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Biocontrol of *Fusarium* wilt of *Capsicum annuum* by rhizospheric bacteria isolated from turmeric endowed with plant growth promotion and disease suppression potential

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Abstract In the present study, 129 rhizospheric bacteria isolated from *Curcuma longa* were screened for their antagonistic potential against six fungal phytopathogens. Among them, 32 isolates that showed significant antagonistic potential were screened for their in vitro plant growth promoting (PGP) traits. The identification of potential isolates was confirmed by 16S rRNA gene sequencing and results revealed *Bacillus* as the dominant genus followed by *Staphylococcus*, *Pseudomonas, Sphingomonas* and *Achromobacter*. Based on the antagonistic activity and PGP traits; two strains (BPSRB4 and BPSRB14), identified as *Bacillus amyloliquefaciens*, were further tested for their in vivo PGP and disease suppression potential on *Capsicum annuum* seedlings under greenhouse conditions. The results demonstrated that BPSRB4 and BPSR14 strains suppress fungal pathogen infection and promote plant growth. Further, the BPSRB4 strain was positive for the production of the phytohormone indole acetic acid (IAA) detected by thin layer chromatography (TLC). In addition, nitrogen fixation and plant growth promotion activity were also confirmed by amplification and sequencing of nitrogen fixation gene (*nif*H) and ACC (1-aminocyclopropane-1-carboxylate) deaminase (*acd*S) gene from strains BPSRB4 and BPSRB14. The present study demonstrated that the *B. amyloliquefaciens* strains BPSRB4 and BPSR14 possess antagonistic activity and PGP potential which could be explored for the development of biofertilizers and biocontrol agents for the growth of chilli seedlings.

Keywords Rhizospheric bacteria · 16S rDNA · *Curcuma Longa* · Biocontrol · Thin layer chromatography (TLC)

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Introduction

Turmeric (*Curcuma longa*) is a widely cultivated rhizomatous perennial crop, belonging to the family Zingiberaceae which can be used as a part of Siddha medicine (Chattopadhyay et al. 2013). It is well known for its medicinal properties, including antibacterial and antiseptic activity for cuts, or for indigestion, osteoarthritis, burns and bruises (Gregory et al. 2008). The

rhizosphere, a narrow region surrounding and influenced by plant roots, contains a huge number of microorganisms capable of having beneficial, neutral or detrimental effects on plant growth (Philippot et al. 2013). Plant growth promoting rhizobacteria (PGPR) can be beneficial for plants by stimulating growth hormones and by suppressing disease prevalence (Shanmugam and Kanoujia 2011). PGPR have been known to enhance availability of nutrients (Drogue et al. 2012) and phytohormone mediated stimulation of root systems (Somers et al. 2004), and induce systematic resistance (Zehnder et al. 2000). Moreover, it has now been demonstrated that microbes obtained from one plant can be applied to other plants for promoting plant growth activity and disease resistance (Abbamondi et al. 2016; Passari et al. 2015b). Therefore, it is important to evaluate the potential of indigenous bacterial populations associated with turmeric rhizosphere soil for growth promotion in this and other crops.

PGPR promote plant growth under both biotic and abiotic stresses; therefore the use of PGPR can assist in increasing eco-friendly practices for sustainable agriculture. For instance, gram-positive and gram-negative bacterial genera such as coryneform bacteria, *B. subtilis, Klebsiella*, *Burkholderia, Pseudomonas gladioli and Pseudomonas cepacia, Azospirillum, Azotobacter, Arthrobacter, Micrococcous*, *Enterobacter*, and *Xanthomonas* have been reported to induce plant growth (El-Sayed et al. 2014; Bal et al. 2013). PGPR have been well documented previously from different agricultural crops like rice (Sudha et al. 1999), tomato (Mena-Violante and Olalde-Portugal 2007), sugarcane (Hassan et al. 2010), maize (Biari et al. 2008) and chilli (Datta et al. 2010). However, PGPR from the turmeric rhizosphere is still poorly explored. Knowledge of indigenous bacterial populations, their characterization and identification is required to understand their assemblages in the rhizosphere of specific crops (Breidenbach et al. 2016). The use of agrochemicals has reduced soil fertility in the northeast region of India by the accumulation of toxic chemicals in the soil. Hence, we endeavored to search for region-specific microbial strains from turmeric rhizosphere with plant growth promoting (PGP) activity and disease suppression potential (Deepa et al. 2010). The main objectives for this work were: (1) to isolate and identify potential plant growth promoting bacterial species from rhizospheric soil of *C. longa;* (2) to screen their in vitro antagonistic and PGP abilities; (3) to test their activity for production of lytic enzymes and detect genes for activities related to plant growth promotion; and (4) to evaluate the in vivo plant growth promotion and disease suppression potential on chilli seedlings.

Materials and methods

The study areas and sample collection

Rhizospheric soil and root samples of Turmeric plants (*C. longa*) were randomly collected from Dampa Tiger Reserve Forest [Dampa TRF] (23°25′N; 92°20′E) and Reiek Mountain hill forest (23°69′N; 92°62′E) district in Mizoram, India. The soil samples were red and yellow in colour with loamy texture. Each plant rhizosphere was carefully removed with the intact root system. The samples were placed in sterile Himedia plastic bags and carried to the laboratory on ice and processed immediately.

Isolation and primary identification of rhizospheric bacteria

Isolation of bacterial populations was carried out using serial dilution technique (Manivannan et al. 2012). Pure cultures were stored in 20% glycerol at -80 °C. Primary identification of bacteria was carried out based on morphological, biochemical and physiological characteristics according to Bergey's Manual of Determinative Bacteriology (Bergey and Holt 2000).

Antagonistic activity of rhizospheric bacterial isolates

All the isolates were screened for their antagonistic activity against six plant pathogens i.e. *Fusarium proliferatum* (MTCC-286), *Fusarium oxysporum* f sp. *pisi* (MTCC-2480), *Fusarium oxysporum* f. sp. *capsici* (ATCC-66421), *F. udum* (MTCC-2755), *Rhizoctonia solani* (MTCC-9666) and *Colletotrichum capsici* (MTCC-8473) by using dual culture method (Bredholdt et al. 2007). Briefly, 5 mm potato dextrose agar (PDA) block with fungal growth was placed at the center of a petri plate containing PDA and the bacterial isolates were streaked at the periphery of the plates. Dual cultures were incubated at 28 °C for 7 days and the diameter of fungal mycellial growth was measured and compared to the control (without any bacterial isolate). The percentage of inhibition was calculated as: % inhibition = [1-(Fungal growth /Control growth)] ×100. The pathogens were obtained from the Microbial

Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India. All experiments were carried out in triplicates and repeated twice.

Plant growth promoting abilities of rhizospheric bacterial isolates

Phosphate solubilization

All isolates were screened for their ability to solubilize insoluble phosphate by spot inoculation onto Pikovskayas agar (PKV) plates supplemented with rock phosphate (P_2O_5), an insoluble phosphate source (Nautiyal 1999).

Characterization of indole acetic acid (IAA) production

Bacterial isolates were screened for IAA production using nutrient broth supplemented with 0.2% L-tryptophan according to Bric et al. (1991). The concentration of IAA was calculated using a standard curve with indole-3-acetic acid. The experiments were repeated twice with three replicates each and means were calculated.

Detection of IAA by using thin layer chromatography (TLC)

Isolate BPSRB14 was grown in LB broth supplemented with 0.01% tryptophan and incubated at 37 °C, 150 rpm for 2 days. The culture was extracted thrice with ethyl acetate. The ethyl acetate extract was evaporated to dryness under pressure at 45 °C using a rotary evaporator (BUCHI, India) to obtain the crude extract. Ten (10) μl of bacterial (BPSRB14) crude ethyl acetate extract and standard IAA (10 mg/100 ml) was spotted onto TLC plates, and separated in a solvent containing, propanol: water (8:2) for 2 h. After the plates were dry, the indole compounds were developed by spraying chromogenic reagent (Salkowski reagent) in an upright position followed by heating to 90 °C. The R_f value was calculated based on mobility as compared to the standard (Mohite 2013).

Screening for ammonia, siderophore and catalase production

The rhizospheric bacterial isolates were tested for the production of ammonia as previously described (Cappucino and Sherman 1992). Siderophore production was evaluated using CAS agar medium supplemented with chrome azurol S (Schwyn and Neilands 1987). Catalase production by all isolates was performed as per Kumar et al. (2012a).

Estimation of Carboxymethyl Cellulase (CMCase), amylase and xylanase activities

Cellulase activity was screened on CMC agar medium containing (K_2HPO_4–0.5 g, $MgSO_4 \cdot 7H_2O$ - 0.25 g, carboxymethyl cellulose - 15.0 g and agar - 20.0 g) per litre of distilled water. The bacterial isolates were streaked onto CMC agar plates and incubated at 30 °C for 3–5 days, then 5 ml of congo red solution (1 mg/ml) was added to the plates. The plates were distained with 1 M NaCl for 15 min. A clear zone of hydrolysis indicated cellulase production (Teather and Wood 1982). Amylase activity was screened on Luria-Bertani (LB) agar medium containing 1% soluble starch. After growing bacteria at 30 °C for 5 days, 5 ml (1%) iodine solution was added to the plates, and a clear zone around the colonies indicated amylase production (Hankin and Anagnostakis 1975). For the xylanase activity, oat spelt agar medium containing 1% oat spelt was used. Each bacterial isolate was streaked onto the medium and incubated at 37 °C for 3–5 days. Thereafter, the plates were flooded with congo-red solution (1 mg/ml) for 10 min. Followed by destaining with 1 M NaCl for 15 min, and the appearance of a clear zone indicated xylanase production.

For quantitative estimation of cellulase, amylase and xylanase, each bacterial isolate was inoculated in tryptone yeast extract (ISP1) broth and incubated at 37 °C for 2–3 days using the 3,5-dinitrosalicylic acid (DNS) method according to Ghose (1987). The culture was centrifuged at 5000 rpm for 10 min and the cell free supernatant was collected for the enzymatic assay. The crude enzyme extract (0.5 ml) was mixed with 0.5 ml of specific substrates (1% of carboxymethyl cellulose for cellulase, soluble starch for amylase or xylan in 50 mM of phosphate buffer, pH -6.9 for xylanase). The mixture was incubated at 50 °C for 30 min and the reaction was stopped by the addition of 3 ml DNS reagent. Following, the mixture was boiled for 5 to 10 min and then 1 ml of sodium potassium tartarate (called Roselle salt) was added. The absorbance was recorded at 540 nm using a spectrophotometer (Thermo scientific Multiskan GO microplate reader). The enzyme activities

were estimated using a standard graph of glucose. The one unit of enzyme activity was defined as the amount of enzyme that released 1 μM of glucose per min (Ariffin et al. 2006).

Molecular characterization and phylogenetic analysis of rhizospheric bacteria

PCR amplification of 16S rRNA gene and sequencing

PCR amplification of the 16S rRNA region of bacterial isolates was carried out by using universal primers- PA: 5′-AGA GTT TGA TCC TGG CTC AG-3′) and PH: 5′-AAG GAG GTG ATC CAG CCG CA-3′ (Qin et al. 2009). The amplified PCR products were analyzed by electrophoresis through 1.5% agarose gels and documented using a Bio-rad Gel Doc XR$^+$ system (Hercules, CA, USA). The PCR products were purified using Pure-link PCR Purification Kit (Invitrogen), and was sequenced commercially at Sci-Genome Labs Pvt. Ltd., India.

Phylogenetic analysis of rhizospheric bacteria

The 16S rRNA gene sequences were compared with the National Center for Biotechnology Information (NCBI) database using the BlastN search program. The species showing 98–100% sequence identity were selected as close matches. The sequences were aligned using Clustal W (Thompson et al. 1997). All the sequences were submitted to NCBI Genbank and accession numbers were obtained. A maximum-likelihood phylogenetic tree was constructed by Kimura 2- parameter model using MEGA 5.05. Sequences from *E. coli* (gram-negative bacteria) and *Kocuria rhizophila* (gram-positive bacteria) were included as outgroups for the analysis (Saitou and Nei 1987). The reliability of the phylogenetic trees was evaluated by bootstrap analysis with 1000 resamplings using *p*-distance model (Felsenstein 1985).

Detection of nitrogen fixation (nifH) and ACC deaminase (acdS) encoding genes in rhizospheric bacteria

The *nif*H gene was amplified using *nif*H1 forward primer (5′-CGTTTTACGGCAAGGGCGGTATCGGCA-3′) and *nif*H2 reverse primer (5′-TCCTCCAGCTCCTC CATGGTGATCGG-3′) as described by Pandey et al. (2004).

The Acc deaminase gene (*acd*S) was amplified using the forward primer 5′- GGCAAGGTCGACAT CTATGC-3′ and reverse primer 5′-GGCT TGCCATTCAGCTATG-3′ as per Antoun and Prevost (2005). The PCR products were purified using a Quick PCR purification kit (GeneiPure, Merk) and sequencing was done commercially at Sci-Genome Pvt. Ltd. Kochin, India.

Ad planta

Preparation of planting material and bacterial inoculum

Chilli (*Capsicum annuum*) seeds (PUSA-Jwala, collected from the Indian Agricultural Research Institute (IARI), New Delhi, India) were surface sterilized in 70% ethanol for 5 min followed by 2% NaOCl for 3 min and then, washed three times with sterile distilled water. The rhizospheric isolates BPSRB 4 and 14, were evaluated for in vivo plant growth promoting activity on chilli seed. Bacterial isolates grown overnight in LB broth at 37 °C at 150 rpm were used as bacterial inoculum for PGP effect.

Plant growth promoting (PGP) assay

Chilli (*C. annuum*) seeds of the cultivar PUSA-Jwala were soaked overnight in sterile distilled water and were planted in a plug tray using nursery substrates until three to four leaves emerged. Individual seedlings were transferred to plastic pots filled with soil. The seedlings were grown under greenhouse conditions, temperatures ranging from 25 to 30 °C and the humidity between 75 and 90%. The experiment was carried out in a randomized design. Three treatments were used in PGP assay: TA1-seedlings without any inoculum (control), **TA2-** seedlings + isolate BPSRB4 inoculum and **TA3-** seedlings + isolate BPSRB14 inoculum. Each treatment included three replications with ten plants per replica. Root length, shoot length and fresh plant weight measurement were recorded after 30–45 days between inoculated and uninoculated plants (Ji et al. 2014). Each treatment included three replications with ten plants per replica. Five plants from each treatment were removed to measure the

growth measuring parameters at 30 and 45 days. All treatments were carried out twice a week and plants were watered once daily until harvesting. Data were statistically analyzed using standard analysis of variance (ANOVA) and Duncan's multiple comparison tests at $P \leq 0.05$.

Biocontrol activity

Chilli (*C. annuum*) seeds of the cultivar PUSA-Jwala were soaked overnight in sterile distilled water and similarly treated as described above. The experiment was carried out in a completely randomized design. Two potential strains (BPSAC4 and BPSAC14), based on their in vitro screening, were selected for in planta activity against *F. oxysporum* f. sp. *capsici,* the major causative agent of *Fusarium* wilt in chilli. Three treatments were performed to promote disease development under greenhouse conditions: **TB1-** seedlings + conidial suspension of *F. oxysporum* f. sp. *capsici* (control); **TB2-** seedlings + isolate BPSRB4 inoculum + conidial suspension of *F. oxysporum* f. sp. *capsici*, **TB3-** seedlings + isolate BPSRB14 inoculum + conidial suspension of *F. oxysporum* f. sp. *capsici.*. Five plants from each treatment were removed to confirm their disease suppression potential of the bacterial strains compared with control after 30 and 45 days. All treatments were carried out twice a week and plants were watered once daily till harvesting.

The percent disease index was calculated according to Shanmugam and Kanoujia (2011) as follows:

$$\textbf{Disease index} = [\mathrm{R}/\textit{Total number of plants} \times \textit{highest rating}] \times 100.$$

Where, R = rating × number of plants rated.

The wilt development of each chilli plant was rated as described; 0 = no symptoms; 1 = <25% of leaves with symptoms; 2 = 26–50% of leaves with symptoms; 3 = 51–75% of leaves with symptoms; 4 = 76–100% of leaves with symptoms. Disease index data were statistically analyzed.

Statistical analysis

The data obtained was analyzed using standard analysis of variance (ANOVA) generalized linear model (GLM) followed by Duncan's multiple comparison test at $p < 0.05$. All treatments were carried out in triplicate and the results are presented as mean ± standard deviation (SD). Mean values were separated by using the least significant difference (LSD) test.

Result

Isolation and morphological identification of rhizospheric bacteria

A total of 129 isolates were obtained from the rhizospheric soil samples collected from Dampa Tiger Reserve forest and Reiek mountain hill forest Mizoram. Based on Gram staining, 96 isolates were found to be Gram positive and 33 isolates were Gram negative. Biochemical analysis revealed that all the isolates were positive for the catalase and starch hydrolysis tests. Relative abundance of rhizospheric bacteria indicated that *Bacillus* sp. was the most dominant genus at Dampa TRF (63.1%) followed by Reiek with 36.8%. The genus *Staphylococcus* was most abundant in Reiek (80.0%) followed by Dampa TRF (20.0%) (Fig. 1). The genus *Achromobacter* was prevalent only in Dampa tiger reserve forest of Mizoram.

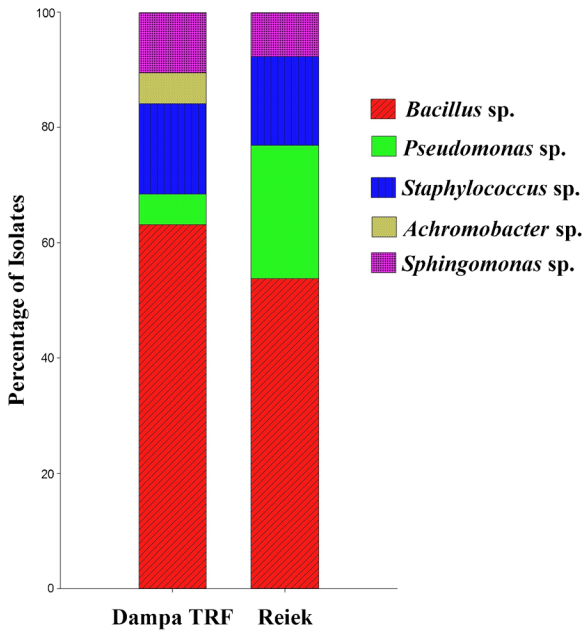


Fig. 1 The relative abundance of rhizospheric bacterial isolates at genus level from Dampa TRF and Reiek mountain hill forest of Mizoram

In vitro antagonistic activity of rhizospheric bacteria

Among, 129 rhizospheric bacterial isolates, 32 showed positive antagonistic activity against more than two fungal phytopathogens out of the six fungal phytopathogens tested with the percentage of inhibition ranging from 31% to 89%. All isolates showed antagonistic activity against *F. oxysporum* f. sp. *capsici*, the major pathogen responsible for the *Fusarium* wilt in chilli, whereas *F. udum* was the most susceptible fungal pathogen against most of the rhizospheric bacteria isolates with an exception of 6 isolates (BPSRB 2, 3, 4, 7, 10 and 14). Isolate BPSRB4 showed strong activity against *F. proliferatum* (85%), whereas isolate BPSAC 14 exhibited the greatest antagonism potential against *F. oxysporum* f. sp. *capsici* (89%) and *F. oxysporum* f. sp. *pisi* (84%) (Table 1).

Plant growth promoting traits of antagonistic rhizospheric bacteria

Phosphate solubilization and IAA production

Among 32 antagonistic positive isolates, 26 showed phosphate solubilization activity based on their solubilizing capacity of rock phosphate (P_2O_5) by producing a clear halo zone around the spotted bacterial colonies on Pikovskaya's medium. The zone of phosphate solubilization of rhizospheric bacteria varied from 1.0 to 12.4 mm. Isolate BPSRB4 showed the highest phosphate solubilization activity with P solubilization zone of 12.4 mm, followed by BPSRB14 (10.5 mm) and BPSRB2 (9.5 mm) (Table 2).

The IAA screening results showed that 28 isolates (87.5%) were positive for IAA production. The quantitative estimation of the IAA production in culture broth in the presence of tryptophan ranged from 16.8 to 60.5 μg/ml. The IAA yields of isolates BPSRB4 and BPSRB14 were 60.5 μg/ml and 59.4 μg/ml, respectively, which was the highest among all the isolates tested (Table 2). Moreover, the Rf value of isolate BPSRB4 on TLC plate was equivalent to standard IAA (0.57).

Ammonia, Siderophore and catalase production

Quantitative estimation of ammonia production by all the isolates showed positive results in peptone water broth, and ranging from 15.4 to 72.3 mg/100 ml. Isolate BPSRB4 produced the greatest amount of ammonia (72.3 mg/100 ml) followed by BPSRB14 (69.8 mg/100 ml) and BPSRB2 (67.4 mg/100 ml). Out of 32 isolates, 23 (71.8%) had a clear orange halo zone around the colonies on CAS agar media. Isolate BPSRB4 showed the highest siderophore production followed by BPSRB14 and BPSRB2. All the isolates were positive for catalase production as indicated by effervescence after the addition of H_2O_2 on bacterial culture which was grown for 48 h on LB agar medium (Table 2).

Cellulase, amylase and xylanase production assays

The 32 antagonistic positive isolates were further screened for the qualitative production of cellulase, amylase and xylanase, out of which 27 (84.3%) isolates were positive for cellulase and xylanase production, whereas, 29 (90.6%) isolates showed amylase production. The cellulase, amylase and xylanase production ranged from 1.84 to 7.86 IU/ml, 1.62 to 8.24 IU/ml and 1.56 to 7.42 IU/ml, respectively. Among the positive isolates, isolate BPSRB4 showed the highest cellulase (7.86 IU/ml) and amylase (8.24 IU/ml) production; however the highest xylanase (7.46 IU/ml) production was detected in isolate BPSRB14 (Table 2).

Identification and molecular characterization of bacterial isolates

In order to confirm the identity of all 32 antagonistic positive isolates, 16S rRNA gene sequencing was carried out. The results of DNA sequencing classified all the isolates into five genera; *Bacillus* (56.25%), *Staphylococcus* (15.6%), *Pseudomonas* (12.5%), *Sphingomonas* (9.3%) and *Achromobacter* (3.1%) (Table 3). The nucleotide sequences were further compared by BLASTn analysis tool to look for 16S rRNA gene homology along with sequences from type strains retrieved from EzTaxon-database (http://www.ezbiocloud.net/eztaxon). The nucleotide sequences were deposited in NCBI Genbank database under the accession numbers KT028674 to KT028688 and KU158218 to KU158234 (Table 3).

The phylogenetic tree of gram positive bacterial nucleotide sequences using Maximum-likelihood method

Table 1 In vitro antagonistic activity of the rhizospheric bacteria recovered from *Curcuma longa* against six fungal phytopathogens

Isolate no.	Percentage of inhibition (PI ± SE)					
	F. oxy. f. sp. *pisi*	*F. oxy.* f. sp. *capsici*	*F. prolifreatum*	*F. udum*	*R. solani*	*C. capsici*
BPSRB1	44.0 ± 0.25	48.2 ± 0.18	55.3 ± 0.25	0.0 ± 0.00	37.6 ± 0.15	47.3 ± 0.25
BPSRB2	60.5 ± 0.50	82.5 ± 0.25	72.5 ± 0.15	38.5 ± 0.32	0.0 ± 0.00	0.0 ± 0.00
BPSRB3	57.8 ± 0.32	76.4 ± 0.00	64.0 ± 0.30	41.3 ± 0.25	0.0 ± 0.00	48.9 ± 0.30
BPSRB4	82.4 ± 0.41	86.8 ± 0.25	85.0 ± 0.32	57.8 ± 0.25	62.5 ± 0.34	51.7 ± 0.50
BPSRB5	0.0 ± 0.00	62.3 ± 0.32	0.0 ± 0.00	53.4 ± 0.32	53.4 ± 0.32	0.0 ± 0.00
BPSRB6	0.0 ± 0.00	55.0 ± 0.40	43.4 ± 0.32	0.0 ± 0.00	45.3 ± 0.25	54.2 ± 0.34
BPSRB7	74.0 ± 0.25	69.6 ± 0.36	56.4 ± 0.25	37.6 ± 0.20	0.0 ± 0.00	0.0 ± 0.00
BPSRB8	71.6 ± 0.25	81.1 ± 0.18	48.9 ± 0.30	0.0 ± 0.00	32.6 ± 0.15	48.9 ± 0.30
BPSRB9	0.0 ± 0.00	41.0 ± 0.34	0.0 ± 0.0	0.0 ± 0.00	50.1 ± 0.40	40.0 ± 0.36
BPSRB10	62.5 ± 0.34	59.9 ± 0.20	54.2 ± 0.34	52.6 ± 0.36	0.0 ± 0.00	0.0 ± 0.00
BPSRB11	0.0 ± 0.00	37.2 ± 0.25	56.4 ± 0.32	0.0 ± 0.00	48.9 ± 0.30	50.1 ± 0.40
BPSRB12	0.0 ± 0.00	45.3 ± 0.25	64.0 ± 0.20	0.0 ± 0.00	56.5 ± 0.28	0.0 ± 0.00
BPSRB13	0.0 ± 0.00	53.4 ± 0.32	0.0 ± 0.00	63.4 ± 0.15	38.6 ± 0.15	44.3 ± 0.25
BPSRB14	84.0 ± 0.15	89.0 ± 0.28	78.4 ± 0.25	48.6 ± 0.25	60.4 ± 0.36	55.0 ± 0.40
BPSRB15	68.8 ± 0.28	74.1 ± 0.40	0.0 ± 0.00	0.0 ± 0.00	49.0 ± 0.27	51.0 ± 0.32
BPSRB16	42.9 ± 0.25	51.7 ± 0.50	62.6 ± 0.15	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
BPSRB17	45.6 ± 0.20	44.0 ± 0.32	71.2 ± 0.00	0.0 ± 0.00	57.0 ± 0.20	0.0 ± 0.00
BPSRB18	0.0 ± 0.00	60.4 ± 0.36	0.0 ± 0.0	37.6 ± 0.15	42.3 ± 0.25	0.0 ± 0.00
BPSRB19	40.8 ± 0.15	49.2 ± 0.25	0.0 ± 0.0	0.0 ± 0.00	40.9 ± 0.25	45.3 ± 0.25
BPSRB20	73.7 ± 0.57	44.0 ± 0.15	47.3 ± 0.25	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
BPSRB21	0.0 ± 0.00	44.0 ± 0.25	56.4 ± 0.25	0.0 ± 0.00	37.2 ± 0.25	44.0 ± 0.32
BPSRB22	37.6 ± 0.15	44.0 ± 0.28	39.2 ± 0.18	0.0 ± 0.00	31.1 ± 0.15	0.0 ± 0.00
BPSRB23	52.5 ± 0.28	44.0 ± 0.32	46.6 ± 0.28	0.0 ± 0.00	0.0 ± 0.00	40.0 ± 0.36
BPSRB24	47.2 ± 0.25	44.0 ± 0.25	0.0 ± 0.00	0.0 ± 0.00	38.0 ± 0.15	0.0 ± 0.00
BPSRB25	41.9 ± 0.25	90.0 ± 0.25	36.0 ± 0.57	0.0 ± 0.00	43.9 ± 0.25	0.0 ± 0.00
BPSRB26	65.0 ± 0.20	72.3 ± 0.32	50.1 ± 0.40	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
BPSRB27	54.1 ± 0.20	85.3 ± 0.15	0.0 ± 0.00	0.0 ± 0.00	41.1 ± 0.10	0.0 ± 0.00
BPSRB28	0.0 ± 0.00	60.0 ± 0.00	0.0 ± 0.00	49.4 ± 0.20	0.0 ± 0.00	35.0 ± 0.36
BPSRB29	0.0 ± 0.00	90.6 ± 0.5	0.0 ± 0.00	42.3 ± 0.12	0.0 ± 0.00	51.9 ± 0.25
BPSRB30	40.67 ± 0.28	74.6 ± 0.15	54.0 ± 0.34	0.0 ± 0.00	41.6 ± 0.28	0.0 ± 0.00
BPSRB31	52.3 ± 0.17	72.3 ± 0.25	0.0 ± 0.00	57.4 ± 0.12	0.0 ± 0.00	0.0 ± 0.00
BPSRB32	43.8 ± 0.50	44.0 ± 0.30	40.0 ± 0.36	0.0 ± 0.00	0.0 ± 0.00	37.2 ± 0.25
LSD	0.110	0.152	0.344	0.362	0.681	0.004
CV	0.023	0.008	0.024	0.041	0.025	0.032

Values are represented as mean of percentage of inhibition ± SE

LSD, least significant difference;

CV, Coefficient of variance

with kimura 2-parameter model (the estimated transition/transversion bias, R = 1.52) is shown in Fig. 2. The model was selected based on lowest BIC values. The phylogenetic tree divided turmeric rhizospheric bacterial isolates into two different clades (Clades I and Clades II) with bootstrap value 74%. The rhizospheric bacterial isolates in Clade I, were further divided into two small clades (Clade IA and Clade IB)

Table 2 Plant growth promoting characteristics of rhizospheric bacterial isolates isolated from *Curcuma longa*

Isolate No.	Siderophore (zone in mm)	IAA (μg/ml)	Ammonia (mg/100 ml)	Cellulase (IU/ml)	Amylase (IU/ml)	Xylanase (IU/ml)	Phosphate (zone in mm)	Catalase
BPSRB1	6.2 ± 0.15	17.2 ± 0.02	31.6 ± 0.02	1.84 ± 0.15	3.62 ± 0.05	2.58 ± 0.15	8.0 ± 0.05	+ve[a]
BPSRB2	7.4 ± 0.15	52.3 ± 0.17	67.4 ± 0.02	2.45 ± 0.25	2.19 ± 0.35	3.24 ± 0.05	9.5 ± 0.25	+ve[a]
BPSRB3	6.2 ± 0.15	0.0 ± 0.00	42.4 ± 0.02	3.16 ± 0.02	3.16 ± 0.05	4.72 ± 0.15	7.5 ± 0.15	+ve[a]
BPSRB4	9.5 ± 0.15	60.5 ± 0.05	72.3 ± 0.02	7.86 ± 0.01	8.24 ± 0.15	7.28 ± 0.05	12.4 ± 0.05	+ve[a]
BPSRB5	5.2 ± 0.05	44.1 ± 0.15	27.2 ± 0.02	4.42 ± 0.05	4.18 ± 0.35	4.42 ± 0.25	4.5 ± 0.26	+ve[a]
BPSRB6	6.5 ± 0.05	0.0 ± 0.00	38.6 ± 0.15	1.94 ± 0.03	2.91 ± 0.05	1.56 ± 0.26	3.0 ± 0.17	+ve[a]
BPSRB7	4.2 ± 0.26	46.3 ± 0.01	45.1 ± 0.05	2.81 ± 0.17	2.89 ± 0.05	3.81 ± 0.17	7.5 ± 0.15	+ve[a]
BPSRB8	3.7 ± 0.17	39.2 ± 0.26	15.4 ± 0.02	3.75 ± 0.02	4.76 ± 0.05	7.03 ± 0.35	4.5 ± 0.05	+ve[a]
BPSRB9	6.8 ± 0.15	45.4 ± 0.02	52.1 ± 0.05	4.86 ± 0.15	6.16 ± 0.02	5.45 ± 0.05	5.0 ± 0.05	+ve[a]
BPSRB10	4.5 ± 0.25	0.0 ± 0.00	38.4 ± 0.37	5.41 ± 0.05	5.21 ± 0.37	3.98 ± 0.25	5.0 ± 0.02	+ve[a]
BPSRB11	5.0 ± 0.37	18.6 ± 0.17	56.3 ± 0.01	5.23 ± 0.15	6.63 ± 0.05	4.73 ± 0.02	6.2 ± 0.15	+ve[a]
BPSRB12	5.5 ± 0.22	49.0 ± 0.05	35.4 ± 0.05	2.09 ± 0.03	3.19 ± 0.04	2.61 ± 0.05	9.0 ± 0.37	+ve[a]
BPSRB13	5.8 ± 0.17	34.1 ± 0.35	41.5 ± 0.02	4.92 ± 0.35	5.72 ± 0.05	3.40 ± 0.15	8.5 ± 0.26	+ve[a]
BPSRB14	8.5 ± 0.05	59.4 ± 0.15	69.8 ± 0.02	7.64 ± 0.27	7.89 ± 0.05	7.42 ± 0.25	10.5 ± 0.05	+ve[a]
BPSRB15	6.4 ± 0.02	29.8 ± 0.02	17.3 ± 0.03	6.58 ± 0.05	6.94 ± 0.35	5.77 ± 0.05	4.1 ± 0.15	+ve[a]
BPSRB16	4.5 ± 0.15	46.9 ± 0.17	48.1 ± 0.17	7.24 ± 0.25	6.81 ± 0.02	5.93 ± 0.04	3.4 ± 0.05	+ve[a]
BPSRB17	0.0 ± 0.00	22.4 ± 0.35	17.5 ± 0.02	5.47 ± 0.25	6.75 ± 0.05	4.85 ± 0.05	2.5 ± 0.05	+ve[a]
BPSRB18	3.8 ± 0.05	31.8 ± 0.02	19.6 ± 0.15	4.21 ± 0.05	5.82 ± 0.26	4.24 ± 0.15	3.5 ± 0.15	+ve[a]
BPSRB19	7.5 ± 0.15	0.0 ± 0.00	54.1 ± 0.05	4.86 ± 0.37	1.62 ± 0.05	1.98 ± 0.15	7.1 ± 0.25	+ve[a]
BPSRB20	0.0 ± 0.00	46.6 ± 0.05	32.3 ± 0.15	5.43 ± 0.01	6.23 ± 0.05	4.42 ± 0.02	6.8 ± 0.37	+ve[a]
BPSRB21	5.2 ± 0.10	21.5 ± 0.02	20.9 ± 0.03	2.54 ± 0.05	4.69 ± 0.04	2.23 ± 0.02	8.0 ± 0.25	+ve[a]
BPSRB22	0.0 ± 0.00	41.4 ± 0.35	39.2 ± 0.35	6.34 ± 0.05	5.98 ± 0.05	4.06 ± 0.15	8.5 ± 0.05	+ve[a]
BPSRB23	6.7 ± 0.01	28.9 ± 0.26	66.4 ± 0.27	3.79 ± 0.17	6.09 ± 0.32	3.22 ± 0.05	3.2 ± 0.15	+ve[a]
BPSRB24	0.0 ± 0.00	39.6 ± 0.17	62.8 ± 0.05	0.0 ± 0.00	4.47 ± 0.17	2.91 ± 0.02	6.0 ± 0.35	+ve[a]
BPSRB25	0.0 ± 0.00	18.3 ± 0.02	57.4 ± 0.25	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	5.1 ± 0.26	+ve[a]
BPSRB26	8.2 ± 0.15	37.8 ± 0.05	34.9 ± 0.25	0.0 ± 0.00	5.02 ± 0.05	1.94 ± 0.01	0.0 ± 0.00	+ve[a]
BPSRB27	0.0 ± 0.00	38.4 ± 0.26	62.3 ± 0.05	4.26 ± 0.26	3.15 ± 0.01	0.0 ± 0.00	0.0 ± 0.00	+ve[a]
BPSRB28	0.0 ± 0.00	22.9 ± 0.03	38.8 ± 0.37	5.56 ± 0.15	0.0 ± 0.00	1.84 ± 0.17	0.0 ± 0.00	+ve[a]
BPSRB29	0.0 ± 0.00	16.8 ± 0.05	64.2 ± 0.01	0.0 ± 0.00	3.86 ± 0.05	0.0 ± 0.00	0.0 ± 0.00	+ve[a]
BPSRB30	2.8 ± 0.05	43.1 ± 0.15	55.6 ± 0.05	5.88 ± 0.27	4.27 ± 0.15	0.0 ± 0.00	3.2 ± 0.05	+ve[a]
BPSRB31	4.5 ± 0.26	45.9 ± 0.02	36.7 ± 0.05	0.0 ± 0.00	0.0 ± 0.00	2.21 ± 0.05	0.0 ± 0.00	+ve[a]
BPSRB32	0.0 ± 0.00	34.2 ± 0.01	64.0 ± 0.15	3.47 ± 0.17	2.91 ± 0.02	0.0 ± 0.00	0.0 ± 0.00	+ve[a]
LSD	0.03	0.049	0.003	0.002	0.090	0.001	0.140	
CV%	5.73	10.19	3.48	5.34	5.09	6.94	7.49	

Values are represented as mean ± SE

LSD, least significant difference; CV, Coefficient of variance

[a] indicates confirmation of catalase activity

under a bootstrap support value of 59%. Most of the isolates formed a major clade IA belonged to the *Bacillus* group, along with type strains from EzTaxon databases with bootstrap value of 69%. All the *Staphylococcus* sp. in Clade IB were closely related with a bootstrap value of 74%. In clade II were all the *Lysinibacillus* sp. with type strains with a bootstrap value of 74%.

The phylogenetic tree with gram negative bacterial nucleotide sequences using Maximum-

Table 3 Molecular identification of rhizospheric bacterial isolates based on 16S rRNA gene sequences and their NCBI GenBank accession numbers

Location	Isolate No	Organism Name	Type Strains	Accession No	% of Similarity
Dampa Tiger Reserve Forest	BPSRB1	*Lysinibacillus varians*	*Lysinibacillus sphaericus* KCTC 3346^T	KT028674	99%
	BPSRB2	*Bacillus* amyloliquefaciens	*Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* DSM ^{T}	KT08675	98%
	BPSRB3	*Bacillus subtilis*	*Bacillus subtilis* subsp. *subtilis* NCIB 3610 ^{T}	KT08676	98%
	BPSRB4	*Bacillus amyloliquefaciens*	*Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* DSM ^{T}	KT08677	99%
	BPSRB5	*Bacillus subtilis*	*Bacillus subtilis* subsp. *subtilis* NCIB 3610 ^{T}	KT08678	100%
	BPSRB6	*Bacillus subtilis*	*Bacillus subtilis* subsp. *subtilis* NCIB 3610 ^{T}	KT08679	98%
	BPSRB7	*Bacillus cereus*	*Bacillus cereus* ATCC14579 ^{T}	KT08680	98%
	BPSRB9	*Bacillus subtilis*	*Bacillus subtilis* subsp. *subtilis* NCIB 3610 ^{T}	KT08682	98%
	BPSRB15	*Bacillus* sp.	*Bacillus licheniformis* ATCC14580 ^{T}	KT08688	98%
	BPSRB17	*Bacillus anthracis*	*Bacillus anthracis* ATCC 14578 ^{T}	KU158219	100%
	BPSRB18	*Bacillus* sp.	*Bacillus anthracis* ATCC 14578 ^{T}	KU158220	100%
	BPSRB19	*Bacillus* sp.	*Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* DSM ^{T}	KU158221	99%
	BPSRB21	*Peudomonas stutzeri*	*Pseudomonas stutzeri* ATCC 17588 ^{T}	KU158223	99%
	BPSRB26	*Staphylococcus* sp.	*Staphylococcus hominis* subsp. *hominis* DSM 20328 ^{T}	KU158228	99%
	BPSRB27	*Staphylococcus* sp.	*Staphylococcus hominis* subsp. *hominis* DSM 20328 ^{T}	KU158229	98%
	BPSRB28	*Staphylococcus* sp.	*Staphylococcus hominis* subsp. *hominis* DSM 20328 ^{T}	KU158230	98%
	BPSRB29	*Achromobacter* sp.	*Achromobacter mucicolens* LMG 26685 ^{T}	KU158231	98%
	BPSRB31	*Sphingomonas* canadensis	*Sphingomonas canadensis* FWC47 ^{T}	KU158233	98%
	BPSRB32	*Sphingomonas* sp.	*Sphingomonas wittichii* RW1 ^{T}	KU158234	98%
Reiek Mountain Hills Forest	BPSRB8	*Lysinibacillus sphaericus*	*Lysinibacillus varians* GY32 ^{T}	KT08681	98%
	BPSRB10	*Bacillus subtilis*	*Bacillus subtilis* subsp. *subtilis* NCIB 3610 ^{T}	KT08683	99%
	BPSRB11	*Bacillus amyloliquefaciens*	*Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* DSM ^{T}	KT08684	98%
	BPSRB12	*Bacillus* sp.	*Bacillus cereus* ATCC 14579 ^{T}	KT08685	99%
	BPSRB13	*Bacillus subtilis*	*Bacillus subtilis* subsp. *subtilis* NCIB 3610 ^{T}	KT08686	99%
	BPSRB14	*Bacillus amyloliquefaciens*	*Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* DSM ^{T}	KT08687	99%
	BPSRB16	*Bacillus pumilus*	*Bacillus pumilus* ATCC 7061 ^{T}	KU158218	100%
	BPSRB20	*Peudomonas* sp.	*Pseudomonas baetica* a390 ^{T}	KU158222	98%
	BPSRB22	*Peudomonas* sp.	*Pseudomonas baetica* a390 ^{T}	KU158224	99%
	BPSRB23	*Peudomonas* sp.	*Pseudomonas baetica* a390 ^{T}	KU158225	98%
	BPSRB24	*Staphylococcus* sp.	*Staphylococcus hominis* subsp. *hominis* DSM 20328 ^{T}	KU158226	99%
	BPSRB25	*Staphylococcus* sp.	*Staphylococcus hominis* subsp. *hominis* DSM 20328 ^{T}	KU158227	99%
	BPSRB30	*Sphingomonas* sp.	*Sphingomonas wittichii* RW1 ^{T}	KU158232	98%

likelihood method with kimura 2-parameter model (the estimated transition/transversion bias, $R = 1.52$) is shown in Fig. 3. All the isolates were separated into two clades (clade I and clade II) with bootstrap support value of 88%. In clade I, all the isolates were closely related and they belonged to *Pseudomonas* sp. and *Achromobacter* sp., whereas clade II consisted of three isolates belong to *Sphingomonas* sp.

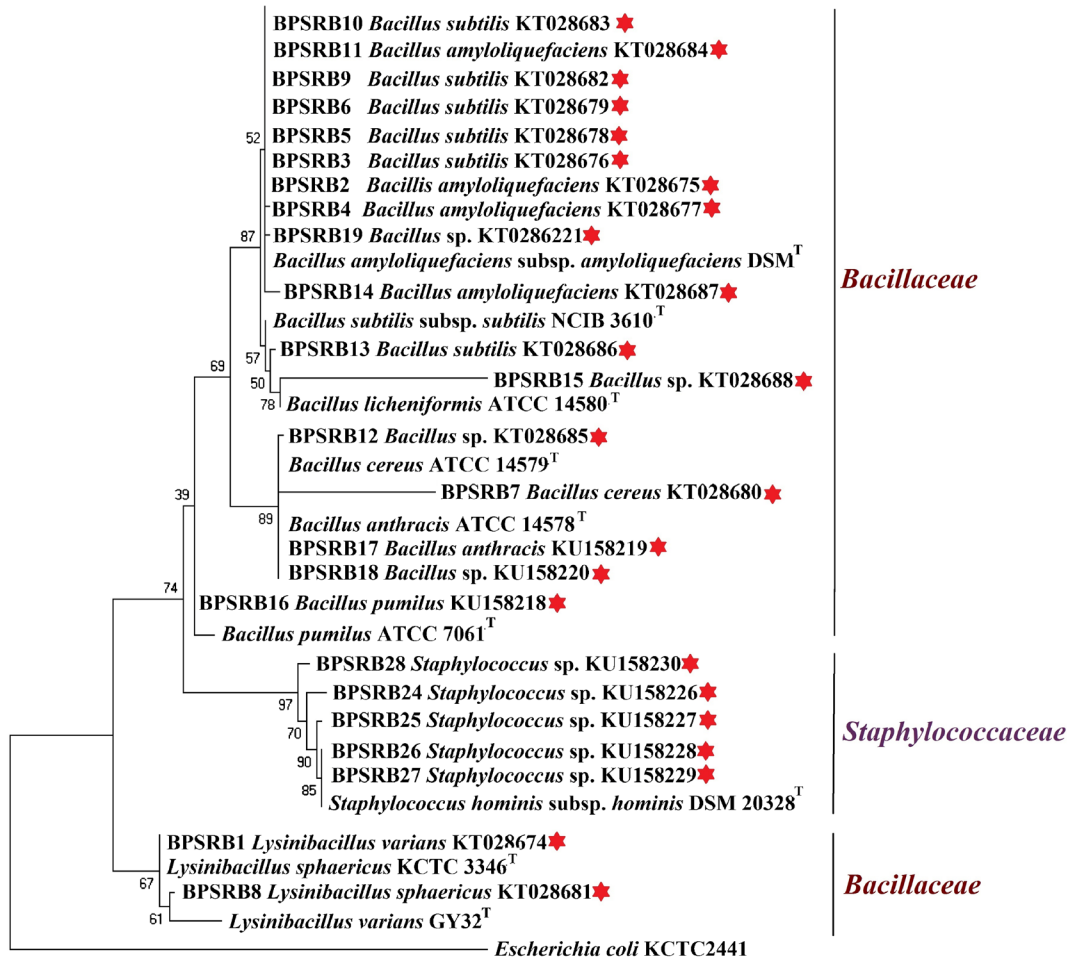


Fig. 2 Phylogenetic relationships based on partial 16S rRNA gene sequences from PGPR rhizospheric Gram positive bacterial isolates with reference strains from EZ-Taxon database. The phylogenetic tree was constructed using maximum-likelihood method. A bootstrap analysis was performed with1000 replicates. The red stars indicate gram-positive rhizospheric bacteria isolated from *C. longa*

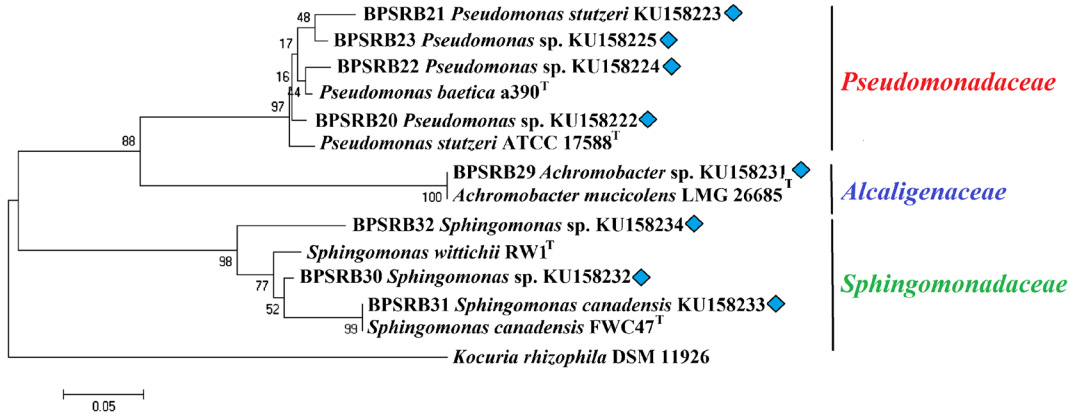


Fig. 3 Phylogenetic relationships based on partial 16S rRNA gene sequences from PGPR rhizospheric Gram negative bacterial isolates with reference strains from EZ-Taxon database. The phylogenetic tree was constructed using neighbor-joining method. A bootstrap analysis was performed with 1000 replicates and the scale bar indicates 0.05 substitutions per site. The blue diamonds indicate gram-negative rhizospheric bacteria isolated from *C. longa*

Detection of nitrogen fixation (nifH) and ACC deaminase (acdS) encoding genes in rhizospheric bacteria

Out of 32 isolates, 12 isolates showed amplification of the *nif*H encoding gene and the desired amplified fragment of about 780 bp was obtained (Fig. 4a). The fragments obtained from strains BPSRB4 and 14 were sequenced and submitted to NCBI GenBank with accession numbers KX709838 and KX709839. The sequenced fragments from strains BPSRB4 and BPSRB14 showed 98% homology with other *nif*H gene using BLAST analysis.

Out of 32 isolates, 12 isolates showed amplification of a fragment size of 1000 bp which is consistent with the *acd*S gene responsible for ACC deaminase activity (Fig. 4b). The fragments from strains BPSRB4 and 14 were sequenced and submitted to NCBI GenBank with accession numbers KX709840 and KX709841. The sequenced fragments from strains BPSRB4 and BPSRB14 showed 97% homology with other *acdS* gene in BLAST analysis.

Plant growth promoting and disease suppression effect

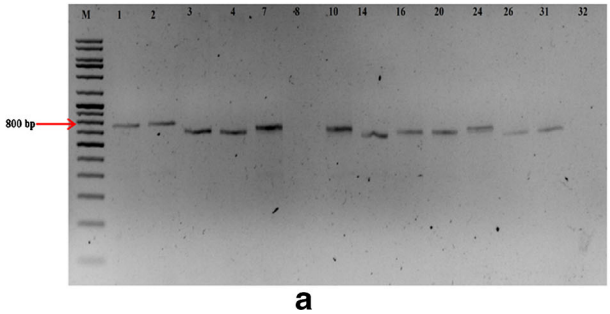
The two isolates BPSRB4 and BPSRB14, which were identified as *Bacillus amyloliquefaciens*, were further evaluated for growth promotion and disease suppression potential on chilli seedlings, under greenhouse conditions (Fig. 5). Application of isolate BPSRB14 resulted in the greatest increase in shoot and root length of chilli plants when compared with the control after 30 and 45 days of sprouting. After 45 days, decrease in disease incidence was observed in both the tested isolates, and strain BSPRB4 showed the greatest decrease in disease index (37.6%) as compared to the control (48.7%) (Fig. 6).

Discussion

The PGPR enhance growth and productivity of agronomically important crops by colonizing on the surface or in the interior of roots (Dutta et al. 2015; Passari et al. 2015a). In the current study, 32 bacteria were isolated from rhizospheric soil of turmeric (*C. longa*) plants from two locations of India. These were screened for a broad spectrum of plant growth promoting attributes and their antagonistic potential against fungal phytopathogens.

Besides showing various PGP activities directly, PGPR can also protect plants indirectly by inhibiting soil-borne pathogens (Bakker et al. 2013). Similar findings were reported previously from bacterial isolates recovered from different rhizospheric soils (Kareem et al. 2014). Furthermore, the obtained isolates demonstrated significant in vitro PGP activity like phosphorous solubilization, ammonia production, IAA production, siderophore production and extracellular enzyme (catalase, amylase, cellulase and xylanase) production.

Among the proposed mechanisms for phosphate solubilization efficiency, Liu et al. (2014) had demonstrated that the production of gluconic acid, lactic acid, malic acid, succinic acid, formic acid, citric acid, malonic acid and tartaric acid are the most common acids which are responsible for phosphate solubilization. In this study, 26 (81.2%) isolates showed phosphate solubilization potential at varying levels by forming clear halo zones surrounding the colony on PKV agar plates and a significant decrease in pH of the medium was recorded, which indicated the production of acids. Previous



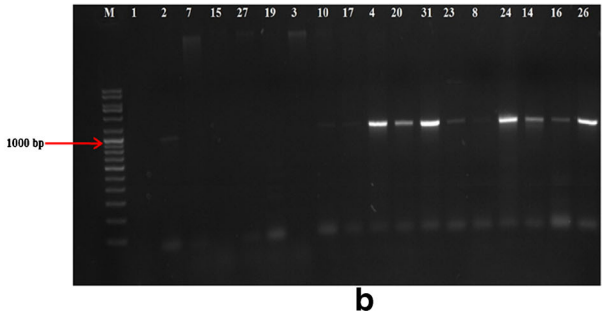


Fig. 4 A Polymerase chain reaction (PCR) based detection of nitrogen fixation (*nif*H) encoding gene; M: low range DNA ruler (3 kb-100 bp); 1 to 32 represents rhizospheric bacterial isolates. 4B Polymerase chain reaction (PCR) based detection of ACC deaminase (*acd*S) encoding gene. M: low range DNA ruler (3 kb-100 bp); 1 to 26 represents rhizospheric bacterial isolates

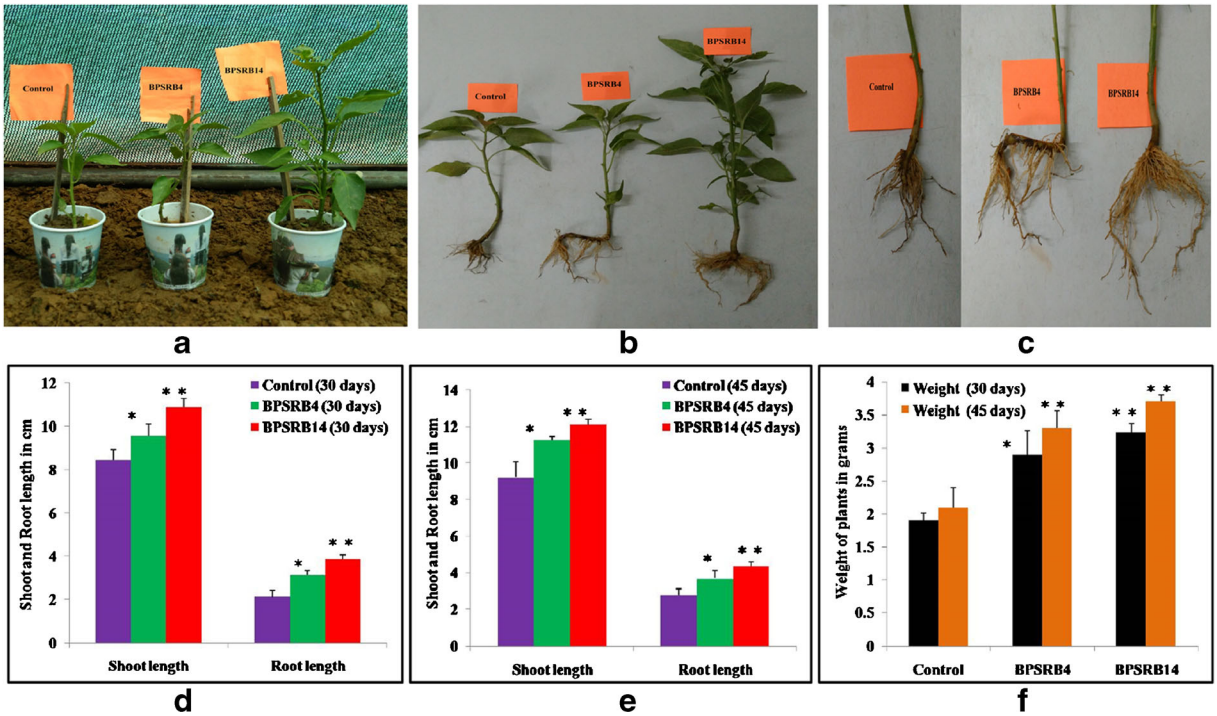


Fig. 5 Evaluation of plant growth promoting potential of isolates *Bacillus amyloliquefaciens* strain BPSRB4 and *Bacillus amyloliquefaciens* strain BPSRB14 to show growth characteristics of chilli seedlings under greenhouse conditions after application of strains. **a** Growth of chilli plants, control, BPSRB4 and BPSRB14, under greenhouse conditions; **b** Comparison of shoot lengths of chilli plants: control, BPSRB4 and BPSRB14; **c** Comparison of root lengths of chilli plants: control, BPSRB4 and BPSRB14; **d** Measurement of shoot and root lengths of plants at 30 days after bacterial inoculation (isolates BPSRB4 and BPSRB14). **e** Shoot and root lengths of treated and control plants at 45 days after bacterial inoculation. **f** Weight of wet plants after 30 and 45 days after bacterial treatment. The results were compared by Students t test (*corresponds to $p < 0.05$, **corresponds to $p < 0.005$). The error bars represent the standard deviation

researchers suggested that phosphate solubilization occurs mainly due to production of microbial metabolites with organic acids which decreases the pH of the culture media (Sahin et al. 2004). Here, the *Bacillus amyloliquefaciens* (strain BPSRB4) showed the highest phosphate solubilization activity after 5 days, similar to the activity of *Bacillus* sp. PnB 1 reported by Jasim et al. (2013) who stated that *Bacillus* sp. strain PnB1 exhibited the greatest phosphate solubilization efficiency, among 12 bacterial strains obtained from *Piper nigrum*.

Siderophore production is another indirect mechanism of PGPR to enhance plant growth along with extracellular enzyme (e.g. catalase, cellulase xylanase and amylase) production (Kavamura et al. 2013) and ammonia production (Marques et al. 2010; Minaxi et al. 2012). Among the isolates tested for PGPR in the current study, 18 (56.25%) showed in vitro siderophore production. Interestingly, all the isolates which showed antagonistic potential were positive for siderophore production. It has been reported that the ability of PGPR to produce siderophore is directly related to the antagonistic potential, as siderophore production deprive the phytopathogenic microflora from iron, thus limiting their growth (Kloepper et al. 1980; Wahyudi et al. 2011). *B. amyloliquefaciens* strain BPSRB 14 produced the highest amount of siderophore, which is in agreement with Clark et al. (2014), who stated that *B. amyloliquefaciens* ATCC 23843 cultures grown with ferric ammonium citrate as the iron source showed production of siderophore.

Among lytic enzymes, cellulase is important for the functioning of depolymerization of cellulase into fermentable sugar (Xing-hua et al. 2009). Screening of cellulase production on CMC agar plates amended with 1% CMC showed a clear zone around the colonies in 27 (84.3%) isolates. Quantitative estimation results showed that cellulase production was ranged from 1.84 to 7.86 IU/ml, which was higher as compare to Kumar

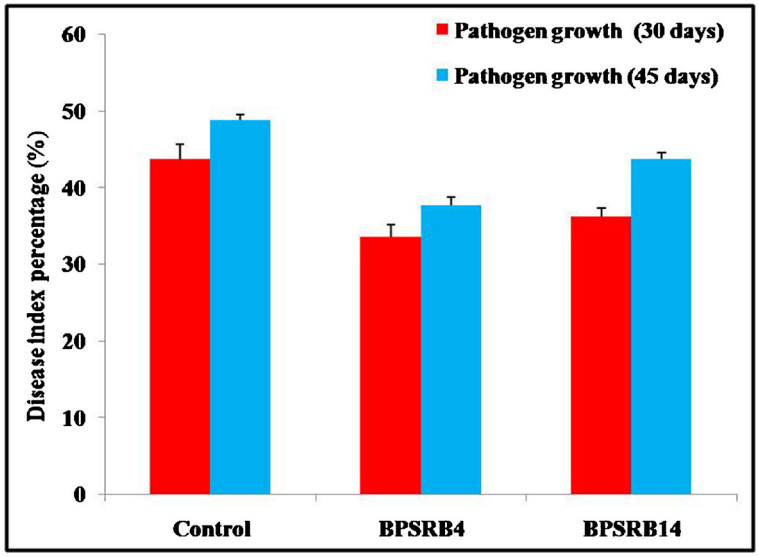


Fig. 6 Disease suppression potential of isolates *Bacillus amyloliquefaciens* strain BPSRB4 and *Bacillus amyloliquefaciens* strain BPSRB14 at 30 and 45 days on chilli seedlings against *F. oxysporum* f. sp. *capsici*. The results were compared by Students t test (*corresponds to $p < 0.05$, **corresponds to $p < 0.005$). The error bars represent the standard deviation

et al. (2012b), who stated that 60% of the isolates showed 0.40 to 0.75 IU/ml of cellulase production. In the current study, *Bacillus amyloliquefaciens* (strain BPSRB4) demonstrated the highest amount of cellulase production activity (7.84 IU/ml). Similarly, several researchers have previously screened rhizospheric bacteria for cellulase production and correlated that activity with their plant growth promoting abilities (Singh et al. 2004). Further, isolate *Bacillus amyloliquefaciens* strain BPSRB4 produced the greatest amount of amylase enzyme (8.24 IU/ml) which is similarly reported by other research on bacteria isolated from other sources (Swain et al. 2006; Deb et al. 2013). In addition, isolate *Bacillus amyloliquefaciens* strain BPSRB14 showed the greatest xylanase enzyme (7.42 IU/ml) production. This finding was similarly reported by Amore et al. (2015), who isolated *Bacillus amyloliquefaciens* strain XR44A from western ghat region, which produced xylanase enzyme (10.5 IU/ml). All the rhizospheric bacteria analyzed in the current study exhibited catalase production which is in agreement with Kumar et al. (2012a) who showed that rhizospheric bacteria having PGP activity produced catalase.

Ammonia production also plays an important role in plant growth by accumulating nitrogen and increasing root and shoots growth as well as plant biomass (Marques et al. 2010). Among the tested isolates in the present study, *Bacillus amyloliquifaciens strains* BPSRB4 and BPSRB14 produced the highest amounts of ammonia.

All isolates screened for PGP activities were identified based on 16S rRNA gene sequence similarity and showed 98–100% similarity with type strains from NCBI GenBank database. Phylogenetic analysis based on 16S rRNA gene sequences clearly showed that the Gram positive *Bacillus* and *Staphylococcus* were clustered separately and formed different clades among the Gram positive bacterial isolates. Likewise, among the Gram negative bacteria three gram negative genera formed different clades. Similar findings were demonstrated by Jasim et al. (2013). To the best of our knowledge, isolate BPSRB31 (*Sphingomonas canadensis*) and BPSRB1 (*Lysinibacillus varians*) were isolated for the first time from rhizospheric soil of *Curcuma longa* plant though, Zhu et al. (2014) isolated *Lysinibacillus varians* from sediments of river and Abraham et al. (2013) isolated *Sphingomonas canadensis* sp. nov. from water.

The results of this study demonstrated that the plants treated with *Bacillus amyloliquifaciens strains* BPSRB4 and BPSRB14 showed increased root length, shoot length and plant weight as compare to the control after 30 and 45 days of inoculation. These results are in agreement with previous reports that stated that rhizosphere bacteria treatment increased plants roots, shoots and biomass (Goudjal et al. 2013; Liu et al. 2016). In addition, in the current study isolates BPSRB4 and BPSRB14 also demonstrated antifungal activity against *F. oxysporum* f. sp. *Capsici,* under green-house condition. These *Bacillus amyloliquefaciens* with effective

biocontrol activity are promising for use in the agricultural sector for plant growth promotion to suppress fungal diseases.

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Compliance with ethical standards Authors declared that this manuscript have not published elsewhere. All the authors have read very carefully and approved current version of this manuscript. All authors also declared that the data or images have not manipulated. This article does not contain any experiments with human participants or animals.

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

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