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4 Environmental uses of plant growth-promoting bacteria

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Abstract

Plant growth-promoting bacteria (PGPB) are commonly used to improve crop yields. In addition to their agricultural usefulness, there are potential benefits in environmental applications. For example, species of Azospirillum can increase bioremediation of wastewater by microalgae by increasing algal proliferation and metabolism. Additionally, these genera and several other bacterial species may prevent soil erosion in arid zones by improving growth of desert plants, which in turn leads to reduced dust pollution. Other PGPB promote plants that extract hazardous materials from soil.

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1. Introduction

Plant growth-promoting bacteria [PGPB; 4] were defined as free-living soil, rhizosphere, rhizoplane, and phylosphere bacteria that, under some conditions, are beneficial for plants [3]. Most of the activities of PGPB have been studied in the rhizosphere, and to lesser extent, on leaf surfaces. Endophytic PGPB have recently emerged as an important area of PGPB study.

PGPB promote plant growth in two ways: (i) they directly affect the metabolism of the plants by providing substances that are usually in short supply. These bacteria are capable of fixing atmospheric nitrogen, solubilize phosphorus and iron, and enhance production of plant hormones. Additionally, they improve the plant tolerance to stresses, such as drought, high salinity, metal toxicity, and pesticide load. One or more of these capabilities mechanisms may contribute to the increased plant growth and development, greater than plants grown under standard cultivation conditions. (ii) A group of PGPB, referred to as biocontrol-PGPB [4], indirectly promote plant growth by preventing deleterious effects of phytopathogenic bacteria, fungi, nematodes and viruses.

Many soil and especially rhizosphere bacteria can stimulate growth of crops, in the absence of a major pathogen, by directly affecting plant metabolism. These bacteria belong to diverse genera, including *Acetobacter, Achromobacter, Anabaena, Arthrobacter, Azoarcos, Azospirillum, Azotobacter, Bacillus, Burkholderia, Clostridium, Enterobacter, Flavobacterium, Frankia, Hydrogenophaga, Kluyvera, Microcoleus, Phyllobacterium, Pseudomonas, Serratia, Staphylococcus, Streptomyces,* and *Vibrio*, as well as the well-known legume symbiont *Rhizobium.*

In addition to their value as crop inoculants, the potential benefits of PGPB have been used in recent years in environmental applications. The limited studies in this emerging field are the focal point of this chapter. For example, *Azospirillum* species enhances bioremediation of wastewater treated with microalgae by increasing proliferation and metabolism of the microalgae; hence increasing the effectiveness of the microalgae to clean wastewater better than when used without PGPB [22, 35].

Inoculation with *Azospirillum* enhances health and survival of cactus seedlings planted in degraded desert areas [7]. Rhizosphere bacteria of diverse genera (mainly Gram-positive species), isolated from extremely dry areas (plants growing in rocks without the benefit of soil) were found to enhance the growth of cacti in the rock [58]. Re-vegetation of eroded and degraded desert areas, aided by PGPB and vesicular-arbuscular mycorrhizal (AM) fungi invigorated desert plants responsible for soil stabilization, prevented soil erosion, and promoted abatement of dust [7, 39]. Finally, plants inoculated with PGPB have reduced contamination from heavy metals and can be used to rehabilitate contaminated wastelands [28].

2. Re-vegetation and re-forestation of eroded desert lands by PGPB

When deserts are cleared to produce agricultural land and later abandoned (a process often called "desertification"), or to build urban neighborhoods lacking paved roads, nothing remains to hold the topsoil against wind-caused erosion. The result is severe soil erosion and subsequent dust pollution. Dust pollution, only indirectly but significantly, increases chronic respiratory illnesses [64, 70]. This phenomenon has been increasing throughout the developing world [25, 54, 60]. In North America, it is common in the semi-arid areas of northern Mexico [33, 65]. Prevention of soil erosion and dust pollution in agricultural and urban, low income, desert communities in northwest Mexico is difficult for various socio-economical reasons [7]. For decades, it has been mistakenly assumed that these deforested and abandoned agricultural lands would revert to their original vegetation on their own [72] as usually occurs in temperate areas where forests return in the absence of domestic grazing. Abandoned fields in northwestern Mexico, under the pressure of grazing, quickly become barren landscapes, with few, if any, annual plants [48]. These areas do not reforest naturally with endemic cacti because nurse trees (mesquite), that create a nutrient-rich soil environment below their canopy, are essential for sustained growth of cactus seedlings [13, 15] have been removed and the topsoil removed by wind erosion. As a consequence, massive clouds of dust occur during wind storms.

Desert plants, especially cacti, are excellent topsoil stabilizers [27], having the potential to prevent soil erosion and reduce dust in abandoned agricultural and urban areas, if revegetation programs are correctly implemented to allow their growth in degraded areas. Local municipalities have tried this, but with meager success because low rates of establishment and slow growth of cacti occur with the transplant methods used to transfer cacti from natural habitats or nurseries to eroded soil. Cacti may, however, like crops, benefit from inoculation with beneficial microbial populations at planting. Since young cacti grow slowly [63] and are subject to competition by aggressive desert trees and shrubs, especially while growing as seedlings under mesquite, PGPB and AM fungi, as soil amendments, may be a key to accelerate their development as part of restoration programs that will by-pass the natural process of succession that may take many decades to accomplish. Of particular interest among these microorganisms are mycorrhizal fungi [5, 15, 18] and diazotrophic bacteria like *Azospirillum brasilense* because they can improve plant nutrient status, reduce environmental stress, and stimulate growth [6]. Giant cardon (*Pachycereus pringlei*), for example, is the dominant tree-like cactus responsible for significant soil stabilization in the deserts of the Baja

California Peninsula of Mexico, and positively responds to artificial inoculation with one species of PGPB (*A. brasilense*). Seed germination, survival, and growth rates are also improved [55]. In other field and laboratory trials, additional responses have been found. In a similar plant-bacteria combination, inoculation of cardon seeds with *A. brasilense* did not affect survival, but resulted in significantly better root and shoot growth, and this effect increased linearly as soil nutrients declined. In a “resource island” soil, *A. brasilense* had no effect on cardon growth, but in the poorest soil (soil incapable of supporting perennial growth), dry mass of the cactus shoot was almost 60% greater and root length over 100% greater if the samples had been inoculated [14].

The feasibility of using bacterial inoculation of cacti to enhance establishment in disturbed areas and stabilize soil was demonstrated with cactus transplants in disturbed urban areas. After transplanting, young plants of three cactus species (*Pachycereus pringlei, Stenocereus thurberi,* and *Lophocereus schottii*), inoculated with *A. brasilense*, had high survival and developed more rapidly than control plants for a test lasting 3.5 years. Soil erosion in the field with inoculated cacti decreased and small, but significant wind blown dust accumulated around cactus roots [7]. One mechanism that anchors dust around inoculated plants is the enhanced upward growth of small “rain roots” (roots produced very rapidly by cacti in response to occasional rain [53]) during the rainy season (Figure 1). Enhanced growth of cacti with *Azospirillum* inoculation was attributed to acidification of the rhizosphere by the inoculated plant from proton extrusion and release of organic acids, followed by uptake of phosphorus [12].

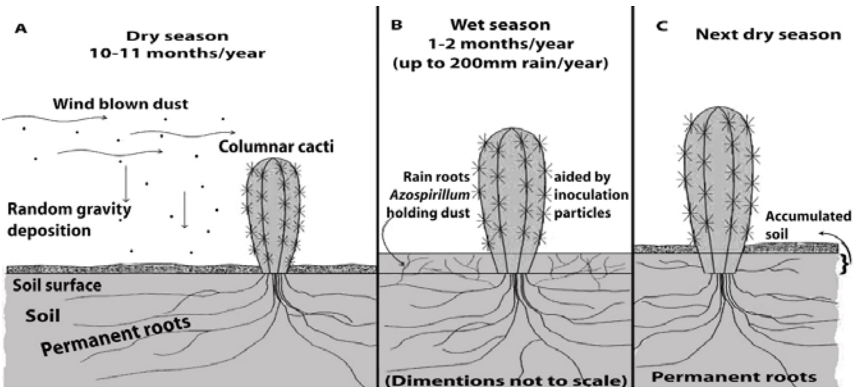

Figure 1. Soil accumulation in cactus vegetation under arid environment (Sonoran desert, Mexico).

Although these *Azospirillum* species can tolerate hot desert temperatures, they are primarily crop enhancers, and require water and an active plant rhizosphere to have a positive effect on growth. Therefore, these bacterial species are not necessarily the best strains for improving growth of native desert plants intended for arid zone reforestation. Native, drought-resistant PGPB strains may possess greater potential for enhancing environmental recovery. A search for these bacteria was undertaken in recent years in Mexico. Conditions where desert reforestation are harsh, such as water deficits even during the rainy season, extreme heat, absence of nitrogen, practically no available minerals, and frequent long droughts, a habitat where desert plants grow in rock, without the benefit of soil, were the environments to search for such bacteria. We assumed consequently that plants growing in such inhospitable environments may harbor microorganisms that assist in supporting growth. Cultivable microorganisms could be used as well for reforestation efforts in arid areas possessing less challenging conditions. While perennial plants that grow directly in rocks are uncommon, several species of cacti and 2-3 species flourish in this extreme environment [8, 9].

In the roots of these rock dwelling plants growing in rocks devoid of soil and responsible for rock weathering in an ancient lava flow in Baja California Sur, Mexico, a multitude of microorganisms were detected, isolated, and studied. Dense multiple layers of bacteria and fungi in the rhizoplane of three cacti (*Pachycereus pringlei, Stenocereus thurberi, Opuntia cholla*) and a wild fig tree (*Ficus palmeri*) were isolated. The dominant bacterial groups colonizing the rhizoplane were fluorescent pseudomonads and many morphotypes of bacilli. Seven of these bacterial species were identified by the 16s rRNA molecular method. Unidentified fungal and actimomycete species were also present in large numbers. During *in vitro* assays, some of the root-colonizing microorganisms fixed N_2, produced volatile and non-volatile organic acids that subsequently reduced the pH of the rock slurry medium in which the bacteria grew, and significantly dissolved insoluble phosphates, extrusive igneous rock, marble, and limestone. The bacteria were able to unbind significant amounts of useful minerals (P, K, Mg, Mn, Fe, Cu, and Zn) from rocks of ancient lava flows. These bacteria were thermo-tolerant, halo-tolerant, and drought-tolerant. The microbial community survived in the rhizoplane of cacti during the 10-month dry season. This study indicated that rhizoplane bacteria on cactus roots growing in rock may be involved in chemical weathering of rocks in hot deserts [57] (Figure 2). Additionally, bacteria that dissolved rock and insoluble phosphates and fix atmospheric nitrogen, when taken in combination, have potential to promote growth of plants in arid regions under a wider variety of arid region conditions. Because few bacterial spores were detected, survival mechanisms of these bacteria are unknown. Since

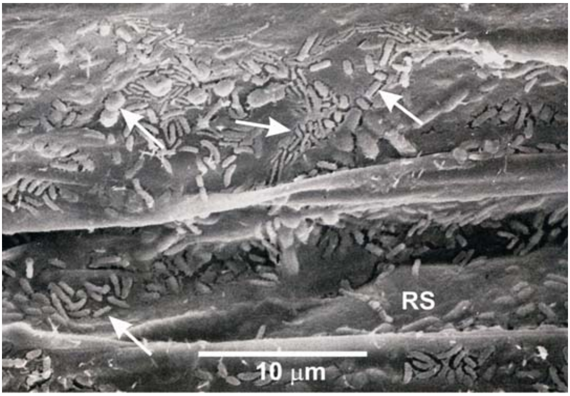

Figure 2. Natural rhizoplane colonization of potential PGPB on cardon roots growing in the absence of soil in rocks. Arrows indicating bacterial layers; RS = root surface.

large populations of live bacteria were detected in the rhizoplane by direct counting and vital staining with fluorescent dyes, we suspect an, as yet, unknown survival mechanisms of sporeless bacteria in arid zones.

Although cacti on the Baja California Peninsula of Mexico are well-adapted to water scarcity and harsh climatic conditions, transplants to urban areas for decoration or to agricultural areas for prevention of soil erosion and dust pollution [7] seldom succeed in the absence of available water, as explained above. The leading theory for explaining this failure is that, apart from some agrotechnical difficulties like transportation to the site and initial irrigation regime onsite, the nursery-reared cacti lacked indigenous microflora, such as mycorrhizal fungi [5, 15] and bacteria that normally assist plant growth under natural conditions and transplanting.

In agriculture, PGPB are well known for their profound impact on vascular plants with which they are associated. For many crops, the bacteria are an integral part of management programs [2, 6]. Yet, desert reforestation aided by native microflora is a young field of research and PGPB has not been used in sponsored reforestation projects. Two familiar examples of absence of knowledge transference are: 1) The diazotrophic, endophytic *Pseudomonas stutzeri*, a PGPB found on the desert epiphyte *Tillandsia recurvata* [56], has never been used to inoculate desert plants; 2) The PGPB *A. brasilense*, widely used in agriculture, improved establishment, growth, and survival of inoculated cardon cactus under controlled greenhouse and field conditions [7, 14, 55] but it is not the optimal PGPB for desert reforestation. The thrust of the hypothesis is that native root-colonizing bacteria from cacti capable of surviving in a harsh environment participate in rock and mineral weathering and supply plants with released inorganic nutrients and fixed nitrogen. Furthermore these particular bacteria have more potential as a PGPB strategy in arid region plants than the PGPB used in agriculture.

Four bacterial species isolated from the rhizoplane of cacti growing naturally in soilless lava rocks were assessed for growth promotion of giant cardon cactus seedlings. These bacterial species fixed N_2, dissolved various chemical forms of phosphorus, weathered extrusive igneous rock, marble, and limestone slurries, and significantly mobilized useful minerals, such as P, K, Mg, Mn, Fe, Cu, and Zn in rock minerals. Cardon cactus seeds inoculated with these bacteria were able to sprout and grow normally without added nutrients in irrigation water for at least 12 months in pulverized extrusive igneous rock (ancient lava flows) mixed with perlite. However, non-inoculated cacti grew less vigorously and some died. The amount of useful minerals (P, K, Fe, Mg) extracted from the pulverized lava, measured after cultivation of the inoculated plants, was significant. This study showed that rhizoplane bacteria isolated from rock-growing cacti promoted growth of cactus and helped to supply essential minerals. These strains may be labeled as rock-weathering PGPB [58].

The accelerated breakdown of rock by plants or associated biological processes, in contrast to chemical and physical breakdown, can be attributed in part to the solubilizing activity of microorganisms that colonize plant roots [34] and the organic acids exuded by roots [47]. Cardon cacti, inoculated with *A. brasilense*, excreted more protons and possibly more organic acids [12]. Others demonstrated that the rhizosphere microflora (endo- or ecto-mycorrhiza and rhizobacteria) of maize, rice, and pine trees promote transformation of minerals [11]. Most rhizoplane bacteria dissolve calcium phosphate, which is easier to dissolve than aluminum and iron phosphates [38]. All three forms of phosphorus are found in ancient lava rock [8]. That this process is universal was demonstrated in a cold extreme climate, where Antarctic lichens weathered andesitic basalt and altered minerals as they grew [1]. It was also assumed in the cacti studies that root-colonizing microorganisms directly participate in rock weathering and perhaps supply plants with released inorganic nutrients and nitrogen from nitrogen-fixation assisting plant development also accelerates soil formation on barren landscapes. A study to evaluate if PGPB can serve as a rock-weathering agent used quantitative image analysis to assess rock weathering by bacteria. Two agriculture-originated PGPB *Pseudomonas putida* R-20 and *A. brasilense* Cd were capable of dissolving, marble, granite, apatite, quartz, limestone, and volcanic rock slurries [59].

Further study revealed an association between cardon cactus and endophytic bacteria, where the seedlings root grow in soilless igneous rocks. These endophytic bacteria moved to the surface of emerging roots and weathered several pulverized rock types and minerals, releasing significant amounts of useful minerals, fixed N_2, produced volatile and non-volatile organic acids, and reduce the size of the rock particles. Large populations of endophytic bacteria was found inside seeds from wild plants, in seeds extracted from guano of bats feeding on cactus fruit and seedlings growing from these seeds, in pulp of fruit, and in small mature wild plants. This presence indicates their ubiquity and probably a symbiotic relationship with this and probably other desert species. The dominant culturable endophytes were several species of bacilli, *Klebsiella* spp. and *Staphylococcus* spp. These strains have molecular similarity with endophytes obtained from seeds and roots and bacteria from the rhizoplane. When inoculated into disinfected cardon seeds, the treated seedlings were capable of growing for one year in pulverized rock without fertilization and without showing stress. This effect was similar to the effect obtained using rhizoplane bacteria from cactus roots for inoculation. These seedlings contain the same endophytic species in their shoots, probably originating from the seed [Puente et al., 2006, unpublished] (Figure 3). Cardon roots contain massive populations of endophytic bacteria that are capable of fixing nitrogen, dissolving essential minerals, and solubilizing insoluble phosphate, traits that characterize these bacterial populations as PGPB of wild plants in arid land that resemble the PGPB phenomenon in agriculture [3, 40].

The knowledge accumulated in several laboratories, growth chambers, and greenhouse experiments led to several field experiments to demonstrate the feasibility of using PGPB and AM fungi for reforestation programs in arid regions. These experiments, conducted as recently as 2004, used native desert

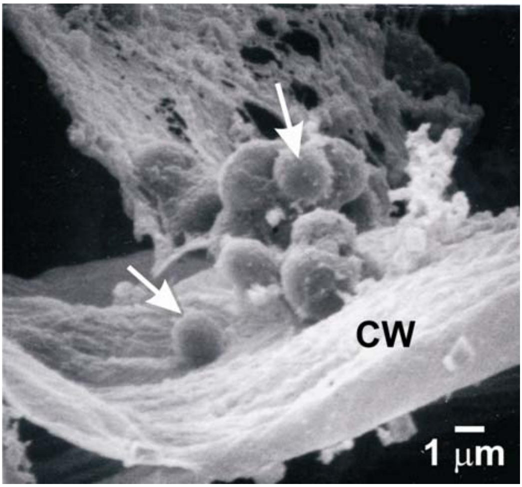

Figure 3. Endophytic PGPB (arrows) in cardon cactus tissue; CW=plant cell wall.

leguminous trees and cardon cactus. Although still in progress, these experiments show that inoculation with biological agents significantly increased survival and growth of the legume trees. On the other hand adding small amount of compost was the decisive factor in survival and growth of cardon cacti. These experiments demonstrate the potential of using biological agents, including PGPB, and organic amendments for reforestation programs in deserts [Bashan et al., 2006 unpublished data].

In conclusion, rock-weathering PGPB is a newly proposed functional group of PGPB that might have potential to assist reforestation programs.

3. Bioremediation of wastewater using PGPB

Microalgae have many uses, including water bioremediation. The unicellular microalgae *Chlorella vulgaris and Scenedesmus dimorphus* are capable of removing half of the phosphates and most of the ammonium from dairy industry and pig farm wastewaters [22, 24, 31]. In these studies, microalgae were in a suspension.

For bioremediation of wastewater, it is usually desirable to establish large populations of microalgae. Their application is severely limited by the difficulties of harvesting enormous microalgal populations developed in wastewater after treatment. Entrapping microalgae for easy removal after sedimentation with spherical gels gained some adherents. For example, *C. vulgaris*, immobilized in two natural polysaccharide gels (carrageenan and alginate), was used to treat primary domestic wastewater. Although algal cells in the carrageenan and alginate beads grew far slower than the suspended cells, the immobilized cells were more metabolically active. In one trial, over 95% of ammonium and 99% of phosphates were removed from wastewater in three days. This was much more efficient than using suspended cells that reduced only 50% of nitrogen and phosphorus in the same time period [42]. Algal uptake of nutrients and adsorption in alginate gels were the major mechanisms involved in the removal of ammonium and phosphate [69].

Apart from the straightforward studies mentioned above, and many more listed in their literature lists, the idea that a combination of two or more microorganisms is better than a single microorganism is gaining acceptance in agriculture and forestry. Combinations of two or more microorganisms (consortia) are common place in contemporary research with PGPB [3, 6]. The most radical combination of microalgae and bacteria suggested so far is the use of PGPB known in agriculture to enhance the growth and nutrient removal capacity of microalgae from wastewater. A candidate microorganism for co-inoculation with microalgae is *A. brasilense* (strain Cd), a common plant growth-promoting bacterium. Bacteria of the genus *Azospirillum* are used as inoculants to promote growth and yield of numerous crop plants, mainly by affecting the hormonal metabolism and mineral absorption of plants [6]. The underlying hypothesis assumed that bacteria will enhance the performance of unicellular plants, like microalgae, and that the single-cell plant will respond similarly to bacterial inoculation of higher plants. The deliberate inoculation of *Chlorella* spp. with a terrestrial PGPB was not reported prior to these studies, perhaps because of the different origins of the two microorganisms. *C. vulgaris* is not known to harbor any associative beneficial bacteria, and *Azospirillum* sp. is rarely used for inoculation in aquatic environments. One difficulty is the technical problem of ensuring that both single-cell microorganisms will stay in close proximity, an especially difficult task with motile *Azospirillum* species.

To improve growth and metabolism of the freshwater microalga *C. vulgaris* and enhance removal of nitrogen and phosphorus, the microalgae was inoculated with *A. brasilense.* The two microorganisms were kept in close proximity in the liquid medium essential for *C. vulgaris* by immobilizing both species in alginate beads and cultivating them under conditions suitable to both species in batch cultures and in continuous flow cultures in a chemostat. Alginate beads of various forms and shapes are convenient inoculant carriers in numerous industrial, environmental, and agricultural applications. Immobilizing both microorganisms in small alginate beads resulted in significantly greater growth of the microalga. Dry and fresh weight, total number of cells, size of the microalgal colonies within the beads, number of microalgal cells per colony, and the levels of microalgal pigments significantly increased [30]. Light and transmission electron microscopy revealed that both microorganisms colonized the same cavities inside the beads, though the microalgae tended to concentrate in the more aerated periphery, and the bacteria colonized the entire bead [32, 43]. Immobilizing *C. vulgaris* or *C. sorokiniana* with *A. brasilense* resulted in significant changes in the microalgae cell morphology and pigment content. The size of *C. sorokiniana* cells did not change, but the population within the beads significantly increased. Cells of another strain, *C. vulgaris* UTEX 395, grew larger, but their number did not increase [21].

The ability of the mixed immobilized culture to remove ammonium and phosphorus from wastewater was analyzed in continuous culture and in step culture, where the wastewater was replaced every 48 hours. In continuous culture, only moderate levels of nutrients were removed. In step culture, almost all of the ammonium was removed. After six consecutive 48-hours cycles, the bioremediation system was saturated and the efficiency of ammonium removal decreased. In comparison, saturation was reached after three cycles with immobilized microalgae alone, and the level of ammonium removal was reduced [23]. In another study, *C. vulgaris* was immobilized and incubated with *A. brasilense* or its natural associative bacterium *Phyllobacterium myrsinacearum* in alginate beads. The interaction between the microalga and the two bacteria species was observed by transmission electron microscopy for 10 days. Most of the small cavities within the beads were colonized by microcolonies of only one microorganism regardless of the bacterial species cultured with the microalga. Subsequently, the bacteria and microalga microcolonies merged to form large, mixed colonies within the cavities. At this stage, the effect of bacterial association with the microalga differed depending on the bacterium present. The microalga entered a senescence phase when associated with *P. myrsinacearum*, but remained in a growth phase when associated with *A. brasilense.* This study suggested that there are commensal interactions between microalga and associative bacteria and that, with time, the bacterial species determines whether the outcome for microalga is senescence or continuous multiplication [32, 43]. These studies and those described below showed that *A. brasilense* strain Cd is a “microalgae growth-promoting bacterium” (MGPB) (Figure 4).

The capacity of microalga-bacterium consortia in an artificial system of bioremediation to remove ammonium and phosphorus from municipal wastewater was demonstrated [22]. While this system showed a potential for treatment of municipal wastewater of changing sources and nutrient concentrations, as is common to any domestic wastewater system, phosphorus removal was not improved and remained at the same level as is common for other biological systems, about 30%. This level is far below the efficiency of phosphorus by precipitation with metals and chelates, common in wastewater treatment worldwide [19]. The challenge to develop a scheme for enhanced phosphorus removal remains.

In synthetic wastewater growth of microalgae and phosphorus absorption by *C. sorokiniana* or *C. vulgaris* was significantly enhanced after a starvation period of three days in saline solution when immobilized in alginate beads with *A. brasilense* Cd. Similar results occurred when *C. sorokiniana* and *A. brasilense* Cd were combined for treating domestic wastewater. A starvation period followed by submersion of the cultures in fresh wastewater led to continued phosphorus absorption. The best phosphorus removal treatment from a batch of synthetic or domestic wastewater was with tandem treatments of wastewater with pre-starved, immobilized microalgae with bacteria and replacement of this culture after one cycle of phosphorus removal with a new, similarly-starved culture. This combination treatment with two cultures was capable of removing up to 72% of the phosphorus in wastewater. There was a direct correlation between the initial load of phosphorus in the domestic wastewater and the efficiency level of removal. Removal was highest at higher loads of phosphorus loads when treated with immobilized cultures. This occurred when either an immobilized microalgal culture was used or an immobilized combination of a microalga-bacterial culture was used. Further, the negative

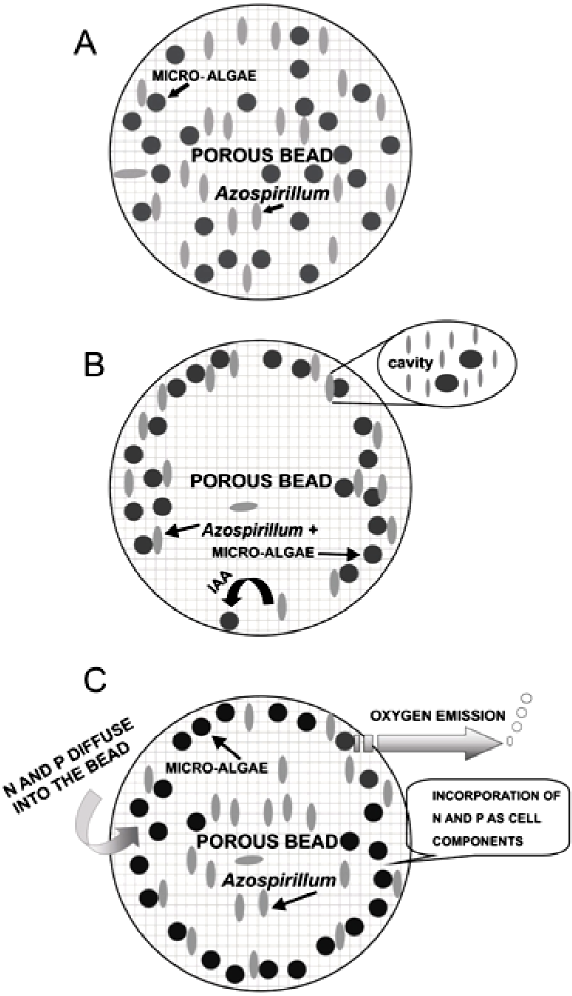

Figure 4. A conceptual model for co-immobilization of microalgae and MGPB in alginate spheres. (A) Both microorganisms are randomly co-immobilized in the polymeric matrix, (B) After co-cultivating for a period of time the two microorganisms share the cavities within the sphere where the MGPB excrete indole-3-acetic acids (and possibly other plant hormones) that enhanced the microalgal growth, (C). Nitrogen and phosphorus from the wastewater diffused inside the porous sphere and incorporated as cell components of the microalgae, increasing its population and releasing oxygen into the medium.

effects of starving microalgae were mitigated by adding the MGPB *A. brasilense* Cd. In summary, starvation combined with immobilizing microalgae and an MGPB have synergistic effects on absorption of phosphorus from wastewater and this kind of biological treatment of wastewater deserves further consideration when designing future facilities [35].

Since microalgae-bacteria immobilized systems have not been found in nature, the search for the cause of the synergistic effects of bacteria on microalgae has concentrated on the parameters of aquatic microbial cultural systems, production of phytohormones by *Azospirillum* (common effect in the production of field crops), and whether enzymes involved in nitrogen metabolism in microalgae are induced or enhanced by the forced artificial association of microalgae with MGPB.

A study of the microalga *C. vulgaris* growth and its capacity to take up nitrogen, treatments with varying concentrations of ammonium and nitrate sources of nitrogen, pH, and different sources of carbon, showed two contrasting phenomena. General analysis showed that in this two-microorganisms system, growth of the microalgal population increased without enhancing the capacity of the individual cells to take up nitrogen or the capacity of cells to take up nitrogen increased without an increase in the total microalgal population. These phenomena were dependent on population density of the microalgae, which, in turn, was affected by culturing conditions. The most evident conclusion is that the size of the microalgal population controls uptake of nitrogen in *C. vulgaris* cells, that is, regardless of experimental parameters, the higher the population, the less nitrogen each cell takes up [20].

Indole-3-acetic acid (IAA), produced by MGPB *A. brasilense* and *A. lipoferum*, was studied for its ability to enhance growth of *C. vulgaris*. Four wild strains of *Azospirillum* (*A. brasilense* Cd, Sp6, and Sp245 and *A. lipoferum* JA4) and their IAA-deficient mutants, *A. brasilense* SpM 7918 (modified *A. brasilense* Sp6), *A. brasilense* FAJ0009 (modified *A. brasilense* Sp245), and *A. lipoferum* JA4*::ngfp*15 (modified *A. lipoferum* JA4) were immobilized with *C. vulgaris* in alginate beads. Results demonstrated significantly more growth in *Chlorella* cultures immobilized with any of the four wild type strains of *Azospirillum*, but very low or no enhanced growth when the four IAA-deficient mutants were present, compared with *C. vulgaris* immobilized without bacteria [de-Bashan et al., 2006, unpublished data].

Because immobilization of *C. vulgaris* with *A. brasilense* affects absorption of ammonium by microalgae, the mechanisms of conversion of ammonia to glutamate by two possible pathways, the enzymes glutamate dehydrogenase and glutamine synthetase were evaluated. In all cases, glutamine synthetase (GS) and glutamate dehydrogenase (GDH) activity in *Chlorella* were affected by immobilization with *A. brasilense.* When immobilized *Chlorella* (in presence of *A. brasilense*) absorbs more ammonium than *Chlorella* without the bacterium, both enzymes are operating at the same time, and activity per cell of GS and GDH are higher. However, when only GS activity increases, greater absorption of ammonium at the culture level does not not occur. The data available so far indicates that higher GS and GDH activity increases ammonium absorption by *Chlorella*. [de-Bashan et al., 2006, unpublished data].

In summary, no new applied technology has emerged from decades of research on intentionally using specific microorganisms for removing nutrients from wastewater. Several proposals, especially entrapment of microorganisms in polysaccharide gels and combinations of several microorganisms for simultaneous treatment of wastewater, have the best potential for commercial use. However, it is still a minor avenue of research. Nevertheless, immobilizing microalgae with MGPB is an effective way to increase populations of microalgae within confined environments and improving wastewater treatment under experimental conditions.

4. Phytoremediation of soils using PGPB

Phytoremediation is a promising, relatively new approach for cleanup of polluted environments. It may be defined as the use of plants to remove, destroy, or sequester hazardous substances from the environment. The technology has so far been used experimentally to remove toxic heavy metals from contaminated soil; expansion of its capacity for applications to remove and degrade organic pollutants in the environment is the next phase.

Both plants and soil microorganisms have some limitations in their individual abilities to remove and break down organic compounds. For example, even plants that are relatively tolerant of various environmental contaminants often remain underdeveloped, or slowly grow in the presence of the contaminants. A technology combining plant and microorganisms, mainly PGPB inoculated into the plant roots, may promote a synergistic action, leading to improved plant growth. The net result of adding PGPB to plants is a significant increase in both the number of germinating seeds and increased biomass of the plants, making phytoremediation a faster and more efficient process. This may overcome many of the limitations of single organisms, and provide a useful and more powerful approach for enhancing remediation of contaminated environments [16, 17, 28, 29].

It is well documented that plants can enhance degradation of certain recalcitrant organic chemical in soil. Removal and degradation of pollutants during remediation with plants are usually slower than physical or chemical methods because plant growth is dependent on environmental parameters that are not always favorable in contaminated areas, such as mine tailings. Some plants are slow growing by nature. Examples of pollutants that can be handled with phytoremediation ranged from chlorinated hydrocarbons and pesticides, petroleum compounds, explosives, dyes, and detergents. The main mechanisms that constitute phytoremediation include phytotransformation, phytoextraction, phytoaccumulation, phytotranspiration, and rhizosphere remediation [16].

Biodegradation of organic compounds is affected by the plant-microbial interactions in the rhizosphere, which apparently offers a favorable environment for co-metabolism of soil-bound and recalcitrant chemicals. The microbial transformation of organic compounds is probably helped by the abundance of energy provided in root exudates. Rhizosphere interaction between plants and microbial communities, including PGPB, benefits both organisms. The bacteria receive nutrients from root exudates and the plants have enhanced nutrient uptake and reduction in the toxicity of soil contaminants. Soil microorganisms are also known to produce biosurfactants that may facilitate removal of organic pollutants. When soils are contaminated with heavy metals, root exudates, besides providing source of carbon for the microbes, may also take part in direct detoxification by forming chelates with metal ions. In these cases, mycorrhiza fungi may improve establishment of plants and their survival in soils contaminated with both heavy metals and organic compounds. Plant-microbial interactions can work on some of the most recalcitrant contaminants (described later), which is of specific interest to researchers intent on enhancing and extending the scope of remediation technologies now focusing on either microorganisms or plants.

In the following sections, we highlight several studies employing PGPB combined with plants to remediate polluted environments. Compared to the volume of literature on phytoremediation in general, these are only a few examples to represent the state-of-the-art. These might be the pathfinders for developing phytoremediating technologies in the near future. These experimental systems are generally separated into several sections: (i) inoculation of plants with a single PGPB or expressing their genes in plants useful for phytoremediation, (ii) inoculation with bacterial consortium, and (iii) general remediation by beneficial microorganisms in the rhizosphere and plant foliage for enhancing contaminant removal.

4.1 Inoculation of plants with a single PGPB (native or genetically-modified) or expressing their genes in plants useful for phytoremediation

Polycyclic aromatic hydrocarbons (PAHs) are particularly recalcitrant contaminants, known to be highly persistent in the environment. Phytoremediation of creosonte-contaminated soil was evaluated in three grasses:

tall fescue, kentucky blue grass, and wild rye were inoculated with one of three PGPB (*Pseudomona putida*, *Azospirillum brasilense*, or *Enterobacter cloacae*) and evaluated for promotion of plant growth and protection from the toxicity of the contaminant toxicity. Some plants were less affected by contaminants possibly as a response from the addition of PGPB. PGPB were able to greatly enhance phytoremediation by accelerating plant growth, especially roots. Increased root biomass led to more effective remediation [36]. To further improve phytoremediation of PAH-contaminated soils, techniques using different aspects of contaminant removal were combined. The multi-process system was composed of volatilization, photo oxidation, PGPB microbial remediation, and plant remediation (contaminant-tolerant tall fescue). Over a four-month trial, the average efficiency of removing 16 high-priority PAHs by the multi-process remediation system was significantly greater. Even more important, the multi-process system was capable of removing most of the highly hydrophobic, soil-bound PAHs. The explanation provided by the investigators was that the key elements for successful phytoremediation were the use of plant species that have the ability to proliferate in the presence of high levels of contaminants when combined with PGPB strains that increase plant tolerance to contaminants and accelerate plant growth. The combination of methods enhanced active metabolic processes in the soil, leading to more rapid and more complete removal of PAHs [37].

Inoculation of the common reed *Phragmites australis*, which is widely used for wastewater treatment in wetlands, with genetically modified *Pseudomonas asplenii* AC, which contains the gene encoding for the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase), significantly increased seed germination. Furthermore, in soils contaminated with copper or creosote, inoculation of *P. australis* seeds with native or transformed *P. asplenii* AC generated larger plants shoots and roots than plants that were not inoculated. The explanation was that plants exposed to heavy metals and other stressors induce production of stress ethylene, which leads to premature plant senescence. In contrast, lowering ethylene levels mitigates harmful effects of many stressors. PGPB containing ACC deaminase, an enzyme that decreases ethylene, can help plants to resist certain stresses and grow in difficult environmental conditions [61].

A procedure for selecting a microbe-plant pair for efficient degradation of naphthalene was based on the rationale that root exudates are the best nutrient source available in soil. The grass *Lolium multiflorum* was chosen because of its abilities to produce a highly-branched, deep root system and support a large population of *Pseudomonas* spp. on its roots. This procedure yielded a *Pseudomonas putida* strain that is stable in the rhizosphere, has superior root-colonizing properties, capable of using root exudates efficiently, and has superior ability to degrade naphthalene. Roots colonized by this strain were able to penetrate an agar layer and degrade naphthalene beneath this layer. Inoculation of the grass seeds or seedlings with this bacteria strain protected the plants against naphthalene toxicity. This plant-microbe combination appeared to degrade naphthalene from soil that was also heavily polluted with a mixture of PAHs [41].

Bioremediation potential of the legume *Galega orientalis* and its rhizosphere symbiont *Rhizobium galegae* was evaluated in soils contaminated with diesel or toluene. *G. orientalis* nodulated well and had good growth in diesel-contaminated soils or in soils containing 2 g/l toluene [44].

Several cadmium-tolerant bacterial strains were isolated from the root zone of Indian mustard (*Brassica juncea*) seedlings grown in soils, sewage sludge, and mining waste that were highly contaminated with cadmium. The strains included *Variovorax paradoxus*, *Rhodococcus* sp., and *Flavobacterium* sp., and were capable of stimulating elongation of *B. juncea* seedling roots in soils with or without toxic concentrations of cadmium. These PGPB may be useful for developing plant-inoculant systems for phytoremediation of polluted soils [10].

A different approach was to employ useful PGPB genes for plant growth promotion and express them in plants used for phytoremediation. Transgenic canola (*Brassica napus*) that constitutively express the ACC deaminase gene, whose effect on plant growth was described earlier, were generated and tested for their ability to proliferate in soils containing high levels of arsenic and accumulate it in plant tissues. The ability of the PGPB *Enterobacter cloacae* to facilitate the growth of non-transgenic and ACC deaminase-expressing canola plants was also tested. In the presence of arsenate, in plants with and without added PGPB, transgenic canola plants grew better than non-transformed canola plants [52]. In a similar attempt, this approach to gain advantages provided by bacterial ACC deaminase, in phytoremediation of soils contaminated with nickel, transgenic canola significantly increased tolerance to nickel, compared to the control plants [66].

4.2 Inoculation with bacterial consortium

Pentachlorophenol (PCP) is a widespread, highly toxic contaminant of soil and water that is generally recalcitrant to microbial breakdown. PCP toxicity and rates of mineralization were compared in crested wheatgrass seedlings that were either sterile or had roots inoculated with a consortia of microbes derived from soil at a PCP-contaminated site. Inoculated seedlings were more tolerant to PCP and mineralized three times more ^{14}C-PCP than microbial-free seedlings. Only 10% of the radioactivity from microbial-free seedlings represented mineralized PCP, indicating that rhizosphere microorganisms are primarily responsible for PCP mineralization. The presence of crested wheatgrass root exudates enhanced the number of PCP-degrading microbes by 100-fold in liquid culture, suggesting that a close association of plants and rhizosphere microorganisms appears to be necessary for survival of crested wheatgrass in PCP-contaminated soil [49].

The alpine pennycress *Thlaspi caerulescens* of the Rocky Mountain States has a remarkable ability to hyperaccumulate cadmium and zinc. In soils containing mostly nonlabile zinc, inoculation of a bacterial consortia into the plants shows that rhizosphere microbes play an important role in increasing availability of water soluble zinc and enhancing accumulation in *T. caerulescens* [73].

4.3 General remediation by beneficial microorganisms in the rhizosphere and in plant foliage for improving removal of contaminants

The bacterial populations associated with the hyperaccumulator *T. caerulescens* subsp. *calaminaria* was grown in soil collected from an abandoned lead-zinc mine and smelter. The rhizosphere populations had significantly higher heavy-metal resistance characteristics than population surviving in the rest of the soil. The endophytic bacterial populations in roots and shoots of these plants differ significantly. Although similar endophytic species were isolated from both parts, those from the rhizoplane and roots showed lower resistance to zinc and cadmium than the endophytic bacteria residing in the shoots. Contrary to the bacteria in the roots, the shoot bacteria represent a niche rich in metal-resistant bacteria and even seemed to contain species that were exclusively abundant there, with some potential to assist the plant in phytoremediation [45].

Lupinus luteus, when grown on a nickel-enriched substrate and inoculated with modified *Burkholderia cepacia* strain L.S.2.4::ncc-nre (modified to harbor the *ncc-nre* nickel-resistance system of *Ralstonia metallidurans*), showed a significant increase (30%) of nickel concentrated in the roots, whereas the nickel concentration in the shoots remained comparable with that of the control plants [46].

The capability of plants to promote microbial degradation of pollutants in the rhizosphere soil is a principal mechanism of phytoremediation. Microbial communities and their degradative potential in the rhizospheres of alfalfa (*Medicago sativa*) and reed (*Phragmites australis*) and in unplanted soil in response to bitumen contamination were studied. Over a period of 27 months, bitumen reduced the total number of microorganisms by 75% in unplanted soil than in rhizosphere soil, only 42% and 7% for soils planted in reeds and alfalfa, respectively. Changes in the structure of the microbial community under bitumen contamination were found to hinge, not merely on the presence of plants, but also the rhizosphere microflora, with alfalfa microflora reducing hydrocarbon pollution more than reed microflora [50]. An identical approach towards PAH-contaminated soil using alfalfa and common reed showed that both alfalfa and reed reduced contaminated soil by degrading most of the PAHs contaminating the soil. However alfalfa stimulated the rhizosphere microflora more effectively than the common reed. Alfalfa clearly enhanced the population of microorganisms and dramatically increased the rate of the PAH-degrading microflora populations. The potential for using the alfalfa-based system to remediate PAH contamination makes it attractive for phytoremediation [51].

5. Using PGPB with aquatic microorganisms to solve environmental problems

PGPB may serve as “helper” bacteria in promoting growth of economically important aquatic microorganisms. Although some positive effects of marine bacteria on marine diatoms were known for decades [62, 67, 71], the knowledge was not applied to practical uses. Only a very small-scale use of these microorganisms is documented and exclusively used in aquaculture. In this case, inoculation of a marine diatom *Chaetoceros gracilis* was used as feed in a Japanese pearl oyster hatchery with the PGPB *Flavobacterium* sp. in mass culture production of the diatom. This resulted in significantly higher growth than the control cultures, and the stationary growth phase in the treated cultures lasted longer, until the end of the culture period [68]. In a different situation, *Azospirillum* sp. and *Azotobacter* sp. significantly increased phytoplankton population and consequently the yield of carp when introduced to freshwater fish aquaculture ponds in India [26]. The knowledge and application of this sub-field is embryonic and does not allow us to reach conclusions or recommendations.

6. Concluding remarks and future research perspectives

Using PGPB and MGPB to solve environmental problems is in its infancy and largely experimental. To date, no technology is available in the marketplace. The ways by which PGPB affects growths of other microorganisms are rarely known. In wastewater treatment where information is more available, immobilization of microalgae and MGPB as an effective means of increasing microalgal population within confined environments and increasing removal of nutrients from wastewater has been demonstrated. However, we suggest that, although *Azospirillum* spp. positively affects *Chlorella* growth and removal of nutrients from wastewater, other PGPB should be tested for efficiency. The man-made association of a microalga with a bacterium is capable of improved removal of nutrients from wastewater and has promise for wastewater treatment.

Reforestation of arid land, aided by PGPB and AM fungi, is a feasible method for reclaiming desert areas outside the northwestern deserts of Mexico, where the techniques were developed. Around the world, PGPB collected from “resource island” soils and from tissues and seeds of natural vegetation are worth exploring.

Phytoremediation of contaminated lands assisted by PGPB is a promising avenue to overcome the limitation imposed on using plants alone that until now did not yield commercial application despite the large volume of knowledge on that topic. A combination of plant, AM fungi, PGPB and small amount of organic matter to clean contaminated land is the best recommendation so far for future research.

Taken together, the experimental technologies described here, although the cutting edge in their fields, are yet to prove useful for the benefit of mankind.

7. References

1. Ascaso, C., L.G. Sancho, and C. Rodriguez Pascual. 1990. The weathering action of saxicolous lichens in maritime Antarctica. Polar Biol. **11:** 33-40.
2. Bashan, Y. 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol. Adv. **16:** 729-770.
3. Bashan, Y., and L.E. de-Bashan. 2005. Bacteria/plant growth-promotion. In Encyclopedia of soils in the environment. Vol. 1, ed D. Hillel, pp. 103-115. Elsevier, Oxford, UK.
4. 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 Biol. Biochem. **30:** 1225-1228.
5. Bashan, Y., E.A. Davis, A. Carrillo-Garcia, and R.G. Linderman. 2000. Assessment of VA mycorrhizal inoculum potential in relation to the establishmentof cactus seedlings under mesquite nurse-trees in the Sonoran Desert. Appl. Soil Ecol. **14:** 165-176.
6. 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.
7. Bashan, Y., A. Rojas, and M.E. Puente. 1999. Improved establishment and development of three cacti species inoculated with *Azospirillum brasilense* transplanted into disturbed urban desert soil. Can. J. Microbiol. **45:** 441-451.
8. Bashan, Y., C.Y. Li, V.K. Lebsky, M. Moreno, and L.E. de-Bashan. 2002. Primarycolonization of volcanic rocks by plants in arid Baja California, Mexico. Plant Biol. **4:** 392-402.
9. Bashan, Y., H. Vierheilig, B.G. Salazar, and L.E. de-Bashan. 2006. Primary colonization and breakdown of igneous rocks by endemic, succulent elephant trees (*Pachycormus Discolor*) of the deserts in Baja California, Mexico. Naturwissenschaften **93:** 344-347.
10. 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 Biol. Biochem. **3:** 241-250.

11. Berthelin, J., C. Leyval, F. Laheurte, and P. de Giudici. 1991. Involvement of roots and rhizosphere microflora in the chemical weathering of soil minerals. In Plant root growth: an ecological perspective, ed D. Atkinson, Blackwell Scientific Publications, pp. 187-200. Oxford, UK.
12. 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.
13. Carrillo-Garcia, A., Y. Bashan, and G.J. Bethlenfalvay. 2000. Resource-island soils and the survival of the giant cactus, cardon, of Baja California Sur. Plant Soil **218:** 207-214.
14. Carrillo-Gracia, 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. Restor. Ecol. **8:** 65-73.
15. Carrillo-Garcia, A., J.-L. Leon de la Luz, Y. Bashan, and G.J. Bethlenfalvay. 1999. Nurse plants, mycorrhizae, and plant establishment in a disturbed area of the Sonoran desert. Restor. Ecol. **7:** 321-335.
16. Chaudhry, Q., M. Blom-Zandstra, S. Gupta, and E.J. Joner. 2005. Utilizing the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. Environ. Sci. Pollut. Res. **12:** 34-48.
17. Chaudhry, Q., P. Schröder, D. Werck-Reichhart, W. Grajek, R. Marecik. 2002. Prospects and limitations of phytoremediation for the removal of persistent pesticides in the environment. Environ. Sci. Pollut. Res. **9:** 4-17.
18. Corkidi, L., and E. Rincón. 1997. Arbuscular mycorrhizae in a tropical sand dune ecosystem on the Gulf of Mexico. II. Effects of arbuscular mycorrhizal fungi on the growth of species distributed in different early successional stages. Mycorrhiza **7:** 17-23.
19. de-Bashan, L.E., and Y. Bashan. 2004. Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003). Water Res. **38:** 4222-4246.
20. de-Bashan, L.E., H. Antoun, and Y. Bashan. 2005. Cultivation factors and population size control uptake of nitrogen by the microalgae *Chlorella vulgaris* when interacting with the microalgae growth-promoting bacterium *Azospirillum brasilense.* FEMS Microbiol. Ecol. **54:** 197-203.
21. de-Bashan, L.E., Y. Bashan, M. Moreno, V.K. Lebsky, and J.J. Bustillos. 2002. Increased pigment 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 brasilenses.* Can. J. Microbiol. **48:** 514-521.
22. 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.
23. de-Bashan, L.E., M. Moreno, J.-P. Hernandez, and Y. Bashan. 2002. Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae

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

24. De la Noüe, J., and N. De Pauw. 1988. The potential of microalgal biotechnology: a review of production and uses of microalgae. Biotechnol. Adv. **6:** 725-770.
25. Ellis, W.S., and D.C. Turnley. 1990. The Aral: a Soviet sea lies dying. National Geograph. **177:** 70-93
26. Garg, S.K., and A. Bhatnagar. 1999. Effect of *Azospirillum* and *Azotobacter* inoculation on pond productivity and fish growth under fresh water conditions. Indian J. Microbiol. **39:** 227-233.
27. Gibson, A.C., and P.S, Nobel, 1986. The cactus primer. Harvard University Press, Cambridge, MA.
28. Glick, B.R. 2003. Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol. Adv. **21:** 383-393.
29. Glick, B.R. 2004. Teamwork in phytoremediation. Nature Biotechnol. **22:** 526-527.
30. Gonzalez, L.E., and Y. Bashan, 2000. Increased growth of the microalga *Chlorella vulgaris* when co-immobilized and co-cultured in alginate beads with the plant growth-promoting bacterium *Azospirillum brasilense*. Appl. Environ. Microbiol. **66:** 1527-1531.
31. Gonzalez, L.E., R.O. Cañizares, and S. Baena. 1997. Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. Biores. Technol. **60:** 259-262.
32. Gonzalez-Bashan, L.E., V.K. Lebsky, J.P. Hernandez, J.J. Bustillos, and Y. Bashan, 2000. Change in the metabolism of the microalga *Chlorella vulgaris* when coimmobilized in alginate with the nitrogen-fixing *Phyllobacterium myrsinacearum*. Can. J. Microbiol. **46:** 653-659.
33. Grover, H.D., and H.B. Musick. 1990. Mexico, U.S.A.: an analysis of desertification processes in the American southwest. Climatic Change, **17:** 305- 330.
34. Gyaneshwar, P., G.N. Kumar, and L.J. Parekh. 1998. Effect of buffering on the phosphate-solubilizing ability of microorganism. World J. Microbiol. Biotechnol. **14:** 669-673.
35. Hernandez, J.P., L.E. de-Bashan, and Y. Bashan. 2006. Starvation enhances phosphorus removal from wastewater by the microalga *Chlorella* spp. co-immobilized with *Azospirillum brasilense.* Enzyme Microb. Technol. **38:** 190-198.
36. Huang, X-D., Y. El-Alawi, D.M. Penrose, B.R.Glick, and B.M. Greenberg. 2004. Responses of three grass species to creosote during phytoremediation. Environ. Pollut. **130:** 453-463.
37. Huang, X-D., Y. El-Alawi, D.M. Penrose, B.R. Glick, and B.M. Greenberg. 2004. A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ. Pollut. **130:** 465-476.
38. Illmer, P., and F. Schinner. 1995. Solubilization of inorganic calcium phosphate-solubilization mechanisms. Soil Biol. Biochem. **27:** 257-263.
39. Jackson, L.L., J.R. McAuliffe, and B.A. Roundy. 1991. Desert restoration. Restor. Manage. Notes **9:** 71-80.
40. Kobayashi, D.Y., and J.D. Palumbo. 2000. Bacterial endophytes and their effects on plants and uses in agriculture. In Microbial endophytes, eds C.W. Bacon, and J.F. Whites Jr., pp. 199-233. Marcel Decker, New York.

41. Kuiper, I., G.V. Bloemberg, and J.J. Lugtenberg. 2001. Selection of a plant-bacterium pair as a novel tool for rhizostimulation of polycyclic aromatic hydrocarbon-degrading bacteria. Mol. Plant Microbe Inter. **14:** 1197-1205.
42. Lau, P., N.F.Y. Tam, and Y.S. Wong. 1997. Wastewater nutrients (N and P) removal by carrageenan and alginate immobilized *Chlorella vulgaris*. Environ. Technol. **18:** 893-899.
43. 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.
44. Lindström, K., M.M. Jussila, H. Hintsa, A. Kaksonen, L. Mokelke, K. Mäkeläinen, J. Pitkäjärvi, and L. Suominen. 2003. Potential of the *Galega-Rhizobium galegae* system for bioremediation of oil-contaminated soil. Food Technol. Biotechnol. **41:** 11-16.
45. Lodewyckx, C., M. Mergeay, J. Vangronsveld, H. Clijsters, and D. Van Der Lelie. 2002. Isolation, characterization, and identification of bacteria associated with the zinc hyperacccumulator *Thlaspi caerulescens* subsp. *calaminaria*. Int. J. Phytoremediat. **4:** 101-115.
46. 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.
47. Lynch, J.M., and J.M. Whipps. 1990. Substrate flow in the rhizosphere. Plant Soil **129:** 1-10.
48. Mata, L.J., and M. Campos (+25 co-authors). 2001. Climate Change 2001: Impacts, Adaptation and Vulnerability: Chapter 14. Latin America. The Intergovernmental Panel on Climate Change (IPCC). The World Meteorological Organization (WMO) and the United Nations Environment Program (UNEP) Geneva, Switzerland. 42 pp.
49. Miller, E.K., and W. Dyer. 2002. Phytoremediation of pentachlorophenol in the crested wheatgrass (*Agropyron cristatum x desertorum*) rhizosphere. Int. J. Phytoremediat. **4:** 223-238.
50. Muratova, A., Th. Hübner, N. Narula, H. Wand, O. Turkovskaya, P. Kuschk, R. Jahn, W. Merbach. 2003a. Rhizosphere microflora of plants used for the phytoremediation of bitumen-contaminated soil. Microbiol. Res. **158:** 151-161.
51. Muratova, A., Th. Hübner, O. Tischer, O. Turkovskaya, M. Möder, and P. Kuschk. 2003b. Plant-rhizosphere-microflora association during phytoremediation of PAH-contaminated soils. Int. J. Phytoremediat. **5:** 137-151.
52. 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.
53. Nobel, P.S. 1996. Ecophysiology of roots of desert plants, with special emphasis on agaves and cacti. In Plants roots. The hidden half, ed Y. Waisel, A. Eshel, and U. Kafkafi, pp. 823-844. Marcel Dekker Inc., New York.
54. Ortega-Rubio, A., A. Naranjo, A. Nieto, C. Arguelles, F. Salinas, R. Aguilar, and H. Romero. 1998. Suspended particles in atmosphere and respiratory health

problems at La Paz City, Baja California Sur, Mexico. J. Environ. Biol. **19:** 381-387.

55. 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.
56. Puente, M.E., and Y. Bashan. 1994. The desert epiphyte *Tillandsia recurvata* harbors the nitrogen-fixing bacterium *Pseudomonas stutzeri*. Can. J. Bot. **72**: 406-408.
57. Puente, M.E., Y. Bashan, C.Y. Li, and V.K. Lebsky. 2004. 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.
58. Puente, M.E., C.Y. Li, and Y. Bashan. 2004. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings. Plant Biol. **6:** 643-650.
59. Puente, M.E., M.C. Rodriguez-Jaramillo, C.Y. Li, and Y. Bashan. 2006. Image analysis for quantification of bacterial rock weathering. J. Microbiol. Meth. **64:** 275-286.
60. Rapp, A. 1986. Introduction to soil degradation processes in drylands. Climatic Change **9:** 19-31.
61. Reed, M.L.E., B. Warner, and B.R. Glick. 2005. Plant growth-promoting bacteria facilitate the growth of the common reed *Phragmites australis* in the presence of copper or polycyclic aromatic hydrocarbons. Curr. Microbiol. **51:** 425-429
62. Riquelm C.E., K. Fukami, and Y. Ishida. 1988. Effects of bacteria on the growth of a marine diatom, *Asterionella glacialis*. Bull. Jap. Soc. Microbial. Ecol. **3:** 29-34.
63. Roberts, N.C. 1989. Baja California Plant Field Guide. Natural History Publ. Co., La Jolla, CA. USA, 180 p.
64. Samet, J.M., F. Dominici, F.C. Curriero, I. Coursac, and S.L. Zeger. 2000. Fine Particulate Air Pollution and Mortality in 20 U.S. Cities, 1987–1994. New England. J. Med. **343:** 1742-1749.
65. Secretaría de Desarrollo Social. 1994. La desertificación en México. Plan de acción para el combate a la desertificación. In Plan de acción para combatir la desertificación en Mexico. *Edited by* Comisión Nacional de Zonas Aridas., Secretaría de Desarrollo Social, Saltillo, Coahuila, Mexico. pp. 73-114 (In Spanish).
66. Stearns, J.C., S. Shah, B.M. Greenberg, D.G. Dixon, and B.R. Glick. 2005. Tolerance of transgenic canola expressing 1-aminocyclopropane-1-carboxylic acid deaminase to growth inhibition by nickel. Plant Physiol. Biochem. **43:** 701-708.
67. Suminto, and K. Hirayama. 1996. Effects of bacterial coexistence on the growth of a marine diatom *Chaetoceros gracilis*. Fish Sci. **62:** 40-43.
68. Suminto, and K. Hirayama. 1997. Application of a growth-promoting bacteria for stable mass culture of three marine microalgae. Hydrobiologia **358:** 223-230.
69. Tam, N.F.Y., and Y.S. Wong. 2000. Effect of immobilized microalgal bead concentration on wastewater nutrient removal. Environ. Pollut. **107:** 145-151.
70. Tiittanen, P., K.L. Timonen, J. Ruuskanen, A. Mirme, J. Pekkanen. 1999. Fine particulate air pollution, resuspended road dust and respiratory health among symptomatic children. Eur. Respir. J. **13:** 266-273.

71. Ukeles, R., and J. Bishop. 1975. Enhancement of phytoplankton growth by marine bacteria. J. Phycol. **11:** 142-149.
72. Weber, F.R. 1986. Reforestation in Arid Lands, VITA Publishing, Riverside, CA.
73. 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.