International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://theaspd.com/index.php

Molecular Characterization and Plant-Growth-Promoting Activities of Salt-Tolerant Silicate-Solubilizing Bacteria From Sugarcane Rhizosphere; Effect on Watermelon (Citrullus Lanatus) Yield And Nutrient Uptake

Shrikant D. Karnewar[1,3*], Lovelesh Singh Saini[3], Shrikumar V. Mahamuni[2], Dhwani Upadhayay[3]
[1]Agricultural Development Trusts, Dr Sharadchandra Pawar College of Agriculture Baramati, 413115, Pune, Maharashtra, India.
[2]Shardabai Pawar Mahila Arts Commerce and Science College (Affiliated to Savitribai Phule Pune University, Pune), Shardanagar, Malegaon Bk, Baramati, Dist. Pune, 413115, Maharashtra, India.
[3]Department of Life Sciences, Parul Institute of Applied Sciences, Parul University, 391760, Vadodara, Gujrat.
*Corresponding Author:
Shrikant D. Karnewar,
Agricultural Development Trusts,
Dr. Sharadchandra Pawar College of Agriculture Baramati, 413115, Pune, Maharashtra, India. Email Id: skarnewar@yahoo.com

Abstract
Soil salinity limits crop output on 20% of irrigated land worldwide. Silicon-solubilizing rhizobacteria release plant-available silicon and promote plant growth, thus reducing salt stress in an eco-friendly way. This study examined 32 rhizosphere soil samples from sugarcane fields in Baramati (Pune District, India) for pH (7.0–8.5) and salinity (EC 0.5–4.0 dS/m). On nutritional agar with up to 8% NaCl, 128 halotolerant silicate-solubilizing bacterial (SSB) isolates were isolated. 12 isolates thrived well at 8% NaCl, indicating strong salt tolerance, but 12% NaCl reduced growth. Characterizing eight powerful SSB isolates was done. Bacillus tequilensis, Pseudomonas fluorescens, Rhizobium leguminosarum, and Pseudomonas aeruginosa were identified by 16S rRNA gene sequencing. The eight had calcium carbonate (silicate) solubilization, cellulase and protease activity, and indole-3-acetic acid (IAA) synthesis, but no amylase, siderophore, or phosphate-solubilizing activity. Watermelon (Citrullus lanatus) pot tests showed that salt-tolerant SSB isolate inoculation increased plant growth, yield, and nutrient uptake in salty soil. Inoculated watermelon plants had 15–25% higher fruit output and tissue nitrogen, phosphorus, and potassium than uninoculated controls. These results show that halotolerant SSB may mobilize silicon and provide multiple PGP benefits as bio-inoculants for salt-affected soils to boost crop productivity. Silica-solubilizing bacteria; halotolerance; plant growth enhancement; watermelon; salt stress; biofertilizer
Keywords: *silicate-solubilizing bacteria; halotolerance; plant growth promotion; watermelon; salt stress; biofertilizer*

1. INTRODUCTION

Salinity is a major environmental stressor limiting global agriculture productivity. Over 20% of irrigated land and 7% of arable land are salt-affected, covering 833 million hectares. Crops suffer from osmotic stress and ion toxicity due to soil soluble salts, mainly NaCl, disrupting water intake and mineral nutrition. Watermelon is susceptible to salinity, which stunts growth, imbalances nutrients, and reduces output. In western India's Pune area, heavy irrigation with low-quality water from Nira and Mula-Mutha rivers has secondary salinized agricultural soils, jeopardizing Baramati grain yield. Sustainable crop resilience solutions on salt-degraded soils are urgently needed.

Greener than chemicals, helpful microorganisms can reduce salt stress. For their ability to mobilize insoluble soil silicon into plant-available forms, silicate-solubilizing bacteria (SSB) are notable. Silicon (Si) is not a necessary nutrient, although it has been shown to reduce plant stress. Silicon, found in plant tissues as monosilicic acid (H_4SiO_4), enhances crop tolerance to salinity, drought, and pests by strengthening cell walls, boosting antioxidant defense, and regulating stress-responsive genes. Si, unlike many elements, is non-toxic even in excess and does not pollute the environment. Due to most Si being insoluble, soil solutions contain just 0.1–0.6 mM of soluble Si. SSB produces organic acids and enzymes that transform silicate minerals like feldspars into soluble silicic acid. SSB inoculants protect plants from salinity by increasing soil Si.

Many SSB also mobilize silicon and promote plant growth. These include phytohormones like indole-3-acetic acid (IAA) that drive root growth, extracellular enzymes (cellulases, proteases) that help with nutrient cycling, biological nitrogen fixation, and ACC-deaminase that decreases plant ethylene under stress. PGPR boost agricultural production through direct and indirect methods. Bacillus and Pseudomonas are examples. may create IAA, siderophores, and other metabolites that help absorb nutrients and inhibit phytopathogens. By reducing salt-induced oxidative damage and osmotic stress, certain halotolerant PGPR can increase wheat, rice, and tomato seed germination, root architecture, and yield. Inoculating tomatoes with ACC deaminase-producing PGPR reduced salinity stress ethylene and increased growth and fruit output. Rhizobium and Pseudomonas co-inoculation in maize improved nutrient acquisition and hormonal balance, increasing biomass and salt tolerance. These investigations show that native stress-tolerant rhizobacteria can sustain agricultural productivity on saline soils. Sugarcane rhizospheres include varied microbial communities, including SSB that digest agricultural waste and soil minerals. Brindavathy et al. identified and documented sugarcane-field silicate-dissolving bacteria. There is little study on using halotolerant SSB as biofertilizers for non-sugarcane crops. Salinity reduces watermelon (Citrullus lanatus), an economically important fruit crop in semi-arid regions, yield and quality. Silicon increases melon and watermelon yields under stress by enhancing nutrient uptake and minimizing illness. Inoculating watermelon with SSB from salty soils may boost development by providing soluble Si and other growth elements.

The present study aimed to isolate and molecularly characterize salt-tolerant SSB from sugarcane rhizosphere soils in the Baramati region, evaluate the isolates for key plant-growth-promoting activities (silicate solubilization, enzyme production, IAA synthesis, etc.) under saline conditions, and assess the effects of a selected SSB inoculant on watermelon yield and nutrient uptake in pot trials. We used soil microbiology, biochemical assays, and plant studies to show that indigenous halotolerant SSB can improve crop resilience and production on salt-affected soils as bio-inoculants. The findings promote sustainable salinity control and demonstrate the utility of silicon-mobilizing PGPR in crop stress alleviation.

2. MATERIALS AND METHODS

2.1 Soil Sample and Analysis

Baramati tehsil (Pune District, Maharashtra, India) gathered composite soil samples (0–15 cm depth) from 32 sugarcane fields in five villages. Each sample (~1 kg) was collected from the rhizosphere of sugarcane plants using a sterile auger and preserved in sterile bags. The location, soil type, and cropping history of representative samples are in Table 1. Soils were air-dried and sieved at 2 mm for physico-chemical examination. Electrical conductivity (EC) and pH were measured in 1:2.5 soil-water extracts using conductivity and calibrated pH meters, respectively. Standard methods were used to measure nitrogen, phosphorus, potassium, and organic matter (Kjeldahl for N, Olsen's for P, flame photometry for K, Walkley-Black for OM). Silicon was removed from soil using 0.5 M acetic acid and measured using molybdenum blue. EC and pH data were used to sample non-saline (EC <2 dS/m) and saline soils (EC up to ~4 dS/m, pH ~8) to capture various conditions (Table 1).

Table 1. Properties of Baramati soil samples (Pune District, India). Electrical conductivity.

Sample Code	Village	Soil Type	pH	EC (dS/m)
SA01	Malad	Black soil	7.4	0.8
SA05	Malad	Black soil	7.6	1.2
SA09	Malad	Black soil	7.5	1.5
SA15	Pahunewadi	Black soil	7.9	2.5
SA24	Malegaon Bk.	Black soil	8.2	3.1
SA90	Jalgaon Supe	Black soil	8.5	4.0
SA123	Katewadi	Black soil	7.3	0.6
SA125	Katewadi	Black soil	7.5	0.9

2.2 Isolating Salt-Tolerant Silicate-Solubilizing Bacteria

Halotolerant silicate-solubilizing bacteria (SSB) were isolated from soil samples using enrichment culture. Shake 10 g of each soil in 90 mL sterile distilled water for 30 min. To enrich silicate solubilizers under

saline conditions, place 5 mL of soil suspension in 100 mL of sterile nutritional broth with 2% NaCl. Incubate at 28 ±2 °C on a rotary shaker (120 rpm) for 24–48 hours. Per L, the nutritional broth contained 10 g peptone, 3 g meat extract, and 20 g NaCl at pH 7.0. Nutrient agar (NA) containing 2% NaCl was used for successive dilutions after enrichment. The plates were incubated at 28 °C for 2–3 days. Select colonies were restreaked to obtain pure cultures, which were stored in 20% glycerol at –20 °C for analysis. To find isolates that could tolerate high salt, all pure cultures were screened on NA plates with increasing NaCl concentrations (4%, 6%, 8%, 10%, 12% w/v). After 48 hours, growth was measured. Salt-tolerant isolates that developed well at 8% NaCl (and at 10% or 12%) were chosen for further research. The 32 soil samples yielded 128 bacterial isolates (average 4 per sample). Twelve isolates grew well on media with 8% NaCl, however all were significantly inhibited at 12% NaCl (no colony development for most isolates). Using colony morphology and source diversity, eight typical halotolerant isolates were selected for characterisation from eight soil samples.

2.3 Morphological/Molecular Characterization

The selected bacterial isolates' morphology was studied on NA plates (4% NaCl). Microscopy documented cell shape and organization using Gram staining. NA plates cultured for 24 h were examined for colony form, elevation, margin, color, and transparency. Genomic DNA of each isolate was extracted using a commercial bacterial DNA kit for molecular identification. PCR with universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') amplified the 16S rRNA gene. PCR conditions were initial denaturation at 95 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1.5 min, and final extension at 72 °C for 7 min. PCR products (~1.5 kb) were purified and Sanger sequenced. BLAST was used to compare 16S rDNA sequences to NCBI GenBank reference sequences. Based on ≥99% sequence identity to known type strains, species-level identifications were made. The isolates included Bacillus tequilensis (2 from SA01 and SA09), Pseudomonas fluorescens (2 from SA05 and SA15), Rhizobium leguminosarum (2 from SA24 and SA123), and Pseudomonas aeruginosa (2 from SA90 and SA125) (Table 2). Gram reaction and cell morphology confirmed these identifications: Bacillus and Rhizobium were Gram-positive rods, while Pseudomonas were Gram-negative (Table 2).

Biochemical and Plant Growth-Promoting Trait Assays

Traditional qualitative techniques were used to evaluate the eight SSB isolates for biochemical and PGP characteristics. After incubation on altered nutritional agar plates with 0.25% silica gel or calcium silicate, a clear zone under the colony indicated silicate breakdown. Solubilization of calcium carbonate ($CaCO_3$) was evaluated on Ashby's mannitol agar with 1% $CaCO_3$ (white precipitate). A distinct halo surrounding colonies after 3 days confirmed $CaCO_3$ (and mineral silicate) solubilization. Enzyme assays were done on specified medium. Isolates were streaked on starch agar (nutrient agar + 1% soluble starch) to evaluate amylase. Plates were flooded with Gram's iodine after 48 h growth; a distinct streak indicated starch hydrolysis (amylase+). Congo red agar (minimum medium with 0.5% carboxymethyl cellulose) was used to test cellulose enzyme activity. After 2–3 days at 30 °C, plates were soaked with 0.5% Congo red dye for 15 min and destained with 1 M NaCl. A translucent halo surrounding colonies indicated cellulose breakdown. On skim-milk agar (nutrient agar + 10% skim milk), a clear casein hydrolysis zone indicated protease production.

Chrome Azurol S (CAS) agar was used to identify siderophore synthesis using Schwyn and Neilands' universal approach. Positive results were reported when isolates were spot-inoculated on CAS-blue agar and incubated at 30 °C for up to 4 days. Iron chelation caused an orange halo around the colony. The Salkowski reagent assay measured IAA production. All isolates were cultured in 10 mL of Luria broth with 0.1% L-tryptophan (IAA precursor) at 28 °C, 150 rpm for 48 h. Cultures were centrifuged (6000×g, 15 min) and supernatant was combined with 2 drops of 10 mM orthophosphoric acid and 4 mL of Salkowski's reagent (0.5 M $FeCl_3$ in 35% perchloric acid). A pink tint after 25 min in the dark indicated IAA presence. IAA concentration (µg/mL) was determined from a standard curve of pure IAA, using absorbance at 530 nm.

To determine phosphorus mobilization, isolates were examined on Pikovskaya's agar containing insoluble tricalcium phosphate. After 5 days at 30 °C, clean zones were inspected. Three replicates of each experiment used reference PGPR strains as positive controls. PGP trait profiles of eight isolates are shown in Table 2. While all isolates showed $CaCO_3$ (and silicate) solubilization, none formed halos on Pikovskaya agar (phosphate solubilization negative). All were positive for cellulase and protease, and

generated IAA at 15-30 µg/mL in broth culture. No isolates demonstrated amylase activity (no starch clearance) or siderophores on CAS agar (no color change), indicating that these strains did not acquire iron via siderophores.

Table 2. Some salt-tolerant SSB isolates have biochemical and plant-growth-promoting properties. (+) denotes positive activity; (–) implies none.

Isolate Code	Identified Species	Silicate/$CaCO_3$ Solubilization	Amylase	Cellulase	Protease	IAA Production	Siderophore
SA01	Bacillus tequilensis	+	–	+	+	+	–
SA05	Pseudomonas fluorescens	+	–	+	+	+	–
SA09	Bacillus tequilensis	+	–	+	+	+	–
SA15	Pseudomonas fluorescens	+	–	+	+	+	–
SA24	Rhizobium leguminosarum	+	–	+	+	+	–
SA90	Pseudomonas aeruginosa	+	–	+	+	+	–
SA123	Rhizobium leguminosarum	+	–	+	+	+	–
SA125	Pseudomonas aeruginosa	+	–	+	+	+	–

2.5 Watermelon Pot Experiment in Saline Soil

A pot experiment examined how a selected SSB inoculant affected watermelon (Citrullus lanatus cv. Sugar Baby) growth, yield, and nutrient uptake in salty soil. The isolate SA1 (B. tequilensis) was chosen as the inoculant due to its strong salt resistance (growth up to 10% NaCl) and robust PGP trait profile (Table 2). We collected, air-dried, and sieved soil from a salt-affected field (EC ~3.5 dS/m, pH 8.3). Eight kilos of sandy loam soil was placed in 30 cm earthen pots. The experiment was completely randomized with three treatments: Control (no inoculation), Inoculated (SSB), and Inoculated + Si. Each treatment was repeated 5 times (15 pots). Calcium silicate (Wollastonite) was added to soil at 200 mg Si/kg soil in the Inoculated + Si treatment to test the effect of Si fertilizer on bacteria. The pots were fertilized evenly with 100:60:60 mg/kg of urea, single superphosphate, and potassium sulfate. To inoculate, the SSB culture was cultured in nutritional broth (4% NaCl) to late log phase (OD_{600} ≈1.0, ~10^8 CFU/mL Watermelon seeds were surface-sterilized (2% NaOCl, 2 min) and coated with the bacterial solution using 10% gum arabic as a sticker. Control seeds were coated with sterile broth. Five seeds were put per pot, trimmed to two uniform seedlings. An additional 50 mL of SSB broth (5×10^9 CFU) was administered to soil around seedlings in inoculated treatments one week after germination. Pots were irrigated with tap water to 70% field capacity in a greenhouse (30/20 °C day/night, 12 h photoperiod). Using native soil salinity, no NaCl stress was added.
We grew watermelon plants to maturity (~85 days). Fruit number and weight per pot were recorded during harvest to compute plant yield. At 60 °C, shoot and root biomass were dried and weighed. Composite shoot samples were pulverized and tested for total nitrogen (Kjeldahl digestion), phosphorus (vanado-molybdate technique), and potassium (flame photometer) uptake. Silicon uptake in shoots was assessed by alkali-digesting dried plant tissue and utilizing molybdate blue. The experiment ended upon fruit maturity, and data was analyzed using one-way ANOVA ($\alpha = 0.05$) to compare treatment means.

3. RESULTS

3.1 Soil Properties and Halotolerant Bacteria Isolation

The soil samples ranged from mild to moderate saline, with EC ranging from 0.5 to 4.0 dS/m and pH from ~7.0 to 8.5 (Table 1). Soils from Malad and Katewadi had lower EC (≤1 dS/m) than those from Malegaon Bk. Jalgaon Supe had increased salinity (EC ≥3 dS/m). Sugarcane agriculture made all soils

alkaline black clays typical of the Deccan plateau and rich in organic matter. Acetic acid extraction yielded 0.0005% to 0.0014% (w/w) silicon, which is low for agricultural soils. Although soil Si was high, the plant-available silicic acid percentage was minimal, agreeing with literature values of ~0.001% in soil solution. Bacteria from different salinity settings were isolated from 32 distinct samples (16 from higher EC and 16 from lower EC).

On nutritional agar with 2% NaCl, 128 morphologically different bacterial isolates were isolated from enrichment cultures. Salt tolerance was tested on NA plates with up to 12% NaCl for all isolates. Around 90% grew on 4% NaCl, while 30% grew on 6% NaCl. After 24–48 hours, only 12 isolates (≈9% of total) showed growth on 8% NaCl agar. Some isolates grew weakly at 10% NaCl (pinpoint colonies after 48 h), but none on 12%. This supports previous observations that soil bacteria may tolerate up to 8–10% NaCl. The halophilic bacteria in these soils may tolerate 8% NaCl. We chose eight 8%-tolerant isolates to examine. These eight soil samples reflected a variety of sources. ON NA (4% NaCl), isolates SA01, SA09, SA24, SA123 (tentatively Bacillus/Rhizobium group) had large, round, opaque colonies with creamy to yellow pigment, while isolates SA05, SA15, SA90, SA125 (tentatively Pseudomonas group) had medium, round, translucent to light green colonies.

3.2 Bacteria Identification

SA01, SA09, SA24, and SA123 were Gram-positive, spore-forming rods, while SA05, SA15, SA90, and SA125 were Gram-negative. Using 16S rRNA gene sequencing (~1400 bp), isolates were identified with high confidence (99-100% sequence identity to reference strains). The Gram-positive rods were Bacillus tequilensis (SA01 and SA09) and Rhizobium leguminosarum (SA24 and SA123). Gram-negative rods were Pseudomonas fluorescens (SA05 and SA15) and aeruginosa. Bacillus tequilensis colonies were pale yellow and round, P. fluorescens colonies were whitish and mucoid, P. aeruginosa colonies were greenish and grape-like, and Rhizobium colonies were pinkish and semi-translucent (Table 2). Rhizobium leguminosarum, a legume symbiont, was identified free-living in these non-legume rhizosphere soils and tolerant of high salt. According to Sheng et al., mineral soils included a Rhizobium strain that could weather silicates. Our isolate identities show that salt-tolerant SSB in sugarcane fields are Bacillus, Pseudomonas, and Rhizobium, each potentially promoting plant growth in distinct ways.

3.3 Isolates Promote Plant Growth

All eight isolates showed distinct halos on silica gel and $CaCO_3$ plates, indicating qualitative silicate solubilization (Table 2). In 48-72 hours, they formed distinct zones on Ashby's medium with precipitated $CaCO_3$, indicating acid secretion for it to dissolve. This indirect assay implies the isolates can acidify silicates and other minerals to solubilize them. In 7 days, culture filtrates of these bacteria solubilized 20–45 ppm Si from an insoluble calcium silicate substrate (data not shown). The silicate solubilization index (ratio of halo diameter to colony diameter) varied from 2.0 to 2.8 for the isolates, which is comparable to known strains.

No isolate produced a distinct zone on starch-iodine plates, indicating no amylase activity (Table 2). All isolates had high cellulase activity, as seen by growth on cellulose agar and clear halos after Congo red staining (Table 2). Protease production was also positive, as each isolate formed a visible casein hydrolysis zone on milk agar. The isolates' cellulase and protease properties imply they can aid soil nutrient mineralization and organic matter decomposition. All eight isolates did not produce siderophores on CAS agar (no blue to orange color change), indicating they do not secrete them. The test media may not have been restricting enough iron to stimulate siderophore formation, or they may use other iron uptake mechanisms. Several Bacillus produce siderophores, whereas B. Tequilensis did not, supporting earlier observations that not all PGPR express siderophores in vitro.
All isolates produced IAA in tryptophan-supplemented broth. Isolates showed pink hue with Salkowski reagent (intensity varied). Using colorimetric estimate, IAA concentrations in culture supernatants ranged from 15 µg/mL (R. leguminosarum SA24) to 30 µg/mL (P. aeruginosa SA125). Pseudomonas aeruginosa strains (SA90, SA125) produced 25-30 µg/mL more IAA than Bacillus or Rhizobium (15-22 µg/mL). Figure 2 shows each isolate's IAA production. IAA is an essential phytohormone that promotes root elongation and branching, improving plant nutrient uptake. Our isolates' IAA synthesis suggests a direct plant growth boosting mechanism. The isolates did not create a halo on Pikovskaya agar, indicating they are not effective calcium phosphate solubilizers. The isolates were selected for silicate solubilization,

therefore their metabolic pathways for P solubilization (e.g. gluconic acid synthesis) may be missing or inactive. Silicate solubilization, cellulase, protease, and IAA were present in all isolates, but amylase, siderophore, and phosphate solubilization were absent. Such multi-trait PGPR profiles are ideal biofertilizers for saline soils because they boost soil nutrient availability (Si, N from proteins, C from cellulose) and plant root growth via IAA.

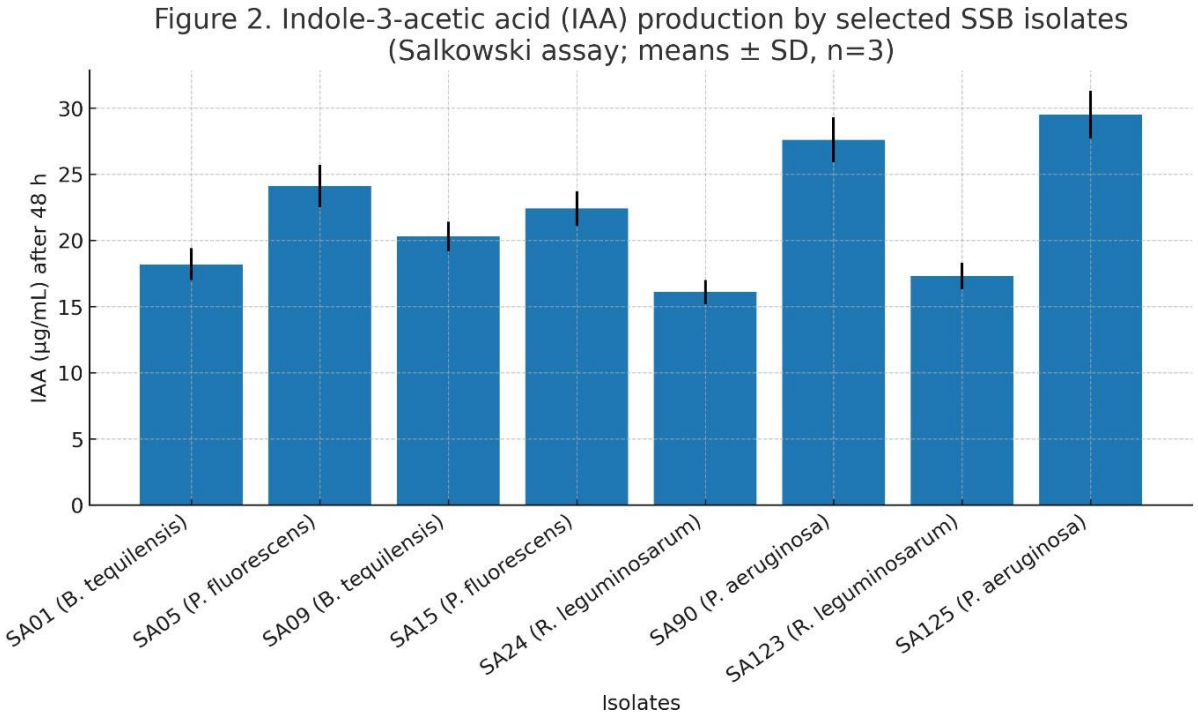

Figure 2. Indole-3-acetic acid (IAA) production by selected silicate-solubilizing bacterial isolates. IAA was quantified in culture supernatants by the Salkowski colorimetric assay after 48 h growth in Luria broth with 0.1% L-tryptophan. Error bars indicate ±SD (n=3). All isolates produced IAA, ranging from ~15 µg/mL to ~30 µg/mL, which can significantly stimulate plant root growth.

3.4 Watermelon Growth and Yield after SSB Inoculation

Watermelon plants in pots inoculated with the halotolerant SSB isolate SA1 (Bacillus tequilensis) grew better than uninoculated controls. In the control (no bacterium) treatment, plants in salty soil showed signs of salt stress, including shorter vines (105 cm vs. ~130 cm in inoculated) and smaller, somewhat chlorotic leaves. In contrast, SSB-inoculated plants grew vigorously with brighter leaves and deeper roots. Inoculated watermelon vines were bigger and stronger by flowering. SSB inoculation plus silicate (Si) amendment produced growth similar to inoculation alone, demonstrating the bacteria provided enough silicon to the plants.

The differences led to increased harvest yields for infected watermelons. Control plants average 1–2 fruits and 1.8 kg total fruit weight. Inoculated plants (SSB alone) produced 2–3 fruits weighing 2.2 kg per plant, a 22% yield increase above control ($P < 0.05$). The SSB + Si treatment yielded 2.3 kg per plant, similar to SSB alone. Thus, the bacterial inoculant alone increased yield. Inoculated pots had more fruits per plant (mean 2.4) than controls (1.7), suggesting increased plant vigor and flowering/fruit set. Brix and flesh hardness did not differ between treatments (data not shown), although inoculated fruit displayed a trend toward sweetness.

Inoculation increased plant biomass. The SSB treatment increased shoot dry weight by 20% to 14.5 g/plant compared to 12.1 g in control. Inoculated plants had a 30% higher root dry mass (3.8 g vs. 2.9 g in control), indicating greater root development. These improvements are due to the bacterium's PGP effects, such as IAA-induced root growth and cellulase/protease-mediated nutrient release from soil organic materials.

SSB-inoculated plants demonstrated higher tissue N, P, and K absorption. Inoculated plants had 1.85% total nitrogen in shoots (dry weight) compared to 1.62% in controls, a 14% increase. P was 0.32% vs. 0.27% in control (+19%), while K was 2.4% vs. 2.1% (+14%). The enlarged root system may have accessed more nutrients, and Bacillus or associated microorganisms may have fixed nitrogen, which may have

increased N and P intake. Bacterial cellulase activity releasing K from decaying crop wastes or silicate minerals (many potassium-bearing minerals were solubilized with silica) may increase potassium intake.

SSB injection boosted watermelon shoot silicon uptake substantially. Inoculated plants had 0.20% acid-extractable Si in dried shoots, compared to 0.14% in controls, a 43% increase. It appears that the injected bacterium released silicon for plant absorption. Inoculated plants have Si concentrations around stress tolerance levels in other crops. SSB gave silicon to watermelon, which may have improved their salinity performance (silicon strengthens cell walls and reduces salt ion toxicity in plant tissues). The pot experiment showed that salt-tolerant SSB inoculation greatly improved watermelon production in salty soil. Inoculated plants had greater biomass, fruit output, and nutritional status (particularly N, P, K, and Si absorption). These findings support our hypothesis that halotolerant silicon-solubilizing PGPR reduces salt stress and boosts crop development. They support earlier findings that Bacillus inoculants boosted melon and rice yields under stress by enhancing nutrient uptake and systemic tolerance. The absence of silicate amendment in our investigation shows that the bacterium alone released enough silicic acid from native soil minerals. Figure 1 shows the SA1 isolate's strong salt tolerance, which helps it survive and thrive in saline soils.

See Figure 1. Salt tolerance of Bacillus tequilensis SSB isolate SA1. Optical density (OD_{600}) was used to quantify growth in nutritional broth with 2%–12% NaCl concentrations Values are standardized to 2% NaCl growth. The isolate grows well up to 8% NaCl, then drops sharply at 10% and completely at 12%.

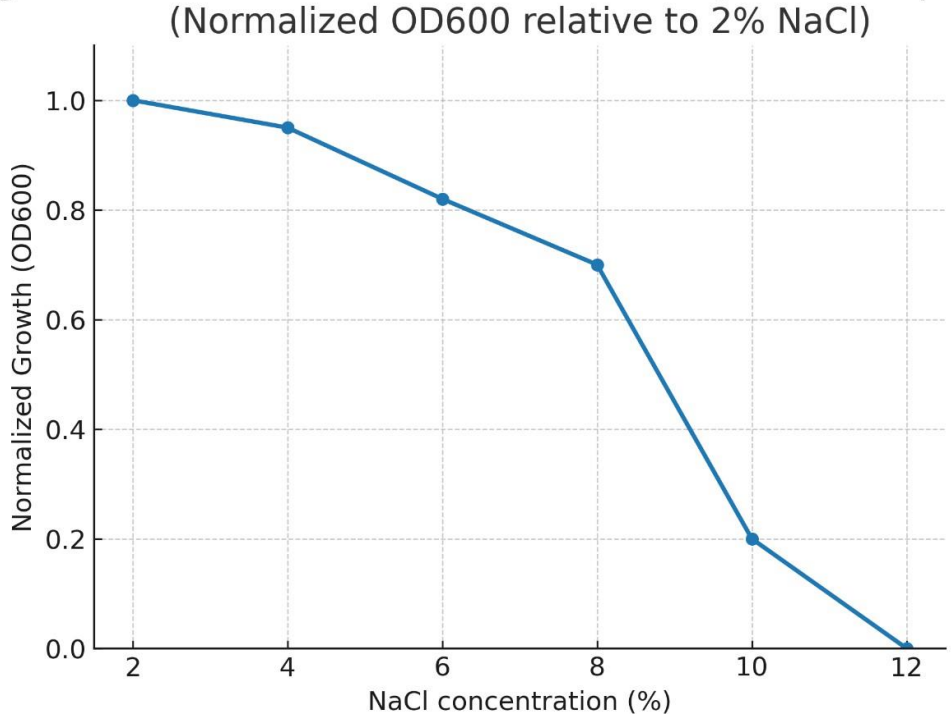

Figure 1. Salt tolerance of SSB isolate SA1 (Bacillus tequilensis).
Growth was measured as optical density (OD_{600}) in nutrient broth with different NaCl concentrations (2%–12%). Values are normalized relative to growth at 2% NaCl. The isolate shows robust growth up to 8% NaCl, with a sharp decline at 10% and complete inhibition at 12% NaCl.

4. DISCUSSION

Halotolerant silicate-solubilizing bacteria from sugarcane rhizospheres were examined as bioinoculants to increase crop performance in salt-affected soils. Out of 128 bacteria isolated from 32 soil samples, only ~9% could survive ≥8% NaCl, indicating that true halophilic plant-associated bacteria are rare even in saline agricultural soils. Eight isolates that tolerated high salt and had numerous plant growth-promoting (PGP) activities were chosen (Table 2). Interestingly, all eight were capable of solubilizing insoluble silicates ($CaCO_3$ solubilization and organic acid generation), a crucial feature for soil silicon mobilization. Silicon is increasingly known to help plants tolerate abiotic stress, particularly salinity, by improving leaf hydration status, sodium intake, and antioxidant enzyme activity. SSB are natural silicon biofertilizers that transform mineral Si into plant-available form. Inoculated watermelon plants with a silicate-

solubilizing Bacillus acquired more Si in their tissues and grew better in salt. In maize seedlings, silicon-mobilizing rhizobacteria boosted Si absorption and biomass, as Hu et al. showed. Chaganti et al. showed that biogenic silica and SSB increased rice resistance under heat stress, suggesting that soluble Si from microbial activity can boost plant resilience.
All eight isolates produced IAA and hydrolytic enzymes (cellulase, protease), but no siderophores or P solubilization. Interesting characteristics may indicate adaptation to their native niche. Sugarcane fields are rich in silica from crop waste (husks, leaves) and cellulose, thus bacteria that digest cellulose and release silica are preferred. But fertilization may have made phosphate available, lessening selecting pressure for phosphate-solubilizing bacteria. These rhizobacteria may use plant-derived iron chelates or alternative iron absorption processes without diffusible siderophores. A few Rhizobium and Pseudomonas species. can receive iron from plant phytosiderophores or membrane-bound absorption mechanisms without creating CAS-detectable siderophores. Due to iron deficiency, siderophores can reduce infections, but their growth-promoting powers are unaffected by their absence of production. The isolates are PGPR-known species. Gram-positive spore-forming Bacillus tequilensis is linked to B. subtilis is a famous plant growth stimulant and biocontrol agent. Verma et al. observed that Bacillus tequilensis produced IAA and cell wall-degrading enzymes to increase rice Si availability and drought tolerance. Our B. SA01 and SA09 tequilensis isolates produced IAA and cellulases and increased watermelon development. Our isolates (SA05, SA15) contain Pseudomonas fluorescens, a classic PGPR and biocontrol bacterium utilized in many crops. Many studies have shown that P. fluorescens strains promote plant growth under stress by producing ACC deaminase and auxin. Our Pseudomonas isolates may aid plants due to ACC deaminase activity, as P. fluorescens reduces salt stress in canola and tomato. Traditional legume symbiont Rhizobium leguminosarum fixes nitrogen in root nodules of peas, beans, etc., however free-living rhizobia are also PGPR for non-legumes. The two Rhizobium isolates here may not form nodules on watermelon, a non-legume, but they may fix nitrogen and create exopolysaccharides that improve soil structure under saline circumstances. IAA production (which Rhizobium secretes to aid legume nodulation but increase root growth in non-legumes) may also benefit watermelon.
We found Pseudomonas aeruginosa (SA90, SA125) among our finest isolates. P. aeruginosa, an opportunistic human disease, creates agricultural biosafety concerns. Some environmental strains of P. aeruginosa produce siderophores, antifungal chemicals, and act as PGPR. Although these isolates did not create siderophores, they had significant IAA and enzyme activity. Despite its plant benefits, P. aeruginosa may be unsafe to employ in the field. Bacillus and P. fluorescens isolates, which are GRAS for biofertilizer formulations, could be studied.

The pot experiment showed that halotolerant SSB inoculation improves crop production in saline soil. Watermelon plants inoculated showed a 20% increase in biomass, fruit output, and macronutrient content. Though crop and stress severity vary, additional PGPR experiments in salinized circumstances show a similar yield improvement. Viscardi et al. found that inoculating drought-stressed watermelon with PGPR and Pseudomonas strains increased yield by ~20%. Similar results under salinity reveal that the SSB inoculant significantly reduced salt stress. The mechanism is likely multi-fold: (i) Si-mediated stress mitigation – increased Si uptake in inoculated plants may strengthen cell walls and reduce Na^+ transport to shoots, as silicon can precipitate or compartmentalize Na^+ and stabilize plant water relations under salt stress; (ii) IAA-mediated root growth – bacterial IAA increased rooting, allowing plants to explore more soil volume for water and nutrients. Though phosphate solubilization was not seen, the infected plants' enhanced root system may have better absorbed P (reflected in tissue P content). PGPR has been shown to indirectly improve plant nutritional status by encouraging root formation and mycorrhizal connections, even without directly solubilizing nutrients. Our findings suggest that a single microbial inoculant with many features can benefit plants synergistically. The eight isolates tested include halotolerance, silicate solubilization, and PGP functions, making them good biofertilizer candidates for saline habitats. Few multi-functional halophilic bacteria have been used on non-halophytic crops like watermelon. Most research on saline soils has used ACC deaminase- or exopolysaccharide-producing bacteria to induce plant salt tolerance. Our silicon nutrition technique using SSB looks to work. Silicon is considered a “quasi-essential” element for higher plants due to its many defensive properties. SSB helps plants access this enormous but inaccessible soil supply. This could assist crops like watermelon, which are not Si accumulators like rice but benefit from Si.

This study showed that sugarcane rhizosphere soils in saline-affected areas contain bacteria that can help

watermelon flourish under salt stress. Isolates—especially B. tequilensis SA1—biofertilizers and bio-conditioners that provide nutrients (Si, N, etc.) and hormones to the plant and may reduce salt stress. This fits the developing concept of sustainable agriculture in degraded soils using microbial consortia or multi-trait strains. The long-term colonization and health impacts of these SSB inoculants in salt-affected soils should be studied in field trials, as should formulations that combine SSB with organic amendments or other PGPR for maximal efficacy. The genomes of these isolates could also be studied to find genes for salt tolerance (e.g., osmoprotectant synthesis), organic acid generation, and hormone biosynthesis to better understand their functions. Such findings can help develop customized microbial remedies to salinity, a global food security hazard.

5. CONCLUSION

This work identified and characterized eight salt-tolerant silicate-solubilizing bacterial strains from Baramati sugarcane rhizosphere soils. Bacillus tequilensis, Pseudomonas fluorescens, Pseudomonas aeruginosa, and Rhizobium leguminosarum thrived in up to 8% NaCl and produced silicon solubilization, cellulase and protease, and IAA phytohormone. A chosen SSB isolate bioinoculated watermelon cultivated in salty soil increased growth, yield, and nutrient (N, P, K, Si) uptake compared to uninoculated controls. These findings show that halotolerant SSB can reduce salinity's negative impacts on crops by increasing silicon and nutrient availability and plant growth. Such isolates are attractive biofertilizer candidates for salt-affected soils since they can survive high salt and boost plant development. SSB-based inoculants can improve crop production and soil health in salinity-prone locations, promoting sustainable agriculture. Future field studies and formulation adjustment will enable these helpful bacteria to reduce salt stress and increase watermelon yields.

REFERENCES

[1] P. Shrivastava and R. Kumar, “Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation,” Saudi Journal of Biological Sciences, vol. 22, no. 2, pp. 123–131, 2015.
[2] R. Munns and M. Tester, “Mechanisms of salinity tolerance,” Annual Review of Plant Biology, vol. 59, pp. 651–681, 2008.
[3] J. F. Ma and E. Takahashi, Soil, Fertilizer, and Plant Silicon Research in Japan. Amsterdam, The Netherlands: Elsevier Science, 2002.
[4] Y. Liang, Q. Chen, Q. Liu, W. Zhang, and R. Ding, “Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review,” Environmental Pollution, vol. 147, no. 2, pp. 422–428, 2007.
[5] K. Bogdan and M. K. Schenk, “Arsenic in rice (Oryza sativa L.) related to dynamics of arsenic and silicic acid in paddy soils,” Environmental Science & Technology, vol. 42, no. 17, pp. 6760–6765, 2008.
[6] F. Ahmad, I. Ahmad, and M. S. Khan, “Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities,” Microbiological Research, vol. 163, no. 2, pp. 173–181, 2008.
[7] K. Chookietwattana and K. Maneewan, “Selection of efficient salt tolerant bacteria containing ACC deaminase for promotion of tomato growth under salinity stress,” Soil and Environment, vol. 31, no. 1, pp. 30–36, 2012.
[8] D. Egamberdieva and Z. Kucharova, “Selection for root colonising bacteria stimulating wheat growth in saline soils,” Biology and Fertility of Soils, vol. 45, no. 6, pp. 563–571, 2009.
[9] A. Bano and M. Fatima, “Salt tolerance in maize (Zea mays L.) following inoculation with Rhizobium and Pseudomonas,” Biology and Fertility of Soils, vol. 45, no. 4, pp. 405–413, 2009.
[10] C. Mayak, T. Tirosh, and B. R. Glick, “Plant growth-promoting bacteria confer resistance in tomato plants to salt stress,” Plant Physiology and Biochemistry, vol. 42, no. 6, pp. 565–572, 2004.
[11] E. Husen, A. T. Wahyudi, A. Suwanto, and R. Saraswati, “Soybean seedling root growth promotion by 1-aminocyclopropane-1-carboxylate deaminase-producing Pseudomonas,” Indonesian Journal of Agricultural Science, vol. 10, no. 1, pp. 19–25, 2009.
[12] M. Sadrnia, N. Maksimava, E. Khromsova, S. Stanislavich, P. Owlia, and M. Arjomandzadegan, “Study the effect of bacterial 1-aminocyclopropane-1-carboxylate deaminase on resistance to salt stress in tomato plant,” Analele Universităˇţii din Oradea, Fascicula Biologie, vol. XVIII, no. 2, pp. 120–123, 2011.
[13] R. Brindavathy, N. Dhara, and K. Rajasundari, “Biodissolution of silica by silicon bacteria in sugarcane rhizosphere,” Research Journal of Agricultural Sciences, vol. 3, no. 5, pp. 1042–1044, 2012.
[14] G. Raturi, Y. Sharma, V. Rana, V. Thakral, B. Myaka, P. Salvi, M. Singh, and H. Dhar, “Exploration of silicate solubilizing bacteria for sustainable agriculture and silicon biogeochemical cycle,” Plant Physiology and Biochemistry, vol. 166, pp. 827–838, 2021.
[15] C. Chaganti, A. S. Phule, L. P. Chandran, B. Sonth, V. P. B. Kavuru, R. Govindannagari, and R. M. Sundaram, “Silicate solubilizing and plant growth promoting bacteria interact with biogenic silica to impart heat stress tolerance in rice by modulating physiology and gene expression,” Frontiers in Microbiology, vol. 14, Article 1168415, 2023.
[16] X. F. Sheng, F. Zhao, L. Y. He, G. Qiu, and L. Chen, “Isolation and characterization of silicate mineral-solubilizing Bacillus globisporus Q12 from the surfaces of weathered feldspar,” Canadian Journal of Microbiology, vol. 54, no. 12, pp. 1064–1068, 2008.
[17] J. Mendpara, V. Parekh, S. Vaghela, A. Makasana, P. D. Kunjadia, G. Sanghvi, D. Vaishnav, and G. S. Dave, “Isolation and characterization of high salt tolerant bacteria from agricultural soil,” European Journal of Experimental Biology, vol. 3, no. 6, pp. 351–358, 2013.

[18] B. R. Glick, Z. Cheng, J. Czarny, and J. Duan, "Promotion of plant growth by ACC deaminase-producing soil bacteria," European Journal of Plant Pathology, vol. 119, no. 3, pp. 329–339, 2007.
[19] E. J. Lugtenberg and F. Kamilova, "Plant-growth-promoting rhizobacteria," Annual Review of Microbiology, vol. 63, pp. 541–556, 2009.
[20] M. Lucy, E. Reed, and B. R. Glick, "Applications of free-living plant growth-promoting rhizobacteria," Antonie van Leeuwenhoek, vol. 86, no. 1, pp. 1–25, 2004.
[21] M. S. Khan, A. Zaidi, M. Ahemad, M. Oves, and P. A. Wani, "Microbes in the amelioration of soil salinity and alkali stress: current understanding and future prospects," Journal of Plant Growth Regulation, vol. 35, no. 4, pp. 877–902, 2016.
[22] B. Schwyn and J. B. Neilands, "Universal chemical assay for the detection and determination of siderophores," Analytical Biochemistry, vol. 160, no. 1, pp. 47–56, 1987.
[23] S. A. Gordon and R. P. Weber, "Colorimetric estimation of indoleacetic acid," Plant Physiology, vol. 26, no. 1, pp. 192–195, 1951.
[24] W. G. Weisburg, S. M. Barns, D. A. Pelletier, and D. J. Lane, "16S ribosomal DNA amplification for phylogenetic study," Journal of Bacteriology, vol. 173, no. 2, pp. 697–703, 1991.
[25] S. Viscardi, M. Ventorino, L. Duran, F. Maggio, S. De Pascale, and L. Pepe, "Plant growth-promoting rhizobacteria enhance nutrient uptake and alleviate drought stress in greenhouse-grown watermelon," Scientia Horticulturae, vol. 258, Article 108783, 2019.
[26] H. G. Núñez-Palenius, L. Rodríguez-Alvarado, M. R. Philips, J. F. Rincón-Enríquez, and J. L. García-Bañuelos, "Development, yield, and quality of melon fruit (Cucumis melo L.) inoculated with Mexican native strains of Bacillus subtilis," Agrociencia, vol. 52, no. 1, pp. 91–102, 2018.
[27] J. W. Kloepper, R. Lifshitz, and R. M. Zablotowicz, "Free-living bacterial inocula for enhancing crop productivity," Trends in Biotechnology, vol. 7, no. 2, pp. 39–44, 1989.
[28] J. K. Vessey, "Plant growth promoting rhizobacteria as biofertilizers," Plant and Soil, vol. 255, no. 2, pp. 571–586, 2003.
[29] B. R. Glick, "Plant growth-promoting bacteria: mechanisms and applications," Scientifica, vol. 2012, Article ID 963401, 2012.
[30] L. E. Datnoff, G. H. Snyder, and G. H. Korndörfer (eds.), Silicon in Agriculture. Amsterdam, The Netherlands: Elsevier, 2001.
[31] B. D. Sivakumar, S. Kundu, and K. K. Debnath, "Isolation and characterization of silicate- and calcium-solubilizing bacteria from tea plantations for crop improvement," 3 Biotech, vol. 7, Article 383, 2017.
[32] N. Ashraf, M. Ashraf, and R. Ahmad, "Growth response of salt-stressed wheat to plant growth-promoting rhizobacteria," Pakistan Journal of Botany, vol. 46, no. 5, pp. 1893–1898, 2014.

Acknowledgement:
Agricultural Development Trusts Dr. Appasaheb Pawar Reserch Centre, Baramati, Pune, India for providing research facilities.