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# Plant growth promoting Rhizobacteria: An overview

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## ABSTRACT

*Numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, stimulate plant growth by a plethora of mechanisms. These bacteria are collectively known as PGPR (plant growth promoting rhizobacteria).Of the microorganisms that parasitize on nematodes and reduce nematode populations by antagonistic behaviour, bacteria hold an important position where some of them have shown great potential as biocontrol agents. Bacteria destroy nematodes continuously in virtually all soils because of their constant association with nematodes in the rhizosphere. Although a large number of bacteria have shown antagonistic effects against nematodes but the most important genera include Rhizobium (R. leguminosorum), Bradyrhizobium japonicum, Mesorhizobium sp., Azorhizobium sp., Pseudomonas (P. fluorescens and P. aeruginosa) and Bacillus (B. subtilis). Application of some of these bacteria has accorded promosing results. There are several reports in the literature indicating that PGPR could be proved a boon in sustainable agriculture. Their beneficial events could be biological control of diseases and pests, plant growth promotion, increase in crop yields and quality improvement that can take place simultaneously and sequentially. There is an urgent need to develop some easy to manage technologies for formulation and mass production of bacteria at a commercial scale for field application.*

**Key words**: PGPR, nematodes, biocontrol.

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## INTRODUCTION

The rhizosphere is the volume of soil surrounding and under the influence of plant roots, where rhizoplane comprises of plant root surfaces and strongly adhering soil particles. There are many species of bacteria which are found in soil reported to promote plant growth by producing growth regulators, inducing root exudation and enhancing the availability of nutrients to plant, besides controlling soil borne plant pathogens [31]. The means by which PGPR enhance the

nutrient status of host plants can be categorized into five areas: (1) biological nitrogen fixation, (2) increasing the availability of nutrients in the rhizosphere, (3) inducing root surface area, (4) enhancing other beneficial symbiosis of the host, and (5) combination of modes of action. The roots of leguminous plants are colonized by numerous rhizospheric microorganisms and which cause definite influence on the survival and nodulation ability of seed inoculated rhizobia [90]. Rhizospheric microorganisms may not only influence the inoculated rhizobia adversely through saprophytic competition, but also help them in survival through synergism resulting in an increase in their nodulation ability and N2 fixing efficiency [77,1]. Several mechanisms such as alteration in the composition of rhizospheric microorganisms, production of plant signaling compounds, bacteriocins, siderophores, plant growth hormones and improving availability of nutrients by rhizospheric microorganisms have been reported for such synergism [105, 49]. Rhizospheric bacterialcommunities however have efficient systems for uptake and catabolism of organic compoundspresent in root exudates [123]. Ramaswami and Oblisami (1986) [95] reported the increase in nodules due to inoculation application. The nodulation process involves signal exchange between the host and the bacterium. Plant growth and nodulation by rhizobia are promoted by certain rhizobacteria [128, 90, 30]. The PGPR can influence plant growth directly through N2 fixation and production of biocontrol agents against soil-borne phytopathogens [66, 92, 70, 110, 15]. In fact, biochemical interactions and exchanges of signal molecules between plants and soil microorganisms have been described and reviewed [99].

In the rhizosphere, bacteria are the most abundant microorganisms. Rhizobacteria are rhizosphere competent bacteria that aggressively colonize plant roots; they are able to multiply and colonize all the ecological niches found on the roots at all stages of plant growth, in the presence of a competing microflora [52]. Associative dinitrogen fixing bacteria when they do not exhibit morphological modification of the host plant are considered as PGPR. However, rhizobia can also behave like PGPR with non-legume plants and some rhizobia are endophytes [3]. PGPR may induce plant growth promotion by direct or indirect modes of action [19, 67, 126, 47].

**Influence of *Pseudomonas* species**

Of the various rhizospheric bacteria, *Pseudomonas* sp. are aggressive colonizers of the rhizosphere of various crop plants (Schroth and Hancock, 1982) and have a broad spectrum of antagonistic activity against plant pathogens [88, 62, 58]. The antibiotic produced by *Pseudomonas fluorescens* was found to control damping-off of cotton seedlings caused by *R. solani* [22]. Among *Pseudomonas* species, *Pseudomonas aeruginosa*, a plant growth promoting rhizobacterium has been found to be an effective biocontrol agent of root pathogens [57, 105]. *Septoria tritici* (*Mycosphaerella graminicola*) was suppressed by *P. aeruginosa* strain leci [38]. Fuhrman and Wollum (1989) [62] reported that co-inoculation of siderophore producing pseudomonads with mixtures of the competing *Bradyrhizobium* typically enhanced nodulation by *Bradyrhizobium japonicum* strain USDA 110. For many pseudomonads, production of metabolites such as antibiotics, siderophores and hydrogen cyanide (HCN) is the primary mechanism of biocontrol [34]. *Pseudomonas fluorescens* CHAO isolated and intensively studied by the group of G. Défago in Switzerland produced several bioactive compounds (antibiotics, siderophores, HCN, indole acetic acid) giving it one of the broadest spectra of potential biocontrol and growth promoting mechanisms of known PGPR [34]. Many strains of pseudomonads can indirectly protect the plants by inducing systemic resistance against various pests and diseases [76, 119, 51]. The beneficial effect on plant shoot dry mass was more

pronounced with HCNproducing *Pseudomonas* strain [9]. PGPR including phosphate-solubilizing bacteria and biocontrol agents. *Pseudomonads* possess many traits that make them well suited as biocontrol and growth-promoting agents [31]. These include the ability to (i) grow rapidly *in vitro* and to be mass produced; (ii) rapidly utilize seed and root exudates; (iii) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant; (iv) produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles, and growth promoting substances); (v) compete aggressively with other microorganisms; and (vi) adapt to environmental stresses. In addition, pseudomonads are responsible for the natural suppressiveness of some soils to soil borne pathogens [33]. The *Pseudomonas* bacteria were inoculated into the rhizosphere and remained spatially separated from the pathogen that was inoculated on the above ground plant parts, either into the stem [100] or on the leaf surface [48].

***Pseudomonas* species**

All species and strains of *Pseudomonas* are Gram negative rods. Exceptions to this classification have recently been discovered in *Pseudomonas* biofilms. A significant number of cells can produce exopolysaccharides known as biofilms [26]. Secretion of exopolysaccharides such as alginate makes it difficult for Pseudomonads to be phagocytosed by mammalian white blood cells [71]. Exopolysaccharide production also contributes to surface colonising biofilms which are difficult to remove from preparation surface.

Growth of Pseudomonads on spoiling foods can generate a “fruity odour”. *Pseudomonas aeruginosa* is a highly relevant opportunistic human pathogen. One of the most worrying characteristics of *P. aeruginosa* is its low antibiotic susceptibility. Certain members of the *Pseudomonas* genus have been applied directly to soils as a way of preventing the growth or establishment of crop pathogens. This practice is known as biocontrol. The biocontrol properties of *P. fluorescens* strains CHAO or Pf-5 are currently best understood, although it is not clear exactly how the plant growth promoting properties of *P. fluorescens* are activated. Theories include that the bacteria might induce systemic resistance in the host plant. So it can better resist attack by the pathogens, the bacteria might out compete other (pathogenic) soil microbes, e.g., by siderophores giving a competitive advantage at scavenging for iron; the bacteria might produce compounds antagonistic to other soil microbes, such as phenazine type antibiotics or HCN. There is an experimental evidence to support all of these theories, in certain conditions, a good review of the topic is written by Haas and Defago, 2005. Some members of genus *Pseudomonas* are able to metabolise chemical pollutants in the environment, and as a result can be used for bioremediation. *P. alcaligenes*, can degrade polycyclic aromatic hydrocarbons [87]. Bacteria make excellent biosorbents because of their high surface volume ratios and a high content of potentially active chemosorption sites such as teichoic acid in their cell wall that contains chemical compounds with sites capable of passively sequestering metals. Different genera of bacteria (*Pseudomonas*, *Bacillus* and *Micrococcus* etc., have been reported as efficient lead reducers.

***Bacillus* species**

*Bacillus subtilis* is a ubiquitous, saprophytic soil bacterium which is thought to contribute to nutrient cycling due to its ability to produce a wide variety of enzymes. It has been used for industrial production of proteases, amylases, antibiotics and chemicals. *B. subtilis* strain QST713 has natural fungicidal activity, and is employed as a biocontrol agent.

**Influence of *Bacillus* species**

*Bacillus subtilis* has shown antagonistic activity towards *Fusarium solani* in vitro [121]. Schonbick *et al*., (1980) [42] isolated a *B. subtilis* strain whose metabolites are able to induce systemic resistance against powdery mildew on Barley. Similarly, *Bacillus* spp. Have been tested on a wide variety of plant species for their ability to control diseases [103]. *Bacillus* spp. are able to form endospores that allow them to survive for extended periods of time under adverse environmental conditions. Some members of the group are diazotrophs, and *B. subtilis* was isolated from the rhizosphere of a range of plant species at a concentration as high as 107 per gram of rhizosphere soil [5]. *Bacillus subtilis* also synthesizes an antifungal antibiotic inhibiting *Fusarium oxysporum* f. sp. *ciceris*, the agent of fusarial wilt in chickpea [16].

***Rhizobium* species**

Lentil has inherent capacity to fix atmospheric nitrogen in association with *Rhizobium leguminosarum* and generally gives poor response to inoculation [11] because of the build up of rhizobial population in the soils. The favourable effect of PGPRs oncompetitiveness of inoculum rhizobia was probably due to better survival of inoculated *Rhizobium* sp. in rhizosphere in presence of PGPRs as reported for Urd bean rhizobia in culture medium [53] and for chickpea rhizobia in soil condition [49]. *Rhizobium* spp. invade the root hairs of mungbean and result in the formation of nodules, where free air nitrogen is fixed. These bacteria, although present in soil vary in number, effectiveness in nodulation and nitrogen fixation [40]. Inoculation of mungbean with *rhizobium* spp. increased plant height, leaf area, photosynthetic rate and dry matter production [10].

Soil inhabiting bacteria developing nitrogen-fixing symbiosis with legumes are classically named rhizobia and currently include more than 50 species distributed in the genera *Rhizobium, Ensifer, Mesorhizobium, Azorhizobium, and Bradyrhizobium* [39]. During Rhizobium–legume interaction, rhizobia induce nodule formation in the root system of host legume by *nod* factors. The rhizobia inside the nodules then convert nitrogen into ammonia for uptake by host plants, while legumes provide nutrients to rhizobia [56]. Application of PSB as inoculant in green gram has also been reported to increase the nodule numbers and nodule dry biomass [4]. Siddiqui *et al*. (2006) [59] reported the effect of rhizobium to the greater colonization and siderophores production. This genus shows high potential in suppressing the root knot nematodes which interfere with the host finding processes of the nematodes. *Rhizobium* helped the plants in growth enhancement by fixing atmospheric nitrogen more effectively, as a result the nitrogen content in seeds increased substantially with subsequent increase in protein content. Effect of *Rhizobium* inoculation was more pronounced on histidine, isoluecine; threonine, valine and total amino acid in black gram [80], *Rhizobium* also has favourable effect on carbohydrate content. Since sulphur is a constituent of sulphur containing amino acids (methionine, cystine and cysteine), rhizobium application increased the amount of these amino acids and protein in black gram grains and also helped in the metabolism of carbohydrates which increased sugar content in seeds. The Rhizobium-legume symbiosis is the most promising plant bacterium association so far known. Inoculated *Rhizobium* sp. strains often fail to compete with indigenous rhizobia and do not increase nodulation [45].

**Mechanism**

Direct mechanisms include the production of stimulatory bacterial volatiles and phytohormones, lowering of the ethylene level in plants, improvement of the plant nutrient status (liberation of phosphates and micronutrients from insoluble sources; non-symbiotic nitrogen fixation) and stimulation of disease-resistance mechanisms (induced systemic resistance). Indirect effects originate for example when PGPR act like biocontrol agents reducing diseases, when they stimulate other beneficial symbioses, or when they protect the plant by degrading xenobiotics in inhibitory contaminated soils [23]. Exposure to the PGPR triggers a defence response by the crop as if attacked by pathogenic organisms. Siderophores produced by some PGPR scavenge heavy metal micronutrients in the rhizosphere (e.g. iron) starving pathogenic organisms of proper nutrition to mount an attack of the crop. Plants commonly excrete soluble organic compounds (chelators and phytosiderophores) which binds Fe+3 and helps to maintain it in solution. Chelators deliver the Fe+3 to the root surface where it is reduced to Fe+2 and immediately absorbed [91].Antibiotic producing PGPR releases compounds that prevent the growth of the pathogens. In the plant-beneficial rhizosphere bacterium *Pseudomonas fluorescens* CHA0, the GacS/GacA system is essential for the production of antibiotic compounds and hence for biological control of root-pathogenic fungi. The differential expression of three small RNAs facilitated the fine tuning of GacS/A-controlled cell population density-dependent regulation in *P. fluorescens* [37]. Early studies on PGPR focussed more on biological control of plant diseases than on growth promotion, and involved bacteria like fluorescent *Pseudomonas* and *Bacillus subtilis* that are antagonistic to soil-borne plant pathogens [69].

**Effect of bacteria with AM fungi**

Bacteria associated to mycorrhizal fungi adhere to fungal spores and hyphal structures and thus spread to the rhizosphere [118]. Bianciotto *et al.* (2004) [117] observed strong evidence of a vertical transmission of endobacteria through the AM fungus vegetative generation. However, antagonistic effects are often reported in the AM fungi-PGPR interactions. Positive interactions often result in plant growth improvement.

A dynamic role performed by PGPR in plant nutrition is by transforming nutrients in soils that are beneficial to plant growth through a process called biogeochemical cycling, and directly transporting these nutrients to the plant [79]. These microbes determine the nutrient pool of soils and facilitate the growth and development of plants [6]. An eco-friendly approach recently advocated to enhance the crop production is the use of PGPR as bio-inoculants. The PGPR is known to facilitate the plant growth through N-fixation; solubilization of insoluble phosphorus (P); production of compounds like siderophores, phytohormones, antibiotics, and antifungal metabolites; and induced systemic resistance [6]. Among PGPR, phosphate solubilizing bacteria (PSB) supply P to the plants [2]. In this regard, numerous PSB have successfully been used as commercial biofertilizer in sustained agricultural production systems [89, 6].

**Induced systemic resistance regarding nematode**

In induce systemic resistance is induced depending upon the type of genus. Regarding, fluorescent Pseudomonads, Wescott and Kluepfel (1990) [114] showed that all these *Rhizobacteria*, *Bacillus* and *Pseudomonas* bacterial sp. inhibited egg hatching whereas it can produce exotoxic compounds as a result of cellular metabolism, and also can affect nematode juveniles. This antagonistic effect against *M. incognita* is due to the permeability changes of

juveniles cuticle which is characterized by its selective permeability and this effect is more pronounced with molting inside eggs. The environment not only influences the growth and longevity of nodule inducing bacteroid, but also the production and behavior of nodules and development of host plant and product [8]. The principal effect of *M. incognita* race-1 was suppression of plant growth and adverse effects of nematode was reduced in the presence of *Bradyrhizobium* as reported earlier [14, 129]. Nematode infestation decreased the number of nodules on primary and secondary roots. Nodules are occasionally found on galls as reported by Hussey and Barker (1976) [104] and Raut (1980) [113]. Mature females, juveniles and egg masses were also detected in nodules as reported by Ali *et al*. [84].

The higher suppressive effects of these selected bacteria (*Pseudomonas*, *Bacillus* and *Micrococcus*) against *M. incognita* may be attributed to the distinctive properties of these genera. As concerning *B. thuringiensis*, it is known that this bacterium produces chitinolytic enzyme i.e., chitinase which is responsible for degrading chitin present in the walls of the nematode egg and the egg masses, so this bacterium is known as chitinolytic bacterium [101]. It is important to remember the above effect of the volatile nematicidal products of genus *Bacillus* against juveniles and egg masses [125]. Reitz *et al*., (2002) [82] showed that lipopolysaccharides, LPS (lipid A) which is defined as an integral part of the outer membrane of the cell, which can be extracted from bacterial cultures has an antagonistic agent against nematodes. Bin *et al*., (2005) [74] mentioned that culture filtrate of rhizobacterium is heat stable and resistant to extreme pH values, which suggested that the antibiotic rather than protein might be responsible for the nematicidal activity. Induced resistance is a state of enhanced defensive capacity developed by a plant reacting to specific biotic or chemical stimuli [76]. Research groups induced systemic resistance (ISR) is a mode of action of plant growth promoting rhizobacteria (PGPR), especially fluorescent *Pseudomonads*, in suppressing disease [100, 48]. The Netherlands, and J. W. Kloepper in Auburn, AL, discovered independently that induced systemic resistance is a mode of action of plant growth promoting rhizobacteria, especially fluorescent pseudomonads, in suppressing diseases [100, 48]. By ensuring spatial separation between the *Pseudomonas* bacteria and the pathogen on the root system, it was demonstrated that ISR is also effective against root infecting pathogens [81, 115]. ISR is phenotypically similar to systemic acquired resistance (SAR) that is triggered by nectrotinizing pathogens in that disease caused by a challenging pathogen is reduced. Accumulation of salicylic acid in the plant is required for SAR [75]. Improving the effectiveness of biological control by fluorescent *Pseudomonas* spp. may be established by using combination of strains that have different mechanisms of disease suppression, such as competition for iron and ISR [68]. Also for plant growth promoting *Bacillus* spp. mechanisms of ISR have been studied [68]. Bacterial production of the volatile 2, 3-butanediol is the trigger of *Bacillus* mediated ISR in *Arabidopsis*. *Bacillus* sp. produces large, spreading, grey white colonies with irregular margins. A unique characteristic of this bacterium is its ability to produce endospores, when environmental conditions are stressful. Although most species of *Bacillus* are harmful saprophytes, two species viz., *B. thuringiensis* and *B. cereus* are considered medically significant. *Bacillus thuringiensis* is a plant growth promoting bacterium which produces bacteriocin compounds [41]. Bankole and Adebanjo (1998) [112] reported that soils inoculated with *B. subtilis* and *B. cereus* reduced seedling infection and that the efficacy of antagonists increased with increase in dose. Lytic enzymes are known to be produced by *B. cereus* [107], these enzymes and other antibiotics produced by *B. cereus* have been reported to have antagonistic effects on some microbes [85].

Application of bacteria either as seed dressing or as soil drench has shown significant suppressive effects on root infecting pathogens on leguminous and non-leguminous plants [109]. Moreover, it is known that the LPS, lipid A is an endotoxin that is released from bacterial cell membrane after their death [50]. Application of PGPR has also been extended to remediate contaminated soil enabling the plants to grow under such conditions [124]. Many soil bacteria and especially rhizosphere bacteria can stimulate plant growth through a number of direct and indirect pathways.

**Bioremediation**

Metal contamination of soil has an important bearing on PGPR functions. Metal homeostasis resistance in bacteria is often maintained by sequestration, active efflux, reduced uptake, detoxification and synthesis of binding protein [97, 98]. In some cases, a few mechanisms may also co-exist. Strain Psd was able to resist Cd, Al and Zn and thus could be able to survive for carrying out its PGPR functions in soil containing high concentration of these metal ions.

Phosphate solubilization by strain Psd is another important property as non-availability of phosphate can be grown limiting for plants. Strain Psd could solubilize minerals, source of complex phosphate as well as release phosphate from organic sources via two phosphatase enzymes. Mineral phosphate solubilization in bacteria occurs by production of organic acids and organic phosphate release is aided by acid and alkaline phosphatases [54]. The complete genome sequence analysis of *P. fluorescens* Pf-5 and detailed molecular genetic analysis of *P. fluorescens* CHAO has firmly established the biocontrol capabilities and its regulation [108, 111]. Phosphate solubilizing bacteria are common in the rhizosphere [24] and secretion of organic acids and phosphatase are common method of facilitating the conversion of insoluble forms of P to plant available forms [73]. The solubilization of P in the rhizosphere is the most common mode of action implicated in PGPR that increase nutrient availability to host plants [7]. More importantly, increases in root length and root surface area are sometimes reported [17, 86, 46, 18, 72]. Fallik *et al*., 1994 [36] reported that inoculation of maize with Azospirillum brasilense resulted in a proliferation of root hairs which could have dramatic effects on increasing root suface area.

**Hormones production by PGPR**

PGPR produce phytohormones that are believed to be related to their ability to stimulate plant growth. Indole-3-acetic acid is a phytohormone which is known to be involved in root initiation, cell division, and cell enlargement [43]. This hormone is very commonly produced by PGPR [93]. Most commonly, IAA-producing PGPR are believed to increase root growth and root length, resulting in greater root surface area which enables the plant to access more nutrients from soil. Cytokinins are a class of phytohormones which are known to promote cell divisions, cell enlargement, and tissue expansion in certain plant parts [43]. Cytokinin is produced by *Pseudomonas fluorescens* isolating from the rhizosphere of the soybean [60]. Gibberellins are a class of phytohormones most commonly associated with modifying plant morphology by the extension of plant tissue, particularly stem tissue [43]. Evidence of GA production by PGPR is rare, however, Gutierrez-Manero *et al*. (2000) [44] provide evidence that four different forms of GA are produced by *Bacillus pumilus* and *B. licheniformis*. Ethylene is the only gaseous phytohormone. It is also known as the wounding hormone because its production in the plant can be induced by physical or chemical perturbation of plant tissues [43]. Glick *et al*. 2003 [15] put

forward the theory that the mode of action of some PGPR was the production of 1-carboxylate deaminase, an enzyme which could cleave ACC, the immediate precursor to ethylene in the biosynthetic pathway for ethylene in plant. The signaling pathway that is activated in this case depends on ethylene but is independent of SA and JA signaling [21]. It would be interesting to investigate the capacity of plant growth promoting *Pseudomonas* spp. to produce 2, 3-butanediol and its possible involvement in ISR. Identification of bacterial traits that are involved in ISR relies on time consuming bioassay in which suppression of disease symptoms and population dynamics of the pathogen are used as parameters.

**Mass production of *Pseudomonas* and *Bacillus* species**

Most of the biocontrol agents have varied performance in different environmental conditions. Some of these variabilities have been attributed to differences in physical and chemical properties found in natural environments where biocontrol agents are applied [78, 12]. The growth medium used to produce these agents, has a profound effect on them and their products. Mass production of biocontrol agents has become a focus of research and industrial development in the search for alternatives to chemical post harvest treatments [102, 122]. The accurate incorporation of nutrients has improved the biomass production of BAs, but unexpectedly did not enhance [94] or even decrease the biocontrol efficacy [65].

On a large scale, the medium should allow a maximum concentration of biomass and the active products to be produced at a low price [64]. Recognition of the environmental factors that regulate the growth and biocontrol efficacy of antagonist bacteria is an essential step towards advancing the level and reliability of their biocontrol potential [13].

**Commercial production**

Commercial production of disease suppressive strains of bacteria such as *P. fluorescens* and *B. subtilis* as biocontrol agents in postharvest diseases requires low cost and high biomass production while maintaining their biocontrol efficacy [35]. Yeast extract as a nitrogen source supports rapid growth and higher cell yields in all of the strains as compared to urea. Yeast extract contains amino acids and peptides, water soluble vitamins and carbohydrates [55, 120], which make it an excellent substrate for many microorganisms [116]. Costa et al., (2001) [35] and Dharani-Aiyer (2004) [96] showed that yeast extract was the best organic nitrogen source for antagonist bacteria. Nohata and Kurane (1997) [127] considered yeast extract to be too expensive for an industrial process, so it should preferably be replaced by a cheaper industrial product having similar growth characteristics, that should be determined in a economic and technological study. Molasses showed good yield efficacy in both strains (*Pseudomonas fluorescens* and *Bacillus subtilis*), which may account for the high biomass obtained, because the combination of yeast extract and commercial sucrose also gave high final growth of bacteria. However, a combination of molasses and urea decreased bacterial growth. Luna *et al*., (2002) [20] and Costa *et al*., (2001) [35] showed that a molasses based medium may be used for production of bacterial Bas. Apparently a C: N :: 1:1 ratio produces optimal growth for bacteria of two different genera (*Pseudomonas and Bacillus*) and this may hold true for other genera. The pH is anotherimportant parameter for bacterial growth. As a general principle [29] bacterial growth decreases at more acidic pH values. These observations are in accordance with the results of Costa *et al*., (2001) [35] and Fuchs et al., (1990). Antagonist bacteria such as *P. fluorescens and B. subtilis* can be produced in different media, using various N and C sources, while maintaining the efficacy of

BAs (Biocontrol agents). By-products such as molasses can serve as an economic culture medium, as suggested by the encouraging results obtained with the present results. Future research will concentrate on optimizing growth conditions and possible incorporation of other nutrients into formulations to obtain an even higher biomass.

**Future prospects**

New insights are certain to be gained from the recently published genomic sequence of *P. fluorescens* Pf-5, which already has revealed biosynthetic potential for many previously undetected compounds likely to contribute to the broad antifungal activity of this strain [61]. Perhaps the greatest remaining challenge facing *Pseudomonas* biocontrol research is the development of new formulations.

## REFEERENCES

[1] A Gupta; AK Saxena; G Murali; KVBR Tilak. *Tropical Agriculture*, **2003**, 80, 28-35.
[2] A Keneni A; F Assefa; PC Prabu. *J Agric Sci Technol*, **2010**, 12, 79–89.
[3] A Sessitsch; JG Howieson; X Perret; H Antoun; E Martinez-Romero. *Crit, Rev. Plant Sci*, **2002**, 21, 323-378.
[4] A Vikram; H Hamzehzarghani. *Res J Microbiol*, **2008**, 3, 62–72.
[5] A Wipat; CR Harwood. *FEMS Microb Ecol*, **1999**, 28, 1-9.
[6] A Zaidi; MS Khan; M Ahemad; M Oves. *Acta Microbiol Immunol Hung*, **2009**, 56, 263–284.
[7] AE Richardson. *Aust J Plant Physiol*, **2001**, 28, 897–906.
[8] AHV Taha. Nematode interactions with root nodule bacteria. In: Khan, MW (Ed) Nematode interactions. Chapman and Hall, London, **1993**, 202.
[9] AK Goel; SS Sindhu; KR Dadarwal. *Biol Fertil Soils*, **2002**, 36, 391-396.
[10] AK Tharkur; JDS Panwar. *Indian J Pl Pathol*, **1995**, 38, 62–5.
[11] AL Khurana; SS Dudeja. Biological Nitrogen Fixation Technology for Pulses Production in India. Indian Institute of Pulses Research, **1997**, Kanpur.
[12] BK Duffy; BH Ownley; DM Weller. *Phytopathology*, **1997**, 87, 1118-1124.
[13] BK Duffy; G Defago. *Applied and Environmental Microbiology*, **1999**, 65, 2429-2438.
[14] BM Bopaiah; RB Patil; DDR Reddy. *Indian journal of Nematology*, **1976,** 6, 124-130.
[15] BR Glick; JJ Pasternak. Plant growth promoting bacteria. In: BR Glick & JJ Pasternak (eds) Molecular Biotechnology – Principles and Applications of Recombinant DNA. ASM Press, Washington, DC, **2003**, 3rd edn, pp. 436–454.
[16] BSD Kumar. *Biol Fert Soils*, **1999**, 29, 87-91.
[17] C Galleguillos; C Aguirre; JM Barea; R Azcon. *Plant Sci*, **2000,** 159, 57–63.
[18] C Jacoud; D Job; P Wadoux; R Bally. *Can J Microbiol*, **1999**, 45, 339–342.
[19] CJ Beauchamp. *Phytoprotection*, **1993**, 71, 19-27.
[20] CL Luna; RLR Mariano RLR; AM Souto-Maior. *Brazilian Journal of Chemical engineering*, **2002**, 19, 133-140.
[21] CM Ryu; MA Farag; CH Hu; MS Reddy; JW Kloepper; PW Pare. *Plant physiol*, **2004**, 134, 1017-1026.
[22] CR Howell; RD Stipanovic. *Phytopathology*, **1979**, *69*, 480-482.
[23] CS Jacobson. *Plant Soil*, **1997**, 189**,** 139–144.
[24] CS Nautiyal; S Bhadauria; P Kumar; H Lal; R Mondal; D Verma. *FEMS Microbiol Lett*, **2000**, 182, 291–296.

[25] D Haas; G Defago. *Nature Reviews in Microbiology*, **2005**, 3, *4*, 307–319.
[26] D Hassett; J Cuppoletti; B Trapnell; S Lymar; J Rowe; S Yoon; G Hilliard; K Parvatiyar; M Kamani; D Wozniak; S Hwang; T McDermott; U Ochsner. *Adv Drug Deliv Rev*, **2002**, 54, *11*, 1425–1443.
[27] D Werner. Signaling in the rhizobia-legumes symbiosis. In: Varma A, Abott L, Werner D, Hampp R (eds) In plant surface microbiology. Springer, New York, **2004**, 99-119.
[28] D Wernwr. Organic signals between plants and microorganisms. In: Pinton R, Varanini Z, and Nannipieri P (eds) The rhizosphere. Biochemistry and organic substances at the soil plant interface. Marcel Dekker, New York, **2001**, 197-222.
[29] DAA Mossel; JEL Corry; CB Striejk; R Baird. Essentials of the microbiology of foods. **1991**, Wiley New York, NY, USA.
[30] DLN Rao; KK Pal. Biofertilizers in oilseeds production: Status and future strategies. National Seminar on Stress Management in Oilseeds for Attaining Self Reliance in Vegetable Oils, Directorate of Oilseeds Research, Indian Council of Agricultural Research, Hyderabad, India, **2003**, 195-220.
[31] DM Weller. *Annual Review of Phytopathology*, **1988**, 26, 379-407.
[32] DM Weller. *Ann Rev Phytopathol*, **1988**, 26, 379-407.
[33] DM Weller; JM Raaijmakers; McSpadden; BB Gardener; LS Thomashow. *Annual Review of Phytopathology*, **2002**, 40, 309-348.
[34] DM Weller; LS Thomashow. *Curr Opin Biotechnol*, **1993**, 4, 306-311.
[35] E Costa; N Teixdo; J Usall; E Atares; I Vinas. *Applied Microbiology and Biotechnology*, **2001**, 56: 367-371.
[36] E Fallik; S Sarig; Y Okon. Morphology and physiology of plant roots associated with *Azospirillum*. In: Okon Y (eds) *Azospirillum* plant associations, CRC press Boca Raton, FL, USA, **1994**, 77-86.
[37] E Kay; C Dubuis; D Haas. Three small RNAs jointly ensure secondary metabolism and biocontrol in *Pseudomonas fluorescens* CHA0. Proceedings of the National Academy of Sciences, **2005**, 102, *47*, 17136-17141.
[38] E Leavy; Z Eyal; I Chet; A Hochman. *Physiol Mol Plant Pathol*, **1992**, 40, 163-171.
[39] E Vela´zquez; P Garc0ıa-Fraile; MH Ramırez-Bahena; R Rivas; E Martınez-Molina. Bacteria involved in nitrogen-fixing legume symbiosis: current taxonomic perspective. In: Khan MS, Zaidi A, Musarrat J (eds) Microbes for legume improvement. Springer-Verlag, **2010**, Wien. doi:10.1007/978-3-211-99753-6_1.
[40] EF Henzell. *Pl. Soil*, **1988**, *108*, 15–21.
[41] EJ Gray; KD Lee; AM Souleimanov; MRD Falco; X Zhou; A Ly; TC Chales; BT Driscoll; DL Smith. *J Applied Microbiology*, **2006**, 100, *3*, 545-554.
[42] F Schonbeck; HW Dehne; W Beicht. *Z Pflk Pflschutz*, **1980**, 87, 654-666.
[43] FB Salisbury. The role of plant hormones. In: Wilkinson RE (eds) Plant–Environment Interactions. Marcel Dekker, New York, USA, **1994**, 39-81.
[44] FJ Gutierrez-Manero; B Ramos-Solano; A Probanza; J Mehouachi; FR Tadeo; M Talon. *Physiol Plant*, **2001**, 111, 206–211.
[45] FY Hafeez; FI Naeem; R Naeem; AH Zaidi; KA Malik. *Environ Exp Bot*, **2005**, 54, 142-147.
[46] G Holguin; BR Glick. *Microbial Ecol*, **2001**, *41*, 281–288.
[47] G Lazarovits G; J Nowak. *HortScience*, **1997**, 32, 188-192.
[48] G Wei; JW Kloepper; S Tuzun. *Phytopathology*, **1991**, 81, 1508-1512.

[49] GG Khot; Tauro; KR Dadarwal. *Indian Journal of Microbiology*, **1996**, 36, 217-222.
[50] GJ Tortora; BR Funke; CL Case. Microbiology-An introduction. Benjamin Cummings, NewYork, 10th edition, **1980**, 810.
[51] GW Zehnder; JF Murphy; EJ Sikora; JW Kloepper. *Eur. J. Plant Pathol*, **2001**, 107, 39-50.
[52] H Antoun; JW Kloepper. Plant growth promoting rhizobacteria (PGPR). In: Brenner S and Miller JM (eds) Encyclopedia of Genetics. New York, Academic press, **2001**, 1477-1480.
[53] H Prasad; R Chandra. *Journal of the Indian society of soil science*, **2003**, 51, 76-78.
[54] H Rodriguez; R Fraga. *Biotechnol Adv*, **1999**, 317-319.
[55] HJ Peppler. Yeast extract. In: Rose AH (eds) Fermented Foods. Academic press, London, UK, **1982**, 293-312.
[56] HP Spaink. *Annu Rev Microbiol*, **2000**, 54, 257–288.
[57] I Izhar; S Ehteshamul-Haque; M Javeed; A Ghaffar. *Pak J Bot*, **1995**, 27, 451-455.
[58] IA Siddiqui; S Ehteshamul-Haque; A Ghaffar. *Pak J Nematol*, **1999**, 17, 67-75.
[59] IA Siddiqui; SS Shaukat; IH Sheikh; S Khan. *World J Microbiology and Biotechnology*, **2006**, 22, *6*, 641-650.
[60] IEG De Salamone; RK Hynes; LM Nelson. *Can J Miccrobiol*, **2001**, 47, 404-411.
[61] IT Paulsen; C Press; J Ravel; DY Kobayashi; GSA Myers; DV Mavrodi; RT DeBoy; R Seshadri R; Q Ren; R Madupu; RJ Dodson; AS Durkin; LM Brinkac; SC Daugherty; SA Sullivan; MJ Rosovitz; ML Gwinn; L Zhou; WC Nelson; J Weidman; K Watkins; K Tran; H Khouri; EA Pierson; LS Pierson; LS III Thomashow; JE Loper. *Nature Biotechnol*, **2005**, 23, 873-878.
[62] J Davison. *Biotechnology*, **1988**, 6, 282-286.
[63] J Fuhrmann; AG Wollum. *Biology and Fertility of Soils*, **1989**, 7, 108–112.
[64] JA Lewis. Formation and delivery systems of biocontrol agents with emphasis on fungi. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer, Rotterdam, The Netherlands, **1991**, 279-287.
[65] JG Fuchs; Y Moenne-Loccoz; G Defago. *Soil Biology and Bio Chemistry*, **1999**, 32, 421-424.
[66] JL Firmin; KE Wilson; L Rossen; AWB Johnston. *Nature London*, **1986**, 324, 90–92.
[67] JW Kloepper. Plant growth promoting rhizobacteria as biocontrol agents. In: Jr FB (eds) Soil Microbiol Ecology. Marcel Dekker inc, New York, **1993**, 255-273.
[68] JW Kloepper; CM Ryu; S Zhang. *Phytopathology*, **2004**, 94, 1259-1266.
[69] JW Kloepper; R Lifshitz; RM Zablotowicz. *Trend Biotechnol*, **1989**, 7, 39-44.
[70] JW Redmond; M Batley; MA Djordjevic; RW Innes; PL Kuempel; BG Rolfe. *Nature, London*, **1986**, 323, 632–635.
[71] KJ Ryan; CG Ray. *Sherris Medical Microbiology*, **2004**, (4th ed.). McGraw Hill.
[72] KM Volkmar; E Bremer. *Can J Plant Sci*, **1998**, 78, 545–551.
[73] KY Kim; D Jordan; GA McDonald. *Biol. Fertil Soils*, **1998**, 26, 79–87.
[74] L Bin; X Guan-li; A Soad; J Coosemans. *Journal Zhejisng University Science*, **2005**, 6B, *6*, 491-501.
[75] L Sticher; B Mauch-Mani; JP Metraux. *Annu Rev Phytopathol*, **1997**, 35, 235-270.
[76] LC Van Loon; PAHM Bakker; CMJ Pieterse. *Annu Rev Phytopathol*, **1998**, 36, 453-483.
[77] LS Rautela; R Chandra; RP Pareek. *Indian journal of Pulses Research*, **2001**, 14, 133-137.
[78] LS Thomashow; DM Weller. Current concept in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites. In: Stracey G, Keen NT (eds) Plant Microbe Interactions. Chapman and Hall, New York, USA, **1996**, 187-235.

[79] M Ahmed; A Zaidi; MS Khan; M Oves. Factors affecting the variations of Microbial communities in different agro-ecosystem. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial Strategies for crop improvement. Springer Berlin, Heidelberg, **2009,** 301-324.
[80] M Dwivedi; RM Upadhyay; GK Dwivedi. *Indian J Agril Chem*, **1993**, 26, (*2* &*3*), 111-122.
[81] M Leeman; JA Van Pelt JA; FM Den Ouden; M Heinsbroek; PAHM Bakker; B Schippers. *Eur J Plant Pathol*, **1995**, 101, 655-664.
[82] M Reitz; P Oger; A Meyer; K Niehaus; SK Farrand; J Hallman; R Sikora; SA Richard. *Nematology*, **2002**, 4, *1*, 73-79
[83] M Vassileva; M Serrano; V Bravo; E Jurado; I Nikolaeva; V Martos; N Vassilev. *Appl Microbiol Biotechnol*, **2010**, 85, 1287–1299.
[84] MA Ali; IY Trabulsi; ME Abd-Elsamea. *Plant Diseases*, **1981**, 65, 432-435.
[85] MA Doherty; TF Preece. *Physiol Plant Pathol*, **1988**, 22, 123-132.
[86] MA German; S Burdman; Y Okon; J Kigel. *Biol Fertil Soils*, **2000**, 32, 259–264.
[87] MM O'Mahony; AD Dobson; JD Barnes; I Singleton. *Chemosphere*, **2006**, 63, *2*, 307-14.
[88] MN Schroth; JG Hancock. *Science*, **1982**, 216, 1376-1381.
[89] MS Khan; A Zaidi; PA Wani; M Oves. *Environ chem Lett*, **2007**, 7, 1-19.
[90] N Dashti; F Zhang; R Hynes; DL Smith. *Plant and soil*, **1998**, 200, 205-213
[91] N Von Wiren; H Khodr; RC Hider. *Plant Physiol*, **2000**, 124, 1149-1157.
[92] NK Peters; JW Frost; SRA Long. *Science*, **1986**, 233, 977–980.
[93] O Barazani; J Friedman. *J. Chem Ecol* , **1991**, 25, 2397-2406.
[94] PJ Slininger; JE Van Cauwenberge; RJ Bothast; DM Weller; LS Thomashow; RJ Cook. *Applied Microbiology and Biotechnology*, **1996**, 45, 391-398.
[95] PPO Ramaswami; G Oblisami. Influence of nitrogen, Phosphorus and inoculation on nitrogen fixation and yield in black gram (*Vigna mungo* L.). Proc. National Seminar on Microbial Ecology. p. 2. Jan., **1986**, 23–4.
[96] PV Dharani-Aiyer. *African Journal of Biotechnology*, **2004,** 10, 519-522.
[97] R Choudhary; S Shrivastava. *Current Science*, **2001**, 768-81.
[98] R Mago; S Shrivastava. *Appl Environ Microbial*, **1994**, 2360-2367.
[99] R Pinton; Z Varanini; P Nannipier. The rhizosphere as a site of biochemical interactions among soil components, plant and microorganisms. In: Pinton R, Varanini Z, & Nannipieri P (eds) The rhizosphere Biochemistry and organic substances at the soil plant interface. Marcel Dekker, New York, **2001**, 1-17.
[100] R Van Peer; GJ Niemann; B Schippers. Induced resistance and Phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. Strain WCS417r. *Phytopathology*, **1991**, 81, 728-734.
[101] RA Muzzarelli. Chitin. Pergamon Press, NewYork, **1977**, 309.
[102] RD Lumsden; JA Lewis. Problems and progress selection, production, formulation and commercial use of plant disease control fungi. In: Whipps JM, Lumsden RD (eds) Biotecnology of fungi for improved plant growth. British Mycological society. London, UK, **1989**, 171-190.
[103] RJ Cook; KF Baker. The Nature and Practice of Biological Control of Plant Pathogens. *American Phytopathological Society*, **1983**, St. Paul, MN, USA.
[104] RS Hussey; KR Barker**.** *Indian Journal of Nematology*, **1976**, 8, 48-52.
[105] S Burdman; H Volpin; J Kigel; Y Kaplunic; Y Okon. *Applied and environmental Microbiology*, **1996**, 62, 3030-3033.
[106] S Buysens; K Heungens; J Poppe; M Hoefte. *Appl Environ Microbiol*, **1996**, 62, 865-871.
[107] S Csuzi. *Acta Biochim Biophys Acad Sci Hung*, **1978**, 13, 41-42.

[108] S Heeb; D Haas. *Mol Plant-Microbe Interact*, **2001**, 1314-1351.
[109] S Shahzad; A Ghaffar. Effect of different populations of *Paecilomyces lilacinus* on the biological control of *M. phaseoline* and *Meloidogyne incognita* infection on mung bean. Expert Consultation on Plant Nematode Problems and their control in the Near East Region, IInd international meeting on plant Nematology, Karachi, **1992**, 77.
[110] S Singh; YD Gaur. *Indian Journal of Microbiology*, **1995**, 35, 317–325.
[111] S Zuber; F Carruthers; C Keel; A Mattart; C Blumer; G Pessi; C Gigot-Bonnefoy; U Schnider-Keel; S Heeb; C Reimmann; D Haas. *Mol plant Microbe Interact*, **2003**, 616-634.
[112] SA Bankole; A Adebanjo. *J. Plant Protect. Tropics*, **1998**, 11, 37-43.
[113] SP Raut. *Indian Journal of Phytopathology*, **1980**, 33, 351-353.
[114] SW Wescott; DA Kluepfel. *Phytopathology*, **1990**, 8, 1245-1249.
[115] T Zhou; TC Paulitz. *J Phytopathol*, **1994**, 142, 51-63.
[116] TJ Smith; AJ Hillier; GJ Lee. *Journal of Dairy Research*, **1975**, 42, 123-138.
[117] V Bianciotto; A Genre; P Jargeat; E Lumini; G Becard; P Bonfante. *Appl Environ Microbiol*, **2004**, 70, 3600-3608.
[118] V Bianciotto; P Bonfante. *Antonie Leewvenhoek*, **2002**, 81, 365-371.
[119] V Ramamoorthy; R Viswanathan; T Raguchander; V Prakasam; R Samiyappan. *Crop Prot*, **2001**, 20, 1-11.
[120] W Crueger; A Cruegr. Substratos para la Fermentation Industrial. Acribia, **1993**, Madrid, Spain.
[121] WB Kendrick*Mycopath Appl*, **1963**, 19, 241-245.
[122] WJ Connick; JA Lewis; PC Quimby. Formulation of biocontrol agents for use in plant pathology. In: Baker R, Dunn P, (eds) New Direction in biological control. **1990**, Liss, New York, USA, 345-372.
[123] WL Barraquio; EM Segubre; MS Gonzalez; SC Verma; EK James; JK Ladha; AK Tripathi. Diazotrophic enterobacteria: what is the role in the rhizosphere? In: Ladha JK, Reddy PM (eds) The Quest of nitrogen fixation in Rice, **2000**, IRRI, Manila, 93-118.
[124] X Zhuang; J Chen; H Shim; Z Bai. *Environ Int*, **2007**, 406-433
[125] Y Huang; C Xu; L Ma; K Zhang; C Duan; M Mo*European J Plant Pathology*, **2009**, 26, *3*, 417-422.
[126] Y Kapulnik. Plant growth promoting rhizosphere bacteria. In: Waisely Y, Eshel A and Kafakfi U (eds) Plant roots. The Hidden Half, Marcel Dekker, New York, **1996**, 769-781.
[127] Y Nohata; R Kurane. *Journal of Fermentation and bio-engineering*, **1997**, 83, 116-117.
[128] YM Bai; F D'Aoust; DL Smith; BT Driscoll. *Canadian Journal of Microbiology*, **2002**, 48, 230–238.
[129] ZA Siddiqui; SI Husain. *Fundamental and Applied Nematology*, **1992**, 15, 491-591.