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Isolation, Screening and Characterization of Silica Solubilizing Bacteria from Direct Sown Paddy (*Oryza sativa* L.) Growing Areas of Andhra Pradesh

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Rice (*Oryza sativa* L.) production in India is an important part of the national economy. India is the world's 2nd largest rice producer with approximately 43 Million hectares planted area, accounting for 22% of the world's rice production. Silica as beneficial element has function for its cell strength in growth and development of paddy, which is accumulated 10% of its dry weight. In the present study was collected twenty (20) direct sown paddy rhizosphere soil samples from four districts of Andhra Pradesh and recorded their geographical position of collected soil samples. From that twenty eight (28) Silica Solubilizing Bacteria (SiSB) isolated by using magnesium trisilicate (0.25 %) as an insoluble source of silica in *Bunt and Rovira*media. Silica solubilizing isolates coded based on their soil sample collected geographical position, recorded the morphological, cultural characteristics of SiSB. SiSB isolates screened based on their silica solubilization efficiency both qualitatively and quantitatively. Among all 28 SiSB isolates SiKPP-1 has the high ability to solubilize silica both qualitatively and quantitatively (12.41 mm & 2.16 ppm) followed by SiPYY-3 (12.02 mm & 2.12 ppm) and lowest was showed by SiAGG-1 (5.20 mm & 0.52 ppm). SiKPP-1 showed the positive for the Starch hydrolysis test, Indole production test, Catalase test, Oxidase test, Gelatine liquefaction test, Methyl red test, Vogues Proskauer test, Citrate Utilization test and Ammonia production test. SiKPP-1 showed the negative result for Hydrogen sulphide test.

Introduction

Several macro and micro nutrients are helps the plants for their growth and development. Silica is a beneficial plant nutrient with a vital role in maintaining plant growth and enhancing tolerance to both biotic and abiotic stresses. The polymeric insoluble silica present in soils is solubilised during weathering to release monosilicic acid into the soil which is the bioavailable form of silicon absorbed by plants. Although silica

(Si) is not considered an essential element for higher plants, it has been proven to be beneficial for the well growth and development of many plant species, particularly tropical graminaceous plants such as rice (Liang *et al.,* 2007). Silicate solubilizing bacteria (SSB) can play an effective role in soil by solubilizing insoluble forms of silicates. In addition to this some SSB can also solubilize potassium and phosphates, hence increasing soil fertility and enhancing plant defence mechanisms. Bacterial strains *Bacillus* spp., *Pseudomonas* spp., *Aspergillus* spp *and Pencillium* spp that have been reported to show silica solubilisation (Gopal *et al.,* 2005). Vasanthi *et al.,* (2018) identified the *Bacillus flexus*, *B. mucilaginosus*, *B. megaterium* and *Pseudomonas fluorescens* are the some of the silica solubilizing microorganisms by 16S r RNA sequencing.

Materials and Methods

Collection of soil samples

Rhizospheric soils were collected from different places of Kurnool, Prakasam, Guntur and Anantapur districts such as soils of direct sown paddy growing areas by using quadratic method of soil sample collecting procedure. In Kurnool District (Atmakur, Kothapalle, Jupadu bungalow, Pamulapadu and Velugodu), Prakasam District (Tripuranthakam, Yerragondapalem, Dornala, Markapuram and Giddalur), Guntur District (Vinukonda, Narasaropet, Chilakaluripet, Sattenapalle and Piduguralla) and Anantapur District (Guntakal, Gooty, Pamidi, Tadipatri and Uravakonda) along with particular GPS coordinates for each sampling area was fixed (Table.1) soil samples were collected from each Mandal from different farmer's fields. Isolation of different bacterial isolates following serial dilution method and plating techniques using suitable media and incubated conditions were followed. Pure cultures must be obtained by the streak plate method. These pure cultures of different bacterial isolates were preserved and used for further analysis.

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

Morphological characterization

All the zinc solubilizing bacterial isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram reaction was also recorded as per the standard procedures given by Barthalomew and Mittewar (1950).

Colony morphology

Morphological characteristics of the colony of each isolate was examined on Nutrient agar and specialized medium and incubated for according to isolate. Cultural characterization of isolates were observed by different characteristics of colonies such as shape, size, elevation, surface, margin, colour, odour, pigmentation etc. were recorded.

Biochemical and physiological characterization of zinc solubilizing bacterial isolates

Starch hydrolysis (MacFaddin, 2000)

Sterile starch agar plates were spotted with 10 µl overnight broth cultures of the isolates and incubated at 28 ± 2^{o}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 (Beishir, 1991)

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

Indole production (Isenberg and Sundheim, 1958)

Sterilized hydrogen sulfide-indole-motility agar (SIM agar) slants were inoculated with the overnight cultures of the isolates and incubated for 48 hours at 28 ± 2^{o}C. Following incubation, 10 drops of Kovac's indole reagent was added to each tube. The isolates showing the production of red colour were recorded as positive for indole production.

Catalase test (Rangaswami and Bagyaraj, 1993)

This test was performed to study the presence of catalase enzyme in bacterial colonies. Pure isolates (24 hours old) 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 (Collins and Lyne, 1970)

The overnight cultures of the test isolate were spotted on plates poured with sterile Trypticase Soy Agar (TSA) and the plates were incubated for 24 hours at 28 ± 2^{o}C. After incubation, 2-3 drops of N, N, N', N'-tetramethyl-p-phenylenediaminedihydro-chloride (Wurster's reagent) was added onto the surface of growth of each test organism. The isolates showing the change of colour to maroon were noted as oxidase positive.

Gelatin liquefaction (MacFaddin, 2000)

The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated for 24 hours at 28 ± 2^{o}C. Then the tubes were kept in the refrigerator for 30 minutes at 4^{o}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 (Crown and Gen, 1998)

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

Vogesprausker's test (MacFaddin, 2000)

To the pre-sterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37^{o}C for 48 hours. After incubation 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 colour in the broth was taken as positive for the test.

Citrate utilization (MacFaddin, 2000)

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

Ammonia production (Juanda, 2005)

The isolates were tested for ammonia production by inoculating the isolates into 10 ml of pre-sterilized peptone water in test tubes. The tubes were incubated for 48-72 hours at 36 ± 2^{o}C. After that Nessler's reagent (0.5 ml) was added in each tube. Change in colour of the medium from brown to yellow colour was taken as a positive test for ammonia production.

Isolation of silica solubilising bacteria

Silica solubilizing bacteria (SSB) were isolated from the rhizosphere soils of the direct sown rice crop of Kurnool, Guntur, Ananthapur and Prakasham districts. The medium used for isolation of silica solubilizing bacteria is modified Bunt and Rovira medium (Bunt and Rovira, 1955) containing (g/l):10 g peptone, 20 g glucose, 0.1 g magnesium chloride, 0.01 g ferric chloride, 1 g yeast extract, 0.5 g ammonium sulphate, 0.4 g disodium hydrogen phosphate and 20 g agar. 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 sterilisation. Filter sterilised cycloheximide (50 mg/l) was added to prevent the growth of fungi in petri plates. Tenfold dilution series of the sieved rhizosphere soil was 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 in the dark at 28-30^{0}C, the plates were observed for the appearance of clearing zone around bacterial colonies which is indicative of silica solubilization. The bacterial colony which displayed the largest solubilization zone was selected for further experimentation.

Qualitative estimation of silica solubilizing activity in Bunt and Rovira agar

The selected bacterial isolates from direct sown paddy rhizosphere were subjected to a silica solubilizing test in *Bunt and Rovira*Agar, incubated at 30^{0}C for 4 days. Silica source (magnesium trisilicate) were air dried and passed through 325 mesh-sieve. The medium contains of (g/750 mL): 20 g glucose; 20 g agar; 1 g peptone; 1 g yeast extract; 0.5 g $(NH_4)_2SO_4$; 0.4 g K_2HPO_4; 0.1 g $MgCl_2$; 0.01 g $FeCl_3$; 250 ml soil extract; 750 ml aquades; pH 6.6-7.0 (Santi L P and Didiek H G. 2017). Isolates that produces a clear zone was recognized has its capability to solubilize 0.25 % magnesium trisilicate in Bunt and Rovira Agar. Clear zone from each isolates was measured by Solubilizing Index (Premono *et al.*, 1996).

Quantitative estimation of silica solubilizing activity in Bunt and Rovira broth

The solubility of silica was investigated in 100 ml *Bunt and Rovira*broth, which each of isolates supplemented by 0.25 % magnesium trisilicate ($Mg_2O_8Si_3$). Source of silica (0.25% magnesium trisilicate) were add separately into Bunt and Rovirabroth. One ml of the bacteria cell 10^8cfu/ml was inoculated into *Bunt and Rovira*broth for 7 days. After incubation periods, the culture was centrifuged at 10,000 rpm for 15 minutes to remove supernatant from debris. One ml of supernatant was added reagents into urine container and analysed by silico-molybdate's method (Santi and Didiek. 2017, Samin and Susanna. 2006).

Results and Discussion

Isolation of silica solubilizing microorganisms from direct sown paddy rhizosphere soils

Rhizospheric soils were collected from different places of Kurnool, Prakasam, Guntur and Anantapur districts such as soils of direct sown paddy growing areas by using quadratic method of soil sample collecting procedure. In Kurnool District (Atmakur, Kothapalle, Jupadu bungalow, Pamulapadu and Velugodu), Prakasam District (Tripuranthakam, Yerragondapalem, Dornala, Markapuram and Giddalur), Guntur District (Vinukonda, Narasaropet, Chilakaluripet, Sattenapalle and Piduguralla) and Anantapur District (Guntakal, Gooty, Pamidi, Tadipatri and Uravakonda) along with particular GPS coordinates for each sampling area was fixed (Table. 1) soil samples were collected from each Mandal from different farmer's fields.

Coding of the isolates based on the GPS location of the collected soil samples (Table.2).

Cultural and morphological characterization

More number of silica solubilizing microorganisms was present in the rhizosphere and they are metabolically more active than others. Morphological and cultural characteristics of all the isolates were studied viz., shape, size, margins and colour etc.,

The morphological features on Bunt and Roviraagar 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 3).

These isolates were found to be gram negative, short stumpy, rod shaped cells when observed under microscope. On the basis of biochemical reactions it was found that *Bacillus* spp. The twenty eight (28) isolates were named based on the geographical position of soil sample collected area. Based on microscopic examination and cultural characteristics, Preeti *et al.*, (2011) identified four isolates as *Pseudomonas* spp. and others as *Bacillus* spp.

Biochemical characterization

All the isolates were tested for biochemical characterization viz., Starch hydrolysis, Hydrogen sulphide test, Indole production, Catalase test, Oxidase test, Gelatine liquification, Methyl red test, Vogues Proskauer test, Citrate Utilization, Ammonia production results (Table.5) were revealed that all the silica solubilizing bacterial isolates were positive for starch hydrolysis expect SiKKK-1, SiPMM-1 and SiGSS-1.

For hydrogen sulphide test all the isolates were positive expect SiKPP-1, SiPYY-1, SiPMM-2 and SiAGG-2.All the isolates were positive for indole production test expect SiKPP-4, SiKKK-2, SiPYY-4 and SiGVV-3.

For catalase test all isolates are showed positive expect SiKAA-2, SiGVV-1 and SiAGG-4 are negative. For oxidase test SiKPP-3, SiPYY-2, SiPYY-3, SiPYY-4, SiGVV-3, SiAGG-2 and SiAGG-3are showed negative remaining all are showed positive.

In the test of gelatine liquefaction all the isolates were positive expect SiKPP-1, SiKKK-4, SiPMM-2 and SiGSS-2.For methyl red test SiKPP-1, SiKKK-3, SiPYY-4, SiGVV-2 and SiAGG-4 were negative remaining were showed the positive. For the test of vogues proskauer all the isolates were showed the positive expect the SiKKK-1 and SiGSS-1 were showed the negative (Table.5).

In the test of citrate utilization SiKAA-1, SiKKK-4 and SiGSS-3 isolates were showed the negative result remaining were all showed the positive result.

All the isolates were showed the positive for ammonia production test expect SiKPP-5, SiPYY-4 and SiGVV-3 isolates were showed the negative.

This results are correlated with Vasanthi *et al.*, (2018) isolated three silicate solubilising bacterial isolates from the different sources. They characterized morphologically and biochemically and concluded as genus *Bacillus* spp.

Screening of isolates for their zinc solubilization efficiency

Qualitative method in plat assay

All the twenty eight silica solubilizing isolates were able to form clear zone of silica solubilization on Bunt and Roviraagar plate ranged from 12.41-5.20 mm (Table.4).

Table.1 Details of soil samples collected from different districts of Andhra Pradesh

S.No.	District	Mandal	Village	Geographical Location		Sample Code	Soil type	Cropping Pattern
				Latitude	Longitude			
1.	Kurnool	Atmakur	Atmakur	$15^0$91'53" N	$78^0$70'84" E	KAA	Black soil	Rice - Rice
2.	Kurnool	Pamulapadu	Pamulapadu	$15^0$81'87" N	$78^0$50'77" E	KPP	Black soil	Rice - Blackgram
3.	Kurnool	Jupadu Bungalow	Jupadu Bungalow	$15^0$85'42" N	$78^0$36'16" E	KJJ	Black soil	Rice - Rice
4.	Kurnool	Kothapalle	Kothapalle	$15^0$97'84" N	$78^0$48'63" E	KKK	Black soil	Rice - Rice
5.	Kurnool	Velugodu	Velugodu	$15^0$72'02" N	$78^0$57'28" E	KVV	Black soil	Rice - Rice
6.	Prakasham	Tripuranthakam	Tripuranthakam	$15^0$97'80"N	$79^0$44'61" E	PTT	Black soil	Rice - Blackgram
7.	Prakasham	Yerragondapalem	Yerragondapalem	$15^0$99'96"N	$79^0$31'54" E	PYY	Black soil	Rice - Rice
8.	Prakasham	Dornala	Dornala	$15^0$90'33"N	$79^0$11'81" E	PDD	Black soil	Rice - Rice
9.	Prakasham	Markapuram	Markapuram	$15^0$74'28"N	$79^0$26'77" E	PMM	Black soil	Rice - Rice
10.	Prakasham	Giddalur	Giddalur	$15^0$37'24"N	$78^0$95'92"E	PGG	Black soil	Rice - Rice
11.	Guntur	Vinukonda	Vinukonda	$16^0$04'85"N	$79^0$75'12"E	GVV	Black soil	Rice - Rice
12.	Guntur	Narasaropet	Narasaropet	$16^0$23'48"N	$80^0$07'04"E	GNN	Black soil	Rice - Blackgram
13.	Guntur	Chilakaluripet	Chilakaluripet	$16^0$09'93"N	$80^0$18'13"E	GCC	Black soil	Rice - Blackgram
14.	Guntur	Sattenapalle	Sattenapalle	$16^0$39'92"N	$80^0$13'70"E	GSS	Black soil	Rice - Blackgram
15.	Guntur	Piduguralla	Piduguralla	$16^0$47'29"N	$79^0$90'63"E	GPP	Black soil	Rice - Blackgram
16.	Anathapuram	Guntakal	Guntakal	$15^0$16'52"N	$77^0$37'54"E	AGG	Black soil	Rice - Rice
17.	Anathapuram	Gooty	Gooty	$15^0$11'23"N	77.63'19"E	AGoGo	Black soil	Rice - Rice
18.	Anathapuram	Pamidi	Pamidi	$14^0$94'40"N	$77^0$58'57"E	APP	Black soil	Rice - Rice
19.	Anathapuram	Tadipatri	Tadipatri	$14^0$91'21"N	$78^0$00'32"E	ATT	Black soil	Rice - Rice
20.	Anathapuramu	Uravakonda	Uravakonda	$14^0$94'74"N	$77^0$22'56"E	AUU	Black soil	Rice - Rice

Table.2 Coding of SiSB isolates collected from different soil samples according to their geographical position of Andhra Pradesh

S. No.	Type of Organism	Number of isolates	Isolate code
1.	SiSB	28	SiKAA-1, SiKAA-2, SiKPP-1, SiKPP-2, SiKPP-3, SiKPP-4, SiKPP-5, SiKKK-1, SiKKK-2, SiKKK-3, SiKKK-4, SiPYY-1, SiPYY-2, SiPYY-3, SiPYY-4, SiPMM-1, SiPMM-2, SiPMM-3, SiGVV-1, SiGVV-2, SiGVV-3, SiGSS-1, SiGSS-2, SiGSS-3, SiAGG-1, SiAGG-2, SiAGG-3, SiAGG-4.

Table.3 Morphological and cultural characterization of Silica solubilizing bacterial (SiSB) isolates of different soil samples

S.No.	Isolate code	Gram reaction	Cell shape	Colony morphology			
				Colour	Form	Elevation	Margin
1.	SiKAA-1	-ve	Rod	Creamy	Circular	Raised	Entire
2.	SiKAA-2	-ve	Rod	Milky white	Irregular	Raised	Irregular
3.	SiKPP-1	+ve	Rod	Creamy white	Irregular	Flat	Undulate
4.	SiKPP-2	-ve	Rod	Milky white	Irregular	Flat	Irregular
5.	SiKPP-3	-ve	Rod	Milky white	Irregular	Flat	Undulate
6.	SiKPP-4	-ve	Rod	Creamy white	Circular	Convex	Entire
7.	SiKPP-5	-ve	Rod	Creamy	Circular	Convex	Irregular
8.	SiKKK-1	-ve	Rod	White	Irregular	Convex	Irregular
9.	SiKKK-2	+ve	Rod	White	Circular	Raised	Irregular
10.	SiKKK-3	-ve	Rod	White	Irregular	Convex	Entire
11.	SiKKK-4	-ve	Rod	Off white	Circular	Raised	Undulate
12.	SiPYY-1	-ve	Rod	Yellow	Circular	Flat	Curled
13.	SiPYY-2	-ve	Rod	Yellow	Irregular	Lobate	Irregular
14.	SiPYY-3	+ve	Rod	White	Circular	Convex	Entire
15.	SiPYY-4	-ve	Rod	White	Irregular	Flat	Undulate
16.	SiPMM-1	-ve	Rod	Off white	Irregular	Raised	Undulate
17.	SiPMM-2	-ve	Rod	White	Circular	Elevated	Curled
18.	SiPMM-3	-ve	Rod	White	Circular	Raised	Entire
19.	SiGVV-1	-ve	Rod	Off white	Circular	Convex	Entire
20.	SiGVV-2	-ve	Rod	White	Irregular	Flat	Irregular
21.	SiGVV-3	-ve	Rod	White	Irregular	Elevated	Irregular
22.	SiGSS-1	-ve	Rod	White	Circular	Flat	Undulate
23.	SiGSS-2	+ve	Rod	White	Round	Umbonate	Irregular
24.	SiGSS-3	-ve	Rod	Light yellow	Irregular	Raised	Irregular
25.	SiAGG-1	+ve	Rod	White	Irregular	Elevated	Undulate
26.	SiAGG-2	-ve	Rod	White	Circular	Elevated	Entire
27.	SiAGG-3	-ve	Rod	White	Irregular	Flat	Entire
28.	SiAGG-4	-ve	Rod	White	Circular	Elevated	Entire

Table.4 Estimation of Silica solubilization quantitatively and qualitatively for the screening of SiSB isolates from different soil samples

S.No.	Isolate name	Soluble Si concentration ppm	Silicasolubilisation		
			Silica solubilization index (SSI)	Zone diameter (mm)	
				Solubilization zone	Culture diameter
1.	SiKAA-1	1.83	3.86	6.14	2.15
2.	SiKAA-2	1.71	3.77	6.79	2.45
3.	SiKPP-1	2.16	4.95	12.41	3.14
4.	SiKPP-2	1.51	3.61	7.15	2.74
5.	SiKPP-3	0.75	2.73	6.14	3.54
6.	SiKPP-4	1.98	4.16	9.21	2.91
7.	SiKPP-5	1.85	3.92	10.14	3.47
8.	SiKKK-1	2.01	4.07	11.02	3.59
9.	SiKKK-2	1.56	3.36	8.14	3.45
10.	SiKKK-3	0.74	2.88	6.49	3.46
11.	SiKKK-4	1.88	3.97	10.15	3.42
12.	SiPYY-1	1.91	3.90	6.99	2.41
13.	SiPYY-2	2.02	4.32	10.42	3.14
14.	SiPYY-3	2.12	4.85	12.02	3.12
15.	SiPYY-4	1.94	3.92	8.12	2.78
16.	SiPMM-1	2.09	4.19	11.02	3.45
17.	SiPMM-2	1.80	3.80	8.79	3.14
18.	SiPMM-3	2.06	4.68	8.54	2.32
19.	SiGVV-1	2.02	4.31	7.15	2.16
20.	SiGVV-2	1.98	4.02	7.48	2.48
21.	SiGVV-3	0.91	2.89	6.15	3.26
22.	SiGSS-1	2.08	4.24	9.01	2.78
23.	SiGSS-2	2.01	4.20	10.12	3.16
24.	SiGSS-3	2.04	4.60	7.64	2.12
25.	SiAGG-1	0.52	2.64	5.20	3.18
26.	SiAGG-2	1.45	3.62	8.79	3.36
27.	SiAGG-3	1.32	3.42	9.15	3.78
28.	SiAGG-4	1.51	3.65	6.53	2.46
SE(m)		0.05		0.08	
CD(P=0.05)		0.12		0.16	
CV		1.76		1.91	

Table.5 Biochemical and physiological characterization of Silica Solubilizing Bacterial (SiSB) isolates collected from different soil samples

S.No.	Isolate code	Starch hydrolysis	Hydrogen sulphide test	Indole production	Catalase test	Oxidase test	Gelatine liquification	Methyl red test	Vogues Proskauer test	Citrate Utilization	Ammonia production
1.	SiKAA-1	+	+	+	+	+	+	+	+	-	+
2.	SiKAA-2	+	+	+	-	+	+	+	+	+	+
3.	SiKPP-1	+	-	+	+	+	+	+	+	+	+
4.	SiKPP-2	+	+	+	+	+	+	+	+	+	+
5.	SiKPP-3	+	+	+	+	-	+	+	+	+	+
6.	SiKPP-4	+	+	-	+	+	+	+	+	+	+
7.	SiKPP-5	+	+	+	+	+	+	+	+	+	-
8.	SiKKK-1	-	+	+	+	+	+	+	-	+	+
9.	SiKKK-2	+	+	-	+	+	+	+	+	+	+
10.	SiKKK-3	+	+	+	+	+	+	-	+	+	+
11.	SiKKK-4	+	+	+	+	+	-	+	+	-	+
12.	SiPYY-1	+	-	+	+	+	+	+	+	+	+
13.	SiPYY-2	+	+	+	+	-	+	+	+	+	+
14.	SiPYY-3	+	+	+	+	-	+	+	+	+	+
15.	SiPYY-4	+	+	-	+	-	+	-	+	+	-
16.	SiPMM-1	-	+	+	+	+	+	+	+	+	+
17.	SiPMM-2	+	-	+	+	+	-	+	+	+	+
18.	SiPMM-3	+	+	+	+	+	+	+	+	+	+
19.	SiGVV-1	+	+	+	-	+	+	+	+	+	+
20.	SiGVV-2	+	+	+	+	+	+	-	+	+	+
21.	SiGVV-3	+	+	-	+	-	+	+	+	+	-
22.	SiGSS-1	-	+	+	+	+	+	+	-	+	+
23.	SiGSS-2	+	+	+	+	+	-	+	+	+	+
24.	SiGSS-3	+	+	+	+	+	+	+	+	-	+
25.	SiAGG-1	+	+	+	+	+	+	+	+	+	+
26.	SiAGG-2	+	-	+	+	-	+	+	+	+	+
27.	SiAGG-3	+	+	+	+	-	+	+	+	+	+
28.	SiAGG-4	+	+	+	-	+	+	-	+	+	+

Plate.1 Silica solubilization of different bacterial isolates on SiSB medium

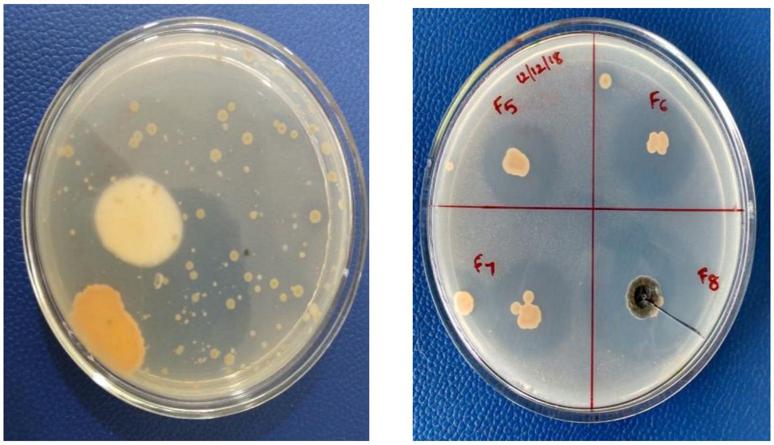


Plate.2 Silica solubilization bySiKPP-1bacterial isolate

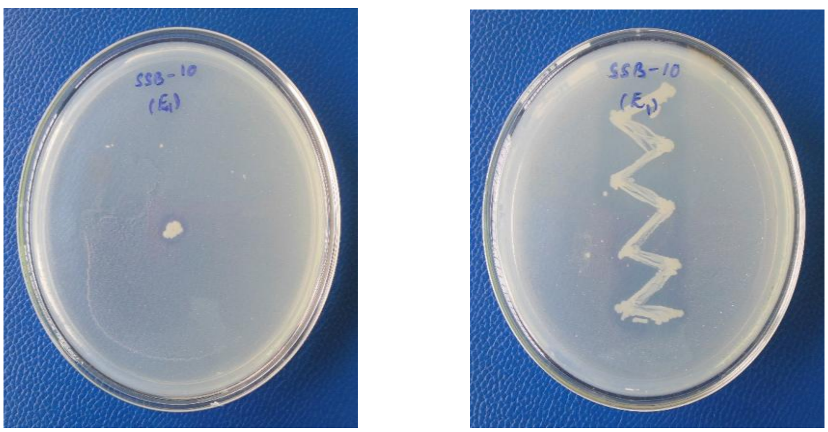


Plate.3 Silica solubilization by SiPYY-3bacterial isolate

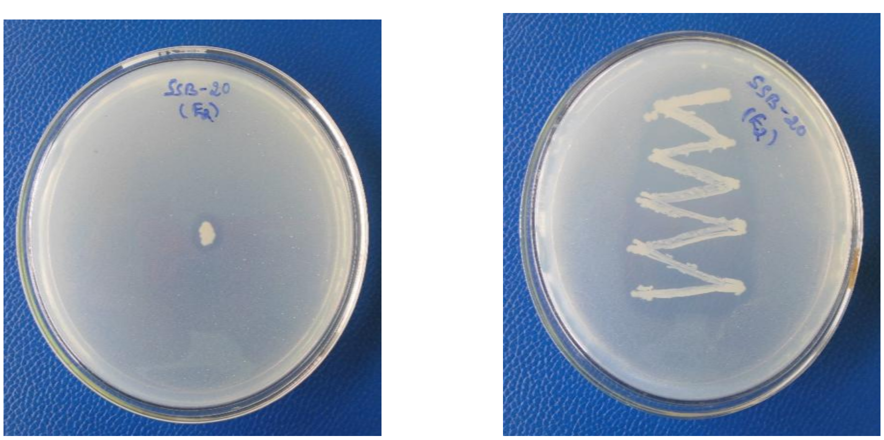
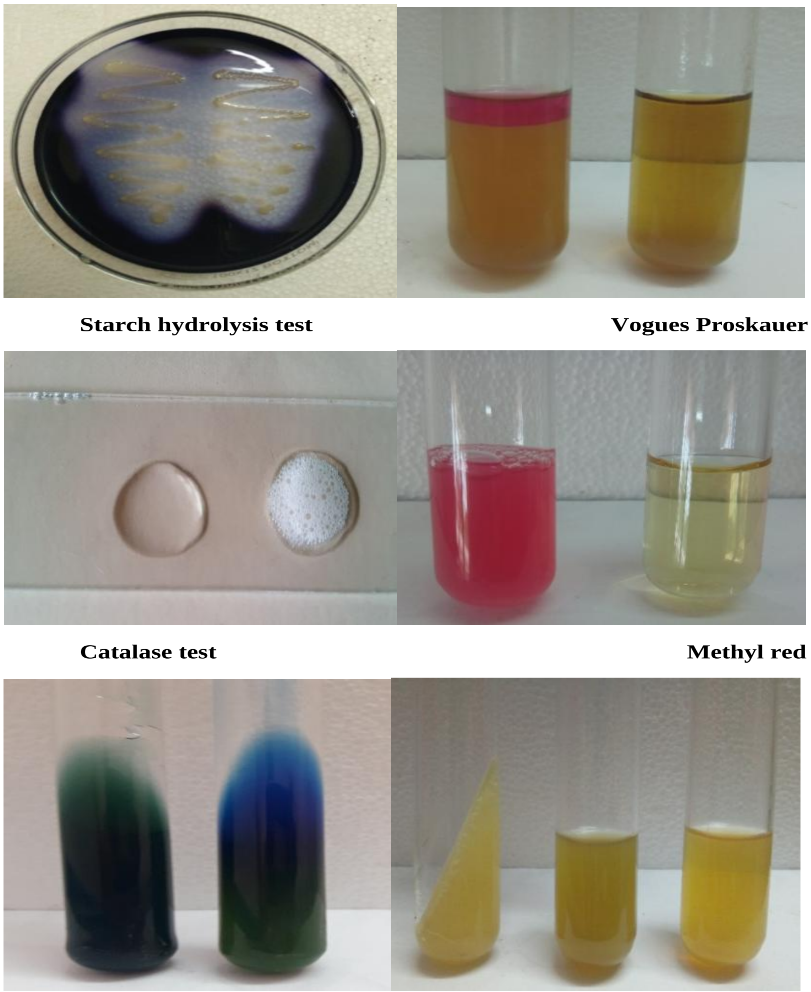


Plate.4 Different biochemical tests for characterization of ZnSB isolates



Starch hydrolysis test **Vogues Proskauer test**

Catalase test **Methyl red test**

Citrate test **Gelatine test**

Among them SiKPP-1of *Bcillus* spp detected the highest solubilization zone (12.41 mm) followed by SiPYY-3(12.02mm) and the lowest solubilization zone was observed with SiAGG-1(5.20mm). Silica solubilization index were also highest in SiKPP-1(4.95) followed by SiPYY-3(4.85) and the lowest solubilization index was observed in SiAGG-1(2.64).

This results were correlated with the Vijayapriya and Muthukkaruppan (2010) isolated ten bacterial isolates from soil, identified and characterized as *Bacillus mucilaginosus.* Among the 10 isolates tested, four isolates viz., SSB-3, SSB-5, SSS-8 and 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 solubilisation.

Quantitative method in broth assay

All the twenty eight silica solubilizing isolates were able to solubilize the available silica in Bunt and Rovirabroth, which each of isolates supplemented by 0.25 % magnesium trisilicate ($Mg_2O_8Si_3$). Among them SiKPP-1 recorded the more available silica content of 2.16 ppm. Second best was showed by

SiPYY-3*i.e.,* 2.12 ppm. The lowest was shown by SiAGG-1with 0.52 ppm. Similar results were observed by Sulizah *et al.,* (2018) isolated silicate bacteria from paddy rhizosphere soil in Bunt and Rovira medium and also characterized 39 characters, which is include of colony morphological, cell morphological and physiological-biochemical test. Five isolates silicate solubilizing bacteria were found OS4, OS5, OS7, OS12 and OS13. The highest Solubilizing Index was gained by OS7 on 1.10, while the highest silicate concentration was solubilized by OS12 on 1.053 ppm in Bunt and Rovira broth.

In conclusion, silica available in soilin various forms it was solubilized by the certain microorganisms known as silica solubilizing microorganisms. Silica solubilizing microorganisms has potential to release soluble silica from insoluble inorganic (Ca, Al, K and Mg) silicates and biogenic materials like diatomaceous earth, siliceous earth, rice husk and rice straw. *Bacillus* spp reported to be active in the silica solubilization process. Initially silica solubilizing bacteria was isolated and grown in Bunt and Roviramedia with insoluble source of magnesium trisilicate. All the isolates were characterized morphologically and biochemically. The efficient isolates were subjected to further characterization. The isolates were selected based on their good performance of PGPR characters. For silica solubilization the individual isolates were able to form a solubilization zone ranged from 5.20 - 12.4 mm. The highest silica solubilization index (4.95) was recorded in SiKPP-1and lowest silica solubilization index was observed in SiAGG-1(2.64).

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