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CHARACTERIZATION OF PLANT GROWTH PROMOTING BACTERIA FROM SOIL OF CENTRAL AND UPPER HIMALAYAN REGION

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ABSTRACT: Natural ecosystems are directly dependent on beneficial microorganisms present in the rhizosphere for soil health and plant productivity. Soil bacteria were isolated from soil of central and upper Himalayan region with a view to screen/evaluate their Plant growth promoting potential. Plant growth-promoting rhizobacteria (PGPRs) are employed as inoculants for biofertilization, phytostimulation and biocontrol. Dominant morphotypes were picked up from King`s B, CAS, YEMA and Pikovaskaya Agar by employing dilution plating. A total of 55 isolates were evaluated for growth promotion using Paper Towel Assay on lentil and 9 isolates were selected for detailed characterization. In dual plate assay all 9 isolates inhibited *Fusarium solani*; few isolates were inhibitory towards *Ganoderma lividense* and *Colletotrichum dematium*.
Key words: Plant growth promotory rhizobacteria, Paper towel assay, lentil

INTRODUCTION

Bacterial diversity is of particular importance in human sustenance since these small creatures comprise the majority of earth's species diversity. Bacterial diversity is considered as one of the most useful resource with considerable significance in the global form of bioremediation and bio-prospecting (Homer-Devin et al 2004).
Interaction between bacteria and roots of plants has been reported to be beneficial, detrimental or neutral and this delicate balance is a consequence of both soil and plant type(Latour et al 1996) . Bacteria, beneficial to plants may be symbiotic or free living, and are abundant near the roots. Such beneficial free-living bacteria have been termed PGPR or plant growth promotory rhizobacteria(Glick ,1995). They benefit plants through, (a) Production of plant hormones, such as auxins by Gutierrez et al 1996 (b) asymbiotic N_2 fixation by Kennedy et al 1997 (c) antagonism against phytopathogenic microorganisms by production of antibiotics (Sharma et al 2003) , siderophroes(Meyer2000), β-(1,3)-glucanase, chitinase(Renwick et al 1991) and cyanide(Flaishman et al 1996) and (d) solubilization of mineral phosphates and other nutrients(de Freitas et al 1997) (e)ability to effectively colonize roots are responsible for plant growth promotion [Burdetal 2000, Duffy BK, Defago G (1999),. A number of PGPR such as *Bacillus (*Holl et al 1988), *Pseudomonas(*O' Neill et al 1992) and *Arthrobacter (*Beall and Tipping 1989) have been used for enhancement of plant performance.
Phytopathogens are major and chronic threats to food production and ecosystem stability worldwide. As agricultural production intensified over the past few decades, producers became more and more dependent on agrochemicals as a relatively reliable method of crop protection helping with economic stability of their operations. Despite inconsistency in field performance, biological control is considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture (Gerhadson 2002)
The Himalayan region represents a unique combination of plant and soil type that changes drastically with altitude however only limited efforts have so far been made to explore the available bacterial diversity. In the present study soil samples were collected from Chaubatia (Ranikhet) Uttaranchal Himalayas and Leh region, for assessment of PGPR characterization.

MATERIAL AND METHODS

Sampling Sites: Five soil samples *viz,* MFA-1, MFB-1, FQP, FMP and FPA were collected from Ranikhet (Chaubatia) at latitude 29.6^{0}C and longitude 79.5^{0}C; soil sample A-2 was from Leh (latitude, 34^{0}C and longitude, 77.5^{0}C). Characteristics of the habitat and their physico-chemical properties are presented in Table 1.

Soil Analysis: Soils were analyzed for pH and organic carbon by Walkely & Black 1934. Available and total 'P' were analyzed according to Olsen 1954. Dehydrogenase activity was measured according to Thalman1968.
Isolation of bacteria: Bacterial population was enumerated using 10-fold serial dilutions (Johnson and Curl1972) and expressed as CFU g^{-1} dry soil; serially diluted soil samples (upto 10^{-5}) were plated on King's B medium for pseudomonads by Buysen1996, yeast extract mannitol agar for rhizobia (Vincet et al 1991), Pikovaskya's agar for phosphate solubilizer (Pickovaskaya1948) and chromeazurol 'S' (CAS) agar for siderophore producers(Schwyn and Neilands 1987). Plates were incubated in triplicates at 28^{0}C and counts (CFU) were recorded after 72 h. A total of 55 colonies representing all the morphotypes were picked up and categorized on the basis of soil type and media used.

Screening of bacteria for plant growth: Lentil (*Lens esculentus* var. PL406) seeds were surface sterilized with 0.1% mercuric chloride for 5 min, rinsed with sterilized distilled water (SDW) and soaked in bacterial suspension (3×10^8 cfu ml^{-1}) using 1% carboxymethyl cellulose (CMC). Air dried seeds were placed on a paper towel (ten seeds per paper) and incubated at 28±2^{0}C for 21 d in a growth chamber. Percentage germination was recorded along with root and shoot length (ISTA1993). Non-bacterized seeds served as control. Five replicates were used.
Functional attributes of the bacterial isolates: On the basis of plant growth performance in paper towel assay, nine promising isolates were tested for their ability to produce indole acetic acid (Gordon and Weber 1951).
IAA production: Bacteria were grown overnight in five ml of M-9 minimal medium(John et al 2005) supplemented with L-tryptophan to achieve a final concentration of 0, 50, 100, 200 and 500 µg ml^{-1}. After incubation for 42 h, bacterial growth was measured spectrophotometrically at 600 nm; cells were removed from culture medium by centrifugation at 7,500 rpm for 10 min. A 1 ml aliquot of supernatant was mixed with 4 ml of Salkowski's reagent (150 ml of concentrated H_2SO_4, 250 ml of D.W, 7.5 ml of 0.5 M $FeCl_3$, $6H_2O$)**.** Samples were left at 28±2^{0}C for 25 min and absorbance was read at 535 nm. The concentration of IAA was determined by referring to a standard curve.

In vitro antifungal assay: Bacteria were tested for antagonism against *Colletotrichum dematium, Fusarium solani* and *Ganoderma lividans* using a dual culture plate assay (Sharma and Johri 2003).

RESULTS AND DISCUSSIONS

Soil analysis: Soil samples (MFA-1, MFB-1, FPA, FQP, FMP and A2) used in this investigation had slightly acidic pH (6.25 to 6.60). Organic matter and organic carbon content was high in all samples except FPA (0.64%) (Table 2) which represents a forest soils. Sample MFB-1 (11.84 kg ha^{-1}), MFA-1 (27.87 kg ha^{-1}) and FMP (50.32 kg ha^{-1}) showed medium to high level of available 'P' whereas others were low (Table 1). Presence of organic matter and availability of nutrients such as phosphorus influences microbial activity of the soil studied by Johri et al 1999; Nautiyal et al 2000. A forest soil with mixed root fragments (FMP) depicted high dehydrogenase activity (448.87 µg TPF ml^{-1} 16 h^{-1}) compared to soil without such material (FPA 52.61 µg TPF ml^{-1} 16 h^{-1}). FMP also had population count (7.0×10^7 CFU g^{-1} soil) Table 1.

Dehydrogenase activity and organic matter content of a soil are correlated with soil health. There was a direct relationship between total biological activity and organic carbon. Microbial counts in FMP were also directly related with biological activity and organic carbon (Lodha et al 2002).

Table 1: Characteristics of soils from Central and Upper Himalayan region

Code	Sample site	pH ±sem*	Organic matter (%) ±sem*	Organic 'C' (%) ±sem*	Available 'P' Kg ha^{-1} ±sem*	Total'P' Kg ha^{-1} ±sem*	Dehydrogenase activity µg TPF ml^{-1} ±sem*
MFA-1	Modern farming upper profile Padhandsyar, Rampur (3100 msl)	6.55±0.12	2.98 ±0.11	1.73±0.15	27.87±0.37	124.0±o.66	352.48±0.33
MFB-1	Modern Farming Lower profile Padhandsyar, Rampur (3100 msl)	6.50±0.19	2.17±0.17	1.26±0.16	11.84±0.89	43.83±0.67	131.54±0.18
FQP	Forest Soil (Oak, Banj) Ganju (3500 msl)	6.45±0.32	3.93±0.53	2.28±0.29	9.15±0.59	258.14±0.11	437.60±0.79
FMP	Forest Soil mixed with roots (5000 msl)	6.40±0.22	4.59±0.41	2.66±0.32	50.32±0.12	196.00±0.17	448.87±0.16
FPA	Forest Soil (Planted 1987, Rampur road (3300 msl)	6.25±0.19	1.10±0.13	0.64±0.37	5.73±0.25	159.46±0.13	52.61±0.27
A-2	Leh cold Desert at an altitude of 12,000-13,000 feet (Mixed farming of wheat Brassica and Potato	6.60±0.43	2.07±0.17	0.92±0.47	33.80±0.32	115.52±0.17	109.50±0.38

***sem: standard error of mean**

Screening of bacteria for plant growth promotion: *A* total of 55 isolates were used to asses their influence on seed germination and root/ shoot length of lentil in a paper towel assay. On the basis of their growth performance, nine isolates namely MFA-1SD-2, MFA-1R-3, MFB-1R-3, FPASD-1, FQPR-2, FQPR-3, FMPPB-3, FA_2K10^5 and FA_2K100^3, were used for further characterization.
Bacterized lentil seeds showed improved plant growth compared to untreated control (Table 2). A significant difference in percentage of germination was observed compared to control; values ranged between 14.18 to 38.36%. Isolates exhibiting improved seed germination also supported improved root and shoot length (Table 3). Seeds coated with bacterial isolates derived from soil representing modern farming practices (MFB-1R-3) showed maximum germination (38.36%), root length (9.41 cm) and shoot length (2.83 cm). On the contrary, seeds coated with bacterial isolates derived from Leh soil (FA_2K100^3) showed minimum seed germination (14.18%), root length (9.41 cm) and shoot length (2.83 cm) compared to control (Table 3). Seed bacterization (or seed coating) has proven to be a method of choice for studying bacterial growth promotion and biological control of plant diseases including pre emergence and post-emergence diseases.In this study, seed treatment with the bacterial isolates significantly improved seed emergence together with plant root and shoot length.
Functional attributes of bacteria: Except MFA-1R3, all other bacterial isolates were positive for siderophore production, phosphate solubilization and indole acetic acid production (Table 2) but were negative for HCN. Isolate MFB-1 R-3 produced highest level of IAA in liquid broth (33.55 µg ml^{-1}) and FA_2K100^3, the least (1.75 µg ml^{-1}).
In vitro fungal growth inhibition assay: Under *in vitro* condition, five isolates restricted growth of test fungus *G. lividans* in dual culture test. Inhibition level was 31.5% for MFB-1 R-3 and 39.4% for FA_2K100^3. Growth of *Colletotrichum dematium* was inhibited by five bacterial isolates with a value of 22.5% for MFB-1 R-3 and 47.5% for FPA SD-1. Growth of *F. solani* was inhibited by all nine bacterial isolates; inhibition level ranged from 7.14% to 52.3% (Table 3).

Table 2: Growth promotory potential of selected bacterial isolates in a paper towel assay on lentil (*Lens esculentus* var. PL 406)

Isolates	***IAA (µg ml^{-1})*** ±sem*	***Seed germination (%) 3d***	***Root length (cm) 7d***	***Shoot length (cm) 7d***
Control		53.252	1.70	3.29
MFA-1 SD-2	1.80±0.21	68.31	4.19	12.60
MFA-1R-3	6.22±0.19	73.62	2.04	10.40
MFB-R-3	33.55±0.37	73.62	4.53	12.70
FPA-SD-1	2.88±0.31	73.62	2.98	11.50
FQP R-2	2.25±0.48	68.31	2.80	10.90
FQP R-3	1.83±0.29	66.17	3.21	12.20
FMP PB-3	6.31±0.27	66.17	3.57	11.50
FA2K10^5	13.55±0.16	68.31	2.45	12.52
FA2K10^5	1.75±0.24	60.77	1.99	9.45
CD at 5%	-	7.79	0.623	1.77
SEm	-	2.80	0.224	0.640

Table 3: *In vitro* antagonistic behaviour of select bacterial isolates against phytopathogenic fungi

Percent inhibition of growth (%)			
Isolates	*G. lividens*	*C. dematium*	*F. solani*
MFA-1 SD-2	ND	ND	35.70±0.77
MFA-1R-3	34.00±0.19	ND	42.80±0.58
MFB-R-3	31.50±0.82	22.50±0.58	45.02±0.24
FPA-SD-1	36.80±0.56	47.50±0.24	52.30±0.16
FQP R-2	ND	ND	19.00±0.29
FQP R-3	ND	ND	23.80±0.17
FMP PB-3	36.80±0.12	25.00±0.16	7.14±0.13
FA2K10^5	ND	32.50±0.29	40.00±0.26
FA2K100^3	39.40±0.77	25.00±0.17	33.30±0.12

Based on dual culture

In vitro fungal growth inhibition assay showed variable antagonism against *G. lividans, C. dematium* and *F. solani*. There is considerable evidence to suggest that there is unlimited diversity of genetically dissimilar microorganisms in the rhizosphere, among which communities, suppressive to plant pathogens are abundant in the soil.

Biological control of pathogenic and other deleterious microorganisms in soil-root interface is often attributed to antibiosis where antibiotics produced by Gram -ve and Gram +ve antagonistic bacteria play direct role in disease suppression. In this study, isolates were prominent antagonistic against *F. solani.* The highly effective disease-suppressive fluorescent pseudomonad species strains owe their suppressive potential to antibiotics produced in the rhizosphere. Phenazine derivatives were the first antibiotics implicated in biocontrol produced by fluorescent pseudomonads, such as *Pseudomonas fluorescens* and *P. aureofaciens*. Several other antibiotics and antimicrobials are produced in the rhizosphere by *P. fluorescens*, including HCN, 2, 4-diacetylphloroglucinol (DAPG), and pyoluteorin, which directly interfere with growth of various pathogens and contribute to disease suppression (Dwivedi and Johri 2003).

From a more general perspective, the diversity within populations of antagonistic microorganisms with a common biocontrol trait is a means to improving biocontrol.

This approach builds on existing knowledge of mechanisms while exploiting genetic differences that have evolved to enable microbial populations to compete successfully in diverse soil and rhizosphere environments. Understanding the diversity within populations of biocontrol agents holds the promise of pairing specific genotypes with their most supportive plant hosts or soil environments to maximize root colonization and disease suppression. Finally, establishing the presence and functionality of individual populations within a particular soil is just one first step toward fully understanding the nature of suppressiveness within that soil. Ultimately, the parameters within which the activities of functionally important microbial populations combine to produce a suppressive soil also must be defined. To identify those parameters, new and more detailed studies will be required to characterize the soil structure and composition, the environmental conditions under which suppression occurs, the molecular interactions among functionally important populations under different conditions, and the biogeography and population dynamics of beneficial as well as pathogenic microbial populations in the field. Because of the complexity of field soils, high through put methods will be required to adequately characterize these populations, but the pay-off will be worth the effort. The future studies of biologically based soil suppressiveness will present new insights in to the microbial ecology of agricultural soils and lay the foundation for the development of creative management strategies for the suppression of soil borne diseases.

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