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Indigenous plant growth promoting rhizobacteria: potential green alternative for capsicum productivity under mid-hill conditions of north-western Himalayan region

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ABSTRACT

Plant growth promoting (PGP) potential of rhizospheric and endophytic bacterial isolates of capsicum (*Capsicum annuum* L) obtained from different agro-climatic zones of Himachal Pradesh was studied. Total of 10 isolates (RS_1-RS_{10}) were morphologically and physiologically characterized and tested for multifarious PGP traits viz phosphate solubilization, auxin and siderophore production. Nutrient broths having 1.5 OD at 540 nm were used as inoculum and 4 hours as seed imbibition time period. Only six isolates (RS_2, RS_3, RS_4, RS_7, RS_8, RS_{10}) possessing maximum of PGP traits were evaluated for seed germination and seedling growth studies under controlled conditions. Isolates were tried singly as seed inoculation in Completely Randomized Block Design replicated thrice. All the selected isolates were Gram positive rod, differed in colony morphology, showed optimum growth at 35°C and 7 pH and possessed various PGP traits. Maximum seed germination (84.4%), increase in shoot length (41.54%), shoot biomass (41.46%), root length (69.64%), root biomass (42.85%) over un-inoculated control were noted for RS_7 isolate inoculated seeds. The RS_2 isolate inoculation in general had the similar effect of studied plant parameter. Present investigations focus on potential use of isolate RS_7 (*Bacillus* spp) and RS_2 (*B subtilis*) as biofertilizer for capsicum under north-western Himalayan region.

Keywords: Capsicum; PGPR; IAA; HCN; inoculation

INTRODUCTION

Capsicum (*Capsicum annuum* L) occupies a place of pride among vegetables in Indian cuisine because of its delicacy and flavour occupied with rich content of ascorbic acid and other vitamins and minerals. Capsicum is the most popular and highly remunerative, commercial vegetable crop in the world. India is the second largest producer of vegetables in the world and capsicum occupies an area of 9,630 hectares with annual production of 1,27,480 metric tons (Anon 2012).

Himachal Pradesh has about 50 per cent share in the country's area and production of capsicum.

In order to meet the growing demand of burgeoning population large amount of herbicides, pesticides and fertilizers are being applied to the fields every year to achieve maximum production. This indiscriminate use of chemical inputs has deleterious effect on agro-ecosystem as well as productivity of the crop.

Plant growth promoting rhizobacteria (PGPR) are recognized as an important factor in sustainable agricultural production (Mrkovacki and Bjelic 2011). PGPR refers to the sub-set (2-5%) of soil and rhizosphere bacteria colonizing the roots in the competitive environment and causing unapparent and asymptomatic infections in plants. Growth promotion by synthesis of some phytoharmones, production of siderophores and disease control by antibiotics, hydrogen cyanide (HCN) and volatile compounds production is an added advantage of these bacteria (Lugtenberg et al 2002).

The application of eco-friendly, non-bulky, beneficial soil organisms that could enhance the plant growth and crop yields by direct or indirect modes of action could be a potential alternative to increase agricultural productivity in a sustainable manner. Therefore the present investigations were undertaken to isolate, screen and characterise the PGPR strains from roots and rhizosphere of bell pepper under mid-hill conditions of Himachal Pradesh falling in north-western Himalayan region.

MATERIAL and METHODS

Isolation of rhizospheric and endophytic bacterial isolates

Capsicum roots along with the adhered soil were collected from different locations of Shimla (Taradevi and Theog) and Sirmour (Rajgarh and Lanacheta) districts representing the mid-hill conditions of the state. Bacterial isolates persisting in the rhizospheric soil samples and roots were obtained by serial dilution and plate count technique by using nutrient agar medium. Enumeration on Jensen's and Pikovskaya's medium (PVK) was done using replica plate technique. Ten most predominant and morphological distinct isolates that were able to form clear halo zone on the PVK media and grow on Jensen's media were selected for screening and were designated as RS_1 to RS_{10}.

Characterization and screening of PGPR isolates

Morphological and physiological characteristics of the isolates were performed by following standard microbiological procedures. Phosphate solubilization capacity of the isolates was estimated using Pikovskaya's medium (Pikovskaya 1944). The production of indole acetic acid (IAA) siderophore was assessed following the methods of Gorden and Palleg (1957) and Schwyn and Neilands [(1987).

Standardization of inoculum density and imbibition time

Seeds of capsicum (*Capsicum annuum* L) variety California Wonder were procured from Seed Technology and Production Centre of Dr YS Parmar University of Horticulture and Forestry Nauni, Solan, HP. Capsicum seeds were surface sterilized in 0.2 per cent mercuric chloride ($HgCl_2$) solution for two minutes and rinsed 6-8 times with sterilized distilled water. The cell suspension of population density i.e 0.5, 1.0 and 1.5 at 540 nm was prepared from 72 h old culture medium in nutrient broth for different bacterial isolates and data on viable counts were recorded. The population density that resulted in formation of 10^8 cfu/ml of bacterial isolate was used as culture broth for seed inoculation. 100 mg air dried capsicum seeds were soaked in the culture broth. After every hour seeds samples were taken, dried at room temperature for one hour and weighed. Per cent seed weight increment over initial was calculated and imbibition time was noted for which there was no seed weight increment afterwards.

Growth chamber studies

Among ten only six isolates (RS_2, RS_3, RS_4, RS_7, RS_8 and RS_{10}) possessing maximum PGP traits were further evaluated in growth chamber studies. 25 sterilized seeds were soaked in individual culture broth (cell density about 10^8 cfu/ml) for 4 hours. Seeds without treatment with any isolate were designated as control; however they were kept in sterilized water for the same time. Thereafter seeds were sown at equidistance at a uniform depth of 2 cm in sterilized vermiculite moistened with Hoagland's nutrient solution in a pot having 25 g of vermiculite. After 3-4 days of seedling emergence thinning was done and 3 plants/pot was maintained. Plant growth response was observed 45 days after sowing in plant growth chamber (25°C, 14/10 h light/dark, 75% humidity) following standard procedures. All treatments were tried in triplicate under completely randomized block design.

RESULTS and DISCUSSION

Isolation of rhizospheric bacterial isolates

The microbial counts in rhizosphere and plant roots varied significantly with location (Table 1). The total microbial counts in general were more in rhizosphere (94.67 to 84.33 × 10^5 cfu/g soil) as compared to endophytic count (86.00 to 70.33 × 10^2 cfu/g root). This variation in rhizosphere and roots may be attributed to positive influence exerted by root exudates, environmental conditions, age of plant, variety/cultivar type, time of sampling and physico-chemical properties of soil (Wieland et al 2011).

Table 1. Endophytic and rhizospheric bacterial population associated with capsicum plants

Location	Site	Rhizospheric microbial count ($\times 10^5$ cfu /g soil)			Endophytic microbial count ($\times 10^2$ cfu/g root)		
		NA	JM	PVK	NA	JM	PVK
Shimla	Taradevi	84.33	30.67	68.67	70.33	30.33	61.67
	Theog	86.67	40.67	79.00	75.67	34.67	56.67
Sirmour	Rajgarh	94.67	42.33	84.00	86.00	44.33	71.67
	Lanacheta	89.33	33.33	74.67	83.67	36.67	58.00
$CD_{0.05}$		1.08	1.09	1.54	1.33	1.09	1.32

Characterization and screening of PGPR isolates for multifarious PGP traits

Morphological and physiological characteristics for each isolate are listed in Table 2. The selected 10 isolates showed heterogeneity with regard to colony morphology. The isolates grew at a wider pH and temperature range ie from 4-8 and 10°C-45°C respectively however showed the optimum growth at 35°C and 7.0 pH. Large proportion of phosphorus in soil is insoluble and hence unavailable to plants. Free-living P-solubilizing bacteria such as *Bacillus* and *Pseudomonas* are able to solubilize the insoluble phosphates in soil and so contribute to increase available phosphate for the plants (Gopalakrishnan et al 2011). All the isolates were phosphorus, auxin and siderophore producers (Fig 1). The PGPR isolates (RS_1-RS_{10}) showed significant variation in phosphate solubilization ranging from 44.4 to 80.0 per cent. Similar release of phosphate by different isolates has been reported by Suresh et al (2010). IAA functions as important signal molecule in regulation of plant development. Further the role of siderophores in control of diseases has been well documented by Bakker and Schippers (1987). The auxin and siderophore production ranged from 29.67 to 17.70 µg/ml and 24.4 to 83.3 per cent respectively. The amount of IAA and siderophore is comparable with the results reported by Jarak et al (2012) for *Bacillus* and *Pseudomonas* spp. The isolates in this study presented several enviable features for PGPR which suggests their potential for growth promotion.

Standardization of inoculum density and imbibition time

The data on viable count (Table 3) reveal a gradual increase in number of viable cells of bacterial isolates with increase in optical density (OD) from 0.5 to 1.50 at 540 nm. The maximum (238.67 x 10^8 cfu/

Table 2. Morphological and pysiological characteristics of rhizospheric and endophytic bacterial isolates of capsicum plants

Characteristic	Isolate									
	RS_1	RS_2	RS_3	RS_4	RS_5	RS_6	RS_7	RS_8	RS_9	RS_{10}
Morphological										
Colour	White	White	Greyish	White	White	Greyish	White	White	Greyish -white	White
Shape	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round
Form	Irregular	Irregular	Circular	Irregular	Circular	Punctiform	Circular	Irregular	Circular	Irregular
Size (mm)	1.0-1.5	0.9-1.1	1.9-2.0	1.5-2.0	1.0-1.5	0.9-1.1	09-1.1	0.5-1.1	0.2-0.5	0.9-1.1
Surface	Smooth	Smooth	Smooth	Rough	Rough	Smooth	Rough	Smooth	Smooth	Smooth
Density	Translucent	Opaque	Opaque	Translucent	Opaque	Opaque	Opaque	Translucent	Opaque	Translucent
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Gram's test	-	+	+	+	+	+	-	-	+	-
Physiological										
	Growth at different temperatures									
10 °C	+	+	+	+	+	+	+	+	+	+
20 °C	+	+	+	+	+	+	+	+	+	+
30 °C	+	+	+	+	+	+	+	+	+	+
35 °C	+	+	+	+	+	+	+	+	+	+
37 °C	+	+	+	+	+	+	+	+	+	+
40 °C	+	+	+	+	+	+	+	+	+	+
47 °C	-	-	-	-	-	-	-	-	-	-
	Growth at different pH									
5	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+
9	-	-	-	-	-	-	-	-	-	-

Table 3. Effect of cell density on viable count of different bacterial isolates

Isolate	# viable cells (x 10^8 cfu/ml)			Mean
	0.50	1.00	1.50	
C	0.00	0.00	0.00	0.00
RS_2	156.33	217.00	240.00	204.44
RS_3	141.33	203.00	218.00	187.44
RS_4	145.00	189.00	222.00	185.33
RS_7	160.00	222.33	238.67	207.00
RS_8	146.00	204.00	227.00	192.33
RS_{10}	135.00	195.00	213.33	181.11
Mean	126.24	175.76	194.14	

$CD_{0.05}$ (T)= 1.66, $CD_{0.05}$ (I)=1.08, $CD_{0.05}$ (T × I)= 2.87

ml) number of viable cells was recorded for RS_7 isolate at 1.50 OD at 540 nm after 72 hours of incubation. Maximum (117%) seed weight increment was noted after four hours of imbibition (Fig 2). Therefore bacterial broths having 1.5 OD at 540 nm were used as inoculum and 4 hours as imbibition time period.

Growth chamber studies

Plant growth promotion is crucial in early developmental stages such as germination and seedling stage (Gholami et al 2009). The selected six isolates (RS_2, RS_3, RS_4, RS_7, RS_8, RS_{10}) significantly increased the seedling growth over control (Table 4). The isolates increased the seed germination by 20 to 27 per cent over control. The maximum seed germination (84.4%) was recorded with RS_7 isolate; however it was statistically at par with RS_2, RS_3 and RS_4. Similarly maximum vigour index (1025.9) was also recorded for RS_7 isolate inoculated seeds. Enhanced cell division, cell enlargement, development of greater amount of fine roots and ultimately the better plant stands may be attributed to enhanced availability and thereof absorption of essential nutrients particularly nitrogen, phosphorus and iron by plant. Sakthivel and Karthikeyan (2009) also reported improved seed germination and vigour index of tomato with *Pseudomonas fluorescens* inoculation over uninoculated control. All selected bacterial isolates registered significant increase in shoot/root length and biomass over control. Maximum shoot length (6.5 cm) was recorded for RS_7

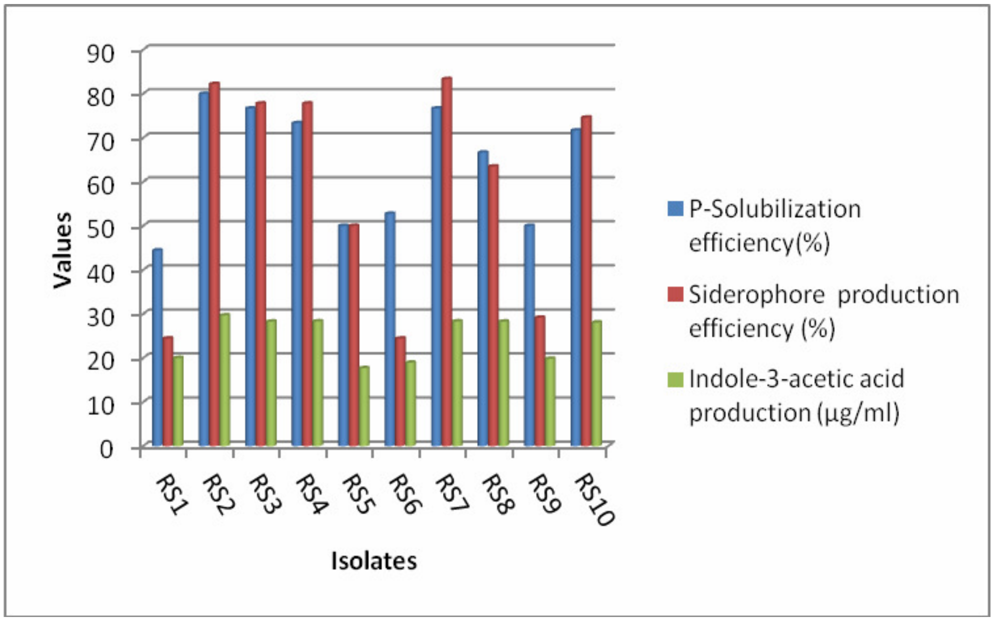

Fig 1. **Phosphate solubilization, siderophore and Indole-3-acetic acid production efficiency of various isolates obtained from roots and rhizosphere of capsicum**

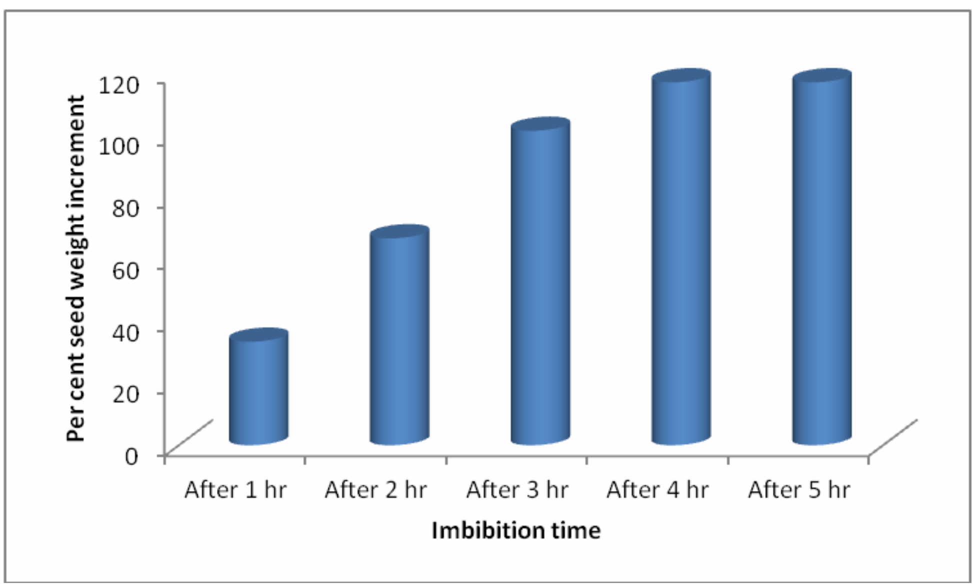

Fig 2. Standardisation of imbibition time for seed treatment

Table 4. Effect of selected bacterial inoculum on the growth parameters of capsicum seedlings

Isolate	Germination (%)	Vigour Index	Shoot		Root	
			length (cm)	biomass (mg/plant)	length (cm)	biomass (mg/plant)
C	57.8^c	320.8^e	3.8^d	45.9^c	1.7^d	3.2^d
RS_2	81.7^a	929.1^b	6.2^a	72.3^a	5.2^a	5.2^a
RS_3	77.8^a	731.8^c	5.4^b	62.3^b	4.0^b	4.8^b
RS_4	80.0^a	679.3^c	5.1^b	61.6^b	3.4^b	4.4^b
RS_7	84.4^a	1025.9^a	6.5^a	78.4^a	5.6^a	5.6^a
RS_8	74.4^b	507.6^d	4.2^c	50.2^c	2.6^c	3.6^c
RS_{10}	69.4^b	506.8^d	4.5^c	51.5^c	2.8^c	3.8^c
$CD_{0.05}$	7.6	92.6	0.5	6.3	0.6	0.5

isolate which was statistically at par with isolate RS_2 (6.2 cm). Similarly maximum shoot biomass (78.40 mg/plant) was also noted for RS_7 isolate however was statistically at par with RS_2 isolate (72.3 mg/plant). Maximum root length (5.6 cm) and root biomass (5.6 mg/plant) were also recorded for RS_7 which however were statistically at par with RS_2 isolate. Similar increased seedling height and seedling biomass with bacterial isolates over control were recorded by Suryanto et al (2010). The isolates (RS_7 and RS_2) in this study presented several enviable features for PGPR which suggests their use as potential bio-fertilizers for sustained crop production of capsicum under mid hill conditions of HP.

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