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Relative plant growth promoting potential of Himalayan Psychrotolerant *Pseudomonas jesenii* strain MP1 against native *Cicer arietinum* (L.)., *Vigna mungo* (L.) Hepper; *Vigna radiata* (L.) Wilczek., *Cajanus cajan* (L.) Millsp. and *Eleusine coracana* (L.)Gaertn.

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Plant growth promoting properties of *Pseudomonas jesenii* strain MP1 were tested against five native crops *viz. Cicer arietinum* (L.) (Chickpea), *Vigna mungo* (L.) Hepper. (Blackgram), *Vignaradiata* (L.) Wilczek. (Greengram), *Cajanus cajan* (L.) Millsp. (Pigeonpea) and *Eleusine coracana* (L.) Gaertn. (Finger millet). The strain significantly ($p<0.05$) stimulated the growth of shoot length, root length, plant fresh weight and plant dry weight of each crop, over their respective untreated controls. Moreover, MP1 treated plant leaves typically showed significant increase in their chlorophyll content, nitrate reductase activity and P content. Chickpea and black gram responded better to MP1 inoculation relatively to other crops. Further, total bacterial and diazotrophic count of MP1 treated soils along with their available phosphorus (P) and nitrogen (N) content were found to increase significantly, in comparison to their respective untreated controls. Microbial community analysis using denaturant gradient gel electrophoresis (DGGE) revealed that the soil bacterial communities were minimally affected by MP1 inoculation. Conclusively, the sustainable agriculture plan in Himalaya may be developed on a strategy of exploring psychrotolerant *P. jesenii* MP1 strain as representative candidate of indigenous biodiversity for individual and/or mixed cropping.

Key words: Psychrotolerant, plant growth promotion, Himalayan agriculture, microbial community analysis.

INTRODUCTION

The need of sustainable agriculture development is revitalizing the interest in plant growth promoting rhizobacteria (PGPR), particularly those involving economically important crops in terms of food and forage. PGPR are able to promote plant growth directly by either assisting in nutrients acquisition (N, P and minerals) or

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modulating plant hormone levels, or indirectly in the forms of biocontrol agents (Glick, 2012). These potential PGPR can fertilize several important agronomic plants such as sugarcane (Mirza et al., 2001), rice (Isawa et al., 2010), maize (Couillerot et al., 2012) or wheat (Upadhyay and Singh, 2014).

Agriculture plays an important role in sustaining livelihood of local people in marginal land of Uttarakhand Himalayas. Being an organic state use of chemical fertilizers for crop production is not recommended. Hence, the application of PGPR as an alternative to chemical fertilizers has emerged as a promising approach. Given the existence of novel and unique gene pool (Soni and Goel, 2010; Suyal et al., 2014), Himalayan soils may be a rich reservoir of novel and diverse microorganisms, as also confirmed by their tremendous potential of biodegradation (Soni et al., 2008) and PGP traits (Selvakumar et al., 2011; Singh et al., 2012). However, despite being potent most of them are not under practice; probably due to their restricted environmental adaptations. In this context, PGP traits of indigenous psychrotolerant *Pseudomonas jesenii* MP1 strain that was able to well adapt to fluctuating temperatures, could be used effectively as a low cost bioinoculant in Himalayan agricultural lands.

Numerous studies conducted over the past three decades have clearly shown that the plant genotype and the soil type are two main drivers that shape the rhizosphere microbiome (Bakker et al., 2012). Moreover, microbial application in an ecosystem may cause tremendous changes in the number and composition of the taxonomic groups (Mendes et al., 2013). These changes may be undesirable if important native species are lost, thus affecting subsequent crops. Therefore, to exert beneficial effects in the root environment, it has to be rhizosphere competent. Various culture independent methods has been used to investigate the effect of bacterial inoculation in soil microbial communities *viz.* denaturant gradient gel electrophoresis (DGGE) for *Rizobium* (Herrmann et al., 2012), quantitative real time PCR (qPCR) for *Rizobium* (Babic et al., 2008), ribosomal intergenic spacers analysis (RISA) for *Azospirillum* (Schumpp and Deakin, 2010), fatty acid methyl ester analysis (FAME) (Kozdroj et al., 2004), etc.

The primary objectives of this study were to evaluate PGP traits of psychrotolerant MP1 strains against four native pulses *viz*. chick pea, black gram, green gram and pigeon pea and one cereal *viz*. finger millet; to investigate native bacterial diversity response to PGPR inoculation and assessment of the effect of MP1 strain inoculation on soil health. Here, it is pertinent to mention that the demand of these pluses and millet produced in Himalayan hills is on an increase, not only for taste and nutritive value but because they are a bio-food. The positive growth response of all the crops signifies the use of *P. jesenii* MP1 strain to the agriculture practices in Himalayan and/or similar agro-ecosystems for improved crop production and sustainability.

MATERIALS AND METHODS

Strain and culture conditions

Strain MP1, originally isolated from agricultural soil sample from Munsyari (2200 m, 30.60°N/80.20°E) from Western Indian Himalayas, was obtained from departmental culture collection. Strain was maintained aerobically in Burk medium at 28°C.

In vitro assessment for plant growth promoting attributes

MP1 was assessed for the presence of important plant growth promoting traits *viz.* N_2 fixation, P solubilization and indole acetic acid (IAA) production. The procedures were taken from respective standard protocols (Supplementary material).

In vitro seed germination assay

In vitro comparative seed germination assay was conducted to determine the effect of MP1 strain on seed germination of local varieties of five crops *viz.* chickpea, blackgram, greengram, pigeonpea and finger millet. In the experiment, 60 seeds of each crop were imbibed separately in 5 ml of a 1×10^8 ml^{-1} bacterial suspension. Controls were imbibed with Burk medium broth only. After 15 min excess suspension was decanted off and the seeds were plated out on to filter paper laid over 0.5% water agar in Petri dishes (20 seeds per Petri dish) at 28°C under a diurnal cycle of white light. The number of seeds germinated was recorded as seedlings with coleoptile lengths >5 mm on day 3 and 6 post-inoculation. The number of germinating seeds was taken as the mean of three Petri dishes so that each value was the mean of 60 imbibed seeds (three Petri dishes × 20 seeds), expressed as a percentage of the controls.

Chemical analysis of the soils

Soil samples were analyzed using “K054 Soil Testing Kit” Himedia Laboratories Pvt Ltd, India pH, organic carbon (% oxidizable OC), available phosphate (P_2O_5), available potassium (K_2O), ammonical-nitrogen (NH_3-N), and nitrate nitrogen (NO_3-N) contents.

Plant growth promotion studies under net house conditions

The pot trial was performed at Pantnagar (244 m, 28.97°N, 79.41°E), a Tarai region of Indian Shiwalik Himalayas, during the month of August to October as described previously by Rani et al. (2012). For the experiment, local varieties of chickpea, blackgram, greengram, pigeon pea and finger millet were used. Seeds sterilized with 1% sodium hypochlorite for 3 min and washed thrice with sterile distilled water were bacterized (10^8 cells/seed) using carboxymethyl cellulose (Katiyar and Goel, 2003; Singh et al., 2012). Seven seeds per pot were sown in 20-cm-diameter pots filled with 3-kg non-sterilized sandy-loam soil having pH 7.5 without any external fertilizer input. Non-bacterized seeds served as control. Pots were kept in a net house having natural fluctuating temperature range from 30 ± 5°C during the day to 15 ± 5°C during the night, for 90 days (Rani et al., 2012). All the analysis was carried out in triplicate. Agronomical parameters (shoot length, root length, fresh weight, dry weight), leaf nitrate reductase activity, total leaf chlorophyll content and leaf P content was measured at 30, 45,

60 and 90 days after sowing (das). Simultaneously, rhizospheric soil samples were collected for bacterial community analysis, using sterile spatula in sterile polythene bags and transported to laboratory under sterile and cold conditions. Each soil sample was collected in triplicates which were later mixed to make a single composed sample per treatment.

Nitrate reductase activity, chlorophyll assay and estimation of leaf P content

The nitrate reductase activity of plant flag leaves was measured according to previous reports (Rani et al., 2012; Singh et al., 2012). The total chlorophyll content of plant flag leaves was measured as described previously (Rani et al., 2012). P content of plant flag leaves was measured according to Fiske and Subbaraw (1925). The plant flag leaves were sampled at 30, 45 and 60 days.

Statistical analysis

The pot experiment was performed with three replicates per treatment. Data were analyzed by ANOVA. Mean difference of the treatments was considered to be significant at the 5% level. STPR-15 software was used to calculate analysis of variance which was programmed by department of Mathematics, Statistics and Computer Science G.B.P.U.A.&T. Pantnagar.

Bacterial community analysis

Effect of bio-inoculant on native micro-flora was assessed by using qPCR and PCR-DGGE techniques. Rhizospheric soil samples (not deeper than 15 cm) were collected from the rhizosphere of each crop plants at 0, 30, 45, 60 and 90 days, using sterile spatula in sterile polythene bags and transported to laboratory under sterile and cold conditions. The soil samples from the replicate pots (3) were mixed to make a single composed sample per site. All the samples were analyzed chemically as per described earlier in the manuscript.

Total soil DNA extraction

Metagenomic DNA was extracted from each 0.5 g (fresh weight) soil sample by using the Powersoil™ DNA isolation kit (Mobio Lab. Inc., USA) according to the manufacturer's instructions. After extraction, DNA samples were quantified spectrophotometrically at 260 nm and used immediately for further analysis.

Real time PCR (qPCR) analysis

Copy number of 16S rDNA and *nif*H from collected soil samples were quantified by the primer set 16S F/R(5' CCTACGGGAGGCAGCAG 3' and 5' ATTACCGCGGCTGCTGG 3', respectively) and PolF/R (5'-TGC GAY CCS AAR GCB GAC TC-3' and 5'-ATS GCC ATC ATY TCR CCG GA-3', respectively), using iCycleriQ™ Multicolor (Bio-Rad Lab, Hercules, USA) real-time polymerase chain reaction (qPCR) machine as described previously (Prema et al., 2009; Soni and Goel, 2010).

Bacterial community analysis

Variable region 3 (V3) of the 16S ribosomal RNA (rRNA) gene was amplified by the primer set 357f-GC (*Escherichia coli* position, 341-357, 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGG GCCTACGGGAGGCAGG-3′, underline of sequence denotes GC clamp) and 518r (*E. coli* position, 518-534, 5′-ATTACCGCGGCTGCTGG-3′). Denaturing gradient gel electrophoresis (DGGE) was performed with 8% (w/v) acrylamide gel containing a linear chemical gradient ranging from 40 to 60% denaturant as described earlier (Soni and Goel, 2010).

Bacterial strain deposition

P. jesenii MP1 strain used in this study has been deposited in the "National Bureau of Agriculturally Important Microorganisms (NBAIM) microbial repository" recognized by Biodiversity Authority of India, under accession number B-01444.

RESULTS AND DISCUSSION

P. jesenii strain MP1 has shown the capability to enhance plant growth as substantiated by the presence of various PGP traits (Table SM1). Moreover, it can grow on N_2 free media and subsequently gave an amplification of 360 bp*nif*H fragment confirming its diazotrophic trait too. Detection of nitrogen fixers, by conventional methods *viz.* acetylene reduction assay, is sometimes ineffectual and inconclusive (Franche et al., 2009). Therefore, *nif*H gene is most often used as biomarker for nitrogen fixation.

In vitro seed germination assay confirm the efficacy of MP1 strain to enhance the seed germination of all the crops (Table 1). The maximum germination rate of treated seeds was observed in black gram (22%), followed by green gram (21%). In pigeon pea and finger millet the increment in seed germination was 18%, while, in case of chickpea, it was minimum (17%). Significant difference in plant growth was observed among inoculated and uninoculated treatments. The effect of MP1 strain on agronomical parameters of chickpea, black gram, green gram, pigeon pea and finger millet was significantly higher with an increase in shoot length upto 41.5, 136.8, 49.0, 20.7 and 39.3%; root length upto 83.3, 81.2, 14.1, 66.7 and 80.0%; fresh weight upto 102.2, 97.4, 81.7, 84.4 and 97.4% and dry weight upto 261.8, 125.0, 150.0, 149.6 and 225.0%, respectively, over control (Table 1). In general, better plant growth was observed in chickpea and black gram than other crops. Moreover, the most prominent effect of bio-inoculant on the crops was observed up to 45 days with a slight deviation in case of black gram and green gram. The increment in agronomical parameters of the MP1 treated plants in comparison with respective control could be correlated with enhanced crop productivity (Rani et al., 2012; Singh et al., 2012). An overwhelming number of studies have revealed the successful implementation of the PGPR having profound effects on seed germination, seedling vigor, plant growth and development, nutrition, diseases and productivity (Kogel et al., 2006; Rani et al., 2012; Singh et al., 2012). However, exploration of cold adapted bacterial strains for plant growth promotion in indigenous plants is still lacking. Katiyar and Goel

Table 1. Two- way ANOVA depicting the effect of *P. jesenii* MP1 strain on growth of various crops under greenhouse conditions.

Crops		*In vitro* seed germination assay (% germination of the seeds)	Days	Shoot length (cm)[a]	Root length (cm)[a]	Plant fresh weight (g plant^{-1})[a]	Plant dry weight (g plant^{-1})[a]
Pigeon pea	C	65 ± 0.52	30	43.00±1.5	6.00±1.10	2.76±0.08	0.75±0.02
			45	58.83±1.1	8.16±.1.80	5.51±0.16	1.37±.0.04
			60	68.16±0.3	9.67±1.31	10.35±0.43	3.01±0.06
			90	68.50±2.0	11.17±1.10	11.67±0.33	4.50±0.09
	T	80 ± 0.27	30	50.00±1.2(16.3)[b]	10.00±0.90(66.7)[b]	4.30±0.28(55.8)[b]	1.41±0.30(88.0)[b]
			45	71.00±1.9(20.7)[b]	11.00±0.84(34.8)[b]	10.16±0.26(84.4)[b]	3.42±0.50(149.6)[b]
			60	73.00±1.5(7.1)[b]	11.50±1.20(18.9)[b]	13.65±0.75(31.9)[b]	5.20±0.19(72.8)[b]
			90	74.60±1.3(8.9)[b]	12.30±0.74(10.1)[b]	18.00±0.8 (54.2)[b]	6.16±0.27(36.9)[b]
Gram	C	70 ± 0.31	30	23.80±0.9	6.00±0.44	1.74±0.15	0.52±0.06
			45	23.80±0.9	6.00±0.44	3.99±0.15	0.62±0.06
			60	30.17±1.4	9.60±0.75	7.37±0.19	1.10±0.03
			90	32.17±1.7	11.17±0.91	8.36±0.29	1.90±0.07
	T	85 ± 0.58	30	31.17±1.8(31.0)[b]	11.00±0.53(83.3)[b]	3.52±0.26(102.2)[b]	1.16±0.12(123.0)[b]
			45	33.67±2.4(41.5)[b]	11.00±0.34(83.3)[b]	10.30±1.20(158.1)[b]	2.00±0.19(222.5)[b]
			60	40.33±1.9(33.7)[b]	11.50±0.50(19.8)[b]	11.5±0.29(56.0)[b]	3.98±0.08(261.8)[b]
			90	41.83±1.3(30.0)[b]	12.30±0.64(10.1)[b]	16.00±0.58(79.4)[b]	5.50±0.13(189.4)[b]
Green Gram	C	75 ± 0.64	30	19.80±1.6	5.30±0.68	4.65±0.18	0.52±0.04
			45	30.00±2.3	6.30±0.45	6.02±0.33	0.75±0.26
			60	32.30±1.7	7.16±0.16	7.50±0.16	1.44±0.49
			90	33.30±0.7	8.00±0.46	10.27±1.20	2.5±0.78
	T	95 ± 0.72	30	29.50±1.1(49.0)[b]	6.00±0.21(13.2)[b]	7.13±1.15(53.3)[b]	1.3±0.07(150.0)[b]
			45	33.30±1.3(11.0)[b]	7.00±0.35(11.1)[b]	8.46±0.46(36.4)[b]	1.35±0.11(80.0)[b]
			60	35.00±2.0(8.4)[b]	8.17±0.36(14.1)[b]	10.89±0.76(45.2)[b]	2.33±0.06(61.8)[b]
			90	35.80±1.2(7.5)[b]	8.50±0.44(6.3)[b]	18.67±0.43(81.7)[b]	2.83±0.07(13.2)[b]
Black Gram	C	70 ± 0.79	30	16.67±1.2	6.00±0.29	4.99±0.02	0.44±0.02
			45	17.67±1.1	8.00±0.29	7.09±0.03	0.58±0.03
			60	19.00±1.4	10.50±0.35	11.13±0.07	0.79±0.07
			90	20.50±2.1	11.30±0.18	12.83±0.10	1.50±0.10
	T	90±0.85	30	17.67±1.1(6.0)[b]	10.00±0.31(66.6)[b]	8±1.53(60.3)[b]	0.99±0.05(125.0)[b]
			45	21.50±1.2(21.7)[b]	14.50±0.17(81.2)[b]	14±1.43(97.4)[b]	1.09±0.08(87.9)[b]
			60	45.00±2.3(136.8)[b]	18.17±0.13(73.0)[b]	18.33±0.67(64.6)[b]	1.13±0.06(43.0)[b]
			90	47.16±1.1 (130.0)[b]	20.00±0.12(76.9)[b]	19.19±0.44(49.4)[b]	1.83±0.18(22.0)[b]

Table 1. Contd.

Ragi	C	72±0.82	30	14.00±0.6	5.00±0.10	0.96±0.15	0.29±0.08
			45	15.83±0.4	6.67±0.11	1.24±0.09	0.32±0.07
			60	19.17±0.7	8.00±0.15	1.37±0.11	0.67±0.04
			90	20.30±0.2	9.50±0.11	1.83±0.08	1.50±0.13
	T	88±0.67	30	19.50±0.2(39.3)[b]	9.00±0.26(80.0)[b]	1.66±0.12(72.9)[b]	0.66±0.12(127.5)[b]
			45	20.00±0.3(26.3)[b]	10.60±0.64(58.9)[b]	1.99±0.15(60.4)[b]	1.04±0.15(225.0)[b]
			60	21.50±0.6(12.2)[b]	11.00±0.23(37.5)[b]	2.06±0.08(50.3)[b]	1.43±0.08(113.4)[b]
			90	24.30±0.8(19.7)[b]	12.83±0.27(35.1)[b]	2.67±0.13(45.9)[b]	1.83±0.13(22.0)[b]
A (Treatment)	SEM			0.37	0.16	0.15	0.06
	CD			1.04	0.44	0.42	0.17
B (Days)	SEM			0.52	0.22	0.21	0.09
	CD			1.47	0.62	0.59	0.24
C (Crops)	SEM			0.58	0.25	0.24	0.09
	CD			1.64	0.69	0.67	0.27
AXB	SEM			0.73	0.31	0.30	0.12
	CD			2.08	0.88	0.85	.34
AXC	SEM			0.83	0.35	0.34	0.13
	CD			2.33	0.98	0.95	0.38
BXC	SEM			1.17	0.49	0.48	0.19
	CD			3.29	1.39	1.34	0.54
AXBXC	SEM			1.65	0.69	0.67	0.27
	CD			4.65	1.96	1.89	0.77

a: Mean of three replicates; b: Values in parentheses indicate percent increase over treatment. Data were analyzed statistically at the 5% (p>0.05) level of significance.

(2003) assessed the inoculation effect of phosphate-solubilizing cold-tolerant mutant of *P. fluorescens* on mungbean in sterilized and unsterilized soil and observed that inoculated plants resulted in better plant growth in both soils. Biochemical parameters of the MP1 treated plants were also found to be enhanced significantly (Figure 1).

The total chlorophyll content of the treated plants of all the crops was maximum at 45 days. Nitrate reductase activity was maximum on 30 days in chick pea, pigeon pea and finger millet, while on 45 days in black gram and green gram. The leaf P content was also found to increase significantly in MP1 treated plants especially in finger millet, followed by chick pea, green gram, pigeon pea and black gram on 60 days (Figure 2). These results showed that MP1 treated plants had better nutrient uptake efficiency in comparison with their respective controls.

Members of the rhizosphere microbiome can significantly influence the nutrient status of plants. Well-known examples are the rhizobia, the mycorrhizal fungi and *Pseudomonas* sp. that facilitate N and P uptake (Miransari, 2011; Rani et al., 2012).

Bacterial application significantly changed soil physiochemical properties ((Table SM2). The initial pH of the soils was neutral (7.5 ±0.1), which was increased to maximum (pH 9.0±0.1) on 45 days, and thereafter, decreased to pH 8.0 to 7.5 (±0.1) on crop maturity, regardless to bacterial treatment. Contrary to earlier reports (Orhan

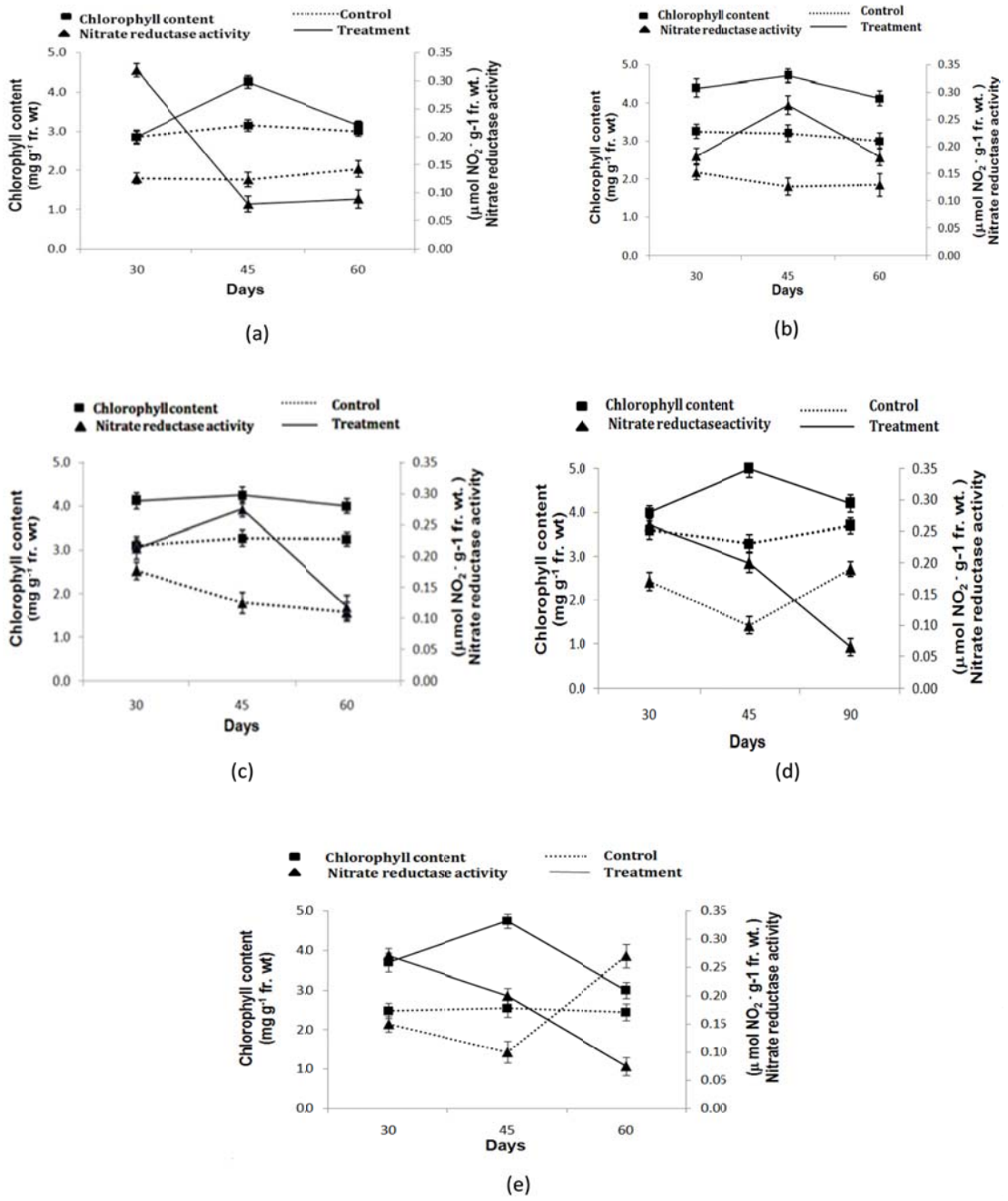

Figure 1. Impact of MP1 strain inoculation on chlorophyll content and nitrate reductase activity of chickpea (a), black gram (b), green gram (c), pigeon pea (d) and finger millet (e), respectively

et al., 2006; Das and Singh, 2014), increase in pH may be explained by the combined effect of different plant exudates and/or bacterial activities. It was observed that bacterial diversity was highest in neutral soils and lower in acidic soils (Fierer and Jackson, 2006).

Most rhizobacterial species are organotrophs and therefore, their growth is greatly affected by the availability and accessibility of the available carbon (Rousk and Baath, 2007). The total organic carbon (TOC) content in soil was “0.505-0.750% oxidizable OC’ which was decreased on 90 days in black gram and finger millet, regardless of bacterial treatment. Contrary to it, MP1 treatment in chickpea, green gram and pigeon pea, increased the TOC on 90 days to “0.750-1.00% oxidizable OC”; thereby, helping the growth of other soil bacteria.

In the case of available P (APH), MP1 treatment in each crop, increase the availability of P from 22-56 to

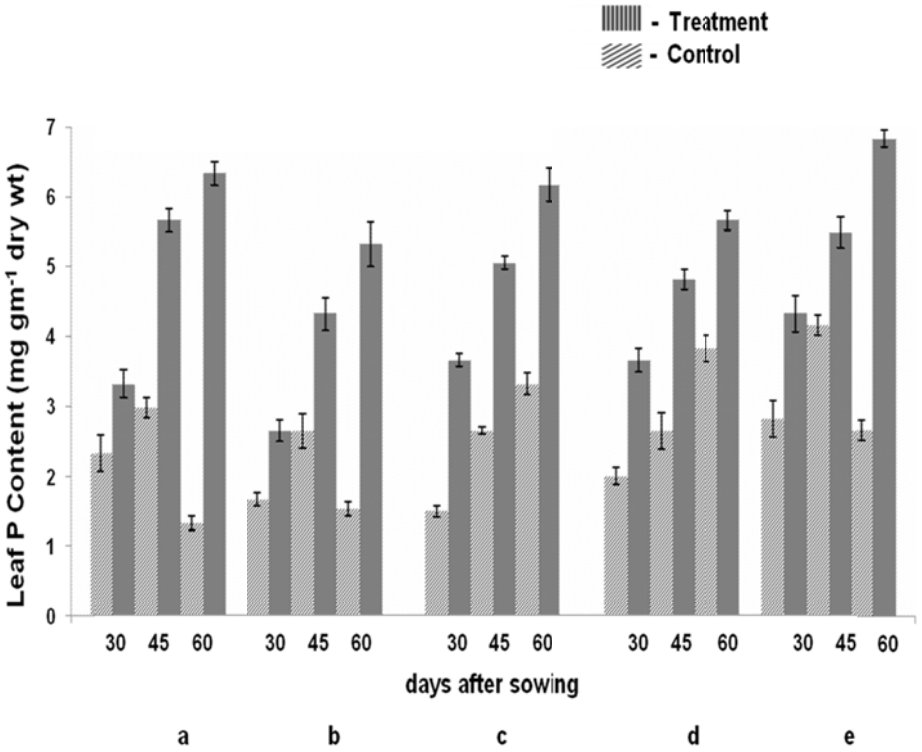

Figure 2. Impact of MP1 strain inoculation on leaf P content of chickpea (a), black gram (b), green gram (c), pigeon pea (d) and finger millet (e), respectively.

56-73 kg ha^{-1} on 45 days and then maintain its concentration at 22-56 kg ha^{-1}; while, in untreated plants a APH concentration remained constant (22-56 kg ha^{-1}) up to 60 days and thereafter decreased to < 22 kg ha^{-1}, excepting finger millet, where it was found to increase to 56-73 kg ha^{-1} on 45 days. Similarly, N content of the soil was analyzed in two forms: "ammonical N (AN) and nitrate N (NN) content". A common trend of increasing AN content from ≤ 15 to 16 ≤ 73 kg ha^{-1} onwards to 60 days was observed in the soils of treated plants; however, that of untreated plants showed no changes in their AN content. Soil NN content of the untreated plants was found to remain constant (≤ 04 kg ha^{-1}) up to 45 days and thereafter increased to 05 ≤ 15 kg ha^{-1}on 60 and 90 days, in all the crops except finger millet, where it remained the same (≤ 04 kg ha^{-1}) till 90 days. Contrary to it, soil NN content of the MP1 treated plants, was found to increase from ≤ 04 kg ha^{-1} (initial) to 05 ≤ 15 kg ha^{-1} (30 and 45 das) to 16 ≤ 20 kg ha^{-1} (60 and 90 das), except finger millet where the increment was only up to 5 ≤ 15 kg ha^{-1}. Increment in soil NN content on later days indicates the lesser requirement of N at crop maturity. Furthermore, its higher amount in the soil of pulses than that of cereal, could be correlated with the fact that the earlier are frequently nodulated by symbiotic N_2 fixers which ultimately contributes to the soil N reservoir. Available potassium (APT)" content of the crops was also found to increase in MP1 treated plants, although, no generalized trend was observed. Orhan et al. (2006) analyzed the effects of PGPR on growth and nutrient contents in organically growing raspberry and observed the significant increase in soil nutrients. Therefore, enhanced N and P content on application of MP1 strain signified its importance in reducing the N and P fertilizers in Himalayan soils.

In the present study, qPCR and PCR-DGGE techniques were explored just to show the effect of MP1 strain inoculation on abundance and composition of native soil bacterial diversity. MP1 strain treatment in all the crops significantly increased the 16S rDNA and *nif*H abundances, calculated on the basis of respective standard curves with slope (0.973 and 0.986) and R^2 value (0.958 and 0.984). In the treated plants of green gram, black gram and finger millet, 16S rDNA abundance was maximum at 45 days; while, in chick pea and pigeon pea, it was maximum at 30 and 60 days, respectively (Figure 3). Untreated controls showed gene abundance maxima at 60 days after sowing in all the crops except black gram, where it was 45 days. Similarly, in the case of *nif*H, abundance was maximum at 30 days after sowing in MP1 treated plants and at 60 days after sowing in untreated controls of each crop. It may be due to the soil NN status which was found to increase after 30 days and therefore, negatively affect the soil diazotrophic

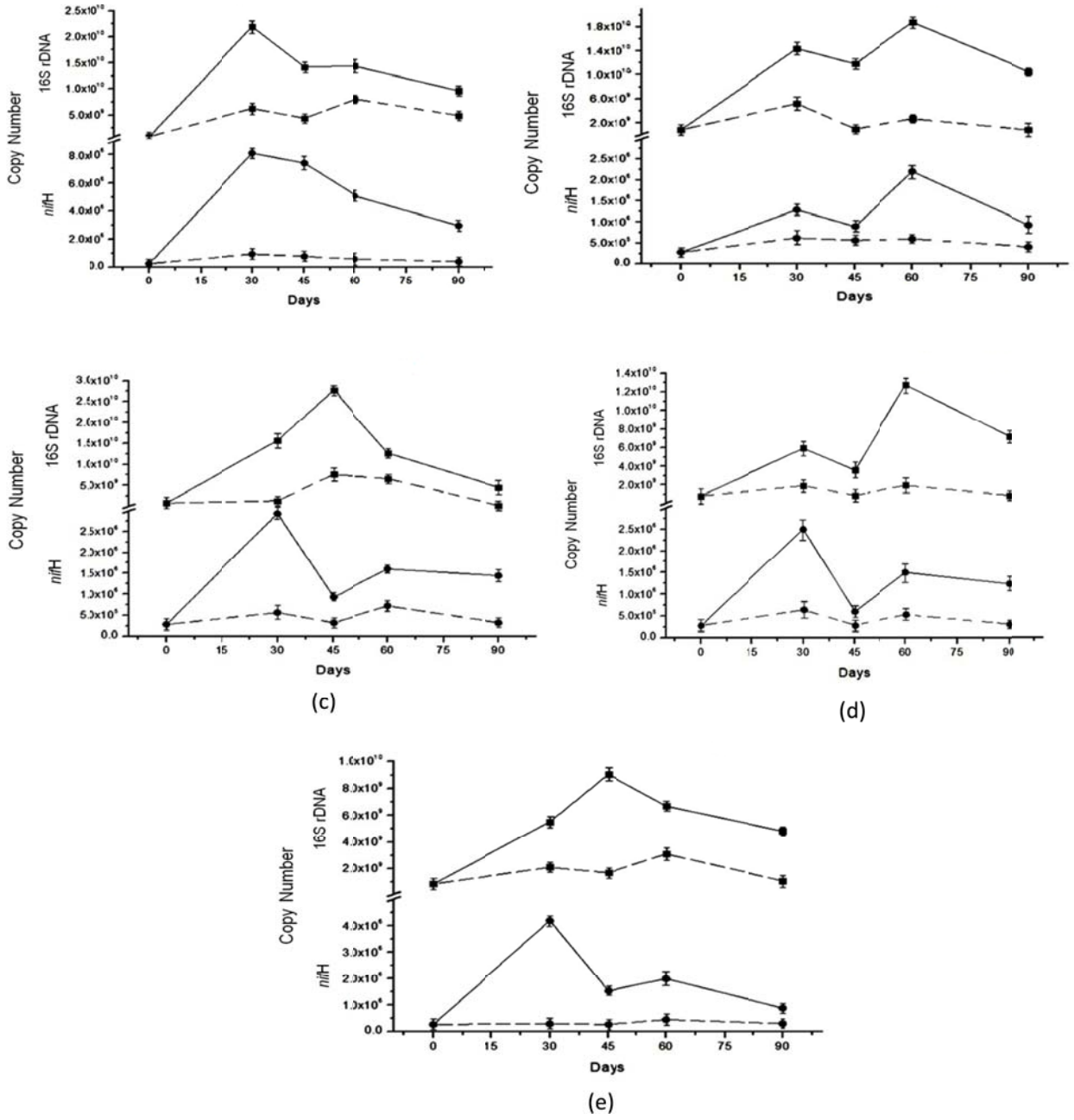

Figure 3. Dynamics of 16S rDNA and *nif*H copy number in the rhizosphere of chickpea (a), black gram (b), green gram (c), pigeon pea (d) and finger millet (e), respectively

population (Parmar and Dufresne, 2011). In all the cases, gene abundances were found to decrease towards harvesting of the crops; yet, greater than their untreated controls. Furthermore, rhizospheric bacterial communities from each crop during different time intervals were compared based on the DGGE patterns of partial 16S rRNA gene amplified using a bacteria-specific primer set. DGGE patterns are shown in Figure 4 along with respective intensity curves (IC), generated by software Quantity One (BioRad). No difference in patterns was observed in sample duplicates (data not shown). The patterns and intensities of bands were mainly affected by number of days after sowing; however, a few bands were influenced by type of crop and bacterial inoculation. Increasing number of peaks in IC revealed the increment in Rhizospheric bacterial communities, significantly after 30 days of sowing except green gram and pigeon pea. It could be attributed to the time required for bacterial adaptation, once adapted, contributes to the diversity.

Another reason could be the soil health. Initially, the

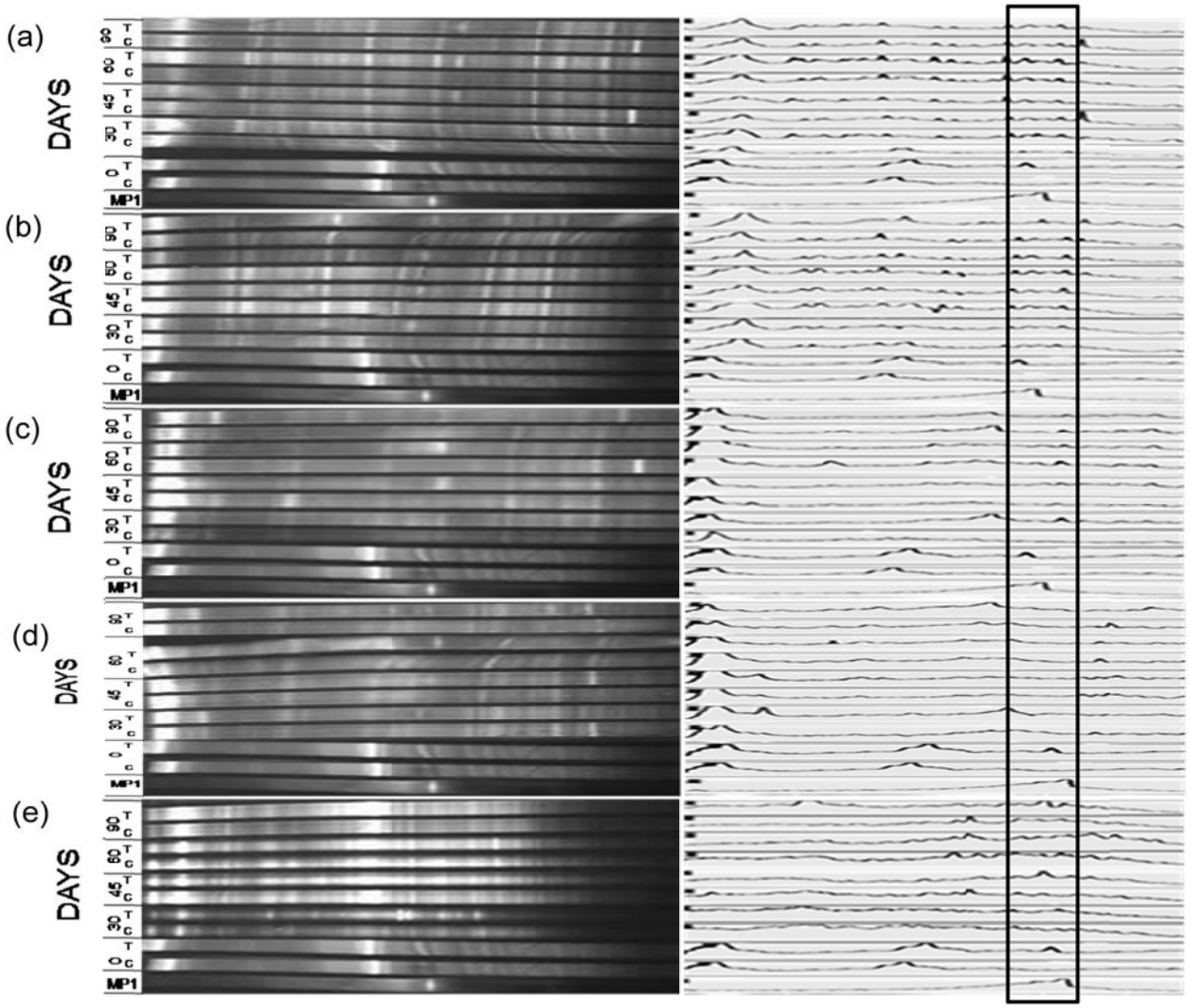

Figure 4. DGGE patterns of partial 16S rDNA of bacterial communities in rhizosphere of five crops *viz*. chickpea (a), black gram (b), green gram (c), pigeon pea (d) and finger millet (e), respectively. C and T are control and treatment, respectively. MP1 represent partial 16S rDNA DGGE pattern of *P. jesenii* strain MP1. Highlighted part indicates the probable persistence of bio-inoculant in respective rhizosphere

nutrient status of the soil was low and therefore, plants restricted its rhizospheric microbial diversity to its minimum; as supported by the fact that at higher diversity the increase in productivity decreases because resources become limiting, resulting in the classic asymptotic diversity-productivity pattern (Schnitzer and Klironomos, 2011). Moreover, during this period, most of the crops require external fertilizers, even nodulating pulses too, due to lack of functional nodules. In this perspective, MP1 strain could be very promising because it was dominant up to 30 days and probably, take care of the plant's growth and development. Once, soil nutrient reservoir increased after 30 days, bacterial diversity was increased and simultaneously, the rate of persistence of MP1 strain was declined, as revealed by aligning its DGGE band position with the crops. Cook et al. (1995) postulated that plants may modulate the rhizosphere microbiome to their benefit by selectively stimulating microorganisms with traits that are beneficial to plant growth and health. The effect of inoculant on soil microbial communities has been studied earlier using qPCR (Babic et al., 2008), ribosomal intergenic spacers analysis (RISA) (Schumpp and Deakin, 2010), DGGE (Herrmann et al., 2012) and other techniques. Trabelsi et al., (2011) revealed that the perturbation of the community due to inoculation with a rhizobial strain is higher than that due to chemical fertilization. It suggests that the introduction of exogenous bacteria in a community is likely to produce more long-term effects than external chemical supply. For future studies, unraveling the rhizosphere microbiome holds

potential to improve crop protection and to uncover numerous yet unknown soil microorganisms, functions and genes for diverse applications.

Conclusion

Application of *P. jesenii* MP1 strain as a bio-inoculant in four important WIH pulses *viz.* chickpea, black gram, green gram and pigeon pea enhanced their growth and respective soil nutrients status. In cereal (finger millet), MP1 strain was not so effective, although better than respective untreated control. Therefore, being a psychrotolerant, *P. jesenii* MP1 strain can withstand extremities of temperature fluctuations and play an important role in growth and yield enhancement of native Himalayan pulses under individual cropping pattern.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Supplementary material

Growth promotory properties

Phosphate solubilization

The bacterial strain was checked for phosphate solubilizing ability on Pikovskaya (PVK) agar medium (Pikovskaya, 1948) incorporated with tricalcium phosphate. Formation of a clear halo zone around the index (S.I.). Formation of a clear halo zone around the

Table SM1. Growth promotory properties of *P. Jesenii* MP1 strain.

S/N	Accession No.	Strain name	Phosphate Solubilization (SI)	IAA Production	Siderophore production
1	JX310329	*Pseudomonas jesenii* strain MP1	+ve (2.54)	+ve	+ve

(+) indicates +ve response; (-) indicates -ve response; SI: solubilization index = colony diameter + halozone diameter/colony diameter (Edi Premono et al., 1996).

Table SM2. Physiochemical characteristics of the soils collected on subsequent days of sowing

Crops		Days	pH	Soil physiological parameters[a]																						
				OC[b]						APH[c]				APT[d]				AN[e]			NN[f]					
				L	ML	M	MH	H	B	L	M	MH	H	L	M	H	VH	L	M	H	VL	L	M	H		
Gram	C[g]	0	7.5			■					■			■				■			■					
		30	8.0				■				■			■				■			■					
		45	9.0		■						■			■				■			■					
		60	8.0	■							■			■				■				■				
		90	8.0	■						■						■		■				■				
	T[h]	0	7.5			■					■			■				■			■					
		30	8.5				■				■					■		■				■				
		45	9.0	■								■			■			■				■				
		60	8.0	■							■				■				■				■			
		90	8.0				■				■						■		■				■			
Black Gram	C[g]	0	7.5			■					■			■				■			■					
		30	8.0			■					■				■			■				■				
		45	9.0	■							■				■			■				■				
		60	8.0	■							■						■	■				■				
		90	7.5	■						■					■			■				■				
	T[h]	0	7.5			■					■			■				■			■					
		30	8.5				■				■				■			■				■				
		45	9.0			■						■			■			■				■				
		60	8.0	■							■						■		■				■			
		90	7.5	■											■				■				■			
Green Gram	C[g]	0	7.5			■					■			■				■			■					
		30	8.0					■			■			■				■			■					
		45	9.0			■					■				■			■				■				
		60	8.0		■						■				■				■				■			
		90	7.5	■						■						■			■				■			
	T[h]	0	7.5			■					■			■				■			■					
		30	8.5				■				■				■			■				■				
		45	9.0	■								■			■			■				■				
		60	8.0		■						■					■				■				■		
		90	7.5				■				■				■					■				■		

phosphate (Ca3(PO4)2) by observing the solubilization bacterial growth after seven days of incubation at 28°C indicates phosphate solubilizing ability. S.I. was calculated on PVK plates by the formula: Solubilization index = Colony diameter + Halozone diameter/Colony diameter (Edi Premono et al., 1996).

Siderophore production

The chrome azurolsulfonate (CAS) assay (Schwyn and Neilands, 1987) was used for screening siderophores production, since; it is comprehensive, exceptionally responsive, and most convenient. For the qualitative assay, MP1 strain was spot inoculated onto the blue agar and incubated at 28°C for 24 to 48 h. The results were interpreted based on the colour change due to transfer of the ferric ions from its intense blue complex to the siderophore. The sizes of yellow orange haloes around the growth indicated siderophore activity.

IAA production

For qualitative estimation of IAA production, Tryptone soy broth is used. Tryptone soy broth (5.0 ml) tubes with and without tryptophan (200 µl/ml) were inoculated with loopful of actively growing bacterial cultures aseptically and incubated for 48 h at 28°C under shaking conditions. Cultures were centrifuged at 10,000 rpm for 10 min. 2 ml of Salkowski reagent was added in 1 ml supernatant. The mixture was incubated at 28°C for 25 min. Development of pink colour shows IAA production.