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Diversity and function of plant growth promoting Rhizobacteria associated with wheat Rhizosphere in North Himalayan Region

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ABSTRACT

Little is known about the composition of bacterial diversity associated with plant roots. The purpose of this study was to investigate the diversity of bacteria associated with the root of wheat. Present study revels, the diversity of bacterial isolates from soils under wheat cultivation in district Uttarkashi. Phenotypic and physiological characteristics of the isolates were record to categorize and identify the bacteria. In all 133 different bacteria isolates were recovered from four different locations of which two were rainfed and two were irrigated. The spread plate technique on nutrient agar was use to isolates and purifies all the strains. The characteristics of the bacterial strains were determined using the colony morphology, gram staining as well as biochemical properties. On the basis of biochemical characterization 44 % were *Bacillus* sp. and 24% belong to *Pseudomonas* sp. Genera identified in the rhizosphere isolates were also found in the rhizoplane isolates. Shannon Winner index of microbial diversity was 1.752 in irrigated crop and 1.594 in rain fed crop but there were no significant differences.

Keywords: Rhizobacteria, Wheat, Himalaya, diversity, Rhizosphere.

1. Introduction

Wheat is the major grain that sustains humanity. Wheat grows in temperate climate and it is staple food for 35% of world's population. On other hand, it provides more calories and protein in the diet than any other crop (Laegreid *et al.,* 1999). To provide food security to the ever increasing population; greater agriculture production is a pressing need in 21st century. The increasing demand for a steady and healthy food supply by a burgeoning human population will require efficient management practices along with controlling disease that reduce crop yield. During last few decades, agricultural production has increased due to the use of high yielding varieties and enhanced consumption of chemicals, which are used both as fertilizers to provide nutrition and as protection agents to control the damage caused by phytopathogens. Excessive use of chemicals and change in traditional cultivation practices has resulted in the deterioration of physical, chemical and biological health of the cultivable soil. Microbial diversity in soil is considered important for maintaining for the sustainability of agriculture production systems. However, the links between microbial diversity and ecosystem processes is not well understood (Stark, 2007).

Region of contact between root and soil where soil is affected by roots is designated as **"rhizosphere"** (Hiltner, 1904). Broadly, there are three separate, but interacting, components recognized in the rhizosphere. These are the rhizosphere (soil), the rhizoplane, and root itself. The rhizosphere is the zone of soil influenced by roots through the release of substrates that

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affect microbial activity. The rhizoplane is the surface, including the strongly adhering soil particles.

Several microorganisms are able to promote the plant growth. Several microbial products either directly promote growth or indirectly protect them from diseases, have been marketed (Lugtenberg, *et al.,* 2004). Root colonizing bacteria (rhizobacteria) that exert beneficial effect on plant development *via* direct or indirect mechanisms have been defined as plant growth promoting rhizobacteria (PGPR) (Nelson, 2004). The concept of plant growth promoting rhizobacteria is now well established; both for growth promotion and biocontrol. Plant growth-promoting rhizobacteria (PGPR) were first defined by Kloepper and Schroth (1978) to describe soil bacteria that colonize the roots of plants following inoculation onto seed and they enhance plant growth The ineffectiveness of PGPR in the field has often attributed to their inability to colonize plant roots (Benizri, *et al.,* 2001, Lugtenberg, *et al.,* 2001). With a view of developing microbial inoculants suitable for field application in the colder mountainous region, a systemic investigation indicates dominance of the species of *the Bacillus megaterum, B. subtilis, Pseudomonas corrugate,* were considered suitable for developing as potential inoculants for wide spread application in the mountains.

2. Materials and Methods

2.1 Research Sites

The experimental fields have the geographically location of 30°44'N 78°27'E and 30°.73'N 78°.45E at an elevation of 1550 meter amsl. Out of these four sites, Bhatwari and Uddar were irrigated and Qyrk and Jangal were rainfed.

2.2 Isolation and enumeration of rhizobacteria from wheat root

Bacteria isolates were isolated from the rhizosphere soil and rhizoplane of wheat plants during the winter of 2008-2009. To estimate the number of soil microflora, counts were calculated on the basis of serial 10-fold dilutions in duplicate, using the pour plate methods and triplicate samples of 1 gm soil, and an appropriate dilutions (Johnson and Curl, 1972); each value presented here is therefore an average of three individual counts. All petri dishes (90 mm diameter) contained 25 ml medium, and plate were incubated at 28-30°C. Colony forming units (CFU) were recorded after 48 hour, the average number per gram oven dry weight of soil was calculated as:

$$\text{CFU} = \frac{\text{Bacterial plate count} \times \text{dilution factor}}{\text{Oven dry weight of soil}}$$

2.3 Plant Growth Promoting Mechanisms

2.3.1 Siderophore detection

Siderophore was detected by the formation of orange halos surrounding bacterial colonies on CAS agar plates after 48 hour at 28°C (Schwyan & Neilands, 1987).

2.3.2 Phosphate solubilization

Phosphate solubilization detected by formation of transparent halos surroundings bacterial colonies on the Pikovskaya agar after 72 hour incubation at 28°C (Pikovskaya, 1948).

2.3.4 Indol acetic acid production

Bacteria cultures were incubated in Luria Bertani broth at 28°C. The bacterial cells were removed from the culture medium by centrifugation at 8000× gm for 10 min. A 1ml of supernatant was mixed vigorously with 2ml of Salkowaski's reagent (4.5 gm of $FeCl_3$ per liter in 10.8 M H_2SO_4) and incubated at room temperature in the dark for 30 min. and observed the color formation. (Fischer, *et al.,* 2007).

2.4. Soil Analysis

For the soil analysis, the samples were mixed well individually before use. The sample dried at 20^0C to 25^0C (Jackson, 1958). Soil organic carbon was determined by Walkley and Black's rapid titration method (Walkley, and Black, 1934). Total nitrogen (%) was measured using kjeldahl's procedure (Bremmer, 1965). Exchangeable phosphorus (P) and available potassium (K) was determined by (Jackson, 1958).

3. Results

3.1 Isolation and enumeration of rhizobacteria from wheat root: A total of 133 isolates were recovered. The organisms identified as *Bacillus* sp. (44%) the most dominant genera followed by *Pseudomonas* sp. (24%), *Serratia* sp. *Flavobacterium* sp. (7% each), *Micrococcus* sp. (3%), *Klebsiella* sp. (4%), *Azotobacter* sp. (6%), *Enterobacter* sp. (4%), *Xanthomonas* sp. *Staphylococcus* sp. and *Micrococcus* sp. were frequently present. All the genera were tentatively identified following biochemical and morphological characterization.

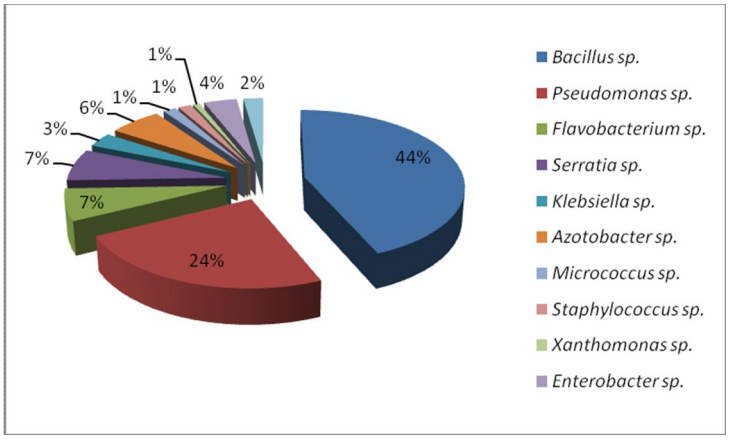

Figure 1: Percentage distribution of isolated microbial population belongs to the various bacterial genera in 0 day (crop sowing time) to 120 day (April).

3.2. Plant Growth Promoting Mechanisms: Each isolates were screened for plant growth promoting traits such as siderophore production, phosphate solubulization, indole acetic acid production, antibiotic production. Plant growth promoting activities compared in fig. 3. Seventeen isolates produce IAA that range from 1.22µg/ml to 21.31µg/ml. The ability to

solubilized phosphate was positively exhibited by 30 isolates and 36 isolates produced siderophores.

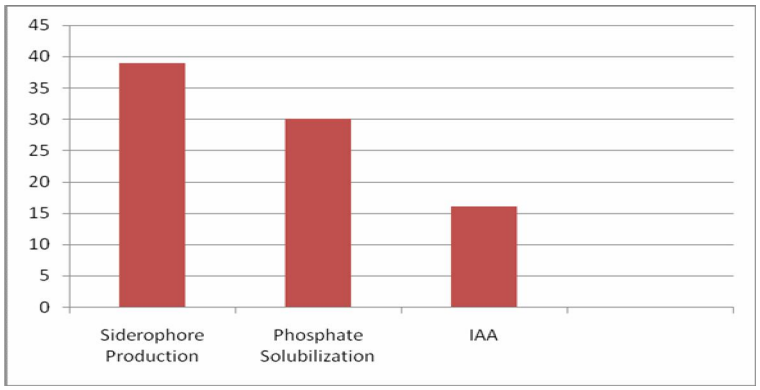

Figure 3: Numbers of plant growth promoting isolates.

The colony forming units trend are shown in fig.2. Initially cfu increased zero to 90 day followed by decrease in 120 day. The mean cfu in zero day was 2.5 $\times 10^3$ and 1×10^3 ; in 30 days it enhanced to 5×10^3 and 4.5×10^3 ; in 60 days to 10×10^6 and 8.5 $\times 10^6$; in 90 days cfu approached a level of 22×10^6 and 14 $\times 10^6$ and 9.5 $\times 10^6$ and 6×10^6 in 120 days in irregited and rainfed fields respectively.

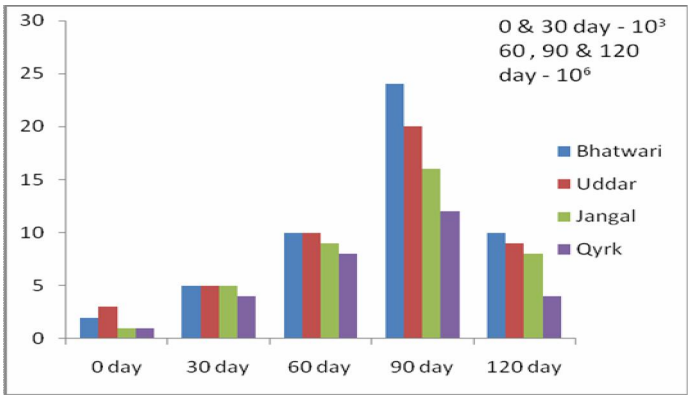

Figure 2: Comparison of colony forming units between irrigated and rainfed fields at different sampling period.

Table 1: Chemical properties of soil.

S. No.	Detail of Samples	pH	Total Nitrogen (%)	Available Phosphorus (ppm)	Exchangeable Potassium (ppm)	Organic Carbon (%)
1	Bhattwari	5.79±0.03	0.104±0.003	25.30±0.12	374±0.58	1.155±0.002
2	Jangal	5.87±0.02	0.224±.002	8.25±0.006	138±0.0.58	2.145±0.006
3	Qyrk	5.50±0.02	0.295±0.002	47.46±0.11	714±1.0	2.430±0.58
4	Uddar	5.98±0.58	0.118±0.05	6.00±0.18	56±1.0	0.953±0.23

3.3 Soil Analysis: Chemical properties of soil are presented in table-1, pH of soil range from 5.50 to 5.98 i.e. soil was acidic in nature. The mean temperature during sampling month varied from 14^{o}C in December to a highest of 31^{0}C in April. The soil moisture content fluctuated between 9.8 to 20.4% during the study period.

3.4. Assessment of the diversity as calculated by Shannon Waver diversity index (H') in wheat rhizosphere: Several indices of community diversity and richness were used to calculate bacterial diversity in the samples. Shannon Winner index of irrigated soil was computed to be 1.752 higher than rainfed soil being 1.594.

Table 2: Diversity index in wheat rhizosphere.

Indices	Rain fed fields	Irrigated fields
Shannon index	1.594	1.752
Simpson index	0.6939	0.7566
Equitability	0.6647	0.7608

4. Discussion

Rhizosphere is a rich habitat of microorganisms and should be explored for obtaining potential PGPR, which can be useful in developing bio-inoculants for enhancement of growth and yield of crop plants. The beneficial effect of plant growth promoting rhizobacteria, particularly those belonging to the genus *Bacillus* or *Pseudomonas*, in enhancing growth and overall plant establishment is well established. This has been attributed to various mechanisms, such as providing fixed nitrogen to the host plant, production of phytohormones, solubulization of insoluble phosphate, production of metabolites, including antibiotics and siderophores (Compant *et al.,* 2005).

The actual composition of the microbial community in the root zone is dependent on root type, plant species, plant age soil type (Campbell, 1985) as well as other selection pressures. Typically, the rhizosphere is colonized by a predominantly Gram-negative microbial community (Atlas & Bartha, 1983).

The results of the present studies, based on the rhizobacteria isolation on the rich media and classification of the isolates on the grounds only of their phenotypic (but not genetic), morphological and biochemical features, can be criticized. In our study, twelve different genera were identified *viz. Bacillus, Pseudomonas, Flavobacterium, Serratia, Micrococcus, Klebsiella, Alkaligenes, Staphylococcus, Enterobacter and Xanthomonas.* Enumeration of total bacteria population was determined on standard plate count (SPC) agar. The data suggest that only *Bacillus sp.* was present at 30^{th} day i.e. January-2009 month. Culturable bacterial population in January differed from the population of other days, although the differences might also have been caused by difference in cell physiology in certain microorganisms. Certain bacterial specie or genera isolated from soil in cold period could experiences shock when plated and incubated at relatively high temperatures, which could prevent them from growing (Smit, 2001).

In our study, *Bacillus* (40%) was dominant group. *Bacillus* species are also a major component of the microbial flora, which live in close association with various types of

agricultural crops. Many authors cite *Pseudomonas* as the dominant genera in the rhizosphere, probably because under favorable environmental conditions, its growth rate is higher than that of *Bacillus* (Bowen & Foster, 1978). Predominance of *Bacilllus* sp. is due to its ability to efficiently use the nutrients provided by the plant through exudates. In additions, *Bacillus* has the ability to inhibit the growth of other strains. Many strains of *Bacillus* have been reported to produce substances that act as growth inhibitors for other microorganisms (Lilinares, *et al.,* 1994). However, previous study shows *Bacillus* as the dominant genera in the rhizosphere of *Elaeagnus angusti folia* L. (Ramos, 1998).

Seasonal change in the number of all group bacterial population showed that with increase in the atmospheric temperature, their numbers increasing (fig. 2). The highest number found in 90 day March, April, and lowest in December. Zou Li *et al.,* (2000) found similar change in microbial population during different seasonal time that in winter the total number of microorganisms decreased. The increase soil moisture content and optimum temperature enhance the development of microflora.

The results show 29.32% isolates have ability to produce siderophore on CAS agar medium, 22.56% isolates solubilise phosphate and 12.03% produce IAA. These results indicate that the tested isolates could exhibit two or three plant growth promoting (PGPR) traits, which may promote plant growth directly, indirectly, or synergistically. Similarly to our findings multiple PGP activities among PGPR have been reported by some other investigators (Gupta *et al.,* 1998; and Dey *et al.,* 2004). Several studies have demonstrated that production of siderophore by PGPR was most effective in controlling the plant root pathogens (Mullen, 1998; Diaz *et al.,* 2002; and Dey *et al.,* 2004). The potential to produce siderophores by microorganisms in improving iron availability to plants was also reported by some workers (Bar-Ness *et al.,* 1992; Rroco *et al.,* 2003 and Sharma *et al.,* 2003). Phosphate solubilisation is considered to be most important attribute of plant growth promoting rhizobacteria (Kloepper *et al*., 1989). Bacterial IAA stimulates the development of the host plant root system. The advantage for root associated bacteria is rich supply of nutrients, as much as metabolic products of carbon fixed by plants is lost from roots and move in rhizosphere as exudates, lysates, mucilage

Hill and mountain agro ecosystems are characterized by difficult terrain; inadequate infrastructure, frague ecosystems and societies entrenched in severe top soil erosion and low input application. The application of external inputs in the form of fertilizers, pesticides etc. is very minimal for various reasons, hence by default, hill agriculture largely remains a low external input based production system. In the context of hill agriculture, cold tolerant microbial inoculants are required since a major part of the crop season is characterized by cold temperatures.

The findings of the present investigation highlighted that plant growth promoting rhizobacteria from local soil could be easily isolated and may be exploited after strain improvement for local use.

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