Isolation and characterization of plant growth promoting bacteria from soybean rhizosphere and their effect on soybean plant growth promotion

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Abstract

Plant growth promoting bacteria (PGPB) possess the ability to aggressively colonize the rhizosphere or plant roots or both when applied to seed or crops that enhance the growth and yield of plants. The aim of the present study was to isolate potent bacteria from the soil and enhance growth of soybean crop by using it. Ten bacterial isolates were purified and screened *in vitro* for plant growth promoting (PGP) characteristics. Among the 10 isolates, 9 found with phosphate solubilizing activity, 5 with IAA producing activity while 3 with N_2 fixation activity. Microbial strains SJ-5 showed positive result for all the PGP properties and further characterized by biochemical and molecular method. Based on biochemical and 16S rRNA gene sequence analysis it was found *Bacillus* sp. Plant inoculation studies indicated that this PGPB strain provided a significant increase in shoot and root length, and shoot and root biomass. A significant increase in number of lateral roots was observed over the un-inoculated control. The study indicates the potential of this PGPB for inoculums production or biofertilizer for enhancing growth and nutrient content of soybean.

Keywords: Plant growth promoting bacteria, Soybean, Phosphate solubilization, 16 rRNA

Introduction

Agricultural products are the primary dietary source of proteins, carbohydrates, fat, vitamins and other nutrients, and soybean (*Glycine max* L. Merrill) is one of the best source of 'all in one' with a very high nutritional value. For the good health, healthy food is the main concern which is directly related to the present agro-ecosystem. With the increasing population, the need of dietary sources increasing day by day drastically that ultimately leads to use of different chemicals in the form of phytohormones and pesticides to promote plants growth and amelioration of pathogens respectively, to get quick result. In the present scenario of agro-world the first priority of the cultivator is to produce a healthy plant, i.e. plant without any infectious disease, and to gain high yield in any adverse condition.

Therefore, there is an urgent requirement to develop a strategy for providing plant protection along with crop yeild improvement in a manner that will not affect ecological balance in agro-ecosystem. soil-borne beneficial bacteria knowna as Plant growth promoting bacteria (PGPB) possess the ability to aggressively colonize the rhizosphere or plant roots or both when applied to seed or crops that enhance the growth and yield of plants (Ashrafuzzaman et al., 2009 and Kaymak, 2011). The plant growth promoting (PGP) properties of the PGPB is because of its ability to synthesize and secrete metabolites directly stimulating plant growth and several mechanisms have been postulated to explain how it can benefit the host plant. Root is the main part of the plant responsible for nutrient acquisition from the soil and form a solid base to grow up shoot. PGPB enhanced root growth and hence nutrient uptake by secreting plant growth hormone indole acetic acid (IAA) (Egamberdieva and Kucharova 2009; Piccoli et al. 2011; Duca et al. 2014). Ethylene, the only gaseous hormone responsible for the plant growth inhibition, level increases in the plant in stressed conditions. Some PGPB reduced this level by degrading 1-aminocyclopropane-1-carboxylate (ACC), immediate precursor of ethylene, into 2-oxobutanoate and ammonia by production of ACC-Deaminase enzyme and indirectly increase plant growth (Kumari et al. 2015). Bacterial polysaccharides can bind soil particles to form microaggregates and macroaggregates. Plants treated with exopolysaccharide (EPS) producing bacteria display increased resistance to water stress due to improved soil structure (Sandhya et al. 2009). EPS can also bind to cations including Na^+ thus making it unavailable to plants under saline conditions. Some microbial strains produce cytokinin and antioxidants, which result in abscisic acid (ABA) reduction and degradation of reactive oxygen species (ROS). In addition, nutrient elements, such as phosphorus, potassium, iron, zinc and copper, possess limited mobility in the soil and are present in insoluble from. PGPB can solubilize insoluble inorganic phosphate compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate by the production of organic acids such as gluconic acid, citric acid etc. and thus convert it into bioavailable phosphorus (Richardson et al. 2009; Khan et al. 2009; Egamberdiyeva 2007). Incapability of plants to use atmospheric nitrogen is one of the major constraints. Some PGPB convert atmospheric nitrogen to ammonia by nitrogenase enzyme complex and thus make it available for plants (Santi et al. 2013). Likewise phosphorus, Iron is also present in the environment in inaccessible form (Fe^{3+}) for plant. PGPB provide this iron to plant by chelating it from the soil through siderophores production (Fouzia et al. 2015; Boukhalfa and Crumbliss 2002). Proteases secreted by PGPB breaks down complex proteins available in soil into plant usable amino acids. They catalyze total hydrolysis of proteins to peptides; there by function as degradative enzymes (López-Otín et al. 2002). Other then these plant growth promoting activities, some PGPB secrets cell wall degrading enzyme, antibiotics and fungicidal compounds that help in get rid of fungal pathogens (Dey et al., 2004; Lucy et al., 2004).

Material and methods

Sampling site and soil sample collection

The sampling site chosen for the sample collection was Mandsaur district of the Malwa region of Madhya Pradesh (M.P.), India, situated between 74◦ 42’30” and 75◦ 50’20” to the East Longitudes and 23◦45’50” and 25◦ 2’55” to the North Latitudes. This is one of the prominent soybean rich region of India. Healthy soybean plant samples with bulky rhizospheric soil were collected by wearing pre-sterilized gloves (Genetix), from different location of

Mandsaur and placed individually in pre-sterilized polybeg (Himedia, India). Followed by collection, plant samples were brought to the laboratory of Mody University, Lakshmangarh, Rajasthan, India for further bacterial isolation.

Isolation of bacterial strains

Isolation of bacterial strains from different soil samples have been done by using serial dilution method. One gm of soil was homogenized in 9 ml of sterilized physiological saline water (0.85%) and incubated at room temperature (RT) for 5 minuts. Serial dilution was done in the saline water up to 10^{-6} and 500 µl of different dilute sample were spreaded on nutrient agar (NA) plates. Plates were incubated at 28± 2^{o}C in BOD incubator for 24 hr and observed for the bacterial growth. Single colony from different soil sample plates have been picked and streaked on NA plates for pure culture.

Screening of potential isolates through characterization of plant growth promoting properties

Phosphate solubilization

The phosphate-solubilizing test was done by using National Botanical Research Institute's Phosphate (NBRIP) growth medium (Nautiyal 1999). All the ten bacterial cultures were spot inoculated at the centre of the NBRIP agar plates and incubated for 5-6 days at 28 ± 2°C. After incubation period, plates were observed for the clear halo zone formed around the bacterial colony and diameter of the colony and clearing zone were measured. P-solubilization index was calculated by the following formula-

$$\text{P-solubilization index} = \frac{\text{Colony diameter + Clearing zone}}{\text{Colony diameter}}$$

IAA production

IAA production was detected according to method described by Patten and Glick (1996) with little modification. 50 µl of overnight grown culture of all the bacterial isolates were inoculated into 20 ml of NB supplemented with 500 ppm filter-sterilized (Millipore membranes, pore size, 0.22 µm, Millipore corporation, Bedford, MA, USA) L-tryptophan and incubated on an orbital shaker at 28 ± 2°C at 150 rpm for 48 h. IAA quantification was done by using bacterial supernatant after centrifugation at 6000 rpm for 10 min. 4 ml of Salkowski's reagent added in 1 ml of supernatant and incubated on RT for 20 minutes. Absorbance of the developed pink/red color were taken at 535 nm and concentration was calculated using standard curve of IAA (10–100 ppm)

ACC deaminase production

Qualitative assay for ACC deaminase activity was determined by employing method described by Glick et al. (1999) with little modification. 100 µl of overnight grown culture of all the bacterial isolates were inoculated into liquid Dworkin and Foster (DF) salt medium supplemented with 3 mM ACC. DF with 2 g $(NH_4)_2SO_4$ was used as positive control while only DF was kept as negative control. Utilization of ACC by PGPB was determined if ACC containing DF salt medium turned turbid which is considered as an indication of the presence of ACC deaminase.

N_2-fixation test

The presence of nitrogen fixation capabilities were checked by allowing the bacterial strains to grow in medium devoid of nitrogen. Bacterial strains were streaked on Jensen's medium (Himedia, India) and incubated at 28 ± 2°C for 4-5 days. After incubation plates were observed for the bacterial growth.

Siderophore production

Siderophore production assay was performed by the method described by Panhwar et al. (2012) using blue indicator dye, crome azurol S (CAS). All the bacterial isolates were spot inoculated in the centre of CAS agar plates and incubated at 28 ± 2°C for 4-5 days. Bacterial isolates exhibiting an orange halo after incubation were considered positive for the production of siderophores. Siderophore production index (SPI) was calculated by using following formula-

$$\text{Siderophore production index} = \frac{\text{Colony diameter} + \text{Orange zone diameter}}{\text{Colony diameter}}$$

EPS production

EPS producing activity of the bacterial isolates was done according to the method described by Qurashi and Sabri (2011) with little modification. 200 µl of overnight grown cultures of all bacterial isolates were inoculated in 100 ml of NB medium and incubated at 28 ± 2°C for 72 h at 150 rpm in shaking incubator. After incubation, supernatant was collected with centrifugation at 12000 rpm for 10 min at 4°C and pre-chilled acetone was added to supernatant in 3:1 ratio. Formation of precipitation was considered as positive result for EPS production. For quantitative analysis, after 48 h incubation, precipitated EPS was collected through centrifugation at 15000 rpm for 20 min at 4°C and pellets were dried at 60 °C in oven for 24 h. EPS was quantified in terms of gm 100 ml $^{-1}$culture through weighing of dried EPS.

Effect of bacterial isolates on plant growth

To check the effect of screened isolates on plant growth, soybean seeds were surface sterilized with 0.1 % $HgCl_2$ and 70 % ethanol for 3 min and washed repeatedly with Milli Q water (Millipore, Germany) prior to sowing. Overnight grown bacterial cultures were centrifuged at 10,000 rpm for 20 min and the pellets were washed with 10 mM phosphate buffer saline (PBS, pH 7.2). Pellets were re-suspended in PBS containing 0.1% carboxymethylcellulose (CMC) and the OD^{600} adjusted to 0.2 (10^8 CFU ml^{-1}). Surface sterilized seeds were placed in PBS containing 0.1% carboxymethylcellulose (CMC) as a binder for 1 h, followed by drying under air in laminar. Sterile conditions were maintained throughout the experiment. Seeds immersed only in autoclaved Milli Q water (non-microbiolized) treated as control. Soybean seeds were sown in the small polypropylene cups containing autoclaved soil and maintained at 26 °C with 16 h/8 h light/dark photoperiod and 80 % humidity in plant growth chamber for 15 days.

Sample collection and plant growth parameter studies

Plants were carefully uprooted without causing any damage to root tissues 15 days after the sowing. Three plants were sampled from each replication of the treatment in randomized fashion and washed with Milli Q water. Different plant growth parameters such as root and shoot length, root and shoot fresh weight, number of lateral roots were analyzed.

Biochemical characterization of plant growth promoting bacterial isolate

Biochemical characteristics of the potent isolate SJ-5, like Gram reaction, catalase reactions, endospore and capsule staining, motility test, mannitol fermentation test, urease activity, amylase test were determined following the

standard procedures. Gram reaction, endospore staining and capsule staining were performed with standard kit (Himedia, India). Catalase test was performed by adding 100 µl overnight grown bacterial culture in 1 ml of H_2O_2 and observed for gas bubble formation. Motility test, mannitol fermentation test, urease activity and amylase test were performed by inculcating bacteria on 0.35 % NA medium, phenol red mannitol broth medium (pH 7.3), urea broth and starch minimal medium respectively.

Molecular characterization of plant growth promoting bacteria

Bacterial genomic DNA was isolated by Bollet et al. (1991) method with little modification. Quantitative estimation of the DNA was done spectrophotometrically by using biophotometer (Eppendorf, India) that is based on Beer Lamberts law while for the qualitative estimation agarose gel electrophoresis was performed and observation for the DNA band was done by visualizing gel for the DNA band in UVITECH gel doc system. PCR amplification of the 16S rRNA gene from genomic DNA of the bacterium isolate SJ-5 was done by using universal 16S rRNA primers: *27F*- AGAGTTTGATCMTGGCTCAG and *1492R*-CGGTTACCTTG TTACGACTT in Thermal cycler (Kyratec, Australia). The 25 µl of reaction mixture consisted of 100 ng of genomic DNA, 1 µl Taq polymerase (3U/ul), 2 µl of 10× buffer, 2 µl dNTP mix (10mM), 2 µl $MgCl_2$ (25mM) and 1 µl of each primer (10 pmol/µl). Amplification was performed under the following PCR conditions: initial denaturation at 94 °C for 4 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1.30 min and a final extension at 72°C for 10 min. For the assessment of gene, PCR product was loaded in the 1 % agarose gel (wt/vol) containing EtBr and run for 45 min at 85 Volts in 1X TAE buffer. Observation for the DNA band was done by visualizing gel for the DNA band in UVITECH gel doc system.

16S rRNA gene sequence homology and phylogeny analysis

Molecular identification of the bacterium SJ-5 was done by homology analysis of 16S rRNA gene sequence obtained through sequencing, by using BLAST tool of NCBI available at the site http://blast.ncbi.nlm.nih.gov/. Based on maximum identity score first ten sequences were selected for further analysis. Selected sequences were aligned using Clustal W and checked for the gap. Phylogenetic and molecular evolutionary analysis was performed using MEGA 6 software (Tamura et al 2013).

Sequence submission and culture deposition

The nucleotide sequence of the 16S rRNA gene was submitted to NCBI Genbank and accession number provided by the NCBI is KJ 184312. Bacterial culture was also deposited to Microbial Culture Collection (MCC), Pune with accession number 'MCC-2069'.

Results

Bacterial isolation

For the preliminary analysis of potential plant growth promoting bacteria 10 different bacterial strains were isolated from the soil sample and designated as SJ-1, SJ-2, SJ-3, SJ-4, SJ-5, SJ-6, SJ-7, SJ-8, SJ-9 and SJ-10.

Plant growth promoting activities

Functional characterization of direct plant growth promoting activities was done by growing all bacterial isolates on different specified medium. Except SJ-4, all isolates were found with P-solubilizing activity while few isolates were

found positive for other properties. Among the all isolates SJ-2 and SJ-5 were found most potent as both of these possess all the PGP properties. Based on the plant growth promoting activities shown by all isolates, SJ-2 and SJ-5 bacterial isolates were selected for further to check effect on soybean plant growth (Fig 1). The solubilization index and presence and absence of functional characteristics measured are shown in Table 1.

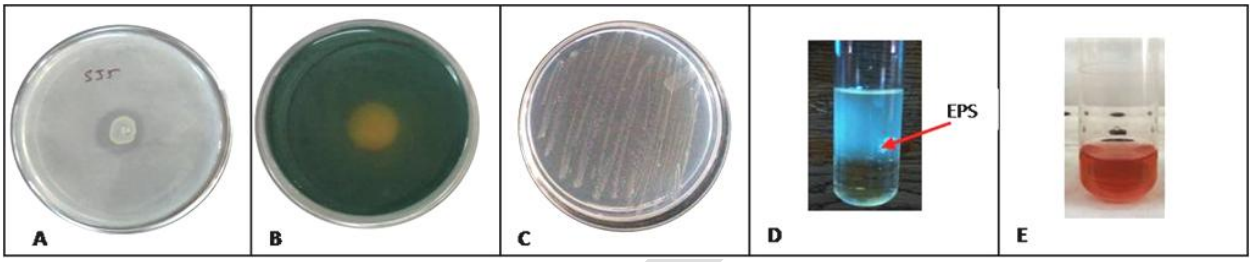

Fig. 1 PGP properties of *Bacillus* sp. SJ-5 **A.** Phosphate solubilization **B.** Siderophore production **C.** Nitrogen fixation **D.** EPS production **E.** IAA production

Table 1 Plant growth promoting characteristics of bacterial isolates

Bacterial strains	Plant growth promoting properties					
	Phosphate solubilization (SI)	Siderophore production (SPI)	EPS production (gm /100ml)	IAA production (µg/ml)	ACC deaminase (+/-)	N_2 fixation (+/-)
SJ-1	1.2±0.18	Nil	Nil	Nil	+	-
SJ-2	1.9±0.65	2.87±0.12	1.2±0.11	15.3±0.58	+	+
SJ-3	1.32±0.37	4.2±0.19	Nil	Nil	-	+
SJ-4	Nil	Nil	0.8±0.09	11.2±0.43	+	-
SJ-5	2±0.25	3.42±0.42	1.5±0.21	19±0.6	+	+
SJ-6	2.41±0.26	3.2±0.32	Nil	Nil	+	-
SJ-7	1.52±0.41	Nil	Nil	8.7±0.29	-	-
SJ-8	2.3±0.14	3.14±0.3	1.6±0.14	9.7±0.32	-	-
SJ-9	1.08±0.23	Nil	0.5±0.17	Nil	+	-
SJ-10	2.2±0.11	1.5±0.17	1.2±0.2	Nil	-	-

Values are the means of three replications ± S.E

Effect of bacterial isolates on plant growth

Plant-microbe interaction in the rhizosphere plays an important role in plant growth. In an experiment conducted in the plant growth chamber, *Bacillus* sp. SJ-5 showed a significant increase in percentage of healthy plants and growth-promotion characteristics such as root, shoot length and number of lateral roots compared to other treatments. SJ-2 also showed plant growth promoting characteristic but less as compare to SJ-5. SJ-5 enhanced shoot length by 26.7 % and root length by 292% over control plant while SJ-2 was found with 11% increase in shoot

length and 211% in root length. Similarly, number of lateral roots in SJ-5 treated plant (20) found 300% more than that of control plant (5) while in SJ-5 (14) it was observed 180% more. Likewise length and fresh weight of the root and shoot of SJ-5 treated plant also found significant higher than the control plant (Table 2). Based on the comparative plant growth parameter results, SJ-5 was found potent growth promoter.

Table 2 Effect of SJ-2 and SJ-5 on soybean plant growth promoting parameters

Treatment	Shoot length (cm)	Root length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Lateral roots
Control	4.5±0.27	2.6±0.2	0.045±0.003	0.425±0.03	5±0.21
SJ-2	5±0.18	8.1±0.11	0.082±0.001	0.45±0.02	14±0.17
SJ-5	5.7±0.3	10.2±0.31	0.093±0.002	0.493±0.02	20±0.28

Values represent the mean of 3 replications

Biochemical characterization of plant growth promoting bacterial isolate

Biochemical characterization was done for preliminary identification of bacterial isolate SJ-5 and it was found rod shaped, gram positive, endospore and capsule forming bacterium. SJ-5 found positive for the catalase test that shows it belong to *Bacillus* genus. Further confirmation was done by checking the endospore and capsule formation properties.

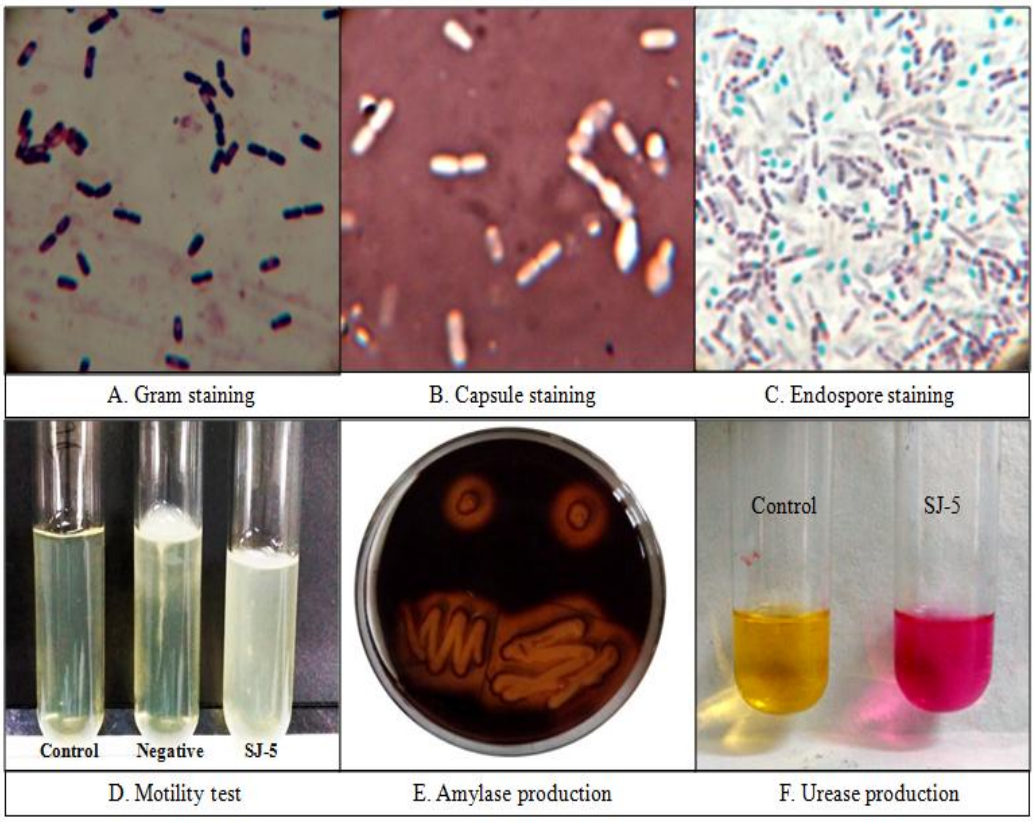

Fig. 2 Biochemical characteristics of *Bacillus* sp. SJ-5

On the motility test medium, it was found motile as growth was observed through the medium instead of growth in inoculation line. It was also found positive for the amylase and urease enzyme test (Fig 2). Hence, based on the biochemical results stain was identified as *Bacillus* sp. and further confirmation was done by molecular characterization.

Molecular characterization of plant growth promoting bacterium and phylogeny analysis

Molecular characterization of plant growth promoting bacterium SJ-5 was done by amplifying 16S rRNA gene from genomic DNA by using universal 16S rRNA primers. On the agarose gel a sharp band around 1.5 kb was observed (Fig 3). Sequence homology and phylogenetic analysis of the obtained sequence, shown similarity of the strain with *B. cereus* and *B. thuringiensis* and hence, bacterium was confirmed as a member of genus *Bacillus* and submitted by the name of *Bacillus* sp. SJ-5 in the NCBI Genbank (Fig 4). Accession number provided by NCBI to bacterium is KJ 184312. Bacterial culture was also deposited to Microbial Culture Collection (MCC), Pune with accession number MCC-2069.

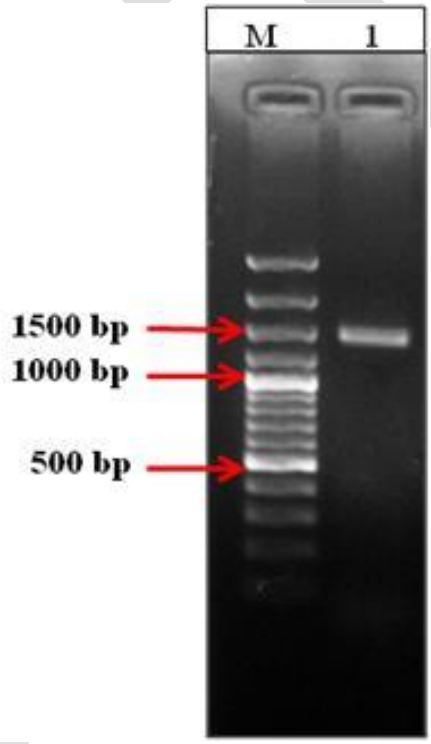

Fig. 3 PCR amplification of 16S rRNA gene of *Bacillus* sp. SJ-5; Lanes **M**-Marker (100 bp plus, Thermo scientific); **1**-16S rRNA gene of SJ-5

Discussion

Interaction of beneficial microbes with plant root in the rhizospheric region help in plant growth promotion resulted in high yield. In the present investigation 10 different bacterial isolates were isolated from the different soil sample collected from Mandsaur (M.P.) and Kota (Rajasthan) district of India. All the isolates were screened for the plant

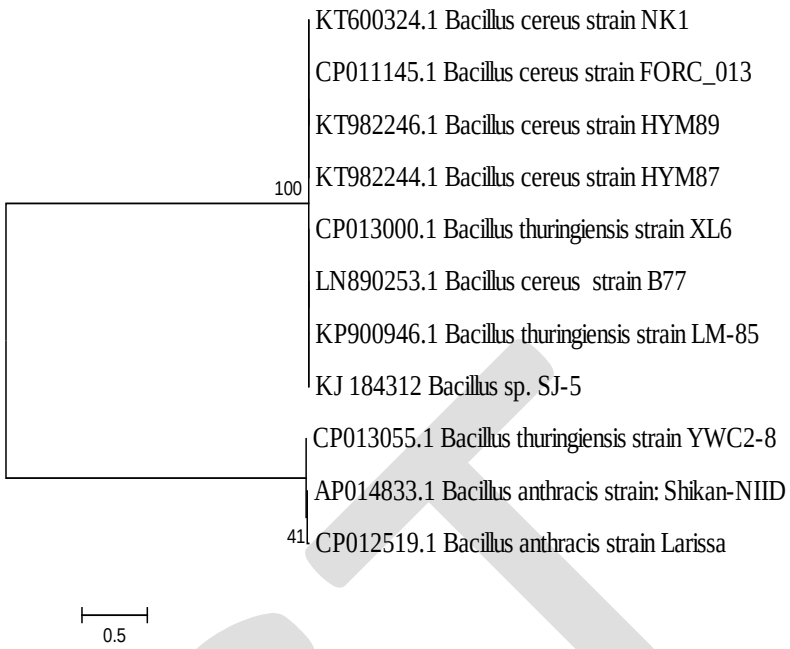

Fig 4 Evolutionary relationships of *Bacillus* sp. SJ-5.The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method

growth promoting activities such as phosphate solubilization, siderophore production, nitrogen fixation, IAA production, ACC deaminase production and EPS production, and were found with the differential PGP activities. About 90% isolates were found with P-solubilizing activities while 60 % for the siderophore and EPS production activities. Only 30% isolates found to have the ability to grow on nitrogen free medium. Isolation and characterization of PGP activities of beneficial microbes is the root step of any plant microbe interaction studies. Yadav (2013) have isolated about 185 bacterial isolates from rhizosphere associated soils of *V. angularis, V. aconitifolia V. mungo* and *V. radiata* and tested their ability for IAA synthesis. Among the 266 bacterial isolates isolated from the 24 rhizospheric soil samples of *Ocimum* sp.l , about 86.46% bacterial isolates showed ammonium production, 89.09% exhibited phosphate solubilization, 87.59% catalase production and 7.14% showed positive reaction for HCN production for the *in vitro* plant growth promoting traits analysis (Saharan and Verma 2015).

Recently Tan et al. (2015) have isolated 13 bacterial strains based on the abilities of phosphate and potassium-solubilizing while earlier Bai et al. (2002) isolated 14 bacterial strains of putative endophytic bacteria, not including endosymbiotic *Bradyrhizobium* strains, from surface-sterilized soybean root nodules. Plant growth parameters such as root and shoot length, root and shoot fresh weight, number of leaves and lateral roots are the measure of plants healthiness and considered for the comparative study between different treatments. PGPB uses different ways to proliferate plants growth in direct manner. PGPB produces organic acid to solubilize insoluble phosphate present in the soil, fix atmospheric nitrogen and supply it to plants; produce siderophores which chelates iron from the soil and provide it to plant cells; synthesize different plant growth phytohormones such as auxins, cytokinins, or gibberellins which enhances plant growth; and synthesize the enzyme ACC deaminase which lowers

plant ethylene levels. At seedling stage, the PGPB may help to provide the plant with a sufficient amount of iron and phosphorus from the soil and also stimulate cell division by providing appropriate levels of phytohormones. Indirectly, PGPB secreted different cell wall degrading enzymes against phytopathogens and elicited induced systemic resistance to promote plant growth (Glick 2015). In the present study SJ-5, confirmed as *Bacillus* sp. SJ-5 has shown most promising results as it found with all the PGP activities. A lot of studies have been done in the past years to characterize plant growth promoting properties of bacteria and reported their role in improving plant growth in normal as well as stressful condition (Belimov et al. 2009; Heidari and Golpayegani 2012; Nadeem et al. 2010; Tank and Saraf 2010; Glick 2012). In our study, among the nine P-solubilizing isolates, three were found with more than 2 solubilization index. An inverse relationship between pH and soluble phosphate was reported earlier by Illmer and Schinner (1995) and Hwangbo et al. (2003). Vyas and Gulatti (2009) showed that phosphate-solubilizing *Pseudomonas* spp. increased both the growth and phosphorus content of maize. Kannahi and Kowsalya (2013) reported phosphate solubilization by *B. subtilis* and *P. fluorescens.*

Iron, which is essential for metabolism of plants and bacteria is present in the soil in largely insoluble ferric form that's how not readily available to bacteria and many host species actively withhold iron from infecting bacteria (Ratledge and Dover 2000). We found SJ-5 with siderophore production activity in CAS agar plates that is a requisite to scavenge iron from the soil and make it available to the host organism. Siderophore produced by the bacteria only under iron-limited conditions. High-affinity iron uptake systems of the PGPRs by siderophore secretion may impede with iron nutrition of many pathogenic bacteria and fungi leading to their suppression (Sharma et al. 2003). Siderophore production activity of the *Bacillus* sp. have been reported by many researchers (Zhu and Yang 2015; Xu et al. 2014; Jikare et al. 2013; Luo et al. 2012). Sivasakthi et al. (2013) have observed siderophore producing activity of *P. fluorescens* and *B. subtilis* isolated from paddy rhizosphere soil.

IAA may act as important signal molecule in the regulation of plant development and hence very essential plant growth hormone. Jangu and Sindhu (2011) stated that IAA produced by bacteria positively affected the plant growth and nodulation in green gram (*V. radiata*) and black gram (*V. mungo*). Idris et al. (2007) have shown that *B. amyloliquefaciens* FZB42 influence plant growth by production of IAA in a tryptophan dependent manner while Mishra and Kumar (2012) have investigated plant growth promoting and phytostimulatory potential of *B. subtilis* and *B. amyloliquefaciens.* Shim et al. (2015) have shown that *Bacillus* sp. JH 2-2 promote mustard plant growth through IAA production. Many other reports were also published on the IAA production activity of *Bacillus* sp. (Boiero et al. 2007; Ab Aziz et al. 2015; Meng et al. 2016). Glick (2005) reported the significance of the role of ACC deaminase in the regulation of a plant hormone ethylene and enhancement of the growth and development of plants. In our studies we found that SJ-5 produces 19 µg/ml IAA which is very significant. SJ-5 growth on DF medium shows its ACC deaminase producing activity which is earlier also reported by many researcher for *Bacillus* and various other PGPR such as *Enteobacter cloacae*, *Klebsiella pneumonie* sp., *Pseudomonas* sp. ACP, *P. putida* strain UW4 and *S. quinivorans* SUD165 (Shah et al. 1998; Belimov et al. 2005). Bacterial isolate SJ-5 significantly increased shoot and root length, shoot and root dry weight, and lateral root number also. The plant growth promotion could be the result of the beneficial functions of applied PGPR isolates, like plant growth hormone production, nitrogen fixation, and P solubilization.

Conclusion

This is a basic study that has provided an insight into the role of bacterial isolate in the soybean plant growth promotion. These studies show that the bacterium SJ-5 having all the PGP properties and can promote soybean plant growth and inhibits the fungal pathogens also. This bacterium may be used as efficient PGPB for soybean production in farmer's fields. It is an environment friendly and cost effective technology.

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