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Isolation, screening and molecular identification of silicate solubilizing bacteria from the rhizosphere soil of Brinjal

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ABSTRACT
Brinjal or eggplant is an important vegetable crop which are grown in most of the parts of the India. The crop is rich in proteins, vitamins, minerals which are needed for human health. Silicate is the second most abundant element occurs in unavailable forms of silicates (SiO_3) of aluminum, magnesium, calcium, sodium, potassium, or iron. Accessibility of silicates to the plant roots is generally governed by chemical and biological reactions of soil. Microorganisms are known to play major role in dissolution of minerals like silicates and phosphates. Several beneficial microbes have been reported for their positive impacts on plant under different stress conditions through better uptake of these minerals. In this study, ten isolates of silicate solubilizing bacteria were isolated from brinjal rhizosphere soil sample and designated as BSSB-1 to BSSB-10. Different parameters viz., characterization, silicate solubilizing efficiency, plant growth hormone production by the isolates were studied. Among the ten isolates, the isolate BSSB-3 showed highest solubilization zone (12.45 mm) and recorded more available silica content of 2.18 ppm and also produced maximum IAA (12.10 µg/ml), HCN (+++) production and ammonia excretion (4.65 µg/ml). The isolate BSSB-3 showed best result compare to the other isolates in terms of IAA, HCN production, ammonia excretion and the isolate BSSB-3 is identified as Bacillus amyloliquefaciens by 16S rRNA sequencing.
Keywords: Brinjal, Silicate solubilizing microorganisms, silicate solubilizing efficiency, plant growth hormone production, *Bacillus amyloliquefaciens*

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INTRODUCTION

Brinjal or eggplant (*Solanum melongena* L.) is an important solanaceous crop of sub-tropics and tropics. The name brinjal is popular in Indian subcontinents and is derived from Arabic and Sanskrit whereas the name eggplant has been derived from the shape of the fruit of some varieties, which are white and resemble in shape to chicken eggs. It is also called aubergine (French word) in Europe. The varieties of brinjal displays a wide range of fruit shapes and colors, ranging from oval or egg-shaped to long club-shape and color from white, yellow, green through degrees of purple pigmentation to almost black. Most of the important varieties of brinjal have been selected from the long-established types of the tropical India and China.

Microorganisms are known to play major role in dissolution of minerals like silicates and phosphates [1]. [2]. Several beneficial microbes have been reported for their positive impacts on plant under different stress condition ns through better uptake of these minerals [2]. Solubilization of both insoluble silicon and phosphate due to organic acid production by microbes is known to enhance their availability to plants [3]. Various studies have reported weathering of silicates by bacteria for its dissolution to make it available to the plant [4].

Plants assimilate silicates as soluble monosilic acid resulting in strengthening of cell wall through various mechanisms [5]. Strengthened cell wall due to higher accumulation of silicon is known to improve plant resistance to diseases, insect attack, and adverse climatic conditions in various plant species like rice, oat, barley, wheat, cucumber,and sugarcane [6].

Silicate solubilizing bacteria (SSB) can play an efficient role here by solubilizing insoluble forms of silicates hence increasing soil fertility and enhancing plant defense mechanisms [7].The accumulated silicate not only improves growth and yield of these plants but is also involved in induction of systemic resistance (ISR) against pests and diseases [8]. Microorganisms like *Burkholderia eburnea, Bacillus globisporous, Bacillus mucilaginosus*, and *Enterobacter ludwigii* are some examples of silicate solubilizing bacteria (SSB) reported in literature [9] [2]. Vasanthi *et al., 10* (2018) identified the *Bacillus flexus*, *B. mucilaginosus*, *B. megaterium*

and *Pseudomonas fluorescens* are the some of the silica solubilizing microorganisms by 16S rRNA sequencing.

In the present study, the soil samples were collected from different locations of Cuddalore districts for the isolation of silicate solubilizing bacteria and their qualitative and quantitative characters were studied.

MATERIAL AND METHODS

Isolation of silicate solubilizing bacteria

The brinjal rhizosphere soil samples were collected from ten different locations of Cuddalore districts of Tamil Nadu. Silicate solubilizing bacteria were isolated by serial dilution and plating technique. The medium used for isolation of silicate solubilizing bacteria is modified Bunt and Rovira medium [2]. Insoluble magnesium trisilicate (0.25 %) was also added to the medium along with 250 ml soil extract and the pH was adjusted to 7.0 before sterilization. Sterilized cycloheximide (50 mg/l) was added to the growth media to prevent the growth of fungi on Petri plates. Tenfold dilution series of the sieved rhizosphere soil sample were prepared and an aliquot of 0.1 ml from 10^4 dilution was plated on to *Bunt and Rovira* medium. After incubating the plates for 72 h at 28-30°C in the incubator, the plates were observed and the appearance of clearing zone around bacterial grown colonies which is an indicative of silicate solubilization. The bacterial colony which displayed the largest solubilization zone on the growth media was selected for further experimentation. Pure cultures of different isolates were obtained by streak plate method. These pure cultures of different bacterial isolates were preserved and used for further analysis. The identified silicate solubilizing isolates were designated as Brinjal Silicate Solubilizing Bacteria (BSSB-1 to BSSB-10)

Characterization of silicate solubilizing bacterial isolates by morphological, cultural and biochemical characters

Morphological characterization

All the silicate solubilizing bacterial isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by [11].

Colony morphology

Morphological characteristics of the colony of each isolate was examined on Nutrient agar medium and specialized medium and the isolates was kept for incubation. Different characteristics of colonies such as shape, size, elevation, surface, margin, colour, odour, pigmentation etc. were recorded.

Biochemical Characterization

Starch hydrolysis

Sterile starch agar plates were prepared and streaked with overnight culture of the isolates and incubated at 28 ± 2°C for 24-48 hours. After incubation, the plates were flooded with an iodine solution. The formation of a transparent zone around the colony was taken as a positive reaction to the test.

Hydrogen sulfide test

Sterilized Hydrogen Sulfide-Indole-Motility agar (SIM agar) were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48 hours at 28 ± 2°C. Visualization of black colour along the line of inoculation indicated a positive reaction to the test.

Indole production

Hydrogen sulfide-indole-motility agar (SIM agar) media were prepared and the slants were inoculated with the overnight cultures of the isolates and incubated for 48 hours at 28 ± 2°C. After incubation, 10 drops of Kovac's indole reagent was added to each tube. Production of red color in the tube were recorded as positive for indole production.

Catalase test

Catalase test was performed to study the presence of catalase enzyme in bacterial colonies.24 hours old pure isolates were taken on glass slides and one drop of H_2O_2 (30%) was added. The appearance of the gas bubble indicated the presence of catalase enzyme.

Oxidase test

The overnight cultures of the isolates were streaked on plates poured with sterile Trypticase Soy Agar (TSA) media and the plates were incubated for 24 hours at 28 ± 2°C. After incubation, 2-3 drops of tetramethyl-p-phenylene di amine di hydro-chloride (Wurster's reagent) was added onto the surface of growth of each test organism. The change of color to maroon were noted as oxidase positive.

Gelatin liquefaction

Overnight cultures of the isolates were inoculated on sterilized nutrient gelatin deep tubes and incubated for 24 hours at 28 ± 2°C. The tubes were kept in the refrigerator for 30 minutes at 4°C. The isolates showing liquefied gelatin were taken as positive and those which resulted in the solidification of gelatin on refrigeration were recorded as negative for the test.

Methyl red test

Sterilized glucose-phosphate broth tubes were inoculated with isolated culture and incubated at 28 ± 2°C for 48 hours. After incubation, five drops of methyl red indicator were added to each tube and gently shaken. Red color production in the broth tube was taken as positive and yellow color production was taken as negative for the test.

Voges prausker's test

On the pre-sterilized glucose-phosphate broth tubes, isolated cultures were inoculated and incubated at 37°C for 48 hours. After incubation period ten drops of Barritt's reagent-A was added and gently shaken followed by the addition of 10 drops of Barritt's reagent-B. The development of pink color in the broth was taken as positive for the test.

Citrate utilization

Isolates were streaked on Simmon's citrate agar slants and incubated at 28 ± 2°C for 24 hours. Change in color from green to blue indicates the positive reaction for citrate utilization.

Nitrate reduction:

Silicate solubilizing isolates were inoculated into 10 ml of nitrate broth taken in test tubes and the tubes were incubated at 30°C. After 14 days, 2 ml of the broth was tested by adding equal amounts of sulfanilic acid and alpha naphthylamine. Development of red color indicates nitrate reduction.

Qualitative estimation of silicate solubilizing activity in Bunt and Rovira agar

The ten selected bacterial isolates from brinjal rhizosphere soil sample were subjected to a silicate solubilizing test in *Bunt and Rovirav* Agar, incubated at 30°C for 4 days. Silicate source (magnesium trisilicate) were air dried and sieved through 325 mesh-sieve. Isolates that produces a clear zone on the media was recognized has the capability to solubilize 0.25 % magnesium trisilicate in Bunt and Rovira Agar. Clear zone from each isolate was measured by Solubilizing Index [12].

Quantitative estimation of silicate solubilizing activity in Bunt and Rovira broth

The solubility of silicate was investigated in 100 ml *Bunt and Rovira* broth. Ten silicate solubilizing isolates supplemented by 0.25 % magnesium trisilicate ($Mg_2O_8Si_3$). Source of silicate (0.25% magnesium trisilicate) was added separately into Bunt and Rovira broth. One ml of the bacterial cells 10^8 cfu/ml was inoculated into *Bunt and Rovira* broth and kept for 7 days. After incubation periods, the culture was centrifuged at 10,000 rpm for 15 minutes to remove supernatant. One ml of the culture supernatant was added to the reagents into container and analysed by silico-molybdate's method [13].

Indole acetic acid production

Ten silicate solubilizing bacterial isolates were tested for their potential of production of indole acetic acid (IAA). Silicate solubilizing bacterial isolates were grown in nutrient broth having 100 µg/ml dl- tryptophan for 72 h at 30±2 °C. Two ml of Salkowaski reagent was added in test tube containing 2 ml of 72 h incubated culture broth supernatant and allowed to develop pink color [14] and the intensity of color developed was measured at 530 nm using Spectrophotometer. IAA production was calculated by reference standard curve (10-100 µg/ml).

HCN production

HCN production was assessed by method proposed by [15]. SSB isolates were grown in test tube containing Bunt and Rovira medium which comprised of 4.4 g/l glycine. Filter paper strips soaked in solution (0.5% picric acid in 2% sodium carbonate solution) were hanged in test tubes containing medium inoculated by bacterial cultures and incubated for 5 days at 30±2°C. The change in color of filter paper from yellow to brown or light red indicated HCN production.

Ammonia excretion:

Five days old culture were taken in Test tubes having 5 ml peptone broth and incubated for 4 days at 30±2°C. After the incubation period, 0.5 ml of Nessler's reagent was added to each test tube and shaken vigorously. Two ml aliquot was taken in Eppendorf and centrifuged at 10,000 rpm for 15 minutes and ammonia excreted was calculated by referring to standard curve (1-10 µg/ml ammonia). The absorbance of supernatant was read at 450 nm with of UV-Vis spectrophotometer [16].

16S rRNA gene sequencing analysis

The selected efficient strain BSSB-3, was identified based on its 16S rRNA gene sequence. The genomic DNA of BSSB-3 was extracted and purified using standard procedures [17]. The 16S ribosomal RNA gene was amplified from the BSSB-3 genomic DNA using specific primers (27F (5′-AGAGTTTGATCCTGGCTCAG-3′) 1492R (5′-TACGGYTACCTTGTTACGACTT-3′).). The bacterial 16S rRNA gene PCR amplicon was eluted and purified according to manufacturer's instructions. Consensus sequence of 16S rRNA gene was generated from forward and reverse sequence 16S rRNA gene sequence was used to carry out NCBI-Basic Local Alignment Search Tool database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program ClustalW multiple alignment. Distance matrix was generated and the phylogenetic tree was constructed by using MEGA 11software. DNA sequence homology

searches were performed using the online BLAST search engine in Gene Bank and the phylogenetic tree of the silicate solubilizing bacteria was built in MEGA 11.

RESULTS AND DISCUSSION

Isolation of silica solubilizing microorganisms

Rhizosphere soil samples were collected from ten different locations of Cuddalore district of Tamil Nadu. Isolate code (BSSB-1 to BSSB-10) was given based on the locations where the samples collected. Isolation of silicate solubilizing bacteria was done by serial dilution and plating technique using Bunt and Rovira medium.

Cultural and morphological characterization

Morphological and cultural characteristics of all the ten isolates were studied *viz.,* Gram reaction, cell shape, colony morphology. The morphological features on Bunt and Rovira agar plate was studied and they showed small to medium size, dull white or off white, flat, smooth, irregular colonies and there was no pigment production (Table 1). These isolates were found to be gram positive, short stumpy, rod shaped cells when observed under microscope.

Biochemical characterization

All the ten isolates were examined for biochemical characterization *viz.,* Starch hydrolysis, Hydrogen sulphide test, Indole production, Catalase test, Oxidase test, Gelatin liquefaction, Methyl red test, Vogues Proskauer test, Citrate Utilization, Nitrate reduction and the results were presented in Table.2. All the silicate solubilizing bacterial isolates were positive for starch hydrolysis expect the isolate BSSB-2, BSSB-4 and BSSB-8. For hydrogen sulphide test all the isolates were positive except the isolates BSSB-1, BSSB-3, BSSB-5, BSSB-9 and BSSB-10. In indole production isolates BSSB-6 and BSSB-7 showed positive result and all other isolates showed negative result.

For catalase test all isolates showed positive result. In oxidase test BSSB-3, BSSB-6, BSSB-7, BSSB-10 showed negative result remaining all are showed positive result. In gelatin liquefaction test all the isolates were positive. For methyl red test the isolates BSSB-2, BSSB-3, BSSB-4 BSSB-8 and BSSB-10 were negative. For vogues proskauer the isolates BSSB-3, BSSB-6, BSSB-7, and BSSB-10 were showed the positive result. In citrate utilization test all the isolates showed positive result. All the isolates were showed the positive for nitrate reduction test.

Based on biochemical characterization the isolates were tentatively identified as *Bacillus* sp, Pseudomonas sp, *Bacillus amyloliquefaciens*, *Penibacillus mucilaginosus.*

Screening of isolates for their Silicate solubilization efficiency

Qualitative method in plate assay

All the ten silica solubilizing isolates were able to form clear zone of silicate solubilization on Bunt and Rovira agar plate ranged from 6.21 -12.45 mm (Table.3). Among the ten isolates, the isolate BSSB-3 showed highest solubilization zone (12.45 mm) followed by BSSB-6 (10.42 mm) and the lowest solubilization zone was observed on isolate BSSB-5 (6.21 mm). Silicate solubilization index were also highest in BSSB-3 (4.92 mm) followed by BSSB-6 (4.60 mm) and the lowest solubilization index was observed in BSSB- 5 (2.77). Similar results were also observed by [8] they were isolated ten isolates of bacteria from soil and identified as *Bacillus mucilaginosus* and the isolates SSB-3, SSB-5, SSB-8 and SSB-9 were found to be very efficient in silicate solubilization and recorded 11.0 mm, 11.5 mm 15.4 mm and 15.0 mm zone of solubilization respectively.

Quantitative method in broth assay

All the ten silicate solubilizing isolates were able to solubilize the available silica in Bunt and Rovira broth, which supplemented by 0.25 % magnesium trisilicate ($Mg_2O_8Si_3$) and the result were presented in Table.3. Among them, the isolate BSSB-3 recorded the more available silica content of 2.18 ppm and followed by BSSB-6 (1.95 ppm). The lowest was shown by BSSB-5 with 0.64 ppm. Similar results were observed by [18] who isolated silicate bacteria from paddy rhizosphere soil in Bunt and Rovira medium and the highest solubilizing index was gained by the isolates OS-7 on 1.10 ppm, while the highest silicate concentration was solubilized by OS-12 on 1.053 ppm in Bunt and Rovira broth.

Indole Acetic Acid (IAA) Production

The IAA production potential of all the ten BSSB isolates was studied and it was found that isolate BSSB-3 produced maximum IAA (12.10 µg/ml) followed by the isolate BSSB-6 which produce IAA of 10.90 µg/ml. The isolate BSSB-5 recorded very low IAA production of 5.85 µg/ml (Table 4). [19] evaluated bacterial isolates for IAA production and found all isolates able to produce IAA ranging from 3.17-13.29 µg/ml. [20] reported 4-10 µg/ml IAA production by rhizobacteria and similar study reported the IAA production of different bacterial isolates in range of 0.38-2.37 µg/ ml.

HCN production

In the present study, among the isolates only six isolates changed the color of filter paper strips confirming HCN production. The isolates BSSB-5 and BSSB-10 doesn't change the color and showed negative for HCN production (Table 4). Similar results were also obtained by [21] they isolated thirteen bacterial isolates from *Crocus sativus* L. rhizospheric soil and 61% bacterial isolates were reported to prouce HCN. Similarly, [20] also reported that five out of ten (50%) bacterial isolates clearly showed HCN production.

Ammonia excretion

In present investigation, ten isolates were tested for ammonia excretion by silicate solubilizing bacterial isolates and the findings ranged from 1.51-4.65 µg/ml of ammonia. The isolate BSSB-3 had produced maximum color change of solution after Nesslerization reaction and produce 4.65 (µg/ml) of ammonia (Table 4). Similarly, [22] observed on their findings that ammonia excretion by silicate solubilizing bacterial isolates varied from 1.75-4.60 µg/ml of ammonia. They also found that the isolate SSB-2 had produced maximum colour change of solution after Nesslerization reaction.

Molecular identification of isolate BSSB-3

The genomic DNA of BSSB-3 was used to sequence the 16S rRNA in order to identify the bacterial isolate. The obtained 16S rRNA sequence for BSSB-3 was submitted to the NCBI GenBank and was assigned Accession No. OQ702756 On the basis of sequence homology and phylogenetic analysis, bacterial isolate BSSB-3 was identified as *Bacillus amyloliquefaciens* and the phylogenetic tree was drawn using MEGA 11 software (Fig.1).

Table.1 Morphological and cultural characterization of Silicate solubilizing bacterial isolates from ten different rhizosphere soil samples of brinjal

S.No.	Isolate name	Gram reaction	Cell shape	Colony Morphology			
				Color	Form	Elevation	Margin
1.	BSSB-1	+ve	Rod	Creamy	Circular	Raised	Entire
2.	BSSB-2	-ve	Rod	Milky white	Irregular	Raised	Swell
3.	BSSB-3	+ve	Rod	Creamy white	Irregular	Flat	Undulate
4.	BSSB-4	-ve	Rod	Milky white	Irregular	Raised	Irregular
5.	BSSB-5	+ve	Rod	Milky white	Irregular	Flat	Undulate
6.	BSSB-6	+ve	Rod	Creamy white	Circular	Convex	Entire
7.	BSSB-7	+ve	Rod	Creamy	Circular	Convex	Irregular
8.	BSSB-8	-ve	Diplococci	Slimy White	Irregular	Low convex	Swell
9.	BSSB-9	+ve	Rod	White	Circular	Raised	Irregular
10.	BSSB-10	+ve	Rod	White	Irregular	Convex	Entire

Table.2 Biochemical and physiological characterization of Silicate Solubilizing Bacterial isolates collected from ten different rhizosphere soil samples of brinjal

S.No.	Isolate name	Starch hydrolysis	Hydrogen sulphide test	Indole production	Catalase test	Oxidase test	Gelatin liquefaction	Methyl red test	Vogues Proskauer test	Citrate Utilization	Nitrate reduction	Tentative identification
1.	BSSB-1	+	-	-	+	+	+	+	-	+	+	*Bacillus* sp
2.	BSSB-2	-	+	-	+	+	+	-	-	+	+	*Pseudomonas* sp
3.	BSSB-3	+	-	-	+	-	+	-	+	+	+	*Bacillus amyloliquefaciens*
4.	BSSB-4	-	+	-	+	+	+	-	-	+	+	*Pseudononas* sp
5.	BSSB-5	+	-	-	+	+	+	+	-	+	+	*Bacillus* sp
6.	BSSB-6	+	+	+	+	-	+	+	+	+	+	*Paenibacillus mucilaginosus*
7.	BSSB-7	+	+	+	+	-	+	+	+	+	+	*Paenibacillus mucilaginosus*
8.	BSSB-8	-	+	-	+	+	+	-	-	+	+	*Pseudomonas* sp
9.	BSSB-9	+	-	-	+	+	+	+	-	+	+	*Bacillus* sp
10.	BSSB-10	+	-	-	+	-	+	-	+	+	+	*Bacillus amyloliquefaciens*

Table.3 Estimation of Silicate solubilization by quantitatively and qualitatively for the screening of silicate solubilizing isolates from ten different rhizosphere soil samples of brinjal

S.No.	Isolate name	Soluble Silicate concentration (ppm) in Bunt and rovira broth	Silicate solubilization		
			Silicate solubilization index	Zone diameter (mm)	
				Solubilization zone	Culture diameter
1.	BSSB-1	1.80	4.17	10.17	3.32
2.	BSSB-2	1.41	3.90	8.76	2.38
3.	BSSB-3	2.18	4.92	12.45	4.92
4.	BSSB-4	1.65	4.13	9.14	3.15
5.	BSSB-5	0.64	2.77	6.21	1.62
6.	BSSB-6	1.95	4.60	10.42	3.60
7.	BSSB-7	0.72	3.20	6.57	1.71
8.	BSSB-8	0.90	3.52	7.45	1.90
9.	BSSB-9	1.70	4.18	10.12	3.20
10.	BSSB-10	1.20	3.60	7.70	2.10

*****Values of mean of three replications ± SD

Table 4.Plant growth promoting substances produced by silicate solubilizing bacteria from ten different rhizosphere soil samples of brinjal

S.No.	Isolate name	IAA (µg/ml)*	HCN Production	Ammonia excretion(µg/ml)*
1.	BSSB-1	9.75	+	3.84
2.	BSSB-2	8.13	++	3.17
3.	BSSB-3	12.10	+++	4.65
4.	BSSB-4	9.06	+	3.63
5.	BSSB-5	5.85	-	1.51
6.	BSSB-6	10.90	+	4.10
7.	BSSB-7	6.20	+	1.70
8.	BSSB-8	6.55	++	2.12
9.	BSSB-9	9.41	+	3.70
10.	BSSB-10	6.70	-	2.44

*****Values of mean of three replications ± SD
(+++ High production, + Low production, - No production)

CONCLUSION

Silicate solubilizing microorganisms has the ability to release soluble silicate from insoluble inorganic silicates. *Bacillus* species are identified as the good silicate solubilizing organisms. In the present study, 10 different rhizosphere soil samples were collected and used for the isolation of silicate solubilizing bacteria. The isolates were screened for plant growth promoting hormones production like IAA, ammonia excretion, HCN production and silicate solubilizing efficiency were studied. Among the 10 isolates the isolate BSSB-3 performed well based on their screening efficiency and identified using 16S rRNA sequencing. Using beneficial silicate solubilizing strain BSSB-3 offers development of organic biofertilizers which improve the growth and development of crops.

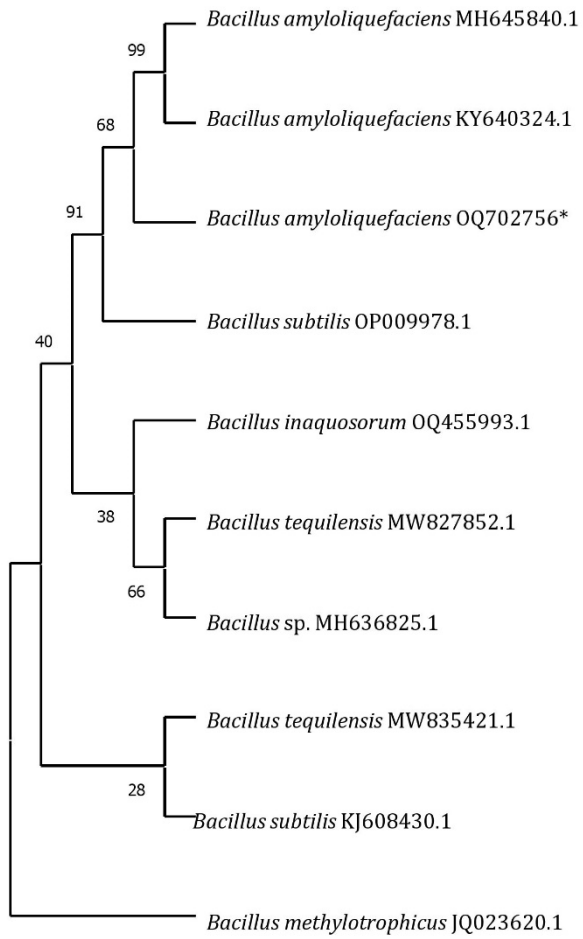

Fig 1.Phylogentic tree of strain BSSB-3 (OQ702756 *) analysis based on 16s rRNA (partial) *sequences after Muscle alignment using MEGA11 software*

>*Bacillus amyloliquefaciens* BSSB-3 (OQ702756)

CCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGGAACTTGAGTGCAGAAGAGG
AGAGGGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGGAGGAACACCAGTGGC
GAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC
AGGATTAGATACCCTGGTAGTCCACCCGTAAACGATGAGTGCTAAGTGTTAGGGGGT
TTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCG
CAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
TTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAATCCTAG
AGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTC
GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCC
AGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGG
ATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGCA
GAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTC
GGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAG
CATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAG
TTTGTAACACCCGAAGTCGGTGAGGTAACCT

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