EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

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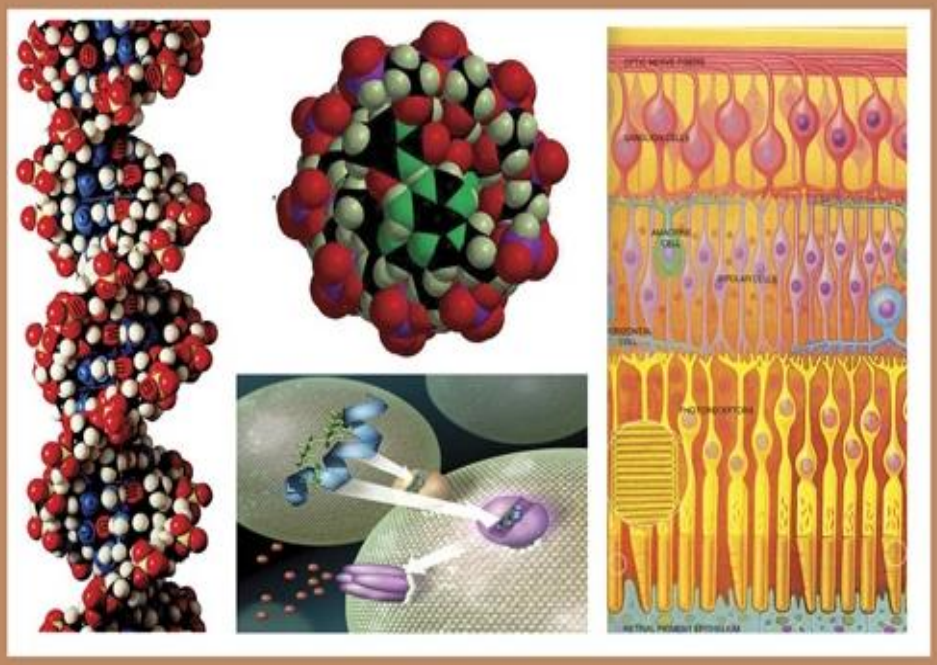

ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 14 No. 2 (2022)

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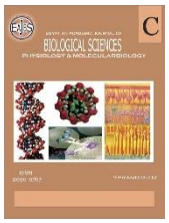
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 14(2) pp267-285 (2022)*
DOI: 10.21608/EAJBSC.2022.270219

Egypt. Acad. J. Biolog. Sci., 14(2):267-285 (2022)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Enhancing Tomato Growth and NaCl Stress Using ACC Deaminase-Producing *Streptomyces* Isolate Alone or In Combination with *Azotobacter vinelandii* MM1

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ARTICLE INFO
Article History
Received:2/10/2022
Accepted:15/11/2022
Available:18/11/2022

Keywords:
Azotobacter, tomato, *Streptomyces*, Indole, salinity, ACC Deaminase.

ABSTRACT

From the rhizosphere of Tomato plants, grown in saline soil in the western region, of Saudi Arabia, twenty-five actinobacterial strains were isolated on starch nitrate agar medium with 5% NaCl. All the isolates were screened on different concentrations of NaCl up 12%. The isolate SA5 was the most resistant isolate, thus, it was selected for detailed studies. The isolate SA5 showed positive results when screened for indole acetic acid production in a broth medium supplemented with 2 mg/ml L-tryptophan. The ability to reduce endogenous levels of ethylene produced by the plant, through the enzyme ACC-deaminase (1-aminocyclopropane-1-carboxylate) was confirmed in the toluenized cells. The isolates SA5 were identified as *Streptomyces* sp. SA5. *Azotobacter vinelandii* can grow in saline and enhance plant growth. Soaking Tomato seeds in *Streptomyces* (ST) or *Azotobacter* both culture filtrates (AZ+ST) increased significantly Tomato seed germination, growth and development. Moreover, soil inoculations with the bacterial cells of AZ, ST, or AZ+ST increased the chlorophyll a, b and carotenoid contents of tomato leaves in normal and under the stress of salinity. There were significant increases in root depth, shoot length and shoot and root dry weights compared to the control under the same level of salinity. The amounts of phosphate, N, Mg, K and proteins present in tomato shoots, grown in normal and saline soil were also increased by soil inoculation. Increasing NaCl concentration increased proline, soluble sugar and esterase contents but soil inoculation decreased the adverse effects of NaCl and decreased them compared to control at the same salinity level. In conclusion, the results of this study indicated that *Streptomyces*, Azotobacter vinelandii or both could be utilized as biofertilizers in saline soils due to the production of plant growth-promoting agents, siderophore, indole acetic acid, and ACC deaminase, phosphate solubilization enzymes and tolerance to NaCl.

INTRODUCTION

Millions of microorganisms were detected in soil and most of these bacteria are significant for plant growth and development in addition these microorganisms provide valuable life to the soil systems. Shahzadi *et al.* (2012) reported a close association between soil microorganisms and plant roots and this association plays a very important direct or indirect role in enhancing plant growth by the production of plant growth regulators (indole acetic acid, gibberellins and cytokines), ACC deaminase enzyme, nitrogenous compounds after nitrogen fixation and many antimicrobial compounds for suppression of different fungal pathogens. In addition to removal of dangerous heavy metals from soil and the environments (Mahmoud *et al.*, 2004, Babaloa, 2010, Aly *et al.*, 2011, Adnan *et al.*, 2018, Backer *et al.*, 2018, Rehman *et al.*, 2019).

It was reported that inoculation of plants with some important bacteria enhanced plant growth, development and production due to increased nutrient availability in soil, enhancing the percentage of seed germination and plant metabolism (Adesemoye and Kloepper, 2009, Abou-Aly *et al.*, 2019). The Gram-negative free-living *Azotobacter vinelandii* had strong beneficial effects and can be used as effective inoculum to improve plant growth due to nitrogen fixation using nitrogenase enzyme which needs molybdenum-iron/sulfido as a cofactor for the previous process and production of many plants' growth promoting substance, especially indolyl acetic acid (Chiu *et al.*, 2001). *Azotobacter* cells were highly isolated from natural habitats and normal soil but their presence decreased in marine soil or waters. Also, species of the genus *Streptomyces* belong to filamentous bacteria and showed different colors on agar media, are abundant in soil and produce many secondary metabolites, like antimicrobial agents and hydrolytic enzymes for agriculture wastes degradation (Aly *et al.*, 2011, 2012, Akladious *et al.*, 2019). In the growth medium, the *Streptomyces* can change calcium phosphate to soluble form and produce IAA but in presence of NaCl, the amount of IAA increased (Sadeghi *et al.*, 2012).

Salinity is the most important environmental stress that affects plant growth by the osmotic effect of salts in the outside solution and it poses a serious problem in food production and plant growth (Munns, 2002; Flowers, 2004, Desoky *et al.*, 2020). Plants grown in saline soil decreased with increasing soil salinity due to induction of nutrient deficiencies, ion toxicity and salt build-up in transpiring leaves, molecular damage and at a high level of salt stress, there is a change in water potential, ion distribution. Finally, the growth of the plant decreased due to disorders in protein synthesis and enzyme activities which led to plant death (Zhu, 2001, Tester and Davenport, 2003, Desoky *et al.*, 2020).

The growth, biomass production and lateral root formation of *Lycopersicon esculentum* (tomato), *Arabidopsis* and *Phaseolus* plants were increased by inoculation of soil with bacteria and this increase was due to phytohormone production (Lopez-Bucio *et al.*, 2007, Ortiz-Castro *et al.*, 2008, García *et al.*, 2017 Gusmiaty *et al.*, 2019). *Azotobacter*, *Arthrobacter*, *Azospirillum* and *Streptomyces* are rhizosphere bacteria that promote plant growth due to nutrient dissolution, nitrogen fixation, and production of antibiotics, plant growth regulators and vitamins and under saline conditions, inoculation of soil with these bacteria increases significantly maize, wheat and tomato growth in addition to total sugars and amino acids, shoot polysaccharides and protein but decreased proline levels (Revillas *et al.*, 2000, Aly *et al.*, 2003, 2012, Chukwuneme *et al.*, 2020). Similarly, soil inoculation with *Streptomyces* increased wheat growth grown in normal and saline soil and there were significant increases in seed germination rate, shoot length and dry weight and concentration of N, P, Fe and Mn plants compared to the control (Aly *et al.*, 2004, Sadeghi *et al.*, 2012, Adnan *et al.*, 2018, Abou-Aly *et al.*, 2019, Akladious *et al.*, 2019). Thus, plants were influenced by salinity but bacterial inoculation resulted in a higher salt-tolerant plant compared to uninoculated plants. The enzymes including glycosyl-hydrolases, phosphatases, esterases and proteases are associated with some biotic and abiotic stresses such as drought and salinity. Esterase and alkali phosphatase are extensively allocated in plants increased as salinity increases (Reyes-Pérez *et al.*, 2019). This study aimed to use the identified bacteria singly or in combination as biofertilizers of tomato plants grown under saline conditions.

MATERIALS AND METHODS

The Used Bacteria:

Cells of the free-living nitrogen fixing bacteria *Azotobacter vinelandii* were kindly provided by Aly *et al.* (2012). The cells were grown on Ashby-Sucrose agar (Agar 1.5%, Sucrose 0.5%, $CaCO_3$ 0.5%, $MgSO_4$ 0.02%, NaCl 0.02%, KH_2PO_4 0.02%, $FeSO_4$

0.0005%). The present investigation was carried out to isolate and identify filamentous bacteria from saline soil samples collected from the rhizosphere region. Randomly, ten soil samples of 100 g each and 10 cm depth were collected from the normal and saline soils from the Western region, Saudi Arabia, dried and sieved. Actinomycetes isolation was carried out on plates of starch nitrate agar with 5% NaCl (Shirling and Gotleib, 1966), incubated for 4 days at 30°C. All isolates were screened on the previous medium with different concentrations of NaCl.

Identification of the Isolates:

The actinomycete isolate SA5 was characterized using many morphological, physiological and biochemical tests after incubation at 30°C for 7 days. The aerial and substrate mycelia and spore chain type and morphology of the selected isolate were examined under light and electron microscopes. It was biochemically characterized by Gram stain, starch hydrolysis, oxidase test, carbohydrate fermentation and color of diffusible pigment (Hoischen *et al.*, 1997, Chukwuneme *et al.*, 2020).

Quantification of Plant Growth Regulators and Phosphate Solubilization:

The isolates SA5 and *A. vinelandii* were screened for IAA production in a medium supplemented with 2 mg/ml of L-tryptophan at a pH of 7.0. After growth, the filtered sterile filtrate was used for IAA extraction with ethyl acetate (Ahmad *et al.* 2005) and the quantity was recorded by measuring the absorbance at 530 nm according to Bano and Musarrat (2003) and the quantity of IAA produced by each bacterium was estimated from a standard curve of IAA. Similarly, the amount of GA3 produced by the two tested isolates was estimated by the method of Holbrook *et al.*, (1961) and a standard curve prepared using gibberellic acid to calculate the GA3 quantities (Ashkan *et al.*, 2021). The bacterial isolates were screened for phosphate solubilization using Pikovskaya's medium which contains tricalcium phosphate and the mean diameter of the clear zone (mm) around the tested bacterial colony was measured (Lavakush and Verma, 2012).

Enzyme Assay of ACC Deaminase:

The activity of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase was measured with some modification by detecting the amount of α-ketobutyrate (absorbance at 540 nm) produced by the action of ACC deaminase on ACC (Louden *et al.*, 2011).269

1-aminocyclopropane-1-carboxylic acid (ACC) $\xrightarrow{\text{ACC deaminase}}$ α-ketobutyrate

The Effect of The Bacterial Culture Filtrates on Tomato Seed Germination:

The filtrates of the two bacterial isolates were filter sterilized (Millipore filter, 0.45 mm) and the sterile filtrate was used for soaking the tomato seeds (*Lycopersicon esculentum* Mill. cv. Harzfeuer) were surface-sterilized by soaking in a 10% NaOCl for 3 min, followed by rinsing in sterile distilled water. In sterile plates, the surface sterilized seeds were separately soaked in sterile culture filtrate of *Azotobacter*, *Streptomyces*, or their mixture (1:1, V/V) or distilled water and all plats were incubated in the dark until the seedlings emerged (10 days) and germination percentage (%) and index were determined as described by Dhamangaonkar and Pragati (2009).

$$\text{Germination Index} = \frac{\text{Sum of germinated seed for a certain period}}{\text{Total days} \times \text{Total seeds}}$$

Preparation of Inoculum:

Azotobacter vinelandii and *Streptomyces* sp. SA5 were grown on Ashby-Sucrose broth and starch nitrate broth media, respectively for 5 days at 80 rpm and 30◦C. The growth of the two isolated bacteria was measured by determining the optical density at 550 nm. The bacterial cells were collected

by centrifugation at 5000 rpm for 10 min and each bacterial inoculum was prepared in a sterile saline solution to give a bacterial suspension of about $8xl0^5$ CFU/ml.

Plant Growth Studies:

The greenhouse experiment was carried out during the period 2019-2020 at 20-22°C. The sterile Tomato seeds were germinated for a week and 5 seedlings were taken to each plastic pot (30x20 cm), filled with 2 kg of steam sterilize sandy soil. The pots were divided into 4 groups (G), G1: control plants (without inoculation and only water was added), G2: the plants inoculated with *Azotobacter* (20 ml of cell suspension of $8x10^5$ CFU/ml), G3: plants inoculated with *Streptomyces* (20 ml of cell suspension of $8x10^5$ CFU/ml), and G4: plants inoculated with both bacteria (40 ml of a mixture of cell suspensions of *Azotobacter* and *Streptomyces*, $8x10^5$ CFU/ml, V/V). After a week, irrigation was applied with 200 ml two times/week of Hoagland nutrient solution, composed of these materials in mM: KH_2PO_4, 1.0; KNO_3, 5; $Ca(NO_3)_2$, 5, $(NH4)Mo_7O_{24}$, 0.0002, $MgSO_4$, 2, Fe/ EDTA, 0.1, H_3BO_3, 0.005, $MnCl_2$, 0.010, $ZnSO_4$, 0.008, $CuSO_4$,0.004 (Hoagland and Arnon, 1950). After 7 days of growth, three levels of NaCl were added to the soil in the nutrient solution and control plants received only distilled water. Sterile distilled water (200 ml/week) can be used to wash each pot and after 60 days, the plants were collected, and the root depth, shoot length and dry weights of shoot and root (dried at 60◦C for three days) were recorded.

Plant Analysis:

The plants were collected, dried grinded and analyzed for protein, proline, soluble sugar, phosphorus and nitrogen concentrations and were estimated according to protocols methods described in Allen *et al.* (1974). After acid digestion, mineral contents including Na^+, K^+, Ca^{++} and Mg^{++}) were determined using Atomic Absorption Flame Photometer (Shimadzu, Model AA-640-12). Chlorophylls and Carotenoids were measured in tomato leaves extracted with 95% ethyl alcohol using UV-VIS Spectroscopy (Hiscox and Israelstam, 1979). Chlorophylls and carotenoid concentrations were calculated using the equations cited by Lichtenthaler (1987).

Esterase Assay:

Esterase assay was carried out using the method described by Junge and Klees (1984). In liquid N_2, plant samples of 1 g of either leaf and roots have homogenized a mixture of 1:10 (w/v) of 0.1M potassium acetate dissolved in 0.1M phosphate buffer (pH 7.0). The extracts were centrifuged at 10,000 g for 5 min at 4◦C and the homogenate was used as the crude enzyme and the enzyme activity was expressed compared to the control.

$$\text{Activity} = \frac{\text{Absorbance x } 0.28\ x\ 100}{\text{time (min)xWt (g)}}$$

The Activity of Peroxidase:

In the shoot sample, peroxidase-specific activity was determined by the method described by Pütter and Becker (1983). From the tested shoot sample, 10 g were weighted, cooled at −80◦C and lyophilized for 24 hrs. To 3 ml of potassium phosphate buffer, 30 mg of each lyophilized sample was added and homogenized and the mixture was centrifuged under cooling at 5000 rpm at 4◦C for 10 min. The cooled supernatant was collected and the absorbance was recorded at 436 nm by UV-VIS spectrophotometer (Double Beam, Indiamart).

Statistical Analysis:

Data were statistically analyzed by *t*-Test to determine the differences between control and treated samples using SPSS software 16 and a Two-way ANOVA test was carried out to detect the effect of different factors, $P<0.05$ are considered significant.

RESULTS

From ten soil samples, 25 bacterial isolates were obtained from soil samples on starch nitrate agar with 5% NaCl and the previous isolates were screened on the previous medium with different concentrations of NaCl, 7 isolates were obtained. All isolates were screened on the previous medium with different concentrations of NaCl and the isolate SA5

was the most resistant isolate to NaCl. The characters of the 7 isolates, shape, color, Gram stain and growth on different concentration of NaCl was summarized in Table 1. All the 7 isolates were screened in liquid medium for IAA production and the detected quantities ranged from 1.21 to 6.6 mg/l and the isolate SA5 was the most active isolate (Table 1), thus it SA5 was selected, characterized and identified by morphological, physiological, biochemical properties. The Gram-positive isolate SA5 has a substrate and aerial mycelia bearing a straight chain of conidia (Figure 1). No zoospore, sporangium, sclerichia, or fragment hyphae were noticed. Isolate SA5 was resistant to some antibiotics, grew aerobically and was catalase and oxidase positive and the physiological characteristics were represented in Table 2. According to the studied characters, the isolate SA5 was identified as *Streptomyces* sp. and identification was confirmed as *Streptomyces* sp. SA5 using molecular methods. The phylogenic tree of isolate SA5 and the most related isolates were found in Figure 2. Table 3 showed phosphate solubilization and siderophore, indole acetic acid, gibberellins and ACC deaminase productions by the isolate Streptomyces sp. SA5 and *Azotobacter* MM1.

Table 1. The growth of the obtained actinomycete isolates from the soil in a medium containing different concentrations of NaCl

Isolate	Source	shape	Color	Gram stain	Concentration of IAA (mg/l)	Growth on NaCl		
						5%	10%	12%
SA1	S. soil	Filamentous	White	Gm+	3.19	+++	++	+
SA2	Soil	Filamentous	Pink	Gm+	2.19	+++	+	-
SA 3	Soil	Filamentous	White	Gm+	1.45	+++	+	-
SA 4	Soil	Filamentous	Gray	Gm+	0.29	+++	+	-
SA 5	S. soil	Filamentous	Yellow	Gm+	6.66	+++	++	++
SA 6	Soil	Filamentous	Gray	Gm+	4.09	+++	+	-
SA 7	Soil	Filamentous	Gray	Gm+	1.22	+++	+	-

S. soil: Saline soil, Gm: Gram positive, +++: high growth, ++; Moderate growth, +: low growth, -: No growth.

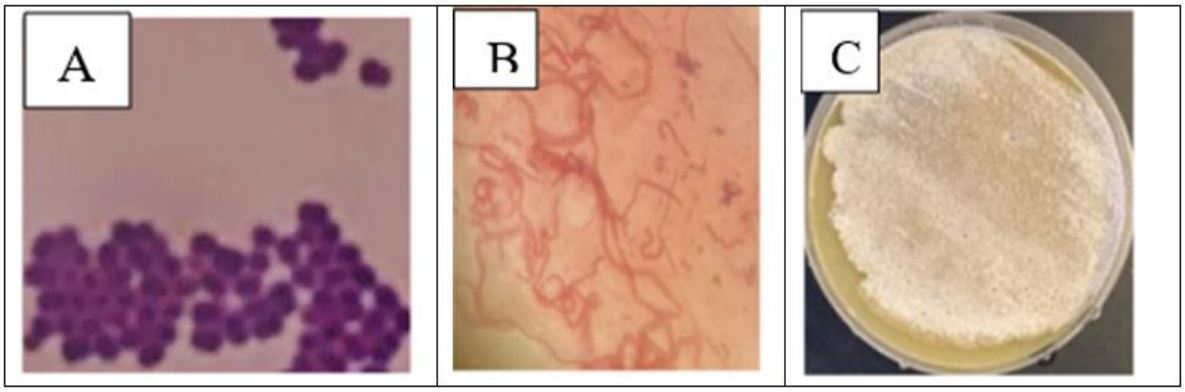

Fig. 1. A: The cell of the selected *Azotobacter* stained with crystal violet, **B**: The isolate SA5 under a light microscope, **C**: The isolate SA5 on starch nitrate agar after 7 days of growth.

Table 2: Physiological properties of the isolate SA5 obtained from the rhizosphere of a tomato plant.

Characteristic	Result	Characteristic	Result
Aerial and substrate mycelia	**Developed**	**Gram stain**	**Gm+**
Decomposition of xanthine, casein, chitin, gelatin, pectin, urea	+	Utilization of valine, phenylalanine, peptone, yeast extract	+
Tolerance to NaCl	5-12%	H_2S production	+
Growth temperature	10 - 45°C	pH range	6-9
Melanin production	+	Nitrate reduction	+
Resistance to Penicillin Cephalosporinem Gentamycin	R	Resistance to Kanamycin, Rifampin Tetracyclines,	S

+: Positive results, Gm+: Gram-positive, R: Resistant, S: Sensitive.

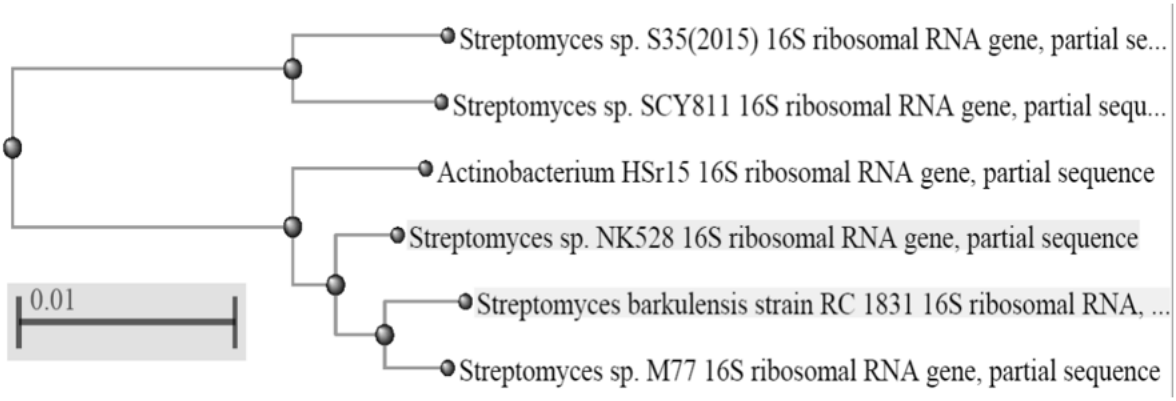

Fig. 2: The phylogenic tree of the isolate SA5 and the most related isolates

Table 3. Phosphate solubilization, siderophore, indole acetic acid (IAA), gibberellins (GA3) and ACC deaminase productions by the bacterial isolates *Azotobacter* and *Streptomyces*.

Bacterial isolates	Phosphate solubilization (mm)	Siderophore production (mm)	Concentration of IAA (mg/l)	Concentration of GA3 (mg/ml)	ACC deaminase activity (mmol)
Azotobacter	4.4±0.21	8.0±0.79	0. 83±0.36*	0.109±0.45	1.01±0.21
Streptomyces	6.0±0.91*	11.4±2.09*	0. 44±0.69	0.104±0.15	1.15±0.01

*Significant results at P<0.05

Soaking sterile tomato seeds in culture filtrates of *Azotobacter* (AZ) or *Streptomyces* (ST) or their mixture (AZ+ST) enhanced significantly the percentage of seed germination and germination index (Table 4). The effect of soil inoculation with the tested bacteria at 0.0, 20, 40 and 60 mM NaCl on the leave contents of chlorophyll a, b, and carotenoids in addition to soluble sugar of shoot was summarized in Table 5. Maximum contents of chlorophyll a, b, and carotenoids were recorded in control plants (0.0 NaCl), inculcation with AZ+ST. At all saline concentrations, pigment contents were decreased with increasing NaCl concentrations while inoculation of soil with AZ, ST or AZ+ST enhanced significantly pigment contents under normal and saline conditions. In contrast, soluble sugars of the shoot system sharply increased with increasing NaCl concentrations while the presence of the used bacterial inoculants treat the bad effects of salinity, thus, the increase was gradual. It is also noted that in control plants, inoculation with *Azotobacter* (AZ) or *Streptomyces* (ST) or their mixture (AZ+ST) significantly improved plant growth, root depth, shoot height, roots and shoots fresh and

dry weights significantly compared to control under normal and saline conditions while under saline conditions there is a clear decrease in root and shoot growth and dry weights particularly at 20 and 40 mM (Table 6). Inoculation of tomato plants with *Azotobacter* (AZ) or *Streptomyces* (ST) or their mixture (AZ+ST) increased phosphate, K^+, Mg^{++}, nitrogen and protein contents of the shoot system while increasing NaCl concentration decrease them and significantly increased both Na^+ ions and proline contents of a shoot system (Table 7). Maximum phosphate and nitrogen contents were found in plants inoculated with both AZ+ST at 20 and 40 and 60 mM but maximum Na content was recorded at 80 mM NaCl. Inoculation of plants with *Azotobacter* (AZ) or *Streptomyces* (ST) or their mixture (AZ+ST) decreased Na^+ ions and proline content in plants grown under different concentrations of NaCl, thus soil inoculation decreased the unfavorable property of NaCl and lowered proline levels in relation to control at the same salinity concentration of NaCl. Figures 3 and 4 showed the relative activity of peroxidase compared to control in root and shoot samples of tomato grown under saline conditions and inoculated with *Azotobacter*, *Streptomyces*, or both. Also, peroxidase activity (µmg of protein/min) was detected in the shoot of tomatoes treated with *Azotobacter*, *Streptomyces*, or their combination and grown under different concentrations of NaCl (Figure 5). Two-way ANOVA test was used to compare the different parameters assayed for tomato plants grown under a combination of inoculation and different concentrations of NaCl (Table 8).

Table 4. Effect of bacterial culture filtrate on a percentage of tomato seed germination

Culture filtrate	% Of germination	Germination index
Control (sterile culture medium)	80.11	0.160
Azotobacter (AZ)	84.98*	0.170
Streptomyces (ST)	82.95	0.164
AZ+ST (V/V)	87.21*	0.173

*Significant results at P<0.05 compared to control.

Table 5. Effect of different concentrations of NaCl on pigment continent of leaves and soluble sugars of tomato shoot grown in sterile soil and inoculated with *Azotobacter*, *Streptomyces* sp SA5, or both.

NaCl level mM	Treatments	Pigment leaves content (mg/g FW)				Soluble sugar (µm/g)
		Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoids	
0.0	control	4.33	1.60	6.1	0.33	18.4
	AZ	5.14*	1.92*	7.06	0.40	!8.6
	ST	5.05*	1.84*	6.89	0.39	18.0
	AZ+ST	5.43*	1.86*	7.29	0.44	18.3
20	control	3.61	1.39	7.00	0.37	33.0
	AZ	3.89	1.30	*5.19	0.40	33.0*
	ST	3.64	1.39	5.03 *	0.40	30.3*
	AZ+ST	4.44*	1.70*	6.14*	0.49*	30.9*
40	control	3.34	1.39	4.82	0.30	38.0*
	AZ	4.04	1.65*	5.69	0.39	34 .5*
	ST	3.66	1.66*	5.32*	0.38	38.3*
	AZ+ST	4.06	1.80 *	5.86*	0.49	30,1*

AZ: Plants treated with Azotobacter, ST: Plants treated with *Streptomyces*, AZ+ST: Plants treated with Azotobacter and *Streptomyces*, * significant results at $p < 0.05$

Table 6. Growth of tomato plants in sterile soil under three levels of salinity and inoculation with *Azotobacter*, *Streptomyces*, or both isolates.

NaCl Concentration (mM)	Inoculum	Root depth (cm)	Shoot length (cm)	Root dry weight g/plant	Shoot dry weight g/plant
0.0	C	12.4	40.5	0.29	2.3
	AZ	14.2	46.0*	0.33	2.3*
	ST	16.2*	49.5 *	0.33	2.4*
	AZ+ST	20.2*	43.5*	0.36*	3.6*
20	C	12.5	36.4	0.29	2.1
	AZ	15.7	38.4	0.30	2.6
	ST	15.6	38.6	0.30	2.6*
	AZ+ST	19.4	40.4*	0.37*	3.4*
40	C	15.3	35.5	0.23	2.0
	AZ	16.0	35.1	0.22	2.3*
	ST	18.4*	35.2	0.23	2.4*
	AZ+ST	18.7*	30.4*	0.36*	3.0*
60	C	13.0	30.7	0.19	1.3
	AZ	15.1	32.7	0.23	1.4*
	ST	15.4	34.7*	0.23	1.5*
	AZ+ST	16.4	35.6*	0.35*	2.2*

Table 7. Effect of tomato inoculation with *Azotobacter*, *Streptomyces*, or both on shoot mineral, protein and proline contents of plants grown in sterile soil under saline. conditions.

NaCl Conc. (mM)	Inoculum type	P mg/g	Na mg/g	K mg/g	Ca mg/g	Mg mg/g	N mg/g	Proline µg/g	Protein mg/g
0.0	C	12.1	4.5	16.4	5.0	4.4	20.4	19.4	20.9
	AZ	12.1	4.6	18.3*	5.1	4.6	28.6*	19.6	29.4*
	ST	12.0	4.6	17.6*	5.4*	4.6	20.0	19.7	20.2
	AZ+ST	15.0*	4.6	20.0*	5.1	5.9*	33.9*	20.0	30.2*
20	C	12.4	6.0	15.4	4.0	4.0	20.3	33.0	23.3
	AZ	14.3*	5.1*	16.8*	4.9*	4.6*	27.7*	33.0	38.5*
	ST	14.5*	5.9*	15.8	4.8*	4.4*	25.7*	30.8*	22.2
	AZ+ST	15.7*	4.3*	19.6*	4.9*	4.4*	34.5*	28.0*	32.3*
40	C	10.9	6.9	15.0	4.1	3.5	18.1	44.6	24.7
	AZ	12.9*	6.0*	15.4*	4.0	4.0*	25.2*	38*	29.7*
	ST	12.3*	5.0*	15.8*	4.2	4.1*	20.2*	36*	24.9
	AZ+ST	13.9*	5.0*	18.8*	4.6*	4.1*	29.5*	34*	29.1*
60	C	10.7	7.0	10.1	3.8	3.5	15.7	70	24.8
	AZ	10.9	6.2*	14.3*	4.0	3.6	19.6*	49*	29.9*
	ST	11.9*	6.0*	14.9*	4.0	3.8*	17.4*	44*	26.7*
	AZ+ST	12.0*	6.0*	14.8*	4.4*	4.0*	22.4*	40*	29.3*

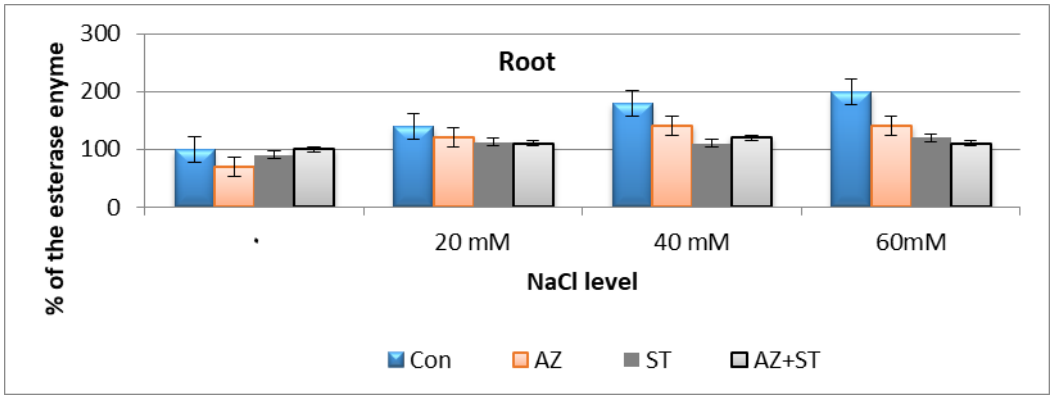

Fig. 3. The percentage of esterase activity of tomato roots, inoculated with *Azotobacter*, *Streptomyces*, or both and grown under saline conditions.

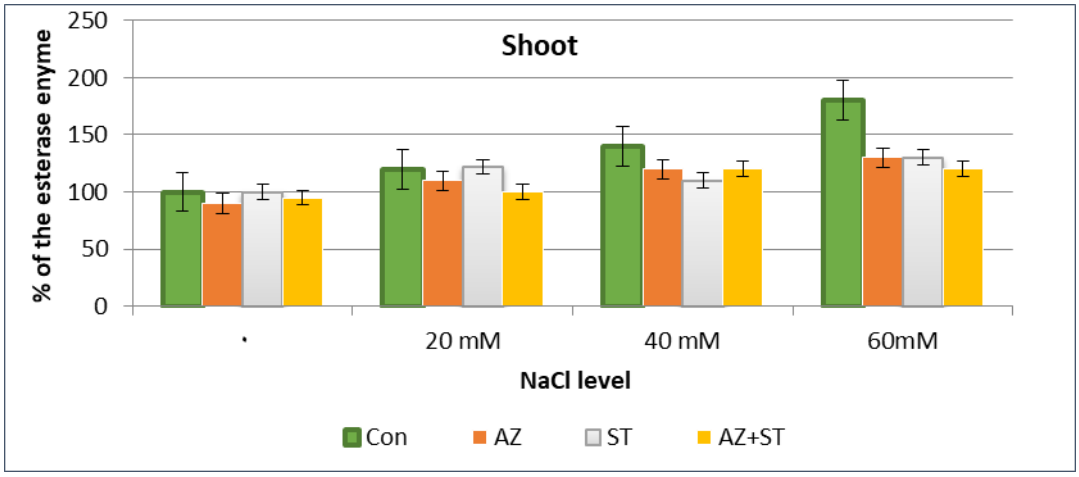

Fig. 4. The percentage of esterase activity in tomato shoots, inoculated with *Azotobacter*, *Streptomyces*, or both and grown under saline conditions.

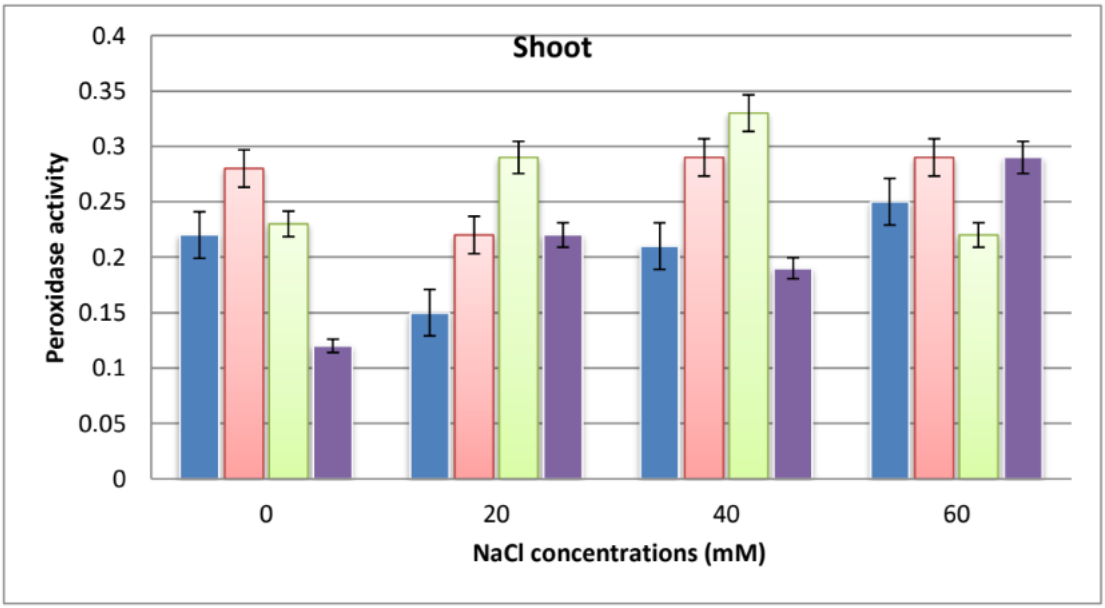

Fig. 5. Peroxidase activity (µmg of Protein/min) was detected in the shoot of tomatoes treated with *Azotobacter*, *Streptomyces*, or their combination and grown under different concentrations of NaCl.

Table 8. The two-way ANOVA table compared the assayed different factors of tomato plants grown under the effect of inoculation and different concentrations of NaCl.

Factors	Df (n-1)	Shoot length		Root depth		Cha a+b		Soluble sugar		Soluble protein		Proline		Peroxidase		Esterase	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Salinity	3	318	L	112	L	199	L	443	L	332	L	311	L	210	L	219	L
Inoculation	3	239	L	229	L	211	L	339	L	394	M	218	M	208	M	210	M
Salinity* Inoculation	15	1229	L	2219	L	632	L	988	L	2334	L	3199	L	2102	L	2109	M

L: $p< 0.05$, M: $p> 0.05$

DISCUSSION

Isolate SA5 was the most resistant isolate to NaCl (12%) and it was identified based on different characteristics and molecular methods (Williams *et al.*, 1994, Santos-Beneit *et al.*, 2022). The phylogenic tree reported that this isolate belongs to the genus *Streptomyces* and is identified as *Streptomyces* sp. SA5. The used nitrogen-fixing soil bacterium *Azotobacter vinelandii* is a rod-shaped Gram-negative motile bacterium that grows at a temperature range of 20 -30°C and produced indole, citrate, catalase and oxidase (Aasfar *et al.*, 2021). In this study, IAA, GA3 and ACC deamiase were detected in the culture filtrate of *Azotobacter vinelandii* and *Streptomyces* sp. SA5 while *A vinelandii* was more active in IAA production compared to *Streptomyces* sp. SA5. Production of IAA in growth media by true bacteria and actinomycetes was confirmed (El-Tarabily and Sivasithamparamb, 2006, Tsavkelova *et al.*, 2006, Patil, 2011). Higher quantities of IAA from actinomycete isolates were recorded by Gangwar *et al*. (2012). The most common natural auxin, indole acetic acid is a product by bacteria during the metabolism of the amino acid L-tryptophan and more than 70% of saline soil bacteria have an excellent ability to form IAA from root exudates (Bhavdish *et al.*, 2003). Additionally, a number of *Streptomyces* species like *S. rochei*, *S. livaceoviridis* and *S. rimosus* obtained from the rhizosphere of tomato were high producers of IAA and enhance the growth of the plant (El-Tarabily, 2008, Aly *et al.*, 2012). The results of this study revealed that *Streptomyces* and *Azotobacter* secrete ACC deaminase enzyme (EC 4.1.99.4) which facilitates plant growth and development by decreasing plant ethylene levels at a variety of abiotic stress such as drought, salinity, temperature water logging, heavy metals, and pH stress (Sumreen *et al.*, 2020). The type of interaction between bacteria and plants seems to be important in increasing the growth and germination of seeds (Phuakjaiphaeo and Kunasakdakul, 2015, Maggini *et al.*, 2017). Moreover, bacteria are well known for their production of enzymes with a significant role in plant growth promotion during biotic and abiotic stresses (Daguerre *et al.*, 2016, Suman *et al.*, 2016). A recent study by Nxumalo *et al.* (2020) showed that 13 isolates of bacteria have the ability to produce siderophores (Musa *et al.*, 2020) while Singh *et al.* (2022) isolated eight bacterial strains which were excellent producers of IAA, siderophore production, and phosphate dissolving bacteria during plant growth.

Promotion of plant growth occurred when the plant is supplied with a compound that is synthesized by the bacteria to facilitate nutrients uptake by the plant from the soil, or through phytohormone and siderophore synthesis, nitrogen fixation, solubilization of minerals to make them available for the plant uptake such as phosphate (Alori *et al.*, 2017; Eid *et al.*, 2021). Soil bacteria produce phytohormones to enhance plant growth and change the morphology and structure of the root (Fadiji and Babalola, 2020). These bacteria are considered eco-friendly biofertilizers, cheap and they provide a renewable source of nutrients to plants which reduce the dependence on chemical fertilizers and play a significant role in increasing nutrient availability which enhances plant growth (Pal

et al., 2015). Ammonia, IAA, cytokinins, and gibberellic acids are produced by soil bacteria to influence plant development through a variety of cellular mechanisms like plant cell division, differentiation, extension, affects photosynthesis process, stimulates seed germination and pigment formation in addition to root and shoot growth and development (Labeeuw *et al.*, 2016). Siderophores produced by soil bacteria are capable of chelating iron to make it available for plants and are of crucial importance for zinc and ferric transport from soils to plants (Kumar *et al.*, 2016). These bacteria can also decompose complex organic compounds to produce strong surface bioactive bio-surfactants with varying chemical properties (Fadiji and Babalola, 2020).

In this study, the filtrates of *Streptomyces* sp. SA5 or *A. vinelandii* or their mixture enhanced seed germination percentage which may be due to the presence of IAA, GA3, vitamins, amino acids, or secondary metabolites. Similarly, the filtrates of *A. vinelandii* and *A. beijerinckii* were rich in IAA and gibberellins and cytokinin-like substances (Brown, 1974, Ahmem *et al.*, 2005, Aly *et al.*, 2012, Ashkan *et al.*, 2020, 2021). The results of this study also reported that the presence of soil microbiota normally or due to inoculation of soil with cells of AZ, ST, or their mixture increased root and shoot growth, straw, pigment, mineral and protein contents and seed yield. These increases may due to nitrogen fixation, ACC deaminase enzyme, auxins and unidentified compounds production. There is a significant increase in growth, indole-3-acetic acid, mineral contents like P, Mg and N and total soluble sugars of wheat plant inoculated with *A. chroococcum, Azospirillum brasilense* and *S. mutabilis* due to the release of IAA and/or nitrogen fixation in soil which significantly enhance roots and leaves dry weights of the wheat plant (El-Shanshoury, 1995, Ahmed *et al.*, 2004, Arzanesh *et al.*, 2014, Cohen *et al.*, 2020). Moreover, Aly et al (2003, 2004) studied the beneficial effect of *Streptomyces* cells on *Zea mays* plants grown under different levels of salinity and attributed this benefit to the secretion of plant growth regulators and some enzymes while wheat and soybean growth were also enhanced after soil inoculation (El-Shanshoury, 1989, 1991, Araujo *et al.*, 2005). Many biologically active compounds from the species of the genus *Streptomyces* are detected to be produced commercially for agricultural uses (Ilic *et al.* 2007, Frankenberger, 1995). Alizadeh et al (2012) in a review reported that in China bacterial inoculation increased the yields of many plants like wheat, rice, maize, beans, sorghum, potato, peanut and some vegetables.

As a response to different stresses at the cellular level, there is an increase in reactive oxygen species due to abiotic and biotic stress leading to reactive oxidative stress which is toxic molecules and signals that control a variety of metabolic pathways and responses (Mhamdi and Van Breusegem, 2018, Kerchev *et al.*, 2020). The major biotic stresses that adversely affect soil are salinity, drought and the presence of heavy metals which also inhibit almost the cell metabolic activities and plant growth (Roychoudury *et al.*, 2008). Our results indicated that salinity mainly decreased plant growth and chlorophyll contents which were clear at high concentrations of NaCl where the cell content of Na^+ ions increased while the cell levels of K+ and Ca^{2+} ions have decreased. The plant responded to the increase of NaCl by increasing proline, soluble sugar and soluble proteins. It was reported that salinity conditions affect the cell membrane which increases important ion leakage leading to ion imbalance and enhanced lipid peroxidation and production of oxidant agents. The presence of growth-promoting bacteria produces or enhanced the plant to produce osmoprotectants agents like soluble sugar, alcohols sugars and amino acids (glycine and betaine, proline and basic amines) which under stress conditions, protect the cell membrane functions and structure (Hasegawa *et al.*, 2000, Summart *et al.*, 2010). Increase proline accumulation in rice plants during stress may have a vital role in protecting the plant cells and reducing the negative effects of salinity by acting as a nitrogen reservoir, a

compatible solute and protectant agent during osmotic stress (Sairam and Tygai, 2004).

The results of this study also confirmed that under saline conditions, inoculation of soil with AZ, ST, or both enhanced plant growth. Several studies reported the successful use of some plant-associated bacteria to raise the resistance of plants to salinity and remove the bad things of salinity (Alizadeh *et al.*, 2012). In saline environment, the inoculation with either *Azotobacter* or *Azospirillum* enhances nitrogen content and produces active metabolites which can osmo-regulate the saline conditions. Salt-tolerant bacteria from wheat rhizosphere can produce IAA, HCN, lipase, or protease which promote root, shoot and leaves dry weights and wheat growth under salt stress (Bacilio *et al.*, 2004, Ashraf and Harris, 2004, Egamberdieva *et al.*, 2008). Also, Gravel *et al.* (2007) used *P. putida* to increase tomato growth under saline conditions and they ascribed this increase to the production of IAA while Woitke *et al.* (2004) found that Bacillus *subtilis* tomato seed inoculation have no effect on tomato yield grown in a saline condition where in high salinity treatment the yield significantly decreased. Similar to our results, Ashry *et al.* (2022) used drought-resistant bacteria, *Bacillus cereus* and *Bacillus albus* to increase plant health and productivity and resistance to drought. They added that these bacteria under the harsh conditions produced plant growth-promoting agents like proline, siderophore, salicylic and gibberellic acids, exopolysaccharides, plant hormones, antioxidants and some enzymes which may affect seed germination, protected the plant from harmful things and the best results were obtained in case of their combination. In recent times, eco-friendly microorganisms are used as bio-stimulating agents to enhance plant growth and yield, defenses against pathogens and fruit quality, or/and reduce biotic stress, thus maintaining the sustainability of soil and environment (Chiaiese *et al.*, 2018, Shukla *et al.*, 2019). The application of biostimulants affects metabolic processes, improves ion transport, and modifies plant hormones. Stress tolerance is perhaps the most significant benefit of bio-stimulants (Backer *et al.*, 2018, Paul *et al.*, 2019, Polo and Mata, 2018). No significant differences were recorded for peroxidase while clear significant differences were recorded for esterase of tomato root and soot. Similar to these results, Reyes-Pérez *et al.* (2019) reported that in shoots of *Solanum*, NaCl increased significantly some enzymatic activity like esterase and alkaline phosphatase but the increase in peroxidase was none significant. Bacterial cultures or their products can be used as bio-fertilizers, bio-pesticides and in the remediation process due to the production of plant hormones, solubilization insoluble minerals, and biocontrol agents for the various pathogens. They can be used to enhance the stress tolerance of the plants by enhancing the root length and growth, availability of water and production of promoter agents for plant growth (Kang *et al.*, 2014, Cohen *et al.*, 2015, Enebe and Babalola, 2018). Finally, it was concluded that the two bacteria *Azotobacter*, *Streptomyces* were isolated from saline soil and they have the potential to be utilized as biofertilizers in normal and saline soils due to high production of plant growth regulators, ACC deaminase, solubilization of phosphate, N_2 fixation and antimicrobial agents.

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ARABIC SUMMARY

تحسين نمو الطماطم وتقليل إجهاد كلوريد الصوديوم باستخدام بكتريا استربتوميسس المنتجه لانزيم ACC deaminaseمنفردة او بالاشتراك مع بكتريا الازتوباكتر فينلانديا

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تم عزل خمسة وعشرين سلالة أكتينوبكتيرية على وسط أجار نترات النشا المحتوي علي 5٪ كلوريد الصوديوم من تربه موجوده حول جذور نباتات الطماطم المزروعة في تربة مالحة في المنطقة الغربية بالمملكة العربية السعودية، تم فحص نمو جميع العزلات علي تراكيز مختلفة من كلوريد الصوديوم حتي 12٪. كانت العزلة SA5 الأكثر مقاومة لذلك تم اختيارها لدراسات تفصيلية .أظهرت العزلة إنتاج عالي من إندول حمض الأسيتيك في وسط الغذائي المحتوي على 2 مجم / مل تريبتوفان. كما كانت هذه السلاله منتجه لإنزيم aminocyclopropane-1-carboxylate deaminase (ACC) الذي يعمل على تقليل المستويات العاليه من الإيثيلين التي ينتجها النبات. تم التعرف على العزله SA5 على أنها تنتمر الي جنس استربتوميسس*Streptomyces*. كما ان بكتريا الازتوباكتر فينلانديا *Azotobacter vinelandii* معروفه بمقاومتها للملوحه وتحسين نمو النبات. أدى نقع بذور الطماطم في راشح *Streptomyces* (ST) او (AZ) *Azotobacter* إلى زيادة إنبات بذور الطماطم ونموها وتطورها بشكل ملحوظ. علاوة على ذلك، أدى تلقيح التربة بالخلايا البكتيرية لـ AZ أو ST أو AZ + ST إلى زيادة محتوى الكلوروفيل a و b والكاروتينات في أوراق الطماطم في التربه العاديه اوتحت ضغط الملوحة. كانت هناك زيادات معنوية في طول الجذر، طول الساق، الأوزان الجافة للنبات والجذر مقارنة مع االنباتات تحت نفس مستوى الملوحة. كما لوحظ زيادة كميات الفوسفات والنيتروجين والماغنسيوم والبروتينات الموجودة في المحموع الخضري للطماطم المزروعة في التربة الطبيعية والمالحة عن طريق تلقيح التربة بالكائنات المختبره. أدت زيادة تركيز كلوريد الصوديوم إلى زيادة محتوى البرولين والسكر الذائب وانزيم الإستريز، لكن التلقيح بالتربة قلل من التأثيرات السلبية لكلوريد الصوديوم بالمقارنةً بالنباتات عند نفس مستوى الملوحة. في الختام، أشارت نتائج هذه الدراسة إلى أنه يمكن استخدام *Streptomyces* و *Azotobacter vinelandii* أو كليهما كسماد حيوي في التربة المالحة لتحسين النمو عن طريق إنتاج العوامل المعززة لنمو النبات و Siderophore و indole acetic acid و ACC deaminase وإنزيمات وإذابة الفوسفات و تقليل مخاطر NaCl علي النبات.