

Review Article

<https://doi.org/10.20546/ijcmas.2017.607.160>

## Plant Growth Promoting Rhizobacteria *Pseudomonas*: A Review

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### ABSTRACT

#### Keywords

Plant Growth-Promoting Rhizobacteria (PGPR), *Pseudomonas*.

#### Article Info

##### Accepted:

17 June 2017

##### Available Online:

10 July 2017

Plant Growth-Promoting Rhizobacteria (PGPR) are naturally occurring soil bacteria that can enhance plant growth by wide variety of mechanisms. PGPR offers an attractive way to replace chemical fertilizer, pesticides, and supplements. Agriculture and horticulture crops inoculated with certain PGPR strains may result in multiple effect right from enhancement of seedling germination to vegetative growth to yield.

### Introduction

Plant growth promoting rhizobacteria (PGPR), a group of root associated bacteria, intimately interact with the plant roots and consequently influence plant health and soil fertility. They offer an excellent combination of traits useful in disease control and plant growth promotion. Plant growth promoting rhizobacteria (PGPR) were first defined by Kloepper and Schroth (1978) as the soil bacteria that colonize the roots of plants. Amongst the PGPRs, fluorescent pseudomonads have emerged as the largest and potentially the most promising group of PGPR with their rapid growth, simple nutritional requirements, ability to utilize diverse organic substrates and mobility.

Fluorescent pseudomonads produce highly potent broad spectrum antifungal molecules against various phytopathogens, thus acting as effective bio control agents.

*Pseudomonas* species are ubiquitous bacteria in agricultural soils and have many traits that make them well suited as PGPR. Fluorescent pseudomonads are gram negative, aerobic rods, motile with polar flagella and have the ability to produce water soluble yellow green pigment (Palleroni *et al.*, 1973). They comprise the species of *P. fluorescens* (four bio types), *P. putida* (two bio types), *P. aeruginosa*, *P. chlororaphis*, *P. aureofaciens* and *P. syringe* (Schippers *et al.*, 1987). They are well adapted to rhizosphere and

rhizoplane, have a fast growth rate in the rhizoplane and are able to utilize a large number of organic substrates (Stolp and Godkari, 1981) including root exudates (Rovira and Davey, 1974). The worldwide interest in this group of rhizobacteria was sparked off by the studies initiated at the University of California, Berkeley, USA during 1970s. Fluorescent pseudomonads exhibit diverse mechanisms of biocontrol which include antibiosis, HCN production, siderophore production, competition for space and nutrients and induced systemic resistance. PGPR are known to induce resistance against fungal, bacterial, viral diseases and insect pests (Chen *et al.*, 2000).

The crucial factor in the success of biological control by fluorescent pseudomonads is their ability to colonize the rhizosphere and their persistence throughout the growing season, because they occur in the natural habitat of rhizosphere and when they are reintroduced to roots through seed or seed-piece inoculation, they colonize root surface profusely (Van-Loon *et al.*, 1998). Fluorescent pseudomonads exert a protective effect on the roots through antagonism against phytopathogenic fungi and bacteria (Dwivedi and Johri, 2003) by suppressing the pathogens adopting various modes of actions. Fluorescent pseudomonads are known to produce plant growth promoting substances like, auxins, gibberellins, cytokinines etc (Suneesh, 2004).

### **Biocontrol activity mediated by the synthesis of allelochemicals**

Plant growth promoting rhizobacteria (PGPR) colonization and defensive retention of rhizosphere niches are enabled by production of bacterial allelochemicals, including iron-chelating siderophores and antibiotics.

### **Siderophore**

Siderophores are low molecular weight ferric iron chelating compounds that are secreted

extracellularly under iron limiting conditions and whose main function is to supply iron to the iron starved cells. Under iron-limiting conditions plant growth promoting rhizobacteria produce low-molecular-weight compounds called siderophores to competitively acquire ferric ion (Whipps, 2001). Siderophores are small, high-affinity iron chelating compounds secreted by microorganisms such as bacteria and fungi (Miller, 2008). *Pseudomonas* siderophores have a high affinity for iron, and when they chelate this micro-nutrient, they make it less available for other micro-organisms, including plant pathogens. This mechanism is considered indirect plant growth promotion by *Pseudomonas*. *Pseudomonas* can synthesise siderophores in iron limiting conditions. It is known that compounds such as siderophores are synthesised mainly during the exponential growth phase, which is the stage in which the population requires more nutrients for cell division (O'Sullivan and O'Gara, 1992). Likewise, the pseudobactin Fe complex has a high stability constant (Chen *et al.*, 1994), suggesting that virtually all excreted pseudobactin molecules bind to Fe present in the medium (Loper and Henkels, 1999). Therefore, in microenvironments such as the rhizosphere, the synthesis of siderophores is important to confer an advantage in the competition for nutrients and space (Loper and Henkels, 1999). Synthesis of iron-chelating compounds, such as siderophores, by *Pseudomonas* is a characteristic feature visible in some isolates from bulk or rhizosphere soils. In culture media with trace amounts of iron, a yellow-green halo can be observed, which may be fluorescent under ultraviolet light (Budzikiewicz, 1993). Kloepper *et al.*, (1980b) proposed that siderophores might be involved in biocontrol of plant pathogens and in plant growth promotion. Since then, the role of siderophores as chelating agents depriving soil pathogens of iron, an essential

element for growth without which the survival of many micro-organisms is affected, has been widely recognised (Loper and Henkels, 1999). Some PGPR strains produce siderophores that bind  $Fe^{3+}$ , making it less available to certain members of native microflora (Kloepper *et al.*, 1980a). The strains of rhizobacteria that produce siderophore under Fe limiting conditions in the rhizosphere chelate  $Fe^{3+}$ , the form that is insoluble in water, hence not available to bacteria. Isolates belonging to *P. fluorescens* were reported to produce extracellular siderophores when grown in Chrome azurol S under iron deficiency (Suryakala *et al.*, 2004). Instant golden yellow colour is a positive test for siderophore production on succinate medium and casamino acid medium (CAA). Most evidences to support the siderophore theory of biological control by rhizobacteria comes from the work with pyoverdine, a class of siderophores that comprise the fluorescent pigment of fluorescent pseudomonads (Demange *et al.*, 1987). Suryakala *et al.*, (2004) suggested that tri-hydroxamate siderophores might be exploited as potent biocontrol compounds against plant pathogens.

### **Antibiosis**

Antibiosis has been postulated to play an important role in disease suppression by rhizobacteria (Gutterson *et al.*, 1986). Pseudomonads suppress the soil-borne fungal pathogens by producing antifungal metabolites such as pyoluteorin, pyrrolnitrin, phenazines and 2, 4-di-acetyl phloroglucinol (Deepti and Johri 2003). The compound 2,4-diacetylphloroglucinol (DAPG) is a phenolic molecule produced by certain plant-associated fluorescent pseudomonads of worldwide origin (Thomashow *et al.*, 1997). Antibiosis is now often implicated as an important mechanism of biological control, resulting from the fact that it is an attractive

mechanism to study and can provide a highly effective mode of action (Handelsman and Stabb, 1996). *Pseudomonas* known to produce the antibiotic 2, 4-diacetylphloroglucinol (DAPG) may also induce host defences. Additionally, DAPG-producer bacterial antagonists can aggressively colonize root, a trait that might further contribute to their ability to suppress pathogen activity in the rhizosphere of plant through competition for organic nutrients (Heydari and Pessaraki, 2010). Antimicrobial compounds produced by *Pseudomonas cepacia* were reported to inhibit the radial growth of some important soil borne pathogens like *F. oxysporum*, *Macrophomina phaseolina*, *Sclerotium rolfsii*, *R. solani*, and *Pythium ultimum* (Baligh *et al.*, 1999).

Several strains of fluorescent pseudomonad produce antifungal metabolites namely phenazines which comprise of a large family of heterocyclic nitrogen containing coloured pigment with broad spectrum antibiotic activity (Thomashow *et al.*, 1997). Pyrrolnitrin (PRN) [3-chloro-4-(2'-nitro-3'-chloro-phenyl) pyrrole] is another broad-spectrum antifungal metabolite produced by many fluorescent and non-fluorescent strains of the genus *Pseudomonas*. A phenyl pyrrol derivative of PRN has been developed as an agricultural fungicide. Pyrrolnitrin persists actively in the soil for at least 30 days, it does not readily diffuse and is released only after lysis of host bacterial cell (Radjacomare *et al.*, 2004). The biological control agent, *P. fluorescens* BL915 is reported to contain four gene clusters involved in the biosynthesis of antifungal molecule PRN from the precursor tryptophan (Hamill *et al.*, 1970). The broad-spectrum activity of pyrrolnitrin, produced by *Pseudomonas* and *Burkholderia* species was noticed by Nishida *et al.*, (1965) who tested and further developed this antibiotic for therapeutic purposes against human pathogenic bacteria and fungi. With respect to

plant pathogenic fungi, pyrrolnitrin has shown activity against a wide range of Basidiomycetes, Deuteromycetes and Ascomycetes, including several economically important pathogens like *Rhizoctonia solani*, *Botrytis cinerea*, *Verticillium dahliae* and *Sclerotinia sclerotiorum* (Ligon *et al.*, 2000).

Hydrogen cyanide (HCN) is produced by many rhizobacteria and is postulated to play a role in biological control of pathogens (Defago *et al.*, 1990). Voisard *et al.*, (1989) presented evidence that HCN was involved in biological control by *Pseudomonas fluorescens* strain CHA0 which stimulated root hair formation, indicating that the strain induced altered plant physiological activities. Ramette *et al.*, (2003) reported that HCN was a broad spectrum antimicrobial compound involved in biological control of root diseases by many plant associated fluorescent pseudomonads. HCN inhibits the electron transport there by energy supply to the cells is disrupted leading to the death of the organism. It affects the proper functioning of the enzymes and natural receptors by reversible mechanisms of inhibition (Corbett, 1974). It is also known to inhibit the action of cytochrome oxidase (Gehring *et al.*, 1993).

### **Indirect plant growth promotion through induced systemic resistance**

Biopriming plants with some PGPR can also provide systemic resistance against a broad spectrum of plant pathogens. Diseases of fungal, bacterial and viral origin and in some instances even damage caused by insects and nematodes can be reduced after application of PGPR (Ryu *et al.*, 2004).

### **Induced systemic resistance**

Induced systemic resistance is broadly defined as activation of latent defence mechanisms in plants prior to pathogenic attack. The mechanism has been hypothesized

to be an operable mechanism in several rhizobacterial systems. Induced systemic resistance is associated with increased synthesis of certain enzymes such as peroxidase (Langrimini and Rothstein, 1987), increased levels of certain acid soluble proteins (Zdor and Anderson, 1992) and the accumulation of phytoalexins in the induced plant tissue (Vanpeer *et al.*, 1991). The seed bacterization of common bean with *P. fluorescens* S97 was reported to suppress the halo blight caused by *P. syringe* pv. *phaseolicola* through induced systemic resistance mechanism (Alstrom, 1991).

### **Influenced of PGPR on agricultural crops**

Tomato, cucumber, lettuce and potato plants bacterized with plant growth promoting *Pseudomonas* strain have shown increased root and shoot fresh weight and simultaneous suppression of deleterious pathogenic microflora (Vanpeer and Schippers, 1989). Walley and Germida (1997) observed enhancement of shoot dry weight from 16 to 48 per cent and root dry weight from 82 to 137 per cent when inoculated with fluorescent pseudomonads. Gupta *et al.*, (2002) reported that peanut seeds bacterized with *Pseudomonas*GRC2 showed a significant increase in germination (83%) under field conditions. *Pseudomonas* do not form a symbiosis similar to that formed by rhizobia with plants, although they are able to penetrate plant tissues and establish themselves as endophytes (Marquez-Santacruz *et al.*, 2010). Inside the plant, they also play an important role as PGPR and inhibit pathogen growth by various mechanisms. By competition and production of antimicrobial compounds, PGPR can reduce populations of plant pathogens and deleterious rhizobacteria, which restrict plant growth. Some of these disease-suppressing activities, such as production of HCN can reduce plant growth as well, but more often

the net effect is improved plant development, resulting in more vigorous growth and increased yield of agricultural crops (Dowling and O'Gara, 1994). The species of fluorescent pseudomonads are grouped into different biovars and subgroups based on similarity in biochemical tests (Barett *et al.*, 1986). Thus, rapid identification of potentially and economically viable bioagents is possible through various methods of biochemical characterization (Weller *et al.*, 2002).

Various phenotypic and biochemical methods have been developed and used for characterizing pseudomonad isolates. Most of the tests conducted for identification of fluorescent pseudomonas have been based on physiological and nutritional tests (Holt *et al.*, 1984). Most of the plant associated *Pseudomonas* spp. belong to *P. fluorescens* and *P. putida* complex and there has been no clear distinction between the two (Sheath *et al.*, 1981). However, these two species are identified based on trehalose utilization and gelatine liquefaction. In this, *P. fluorescens* exhibits positive for both the tests, whereas, *P. putida* shows negative response (Hildebrand *et al.*, 1992). *P. fluorescens* B16 is a plant growth-promoting rhizobacterium and produces pyrroloquinoline quinone which is a plant growth promotion factor (Choi *et al.*, 2008). Burr *et al.*, (1978) reported that strains of *P. fluorescens* and *P. putida* applied to seed tubers improved the growth of potato. These findings were confirmed and later exemplified in the tomato and eggplant (Kumar and Dubey, 1993), and lentil (Rao *et al.*, 1999). Vransky and Ficker (1984) recorded 4 to 30 per cent improvement in plant growth and tuber yield of potato inoculated with *P. fluorescens* under field conditions. Introduction of *sss* gene encoding rhizosphere colonization ability into poor colonizer strain of *P. fluorescens* WCS 307 has exhibited increased competitive rhizosphere colonization ability in tomato roots resulting in increased protection against *F. oxysporum*

f.sp. *radicis-lycopersici* (Dekkers *et al.*, 2000). The fluorescent pseudomonads in addition to their ability to aid plant growth promotion are also good biocontrol agents. They have emerged as the biggest and potentially the most promising group amongst the PGPRs involved in biocontrol of diseases. *P. fluorescens* is adapted to survival in soil and colonization of plant roots and this applies also to the particular case of biocontrol agents from this species (Kiely *et al.*, 2006). Biocontrol strains have noticeably been observed at the root surface, (*i.e.* the rhizoplane) often forming micro colonies or discontinued bio films in the grooves between epidermal cells. Certain strains are also capable of endophytic colonization. Within root tissues, they are mostly found in the intercellular spaces of the epidermis and the cortex (Duijff *et al.*, 1997). They are effective in utilizing seed and root exudates for growth and can colonize the rhizosphere aggressively. Strains with biocontrol ability may represent in the order of 10 per cent of all rhizosphere strains and they have been isolated from a very wide range of soils, climatic regions and host plants (Rezzonico *et al.*, 2007). There are several species of *Pseudomonas* which are effective antagonists of fungal pathogens and act as plant-promoting rhizobacteria (De Curtis *et al.*, 2010), a strain of *Pseudomonas fluorescens* was reported to have antagonistic property against *Rhizoctonia solani* (Howell and Stipanovic, 1979).

Studies have indicated that seed treatment with *P. fluorescens* isolate 63-28 prevented the entry of *Fusarium* wilt pathogen (*F. oxysporum* f. sp. *lycopersici*) in the vascular tissue by strengthening cell wall structures and accumulation of phenolic substances and chitinases (M'Piga *et al.*, 1997). *Pseudomonas* sp. RSB29 showing significant inhibition of fungal pathogens such as *F. oxysporum* f. sp. *ciceri* RS1, *Macrophomina phaseolina* RSB9, *Fusarium udum* RSB19, *Fusarium solani*

RSB38 and *R. solani* BH49 has been reported by Saikia *et al.*, (2004). Fusarium wilts have been reported to be suppressed by the activity of species and non pathogenic strains of *F. oxysporum* by Boer *et al.*, (2003).

Studies implies that prior application of fluorescent pseudomonads strengthen host cell wall structures resulting in restriction of pathogen invasion in plant tissue (Chen *et al.*, 2000). The study also indicated that a PO1 isoform was prominently expressed in *P. fluorescens* isolate Pf1-treated root tissues against *F. oxysporum* f. sp. *lycopersici*. This unique isoform induced by *P. fluorescens* isolate Pf1 might have contributed to induced defense in tomato root tissue against the invasion by *F. oxysporum* f. sp. *lycopersici*. Accumulation of phenolics, PAL,  $\beta$ -1,3-glucanase and induction of PO1 isoform, PPO1 and PPO2 isoforms and Chi2 isoform by *P. fluorescens* isolate Pf1 in tomato root tissues might have collectively contributed to induced resistance in tomato plants against *F. oxysporum* f. sp. *lycopersici*.

Like *Bacillus*, *Pseudomonas* also induces systemic resistance in plants. Studies have reported that *P. fluorescens* EP1, *P. putida* 5-48 and *P. fluorescens* can protect sugarcane, oak and tomato plants from pathogens such as *Colletotrichum falcatum*, *Ceratocystis fagacearum* and *F. oxysporum*, respectively. *P. fluorescens* isolates were obtained from the rhizosphere, checked for their *in vitro* antagonistic activity, formulated and evaluated for their ability to control Fusarium wilt and promote growth of tomato plants under greenhouse conditions. The fresh cultures of *P. fluorescens* isolate increased seedling emergences up to 90 per cent, when compared to the control in pots (Asha *et al.*, 2011). The results indicated that there was no negative effect when the *P. fluorescens* was applied as biocontrol agent, on the contrary they exhibited synergism in promoting crop growth and yield of tomato besides controlling the Fusarium wilt disease. Efri (1994) reported that, *P. fluorescens* could inhibit the development of tomato wilt by

71.7 per cent compared to plants without application of the bacteria. In the laboratory test, appearance of empty zone in the Petri dish that contained *F. oxysporum* and *P. Fluorescens* was observed, proving that there was a competition between the parasitic pathogen and the antagonistic pathogen for iron, because *P. fluorescens* has a high affinity to bind iron. The bacterium takes the iron and binds it to its necessity, thus *F. oxysporum* lacks the iron for its proliferation and development of the disease is suppressed. The reduction of pathogenic activity of *F. oxysporum* by pseudomonads could also be related to the detoxification of fusaric acid (Harbone, 1983). Peitr (1991) reported that *P. fluorescens* suppressed Fusarium wilt by detoxifying the culture filtrates of different *Fusarium* species and inactivating the enzymes from the fungal cultures. Borowitz *et al.*, (1992) reported that extracellular proteases of *P. fluorescens* strains were able to inactivate hydrolases and phytotoxins of phytopathogenic *Fusarium* spp. The talc and sodium alginate formulations of *P. fluorescens* was recommended to the farmers as one of the crop protection strategies for the management of Fusarium wilt of tomato and this practice was also extended to other crops by Asha *et al.*, (2011).

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#### How to cite this article:

Stanzin Dorjey, Disket Dolkar and Richa Sharma. 2017. Plant Growth Promoting Rhizobacteria *Pseudomonas*: A Review. *Int.J.Curr.Microbiol.App.Sci*. 6(7): 1335-1344. doi: <https://doi.org/10.20546/ijcmas.2017.607.160>