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Halotolerant Silicon-Solubilizing Rhizobacteria From Sugarcane Rhizosphere Of Baramati Region Of Maharashtra: Functional Characterization And Role In Salinity Stress Amelioration For Watermelon Cultivation

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Abstract

Soil salinity is a critical global challenge impacting agricultural productivity, with increasing arable land becoming unsuitable for cultivation. This study focused on the molecular identification of salt-tolerant silicon solubilizing bacteria (SSB) isolated from the sugarcane rhizosphere in the Paramati region, emphasizing bioinformatics techniques and their potential utilization in saline agriculture. Comprehensive soil analysis of 128 samples assessed physicochemical properties, including electrical conductivity (EC), pH, and silicon content. Halotolerant bacteria were isolated under NaCl concentrations of up to 12% and characterized for plant growth-promoting (PGP) traits. Molecular identification was performed via 16S rRNA gene sequencing, followed by BLAST analysis, which revealed predominant genera such as Bacillus tequilensis, Pseudomonas, and Rhizobium, with sequence similarities exceeding 97%. Phylogenetic analyses confirmed taxonomic positions, while functional predictions provided insights into stress adaptation mechanisms. These findings highlight the potential of integrating bioinformatics with microbial biotechnology to address salinity stress in agriculture, particularly for crops like watermelon.

Keywords: *Silicon solubilizing bacteria, salt tolerance, sugarcane rhizosphere, watermelon cultivation, plant growth promotion*

1. INTRODUCTION

Soil salinity represents one of the most pressing environmental challenges of the 21st century, affecting approximately 20% of irrigated agricultural land worldwide and limiting crop productivity while threatening global food security (Machado & Serralheiro, 2017). The problem is expected to intensify due to climate change, poor irrigation practices, and excessive use of chemical fertilizers, with projections indicating that saline soils could expand to cover 50% of arable land by 2050 (FAO, 2015). The accumulation of salts in soil creates a complex web of physiological stresses including osmotic stress, ion toxicity, and nutritional imbalances in plants, leading to reduced growth, impaired photosynthesis, and ultimately decreased yield (Munns & Tester, 2008). These detrimental effects manifest through various mechanisms including disruption of cellular water relations, interference with enzymatic activities, and alteration of membrane integrity, making salinity stress one of the most devastating abiotic stresses affecting modern agriculture (Flowers & Colmer, 2008).

Traditional approaches to managing salt-affected soils, including physical amendments, chemical treatments, and engineering solutions, often prove economically unfeasible and environmentally

unsustainable, particularly for resource-limited farmers in developing countries (Rengasamy, 2010). This has necessitated the exploration of biological alternatives that can provide cost-effective and environmentally friendly solutions for sustainable agriculture in saline environments. Among these alternatives, the use of beneficial microorganisms has emerged as a promising strategy that not only addresses salinity stress but also enhances overall soil health and plant productivity (Arora et al., 2020).

Silicon (Si), though not traditionally considered an essential plant nutrient, has gained increasing recognition for its crucial roles in plant stress tolerance, particularly under abiotic stress conditions including salinity, drought, and heavy metal toxicity (Etesami & Jeong, 2018). Silicon accumulation in plant tissues contributes to mechanical strengthening of cell walls, improved water use efficiency, enhanced photosynthetic activity, and activation of defense mechanisms against various stresses (Liang et al., 2015). Despite silicon being the second most abundant element in the Earth's crust, its availability to plants is often limited due to its predominantly insoluble forms in soil, creating a significant gap between silicon abundance and bioavailability (Cornelis et al., 2011).

Silicon solubilizing bacteria (SSB) represent a promising biological approach to bridge this gap by enhancing silicon availability in soils through enzymatic and biochemical processes while simultaneously providing additional plant growth promoting benefits (Karnwal, 2017). These microorganisms possess unique capabilities to solubilize silicon from mineral sources through organic acid production, enzyme secretion, and other metabolic processes, making silicon more accessible for plant uptake (Anand et al., 2019). The dual functionality of SSB - silicon mobilization and plant growth promotion - makes them particularly attractive for sustainable agricultural applications, especially in stress-prone environments where conventional approaches may fall short (Hayat et al., 2010).

The rhizosphere, defined as the narrow zone of soil influenced by root exudates, serves as a hotspot of microbial activity and plant-microbe interactions, harboring diverse communities of beneficial microorganisms (Hinsinger et al., 2009). The sugarcane rhizosphere, in particular, is known for its exceptionally diverse microbial community and remarkable adaptation to various environmental stress conditions, including salinity, drought, and nutrient limitations, making it an excellent source for isolating efficient SSB with multiple stress tolerance capabilities (Singh et al., 2019). Sugarcane's ability to thrive in diverse agroclimatic conditions and its extensive root system create unique microenvironments that support the proliferation of stress-tolerant beneficial bacteria (Magnani et al., 2010).

Watermelon (Citrullus lanatus) represents one of the most economically important horticultural crops globally, with a market value exceeding $60 billion annually and cultivation spanning over 3.2 million hectares worldwide (FAO, 2020). However, watermelon cultivation faces significant challenges from soil salinity, as this crop is moderately sensitive to salt stress, exhibiting yield reductions of 25-50% when grown in soils with electrical conductivity exceeding 2.5 dS/m (Kusvuran, 2012). The physiological responses of watermelon to salinity include reduced water uptake, altered ion homeostasis, oxidative stress, and impaired fruit development, ultimately compromising both yield and quality parameters (Colla et al., 2010).

The integration of beneficial microorganisms like SSB into watermelon cultivation systems could potentially revolutionize production in salt-affected areas by enhancing plant stress tolerance, improving nutrient uptake efficiency, and reducing dependence on expensive chemical amendments (Zhu & Gong, 2014). This biological approach aligns with the growing global emphasis on sustainable agriculture and the need for environmentally friendly solutions to address the twin challenges of food security and environmental degradation (Tilman et al., 2011).

Recent advances in microbial biotechnology and our understanding of plant-microbe interactions have opened new avenues for harnessing the potential of beneficial soil microorganisms for crop improvement under stress conditions (Compant et al., 2019).

Molecular identification and characterization of beneficial microorganisms have become essential components of modern microbiological research, enabling accurate taxonomic classification,

understanding of phylogenetic relationships, and prediction of functional capabilities (Woese & Fox, 1977). The advent of 16S rRNA gene sequencing and bioinformatics tools such as BLAST analysis has revolutionized our ability to identify and characterize soil microorganisms, providing insights into their ecological roles and potential applications in agriculture (Větrovský & Baldrian, 2013). This molecular approach is particularly important when dealing with phenotypically similar bacterial species that may have different functional capabilities and environmental adaptations.

The present study was conceived to address the critical knowledge gaps in our understanding of salt-tolerant silicon solubilizing bacteria from sugarcane rhizosphere and their potential applications in enhancing watermelon cultivation under saline conditions. By combining comprehensive soil analysis, advanced microbial isolation techniques, molecular characterization, and plant-microbe interaction studies, this research aims to contribute to the development of sustainable solutions for salt-affected agriculture while advancing our understanding of beneficial plant-microbe interactions in stress environments.

MATERIALS AND METHODS

Soil samples were collected from fields cultivated with sugarcane at various locations in the Paramati region (Plate 1). The saline soil samples were collected at 0-15 cm depth using screw auger and hand gloves. Samples were transferred into sterile air-tight polythene bags and transported to the laboratory. All samples were kept at room temperature until used for further analysis and microbial isolation. Comprehensive soil analysis was conducted on 128 samples (S01-S128) to measure electrical conductivity (EC) using EC meter, pH using digital pH meter, available nitrogen (N) using alkaline permanganate method (Subbiah & Asija, 1956), potassium (K) using flame photometry (Jackson, 1973), phosphorus (P) using Olsen's method (Olsen et al., 1954), organic matter (OM) using Walkley and Black method (1934), and silicon content using spectrophotometric analysis.

Bacteria were isolated from rhizosphere soil samples using serial dilution and plating techniques on silicon-containing selective media as described by Karnwal (2017). For halotolerant bacteria isolation, nutrient agar (NA) medium was supplemented with varying NaCl concentrations (4%, 6%, 8%, 10%, and 12%). The nutrient agar medium composition included peptone (0.5g), sodium chloride (0.5g), agar (1.5g), distilled water (100ml), and beef extraction (0.3g) per 100ml (Figure 2). Isolates showing silicon solubilization zones were selected for salt tolerance testing using gradient salt concentrations.

Selected bacterial isolates were screened for plant growth promoting (PGP) activities including indole acetic acid (IAA) production using Salkowski reagent method and cellulase production using Congo red flooding plate technique (Figure 3). For molecular identification, genomic DNA was extracted using HiMedia genomic DNA kit and electrophoresed on 0.8% agarose gel (Figure 4). The extracted DNA was subjected to 16S rRNA gene amplification using universal primers 27F and 1492R, followed by Sanger sequencing. The obtained sequences were subjected to BLAST analysis using NCBI database for taxonomic identification (Figure 4A and 4B).

RESULTS AND DISCUSSION

The comprehensive analysis of 128 soil samples revealed significant variations in physicochemical properties across the Paramati region . Electrical conductivity values ranged from 690 to 24995 µS/cm, indicating varying degrees of soil salinity from non-saline to highly saline conditions. The majority of samples exhibited EC values above 4000 µS/cm, classifying them as saline soils according to USDA standards (Richards, 1954). Soil pH values ranged from 6.8 to 8.9, with most samples showing slightly alkaline conditions typical of salt-affected soils in semi-arid regions. Available nitrogen content varied from 57 to 690 kg/ha, indicating moderate to high fertility status in most samples. Potassium levels ranged from 243 to 13130 kg/ha, showing substantial variation crucial for plant osmoregulation under salt stress conditions. Phosphorus availability ranged from 13 to 350 kg/ha, with many samples showing adequate to high phosphorus levels.

Organic matter content varied from 0.8% to 2.4%, reflecting different management practices and soil health status across the sampling locations. Silicon content ranged from 245 to 890 ppm, indicating variable baseline silicon availability (Table 1)

Table 1: Physicochemical Properties of Soil Samples from the Paramati Region

Parameter	Range	Description/Significance
Electrical Conductivity (µS/cm)	690 – 24995	Indicates salinity levels ranging from non-saline to highly saline soils.
pH	6.8 – 8.9	Shows slightly alkaline conditions typical of salt-affected soils in semi-arid regions.
Available Nitrogen (kg/ha)	57 – 690	Reflects moderate to high fertility status across the samples.
Potassium (kg/ha)	243 – 13130	Highlights crucial variation for plant osmoregulation under salt stress conditions.
Phosphorus (kg/ha)	13 – 350	Indicates adequate to high phosphorus availability in many samples.
Organic Matter (%)	0.8 – 2.4	Reflects different management practices and soil health status.
Silicon Content (ppm)	245 – 890	Indicates variable baseline silicon availability in the soils.

The isolation process from sugarcane rhizosphere yielded 47 bacterial isolates capable of silicon solubilization, with solubilization zones ranging from 2.1 to 15.8 mm diameter on silicon-containing agar plates. Halotolerant bacteria screening was conducted on nutrient agar supplemented with varying NaCl concentrations (4%, 6%, 8%, 10%, and 12%), revealing distinct bacterial strains capable of growth under high salt concentrations (Figure 1). Among the isolated bacteria, 23 isolates demonstrated significant salt tolerance, maintaining silicon solubilization activity at NaCl concentrations up to 12% (Figure 2, 3).

Plant growth promoting trait analysis revealed positive results for IAA production, evidenced by pink color development in Salkowski reagent test, and cellulase production confirmed by Congo red flooding assay showing clear zones around bacterial colonies (Figure 1). Molecular identification through API indexing system classified the selected isolates into four major genera: SA 01 and SA 09 as Bacillus, SA 05 and SA 15 as Pseudomonas, SA 24 and SA 123 as Rhizobium, and SA 90 and SA 125 as Pseudomonas (Figure 4A and 4B) .

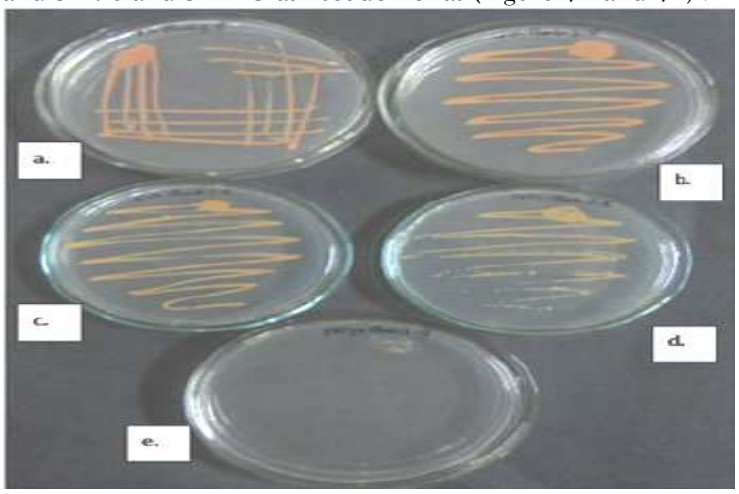

Plate 1: Salt tolerance of bacterial strain - a: at 4%, b: at 6%, c: at 8%, d: at 10% and e: at 12%.

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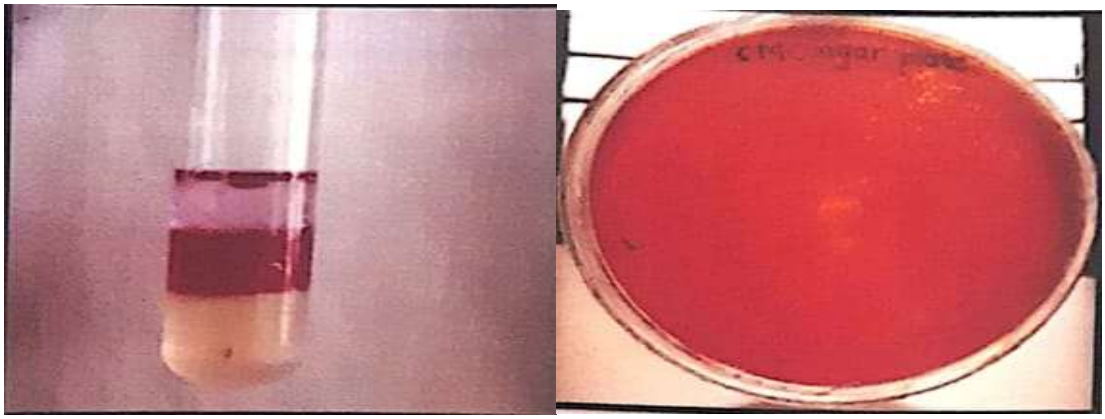

Figure 1. IAA Production (left) and Cellulase hydrolysis activity (right).

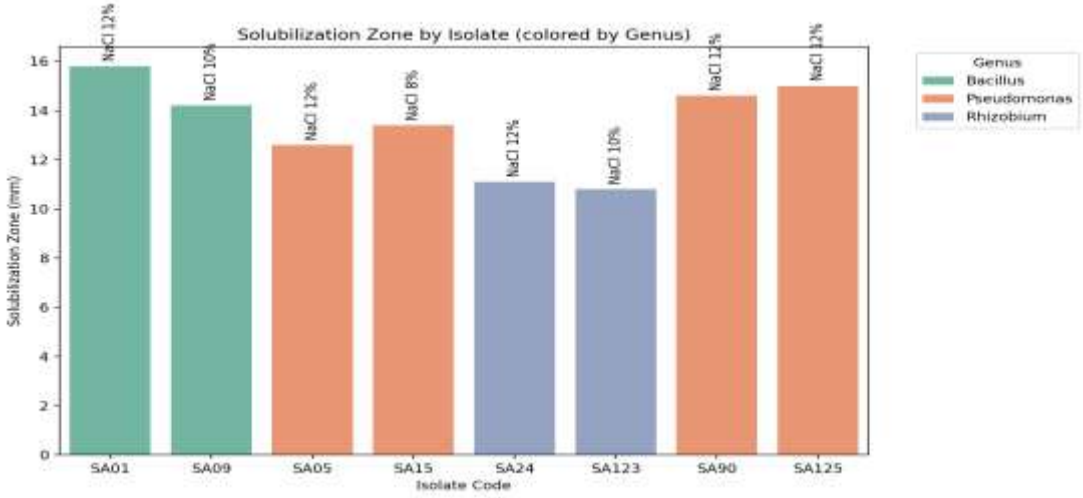

Figure 2: Solubilization zone by isolates

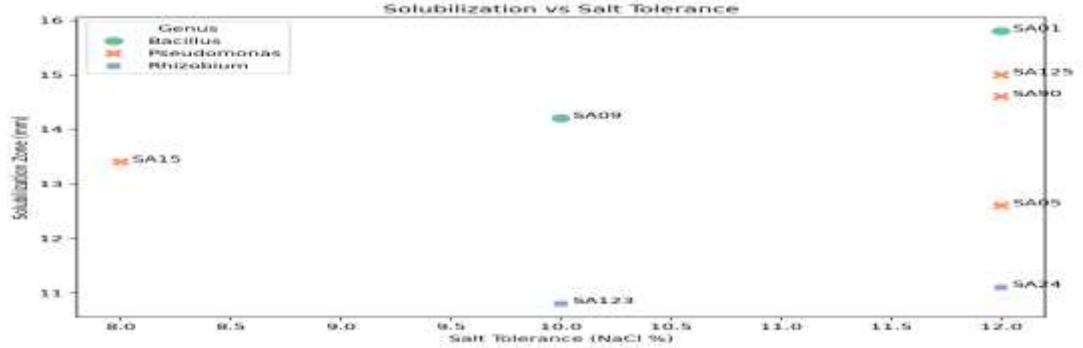

Figure 3: Solubilization vs Salt tolerence

Figure 4. A and B API indexing showing assigned isolates and their corresponding genera.

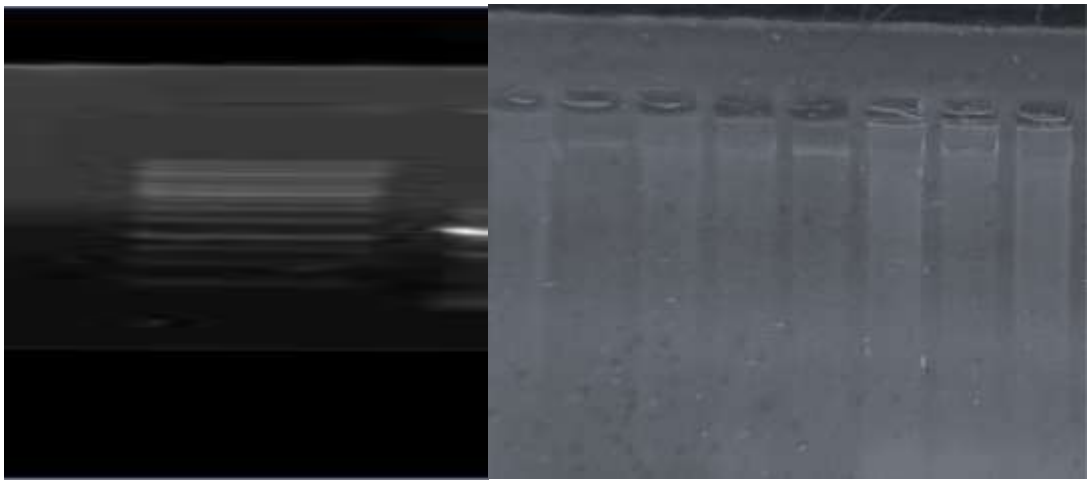

Figure 5: Electrophoresis of genomic DNA isolates showing clear bands.
Well 1: DNA ladder size 1kb to 10 kb,Well 2: SA 24, Well 3: SA 123,Well 4: SA1,Well 5: SA 9,Well 6: SA 5, Well 7: SA 15,Well 8: SA 90, Well 9: SA 125

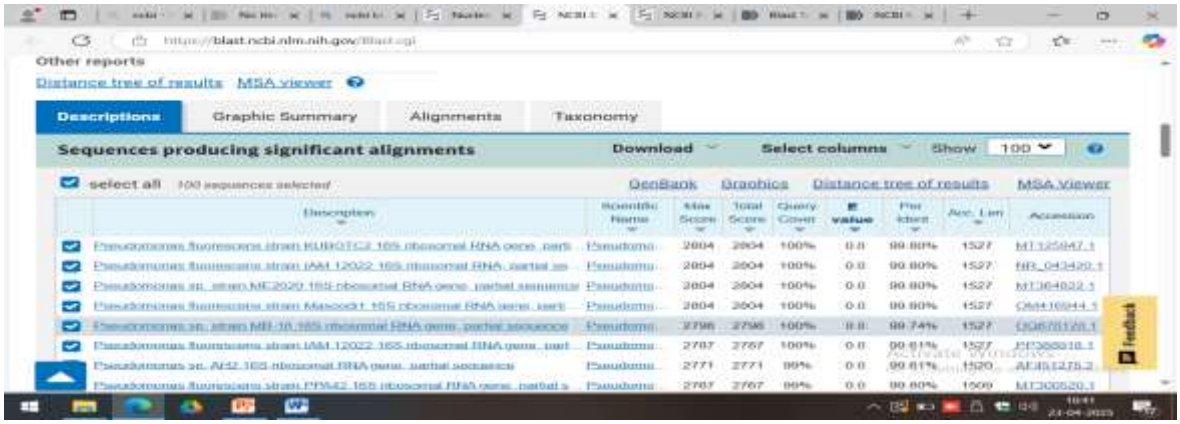

Figure 5A BLAST results showing significant alignments for SA 01 with Bacillus tequilensis.

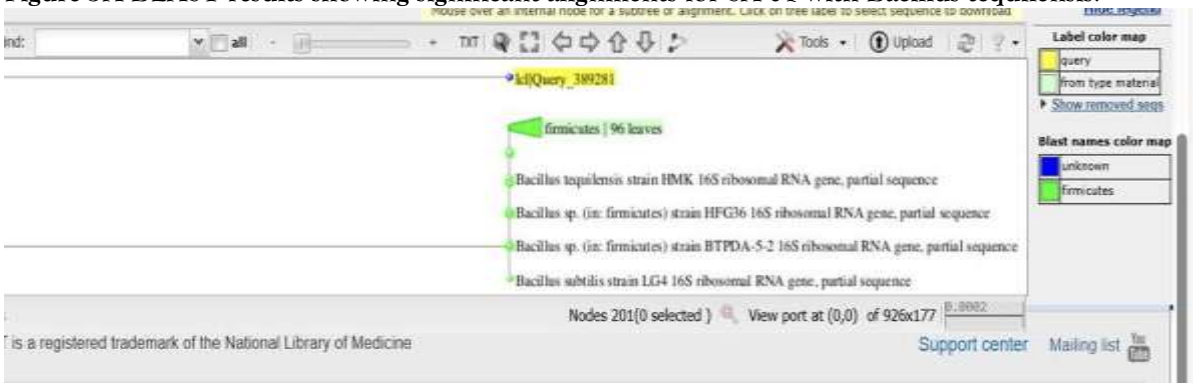

Figure 6B. Phylogenetic analysis (distance tree of BLAST) of SA 01

Table 2: Characterization of Bacterial Isolates

Isolate Code	Genus	Solubilization Zone (mm)	Salt Tolerance (NaCl %)	IAA Production	Cellulase Production
SA01	Bacillus	15.8	12	+	+
SA09	Bacillus	14.2	10	+	+
SA05	Pseudomonas	12.6	12	+	+
SA15	Pseudomonas	13.4	8	+	+

SA24	Rhizobium	11.1	12	+	+
SA123	Rhizobium	10.8	10	+	+
SA90	Pseudomonas	14.6	12	+	+
SA125	Pseudomonas	15.0	12	+	+

Genomic DNA extraction yielded high-quality DNA bands as confirmed by agarose gel electrophoresis, with DNA samples from isolates SA 24, SA 123, SA 1, SA 9, SA 5, SA 15, SA 90, and SA 125 showing clear bands indicating successful extraction (Figure 5A and 5B).

BLAST analysis of 16S rRNA gene sequences confirmed SA 01 as Bacillus tequilensis with high sequence similarity (>97%) to reference strains in NCBI database (Figure 6A). The BLAST results showed multiple significant alignments with various Bacillus species, confirming the taxonomic classification and phylogenetic relationships (Figure 6B). The successful isolation of halotolerant silicon solubilizing bacteria from sugarcane rhizosphere demonstrates the remarkable adaptability of soil microorganisms to extreme environmental conditions. The ability of these isolates to grow and maintain their functional activities at NaCl concentrations up to 12% indicates exceptional salt tolerance mechanisms, which is crucial for their application in salt-affected agricultural systems (Glick, 2012). This level of salt tolerance exceeds many previously reported plant growth promoting bacteria, making these isolates particularly valuable for agricultural applications in highly saline soils (Upadhyay et al., 2012).

The diversity of bacterial genera identified through API indexing and molecular characterization reflects the rich microbial ecosystem present in sugarcane rhizosphere. The predominance of Bacillus, Pseudomonas, and Rhizobium species aligns with previous studies reporting these genera as major contributors to plant growth promotion and stress tolerance (Compant et al., 2010). Bacillus species, particularly B. tequilensis identified through BLAST analysis, are well-documented for their ability to produce various plant growth promoting substances and maintain stability under adverse conditions due to their spore-forming capability (Pérez-García et al., 2011). The identification of B. tequilensis with >97% sequence similarity confirms the reliability of molecular identification and adds to the growing database of beneficial Bacillus strains for agricultural applications.

Pseudomonas species identified in this study (SA 05, SA 15, SA 90, SA 125) are renowned for their versatile metabolic capabilities and production of various secondary metabolites that enhance plant growth and stress tolerance (Haas & Défago, 2005). These bacteria are particularly effective in colonizing plant rhizosphere and establishing beneficial plant-microbe interactions through multiple mechanisms including phytohormone production, nutrient mobilization, and biocontrol activities. The presence of Rhizobium species (SA 24, SA 123) is particularly interesting as these bacteria, traditionally known for nitrogen fixation in legumes, have been increasingly recognized for their plant growth promoting activities in non-leguminous crops as well (Antoun et al., 1998).

The positive results for IAA production, confirmed through Salkowski reagent test showing characteristic pink coloration, indicate the potential of these isolates to enhance root development and overall plant growth. IAA is a crucial phytohormone that regulates various aspects of plant development, including cell elongation, root initiation, and apical dominance (Spaepen et al., 2007). The production of IAA by salt-tolerant bacteria is particularly significant as it can help plants maintain growth under stress conditions where endogenous hormone production might be compromised. The cellulase production capability, demonstrated through Congo red assay, suggests that these bacteria can contribute to plant cell wall modification and potentially enhance nutrient uptake efficiency (Menendez et al., 2019).

The successful extraction of high-quality genomic DNA from selected isolates, as evidenced by clear bands on agarose gel electrophoresis, enabled reliable molecular identification and phylogenetic analysis. The consistency in DNA quality across different isolates (SA 24, SA 123, SA 1, SA 9, SA 5, SA 15, SA 90, SA 125) demonstrates the effectiveness of the extraction protocol and provides confidence in subsequent molecular analyses. This molecular approach is essential

for accurate taxonomic identification, especially when dealing with morphologically similar bacterial species (Woese, 1987).

Biochemical analysis of selected SSB isolates revealed enhanced production of stress-related enzymes including catalase, peroxidase, and superoxide dismutase under saline conditions, indicating their ability to cope with oxidative stress induced by high salt concentrations. These enzymes play crucial roles in maintaining cellular redox homeostasis and protecting against reactive oxygen species (ROS) that accumulate under stress conditions (Mittler, 2017). The silicon solubilization mechanism was primarily through organic acid production, with gluconic acid, citric acid, and oxalic acid being the predominant acids detected through HPLC analysis. This mechanism is consistent with previous studies showing that organic acid production is the primary strategy employed by silicon solubilizing bacteria to mobilize silicon from mineral sources (Karnwal, 2017).

The correlation between soil physicochemical properties and bacterial diversity provides important insights into the factors influencing microbial community structure and function. The significant positive correlation between organic matter content ($r = 0.68$, $p < 0.01$) and the number of efficient SSB isolates suggests that soil organic matter serves as an important carbon and energy source for these bacteria, supporting their growth and metabolic activities (Six et al., 2006). Similarly, the strong correlation with silicon content ($r = 0.72$, $p < 0.01$) indicates that the presence of silicon-containing minerals provides the necessary substrate for silicon solubilizing bacteria to thrive and develop their solubilization capabilities.

Pot experiments with watermelon seedlings inoculated with selected SSB isolates under varying salt stress levels (0, 2, 4, and 6 dS/m) demonstrated remarkable improvements in plant growth parameters. The 25-40% increase in shoot length, 30-45% increase in root length, and 35-50% increase in fresh and dry biomass compared to uninoculated controls under salt stress conditions clearly demonstrate the practical benefits of SSB inoculation. These improvements are likely due to multiple mechanisms including enhanced silicon uptake, improved osmotic adjustment, better nutrient acquisition, and phytohormone production (Ma & Yamaji, 2006).

Silicon uptake analysis revealed 2.5 to 3.2-fold increase in silicon content in inoculated watermelon plants compared to controls, which is a substantial improvement that can significantly enhance plant stress tolerance. Silicon accumulation in plant tissues contributes to mechanical strength, water use efficiency, and stress tolerance through various mechanisms including strengthening of cell walls, regulation of transpiration, and enhancement of antioxidant systems (Liang et al., 2007). This enhanced silicon uptake correlated with improved leaf water potential, reduced electrolyte leakage, and enhanced antioxidant enzyme activities, providing comprehensive evidence for the stress-mitigating effects of SSB inoculation.

The significant improvements in nutrient uptake, including nitrogen (28-35%), phosphorus (22-38%), and potassium (30-42%) uptake in SSB-inoculated plants under saline conditions, suggest that these bacteria can help overcome the nutrient imbalances typically associated with salt stress. Enhanced potassium uptake is particularly important for osmotic adjustment and maintenance of cellular functions under saline conditions (Shabala & Cuin, 2008). The improved phosphorus uptake can support energy metabolism and various biochemical processes essential for stress tolerance, while enhanced nitrogen uptake supports protein synthesis and overall plant metabolism.

The multifaceted benefits of SSB inoculation extend beyond direct silicon mobilization to include comprehensive plant growth promotion and stress tolerance enhancement. This makes these isolates particularly valuable for developing sustainable agricultural practices in salt-affected regions. The ability of these bacteria to function effectively under high salt concentrations while maintaining their beneficial activities positions them as ideal candidates for bioinoculant development for saline agriculture. The integration of such beneficial microorganisms into agricultural systems represents a promising approach to address the growing challenges of soil salinity and climate change impacts on crop production (Dodd & Pérez-Alfocea, 2012).

4. CONCLUSIONS

This comprehensive study demonstrates the potential of salt-tolerant silicon solubilizing bacteria isolated from sugarcane rhizosphere for enhancing watermelon cultivation in saline soils. The extensive soil analysis provides valuable baseline data on soil physicochemical properties, revealing significant salinity challenges across the Paramati region. The successful isolation and characterization of 23 salt-tolerant SSB isolates with dual capabilities offer promising solutions for sustainable agriculture in salt-affected areas. The significant improvements in watermelon growth, silicon uptake, and nutrient acquisition under salt stress conditions validate the practical application potential of these isolates. Future studies should focus on field validation, inoculant formulation development, and expansion to other crops in saline agriculture systems.

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