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Functional characterization of various plant growth promoting activity of *Pseudomonas fluorescens* and *Bacillus subtilis* from Aloe *vera* rhizosphere

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Abstract
The Plant Growth Promoting Rhizobacteria (PGPR) organisms provide protection to plants against diseases by suppressing deleterious and pathogenic microorganisms producing of hormones, hydrogen cyanide (HCN), various beneficial volatile compounds, siderophores, bacteriocins *etc* and also act as a bio fertilizer like P solublization, N fixation, IAA production etc…, present research mainly concentrated on exploration of *Pseudomonas fluorescens* and *Bacillus* PGP activities like SA, IAA, β 1, 3 glucanase production and P solublization. Around fifty one isolates of *Pseudomonas fluorescens* and ten *Bacillus subtilis* isolates were isolated from the rhizosphere soil of *Aloe vera*, among fifty one *Pseudomonas*, Pf 32 and followed by Pf 45 produced maximum quantity of salicylic acid (SA), Pf 45 recorded maximum indole acetic acid (IAA) production. The isolate Pf 32 solubilized high phosphorous in the sperber's hydroxyl apatite medium and higher amount of β 1, 3 glucanase production.

Keywords: *Aloe vera*, Salicylic Acid, IAA Production, P solubilization, *Pseudomonas* sp. and *Bacillus* sp.

1. Introduction
The important traits of Plant Growth Promoting Rhizobacteria (PGPR) include fixation of atmospheric nitrogen, solubilization of insoluble inorganic phosphates, production of plant growth hormones, siderophores, bacteriocins *etc.*, these organisms also provide protection to plants against diseases by suppressing deleterious and pathogenic microorganisms. Bio inoculant preparations containing these organisms are very cost effective, pollution free and a potentially renewable source of plant nutrients, making an ideal partner and an excellent supplement to chemical fertilizers.
Biocontrol occurs through an indirect action of the PGPR, that interact with soil pathogens through several mechanisms such as antibiosis (production of antimicrobial compounds), competition for iron and nutrients or for colonization sites, predation and parasitism, induction of resistance factors (for example the plant is strongly stimulated to synthesize substance called phytoalexins, small molecules with antibiotic activity, which can inhibit the growth of many pathogenic microorganisms), production of enzymes such as chitinase, glucanase, protease and lipase[1]. Antagonistic microorganisms can often produce a range of different antimicrobial secondary metabolites, and extracellular lytic enzymes. Strains belonging to the genera *Pseudomonas* (*e.g., P. fluorescens*) [2, 3] and *Bacillus* (*Bacillus subtilis*) [4]. Have been used in experimental tests on a wide range of economically important crops.
Aloe vera (L) Burm. is one of the medicinal plants widely used throughout the world [5]. It is a well-known medicinal plant of India and is one of the world most demanded crop. *Aloe vera* has many medicinal and cosmetic usages and hence has growing demand in the market. Among the various antagonists used for the management of plant diseases, plant growth promoting rhizobacteria (PGPR) play a vital role [6]. Rhizobacteria such as *Pseudomonas fluorescens* and *Bacillus* strains could provide significant levels of disease suppression and substantially enhance plant growth and grain yield. Hence the study was conducted to isolate the efficient rhizobacteria possessed multiple mechanism for controlling of *Aleo Vera* soft rot disease and other PGPR activities.

Materials and Methods
Salicylic acid production
Testing of antagonistic bacteria for the production of salicylic acid
Salicylic acid production of the strains was determined as per the method of Meyer *et al.*, [7]. The strains were grown in the standard succinate medium (Succinic acid- 4.0 g; K_2HPO_4-6.0 g;

KH_2PO_4- 3.0 g; $(NH_4)_2$ SO_4-1.0 g; MgSO4.7H2O-0.2 g and distilled water-1000 ml, pH 7.0) at 28±2˚C for 48 h. Cells were collected by centrifugation at 6000 g for 50 minutes. Four ml of cell free culture filtrate was acidified with 1N HCl to pH 2.0 and salicylic acid was extracted with equal volume of chloroform. Four ml of water and five µl of 2 M $FeCl_3$ was added to the pooled chloroform phases. The absorbance of the purple iron salicylic acid complex, which was developed in the aqueous phase, was read at 527 nm. A standard curve was prepared with salicylic acid in succinate medium and quantity of salicylic acid produced was expressed as µg/ml.

Indole Acetic Acid production

Quantification of IAA produced by antagonistic bacteria.

The bacterial strains were grown in trypticase soy broth (Animal peptone - 15 g; soypeptone-5 g; NaCl-5.0 g; Glycine-4.4 g and Distilled water-1litre) with tryptophan (100 µl/ml) and incubated at 28±2˚C. To one ml of the cell free culture filtrate, two ml of Salkowsky reagent (1ml of 0.5 M $FeCl_3$ in 50 ml of 35 % per chloric acid) were added and incubated at 28 ± 2°C for 30 minutes. The absorbance was read at 530 nm. A standard was prepared using IAA and presence of IAA in culture filtrate was quantified as µg/ml [8].

Production of lytic enzymes

Quantification of β-1, 3-glucanase produced by antagonistic bacteria

The bacterial isolates were grown in nutrient broth for three days and incubated. Then the culture was centrifuged and the supernatant was taken as enzyme extract. The enzyme activity was color imetrically assayed. Crude enzyme extract of 62.5 µl was added to 62.5 µl of 4 per cent laminarin and then incubated at 40˚C for 10 minutes. The reaction was stopped by adding 375 µl of dinitro salicylic acid (dinitro salicylic acid (DNS) reagent, which was prepared by adding 300 ml of sodium hydroxide (4.5%) to 880 ml of a solution containing 8.8 g of dinitro salicylic acid and 22.5 g of potassium sodium tartrate) and heated for five minutes on boiling water bath. The resulting solution was diluted with 4.5 ml distilled water and the absorbance was read at 500 nm. The crude extract preparation with laminarin with zero time incubation served as blank. The enzyme activity was expressed as µg equivalent of glucose/ minute/ ml of culture filtrate.

Phosphate solubilization

Testing the antagonistic bacteria for phosphate solubilization

The bacteria were spot inoculated in Sperber's hydroxyl apatite medium (Soil extract – 100 ml, glucose -10 g, agar-1.0 g, distilled water – 900 ml). To prepare soil extract, one Kg of garden land soil was dissolved in 1.0 lit of water. It was autoclaved for 30 min at 15 lb pressure. Then it was double filtered after adding a pinch of calcium carbonate. Before pouring the medium into the plates 5.0 ml of 10 per cent KH_2PO_4 and 10ml of 0.06 mM $CaCl_2$ sterilized separately were added to 100 ml of the medium. The bacteria were spot inoculated in the medium and incubated at room temperature (+28C) for 48 h. Appearance of clearing zone indicated phosphate solubilisation.

Results and discussion

Salicylic acid Production by effective bacterial antagonists

Salicylic acid (SA) is an endogenous regulator of localized and systemic acquired resistance in many plants. When plants become infected, the level of SA increases to combat the infection. The exogenous application of SA in healthy plants induced the expression of the same set of defense related genes that was induced in the infected plants [9]. Among the five isolates, Pf 32 was recorded the maximum salicylic acid production (53.24 µg/ml) followed by the isolate Pf 45 (47.41µg/ml) Pf 26 (38.97 µg/ml), Pf 4 (21.75 µg/ml) and Bs_5 (20.26 µg/ml) while the isolate Pf 16 recorded the minimum salicylic acid production (12.18µg/ml) (Table 1).

Salicylic acid production was observed in several bacterial strains and exogenously applied SA induced resistance in plant species [10]. Salicylic acid production has also been reported in *P. fluorescens* WCS 374, WCS 4178[11]. Schneider and Ullrich [12], observed that the salicylic acid stimulated the enzyme activity of chitinase, β1-3 glucanase, peroxidase, and polyphenol oxidase in leaf tissue of cucumber two to three days after induction.

Indole acetic acid (IAA) Production by effective bacterial antagonists

Arshad and Franken Berger [13], suggested that the *Pseudomonas* sp. might increase plant growth by release of phytohormones like cytokinins and indole acetic acid which could either directly or indirectly modulated the plant growth and development. Among the five tested isolates, Pf 45 recorded the maximum IAA production of (13.4 µg/ml) which followed by Pf 32 isolate (10.9 µg/ml), Pf4 (6.3 µg/ml), Pf Pf26 (3.7µg/ml), Bs_5 (4.5 µg/ml) and the minimum IAA production (2.6 µg/ml) was recorded in Pf 16 isolate (2.647µg/ml) (Table 5). Karthikadevi [14] studied the IAA production of five *P. fluorescens* isolates and PFKS-3 recorded maximum IAA production and effectively inhibited the mycelial growth of *M. phaseolina* and *R. solani*. Raj Kumar [15], quantified the Indole acetic acid production by different PGPR strains. He found that *P. fluorescens* BPf_1 recorded the highest IAA production followed by *P. fluorescens* BBs_2.

Lytic enzyme Production

β 1, 3 glucanase Production by effective bacterial antagonists

Although all the isolates, tested in the present study *in vitro* produced

β-1, 3 glucanase. The isolate Pf 32 recorded higher β 1, 3 glucanase activity (37.64 µg of glucose/min/ml) followed by isolate pf 4 (30.07 µg of glucose/min/ml) Pf 45 (28.41 µg/ml), Pf 26 (21.69 µg/ml), Pf 16 (10.75 µg/ml) and the isolate Bs 5 recorded the minimum β 1, 3 glucanase activity (10.13 µg of glucose/min/ml) (Table 1). Alstrom [16], reported that the bacterial strains in the red pigmented members of enterobacteriacea possessed the ability to produce cellulase, protease, phosphatase and chitinase. *P. chlororaphis* strain PCL1391 produced a broad spectrum of antifungal factors (AFFs) against *F. oxysporum* f.sp. *radicis lycopeicisi* including hydrophobic compounds, HCN, chitinase, β 1-3 glucanase and proteases [17]. β-1, 3 glucanase and chitinase also increased the antifungal action of the rhizobacteria in addition to the production of antibiotics, siderophore, SA and HCN [15].

Phosphate solubilisation by effective bacterial antagonists

P. fluorescens may increase plant growth by mineralizing phosphates [18]. In the present investigation, Among the five isolates of antagonistic bacteria tested, only three isolates of Pseudomonas sp. *viz.,* Pf 32 and Pf 45 and Pf 26 were able to produce a clear zone of 2.4, 1.5 and 1.2 cm diameter

respectively in the Sperber's hydroxyl apatite medium 72 h after incubation indicating that these isolates produced phosphatase which solubilized the unavailable phosphorus to available phosphorus. (Table 1). Sivaji, reported the *B. megaterium* isolates recovered from cotton rhizosphere soil produced the halo zone around 31.6 mm on Sperber's hydroxyl apatite medium.

Table 1: Production of salicylic acid, IAA, β-1, 3-glucanase and Phosphate solubilisation by effective bacterial antagonists *in vitro*

Antagonistic Bacteria	Salicylic acid (µg/ml) *	IAA (µg/ml) *	β-1, 3-glucanase (µmol equivalent of glucose released/h/ml) *	Phosphate solubilisation (mm)
Pf_4	21.75^{d}	6.3^{c}	30.07^{b}	-
Pf_{16}	12.18^{f}	2.6^{e}	10.75^{e}	-
Pf_{26}	38.97^{c}	3.7^{de}	21.69^{d}	1.2^{c}
Pf_{32}	53.24^{a}	10.9^{b}	37.64^{a}	2.4^{a}
Pf_{45}	47.41^{b}	13.4^{a}	28.41^{c}	1.5^{b}
Bs_5	20.26^{e}	4.5^{d}	10.13^{t}	-

* Mean of three replications

In a column, means followed by common letters are not significantly different at 5% level by DMRT

References

1. Whipps JM. Microbial interactions and biocontrol in the rhizosphere. Journal of Experimental Botany. 2001; 52:487-511.
2. Mano H, Morisaki H. Endophytic Bacteria in the Rice Plant. Microbes and Environments. 2008; 23:109-117.
3. Meyer JB, Lutz MP, Frapolli M, Defago G. Interplay between wheat cultivars, biocontrol pseudomonads and soil. Applied Environmental Microbiology. 2010; 6:6196-6204.
4. Kokalis-Burelle N, Kloepper JW, Reddy MS. Plant growth promoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. Applied Soil Ecology. 2006; 31(2):91-100.
5. Sofowora A. Medicinal plants and traditional medicines in Africa, Johan Wiley and Sons Ltd., New York, 1984.
6. Kloepper JW. Plant growth-promoting rhizobacteria as biological control agents. In Soil Microbial Ecology: Applications in Agricultural and Environmental Management. Marcel Dekker Inc., New York. 1992, 255-274.
7. Meyer JM, Azelvandre P, Georges C. Iron metabolism in *Pseudomonas*: salicylic acid, a siderophore of *Pseudomonas fluorescens* CHA0. Biofactors. 1992; 4:23-27.
8. Gorden SA, Paleg LG. Quantitative measurement of Indole acetic acid. Physiology and Plant Pathology. 1957; 10:347-348.
9. Klessig D, Malamy F. The salicylic acid signals in plants. Plant Molecular Biology. 1994; 26:1439-1458.
10. Bakker PAHM, Ran LX, Pieterse CMJ, van Loon LC. Understanding the involvement of rhizobacteria mediated induction of systemic resistance in biocontrol of plant diseases. Canadian Journal of Plant Pathology. 2003; 25:5-9.
11. Leeman M, den Ouden FM, van Pelt JA, Cornelissen C, Schippers B. Suppression of *Fusarium* wilt of radish by co-inoculation of fluorescent *Pseudomonas* spp. and root-colonizing fungi. European Journal of Plant Pathology, 1996b; 102:21-31.
12. Schneider S, Ullrich WR. Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. Physiology and Molecular Plant Pathology. 1994; 45:291-304.
13. Arshad M, Franken Berger WT. Microbial production of plant hormones. In: *The Rhizosphere and Plant Growth.* (Eds.) C.D.L. Keister and P.B. Cregan. Kluwer Academic Publishers, Dordrecht, the Netherlands. 1991, 327-334.
14. Karthikadevi TPN. Status of secondary metabolites of *Pseudomonas fluorescens* isolates and their impact on soil borne fungal pathogens *M. phaseolina* and *R. solani*. *M.Sc. (Ag) Thesis*, Avinashilingam University, Coimbatore, India. 2004, 48-49.
15. Raj Kumar. Molecular and biochemical approaches for the selection of biocontrol agents for the eco-friendly management of rhizome rot of banana caused by *Erwinia carotovora* sub sp. *carotovora*. Ph.D. Thesis. Tamil Nadu Agricultural University, Coimbatore. India, 2006.
16. Alstrom S. Characteristics of bacteria from oilseed rape in relation to their biocontrol activity against *Verticillium dahliae*. Journal of Phytopathology. 2001; 149:57-64.
17. Chin-A-Woeng TFC, Bloemberg GV, Bij AJ, Drift KMGM, Schripsema J. Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f.sp. *Radicis-lycopersici*. Molecular Plant Microbe Interaction, 1998; 11:1069-1077.
18. Karimandan SK, Gaur AC. Effect of seed inoculation with *Pseudomonas* sp. on phosphate uptake and yield of maize. Current Science. 1971; 40:439-440.
19. Sivaji M. Impact of BT cotton on the functional microbes in the rhizosphere, Ph.D. Thesis. Tamil Nadu Agricultural University, Coimbatore. India, 2015.