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Structural and functional diversity of Rhizobacteria of Pearl Millet in Semi-arid Agroclimatic Zone

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ABSTRACT

A study was carried out to examine the structural and functional diversity of rhizobacteria associated with pearl millet cultivated in semi arid agroclimatic zone, district Faridabad, Haryana, India. Twenty two rhizospheric soil samples of pearl millet plants were randomly collected, from seven different villages of district Faridabad and rhizobacteria were isolated from them using spread plate technique, on three different nutrient media. A total of sixty two isolates were obtained out of which 27.4 percent isolates have been identified as belonging to genus Streptomyces, 22.5 percent to genus Pseudomonas, 16 percent to genus Flavobacterium, 13 percent to genus Streptococcus, 14.5 percent to genus Bacillus and 6.5 percent to genus Staphylococcus on the basis of their morphological and biochemical characteristics. Various diversity indices were calculated for the bacterial population, obtained. The Simpson index (D) was 0.81, Shannon-Wiener index was 1.71 and equitability was 0.95. More than sixty nine percent of the isolates were found to produce indole acetic acid (IAA), 41.9 percent were phosphate solubilizers, 71 percent produced hydrogen cyanide and 82.3 percent produced ammonia under in-vitro conditions. Average production of IAA was observed as 1.73µg/ml and the average phosphate solubilization index obtained was 2.24. Maximum plant growth promoting potential was exhibited by genus Pseudomonas while isolates belonging to genus Streptomyces and genus Staphylococcus showed minimal potential for the same.

Key Words: Rhizobacteria, Pearl-millet, Diversity, Plant growth promotion

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INTRODUCTION

There is an ever-increasing demand of food worldwide. According to a report of FAO, seventy percent more food is required for an additional 2.3 billion people by 2050. Innovative agricultural tools and methodologies are required to combat these demands. In the last few decades, dramatic improvements in the production of crop plants have been obtained. But at the same time, the dependency of agriculture on various kinds of chemical tools including the chemical pesticides and fertilizers has also increased manifolds. Unwarranted use of such chemical tools to enhance the crop yield has resulted in stern problems like accumulation of chemicals in plant products and thus their entry into the food chain, depletion of soil quality and fertility, contamination of water resources, reduction in population of naturally occurring beneficial organisms [1]. Of late, consumers have shown their concern for food quality and safety as well as for the effects of current farming methods on the environment. The key challenge, thus, is to attain the twin goals of agricultural growth along with maintenance and enhancement of environmental quality.

Use of microbial agents to improve agricultural productions and plant health offers an attractive option to practice and develop sustainable agriculture. The agriculturally important microorganisms have thus been the focus of

research and gained much attention for developing sustainable agriculture, during the last decades. Worldwide, various studies have reported the efficient use of rhizosphere microorganisms to improve plant growth. Rhizosphere, the narrow zone of soil that surrounds and gets influenced by the roots of the plants, is rich in nutrients when compared with the bulk soil, due to the accumulation of a variety of organic compounds released from the roots by exudation, secretion and deposition [2]. As a result it has an enormously rich variety of microorganisms including bacteria, fungus, algae and protozoa. However bacteria are the most numerous amongst them [3] thus influencing the plant in most significant manner. Rhizobacteria have long been known for their role in the promotion of plant growth and health. Beneficial rhizobacteria that stimulates plant growth are usually referred to as Plant–Growth-Promoting Rhizobacteria (PGPR) [4]. They include variety of bacterial genera such as *Pseudomonas*, *Bacillus*, *Acetobacter*, *Arthrobacter*, *Rhizobia*, *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Mycobacterium*, *Flavobacter*, *Cellulomonas* and *Micrococcus* etc.

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the most extensively cultivated cereal crops in the world, after rice, wheat and sorghum, particularly in arid to semi-arid regions. Around 93% of total millet production in the world is contributed by developing countries of Asia and Africa while Asia alone produces forty three percent of total world millet production. India is the world's largest producer of pearl millet, both in terms of area (9.3 m ha) and production (9.5 m ton) with an average productivity of 1044 kg/ha. It is the only cereal crop that is capable of producing a reliable yield under the marginal environments and simultaneously responds to high management conditions [5].

The present study was, therefore aimed at isolation and characterization of rhizobacteria form pearl millet rhizosphere and their *in- vitro* evaluation for plant growth promotion potential.

MATERIALS AND METHODS

Site description and Sampling:
The study site is located at south-east of Haryana state in northern India and lies at 28°25′16″N latitude, 77°18′28″E longitude and at an elevation of 198 meters. It falls under the semi – arid and hot climatic zone and the soil used for this study was sandy loam.

Twenty-two samples of rhizosphere soil were randomly collected during the period of September–October 2010, from pearl-millet fields that had been cultivated with pearl-millet for at least two consecutive years, in seven different villages of Faridabad district. The selected plants were healthy and their age was less than thirty days. The samples were collected in plastic bags and stored at 4^0C till further processing.

Isolation of Rhizobacteria:
Rhizobacteria were isolated from the collected soil samples according to previously described method [3]. Non-rhizospheric, loosely bound soil was detached by vigorous shaking of the roots. After that the roots were washed thoroughly, first with running tap water, then with sterile distilled water and finally soaked in Phosphate buffer saline. The flask containing this root material was shaken at 250 rpm for thirty minutes. The resulting suspension was appropriately diluted and used for isolation of root surface bacteria (rhizospheric soil). In order to obtain the rhizoplane soil sample, roots were surface-sterilized by soaking in 95% (v/v) ethanol for one minute and then washing with sterile tap water, for a minimum of ten times. The washed roots were suspended in 0.05 M phosphate buffer saline and then grinded with the help of a mortar and pestle. The slurry so obtained was filtered through sterile cotton wool. The resulting suspension was appropriately diluted and used for isolation of rhizobacteria on selective and nonselective media. Inoculation was carried out by spread plate technique and the plates were incubated at 37^0C for 24 to 48 hours or more (one or more weeks in case of glycerol-arginine agar plates) and then observed for colony formation.

Purification and Maintenance of isolates:
The isolated colonies of rhizobacterial strains, so obtained, were randomly selected on the basis of colony morphology while colonies obtained on King's B agar plates were observed under UV light on a transilluminator for fluorescence and fluorescent colonies were picked up and further purified by streaking. Pure isolates were maintained as glycerol stocks at -80^0C for further use.

Identification of isolates:
All the rhizobacterial isolates were characterized on the basis of various morphological and biochemical features as per Bergey,s manual of systematic bacteriology according to the standard procedures [6]. Rhizobacterial isolates were examined for their colony and cell morphology including size, shape, margins, elevation, edge, texture, degree of opacity, color and consistency of colony along with growth, pigmentation, cell shape, arrangement, gram reaction and motility. Biochemical characteristics examined were fermentation of various sugars, Starch hydrolysis, Gelatin hydrolysis, Lipid hydrolysis, Hydrogen sulfide (H_2S) production, MRVP, Oxidase test, Indole production, Catalse test, Urease and Nitrate reduction.

***In-vitro* Plant growth promoting activities:** All the rhizobacterial isolates were evaluated for various plant growth promoting activities as discussed below.

Indole acetic acid production: The capacity of the isolates to produce indole acetic acid **(**IAA) was determined following the method of Koo and Kyung [7]. Test tubes containing 5ml of modified DF minimum salt medium supplemented with 0.5mg/ml of *L*-tryptophan were prepared and sterilized. Twenty four hours old bacterial isolates were inoculated into them and incubated at 30^0C for five days under shaking conditions. After five days of incubation, Salkowski's reagent (concentrated H_2SO_4:150 ml; distilled H_2O: 250ml; 0.5M $FeCl_3 \cdot 6H_2O$; 7.5ml) was added to the culture broth in a ratio of 1:2. The resulting mixtures was incubated at room temperature for 20-25 minutes and observed for the development of pink colour. Estimation of pink color was carried out at 530nm and the absorbance was converted into the concentration of -ndole acetic acid using a standard curve prepared with 3- indole acetic acid.

Phosphate solubilization: Rhizobacterial isolates were tested for their phosphate solubilization capacity on the basis of formation of halo zones on Pikovskaya agar plates [8]. Overnight activated cultures were inoculated on Pikovskaya agar plates and incubated at 30^0C up to five days and thereafter observed for the presence of a clear zone of phosphate solubilization around the colony. Diameter of the halozone was measured and solubilization index was calculated as the ratio of the total diameter (colony + halozone) to the colony diameter [9].
SI = total diameter (colony + halozone) / colony diameter

Hydrogen Cyanide (HCN) production: HCN production by rhizobacterial isolates was tested according to previously described method [10]. Plates with Whatman No.1 filter paper pads inside their lids were poured with glycine supplemented (4.4 g/l) Trypticase soy agar (TSA) medium and streak inoculated with twenty four hours old bacterial isolates. The filter paper padding was soaked with sterile picric acid solution and the lid was closed. Inoculated plates were sealed properly, with the help of parafilm in order to avoid the escape of gaseous metabolite (HCN). Sealed plates were given an incubation of five days at 30ºC and then observed for colour change in the filter paper padding. Degree of HCN production was evaluated according to the colour change, ranging from yellow to dark brown.

Production of ammonia: Rhizobacterial isolates were screened for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10ml peptone water in each tube and incubated for 48- 72h at 37^0C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive for ammonia production [6].

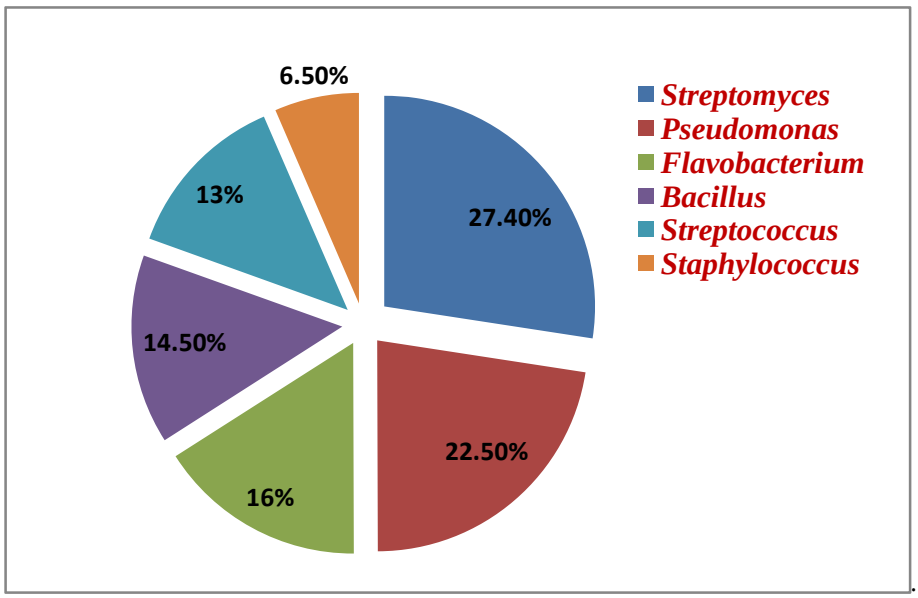
Statistical Analysis: The data obtained for this study was subjected to analysis of variance (ANOVA) and the means were compared using Tukey multiple range test with $P < 0.05$ being accepted as significant.

RESULTS

Isolation and *Identification* of Rhizobacteria: A total of sixty two isolates of rhizobacteria were obtained from healthy rhizosphere and rhizoplane of pearl millet from different locations of district Faridabad, Haryana, India. Figure 1 shows the detailed structural diversity of the isolated rhizobacteria. All isolates on King's B agar were identified as genus *Pseudomonas* on the basis of morphological and biochemical characteristics. They were found to be gram negative; rod shaped; motile and showed fluorescence when observed under UV light (366 nm). Amongst the thirty one isolates on nutrient agar medium, twenty two were coccus or coccobacilli and nine isolates were rods. All the rods were gram positive, spore forming and motile while coccobacilli were non-spore forming and most of them were non motile. On the basis of these morphological as well as biochemical characteristics rods were

identified as genus *Bacillus*. The combined observations of morphological and biochemical properties of the coccus and coccobacilli shaped isolates indicated that they belonged to genus *Flavobacterium, Staphylococcus* and *Streptococcus*. The isolates on glycerol-arginine agar medium showed similarity to *Streptomyces*. All the seventeen isolates were gram positive and spore forming.

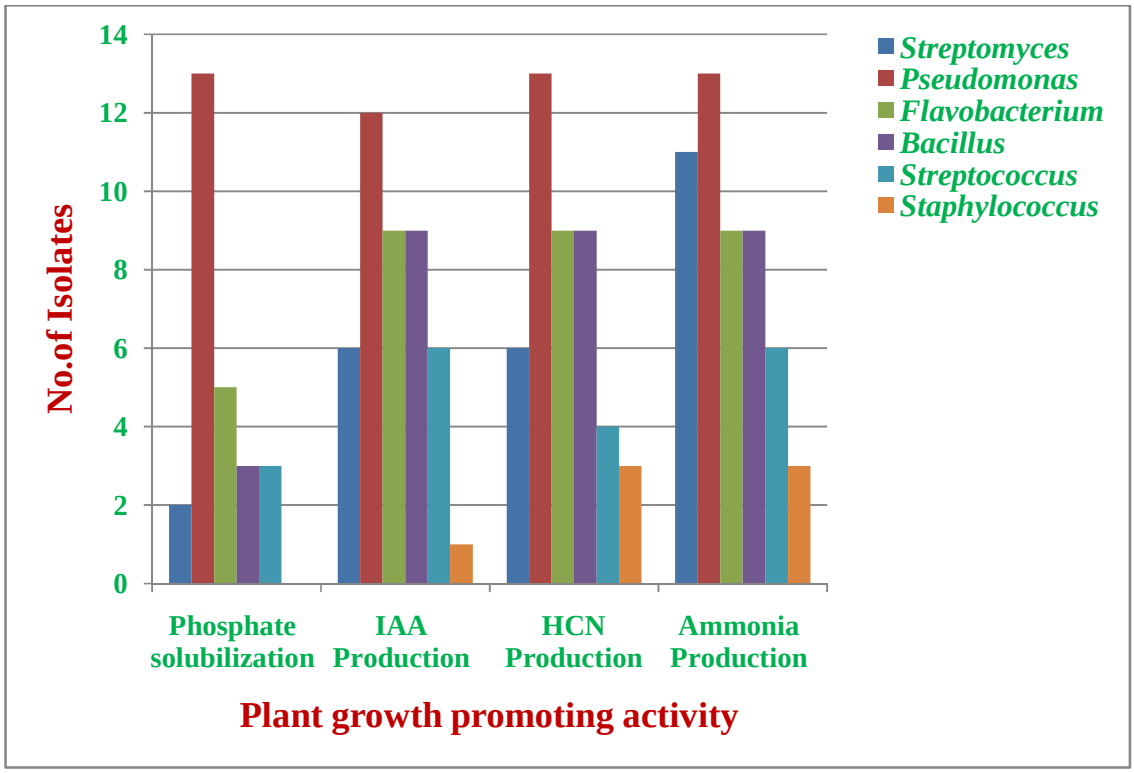
Figure 1: Percentage composition of rhizobacterial diversity isolated from Pearl Millet.



Assessment on the basis of diversity indices: Various diversity indices were calculated to assess the community diversity and richness in the tested soil samples. Simpson index (D) was found to be 0.8059, Shannon Winner index (H) was 1.706 and the Equitability (J) was 0.9523.

Plant growth promoting activities: Amongst the sixty two rhizobacterial isolates of pearl millet, 82.3 percent of them produced ammonia; 71 percent tested positive for hydrogen cyanide production; 69.4 percent for indole acetic acid production and nearly 42 percent were phosphate solubilizers under *in-vitro* conditions (Figure 2). Maximum plant growth promoting potential was exhibited by genus *Pseudomonas*, as 91.07 percent of the isolates were found to be positive for various tested plant growth promoting activities while isolates belonging to genus *Streptomyces* and genus *Staphylococcus* showed minimal potential with respectively 36.76 percent and 43.75 percent of the isolates being tested positive for various plant growth promoting activities. Average IAA concentration given by the rhizobacterial isolates was 1.73 µg/ml and varied significantly among the isolates ($P<0.05$) (Table 1). Highest IAA concentration was given by nutrient agar isolates including *Bacillus*; *Flavobacterium; Staphylococcus* and *Streptococcus* with an average of 1.86 µg/ml, followed by King's B agar isolates(*Pseudomonas*) with average IAA production of 1.78 µg/ml while isolates of arginine-glycerol agar (*Streptomyces*) gave lowest average IAA production of 0.91 µg/ml (Figure 3). Similarly Phosphate solubilization index for the isolates varied between 2.08 to 2.8 ($P<0.05$) with an average of 2.24 (Table 2). Maximum phosphate solubilization activity was shown by genus *Pseudomonas* with an average index of 2.34 and minimum phosphate solubilization index of 2.09 was given by genus *Streptomyces*, while no phosphate solubilization was observed in case of *Staphylococcus* sp (Figure 4). HCN production capacity of the isolates was evaluated as weak, moderate and strong according to the colour change. Amongst the HCN producing isolates, almost 27 percent were weak HCN producers, 66 percent were moderate HCN producers while 7 percent were the strong HCN producers.

Figure 2: Plant growth promoting activities of rhizobacterial isolates of Pearl Millet.



IAA: Indole acetic acid, HCN: Hydrogen Cyanide.

Table 1: Indole acetic acid (IAA) production details

S. No.	Isolate	Concentration of IAA(µg/ml)	S. No.	Isolate	Concentration of IAA(µg/ml)
1	PAG3	0.75±0.02u	23	PNAM22	0.86±0.01st
2	PAG4	0.53±0.01w	24	PNAM21	0.96±0.01r
3	PAG7	1.22±0.02p	25	PNAM7	3.45±0.01a
4	PAG8	1.12±0.03q	26	PNAM27	2.76±0.01c
5	PAG9	0.86±0.01st	27	PNAM18	2.48±0.04ef
6	PAG14	0.97±0.02r	28	PNAM 23	2.34±0g
7	PKB 6	0.93±0.01rs	29	PNAM3	1.76±0.01l
8	PKB 5	1.15±0.01q	30	PNAM25	1.85±0.01k
9	PKB 4	1.3±0.01o	31	PNAM15	2.24±0.02h
10	PKB 1	1.13±0.01q	32	PNAM6	2.58±0.02d
11	PKB 9	2.22±0.01h	33	PNAM17	3.34±0.01b
12	PKB 3	1.54±0.02m	34	PNAM28	0.86±0.01st
13	PKB 12	2.03±0.06j	35	PNAM29	1.24±0.01p
14	PKB 7	2.22±0.02h	36	PNAM10	1.1±0q
15	PKB 10	1.57±0.03m	37	PNAM11	1.41±0.01n
16	PKB 8	2.57±0.1d	38	PNAM12	0.66±0.01v
17	PKB 13	2.78±0.01c	39	PNAM31	0.77±0.01u
18	PKB 14	1.86±0.01k	40	PNAM24	0.85±0t
19	PNAM9	3.43±0.02a	41	PNAM5	0.98±0.01r
20	PNAM4	2.31±0.01g	42	PNAM14	1.15±0q
21	PNAM16	2.13±0.01i	43	PNAM30	2.44±0.02f
22	PNAM13	2.53±0.01de			

Experiment was performed in triplicates and values given are the mean with standard deviation. Means with the same letter are not significantly different at P <0.05 (Tukey's studentized range test).

Figure 3: Indole acetic acid production by different genera of Pearl millet isolates.

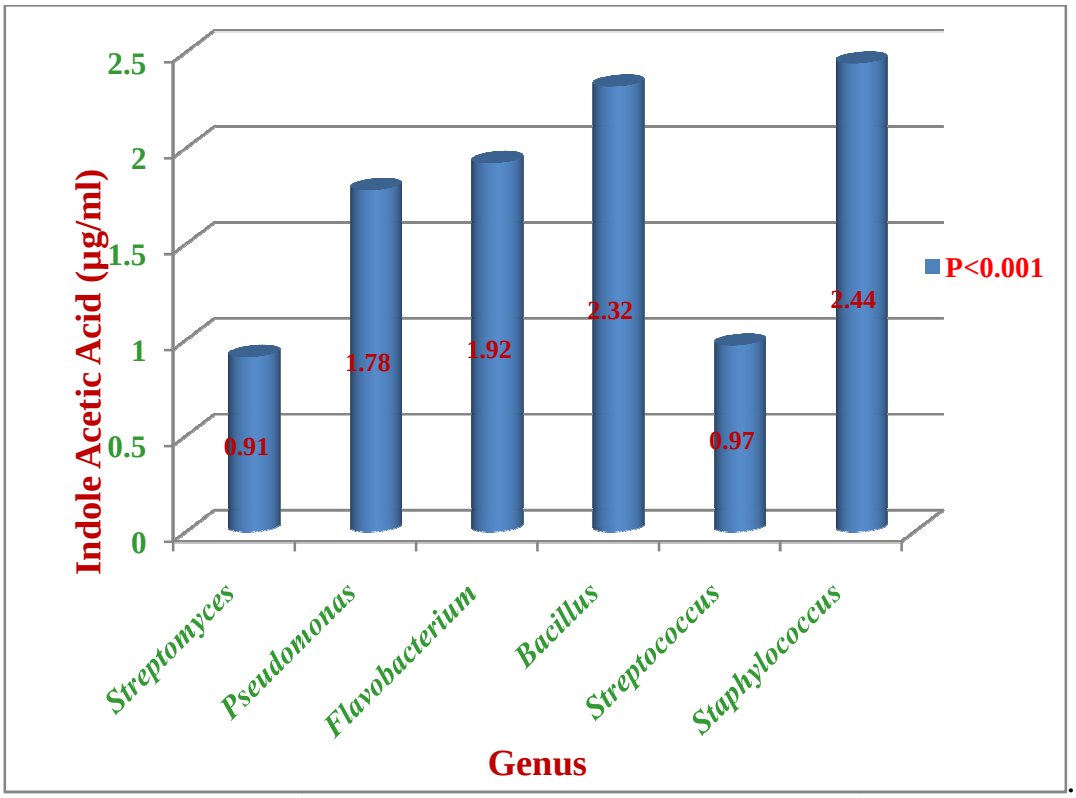
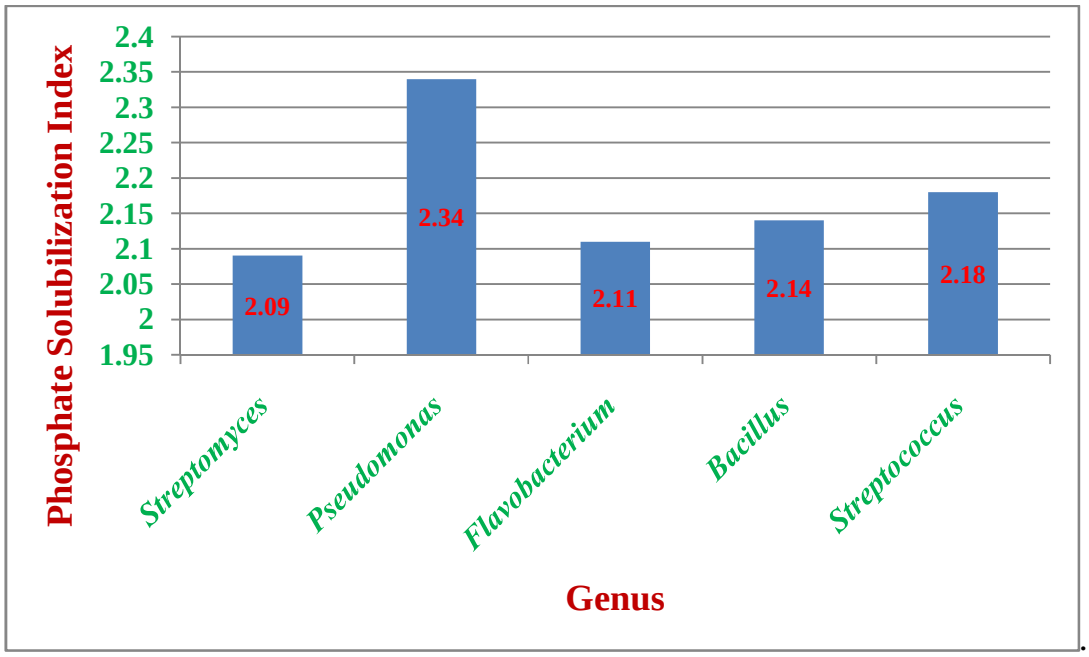


Table 2: Phospahate solubilization by rhizobacterial isolates in Pikovskaya,s medium.

S.No.	Isolate	Colony Diameter (cm)	Halozone Diameter (cm)	Phosphate solubilization Index
1	PAG3	2.43±0.06b	2.63±0.06b	2.08±0.05h
2	PNAM6	1.43±0.05f	1.56±0.02f	2.09±0.05h
3	PNAM10	1.52±0.02e	1.69±0.01e	2.11±0.01h
4	PAG 9	2.5±0.15a	2.78±0.01a	2.1±0.06h
5	PNAM15	1.22±0.02h	1.37±0.01h	2.12±0.02gh
6	PNAM17	1.76±0.02c	1.98±0.02c	2.12±0.001gh
7	PNAM 23	1.21±0.005h	1.37±0.03h	2.13±0.01gh
8	PKB 9	0.86±0.02j	0.98±0.02k	2.13±0.01gh
9	PNAM9	1.63±0.02d	1.86±0.01d	2.14±0.004fgh
10	PNAM4	1.27±0.02gh	1.45±0.05g	2.14±0.05fgh
11	PNAM16	1.25±0.02gh	1.43±0.01gh	2.15±0.02efgh
12	PNAM14	1.63±0.02d	1.87±0.03d	2.15±0.03efgh
13	PNAM24	1.34±0.03fg	1.54±0.04f	2.15±0.04efgh
14	PKB 6	0.970.02i	1.12±0.01j	2.15±0.02efgh
15	PKB 5	1±0i	1.23±0.02i	2.23±0.02defg
16	PKB 4	0.78±0.02jk	0.97±0.02k	2.25±0.04def
17	PKB 1	0.98±0.02i	1.22±0.03i	2.24±0.01def
18	PNAM12	1.32±0.02g	1.65±0e	2.25±0.02def
19	PKB 3	0.54±0.01m	0.68±0.02m	2.26±0.07de
20	PKB 12	0.75±0kl	0.95±0.02k	2.27±0.03d
21	PKB 7	0.96±0.03i	1.23±0.02i	2.28±0.06d
22	PKB 10	0.67±0.01lm	0.87±0.02l	2.32±0.03d
23	PKB 8	0.58±0.01m	0.85±0l	2.46±0.04c
24	PKB 13	0.65±0lm	0.96±0.04k	2.48±0.05c
25	PKB 14	0.73±0.02kl	1.12±0.03ij	2.6±0.06b
26	PKB 11	0.56±0.02m	0.96±0.02k	2.8±0.04a

Experiment was performed in triplicates and values given are the mean with standard deviation. Means with the same letter in the same column are not significantly different at P <0.05 (Tukey's HSD test).

Figure 4: Solubilization of Tri-calcium phosphate by different genus of Pearl millet isolates in Pikovskaya,s medium.



DISCUSSION

Rhizosphere microflora forms a crucial part of soil ecosystem, affecting the plant in a variety of ways including plant health as well as its growth and nutrition. Structural diversity of the rhizosphere in terms of microflora depends upon a variety of factors such as the kind of plant and cultivars, age of plant, soil characteristics including ph, organic matter content, nutrient availability, seasonal effects, root characteristics and management practices like irrigation, tillage, cropping, fertilizer application, residue incorporation etc. [11, 12]. Here we have studied the structural and functional diversity of pearl millet rhizosphere. In this study genus *Streptomyces* has been found to be the most predominant inhabitant forming 27.40 percent of the total rhizospheric population, followed by genus *Pseudomonas* with a population of 22.50 percent while genus *Staphylococcus* was observed to have the minimal population of 6.50 percent only, in the tested rhizosphere samples. Gram-negative, rod shaped, non-sporulating bacteria belonging to the groups Proteobacteria and Actinobacteria are generally considered as predominant bacterial strains in the rhizosphere [13] and *Pseudomonas* are generally found as the most abundant [14]. However, we have found gram-positive actinomycetes i.e. *Streptomyces* sp to be more populated than *Pseudomonas* sp. Here the predominance of genus *Streptomyces* may be attributed to their spore forming capacity, ability to produce a variety of antibiotics, their nutritionally versatile nature which enables them to grow in soils containing a low or even in apparent lack of nutrients and the fact that they are more abundantly found in dry than in wet soils [15, 16] as found in the tested climatic zone. Wide range of bacterial strains have been reported for their plant growth promoting potential including *Bacillus, Pseudomonas*, *Agrobacterium*, *Azospirillum*, *Azotobacter*, *Alcaligenes*, *Arthrobacter*, *Flavobacterium* and *Streptomyces* [17, 18, 19, 20]. PGPR promote growth by a variety of direct and indirect mechanisms like atmospheric nitrogen fixation [21], siderophore production [22], phosphate solubilization [23], synthesis and release of phytohormones like IAA [24] , controlling the phytopathogens by variety of mechanisms like production and release of HCN, ammonia and antibiotics [25], cell wall degrading enzymes[26] etc. In this study, all the isolated rhizobacteria were screened for various plant growth promoting activities and it was found that a significantly large number of the rhizobacteria possess one or more of these traits under *in-vitro* conditions. Most of these isolates exhibited moderate to strong range of one or more plant growth promotion activities and hence may improve the plant health and growth through individual or combined effect of various traits.

CONCLUSION

On the basis of results obtained for this study, it may be concluded that rhizobacteria are an attractive and potential option for developing PGPR inocula for biocontrol and plant growth promotion of various crops. The study should be further extended to check the response of these isolates under field conditions so that such rhizobacterial isolates may be developed as successful inoculants for improvement of plant growth.

REFERENCES

[1] J.M. Kuhajek, S. N. Jeffers, M.Slattery, D.E.Wedge. *Phytppathology*, **2003**, 93, 46-53.
[2] E.A Curl, B. Truelove. The rhizosphere. Springer- Verlag,Berlin, **1986.**
[3] B.R. Glick, J.E Thompson. (Ed), Methods in plant molecular biology and biotechnology.
(CRC Press, Boca Raton, Fla.,**1993**), 331-345.
[4] J.W. Kloepper, R. Lifshitz, R.M. Zablotowicz. *Trends Biotechnol*., **1989**, 7**,** 39-43.
[5] O.P. Yadav. All India Coordinated Pearl Millet Improvement Project Workshop, 12-14 March (Hisar **2011),** 1-9.
[6] J. Cappuccino, N. Sherman. Microbiology: A Laboratory Manual, 9th Edn, Benjamin Cummings, **2010**.
[7] S.Y. Koo, C. Kyung-Suk. *J. Microbiol. Biotechnol*., **2009,** 19, 1431–1438.
[8] R.I. Pikovskaya. *Microbiologiya*, **1948,** 17**,** 362–370.
[9] M.A Edi-Premono, P.L.G. Vleck. *Indonesian J. Crop Sci*., **1996,** 11, 13-23.
[10] G.Wei, J.W. Kloepper, T. Sadik, *Phytopathology*, **1991,** 81, 1508-1512.
[11] S.J Grayston, S.Q Wang, C.D.Campbell, A.C.Edwards. *Soil Biol. Biochem.*, **1998,** 30, 369–378.
[12] L.M. Macdonald, E. Paterson, L.A. Dawson, A.J.S. McDonald. *Soil Biol. Biochem.*,**2004,** 36, 489–498.
[13] R.M. Atlas, R. Batrha (Ed). Microbial Ecology: Fundamentals and Applications. (Menlo Park CA Benjamin/Cummings, **1993**), 563.
[14] I. Ahmad, J. Pichtel, S. Hayat (Eds) Plant-Bacteria Interactions. Strategies and Techniques to Promote Plant Growth. (Wiley-VCH Verlag GmbH and Co. KGaA, Weinheim, **2008**) 1-17.
[15] B. Intra, I. Mungsuntisuk, T. Nihira, Y. Igarashi, W. Panbangred. *BMC Res. Notes*, **2011**,4, 98.
[16] E.M.H. Wellington, E. Stackebrandt, D. Sanders, J. Wolstrup, N.O.G. Jorgensen *Int. J. Syst. Bacteriol*., **1992,** 42, 156-160.
[17] S.E. Fischer, S.I. Fischer, S. Magris, G.B. Mori, *World J Microbiol Biotechnol*., **2005,** 23(7) 895-903.
[18] P. Joshi, V. Tyagi, A.B. Bhatt, *Adv. Appl. Sci. Res*., **2011**, 2 208-216.
[19] G. Berg, N. Roskot, A. Steidle, L. Eberl, A. Zock, K. Smalla. K *Appl. Environ. Microbiol*., **2002,** 68: 3328–3338.
[20] R.C. Gomes, L.T. Semedo, R.M. Soares, L.F. Linhares, C.J. Ulhoa, C.S. Alviano, R.R. Coelho. *J. Appl. Microbiol.*, **2001**, 90, 653-661.
[21] K.V.B.R. Tilak, N. Ranganayaki, K.K. Pal, R. De, A.K. Saxena, C.S. Nautiyal, S. Mittal, A.K. Tripathi, B.N. Johri. *Curr. Sci***., 2005** 89, 136-150.
[22] G.K. Sahu, S.S. Sindhu. *Res. J. Microbiol*., **2011**, 6, 735-749.
[23] H. Rodríguez, R. Fraga. *Biotechnol. Adv*., **1999,** 17, 319–339.
[24] W.T.J. Frankenberger, M.Arshad. Phytohormones in Soil: Microbial Production and Function. (Dekker, New York, USA, **1995**), 503.
[25] G. Berg. *Appl. Biotechnol*., **2009,** 84, 11–18.
[26] D. P. Kumar, A.P. D, R.K.Singh, R. Thenmozhi, A.Nagasathya, N. Thajuddin, A. Paneerselvam. *J. Microbiol. Biotech. Res.*, **2012**, 2 (1):129-137.