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INFLUENCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON THE PRODUCTIVITY OF *PELARGONIUM GRAVEOLENS* L. HERIT

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Abstract

In the revival of herbal age, aromatic crops are being commercially cultivated in order to fetch the great demand of essential oils used by food, pharmaceutical flavour, perfumery and cosmetics industries. Two isolates of plant growth promoting rhizobacteria (PGPR) which were isolated from the rhizosphere soil of Pyrethrum (*Chrysanthemum cineraefolium*) designated as MA-2 and MA-4, and identified as *Bacillus subtilis* and *Pseudomonas fluorescens* on the basis of cultural as well as biochemical testing. They gave excellent result on the productivity of *Pelargonium graveolens*, increased herb yield over control by 9 and 27.6% respectively.

Keywords: PGPR, *Bacillus subtilis, Pseudomonas fluorescens,* Productivity, *Pelargonium graveolens*

Introduction

Plant growth promoting rhizobacteria (PGPR) are originally defined as root- colonizing bacteria *i.e. Bacillus subtilis* and *Pseudomonas fluorescens* that cause either plant growth promotion or biological control of plant diseases (1). The potential to use PGPR in integrated strategies to reduce N and P fertilizers offers an appealing research area for those scientists engaged in growth promotion studies in dependable of biological control. As with attempts to employ PGPR for biological control, practical use of growth promoting PGPR will be aided by clear elucidation of mechanisms for growth promotion. There are several reports that PGPR's have promoted the growth of reproductive parameters of plants ranging from cereals, pulses, ornamentals, medicinal and aromatic plants, vegetable crops, and even tree species.

Treatment with PGPR has increased the germination percentage, seedling vigor, emergence, plant stand, root growth, shoot growth, total biomass of the plants, seed weight, early flowering, increased grain, fodder, fruit yields *etc.* (2,3). The exact mechanism involved in growth promotion when agronomic crops are inoculated with rhizobacteria include, increase in the nitrogen fixation, the production of auxin, gibberellins, cytokinin, ethylene, the solubilization of phosphorus and oxidation of sulfur, increase in nitrate availability, the extracellular production of antibiotics, lytic enzymes, hydrocyanic acid, increases in root permeability (4). ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, siderophore production, enhancing biological nitrogen fixation and enhancement in the uptake of essential plant nutrients could be the best possible explanations. It has been widely reported in numerous microbial species of gram negative bacteria (5). It is extensively studied in numerous species of plant growth promoting bacteria like *Bacillus* (6) and *Pseudomonas* (7).

Furthermore, the plants grow faster and greener with longer roots and shoots than the untreated plants. It has been established that *fluorescent Pseudomonas* enhance plant growth in several ways viz., producing plant growth regulators, such as gibberellins, cytokinins and indole acetic acid, which can either directly or indirectly modulate the plant growth and development (8,9).

Geranium Plant (*Pelargonium graveolens* L. Herit)

Geranium is an erect, much-branched shrub, that can reach a height of up to 1,3 m and a spread of 1 m. The hairy stems are herbaceous when young, becoming woody with age. The deeply incised leaves are velvety and soft to the touch due to the presence of numerous glandular hairs. The leaves are strongly rose-scented. The showy white to pinkish flowers is borne in an umbel-like inflorescence and is present from late winter to summer (August-January).

This plant is confined to two separate areas in Southern Africa, one in Limpopo Province, where it receives summer rain, and the other in the south-eastern part of the Western Cape, where it receives rain throughout the year. In both these regions, the

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summer is hot and the winter is mild, and *Pelargonium graveolens* is found growing on the mountains, in sheltered positions such as kloofs, usually in relatively moist habitats. *Pelargonium graveolens* has also been recorded in Zimbabwe and Mozambique. *Pelargonium graveolens* oil is used extensively in high class of perfumes, soaps and cosmetics because of its pronounced and lasting rose like odour due to rhodinal content. (10). The oil of *Pelargonium graveolens* is also used in aromatherapy.

Materials and Methods

Isolation of rhizobacteria

The soil samples were randomly collected from rhizospheric soil of Pyrethrum (*Chrysanthemum cineraefolium*) at Central Institute of Medicinal and Aromatic Plant (CIMAP), Lucknow, India, and dried at room temperature for 24 hours. 1 gram of soil was dissolved in 10 ml of sterilized water in a test tube, vortexed at high speed and serially diluted to 1:10, 1:100, 1:1000 and 1:10000. An amount of 500 µL of each dilution was separately spread on petri plates containing nutrient agar and King's B medium (11). Three replicates were maintained for each sample. The plates were then sealed with parafilm, incubated at 30±2°C and growth was examined after 24-72 hours. Each of the colonies produced in petridishes were transferred in to fresh nutrient agar slant and cultures were stored at -20°C in refrigerator (12).

Biochemical characterization of rhizobacteria

Selected isolates of *Bacillus* (MA-2) and *Pseudomonas* (MA-4) were biochemically characterized by Gram's reaction, carbohydrate fermentation, oxidase test, O-F test, H2S production, IMViC tests, NO2 reduction, starch hydrolysis, phosphate solublization test (TCP) and gelatin hydrolysis as per the standard methods (13,14).

Production of Indole acetic acid

Indole acetic acid (IAA) production was detected as described by Brick et al., (15). Bacterial cultures were grown for 48h (*Bacillus* and *Pseudomonas*) on their respective media at 36±2ºC. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2ml) was mixed with two drops of orthosporic acid and 4ml of the Salkowski reagent (50ml, 35% of perchloric acid, 1 ml 0.5M FeCl3 solution). Development of pink colour indicates IAA production.

Production of ammonia

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 48-72 h at 36±2ºC. Nessler's reagent (0.5 ml) was added in each tube. Development of brown yellow colour was a positive test for ammonia production production (13).

Siderophore production

Siderophore production was detected by the universal method of Schwyn and Neilands (16) using blue agar plates containing the dye chrom azurol S (CAS). Orange halos around the colonies on blue were indicative for siderophore production.

Application of rhizobacteria in *Pelargonium graveolens*

Single pure isolated colony of selected rhizobacteria was multiplied in nutrient broth medium by incubation for 4-5 days over rotatory shaker at 110 rpm. The bacterial culture was centrifuged at 10,000 rpm for 10 min. The pellet was collected and mixed in 0.01 MgSO4 with the help of magnetic stirrer. An amount of 10-ml bacterial culture was mixed in 100 g of vermicompost, which was applied around the root zone of *Pelargonium graveolens* L'Hérit. In control only 100g vermicompost was added. The effect of PGPR on the growth of *Pelargonium graveolens* was recorded after three months of inoculation.

Results and Discussion

In present study strain MA-2 and MA-4, isolated from *Crysanthemum cineraefolium* rhizosphere and also release inorganic phosphate in tri calcium phosphate medium, which were identified as *Bacillus subtilis* and *Pseudomonas fluorescens* respectively on the basis of cultural as well as biochemical testing (Table 1 and 2).

Table 1. Morphological, cultural and biochemical characteristic of rhizobacteria

Characteristics	Isolate No. MA-2	Isolate No. MA-4
Colony on nutrient agar	Irregular, undulate creamish dull, with ground glass appearance	Round with entire margin, creamish
Fluorescent on Kings B	No pigment	Yellowish green
Grams reaction	Gram (+)	Gram (–)
Shape	Short rods	Rods
Arrangement	Mostly single	Single and in chains
Endospore position	Sub terminal	No spore
Growth at 30°C	Good	Good
Starch hydrolysis	+	+
Haemolysis in Blood agar	Clear zone (β -haemolysis)	No
Indole production	–	–
Methyl-red test	+	–
Voges-Proskauer	–	–
Citrate utilization	–	+
T.S.I.	+	–
O.F. media	+	+
Nitrate reduction	+	+
Urease hydrolysis	+	+
Gelatin liquefaction	+	+
Motality test	Inverted tree +	+
Litmus milk reduction	Acid production	Acid by reduction
Dextrose fermentation	A (-), G (-)	A (-), G (-)
Sucrose fermentation	A (+), G (-)	A (+), G (-)
Lactose fermentation	A (-), G (-)	A (+), G (-)
Growth on PDA	Heavy	Heavy
Identification	*Bacillus subtilis*	*P. fluorescens*

A = Acid, G = Gas

Similarly 44 bacterial isolates from the rhizosphere of tomato were screened for their plant growth promoting activities (17) are able to solubilize sparingly soluble phosphate, usually by releasing chelating organic acids (18). Our strain MA-4 produces fluorescent pigment on King's B agar medium, as reported for production of fluorescent pigment by *Pseudomonas fluorescens* (11).

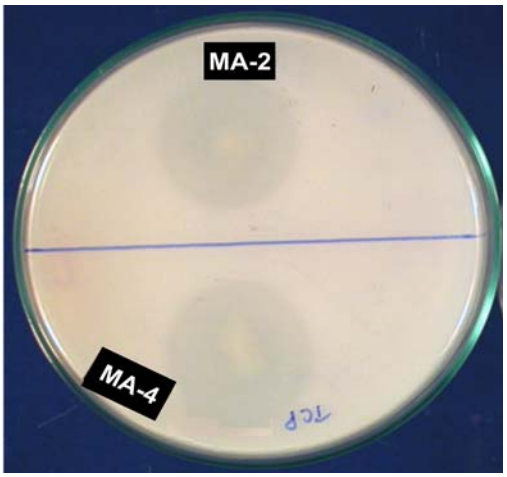
Table 2. Plant growth promoting characteristics of rhizobacterial isolates

Characteristics	Isolate No. MA-2	Isolate No. MA-4
Oxidase activity	+	+
Catalase activity	+	+
Phosphate solubilizing test (TCP)	Clear zone	Clear zone
IAA production	+	+
Ammonia production	+	+
Siderophore production	-	+

In our study both plant growth promoting bacterial isolates isolated from *Chrysanthemum cineraefolium* of Asteraceae and used against the member of Geraniaceae. While 107 rhizobacterial isolates, obtained from the rhizosphere of *Eucalyptus* spp. belongs to family Myrtaceae (19). Some plant growth-promoting rhizobacteria such as *Bacillus subtilis* RC11 were efficient in phosphate solubilization and indole acetic acid (IAA) production and significantly increased growth of wheat and spinach (20).

Therefore, in present study plant growth promoting rhizobacteria *Bacillus subtilis* strain MA-2 and *Pseudomonas fluorescens* strain MA-4 were efficient in phosphate solubilization and indole acetic acid (IAA) (Fig. 1) production and significantly increased biomass 9% and 27.6% respectively of medicinal and aromatic plant such as *Geranium*.

Figure 1. Phosphate solublization test of *Bacillus subtilis* (MA-2) and *Pseudomonas flurescens* (MA-4) on Tri calcium phosphate medium



Increased in biomass production leads to the essential oil yield (Table 3) (Fig. 2). The effect of some bacteria isolates on root formation, root length and dry matter content of roots of mint (*Mentha piperita* L.). Mint and *Agrebacterium rubi* (strain A16), *Burkholderia gladii* (strain BA7), *Peseudomonas putidea* (strain BA8), *Bacillus subtilus* (strain OSU142) *Bacillus megatorium* (strain M3) were used as rooting agent, respectively (21).

According to Kohler *et al.* (22) inoculation with *B. subtilis* increased significantly the urease, protease and phosphatase activities of the rhizosphere soil of the lettuce plants and also increased foliar P and K contents. The mechanisms action of PGPR, namely, induced systemic resistance (ISR) and induced systemic tolerance (IST) were elaborated (23).

Table 3. Influence of PGPR's (*Bacillus subtilis* and *Pseudomonas fluorescens*), Isolate No. MA-2 and MA-4 on the productivity of Geranium (*Pelargonium graveolens*) in pots (unsterile soil)

S. No.	Treatment	Average plant height (cm)	Average no. of branches	Average herb yield fresh wt. in (g)	%increase average herb yield fresh weight over control in (g)	% Oil yield
1	Control	48	16	295	-	0.16
2	*Bacillus subtilis*	50.4	18	321.7	9.0	0.18
3	*Pseudomonas fluorescens*	51.50	19	376.7	27.6	0.19

Figure 2. Effect of PGPR on the growth of *Pelargonium graveolens* (A) *Bacillus subtilis* (B) *Pseudomonas fluorescens* (C) Control

Statistical analysis

$F_{calculated}$ value of *Pelargonium graveolens* L'Hérit plant height due to treatment is 1.497 where as the F $_{table}$ value at 5% probability level is 4.10, treatment is statistically non significant because the calculated value of treatment is lower than the value of $F_{(5\%)}$ table value.

$$F_{cal} \leq F_{(5\%)} \text{ (Non significant)}$$

$F_{calculated}$ value of *Pelargonium graveolens* L'Hérit herb yield fresh wt. Due to treatment is 11.39 where as the F_{table} value at 5% probability level is 6.94 treatment is statistically significant because the calculated value of treatment is higher than the value of $F_{(5\%)}$ table value.

$$F_{cal} \geq F_{(5\%)} \text{ (Significant)}$$

Conclusions

Research in last decade has opened up new horizons for the inoculation industry. Agriculture in developed countries is definitely the major promoter of microbial inoculants that are 'environmentally friendly'. In recent years *fluorescent pseudomonads* have drawn attention world wide due to production of secondary metabolites such as siderophores, antibiotics, volatile compounds, enzymes and phytohormones. Strains of *Bacillus subtilis* and *Pseudomonas fluorescens*, gave effective result in growth promotion in medicinal and aromatics plant such as Geranium (*Pelargonium*

graveolens), therefore called plant growth-promoting rhizobacteria. Isolate MA-4 (*Pseudomonas fluorescens)* gave better result on the productivity of Geranium in comparison to MA-2 (*Bacillus subtilis*). They also possessed biological control activities. Thus, they could be further exploited for commercial scale up.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the manuscript.

References

1. Kloepper, J.W., Schorth, M.N. 1978. Plant growth promoting rhizobacteria on radishes in proceedings of the fourth international conference on plant pathogenic bacteria, Vol 2, INRA, Angers (Ed., Gibert-clareyTours); 879-882.
2. Van Loon, L.C., Bakker, P.A., Pieterse, C.M.J. 1998. Systemic resistence induced by rhizosphere bacteria. Ann Rev Phytopathol. 36:453-483.
3. Ramamoorthy, V., Viswanathan, R., Raghuchander, T., Prakasam, V., Samiyappan, R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pest and diseases. Crop Protection. 20: 1- 11.
4. Enebak, S.A., Carey, W.A. 2000. Evidence for induced systemic protection to Fusarium rust in Loblolly pine by plant growth promoting rhizosphere. Plant Disease. 84: 306-308.
5. Babalola, O.O., Osir, E.O., Sanni, A.I., Odhaimbo, G.D., Bulimo, W.D. 2003. Amplification of 1-aminocyclopropane-1-carboxylic (ACC) deaminase from plant growth promoting rhizobacteria in Striga-infested soils. Afr J Biotechnol. 2:157-160.
6. Belimov, A.A., Dodd, I.C., Safronova, V.I., Hontzeas, N., Davies, W.J. 2007. *Pseudomonas brassicacearum* strain Am3 containing 1-aminocyclopropane-1-carboxylate deaminase can show both pathogenic and growth-promoting properties in its interaction with tomato. J Exp Bot. (doi:10.1093/jxb/erm010).
7. Blaha, D., Combaret, C.P., Mirza, M.S., Loccoz, Y.M. 2006. FEMS, Microbiol. Ecol. 56: 455–470.
8. Dubeikovsky, A.N., Mordukhova, E.A., Kochethov, V.V., Polikarpova, F.Y., Boronin, A.M. 1993. Growth promotion of black currant soft woodcuttings by recombinant strain *Pseudomonas fluorescens* BSP 53 a synthesizing an increased amount of indole-3-acetic acid. Soil Biol. Biochem. 25: 1277-1281.
9. Glick, B.R. 1994. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol.41:109-117.
10. Narayana, M.R., Prakasa Rao, E.V.S., Rajeswara Rao, B.R., Sastri, K.P. 1986. Geranium cultivation in India: potentials and prospects pafai journal. 8: 25-30.
11. King, E.O., Ward, M.K., Raney, D.C. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44: 301-307.
12. Aneja, K.R. 1993. Experiments in Microbiology,Plant Pathology and Tissue Culture. Wishwa Prakashan Publication pp.117-120.
13. Cappuccino, J.C., Sherman, N. 1992. In: Microbiology: A Laboratory Manual, New York, pp. 125-179.
14. Mackie, T.J., Mc Cartney, J.E. 1956. Handbook of practical bacteriology, 9th edition.
15. Brick, J.M., Bostock, R.M., Silverstone, S.E. 1991. Rapid insitu assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Appl. Environ. Microbiol. 57: 535-538.
16. Schwyn, B., Neilands, J.B. 1987. Universal chemical assay for detection and determination of siderophore. Anal. Biochem. 160: 47-56.
17. Mahalakshmi, S., Reetha, D. 2009. Assessment of Plant Growth Promoting Activities of Bacterial isolates from the Rhizosphere of Tomato (*Lycopersicon esculentum* L.) Recent Research in Science and Tech. 1(1): 026–029.
18. Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255: 571-586.
19. Teixeira, D.A., Alfenas, A.C., Mafia, R.G., Ferreira, S.L.D., Mounteer, A.H. 2007. Rhizobacterial growth promotion of eucalypt rooting and growth. Braz.J. Microbiol. Vol.38 no.1 Sao. Paulo
20. Cakmakc, R., Erat, M., Donmez, M.F. 2007. The influence of plant growth-promoting rhizobacteria on growth and enzyme activities in wheat and spinach plants. J. Plant Nutr Soil Sci. 170(2): 288-295.
21. Kaymak, H.C., Yarali, F., Guvenc, I., Donmez, M.F. 2008.The effect of inoculation with plant growth rhizobacteria (PGPR) on root formation of mint (*Mentha piperita* L.) cuttings. African Journal of Biotech. 7(24): 4479-4483.
22. Kohler, J., Caravaca, F., Carrasco, L., Roldan, A. 2007. Interactions between a plant growth-promoting rhizobacterium, an AM fungus and a phosphate-solubilising fungus in the rhizosphere of *Lactuca sativa.* Applied Soil Eco. 35(3): 480-487.
23. Kang, Y.J., Cheng, J., Mei, L.J., Hu, J., Piao, Z., Yin, S.X. 2010. Action mechanisms of plant growth-promoting rhizobacteria (PGPR): a review. Ying Yong Sheng Tai Xue Bao. 21(1): 232-238.