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Plant growth promoting rhizobacteria enhance growth and yield of chilli (*Capsicum annuum* L.) under field conditions

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Abstract

Plant growth promoting rhizobacteria (PGPR) can enhance the growth and productivity by exerting beneficial effects through direct and indirect mechanisms. The effect of PGPR on the growth and yield of chilli under field conditions has to date, not been substantiated. In this study, 15 bacteria were isolated from chilli rhizosphere and their morphological, biochemical, plant growth-promoting, and biocontrol characteristics were elucidated. Plant growth and yield attributes increased significantly when the 15 rhizospheric isolates were applied to a local chilli cultivar 'Suryamukhi' in pots. On the basis of their performance in the pot experiment, three rhizobacteria (C2, C25, and C32) were selected for further study in field. The 16S rDNA sequencing has identified C2 and C25 strains as *Bacillus* spp. and C32 strain as *Streptomyces* sp. Remarkable increase in growth characteristics such as total number of fruits, fruit-weight, and yield was recorded in plants with combined inoculation under field conditions. The results clearly demonstrate the rhizocompetence and plant growth enhancing efficacy of these strains. It can be surmised that the isolated strains have strong potential to be successful biofertilizers and bioenhacers.

Keywords: Chilli, plant growth promoting rhizobacteria, growth, yield, biofertilizer.
Abbreviations: PGPR (plant growth promoting rhizobacteria), IAA (indole acetic acid), HCN (hydrocyanic acid).

Introduction

Chilli, the fruit of *Capsicum annuum* L, is one of the most important commercial crops in India. With an annual production of 1.1 million tones, India is the largest producer of chilli in the world (Khan and Raj, 2006). Owing to its high cash value and consumption rate the annual trade of chilli is approximately 17% of total spice trade in the world (Ahmed et al., 2000) and is about 33% in India. However, the yield of chilli in India is substantially low when the large area (930,000 hectares) of production is considered (Bharathi et al., 2004). A large amount of herbicides, pesticides, and fertilizers is applied every year to achieve maximum productivity of chilli and to meet the growing demand, the use of chemical fertilizers in India has increased 170 times in last 50 years (FAO, 2010). This is a major environmental and health concern considering the deleterious impact of these chemical compounds on terrestrial and aquatic ecosystems. Plant growth promoting rhizobacteria (PGPR) constitute approximately 2-5% of the total rhizomicrobial population (Antoun and Kleopper, 2001; Kloepper et al. 1980). Evidence of the beneficial effects of PGPR has been accumulating for the past 150 years (Berg, 2009). PGPR have been demonstrated to increase growth and productivity of many commercial crops including rice (Ashrafuzzaman et al., 2009), wheat (Khalid et al., 2004), cucumber (Maleki et al., 2010), maize (Sandhya et al., 2010), cotton (Anjum et al., 2007), black pepper (Dastager et al., 2010), and banana (Mia et al., 2010). However, a few studies have isolated and characterized the PGPR and phosphate solubilizing bacteria from chilli rhizosphere (Ponmurugan and Gopi, 2006), the effect of PGPR on chilli growth and productivity under field conditions has hitherto not been investigated. Co-inoculation of PGPR has been demonstrated as a sustainable approach in plant health management. Prudent application of binary or multiple mixtures of PGPR inoculants can expand the spectrum of biocontrol activity (Felici et al., 2008). Therefore, individual and combined effects of PGPR on the yield and productivity of host plant should also be assessed. In this study, PGPR from chilli rhizosphere was isolated and characterized, and their effect on the growth and yield of chilli plants in pot and field experiments was evaluated.

Materials and methods

Isolation and characterization of PGPR strains

Soil samples adhered to chilli roots were collected from four local farmer's field in Kalyani, India (22°59′N, 88°28′E). Bacterial strains persisting in the rhizospheric soil samples were obtained by dilution plate count technique using four different media: Plate Count Agar,Yeast Mannitol Agar, Thronton's Medium, and a synthetic medium (NH_4Cl 5.0 g; K_2HPO_4 3.0 g; Na_2SO_4 2.0 g; KH_2PO_4 1.0 g; NH_4NO_3 1.0 g; $MgSO_4,7H_2O$ 0.1 g; glucose 2.0 g; distilled water 1 litre; pH 7.0±0.2). Biochemical characterization of the isolates was performed following standard microbiological procedures. The production of ammonia and indole acetic acid (IAA) was determined by the method of Dye (1962) and Gordon and

Table 1. Colony morphological characteristics of the isolated plant growth promoting rhizobacteria

Name of isolate	Gram test	Colony characters				
		Colour	Form	Margin	Elevation	Density
C2	+	White	Irregular	Erose	Raised	Opaque
C4	+	White	Irregular	Erose	Flat	Opaque
C6	+	White	Irregular	Lobate	Flat	Opaque
C9	+	White	Irregular	Lobate	Raised	Opaque
C11	+	White	Circular	Erose	Raised	Opaque
C19	+	White	Irregular	Lobate	Flat	Translucent
C25	+	Greyish-white	Circular	Entire	Pulvinate	Opaque
C28	+	White	Irregular	Filamentous	Flat	Opaque
C29	+	Glistering	Circular	Entire	Flat	Opaque
C30	-	White	Spindle	Entire	Raised	Opaque
C31	-	Glistering	Circular	Entire	Flat	Translucent
C26	+	White	Spindle	Entire	Flat	Opaque
C32	+	White	Irregular	Curled	Flat	Opaque
C34	+	White	Circular	Erose	Pulvinate	Opaque
C36	-	White	Circular	Undulate	Flat	Translucent

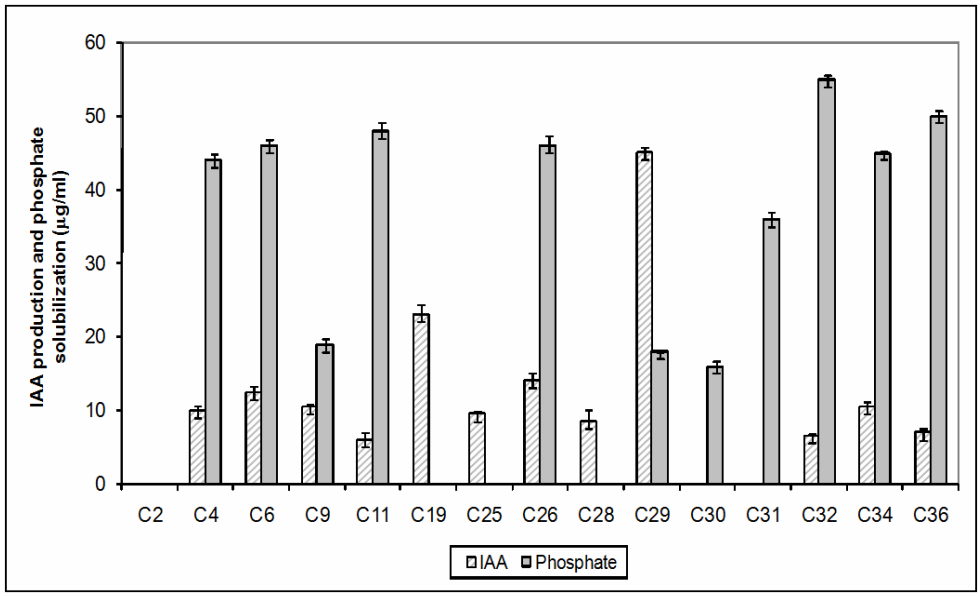

Fig 1. Indole acetic acid production and phosphate solubilization activities of various isolates obtained from chilli rhizosphere. Absence of bar indicates no activities.

Weber (1951) respectively. The biocontrol potential of the bacterial isolates was ascertained by introducing to virulent phytopathogenic strains of *Xanthomonas* sp., *Pseudomonas* sp. and *Fusarium* sp. The interaction was performed on agar plate in aseptic condition initially by streak methods followed by cup method on petriplates. Phosphate solubilizing capacity of the isolates was estimated using Pikovskaya's medium (Pikovskaya, 1948). The ability of the isolates to produce siderophore and hydrocyanic acid (HCN) was assessed following the methods of Schwyn and Neilands (1987) and Bakker and Schipper (1987) respectively.

Pot experiment

Chilli seeds of a local cultivar, popularly known as 'Suryamukhi', were separately sown in a seed bed and regularly maintained. After 45 days of sowing, seedlings were carefully uprooted and the root portion was dipped into individual culture broth (cell density about 10^6cells/ml) for 30 minutes. Pots (14 inch diameter) were prepared with fine soil and sand. The seedlings were subsequently transferred into pots and allowed to grow for 135 days. The plant-height (cm), canopy-width (cm), number of fruits per plant, fruit-weight (g), fruit-length (cm), fruit-width (cm), number of seeds, and yield were recorded. Three rhizobacteria (C2, C25, and C32) were selected for further study in field on the basis of their performance in the pot experiment. Based on 16S rDNA sequencing (using 338f and 518r primers; Ovreas et al., 1997), the PGPR strains C2 and C25 was identified as *Bacillus* spp. and the C32 strain was identified as *Streptomyces* sp (NICED, India).

Field experiment

The field experiment was carried out during the autumn-winter season. The research site predominantly comprised of sandy loam textured soil with a pH of 6.5. The soil has good fertility status and drainage facility. The antagonistic effects

Table 2. Plant growth promoting and biocontrol activities of various isolates obtained from chilli rhizosphere

Characteristics	Isolates from chilli rhizosphere														
	C2	C4	C6	C9	C11	C19	C25	C26	C28	C29	C30	C31	C32	C34	C36
Biochemical															
Amylase production	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
Catalse production	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+
Urease production	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-
Gelatin Hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl Red test	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate utilization	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+
Biocontrol and PGPR characteristics															
Siderophore production	+	+		+		+	+		+	+		+	+	+	
HCN Production	+									+			+		
Ammonia production	+			+				+		+				+	
Biocontrol to Xanthomonas	+		+	+	+	+	+	+	+		+	+	+	+	+
Biocontrol to Fusarium	+	+	+		+	+	+		+		+		+		
Biocontrol to Pseudomonas	+										+	+			+

of the isolates with each other were checked before starting this experiment. Seedlings were treated with C2, C25 and C32 strains, either alone or in combinations, along with two controls (water and medium). Nine treatment combinations were laid out in a randomized block design with three replications. Individual plot size was 1.5 m x 2 m with a plant to plant and row to row spacing of 30 cm x 60 cm respectively, accommodating 35 plants per plot. In between the plots irrigation and drainage channels were made alternatively. Ten plants of each plot were randomly selected for recording observations in each replication. The mean value was used for statistical analysis. Data were recorded on the following parameters: plant-height (cm), canopy-width (cm), total number of fruits per plant, average weight of fruit (g), fruit-length (cm), fruit-width (cm), number of seeds per fruit, and fruit yield per plant (g). The data obtained were statistically analyzed by analysis of variance following the methods of Panse and Sukhatme, (1985) and Gomez and Gomez (1984).

Results

The bacterial population in chilli rhizosphere did not show any heterogeneity with regard to colony morphology and Gram nature (Table 1). Chilli rhizosphere was dominated by Gram positive bacterial population with white, irregular, opaque colonies. Majority of the bacterial isolates were able to produce amylase and catalase enzymes and showed negative response to other biochemical tests (Table 2; Fig 1). Furthermore, the isolates had at least four plant growth promoting and biocontrol characteristics, and among fifteen bacterial isolates, C2 and C32 performed consistently. Albeit the selected 15 isolates showed promising plant growth promoting and biocontrol activities in laboratory but their direct effects on the host plant also need to be substantiated. For this purpose, these isolates were applied to a local cultivar 'Suryamukhi' (Table 3). The height of plant after 90 days of transplanting increased significantly with rhizospheric isolates C2, C4, C9, C11, C19, C25, C30, C32, and C34 as compared to control set whereas isolates C36, C11, C19, C2, C32, and C25 significantly enhanced the canopy width. The total number of fruits per plant and fruit-weight were significantly higher in the majority of PGPR treated plants and the plants treated with C2 showed highest fruit-weight and total number of fruits per plant. Bacterial strains C28 and C32 produced the highest fruit length (6.86 cm), however, the fruit width was not noticeably influenced by the treatments. The plants treated with C2 and C25 strains produced the highest number of seeds per fruit. Overall, the results demonstrate that the isolates C2, C25 and C32 were better performers than the others and hence were selected for further experimentation. The maximum plant height recorded was 54.17 cm in the combined treatment of PGPR C2 and C32 and the next best height was 46.12 cm for the combination of C25 and C32 (Table 4). However, individual treatment did not generate any significant effect on plant height. Number of fruits per plant is an important yield attribute and the C2 and C32 combination critically excelled over all other treatments in producing significantly higher number of fruits per plant (129.77). It is immediately followed by the C2 treatment and the combination of C2, C32 and C25 (82.36). Fruit weight is one of the main yield components because of its direct impact on yield. The weight of fruits for combination of C2 and C32 was significantly different from all other treatments and produced the highest weight of fruit (2.92 g). The combined treatment of isolates C2, C32 and C25 and C2 treatment alone also performed superiorly, having no significant difference there of. However, the treatments had no effect on the length and width of fruits in Suryamukhi cultivar. Yield is the main criterion for any crop and the combined treatment of C2 and C32 showed the maximum yield per plant.

Discussion

Similar to Antoun et al. (1998) who observed that out of 266 strains of rhizobia 54% was able to solubilize insoluble phosphates while 58% and 83% produced IAA and siderophores respectively, it was found that the majority of the rhizospheric isolates regularly produced IAA and siderophores and solubilized tricalcium phosphate. Studies performing isolation and characterization of PGPR from chilli rhizosphere are extremely limited. The amount of IAA

Table 3. Effect of all bacterial isolates on growth and yield characteristics of chilli in pot experiment

Treatments[a]	Average plant height (cm)	Plant canopy width (cm)	Total number of fruits/ plant	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Number of seeds
C2	44.5[b]	65.25	64.75	2.23	6.56	0.48	53.75
C4	43.25	50	48.75	1.86	4.33	0.62	44.5
C6	40.25	50.75	50	1.86	5.9	0.48	43.75
C9	42	49.25	43.5	1.79	5.93	0.505	42
C11	45.5	78.25	46.5	1.25	5.23	0.47	32.5
C19	46.75	70.25	50.75	1.66	5.67	0.565	37.5
C25	44	65.75	54.25	2.19	6.64	0.455	53.75
C26	40.5	58	42.25	1.72	6.5	0.55	40.25
C28	35.75	54.75	44.25	1.91	6.86	0.46	41
C29	36.75	50.25	40.25	1.55	5.83	0.57	32.5
C30	42.25	54.25	44.5	1.88	5.50	0.57	44.75
C31	40	52.25	41.5	1.57	4.5	0.49	34.75
C32	45.5	68.25	52.75	2.10	5.83	0.48	52.5
C34	45.5	54.75	50.25	1.90	5.10	0.6	40.25
C36	38.25	62.5	46	1.78	5.03	0.43	38
Medium control	36.25	48.5	40.25	1.38	5.03	0.575	31.5
Water control	34	47.25	39.25	1.27	5.12	0.58	27.5
Standard error of mean (±	3.73	4.54	2.06	0.06	0.56	0.04	4.07
Critical Difference at 5%	7.5	9.12	4.15	0.12	1.13	0.08	8.18

[a]Name of the isolates [b]Average of four pots with one plant each

Table 4. Effect of three bacterial isolates (C2, C25 and C32) alone or in combination on growth and yield parameters of chilli under field conditions.

Treatments	Plant height (cm)	Plant canopy width (cm)	Total number of fruits/plants	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Number of seeds	Yield/ plant (g)
C2	37.40[a]	47.78	85.42	2.56	6.31	0.49	57.86	223.33
C32	38.27	51.67	39.79	2.46	5.61	0.46	56.00	103.33
C25	33.00	49.14	67.58	2.09	6.37	0.47	48.83	143.33
C2+C32	54.17	50.22	129.77	2.92	6.86	0.61	63.33	386.67
C2+C25	43.14	57.22	53.44	2.14	6.25	0.47	49.83	116.67
C25+C32	46.12	65.44	49.78	2.33	5.77	0.56	50.67	120
C2+C25+C32	38.43	64.11	82.36	2.57	5.91	0.50	57.00	216.67
Medium control	36.34	43.33	40.58	1.52	4.81	0.57	32.17	65
Water control	31.76	42.66	60.55	1.85	4.85	0.59	40.83	116.67
Standard error of mean (±)	2.28	9.15	5.02	0.2	1.2	0.05	3.87	16.71
Critical Difference at 5%	3.98	NS	8.76	0.35	NS[b]	NS	6.76	29.18

[a]Mean of three replicates of ten plants, [b]NS- non significant

and solubilized phosphate by the isolated strains in this study is comparable to results reported by Ponmurugan and Gopi (2006). However, the rate of phosphate solubilization was considerably low when compared to Banerjee et al. (2010) who isolated stress tolerant phosphate solubilizing bacteria from similar soils in Kalyani.Plant growth promoting and biocontrol activities of rhizobacteria have been reported by numerous studies in last three decades (Cattelan et al., 1999; Kloepper et al., 1980; Mia et al., 2010), however, the isolated strains have seldom been applied to elevate the growth and yield of host pants. The application has also been limited due to inconsistency in results of laboratory, greenhouse and field studies (Mishustin and Naumova, 1962). Production of IAA and soluble phosphate are the most common mechanisms of action implicated in PGPR and indeed microbes demonstrating these attributes are widespread in rhizosphere (Vessey et al., 2003). Nonetheless, an isolate producing considerable amount of IAA and solubilized phosphate in laboratory may not exhibit these attributes under field conditions and conversely, some PGPR may not produce soluble phosphate or IAA in the laboratory but can extensively stimulate the growth and yield of the host plant in field. Several mechanisms of action have been proposed for PGPR increasing nutrient level, nitrogen fixation, increasing beneficial symbioses, enhancing root surface area, and combination of multiple modes; IAA and soluble phosphate production are among those several mechanisms of action and a single PGPR may demonstrate several modes of action (Vessey, 2003). Cattelan et al. (1999) observed that only two of five rhizospheric isolates able to solubilize phosphate actually showed a positive effect on soybean seedling growth. Resembling this, the C2 and C25 strains in this study, despite being inconsistent IAA and soluble phosphate producers, substantially increased growth and productivity of chilli in pots and in the field. Performance of a PGPR under field conditions is the central criterion of selection as a commercial biofertilizer. Soil is a highly heterogeneous and unpredictable environment and anticipated results are often difficult to achieve (Bashan, 1998; Lucy et al., 2004). Climatic variability, soil type, soil fertility level, soil moisture content, and the number of PGPR cells applied to the plant are the major determinants of the effectiveness of PGPR under field conditions (Lucy et al., 2004). Furthermore, isolated rhizobacterial strains may explicitly stimulate growth only in certain crops.

Role of *Bacillus* spp. as successful PGPR has been well investigated, however, application of *Streptomyces* spp. as PGPR is considerably uncommon (for a comprehensive list see Chet and Chermin, 2002; Glick et al., 1999). Combined or mixed inoculants that interact synergistically are currently popular as biofertilizer and bioenhancer. The synergistic associations among the rhizobacterial inoculants can play a critical role in alleviating inhibitory products, providing nutrients, and in stimulating each other via physical or biochemical activities that may increase the beneficial attributes of their physiology (Bashan, 1998). Son et al. (2006) found that combined treatment of bradyrhizobia (*Bradyrhizobium japonicum*) and pseudomonads *(Pseudomonas* spp.) increased the number of pods bearing 3 seeds on soybean. The three selected PGPR viz. C2 (*Bacillus* sp.), C25 (*Bacillus* sp.) and C32 (*Streptomyces* sp.) were applied either alone or in all possible combination in pots as well as in field conditions. The combination of isolate C2 and C32 showed remarkable performance in various growth and yield parameters in both pot and field conditions. The consistent performance of these PGPR strains *Bacillus* spp. and *Streptomyces* sp. indicates their potential to be used as commercial biofertilizer for the enhancement of growth and yield of chilli in India. The combination of rhizospheric isolates C2 and C32 is particularly recommended for maximum yield in chilli.

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