

Mechanism of plant growth promotion by rhizobacteria

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Plant growth results from interaction of roots and shoots with the environment. The environment for roots is the soil or planting medium which provide structural support as well as water and nutrients to the plant. Roots also support the growth and functions of a complex of microorganisms that can have a profound effect on the growth and survival of plants. These microorganisms constitute rhizosphere microflora and can be categorized as deleterious, beneficial, or neutral with respect to root/plant health. Beneficial interactions between roots and microbes do occur in rhizosphere and can be enhanced. Increased plant growth and crop yield can be obtained upon inoculating seeds or roots with certain specific root-colonizing bacteria- 'plant growth promoting rhizobacteria'. In this review, we discuss the mechanisms by which plant growth promoting rhizobacteria may stimulate plant growth.

The term "plant growth promotion" is used¹ to describe the increased plant growth and crop yield that occurred upon inoculating seeds or roots with certain specific root-colonizing bacteria²⁻⁵. The term "plant growth promoting rhizobacteria" (PGPR) is coined¹ to refer to root colonizing bacteria that cause the increase in growth and yield and to differentiate them from other microorganisms found in rhizosphere that do not colonize roots or enhance plant growth. Plant growth promoting microorganisms have been studied intensively in the last few years because of their potential impact on agricultural and forest productivity^{6,7}. A large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter* and *Serratia* have been shown to enhance plant growth.

Direct effects

Plant growth regulating substances (PGRS)— Many rhizosphere bacteria produce indole acetic acid (IAA) in culture media specially in the presence of tryptophan⁸⁻¹⁰ in rhizosphere and rhizoplane of forage grasses and many economically important cereals like wheat, barley and pearl millet¹¹, vegetables, tomato and bean plants¹² under cultural conditions. Plant growth substances of bacterial origin accounts for

favourable effect on growth^{13,14}. Production of plant growth substances by bacteria has been confirmed in other investigations^{11,15,16} and that PGRS synthesized by bacteria is absorbed by roots¹⁷. The culture supernatant fluids of phosphate – dissolving bacteria, isolated from rhizospheres of crop plants have shown the presence of IAA, gibberellins and cytokinins¹⁸. Prikryl and coworkers¹⁹ have also reported production of IAA and some other auxins in liquid culture of *Pseudomonas cepacia* and *P.fluorescens* isolated from maize and bean rhizosphere. Mycorrhizal fungi, bacteria and actinomycetes isolated from soil and mycorrhizosphere of pine (*Pine sylvestris*) also produce auxins and gibberellin-like substances⁸. Three gibberellin-like substances have been detected in the culture of *Azotobacter chroococcum* strain A6¹³. When an inoculum of *Azotobacter* was added to seeds or roots, the later stages of development of tomato plants was altered, possibly PGR produced by *Azotobacter* was taken up by the seedlings at a critical stage of development, when vegetative and reproductive primordia were differentiating. Rhizobacterial isolates from the rhizosphere of sugar beet accumulate IAA in the culture filtrate, which on inoculation increase shoot : root ratio of sugar beet²⁰. Bacterization of pearl millet with *Azospirillum brasilense*, which produces small amounts of IAA, gibberellin and cytokinin-like substances, enhanced number of lateral roots densely covered with root hairs¹¹ and also root biomass²¹. Production of bacterial indole-3-acetic acid has been shown to have a stimulatory effect on development of

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black current soft wood cuttings by a recombinant *Pseudomonas* strain²². Microbially produced ethylene by soil fungus *Acremonium falciforme* affected plant growth of etiolated pea seedlings²³. While ethylene is a common product of fungi²⁴, the soft rot bacterium, *Pseudomonas solanacearum* has also been shown to produce the gas in pure culture. *Enterobacter* sp. is also found to produce ethylene in laboratory cultures¹⁰. Therefore, it seems reasonable to suggest that growth hormones (PGRS) are also produced by various bacteria, which live in association with plants, and in some instances can increase growth and improve yields of the host plants.

Nutrient uptake—The extent to which microorganisms promote uptake of mineral nutrients is a topic of considerable disagreement. Microorganisms may alter nutrient uptake rates by direct effects on the roots²⁵, by effects on the environment which in turn modify root behaviour, and by competing directly for nutrients²⁶. Many investigators agree that rhizosphere organisms promote uptake of minerals by roots^{27,28}. Bacteria which reduce the soil pH by production of organic acids or other acidic materials have been studied extensively for their possible role in solubilizing phosphates and other minerals²⁹. PGPR produce PGRS which promote root growth, the active root system then explores more soil zones for nutrients²⁵.

Nitrogen fixation—Root associated bacteria capable of fixing nitrogen, occur regularly in diverse soils which vary widely in nitrogen content and the capability to metabolize dinitrogen, provide a strong competitive advantage in soils of low organic nitrogen content. Non-symbiotic nitrogen fixing bacteria are important in the natural environment. Long term nitrogen balance studies have shown them to be capable of significant rates of nitrogen accumulation³⁰. Several studies indicate that nitrogen fixation rate is about 30-40 kg/ha/yr by the associative bacteria. Crop yields have also been increased in many locations³¹⁻³³.

Denitrification—Removal of soil nitrogen by denitrifying bacteria is normally considered detrimental to crop production, because in most instances nitrogen is the element which most severely limits plant growth. However, these bacteria are useful in some instances since they prevent accumulation of nitrogen compounds to toxic levels, particularly in poorly drained areas. Also, denitrification activity beneath the root zone may be considered beneficial since it will reduce the nitrate load in ground water³⁴. Denitrification tends to

maintain a balance between soil and atmospheric nitrogen, and thus avoid problems that would become serious if no mechanism is available to return nitrogen to the atmosphere³⁵.

Phytoalexins—Production of phytoalexins in response to microbial interaction with plants is considered to be an important mechanism of disease resistance in plant – pathogen relationships³⁶. However, reports on phytoalexin elaboration by rhizobacteria are scanty. Many plants respond to an invasion by a pathogenic or non-pathogenic microorganism, whether a fungus or bacterium or virus, by accumulating phytoalexins at interaction site³⁷. Phytoalexin synthesis can be used as an indicator of enhanced defence mechanism in bacteria-treated plants. Bochow and Fritzsche³⁸ reported an increase in the production of three phytoalexins, *viz.* rishitin, lubimin and solavetivon in potato slices and showed inhibition in mycelial growth of *Phytophthora infestans* by culture filtrate of *Streptomyces*. The defence reactions are established in *Vicia faba* – *Rhizobium leguminosarum* symbiosis by a variable phytoalexin concentration in the nodules³⁹. A study by van Peer and coworkers⁴⁰ has shown involvement of induced resistance and phytoalexin accumulation in biological control of *Fusarium oxysporum* f. sp. *dianthi* in carnation by *Pseudomonas* sp. strain WCS 4178. Phytoalexin production has also been observed in white bean *Phaseolus vulgaris* in response to inoculation by rhizobacteria and in cowpea roots infected with mycorrhizal fungus *Glomus fasciculatum*⁴¹. Inoculation of VAM fungus improves plant growth of cowpea and imparts resistance to *Fusarium* wilt disease⁴¹. Marley and Hillocks⁴² while studying the mechanism of resistance to *Fusarium* wilt in pigeonpea, have isolated four fungitoxic, isoflavonoid phytoalexins, *viz.* hydroxygenistine, genistine, cajanin and cajanal from wilt-resistant (ICP 9145) and wilt-susceptible plants after inoculation with *Fusarium udum*. Cajanal has been identified as the main antifungal compound which totally inhibits conidial germination of *F. udum*.

Indirect effects

Antibiotics—Many rhizobacteria are reported to produce antibiotics *in vitro* as well as under *in vivo* conditions. Compounds such as phenazines⁴³, pyoluteorin⁴⁴, pyrrolnitrin⁴⁴, tropolone, pyocyanin, 2,4-diacetylphloroglucinol⁴⁵ and bulbiformin have been isolated from soil fluorescent pseudomonads, having activity against many bacteria and fungi.

Some unidentified antibiotic compounds have also been implicated in disease suppression in potato⁴⁶, groundnut⁴⁷ and cotton⁴⁸. Weller and coworkers⁴⁹ have isolated and characterized an antibiotic produced by a strain of *Pseudomonas fluorescens* 2-79, which is suppressive towards *Gaeumannomyces graminis* var. *tritici*. The antibiotic is active against several species of fungi like *Pythium* sp. and *Rhizoctonia solani*. *Pseudomonas aureofaciens* Q 2-87, isolated from the rhizosphere of wheat and grown in "take-all" suppressive soil produced an antibiotic, 1,3,6-trihydroxy-2,4-diacetophenone, which is effective against *G. graminis* var. *tritici*⁵⁰. However, the first direct evidence for production of an antibiotic by a fluorescent pseudomonad in the rhizosphere and its involvement in control of a plant root disease has been reported by Thomashow and coworkers⁴³. Using HPLC-based assay, they have reported phenazine antibiotic in the rhizosphere of wheat roots colonized by phenazine-producing fluorescent pseudomonad but detected no phenazine in the rhizosphere of wheat roots colonized by phenazine-negative mutant.

Secondary metabolites other than antibiotics—A volatile alkyl pyrrole produced by *Trichoderma harzianum* inhibits a number of fungi *in vitro*⁵¹. It can reduce the incidence of *Rhizoctonia solani* - induced damping off on lettuce, when added to peat soil mixture⁵¹. Three different antifungal compounds have been reported to be produced by *T. harzianum*, out of which one is identified as an octaketide - derived acetal-diol⁵². *Pseudomonas fluorescens* strain CHAO produces an antifungal and antibacterial compound, 2,4-diacetylphloroglucinol, active against *Thielaviopsis basicola*, which causes black rot in tobacco, and *G. graminis* var. *tritici*, which causes 'take-all' disease in wheat. Mutant strains lacking in production of metabolite reduce suppressive effect on both the diseases⁴⁵.

Bacteriocins—Bacteriocins are the antimetabolites of microorganisms which inhibit the growth of related species or strains. Bacteriocin production by strains of *Rhizobium* has been considered as an additive advantageous character in an inoculant strain to compete with existing population of rhizobia. Strains of *R. trifolii*, *Rhizobium* sp. cowpea and *R. leguminosarum* are few such producers which have been found to compete better for nodulation over a non-producer strain⁵³. Reports on bacteriocin production by rhizobacteria are scanty, however, bacteriocinogenic rhizobacteria have been found to

suppress pathogenic species by virtue of antagonistic action^{54,55}.

Hydrogen cyanide (HCN)—Hydrogen cyanide, which is produced by many fluorescent pseudomonads in the exponential growth phase in media containing FeCl₃ or inorganic phosphate may also influence plant root pathogens⁵⁶ and suppress the disease caused by them^{57,58}. Role of HCN production by fluorescent pseudomonads in control of root pathogens is as yet unclear. It is possible that HCN production in rhizosphere has different effects on different plant types. For tobacco plants, it has been shown that HCN production by fluorescent pseudomonads stimulated root hair formation⁵⁶. No adverse effect on plant growth by HCN-producing pseudomonads could be observed in experiments with neutral or artificial soil under normal plant nutrition⁵⁷.

Competitive interactions among rhizosphere microorganisms—Since the pool of nutrients provided by rhizosphere must be shared by the roots and all microorganisms in the root zone, growth of any one of these will be significantly affected by the competitive ability of other. It is possible that purposeful manipulation of environmental conditions can be used as a method for increasing competitive advantage, and thereby promoting growth of beneficial rhizosphere microorganisms. Inoculation efforts in the past have not given specific attention to altering conditions in the soil to promote development of the inoculant organism. Chen and coworkers⁴⁸ have discussed use of endophytic bacteria to suppress pathogenic fungi attacking cotton. *Bacillus* or *Chaetomium* when coated on maize seeds before planting could suppress *Fusarium* in maize rhizosphere⁵⁹. Defago and coworkers⁶⁰ have used strains of *Pseudomonas fluorescens* to suppress fungal pathogens of tobacco. It is suggested that in some circumstances rhizosphere inoculants were strongly competitive against indigenous pathogenic organisms.

Siderophores—Availability of iron for microbial assimilation in rhizosphere is extremely limiting, because almost all living organisms require iron for growth and survival in a heterogenous environment such as rhizosphere and depend largely on the ability to scavenge sufficient iron from limiting pool. Consequently, to survive in such environments, organisms secrete iron-binding ligands called siderophores which can bind the ferric iron and make it available to the host organisms. These compounds are involved in plant growth promotion and disease suppression by fluorescent pseudomonads^{1,58,61-63} in

potato^{64,65}, cotton⁶⁶, tomato⁶⁷, wheat⁶⁸, carnation⁶⁹. Some plant species are capable of obtaining iron from certain microbial siderophores^{70,71}.

Competition for nutrients and suitable niches—

Other possible mechanisms by which fluorescent pseudomonads can protect plant roots from disease is the competition for nutrients and suitable niches on a root surface. Unless an organism can compete favourably with other organisms and effectively scavenge and utilize the available nutrients, it will not constitute a significant proportion of rhizosphere-rhizoplane population. Nutrient competition varies at different rhizospheres depending on the available sources of carbon, nitrogen, sulfur, phosphate and micronutrients. It is not yet clear whether better capabilities to utilize a particular type of nutrient or nutrients can confer a significant competitive advantage on a fluorescent *Pseudomonas* strain. The concept of direct competition for available habitable niches on a root surface has also not yet been clearly demonstrated for rhizosphere fluorescent pseudomonads. However, a very elegant example of this concept has been shown for a leaf-colonizing *P. syringae* strain⁷². The competitive exclusion of deleterious organisms by fluorescent pseudomonads at the plant root may also be a significant suppressive trait of these biocontrol agents.

Successful root colonization—The competitive exclusion of deleterious rhizosphere organisms is directly linked to an ability to successfully colonize a root surface^{3,73}. Bahme and Schroth⁷⁴ have reported that colonization of a fluorescent *Pseudomonas* strain in potato rhizosphere was 10-fold greater in a sandy loam soil than in a clay loam soil. Another strain performed better in a sandy soil than in a peat soil⁷⁵ suggesting that soil texture may have a direct influence on the colonization of these strains. Strain WCS 365 (good colonizer) of *Pseudomonas* sp. adhere in large number to sterile potato roots than other inferior colonizing strains⁷⁶, suggesting that attachment to the root surface enhances colonization.

Induced systemic resistance (ISR)—

Preinoculation of plants with fluorescent pseudomonads protects the plants against infection with pathogens through the mechanism of “induced” or “acquired” systemic resistance. The induced systemic resistance is defined as “the process of active resistance dependent on the host plant’s physical or chemical barriers, activated by biotic or abiotic agents”⁷⁷. ISR has been reported in cucumber-*Pythium* path system⁷⁸. The levels of

salicylic acid generally increase in correlation with ISR, the compound being involved in the signal transduction pathway that induces systemic resistance. Endophytic pseudomonads when introduced into rice, induced a systemic resistance response, doubling the levels of salicylic acid, which led to the suppression of sheath blight and blast in rice⁷⁹.

ISR is a general phenomenon in plants with neither the inducer nor the host response being specific. Systemic resistance by application of plant growth promoting rhizobacteria to seeds or roots can be induced not only against root diseases causing agents^{80,81} but also against leaf and shoot diseases⁸².

Other mechanisms—Some mechanisms have also been shown to be involved in suppression of growth of pathogenic microorganisms. Adetuyi and Cartwright⁸³ have identified antagonistic agent of *Pseudomonas syringae* and a member of *Erwinia herbicola* as mycotoxins. A relationship between *Fusarium* wilt suppression and agglutinability of *Pseudomonas putida* has been observed⁸⁴. Co-inoculation with parental bacterium (Agg⁺) offers higher level of protection against *Fusarium* wilt in cucumber plants than mutant strain (Agg⁻). Production of ammonia is implicated in inhibition of growth of *Rhizoctonia solani* and *Pythium ultimum* by *Enterobacter cloacae*⁸⁵. *Pseudomonas stutzeri* YP-1 produces lytic enzymes like chitinase and laminarinase against *Fusarium solani*⁸⁶. Some other uncharacterized compounds/mechanisms are also responsible for biocontrol activity of *Pseudomonas*^{87,88}.

In the past two decades, research in the field of plant growth promoting rhizobacteria has provided a better understanding of the mechanisms involved in plant growth promotion by these bacteria. All possible mechanisms of action by plant growth promoting rhizobacteria are currently being investigated at the molecular level by many groups all over the world using DNA recombinant techniques, which would enable to improve effectiveness of plant growth promoting rhizobacteria or in other words, develop “superior” plant growth promoting rhizobacterial strains by genetic manipulation of existing traits or by addition of new traits. However, due to the complexity of the rhizosphere environment and the infinite possibilities of plant – soil – microbial (tripartite) interactions, these mechanisms have not yet been fully elucidated.

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