Review Article

Plant Growth-Promoting Bacteria: Mechanisms and Applications

Bernard R. Glick

Department of Biology, University of Waterloo, 200 University Avenue South, Waterloo, ON, Canada N2L 3G1

Correspondence should be addressed to Bernard R. Glick; glick@uwaterloo.ca

Received 29 July 2012; Accepted 13 September 2012

Academic Editors: T. Ano, G. Comi, and M. Shoda

Copyright © 2012 Bernard R. Glick. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The worldwide increases in both environmental damage and human population pressure have the unfortunate consequence that global food production may soon become insufficient to feed all of the world’s people. It is therefore essential that agricultural productivity be significantly increased within the next few decades. To this end, agricultural practice is moving toward a more sustainable and environmentally friendly approach. This includes both the increasing use of transgenic plants and plant growth-promoting bacteria as a part of mainstream agricultural practice. Here, a number of the mechanisms utilized by plant growth-promoting bacteria are discussed and considered. It is envisioned that in the not too distant future, plant growth-promoting bacteria (PGPB) will begin to replace the use of chemicals in agriculture, horticulture, silviculture, and environmental cleanup strategies. While there may not be one simple strategy that can effectively promote the growth of all plants under all conditions, some of the strategies that are discussed already show great promise.

1. Introduction

There are currently around 7 billion people in the world and this is expected to increase to approximately 8 billion some time around the year 2020. When one considers both the expected worldwide population increase and the increasing environmental damage that is a consequence of ever greater levels of industrialization, it is clear that in the next ten to twenty years it will be a significant challenge to feed all of the world’s people, a problem that will only increase with time. There is absolutely no time to lose; to feed this growing population, the world needs to begin to greatly increase agricultural productivity, and to do so in a sustainable and environmentally friendly manner. To feed the growing world, it is necessary to re-examine many of the existing approaches to agriculture that includes the use of chemical fertilizers, herbicides, fungicides, and insecticides. Instead, sustainable agriculture will likely make much greater use of both transgenic plants (for example, see http://www.isaaa.org/inbrief/default.asp) and plant growth-promoting bacteria, or PGPB [1].

It has been estimated that around “40% of deaths worldwide are caused by water, air, and soil pollution” and that “environmental degradation, coupled with the growth in world population, are (considered to be) major causes behind the rapid (global) increase in human disease” (http://www.sciencedaily.com/releases/2007/08/070813162438.htm). That is, as a consequence of both increasing population and industrialization, the earth’s atmospheric, terrestrial, and aquatic systems are no longer sufficient to absorb and break down the increasing amount of waste that we produce. As a result, the environment is increasingly contaminated with a range of toxic metals and organic compounds [2–4]. Recognizing the nature and magnitude of the problem is an important first step. However, even if all environmental pollution were to cease tomorrow, it is still essential that all of the contaminated lands and waters be remediated. One way to address this problem is through the use of phytoremediation, the purposeful use of plants to take up and concentrate or degrade a wide range of environmental pollutants [5–8]. Moreover, the addition of PGPB to plants that are used in phytoremediation protocols typically makes the entire remediation process much more efficacious [3, 9, 10].

2. Plant Growth-Promoting Bacteria (PGPB)

Soil is replete with microscopic life forms including bacteria, fungi, actinomycetes, protozoa, and algae. Of these different
Historically, *Rhizobia* spp. were studied extensively, from physiological, biochemical, and molecular biological perspectives, before much interest was shown in trying to understand or utilize other PGPB to facilitate plant growth [21–23]. Thus, these early studies became a conceptual starting point for mechanistic studies of PGPB. However, since unlike *Rhizobia* spp., most PGPB fix no or only a limited amount of nitrogen, studies to better understand some of the mechanisms used by PGPB have addressed a wide range of different mechanisms [13, 20, 24].

2.1. Commercialization. Despite the, still, limited understanding of PGPB-plant interactions, a number of these bacteria are nevertheless used commercially as adjuncts to agricultural practice [1, 25]. Commercialized PGPB strains include *Agrobacterium radiobacter*, *Azospirillum brasilense*, *Azospirillum lipoferm*, *Azotobacter chroococcum*, *Bacillus firmus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus mucilaginosus*, *Bacillus pumilus*, *Bacillus subtilis*, *Bacillus subtilis* var. *amyloliquefaciens*, *Burkholderia cepacia*, *Delftia acidovorans*, *Paenobacillus macerans*, *Pantoea agglomerans*, *Pseudomonas aureofaciens*, *Pseudomonas chlororaphis*, *Pseudomonas fluorescens*, *Pseudomonas solanacearum*, *Pseudomonas* spp., *Pseudomonas syringae*, *Serratia entomophila*, *Streptomyces griseoviridis*, *Streptomyces* spp., *Streptomyces lydicus* and various *Rhizobia* spp. However, PGPB inoculated crops represent only a small fraction of current worldwide agricultural practice.

For the more extensive commercialization of PGPB strains, a number of issues need to be addressed. These include (i) determination of those traits that are most important for efficacious functioning and subsequent selection of PGPB strains with appropriate biological activities; (ii) consistency among regulatory agencies in different countries regarding what strains can be released to the environment, and under what conditions genetically engineered strains are suitable for environmental use; (iii) a better understanding of the advantages and disadvantages of using rhizospheric versus endophytic bacteria; (iv) selection of PGPB strains that function optimally under specific environmental conditions (e.g., those that work well in warm and sandy soils versus organisms better adapted to cool and wet environments); (v) development of more effective means of applying PGPB to plants in various settings (e.g., in the field versus in the greenhouse); (vi) a better understanding of the potential interactions between PGPB and mycorrhizae and other soil fungi.

3. Direct Mechanisms

3.1. Facilitating Resource Acquisition. The best-studied mechanisms of bacterial plant growth promotion include providing plants with resources/nutrients that they lack such as fixed nitrogen, iron, and phosphorus. Many agricultural soils lack a sufficient amount of one or more of these compounds so that plant growth is suboptimal. To obviate this problem and obtain higher plant yields, farmers have become increasingly dependent on chemical
sources of nitrogen and phosphorus. Besides being costly, the production of chemical fertilizers depletes nonrenewable resources, the oil and natural gas used to produce these fertilizers, and poses human and environmental hazards. It would obviously be advantageous if efficient biological means of providing nitrogen and phosphorus to plants could be used to substitute for at least a portion of the chemical nitrogen and phosphorus that is currently used.

3.1.1. Nitrogen Fixation. In addition to Rhizobia spp., a number of free-living bacteria, for example Azospirillum spp., are also able to fix nitrogen and provide it to plants [26]. However, it is generally believed that free-living bacteria provide only a small amount of what the fixed nitrogen that the bacterially-associated host plant requires [27]. Nitrogenase (nif) genes required for nitrogen fixation include structural genes, genes involved in activation of the Fe protein, iron molybdenum cofactor biosynthesis, electron donation, and regulatory genes required for the synthesis and function of the enzyme. In diazotrophic (nitrogen fixing) bacteria, nif genes are typically found in a cluster of around 20–24 kb with seven operons encoding 20 different proteins. Because of the complexity of this system, genetic strategies to improve nitrogen fixation have been elusive. At one time, some scientists believed once nif genes were isolated and characterized, that it would be possible to genetically engineer improvements in nitrogen fixation. And, a few individuals argued that it might be possible to genetically engineer plants to fix their own nitrogen. Today, these ideas seem somewhat naive.

Since the process of nitrogen fixation requires a large amount of energy in the form of ATP, it would be advantageous if bacterial carbon resources were directed toward oxidative phosphorylation, which results in the synthesis of ATP, rather than glycogen synthesis, which results in the storage of energy in the form of glycogen. In one experiment, a strain of Rhizobium tropici was constructed with a deletion in the gene for glycogen synthase [28]. Treatment of bean plants with this engineered bacterium resulted in a significant increase in both the number of nodules that formed and an increase in the plant dry weight in comparison with treatment with the wild-type strain. This is one of the very few examples of scientists genetically modifying the nitrogen fixation apparatus of a bacterium and obtaining increased levels of fixed nitrogen. Unfortunately, while this mutant increased nodule number and plant biomass in the field, it does not survive well in the soil environment.

Oxygen is both inhibitory to the enzyme nitrogenase and is also a negative regulator of nif gene expression; however, it is required for Rhizobium spp. bacteroid respiration. To prevent oxygen from inhibiting nitrogen fixation while at the same time providing sufficient oxygen for the bacteroids within the nodule to respire, it is possible to introduce bacterial hemoglobin, which binds free oxygen. Following transformation of Rhizobium etli with a Vitreoscilla sp. (a gram negative bacterium) hemoglobin gene, at low levels of dissolved oxygen, the rhizobial cells had a two- to threefold higher respiratory rate than the nontransformed strain. In the greenhouse, following inoculation of bean plants with hemoglobin-containing R. etli the plants had 68% more nitrogenase activity than plants inoculated with wild-type R. etli. This difference led to a 25–30% increase in leaf nitrogen content and a 16% increase in the nitrogen content of the resultant seeds [29].

A small and localized rise in plant ethylene levels is often produced following the infection of legumes by Rhizobium spp. This increased ethylene concentration can inhibit subsequent rhizobial infection and nodulation [30]. Some rhizobial strains can increase the number of nodules that form on the roots of a host legume by limiting the rise in ethylene by synthesizing a small molecule called rhizobitoxine [31] that chemically inhibits the functioning of the enzyme ACC synthase, one of the ethylene biosynthetic enzymes. Alternatively, some rhizobial strains produce the enzyme ACC deaminase which removes some of the ACC (the immediate precursor to ethylene in plants) before it can be converted to ethylene [30]. The result of lowering the level of ethylene in legume hosts is that both the number of nodules and the biomass of the plant may be increased by 25–40% [32, 33]. In the field, approximately 1–10% of rhizobial strains naturally possess ACC deaminase [34] thus it is possible to increase the nodulation efficiency of Rhizobia strains that lack ACC deaminase by engineering these strains with Rhizobia ACC deaminase genes (and regulatory regions) isolated from other strains. In one instance, insertion of an ACC deaminase gene from R. leguminosarum bv. viciae into the chromosomal DNA of a strain of Sinorhizobium meliloti that lacked this enzyme dramatically increased both the nodule number and biomass of host alfalfa plants [33]. However, because of political/regulatory considerations, genetically engineered strains of Rhizobia are currently not acceptable for use in the field in most jurisdictions. This political/regulatory constraint notwithstanding, several commercial inoculant producers have already begun to screen/test their more recently isolated Rhizobia strains for active ACC deaminase.

3.1.2. Phosphate Solubilization. Despite the fact that the amount of phosphorus in the soil is generally quite high (often between 400 and 1,200 mg kg⁻¹ of soil) most of this phosphorus is insoluble and therefore not available to support plant growth. The insoluble phosphorus is present as either an inorganic mineral such asapatite or as one of several organic forms including inositol phosphate (soil phytate), phosphomonoesters, and phosphotriesters [35]. In addition, much of the soluble inorganic phosphorus that is used as chemical fertilizer is immobilized soon after it is applied so that it then becomes unavailable to plants and is therefore wasted.

The limited bioavailability of phosphorus from the soil combined with the fact that this element is essential for plant growth means that the inability to obtain sufficient phosphorus often limits plant growth [36]. Thus, solubilization and mineralization of phosphorus by phosphate-solubilizing bacteria is an important trait in PGPB as well as in plant growth-promoting fungi such as mycorrhizae [37, 38].

Typically, the solubilization of inorganic phosphorus occurs as a consequence of the action of low molecular
weight organic acids such as gluconic and citric acid, both of
which are synthesized by various soil bacteria [38–40]. On
the other hand, the mineralization of organic phosphorus
occurs through the synthesis of a variety of different phos-
phatases, catalyzing the hydrolysis of phosphoric esters [38].
Importantly, phosphate solubilization and mineralization
can coexist in the same bacterial strain [42].

Unfortunately, because of variable results, the commer-
cial application of phosphate-solubilizing PGPB has been
quite limited. In fact, the most consistent positive effects of
applying phosphate-solubilizing bacteria are seen when these
bacteria are coinoculated with bacteria with other physiologi-
cal capabilities such as N fixation, or with mycorrhizal or
nonmycorrhizal fungi [42].

3.1.3. Sequestering Iron. Despite the fact that iron is the
fourth most abundant element on earth, in aerobic soils,
iron is not readily assimilated by either bacteria or plants
because ferric ion or Fe$^{3+}$, which is the predominant form in
nature, is only sparingly soluble so that the amount of iron
available for assimilation by living organisms is extremely low
[43]. Both microorganisms and plants require a high level of
iron, and obtaining sufficient iron is even more problematic
in the rhizosphere where plant, bacteria and fungi compete
for iron [44, 45]. To survive with such a limited supply of
iron, bacteria synthesize low-molecular mass siderophores
(\(\sim 400–1500\) Da), molecules with an exceptionally high affinity
for Fe$^{3+}$ \((K_a\) ranging from \(10^{23}\) to \(10^{52}\)) as well as mem-
brane receptors able to bind the Fe-siderophore complex,
thereby facilitating iron uptake by microorganisms [46, 47].
At the present time, there are over 500 known siderophores;
the chemical structures of 270 of these compounds have been
determined [46].

The direct benefits of bacterial siderophores on the
growth of plants have been demonstrated in several different
types of experiments. For example, (i) several studies using
radiolabeled ferric-siderophores as a sole source of iron
showed that plants are able to take up the labeled iron [48–
55]; (ii) mung bean plants, inoculated with the siderophore-
producing \textit{Pseudomonas} strain GRP3 and grown under iron-
limiting conditions, showed reduced chlorotic symptoms and
an enhanced chlorophyll level compared to uninoculated
plants [56]; (iii) the Fe-pyoverdine complex synthesized by
\textit{Pseudomonas fluorescens} C7 was taken up by \textit{Arabidopsis
thaliana} plants, leading to an increase of iron inside plant
issues and to improved plant growth [57].

The provision of iron to plants by soil bacteria is even
more important when the plants are exposed to an environ-
mental stress such as heavy metal pollution. In this case,
siderophores help to alleviate the stresses imposed on plants
by high soil levels of heavy metals [58–62].

Plant iron nutrition can affect the structure of bacterial
communities in the rhizosphere. For example, transgenic
tobacco that overexpresses ferritin and accumulates more
iron than nontransformed tobacco has less bioavailable iron
in the rhizosphere [63]. As a consequence, the composition
of the rhizosphere bacterial community differed significantly
when compared to nontransformed tobacco lines.

3.2. Modulating Phytohormone Levels. Plant hormones play
key roles in plant growth and development and in the
response of plants to their environment [64]. Moreover,
during its lifetime, a plant is often subjected to a number
of nonlethal stresses that can limit its growth until either
the stress is removed or the plant is able to adjust its
metabolism to overcome the effects of the stress [65]. When
plants encounter growth limiting environmental conditions,
they often attempt to adjust the levels of their endogenous
phytohormones in order to decrease the negative effects
of the environmental stressors [66]. While this strategy is
times successful, rhizosphere microorganisms may
also produce or modulate phytohormones under in vitro
conditions [66] so that many PGPB can alter phytohormone
levels and thereby affect the plant's hormonal balance and its
response to stress [65].

3.2.1. Cytokinins and Gibberellins. Several studies have
shown that many soil bacteria in general, and PGPB in par-
cular, can produce either cytokinins or gibberellins or both
[67–72]. Thus, for example, cytokinins have been detected in
the cell-free medium of some strains of \textit{Azotobacter} ssp., \textit{Rhizobium}
spp., \textit{Pantoea agglomerans}, \textit{Rhodospirillum rubrum},
\textit{Pseudomonas fluorescens}, \textit{Bacillus subtilis}, and \textit{Paenibacil-
lus polymyxa}. Moreover, plant growth promotion by some
cytokinin- or gibberellin-producing PGPB has been reported
[73–77]. However, a detailed understanding of the role of
bacterially-synthesized hormones and how the bacterial pro-
duction of these plant hormones is regulated is not currently
available. Thus, much of what we believe to be the role of
bacterially-produced cytokinins and gibberellins is based on
our knowledge of plant physiological studies following the
exogenous addition of purified hormones to growing plants.
Finally, some strains of phytopathogens can also synthesize
cytokinins. However, it appears that PGPB produce lower
cytokinin levels compared to phytopathogens so that the
effect of the PGPB on plant growth is stimulatory while the
effect of the cytokinins from pathogens is inhibitory.

3.2.2. Indoleacetic Acid. Although several naturally occur-
ring auxins have been described in the literature, indole-3-
acetic acid (indoleacetic acid, IAA) is by far the most common
as well as the most studied auxin, and much of the scientific
literature considers auxin and IAA to be interchangeable
terms [78, 79]. IAA affects plant cell division, extension,
and differentiation; stimulates seed and tuber germination;
mediates responses to light, gravity and florescence; affects
photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful
conditions [80, 81].

It has been known for more than 70 years that different
IAA concentrations affect the physiology of plants in dra-
matically different ways. Plant responses to IAA vary from
one type of plant to another, where some plants are more
or less sensitive to IAA than other plants; according to the
particular tissue involved, for example, in roots versus shoots

SCIENTIFICA
IAA synthesized by bacteria may be involved at different levels in plant-bacterial interactions. In particular, plant growth promotion and root nodulation are both affected by IAA. The role of IAA that was synthesized by the PGPB *Pseudomonas putida* GR12-2 in the development of canola roots was studied following the construction of an IAA-deficient mutant of this strain [83]. Seed inoculation with wild-type *P. putida* GR12-2 induced the formation of roots that were 35–50% longer than the roots from seeds treated with the IAA-deficient mutant and the roots from uninoculated seeds. On the other hand, inoculation of mung bean cuttings with a mutant of the same strain [84], which overproduces IAA, yielded a much greater number of shorter roots compared with controls [85]. This result was explained by the combined effect of auxin on growth promotion and inhibition of root elongation by ethylene [86]. The bacterial IAA that was incorporated by the plant stimulated the activity of the enzyme ACC synthase, resulting in increased synthesis of ACC [86], and a subsequent rise in ethylene that inhibited root elongation [87]. Overall, bacterial IAA increases root surface area and length, and thereby provides the plant with greater access to soil nutrients. In addition, bacterial IAA loosens plant cell walls and as a result facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria.

Most *Rhizobium* strains that have been examined have been found to produce IAA [88] and several studies have suggested that increases in auxin levels in the host plant are necessary for nodule formation [89]. Thus, mutants of the bacterium *Bradyrhizobium elkanii* that had a decreased level of IAA synthesis induced fewer nodules on soybean roots than did the wild-type strain [90]. In addition, in nodules induced by low IAA-producing mutants of *Rhizobium* sp. NGR234, the IAA content was found to be lower than in nodules induced by the wild-type strain, supporting the idea that part of the IAA found in nodules is of prokaryotic origin and that this IAA facilitates nodulation [91].

3.2.3. Ethylene. The plant hormone ethylene is one of the simplest molecules with biological activity. According to the Hebrew Bible, the prophet Amos was a "herdsman and a nipper of figs." This statement is interpreted as indicating that as early as the ninth century B.C.E., an awareness existed that nipping or piercing figs produced ethylene gas thereby hastening the ripening process and making the figs sweeter.

The plant hormone ethylene has a wide range of biological activities and is active at concentrations as low as 0.05 μL/L although ripening fruit may have ethylene levels of ~200 μL/L [92]. Ethylene can affect plant growth and development in a large number of different ways including promoting root initiation, inhibiting root elongation, promoting fruit ripening, promoting flower wilting, stimulating seed germination, promoting leaf abscission, activating the synthesis of other plant hormones, inhibiting *Rhizobia* spp. nodule formation, inhibiting mycorrhizae-plant interaction, and responding to both biotic and abiotic stresses [92]. The ethylene that is synthesized as a response to various stresses is called "stress ethylene" [92] and it describes the increase in ethylene synthesis that is typically associated with various environmental stresses including extremes of temperature; high light; flooding; drought; the presence of toxic metals and organic pollutants; radiation; wounding; insect predation; high salt; various pathogens including viruses, bacteria, and fungi [93]. The increased amount of ethylene that is formed in response to various environmental stresses can exacerbate some of the symptoms of the stress or it can lead to responses that enhance plant survival under adverse conditions. This seemingly contradictory behavior may be explained by a model wherein plants that are exposed to stress quickly respond by producing a small peak of ethylene that initiates a protective response by the plant, for example, transcription of genes encoding defensive proteins [65, 94]. If the stress persists or is intense, a second much larger peak of ethylene occurs, often several days later. This second ethylene peak induces processes such as senescence, chlorosis, and abscission that may lead to a significant inhibition of plant growth and survival.

Following the discovery in soil bacteria of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase [95], several studies indicated that this enzyme was a common feature of plant growth by PGPB was elaborated [98]. In this model, PGPB colonize the seed or root of a growing plant and, in response to tryptophan and other small molecules in seed or root exudates, the bacteria synthesize and secrete IAA [78, 83]. This bacterial IAA, together with endogenous plant IAA, can either stimulate plant growth or induce the synthesis of the plant enzyme ACC synthase that converts the compound S-adenosyl methionine to ACC, the immediate precursor of ethylene in all higher plants. A portion of the newly synthesized ACC is excluded from seeds or plant roots [99], taken up by the PGPB, and converted by the enzyme ACC deaminase to ammonia and α-ketobutyrate, compounds that are readily assimilated. As a direct consequence of this enzyme’s activity, the amount of ethylene produced by the plant is reduced. Therefore, root or seed colonization by PGPB that synthesize ACC deaminase prevents plant ethylene levels from becoming growth inhibitory [20, 98]. In the short term, the main visible effect of seed or root inoculation with ACC deaminase-producing bacteria is the enhancement of plant root elongation; promotion of shoot growth is generally seen in longer term experiments [13, 100–107]. In addition, other processes such as nodulation...
of legumes and mycorrhizal establishment in the host plant induce local increases in ethylene content. As a result, by lowering the local ethylene content in these plants, ACC deaminase-producing bacteria can increase the extent of both rhizobial noduleation and mycorrhizal colonization, in various legumes such as pea, alfalfa, mung bean, and chickpea [32, 33, 107, 108] and cucumber [109], respectively.

4. Indirect Mechanisms

The ability of biocontrol bacteria to indirectly promote plant growth has been the source of considerable interest, both in terms of (i) developing an understanding of some of the underlying mechanisms used by the biocontrol bacteria and (ii) utilizing these bacteria commercially instead of chemical pesticides. In fact, these two objectives are largely complementary. That is, understanding the mechanisms that are employed by biocontrol bacteria should facilitate the subsequent efficacious use of these bacterial strains in an applied setting.

4.1. Antibiotics and Lytic Enzymes. The synthesis of a range of different antibiotics is the PGPB trait that is most often associated with the ability of the bacterium to prevent the proliferation of plant pathogens (generally fungi) [110–115]. Many of these antibiotics together with their specificity and mode of action have been studied in detail, and some of these biocontrol strains have been commercialized. One problem with depending too much on antibiotic-producing bacteria as biocontrol agents is that with the increased use of these strains, some phytopathogens may develop resistance to specific antibiotics. To prevent this from happening, some researchers have utilized biocontrol strains that synthesize hydrogen cyanide as well as one or more antibiotics. This approach is effective because, while hydrogen cyanide may not have much biocontrol activity by itself, it appears to act synergistically with bacterially encoded antibiotics.

Some biocontrol bacteria produce enzymes including chitinases, cellulases, β-1,3 glucanases, proteases, and lipases that can lyse a portion of the cell walls of many pathogenic fungi. PGPB that synthesize one or more of these enzymes have been found to have biocontrol activity against a range of pathogenic fungi including Botrytis cinerea, Sclerotium rolfsii, Fusarium oxysporum, Phytophthora spp., Rhizoctonia solani, and Pythium ultimum [116–119].

4.2. Siderophores. Some bacterial strains that do not employ any other means of biocontrol can act as biocontrol agents using the siderophores that they produce. In this case, siderophores from PGPB can prevent some phytopathogens from acquiring a sufficient amount of iron thereby limiting their ability to proliferate [120, 121]. It has been suggested that this mechanism is effective because biocontrol PGPB produce siderophores that have a much greater affinity for iron than do fungal pathogens [122] so that the fungal pathogens are unable to proliferate in the rhizosphere of the roots of the host plant because of a lack of iron [123]. In this model, the biocontrol PGPB effectively out-compete fungal pathogens for available iron.

On the other hand, the growth of plants is generally not affected by the depletion of iron in the rhizosphere caused by the siderophores produced by biocontrol PGPB because most plants can grow at much lower iron concentrations than most microorganisms [123]. In addition, many plants can bind, take up and then utilize the biocontrol PGPB iron-siderophore complex [124, 125].

Experimental evidence that is consistent with the involvement of biocontrol PGPB siderophores in the suppression of fungal pathogen-caused plant disease comes from several different studies. For example, some studies have included the use of mutants that were defective in siderophore production and found that these strains were less effective than the wild-type strains at protecting plants against fungal pathogens [126–128]. On the other hand, one study observed that siderophore overproducing mutants were more effective at protecting plants against fungal pathogens [129].

4.3. Competition. Although it is difficult to demonstrate directly, some indirect evidence indicates that competition between pathogens and nonpathogens (PGPB) can limit disease incidence and severity. Thus, for example, abundant nonpathogenic soil microbes rapidly colonize plant surfaces and use most of the available nutrients, making it difficult for pathogens to grow. For example, in one series of experiments, researchers demonstrated that treatment of plants with the leaf bacterium Sphingomonas sp. prevented the bacterial pathogen Pseudomonas syringae pv. tomato from causing pathogenic symptoms [130].

4.4. Ethylene. Plants typically respond to the presence of phytopathogens by synthesizing stress ethylene that exacerbates the effects of the stress on the plant [92]. Thus, one way to decrease the damage to plants caused by a wide range of phytopathogens is to lower the plant’s ethylene response [131]. The simplest way to do this is to treat plants (generally the roots or seeds are treated) with ACC deaminase-containing PGPB [98]. To date, this strategy has been shown, in greenhouse and growth chamber experiments, to lower the damage to cucumber, potato, castor bean, tomato, carrot, and soybean plants [132–136]. Importantly, these studies have tested several different phytopathogens including Pythium ultimum, Fusarium oxysporum, Erwinia carotovora, Agrobacterium tumefaciens, Agrobacterium vitis, Sclerotium rolfsii, and Rhizoctonia solani. In addition, transgenic plants that express a bacterial ACC deaminase are protected to a significant level against damage from various phytopathogens [137, 138]. Notwithstanding these potentially exciting results, the ability of ACC deaminase-containing PGPB to decrease the damage to plants from pathogens, in the field, has not been tested. This likely reflects a reluctance of many individuals to deal with the potentially difficult regulatory approval process for doing this sort of field testing.

4.5. Induced Systemic Resistance. PGPB can trigger a phenomenon in plants known as induced systemic resistance
(ISR) that is phenotypically similar to the systemic acquired resistance (SAR) that occurs when plants activate their defense mechanisms in response to infection by a pathogenic agent [139]. ISR-positive plants are said to be “primed” so that they react faster and more strongly to pathogen attack by inducing defense mechanisms. ISR does not target specific pathogens. Rather, it may be effective at controlling diseases caused by different pathogens. ISR involves jasmonate and ethylene signaling within the plant and these hormones stimulate the host plant’s defense responses to a range of pathogens [140]. ISR does not require any direct interaction between the resistance-inducing PGPB and the pathogen [141]. Besides ethylene and jasmonate, other bacterial molecules such as the O-antigenic side chain of the bacterial outer membrane protein lipopolysaccharide, flagellar proteins, pyoverdine, chitin, β-glucans, cyclic lipopeptide surfactants, and salicylic acid have all been reported to act as signals for the induction of systemic resistance.

5. Modulating the Effects of Environmental Stress

Under ideal circumstances, a large portion of a plant’s growth and development may be thought of as proceeding in a more or less linear fashion over time [65]. However, in the field, the growth of plants may be inhibited by a large number of different biotic and abiotic stresses. These stresses include extremes of temperature, high light, flooding, drought, the presence of toxic metals and environmental organic contaminants, radiation, wounding, insect predation, nematodes, high salt, and various pathogens including viruses, bacteria and fungi. Therefore, as a consequence of these many different environmental stresses, plant growth is invariably lower than it would be in their absence. Moreover, during its life, a plant may be subjected to a number of nonlethal stresses that limit its growth until either the stress is removed or the plant is able to adjust its metabolism to overcome the stress. Thus, in practice, plant growth typically consists of periods of maximal growth interspersed with periods of various levels of growth inhibition. When they are added to plants, PGPB may employ any one or more of several different mechanistic strategies in an effort to overcome this growth inhibition.

5.1. Ethylene. Most of the aforementioned environmental stresses result in the production of inhibitory levels of stress ethylene. As mentioned above when discussing the stress ethylene produced as a consequence of phytopathogen infection, high levels of ethylene and the damage that it causes may be at least partially avoided by employing ACC deaminase-containing PGPB [142]. Some of the abiotic stresses whose effects can be ameliorated in this way include temperature extremes [143], flooding [144], drought [145, 146], metals and metalloids [60, 61, 147–152], hypoxia [153], salt [154–156], and organic contaminants [150, 151, 166–168].

The above mentioned reports from all over the world indicating that numerous different ACC deaminase-containing PGPB can provide significant protection to plants from a range of abiotic stresses suggests that this technology is ready to be utilized commercially in the field and that this approach could make a significant impact on agricultural practice. However, given the reluctance in many jurisdictions to utilize bacteria in agriculture on a large scale, it is likely that ACC deaminase-containing bacteria are more likely to find their first large scale commercial uses as components of phytoremediation protocols, that is, the simultaneous use of bacteria and plants to remove metals and organic contaminants from the environment [3, 9].

5.2. IAA. There are several reports indicating that some PGPB that do not contain ACC deaminase are nevertheless able to protect plants against the deleterious effects of abiotic stresses. In the more recent scientific literature, it has been suggested that PGPB may help plants to overcome abiotic stresses by providing the plant with IAA that directly stimulates plant growth, even in the presence of otherwise inhibitory compounds [169–176].

In addition to the above mentioned reports, a large number of studies have suggested that the bacteria that most effectively protect plants against a wide range of different stresses produce both IAA and ACC deaminase [65, 177–179]. One model that describes how IAA and ACC deaminase synergistically promote plant growth may described as follows [20, 65, 178]: the amino acid tryptophan is excluded by plant roots and then taken up by PGPB bound to the roots, where it is converted into IAA. The bacterially produced IAA is secreted, taken up by plant cells and, together with the plant’s pool of IAA stimulates an auxin signal transduction pathway, including various auxin response factors. As a consequence, plant cells grow and proliferate; at the same time, some of the IAA promotes transcription of the gene encoding the enzyme ACC synthase, thereby yielding an increased concentration of ACC and eventually ethylene (as catalyzed by the enzyme ACC oxidase since ACC is the immediate precursor of ethylene). Various biotic and abiotic stresses may also either increase the synthesis of IAA or stimulate the transcription of the gene for ACC synthase. In the presence of a bacterium that contains the enzyme ACC deaminase, some ACC may be taken up the PGPB bound to the plant, and degraded to ammonia and α-ketobutyrate. Thus, an ACC deaminase-containing PGPB acts as a sink for ACC with the consequence that, following an environmental stress, a lower level of ethylene is produced by the plant and the stress response of the plant is decreased. As the level of ethylene in a plant increases, the transcription of auxin response factors is inhibited [65, 180–182]. In the absence of bacterial ACC deaminase, by limiting transcription of auxin response factors, ethylene limits both cell growth and proliferation, and (important for plant survival) IAA stimulation of the synthesis of additional ethylene. In the presence of ACC deaminase, less ethylene is formed. Thus, when ACC deaminase is present, transcription of auxin response factors is not inhibited, and IAA can stimulate cell growth and proliferation without simultaneously causing a buildup of ethylene. Consequently, ACC deaminase both decreases ethylene inhibition of plant growth, and allows IAA...
to maximally promote plant growth, both in the presence and absence of plant stress.

5.3. Cytokinin. Cytokinins are compounds with a structure resembling adenine (Sakakibara 2006) that are named based on their ability to promote cytokinesis or cell division in plants. They are produced by plants, some yeast strains and by a number of soil bacteria, including PGPB [66, 68]. Transgenic plants that overproduce cytokinins, especially during periods of abiotic stress, are significantly protected from the deleterious effects of those stresses [183]. Unfortunately, there are not yet any definitive studies indicating whether bacterially-produced cytokinins can also protect plants from abiotic stresses. This would involve a detailed comparison of the biological activity of cytokinin-producing PGPB with cytokinin minus mutants of those bacteria.

5.4. Trehalose. Trehalose is a nonreducing disaccharide, an α,α-1,1-glucoside, consisting of two molecules of α-glucose, that is widely distributed in nature. It is found in bacteria, yeast, fungi, plants, insects, and invertebrates. High levels of trehalose can act as a protectant against several different abiotic stresses including drought, high salt, and extremes of temperature. Trehalose, a highly stable molecule that is resistant to both acid and high temperature and can form a gel phase as cells dehydrate, replacing water and, as a result, decreasing damage from drought and salt. In addition, trehalose can prevent some of the protein degradation and aggregation that often occurs under both high and low temperature stresses.

One way to confer drought (and other stress) tolerance onto plants is to treat the plants with PGPB that have been engineered to overproduce trehalose [184, 185]. Thus, when bean plants were treated with the symbiotic bacterium Rhizobium etli that had been genetically engineered to overproduce trehalose, the host plants had more nodules, fixed more nitrogen, had more biomass and recovered to a greater extent from drought stress than plants inoculated with wild-type R. etli [185]. Similarly, when maize plants were treated with the PGPB Azospirillum brasilense that had previously been modified to overproduce trehalose, the treated plants were more drought resistant and produced more biomass than plants treated with wild-type A. brasilense [184]. Although it is also possible to engineer plants to overproduce trehalose, it is much simpler to use genetically manipulated PGPB to achieve the same end. In addition, a single engineered bacterial strain may effectively protect a large number of different crop plants.

5.5. Antifreeze. To function effectively in the field, a PGPB must be to persist and proliferate in the environment [186]. In addition, in cold and temperate climates many fungal phytopathogens are most destructive when the soil temperature is low. In those environments, cold tolerant (psychrotrophic) biocontrol PGPB are likely to be more effective in the field than mesophilic biocontrol strains. Moreover, in countries such as Canada, Sweden, Finland, and Russia, PGPB must be functional at the cool soil temperatures that are common in the spring (i.e., −5–10°C). It would also be advantageous if added PGPB were able to survive repeated freeze thaw cycles that are common during the winter in many places. Nearly twenty years ago, several workers reported for the first time that some psychrophilic and psychrotrophic bacteria, including PGPB, secrete antifreeze proteins into the surrounding medium when the bacteria are grown at low temperatures [187–189]. Bacterial antifreeze proteins, some of which may also have ice-nucleation activity, appear to regulate the formation of ice crystals outside of the bacterium, thereby protecting the bacterial cell wall and membrane from potentially lethal damage (piercing) from the formation of large ice crystals that might otherwise occur at freezing temperatures.

Since the initial reports of bacterial antifreeze proteins, there have been several additional reports documenting the isolation and characterization of bacterial antifreeze proteins [155, 190–195]. However, none of these studies have explored the possibility of using this activity to facilitate the functioning of PGPB in environments that include cold temperatures.

6. Conclusions

The use of PGPB as an integral component of agricultural practice is a technology whose time has come. These bacteria are already being used successfully in a number of countries in the developing world and this practice is expected to grow. In the more developed world, where agricultural chemicals remain relatively inexpensive, the use of PGPB occupies a small but growing niche in the development of organic agriculture. In addition, it is reasonable to expect the increased use of PGPB in various phytoremediation strategies.

However, the more widespread utilization of PGPB will necessitate that a number of issues be addressed. In the first instance, going from laboratory and greenhouse experiments to field trials to large scale commercial field use will require a number of new approaches for the growth, storage, shipping, formulation and application of these bacteria. Second, it will be necessary to educate the public about the use of PGPB in agriculture on a large scale. Much popular mythology is directed toward thinking about bacteria only as agents of disease. This misconception needs to be corrected before the public accepts the deliberate release of beneficial bacteria into the environment on a large scale. Third, while initial PGPB are likely to be nontransformed bacterial strains that have been selected for certain positive traits, it is likely in the future, as a greater understanding of the mechanisms at play in the bacterial stimulation of plant growth is gained, that scientists will genetically engineer more efficacious strains. Scientists will need to prove to both the public and to regulatory agencies worldwide that genetically engineered PGPB do not present any new hazards or risks. Fourth, scientists will need to determine whether future research should be directed toward developing PGPB that are rhizospheric or endophytic. Fifth, it will be necessary to better understand and then to optimize the relationship between PGPB and mycorrhizae [109].
Notwithstanding the above-mentioned constraints, there is every reason to believe that agricultural practice will slowly be able to shift its focus to the efficacious use of PGPB. Thus, the future of this technology looks extremely bright.

References

[1] M. Lucy, E. Reed, and B. R. Glick, "Applications of free living plant growth-promoting rhizobacteria," Antonie van Leeuwenhoek, vol. 86, no. 1, pp. 1–25, 2004.

[2] C. T. de Rosa, B. L. Johnson, M. Fay, H. Hansen, and M. M. Muntaz, "Public health implications of hazardous waste sites: findings, assessment and research," Food and Chemical Toxicology, vol. 34, no. 11–12, pp. 1131–1138, 1996.

[3] B. R. Glick, "Using soil bacteria to facilitate phytoremediation," Biotechnology Advances, vol. 28, no. 3, pp. 367–374, 2010.

[4] J. Ziegler, "Health risk assessment research: the OTA report," Environmental Health Perspectives, vol. 101, no. 5, pp. 402–406, 1993.

[5] I. Alkorta and C. Garbisu, "Phytoremediation of organic contaminants in soils," Bioresource Technology, vol. 79, no. 3, pp. 273–276, 2001.

[6] E. Pilon-Smiths, "Phytoremediation," Annual Review of Plant Biology, vol. 56, pp. 15–39, 2005.

[7] E. Pilon-Smiths and J. L. Freeman, "Environmental cleanup using plants: biotechnological advances and ecological considerations," Frontiers in Ecology and the Environment, vol. 4, no. 4, pp. 203–210, 2006.

[8] D. E. Salt, M. Blaylock, N. P. B. A. Kumar et al., "Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants," Nature Biotechnology, vol. 13, no. 5, pp. 468–474, 1995.

[9] E. Gamalero and B. R. Glick, "Plant growth-promoting bacteria and metal phytoremediation," in Phytotechnologies, N. A. Anjum, M. E. Pereira, I. Ahmad, A. C. Duarte, S. Umar, and N. A. Khan, Eds., pp. 359–374, Taylor & Francis, Boca Raton, FL, USA, 2012.

[10] B. R. Glick and J. C. Stearns, "Making phytoremediation work better: maximizing a plant’s growth potential in the midst of adversity," International Journal of Phytoremediation, vol. 13, no. 1, pp. 4–11, 2011.

[11] L. Schoenborn, P. S. Yates, B. E. Grinton, P. Hugenholtz, and P. H. Janssen, "Liquid serial dilution is inferior to solid media for isolation of cultures representative of the phylum-level diversity of soil bacteria," Applied and Environmental Microbiology, vol. 70, no. 7, pp. 4363–4366, 2004.

[12] S. Timmusk, V. Paalme, T. Pavlicek et al., "Bacterial distribution in the rhizosphere of wild barley under contrasting microclimates," PLoS One, vol. 6, no. 3, Article ID e17968, 2011.

[13] B. R. Glick, C. L. Patten, G. Holguin, and D. M. Penrose, Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria, Imperial College Press, London, UK, 1999.

[14] D. V. Badri, T. L. Weir, D. van der Lelie, and J. M. Vivanco, "Rhizosphere chemical dialogues: plant-microbe interactions," Current Opinion in Biotechnology, vol. 20, no. 6, pp. 642–650, 2009.

[15] D. V. Badri and J. M. Vivanco, "Regulation and function of root exudates," Plant, Cell and Environment, vol. 32, no. 6, pp. 666–681, 2009.

[16] H. P. Bais, T. L. Weir, L. G. Perry, S. Gilroy, and J. M. Vivanco, "The role of root exudates in rhizosphere interactions with plants and other organisms," Annual Review of Plant Biology, vol. 57, pp. 233–266, 2006.

[17] J. M. Whipps, "Carbon utilization," in The Rhizosphere, J. M. Lynch, Ed., pp. 59–97, Wiley-Interscience, Chichester, UK, 1990.

[18] J. M. Lynch, Ed., The Rhizosphere, Wiley-Interscience, Chichester, UK, 1990.

[19] A. N. Dubeikovsky, E. A. Mordukhova, V. V. Kochetkov, F. Y. Polikarpova, and A. M. Boronin, "Growth promotion of blackcurrant softwood cuttings by recombinant strain Pseudomonas fluorescens BSP53a synthesizing an increased amount of indole-3-acetic acid," Soil Biology and Biochemistry, vol. 25, no. 9, pp. 1277–1281, 1993.

[20] B. R. Glick, "The enhancement of plant growth by free-living bacteria," Canadian Journal of Microbiology, vol. 41, no. 2, pp. 109–117, 1995.

[21] R. O. D. Dixon and C. T. Wheeler, Nitrogen Fixation in Plants, Blackie and Son, Glasgow, UK, 1986.

[22] H. M. Fischer, "Genetic regulation of nitrogen fixation in rhizobia," Microbiological Reviews, vol. 58, no. 3, pp. 352–386, 1994.

[23] S. R. Long, W. J. Buikema, and F. M. Ausubel, "Cloning of Rhizobium meliloti nodulation genes by direct complementation of Nod mutants," Nature, vol. 298, no. 5873, pp. 485–488, 1982.

[24] J. W. Klopper, R. Lifshitz, and R. M. Zabloutowicz, "Free-living bacterial inocula for enhancing crop productivity," Trends in Biotechnology, vol. 7, no. 2, pp. 39–44, 1989.

[25] M. R. Banerjee, L. Yesmin, and J. K. Vessey, "Plant-growth-promoting rhizobacteria as biofertilizers and biopesticides," in Handbook of Microbial Biofertilizers, M. K. Rai, Ed., pp. 137–181, Food Products Press, Binghamton, NY, USA, 2006.

[26] Y. Bashan and H. Levanony, "Current status of Azospirillum inoculation technology: Azospirillum as a challenge for agriculture," Canadian Journal of Microbiology, vol. 36, no. 9, pp. 591–608, 1990.

[27] E. K. James and F. L. Oliveira, "Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs," Critical Reviews in Plant Sciences, vol. 17, no. 1, pp. 77–119, 1997.

[28] S. Marroquín, A. Zorreguieta, C. Santamaria et al., "Enhanced symbiotic performance by Rhizobium tropici glycogen synthase mutants," Journal of Bacteriology, vol. 183, no. 3, pp. 854–864, 2001.

[29] M. Ramírez, B. Valderrama, R. Arredondo-Peter, M. Soberón, J. Mora, and G. Hernández, "Rhizobium etli genetically engineered for the heterologous expression of Viteoscilla sp. hemoglobin: effects on free-living and symbiosis," Molecular Plant-Microbe Interactions, vol. 12, no. 11, pp. 1008–1015, 1999.

[30] W. Ma, D. M. Penrose, and B. R. Glick, "Strategies used by rhizobia to lower plant ethylene levels and increase nodulation," Canadian Journal of Microbiology, vol. 48, no. 11, pp. 947–954, 2002.

[31] K. I. Yuhashi, N. Ichikawa, H. Ezura et al., "Rhizobitoxine production by Bradyrhizobium elkanii enhances nodulation and competitiveness on Macroptilium atropurpureum," Applied and Environmental Microbiology, vol. 66, no. 6, pp. 2658–2663, 2000.

[32] W. Ma, F. C. Guinel, and B. R. Glick, "Rhizobium leguminosarum biovar viciea 1-aminoacyclopropane-1-carboxylate deaminase promotes nodulation of pea plants," Applied and Environmental Microbiology, vol. 69, no. 8, pp. 4396–4402, 2003.
[98] B. R. Glick, D. M. Penrose, and J. Li, "A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria," *Journal of Theoretical Biology*, vol. 190, no. 1, pp. 63–68, 1998.

[99] D. M. Penrose and B. R. Glick, "Levels of ACC and related compounds in exudate and extracts of canola seeds treated with ACC deaminase-containing plant growth-promoting bacteria," *Canadian Journal of Microbiology*, vol. 47, no. 4, pp. 368–372, 2001.

[100] C. Contesto, G. Desbrosses, C. Lefoulon et al., "Effects of rhizobacterial ACC deaminase activity on Arabidopsis indicate that ethylene mediates local root responses to plant growth-promoting rhizobacteria," *Plant Science*, vol. 175, no. 1–2, pp. 178–189, 2008.

[101] R. Dey, K. K. Pal, D. M. Bhatt, and S. M. Chauhan, "Growth promotion and yield enhancement of peanut (Arachis hypogaea L.) by application of plant growth-promoting rhizobacteria," *Microbiological Research*, vol. 159, no. 4, pp. 371–394, 2004.

[102] J. A. Hall, D. Peirson, S. Ghosh, and B. R. Glick, "Root elongation in various agronomic crops by the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2," *Israd Journal of Plant Sciences*, vol. 44, no. 1, pp. 37–42, 1996.

[103] F. Nascimento, C. Brígido, L. Alho, B. R. Glick, and S. Oliveira, "Enhanced chickpea growth promotion ability of a mesorhizobia expressing an exogenous ACC deaminase gene," *Plant and Soil*, vol. 353, no. 1–2, pp. 221–230, 2012.

[104] M. Naveed, Z. A. Zahir, M. Khalid, H. N. Asghar, M. J. Akhtar, and M. Arshad, "Rhizobacteria containing acc-deaminase for improving growth and yield of wheat under fertilized conditions," *Pakistan Journal of Botany*, vol. 40, no. 3, pp. 1231–1241, 2008.

[105] J. Onofre-Lemus, I. Hernández-Lucas, L. Girard, and J. Caballero-Mellado, "ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, a widespread trait in *Burkholderia* species, and its growth-promoting effect on tomato plants," *Applied and Environmental Microbiology*, vol. 75, no. 20, pp. 6581–6590, 2009.

[106] S. Shah, J. Li, B. A. Moffatt, and B. R. Glick, "Isolation and characterization of ACC deaminase genes from two different plant growth-promoting rhizobacteria," *Canadian Journal of Microbiology*, vol. 44, no. 9, pp. 833–843, 1998.

[107] B. Shaharoona, M. Arshad, and Z. A. Zahir, "Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (Zea mays L.) growth under axenic conditions and on nodulation in mung bean (Vigna radiata L.)," *Letters in Applied Microbiology*, vol. 42, no. 2, pp. 155–159, 2006.

[108] F. X. Nascimento, C. Brígido, B. R. Glick, S. Oliveira, and L. Alho, "Mesorhizobium ciceri LMS-1 expressing an exogenous ACC deaminase increases its nodulation abilities and chickpea plant resistance to soil constraints," *Letters in Applied Microbiology*, vol. 55, pp. 15–21, 2012.

[109] E. Gamalero, G. Berta, N. Massa, B. R. Glick, and G. Lingua, "Synergistic interactions between the ACC deaminase-producing bacterium *Pseudomonas putida* UW4 and the AM fungus *Gigaspora rosea* positively affect cucumber plant growth," *FEMS Microbiology Ecology*, vol. 64, no. 3, pp. 459–467, 2008.

[110] S. Compton, B. Duffy, J. Nowak, C. Clément, and E. Ait Barka, "Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects," *Applied and Environmental Microbiology*, vol. 71, no. 9, pp. 4951–4959, 2005.

[111] D. Haas and C. Keel, "Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant diseases," *Annual Review of Phytopathology*, vol. 41, pp. 117–153, 2003.

[112] S. Mazurier, T. Corberand, P. Lemanceau, and J. M. Raaijmakers, "Phenazine antibiotics produced by fluorescent pseudomonads contribute to natural soil suppressiveness to *Fusarium wilt*," *ISME Journal*, vol. 3, no. 8, pp. 977–991, 2009.

[113] K. K. Pal and B. McSpadden Gardener, "Biological control of plant pathogens," *The Plant Health Instructor*. In press http://www.apsnet.org/edcenter/advanced/topics/Documents/PHI-BiologicalControl.pdf.

[114] J. M. Raaijmakers, M. Vlam, and J. T. de Souza, "Antibiotic production by bacterial biocontrol agents," *Antonie van Leeuwenhoek*, vol. 81, no. 1–4, pp. 537–547, 2002.

[115] J. M. Whipp, "Microbial interactions and biocontrol in the rhizosphere," *Journal of Experimental Botany*, vol. 52, pp. 487–511, 2001.

[116] J. Frankowski, M. Lorito, F. Scala, R. Schmid, G. Berg, and H. Bahl, "Purification and properties of two chitinolytic enzymes of *Serratia plymuthica* HRO-C48," *Archives of Microbiology*, vol. 176, no. 6, pp. 421–426, 2001.

[117] Y. C. Kim, H. Jung, K. Y. Kim, and S. K. Park, "An effective biocontrol bioformulation against *Phytophthora* blight of pepper using growth mixtures of combined chitinolytic bacteria under different field conditions," *European Journal of Plant Pathology*, vol. 120, no. 4, pp. 373–382, 2008.

[118] A. Ordentlich, Y. Elad, and I. Chet, "The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*," *Phytopathology*, vol. 78, pp. 84–88, 1988.

[119] P. P. Singh, Y. C. Shin, C. S. Park, and Y. R. Chung, "Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria," *Phytopathology*, vol. 89, no. 1, pp. 92–99, 1999.

[120] D. N. Dowling, R. Sexton, A. Fenton et al., "Iron regulation in different field conditions," *using growth mixtures of combined chitinolytic bacteria under different field conditions," *European Journal of Plant Pathology*, vol. 120, no. 4, pp. 373–382, 2008.

[121] S. Mazurier, T. Corberand, P. Lemanceau, and J. M. Raaijmakers, "Phenazine antibiotics produced by fluorescent pseudomonads contribute to natural soil suppressiveness to *Fusarium wilt*," *ISME Journal*, vol. 3, no. 8, pp. 977–991, 2009.

[122] J. W. Kloepper, J. Leong, M. Teintze, and M. N. Schroth, "Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria," *Molecular Biology of Pseudomonads*, T. Nakazawa, K. Furukawa, D. Haas, and S. Silver, Eds., pp. 502–511, American Society for Microbiology Press, Washington, DC, USA, 1996.

[123] J. W. Klopper, J. Leong, M. Teintze, and M. N. Schroth, "Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria," *Nature*, vol. 286, no. 5776, pp. 885–886, 1980.

[124] B. Schippers, A. W. Bakker, and A. H. M. Bakker, "Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practice," *Annual Review of Phytopathology*, vol. 25, pp. 339–358, 1987.

[125] D. J. O’Sullivan and F. O’Gara, "Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens," *Microbiological Reviews*, vol. 56, no. 4, pp. 662–676, 1992.

[126] S. Buysens, K. Heungens, J. Poppe, and M. Höfte, "Involvement of pyochelin and pyoverdin in suppression of *Pythium*-induced
damping-off of tomato by Pseudomonas aeruginosa 7NSK2,” *Applied and Environmental Microbiology*, vol. 62, no. 3, pp. 865–871, 1996.

[127] M. Elsherif and F. Grossmann, “Comparative investigations on the antagonistic activity of fluorescent pseudomonads against *Gaeumannomyces graminis* var. tritici in vitro and in vivo,” *Microbiological Research*, vol. 149, no. 4, pp. 371–377, 1994.

[128] G. Martinetti and J. E. Loper, “Mutational analysis of genes determining antagonism of *Alcaligenes* sp. strain MFA1 against the phytopathogenic fungus *Fusarium oxysporum*,” *Canadian Journal of Microbiology*, vol. 38, no. 3, pp. 241–247, 1992.

[129] P. A. Vandenbergh and C. F. Gonzalez, “Method for protecting the growth of plants employing mutant siderophore producing strains of *Pseudomonas putida*,” United States Patent Number: 4, 479, 936, 1984.

[130] G. Innererbner, C. Knief, and J. A. Vorholt, “Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system,” *Applied and Environmental Microbiology*, vol. 77, no. 10, pp. 3202–3210, 2011.

[131] B. R. Glick and Y. Bashan, “Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens,” *Biotechnology Advances*, vol. 15, no. 2, pp. 353–378, 1997.

[132] Y. Hao, T. C. Charles, and B. R. Glick, “ACC deaminase from plant growth-promoting bacteria affects crown gall development,” *Canadian Journal of Microbiology*, vol. 53, no. 12, pp. 1291–1299, 2007.

[133] Y. Hao, T. C. Charles, and B. R. Glick, “An ACC deaminase containing *A. tumefaciens* strain D3 shows biocontrol activity to crown gall disease,” *Canadian Journal of Microbiology*, vol. 57, no. 4, pp. 278–286, 2011.

[134] E. Husen, A. T. Wahyudi, A. Suwanto, and G. I. Burd, “Growth enhancement and disease reduction of soybean by 1-amino-cyclopropane-1-carboxylic acid deaminase-producing *Pseudomonas*,” *American Journal of Applied Sciences*, vol. 8, no. 11, pp. 1073–1080, 2011.

[135] N. Toklikishvili, N. Dandurishvili, M. Tediashvili et al., “Inhibitory effect of ACC deaminase-producing bacteria on crown gall formation in tomato plants infected by *Agrobacterium tumefaciens* or *A. vitis*,” *Plant Pathology*, vol. 59, no. 6, pp. 1023–1030, 2010.

[136] C. Wang, E. Knill, B. R. Glick, and G. Défago, “Effect of transferring 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase genes into *Pseudomonas fluorescens* strain CHA0 and its gacA derivative CHA96 on their growth-promoting and disease-suppressive capacities,” *Canadian Journal of Microbiology*, vol. 46, no. 10, pp. 898–907, 2000.

[137] S. T. Lund, R. E. Stall, and H. J. Klee, “Ethylene regulates the susceptible response to pathogen infection in tomato,” *Plant Cell*, vol. 10, no. 3, pp. 371–382, 1998.

[138] M. M. Robison, S. Shah, B. Tamot, K. P. Pauls, B. A. Moffatt, and B. R. Glick, “Reduced symptoms of Verticillium wilt in transgenic tomato expressing a bacterial ACC deaminase,” *Molecular Plant Pathology*, vol. 2, no. 3, pp. 135–145, 2001.

[139] C. M. J. Pieterse, A. Leon-Reyes, S. van der Ent, and S. C. M. van Wees, “Networking by small-molecule hormones in plant immunity,” *Nature Chemical Biology*, vol. 5, no. 5, pp. 308–316, 2009.

[140] B. W. M. Verhagen, J. Glazebrook, T. Zhu, H. S. Chang, L. C. van Loon, and C. M. J. Pieterse, “The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*,” *Molecular Plant-Microbe Interactions*, vol. 17, no. 8, pp. 895–908, 2004.

[141] P. A. H. M. Bakker, C. M. J. Pieterse, and L. C. van Loon, “Induced systemic resistance by fluorescent *Pseudomonas* spp,” *Phytopathology*, vol. 97, no. 2, pp. 239–243, 2007.

[142] B. R. Glick, “Bacterial ACC deaminase and the alleviation of plant stress,” *Advances in Applied Microbiology*, vol. 56, pp. 291–312, 2004.

[143] B. R. Glick, C. Liu, S. Ghosh, and E. B. Dumbroff, “Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2,” *Soil Biology and Biochemistry*, vol. 29, no. 8, pp. 1233–1239, 1997.

[144] V. P. Grichko and B. R. Glick, “Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria,” *Plant Physiology and Biochemistry*, vol. 39, no. 1, pp. 11–17, 2001.

[145] S. Mayak, T. Tiross, and B. R. Glick, “Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers,” *Plant Science*, vol. 166, no. 2, pp. 525–530, 2004.

[146] Z. A. Zahir, A. Munir, H. N. Asghar, B. Shaharoon, and M. Arshad, “Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions,” *Journal of Microbiology and Biotechnology*, vol. 18, no. 5, pp. 958–963, 2008.

[147] Z. Cheng, Y. Y. C. Wei, W. W. L. Sung, B. R. Glick, and B. J. McConkey, “Proteomic analysis of the response of the plant growth-promoting bacterium *Pseudomonas putida* UW4 to nickel stress,” *Proteome Science*, vol. 7, article 18, 2009.

[148] A. J. Farwell, S. Vesely, V. Nero et al., “The use of transgenic canola (*Brassica napus*) and plant growth-promoting bacteria to enhance plant biomass at a nickel-contaminated field site,” *Plant and Soil*, vol. 288, no. 1–2, pp. 309–318, 2006.

[149] L. Nie, S. Shah, A. Rashid, G. I. Burd, D. G. Dixon, and B. R. Glick, “Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth-promoting bacterium *Enterobacter cloacae* CAL2,” *Plant Physiology and Biochemistry*, vol. 40, no. 4, pp. 355–361, 2002.

[150] M. L. E. Reed and B. R. Glick, “Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons,” *Canadian Journal of Microbiology*, vol. 51, no. 12, pp. 1061–1069, 2005.

[151] M. L. E. Reed, B. G. Warner, and B. R. Glick, “Plant growth-promoting bacteria facilitate the growth of the common reed *Phragmites australis* in the presence of copper or polycyclic aromatic hydrocarbons,” *Current Microbiology*, vol. 51, no. 6, pp. 425–429, 2005.

[152] V. I. Safronova, V. V. Stepanok, G. L. Engvist, Y. V. Alekseyev, and A. A. Belimov, “Root-associated bacteria containing 1-amino-cyclopropane-1-carboxylic acid deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil,” *Biology and Fertility of Soils*, vol. 42, no. 3, pp. 267–272, 2006.

[153] J. Li, J. Sun, Y. Yang, S. Guo, and B. R. Glick, “Identification of hypoxic-responsive proteins in cucumber using a proteomic approach,” *Plant Physiology and Biochemistry*, vol. 51, pp. 74–80, 2012.
[149] M. Ahmad, Z. A. Zahir, H. N. Asghar, and M. Asghar, "Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase," Canadian Journal of Microbiology, vol. 57, no. 7, pp. 578–589, 2011.

[152] Z. Cheng, E. Park, and B. R. Glick, "1-Aminocyclopropane-1-carboxylate deaminase from Pseudomonas putida UW4 facilitates the growth of canola in the presence of salt," Canadian Journal of Microbiology, vol. 53, no. 7, pp. 912–918, 2007.

[153] Z. Cheng, O. Z. Woody, B. J. McConkey, and B. R. Glick, "Combined effects of the plant growth-promoting bacterium Pseudomonas putida UW4 and salinity stress on the Brassica napus proteome," Applied Soil Ecology, vol. 61, pp. 255–263, 2012.

[156] E. Gamalero, G. Berta, N. Massa, B. R. Glick, and G. Lingua, "Interactions between Pseudomonas putida UW4 and Gigaspora rosea BEG9 and their consequences for the growth of cucumber under salt-stress conditions," Journal of Applied Microbiology, vol. 108, no. 1, pp. 236–245, 2010.

[157] F. Jalili, K. Khavazi, E. Pazira et al., "Isolation and characterization of ACC deaminase-producing fluorescent pseudomonads, to alleviate salinity stress on canola (Brassica napus L.) growth," Journal of Plant Physiology, vol. 166, no. 6, pp. 667–674, 2009.

[158] S. Mayak, T. Tiros, and B. R. Glick, "Plant growth-promoting bacteria confer resistance in tomato plants to salt stress," Plant Physiology and Biochemistry, vol. 42, no. 6, pp. 565–572, 2004.

[159] S. M. Nadeem, Z. A. Zahir, M. Naveed, and M. Arshad, "Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminate activity," Canadian Journal of Microbiology, vol. 53, no. 10, pp. 1141–1149, 2007.

[160] M. Sadnina, N. Maksimava, E. Khromsova, S. Stanislavich, P. Owilia, and M. Arjomandzadegan, "Study the effect of bacterial 1-aminocyclopropane-1-carboxylated deaminase (ACC deaminase) on resistance to salt stress in tomato plants," Annals of University of Oradea, vol. 18, pp. 120–123, 2011.

[161] D. Saravanakumar and R. Samiyappan, "ACC deaminase from Pseudomonas fluorescens mediated saline resistance in groundnut (Arachis hypogea) plants," Journal of Applied Microbiology, vol. 102, no. 5, pp. 1283–1292, 2007.

[162] M. A. Siddiquee, P. S. Chauhan, R. Anandham, G. H. Han, and T. Sa, "Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil," Journal of Microbiology and Biotechnology, vol. 20, no. 11, pp. 1577–1584, 2010.

[163] M. A. Siddikee, B. R. Glick, P. S. Chauhan, W. J. Yin, and T. Sa, "Enhancement of growth and salt tolerance of red pepper seedlings (Capsicum annuum L.) by regulating stress ethylene synthesis with halotolerant bacteria containing 1-aminocyclopropane-1-carboxylic acid deaminase activity," Plant Physiology and Biochemistry, vol. 49, no. 4, pp. 427–434, 2011.

[164] H. T. Yue, W. P. Mo, C. Li, Y. Y. Zheng, and H. Li, "The salt stress relief and growth promotion effect of Rs-5 on cotton," Plant and Soil, vol. 297, no. 1-2, pp. 139–145, 2007.

[165] J. Gurska, W. Wang, K. E. Gerhardt et al., "Three year field test of a plant growth promoting rhizobacteria enhanced phytoremediation system at a land farm for treatment of hydrocarbon waste," Environmental Science and Technology, vol. 43, no. 12, pp. 4472–4479, 2009.

[166] X. D. Huang, Y. El-Alawi, D. M. Penrose, B. R. Glick, and B. M. Greenberg, "A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils," Environmental Pollution, vol. 130, no. 3, pp. 465–476, 2004.

[167] X. D. Huang, Y. El-Alawi, J. Gurska, B. R. Glick, and B. M. Greenberg, "A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils," Microchemical Journal, vol. 81, no. 1, pp. 139–147, 2005.

[168] C. Bianco and R. Defez, "Medicago truncatula improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing Sinorhizobium meliloti strain," Journal of Experimental Botany, vol. 60, no. 1, pp. 3097–3107, 2009.

[169] C. Bianco and R. Defez, "Improvement of phosphate solubilization and Medicago plant yield by an indole-3-acetic acid-overproducing strain of Sinorhizobium meliloti," Applied and Environmental Microbiology, vol. 76, no. 14, pp. 4626–4632, 2010.

[170] L. E. de-Bashan, J. P. Hernandez, K. N. Nelson, Y. Bashan, and R. M. Maier, "Growth of quailbush in acidic, metalliferous desert mine tailings: effect of Azospirillum brasilense Sp6 on biomass production and Rhizosphere community structure," Microbial Ecology, vol. 60, no. 4, pp. 915–927, 2010.

[171] D. Egamberdieva, "Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat," Acta Physiologiae Plantarum, vol. 31, no. 4, pp. 861–864, 2009.

[172] M. Rajkumar, R. Nagendra, K. J. Lee, W. H. Lee, and S. Z. Kim, "Influence of plant growth promoting bacteria and G6 on the growth of Indian mustard," Chemosphere, vol. 62, no. 5, pp. 741–748, 2006.

[173] X. F. Sheng and J. J. Xia, "Improvement of rape (Brassica napus) plant growth and cadmium uptake by cadmium-resistant bacteria," Chemosphere, vol. 64, no. 6, pp. 1036–1042, 2006.

[174] P. A. Wani, M. S. Khan, and A. Zaidi, "Effect of metal tolerant plant growth promoting Bradyrhizobium sp. (vigna) on growth, symbiosis, seed yield and metal uptake by greengram plants," Chemosphere, vol. 70, no. 1, pp. 36–45, 2007.

[175] P. A. Wani, M. S. Khan, and A. Zaidi, "Effect of metal-tolerant plant growth-promoting Rhizobium on the performance of pea grown in metal-amended soil," Archives of Environmental Contamination and Toxicology, vol. 55, no. 1, pp. 33–42, 2008.

[176] D. Egamberdieva and Z. Kucharova, "Selection for root colonizing bacteria stimulating wheat growth in saline soils," Biology and Fertility of Soils, vol. 45, no. 6, pp. 563–571, 2009.

[177] E. Gamalero and B. R. Glick, "Bacterial ACC deaminase and IAA: interactions and consequences for plant growth in polluted environments," in Handbook of Phytoremediation, I. A. Golubev, Ed., pp. 763–774, Nova Science, New York, NY, USA, 2010.

[178] V. Sgroy, F. Cassán, O. Masciarelli, M. F. Del Papa, A. Lagares, and V. Luna, "Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte Prosopis strombulifera," Applied Microbiology and Biotechnology, vol. 85, no. 2, pp. 371–381, 2009.

[179] I. C. Czarnecki, S. Shah, and B. R. Glick, "Response of canola plants at the transcriptional level to expression of a bacterial ACC deaminase in the roots," in Advances in Plant Ethylene Research, A. Ramina, C. Chang, J. Giovannoni, H. Klee, P. Perata, and E. Woltering, Eds., pp. 377–382, Springer, Dordrecht, The Netherlands, 2007.
[181] N. Dharmasiri and M. Estelle, “Auxin signaling and regulated protein degradation,” *Trends in Plant Science*, vol. 9, no. 6, pp. 302–308, 2004.

[182] J. C. Stearns, O. Z. Woody, B. J. McConkey, and B. R. Glick, “Effects of bacterial ACC deaminase on *Brassica napus* gene expression measured with an *Arabidopsis thaliana* microarray,” *Molecular Plant-Microbe Interactions*, vol. 25, pp. 668–676, 2012.

[183] R. M. Rivero, M. Kojima, A. Gepstein et al., “Delayed leaf senescence induces extreme drought tolerance in a flowering plant,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 49, pp. 19631–19636, 2007.

[184] J. Rodríguez-Salazar, R. Suárez, J. Caballero-Mellado, and G. Iturriaga, “Trehalose accumulation in *Azospirillum brasilense* improves drought tolerance and biomass in maize plants,” *FEMS Microbiology Letters*, vol. 296, no. 1, pp. 52–59, 2009.

[185] R. Suárez, A. Wong, M. Ramirez et al., “Improvement of drought tolerance and grain yield in common bean by overexpressing trehalose-6-phosphate synthase in rhizobia,” *Molecular Plant-Microbe Interactions*, vol. 21, no. 7, pp. 958–966, 2008.

[186] B. R. Glick and Y. C. Skof, “Environmental implications of recombinant DNA technology,” *Biotechnology Advances*, vol. 4, no. 2, pp. 261–277, 1986.

[187] J. G. Duman and T. M. Olsen, “Thermal hysteresis protein activity in bacteria, fungi, and phylogenetically diverse plants,” *Cryobiology*, vol. 30, no. 3, pp. 322–328, 1993.

[188] X. Sun, M. Griffith, J. J. Pasternak, and B. R. Glick, “Low temperature growth, freezing survival, and production of antifreeze protein by the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2,” *Canadian Journal of Microbiology*, vol. 41, no. 9, pp. 776–784, 1995.

[189] H. Xu, M. Griffith, C. L. Patten, and B. R. Glick, “Isolation and characterization of an antifreeze protein with ice nucleation activity from the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2,” *Canadian Journal of Microbiology*, vol. 44, no. 1, pp. 64–73, 1998.

[190] C. P. Garnham, J. A. Gilbert, C. P. Hartman, R. L. Campbell, J. Laybourn-Parry, and P. L. Davies, “A Ca$^{2+}$-dependent bacterial antifreeze protein domain has a novel β-helical ice-binding fold,” *Biochemical Journal*, vol. 411, no. 1, pp. 171–180, 2008.

[191] J. A. Gilbert, P. L. Davies, and J. Laybourn-Parry, “A hyperactive, Ca$^{2+}$-dependent antifreeze protein in an Antarctic bacterium,” *FEMS Microbiology Letters*, vol. 245, no. 1, pp. 67–72, 2005.

[192] J. A. Gilbert, P. J. Hill, C. E. R. Dodd, and J. Laybourn-Parry, “Demonstration of antifreeze protein activity in Antarctic lake bacteria,” *Microbiology*, vol. 150, no. 1, pp. 171–180, 2004.

[193] H. Kawahara, Y. Iwanaka, S. Higa et al., “A novel, intracellular antifreeze protein in an antarctic bacterium, *Flavobacterium xanthum*,” *Cryo-Letters*, vol. 28, no. 1, pp. 39–49, 2007.

[194] N. Muryoi, M. Sato, S. Kaneko et al., “Cloning and expression of *afpA*, a gene encoding an antifreeze protein from the arctic plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2,” *Journal of Bacteriology*, vol. 186, no. 17, pp. 5661–5671, 2004.

[195] J. A. Raymond, C. Fritsen, and K. Shen, “An ice-binding protein from an Antarctic sea ice bacterium,” *FEMS Microbiology Ecology*, vol. 61, no. 2, pp. 214–221, 2007.