




Comparative Evaluation of Different Salt-Tolerant Plant Growth-Promoting Bacterial Isolates in Mitigating the Induced Adverse Effect of Salinity in *Pisum sativum*

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Abstract: Due to climate change, salinity has become a limiting factor for many leguminous crops. Therefore, we have explored the comparative study of salt-tolerant plant growth-promoting rhizobacteria (PGPR) for the promotion of plant growth. These PGPR (*Bacillus subtilis* RhStr_71, *Bacillus safensis* RhStr_223, and *Bacillus cereus* RhStr_JH5) were *in vitro* screened for plant growth-promoting (PGP) traits such as IAA, P-solubilization, siderophore, and ammonia production. They were further selected to evaluate the maximum NaCl tolerant level (MTL). Selected salt-tolerant PGP bacteria were further characterized to evaluate their PGP activity on seedlings of *Pisum sativum* under 1% NaCl stress. They were further selected to perform the greenhouse experiments under 1% NaCl stress to compare these isolates on morphological (like plant height and weight) and biochemical parameters (such as carbohydrate, reducing sugar, protein, phenol, flavonoids, chlorophylls, and carotenoids). In a pot experiment, NaCl significantly reduced the plant growth parameters compared to un-inoculated and inoculated. Additional analysis also had shown that these strains also enhanced the antioxidant enzymes, thereby preventing oxidative damage caused due to reactive oxygen species (ROS). The result revealed that these salt-tolerant PGP bacteria exert their beneficial effects on plant growth and play a necessary role in attenuating the salinity stress in agriculture.

Keywords: PGPR; plant growth-promoting (PGP) traits; NaCl; salt tolerance; antioxidants enzymes; *Pisum sativum*.

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1. Introduction

Worldwide, salinity led to the decline of crop production and plant growth in many cultivated areas [1], leading to a 65% loss of crop yield [2]. In India, salinity affects a complete of 9.38 Mha land, while specifically, the Gujarat state has a major share of the saline-affected vicinity of approximately 2.23 Mha [3]. Amongst crop vegetation, cereals and legumes are the foremost sensitives to salt. Even 100mM of salinity in legumes is sufficient to inhibit the nodule formation and eventually the nitrogen fixation [4]. *Pisum sativum* is an important leguminous crop sensitive to salinity, and because of its salinity, it is widely used as an indicator for

research analysis [5]. Salinity stress is well-defined as the existence of excess amounts of salts (soluble) in the soil, which hinders the growth and functioning of the Pea plant [6]. During the first step of salinity stress, the potential of rootage to soak up water decreases, and therefore the water loss from the leaves increases. It is due to the accumulation of high salt caused by osmotic pressure [7]. This osmotic stress causes diverse physiological adjustments, including nutrient imbalance, interruption of membranes, impairing the capability to detoxify the reactive oxygen species (ROS), fluctuations in antioxidant enzymes, and reduction in stomatal aperture, and minimize the photosynthetic activity [8]. Beneath high salinity, the elevated sodium (Na^+) and chloride (Cl^-) concentrations result in an ionic imbalance in plant tissues, which ends up in decreasing nutrient uptake [9].

Determination of seeds' germination ability under saline situations should appear as the beneficial and simple parameter for selecting salt-resistant populations [10]. Furthermore, salinity stress is broadly said to negatively affect seed germination, plant growth and development, nutrient uptake, yield, and crop productivity [11, 12]. It is due to the statement that germination typically takes place in surface soils, which gather soluble salts due to evaporation and capillary push of water content in the soil, thereby decreasing numerous factors along with phytohormones and ROS signaling [13]. It has been documented that the photosynthetic pigment concentrations were drastically reduced in salt-stressed plants [14]. A reduction in the chlorophyll contents signifies that the plant is experiencing abiotic stress [15], and negatively influences the photosynthetic rate. Lowering of photosynthetic rates in crops under abiotic saline stress are correlated with the extreme accumulation of Na^+ and Cl^- concentrations and reduced water potential in the chloroplasts and chlorophyll, which immediately impact plant health [9]. The deleterious effects of salinity on photosynthesis and protein synthesis have also been notified by many other researchers [16, 17]. The seedlings subjected to low-salt stress confirmed a remarkable decline in superoxide dismutase (SOD) and catalase (CAT) activity in comparison to non-stressed control groups [18].

The plant's defense mechanism to the osmotic component of salt stress includes the accumulation of osmoprotective materials, including proline, sucrose, and mannitol [19]. Proline, a low molecular weight (m.w.) osmoprotectant is a biochemical indicator of osmotic adjustment in plant cells and organs [20]. One of the foremost devastating aspects of oxidative stress is lipid peroxidation, which disrupts the cellular-membrane structure and ultimately provokes necrobiosis. To enhance the plant salinity tolerance, several tactics towards sustainable development of agriculture become accomplished. As innovative technologies, RDT (Recombinant DNA Technology) and plant breeding are often accustomed to increase salt-tolerant varieties efficiently but are very time-consuming. In agriculture, microbial technology is one of the technologies wished nowadays and within the future for sustainable crop productiveness [21]. Seeing that Hiltner explained on the rhizosphere concept in 1904 that plant growth-promoting rhizobacteria (PGPR) are used as bio-agents for promoting plant health to conquer salt stress management [22]. Its utilization is one of the most alternative, promising, and economic approaches to revamp crop production in saline stress soils [23, 24]. *Bacillus* and *Pseudomonas* species are the foremost extensively investigated rhizobacteria that facilitate plant health [21].

To our knowledge, no other studies are implemented before using these isolates that have been diagnosed for their salt tolerance property in Pea plants. Thus, this research mainly aimed towards estimating the potential impacts of these three salt-tolerant isolates on positive changes in various morphological parameters of growth, and biochemistry, including the

carbohydrate, protein, reducing sugars, chlorophylls, phenols, flavonoids, and antioxidant defense system, in Pea plants grown under NaCl conditions.

2. Materials and Methods

2.1. Bacterial strains.

Bacillus sp. such as RhStr_71, RhStr_223, and RhStr_JH5, were previously isolated from rhizospheric soil from North India and was used throughout this study.

2.2. *In vitro* characterization of bacterial isolates for plant growth-promoting (PGP) traits and determination of maximum tolerance level (MTL).

The selected three strains were characterized for their PGP traits. IAA assay was done using L-tryptophan/mL and Salkowski's reagent earlier described by [25]. Tri-calcium phosphate (TCP) solubilization quantitatively for phosphate solubilization in a liquid medium is also described [26]. Ammonia production was determined using Nessler's reagent with slight modifications as per Goswami's method [27]. Siderophore production was detected using the Chrome azurol S agar (CAS) medium described by [28].

The MTL level of the selected isolates toward salinity (NaCl) stress was screened using nutrient broth (NB) with dissimilar levels of NaCl salt (1, 2, and 3% NaCl w/v) with varying time intervals at 600nm [29].

2.3. Evaluation of PGP activity of PGPR under NaCl stress condition in *Pisum sativum*.

Based on *in vitro* plant growth-promoting assays, these bacterial isolates were further selected to evaluate their effect on Pea seed at the germination phase in the presence of different salinity levels. For Bacterial inoculum preparation, overnight grown bacterial cells having CFU 1×10^8 cells/ml were harvested by centrifugation (6000 rpm for 10 minutes) and were washed twice with 50mM phosphate saline buffer (PBS) and re-suspended thereof in 1:1 in distilled water (d/w) to maintain the uniform cell density of 1×10^8 CFU ml^{-1} . For plant trial assay, Pea seeds were then surface sterilized with d/w followed by 0.1% of HgCl_2 . The seeds were bacterized by coating with the culture suspension of selected strains. The seeds were air-dried overnight in a Laminar Air Flow under sterile conditions to perform pot experiments.

2.4. Seedlings parameters.

The germinated seeds were measured once per day (simultaneously) until the end of the 10th day after seeding. The germination percentage (GP) was calculated according to the International Seed Testing Association (ISTA) method:

$$\text{GP} = \text{number of normally germinated seeds} / \text{total number of seeds sown} \times 100$$

The germination index (GI) was calculated using the following formula:

$$(\text{GI}) = \Sigma(\text{Gt}/\text{Tt}),$$

where, Gt is the number of seeds germinated on day t, and Tt is the number of days [30].

Other growth parameters such as Mean germination time (MGT), total germination (TG)%, coefficient of the velocity of germination (CVG), Germination rate index (GRI), and Vigour index were also measured [31].

2.5. Greenhouse experiment.

The tyndallized autoclaved soil was then filled, and seeds were then sown in sterile thermo coal pots (5.95 cm x 5.3 cm x 4.2 cm). There were five replicates of each treatment, such as control (normal) and bacteria-inoculated (RhStr_71, RhStr_223, RhStr_JH5) pots in normal and bacteria with salt amended soil. The salinity stress was artificially induced by adding 1% NaCl to the soil. The pots were kept in greenhouse conditions and irrigated with distilled water when required. The plants were monitored regularly, and after 30 days of sowing, plants were harvested to examine the shoot and root length; fresh and dry weights. The harvested plants were further studied to determine the changes in biochemical parameters.

2.6. Biochemical analysis.

A 100mg of the fresh shoot were homogenized in 2ml of a 50 mM ice-chilled phosphate buffer (pH 8.0) in a cool mortar and pestle. The homogenate was further centrifuged at 12,000 (x g) for 12 minutes at 4°C. The supernatant was additionally used for enzymatic assays. Total sugar content was evaluated using the method of [32], and reducing sugars were estimated by following Somogy's method as modified by [33] taking glucose as a standard. The protein concentration was estimated as per the method given by [34] using bovine serum albumin (BSA) as a standard. The chlorophyll content of the leaves was estimated according to [35]. The proline content in the shoot was measured via reaction with ninhydrin [36]. It was estimated by equating with a standard curve of L-proline (Sigma-Aldrich, USA) standard. The total phenols in plant samples were determined by the Folin– Ciocalteu method given by [37] using gallic acid as a standard. The quantitative estimation was performed spectrophotometrically by the aluminum chloride method based on the formation of complex flavonoid- aluminum [38], taking quercetin as a standard.

2.7. Antioxidant enzymes activity.

The enzymatic activities of antioxidants such as Superoxide dismutase (SOD), catalase (CAT), Ascorbate peroxidase (APX), and Peroxidase (POX) were determined. SOD activity was estimated as per the method described by [39]. SOD activity was determined by [40] and the enzyme's ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT) on blue formazan, followed by monitoring the absorbance of the reaction mixture at 560 nm. The catalase activity was determined by observing the disappearance of H₂O₂ at 240nm after adding enzyme extract to the reaction mixture [41] and was quantified by using the extinction coefficient (3.99 mm⁻¹cm⁻¹). Peroxidase assay was determined by [42] and quantified using the extinction coefficient (2.13 mm⁻¹cm⁻¹). POX activity was defined as mmol H₂O₂ decomposed ml⁻¹min⁻¹. APX activity of total shoot was estimated at 290 nm by the method described by [43]. The activity of ascorbate peroxidase was determined based on the reduction in absorbance at 290nm (absorbance coefficient 2.8mM⁻¹cm⁻¹) as ascorbate was oxidized. Lipid peroxidation is easily detected by quantifying the concentration of malonyl di-aldehyde (MDA), a by-product of the process as per the method prescribed by [44].

2.8. Statistical analysis.

The experimental values are presented as the mean of three replicates. The results were expressed as mean, and error bars represent the standard deviation (\pm SD) and the mean (\pm SEM) standard error. Multiple comparisons were conducted using one-way ANOVA analysis, and <https://biointerfaceresearch.com/>

data were compared using "Tukey's Multiple Comparison Test" performed by using GraphPad Prism (GraphPad In-Stat version 5.00, GraphPad Software, San Diego, CA, USA). Different letters in graphs indicate significant differences between treatments ($P < 0.05$), while the same values indicate the non-significant between the samples.

3. Results and Discussion

3.1. PGP traits of bacterial strains.

The selected bacterial strains were positive for PGP traits. Among them, bacterial isolates RhStr_71 (*Bacillus subtilis*) shows the highest activity towards PGP traits compared with other strains (Table 1).

Table 1. Characteristics of PGPR. RhStr_71 (*Bacillus subtilis*), RhStr_223R (*Bacillus safensis*), RhStr_JH5 (*Bacillus cereus*).

Bacterial strains	Test for salt-tolerant bacterial isolates				NCBI gene accession No.
	IAA (with tryptophan) ($\mu\text{g mL}^{-1}$)	P-solubilization ($\mu\text{g/mL}^{-1}$)	Ammonia production	Siderophore production (%)	
RhStr_71	79.0 \pm 2.68	84 \pm 3.1	+	17.1 \pm 0.9	KT429585.1
RhStr_223R	57.6 \pm 3.14	72 \pm 2.2	+	26.7 \pm 0.8	KT751329.1
RhStr_JH5	36.8 \pm 3.65	60 \pm 1.7	+	30.6 \pm 0.4	KT429594.1

(+) = Positive production; Indole acetic acid (IAA); Phosphate solubilization. Values are mean of three replicates \pm standard deviation (SD).

3.2. Determination of maximum tolerance level (MTL) of NaCl by PGP bacteria.

The result shows that 2% NaCl (w/v) did not affect the bacterial count on the growth of *Bacillus* isolates, whereas on increasing the salt concentration, a decrease in OD value was obtained, resulting in the substantial inhibition of bacterial growth (Figure 1) and on inoculation of 3% NaCl in NB some of the bacterial isolates inhibit the bacterial growth.

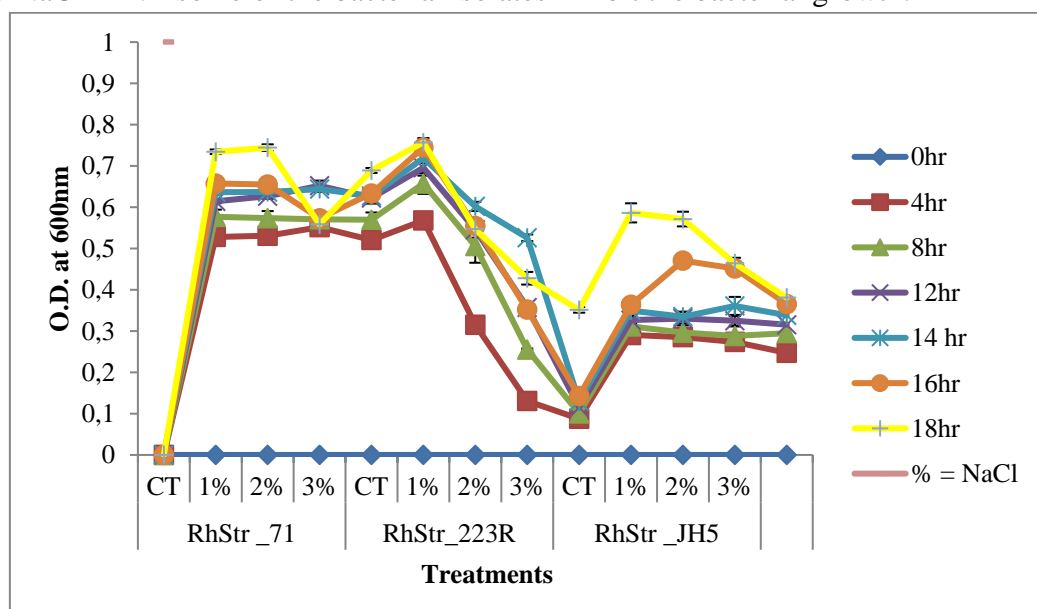


Figure 1. Growth curve analysis of bacterial strains (RhStr_71, RhStr_223R, RhStr_JH5) under different NaCl stress. CT: no salt inoculation; RhStr_71 (*Bacillus subtilis*), RhStr_223R (*Bacillus safensis*), RhStr_JH5 (*Bacillus cereus*): Bacterial treatments; 1%, 2%, and 3%: different NaCl concentration. Values are the mean of three replicates. Error bars represent the standard deviation.

3.3. Seedlings parameters.

Results showed that salinity had an adverse effect on seedling in *Pisum sativum*. The bacterial isolates further increase the plant growth-promoting attributes such as G%, GI, MGT, TGP, CVG, GRI, and VI in Pea seedlings (Table 2) and RhStr _71 isolates in most significant among them.

Table 2. Effect of PGPRs on seedling parameters of Pea seedlings at 1% NaCl concentration along with uninoculated (control). CT: Control without bacterial inoculation; RhStr _71, RhStr _223R, RhStr _JH5: *Bacillus* sp. isolates; S1: 1% NaCl.

S.No	Treatments	Germination % (G%)	Germination index (GI)	Mean Germination Time (MGT)	Total Germination % (TGP)	Coefficient of Velocity of Germination (CVG)	Germination Rate Index (GRI)	Vigour Index (VI)
1.	CT	48	166.67	4.17	24	5.6	1593.26	1121.09
2.	RhStr _71	104	366.67	9.01	60	24.78	3333.33	4809.04
3.	RhStr _223R	60	200.01	5.01	32	6.49	1829.37	3496.41
4.	RhStr _JH5	53	191.67	4.21	28	8.28	1809.52	2248.81
5.	S1	19	83.33	1.67	16	1	543.65	370.29
6.	RhStr _71+S1	59	233.33	5.37	44	9.88	1970.24	2655.58
7.	RhStr _223R+S1	24	100.06	2.15	20	1.55	682.54	1324.57
8.	RhStr _JH5+S1	18	83.33	1.64	16	1.35	562.32	603.022

3.4. Greenhouse experiments.

The greenhouse (pot experiment) experiment was conducted to determine the effect of selected strains on the growth of *Pisum sativum* under salinity stress.



Figure 2. Effect of inoculated PGPRs on *Pisum sativum* under 1% NaCl saline condition. (a) CT- control without bacterial inoculation, RhStr _71- PGPR strain (*Bacillus subtilis*), S1- 1% salt stress, RhStr _71+S1- PGPR+NaCl salt; (b) CT- control without bacterial inoculation, RhStr _223R- PGPR strain (*Bacillus safensis*), S1- 1% salt stress, RhStr _223R+S1- PGPR+NaCl salt; (c) CT- uninoculated; RhStr _JH5- PGPR (*Bacillus cereus*); S1- 1% salt stress, RhStr _JH5+S1- PGPR+NaCl salt.

Table 3. Effect of bacterial isolates on Pea plants after 30 DAS at 1% NaCl concentration compared to uninoculated (control). CT- Control without bacterial inoculation and salt; RhStr_71, RhStr_223, and RhStr_JH5 - PGP isolates; S1- 1% Salt stress, RhStr_71+S1, RhStr_223+S1, and RhStr_JH5+S1- bacterial isolates+salt inoculated plants with salinity stress.

S.No.	Treatments	Fresh weight (gm)		Dry weight (gm)		Length (cm)	
		Shoot	Root	Shoot	Root	Shoot	Root
1.	CT	0.65±0.04 ^a	0.58±0.04 ^a	0.07±0.007 ^a	0.062±0.002 ^a	9.98±0.27 ^a	7.33±0.88 ^a
2.	RhStr _71	1.22±0.14 ^b	1.27±0.07 ^b	0.15±0.004 ^b	0.16±0.01 ^b	14.73±0.37 ^b	17.41±0.82 ^b
3.	RhStr _223R	0.94±0.11 ^{abc}	0.89±0.06 ^{ac}	0.08±0.002 ^a	0.07±0.005 ^a	11.51±0.36 ^{ac}	12.41±0.96 ^c
4.	RhStr _JH5	0.89±0.03 ^{abc}	0.65±0.05 ^{ad}	0.07±0.003 ^{ac}	0.061±0.002 ^a	10.70±0.81 ^{acd}	10±0.58 ^{ac}
5.	S1	0.36±0.05 ^{ad}	0.27±0.09 ^e	0.04±0.005 ^d	0.04±0.002 ^a	4.41±0.66 ^e	4.41±0.37 ^{ae}
6.	RhStr _71+S1	0.88±0.06 ^{abc}	0.92±0.05 ^{cd}	0.08±0.003 ^{ac}	0.11±0.009 ^c	11.10±0.49 ^{acd}	13.15±1.16 ^c
7.	RhStr _223R+S1	0.71±0.08 ^{acd}	0.47±0.05 ^{ad}	0.05±0.003 ^{cd}	0.07±0.002 ^a	8.67±0.33 ^{ad}	8.33±1.45 ^{ac}
8.	RhStr _JH5+S1	0.48±0.07 ^{ad}	0.36±0.06 ^{ae}	0.05±0.003 ^d	0.05±0.007 ^a	7.47±0.29 ^f	7.15±0.46 ^a

Values are the mean of three replicates ± standard error of the mean (SEM). Different letters indicate statistically significant differences between treatments (Tukey's multiple range test $P < 0.05$).

Pisum sativum L. fresh weight, dry weight, and Length on root and shoot were evaluated in salt stress's appearance and non-appearance. Results showed that 1% NaCl had an adverse effect on plant growth parameters, thereby reducing the fresh weight (FW) and dry weight (DW) and shoot (SL), and root length (RL) of Pea plant (Figure 2 and Table 2). Among these isolates, RhStr_71 strain showed a significant difference in plant growth characteristics (Table 2) compared to non-inoculation as a control. Thus we concluded that salinity stress could adversely affect plant growth, but on inoculation, these isolates may reduce the inhibitory effect of the salt.

3.5. Biochemical parameters analysis.

3.5.1. Total sugar, reducing sugar and protein.

Analysis of total sugar, reducing sugar, and protein content showed that NaCl salt's presence causes a reduction in the total sugar, reducing sugar, and protein contents in *Pisum sativum*. While on bacterial inoculation, they were considerably higher compared to stressed control, which led to the improvement of plant growth and biomass production.

3.5.2. Photosynthetic pigments (chlorophyll a, chl b total chl and carotenoids).

When the plants are grown with PGP bacteria under saline conditions, the plants have the highest chlorophylls, carotenoid contents, and healthy leaves compared to NaCl induced plants. The chlorophyll a, chlorophyll b, total chlorophyll and carotenoids contents were 0.35 mg g⁻¹ FW, 0.27 mg g⁻¹ FW, 0.45 mg g⁻¹ FW and 0.63 mg g⁻¹ FW (Figure 3b) respectively in plants with PGP bacteria under salt-stress conditions.

3.5.3. Flavonoids, phenol, and proline.

The application of these PGP bacteria resulted in increasing the flavonoids and phenolic contents in *Pisum sativum*. Among the 3 isolates, RhStr_71 significantly increases the phenolic content. The data conferring in Figure 3(c) also shows that salinity stress significantly induced the proline biosynthesis. The high level of proline accumulation leads to the induction of osmotic stress, thereby causing damage to the plant membrane. However, a significant increase was observed when the plants were exposed to NaCl stress. Therefore, bacterial inoculation with RhStr_71, RhStr_223, and RhStr_JH5 was much more effective in alleviating salinity's adverse effect in *Pisum sativum*.

3.6. Antioxidants assay.

To overcome the deleterious effect of ROS, the plant cells have developed the antioxidants machinery. The antioxidants enzymatic activities were evaluated in un-inoculated and inoculated plants under salinity stress treatment. The antioxidants enzymatic activities of SOD, CAT, POX, and APX in Pea plants were lower in un-inoculated and salt inoculated plants than PGPR inoculated. Application of NaCl (salt) stress leads to induction of antioxidant enzymes irrespective of PGP bacterial inoculation. However, antioxidant activity was higher when treated with PGPR isolates (Figure 4) than in un-inoculated plants. The plant growth-promoting bacteria inoculation of the Pea plant had significantly decreased the lipid peroxidation (LPX) level in the shoot (1% NaCl), which proposes that the membrane structures in Pea plant tissues are significantly less prone to the factors which are responsible for peroxidation.

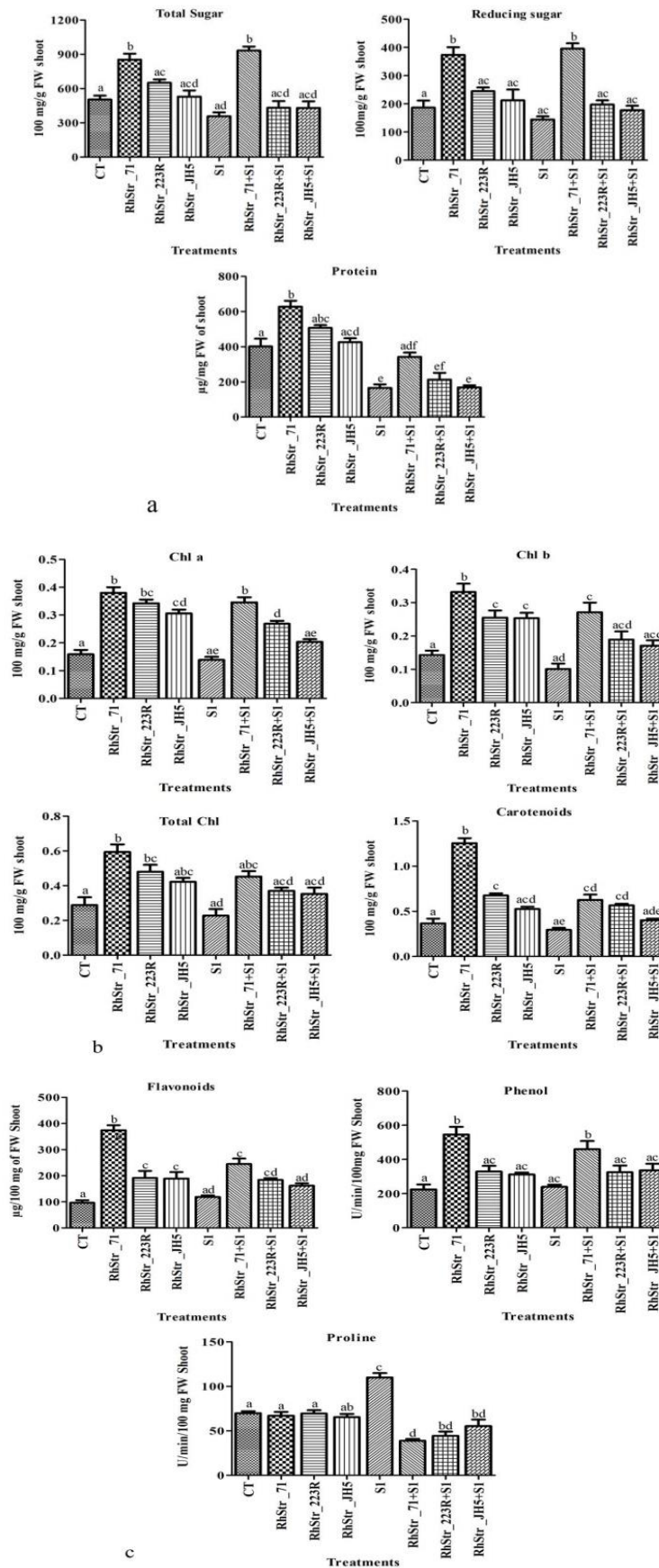


Figure 3. Effect of PGP bacteria on (a) total sugar, reducing sugar and protein; (b) chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids; (c) flavonoids, phenol, and proline content in Pea shoot under salt-stress conditions. Similar letters represent the non-significant among the sample. Error bars represent \pm standard error mean. CT-control; RhStr_71, RhStr_223, and RhStr_JH5 - PGP isolates; S1- 1% Salt stress.

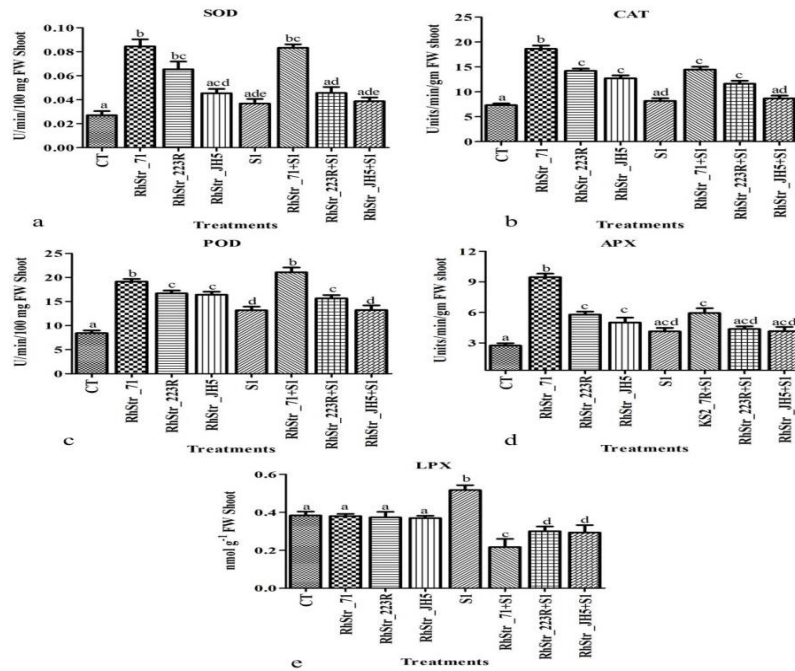


Figure 4. Effect of beneficial microbes and salt on antioxidants enzymes of *Pisum sativum* plants. (a) Superoxide Dismutase (SOD) activity, (b) Catalase (CAT) activity, (c) Peroxidase (POD) activity, (d) Ascorbate Peroxidase (APX) activity, and (e) Lipid peroxidase (LPX) activity. Data are presented as a mean of 3 replicates. Different letters denote significance amongst treatment groups according to Tukey’s test ($P \leq 0.05$). Error bars represent \pm standard error mean. CT-control; RhStr_71, RhStr_223, and RhStr_JH5 - PGP strains; S1- 1% salt stress.

3.7. Discussion.

Our results discovered that these PGP isolates did not decrease their bacterial count at 2% NaCl (w/v), but the bacterial count decreased by increasing the NaCl concentration. Thus, it shows the adverse effect of NaCl salt on bacterial cell counts. As described in this work, the inoculation of *Bacillus* sp. is found to increase the entire seedling, morphological, and biochemical parameters under NaCl stress conditions. These bacterial isolates' beneficial effect in promoting plant growth is due to their PGP traits like IAA, P-solubilization, ammonia, and siderophore production. Similar PGP activities were also reported by [45].

Salinity has an adverse inhibited effect on seed germination due to osmotic/ionic effects [46], resulting in the disturbed water uptake, thereby causing the [47]. The unfavorable effect of NaCl on seed germination and its growth parameters has also been committed by many other scientists [47, 48]. By observing our study, salinity has a considerable effect on seedlings, their germination, germination time and rate, seedling vigor index, fresh and dry biomass, root and shoot lengths, [49], etc. and so the hypocotyl and plant shoot length is noted both in the bacterial inoculated and un-inoculated treatments. This work may have actual consequences in the agriculture sector as these factors are responsible for determining plant productivity [50]. It has also been reported by [51, 52] that salinity has more adverse effects on shoot compared to roots [53]. Many research also validated that PGPR inoculation on pepper seeds exhibited higher morphological parameters such as plant height, greater root length, larger leaf size, and an increase in dry matter in saline soils [54].

These bacterial inoculations can also increase the chlorophyll contents in the host (Pea) plant. A similar result has also been informed by [55] and thereby confirming the presented

results in this work. Enhanced chlorophylls content in the PGPR inoculated plant also led to increased synthesis of photo-assimilates [56], as well as changes in Nitrogen, Phosphorous, and Potassium uptake [57].

To understand their inhibitory effect on plant growth, we further perform several biochemical assays such as total sugar, reducing sugar, protein, chlorophylls, carotenoid, phenol, and flavonoids content. The antioxidants enzymatic activity was also performed to analyze the level of proline, SOD, CAT, POD, APX, and LPX. Each of the assays considered different mechanisms to respond towards salinity stress. The salt stress may lead to oxidative stress, thereby causing photo-oxidative damage, which leads to photo-inhibition in leaves [58].

The increase in phenol and flavonoid contents under NaCl stress revealed its protective role towards plants by activating the cell signaling process, thereby up-regulating the phenylpropanoid pathway and increasing the ROS scavenging enzymes. The increased flavonoid level in plants also reduces the harmful hydroxyl radicals in plant cells generated due to abiotic (saline) stress. The increased proline concentrations under NaCl stress have also been reported in many plant species, such as tomatoes [59], *B. juncea* [60], and are considered as a primary defense response linked with the changing osmotic pressure in the plant cells [61]. Inoculation of *Bacillus* isolates (RhStr_71, RhStr_223, and RhStr_JH5) revealed a decreased proline level, in-plant shoot growing under saline condition. Lowering of proline content in Pea plants by PGP bacterial inoculation has also been reported by [56]. In order to curb the oxidative damages, plants had developed antioxidant mechanisms involving enzymes such as SOD, CAT, POD, APX, and LPX [62, 63]. Inoculation of bacterial strains had a similarly significant effect on antioxidant machinery, thereby protecting the plants from oxidative burst [64, 65]. This is the first study to examine the effect of these isolates on plant-microbe interactions under salinity stress conditions. The hypothesis tested during this study estate that RhStr_71 (*Bacillus subtilis*), RhStr_223R (*Bacillus safensis*), and RhStr_JH5 (*Bacillus cereus*) supplementation will boost Pea plant growth, thereby correcting ion imbalance, conquering the oxidative stress level, augmenting osmoprotectants, and antioxidants activities. These obtainable results will play an indispensable role in attenuating salinity stress.

4. Conclusions

Inoculation of these PGP *Bacillus* isolates under abiotic (salt) stress conditions leads to increased plant growth parameters with a simultaneous increase in antioxidants enzymes, thereby decreasing the oxidative stress. The work further indicated the potential activity of PGP bacteria for Pea growth promotion under NaCl stress. Therefore we concluded that PGP bacterial isolates such as *Bacillus subtilis* RhStr_71, *Bacillus safensis* RhStr_223, and *Bacillus cereus* RhStr_JH5 supplementation will boost the Pea plant growth by correcting ion imbalance, suppressing oxidative stress level, maximizing osmoprotectants and antioxidants activities. These obtainable results play an essential role in attenuating salinity stress in agriculture. The knowledge gained from this study will help to boost the salt stress control management strategies for essential crops in difficult or stressed environments.

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Conflicts of Interest

The authors declare no conflict of interest.

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