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Efficacy of Silica Solubilizing Bacteria as Plant Growth Promoting Rhizobacteria and Their Biochemical Characteristics

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Twenty eight isolates of silica solubilising bacterial inoculants were collected from rhizosphere soils of rice crop in Kurnool, Prakasam, Guntur and Anantapur districts of Andhra Pradesh by using insoluble source of silica. These isolates were verified for plant growth promoting rhizobacteria (PGPR) activity and then subjected biochemical tests in the Department of Agricultural microbiology, Advanced Post Graduate Centre, ANGRAU, Lam, Guntur, Andhra Pradesh, India. Two isolates, SiKPP-1 (Silica solubilizing isolate from Kurnool district, Pamulapadu Mandal and

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Pamulapadu Village - 1) & SiPYY-3 (Silica solubilizing isolate from Prakasam district, Yerragondapalem Mandal and Yerragondapalem Village - 3) showed the highest efficiency in the aspects of silica solubilization, phosphate solubilization, potassium release, exopolysaccharide production activity, indole acetic acid production activity, siderophore production activity and biochemical tests including starch hydrolysis, Hydrogen sulphide test, Indole production, Catalase test, Oxidase test, Gelatine liquification, Methyl red test, Vogues Proskauer test, Citrate Utilization and Ammonia production. Therefore these two isolates (SiKPP-1&SiPYY-3) were selected for further pot and field studies in direct sown paddy crop.

Keywords: Oryzasativa L.; solubilizing bacteria; PGPR.

1. INTRODUCTION

"Rice (*Oryzasativa* L.) is one of the vital staple foods for more than 50 % of the world's population providing major source of food energy. It is cultivated in 114 countries across the world in an area of 192 million hectares with annual production of 546.2 million tons. In India Export value of rice in 2011 was 115.86 billion rupees now in 2022 it was reached to 721.16 billion rupees" [1].

"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 as 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" [2]. Paddy is the greatest accumulator of Si up to 10 % of its dry weight, but can't absorb Siby itself.

"Silica nutrition also reduces many abiotic stresses including physical stress like lodging, drought, radiation, high temperature, freezing, UV and chemical stresses like salt metal toxicity, nutrient imbalance of many others" [3]. The positive effects are credited to Si deposition in cell wall of roots, leaves, culms and hulls. Soluble silica reduces Aluminium (Al) toxicity because monosilica acid reacts with mobile Al and forms slightly soluble aluminosilicates.

"Si is accumulated in the form of silica gel and is deposited in epidermal cells, scelerenchyma, vascular bundles and inflorescence brackets in cereals" [4]. "The accumulated Si not only improves growth and yield of these plants but is also involved in induction of systemic resistance (ISR) against pests and diseases" [5].

"Silicate solubilizing bacteria (SSB) can play an effective role in soil by solubilizing insoluble forms of silicates. In addition, some SSB can also solubilize potassium and phosphates, hence increasing soil fertility and enhancing plant defence mechanisms. Natural existence of silica solubilizing bacteria in rice ecosystem, wheat, sugarcane, bamboo rhizosphere region" [6]. Hence this study was conducted for identification of efficient silica solubilizing isolates for further pot and field investigations on direct sown paddy.

2. MATERIALS AND METHODS

2.1 Isolation of Silica Solubilizing Microorganisms from Direct Sown Rice Rhizospheric Soils

Isolation of silica solubilizing bacteria: Silica solubilizing bacteria (SiSB) were isolated from the rhizosphere soils of the direct sown rice crop of Kurnool (Atmakur, Kothapalle, Jupadu bungalow, Pamulapadu and Velugodu Mandals and villages), Prakasam (Tripuranthakam, Yerragondapalem, Dornala, Markapuram and Giddalur Mandals and villages), Guntur (Vinukonda, Narasaraopet, Chilakaluripet, Sattenapalle and Piduguralla Mandals and villages) and Anantapur (Guntakal, Gooty, Pamidi, Tadipatri and Uravakonda Mandals and villages) and districts. "Modified Bunt and Rovira medium 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 was used for isolation of silica solubilizing bacteria. 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. 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˚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" [7].

Silica solubilizing activity in Bunt and Rovira agar: "The selected bacterial isolates from direct sown paddy rhizosphere were subjected to silica solubilizing test in Bunt and Rovira Agar, incubated at 30˚C for 4 days. Silica source (magnesium trisilicate) was air dried and passed through 325 mesh-sieve. The medium was composed of (g 750 mL^{-1}): 20 g glucose; 20 g agar; 1 g peptone; 1g 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; 750ml aquades; pH 6.6-7.0 [8]. Isolates that produced a clear zone was recognized forits capability to solubilize 0.25% magnesium trisilicate in Bunt and Rovira Agar. Clear zone from each isolate was measured by Solubilizing Index" [9].

Silica solubilizing activity in Bunt and Rovira broth: "The solubility of silica was investigated in 100 ml Bunt and Rovira broth, supplemented with 0.25% magnesium trisilicate ($Mg_2O_8Si_3$). One ml of the bacteria cell 10^8 cfu ml^{-1} was inoculated into Bunt and Rovira broth and incubatedfor 7days. The culture was then centrifuged at 10,000 rpm for 15 minutes to remove supernatant from debris. One ml of supernatant was added to the reagents and analysed by silico-molybdate's method" [10].

2.2 Characterization of Silica Solubilizing Bacterial Isolates by Morphological, Cultural and Biochemical Characters

Morphological characterization: "All the zinc and silica solubilizing bacterial isolates were checked for their purity and then studied for the colony morphology and pigmentation" [11]. The cell shape and gram reaction was also recorded as per the standard procedures given by [12].

Colony morphology: Morphological characteristics of the colony of each isolate were examined by inoculatingon Nutrient agar and specialized medium and incubating according to the nature of 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.

Gram's staining: "A drop of sterile distilled water was placed in the centre of glass slide. A loopful of inoculum from young culture was taken, mixed with water and placed in the centre of the slide. The suspension was spread out on slide using the tip of inoculation needle to make a thin suspension. The smear was dried in air and fixed through mild heating by passing the slide 3 to 4 times over the flame. The smear was then flooded with crystal violet solution for 30 seconds and washed gently with flow of tap water. Then the slide was flooded with iodine solution. After incubation at room temperature for 30 seconds, Iodine solution was drained out by washing with 95% ethanol. After that, it was washed with water within 15 to 30 seconds and blot carefully. The smear was incubated with safranin solution for 30 seconds. The slide was washed gently in flow of tap water and dried in air" [12]. The slide was examined under microscope at 100 X power with oil immersion and data was recorded [12].

Biochemical and physiological characterization of silica solubilizing isolates: Different biochemical tests performed and the protocols followed are briefly outlined below.

Starch hydrolysis: "Sterile starch agar plates were spotted with 10 µl overnight broth cultures of the isolates and incubated at 28 ± 2˚C for 24-48 hours. After incubation, the plates were flooded with iodine solution. The formation of a transparent zone around the colony was taken as a positive reaction to the test" [13].

Hydrogen sulfide test: "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˚C. Visualization of black colour along the line of inoculation indicated a positive reaction to the test" [14].

Indole production: "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˚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" [15].

Catalase test: "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" [16].

Oxidase test: "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˚C. After incubation, 2-3 drops of N, N, N', N'- tetramethyl-p-phenylene diamine dihydrochloride (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" [17].

Gelatin liquefaction: "The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated for 24 hours at 28 ± 2˚C. Then 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" [13].

Methyl red test: "Sterilized glucose-phosphate broth tubes were inoculated with the test 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 colour development was taken as positive and yellow colour production was taken as negative for the test" [18].

Vogues Proskauer's test: "To the pre-sterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37˚C for 48 hours. After incubation ten drops of Barritt's reagent-A was added and gently shaken, followed by10 drops of Barritt's reagent-B. The development of pink colour in the broth was taken as positive for the test" [13].

Citrate utilization: "Isolates were streaked on Simmon's citrate agar slants and incubated at 28 ± 2˚C for 24 hours. Change in colour from green to blue indicated the positive reaction for citrate utilization" [13].

Ammonia production: "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˚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" [19].

Screening of isolates for plant growth promoting (PGP) properties: Pure isolates were isolated by streaking isolates on respective media plates and screened for thefollowing Plant growth promoting properties.

Exo Polysaccharide production (EPS): "Initially TSP broth with -0.30 Mpa osmotic stress, 15% PEG 6000 was prepared, then inoculated with test culture and incubated for 3 days. After incubation culture was centrifuged at 20,000 g in therefrigerated centrifuge for 25 min. The supernatant was filtered through 0.45 µm nitrocellulose membrane, dialysed against 4˚C and then centrifuged at 20,000 g for 25 min to remove any insoluble material; and mixed with 3 volumes ice cold absolute alcohol and kept overnight at 4˚C. Precipitated EPS was obtained by centrifuging at 10,000 g for 15 min and then suspended in water" [20].

Siderophore production: "Siderophore production was estimated qualitatively by using Chrome Azurol S (CAS) Agar medium" [21]. For the detection of siderophores, each isolate was grown in synthetic medium, containing 0.5 µM of iron and incubated for 24 h in a rotary shaker at room temperature. Chrome Azurol S (CAS) assay was used to detect the siderophores. The CAS plates were used to check the culture supernatant for the presence of siderophores. Culture supernatant was added to the wells made on the CAS agar plates and incubated at room temperature for 24 h. Formation of yellow to orange coloured zone around the well indicated the siderophore production.

Indole acetic acid production: Indole Acetic acid Production was tested according to [22]. The active culture of each test isolate was raised in 5 ml respective broth tubes and incubated at determined temperature and time. After incubation these cultures were centrifuged at recommended rpm and time. Two drops of Orthophosphoric acid was added to 2 ml of supernatant and incubated for 30 min to develop the colour. Development of pink colour considered as positive for IAA production.

Phosphate solubilisation: "For this test sterilized Pikovskaya's agar was poured as a thin layer on to the sterilized petriplates and incubated for 24 h. After incubation the Pikovskaya's plates were spot inoculated with isolates and incubated at 28 ± 1˚C for 4-5 days" [23]. Formation of a clear zone around the colonies was considered as positive result for phosphate solubilization.

PSE (Phosphate Solubilization Efficiency) = Z / C x 100

Z = Clearance zone including bacterial growth
C = Colony diameter

Potassium solubilisation: The isolates were inoculated on agar medium containing 0.5 percent insoluble potassium compounds *viz.,* mica (potassium aluminium silicate) or Aleksandrov's agar mediumhaving0.5 percent potassium aluminium silicate. The test organisms were inoculated on these media and incubated at 30˚C for 48-72 hours. The diameter of the clearing zones around the colonies were measured [24].

3. RESULTS AND DISCUSSION

3.1 Screening of Silica Solubilizing Bacteria

3.1.1 Screening of silica solubilizing bacteria for their silica solubilisation efficiency

3.1.1.1 Qualitative method of silica solubilizing efficiency in plate assay

All the twenty eight silica solubilizing isolates were able to form clear zone of silica solubilizationin Bunt and Rovira agar plate ranged from 5.20 - 12.41 mm (Table 1). Among them SiKPP-1 of *Bacillus* sp. statistically recorded the highest solubilization zone (12.41 mm) followed by SiPYY-3 (12.02 mm) and the lowest solubilization zone was observed with SiAGG-1 (5.20 mm). Silica solubilization index was 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). These results were correlated with [5] where ten bacterial isolates from soil were identified and characterized as *Bacillus mucilaginosus.* Among the 10 isolates tested, four isolates *viz.,* SSB-3, SSB-5, SSS-8 and SSS-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.

3.1.1.2 Quantitative method of silica solubilizing efficiency in broth assay

All the twenty eight silica solubilizing isolates were able to solubilize the available silica in Bunt and Rovira broth, supplemented with 0.25 % magnesium trisilicate ($Mg_2O_8Si_3$). Among them SiKPP-1 recorded the most available silica content of 2.16 ppmfollowed by SiPYY-3 (2.12ppm), SiPMM-1 (2.09mm), SiGSS-1 (2.08mm), SiPMM-3 (2.06mm), SiGSS-3 (2.04mm), SiPYY-2 (2.02mm), SiGVV-1(2.02mm) and SiGSS-2 (2.01mm) which were statistically on par. The lowest was shown by SiAGG-1 with 0.52 ppm.

Similar results were observed by [25] who isolated silicate bacteria from paddy rhizosphere soil in Bunt and Rovira medium and also studied 39 characters, including colony morphology, cell morphology and physiological-biochemical characters. Five isolates of silicate solubilizing bacteria were found *viz.,* OS4, OS5, OS7, OS12 and OS13. The highest Solubilizing Index was attained by OS7 (1.10), while the highest silicate concentration was solubilized by OS12 (1.053 ppm) in Bunt and Rovira broth (Table 1).

Screening of silica solubilizing bacteria for phosphate solubilization efficiency: Bacterial isolates were screened for phosphate solubilization potential in Pikovskayas broth supplemented with 0.1 % tricalcium phosphate quantitatively and qualitatively on Pikovskayas agar plate.

Among all the isolates, twenty eight silica solubilizing bacterial isolates were positive for phosphate solubilisation qualitatively. Statistically significant and the highest solubilization was recorded in the isolate SiKPP-1 (10.42 mm), followed by the isolate SiPYY-3 (10.03 mm) and least by SiPMM-1 (5.02 mm). All the bacterial isolates were found positive for phosphate solubilisation quantitatively. The highest solubilization was recorded in the isolate SiKPP-1 (6.46 % of Pi), statistically superior, followed by SiPYY-3 (6.12 % of Pi) and least in SiPMM-1 (3.94 % of Pi) (Table 2). Both qualitative and quantitative values were taken intoconsideration for screening. Insoluble phosphate compounds can be solubilized by organic acids and phosphatase enzymes produced by plants and microorganisms. Phosphate solubilisation was also showed by most of the PGPR. Phosphorous is essential for plant health and is typically insoluble or poorly soluble in soils under salt stress conditions. Some of the bacteria improve the solubilization of unavailable phosphorous and applied phosphates, resulting in higher yields even under unfertilized conditions.

Similar findings were observed by [26] where 111 Silicate solubilizing bacteria (SSB) were isolated from various habitats in Pakistan. Some SiSB also solubilized phosphate thus increasing soil fertility and enhancing plant defence mechanisms. Out of these, 35 bacterial isolates were capable of solubilizingboth silicate and phosphate. The highest phosphate solubilization

(zone diameter 55 mm) was observed for bacterial isolate NR-2.

Screening of silica solubilizing bacteria for potassium solubilization efficiency: All the silica solubilizing bacterial isolates recorded positive for potassium releasing activity. Statistically, the highest amount was recorded in the isolate SiKPP-1 (10.04 mm), followed by SiPYY-3 (10.02 mm) the least was recorded by SiKPP-5 (5.06 mm).

Among the isolates highest amount of potassium releasing activity was recorded with SiKPP-1 (2.52 µg ml^{-1}), followed by SiPYY-3 (2.46 µg ml^{-1}), SiKPP-4 (2.41 µg ml^{-1}), SiPYY-4 (2.32 µg ml^{-1}) and SiGSS-3 (2.31 µg ml^{-1}) which were statistically on par and the least by SiKPP-5 (1.06 µg ml^{-1}) (Table 3). "Potassium releasing activity is the most important mechanism for the microorganisms to solubilize a fixed form of potassium in the soil. The main mechanism of potassium releasing bacteria is acidolysis, chelation, exchange reactions, complexolysis and production of organic acids. The utilization of potassium releasing bacteria to increase the soluble form of potassium and has been regarded as a desirable pathway to increase plant yields" [27].

Comparable results were observed with [28] who isolated silicate solubilizing bacterial isolate (SSB) IIRR-1 from rhizopshere soil of rice and characterized for its potential to release soluble silica from insoluble inorganic (Ca, Al, K and Mg) silicates and biogenic materials. Incubation studies revealed solubilization of potassium besides silica by the SSB isolate. The range of potassium solubilisation by the isolates was 2.52 - 8.45 µg ml^{-1}.

Screening of silica solubilizing bacteria for Exo Polysaccharide production (EPS) efficiency: Exopolysaccharide was produced by all most all the silica solubilizing bacterial isolates. The data on EPS production of all silica solubilizing bacterial isolates were presented in Table 4. The bacterial isolates producing the highest EPS production werefurther tested for biochemical characteristics and regarded as coherent bacteria. Among twenty eight isolates maximum amount of EPS production was observed in the isolate SiKPP-1 (9 mg ml^{-1}) followed by SiPYY-3 (8 mg ml^{-1}).No EPS production was observed in the isolates SiKKK-3, SiPYY-4, SiPMM-1 and SiGVV-3. Exopolysaccharides possess unique cementing and water holding properties resulting inbiofilm formation.

Xia et al. [29] isolated "silicate mineral-solubilizing bacterial strain Q12 from the surfaces of weathered feldspar and identified as *Bacillus globisporus* Q12 based on the 16S rDNA gene sequence analysis. Three silicate minerals (feldspar, muscovite and biotite) were used to investigate potassium and silicon mobilization by strain Q12". Apart from silica mobilization, exopolysaccharide production was also observed in *Bacillus globisporus* Q12. The range of exopolysaccharide production by the isolates was from 0.46 to 8.4 mg ml^{-1}.

Screening of silica solubilizing bacteria for Siderophore production efficiency: Among all twenty eight isolates siderophore production was observed in the isolates of SiKAA-1, SiKPP-1, SiKPP-2, SiKPP-3, SiKKK-1, SiKKK-2, SiKKK-3, SiKKK-4, SiPYY-1, SiPYY-3, SiPYY-4, SiPMM-1, SiPMM-3, SiGVV-1, SiGVV-3, SiGSS-1, SiGSS-3, SiAGG-1, SiAGG-3 and SiAGG-4 (Table 4). Siderophores are produced and utilized by bacteria as iron (Fe)-chelating agents in response to iron deficiency which normally occurs in neutral to alkaline pH soils. Although siderophore production is mainly achieved under iron deficiency, other factors such as carbon source, nitrogen source, pH, and temperature are essential to the synthesis of siderophores.

Siderophores of pseudobacin and pyoverdin represent antimicrobial activity and affinity to trivalent iron [30]. Tailor [31] screened seven bacteria from the sugarcane rhizosphere. Isolates produced more than 85 % siderophore units. Amongst them, S-11 was the most efficient siderophore producer (96 % SU). Physico-chemical parameters were evaluated for the optimum production of siderophores by *Pseudomonas fluorescens*strain. DAPG is a low-molecular-weight polyketide known to inhibit many pathogenic fungi and is responsible for the biocontrol ability of many *P. fluorescens*isolates [32].

Screening of silica solubilizing bacteria for Indole Acetic Acid (IAA) Production efficiency: The results for IAA test revealed that all the fifty one bacteria produced IAA. Among all the isolates *viz*., SiKPP-1, SiKPP-4, SiKKK-2, SiKKK-4, SiPYY-3, SiGVV-2, SiGSS-1 and SiAGG-4exhibited strong (**) IAA production activity, where remaining isolates were weak (*) in IAA production (Table 4).IAA production varieswith different physiological parameters

such as pH, temperature, carbon and nitrogen sources of culture media. So that the intended conditions at which the IAA production is maximized. Similar findings were recorded by [33] who isolated, identified japonica rice (*Oryzasativa* L. cv. Dongjin) root-associated rhizobacteria and investigated their ability to solubilize silicate, produce indole acetic acid (IAA), promote plant growth, and encourage silicon (Si) uptake and deposit in plants. A single bacterial isolate was selected on the basis of its silica-solubilizing ability and IAA production.

Biochemical and Physiological Characterization of silica solubilizing bacterial isolates: "All the isolates were subjected to biochemical characterization *viz.,* Starch hydrolysis, Hydrogen sulphide test, Indole production, Catalase test, Oxidase test, Gelatine liquification, Methyl red test, Vogues Proskauer test, Citrate Utilization and Ammonia production. Results (Table 5) revealed that all the silica solubilizing bacterial isolates were positive for starch hydrolysis expect SiKKK-1, SiPMM-1 and SiGSS-1" [11].

"For hydrogen sulphide test all the isolates were positive except SiKPP-1, SiPYY-1, SiPMM-2 and SiAGG-2. All the isolates were positive for indole production test except SiKPP-4, SiKKK-2, SiPYY-4 and SiGVV-3. For catalase test all isolates are showed positive reaction except SiKAA-2, SiGVV-1 and SiAGG-4. For oxidase test SiKPP-3, SiPYY-2, SiPYY-3, SiPYY-4, SiGVV-3, SiAGG-2 and SiAGG-3 showed negative reaction and remaining isolates were positive" [11].

Table 1. Quantitative and qualitative estimation of silica solubilisation of SiSB isolates from different districts of Andhra Pradesh

S. No.	Isolate name	Soluble Si concentration ppm	Silica solubilization		
			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)		0.056		0.086	
CD(*p*=0.05)		0.168		0.258	
CV (%)		1.486		1.645	

Table 2. Screening of phosphate solubilization efficiency of SiSB isolates from different soil samples

S.No.	Isolate name	% of Inorganic Phosphorus Released	Phosphate solubilization		
			P solubilization index (PSI)	Zone diameter (mm)	
				Solubilization zone	Culture diameter
1.	SiKAA-1	4.11	3.38	7.89	3.32
2.	SiKAA-2	5.07	3.78	8.91	3.21
3.	SiKPP-1	6.46	4.64	10.42	2.86
4.	SiKPP-2	4.18	3.27	7.13	3.14
5.	SiKPP-3	5.22	3.56	9.12	3.56
6.	SiKPP-4	4.25	3.61	8.13	3.12
7.	SiKPP-5	5.03	2.44	5.14	3.58
8.	SiKKK-1	4.05	3.91	9.13	3.14
9.	SiKKK-2	4.92	3.91	7.16	2.46
10.	SiKKK-3	5.06	3.57	8.12	3.16
11.	SiKKK-4	5.16	3.81	8.76	3.12
12.	SiPYY-1	4.19	3.91	9.13	3.14
13.	SiPYY-2	5.08	3.26	8.49	3.76
14.	SiPYY-3	6.12	4.54	10.03	2.83
15.	SiPYY-4	5.13	3.62	8.16	3.12
16.	SiPMM-1	3.94	2.34	5.02	3.74
17.	SiPMM-2	4.25	4.04	9.61	3.16
18.	SiPMM-3	4.21	3.72	9.46	3.48
19.	SiGVV-1	4.81	3.06	7.14	3.46
20.	SiGVV-2	5.61	3.72	7.02	2.58
21.	SiGVV-3	4.16	3.83	7.86	2.78
22.	SiGSS-1	4.06	3.60	8.16	3.14
23.	SiGSS-2	5.42	3.42	9.16	3.78
24.	SiGSS-3	5.61	3.58	8.16	3.16
25.	SiAGG-1	4.16	4.20	7.85	2.45
26.	SiAGG-2	5.46	3.36	8.16	3.46
27.	SiAGG-3	4.46	3.80	8.01	2.86
28.	SiAGG-4	5.18	3.87	8.14	2.84
SE(m)		0.046		0.084	
CD (*p*=0.05)		0.138		0.252	
CV (%)		1.562		1.786	

Table 3. Screening of potassium solubilization efficiency of SiSB isolates from different districts of Andhra Pradesh

S. No.	Isolate name	Soluble K concentration ug ml^{-1}	Potassium solubilization		
			K solubilization index (KSI)	Zone diameter (mm)	
				Solubilization zone	Culture diameter
1.	SiKAA-1	2.05	3.88	8.14	2.83
2.	SiKAA-2	2.02	3.30	7.12	3.10
3.	SiKPP-1	2.52	4.51	10.04	2.86
4.	SiKPP-2	1.32	4.10	8.19	2.64
5.	SiKPP-3	1.23	4.02	9.47	3.14
6.	SiKPP-4	2.41	4.21	9.17	2.86
7.	SiKPP-5	1.06	2.60	5.06	3.16
8.	SiKKK-1	2.12	3.93	9.16	3.13
9.	SiKKK-2	1.13	3.77	8.49	3.06
10.	SiKKK-3	1.23	3.93	8.14	2.78
11.	SiKKK-4	1.31	3.88	9.46	3.28
12.	SiPYY-1	2.01	3.91	9.13	3.14

S. No.	Isolate name	Soluble K concentration ug ml^{-1}	Potassium solubilization		
			K solubilization index (KSI)	Zone diameter (mm)	
				Solubilization zone	Culture diameter
13.	SiPYY-2	1.23	3.74	9.48	3.46
14.	SiPYY-3	2.46	4.36	10.02	2.98
15.	SiPYY-4	2.32	3.78	9.68	3.48
16.	SiPMM-1	1.22	3.59	8.13	3.14
17.	SiPMM-2	1.56	4.26	8.03	2.46
18.	SiPMM-3	1.35	3.39	9.13	3.82
19.	SiGVV-1	2.02	3.85	8.15	2.86
20.	SiGVV-2	1.29	3.93	9.13	3.12
21.	SiGVV-3	1.46	3.79	8.81	3.16
22.	SiGSS-1	1.02	3.85	7.95	2.79
23.	SiGSS-2	1.48	3.94	7.23	2.46
24.	SiGSS-3	2.31	4.02	8.84	2.93
25.	SiAGG-1	1.58	3.37	7.35	3.10
26.	SiAGG-2	1.48	3.99	9.13	3.05
27.	SiAGG-3	1.56	3.58	8.19	3.18
28.	SiAGG-4	2.02	3.89	9.10	3.15
SE(m)		0.074		0.089	
CD (*p*=0.05)		0.222		0.267	
CV (%)		1.861		1.468	

Table 4. Performance of SiSBisolates against plant growth promoting characteristics

S.No.	Isolate code	Exopolysaccharide production (mg ml$^{-1)}$	Siderophore production	Indole acetic acid production (IAA)
1.	SiKAA-1	2.0	+	*
2.	SiKAA-2	3.0	-	*
3.	SiKPP-1	9.0	+	**
4.	SiKPP-2	2.0	+	*
5.	SiKPP-3	1.0	+	-
6.	SiKPP-4	3.0	-	**
7.	SiKPP-5	5.0	-	-
8.	SiKKK-1	6.0	+	*
9.	SiKKK-2	3.0	+	**
10.	SiKKK-3	0.0	+	-
11.	SiKKK-4	2.0	+	**
12.	SiPYY-1	4.0	+	*
13.	SiPYY-2	4.0	-	*
14.	SiPYY-3	8.0	+	**
15.	SiPYY-4	0.0	+	-
16.	SiPMM-1	0.0	+	*
17.	SiPMM-2	2.0	-	*
18.	SiPMM-3	2.0	+	-
19.	SiGVV-1	1.0	+	-
20.	SiGVV-2	2.0	-	**
21.	SiGVV-3	0.0	+	*
22.	SiGSS-1	2.0	+	**
23.	SiGSS-2	2.0	-	*
24.	SiGSS-3	2.0	+	*
25.	SiAGG-1	3.0	+	*
26.	SiAGG-2	2.0	-	-
27.	SiAGG-3	1.0	+	*
28.	SiAGG-4	1.0	+	**

***:** *Weak*
****:** *Strong*

Table 5. Biochemical and physiological characterization of Silica solubilizing bacterial (SiSB) isolates from different districts of Andhra Pradesh

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	+	+	+	-	+	+	-	+	+	+

"In the test of gelatine liquefaction all the isolates were positive except 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 and remaining isolates showed positive reaction. For the test of Vogues Proskauer all the isolates were positive expect SiKKK-1 and SiGSS-1.In the test of citrate utilization SiKAA-1, SiKKK-4 and SiGSS-3 isolates showed negative result whereas the remaining isolates were positive" [11]. All the isolates reacted positively for ammonia productionexcept SiKPP-5, SiPYY-4 and SiGVV-3. These results are correlated with [34] who characterized three silicate solubilising bacterial isolates from the different sources and identified as *Bacillus sp.* based on morphological and biochemical characteristics.

4. CONCLUSION

In the conclusion two (SiKPP-1&SiPYY-3) efficient silica solubilizing bacteria were identified among twenty eight (28) isolates based on silica solubilizing efficiency, PGPR characters and biochemical tests. Further these two efficient isolates with maximum plant growth promoting characteristics will be evaluated as biofertilizer under pot culture and field conditions in paddy crop by using suitable carrier material.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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