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Mitigating salt stress in tomato (*Solanum lycopersicum*) through plant growth-promoting bacteria

V. Revathi[1], A.N. GANESHMURTHY[1], V. Rajesh Kannan[1], P. Pannerselvam[2] and D. KALAIVANNAN[2]

[1]**Department of Microbiology, Bharthidasan University, Tamil Nadu, India**
[2]**Department of Soil Science, IIHR Hessaraghatta, Bangalore, India**

ABSTRACT

The amount of agricultural land has extenuate in recent times due to factors such as deforestation, pollution, erosion, waterlogging or flooding, population growth, alterations in land use, economic development, excessive use of fertilizers, salinity, alkalinity, and acidification. Approximately 6.4 million hectares of land are influenced by salinity, alkalinity, or acidification. Currently, plant growth-promoting bacteria (PGPB) are employed to maintain the nutritional quality of the soil and enhance plant growth in areas affected by salinity, alkalinity, and acidification. They are pivotal for sustainable agriculture in terms of nutrient uptake, stress tolerance, and higher crop yields. Salinity of soil also poses a major challenge to the growth of plants repercussion in osmosis and dehydration, which eventually causes the plant to dry up due to high concentrations of K^+ and Na^+ ions. Therefore, PGPB are useful to the growth of plants and are progressively applied to improve the growth of plants. This paper is devoted to the use of plant growth-promoting bacteria (PGPB) to promote tomato (*Solanum lycopersicum*) growth through high salt stress conditions. It is noted that the higher the salinity in the soil, the more the PGPB promotes the synthesis of the micro and macronutrients in the soil as well as plants that are exposed to high salinity tempos.

KEY WORDS

PGPB; Siderophore; Cellulase; Protease; ACC deaminase; IAA

ARTICLE HISTORY

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Introduction

Solanum lycopersicum, or tomato, is one of the most common vegetable crops in the world that is known to have a great nutritional value and has economic significance. They contain the necessary nutrients, abound in antioxidants and can be grown freshly or processed, which gives substantial health benefits. These multifunctional superfoods have advantages in terms of cancer, heart, brain, skin, digestive and post-exercise recovery. The possible health benefits of tomatoes are the anticancer effect of lycopene which is connected with the inability of this substance to cause the formation of blood vessels, the decreasing insulin-like growth factor (IGF) in the blood, and the regulation of pathways in the cell that are related to cancer [1,2].

Although it is an important horticultural crop in most parts of the world, the ability to reproduce is indirectly challenged by soil salinity. Saline soils also cause abiotic stress, which affects large swathes of arable land, particularly in arid and semi-arid areas. These salty soils are also typified by an excess of soluble salts that interfere with the osmotic regulation of the root zone, and thus plants find it hard to absorb water and other essential nutrients. This discourages the growth of plants.

Besides causing significant losses in yield, salinity is a major problem in tomato cultivation. Salinity stress of tomato plants impairs water and nutrient uptake capacities of plants, triggers oxidative stress, and eventually results in reduced productivity and fruit quality. Heightened concentration of sodium and chloride ions disrupts the uptake of vital nutrients such as potassium, calcium and magnesium, resulting in nutrient imbalances that disrupt physiological activities. Water is constrained by the osmotic stress due to the high level of salt, hence leading to inhibited growth, limited expansion of the leaves and flowering. Thus, salinity causes oxidative stress because of reactive oxygen species accumulation, which may damage membranes, proteins and DNA. Thus, tomatoes planted in salty areas often experience a reduced size of fruits, lower productivity, and deteriorated quality, including changes in taste and nutritional content [3-5]. Therefore, plant growth-promoting bacterium (PGPB) is gaining popularity to protect crops against salinity stress in sodic soils as well as boosting fruit production and nutritional quality.

PGPB resemble unsung heroes in saline soils. They are natural associates of plants that are under extreme saline stress, and they are the key to the improvement of soil quality and plant resilience. They absorb nitrogen in the atmosphere, dissolve phosphorus and mobilize vital minerals. They also help plants to survive in drought, salt, and pressure of pathogens by inducing systemic resistance. In many cases.

PGPB are symbiotic to mycorrhizal fungi and increase nutrient exchange and root growth by their association. They increase crop yields by using low levels of synthetic inputs, which encourages the diversity of the microorganisms and recycling of nutrients. They assist in balancing osmotics in crops by synthesizing exopolysaccharides which enhance soil structure and renew roots. They generate hormones (auxins, gibberellins and cytokinins) that promote the development of roots and shoots. ACC deaminase activity dismantles the ethylene associated with stress, relieving it of its negative influence on the growth of plants. They enhance the supply of phosphorus, nitrogen, and micronutrients as well as triggering the plant defence mechanisms to manage salinity. Osmotic stress reduces the seed germination, causes stunted growth in plant (shorter stems, smaller leaves, and its area) and root damage, which

***Correspondence:** Ms. V. Revathi, Department of Microbiology, Bharthidasan University, Tamil Nadu, India, e-mail: rev25.11@gmail.com

makes roots less efficient in the absorption of water and nutrients [6-8].

Materials and Methods

Bacteria

The bacteria were grown at different NaCl concentration 5%, 10, &15% concentrations in Nutrient Agar Medium (Peptone-5g; Beef Extract-3g; NaCl-5g; Agar-2%; Distilled H_2O-1000ml; pH- 7). Sal-A (*Lycinibacillus spp.*), Sal-B (*Bacillus circulans*), Sal-C (*Pseudomonas plecoglossicida*), Sal- D (*Pseudomonas spp.*) & Sal-E (*Pseudomonas stutzeri*).

Then, the enzymatic activity of the bacteria was conducted by using the following protocols:

IAA & GA3

IAA & GA3 production Nutrient Broth with L-Tryptophan(Peptone-5g; Beefe Extract-3g; NaCl-5g; Distilled H_2O-1000ml; pH-7; L-Tryptophan-100mg) studied using Horemans, S.K. Vlassak 1985 method. All five selected five bacterial isolates were evaluated for IAA and GA3 production. A 100 ml of nutrient broth was prepared and sterilized. Freshly prepared, filtered and sterilized solution of L-Tryptophan was added to each flask to a final concentration of 100µL. Control was also maintained without adding L-tryptophan. The 2 ml of inoculated broth culture was added to each nutrient broth. The cultures were incubated in the tryptophan medium for 7 days. After the incubation, the broth cultures were centrifuged at 6000 rpm to remove the bacterial culture. The pH of the supernatant was adjusted to 2.8 with 0.1N HCl. 15 ml acidified supernatant was taken in a 100 ml conical flask, and an equal volume of diethyl ether was added to the supernatant and incubated in the dark for 4 hrs. The mixture of diethyl ether and the supernatant was incubated for overnight at 4°C in a separating funnel. The organic phase was discarded, and the solvent phase was collected. The solvent phase was poured into a sterile plate and allowed to evaporate. To the dried material 2ml of methanol was added, and the IAA present in the extract was determined by using HPLC.

ACC deaminase

ACC deaminase activity was studied using the Penrose & Glick (2003) method. All five bacterial isolates were tested for ACC deaminase activity. The isolates were grown in freshly prepared Luria Broth and incubated for 48 hrs. After incubation, the LB was centrifuged at 8000 rpm for 10 min. The supernatant was discarded and cell pellets were washed with sterile water followed by DF media (K_2HPO_4 – 6 g; $MgSO_4$ – 0.2 g; Glucose – 2 g; Gluconic acid – 2 g; Citric acid – 2 g; $FeSO_4$ – 1 mg; H_3BO_3 – 10 mg; $MnSO_4$ – 11.19 mg; $ZnSO_4$ – 78.22 µg; MoO_3 – 10 µg; $(NH_4)_2SO_4$ – 2 mg) (N-free media) and then suspended in 50 mL DF media containing ACC (3.0 mM), which was prepared by filtration and sterilization of 0.5 M solution of ACC using a 0.2 µm membrane filter.

The filtrate was collected, aliquoted, and frozen at –20 °C. Just prior to inoculation, the ACC solution was thawed and 300 µL was added to 50 mL DF media after centrifugation at 8000 rpm for 10 min. About 30 µL of ACC was spread on bacto agar plates and the individual pellet samples were spotted. ACC deaminase activity was determined by measuring the production of α-ketobutyrate generated from ACC by ACC deaminase.

Lignase

Lignase activity was determined by Stephen B. (1991) using basal media (KH_2PO_4 – 1 g; $MgSO_4$ – 0.5 g; $C_4NH_{12}O_6$ – 0.5 g; $CaCl_2$ – 0.01 g; Yeast extract – 0.01 g; $CuSO_4{\cdot}5H_2O$ – 0.001 g; Fe_2SO_4 – 0.001 g; $MnSO_4{\cdot}H_2O$ – 0.001 g; Dist. H_2O – 1000 mL). Lignin basal medium contained 0.25% (w/v) lignin + 1.6% agar + 20% glucose in 1000 mL H_2O.

Cellulase

Activity ($NaNO_3$ – 0 g; $MgSO_4$ – 0.1 g; KCl – 0.05 g; CMC – 0.2%; Peptone – 0.02 g; Distilled H_2O – 100 mL; pH 7) by Stephen B. (1991).

Tannase

Tannase activity (Malt extract – 1.5 g; Tannic acid – 0.5%; Agar – 2 g; Distilled H_2O – 100 mL) by Stephen B. [9].

Pectinolytic

Tannase activity (Malt Extract – 1.5 g; Tannic acid – 0.5%; Agar – 2 g; Distilled H_2O – 100 mL) by Stephen B. [9].

Starch hydrolysis

Starch hydrolysis activity (Starch – 20 g; Peptone – 5 g; Beef extract – 3 g; Agar – 15 g; Distilled H_2O – 1000 mL).

Chitinase activity

Chitinase activity by Stephen B. (1991) (Colloidal chitin – 1%; Na_2HPO_4 – 6 g; K_2HPO_4 – 1 g; NH_4Cl – 0.5 g; KH_2PO_4 – 3 g; $MgSO_4{\cdot}7H_2O$ – 0.12 g; NaCl – 0.5 g; $CaCl_2$ – 0.05 g; Yeast extract – 0.05 g; Agar – 2%; Distilled H_2O – 1000 mL).

Urease activity

Urease activity (Peptone – 1 g; NaCl – 5 g; KH_2PO_4 – 2 g; Glucose – 1 g; Phenol red – 0.02%; Urea – 20%; Distilled H_2O – 1000 mL).

Protease

Protease activity by Stephen B. (1991) (Skim milk agar powder – 2.8 g; Yeast extract – 0.25 g; Casein enzymatic hydrolysate – 0.5 g; Dextrose – 0.1 g; Agar – 2%; Distilled H_2O – 100 mL).

IMVC

IMVC Readymade broth medium by HiMedia was used. In the IMVC broth, the cultures were inoculated and observed for colour change after 7days.
(https://microbenotes.com/imvic- tests/)

Siderophore activity

Siderophore activity (a. Nutrient agar composition: 200 mL distilled water added to 180 mL of distilled water; b. Dye solution: (i) Chrome azurol dye – 60.5 g; Distilled water – 50 mL; (ii) Iron source (1 mM $FeCl_2$) $FeCl_2$ – 0.0162 g; Distilled water – 100 mL; (iii) HDTMA solution – HDTMA – 72.9 g; Distilled water – 40 mL. Mix solution (i) with solution (ii) slowly, then add to solution (iii) and mix gently. Add 20 mL of the dye solution into 180 mL of NA medium and mix gently) by Rajat Maheshwari et al. [10].

Pot experiment

The tomato seedlings were raised in sterile cocopeat and transferred to pots in order to observe the effect of Pseudomonas spp (Sal D) on enhancement of growth of plants at different salt concentration. After transplanting, the water containing different EC i.e., 1,2,3,4 and 5 dSm- 1 was given separately with and without bacterial inoculation (@ 20 g inoculum containing 12 X 109 cfu g-1 per pot). Control plants were also maintained with and without the bacterial culture, wherein normal irrigation was given. All the plants were maintained under glass house condition and

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observation was taken at the intervals of twenty days.

Soil analysis

Soil samples were collected from each treatment, dried and sieved in 2mm sieve. All these soil samples were subjected for the following nutrients analysis viz., N, P, K, Na, Ca, Mg, Fe, Cu, Zn and Mn. Similarly, all the samples were analysed for enzyme activities viz., phosphatase and dehydrogenase, soil respiration, pH, EC and microbial population [11].

pH

20g of soil was taken; 50ml of distilled water was added to the soil. The sample was stirred properly and incubated for 30 min. The pH of the soil sample was taken with the help of a pH meter [11].

EC

20g of soil was taken; 50ml of distilled water was added in the soil. The sample was stirred properly and incubated for 30 min. The EC of the soil was measured [11].

Soil respiration

In 50 g of soil, 10 mL of distilled water was added and stirred properly. 25 mL of 0.05 M NaOH solution was added into a closed jar, and a control was also maintained. The samples were incubated for 24 h to 3 days. After incubation, one or two drops of phenolphthalein indicator were added along with 5 mL of 0.5 M $BaCl_3$ [12].

Organic carbon estimation

0.5 g of soil was sieved through a 0.2 mm sieve and taken in a 250 mL conical flask. 10 mL of 1 N $K_2Cr_2O_7$ solution along with 20 mL of concentrated H_2SO_4 was added to the sieved soil. The contents were kept undisturbed for 30 min. After incubation, 100 mL of distilled water was added. A drop of ferroin indicator was added and the solution was titrated against 0.5 N FAS [13].

Dehydrogenase

1g of soil was taken in a screw cap tube. 0.2ml of 3% TTC was added along with 0.5ml of 1% glucose. The contents were mixed properly and incubated for 24hrs at 30°C. 10ml of methanol was added after incubation and stored in a refrigerator for 3hrs. Trphenylformazon was determined by specrophotometric reading at 485nm [14].

Phosphatase

1. Acid phosphatase

1 g of soil sample was taken in a 250 mL Erlenmeyer flask. 0.25 mL of toluene was added along with 4 mL of MUB (modified universal buffer) at pH 6.5, followed by 1 mL of p-nitrophenyl phosphate (prepared in MUB solution). The contents were mixed properly and incubated for 1 h at 37 °C. After incubation, 1 mL of $CaCl_2$ and 4 mL of 0.5 M NaOH were added and mixed properly. The suspension was filtered through Whatman filter paper, and the absorbance was measured at 420 nm [15].

2. Alkaline phosphatase

1 g of soil sample was taken in a 250 mL Erlenmeyer flask. 0.25 mL of toluene was added along with 4 mL of MUB (modified universal buffer) at pH 9, followed by 1 mL of p-nitrophenyl phosphate (prepared in MUB solution). The contents were mixed properly and incubated for 1 h at 37 °C. After incubation, 1 mL of $CaCl_2$ and 4 mL of 0.5 M NaOH were added and mixed properly. The suspension was filtered through Whatman filter paper, and the absorbance was measured at 420 nm [15].

Phosphorous estimation

3. Brays method

5g of soil was taken in a 250ml conical flask. 50ml of Bray's reagent was added and was kept in a shaker for half an hour and filtered. Blank was also prepared. 5ml of filtrate was taken in 25ml of volumetric flask, further added 5 ml of ammonium molybdate. 10ml of distilled water was added to wash the neck of the flask, so as to remove the adherent ammonium molybdate. 1 ml of Stanneous chloride was added and the solution was made up to 25ml. OD was taken at 660 nm [16].

4. Olsens method

5g of soil was taken in a 250 ml conical flask. 50 ml of Olsen's reagent was added and was kept in a shaker for half an hour and filtered. Blank was also prepared. 5ml of filtrate was taken in 25 ml of volumetric flask, further added 5 ml of ammonium molybdate. 10ml of distilled water was added to wash the neck of the flask, to remove the adherent ammonium molybdate. 1ml of Stanneous chloride was added and the solution was made up to 25ml. OD was taken at 660nm [17].

Potassium estimation

5g of soil was taken in a 250ml conical flask. 25ml of neutral normal ammonium acetate was added and kept in a shaker for 5 min. 0.5ml of filtrate was made up to 50ml in a volumetric flask. Then the sample was feed to flame photometer [18].

Micronutrients estimation

20g of soil was taken in a 100ml of conical flask. 40ml of DTPA was added and kept in a shaker for 2hrs. The solution was filtered with a whatman filter paper. 2ml of the sample was made to 25ml in a volumetric flask. The sample was feed to the AAS [19].

Initial Microbial Population in Soil

1g of soil sample was taken in 9ml of sterile distilled water and thoroughly mixed. From this tube 1ml of the sample was transferred to another test tube containing 9ml of distilled water. In the same manner dilutions up to 10-10 were made up. From the dilutions 100µL of the sample was taken from 10-7 for bacteria and from 10-5 for actinobacteria, fungi and yeast spread on the plates with the help of a sterile glass rod.

Plant Growth Analysis

The plant growth was observed at 20 days intervals up to 60 days in plantation. After the saline treatment the height and the girth of the plants has been decreased, on 40th day, but the plant height and girth were found to be increasing on the 60th day. The growth of plant under salt stress has been tabulated in a table.

Leaf Analysis

Fresh leaf samples were taken, dried and grinded. These leaf samples were used to determine the availability of nutrients in plants after saline treatment.

Phosphorous estimation was done by Jones, J. B., method [20]. 1g of the leaf sample was taken in a 100 ml volumetric flask, 10ml of diacid was added to the leaf sample and was incubated overnight. The leaves were digested with fume wood. After digestion the samples were made up to 100ml with

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milli-q water. From the extract, 5ml was taken in a 25ml volumetric flask along with 5ml of vanadium molybdate which was made up in to 25ml. Then the reading was taken in a spectrophotometer at 470nm.

Nitrogen estimation was carried out using the Kjeldahl method [21]. 0.3 g of leaf sample was taken in a Kjeldahl digestion flask. 2 g of digestion mixture was added along with 10 mL of concentrated sulphuric acid (H_2SO_4). The samples were digested in a fume hood by adding 1–2 drops of hydrogen peroxide at intervals of 30 min. After digestion, the samples were allowed to cool and made up to 100 mL in a volumetric flask. 10 mL of 4% boric acid was added to a receiving conical flask. Then 5 mL of the sample and 5 mL of 40% NaOH were added to the distillation flask and the contents were distilled. After complete distillation, the receiving conical flask was removed and the contents were titrated against 0.4 N H_2SO_4 until the colour changed from light green to pink [21].

Results

Bacteria & treating of bacteria for the growth of tomato plants

In the current study, Sal-D (*Pseudomonas spp.*) and Sal-E (*Pseudomonas stutzeri*) were more tolerant to NaCl than Sal-A (*Lysinibacillus spp.*), Sal-B (*Bacillus circulans*), and Sal-C (*Pseudomonas plecoglossicida*) at 5%, 10%, and 15% NaCl concentrations, as depicted in Figure 1. The growth of bacteria was noted to be low at pH 5, but the growth rate improved at pH 7 and 10, as shown in Figure 2. Table 1 presents the identification of the bacteria Sal-A, Sal-B, Sal-C, Sal-D, and Sal-E. These bacteria were found to exhibit the following enzymatic activities, as shown in Figures 3–6 and Table 2: Sal-E was IMViC positive (Figure 3 and Table 3); Sal-A and Sal-B were urease positive (Figure 4 and Table 4); all bacteria (Sal-A, Sal-B, Sal-C, Sal-D, and Sal-E) were protease active (Figure 5 and Table 5). Cellulase, lignolytic, pectinase, and starch hydrolysis activities were observed in Sal-D. Figure 6 and Table 6 show the activity of IAA and GA_3 in the presence and absence of NaCl, as well as the ACC deaminase activity of Sal-A, Sal-B, Sal-C, Sal-D, and Sal-E.

Note: Sal-A (*Lycinibacillus spp.*), Sal-B (*Bacillus circulans*), Sal- C (*Pseudomonas plecoglossicida*), Sal-D (*Pseudomonas spp.*) and Sal-E (*Pseudomonas stutzeri*).

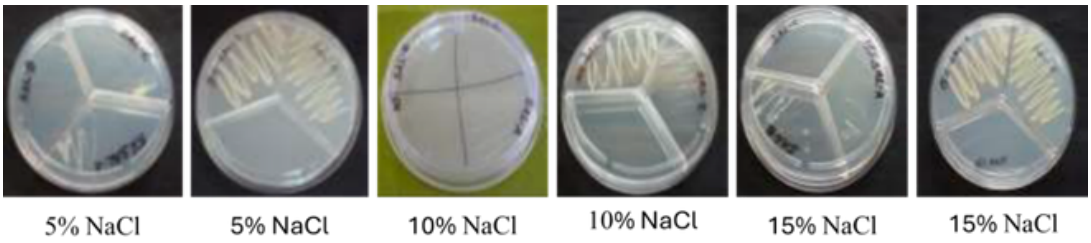

Figure 1. Growth of bacteria under NaCl.

Figure 2. Growth at different pH.

Table 1. Nomenclature of the bacteria.

S.No.	Code No.	Name of the bacteria's
1	SAL-A	Lycinibacillus spp.
2	SAL-B	Bacillus circulans
3	SAL-C	Pseudomonas plecoglossicida
4	SAL-D	Pseudomonas spp.
5	SAL-E	Pseudomonas stutzeri

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Table 2. Enzyme activity.

Organisim	CMC	Pectinase	Lignase	Protease	Starch	Urease	Tannase	Chitinase	Indole	VP	MR	Catalase
SAL-A	-	-	-	+	-	++	-	-	-	-	-	-
SAL-B	-	-	-	+	-	++	-	-	-	-	-	-
SAL-C	-	-	-	+	-	+	-	-	-	-	-	-
SAL-D	+	+	+	+	+	-	-	-	-	+	-	-
SAL-E	-	-	-	+	-	-	-	-	-	+	+	-

Figure 3. IMVIC test.

Table 3. Siderophore production by salt-tolerant bacteria.

Bacteria	Bacterial colony diameter	Zone diameter
SAL – A	4mm	4mm
SAL – D	13mm	5mm
SAL – E	4mm	2mm

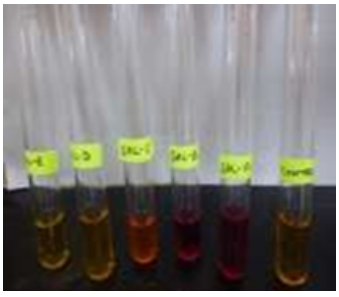

Figure 4. Urease Activity.

Table 4. Enzyme activity for SAL – D.

Enzymes	Bacterial colony diameter	Zone diameter
Starch Hydrolysis	17mm	18mm
Cellulase	20mm	14mm
Lignase	19mm	24mm
Pectinase	20mm	17mm
Protease	18mm	7mm

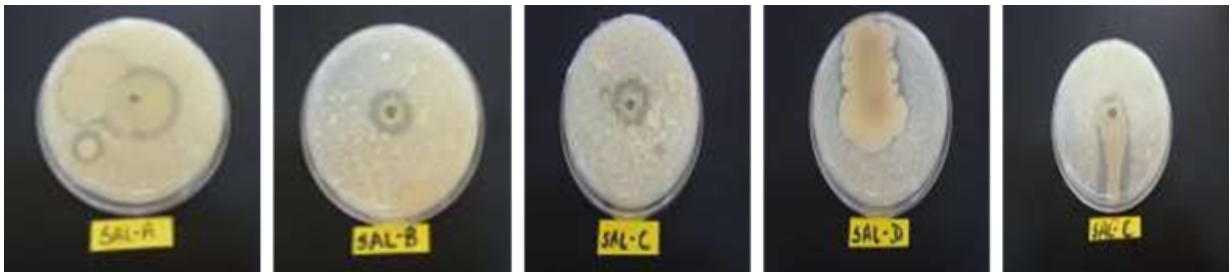

Figure 5. Protease Activity.

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Table 5. Protease activity for other organisms.

Bacteria	Bacterial colony diameter	Zone diameter
SAL – A	20mm	20mm
SAL – B	19mm	18mm
SAL – C	16mm	25mm
SAL – E	20mm	15mm

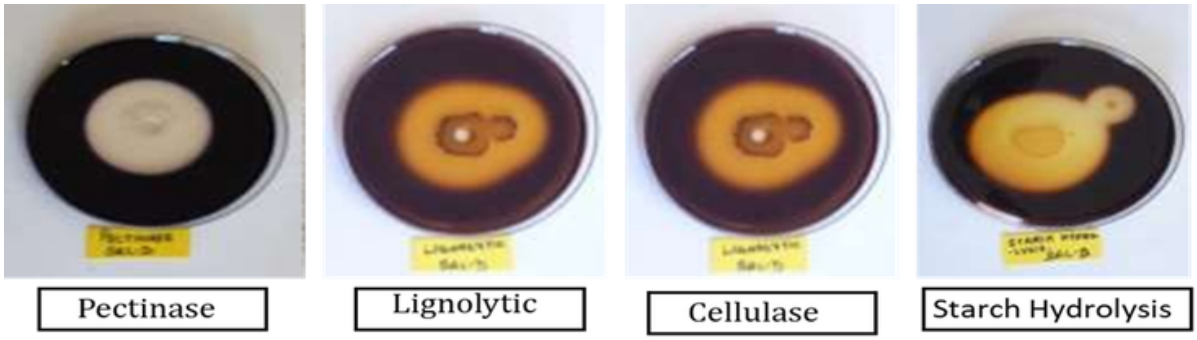

Figure 6. SAL-D Enzymatic activity.

Table 6. IAA and GA_3 production (control and 5 % salt stress) and ACC deaminase activity.

Treatment	Bacteria	IAA (µg/ml)	GA3 (µg/ml)
Production of IAA & GA3	SAL-A	435.33	432.97
	SAL-B	563.79	623.74
	SAL-C	497.43	402.02
	SAL-D	532.72	665.65
	SAL-E	487.33	0
IAA & GA3 at 5% Salt Stress	SAL-A	308.9	369.78
	SAL-B	439.78	426.93
	SAL-C	526.98	617.91
	SAL-D	231.83	303.96
	SAL-E	362.51	396.88
		With ACC	Without ACC
ACC Deaminase Activity	SAL-A	+ve	+ve
	SAL-B	+ve	+ve
	SAL-C	+ve	+ve
	SAL-D	+ve	+ve
	SAL-E	+ve	+ve

Development of tomato in sodic soil treated with and without PGPB

Since Sal-D showed to be the best enzyme and salt-tolerant, it was used as an inoculum to promote the growth of tomato plants in the presence of charcoal impregnated with PGPB and saline water. The EC levels were measured as 1 dS/m, 2 dS/m, 3 dS/m, 4 dS/m and 5 dS/m, which are the salinity of the soil. This research shows that tomato plants require time to become used to salty conditions. The plants that were treated with saline had their internodes that were shorter compared to the control plants. The amount of height of the plants reduced, leading to a wilt at the salinity of 1 dS/m, 2 dS/m and 3 dS/m; whereas height of the plants rose and slight wilt was noted at 4 dS/m and 5 dS/m. The flowering and fruiting were also slowed down by salinity stress. Table 7 and Figure 7 present the growth of the plants, whereas Figure 8 and Table 8 show the physicochemical properties of the soil. Table 9 summarizes the microbial activity of the soil after planting tomato plants and the treatment with NaCl and bacteria.

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Table 7. Growth of plants: Plant growth analysis in sodic soil with and without treatment of PGPB.

Heights of plant				
Sl No	**Treatment**	**20th Day**	**40th Day**	**60th Day**
1	1 dS/m	26.00	44.10	59.43
2	1 dS/m + SAL-D	23.90	41.77	61.80
3	2 dS/m	22.77	44.43	57.63
4	2 dS/m + SAL-D	26.57	37.00	50.80
5	3 dS/m	25.90	38.20	53.93
6	3 dS/m + SAL-D	23.10	38.07	53.30
7	4 dS/m	26.40	41.37	59.67
8	4 dS/m + SAL-D	21.80	39.30	52.67
9	5 dS/m	23.50	44.70	59.10
10	5 dS/m + SAL-D	21.17	37.73	57.83
11	Control	25.70	43.07	65.30
12	Control + SAL-D	22.40	44.83	63.03
Girth of plants				
1	1 dS/m	1.05	1.18	1.15
2	1 dS/m + SAL-D	1.48	0.93	1.18
3	2 dS/m	1.45	1.17	1.42
4	2 dS/m + SAL-D	1.38	0.97	1.18
5	3 dS/m	1.22	1.00	1.05
6	3 dS/m + SAL-D	1.28	0.95	1.13
7	4 dS/m	1.17	0.97	1.05
8	4 dS/m + SAL-D	1.47	1.10	1.08
9	5 dS/m	1.43	1.00	1.25
10	5 dS/m + SAL-D	1.35	0.92	1.17
11	Control	1.47	1.57	1.13
12	Control + SAL-D	1.12	1.07	1.22
Number of Leaves				
1	1 dS/m	31.67	67.00	152.33
2	1 dS/m + SAL-D	26.33	67.33	192.00
3	2 dS/m	31.67	79.00	206.33
4	2 dS/m + SAL-D	32.33	76.33	200.33
5	3 dS/m	25.00	49.33	200.00
6	3 dS/m + SAL-D	28.00	84.67	166.00
7	4 dS/m	27.00	88.67	192.67
8	4 dS/m + SAL-D	33.00	97.00	162.67
9	5 dS/m	31.33	81.67	173.67
10	5 dS/m + SAL-D	30.00	82.33	152.33
11	Control	32.67	116.67	202.67
12	Control + SAL-D	35.00	86.67	193.67

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		Number of Branches		
1	1 dS/m	9.67	14.00	36.67
2	1 dS/m + SAL-D	9.33	13.00	38.00
3	2 dS/m	10.00	13.67	32.33
4	2 dS/m + SAL-D	8.67	11.33	26.67
5	3 dS/m	9.00	13.00	30.33
6	3 dS/m + SAL-D	12.67	12.00	36.67
7	4 dS/m	10.00	14.00	34.33
8	4 dS/m + SAL-D	10.67	14.00	32.33
9	5 dS/m	9.33	13.33	34.33
10	5 dS/m + SAL-D	9.33	14.67	40.00
11	Control	9.67	13.00	41.33
12	Control + SAL-D	9.67	16.67	39.67

		Number of Flowers			
		40th Day		60th Day	
		Open	Closed	Open	Closed
1	1 dS/m	1.67	1.33	15.33	11.67
2	1 dS/m + SAL-D	1.00	6.00	26.00	8.00
3	2 dS/m	2.67	7.33	17.33	6.67
4	2 dS/m + SAL-D	0.67	6.33	16.67	3.00
5	3 dS/m	1.33	7.67	14.33	6.33
6	3 dS/m + SAL-D	2.33	8.33	11.67	6.33
7	4 dS/m	1.00	7.67	16.33	5.00
8	4 dS/m + SAL-D	2.67	8.00	21.67	11.00
9	5 dS/m	1.33	8.33	23.67	9.00
10	5 dS/m + SAL-D	1.00	8.33	23.67	10.00
11	Control	1.33	10.00	32.33	12.00
12	Control + SAL-D	0.67	9.00	18.67	9.33

		Number of Flowers
		60th Day
1	1 dS/m	1.00
2	1 dS/m + SAL-D	1.33
3	2 dS/m	1.67
4	2 dS/m + SAL-D	1.33
5	3 dS/m	1.67
6	3 dS/m + SAL-D	2.33
7	4 dS/m	0.67
8	4 dS/m + SAL-D	1.67
9	5 dS/m	1.67
10	5 dS/m + SAL-D	0.00
11	Control	1.33
12	Control + SAL-D	1.00

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Figure 7. Tomato plants grown under salt stress with and without SAL-D.

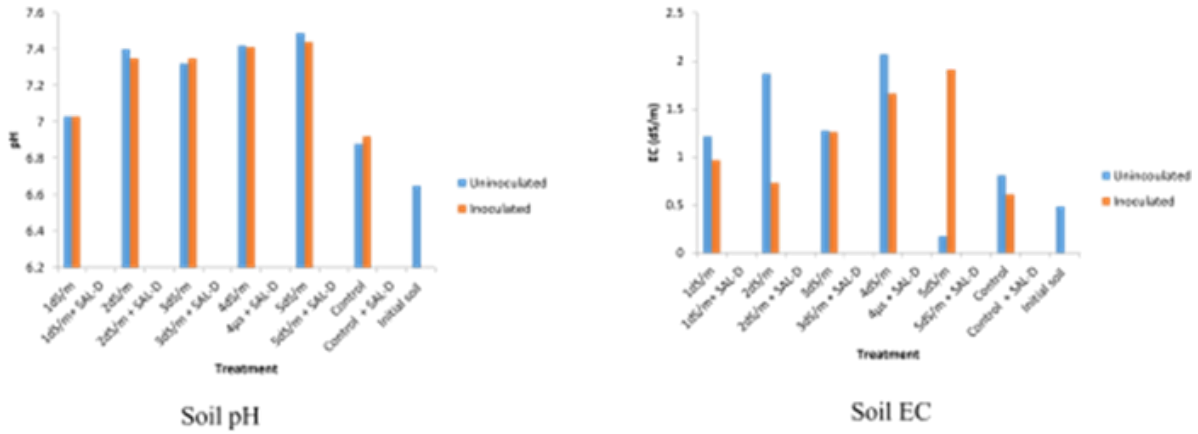

Figure 8. Physiochemical properties of soil.

Table 8. Soil respiration.

Treatment	Soil Respiration (CO2/kg/hr)	
	Uninoculated	Inoculated
1dS/m	34.29	32.08
2 dS/m	26.51	26.1
3 dS/m	28.58	28.2
4 dS/m	20.02	26.41
5 dS/m	24.09	27.97
Control	36.83	22.04

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Table 9. Microbial activity after treating the soil with saline water and Sal-D.

Treatment	Bacteria (107) cfu x107/g soil		Fungi (105) cfu x105/g soil		Yeast (105) cfu x105/g soil		Actinobacteria (105)cfu x105/g soi	
	Uninocul ated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
1dS/m	15.6	36.4	11.5	11.5	14.8	49.2	20.3	5
2 dS/m	25.2	60	44.4	10.2	24	48	29.2	33.2
3 dS/m	7.1	15.8	45.6	12	48	43.2	44	3.8
4 dS/m	24.6	11.8	14.4	10.8	13.5	50	8.8	10.5
5 dS/m	21.6	12.2	12	28.8	16.4	40.8	4.2	17
Control	13.5	23.5	22.6	22.3	61.2	39.8	3.9	5.9

Macro & micronutrient analysis in soil and leaf

The results show that the level of nutrients declined with the rise of salinity of soil in untreated samples with PGPB. On the other hand, PGPB condition enhanced the nutrient levels with the temperature of salinity increasing. Nutrients were observed to be reduced in low salinity of pots. Plant growth was affected negatively when plants were subjected to different treatments of salinity compared to the control group that was not subjected to saline. The culturing of the soil with bacteria led to the highest level of plant height and number of leaves on tomato plants at salinity levels of less than 4.0 dSm^{-1}. An increased count of branches and girth measurements were observed when there was bacterial inoculation of 5.0 dSm^{-1}. The nutrient movement across the soil to leaves was increased during pots that were exposed to the salinity of 4.0 dSm^{-1} and 5.0 dSm^{-1} (Figure 9). The macro and micronutrients composition of the soil is shown in Figure 10, and in the leaves in Figure 7. These findings highlight the potential of specific PGPM strains to enhance crop resilience and productivity in saline soils, supporting sustainable agricultural practices.

Note: Sal-A (*Lycinibacillus spp.*), Sal-B (*Bacillus circulans*), Sal- C (*Pseudomonas plecoglossicida*), Sal-D (*Pseudomonas spp.*) and Sal-E (*Pseudomonas stutzeri*).

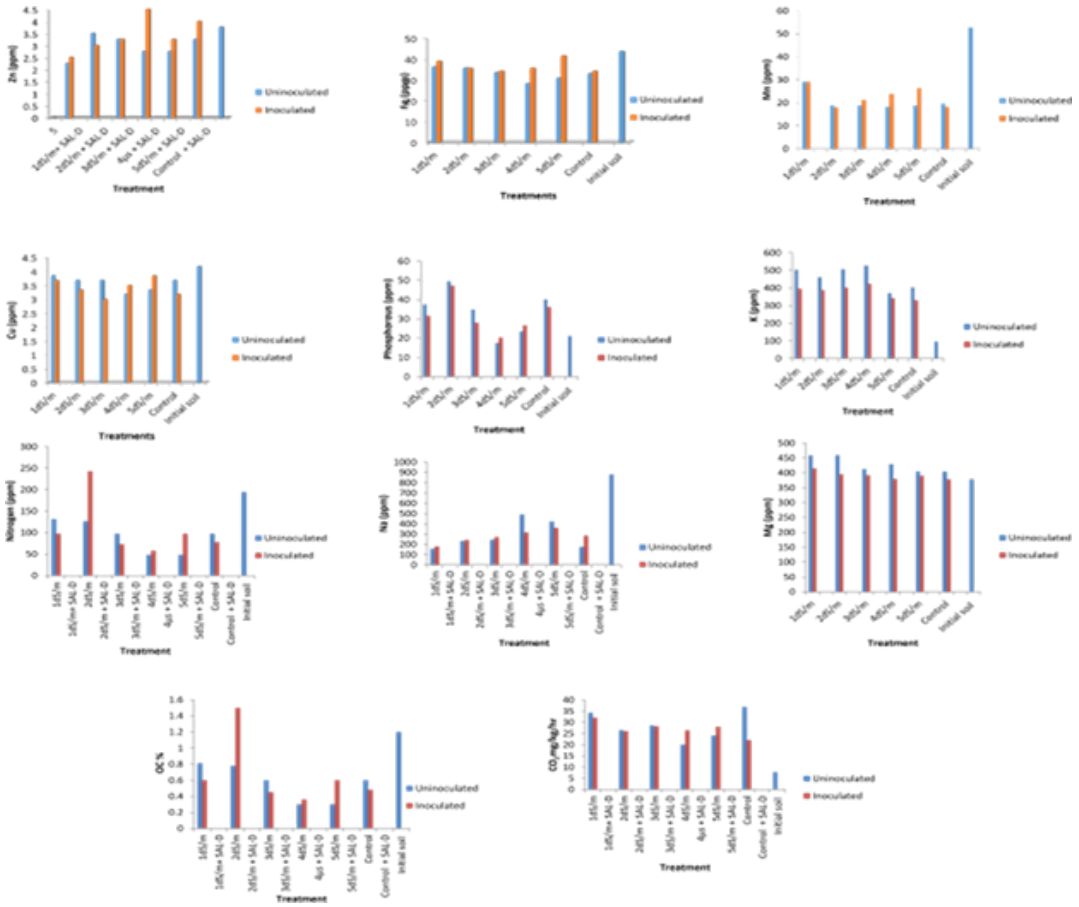

Figure 9. Macro & Micronutrients in soil after saline treatment with PGPB.

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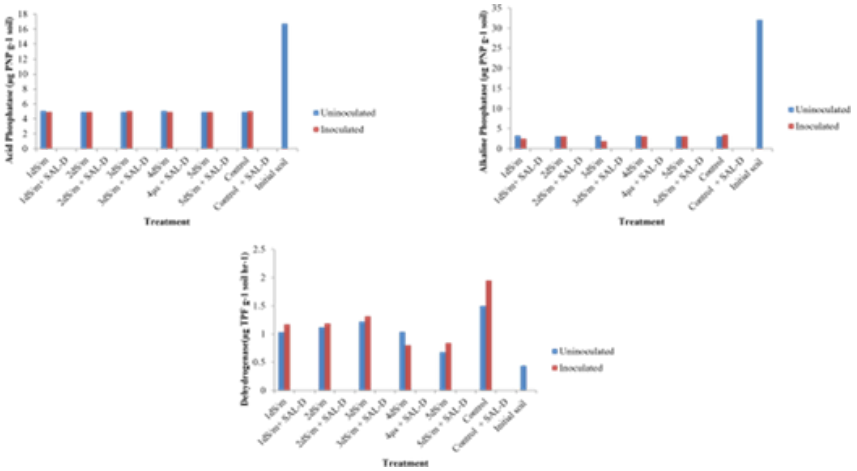

Figure 10. Phosphatase, Alkaline Phosphatase, & Dehydrogenase Activity in Soil with and without Treatment of PGPB.

Discussion

As a research study by Shimon Mayak et al [22]. in 2004 ascertained, rhizobacteria in arid salty soils increase the salt tolerance of tomatoes. With the increase in salt concentration, the ethylene production of tomato seedlings reduced but the same situation was recorded with low saline conditions. Phosphorus uptake was enhanced, which could contribute to the reduction of salt stress, but sodium did not decrease. With the presence of salt, there was an increase in the water productivity because of the activity of the bacterium. The results reveal that this bacterium plays a very crucial role in regulating the salt levels as well as as a photosynthetic promoter.

The other study involved eight bacterial isolates to determine the most useful salt-tolerant bacterium with the production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which measures tomato (*Lycopersicon esculentum Mill. cv. Seeda*) germination and growth in the presence of saline. Bacillus licheniformis B2r was chosen because it uses ACC as the only source of nitrogen during salt stress and exhibited high levels of the ACC deaminase activity at 0.6 M NaCl salinity. When tomato plants were inoculated with the said bacterium under different levels of saline conditions (0, 30, 60, 90 and 120 mM NaCl), it was found that there are increases in the percentage of germination, the germination index, the root length, and the seedling dry weight, especially at 30–90 mM NaCl salinity [23].

A study by Maria F. Valencia-Marin et al. investigated the danger of Fusarium oxysporum F.lycopersici, the cause of Fusarium wilt [24]. The ability of Bacillus velezensis AF12 and Bacillus halotolerans AF23 to survive salinity (up to 1000 mM NaCl) and biocontrol and plant growth-promoting activities were the selection criteria based on the ability to withstand salinity and the presence of biocontrol and plant growth-promoting potential. Both strains synthesized siderophores, indole-3-acetic acid (IAA), proteins and were able to solubilize phosphates under saline stress (100-200 mM NaCl). They had a profound impact on causes of shoot and root weight, chlorophyll content and biomass of soil in non-saline conditions.

Which were beneficial to the growth and decreased infection rates caused by salinity when either strain was applied, or both applied together to high salinity soil (100 mM NaCl). Bacillus AF23 and AF12 have great potential as bioinoculants to enhance tomato growth and control F. oxysporum in saline contaminated soils.

Lucas Arminjon and Francois Lefort carried out an exploration study in 2025 where twenty-three bacterial strains, one yeast and one fungal strain were identified and tested on plant growth hormone production and their effect on tomato growth under saline conditions. The sources of these were Salicornia plants, sandy soils, tomato stems or seeds and tree leaves. The strain of Bacillus sp. 44 had the maximum production of auxin and Bacillus megaterium MJ had the strongest phosphate solubilization parameters. Bacillus sp. strain 44 was observed to produce the highest amount of auxin and Bacillus megaterium MJ to have great phosphate solubilization capacity. In a co-culture assay of salt-enhanced media in vitro, Cryptococcus sp. STSD 4 and Gliomastix murorum (4)10^{-1} (iso1) aided the germination and growth of tomato seedlings. The findings suggested significant positive change in the growth of plants in terms of fresh and dry biomass and the stem length. One of the highest improvements was observed in Gliomastix murorum (4)10^{-1} (iso1), which led to an increase in the fresh biomass by 94% as compared to the negative control subject to saline conditions.

Zhian Dai et al. researched the presence of endophytic bacteria that react to a salinity change to determine the microbial community within the propagules of Kandelia obovate [25]. Delftia tsuruhatensis DYX29 has been reported to grow successfully in high salinity, in the presence of a sodium chloride (NaCl) solution of 5% (w/v). A siderophores-producing DYX29 was able to give a siderophore unit value of 87.6% and has a 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of 29 U L^{-1}. High salinity level promotes its synthesis of intracellular amino acids and auxin. DYX29 inoculation of rice has a great promise to improving salt tolerance, leading to a 32.9% growth in dry weight biomass of rice seedlings and a 23.1% increment in the content of soluble sugars. Moreover, the enzyme activities of the catalase (CAT), and peroxidase (POD) in rice leaves increased by 37.8% and 88.2%, respectively. Also, DYX29 is associated with ionic regulation of both rice roots and leaves. It also enhances the growth-promoting hormones (indole-3-acetic acid (IAA), brassinolide (BL), abscisic acid (ABA), and salicylic acid (SA)) of rice roots by 27.8%, 69.5%, 123.7%, and 28.6%, respectively.

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In tomato breeding, a pot experiment was done on nine halotolerant isolates that were measured on the basis of their plant growth-promoting properties and biomass production in sodic soil with pH 9.23. A group of halotolerant rhizobacteria (PGPR-C3) that includes Lysinibacillus spp. and Bacillus spp. expressed extracellular enzymes, including amylase, protease, cellulase, and lipase, leading to significantly increased vegetative parameters, yield, and lycopene content of tomato hybrid NS585 in salt-stressed sodic soils. The consortium of PGPR-C3 had revealed considerably better plant growth-promoting activities and halo tolerance such as high production of IAA, ACC deaminase, and antioxidative enzyme activity as compared to uninoculated control. The exclusion of Na^+ ions in the rhizosphere was achieved by inoculation with PGPR-C3 that enhanced absorption of K^+. The results of the research findings suggest that the salt stress in tomatoes can be alleviated by inoculation with PGPR-C3, and it helps grow tomatoes prosperously in sodic soils [26].

Nurul Aini et al. conducted research to examine how the frequency of applying a combination of saline-tolerant bacterial isolates and various types of soil ameliorants affected the growth and yield on saline-infected lands [27]. The bacterial consortium comprised of SN13 (*Streptomyces sp.*), SN22 (*Bacillus sp.*), and SN23 (*Corynebacterium sp.*), which were obtained in the soil of saline-prone regions in Lamongan, coastal East Java, Indonesia. The experiment has shown that the application of gypsum and cow manure as soil ameliorants positively influenced the yield and nutrient uptake of tomato, and the use of saline-tolerant bacteria positively influenced nutrient uptake and yield. The mixture of gypsum and cow manure had a negative impact on the yield and nutrient uptake as compared to the lettuce treated with gypsum and cow manure only. Nevertheless, the saline-tolerant bacteria was used four times which significantly improved the height of the plants (by 23.36%), leaf area (by 96.49%), amount of chlorophyll in the plants (by 11.86%), weight of the plants (by 103.59%), weight of the fruit (by 85.51%), amount of nitrogen in plants absorbed (by 135.22%), amount of phosphorus in plants absorbed (by 132.99%). To conclude, salinity stress can be relieved in the tomato plants cultivated in saline conditions by use of the saline-tolerant bacteria four times.

The study by T. Damodaran et al. employed 16 rhizobacteria that were selected by natural selection in saline sodic soils and used to classify them using morphological and biochemical features [28]. The two stress-tolerant rhizobacteria called B-1 and B-3, which were identified as Bacillus pumilus and Bacillus subtilis, had all the qualifications of plant growth-promoting rhizobacteria (PGPR) and tolerance to salinity. These isolates also caused a significantly increased vigor index to tomato seedlings developed under pot culture experiment on salty sodic soils of a pH value of 9.35 and EC of 4.2.

Siderophores are low-weight iron-binding secondary metabolites which are synthesized by a variety of microbial communities to assist in iron-limited conditions. Siderophores produced by endophytic bacteria help in the growth of plants by providing them with iron. This paper was based on the isolation and screening of the siderophore-producing endophytes of the nodules and roots of the Cicer arietinum and Pisum sativum plants. The top ten siderophore producers (those having over 65% siderophore units) were then selected very keenly on the siderophores type they produced. Majority of these isolates generated hydroxamate and carboxylate forms of siderophores, and they were tested on other plant growth-promoting (PGP) attributes in vitro. Many of them generated ammonia and indole-3- acetic acid (IAA). Isolate CPFR10 was positive in all the PGP characteristics such as ammonia, organic acid, production of HCN and IAA. Amplified rDNA Restriction Analysis of these ten isolates showed that there are nine genotypes with a 90% similarity [10].

Conclusions

The article illustrates that halotolerant bacteria, Pseudomonas spp., can support the growth of tomato plants subjected to saline environments in either direct or indirect ways, hence fitting the use of bioinoculants in saline soils. To recap it all, we found that with increase in salinity, there was an improvement in the growth of plants and uptake of nutrients and when salinity was low, there was a decline in plant growth and nutrient uptake. Also, more research is still required to comprehend the mechanism of Pseudomonas spp. in supporting growth of tomato plants when subjected to severe saline stress. More research should also be conducted on the morphological, anatomical and genetic variations of the bacteria, as well as tomato plants. Saline soil also affects the ecosystem stability, quality of groundwater, and biodiversity, besides agriculture. These soils require proper management to have sustainable land use. The process of improving drainage to eliminate salt in the root zone, using soil amendments such as gypsum to substitute sodium ions and the use of salt-tolerant crop varieties can also assist in fighting the effects of salinity in the farms. In addition, salt can be decreased by embracing effective irrigation methods. Saline soils are both a challenge and an opportunity; they limit the usual ways of farming, but they also encourage creativity in the management of soils and the selection of crops.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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