B R Glick
C L Patten
G Holguin
D M Penrose

Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria

B R Glick
C L Patten
G Holguin
D M Penrose

Department of Biology
University of Waterloo
Ontario, Canada

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PREFACE

Plant growth-promoting bacteria are of interest to a wide range of individuals from agronomists and plant physiologists to microbial biochemists and ecologists, and from soil scientists to molecular biologists. This fascinating area of research has an impact on the study of a number of fundamental aspects of both plant growth and development and the strategies employed by soil microorganisms, while at the same time promises to provide us with approaches that have the potential to dramatically alter both agricultural and horticultural practices.

The conflicting and often inconsistent field results that were obtained by workers employing plant growth-promoting bacteria in agriculture on a large scale in the early and middle part of the twentieth century probably reflect an incomplete understanding of the mechanisms that these organisms employed. However, researchers who study and attempt to utilize plant growth-promoting bacteria have come a long way since those early days. Current research with plant growth-promoting bacteria includes a large number of scientists from dozens of countries around the world employing a range of different techniques and approaches.

This small volume endeavors to provide an overview, at a biochemical and molecular biological level, of the most important mechanisms that are utilized by plant growth-promoting bacteria. The assumption underlying this endeavor is that a more profound understanding of these fundamental mechanisms will eventually permit scientists to manipulate these bacteria and to use them more effectively as a regular component of agricultural and/or horticultural practice. Therefore, while all of the topics in this volume are discussed in as comprehensive a manner as possible, the book emphasizes a critical overview of the field rather than a mere compendium of data. Moreover, we have made every effort to make this information accessible to as wide a range of readers as possible by writing this book in a clear, concise, and easy to follow style with complex concepts explained using simple jargon-free language.

Since this is a dynamic and growing field, it is inevitable, as new knowledge is acquired and new studies are published, that some of the information that we have presented will go out of date. While this is unavoidable, we trust that most of the examples that we have chosen to illustrate particular points will continue to stand up to scrutiny with time.

> Bernard R. Glick
> Cheryl L. Patten
> Gina Holguin
> Donna M. Penrose
>
> Waterloo, Ontario, Canada
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Chapter 1

OVERVIEW OF PLANT GROWTH-PROMOTING BACTERIA

Introduction

Annually, more than 100 million tonnes of nitrogen fertilizers and more than 90 million tonnes of potash and phosphate fertilizers are used worldwide to increase the yield of crop plants. Despite the success in using these compounds to improve crop yields, they can, under certain circumstances, pollute the environment and may contribute to a number of human and animal health problems. For example, although nitrates are generally considered to be benign, they can be converted by bacteria to nitrites that can combine with secondary amines or amides to produce N-nitroso compounds which are known carcinogenic agents.

Over the past twenty years global fertilizer consumption has increased on average approximately 3% a year which further exacerbates the problems associated with the use of chemical fertilizers. Moreover, with time the cost of chemical fertilizers has continued to rise. The potential negative environmental impact of the large scale use of chemical fertilizers together with their increased cost has prompted a number of scientists worldwide to seriously consider the possibility of at least partially replacing chemical fertilizers with bacterial inoculants. In the past, when bacterial inoculants were used under field conditions they suffered from a lack of consistency in demonstrating their effectiveness which caused many researchers to conclude that bacterial inoculants would never become a significant component of modern agricultural practice. However, as we enter a new millennium, the desirability of using bacterial inoculants to facilitate plant growth has become apparent once again. In addition, using the techniques of modern molecular biology, we have the tools to both hasten a better understanding of the mechanisms utilized by these organisms, and to rapidly develop new and better strains for use in agriculture.

Soil contains a large number of different types of microorganisms including bacteria, fungi, actinomycetes, protozoa and algae (Paul and Clark, 1989). Of these, bacteria are by far the most common; they can grow rapidly and have the ability to utilize a wide range of different substances as either carbon or nitrogen sources. While many soil bacteria are bound to the surface of soil particles and are found in soil aggregates, many more interact specifically with the roots of plants. In fact, the concentration of bacteria that is found around the roots of plants, i.e., in the rhizosphere, is generally much greater than the bacterial density, or concentration, that is found in the rest of the soil (Lynch, 1990). The high concentration of bacteria around the roots of plants presumably occurs because of the presence of high levels of nutrients (especially small molecules such as amino acids, sugars and organic acids) that are exuded from the roots of most plants, and can then be used to support bacterial growth and metabolism (Whipps, 1990).

The interaction between bacteria and plant roots may be beneficial, harmful or neutral for the plant, and sometimes the effect of a particular bacterium may vary as the soil conditions change (Lynch, 1990). For example, a bacterium that facilitates growth by providing plants with fixed nitrogen, which is usually present in only limited amounts in the soil, is unlikely to provide any benefit to plants when large amounts of chemical nitrogen fertilizer is added to the soil.

While there are some soil bacteria that are pathogenic to plants, most of the more agronomically important plant disease-causing soil microorganisms are fungi, including the genera *Fusarium, Pythium* and *Rhizoctonia*. Most of the research in the area of microbial phytopathogenesis is directed toward ameliorating the negative impact that these organisms have on plants. However, an understanding of the mechanism(s) of microbial phytopathogenesis may indirectly contribute to a better understanding of some of the mechanisms that are used by plant growth-promoting bacteria since phytopathogens and plant growth-promoting bacteria utilize many of the same mechanisms.

The bacteria that provide some benefit to plants are of two general types: those that form a symbiotic relationship, which involves formation of specialized structures or nodules on host plant roots, and those that are free-living in the soil; the latter are often found near, on or even within the roots of plants (Kloepper et al., 1988; van Peer and Schipper, 1989; Frommel et al., 1991). The symbiotic bacteria, especially *Rhizobia*, have been studied extensively and, although the world market for these microorganisms is relatively small at the present time, they have been developed as a "biological" means of increasing crop yields (Vance, 1983; Bohlool, 1990; Paau, 1991; Sharma, 1993). Beneficial free-living soil bacteria are generally referred to as plant growth-promoting rhizobacteria or PGPR and are found in association with the roots of many different plants (Kloepper et al., 1989; Table 1). While numerous free-living soil bacteria are considered to be plant growth-promoting bacteria, not all bacterial strains of a particular genus and species have identical metabolic capabilities. Thus, for example, some *P. putida* strains actively promote plant growth while others have no measurable effect on plants.

Conceptually, plant growth-promoting bacteria can have an impact on plant growth and development in two different ways: indirectly or directly (Table 2). The indirect promotion of plant growth occurs when these bacteria decrease or prevent some of the deleterious effects of a phytopathogenic organism (usually a fungus) by any one or more of several different mechanisms. On the other hand, the direct promotion of plant growth by plant growth-promoting bacteria generally entails providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment.

Up-to-date information, including references to the literature dealing with plant growth-promoting bacteria may be found on the Internet at: www.ag.auburn.edu/pgpr/. Currently, the information on this web site is updated every few months.

At present, phytopathogens cause an enormous loss of agricultural and horticultural crop productivity which is dealt with for the most part by the use of

chemical agents, although fumigation, steam-treatment and solarization of soils have also been employed. Until recently it was believed that the advantages of using chemicals could be obtained without any deleterious environmental effects. However, with the realization that many of these agents are both hazardous to animals and humans, and can persist and accumulate in natural ecosystems, came the desire to replace the use of chemicals with biological approaches that were potentially more "friendly" to the environment, especially in the long term. These biological approaches for the control of different phytopathogenic agents include the development of transgenic plants that are resistant to one or more plant pathogens, and the use of biocontrol plant growth-promoting bacteria that can suppress or prevent the phytopathogen damage (see Chapter 7).

Table 1. Some free-living soil bacteria that can function as plant growth-promoting bacteria. Note that not all strains of the listed organism can act as plant growth-promoting bacteria.

Azorhizobium caulinodans	*Citrobacter freundii*
Azospirillum amazonense	*Curtobacterium flaccumfaciens*
Azospirillum halopraeferens	*Enterobacter agglomerans*
Azospirillum irakense	*Enterobacter cloacae*
Azospirillum lipoferum	*Erwinia herbicola*
Azospirillum brasilense	*Flavomonas oryzihabitans*
Azotobacter chroococcum	*Hydrogenophaga pseudoflava*
Bacillus cereus	*Klebsiella planticola*
Bacillus coagulans	*Kluyvera ascorbata*
Bacillus laterosporus	*Kluyvera cryocrescens*
Bacillus licheniformis	*Phyllobacterium rubiacearum*
Bacillus macerans	*Pseudomonas aeruginosa*
Bacillus megaterium	*Pseudomonas aureofaciens*
Bacillus mycoides	*Pseudomonas corrugata*
Bacillus pasteurii	*Pseudomonas fluorescens*
Bacillus polymyxa	*Pseudomonas marginalis*
Bacillus pumilus	*Pseudomonas putida*
Bacillus sphaericus	*Pseudomonas rubrilineans*
Bacillus subtilis	*Rathayibacter rathayi*
Burkholderia cepacia	*Serratia marcescens*
Burkholderia gladioli	*Stenotrophomonas sp.*
Burkholderia graminis	*Streptomyces griseoviridis*
Burkholderia vietnamensis	

A perusal of the scientific literature indicates that most of the interest in plant growth-promoting bacteria relates to the ability of some of these organisms to function (indirectly) as biocontrol agents. On the other hand, the bacteria that act by directly stimulating plant growth have received much less attention. This probably is a reflection of the generally held view that in the field it is easier to reproducibly demonstrate the efficacy of biocontrol plant growth-promoting bacteria rather than

bacteria that act by directly stimulating plant growth. Technical problems notwithstanding, the use of bacteria that promote plant growth directly represents an enormous potential opportunity for both agriculture and horticulture.

Table 2. Some mechanisms used by plant growth-promoting bacteria to stimulate plant growth

Indirect Mechanisms
- Antibiotic Production
- Depletion of Iron From the Rhizosphere
- Induced Systemic Resistance
- Synthesis of Antifungal Metabolites
- Production of Fungal Cell Wall Lysing Enzymes
- Competition For Sites on the Root

Direct Mechanisms
- Solubilization of Phosphorus
- Nitrogen Fixation
- Sequestering Iron by Siderophores
- Production of Phytohormones (Auxin, Cytokinin, Gibberelin)
- Lowering Ethylene Concentration

Mechanisms of direct growth promotion

There are several ways in which different plant growth-promoting bacteria have been reported to directly facilitate the proliferation of their plant hosts (see Chapters 2-5). Plant growth-promoting bacteria may fix atmospheric nitrogen and supply it to plants; they may synthesize siderophores which can solubilize and sequester iron from the soil and provide it to plant cells; they may synthesize several different phytohormones, including auxins and cytokinins, which can act to enhance various stages of plant growth; they may have mechanisms for the solubilization of minerals such as phosphorus which then become more readily available for plant growth; and they may synthesize some less well characterized low molecular mass compounds or enzymes that can modulate plant growth and development (Brown, 1974; Kloepper et al., 1986; Davison, 1988; Kloepper et al., 1989; Lambert and Joos, 1989; Patten and Glick, 1996). From the numerous studies concerning the mechanisms that are used by plant growth-promoting bacteria, it has become apparent that a particular bacterium may affect plant growth and development using any one, or more, of these mechanisms. Moreover, since many plant growth-promoting bacteria possess several traits that enable them to facilitate plant growth, a bacterium may utilize different traits at various times during the life cycle of the plant. For example, following seed germination a plant growth-promoting bacterium may lower the plant's ethylene concentration thereby decreasing the ethylene inhibition of seedling root length. Once the seedling has depleted the resources that are contained within the seed, a plant growth-promoting bacterium may help to provide the plant with a sufficient

amount of iron and phosphorus from the soil. Through early plant development, plant growth promoting bacteria may stimulate cell division by providing appropriate levels of auxins, cytokinins or gibberelins and help plants to tolerate a variety of environmental stresses such as flooding and drought by lowering the potentially detrimental level of stress ethylene that can form as a response to these stresses.

The extensive biochemical and molecular biological studies of symbiotic diazotrophs, particularly various strains of *Rhizobia*, have served as a conceptual starting point for understanding the mechanisms of growth promotion by plant growth-promoting bacteria. Since one of the major benefits that *Rhizobia* provide to plants is fixed nitrogen in exchange for fixed carbon (photosynthate), it was naively thought that diazotrophic plant growth-promoting bacteria might also function in this way. However, not all plant growth-promoting bacteria are diazotrophic. In addition, many of the plant growth-promoting bacteria that are diazotrophic fix only limited amounts of nitrogen, and not nearly enough to provide for their own as well as the host plant's nitrogen requirements (Hong et al., 1991a). Other diazotrophic plant growth-promoting bacteria can fix high levels of nitrogen; however, most researchers believe that even these organisms provide the plant with only small amounts of fixed nitrogen. Thus, for the majority of plant growth-promoting bacteria nitrogen fixation is only a minor component of the benefit that the bacterium provides to the plant, although under certain circumstances, some diazotrophs may provide their plant hosts with a significant portion of the fixed nitrogen that they require.

It is likely that the impact of all of the mechanisms by which the bacterium provides a compound or nutrient such as fixed nitrogen, phosphorus or iron to the plant, varies considerably depending upon the soil composition. For example, the growth of cactus plants that are cultivated in extremely nutrient-poor sandy soils is dramatically enhanced when the plants are inoculated with *Azospirillum* (Y. Bashan, submitted for publication). On the other hand, numerous researchers have observed that plant growth-promoting bacteria have little or no measurable effect on plant growth when the plants are cultivated in nutrient-rich soil and grown under optimal conditions.

It is generally assumed that bacterial stimulation of plant growth requires the binding of the bacterium to the plant root. While this is undoubtedly true for most plant growth-promoting bacteria effects, binding may not always be required (Reddy and Rahe, 1989; Hong et al., 1991b). For example, in one study of onion in the field, significant growth promotion was observed even though only very low levels of root colonization by plant growth-promoting bacteria were detected (Reddy and Rahe, 1989). In addition, electron microscopy studies of the roots of young canola seedlings grown in growth pouches, under gnotobiotic conditions in the presence of the plant growth-promoting bacterium *Pseudomonas putida* GR12-2, indicated that bacterial adherence to the seed coat alone was sufficient to enhance root elongation during the first few days of seedling development (Hong et al., 1991b). In the latter case, root colonization may play another, and later, role in the plant growth-promoting activity of this bacterium (Glick et al., 1997). That is, root

colonization may become more important once the developing seedling has exhausted the nutrients that are available in the seed.

The mechanism that has been most often invoked to explain the various effects of plant growth-promoting bacteria on plants is the production of phytohormones, and most of the attention has focused on the role of the phytohormone auxin (Brown, 1974; Tien et al., 1979; Patten and Glick, 1996). Since plants as well as plant growth-promoting bacteria can synthesize auxin, it is important when assessing the consequences of treating a plant with a plant growth-promoting bacterium to distinguish between the bacterial stimulation of plant auxin synthesis on the one hand, and auxin that is synthesized by the plant growth-promoting bacterium, on the other (Gaudin et al., 1994). It would seem to be relatively straightforward to monitor the effects of bacterially synthesized auxin by comparing the growth and development of plants treated with either wild-type plant growth-promoting bacteria or with mutant strains of plant growth-promoting bacteria that either do not produce, or else overproduce, auxin. However, scientists have found it extremely difficult to select auxin minus bacterial strains because of the complexity and multiplicity of bacterial auxin biosynthetic pathways (Patten and Glick, 1996). This notwithstanding, mutant strains of the plant growth-promoting bacterium *Azospirillum brasilense* that synthesize only very low levels of auxin have been selected. When these mutant strains were compared to the wild-type they were found to no longer promote the formation of lateral roots of wheat seedlings (Barbieri et al., 1986; Barbieri and Galli, 1993). On the other hand, a mutant strain of the plant growth-promoting bacterium *Pseudomonas fluorescens* BSP53a that overproduced auxin had a stimulatory effect on the root development of black currant softwood cuttings and an inhibitory effect with cherry tree plants (Dubeikovsky et al., 1993). Thus, the growth of plants treated with an auxin-secreting plant growth-promoting bacterium can be affected by the amount of auxin that the bacterium produces such that the observed response may vary from one species of plant to another depending upon the existing hormonal levels within the treated plant.

A relatively recent discovery implicates the plant hormone ethylene in a mechanism of plant growth promotion. It has been found that a number of plant growth-promoting bacteria contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Jacobson et al., 1994; Shah et al., 1997; Glick et al., 1998; Shah et al., 1998; Belimov et al., 1998 submitted for publication) and that this enzyme can cleave the plant ethylene precursor ACC, and thereby lower the level of ethylene in a developing or stressed plant. For many plants a burst of ethylene is required to break seed dormancy (Eashi, 1991) but, following germination, a sustained high level of ethylene may inhibit root elongation (Jackson, 1991). Thus, plant growth-promoting bacteria that contain the enzyme ACC deaminase, when bound to the seed coat of a developing seedling, may act as a mechanism for insuring that the ethylene level does not become elevated to the point where root growth is impaired. By facilitating the formation of longer roots, these bacteria may enhance the survival of some seedlings, especially during the first few days after the seeds are planted.

Strategies to improve plant growth-promoting bacteria

As more and better strains are developed by conventional mutagenesis and selection procedures, and are shown to be effective, the use of plant growth-promoting bacteria in agriculture will increase. Unfortunately however, strain development by conventional mutagenesis and selection procedures is intrinsically limited by the genetic material contained within the target bacterium. Thus, it is only through the use of genetic engineering that truly novel and superior plant growth-promoting bacterial strains can be developed. One can envision a number of different ways in which plant growth-promoting bacteria might be improved by genetic engineering. Some of the strategies are relatively straightforward and require the addition of only a single functional gene (e.g., the gene for ACC deaminase), while others are more complex and require these organisms to be transformed with several genes (e.g., the genes encoding all of the enzymes involved in siderophore biosynthesis) at once.

Plants use a number of different strategies to limit root colonization by phytopathogens. One such strategy is the production of active oxygen species such as the hydroxyl radical, the superoxide anion and hydrogen peroxide (Doke, 1983; Klotz et al., 1989; Sutherland, 1991). Plant roots may also respond to colonization by plant growth-promoting bacteria by producing active oxygen species (Katsuwon and Anderson, 1989; Katsuwon and Anderson, 1990). Previously it was observed that phytopathogens with higher levels of the enzymes that reduce the amount of active oxygen species, i.e., superoxide dismutase, catalase and peroxidase, are more effective pathogens presumably because they are not inhibited by the active oxygen species that are produced by the plant (Klotz and Hutcheson, 1992). It is therefore reasonable to expect that increasing the level or activity of one or more of these enzymes in a plant growth-promoting bacterium should help to protect the plant growth-promoting bacterium from inhibition by active oxygen species. This alteration of enzyme activity should increase the ability of the bacterium to colonize plant roots, increasing the effectiveness of that bacterial strain.

The soil contains a large number and variety of microorganisms, and those microbial strains that are able to utilize an unusual carbon or nitrogen source such as an opine, ACC or a xenobiotic compound should be able to proliferate and then persist longer than other microorganisms in soils that contain such unusual compounds. For example, the ability of some bacteria to hydrolyze ACC may provide these strains with a competitive advantage over other microorganisms in the rhizosphere, although not in the rest of the soil, because they can use ACC as a source of nitrogen (Jacobson et al., 1994; Glick et al., 1994 & 1998).

One group of researchers produced a more soil persistent biocontrol bacterium by transferring the NAH7 plasmid, which carries the genes encoding the enzymes of the naphthalene and salicylate biodegradative pathway, into an established biocontrol strain (Colbert et al., 1993). The introduced plasmid was stably maintained and conferred increased persistence upon the host bacterium when salicylate was present in the soil (Colbert et al., 1993). Similarly, the presence of a herbicide or pesticide in

soil may not only facilitate the proliferation of bacteria that have been genetically engineered to degrade these compounds, but at the same time, these chemicals may suppress the proliferation of the other microorganisms in the same soil thereby providing the engineered biodegradative plant growth-promoting bacterium with a significant competitive advantage. In addition to engineering plant growth-promoting bacteria to utilize unusual compounds, these organisms may be genetically manipulated to permit them to utilize a wider range of compounds exuded by plant roots thereby potentially expanding the range of host plants with which a particular organism can associate. For example, azospirilla are able to utilize the organic acids that are exuded by C4 plants such as maize and sorghum while most pseudomonads cannot metabolize these compounds.

A plant growth-promoting bacterium that can stimulate plant growth in the laboratory will not necessarily have any significant impact on plants in the field unless the bacterium is able to persist and grow in the natural environment. In Canada for example, these bacteria must be able to survive long cold winters and then grow at cool temperatures in the spring ($\sim$5-10°C). It was recently reported that some bacteria can secrete antifreeze protein(s) into the surrounding medium when they are grown at low temperatures (Sun et al., 1995; Xu et al., 1998). These antifreeze protein(s) appear to regulate the formation of ice crystals outside of the bacterium, thereby protecting it from damage that might otherwise occur at freezing temperatures. Thus, the addition, by genetic engineering, of antifreeze protein(s) to bacteria that are otherwise unable to persist and proliferate at cold temperatures may make a bacterium more effective at promoting plant growth because it can thrive under these otherwise adverse conditions.

Antibiotic biosynthesis in the soil is not restricted to plant growth-promoting bacteria. In fact, in the presence of another antibiotic-synthesizing microorganism it may be difficult for a plant growth-promoting bacterium to survive unless it has antibiotic resistance genes that, when expressed, prevent the antibiotic from inhibiting its growth. However, it is impossible to protect a plant growth-promoting bacterium against all possible antibiotics. It may be better, if possible, to avoid the use of antibiotic resistance genes when introducing a trait into a plant growth-promoting bacterium since those bacteria with genetically engineered antibiotic resistance may transfer that resistance to other bacteria in the environment. Moreover, the presence of highly expressed antibiotic resistance genes is likely to decrease the environmental persistence of a genetically engineered plant growth-promoting bacterium as their expression places a greater demand on the energy resources of the bacterium (Glick, 1995).

Diazotrophic plant growth-promoting bacteria are better able to survive in nitrogen-poor soils compared to non-diazotrophic strains. Under these conditions, bacteria that aren't diazotrophic are not able to proliferate. However, the very large amount of energy, in the form of ATP, that is required for nitrogen fixation may put the diazotrophs at a competitive disadvantage in soils where a small amount of fixed nitrogen is available to support bacterial growth and persistence. Therefore, while it is technically possible by genetic engineering to convert a non-diazotrophic plant

growth-promoting bacterium to a diazotrophic one, and this genetic change could enhance the competitiveness of the organism in nitrogen-poor soils, it could debilitate the engineered bacterium rendering it unable to stimulate plant growth in soils where nitrogen is not as limited (Glick et al., 1986 & 1989; Hong et al., 1991a, Glick, 1995).

The enzyme ACC deaminase allows a bacterium to utilize an unusual source of nitrogen enabling it to persist and proliferate in the rhizosphere in competition with other bacteria. While the cleavage of plant ACC by bacterial ACC deaminase lowers root elongation-inhibiting levels of ethylene, in does not completely abolish ethylene in associated plants. Moreover, the low level of ACC deaminase that is normally present in many plant growth-promoting bacteria may not be sufficient to cope with the large pulses of ACC—which may lead to large pulses of ethylene—that are often a component of a plant's response to various stresses. The simplest way to engineer plant growth-promoting bacteria that can respond more efficiently to different environmental stresses (both biotic and abiotic) would be to modify ACC deaminase genes encoded within such bacteria so that they are regulated differently than they are at present. Whether this might be accomplished by placing ACC deaminase genes under the control of a constitutive promoter or a promoter that is strongly induced under stress conditions such as a heat shock promoter, remains to be determined.

In situations where phosphate fertilizers are unavailable or too expensive, it may be possible to genetically engineer plant growth-promoting bacteria to more effectively provide phosphorus to the plant. This might be achieved in either of two different ways. Increased expression of bacterial phosphatase genes might be expected to augment the ability of a bacterium to solubilize organic phosphate in locales where organic fertilizers (e.g., manure) are common. On the other hand, to solubilize inorganic phosphate it may be necessary to genetically engineer plant growth-promoting bacteria so that they overproduce, and secrete from the cell, organic acids.

Conclusions

Research efforts worldwide over the past 15-20 years, which have provided a better understanding of plant growth-promoting bacteria and the mechanisms that they use, have renewed commercial interest in these organisms. Although very few plant growth-promoting bacteria are commercially available at the present time, and the sales of those that are available make up only a tiny fraction of the sales of chemical fertilizers and pesticides, it is expected that within the next few years there will be many more, especially biocontrol plant growth-promoting bacteria. In the past few years, the isolation of thousands of new strains of plant growth-promoting bacteria has been reported. These new strains, as well as many of the already known plant growth-promoting bacteria, are currently being tested and characterized in both laboratory and field settings. The next phase in the development of what promises to eventually become a major industry worldwide will likely be the selection and testing of "improved" variants of existing plant growth-promoting bacteria strains.

Ultimately, significantly better strains will have to be constructed by genetic engineering. Despite the fact that these efforts are still at a very early stage and that strict regulations currently exist in many jurisdictions regarding the deliberate release of genetically engineered organisms into the environment, this approach should have the effect of accelerating progress in this field so that it may not be unreasonable to expect improved genetically engineered strains of plant growth-promoting bacteria to be in widespread commercial use for crop improvement within the next 10-15 years.

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