Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 08 | 2024
DOI: 10.5281/zenodo.13269084

THE MITIGATION OF SALT-STRESS OF *VIGNA RADIATA* (L.) BY AUXIN-PRODUCING SALT-TOLERANT RHIZOBACTERIA

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Abstract

Halotolerant microorganisms have the ability to mitigate the deleterious impacts of salinity on the plant's physiological attributes. The major objective of this study was to evaluate the bacterial diversity associated with *Suaeda fruticosa* (Forssk L.) growing in saline soils. Salt-tolerant bacteria were screened for their potential to promote the growth of *Vigna radiata* under saline conditions. Bacterial strains isolated in this study were found to tolerate NaCl up to 1.5M. These strains were found positive for the production of indole-3 acetic acid (IAA), HCN production, cellulose activity, nitrate reduction, and nitrogenase activity. 16S rRNA sequencing showed that strains belong to eight different bacterial genera including *Brachybacterium, Oceanobacillus, Halomonas, Microbacterium, Kushneria, Kocuria, Rothia, and Staphylococcus*. Bacterial strain *Halomonas caseinilytica* DO, *Halomonas elongate* S3W and *Oceanobacillus picturae* Ra6S3 showed 70.46 µg/ml, 113.53 µg/ml and 73.25 113.53 µg/ml of auxin production under 1M salinity stress in the presence of 1000 µg/ml of L-tryptophane whereas, at 1.5M salinity stress *Halomonas elongate* S3W showed 93.17 113.53 µg/ml of IAA production. In pot trials with *Brachybacterium paraconglomeratum* (Ra11S4), *Staphlococcus equorm* (Rb8S4), *Rothia endophytica* (Rb2S6) recorded increases in fresh biomass up to 2.4 folds and number of pods up to 2.1folds for the plant *Vigna radiata.* These strains can be used as promising bacterial inoculants in sustainable agriculture for the mitigation of salinity stress.

Keywords: Auxin Production, Halo Tolerance, Rhizobacteria, Proline, Salinity, *Vigna Radiata*, Biodiversity.

INTRODUCTION

Pakistan is a country with a semi-arid climate where agriculture is dependent on irrigation from Indus basins or rain falls. The total (cultivable and non-cultivable) land area is 79.61 million hectares (MH) out of which 21.17 M hectares are cultivable and 6 million hectares salt affected with 27% surface salinity and 39% profile salinity [1]. Soil that has an electrical conductivity of over 4ds/m^{-1} in the saturated paste of the soil is known as saline soil. It affects plant growth by hampering their growth by causing a disturbance in photosynthesis, delayed flowering, and lower yield. Soil salinity is an emerging concern in this era of a rapidly growing population [2].

Salinity affects the plant growth by interfering with the osmotic balance in the cells. Increased uptake of Na^+ disturbs the Na^+/K^+ balance. Na^+ ions also impede the uptake of Ca^+ and K^+ which are the major and important parts of the plant photosynthetic machinery [3]. In addition, higher salinity causes a disturbance in CO_2 fixation which is ultimately linked to photosynthesis which is the key life thriving process of the plant. Salinity also disrupts the thylakoid membrane which influences the Electron Transport chain,

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enzymatic activity, protein synthesis, and Calvin cycle which is important for ATP synthesis [4].

In nature, microorganisms are diversified and have several adaptations to overcome abiotic stresses. Bacteria in general have the potential to combat salinity either by efflux mechanisms or via the production of osmoprotectants. Salt-tolerant PGPR diversity mainly includes *Bacillus subtilis, Bacillus amyloliquifaciens,* and *Pseudomonas fluorescence. Kocuria rhizophila, Azotobacter chrococcum, Pseudomonas putida, Halomonas, Oceanobacilius* and *Streptomycetes [5].*

Plant phytohormones are the key to salinity stress tolerance they act in conjunction with other pathways for mitigation and growth development of plants. Two key phytohormones are auxins and gibberellic acid (GA). Auxins are associated with root hair ultra-structure formation whereas GA is associated with seed germination, stem elongation, and flowering in plants [6].

Saudea fruticosa is the main inhabitant of Pakistani saline soils. This plant can tolerate 1M salt with an optimal growth range between 0.3-0.4 M [7]. The rhizopsheric zone of this plant acts as a great hub for the halo-tolerant bacterial diversity *Bacillus* and *Pseudomonas* being the most predominant [8]. Whereas, *brachybacterium, Erwinia persicina, Zhihengliuella halotolerans,* have also been reported as inhabitants of *Suaeda fruticosa* [9].

In this study, we have targeted to screen the bacterial diversity associated with *S. fruticosa*. We hypothesized that microorganisms associated with plants growing in saline habitats may have adapted to tolerant high salt concentrations. Hence bacterial strains were screened for phytohormones production as a major attribute to use them as biofertilizers to grow a glycophytic plant (*Vigna radiata* L.) under saline conditions.

MATERIALS AND METHODS

Isolation and Identification of Salt-tolerant Bacteria

Salt-tolerant bacterial strains were isolated from the soil collected from the rhizosphere of *Suaeda fruticosa*. Soil samples were collected from the vicinity of the Khewra salt mine at the altitude and latitude of 32°38′52.58″N 73°00′30.22″E. Bacterial strains were isolated from serial dilutions of soil samples as mentioned in [10]. About 50µl of suspension was plated on L-agar supplemented with different NaCl concentrations i.e., 50 mM, 100 mM, and 150 mM. Plates were incubated at 37º C for 48 h. Finally, 60 salt-tolerant isolates were selected by several rounds of streaking.

Analysis of Saline Soil

The soil was analyzed for electrical conductivity (EC), pH, and soil texture. In addition, its organic content and available ions including phosphate, potassium, zinc, copper, manganese, iron, and boron were also quantified by sending samples to the “Soil and

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Water Testing Laboratory for research, Agriculture Department, Government of the Punjab, Pakistan".

16S rRNA gene Sequencing

Bacterial DNA was isolated by Favoprep™ tissue genomic DNA extraction mini kit (Favorgen Biotech Corporation). PCR amplification of the 16S rRNA gene was done with a universal set of primers 27f forward 5′-(AGAGTTTGATCCTGGCTCAG-3′) and 1522r reverse (5′-AAGGAGGTGATCCA(AG)CCGCA-3′) as described by Aslam and Ali (2018).. The PCR product was purified by Favoprep™ gel purification mini kit (favogene Biotech Corporation). The purified product was sequenced by sending samples to the ABI base (Singapore).

Bacterial Auxin Production

Bacterial auxin was quantified by growing bacterial strains in 25 ml of L-broth having 0.5M NaCl supplemented with 600µg/ml of L-tryptophan. Controls included the un-supplemented media (0 mM salt: 0 µg/ml L-tryptophan). Media flasks were incubated for "72 h" at 30º C on a shaker (120rpm/min) in triplicates. After incubation, cells were removed from the supernatant by centrifugation at 12000 rpm for 2 min. Auxin production was determined by treating 2 ml of supernatant with 1 ml of Salkowski's reagent [11]. Quantification of bacterial IAA through GC-MS analysis was based on the method described by Andersen [12] with slight modification.

Quantification of Bacterial Gibberellic Acid

HPLC Analysis of Gibberellic Acid

The methanolic extracts were prepared by the method of Desai *et al.* (2012) and analyzed for the detection of GA3 using high-performance liquid chromatography (HPLC) (Waters Alliance 2695) equipped with reversed-phase column crestpak C18. The mobile phase of acetonitrile:-water (70:30 %; V/V) was used with pH 4.5 and a flow rate of 1 ml/min. an injection volume of 10 µl was used for analysis at the wavelength of 208 nm.

Halophility Assay

Salinity tolerance of rhizobacteria was estimated by growing bacterial cells in 20 ml of L-broth amended with 0M, 0.5M, 1M, 1.5M NaCl. Cultures were incubated at 30º C for 48 h on a shaker (120rpm/min) in triplicates. After incubation, optical density was measured at 600 nm to determine the bacterial salt tolerance.

Additional Plant Growth-Promoting (PGP) Attributes

The additional plant growth-promoting attributes of selected strains were also determined by performing in vitro screening. For example, the phosphate solublization potential was evaluated by growing isolates on Pikovskaya's media supplemented with 0M and 0.25M salt concentrations [13]. Hydrogen cyanide (HCN) production was estimated by preparing L-agar supplemented with 0.44% glycine and amended with NaCl (Reetha et al., 2014). Bacterial nitrogenase activity was estimated on nitrogen-free mineral salt medium [14].

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Bacterial nitrate reduction was determined in nitrate broth supplemented with 0 and 0.5M NaCl (G Cappuccino and Sherman, 2014). Cellulase activity was also determined by inoculating strains in a medium supplemented with NaCl [15]. The biofilm formation ability of bacterial strains was determined by the method of O'Toole (2011). Bacterial proline content was estimated by following the method of Bates *et al*. (1973). [16].

Pot Trails under Axenic Conditions

For pot trials in controlled conditions, the inoculum was prepared by growing bacterial strains in L-broth for 24 h at 37 ºC. The cell pellet was obtained by centrifugation at 12000 rpm for 5 min and re-suspended in 10 ml water to obtain CFU of 1.5 x 10^8. Seeds were sterilized in 0.1% $HgCl_2$ for 2 min and incubated with bacterial cultures for 25 min. *Brachybacterium paraconglomeratum* Ra11S4 *Kocuria oceani* C, *Oceanobacillus picturae* Ra6S3, *Staphylococcus equorum* Rb8S4, *Rothia endophytica* Rb2S6, and *Microbacterium indicum* WS6 *Halomonas caseinilytica* DO, *Kushneria aviccenia* R2S4 and *Halomonas elongate* S3W were used as single culture. Bacterial strains were also used in consortium i.e., CS1 (Ra6S3, Ra1S7, Ra11S4), and CS5 (S3W, R2S4, Rb8S4, C). Seeds were transferred to pots (6×6 cm) containing sterilized soil and peat moss (180:20 g). Ten pots were sown with each bacterial strain and salt stress. For control, pots with water-treated seeds and respective salt stress were also kept for comparison. In each pot, 3 seeds were sown in triplicates. After sowing, pots were treated with respective salt stress i.e.; 0 mM, 50 mM, 100 mM, and 150 mM. Pots were incubated in plant growth chamber for a 16 h photoperiod. After 10 days, plants were harvested to record shoot length, root length, and fresh and dry biomass.

Pot Trials under Wire House Conditions

For plant experiments under natural conditions, 9 bacterial monocultures and 2 mixed cultures were used as mentioned above. Large earthen pots (12×24 cm) were filled with 16 kg of fertile sieved soil. The cultures used were the same as mentioned above. Seeds were bacterized as described earlier for lab trials. The experiment was conducted in duplicate. In each pot, 10 seeds were sown and maintained to 5 after thinning. Pots were moistened with normal water after sowing and allowed to germinate. On the 10^{th} and 17^{th} day of germination, pots were saturated with respective molarities of 0 mM, 50 mM, 100 mM, and 150 mM saline water. Plants were allowed to grow to their full maturity. At maturity, plants were harvested, shoot length, fresh and dry biomass, and number of leaves and pods per plant were measured.

Atomic absorption spectroscopy (AAS)

Contents of sodium and potassium in plants grown under different salinity stresses were quantified on the graphite furnace atomic absorption spectroscopy (Hitachi za 3000) by the method of Ali *et al.,* (2014) with slight modifications. One g of dried leave sample, grown to full maturity was taken, ground, and digested in 20 ml of 1M Nitric acid (HNO_3). Then, this material was incubated at 70 ºC for 4 hours for 1 h followed by centrifugation at 10,000 rpm for 1 min. An aliquot of 500 µl was diluted to 5 ml with distilled water.

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Contents were measured by Atomic absorption spectroscopy (AAS) and the concentration in leaves was calculated through a standard curve constructed using 1 to 100 mg of Na^+ and K^+ [17].

Statistical Analysis

Data for auxin production, halophility assay, biofilm formation, and plant growth parameters were subjected to analysis of variance (ANOVA) and means were separated by using Duncan's multiple range test ($P \leq 0.05$).

RESULTS

In total 60 salt-tolerant bacterial strains were isolated from the root zone of *Suaeda fruticosa* plant. Fifty-eight were selected based on their varied colony morphology for further identification by sequencing analysis. Finally, 16 strains were maintained and further analyzed for plant growth-promoting (PGP) attributes.

Soil Analysis

Soil samples collected from bulk soil showed higher EC ranging from 72.7-88.7 (ds/m) as compared to rhizospheric soil which showed 40.6 EC. Similarly, 7.5 pH was noted for rhizospheric soil whereas bulk soil showed a pH range of up to 8.2. For organic matter, all the soils either bulk or rhizospheric soil range from 0.8% to 1.2% which showed medium fertility for plant growth (Table 1).

Table 1: Analysis of Saline Soil taken from Salt Affected Area of Potohar Region

Sample	EC ms/m	pH	Organic matter	Avail. P mg/kg	Avail. K mg/kg	Saturation %	Texture	Zn	Cu	Mn	Fe	B
Plot 1	80.6	8.2	1.2	2.5	95	38	loamy	3.7	5.7	3.1	15.7	0.62
Plot 2	88.7	7.8	1.10	1.5	100	40	loamy	-	-	-	-	-
Plot 3	72.7	7.7	0.90	2.0	105	40	loamy	2.9	3.8	5.3	18.9	0.28
Plot 4	76.9	7.6	0.85	1.5	85	38	loamy	-	-	-	-	-
Rhizo-soil	40.6	7.5	0.90	2.5	90	40	loamy	-	-	-	-	-

16S rRNA Identification of Bacterial Strain

The nucleotide sequences were submitted to the online database NCBI to find homology with respective strains. It was observed that bacterial strains belong to 13 different genera. Nine strains used in this study showed similarity with *B. paraconglomeratum*

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(Ra11S4), *O. picturae* (Ra6S3), *S. equorum* (Rb8S4), *M. indicum* (WS6), *K. avicenniae* (C) and *R.endophytica* (Rb2S6), *H. caseinilytica* DO, *Kushneria aviccenia* R2S4 and *Halomonas elongate* S3W. The sequences were submitted in Gene Bank under accession numbers that are given in Table 2.

Table 2: 16S rRNA gene sequencing of salt-tolerant Bacteria Isolated from *S. Fruticosa*.

S. No	Strains	Identification	Accessions
1	WS6	*Microbacterium indicum*	MK342502
2	C	*Kocuria oceani*	MK342507
3	Ra6S3	*Oceanobacillus picturae*	MK342508
4	DO	*Halomonas caseinilytica*	MK342509
5	Rb8S4	*Staphylococcus equorum*	MK342519
6	Ra11S4	*Brachybacterium paraconglomeratum*	MH844968
7	R2S4	*Kushneris avicenniae*	MH844971
8	S3W	Halomonas elongate	MK342512
9	Rb2S6	*Rothia endophytica*	MH844966

Bacterial plant-growth-promoting attributes

In vitro screening showed that bacterial strains have variable potential to solubilize phosphate, degrade cellulose, HCN production and nitrogen fixation. *K. oceani* C was positive for all the above-mentioned attributes. Whereas, *V.salaries* R10S3 and *B. paraconglomeratum* Ra11S4 were positive for all except nitrogen fixation. While *Staphylococcus equorum* Rb8S4 was positive for all traits except for phosphate solubilization (Table 3). For biofilm formation, O. *picturae* Ra6S3, *R. endophytica* Rb2S6 promising results at 0.5M NaCl as compared to other strains (Fig 1).

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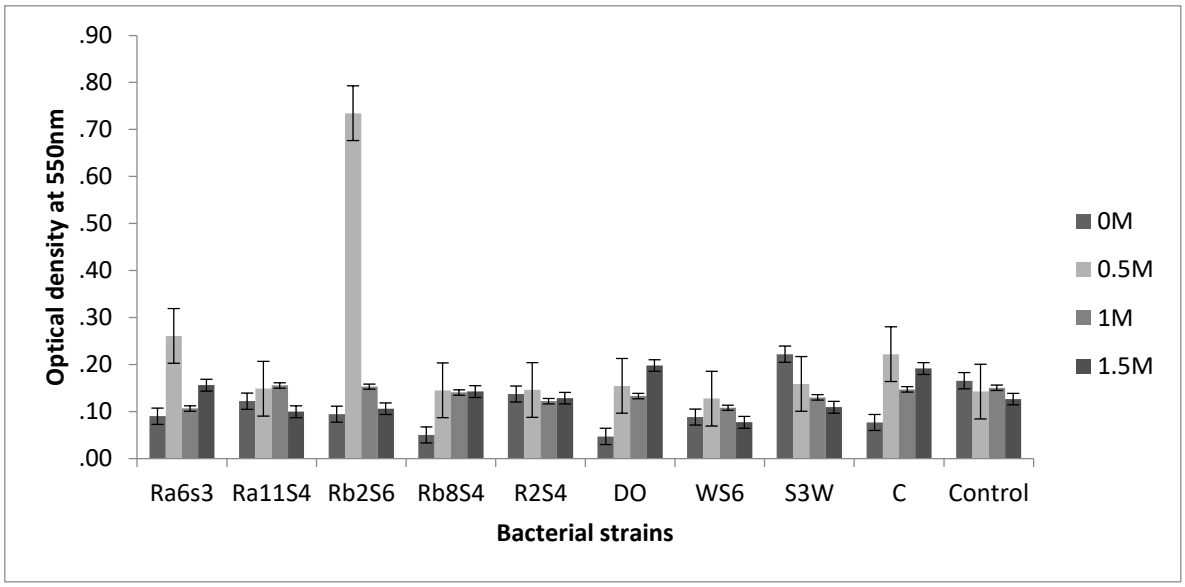

Figure 1: Bacterial Biofilm Formation at Various Salinity Stresses

Table 3: Bacterial PGPR Attributes

S. No.	Bacterial Strains	HCN production				Phosphate solubilization		Cellulose degradation				Nitrogen fixation	Nitrate reduction	
-	-	NaCl (M)												
-	-	0	0.5	1	1.5	0	0.25	0	0.25	1	1.5	1	0	0.5
1	Ra6S3	++	+	++	++	+	-	-	-	-	-	-	-	-
2	WS6	-	-	-	-	-	-	-	+++	+	+	-	++	++
3	DO	-	-	-	-	-	+	-	-	+	+	+	+	-
4	C	-	-	-	+	++	+++	+	+	+	+	+	+	-
5	S3W	-	-	+	++	-	-	-	-	-	++	+	-	+
6	Rb8S4	+	+	++	++	-	-	-	-	++	+	++	++	++
7	Rb2S6	-	+	+	-	-	-	+	-	-	-	-	-	-
8	Ra11S4	++	++	++	++	+	++	-	+	++	+	-	+	+
9	R2S4	-	+	+	+	+	+++	+	-	++	+	-	+	+

(+weak positive, ++ mild positive, +++ strong positive)

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Bacterial Auxin Production

Bacterial strains were grown in the presence and absence of L-tryptophan at 0M and 0.5M salt stress. In the absence of a precursor (L-tryptophan), the highest recorded IAA production was 7.59µg/ml by *R. endophytica* Rb2S6 in non-salinized conditions. Whereas, increasing the salinity from 0M to 0.5M, causes an increase of 2.1-fold with *B. paraconglomeratum* Ra11S4. Moreover, supplementation of media with precursor enhanced IAA production from 1.27 µg/ml to 21.05 µg/ml in non-salinized media. Whereas, increase in salinity and precursor simultaneously increases the levels up to 89% (39.84 µg/ml) in *S. equorum* Rb8S4 (Table 4).

Table 4: Indole-3-acetic acid (IAA) production under 0M and 0.5M Salinity Stress

Sr.No.	Strains	0µg 0M	600µg 0M	0µg 0.5M	600µg 0.5M
1	R2S4	6.00±0.10 (b)	11.12±0.12 (b)	2.78±0.32 (c)	18.98±0.02 (e)
2	S3W	7.37±0.13 (d)	27.74±0.36 (k)	3.19±0.01 (c)	32.96±0.14 (h)
3	WS6	7.82±0.28 (ef)	19.03±0.07 (g)	1.91±0.09 (b)	27.00±0.10 (g)
4	Ra11S4	9.39±0.01 (g)	17.03±0.07 (f)	17.89±0.22 (i)	14.98±0.02 (d)
5	DO	13.49±0.11 (i)	8.24±0.14 (a)	3.89±0.22 (d)	58.18±0.07 (m)
6	Ra6S3	6.44±0.06 (c)	16.67±0.43 (ef)	29.00±0.10 (k)	37.00±0.10 (i)
7	Rb8S4	1.27±0.03 (a)	21.05±0.05 (h)	4.01±0.02 (d)	39.84±0.16 (j)
8	Rb2S6	7.59±0.09 (ef)	23.24±0.14 (e)	7.47±0.07 (f)	20.19±0.06 (f)
9	C	6.29±0.09 (b)	18.59±0.09 (g)	10.07±0.17 (h)	14.05±0.05 (c)

Mean of 3 replicates. Different letters in a column indicate significant differences in treatments using Duncan's multiple range test (P≤0.05).

Gibberellic Acid (GA) Analysis

Strains of *O. picturae* Ra6S3, *B. paraconglomeratum* Ra11S4, *K. oceani* C, *H.caseinilytica* DO showed a higher level of GA in spectrometric analysis. At 0.5M salinity GA production ranged from 10.39 to 47.49 µg/ml. About 2.8-fold increase in GA production was recorded in salinized media (0.5M) as compared to non-stressed controls. Production of GA in bacterial crude extracts was also confirmed by UPLC analysis. Fig 2 shows the presence of GA in the crude extract of *Halomonas caseinilytica* DO at retention time of 2.6.

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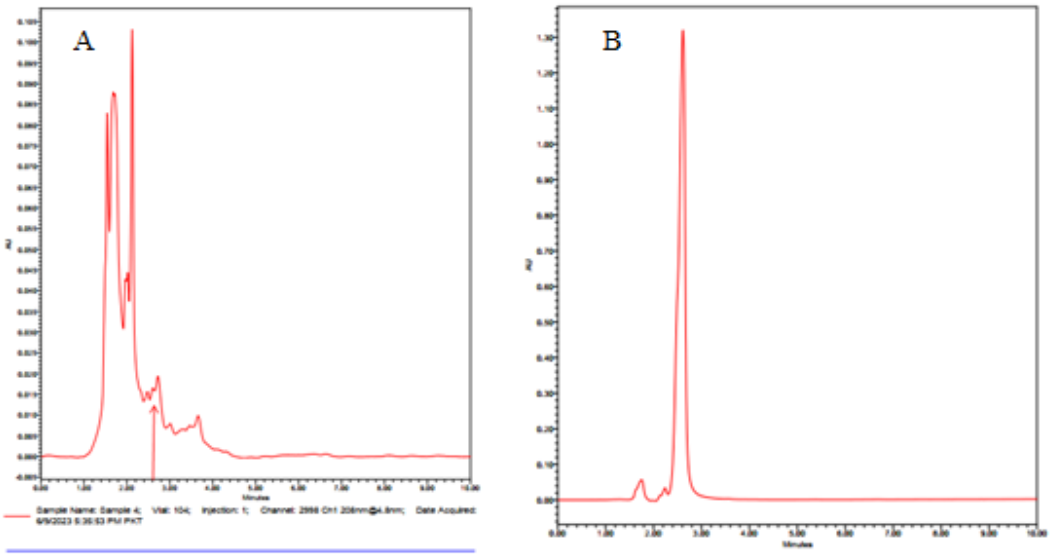

Figure 2: UPLC Analysis of bacterial Gibberellic acid production. (a) Extract from *Halomonas caseinilytica* DO, (b) GA standard 10 ppm

Halophility Assay

Salt tolerance was best exhibited by *B. paraconglomeratum* Ra11S4, *O. picturae* Ra6S3, and *Kocuria oceani* C at 0.5M and 1M NaCl as compare to 0M.

Bacterial Proline

Bacterial strains showed the highest proline production at 1 M salt stress which was 37.32 µg/ml by *K. oceani* C. whereas at 0.5M salinity highest proline value obtained was 21.08 µg/ml by *O. picturae* Ra6S3. Moreover, at 1.5 M stress, *K. oceani* C performed well by producing 18.43 µg/ml proline as compared to 0 M salinity that recordedproline production between 1.41- 4.07 µg/ml.

Pot trials under Axenic Condition

Bacterial strains were evaluated under axenic conditions to determine their ability to enhance plant growth under salt stress. Water-treated seeds showed reduction in growth with increasing NaCl levels. Bacterial strains showed positive growth enhancement under salinity stress, at 50 mM salt stress. For instance, the enhancement in shoot length was 37% by *S. equorum* Rb8S4 and 8.3% by mixed culture combination CS1 (Table 5). For root length, *S. equorum* Rb8S4 showed a 1.2-fold increase at 150 mM salinity while co-inoculation with *B. paracoglomeratum* Ra11S4 enhanced the growth by 80%. Whereas, mixed culture combination CS4 enhanced root length by 17.2-folds at 100 mM salinity (Table 6).

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Table 5: Effect of salt-tolerant bacterial strains on shoot length of *V. radiata* under different saline conditions under axenic conditions

S. No.	Strains	NaCl (mM)			
		0 mM	50 mM	100 mM	150 mM
1.	Controls	20.62±5.36 (e)	12.75±1.84 (bcd)	11.25±2.07 (a-d)	13.12±5.39 (b-d)
2.	Ra11S4	18.25±0.95 (g-m)	14.125±0.47(e-l)	8.62±1.43 (a-f)	12.87±2.52 (c-l)
3.	Ra6S3	15.75±4.57 (f-m)	13.375±9.14 (d-l)	12.12±1.93 (c-k)	4.37±4.85 (ab)
4.	Rb8S4	18.5±1.0 (k-m)	18.375±0.48 (j-m)	15.37±1.75 (f-m)	6.5±6.56 (a-d)
5.	S3W	18.625±1.49 (k-m)	8.8±9.48 (a-f)	10.5±4.5 (a-h)	3.62±4.5 (a)
6.	C	20±2.16 (l-m)	6.75±7.23 (a-e)	10.37±7.59 (a-h)	13.5±1.91 (d-l)
7.	WS6	18.625±3.64 (k-m)	12.75±4.65 (c-l)	10.25±6.89 (a-g)	12.5±1.3 (c-l)
8.	DO	18.45±.63 (efg)	14.0±2.12 (b-f)	14.45±.77 (b-f)	16.50±.70 (d-g)
9.	CS1*	17.875±1.81 (c-e)	13.625±2.5 (b-d)	13.25±0.96 (b-d)	14.12±1.18(b-d)
10.	CS5*	14.125±8.00 (b-d)	14.5±1.68 (bcd)	13.125±8.75 (b-d)	9.37±2.05 (ab)

Mean ± S.E. of 5 replicates. Different letters in a column indicate significant differences in treatments using Duncan's multiple range test (P≤0.05).

Table 6: Effect of salt-tolerant bacterial strains on root length of *V. radiata* in saline conditions under axenic conditions

S. No.	Strains	NaCl (mM)			
		0 mM	50 mM	100 mM	150 mM
1.	Controls	2.37±0.024 (n)	1.25±0.08 (g-m)	2.12±0.02 (c-k)	0.62±0.04 (g-m)
2.	Ra11S4	2.75±1.55 (abcd)	1.5±2.3 (ab)	1.12±0.25 (g-m)	1.12±0.25 (a)
2.	Ra6S3	12.22±4.45 (i-m)	11.9±8.49 (h-n)	8.9±1.46 (e-k)	3.27±4.13 (a-e)
1.	Rb8S4	13.45±1.03 (j-n)	13.82±1.86 (k-m)	11.35±3.72 (h-m)	4.82±3.92 (a-g)
2.	S3W	15.05±2.37 (l-n)	6.55±7.05 (a-h)	6.17±2.88 (a-h)	2.1±3.56 (abc)
3.	C	15.25±2.5 (mn)	3.72±4.02 (a-f)	6.45±4.41 (a-h)	9.52±1.35 (f-m)
4.	WS6	13±3.5 (i-n)	8.35±3.08 (d-k)	7.475±4.98 (b-k)	11.47±5.66 (h-m)
5.	DO	8.15±1.0 (b)	6.50±.70 (ab)	12.65±3.74 (c)	6.50±2.12 (ab)
6.	CS1*	12.075±1.63 (c-j)	8.75±2.21 (a-e)	10.8±0.94 (cde)	10.85±1.67 (cde)
7.	CS5*	9.025±5.7 (b-e)	8.7±1.71 (a-e)	8.475±5.68 (a-e)	6.25±2.26 (a-e)

Mean ± S.E. of 5 replicates. Different letters in a column indicate significant differences in treatments using Duncan's multiple range test (P≤0.05).

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Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 08 | 2024
DOI: 10.5281/zenodo.13269084

Experiment under Natural (wire house) Conditions

At 100 mM salt stress, *B. paraconglomeratum* Ra11S4 and *O. picturae* Ra6S3 showed 2.1-fold and 1.4-fold increases in shoot length (Fig 3), whereas, 40% and 1-fold increase in the number of leaves was recorded by these strains, respectively (Fig 4).

Bacterial treatment also has a positive impact on plant pod development. Plants treated with *B. paraconglomeratum* Ra11S4 and *O. picturae* Ra6S3 showed the best results in increasing the number of pods by 1.2-fold and 78% (Fig 5).

In fresh biomass, bacterial priming also increased the plant responses towards salinity. *B. paraconglomeratum* Ra11S4 increased 30.60% at 100 mM (Fig 6). Similarly, a 42.65% increase in dry weight was observed with Ra11S4 respectively (Fig 7).

Bacterial mixed culture combination with CS5 caused a significant increase in a number of leaves by 76.67%. Shoot length increased with CS5 1.4 folds at 100mM while at 150 Mm increase was up to 16.39 with CS5. Similar results were with pod development, CS5 caused a 22.22 % increase at 100 mM and a 30% increase at 150 mM salt stress (Fig 3-7).

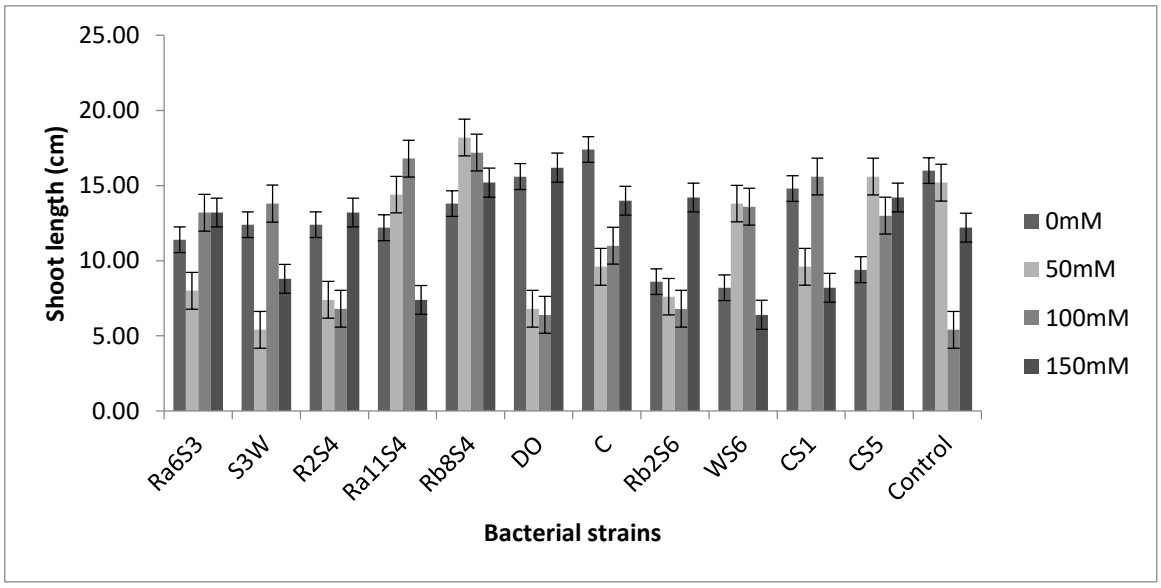

Figure 3: Effect of salt-tolerant bacterial strains on shoot length of *Vigna radiata* at different salinities (mM) under natural conditions. Bars indicate the mean of 5 plants

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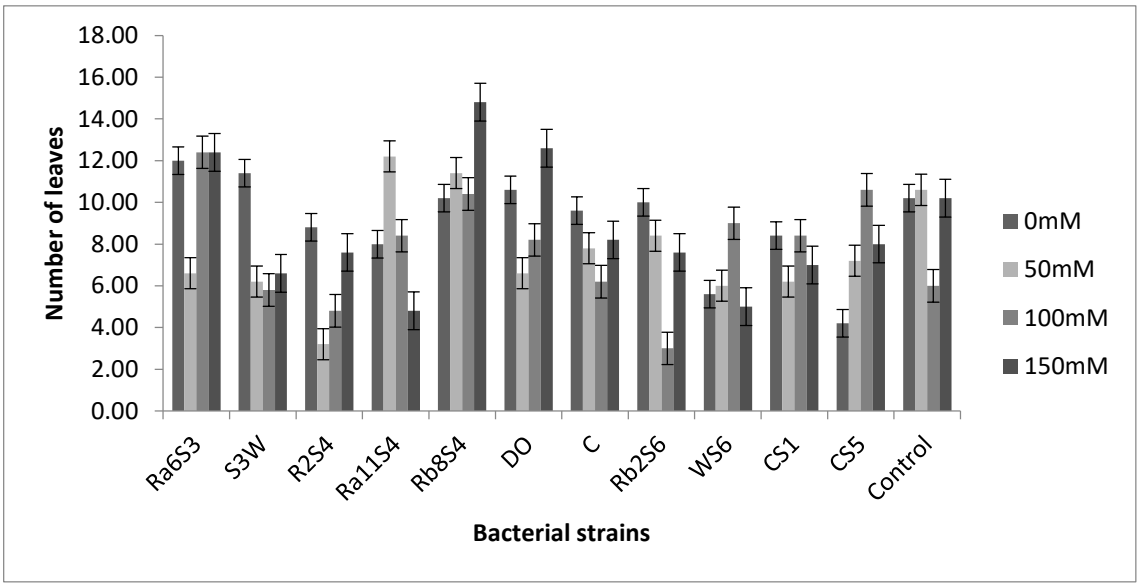

Figure 4: Effect on number of leaves of *Vigna radiata* by salt-tolerant bacterial strains at different salinities (mM) under natural conditions. Bars indicate mean of 5 plants

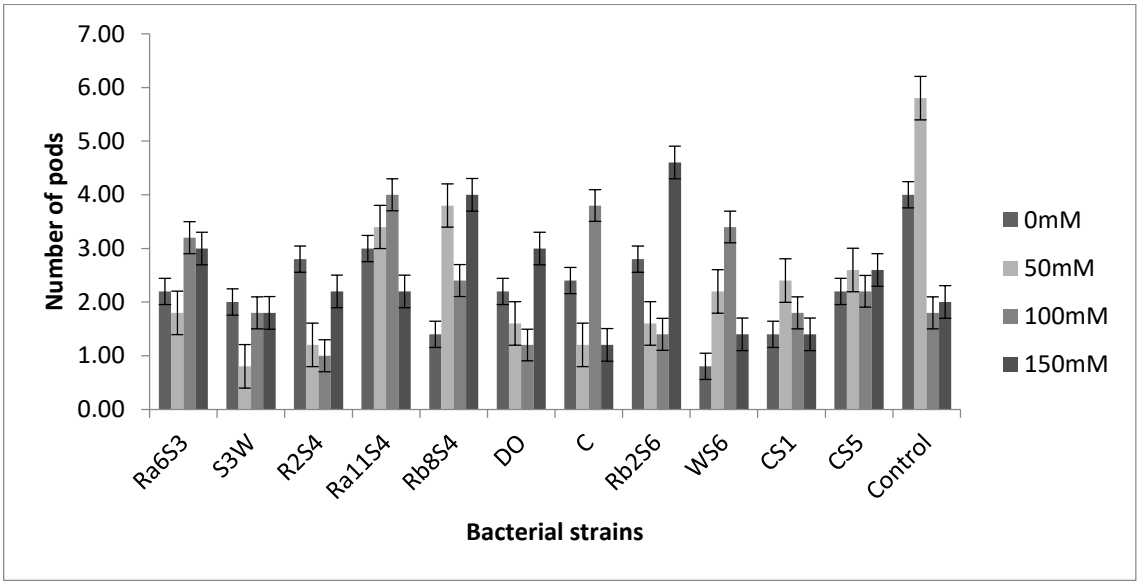

Figure 5: Salt-tolerant bacteria's impact on *Vigna radiata* pod production at varying salinity levels. Bars indicate mean of 5 plants

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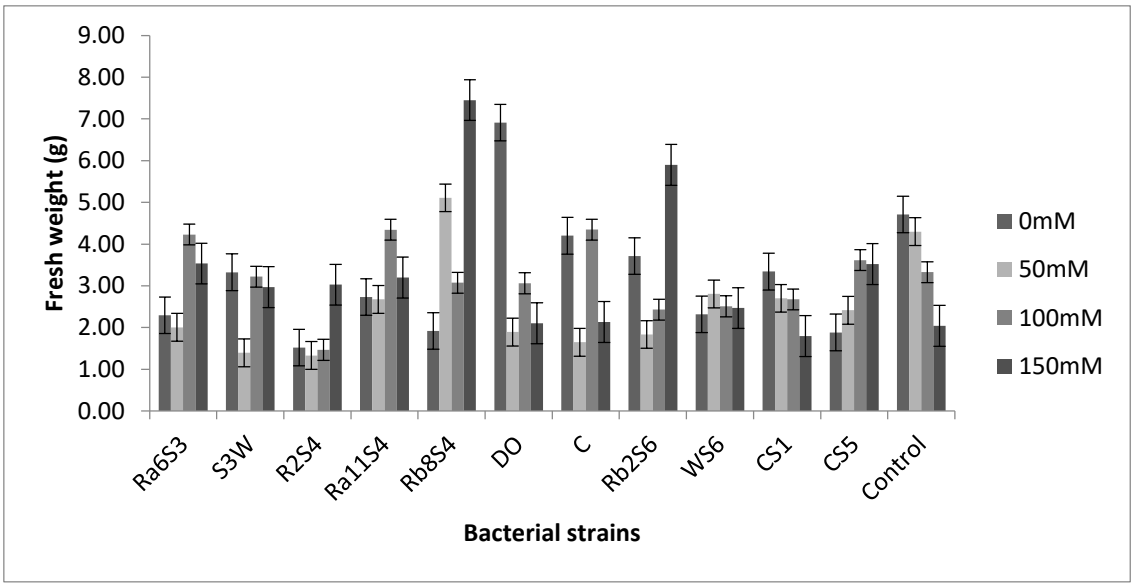

Figure 6: Salt-tolerant bacteria's effect on *Vigna radiata* biomass at different salinity. Bars indicate the mean of 5 plants

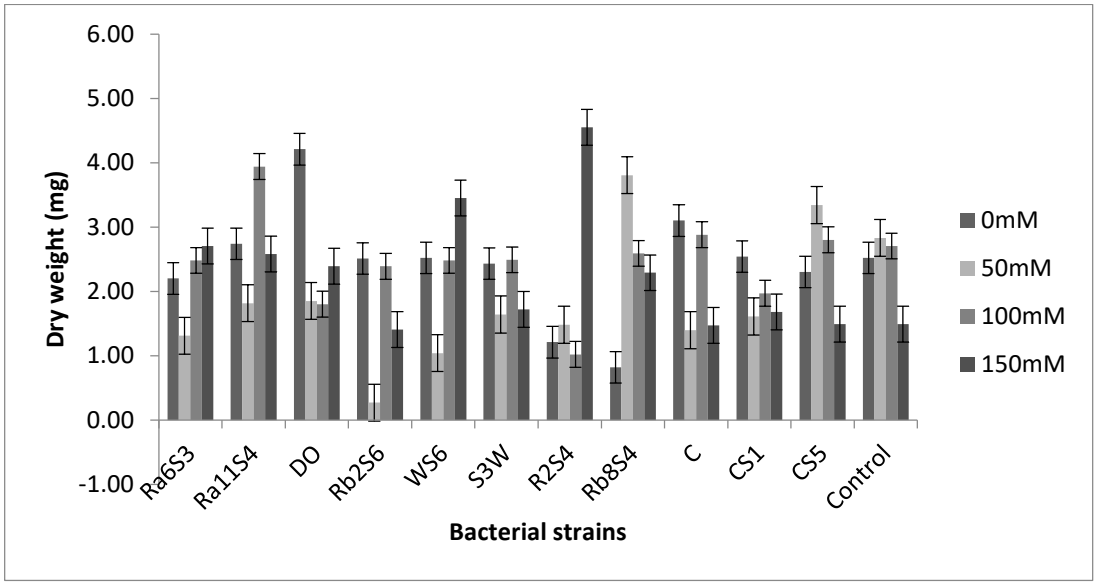

Figure 7: Effect of salt-tolerant bacterial strains on dry biomass of *Vigna radiata* at different salinities (mM) under natural conditions. Bars indicate the mean of 5 plants

Atomic Absorption Spectroscopy

In this experiment, plants co-inoculated with strain Rb8S4 showed lower levels of Na^+ ions (up to 1.98-fold) as compared to the control at 100 mM salt stress. Similarly, at 150 mM salt stress, the uptake of K^+ ions increased from 0.47mg/g to 1.11mg/g DW^{-1}.

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DISCUSSION

In this present study, 9 salt-tolerant bacterial strains belonging to different genera were selected on the basis of exhibiting different plant growth-promoting attributes. Sequencing analysis revealed the strains belonging to *Oceanobacillus*, *Micobacterium*, *Kocuria*, *Staphylococcus*, *Rothia,* and *Brachybacterium* genera.

Aslam *et al*., 2018 have reported *Bacillus* and *Oceanobacillus species, Exiguobacterium* sp., *Staphylococcus jettensis, Salinicoccus sesuvii, Arthrobacter bergerei* and *Pseudomonas rhizosphaerae* associated with the root zone of *S. fruticosa [18].* Whereas, in another study conducted in Turkey, *Bacillus* is reported as the predominant species found in the rhizosphere of *S. fruticosa* with *Providencia, pantoea, pseudomona,s* and *Klebsiella* being the minor diversity [8].

Strains used in this study showed promising results for the production of IAA under saline conditions, *Halomonas caseinilytica* DO, *Halomonas elongate* S3W and *Oceanobacillus picturae* Ra6S3 showed 70.46 µg/ml, 113.53 µg/ml and 73.25 113.53 µg/ml of auxin production under 1M salinity stress in the presence of 1000 µg/ml of L-tryptophane whereas, at 1.5M salinity stress *Halomonas elongate* S3W showed 93.17 113.53 µg/ml of IAA production.

Sadeghi *et al* (2017) have reported the increase in salinity to have an impact on auxin production in positive terms, the reported increase was 4.7µg/ml in 300mM salt stress from 2.4 µg/ml in a non-stressed medium [19]. Bacterial strains were shown to show an increased tendency in IAA production under higher salinity stress as compared to normal conditions, the recorded increase was 29 µg/ml from 6.44 µg/ml by *O. picturae* Ra6S3.

As reported in the literature, an increase in bacterial growth has a direct relation with IAA production as *Enterbacter cloacae* A9G produces 114 µg/ml IAA after 48h as compared to *Enterbacter* SPP. producing 70 µg/ml, similarly increasing tryptophan has a direct impact on IAA synthesis as *Cronobacter* AL2, *Arthrobacter* AHA, and *Halomonas* APA produced the highest levels at 1000 µg/ml L-tryptophan provided [20].

Bacterial strains showed biofilm formation potential at higher salinity stress, O. *picturae* Ra6S3, *K. Oceani* C, and *S. equorum* Rb8S4 showed increased biofilm formation at 1.5M salinity stress as compared to 0M salinity. It was found that the biofilm formation in the root zones of plant inhabitants of saline areas increase plant growth either by inhabiting the higher salts to enter the root cells or producing antioxidant enzymes as scavengers of oxidative stress causing nascent O2.

Bacillus cereus WT wild-type strain showed viable growth with 50uM paraquat as compared to *SigB* and *SodA* mutants [21]. *B. tequilensis* was found to produce varied biofilm formation while the highest biofilm formation was recorded at 100mM salinity stress (Haroon *et al.,* 2023). Whereas *Glutamicibacter arilaitensis* ESM7 was found to produce 86.2 mg/ml of biofilm (Haque *et al,* 2022).

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In this study, strains were positive for the production of HCN, cellulose hydrolysis, and phosphate solubilization under salt stress. It is evident from studies that phosphate solubilizes microbes enhanced plant growth as phosphate is an important component of the photosynthetic machinery. Phosphate present in fertilizers becomes insoluble due to the formation of bonds between phosphate and calcium in alkaline soils. Alkaline phosphatase present in bacterial cells converts phosphate into a usable form [22].

Strains used in this study enhanced the plant growth under natural conditions by means of different parameters, at salt concentrations of 100mM and 150mM. *Brachybacterium* (Ra11S4) increased the shoot length up to 211% and the number of pods up to 122% at 100mM stress. While at 150mM salinity, Rb8S4 increased the fresh and dry biomass up to 2.7 and 1.9 folds and a number of pods up to 100% at 150Mm salinity, while Rb2S6 increased dry biomass up to 1.8-fold whereas an increase in pods was 1.3-fold respectively.

It has been reported that *K. rhizophilla* with 75 salt stress imparted an effect on root and shoot length and fresh weight causing 13.3%, 15.5.%, and 1.3% increase respectively. Chang p. *et al* have reported the same result, inoculation of bacterial strains in single and consortium cultures in saline soil promoted the shoot growth and root biomass up to 150% and 200%, respectively [23].

PGPR-induced growth promotion was also observed for Rice variety BRRI dhang67, with a 56% increase in total dry matter with *B. methylotrophicus* UPMR B9 under salinity stress [24]. Similar were the results observed for beet plant with co-inoculation of *K. mariflavi* that showed 3 times increase in Root length, 2 times increase in fresh weight, and 2.5 times increase in DW with respect to their control treatments under salinity stress of 0, 50, and 300 mM [25].

CONCLUSION

Bacterial strains isolated from the saline environment have already been adapted to tolerate higher salinity stress and have evolved certain survival mechanisms to thrive better in harsh environments, these strains have the higher scope and potential to be used in agricultural systems to mitigate the salinity stress and retain the soil health. Bacterial strains *O. picturae* Ra6S3 *and B. paraconglomeratum* Ra11S4 are promising in terms of IAA production as well as have a high impact on plant growth promotion.

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