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Characterization of effective bio-control agent *Bacillus* sp. SRB 27 with high salt tolerance and thermostability isolated from forest soil sample

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A potential antagonist *Bacillus* sp. SRB 27, against *Fusarium oxysporum*, *Rhizoctonia solani* and *Alternaria solani* was obtained from forest soil sample by carrying out *in vitro* and *in vivo* screening techniques. This study reports the identification and characterization of a *Bacillus* sp. SRB 27 that may be used as a bio-control agent against the plant diseases in crop plants. It was identified as *Bacillus* sp. SRB 27 based on 16S rDNA sequence analysis and biochemical tests. The isolate showed a wide range of antifungal activity *in vitro* against a number of phytopathogens such as *F. oxysporum*, *Alternaria* and *Rhizoctonia* in terms of percentage of growth inhibition which were 76.78, 78.57 and 77.55%, respectively. Strain SRB 27 was tolerant to high salt concentration up to 13%, was phosphate solubilizer, proteolytic and amylase positive and coagulase negative. It had compatibility with broad spectrum of fungicides with field recommended dose. Apart from the antagonistic activities, it showed a positive effect on the growth of the castor and cotton plants (both under seed and soil treatments) in comparison with control (non-inoculated). The growth parameters of the test plant surpassed the control in all the cases.

Key words: *Bacillus* sp. SRB 27, biological control, *Fusarium*, *Rhizoctonia*, *Alternaria*, 16S rRNA gene sequence.

INTRODUCTION

Microorganisms can colonize the tissues of healthy plants. Such endophytic bacteria have been reported to prevent disease development by controlling the spread of plant pathogens or by enhancing plant resistance (Stein, 2005; Ryan et al., 2008). Therefore, there is a considerable potential for finding new and beneficial

endophytic bacteria that can serve as bio-control agents. *Bacillus* sp. were considered as potential bio-control agents due to their high spore production ability, resistance and ability to survive desiccation, heat, ultraviolet (UV) irradiation and organic solvents (Romero et al., 2007). *Bacillus* sp. (Gram positive) form biofilms on

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root surfaces, which are multicellular matrixes of bacteria surrounded by extra cellular polysaccharides called a glycocalyx. The glycocalyx acts as a physical barrier and is strongly anionic, thereby can protect the microcolony from external agents (Jeyasekaran and Karunasagar, 2000). Recent studies suggested that the biofilm mode is important for the bacteria's ability to act as bio-control agents (Bais et al., 2004).

According to Backman et al. (1997), the effectiveness of endophytes as biological control agents (BCAs) is dependent on many factors. These factors include: host specificity, the population dynamics and pattern of host colonization, the ability to move within host tissue, and the ability to induce systemic resistance. For example, *Pseudomonas* sp. strain *PsJN*, an onion endophyte, inhibited *Botrytis cinera* pers. *Pseudomonas putida* 89B-27 and *Serratia marcescens* 90-166 reduced cucumber mosaic virus in tomatoes and cucumbers (Raupach et al., 1996) as well as anthracnose and Fusarium wilt in cucumber (Liu et al., 1995). Jetiyanon (1994) established that cabbage colonized by endophytes in the green house had season-long reduced black rot in the field due to induction of defense mechanisms. Non-treated cabbage plants reached the economic threshold (symptoms of systemic disease) approximately 33 days after inoculation with *Xanthomonas campestris* pv. *campestris*.

Research concluded that *Bacillus mycoides* isolate *BacJ* (Bargabus et al., 2002) and *Bacillus pumilis* isolate 203-7 (Bargabus et al., 2004) suppressed *Cercospora* isolates BT8, and BP24 has successfully been used to experimentally manage diseases on several crop species including tomato, potato and pecan.

The objective of present study was to investigate the antagonistic properties, salt and heat tolerance, phosphate solubilization, compatibility with broad spectrum of fungicides and growth promoting ability of *Bacillus* sp. SRB 27.

MATERIALS AND METHODS

Isolation

The forest soil sample was collected in air tight polythene bags from Nallamala forest located at Munnanoor in Mahabubnagar district, Andhra Pradesh, India. The processed soil sample was serially diluted, spread plated on full strength nutrient agar and incubated at 28°C for 48 h. A total of 46 different colonies were isolated on nutrient agar (NA). Pure cultures were developed with repeated culturing which were maintained in 20% glycerol at -20°C. A potential isolate was screened and selected based on the antagonistic properties, phosphate solubilization and salt tolerance. Further, the isolate was identified based on phenotypic and 16S rDNA sequence.

Antagonistic ability

The *in vitro* antagonistic assay was performed using dual culture method on potatoes dextrose agar (PDA) medium. Seven days old

culture agar discs (5 mm) of *Fusarium oxysporum* f.sp. *ricini*, *Rhizoctonia solani* and *Alternaria solani* were disposed at the center of Petri dishes and the bacterial strain was streaked in a square form around the agar disc at 4 cm distance. The antagonistic activity of the studied bacterial strain was estimated by the inhibition of the fungal growth monitored by measuring the diameter in millimeter of the colony until seven days at 28°C in biochemical oxygen demand (BOD) incubator. The experiment was replicated thrice. The percentage of growth inhibition of the fungus was calculated according to the formula given by Whipps (1987).

$$\text{Per cent growth inhibition} = ((R_1 - R_2) / R_1) \times 100$$

Where, R_1 is the farthest radial distance (measured in millimeter grown by the fungus after seven days of incubation in the direction of the antagonist (a control value). R_2 is the distance of fungal growth from the point of inoculation to the colony margin in the direction of the antagonist. All *in vitro* antagonism assays were replicated thrice. The bacterial strains that showed obvious inhibition to *F. oxysporum*, *R. solani* and *A. solani* were selected for further evaluation and stored in 20% glycerol at -20°C.

Fungicide susceptibility test by agar well diffusion method

Agar well diffusion assay is the key process used to evaluate the antifungal potential of fungicides. In the present study, six systemic fungicides; that is, carbendazim, tebuconazole + trifloxystrobin, hexaconazole, propiconazole, azoxystrobin and benomyl were used. Petri dishes (100 mm) containing 20 ml of Mueller Hinton agar (MHA) were seeded with approximately 100 µl inoculums of bacterial strains (inoculums size was adjusted so as to deliver a final inoculum of approximately 10⁸ CFU/ml). After solidification of the media, wells of 6 mm diameter were cut into media using a sterilized cork borer. 100 µl of each fungicide was poured into respective well and the plates were incubated at 32°C overnight. The experiment was replicated thrice under strict aseptic conditions to ensure consistency of all findings. Fungicidal activity on inoculums was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced at the end of the incubation period.

Phosphate solubilization

The isolate was screened for phosphate solubilization as per the methodology described by Gupta et al. (1994) on modified Pikovskaya agar (Glucose - 10 g, Ca₃(PO₄)₂ - 5 g, KCl - 0.2 g, MgSO₄ - 0.1 g, MnSO₄ - trace, FeSO₄ - trace, Yeast extract 0.5 g, Agar - 15 g, Distilled water- 1 L, pH - 7.0) and the plates were incubated at 30±1°C for 48-96 h. Phosphate solubilization is indicated by the formation of a solubilization or a clear zone around the bacterial colony. A loop full of SRB 27 culture was placed on the center of agar plates and incubated at 30±1°C for 5 days. The solubilization zone was determined by subtracting the diameter of bacterial colony from the diameter of total zone. Halo surrounding the colonies were measured and the solubilizing efficiency (SE) was calculated by the following formula: SE= (Solubilization diameter (S)/Growth diameter (G)) x 100.

Stress tolerance study

Salt tolerance

The bacterial isolates were inoculated separately on specific agar medium containing NaCl at 4 to 13% concentration. Four replications of the plates for each isolate and concentration were maintained along with control. After 48 h of incubation, observations

Table 1. Antifungal activity shown by the isolate *Bacillus* sp. SRB 27.

Fungi	Control (R ₁)	SRB (R ₂)	Percentage of growth inhibition $((R_1 - R_2) / R_1) \times 100$
<i>Fusarium oxysporum</i>	5.6	1.3	76.78
<i>Rhizoctonia solani</i>	4.2	0.9	78.57
<i>Alternaria solani</i>	4.9	1.1	77.55

for survival and growth of inoculum started (Benson, 1990). The test was repeated for the promising isolates for the salt tolerance confirmation.

Temperature tolerance study

SRB 27 culture was streaked on nutrient agar plates and incubated at different temperature (40, 50 and 60°C) for 24 h with four replications. Control was also maintained for comparison. All the inoculated Petri plates were incubated in BOD incubator at 28±2°C up to 2 to 15 days (Benson, 1990). Observation was recorded for survival and growth of inoculum.

Bacterial identification and characterization

The isolate was subsequently differentiated by gram reaction, microscopic observation, biochemical tests ortho-nitrophenyl-β-galactoside (ONPG), lysine utilization, ornithine utilization, urease, phenylalanine deamination, nitrate reduction, H₂S production, citrate utilization, voges proskauer's, methyl red, indole, malonate utilization, esculin hydrolysis, arabinose, xylose, adonitol, rhamnose, cellobiose, melibiose, saccharose, raffinose, trehalose, glucose, lactose, oxidase, catalase, coagulase, amylase, protease (casein hydrolysis), lipase (Tween 20), HCN and gelatinase (using Hi-25 Kit, Himedia, Mumbai).

Amplification, sequencing and phylogenetic analysis of 16S rRNA gene

The cells of isolate SRB 27 were harvested after incubation in liquid Luria Bertani (LB) medium for 24 h. After centrifugation at 10,000 rpm for 5 min, cells were collected and washed several times by Tris-HCl-EDTA (TE) (pH 8.0) buffer. Genomic DNA was extracted by X-pert bacterial genomic DNA extraction teaching kit (solution based Hi-media kit). Amplification of 16S rRNA gene of the isolate was carried out in PCR cyler using universal primers FD1 (5'-AGA-GTT-TGA-TCC-TGG-CTC-AG-3') and RP2 (5'-ACG-GCT-ACC-TTG-TTA-CGA-CTT-3'). Amplification reactions were performed in a total volume of 50 µl of 10 X PCR buffer, 5 µl of 25 mmol/l, MgCl₂, 5 µl of 2 mmole/l dNTPs mixture, 2 µl of primer FD1, and 2 µl of RP2, 1 µl of Taq DNA polymerase (5 U/µl) and 2 µl of template DNA. Thermo cycling procedure was as follow: an initial keeping at 94°C for 45 s, 55°C for 45 s and 72°C for 90 s, then an extension in the last cycle at 72°C for 5 min. The PCR product was purified using X-Pert gel extraction teaching kit (solution based Hi-media gel extraction kit). The PCR product was sequenced at Bioserve Pvt. Ltd, Hyderabad.

Coagulase test (method for tube test)

The plasma was diluted 1 in 10 in physiological saline (mix 0.2 ml of plasma with 1.8 ml of saline). In this test, three small test tubes were labeled as follows T = test organisms, Pos = positive control

(*Staphylococcus aureus* broth culture), neg = negative control (sterile broth). To each tube, 0.5 ml of the diluted plasma was added and later five drops (about 0.1 ml) of the test organism culture was added to the tube labeled 'T'. To the tube labeled 'pos', five drops of the *S. aureus* culture was added. Later, five drops of sterile broth was added to the tube labeled 'neg' after mixing gently, after which the three tubes were incubated at 35-37°C. The test tubes were examined for clotting after 1 h and if no clotting occurred, examined at 30 min intervals for up to 6 h; each tube was gently tilted upon observing the clotting.

Protease production

Bacterial isolates were tested for production of protease by growing them on skim milk agar (SKM) (Chantawannkul et al., 2002). An ability to clear the skim milk suspension in the agar was taken as evidence for the secretion of protease. Petri plates with no bacterial inoculation were used as the control.

Green house experiments

Growth promotion efficacy

Castor and cotton seeds were treated with *Bacillus* sp. SRB 27. For control treatments, seeds were not treated. Treated and controlled seeds were sown in pots. Root length, shoot length, root dry weight and shoot dry weight were measured 30 days after sowing. In another experiment, soil was treated with the test bacteria.

RESULTS AND DISCUSSION

In the present investigation, an attempt was made to isolate the potential bio-control agent with high salt tolerance and thermo-stability properties for which the bacteria was isolated. The isolate was characterized and identified using morphological, biochemical and 16S rDNA sequence analyses. Further, its effect on growth parameters of test plants under green house conditions was evaluated.

Assessment of *in vitro* antifungal activity

The isolate showing bio-control property was identified as *Bacillus* sp. SRB 27 by preliminary morphological and biochemical observations. The data presented in Table 1 explicates the antifungal activity shown by the *Bacillus* sp. SRB 27 against the fungi *F. oxysporum*, *R. solani*, *A. solani* (Figure 1). The *Bacillus* sp. SRB 27 showed the antagonistic activity in terms of growth inhibition in the

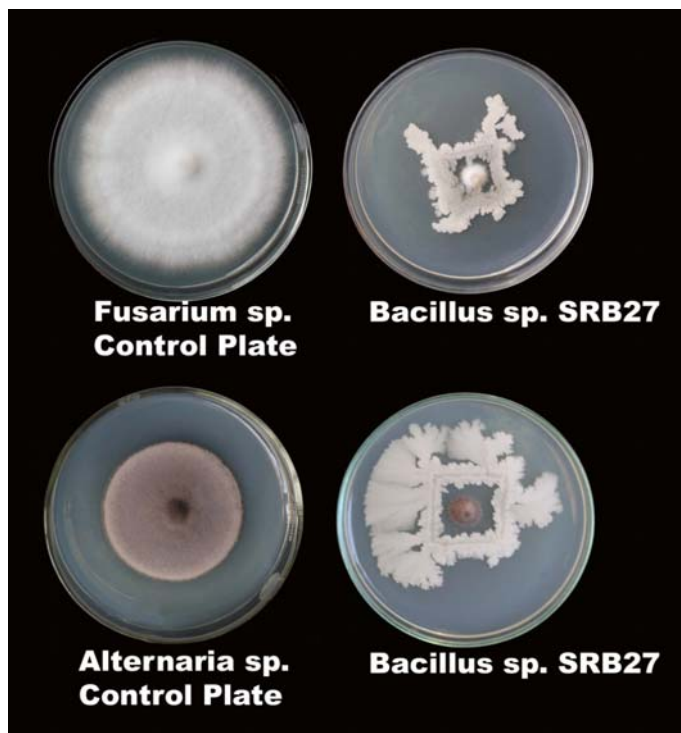


Figure 1. Inhibition of the growth of *Fusarium* sp. and *Alternaria* sp by *Bacillus* sp. SRB 27. Control plate without *Bacillus* sp. SRB 27 shows a luxuriant growth of fungi.

Table 2. Effect of fungicides on *Bacillus* sp. SRB 27.

Fungicide	Concentration of the fungicide in ppm			
	1000	2000	3000	4000
Carbendazim	R	R	11 mm	14 mm
Tebuconazole + Trifloxystrobin	R	R	10 mm	18 mm
Hexaconazole	R	R	28 mm	35 mm
Propiconazole	R	R	25 mm	31 mm
Azoxystrobin	R	R	R	R
Benomyl	R	R	R	R

R= Resistant; susceptibility indicated by the halo zone (mm)

above mentioned fungi. The growth size of the *F. oxysporum*, *R. solani* and *A. solani* in the control plate was found to be 5.6, 4.2 and 4.9 mm, respectively whereas their growth was restricted to 1.3 (*F. oxysporum*), 0.9 (*R. solani*) and 1.1 mm (*A. solani*) in presence of the test organism. The growth inhibition was found to be 76.78, 78.57 and 77.55% for *F. oxysporum*, *R. solani*, and *A. solani*, respectively.

Assessment of anti fungicide susceptibility of the *Bacillus* sp. SRB 27

In agriculture, the bio-control agents are supposed to be

mixed with the fungicides for seed/soil treatment. Such kind of treatments may enhance the effective elimination of fungal pathogens. In light of the above, the *Bacillus* sp. SRB 27 was assayed for the fungicide susceptibility. The data pertaining to Table 2 describes effect of fungicides namely: carbendazim, tebuconazole + trifloxystrobin, hexaconazole, propiconazole, azoxystrobin and benomyl on *Bacillus* sp. SRB 27. All the fungicides were applied at different concentrations; that is, 1000, 2000, 3000 and 4000 ppm. The fungicidal activity was recorded in terms of the mean of diameter of zone of inhibition (in mm) produced at the end of the incubation period. The test organism was completely resistant to the two concentrations that is, 1000 and 2000 ppm for all the

Table 3. *Bacillus* sp. SRB 27 showing tolerance to NaCl (4 to 13 %).

Salt concentration (%)	Level of tolerance to NaCl
4	+++
5	+++
6	++
7	++
8	++
9	+
10	+
11	+
12	+
13	+

+++ = High tolerance; ++ = moderate tolerance; + = low tolerance.

Table 4. Temperature tolerance shown by the *Bacillus* sp. SRB 27 at the range of 30 to 50°C.

Temperature (°C)	Level of growth
30	++
35	+++
40	+++
45	++
50	+
55	+
60	-
70	-

+++ = High tolerance; ++ = Moderate tolerance; + = Low tolerance; - = No tolerance.

Table 5. Phenotypic characteristics of strain *Bacillus* sp. SRB 27.

Characteristic	<i>Bacillus</i> sp. SRB 27
Colony size	Big
Surface	Mucoid
Colony color	White
Margin	Irregular edge
Bacterial cell shape	Bacilli
Endospore formation	Present (Sub terminal)
Elevation	Flat
Growth in liquid medium	Pellicle
Motility	+
Growth optimum temperature	40
Growth pH	7

whereas it was resistant for azoxystrobin and benomyl at concentrations 3000 and 4000 ppm. *Bacillus* sp. SRB 27 showed susceptibility reaction for tebuconazole +

trifloxystrobin, hexaconazole, propiconazole, azoxystrobin and benomyl at 3000 and 4000 ppm.

Evaluation of NaCl and temperature tolerance shown by *Bacillus* sp. SRB 27

The data pertaining to Tables 3 and 4 describes tolerance levels shown by *Bacillus* sp. SRB 27 towards high NaCl concentrations and temperatures, respectively. The tolerance range was designated in connection with growth of the bacteria (in terms of optical density) in the broth at various concentrations. The isolate was tolerant for a wide range of NaCl concentrations (4 to 13%). High tolerance (+++) was shown at 4 and 5% whereas moderate tolerance (++) at 6 to 8% and low tolerance at 9 to 13% (Table 3). The test organism tolerated a wide range of temperatures (45 to 60°C) and exhibited high tolerance at 45°C (+++); whereas moderate tolerance (++) at 50°C and low tolerance at 55 and 60°C (Table 4).

Phenotypic and biochemical characterization

In light of the results obtained for the assessment of fungicide susceptibility, high NaCl concentrations and temperatures tolerance shown by the test organism, the phenotypic and biochemical characterization of the bacteria was carried out and the results given in Tables 5 and 6. The isolate was found to be Gram positive, aerobic and motile aerobic rod that produced a big size colony with mucoid surface in white colour with irregular margin. The bacteria produce endospores (sub terminal) and form a pellicle in liquid medium. It grows better at temperature 40°C and pH 7.

The results pertaining to the biochemical tests performed are presented in Table 6 which explicates that, the *Bacillus* sp. SRB 27 utilized ornithine as the nitrogen source and did not utilized ONPG, lysine and urea. It showed positive response for nitrate reduction whereas negative for phenylalanine deamination and H₂S production. Response for citrate, voges proskauer's and indole tests was positive for the bacteria. The *Bacillus* sp. SRB 27 utilized some carbon sources (arabinose, cellobiose, saccharose, trehalose, glucose and lactose) and rejected the other (xylose, adonitol, rhamnose, melibiose and raffinose). The test organism showed positive activity for oxidase, amylase, protease, lipase and gelatinase whereas negative for catalase, coagulase and HCN production.

Phylogentic analysis of 16S rRNA gene and deposition in NCBI

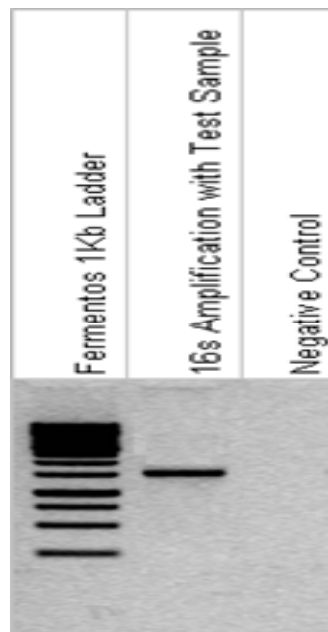
The PCR amplified product was gel electrophoresed and showed the band with 1.5 kb on the gel (Figure 2). The 16S rDNA gene sequencing of the isolate was amplified

Table 6. Biochemical tests carried out for the isolate *Bacillus* sp. SRB 27.

Test	SRB
ONPG	-
Lysine utilization	-
Ornithine utilization	+
Urease	-
Phenylalanine deamination	-
Nitrate reduction	+
H ₂ S production	-
Citrate utilization	+
Voges Proskauer's	+
Methyl red	-
Indole	+
Malonate utilization	+
Esculin hydrolysis	-
Arabinose	+
Xylose	-
Adonitol	-
Rhamnose	-
Cellobiose	+
Melibiose	-
Saccharose	+
Raffinose	-
Trehalose	+
Glucose	+
Lactose	+
Oxidase	+
Catalase	-
Coagulase	-
Amylase	+
Protease (Casein hydrolysis)	+
Lipase (Tween 20)	+
HCN	-
Gelatinase	+
IAA	+

+ = Positive; - = negative

analysed (NCBI Accession no. JX276739). The data pertaining to the basic local alignment search tool (BLAST) analysis is shown in Table 7. Neighbor-joining phylogenetic tree based on the alignment of the nearly complete 16S rDNA gene sequence of the *Bacillus* sp. SRB 27 with the 16S rDNA sequences of the seven described *Bacillus* type strains available in GenBank and NCBI databases is shown in Figure 3. BLAST analysis and a neighbor-joining dendrogram constructed using MEGA revealed that the bacterium belongs to the genus *Bacillus* and as closely clustered together with *Bacillus amyloliquefaciens*. The amplified 16S rRNA gene sequence of isolate *Bacillus* sp. SRB 27 was most closely

**Figure 2.** Gel image showing the PCR product (1.5kb) of the 16SrRNA gene.

related to that of *Bacillus* sp. HNR03 (GenBank accession number, EU373340.1) and showed 99% identity with the sequence from *Bacillus* sp. HNR03. The sequence showed 99% identity with the sequences obtained from most of the *B. amyloliquefaciens* strains and *Bacillus subtilis* strains. On the basis of the results of the classical bacteriological tests and the analysis of the 16S rRNA gene, it was concluded that the isolate was a strain of *B. amyloliquefaciens* and *Bacillus* sp. SRB 27.

Phosphate solubilization

The *Bacillus* sp. SRB 27 has the ability of phosphate solubilization (Table 8) the solubilizing efficiency (SE) of the bacteria was found to be 163.63%.

Effect of the isolate on growth of castor and cotton plants in soil and seed treatments under green house conditions

The results pertaining to the effect of the *Bacillus* sp. SRB 27 for growth promotion of castor and cotton when treated to soil and seed under green house condition are mentioned in Table 9. The test organisms was able to influence the growth of castor and cotton in terms of the shoot length (LS), root length (LR), dry weight of shoot (DWS) and dry weight of root in connection with the seed and soil treatment when compared with control at 30 days after sowing. The shoot length was recorded as 25.5±0.2 and 28.4±0.6 cm for castor and cotton,

Table 7. Strain types of *Bacillus* as reference for 16s rDNA sequence determination.

Species number	Gene bank accession
<i>Bacillus</i> sp. TPR06 16s ribosomal RNA gene	EU373402.1
<i>Bacillus</i> sp. TPL08 16S ribosomal RNA gene	EU373378.1
<i>Bacillus</i> sp. HNR03 16S ribosomal RNA gene	EU373340.1
<i>Bacillus subtilis</i> Strain AQ1 16S ribosomal gene	FJ644629.1
<i>Bacillus amyloliquefaciens</i> subsp. Plantanum CAUB946 complete	HE617159.1
<i>Bacillus subtilis</i> strains Aj080718IA-25 16s ribosomal RNA gene	HQ727971.1
<i>Bacillus subtilis</i> strain BL4 16S ribosomal RNA gene HQ	GU826160.1
<i>Bacillus amyloliquefaciens</i> strain SB 3200 16S ribosomal RNA	GU191911.1
<i>Bacillus amyloliquefaciens</i> strain SB 3195 16S ribosomal RNA	GU191910.1
<i>Bacillus subtilis</i> strain En7 16S ribosomal RNA gene	GU258545.1

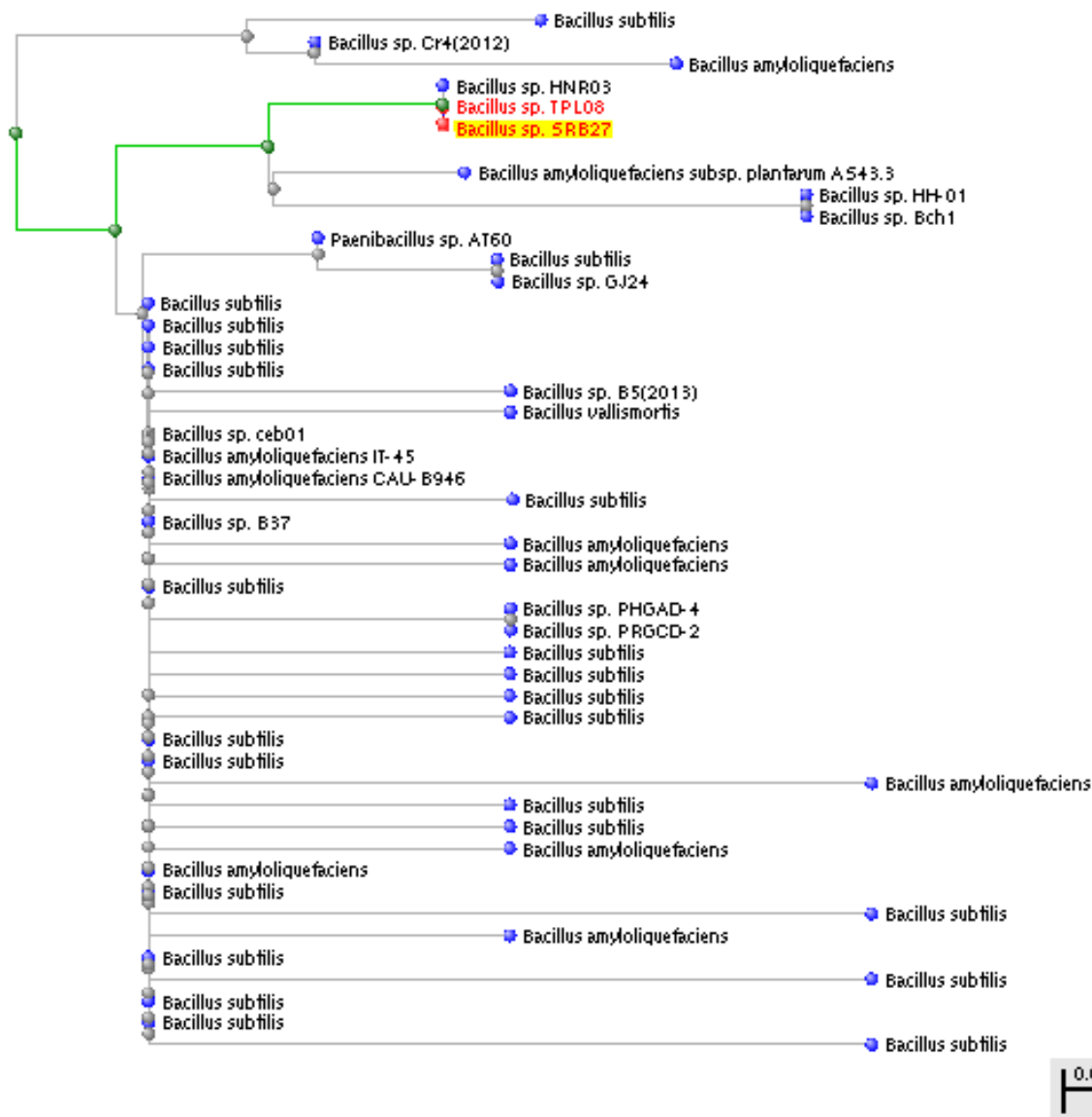


Figure 3. The evolutionary history of *Bacillus* sp. using the neighbor-Joining method. The optimal tree is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option).

Table 8. Phosphate solubilizing ability of *Bacillus* sp. SRB.

Organism	Growth diameter (mm)	Solubilization diameter (mm)	Solubilizing efficiency (SE)
SRB	11±0.12*	180.32	163.63%

*Standard error (SE).

Table 9. The effects of bacterial isolates on growth parameters of plants in soil and seed treatments under greenhouse conditions.

Growth parameter	Castor		Cotton	
	Control	<i>Bacillus</i> sp. SRB 27	Control	<i>Bacillus</i> sp. SRB 27
Seed treatment				
LS (cm)	18.3 ± 0.3*	25.5 ± 0.2	24.5 ± 0.32	28.4 ± 0.6
LR (cm)	4.7 ± 0.1	7.6 ± 0.4	8.2 ± 0.23	12.6 ± 0.45
DWS (g)	1.4 ± 0.21	2.5 ± 0.11	1.05 ± 0.51	1.8 ± 0.36
DWR (g)	0.7 ± 0.32	1.5 ± 0.3	0.54 ± 0.11	0.9 ± 0.5
Soil treatment				
LS (cm)	18.3 ± 0.22	26.2 ± 0.4	24.5 ± 0.14	30.2 ± 0.4
LR (cm)	4.7 ± 0.15	8.1 ± 0.13	8.2 ± 0.32	14.6 ± 0.1
DWS (g)	1.4 ± 0.54	3.2 ± 0.52	1.05 ± 0.15	2.3 ± 0.42
DWR (g)	0.7 ± 0.4	1.9 ± 0.1	0.54 ± 0.33	1.2 ± 0.54

*Standard error (SE).

respectively under seed treatment whereas it was recorded as 26.2±0.4 and 30.2±0.4 cm for castor and cotton, respectively under soil treatment similarity; the root length was recorded as 7.6±0.4 (castor), 12.6±0.45 cm (cotton) under seed treatment and 8.1±0.13 (castor), 14.6±0.1 cm (cotton) under soil treatment. In case of dry weight of shoot, 2.5±0.11 (castor) and 1.8±0.36 g (cotton) under seed treatment and 3.2±0.52 (castor) and 2.3±0.12 g (cotton) under soil treatment was recorded. The dry weight of castor and cotton was recorded as 1.5±0.3 and 0.9±0.5 g, respectively; whereas for soil treatment 1.9±0.1 and 1.2±0.54, respectively.

The results obtained in the present investigation are found to be similar with studies of Stein (2005); Romero et al. (2007) in which, the species of the genus *Bacillus*, particularly *B. amyloliquefaciens* and *B. subtilis*, have been shown to produce a range of antimicrobial dipeptides or cyclic lipopeptides. Some of the metabolites are strain-specific and may be associated with certain species and subspecies of *B. amyloliquefaciens* and *B. subtilis*. In the studies reported by Earl et al. (2008) and Rosas-Garcia (2009), it was revealed that the *in vitro* prescreening test of dual culture selected four *B. subtilis* strains, PLC1605, PCL1608, PCL1610 and PCL1612 with noticeable antifungal activity against *R. necatrix* and other soil-borne phytopathogenic fungi. *B. subtilis* possess several characteristics that enhance its survival in the rhizosphere and thus its effectiveness as a biopesticide. Compant et al. (2005) noticed that many

root associated bacteria have a direct positive influence on plant growth and can indirectly stimulate plant health. Plant bacterial endophytic populations correlate to a certain extent with plant growth performance (Sessitsch et al., 2004). Studies conducted by Yun et al. (2011) revealed that *B. amyloliquefaciens* PEBA20 showed antimicrobial activity against a wide range of fungi and bacteria. The efficacy of the bacterial suspension in inhibiting fungal growth *in vitro* ranged from 38.44 to 89.37%, while that of the fermentation filtrate ranged from 19.2 to 82.62%. The diameter of the inhibition zone after treatment with bacterial suspension and fermentation filtrate of PEBA20 ranged from 9.97 to 19.14 mm and from 5.1 (very weak effect) to 19.05 mm. Nitrogen-fixing *Bacillus* sp. isolated by Jadhav et al. (2010) has 15% salt-tolerance, indicating they are new novel strains qualified by adaptation to environment and thereby acquiring additional traits. *B. subtilis* subsp. *Subtilis* NCIB 3610T and *Bacillus sonorensis* NRRL B-23154T isolated by Jadhav et al. (2010) tolerated very high salt concentration (10 and 15%, respectively). The inhibitory potential and antibiotic production of *B. subtilis* was investigated *in vitro* against phytopathogen fungus and bacteria (Földes et al., 2000).

Touré et al. (2004) was attributed to lipopeptides produced by one isolate of *Bacillus subtilis*, the antagonistic action over mycelia growth, which they obtained over many phytopatogenic fungi from soil, as *Fusarium*, *Pythium ultimum*, *Rhizoctonia solani* and

Rhizopus. Mariana et al. (2009) observed the differences in susceptibility for each fungal isolate, in relation to the antagonist action of *Bacillus* E164 which may be related to variations in the production of inhibitory metabolites by the bacterial strains. The phosphate solubilization of *Bacillus cereus* [0.32+0.05] and *B. subtilis* [0.38+0.01] were analyzed using various parameters such as pH [7 to 9], temperature (30 to 45°C), and nutrient supplementation. The role of phosphorus in increasing the yield and improving the quality of *Bacillus* sp. is well known. Phosphorous, next to nitrogen is a vital nutrient for plant and microorganisms.

In conclusion, the isolated bacterial species was identified as *Bacillus* sp. based on the phenotypic, biochemical and phylogenetic characterization. The *Bacillus* sp. SRB 27 was found to be tolerant to high temperatures and high salt conditions which is a positive aspect of the strain to grow better for the crops that grow under high saline conditions and withstand high temperatures. Apart from that, the *Bacillus* sp. SRB 27 has the superior quality of antagonistic activity against phytopathogenic fungal genera like *Fusarium* sp., *Rhizoctonia* sp. and *Alternaria* sp. The phosphate solubilization efficiency was found to be very high which helps in better uptake of the phosphorous under field conditions. Moreover, *Bacillus* sp. SRB 27 has shown proven effect on the growth of castor and cotton in specific and many plant species in general.

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