes	Yes	No	0	4.7	0
MTR-38	Gram (-)	No	No	Yes	Yes	0	1.4	0
MTR-39	Gram (+)	Yes	Yes	Yes	No	200	1.1	No
MTR-40	Gram (-)	Yes	No	Yes	No	139	4.4	No
MTR-41	Gram (+)	Yes	No	No	No	0	8.2	No
MTR-42	Gram (+)	Yes	No	Yes	No	0	11.2	No
MTR-43	Gram (-)	No	No	Yes	Yes	0	49	No
MTR-44 *Arthrobacter sp.*	Gram (+)	Yes	No	No	Yes	58	86.8	Yes
MTR-45A	Gram (-)	Yes	No	Yes	No	0	16	No
MTR-45B *Rhodanobacter lindaniclasticus*	Gram (-)	Yes	Yes	Yes	No	0	11	No
MTR-45C	Gram (-)	Yes	No	No	No	0	0	No
MTR-46	Gram (+)	Yes	No	No	Yes	0	7.1	No
MTR-47	Gram (+)	No	No	Yes	No	31	1.3	No
MTR-48	Gram (+)	No	No	No	No	0	0	No
MTR-49	Gram (+)	Yes	No	Yes	No	48	76	No
MTR-50	Gram (+)	Yes	No	No	Yes	0	30.9	No
MTR-51	Gram (+)	Yes	No	Yes	Yes	9	6.6	No
MTR-52	Gram (+)	Yes	No	No	No	35	7.1	Yes
MTR-53 *Arthrobacter histidinolovorans*	Gram (+)	Yes	Yes	No	Yes	165	0.9	No
MTR-54	Gram (+)	Yes	No	No	No	0	2.4	No
MTR-55A	Gram (-)	No	No	Yes	No	0	18.7	No
MTR-55B *Microbacterium sp.*	Gram (+)	No	No	Yes	Yes	0	4.2	No
MTR-56	Gram (+)	Yes	No	Yes	Yes	4	3.3	Yes
MTR-56B	Gram (+)	Yes	No	No	No	0	14.7	No
MTR-57	Gram (+)	Yes	No	Yes	No	162	5	No

MTR-58	Gram (+)	Yes	No	No	Yes	0	8	Yes
MTR-59	Gram (+)	Yes	No	Yes	No	0	1.3	No
MTR-60	Gram (-)	Yes	No	Yes	Yes	200	12.7	Yes
MTR-61 *Pseudomonas sp.*	Gram (-)	Yes	No	Yes	Yes	188	7.8	Yes
MTR-62	Gram(+/-)	Yes	No	No	Yes	0	9.1	No
MTR-63	Gram (+)	Yes	No	Yes	Yes	0	2.8	No
MTR-64	Gram (-)	No	No	No	Yes	0	12.9	No
MTR-65	Gram (-)	No	No	No	No	0	14.6	No
MTR-66 *Methylobacterium fujisawaens*	Gram (+)	Yes	No	Yes	No	0	13.5	Yes
MTR-67	Gram (+)	Yes	No	No	Yes	0	57.7	Yes
MTR-68	Gram (+)	Yes	No	Yes	Yes	12	2	Yes
MTR-69	Gram (-)	Yes	No	Yes	Yes	0	7.8	No
MTR-70 *Rhodococcus erythropolis*	Gram (+)	Yes	No	No	Yes	4	0	Yes
MTR-71 *Erythromonas ursincola*	Gram (-)	No	No	Yes	No	0	1.5	No
MTR-72	Gram (+)	Yes	No	No	Yes	0	3.5	No
SP-1 *Microbacterium sp.*	Gram (+)	No	No	Yes	No	0	12.8	No
SP-3	Gram (-)	Yes	No	Yes	No	0	17.4	No
SP-4	Gram (+)	Yes	No	Yes	No	0	6.8	No
SP-5	Gram (-)	No	No	No	No	0	6.8	No
SP-7 *Arthrobacter sp.*	Gram (+)	Yes	No	Yes	No	5	72.1	No

Table 2 Plant Growth-Promoting Mechanisms from Bacterial Isolates Collected from Wild Native Desert Plants

Isolate	Source	pH 5	pH 4	DCP	IAA	CAS	Siderophore	ACC	Pb 1 mM	Zn 1 mM	Motility
AL-01A	CAS	yes	no	positive	16.0 ± 2.3	positive	15.2 ± 5.0	no	yes	no	yes
AL-01B	CAS	yes	no	positive	9.3 ± 0.2	positive	61.8 ± 16.2	no	yes	no	yes
AL-02	1/100 TSA	yes	no	positive	12.3 ± 0.5	negative	0	no	yes	**********	no
AL-03A	1/100 TSA	yes	no	negative	6.7 ± 0.7	positive	11.0 ± 10.0	no	yes	no	yes
AL-04	1/100 TSA	yes	no	negative	5.8 ± 0.1	negative	0	no	yes	**********	yes
AL-05	1/100 TSA	yes	no	negative	2.7 ± 0.3	negative	0	no	yes	no	no
AL-06	1/10 TSA	yes	no	negative	0	negative	4.2 ± 0.6	**********	no	**********	yes
AL-07	1/10 TSA	yes	no	negative	4.3 ± 1.3	negative	0	no	yes	yes	no
AL-09A	Pb+	yes	yes	negative	62.0 ± 3.6	negative	0	yes	yes	**********	no
AL-10	Bashan TSA	yes	no	negative	1.9 ± 1.4	negative	19.2 ± 12.5	no	yes	yes	no
AL-11	Pb+	yes	no	negative	7.1 ± 0.6	negative	0	no	yes	**********	yes
AL-12	Pb+	no	no	negative	0.1 ± 0.1	positive	0	**********	yes	**********	yes
AL-13A	Bashan TSA	yes	yes	positive	49.1 ± 1.9	positive	0	yes	yes	yes	yes
AL-13B	Bashan TSA	yes	yes	positive	0	positive	19.6 ± 34.0	no	yes	no	yes
AL-15	NaCl/Pb	yes	no	negative	6.3 ± 0.2	negative	0	no	yes	**********	yes
AL-16	NaCl	yes	yes	negative	1.5 ± 0.1	negative	0	yes	yes	yes	no
AL-17	NaCl	yes	no	negative	4.0 ± 0.7	negative	0		yes	no	no

AL-18	NaCl	yes	no	negative	1.7 ± 0.1	negative	0	**********	no	no	yes
AL-19	Pb+	yes	no	negative	1.3 ± 1.0	negative	0	no	no	**********	yes
AL-20A	Pb+	yes	no	negative	2.5 ± 0.0	negative	1.2 ± 1.3	**********	no	**********	no
AL-20B	Pb+	yes	no	negative	2.4 ± 0.0	negative	20.3 ± 17.7	no	yes	no	yes
AL-21	Pb+	no	no	negative	1.6 ± 0.4	negative	**********	no	**********	**********	no
AL-22	NFb	yes	yes	negative	0.2 ± 0.2	negative	0	no	no	no	yes
AL-23B	Bashan TSA	yes	yes	positive	0.4 ± 0.2	negative	29.3 ± 9.7	yes	yes	**********	yes
AL-23C	Bashan TSA	yes	yes	positive	39.7 ± 2.5	positive	47.8 ± 19.1	yes	yes	no	no
AL-24	1/100 TSA	yes	no	negative	6.0 ± 0.3	negative	0	**********	yes	**********	yes
AL-26	NaCl/Pb	yes	no	negative	5.8 ± 0.5	negative	0.5 ± 0.9	no	yes	yes	yes
AL-27	1/10 TSA	yes	no	negative	44.3 ± 36.4	negative	0	no	no	**********	yes
AL-28	1/10 TSA	yes	no	negative	48.6 ± 3.1	positive	0.3 ± 3.1	no	yes	yes	yes
AL-29A	NFb	no	yes	negative	0.5 ± 0.1	positive	11.0 ± 9.6	no	no	no	yes
AL-29B	NFb	yes	no	positive	0.4 ± 0.1	negative	59.5 ± 0.5	no	no	no	no
AL-30	CAS	yes	yes	positive	51.7 ± 5.1	positive	52.3 ± 7.6	yes	yes	no	yes
AL-31	R2A	yes	no	positive	1.2 ± 0.2	positive	0	no	yes	yes	yes
AL-32	Pb+	yes	no	negative	5.8 ± 0.4	negative	0	no	yes	**********	yes
AL-33	CAS	yes	yes	positive	16.1± 1.3	positive	18.4 ± 32.0	no	yes	**********	yes

AL-35	Pseudo-Selective	yes	yes	negative	1.5 ± 0.1	negative	18.9 ± 0.5	no	no	**********	no
AL-36A	1/100 TSA	no	no	positive	6.4 ± 0.1	positive	13.8 ± 6.5	no	yes	**********	yes
AL-36B	1/100 TSA	no	no	positive	7.8 ± 0.1	positive	5.7 ± 5.8	no	yes	**********	yes
AL-37	1/100 TSA	no	no	positive	7.0 ± 1.0	positive	11.6 ± 3.6	no	yes	**********	yes
AL-38	R2A	yes	no	negative	1.1 ± 1.9	positive	0	no	yes	yes	yes
AL-39A	Bashan TSA	yes	no	negative	6.1 ± 0.2	negative	8.7 ± 7.5	no	yes	no	yes
AL-39B	Bashan TSA	yes	no	negative	9.0 ± 4.4	negative	2.4± 1.2	no	no	no	yes
AL-40	Bashan TSA	yes	no	negative	1.1 ± 0.3	negative	24.8 ± 33.1	**********	yes	yes	no
AL-41	1/100 TSA	yes	no	negative	7.2 ± 0.9	positive	0	no	**********	**********	yes
AL-42	NaCl/Pb	yes	no	negative	4.8 ± 0.4	negative	0	no	yes	**********	yes
AL-43	1/100 TSA	yes	yes	negative	0.2 ± 0.1	positive	51.3 ± 8.0	no	yes	no	yes
AL-44	NaCl/Pb	yes	yes	positive	2.0 ± 0.2	negative	9.9 ± 9.4	no	**********	**********	yes
AL-45	1/10 TSA	yes	no	negative	0.2 ± 0.2	negative	12.2 ± 0.4	no	**********	**********	yes
AL-46	1/10 TSA	no	no	negative	7.4 ± 1.5	negative	4.8 ± 0.8	no	**********	**********	no
AL-47	1/100 TSA	yes	no	negative	70.0 ± 2.2	negative	0	no	no	**********	yes
AL-48	1/100 TSA	yes	yes	negative	6.9 ± 3.7	positive	0	no	no	no	yes
AL-49	CAS	yes	yes	positive	18.5 ± 1.9	positive	39.1 ± 33.9	no	yes	**********	yes
AL-50A	1/100 TSA	yes	yes	positive	28.2 ± 2.1	positive	24.8 ± 1.3	no	yes	**********	yes

AL-50B	1/100 TSA	yes	no	negative	37.9 ± 4.3	positive	3.2 ± 5.6	no	yes	**********	yes
AL-51B	1/100 TSA	yes	no	positive	30.4 ± 6.1	positive	12.8 ± 11.2	no	yes	yes	yes
AL-52	1/100 TSA	yes	no	negative	0	positive	0	no	no	**********	yes
AL-53	1/100 TSA	yes	no	negative	47.6 ± 3.1	negative	0	no	**********	yes	yes
AL-54	1/100 TSA	yes	yes	negative	17.7 ± 0.8	positive	28.2 ± 27.3	no	no	**********	yes
AL-55	1/100 TSA	yes	no	negative	38.9 ± 1.0	positive	0	no	yes	yes	yes
AL-56	1/100 TSA	yes	no	positive	15.1 ± 1.2	negative	**********	**********	yes	**********	no
AL-57A	1/100 TSA	yes	no	negative	6.0 ± .01	negative	15.2 ± 21.1	**********	**********	no	yes
AL-57B	1/100 TSA	yes	no	negative	46.7 ± 2.4	positive	3.4 ± 0.4	no	yes	**********	no
AL-58	1/10 TSA	yes	no	negative	14.3 ± 0.6	positive	0	no	yes	**********	no
AL-59	Unknown	yes	no	negative	1.2 ± 0.5	negative	0	no	yes	yes	yes
GR-01	NaCl/Pb	yes	no	negative	0.7 ± 1.0	negative	0	no	no	**********	yes
GR-02	Pb+	yes	no	negative	1.0 ± 1.0	negative	**********	**********	no	**********	no
GR-03	CAS	yes	yes	positive	54.3 ± 7.1	positive	0.2 ± 16.0	no	yes	yes	yes
GR-04	R2A	yes	no	negative	1.1 ± 1.4	negative	0	no	yes	yes	no
GR-05	CAS	yes	yes	negative	0	negative	52.5 ± 0.6	yes	yes		yes
GR-06	CAS	yes	yes	positive	0	positive	44.8 ± 2.6	yes	no	no	yes
GR-07	Pb+	yes	no	negative	20.6 ± 2.5	negative	does not grow	no	yes	**********	no

							on MM9				
GR-08A	R2A	yes	yes	negative	17.2 ± 13.0	negative	0	no	yes	no	no
GR-08B	R2A	yes	no	negative	2.3 ± 0.1	negative	16.3 ± 1.6	**********	**********	**********	no
GR-09	R2A	no	no	negative	2.2 ± 0.2	negative	**********	**********	**********	**********	no
GR-10A	R2A	yes	no	negative	0	negative	**********	**********	**********	**********	no
GR-10B	R2A	yes	no	negative	2.6 ± 2.4	negative	5.8 ± 0.3	no	no	**********	yes
GR-11	1/100 TSA	no	no	negative	0.3 ± 0.5	negative	29.3 ± 2.9	no	no	**********	no
GR-12	1/100 TSA	yes	no	negative	0.2 ± 0.4	negative	0	no	**********	**********	no
GR-13	1/100 TSA	no	no	positive	8.0 ± 0.9	positive	14.8 ± 1.4	no	no	**********	yes
GR-14	Pb+	yes	yes	negative	15.0 ± 5.4	negative	0	no	no	**********	yes
GR-15	1/100 TSA	no	no	negative	5.3 ± 0.3	positive	21.2 ± 0.2	no	no	**********	yes
GR-16	Bashan TSA	no	no	negative	1.4 ± 0.3	negative	0	yes	yes	no	no
GR-17A1	1/10 TSA	yes	no	negative	0.7 ± 0.2	negative	12.5 ± 3.1	no	yes	no	no
GR-17A2	1/10 TSA	yes	no	negative	0.4 ± 0.0	negative	3.2 ± 2.8	no	yes	no	no
GR-17B	1/10 TSA	yes	no	negative	0	negative	0	no	no	no	no
GR-18A	1/10 TSA	yes	yes	positive	59.7 ± 1.7	positive	15.4 ± 5.3	no	yes	yes	no
GR-18B	1/10 TSA	yes	no	positive	13.8 ± 21.8	positive	17.9 ± 2.2	no	no	**********	no
GR-19A2	NFb	yes	no	negative	4.1 ± 0.8	positive	0	**********	**********	**********	yes

GR-20A2	NFb	yes	yes	negative	34.6	positive	7.1 ± 5.5	yes	yes	yes	yes
GR-20B	NFb	yes	no	negative	2.6 ± 0.8	positive	17.0± 0.9	no	**********	yes	yes
GR-21	Pb+	yes	no	negative	3.5 ± 0.7	positive		no	no	yes	yes
GR-22	Pb+	no	no	negative	1.9 ± 0.1	positive	24.4 ± 11.4	no	**********	**********	no
GR-23	1/10 TSA	no	no	negative	11.8 ± 1.8	negative	3.5 ± 3.0	no	**********	**********	yes
GR-24	1/10 TSA	yes	no	positive	2.6 ± 0.5	negative	0	no	**********	**********	no
GR-25	1/10 TSA	no	no	negative	6.8 ± 2.6	negative	8.6 ± 0.6	no	**********	**********	yes
GR-26	1/10 TSA	yes	no	negative	2.3 ± 1.1	negative	0	no	**********	**********	no
GR-27	1/10 TSA	yes	no	negative	1.6 ± 1.9	negative	14.2 ± 10.7	no	**********	no	no
GR-28A	1/10 TSA	no	no	positive	2.5 ± 2.3	negative	17.7 ± 2.2	no	no	**********	no
GR-28B	1/10 TSA	no	no	positive	19.4 ± 14.5	negative	25.4 ± 19.4	no	**********	no	no
GR-29	1/10 TSA	yes	no	positive	17.7 ± 0.1	negative	**********	no	no	**********	no
GR-30	Pseudo-Selective	yes	yes	negative	3.1 ± 0.5	negative	12.5 ± 4.1	yes	yes	yes	no
GR-31	Pseudo-Selective	yes	yes	positive	2.5 ± 0.1	negative	1.0 ± 0.9	no	no	**********	no
GR-32	Pseudo-Selective	yes	no	negative	0.3 ± 0.5	negative	0	no	**********	**********	no
GR-33	Pb+	yes	yes	positive	5.8 ± 0.2	negative	0	**********	no	no	no
GR-34	Pb+	no	no	positive	29.4 ± 1.3	negative	0.3 ± 0.2	no	no	yes	no
GR-35A	1/100 TSA	yes	yes	negative	2.4 ± 0.1	negative	1.1 ± 7.2	no	**********	**********	no

GR-36	1/100 TSA	yes	no	positive	3.3 ± 0.4	negative	11.7 ± 4.5	no	**********	**********	yes
GR-37	NaCl/Pb	yes	no	negative	9.1 ± 1.2	negative	0	no	no	**********	yes
GR-38	NaCl	no	no	positive	2.4 ± 2.3	negative	20.3 ± 2.1	no	no	**********	yes
GR-39	NaCl	yes	no	negative	1.9 ± 0.2	negative	47.0 ± 1.6	no	**********	**********	no
GR-40	R2A	yes	no	negative	0	negative	49.2 ± 0.8	no	no	yes	no
GR-41	R2A	no	no	negative	26.5 ± 1.3	negative		no	**********	**********	no
GR-42	NaCl/Pb	yes	no	negative	5.2 ± 0.8	negative	0	no	yes	**********	yes
GR-43	1/100 TSA	yes	no	negative	3.2 ± 0.0	negative	49.1 ± 3.4	no	**********	**********	yes
GR-44	1/100 TSA	yes	no	negative	5.3 ± 1.5	negative	0	no	no	**********	yes
GR-45	R2A	yes	no	negative	1.1 ± 0.1	negative	39.9 ± 6.0	no	no	**********	no
GR-46	R2A	yes	no	negative	0	positive	0	no	no	yes	no
GR-47	R2A	yes	no	negative	2.2 ± 0.1	negative	0	no	no	yes	no
GR-48	R2A	yes	yes	negative	1.4 ± 0.3	negative	**********	**********	no	**********	no
GR-49A1	R2A	yes	no	positive	1.8 ± 0.2	negative	0	no	yes	**********	no
GR-49A2	R2A	yes	yes	negative	3.2 ± 0.0	negative	**********	**********	yes	**********	no
GR-50	R2A	yes	no	negative	0.3 ± 0.2	positive	0	**********	yes	yes	no
GR-51	R2A	yes	no	negative	9.3 ± 1.6	negative	34.8 ± 10.3	no	no	yes	no
GR-53	R2A	no	no	negative	2.7 ± 0.8	negative	37.9 ± 15.0	no	**********	**********	no

GR-54	R2A	yes	no	negative	0.4 ± 0.8	negative	0	no	no	yes	no
MQ-01	1/10 TSA	no	no	negative	12.1 ± 2.1	negative	0	**********	**********	**********	no
MQ-02	Bashan TSA	yes	no	negative	6.2 ± 0.8	negative	17.8 ± 22.3	no	no	no	no
MQ-03	Bashan TSA	yes	yes	positive	12.3 ± 0.9	negative	0	no	yes	yes	no
MQ-05	Bashan TSA	yes	no	positive	20.7 ± 1.8	positive	21.2 ± 1.2	no	yes	**********	no
MQ-06	Bashan TSA	yes	yes	positive	31.7 ± 1.1	positive	0	**********	**********	**********	no
MQ-07	1/100 TSA	yes	no	negative	**********	negative	2.0 ± 1.8	**********	**********	**********	no
MQ-08	CAS	yes	yes	negative	11.8 ± 0.8	negative	0	**********	no	no	no
MQ-09A	R2A	yes	no	negative	3.1 ± 0.2	negative	**********	**********	no	no	no
MQ-09B	R2A	yes	no	positive	6.9 ± 0.6	positive	6.4 ± 0.5	no	yes	**********	yes
MQ-10A	R2A	yes	no	negative	2.5 ± 0.3	negative	0	no			no
MQ-10B	R2A	no	no	negative	1.3 ± 0.3	negative	44.1 ± 2.9	no	no	yes	yes
MQ-11A	R2A	no	no	negative	1.0 ± 0.0	negative	0	no	no	**********	yes
MQ-12	Bashan TSA	yes	no	negative	14.6 ± 0.8	negative	0	**********	no	yes	no
MQ-13	R2A	yes	no	negative	2.9 ± 0.1	negative	**********	**********	**********	**********	no
MQ-14A	R2A	yes	no	negative	**********	negative	**********	**********	**********	**********	no
MQ-14B	R2A	yes	no	negative	4.8 ± 3.4	negative	2.2 ± 1.9	no	yes	yes	no
MQ-15	R2A	no	no	negative	22.8 ± 2.3	negative	0	no	**********	**********	no

MQ-16	R2A	yes	no	negative	12.9 ± 0.5	negative		no	**********	**********	no
MQ-17	R2A	yes	no	negative	3.2 ± 0.5	negative	**********	no	**********	no	yes
MQ-18	Pb+	yes	no	negative	2.1 ± 0.2	negative	4.8 ± 0.7	**********	**********	**********	no
MQ-19	Pb+	yes	no	negative	23.3 ± 0.9	negative		**********	yes	**********	no
MQ-20	Pb+	yes	no	negative	0.8 ± 0.1	negative	5.2 ± 5.1	**********	**********	no	yes
MQ-21	Pb+	yes	no	negative	13.9 ± 0.7	negative		no	no	**********	no
MQ-22	1/10 TSA	yes	no	negative	11.4 ± 0.6	negative		**********	no	**********	no
MQ-23B	1/10 TSA	no	no	negative	25.9 ± 0.9	negative	0	**********	no	**********	yes
MQ-24	1/10 TSA	yes	no	negative	14.1 ± 0.7	negative	25.2 ± 0.2	**********	no	**********	no
MQ-25	1/10 TSA	yes	no	negative	1.1 ± 0.7	negative	11.6 ± 15.8	**********	**********	no	no
MQ-26	1/10 TSA	yes	no	positive	19.7 ± 0.7	positive	15.2 ± 5.1	**********	yes	no	yes
MQ-27A	NaCl	yes	no	positive	0.5 ± 0.4	negative	**********	**********	**********	yes	no
MQ-27B	NaCl	yes	yes	negative	0.9 ± 0.1	negative	4.2 ± 1.6	**********	yes	**********	no
MQ-28	NaCl	yes	no	negative	10.6 ± 1.2	negative	3.8 ± 5.1	**********	**********	**********	no
MQ-29	Bashan TSA	yes	no	negative	8.7 ± 2.9	negative	12.8 ± 11.7	**********	**********	**********	yes
MQ-30	Bashan TSA	yes	no	negative	**********	negative	0	**********	**********	**********	no
MQ-31	Bashan TSA	yes	no	negative	12.3 ± 0.3	positive	0	no	yes	yes	no
MQ-32	NaCl	yes	yes	positive	27.2 ± 1.0	positive	12.8 ± 11.7	**********	**********	**********	no

MQ-33	NaCl	yes	yes	positive	22.4 ± 1.3	positive	47.8 ± 0.1	no	yes	yes	yes
MQ-35A	Bashan TSA	yes	no	negative	33.6 ± 3.9	positive	10.5 ± 9.1	**********	**********	**********	no
MQ-35B	Bashan TSA	yes	yes	negative	44.4 ± 18.3	positive	20.9 ± 0.6	**********	**********	**********	no
MQ-36	Bashan TSA	yes	no	negative	6.8 ± 0.5	negative	2.7 ± 0.3	no	yes	yes	no
MQ-37	Bashan TSA	no	no	negative	**********	negative		**********	**********	**********	no
MQ-38	Bashan TSA	yes	no	negative	11.2 ± 1.0	positive	0	no	**********	**********	no
MQ-39A	1/100 TSA	yes	no	negative	6.1 ± 0.2	negative	9.6 ± 16.6	no	yes	yes	no
MQ-39B	1/100 TSA	yes	no	negative	0.5 ± 0.1	negative		**********	no	no	no
PV-01	R2A	yes	no	negative	2.8 ± 0.2	negative	**********	**********	**********	**********	yes
PV-02	1/100 TSA	yes	no	negative	2.1 ± 0.1	negative	does not grow on MM9	**********	**********	**********	no
PV-03A	R2A	no	no	negative	1.0 ± 0.1	negative		**********	**********	**********	no
PV-03B	R2A	no	no	negative	2.3 ± 0.8	negative		**********	**********	**********	no
PV-04A	R2A	yes	no	negative	44.5 ± 1.6	negative	does not grow on MM9	no	**********	**********	no
PV-04B	R2A	no	no	negative	44.0 ± 1.4	negative	does not grow on MM9	no	**********	**********	no
PV-05A	R2A	no	no	negative	40.7 ± 8.2	negative	does not grow on MM9	no	**********	**********	no
PV-05B	R2A	no	no	negative	48.1 ± 3.0	positive	does not grow	no	**********	**********	no

							on MM9				
PV-06A	R2A	no	no	negative	43.3 ± 3.1	negative	does not grow on MM9	no	no	**********	no
PV-06B	R2A	yes	no	negative	46.8 ± 4.3	negative	does not grow on MM9	no	**********	**********	no
PV-06C	R2A	no	no	negative	44.1 ± 1.7	positive	0	no	**********	**********	no
PV-07	1/10 TSA	yes	no	negative	2.8 ± 0.2	negative	0	**********	**********	**********	no
PV-08	Pseudo-Selective	yes	no	negative	2.8 ± 0.1	negative	does not grow on MM9	**********	no	**********	no
PV-09	CAS	yes	no	negative	4.7 ± 0.6	negative	does not grow on MM9	**********	**********	**********	yes
PV-10	CAS	yes	yes	positive	2.7 ± 0.1	positive	0	no	yes	yes	no
PV-11	1/10 TSA	yes	no	negative	**********	negative	**********	**********	**********	**********	no
PV-11B	1/10 TSA	yes	yes	positive	1.4 ± 0.1	negative	0	no	yes	no	no
PV-12	1/10 TSA	yes	no	negative	1.4 ± 0.9	negative	does not grow on MM9	**********	**********	**********	no
PV-13	R2A	no	no	negative	2.8 ± 0.2	negative	does not grow on MM9	**********	**********	**********	no
PV-14	NaCl/Pb	yes	yes	positive	68.6 ± 1.2	positive	37.2 ± 26.7	yes	no	yes	no
PV-15	1/10 TSA	yes	no	negative	1.0 ± 0.5	negative	0	**********	**********	**********	no
PV-16A	1/10 TSA	yes	yes	negative	3.0 ± 0.6	negative	0	no	**********	**********	no

PV-17	1/10 TSA	yes	yes	negative	2.9 ± 0.7	negative	0	no	**********	no	no
PV-18	Pseudo-Selective	yes	yes	negative	2.8 ± 0.1	negative	0	no	**********	**********	no
PV-19	Bashan TSA	yes	no	negative	1.3 ± 0.4	positive	0	**********	no	yes	no
PV-20A	Bashan TSA	yes	yes	positive	0.9 ± 1.0	positive	7.7 ± 7.4	no	yes	yes	yes
PV-20B	Bashan TSA	yes	yes	positive	19.3 ± 0.5	negative	6.5 ± 1.2	no	no	yes	no
PV-21	1/10 TSA	no	no	negative	27.1 ± 0.4	negative	does not grow on MM9	**********	**********	**********	no
PV-22	Bashan TSA	yes	no	negative	21.2 ± 2.1	negative	**********	**********	no	yes	yes
PV-23	1/10 TSA	yes	yes	negative	3.8 ± 0.1	positive	0	no	**********	yes	no
PV-24A	1/10 TSA	yes	no	negative	2.3 ± 0.1	negative	17.5 ± 0.2	no	yes	yes	no
PV-24B	1/10 TSA	yes	yes	negative	2.6 ± 0.1	negative	0	no	yes	yes	no
PV-25	CAS	yes	no	negative	14.0 ± 1.5	negative	15.5 ± 32.3	**********	**********	**********	yes
PV-27	R2A	yes	no	negative	7.1 ± 1.2	positive	does not grow on MM9	**********	no	**********	no
PV-28A	1/10 TSA	no	no	negative	35.6 ± 9.1	negative	0	no	**********	**********	no
PV-28B	1/10 TSA	yes	no	negative	6.9 ± 1.8	positive	29.5 ± 23.4	no		yes	no
PV-29A	R2A	yes	no	negative	**********	positive	**********	no	**********	**********	no
PV-29B1	R2A	yes	no	negative	1.0 ± 0.0	negative	**********	**********	**********	**********	no
PV-29B2	R2A	no	no	negative	2.0 ± 0.2	negative	0	no	**********	**********	no

PV-30	Bashan TSA	yes	no	negative	4.8 ± 0.4	positive	0	no	yes	yes	no
PV-31	1/10 TSA	yes	no	negative	9.8 ± 0.6	negative	0	no	**********	**********	no
PV-33	Bashan TSA	yes	no	positive	1.8 ± 0.6	negative	0	no	**********	**********	yes
PV-34	1/10 TSA	yes	no	negative	1.3 ± 0.2	negative	0	no	**********	yes	yes
PV-35A	1/10 TSA	yes	no	negative	1.3 ± 0.3	negative	0	no	**********	**********	no
PV-35B	1/10 TSA	yes	no	negative	1.4 ± 0.6	negative	0	yes	yes	yes	yes
PV-36A	1/10 TSA	yes	no	negative	1.3 ± 0.2	negative	1.7 ± 16.0	yes	**********	**********	yes
PV-36B	1/10 TSA	yes	no	negative	0.8 ± 0.3	negative	0	no	no	yes	yes
PV-37	1/10 TSA	yes	yes	negative	1.3 ± 0.2	negative	0	**********	**********	**********	yes
PV-38	1/10 TSA	no	no	negative	47.3 ± 4.4	negative	does not grow on MM9	**********	**********	**********	no
PV-39	1/10 TSA	yes	no	negative	2.4 ± 0.2	negative	**********	**********	**********	**********	yes
PV-40	1/10 TSA	yes	no	negative	2.3 ± 0.2	negative	**********	**********	**********	**********	yes
PV-41	R2A	yes	no	negative	1.0 ± 0.1	negative	0	**********	no	no	yes
PV-42	R2A	yes	no	negative	7.6 ± 1.0	negative	**********	**********	**********	**********	yes
PV-43	Bashan TSA	yes	no	negative	14.6 ± 1.1	positive	13.0 ± 13.8	no	**********	**********	yes
PV-44	Bashan TSA	yes	no	negative	5.7 ± 0.8	negative	**********	**********	**********	**********	no
PV-45	Bashan TSA	no	no	negative	0.7 ± 0.2	negative	0	**********	**********	no	yes

REFERENCES

Abd El-Samad Hamdia, M., M.A.K. Shaddad, and M.M. Doaa. 2004. Mechanisms of salt tolerance and interactive effects of *Azospirillum brasilense*. Plant Growth Regulation. **44:**165-174.

Adams, F. 1981. Nutritional imbalances and constraints to plant growth on acid soils. J. Plant Nutr. **4:**81.

Alexander, D.B., and D.A. Zuberer. 1991. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol. Fertil. Soils. **12:**39-45.

Amacher. 1996. Nickle, cadmium and lead. p. 754-756. *In* J.M. Bartels (ed.) Methods of soil analysis part 3: Chemical methods. Soil Science Society of America, Madison, WI.

Andersson, M. 1988. Toxicity and tolerance of aluminum in vascular plants. Water, Air and Soil Poll. **39:**439-462.

Andrade G., Linderman R.G. and Bethlenfalvay G.J. 1998. Bacterial associations with the mycorrhizosphere and hyphosphere of the arbuscular mycorrhizal fungus *Glomus mosseae*. Plant and Soil 202: 79-87

Antoun, H., C. J. Beauchamp, N. Goussard, R. Chabot, and R. Lalande. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). Plant and Soil. **204:**57-67.

Arshad, M., M. Saleem, and S. Hussain. 2007. Perspectives of bacterial ACC deaminase in phytoremediation. TRENDS Biotech. **25:**356-362.

Azcon, R., and J. M. Barea. 1997. Mycorrhizal dependency of a representative plant species in Mediterranean shrublands (*Lavandula spica* L.) as a key factor to its use for revegetation strategies in desertification-threatened areas. **7:**83-92.

Bacilio M., Hernandez, J.-P., and Bashan, Y. 2006. Restoration of giant cardon cacti in barren desert soil amended with common compost and inoculated with *Azospirillum brasilense.* Biology and Fertility of Soils 43: 112-119.

Bacilio M., P. Vazquez, and Y. Bashan. 2003. Alleviation of noxious effects of cattle ranch composts on wheat seed germination by inoculation with *Azospirillum* spp. Biol. Fert. Soils. **38:**261-266.

Barac, T ., Taghavi, S ., Borremans, B ., Provoost, A ., Oeyen, L ., Colpaert, JV ., Vangronsveld, J ., van der Lelie, D. 2004. Engineered endophytic bacteria improve

phytoremediation of water-soluble, volatile, organic pollutants. Nat. Biotechnol. 22, 583-588.

Bar-Ness, E. Y. Chen, Y. Hadar, H. Marchner, and V. Römheld. 1991. Siderophores of *Pseudomonas putida* as an iron source for dicot and monocot plants. Plant Soil. **130:**231-241.

Bar-Ness, E., Y. Hadar, Y. Chen, A. Shanzer, and J. Libman. 1992. Iron uptake by plant from microbial siderophores. Plant Physiol. **99:**1329-1335.

Barriuso, J., M.T. Pereyra, J.A. Lucas Garcia, M. Megias, F.J. Gutierrez Manero, and B. Ramos. 2005. Screening for putative PGPR to improve establishment of the symbiosis *Lactarius deliciosus-Pinus* sp. Microbial Ecology. **50:**82-89.

Bashan, Y. 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol. Adv. **16:**729-770.

Bashan, Y., E.A. Davis, A. Carrillo, and R.G. Linderman. 2000. Assessment of VA mycorrhizal inoculum potential in relation to the establishment of cactus seedlings under mesquite nurse-trees in the Sonoran desert. Applied Soil Ecology **14**:165-176

Bashan, Y. and de-Bashan, L.E. 2005. Bacteria / Plant growth-promotion. In: Encyclopedia of soils in the environment. (Ed.) D. Hillel, Elsevier, Oxford, U.K. Vol. 1., pp. 103-115.

Bashan, Y., Harrison, S.K. and Whitmoyer, R.E. 1990. Enhanced growth of wheat and soybean plants inoculated with *Azospirillum brasilense* is not necessarily due to general enhancement of mineral uptake. Applied and Environmental Microbiology 56: 769-775.

Bashan, Y. and Holguin, G. 1993. Anchoring of *Azospirillum brasilense* to hydrophobic polystyrene and wheat roots. Journal of General Microbiology 139: 379-385.

Bashan, Y. and Holguin, G. 1997. Azospirillum/plant relationships: environmental and physiological advances (1990-1996). Canadian Journal of Microbiology 43: 103-121.

Bashan, Y., and Holguin, G. 1998. Proposal for the division of Plant Growth-Promoting Rhizobacteria into two classifications: biocontrol-PGPB (Plant Growth-Promoting Bacteria) and PGPB. Soil Biology and Biochemistry 30:1225-1228

Bashan, Y., A. Rojas, and M.E. Puente. 1999. Improved establishment and development of three cactus species inoculated with *Azospirillum brasilense* transplanted into disturbed urban soil. Can. J. Microbiol. **45:**441-451.

Bashan, Y., and G. Holguin. 2002. Plant growth-promoting bacteria: a potential tool for arid mangrove reforestation. Trees Struc. Func. **16:**159-166.

Bashan, Y., G. Holguin, and L.E. de-Bashan. 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Can. J. Microbiol. **50:**521-577.

Bashan, Y., J.-P. Hernandez, L.A. Leyva, and M. Bacilio. 2002. Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. Biol. Fertil. Soils. **35:**359-368.

Bashan, Y., M. Moreno, and E. Troyo. 2000b. Growth promotion of the oilseed halophyte *Salicornia bigelovii* in seawater inoculated with mangrove rhizosphere bacteria and *Azospirillum* . Biol. Fert. Soils. **32:** 265-272.

Bashan, Y., M.E. Puente, D.D. Myrold, G. Toledo. 1998. In vitro transfer of fixed nitrogen from diazotrophic filamentous cyanobacteria to black mangrove seedlings. FEMS Microbiol. Ecol. **26:**165-170.

Belimov, A.A., A.M. Kunakova, N.D. Vasilyeva, T.S. Kovatcheva, V.F. Dritchko, S.N. Kuzovatov, I.R. Trushkina, Yu.V. Alekseyev. 1998. Accumulation of radionucleotides by associative bacteria and the uptake of 134Cs by the inoculated barley plants in: Malik, K.A., M.S. Mizra, J.K. Ladha (Eds.), Nitrogen fixation with non-legumes. Kluwer Academic Publishers, Dordrecht, pp 275-280.

Belimov, A.A., A.M. Kunakova, V.I. Safronova, V.V. Stepanok, L.V. Yudkin, Y.V. Alekseev, and A.P. Kozhemyakov. 2004. Employment of rhizobacteria for the inoculation of barley plants cultivated in soil contaminated with lead and cadmium. Microbiol. **73:**99-106.

Belimov, A.A., A.Y. Ivanchikov, L.V. Yudkin, O.F. Khamova, S.M. Postavskaya, P.V. Popolzukhin, A.A. Shmakova, G.Y. Kozlova. 1999. New strains of associative growth-stimulating bacteria dominating the rhizoplane of barley seedlings: characterization and introduction. Mikrobiologiya. **47:**337-342.

Belimov, A.A., N. Hontzeas, V.I. Safronova, S.V. Demchinskaya, G. Piluzza, S. Bullitta, and B.R. Glick. 2005. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). Soil Boil. Biochem. **37:**241-250.

Belimov, A.A., V.I. Safronova, T. Mimura. 2002. Response of spring rape to inoculation with plant growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase depends on nutrient status of the plant. Can. J. Microbiol. **48:**189-199.

Belimov, A.A., V.I. Safronova, T.A. Sergeyeva, T.N. Egorova, V.A. Matveyeva, V.E. Tsyganov, A.Y. Borisov, I.A. Tikhonovich, C. Kluge, A. Preisfeld, K.-J. Dietz, and V.V. Stepanok. 2001. Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. Can. J. Microbiol. **47:**642-652.

Benizri, E., E. Baudoin, and A. Guckert. 2001. Root colonization by inoculated plant growth-promoting rhizobacteria. Biocontrol Sci. Technol. **11:**557-574.

Berti W.W.R., and S.D. Cunningham. 2000. Phytostabilization of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals – using plants to clean up the environment. John Wiley & Sons, Inc. New York. pp 71–88.

Bertrand, H., R. Nalin, R. Bally, and J.-C. Cleyet-Marel. 2001. Isolation and identification of the most efficient plant growth-promoting bacteria associated with canola (*Brassica napus)*. Biol. Fertil. Soils. **33:**152-156.

Bertsch and Bloom. 1996. Aluminum. p. 524-531. *In* J.M. Bartels (ed.) Methods of soil analysis part 3: Chemical methods. Soil Science Society of America, Madison, WI.

Bethlenfalvay, G. J., I. C. Cantrell, K. L. Mihara, and R. P. Schreinre. 1999. Relationships between soil aggregation and mycorrhizae as influenced by soil biota and nitrogen nutrition. Biol. Fertil. Soils. **28:**356-363.

Burd, G.I., D.G. Dixon, and B.R. Glick. 1998. A plant growth-promoting bacterium that decreases nickel toxicity in seedlings. Appl. Environ. Microbiol. **64:**3663-3668.

Burd, G.I., D.G. Dixon, and B.R. Glick. 2000. Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can. J. Microbiol. **46:**237-245.

Burr, T.J., M.N. Schroth and T. Suslow. 1978. Increased potato yields by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathol. **68:** 1377–1383.

Burt, R. 2004. Ed. Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report No. 42. Version 4.0, NRCS.

Buyer, J.S., D.P. Roberts, and E. Russek-Cohen. 2002. Soil and plant effects on microbial community structure. Can. J. Microbiol. **48:**955–964.

Çakmakçi, R., F. Donmez, A. Aydin, and F. Sahin. 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biol. Biochem. **38:**1482-1487.

Canbolat, M., S. Bilen, R. Cakmakc, F. Sahin, and A. Aydin. 2005. Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol. Fertil. Soils. In Press.

Carrillo-Garcia, A., Y. Bashan, E. Diaz Rivera, and G.J. Bethlenfalvay. 2000. Effects of resource island soils, competition, and inoculation with *Azospirillum* on survival and growth of *Pachycereus pringlei*, the giant cactus of the Sonoran Desert. Res. Ecol. **8:**65-73.

Carrillo, A.E., C.Y. Li, and Y. Bashan. 2002. Increased acidification in the rhizosphere of cactus seedlings induced by *Azospirillum brasilense.* Naturwissenschaften. **89:**428-432.

Castro-Larragoitia J., U. Kramar, and H. Puchelt. 1997. 200 years of mining activities at La Paz, San Luis Potosi, Mexico – consequences for environment and geochemical exploration. J. Geochem. Explor. **58:**81–91.

Cattelan, A.J., P.G. Hartel, J.J. Fuhrmann. 1999. Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci. Soc. Am. J. **63:**1670-1680.

Chen, Y.P., P.D. Rekah, A.B. Arun, F.T. Shen, W.-A. Lai, and C.C. Young. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl. Soil Ecol. **34:**33-41.

Chung, H., M. Park, M. Madhaiyan, S. Seshadri, J. Song, H. Cho, and T. Sa. 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil. Biol. Biochem. **37:**1970-1974.

Coelho, P., S. Silva, J. Roma-Torres, C. Costa, A. Henriques, J. Teixeira, M. Gomes, and O. Mayan. 2007. Health impact of living near an abandoned mine – case study: Jales mines. International J. Hygiene and Environmental Health. **210:**399-402.

Colores, G.M., R.E. Macur, D.M. Ward, and W.P. Inskeep. 2000. Molecular analysis of surfactant-driven microbial population shifts in hydrocarbon-contaminated soil. Appl. Environ. Microbiol. **66:**2959–2964.

Compant, S., B. Duffy, J. Nowak, C. Clément, and E. Ait. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action and future prospects. Appl. Environ. Microbiol. **71:**4951-4959.

Creus, C.M., R.J. Sueldo, and C.A. Barassi. 2004. Water relations and yield in *Azospirillum*-inoculated wheat exposed to drought in the field. Can. J. Bot. **82:**273-281.

Cunningham, S.D., W.R. Betri, J.W. Huang. 1995. Phytoremediation of contaminated soils. Trends Biotechnol. **13:**393-397.

Custer, K. 2003. Cleaning Up Western Watersheds; Mineral Policy Center; EarthWorks: Washington DC.

Dalmastri, C., L. Chiarini, C. Cantale, A. Bevivino, S. Tabacchioni. 1999. Soil type and maize cultivar affect the genetic diversity of maize root-associated *Burkholderia cepacia* populations. Microbial Ecol. **38:**273–284.

de-Bashan, L.E., J.-P. Hernandez, T. Morey, and Y. Bashan. 2004. Microalgae growth-promoting bacteria as "helpers" for microalgae: a novel approach for removing ammonium and phosphorus from municipal wastewater. Water Res. **38:**466-474.

de-Bashan, L.E., Antoun, H., and Bashan Y. 2008. Involvement of indole-3-acetic-acid produced by the microalgae growth-promoting bacterium *Azospirillum* spp. in growth promotion of *Chlorella vulgaris*. **Journal of Phycology 44:** (In press)

de-Bashan, L.E., M. Moreno, J.-P. Hernandez, and Y. Bashan. 2002a. Removal of ammonium and phosphorous ions from synthetic wastewater by the microalgae *Chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillum brasilense*. Water Res. **36:**2941-2948.

de-Bashan, L.E., Y. Bashan. 2004. Recent advances in removing phosphorous from wastewater and its future use as fertilizer (1997-2003). Water Res. **38:**4222-4246.

de-Bashan, L.E., Y. Bashan, M. Moreno, V.K. Lebsky, and J.J. Bustillos. 2002. Increased pigement and lipid content, lipid variety, and cell and population size of the microalgae *Chlorella* spp. when co-immobilized in alginate beads with the microalgae-growth-promoting bacterium *Azospirillum brasilense*. Can. J. Microbiol. **48:**514-521.

de-Bashan, L.E., Magallon, P., Antoun, H., and Bashan Y. 2008. Participation of glutamate dehydrogenase and glutamine synthetase in ammonium assimilation by *Chlorella vulgaris* jointly immobilized with the microalgae growth-promoting bacterium *Azospirillum brasilense.* **Journal of Phycology 44:** (In press).

de-Bashan L.E., Trejo A., Huss V.A.R., Hernandez J.-P. and Bashan, Y. 2008. *Chlorella sorokiniana* UTEX 2805, a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater. **Bioresource Technology** 99: 4980–4989

de Freitas, J.R. and J.J Germida. 1990. Plant growth promoting rhizobacteria for winter wheat. Can. J. Microbiol. **36:**265–272.

de Freitas, J. R., M. R. Banerjee, J. J. Germida. 1997. Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus L.*). Biol. Fertil. Soils. **24:**358-364.

Dekkers, L. C., A. J. van der Bij, I. H. M. Mulders, C. C. Phoelich, R. A. R. Wentwoord, D. C. M. Glandorf, C. A. Wijffelman, and B. J. J. Lugtenberg. 1998. Role of the O-antigen of lipopolysaccheride, and possible roles of growth rate and of NADH:ubiquinone oxidoreductase (*nuo*) in competitive tomato root-tip colonization by *Pseudomonas fluorescens* WCS365. Mol. Plant-Microbe Interact. **11:**763–771.

Dell' Amico, E., L. Cavalca, and V. Andreoni. 2005. Analysis of rhizobacterial communities in perennial *Graminaceae* from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. Microbial Ecol. **52:**153-162.

Delhaize, E., and P.R. Ryan. 1995. Al toxicity and tolerance in plants. Plant Physiol. **107:**315-321.

De-Ridder-Duine, A.S., G.A. Kowalchuk, P.J.A. Klein Gunnewiek, W. Smant, and J.A. van Veen. 2005. Rhizosphere bacterial community composition in natural stands of *Carex arenaria* (sand sedge) is determined by bulk soil community composition. Soil Biol. Biochem. **37:**349-357.

de Souza, M.P., D. Chu, M. Zhao, A.M. Zayed, S.E. Ruzin, D. Schichnes, and N. Terry. 1999a. Rhizosphere bacteria enhance selenium accumulation and volatilization by Indian mustard. Plant Physiol. **119:**565-573.

de Souza, M.D., C.P. Huang, N. Chee, N. Terry. 1999b. Rhizosphere bacteria enhance the accumulation of selenium and mercury in wetland plants. Planta. **209:**259-263.

De Troch, P., and J. Vanderleyden. 1996. Surface properties and motility or *Rhizobium* and *Azospirillum* in relation to plant root attachment. Microb. Ecol. **32:**149-169.

Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, Y. Okon, and J. Vanderleyden. 2002. Effects of inoculation on wild type *Azospirillum brasilense* and *A. irakense* strains in development and nitrogen uptake of spring wheat and grain maize. Biol. Fertil. Soils. **36:**284-297.

Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, J. Vanderleyden, P. Dutto, C. Labandera-Gonzalez, C. Mellado, J.F. Aguirre, Y. Kapulnik, S. Brener, S. Burdman, D. Kadouri, S. Sarig and Y. Okon. 2001. Responses of agronomically important crops to inoculation with *Azospirillum*. Aust J. Plant Physiol. 28: 871–879.

Döbereiner, J., Marriel, I. E., and Nery, M. 1976. Ecological distribution of *Spirillum lipoferum* Beijerinck. Can. J. Microbiol. **22**: 1464-1473.

Donate-Correa, J., M. Leon-Barrios, and R. Perez-Galdona. 2004. Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. Plant and Soil. **266:**261-272

Donnelly, P.K., R.S. Hedge, and J.S. Fletcher. 1994. Growth of PCB-degrading bacteria on compounds from photosynthetic plants. Chemosphere. **28:**981-988.

Duponnois, R., M. Kisa, K. Assigbetse, Y. Prin, J. Thioulouse, M. Issartel, P. Moulin, and M. Lepage. 2006. Fluorescent pseudomonads occurring in *Macrotermes subhyalinus* mound structures decrease Cd toxicity and improve its accumulation in sorghum plants. Science Total Environ. **370:**391-400.

Egamberdiyeva, D. 2007. The effect of nutrient uptake of maize in two different soils. Appl. Soil Ecology. **36:**184-189.

Egamberdiyeva, D., and G. Höflich. 2002. Root colonization and growth promotion of winter wheat and pea by *Cellulomonas spp.* at different temperatures. Plant Growth Reg. **38:**219-224.

El-Komy, H.M.A. 2005. Coimmobilization of *A. lipoferum* and *B. megaterium* for plant nutrition. Food Technol. Biotech. **43:**19–27.

Ferris, M.J., G. Muyzer, and D.M. Ward. 1996. Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring mat community. Appl. Environ. Microbiol. **62:**340–346.

Frey-Klett, P., J. C. Pierrat, and J. Garbaye. 1997. Location and survival ofmycorrhiza helper *Pseudomonas fluorescens* during establishment of ectomycorrhizal symbiosis between *Laccaria bicolor* and Douglas Fir. Appl. Environ. Microbiol. **63:**139-144.

Fridlender, M., J. Inbar, and I. Chet. 1993. Biological control of soilborne plant pathogens by a β – 1,3 glucanase-producing Pseudomonas cepacia. Soil Biol. Biochem. **25:**1211-1221.

Garbaye J.1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytologist 128, 197-210

Garcia de Salamone, I. E., R. K. Hynes, and L. M. Nelson. 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can. J. Microbiol. **47:**404-411.

Gratão P. L.; Prasad M. N. V.; Cardoso P. F.; Lea P. J.; Azevedo R. A. 2005. Phytoremediation: green technology for the clean up of toxic metals in the environment. Braz. J. Plant Physiol. 17: 53-64.

Gupta, A., V. Rai, N. Bagdwal, R. Goel. 2005. In situ characterization of mercury-resistant growth-promoting fluorescent pseudomonads. Microbiol. Res. **160:**385-388.

Farwell, A.J., S. Vesely, V. Nero, H. Rodriguez, K. McCormack, S. Shah, D.G. Dixon, B.R. Glick. 2007. Tolerance of transgenic canola plants (*Brasica napus*) amended with plant growth-promoting bacteria to flooding stress at a metal-contaminated site. Environ. Poll. **147:**540-545.

Favero-Molina, C., C.M. Creus, M. Simontacchi, S. Puntarulo, and L. Lamattina. 2008. Aerobic nitric oxide production by *Azospirillum brasilense* Sp245 and its influence on root architecture in tomato. Mol. Plant-Microbe Interactions. **21:**1001-1009.

Felker P., D. Medina, C. Soulier, G. Velicce, M. Velarde, and C. Gonzalez. 2005. A survey of environmental and biological factors (*Azospirillum* spp*, Agrobacterium rhizogenes, Pseudomonas aurantiaca*) for their influence in rooting cuttings of *Prosopis alba* clones. J Arid Environ. **61:**227-247.

Flores-Mireles, A.L., S.C. Winans, and G. Holguin. 2007. Molecular Characterization of Diazotrophic and Denitrifying Bacteria Associated with Mangrove Roots. Applied and Environmental Microbiology 73: 7308–7321.

Frey-Klett, P., J.C. Pierrat, and J. Garbaye. 1997. Location and survival of mycorrhiza helper *Pseudomonas fluorescens* during establishment of ectomycorrhizal symbiosis between *Laccaria biocolor* and douglas fir. Appl. Environ. Microbiol. **63:**139-144.

Frommel. M.I., J. Nowak, and G. Lazarovitis. 1991. Growth enhancement and developmental modifications of in vitro grown potato *Solanum tuberosum* ssp. *tuberosum*. Plant Physiol. **96:** 928–936.

Garbaye, J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol. **128:**197-210.

Garcia de Salamone, I.E., R.K. Hynes, and L.M. Nelson. 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can. J. Microbiol. **47:**404-411.

Glick, B.R. 2003. Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol. Adv. **21:** 383-393.

Glick, B.R. 2004. Teamwork in phytoremediation. Nature Biotechnol. **22:** 526-527.

Glick, B.R., and Bashan, Y. 1997. Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnological Advances 15: 353-378.

Glick, B.R., D.M. Penrose, and J. Li. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J. Theo. Biol. **190:**63-68.

Goldstein, A. H. 1987. Bacterial solubilization of mineral phosphates: historical perspective and future prospects. Am. J. Altern. Agric. **1:**51-57.

Gonzalez-Acosta, B., Y. Bashan, N.Y. Hernandez-Saavedra, F. Ascencio, and G. De la Cruz-Agüero. 2006. Seasonal seawater temperature as the major determinant for populations of culturable bacteria in the sediments of an intact mangrove in an arid region. FEMS Microbiol. Ecol. **55:**311-321.

Gonzalez L.E., and Y. Bashan. 2000. Increased growth of the microalgae *Chlorella vulgaris* when coimmobilized and cocultured in alginate beads with the plant growth-promoting bacterium *Azospirillum brasilense.* Appl. Environ. Microbiol. **66:**1527-1531.

Grandlic, C.J., M.O. Mendez, J. Chorover, B. Machado, and R.M. Maier. 2008. Plant growth-promoting bacteria for phytostabilization of mine tailings. Environ. Sci. Tech. *In Press.*

Grichko, V.P., and B.R. Glick. 2001. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol. Biochem. **39:**11-17.

Halsall, D. M., and A. H. Gibson. 1989. Nitrogenase activity of a range of diazotriphic bacteria on straw, straw breakdown products and related compounds. Soil. Biol. Biochem. **21:** 291-298.

Hargrove, T. 2008. World fertilizer prices soar as food and fuel economies merge. International Center for Soil Fertility and Agricultural Development. http://ifdc.org.

Hernandez, J.-P., L.E. de-Bashan, and Y. Bashan. 2006. Starvation enhances phosphorous removal from wastewater by the microalga *Chlorella* spp. co-immobilized with *A. brasilense*. Enzyme Microbial Tech. **38:**190-198.

Herrera, M. A., C. P. Salamanca, and J. M. Barea. 1993. Inoculation of woody legumes with selected arbuscular mycorrhizal fungi and rhizobia to recover desertified Mediterranean ecosystems. Applied and Environmental Microbiology 59:129-133.

Hetrick, B. A. D., G. W. T. Wilson, and D. A. H. Figge. 1994. The influence of mycorrhizal symbiosis and fertilizer amendments on establishment of vegetation in heavy metal mine spoil. Enviorn. Poll. **86:**171-179.

Hoflich, G. R. and Metz. 1997. Interactions of plant-microorganism associations in heavy metal containing soils from sewage farms. Bodenkultur. **48:**239–247.

Holguin, G. and Bashan, Y. 1996. Nitrogen-fixation by *Azospirillum brasilense* Cd is promoted when co-cultured with a mangrove rhizosphere bacterium *(Staphylococcus* sp.) **Soil Biology and Biochemistry 28**:1651-1660.

Holguin, G., and Glick, B.R. 2003. Transformation of *Azospirillum brasilense* Cd with an ACC deaminase genes from *Enterobacter cloacae* UW4 fused to the Tet^R gene promoter improves its fitness and plant growth promoting ability. Microbial Ecol. **46:**122-133.

Holguin, G., P. Gonzalez-Zamorano, L.E. de-Bashan, R. Mendoza, E. Amador, and Y. Bashan. 2006. Mangrove health in an arid environment encroached by urban development – a case study. Sci. Total Environ. **363:**260-274.

Holguin, G., Guzman, M.A. and Bashan, Y. 1992. Two new nitrogen-fixing bacteria from the rhizosphere of mangrove trees, isolation, identification and in vitro interaction with rhizosphere *Staphylococcus* sp. **FEMS Microbiology Ecology 101**: 207-216.

Holguin, G. Vazquez, P., and Bashan, Y. 2001. The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. Biology and Fertility of Soils **33**: 265-278.

Hontzeas, N., S.S. Saleh, and B. Glick. 2004. Changes in gene expression in canola roots induced by ACC-deaminse-containing plant growth-promoting bacteria. Mol. Plant-Microbe Interactions. **17:**865-871.

Huang, X.-D., Y. El-Alawi, D.M. Penrose, B.R. Glick, and B.M. Greenberg. 2004. A multi-process system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ. Poll. **130:**465-476.

Idris E.E., D.J. Iglesias, M. Talon, and R. Borris. 2007. Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. Mol. Plant-Microbe Interactions. **20:**619-626.

Ikner, L.A., R.S. Toomey, G. Nolan, J.W. Neilson, B.M. Pryor, and R.M. Maier. 2007. Cultural microbial diversity and the impact of tourism in Kartchner Caverns, Arizona. Microbial Ecol. **53:**30-42.

Illmer, P., and F. Schinner. 1995. Solubilization of inorganic calcium phosphates – solubilization mechanisms. Soil Boil. Biochem. **27:**257-263.

Illmer, P., A. Barbato, and F. Schinner. 1995. Solubilization of hardly soluble AlPO4 with P-solubilizing microorganisms. Soil. Biol. Biochem. **27:**265-270.

Jackson, M.L. 1985. *Soil chemical analysis*. Parallel Press, Madison, Wisconsin.

Jankong, P., P. Visoottiviseth, S. Khokiattiwong. 2007. Enhanced phytoremediation of arsenic contaminated land. Chemosphere. **68:**1906-1912.

Jayachandran, K., A. P. Schwab, and B. A. D. Hetrick. 1989. Mycorrhizal mediation of phosphorous availability: synthetic iron chelate effects on phosphorous availability. Soil Soc. Amer. J. **53:**1701-1706.

Jiang, L.Y., X.E. Yang, Z.L. He. 2004. Growth response and phytoextraction of copper at different levels in soils by *Elsholtzia splendens*. Chemosphere. **55:**1179-1187.

Jjemba, P.K., and M. Alexander. 1999. Possible determinants of rhizosphere competence of bacteria. Soil Biol. Biochem. **31:**623-632.

Johansen, J. E., and S. J. Binnerup. 2002. Contribution of Cytophage-like bacteria to the potential of turnover of carbon, nitrogen, and phosphorous by bacteria in the rhizosphere of barley (Hordeum vulgare L.). Microb. Ecol. **43:**289-306.

Jordan, F.L., S.K. Sandrin, R.J. Frye, M.L. Brusseau, and R.M. Maier. 2004. The influence of system complexity on bacterial transport in porous media. J. Contam. Hydrol. **74:**19-38.

Kathiresan, K., and M. Masilamani Selvam. 2006. Evaluation of beneficial bacteria from mangrove soil. Botanica Marina. **49:**86-88.

Kennedy, I. R., L. L. Pereg-Gerk, C. Wood., R. Deaker, K. Gilchrist, and S. Katupitiya. 1997. Biological nitrogen fixation in non-leguminous field crops: facilitating the evolution of an effective association between *Azospirillum* and wheat. Plant and Soil. **194:**65-79.

Khan, A.G. 2005. Role of soil microbes in the rhizospheres of plants growing on trace metals contaminated soils in phytoremediation. J Trace Elem. Med. Biol. **18:**355-364.

Kim, K.Y., D. Jordan, and H.B. Krishnan. 1997. *Rahnella aquatillis*, a bacterium isolated from soybean rhizosphere, can solubilize hydroxyapatite. FEMS Microbiol. Lett. **153:**273-277.

King, A.K., and M. Martinez. 1998. *Metals* in Fish Collected from Aravaipa Creek; U.S. Fish and Wildlife Service; Arizona Ecological Services Field Office: Phoenix, AZ.

Kloepper, J.W., J. Leong, M. Teintze, M.N. Schroth. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature. **286:**885-886.

Kloepper, J.W., R. Lifshitz, and R. Zablotowicz. 1987. Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol. **7:**39-44.

Kumar, P.B.A., V. Dushenkov, H. Motto, and I. Raskin. 1995. Phytoextraction: the use of plants to remove heavy metals from soils. Environ. Sci. Tech. **29:**1232-1238.

Kuklinsky-Sobral, J., W. L. Araujo, R. Mendes, I. O. Geraldi, A. A. Pizzirani-Kleiner, and J. L. Azevedo. 2004. Isolation and characterization of soy bean-associated bacteria and their potential for plant growth promotion. **6:**1244-1251.

Lande, J., S.M. Webb and A. Mehta. Area diffraction machine. Http://groups.google.com.ezproxy2.library.arizona.edu/group/area-Diffraction-Machine.

Lebeau, T., A. Braud, and K. Jezequel. 2007. Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. Environ. Poll. dio:10.1016/j.envpol.2007.09.015. better put the pages as it was published last year

Lebsky, V.K., L.E. Gonzalez-Bashan, and Y. Bashan. 2001. Ultrastructure of interaction in alginate beads between the microalga *Chlorella vulgaris* with its natural associative bacterium *Phyllobacterium myrsinacearum* and with the plant growth-promoting bacterium *Azospirillum brasilense.* Can. J. Microbiol. **47:**1-8.

Leyva L.A. and Bashan, Y. 2008. Activity of two catabolic enzymes of the phosphogluconate pathway in mesquite roots inoculated with *Azospirillum brasilense* Cd **Plant Physiology and Biochemistry** (In press)

Lodewyckx, C., S. Taghavi, M. Mergeay, J. Vangronsveld, H. Clijsters, and D. Van Der Lelie. 2001. The effect of recombinant heavy metal-resistant endophytic bacteria on heavy metal uptake by their host plant. Int. J. Phytoremediat. **3:** 173-187.

Lucas Garcia, J., J. Domenech, C. Santamaria, M. Camacho, A. Daza, F.J. Gutierrez Manero. 2004. Growth of forest plants (pine and holm-oak) inoculated with rhizobacteria: relationship with microbial community structure and biological activity of its rhizosphere. Environ. Experimental Botany. **52:**239-251.

Lucy, M. Reed E.and Glick B. R. 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie van Leeuwenhoek J. G. **86:** 1–25.

Macková, M., B. Vrchotová, K. Francová, M. Sylvestre, M. Tomaniová, P. Lovecká, K. Demnerová, and T. Macek. 2007. Biotransformation of PCBs by plants and bacteria – consequences of plant-microbe interactions. European J. Soil Biology. **43:**233-341

Marschner, P., C.H. Yang, R. Lieberei, and D.E. Crowley. 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol. Biochem. **33:**1437–1445.

Mayak, S., T. Tirosh, and B.R. Glick. 2004. Plant growth-promoting bacteria that confer resistance in tomato to salt stress. Plant Physiol. Biochem. 42:565-572.

McCutcheon, S.C. and Schnoor, J.L. 2003. Phytoremediation: Transformation and control of contaminants. Wiley-Interscience, 1024 p.

Mendez, M.O., E.P. Glenn, and R.M. Maier. 2007. Phytostabilization potential of quailbush for mine tailings: growth, metal accumulation, and microbial community changes. J. Environ. Qual. **36:**245-253.

Mendez MO and Maier RM. 2008a. Phytoremediation of mine tailings in temperate and arid environments. Rev. Environ. Sci. Biotechnol. 7:47-59.

Mendez, M.O., and R.M. Maier. 2008b. Phytostabilization of mine tailings in arid and semiarid environments – an emerging remediation technology. Environ. Health Perspect. **116:**278-283.

Miché, L., and J. Balandreau. 2001. Effects of rice seed surface sterilization with hypochlorite on inoculated *Burkholderia vietnamiensis*. Appl. Environ. Microbiol. **67:**3046-3052.

Moynahan O.S., C.A. Zabinski, and J.E. Gannon. 2002. Microbial community structure and carbon utilization diversity in a mine tailings revegetation study. Restor. Ecol. **10:**77-87.

Munshower, F.F. 1994. Practical handbook of disturbed land revegetation. Boca Ratton, FL. Lewis Publishing.

Nagendran, R., A. Selvam, K. Joseph, and C. Chiemchaisri. 2006. Phytoremediation and rehabilitation of municipal solid waste landfills and dumpsites: a brief review. Waste Management. **26:**1357-1369.

Navarro, M.C., C. Pérez-Sirvent, M.J. Martínez-Sánchez, J. Vidal, and J. Marimón. 2006. Lead, cadmium and arsenic bioavailability in the abandoned mine site of Cabezo Rajao (Murcia, SE Spain). Chemosphere. **63:**484-489.

Navarro, M.C., C. Pérez-Sirvent, M.J. Martínez-Sánchez, J. Vidal, P.J. Tovar, and J. Bech. 2008. Abandoned mine sites as a source of contamination by heavy metals: a case study in a semi-arid zone. Geochemical Exploration. **96:**183-193.

Nie, L., S. Shah, A. Rashid, G.I. Burd, D.G. Dixon, and B. Glick. 2002. Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth-promoting bacterium *Enterobacter cloacae* CAL2. Plant Physiol. Biochem. **40:** 355-361.

Oliveira, A.L.M., E.L. Canuto, E.E. Silva, V.M. Reis, and J.I. Baldani. 2004. Survival of endophytic diazotrophic bacteria in soil under different moisture levels. Brazilian J. Microbiol. **35:**295-299.

Patten, C.L., and B.R. Glick. 2002. Role of *Psuedomonas putida* indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. **68:**3795-3801.

Penrose, D.M., and B.R. Glick. 2001. Levels of ACC and related compounds in exudate and extracts of canola seeds treated with ACC deaminase-containing plant growth-promoting bacteria. Can. J. Microbiol. **47:**368-372.

Petrisor, I., S. Dobrota, K. Komnitsas, I. Lazar, J.M. Kuperberg, and M. Serban. 2004. Artificial inoculation – perspectives in tailings phytostabilization. Int. J. Phytoremediation. **6:**1-15.

Pilon-Smits E. 2005. Phytoremediation. Annu. Rev. Plant Biol. 56:15-39**.**

Pishchik, V.N., N.I. Vorobeyv, I.I. Chernyaeva, S.V. Timofeeva, A.P. Kozhemyakov, Y.V. Alexeev, S.M. Lukin. 2002. Experimental and mathematical simulation of plant growth promoting rhizobacteria and plant interactions under cadmium stress. Plant and soil. **243:**173-186.

Prasad MNV and Freitas H. 2003. Metal hyperaccumulation in plants-biodiversity prospecting for phytoremediation technology. Electronic Journal of Biotechnology 6:275-321.

Press, C.M., J.E. Loper, and J.W. Kloepper. 2001. Role of iron in rhizobacteria-mediated induced systemic resistance of cucumber. Phytopathology. **6:**593-598.

Primavera J.H., R.S. Sadaba, M.J.H.L. Lebata, and J.P. Altamirano. 2004. Handbook of mangroves in the Philippines-Panay. Iloilo, Philippines. Southeast Asian Fisheries Development Center Aquaculture Department; 106 pp.

Puente, M.-E., C.Y. Li, and Y. Bashan. 2004a. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings. Plant Biol. **6:**643-650.

Puente, M.-E., and Y. Bashan. 1993. Effect of inoculation with *A. brasilense* strains on the germination and seedlings growth of the giant columnar Cardon cactus (*Pachycereus pringlei*). Symbiosis. **15:**49-60.

Puente, M.-E., Y. Bashan, C.Y. Li, and V.K. Lebsky. 2004b. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. root colonization and weathering of igneous rocks. Plant Biol. **6:**629-642.

Puente, M.-E., G. Holguin, B.R. Glick, and Y. Bashan. 1999. Root surface colonization of black mangrove seedlings by *Azospirillum halopraeferens* and *Azospirillum brasilense* in seawater. FEMS Microb. Ecol. **29:**283-292.

Radwan, S.S., N. Dashti, and I.M. El-Nemr. 2005. Enhancing the growth of *Vivia faba* plants by microbial inoculation to improve their potential for oily desert areas. Int. J. Phytoremediation. **7:**19-32.

Rai, U.N., K. Pandey, S. Sinha, A. Singh, R. Saxena, and D.K. Gupta. 2004. Revegetating fly ash landfills with *Prosopis juliflora* L.: impact of different amendments and *Rhizobium* inoculation. Environment International. **30:**293-300.

Rajkumar, M., and H. Freitas. 2007. Effects of inoculation of plant growth-promoting bacteria on Ni uptake by Indian mustard. Bioresour. Technol. doi:10.1016/j.biortech.2007.07.046.

Rajkumar, M., and H. Freitas. 2008. Influence of metal-resistant plant growth-promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. Chemosphere. **71:**834-842.

Rao, A.V., and R. Tak. 2001. Effect of rhizobial inoculation on *Albizia lebbeck* and its rhizosphere activity in mine spoils. Arid Land Res. Management. **15:**157-162.

Raupach, G.S., and J.W. Kloepper. 1998. Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology. **88:**1158-1164.

Reed, M.L.E., B.G. Warner, and B.R. Glick. 2005. Plant growth-promoting bacteria facilitate the growth of the common reed *Phargmites australis* in the presence of copper or polycyclic aromatic hydrocarbons. Current Microbiol. **51:**425-429.

Reed, M.L.E., and B.R. Glick. 2005. Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can. J. Microbiol. **51:**1061-1069.

Reichman, S.M. 2007. The potential use of legume-rhizobium symbiosis for the remediation of arsenic contaminated sites. Soil Biol. Biochem. **39:**2587-2593.

Requena, N., E. Perez-Solis, C. Azcon-Aguilar, P. Jefferies, and J.-M. Barea. 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Appl. Environ. Microbiol. **67:**495-498.

Requena, N., I. Jimenez, M. Toro, and J.M. Barea. 1997. Interactions between plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and *Rhizobium* spp. in the rhizosphere of *Anthyllis cytisoides*, a model legume for revegetation in Mediterranean semi-arid ecosystems. New Phytol. **136:**667-677.

Richardson, A. E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorous by plants. Aust. J. Plant Physiol. **28:**897-906.

Rodriguez, H., and R. Fraga. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. **17:**319-339.

Rodriguez, H., Fraga, R., Gonzalez, T., and Bashan, Y. 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant and Soil 287: 15-21

Rojas, A., G. Holguin, B.R. Glick, and Y. Bashan. 2001. Synergism between *Phyllobacterium* sp. (N-fixer) and *Bacillus licheniformis* (P-solubilizer), both from a semiarid mangrove rhizosphere. FEMS Microb. Ecol. **35:**181-187.

Rosario, K., S.L. Iverson, D.A. Henderson, S. Chartrand, C. McKeon, E.P. Glenn, and R.M. Maier. 2007. Bacterial community changes during plant establishment at the San Pedro River mine tailings site. J. Environ. Qual. **36:**1249-1259.

Rönnbäck P. 1999. The ecological basis for economic value of seafood production supported by mangrove ecosystems. Ecol Econ. 29:235–252.

Ryu, C.-M., M.A. Farag, C.-H. Hu, M.S. Reddy, H.-X. Wei, P.W. Pare, and J.W. Kloepper. 2003. Bacterial volatiles promote growth in Arabidopsis. Proc. Natl. Acad. Sci. USA. **100:**4927-4932.

Saravanan, V.S., M. Madhaiyan, and M. Thangaraju. 2007. Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. Chemosphere. **66:**1794-1798.

Schwyn, B., and J.B. Neilands. 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. **160:**47-56.

Sessitsch, A., B. Reiter, and G. Berg. 2004. Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. Can. J. Microbiol. **50:**239-249.

Sevilla, M., R.H. Burris, N. Gunapala, and C. Kennedy. 2001. Comparison of benefit to sugarcane plant growth and 15N2 incorporation following inoculation of sterile plant with *Acetobacter diazotrophicus* wild-type and Nif- mutant strains. Mol. Plant-Microbe Interactions. **14:**358-366.

Sheng, X.-F., and J.-J. Xia. 2006. Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. Chemosphere. **64:**1036-1042.

Shetty, K. G., B. A. D. Hetrick, D. A. H. Figge, and A. P. Schwab. 1994. Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. **86:**181-188.

Shishido M., Petersen D. J., Massicotte H. B., Chanway, C. P. 1996. Pine and spruce seedling growth and mycorrhizal infection after inoculation with plant growth promoting Pseudomonas strains. FEMS Microbiology Ecology 21 , 109–119

Simons, M., A. J. van der Bij, L. A. de Weger, C. A. Wijffelman, and B. J. Lugtenberg. 1996. Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. Mol. Plant-Microbe Interact. **9:**600–607.

Simons, M., H. P. Permentier, L. A. de Weger, C. A. Wijffelman, and B. J. J. Lugtenberg. 1997. Amino acid synthesis is necessary for tomato root colonization by *Pseudomonas fluorescens* strain WCS365. Mol. Plant-Microbe Interact. **10:**102–106.

Singh, U.P. B.K. Sarma, D.P. Singh, and A. Bahadur. 2002. Plant growth-promoting rhizobacteria mediated induction of phenolics in pea (*Pisum sativum*). Curr. Micorbiol. **44:**396-400. .

Spaepen, S., Vanderleyden, J. & Remans, R. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol. Rev. 31, 425-448.

Strangmann, A., Bashan, Y. and Giani, L. 2008. Methane in pristine and impaired mangrove soils and its possible effect on establishment of mangrove seedlings. Biology and Fertility of Soils 44: 511–519

Sturz, A.V., B.G. Matheson, W. Arsenault, J. Kimpinski, and B.R. Christie. 2001. Weeds as a source of plant growth promoting rhizobacteria in agricultural soils. Can. J. Microbiol. **47:**1013-1024.

Tam, N.F.Y., and Y.S. Wong. 2000. Effect of immobilized microalgal bead concentration on wastewater nutrient removal. Environ. Pollut. **107:**145-151.

Thakuria, D., N.C. Talukdar, C. Goswami, S. Hazarika, R.C. Boro, and M.R. Khan. 2004. Characterization and screening of bacteria from the rhizosphere of rice grown in acidic soils of Assam. Current Science. **86:**978-985.

Toledo, G., Y. Bashan, A. Soeldner. 1995. In vitro colonization and increase in nitrogen fixation of seedling roots of black mangrove inoculated by a filamentous cyanobacteria. Can. J. Microbiol. **41:**1012-1020.

Toledo, G., Rojas, A., and Bashan, Y. 2001. Monitoring of black mangrove restoration with nursery-reared seedlings on an arid coastal lagoon. **Hydrobiologia 444**: 101-109

Tordoff, G.M., A.J.M. Baker, A.J. Willis. 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere. **41:**219-228.

Tummala, P.S.L., and W. Humble. 1998. Public Health Assessment; Klondyke Mine Tailings; Arizona Department of Health Services.

Ulrich, B. 1983. *In* A. Cammerer (ed.), Pollution Stresses on Forest Ecosystems, Transl. From LOLF-Mitteilun- gen 1982 Sonderheft. Translation - Environment Canada.

US Environmental Protection Agency. 2004. Test Methods for Evaluating Solid Waste; EPA SW-846: Washington, DC.

Velázquez-Rodríguez AS, Flores-Román D and Acevedo-Sandoval OA. 2001. Aggregate formation in tepetate by effect of plant species. Agrociencia 35: 311-320.

Valdenegro, M., J.M. Barea, and R. Azcón. 2001. Influence of arbuscular-mycorrhizal fungi, *Rhizobium meliloti* strains and PGPR inoculation on the growth of *Medicago arborea* used as model legume for re-vegetation and biological reactivation in a semi-arid Mediterranean area. Plant Growth Reg. **34:**233-24

Valderrama, L.T., C.M. Del Campo, C.M. Rodriguez, L.E. de-Bashan, and Y. Bashan. 2002. Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga *Chlorella vulgaris* and the macrophyte *Lenna minuscula.* Water Res. **36:**4185-4192.

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature. **396:**69-72.

Van Loon LC and Glick BR 2004. Increased plant fitness by rhizobacteria. In; Molecular ecotoxicology of plants. Ecological Studies, Vol. 170. (Ed.) H. Sandermann. Springer-Verlag, Berlin, pp. 177-205.

Vazquez, P., G. Holguin, M.E. Puente, A. Lopez-Cortez, and Y. Bashan. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol. Fertil. Soils. **30:**460-468.

Venables, W.N., and B.D. Ripley. 2002. Modern applied statistics with S. 4th ed. p. 385–389. Springer Statistics and Computing Series, New York.

Vessey, J.K. 2003. Plant growth-promoting rhizobacteria as biofertilizers. Plant Soil. **255:**571-586.

Vivas, A., B. Biro, J.M. Ruiz-Lozano, J.M. Barea, and R. Azcon. 2006. Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zu-toxicity. Chemosphere. **62:**1523-1533.

Wenbo, M., S. B. Sebestinaova, J. Sebestian, G. I. Burd, E. C. Guinel, and B. Glick. 2003. Prevalence of 1-aminocyclopropane-1-carboxylate deaminase in *Rhizobium* spp. Leeuwenhoek. **83:**285-291.

Whiting, S.N., M.P. De Souza. and N. Terry. 2001. Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*. Environ. Sci. Technol. **35:**3144–3150.

Wilson, D.O. 1976. Evaluation of chemical seed coat sterilants. Plant Soil. **44:**703-707.

Wright, S. F., and A. Upadhyaya. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil. **198:**97-107.

Wu, S.C., K.C. Cheung, Y.M. Luo, and H.M. Wong. 2006a. Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by *Brassica juncea*. Environmental Pollution. **140:** 124-135.

Wu, S.C., Y.M. Luo, K.C. Cheung, and M.H. Wong. 2006b. Influence of bacteria on Pb and Zn speciation, mobility and bioavailability in soil: a laboratory study. Environ. Poll. **144:**765-773.

Yabur, R., Y. Bashan, G. Hernandez-Carmona. 2007. Alginate from the macroalgae *Sargassum sinicola* as a novel source for microbial immobilization material in wastewater treatment and plant growth promotion. J. Appl. Phycol. **19:**43-53.

Yang, X.E., X.X. Long, W.Z. Ni, Z.L. He, P.J. Stoffella, D.V. Calvert. 2002. Assessing copper thresholds for phytotoxicity and potential toxicity in selected crops. J. Environ. Sci. Health B **37:**625–635.

Zaady, E., Y. Okon, and A. Perevolotsky. 1994. Growth response of Mediterranean herbaceous swards to inoculation with *Azospirillum brasilense*. Journal of Range Management. **47:**12-15.

Zhuang, X., J. Chen, H. Shim, and Z. Bai. 2007. New advances in plant growth-promoting rhizobacteria for bioremediation. Environ. Inter. **33:**406-413.