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Phosphate-Solubilizing Salt-Tolerant Bacteria to Support Soybean Growth (*Glycine max* (L.) Merr.) On Saline Soils

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Abstract: Phosphorus is one of the important elements of plant nutrition that affect crop productivity. Increasing the availability of phosphorus through the use of microbial inoculants capable of stimulating soybean growth on saline soils is a promising solution to the problem. However, most phosphate-solubilizing bacteria are not capable of the growth and mobilization of phosphates on saline soils. The purpose of this study was to isolate phosphate-solubilizing salt-tolerant bacteria from the soybean rhizosphere and to study their salt tolerance, phosphate solubilization, and ability to influence soybean growth in saline soils. Phosphate-solubilizing isolates were obtained from the soybean rhizosphere. Their ability to grow and mobilize phosphates under conditions of high salt stress was studied, after which five strains were selected. Identification by the Sanger molecular genetic method showed that the strains were *Pseudomonas rhizosphaerae* FT2, *Ps. koreensis* FT4, *Ps. sp.* FM9, *Bacillus pumilus* FM12, and *B. sp.* FC11. The production of the phytohormone indole-3-acetic acid under high salt stress was studied and three strains (FT4, FM12, and FC11) were selected in which the production of indole-3-acetic acid under salt stress decreased slightly by 20-22%. Inoculation with bacteria significantly mitigated salt stress, as evidenced by the growth indicators of soybeans; the weight of roots increased by 1.9-2.1 times, the weight of soybean shoots by 2.0-2.2 times, the weight of leaves by 2.5-3.5 times and the number of nodules by 1.7-2.0 times compared to the control variant without inoculation. The study showed that inoculation with phosphate-solubilizing salt-tolerant bacteria increased plant resistance to salt stress, improved growth, and promoted the ecological adaptation of soybeans. Based on the isolated strains, it is possible to develop inoculants for crops growing on saline soils, which is a promising strategy.

Keywords: Phosphate-Solubilizing Bacteria, Salt Tolerance, Inoculation, Resistance, Growth Stimulation

Introduction

Soy proteins account for half of the global consumption of vegetable proteins. The nodules on the roots of soybeans fix atmospheric nitrogen and contribute to an increase in the nitrogen content in the soil. Therefore, soy is used for both food production and crop rotation, too (Cai *et al*., 2019). At the same time, soy does not belong to salt-resistant crops and soil salinity is one of the main factors constraining its production, since salinity causes physiological and biochemical disorders of plant development, such as inhibition of seed germination and plant growth, exacerbates chlorosis and inhibits nodule formation and nitrogen fixation (Ren *et al*., 2016; Sadak *et al*., 2020). The method of mitigating the salt stress of plants is inoculation with salt-resistant bacteria present in the rhizosphere of plants and stimulating their growth Plant Growth Promoting Rhizobacteria, (PGPR).

The main elements of plant nutrition are nitrogen and phosphorus (Chen *et al*., 2020; 2022). Phosphorus is important for plants, as it participates in photosynthesis, enzyme activation, and Adenosine Triphosphate (ATP) formation (Savala *et al*., 2021). Its deficiency causes a decrease in nitrogen fixation and crop yield (Matse *et al*., 2020). In soil, phosphorus compounds are mainly found in a form inaccessible to plants (Bhat *et al*., 2017; Bekele *et al*., 2020). The problem of phosphorus deficiency in highly saline soils is particularly serious (Alori *et al*., 2017). It has been proven that soil salinity significantly reduces its bioavailability to plants (Penn and Camberato 2019; Dey *et al*., 2021; Xie *et al*., 2022). Phosphorus deficiency in saline soils causes symptoms of phosphorus deficiency in plants much faster than in unsalted soils (Isidra-Arellano *et al*., 2018; Tang *et al*., 2019).

For Kazakhstan, soy is one of the main protein/oilseed crops with a wide range of applications in the food, feed, technical, and medical industries. Taking into account the high nutritional value and protein content, soy is defined as a strategically important crop (Didorenko, 2021). The main region of soybean production is the Almaty region of Kazakhstan. The soils of this region are characterized by varying degrees of salinity and low content of available phosphorus, which reduces its yield. To increase the productivity of soybeans, chemical phosphorus fertilizers are applied. However, their high cost and the threat to the environment (pollution of water, soil, etc.,) motivate the search for an alternative in which natural sources of phosphorus will serve as its reservoir (Ali *et al*., 2019).

The main research on the mitigation of the effect of salinity on legumes, in particular soybeans, focuses on the use of salt-resistant soy symbionts, i.e., rhizobia (Etesami and Beattie, 2018; Ali *et al*., 2022). However, there are other useful rhizobacteria in the soybean rhizosphere that promote plant growth and help with salt stress. One of these is phosphate-solubilizing bacteria. These bacteria can serve as an alternative to phosphorus-based fertilizers. Phosphate-solubilizing bacteria present in the plant rhizosphere can convert insoluble phosphates into a form accessible to plants, thus improving the phosphorus nutrition of plants and stimulating their growth (Timofeeva *et al*., 2021). In addition, phosphorus is especially important for soy symbionts (rhizobia), since with phosphorus deficiency, rhizobia cannot colonize roots, form nodules, and actively fix nitrogen (Li *et al*., 2021). An increase in root growth and the number of soybean nodules have been reported during inoculation with phosphate-solubilizing bacteria (Janati *et al*., 2021; Berza *et al*., 2022; Shome *et al*., 2022).

In this regard, the search for inexpensive and environmentally friendly microbial inoculants to increase the availability of phosphorus is very relevant. However, not all phosphate-solubilizing microorganisms can actively grow and mobilize phosphorus compounds in saline soils. Most of them have a low resistance to salinization and cannot be applied as inoculants for use in saline soil (Joshi *et al*., 2021). Thus, although the use of phosphate-solubilizing bacteria can be an efficient, environmentally friendly, and cost-effective replacement for phosphorus fertilizers, their potential in saline soils has not been sufficiently studied.

The purpose of this study was to isolate phosphate-solubilizing salt-tolerant bacteria from the soybean rhizosphere and to study their salt tolerance, phosphate mobilization, and ability to influence soybean growth in saline soils.

Materials and Methods

Location of the Study

Soil samples were collected from June 28-July 30, 2021, in the South-East of Kazakhstan in the Almaty region, Enbekshikazakh district, in the fields in the vicinity of the Turgen village, with the following coordinates: N 43°39'36.24", E 77°58'87.21", 365 m above sea level. Soil samples were taken in the rhizosphere of the Eureka soybean variety growing in a saline field during the flowering phase of soybeans. The type of soil is ordinary serozem and the main physicochemical properties of the soil are given in Table 1.

It follows from Table 1 that the soil is highly saline, the total salt content in the water extract is 1.051±0.06% and the pH value is 9.3±0.02.56 soil samples were collected from the rhizosphere of healthy soybean plants. Soil samples for the isolation of microorganisms were collected in compliance with the rules of asepsis and stored in sterile conditions before analysis.

Isolation and studies of phosphate-solubilizing bacteria were carried out in laboratory conditions in 2021-2022 in Almaty, Kazakhstan.

The present study included the following tasks: Obtaining phosphate-solubilizing isolates from the rhizosphere of soybeans growing on saline soils; primary screening of isolates for the ability to mobilize phosphates under salinization conditions; study of growth and resistance of isolates to salt stress in a range of salt concentrations; determination of the phosphate-solubilizing ability of isolates under salt stress of varying degrees; production of metabolites that promote plant growth; study of the main morphological and biochemical properties of bacteria and identification of bacteria by molecular genetic method; and study of the ability of phosphate-solubilizing salt-tolerant bacteria to enhance plant growth, stimulate nodulation and increase the resistance of soybeans to salt stress by evaluating growth parameters.

Table 1: Basic physical and chemical properties of the soil

Indicators	Content
Total humus (%)	0.860±0.050
Hydrolyzed nitrogen (mg/kg)	56.400±0.030
Available phosphorus (mg/kg)	25.200±0.020
Available potassium (mg/kg)	473.400±9.100
Soil temperature (°C)	26.400±0.130
Moisture content (%)	35.800±0.910
pH	9.300±0.010
Cl^- (mm (eq)/100 g)	0.032±0.001
SO_4^{-2} (mm (eq)/100 g)	0.517±0.001
Ca^{+2} (mm (eq)/100 g)	0.095±0.002
Mg^{+2} (mm (eq)/100 g)	0.051±0.002
Na^{+2} (mm (eq)/100 g)	0.064±0.001
Total salt (%)	1.054±0.060

Phosphate Solubilizing Activity of Bacteria Under Salt Stress

To study the phosphate-solubilizing activity, the bacteria were grown on an NBRIP liquid medium at 28°C 180 rpm for three days. NBRIP agarized medium was poured into Petri dishes with the addition of 100, 250, or 500 mm NaCl, after solidification, wells were punched in the agar, where an equal amount of a suspension of bacteria (0.1 mL) with a concentration of 1×10^8 Colony-Forming Units (CFU)/mL was introduced. The cups were placed in a thermostat at 28°C and kept until clear transparent zones of tricalcium phosphate solubilization (halo zones) appeared around the wells (3 days). The presence of a clear halo around bacterial colonies indicates the ability of the strain to dissolve mineral phosphates. Negative control was provided by wells into which a medium without bacteria was introduced. The study was carried out three times.

Salt Tolerance of Phosphate-Solubilizing Bacteria

The salt tolerance of phosphate-solubilizing bacteria was determined in a liquid medium with NBRIP containing 100, 250, and 500 mm of NaCl. The bacteria were grown for 3 days at 28°C and 180 rpm. Bacterial growth was determined on a spectrophotometer (PD-303, "Apel", Japan) at 540 nm. Bacterial growth was evaluated on a scale where-no growth, + presence of growth and the addition of + indicates the intensity of growth. The study was carried out three times.

Production of Indole-3-Acetic Acid (IAA) by Phosphate Solubilizing Bacteria

The bacteria were grown on a Lysogeny Broth (LB) medium under salt stress, which was created by NaCl concentrations of 100, 250, and 500 mm a medium without the addition of NaCl was used as the control variant. Tryptophan was added to bacterial cultures and incubated at 28°C on a shaker at 180 rpm for 5 days. Indole-3-Acetic Acid (IAA) was determined using a gas chromatograph (Agilent technology 7890B, USA) following the protocol given by Hernández-León *et al*. (2015). The experiments were carried out in five-fold repetition.

Effect of Phosphate-Solubilizing Bacteria on Soybean Growth under Soil Salinity Conditions

The seeds were planted in 5,000 mL vegetative vessels with saline soil, three plants per vessel. The soil type is ordinary serozem, humus content 0.86±0.03%, hydrolyzed nitrogen 58.6±0.01 mg/kg, available phosphorus 24.8±0.01 mg/kg, and available potassium 468.7 mg/kg. The total salt content is 1.058±0.04%, and the pH value is 9.2±0.01. After 3 months, the plants were collected, the number of nodules was counted, the nodules, roots, shoots, and leaves were dried to a constant weight and their dry weight was determined. The studies were carried out in 5-fold repetition.

Statistical Analysis

The statistical significance of the results obtained was analyzed using the statistica 10.0, var. 6.0 software package. The differences were considered significant at $p<0.05$ and the values are presented as Mean (M) ± standard deviation (± SEM) (Gómez-de-Mariscal *et al*., 2021).

Results

Active Phosphate-Solubilizing Salt-Tolerant Isolates

56 soil samples were collected to isolate phosphate-solubilizing salt-resistant bacteria from the rhizosphere of healthy soybean plants growing on saline soils. When seeding on an NBRIP medium containing tricalcium phosphate and 10% NaCl, colonies showing clear zones of phosphate mobilization (halo zones) were selected (Fig. 1).

Fig. 1: Obtaining isolates of phosphate-solubilizing salt-tolerant bacteria on NBRIP medium with 10% NaCl (zones of phosphate mobilization (halo) around the colonies are visible

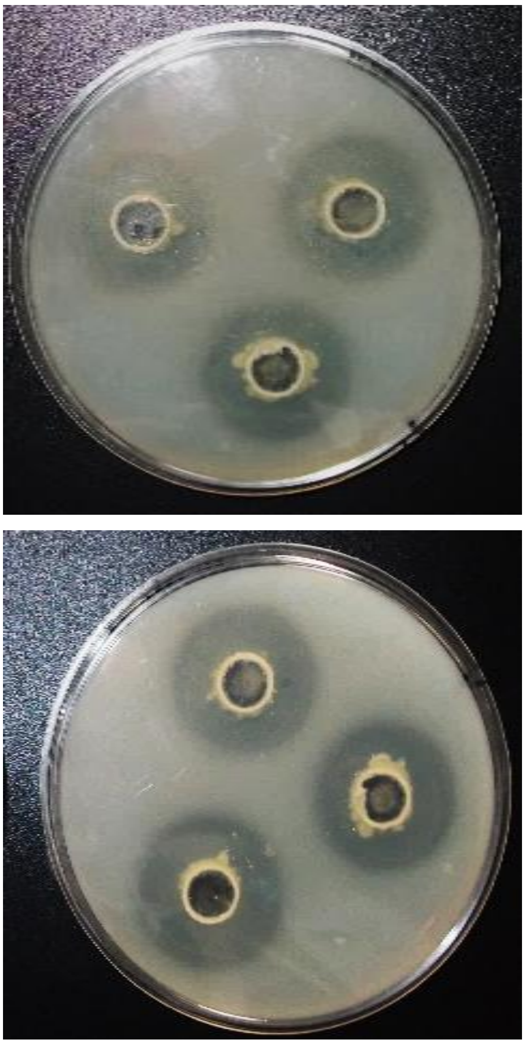

Fig. 2: Screening of active phosphate-solubilizing salt-tolerant isolates on NBRIP medium with 10% NaCl

As a result of the work carried out, 32 isolates of salt-resistant phosphate-solubilizing bacteria were isolated. The initial screening of isolates allowed us to select 12 isolates that formed the largest phosphate mobilization zone in a shorter period Fig. 2.

The study of the main morphological and biochemical features of phosphate-solubilizing salt-tolerant bacteria showed that the colonies of isolated isolates were round with a smooth profile and a smooth edge, from 0.9-2.5 mm in diameter, with a fine-grained structure. The color of the colonies was mainly milky, creamy, and pale yellow and the consistency varied. In the study of cell morphology, it was found that the bacteria were both spore-forming and non-spore-forming, mainly rod-shaped. Some bacteria acquired a coccoid shape with age. The bacteria were gram-positive and gram-negative and were characterized by cell mobility. The study of biochemical properties showed that the isolated bacteria were aerobic and catalase positive. It was found that the isolates had a different ability to use carbon compounds, form indole, hydrogen sulfide, and dilute gelatin. According to the main morphological and biochemical characteristics, the isolated phosphate-mobilizing bacteria were assigned to the genera *Bacillus* and *Pseudomonas*.

Salinity Tolerance of the Isolates

Further, the resistance of isolates to salt stress was studied at different concentrations of NaCl (100, 250, and 500 mm). To study the phosphate-solubilizing activity, the bacteria were grown on an NBRIP liquid medium at 28°C and 180 rpm for three days. Table 2 shows the data of the 12 most salt-resistant isolates.

It follows from the data in Table 2 that not all isolates had high salt tolerance. Thus, at a concentration of 100 mm NaCl in the medium almost all isolates grew, while at a concentration of 500 mm NaCl, only five (FT2, FT4, FM9, FM12, and FC11) showed growth. These five isolates were selected as the most salt-resistant ones.

Table 2: Screening for salinity tolerance of the isolates

	NaCl concentration (mm)		
Isolates	100	250	500
FT-1	++	+	-
FT2	++++	+++	++
FT-15	++	+	-
FT4	+++	+++	++
FM9	++++	+++	++
FM12	++++	+++	+++
FL-27	++	+	-
FY-36	+++	+	-
FC11	++++	+++	+++
FT-34	++	+	-
FM-22	+++	+	-
FM-19	+++	+	-

Qualitative assessment: -Indicates the absence of the growth; + indicates the presence of the growth; additional + indicates the intensity of the growth exhibited by the isolates

Table 3: Phosphate solubilizing activity as influenced by the concentration of NaCl

Isolates	Concentration NaCl (mm) Zone of solubilization (mm) 0	100	250	500
FT2	32.6±0.2	26.6±0.7	21.9±0.1	15.8±0.2
FT4	37.4±0.1	33.4±0.2	28.4±0.3	26.9±0.3
FM9	32.9±0.4	28.9±0.1	22.9±0.1	15.9±0.1
FM12	36.2±0.2	30.2±0.4	26.8±0.2	24.8±0.1
FC11	36.2±0.2	32.5±0.2	27.7±0.2	25.7±0.2

Note: Values are means ± Standard Error of the Mean (SEM), n = 5; p< 0.05

Phosphate-Solubilizing Ability of Selected Isolates

The study of the phosphate-solubilizing ability of selected isolates under salt stress of various degrees was carried out on an NBRIP medium with different concentrations of NaCl (100, 250, and 500 mm). A medium without the addition of NaCl was used as the control variant. The data obtained are given in Table 3.

Table 3 shows that the bacteria could solubilize phosphates under salt stress. At a salt concentration of 100 mm in the medium, all isolates were characterized by high solubilization of phosphates and the zones of dissolution of phosphates were 31.4±1.2-37.4±1.1 mm (p<0.05). It was found that with an increase in the concentration of NaCl in the medium, the solubilization activity decreased. Thus, at high salt stress (500 mm NaCl) in two FT2, FM9 isolates, the phosphate solubilization potential decreased significantly (by 52-53%) compared to the control variant (p<0.05). However, in FT4, FM12, and FC11 isolates, salt stress did not have such a strong effect and solubilization activity decreased by only 28-30%.

The identification of FT2, FT4, FM9, FM12, and FC11 isolates was carried out by the Sanger molecular genetic method, by sequencing the 16S rRNA gene and comparing the nucleotide sequences of the 16S rRNA gene with strains from the GenBank National Center for Biotechnology Information (NCBI) database. It is shown that in phylogenetic terms, the strains FT2, FT4, and FM9 were closest to the *Pseudomonas* genus and were defined as *Pseudomonas rhizosphaerae* FT2, *Ps. koreensis* FT4 and *Ps. sp.* FM9, FM12, and FC11 strains belonged to the *Bacillus* genus and were identified as *Bacillus pumilis* FM12 and *B. sp.* FC11.

Production of IAA by Strains of Bacteria under Salt Stress

An important indicator in the selection of strains for use in agriculture is their ability to stimulate crop growth and development. One of the main phytohormones affecting plant growth is IAA. Therefore, strains FT2, FT4, FM9, FM12, and FC11 were analyzed for their ability to produce IAA under salinity conditions. To create salt stress, the strains were grown on a medium with different concentrations of NaCl (100, 250, and 500 mm). A medium without the addition of NaCl was used as the control variant. The data of the three strains with the highest IAA production are presented in Fig. 3.

Figure 3 shows that FT4, FM12, and FC11 strains were able to produce a significant amount of IAA phytohormone at salinity. At the same time, it was found that under conditions of severe salt stress (500 mm NaCl), the production of IAA by bacterial strains decreased slightly, by 20-22% compared to the control variant (salt-free medium). The maximum production of phytohormone with a high degree of salinity was shown by the FT4 strain (p<0.05). This indicates that FT4, FM12, and FC11 strains can be used as potential microbial inoculants for agriculture on saline soils.

Effect of Inoculation on the Number of Nodules

The study of the effect of inoculation of phosphate-solubilizing salt-tolerant bacteria on the growth of soybean plants and the formation of nodules under salt stress was carried out on highly saline soil. FT4, FM12, and FC11 strains were used for seed inoculation, and seeds without inoculation served as the control variant. The results obtained are presented in Fig. 4.

It was found that seed inoculation significantly reduced the negative effect of salt stress on soybean plants. After three months of growing soybeans in the inoculated variants, the plants looked taller, stronger, and greener compared to the control plants. It was found that the length of plants and the dry weight of shoots and leaves increased significantly. Thus, the dry weight of shoots had grown 2.0±0.02-2.2±0.04 times and the dry weight of the leaves had grown 2.5±0.2-3.5±0.4 times (p<0.05) compared to the control variant Fig. 4a. Besides, a significant increase in the dry weight of roots was found by 1.9±0.02 to 2.1±0.03 times and the number of nodules by 1.7±0.01 to 2.0±0.02 times per plant (p<0.05) Fig. 4b. The *Ps. koreensis* FT4 strain had the greatest positive effect on the formation of nodules, growth, and development of soybean plants. In this variant, the highest weight of roots, shoots, and leaves was noted and the number of nodules increased by 2.0±0.1 times Fig. 4b. Thus, it has been shown that inoculation with phosphate-solubilizing salt-tolerant bacteria promotes plant growth, stimulates root development and increases the number of nodules on them, which indicates significant mitigation of salt stress and ecological adaptation of soybean plants to soil salinity.

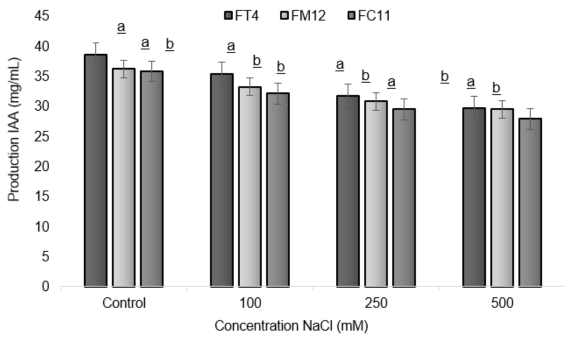

Fig. 3: Production of IAA by strains of bacteria under salt stress. Control without NaCl

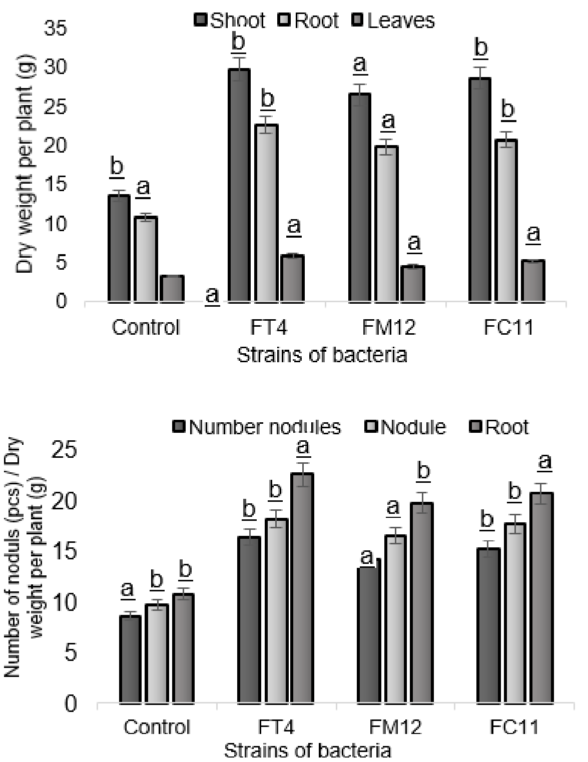

Fig. 4: Effect of inoculation on the number of nodules. The dry weight of nodules (a), dry weight of shoots, roots, and leaves (b) in highly saline soils. Control without inoculation by bacteria

Discussion

In our study, the primary screening of the obtained isolates demonstrated their ability to mobilize phosphates and allowed us to select 12 of the most promising ones, characterized by high solubilization. The study of the ability of the selected isolates to grow at a high degree of salinity allowed us to select five isolates (FT2, FT4, FM9, FM12, and FC11). These isolates not only withstood high salt stress but were characterized by active growth during salinization. Studies by several authors have also shown the resistance of bacteria isolated from soils and the rhizosphere of different agricultural cultures to salinization. Thus, out of 81 isolates obtained from saline soils, only three strains of *Bacillus paramycoides* HB6J2, *Bacillus amyloliquefaciens* HB8P1 and *Bacillus pumilus* HB4N3 were significantly salt-resistant (Sharma *et al*., 2021). In a similar study of 152 isolates, 50% demonstrated resistance to soil salinization, but only two strains of *Streptomyces* sp. PR-3 and *Bacillus* sp. PR-6 was selected as effective (Mahmood *et al*., 2019). This indicates that finding active strains of phosphate-solubilizing salt-resistant microbes is not always easy.

One of the important properties in the selection of bacteria is their ability to solubilize phosphates under salinization conditions. Behavioral studies have shown that FT2, FT4, FM9, FM12, and FC11 isolates actively mobilize insoluble phosphates even with a high degree of salinity. The most promising were three isolates (FT4, FM12, and FC11), which gave isolates that actively mobilized phosphates even under high salt stress (500 mm NaCl). In this case, their activity decreased by only 28-30%, compared with activity in unsalted conditions. This property of isolates is very interesting and important for use in agriculture. It can be assumed that they will be more successful as inoculants, which is due to their ability to survive under stress. In the future, it is planned to use these bacteria as effective biofertilizers to improve the phosphorus nutrition of crops, which will minimize the use of phosphate fertilizers.

Isolates FT2, FT4, FM9, FM12, and FC11, which showed active growth, high salt tolerance, and phosphate mobilization under high salt stress, were identified by the Sanger molecular genetic method. Amplification of the 16SrRNA gene sequence revealed that the FT2, FT4, and FM9 isolates were closest to the *Pseudomonas* genus and were identified as *Pseudomonas rhizosphaerae* FT2, *Ps. koreensis* FT4 and *Ps. sp.* FM9, FM12, and FC11 strains belonged to the *Bacillus* genus and were identified as *Bacillus pumilis* FM12 and *B. sp.* FC11.

Salt stress causes significant changes in plant physiology: It reduces the absorption of nutrients and the production of physiologically active metabolites and slows down the growth and development of crops (Yaghoubi Khanghahi *et al*., 2021). Several authors have proved that phosphate-solubilizing microorganisms have a positive effect on crops, promote their growth, stimulate disease resistance, and increase yields (Bargaz *et al*., 2021; Sarmah and Sarma, 2022). It is believed that they can stimulate plant growth both by increasing phosphorus nutrition

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and by producing growth-promoting metabolites (vitamins, amino acids, phytohormones, etc.) (Shemshura *et al*., 2019; Rafique *et al*., 2022).

In most studies, the production of IAA by bacteria was analyzed in the absence of salinity (Elhaissoufi *et al*., 2020; Lebrazi *et al*., 2020; Shahzad *et al*., 2022). Therefore, FT4, FM12, and FC11 isolates were tested for the ability to produce IAA at different degrees of salt stress. In our study, it was found that the isolates were able to produce IAA phytohormone under salinity conditions. Moreover, with high salt stress (500 mm NaCl), IAA production decreased slightly by only 20-22% compared to the non-salinity variants. The maximum production of phytohormone was shown by the FT4 strain. The results obtained show that these isolates can promote the growth of soybean plants in saline conditions since they produce a phytohormone of growth in stressful situations and they can be used as potential microbial inoculants for agriculture on saline soils.

Our study showed that inoculation with phosphate-solubilizing salt-tolerant bacteria mitigated the negative effect of salinization on soybean plants: Inoculated plants had a higher stem and root length and a larger number of leaves compared to non-inoculated plants. Thus, the dry mass of shoots and roots increased by two or more times and leaves by 3.5 times. We also found that under salinity conditions, inoculation contributed to the formation of nodules on soybean roots, which is consistent with (Janati *et al*., 2021; Shome *et al*., 2022). At the same time, it was found that the number of nodules on the roots increased almost twice and the size and weight of the roots more than doubled. This is explained by the fact that the formation and functioning of nodules in legumes are affected by the phosphate status of plants and phosphorus deficiency reduces the colonization of roots by rhizobia and their nitrogen fixation (Bargaz *et al*., 2018; Li *et al*., 2021; Berza *et al*., 2022). Inoculation with phosphate-solubilizing salt-tolerant bacteria contributed to an increase in the bioavailability of phosphorus and improved phosphorus nutrition of both soybean rhizobia symbionts and plants themselves (El-Rahman *et al*., 2019; Lebrazi *et al*., 2020; Shome *et al*., 2022). It is also possible that the positive effect of inoculation is associated with the production of IAA phytohormone bacteria, which stimulates plant growth and has a positive effect on rhizobia. The *Ps koreensis* FT4 strain showed the greatest positive effect on soybean plants. In this variant, the largest number of nodules was 17.4±0.1 (control variant: 8.6±0.04).

Conclusion

Inoculation with the phosphate-solubilizing salt-tolerant bacteria isolated by us improved the growth of soybean plants under conditions of high salt stress. These growth improvements are recorded by increasing the number of nodules, plant length, and dry weight of roots, shoots, and leaves. Our results showed that the isolated bacteria had great potential for maintaining soybean plants and their survival in stressful conditions on saline soils. After evaluating the strains in the field on saline soil, the bacteria may be considered suitable for commercial use to replace the use of expensive phosphorus fertilizers. Further studies of such stress-resistant phosphate-solubilizing salt-tolerant cultures are very interesting and important.

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Author’s Contributions

All authors equally contributed to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

References

Ali, B., Wang, X., Saleem, M. H., Sumaira, Hafeez, A., Afridi, M. S., Khan, S., Zaib-Un-Nisa, Ullah, I., Amaral Júnior, A. T. d., Alatawi, A., & Ali, S. (2022). PGPR-mediated salt tolerance in maize by modulating plant physiology, antioxidant defense, compatible solutes accumulation and bio-surfactant producing genes. *Plants, 11*(3), 345. https://doi.org/10.3390/plants11030345

Ali, W., Nadeem, M., Ashiq, W., Zaeem, M., Gilani, S. S. M., Rajabi-Khamseh, S. ... & Cheema, M. (2019). The effects of organic and inorganic phosphorus amendments on the biochemical attributes and active microbial population of agriculture podzols following silage corn cultivation in boreal climate. *Scientific Reports, 9*(1), 17297. https://doi.org/10.1038/s41598-019-53906-8

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology, 8*, 971. http://dx.doi.org/10.3389/fmicb.2017.00971

Bargaz, A., Elhaissoufi, W., Khourchi, S., Benmrid, B., Borden, K. A., & Rchiad, Z. (2021). Benefits of phosphate solubilizing bacteria on belowground crop performance for improved crop acquisition of phosphorus. *Microbiological Research, 252*, e126842. https://doi.org/10.1016/j.micres.2021.126842

Bargaz, A., Lyamlouli, K., Chtouki, M., Zeroual, Y., & Dhiba, D. (2018). Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system *Frontiers in Microbiology, 9*, 1606. https://doi.org/10.3389/fmicb.2018.01606

Bekele, M., Kebede, F., & Haile, W. (2020). Phosphorus Adsorption-Desorption Isotherm of Lime Treated and Untreated Acid Soils of Assosa and Bambasi Districts, West Ethiopia. *Communications in Soil Science and Plant Analysis*, *51*(15), 1979-1990. https://doi.org/10.1080/00103624.2020.1808011

Berza, B., Sekar, J., Vaiyapuri, P., Pagano, M. C., & Assefa, F. (2022). Evaluation of inorganic phosphate solubilizing efficiency and multiple plant growth promoting properties of endophytic bacteria isolated from root nodules Erythrina brucei. *BMC Microbiology*, *22*(1), 276. https://doi.org/10.1186/s12866-022-02688-7

Bhat, N. A., Riar, A., Ramesh, A., Iqbal, S., Sharma, M. P., Sharma, S. K., & Bhullar, G. S. (2017). Soil biological activity contributing to phosphorus availability in vertisols under long-term organic and conventional agricultural management. *Frontiers in Plant Science, 8*, 1523. http://dx.doi.org/10.3389/fpls.2017.01523

Cai, Z., Udawatta, R. P., Gantzer, C. J., Jose, S., Godsey, L., & Cartwright, L. (2019). Economic impacts of cover crops for a Missouri wheat-corn-soybean rotation. *Agriculture*, *9*(4), 83. https://doi.org/10.3390/agriculture9040083

Chen, J., Liu, L., Wang, Z., Zhang, Y., Sun, H., Song, S., ... & Li, C. (2020). Nitrogen fertilization increases root growth and coordinates the root–shoot relationship in cotton. *Frontiers in Plant Science*, *11*, 880. http://dx.doi.org/10.3389/fpls.2020.00880

Chen, L. H., Cheng, Z. X., Xu, M., Yang, Z. J., & Yang, L. T. (2022). Effects of nitrogen deficiency on the metabolism of organic acids and amino acids in Oryza sativa. *Plants*, *11*(19), 2576. https://doi.org/10.3390/plants11192576

Dey, G., Banerjee, P., Sharma, R. K., Maity, J. P., Etesami, H., Shaw, A. K., ... & Chen, C. Y. (2021). Management of phosphorus in salinity-stressed agriculture for sustainable crop production by salt-tolerant phosphate-solubilizing bacteria-A review. *Agronomy*, *11*(8), 1552. https://doi.org/10.3390/agronomy11081552

Didorenko, S. V. (2021). Domestic selection of soybeans during the years of independence of Kazakhstan as the basis of the country's food security. https://kazniizr.kz/otechestvennaya-selektsiya-soi-v-gody-nezavisimosti-kazahstana-kak-osnova-prodovolstven

Elhaissoufi, W., Khourchi, S., Ibnyasser, A., Ghoulam, C., Rchiad, Z., Zeroual, Y., Lyamlouli, K., & Bargaz, A. (2020). Phosphate solubilizing rhizobacteria could have a stronger influence on wheat root traits and aboveground physiology than rhizosphere P solubilization. *Frontiers in Plant Science, 11*, 979. http://dx.doi.org/10.3389/fpls.2020.00979

El-Rahman, L. A. A., Sayed, D. A., & Ewais, M. A. (2019). Effect of different sources of phosphorus and bio fertilizers on yield and seeds quality of soybean. *Menoufia Journal of Soil Science*, *4*(1), 15-35. http://dx.doi.org/10.21608/mjss.2019.174207

Etesami, H., & Beattie, G. A. (2018). Mining Halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. *Frontiers in Microbiology*, *9*, 148. http://dx.doi.org/10.3389/fmicb.2018.00148

Gómez-de-Mariscal, E., Guerrero, V., Sneider, A., Jayatilaka, H., Phillip, J. M., Wirtz, D., & Muñoz-Barrutia, A. (2021). Use of the p-values as a size-dependent function to address practical differences when analyzing large datasets. *Scientific Reports, 11*, 20942. https://doi.org/10.1038/s41598-021-00199-5

Hernández-León, R., Rojas-Solís, D., Contreras-Pérez, M., Orozco-Mosqueda, M. D. C., Macías-Rodríguez, L. I., Reyes de La Cruz, H., Valencia-Cantero, E., & Santoyo, G. (2015). Characterization of the antifungal and plant growth-promoting effects of diffusible and volatile organic compounds produced by Pseudomonas fluorescens strains. *Biological Control, 81*, 83-92. https://doi.org/10.1016/j.biocontrol.2014.11.011

Isidra-Arellano, M. C., Reyero-Saavedra, M. D. R., Sánchez-Correa, M. D. S., Pingault, L., Sen, S., Joshi, T., Girard, L., Castro-Guerrero, N. A., Mendoza-Cozatl, D. G., Libault, M., & Valdés-López, O. (2018). Phosphate deficiency negatively affects early steps of the symbiosis between common bean and rhizobia. *Genes*, *9*(10), 498. http://dx.doi.org/10.3390/genes9100498

Janati, W., Benmrid, B., Elhaissoufi, W., Zeroual, Y., Nasielski, J., & Bargaz, A. (2021). Will phosphate bio-solubilization stimulate biological nitrogen fixation in grain legumes? *Frontiers in Agronomy, 3*, 637196. http://dx.doi.org/10.3389/fagro.2021.637196

Joshi, G., Kumar, V., & Brahmachari, S. K. (2021). Screening and identification of novel halotolerant bacterial strains and assessment for insoluble phosphate solubilization and IAA production. *Bulletin of the National Research Centre, 45*, 83. https://doi.org/10.1186/s42269-021-00545-7

Lebrazi, S., Niehaus, K., Bednarz, H., Fadil, M., Chraibi, M., & Fikri-Benbrahim, K. (2020). Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from Acacia cyanophylla root nodules and their effects on its plant growth. *Journal of Genetic Engineering and Biotechnology, 18*(1), 71. http://dx.doi.org/10.1186/s43141-020-00090-2

Li, H., Wang, X., Liang, Q., Lyu, X., Li, S., Gong, Z., Dong, S., Yan, C., & Ma, C. (2021). Regulation of phosphorus supply on nodulation and nitrogen fixation in soybean plants with dual-root systems. *Agronomy, 11*(11), 2354. https://doi.org/10.3390/agronomy11112354

Mahmood, A., Amaya, R., Turgay, O. C., Yaprak, A. E., Taniguchi, T., & Kataoka, R. (2019). High salt tolerant plant growth promoting rhizobacteria from the common ice-plant Mesembryanthemum crystallinum L. *Rhizosphere, 9*, 10-17. https://doi.org/10.1016/j.rhisph.2018.10.004

Matse, D. T., Huang, C.-H., Huang, Y.-M., & Yen, M.-Y. (2020). Effects of coinoculation of Rhizobium with plant growth-promoting rhizobacteria on the nitrogen fixation and nutrient uptake of Trifolium repens in low phosphorus soil. *Journal of Plant Nutrition, 43*(5), 739-752. http://dx.doi.org/10.1080/01904167.2019.1702205

Penn, C. J., & Camberato, J. J. (2019). A Critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agriculture, 9*(6), 120. https://doi.org/10.3390/agriculture9060120

Rafique, E., Mumtaz, M. Z., Ullah, I., Rehman, A., Qureshi, K. A., Kamran, M., Rehman, M. U., Jaremko, M., & Alenezi, M. A. (2022). Potential of mineral-solubilizing bacteria for physiology and growth promotion of *Chenopodium quinoa* Willd. *Frontiers in Plant Science, 13*, 1004833. https://doi.org/10.3389/fpls.2022.1004833

Ren, S., Lyle, C., Jiang, G.-L., & Penumala, A. (2016). Soybean salt tolerance 1 (GmST1) reduces ROS production, enhances ABA sensitivity and abiotic stress tolerance in arabidopsis thaliana. *Frontiers in Plant Science, 7*, 445. http://dx.doi.org/10.3389/fpls.2016.00445

Sadak, M. S., Abd El-Hameid, A. R., Zaki, F. S. A., Dawood, M. G., & El-Awadi, M. E. (2020). Physiological and biochemical responses of soybean (*Glycine max* L.) to cysteine application under sea salt stress. *Bulletin of the National Research Centre, 44*, 1. https://doi.org/10.1186/s42269-019-0259-7

Sarmah, R., & Sarma, A. K. (2022). Phosphate solubilizing microorganisms: A review. *Communications in Soil Science and Plant Analysis*. http://dx.doi.org/10.1080/00103624.2022.2142238

Savala, C., Wiredu, A., Okoth, J., & Kyei-Boahen, S. (2021). Inoculant, nitrogen and phosphorus improve photosynthesis and water-use efficiency in soybean production. *The Journal of Agricultural Science, 159*(5-6), 349-362. http://dx.doi.org/10.1017/S0021859621000617

Shahzad, K., Siddiqi, E. H., Ahmad, S., Zeb, U., Muhammad, I., Khan, H., Zhao, G.-F., & Li, Z.-H. (2022). Exogenous application of indole-3-acetic acid to ameliorate salt-induced harmful effects on four eggplants (*Solanum melongena* L.) varieties. *Scientia Horticulturae, 292*, 110662. https://doi.org/10.1016/j.scienta.2021.110662

Sharma, A., Dev, K., Sourirajan, A., & Choudhary, M. (2021). Isolation and characterization of salt-tolerant bacteria with plant growth-promoting activities from saline agricultural fields of Haryana, India. *Journal of Genetic Engineering and Biotechnology, 19*, 99. https://doi.org/10.1186/s43141-021-00186-3

Shemshura, O. N., Shemsheyeva, Zh. N., Sadanov, A. K., Alimzhanova, M. B., Daugaliyeva, S. T., Mombekova, G. A., Rakhmetova, Zh. K. (2019). Plant growth promotion by volatile organic compounds produced by Chryseobacterium rhizoplanae isolated from Vigna radiate. *Ecology, Environment and Conservation Paper, 25*(2), 807-812.

Shome, S., Barman, A., & Solaiman, Z. M. (2022). Rhizobium and phosphate solubilizing bacteria influence the soil nutrient availability, growth, yield and quality of soybean. *Agriculture, 12*(8), 1136. https://doi.org/10.3390/agriculture12081136

Tang, H., Niu, L., Wei, J., Chen, X., & Chen, Y. (2019). Phosphorus limitation improved salt tolerance in maize through tissue mass density increase, osmolytes accumulation and Na + uptake inhibition. *Frontiers in Plant Science, 10*, 856. https://doi.org/10.3389/fpls.2019.00856

Timofeeva, A., Galyamova, M., & Sedykh, S. (2022). Prospects for using phosphate-solubilizing microorganisms as natural fertilizers in agriculture. *Plants*, *11*(16), 2119. https://doi.org/10.3390/plants11162119

Xie, W., Yang, J., Gao, S., Yao, R., & Wang, X. (2022). The effect and influence mechanism of soil salinity on phosphorus availability in coastal salt-affected soils. *Water*, *14*(18), 2804. https://doi.org/10.3390/w14182804

Yaghoubi Khanghahi, M., Strafella, S., Allegretta, I., & Crecchio, C. (2021). Isolation of bacteria with potential plant-promoting traits and optimization of their growth conditions. *Current Microbiology*, *78*(2), 464-478. http://dx.doi.org/10.1007/s00284-020-02303-w