

Bacillus sonorensis, a Novel Plant Growth Promoting Rhizobacterium in Improving Growth, Nutrition and Yield of Chilly (*Capsicum annuum* L.)

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Abstract Plant growth promoting rhizomicroorganisms (PGPR) play an important role in improving plant growth, nutrition and yield of different crops. Chilly is one of the major commercial crops of India with large export potential. The first experiment was conducted with 10 PGPR to investigate their effects on growth and yield of chilly. Although most of the plant parameters studied were statistically not significant, 2 PGPR viz. *Paenibacillus polymyxa* and *Pantoea dispersa* showed agronomic improvement in plant growth as compared to control and other treatments. The second experiment was conducted with these 2 PGPR plus 8 more PGPR in order to select the best PGPR for inoculating chilly. Inoculation significantly improved the growth, nutrition and fruit yield as compared to uninoculated control. Considering plant dry biomass and fruit yield, *Methylobacterium radiotolerans* proved to be the best PGPR. Further screening with *M. radiotolerans* plus 2 more PGPR viz. *Bacillus sonorensis* and *Paenibacillus elgii* on 2 common varieties of chilly resulted in enhanced plant dry biomass, nutrition and fruit yield. The results clearly brought out that *B. sonorensis* is the most promising PGPR inoculant for chilly. The plant growth promoting traits revealed that *B. sonorensis* is a P-solubilizer and able to produce indole acetic acid, siderophore, chitinase, hydrogen cyanide and good in biofilm formation.

Keywords *Bacillus sonorensis* · *Methylobacterium radiotolerans* · Microbial inoculants · Plant growth response

Introduction

Over the last few decades, the high increase in crop yield was achieved through high input of inorganic fertilizers and pesticides. However, currently emphasis is on sustainable agriculture, which uses less of chemical inputs like fertilizers/pesticides having adverse effect on soil and environment. Microbial inoculants can fulfill diverse beneficial interactions in plants leading to promising solutions for sustainable and environment-friendly agriculture. It is well known that PGPR play an important role in maintaining crop and soil health through nutrient cycling and uptake, suppression of plant pathogens, induction of resistance in plant host and direct stimulation of plant growth [1, 2]. Application of PGPR as biofertilizer reduces the cost of crop production. A large number of bacteria like *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Klebsiella* and *Paenibacillus* have been isolated from rhizosphere of various crops and used as PGPR [3, 4]. PGPR can be the best alternative to chemical fertilizers for sustainable and eco-friendly agriculture.

Chilly (*Capsicum annuum* L.) belonging to the family Solanaceae is one of the major commercial crop of India with large export potential. India is the largest producer of chilly in the world producing about 1.2 million tons every year [5]. However the production of chilly in India is considerably low when compared to the large area (930,000 hectares) of its production [6]. The application of chemical fertilizers and pesticides is increasing every year

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to attain the maximum production of chilly. The use of chemical fertilizers in India has increased up to 170 times in the last 50 years [7]. Different varieties are grown for vegetables, spices, condiments, sauces, pickles, etc. Chilly powder is the most important ground spice item exported from India. Since, chilly being a major spice with tremendous export potential, the emphasis needs to be given for sustainable cultivation of chilly. The main objective of the present study was to screen and select the efficient PGPR which can be used as an inoculant to improve growth, nutrition and yield of chilly.

Material and Methods

The investigations were carried out under pot culture conditions. Three different sets of pot culture experiments were conducted to screen and select the efficient PGPR for inoculating chilly. In the first pot culture trial 10 PGPR viz. *Paenibacillus polymyxa*, *Pantoea dispersa*, *Pantoea agglomerans*, *Pseudomonas* sp., *Bacillus* sp., *Bacillus pumilus*, *Pseudomonas putida*, *Trichoderma viride*, *Bacillus* sp. EB 02 and *Bacillus* sp. EB 04 were screened. In the second experiment 2 promising PGPR selected from the first trial (*Paenibacillus polymyxa* and *Pantoea dispersa*) plus 8 new PGPR (*Paenibacillus* sp., *Pantoea agglomerans*, *Methylobacterium radiotolerans*, *Exiguobacterium acetylicum*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Azospirillum brasilense* and *Azotobacter chroococcum*) were screened, and in the third experiment the best PGPR from the second experiment (*Methylobacterium radiotolerans*) plus 2 newer PGPR (*Bacillus sonorensis* and *Paenibacillus elgii*) were used. The PGPR used in the first and second experiments were obtained from culture collection centers and from scientists working with PGPR. The 2 new PGPR used in the third experiment were isolated by the last two authors. *Bacillus sonorensis* was isolated from the tomato rhizosphere and *Paenibacillus elgii* was isolated from chitin enriched soil [8].

The soil used in the present study was collected from an uncultivated field from a depth of 0–15 cm and has been classified as a fine, kaolinitic isohyperthermic kanhaplustulf. In the first and second experiments, variety NS-1701 was used only. In the third experiment, 2 varieties of chilly seeds commonly cultivated in this region viz, NS-1701 and Ujala F1 hybrid were used. Chilly seeds were sown in the pro-trays containing coco-peat enriched with vermicompost as substrate. After 30 days, uniform size seedlings were transplanted to polythene bags (21 × 14 cm) filled with soil, sand and vermicompost mix in the ratio 1:1:0.25 (v/v/v basis). The PGPR used in the present study were grown in liquid media and 10 ml of inoculum with 10⁸ cfu/ml culture was added to the respective pot according to the treatment. Ten

replications were maintained for each treatment and plants were watered when necessary.

Plant Parameters Studied

The height of the plant and stem girth were recorded at 60 days after transplanting (DAP). Biovolume index (BI) was calculated using the formula given below [9].

$$\text{Biovolume index} = \frac{\text{Plant height (cm)}}{\text{Stem girth (mm)}}$$

Chilly fruits were harvested 70 DAP and fresh weight of the fruits was taken for each plant separately. The shoot of the plants were cut and removed from soil surface and then roots of the plants were collected from soil, washed thoroughly and kept for drying at 60 °C in an oven. The dry weight of shoot and root was determined after drying to a constant weight. Dried shoot and root samples were then powdered using a blender. The major nutrients (N, P and K) in powdered plant samples were analyzed using the methods outlined by Jackson [10]. The data was analyzed using one way analysis of variance. The means were compared by Duncan's multiple range test at 5% level [11].

PGP Traits of *Bacillus Sonorensis*

Bacillus sonorensis was found to be the best in increasing the growth, nutrition and yield of chilly and hence it was characterized for its PGP traits. Phosphate solubilization was checked on Pikovskaya's and National Botanical Research Institute's phosphate (NBRIP) medium agar plates. Overnight culture was spotted on both plates, incubated at 30 °C for 72 h and checked for zone of solubilization [12]. Indole acetic acid production was detected as described by Brick et al. [13]. Siderophore production was checked on Chrome Azurol S agar plates as described by Schwyn and Neilands [14]. Chitinase assay was performed as described by Das et al. [15]. Qualitative hydrogen cyanide (HCN) production was checked using nutrient agar supplemented with 0.44% of glycine [16]. Biofilm formation by *Bacillus sonorensis* during growth was examined in borosilicate glass tubes, as described by Yousef et al. [17] and O'Toole and Kolter [18]. The ability of the bacterium to utilize 1-aminocyclopropane-1-carboxylate (ACC) as a sole source of nitrogen was assayed as described by Li et al. [19].

Results and Discussion

Under pot culture studies, chilly plants varied in their response to inoculation with different PGPR. Significant increases in growth and yield of agronomically important

crops in response to inoculation with PGPR have been reported [20, 21]. Studies have also shown that the growth-promoting ability of some bacteria may be highly specific to certain plant species, cultivar and genotypes [22]. Poi and Kabi [23] reported that inoculation with *Azotobacter* strains isolated from the rhizosphere soils of squash, wheat and jute improved the yield but the strains were crop specific. The importance of screening of different PGPR and their potential features on plant growth and yield for different plants have been studied earlier [20, 24]. Main objective of this study was to screen and select the efficient PGPR to be used as an inoculant to increase the growth, yield and nutrition of chilly.

In the first experiment, 10 different PGPR were used. It was found that PGPR generally increased the plant height and stem girth as compared to uninoculated plants. Increased fruit yield was observed in six treatments viz, *Paenibacillus polymyxa*, *Pantoea dispersa*, *Pseudomonas* sp., *Trichoderma viride* and *Bacillus* sp. (EB 04) over control. The total plant (shoot + root) dry biomass was high in treatments inoculated with PGPR as compared to the uninoculated treatment. Though most of the plant parameters studied were statistically not significant, 2 PGPR viz., *P. polymyxa* and *P. dispersa* showed agronomic improvement in plant growth as compared to control; the increase in fruit yield in these two treatments being 33 and 17%, respectively.

The second experiment was conducted with 2 PGPR selected based on the results of the first experiment plus 8 more PGPR. Results showed that the plant height and stem girth of the chilly plants 60 DAP increased significantly with the PGPR, *M. radiotolerans* followed by *P. fluorescens*, *B. subtilis* and *A. chroococcum*. Other treatments

did not differ significantly from the uninoculated control. The plants inoculated with *M. radiotolerans* and *P. fluorescens* had significantly greater biovolume-index and fruit yield as compared to other treatments and uninoculated control (Table 1). *Methylobacterium* sp., playing an important role in promoting the germination and plant growth, has been reported by many workers [25–27]. The dry weight of shoot was significantly more in 6 out of 10 PGPR inoculation treatments. The highest dry weight of the root was obtained in the treatment of *M. radiotolerans* which was statistically at par with *A. chroococcum* and *A. brasilense* treatments. The increase in total dry biomass was found in most of the PGPR inoculated plants as compared to uninoculated plants. However highest total dry biomass was recorded in plants treated with *M. radiotolerans* which was statistically at par with *B. subtilis* treatment (Table 2). Giving weightage to biovolume index, plant dry biomass and fruit yield, *M. radiotolerans* proved to be the best.

In the third pot experiment, further screening with *M. radiotolerans* plus 2 more new PGPR viz. *Bacillus sonorensis* and *Paenibacillus elgii* was done using 2 varieties of chilly viz. NS-1701 and Ujala F1 hybrid. Due to enhanced plant height and stem girth, the biovolume index was significantly higher in plants treated with *B. sonorensis* (Fig. 1) followed by *M. radiotolerans* in both the varieties of chilly. Inoculation of chilly plants (both varieties) with *Bacillus sonorensis* also showed significant increase in fruit yield. The increase in plant dry biomass and fruit yield because of inoculation with *B. sonorensis* was 41 and 57.74% in NS-1701, and 36 and 37.07% in Ujala F1 hybrid, respectively. The increase in plant dry biomass and fruit yield because of

Table 1 Effect of PGPR inoculation on plant height, stem girth, biovolume index and yield of chilly variety NS-1701 (second experiment)

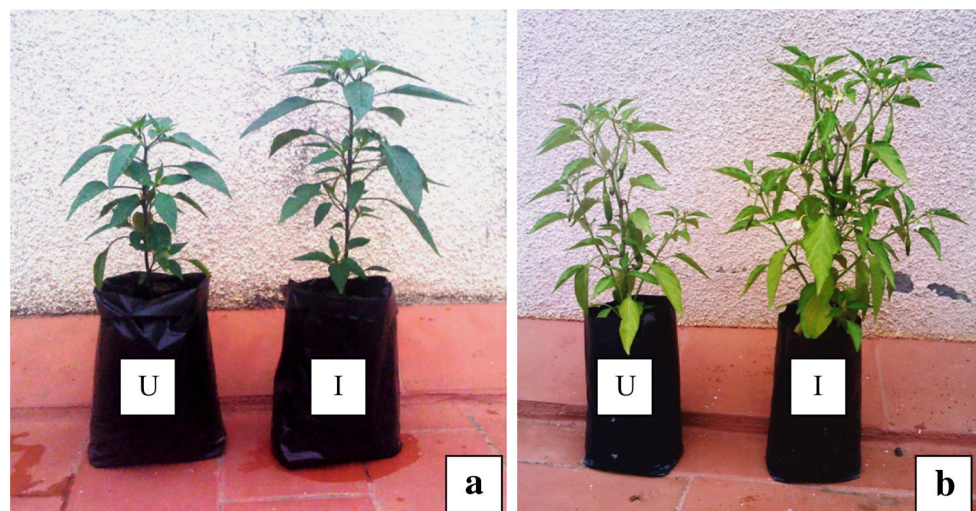
Treatments	Plant height (cm/plant)	Stem girth (mm/plant)	Biovolume index (BI)	Fruit yield (g/plant)
Uninoculated	32.07 ^{bc}	12.12 ^{bc}	389.11 ^{bc}	6.35 ^{bcd}
<i>Paenibacillus</i> sp.	26.29 ^d	11.56 ^{cd}	305.63 ^d	5.41 ^{ce}
<i>Paenibacillus polymyxa</i>	26.64 ^d	11.33 ^{de}	306.0 ^d	4.81 ^{cde}
<i>Pantoea dispersa</i>	24.79 ^{de}	10.7 ^{ef}	269.17 ^{de}	3.11 ^e
<i>Pantoea agglomerans</i>	20.57 ^e	10.33 ^f	216.29 ^e	4.44 ^{de}
<i>Methylobacterium radiotolerans</i>	37.64 ^a	12.9 ^a	485.74 ^a	9.75 ^a
<i>Exiguobacterium acetylicum</i>	27.64 ^{cd}	11.58 ^{cd}	327.47 ^{cd}	6.02 ^{bcd}
<i>Bacillus subtilis</i>	32.92 ^{ab}	12.48 ^{ab}	411.12 ^b	6.74 ^{bcd}
<i>Pseudomonas fluorescens</i>	33.28 ^{ab}	12.55 ^{ab}	417.74 ^{ab}	7.3 ^{abc}
<i>Azotobacter chroococcum</i>	32.93 ^{ab}	12.4 ^{ab}	408.88 ^b	7.25 ^{bc}
<i>Azospirillum brasilense</i>	32.07 ^{bc}	11.99 ^{bcd}	385.81 ^{bc}	6.41 ^{bcd}
SEd	2.4	0.34	34.76	1.25
CD (0.05)	4.79	0.69	69.3	2.5

Values in each column followed by the same letter are not significantly different ($P = 0.05$)

Table 2 Effect of PGPR inoculation on dry weight of shoot and root and total dry biomass of chilly variety NS-1701 (second experiment)

Treatments	Dry weight of shoot (g/plant)	Dry weight of root (g/plant)	Total dry biomass (g/plant)
Uninoculated	3.4 ^{bc}	1.46 ^b	4.86 ^b
<i>Paenibacillus</i> sp.	2.17 ^d	0.99 ^c	3.17 ^c
<i>Paenibacillus polymyxa</i>	2.46 ^{cd}	1.00 ^c	3.47 ^c
<i>Pantoea dispersa</i>	1.73 ^{de}	0.69 ^{cd}	2.43 ^{cd}
<i>Pantoea agglomerans</i>	1.04 ^e	0.41 ^d	1.45 ^d
<i>Methylobacterium radiotolerans</i>	4.88 ^a	2.49 ^a	6.94 ^a
<i>Exiguobacterium acetylicum</i>	3.94 ^{ab}	1.59 ^b	5.53 ^b
<i>Bacillus subtilis</i>	4.17 ^{ab}	1.55 ^b	5.72 ^{ab}
<i>Pseudomonas fluorescens</i>	3.85 ^{ab}	1.63 ^b	5.48 ^b
<i>Azotobacter chroococcum</i>	3.86 ^{ab}	1.7 ^{ab}	5.56 ^b
<i>Azospirillum brasilense</i>	4.35 ^{ab}	1.73 ^{ab}	5.65 ^b
SEd	0.52	0.2	0.63
CD (0.05)	1.03	0.39	1.26

Values in each column followed by the same letter are not significantly different ($P = 0.05$)

Fig. 1 Effect of inoculation with *B. sonorensis* on growth of chilly varieties **a** NS-1701 and **b** Ujala F1 hybrid. (U uninoculated; I inoculated with *B. sonorensis*)**Table 3** Effect of PGPR inoculation on biovolume index, fruit yield, total dry biomass, N, P and K concentration of chilly variety NS-1701 (third experiment)

Treatments	Bio-volume Index	Fruit yield (g/plant)	Total biomass (g/plant)	N (%)	P (%)	K (%)
Uninoculated	182.69 ^b	12.99 ^c	6.45 ^b	2.76 ^d	0.79 ^c	4.77 ^d
<i>Methylobacterium radiotolerans</i>	215.49 ^a	16.33 ^{bc}	8.76 ^a	3.51 ^b	0.86 ^b	5.86 ^b
<i>Paenibacillus elgii</i>	172.62 ^b	14.02 ^c	6.4 ^b	3.1 ^c	0.80 ^c	5.17 ^c
<i>Bacillus sonorensis</i>	215.29 ^a	20.49 ^a	9.15 ^a	3.72 ^a	0.94 ^a	6.18 ^a
SEd	13.75	1.94	0.74	0.04	0.01	0.01
CD (0.05)	28.08	3.97	1.51	0.08	0.02	0.02

Values in each column followed by the same letter are not significantly different ($P = 0.05$)

inoculation with *B. sonorensis* was 6.31 and 19.02% respectively as compared to that of *M. radiotolerans* treatment. *B. sonorensis* enhancing the growth and plant dry weight of tomato and sorghum has also been

observed earlier by the authors [8]. The results of the major plant nutrient analysis brought out that N, P and K concentration of chilly samples was significantly high in plants inoculated with *Bacillus sonorensis* (Tables 3, 4).

Table 4 Effect of PGPR inoculation on biovolume index, fruit yield, total dry biomass, N, P and K concentration of chilly variety Ujala F1 hybrid (third experiment)

Treatments	Bio-volume index*	Yield (g/plant)	Total biomass (g/plant)	N (%)	P (%)	K (%)
Uninoculated	207.43	14.97 ^b	5.48 ^c	2.95 ^e	0.80 ^d	5.03 ^d
<i>Methylobacterium radiotolerans</i>	211.73	18.23 ^{ab}	6.87 ^{ab}	3.62 ^b	0.92 ^b	6.09 ^b
<i>Paenibacillus elgii</i>	206.51	14.3 ^b	6.02 ^{bc}	3.26 ^d	0.89 ^c	5.31 ^c
<i>Bacillus sonorensis</i>	229.46	20.52 ^a	7.43 ^a	4.00 ^a	0.98 ^a	6.27 ^a
SEd	NS	2.02	0.65	0.01	0.01	0.01
CD (0.05)	NS	4.13	1.32	0.02	0.02	0.02

Values in each column followed by the same letter are not significantly different ($P = 0.05$)

* Not significant

Table 5 PGPR traits of *Bacillus sonorensis*

PGPR traits	<i>Bacillus sonorensis</i>
IAA Production	+ (3.1 µg/ml at 12 h, 4.3 µg/ml at 24 h)
Phosphate solubilization	+++
Siderophore production	+
Chitinase production	++
HCN production	-
ACC deaminase production	++

+ = positive; - = negative result for the test

For phosphate solubilization, siderophore production and chitinase production: + = zone of clearance <0.2 cm; ++ = zone of clearance 0.2–0.4 cm; +++ = zone of clearance >0.4 cm

For ACC deaminase production: + = slight; ++ = medium; +++ = good

Hence, it can be concluded from the present study that *B. sonorensis* is the most promising PGPR for inoculating both the varieties of chilly. Since *Bacillus sonorensis* proved to be the best bacterium among 20 PGPR screened for inoculating chilly, its PGPR traits were studied and the results are given in Table 5. The traits such as P-solubilization, production of indole acetic acid (IAA), siderophore, chitinase, HCN, ACC deaminase, and biofilm formation confirmed that it is a PGPR.

Bacillus sp. are known to be P solubilizers and IAA producers and inoculation with *Bacillus* spp. improving growth of maize [28] and wheat [29] have been reported. Husen [30] reported that soil bacterial isolate *Bacillus cereus* produces siderophores and hence it can be used as efficient PGPR to increase crop yield. Shobha and Kumudini [31] isolated a *Bacillus* sp. which produced IAA, ammonia, HCN and siderophore, and inhibited the pathogen *Fusarium oxysporum* and suggested that it can be used as a PGPR. Zahir et al. [32] reported that the inoculation with rhizobacterial strains containing ACC-deaminase activity significantly promoted root, shoot and other growth contributing parameters of wheat at all salinity levels both under axenic and pot conditions. ACC deaminase is a master regulator of ethylene synthesis in plants. Bacteria

possessing ACC deaminase activity reduce the level of stress hormone ethylene resulting in plant growth promotion under various abiotic stresses such as salt, drought, flooding, and heavy metal [33]. Thus, it is believed that the strain of *B. sonorensis* used in the present study will also protect plants against abiotic stresses. This aspect needs further investigation.

Conclusions

In conclusion, the present study demonstrates that PGPR are not host specific, however they exhibit host preference, thus emphasizing the need for screening different PGPR in order to select the best to be used as an inoculant for a particular crop. Out of the 3 pot experiments conducted using 20 PGPR, the results clearly brought out that *B. sonorensis* is the most promising PGPR inoculant for chilly. Studies also confirmed that *B. sonorensis* possessed all the PGPR traits.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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