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Effects of *Exiguobacterium* sp. DYS212, a Saline-Alkaline-Tolerant P-Solubilizing Bacterium, on *Suaeda salsa* Germination and Growth

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Abstract: Soil nutrient availability under saline-alkali stress limits plant primary productivity. P-solubilizing bacteria (PSB) improve inorganic P dissolution and promote plant growth. However, the application studies of saline-alkaline-tolerant PSB are still scarce. We isolated one PSB strain from bird droppings in saline-alkali regions and identified its growth characteristics and resistance to salt and alkali. A potting experiment with PSB addition was performed to analyze the effect of this strain on the germination and growth of *Suaeda salsa*. The PSB were identified as *Exiguobacterium* sp. DYS212 strain, and it utilized glucose, ammonium sulfate, and yeast extract powder well. The strain is halophilic, has the ability to dissolve inorganic P, and improved P-solubilization under 1–5.5% salinity (available P > 200 mg L^{-1}), reached a maximum at 2.5% NaCl concentration yielding 410.73 mg L^{-1} of available P. The PSB promoted seed germination, especially under high alkaline stress, wherein the growth promoting rate increased to 5.26%. The PSB improved the growth of *S. salsa*, in terms of plant height, stem diameter, and biomass (up to 2.5 times), under saline and alkaline conditions. This study highlights the potential of *Exiguobacterium* sp. isolates as biofertilizers, and provides reference for environment sustainability of saline-alkali region.

Keywords: available phosphorus; phosphate-solubilizing bacteria; *Suaeda salsa*; seed germination; plant biomass

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1. Introduction

Phosphorus accounts for approximately 0.2% of plant dry weight and is the second most important macronutrient for plant metabolism, growth, and development after N [1,2]. Phosphorus is a component of nucleic acids, ATP, and phospholipids, and inorganic P regulates several important enzymatic reactions and metabolic pathways [3]. Thus, P unavailability is a stressful condition for plants that can severely limit their yield [4]. Plant P is acquired mainly from the soil. The total amount of P in soil is generally very high (400–1200 mg kg^{-1}), but only 0.1% of the total P content available for plant uptake [5].

Soil inorganic P is generally immobilized by iron, aluminum, and calcium to form insoluble P complexes, a process strongly dependent on pH [6]. Moreover, soil organic P is not easily absorbed by plants and forms complexes with these metal ions [7]. When the soil is alkaline, P mainly exists in the inaccessible form of $Ca_3(PO_4)_2$. Therefore, saline-alkali areas, which inherently have a higher soil pH, are detrimental to the transformation and utilization of soluble P [8,9].

To solve the problem of P shortage, fertilizers are typically applied, but approximately 80% of the P in the fertilizer is quickly fixed in the soil, made unavailable to plants, resulting

in environmental concerns such as soil fertility reduction, water eutrophication, and non-point source pollution [10,11]. Therefore, how to utilize insoluble P in situ soil is the key to improve te available P of saline-alkali soil in a long time.

Microorganisms play an important role in plant growth and stress tolerance [12]. Various beneficial bacteria and fungi establish complex mutualistic relationships with plants, including the formation of specialized symbiotic structures such as nodules and mycorrhizae [13,14], thereby increasing resource availability [15]. P-solubilizing bacteria (PSB) are some of the most important plant growth-promoting microorganisms through the dissolution or mineralization of soil P [16–18]. PSB convert insoluble P into soluble forms playing a crucial role in P mobility [19,20]. Studies have found that inoculation with PSB improves plant P uptake, increases N and P content in leaves, promotes plant growth, and contributes to increased crop yield [21–24]. This is an effective way to solve the low available P problem and to alleviate the negative effects of chemical fertilizers.

The phosphorolytic mechanism of PSB mainly includes acid hydrolysis of inorganic P and enzymatic hydrolysis of organic P [6]. The dissolution of inorganic P occurs mainly through the release of organic acids whose hydroxyl or carboxyl groups chelate the cations previously bound to phosphate; the release of acids reduces the pH, resulting in the conversion of PO_4^{3-} (a P source not available to plants) to HPO_4^{2-} and $H_2PO_4^-$ (a P source available to plants) [25,26]. Some PSB, like *Enterobacter* and *Burkholderia*, produce phosphate hydrolyzing enzymes such as phosphohydrolases and phosphomonoesterases, which act to, mineralize organic P from, for example, phytic acid and other associated organic P sources [27,28]. However, P solubilizing performance varies with bacterial growth conditions, such as temperature, salinity and pH [22]. The screening by plate, morphological observation, and molecular biological identification by the homology of 16SrDNA sequence comparison of PSB have been a topic of great interest in recent years [18,26], and studies on the application of PSB in P-deficient soils are still in their infancy [29].

Strains have various environment adaptations. For example, bacteria of the genus *Exiguobacterium* have been frequently isolated from various habitats, thus, it has a variety of unique properties, including alkali resistance and salt resistance, etc. [30]. For saline-alkali regions, saline-alkaline-resistant PSB are considered as a potential biofertilizer, which would improve soil conditions and promote plant [21,23,31]. When used as a biofertilizer in the field, plants respond differently to the addition of PSB depending on soil pH, salinity, inoculation method, and the bacterial strain [32]. *Suaeda salsa* is an annual saline-tolerant herbaceous plant with a wide ecological amplitude and adaptability in coastal wetland [33], and it commonly used in salinized land restoration [34,35]. *S. salsa* absorbs soluble soil salt [36], and also plays an important role in C sequestration and coastline protection [37]. We screened saline-alkaline-resistant PSB, tested their characteristics, and inoculated onto *S. salsa* to assess seed germination rates and plant growth. This study provides a reference for the PSB application for vegetation restoration in saline areas.

2. Materials and Methods

2.1. Isolation and Identification of PSB

In December 2021, bird droppings were collected from a tidal flat of the Yellow River Delta in Dongying City, Shandong Province, China (37°43′12″ N, 113°12′40″ E), with an average total P content of 0.55 g kg^{-1} and available P content of 4.5 mg kg^{-1}. The standard serial dilution method was used to screen for saline-alkaline-resistant PSB. Five grams of the collected sample and 45 mL of sterilized water were added to a sterilized conical flask. The mixture was shaken for 30 min. The supernatant was serially diluted to 10^{-6}. A diluted solution (0.1 mL) with a concentration of 10^{-3}–10^{-5} was selected and used to coat a plate medium of tricalcium phosphate inorganic P and lecithin organic P. After 2–3 days of incubation at 28 °C, single colonies with obvious transparent encircling boundaries were selected using a sterilized inoculation ring and purified by treatment on a lysogeny broth (LB) solid medium (Peptone, 10 g L^{-1}; Yeast Extract, 5 g L^{-1}; NaCl, 10 g L^{-1}; Agar Powder, 15 g L^{-1}). The dominant PSB colony was isolated three times and

subjected to 16S rRNA sequencing with subsequent comparison to the GenBank database (NCBI). Sequences of several strains with the highest comparative similarity were derived. A phylogenetic tree was established using the GTR+GAMMA model of RAxML v8.2.11 with 1000 bootstrap replications.

The selected strain was amplified by PCR using 16S rDNA universal primers F27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and R1492 (5′-TACGACTTAACCCCAATCGC-3′) [38], and after completion, sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing. The gene sequence was logged into the US National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov (accessed on 29 March 2023)) for nucleotide sequence Blast alignment [39]. Sequences with the highest homology were selected to identify the strains and were combined with the results of physiological and biochemical identification.

2.2. Physiological, Biochemical, and Strain Identification of PSB

The selected strain was cultured in a 2216E medium (Peptone, 10 g L^{-1}; Yeast Extract, 5 g L^{-1}; $C_6H_8FeNO_7$, 0.1 g L^{-1}; NaCl, 19.45 g L^{-1}; $MgCl_2$, 5.98 g L^{-1}; Na_2SO_4, 3.24 g L^{-1}; $CaCl_2$, 1.8 g L^{-1}; KCl, 0.55 g L^{-1}; Na_2CO_3, 0.16 g L^{-1}; KBr, 0.08 g L^{-1}; $SrCl_2$, 0.034 g L^{-1}; H_3BO_3, 0.0022 g L^{-1}; Na_2SiO_3, 0.004 g L^{-1}; NaF, 0.0024 g L^{-1}; NH_4NO_3, 0.0016 g L^{-1}; Na_2HPO_4, 0.008 g L^{-1}; Agar Powder, 15 g L^{-1}) at 28 °C, and colony characteristics were observed under light and transmission electron (JEM-1200EX, JOEL, Tokyo, Japan) microscopes. Biochemical identification was performed using microbial biochemical tubes (Hopebio Microbe Reagent) according to the Berger's Manual of Determinative Bacteriology. After inoculation, the samples were sealed with a sealing film wiped with 75% alcohol and incubated at 28 °C. The strain was identified according to the Bergey Manual of Systematic Bacteriology [40].

2.3. Analysis of P Dissolution by PSB

2.3.1. Effect of PSB in Solid Medium

The test strain was inoculated onto inorganic P (tricalcium phosphate) and organic P (lecithin) media using the point-inoculation method. After 5 days of culture at 28 °C in an incubator, the P solubilizing ability of the strain was evaluated based on the presence and size of a P solubilizing ring around the colony. Three replicates were conducted. The strain's P solubilizing ability was calculated using Digimizer Image Analysis Software, and the formula:

$$\text{P solubilizing coefficient}\left(\frac{\text{D}}{\text{d}}\right) = \text{Diameter of P solubilizing ring (D)} \div \text{PSB diameter (d)}.$$

2.3.2. Effect of PSB in Liquid Medium

After culturing PSB in LB liquid medium (Peptone, 10 g L^{-1}; Yeast Extract, 5 g L^{-1}; NaCl, 10 g L^{-1}), a 1% stock culture (A_{600}~1.0) was inoculated into organic P and inorganic P liquid media (Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China). The culture was placed into an orbital-shaking incubator set to 28 °C and 180 r/min. Samples were retrieved every 4 h for seven consecutive days to determine PSB growth and available P content. Five replicates were performed for each experiment. The available P content in the PSB fermentation broth was determined using the molybdenum blue colorimetric method [41].

2.4. Determination of Salt and Alkali Resistance of PSB

An inorganic P liquid medium (Glucose, 10 g/L; $(NH_4)_2SO_4$, 0.5 g/L; Yeast Extract, 0.5 g/L; NaCl, 0.3 g/L; KCl, 0.3 g/L; $MgSO_4$, 0.3 g/L; $FeSO_4$, 0.03 g/L; $MnSO_4$, 0.03 g/L; $Ca_3(PO_4)_2$, 5 g/L) was used as the base medium for these experiments. The PSB strain was multiplied, and then, a 1% stock culture (A_{600}~1.0) was inoculated into the LB liquid medium. The incubation duration was determined based on the peak values observed in the growth curve. PSB was cultured in the same medium (tryptone, 10 g L^{-1}; yeast

extract, 5 g L^{-1}) under different NaCl concentrations (0, 1, 2.5, 4, 5.5, 7, 8, 9, 10, 20, and 30%) and pH (7, 8, 9, 10, 11, and 12) (five replicates in each group) in an orbital-shaking incubator at 28 °C and 180 r/min. After the incubation period, 1 mL of the culture was collected and centrifuged at 6000 rpm for 12 min. The available P content and terminal pH were determined.

2.5. Optimization of Culture Conditions of PSB

Four C sources (glucose, sucrose, lactose, and soluble starch) and six N sources (potassium nitrate, ammonium nitrate, ammonium sulfate, urea, sodium nitrate, and yeast powder) were used to replace the C and N sources in the basal medium with an equal mass, respectively. To ensure the rationality of the test, the concentration of each substance was designed to be the same, with the C source added at 10.0 g L^{-1} and the N source added at 0.5 g L^{-1}.

2.6. Effect of PSB on S. salsa Germination Characteristics

Fully mature and uniform seeds of *S. salsa*, collected from *S. salsa* planted on the tidal flat of the Yellow River Delta in Dongying City, Shandong Province, China, were selected for the experiment. Seeds were surface-sterilized with 75% ethanol for 1 min, washed three times with sterile distilled water, surface-sterilized with 10% sodium hypochlorite for 10 min, and washed five times with sterile distilled water. The seeds were soaked in the bacterial stock culture (A_{600}~1.0) for 3 h, washed five times with sterile distilled water, and blot-dried on sterile filter paper.

The experiment was conducted using a two-factor design (Table 1), including salt and alkali stress (ten levels) and the PSB strain inoculation (two levels). One factor was the NaCl, alkali, or NaCl-alkali mixed stress treatment, and the control group (Control 1) was treated with sterile distilled water. The other factor was the strain inoculation treatment, and in the control group (Control 2), we used an uninoculated LB medium instead of the strain suspension. The experimental design is presented in Table 1. Ten milliliters of the prepared liquid with different salinity and alkalinity concentrations (Table 1) were added to a single layer of sterilized filter paper on a sterilized plate. Three replicates were performed for each treatment. Thirty pretreated seeds were evenly placed on each plate, and the plates were sealed with a sealing film to avoid evaporation and maintain the concentration of each treatment. The experiment was carried out in an artificial climate chamber, and the following parameters were maintained: light duration, 12 h day^{-1}; daytime light intensity, 80%; relative humidity, 70%; and ambient temperature, 25 °C during the day and 20 °C at night.

Table 1. Two-factor experimental design.

Treatment	Name	Uninoculated Medium (Control 2)	Strain Inoculation Medium
Sterile water	Control 1	/	/
NaCl stress	N1	150 mM	150 mM
	N2	300 mM	300 mM
	N3	450 mM	450 mM
Alkali stress (molar ratio of Na_2CO_3:$NaHCO_3$ = 1:1)	A1	50 mM	50 mM
	A2	100 mM	100 mM
	A3	150 mM	150 mM
NaCl–Alkali (molar ratio of Na_2CO_3:$NaHCO_3$ = 1:1) mixed stress	M1	150 mM–50 mM NaCl-Alkali	150 mM–50 mM NaCl-Alkali
	M2	300 mM–100 mM NaCl-Alkali	300 mM–100 mM NaCl-Alkali
	M3	450 mM–150 mM NaCl-Alkali	450 mM–150 mM NaCl-Alkali

Seeds were considered germinated when the radicle extended 1 mm from the seed coat, and the germination number was recorded at a fixed time daily. Germination energy was calculated on day 4. All observations were recorded for five days. The germination rate, inhibition rate (relative NaCl injury rate), growth promotion rate, germination energy, and germination index of the seeds were calculated using the following formulae:

$$\text{Seed germination rate } (\%) = \frac{\text{actual germination number}}{\text{total number of seeds}} \times 100$$

$$\text{Inhibition rate } (\%) = \times 100$$

$$\text{Growth promotion rate } (\%) = \frac{\text{treatment group germination rate} - \text{control group germination rate}}{\text{control group germination rate}} \times 100$$

$$\text{Germination energy } (\%) = \frac{\text{germination number of tested seeds within 4 days}}{\text{number of tested seeds}} \times 100$$

$$\text{Germinating index GI} = \frac{\sum \text{Gt}}{\text{Dt}}$$

where Gt is the germination number of "t" days and Dt refers to the corresponding germination days.

2.7. Effect of PSB on S. salsa Growth

Pot experiments with *S. salsa* were designed using two factors, similar to the seed germination experiment (Table 1). The planting density was 10 seeds per pot, and the soil mass in each pot was 300 g. The soil was irrigated with sterile distilled water or sodium solution every 3 days to keep it moist. The experiment was conducted in an artificial climate chamber under the same conditions as those used for the seed germination experiment. Five replicates were analyzed for each treatment group.

Six days after sowing (DAS), the seedlings were subjected to the first saline stress treatment. To prevent the seedlings from burning due to high salinity, saline stress was applied at 150 mM increments until the target concentration was reached, and under alkali stress (1:1 molar ratio of Na_2CO_3:$NaHCO_3$; Table 1), alkali solutions were applied at 50 mM increments until the target concentration was reached. Saline (NaCl)-alkali (molar ratio of Na_2CO_3:$NaHCO_3$ = 1:1) combinatorial stress was provided by irrigating the plants at a concentration of 150 mM NaCl-50 mM alkali until the target concentration was reached. Based on the recovery and growth of plants after NaCl and alkali addition, the plants were subjected to the second and third NaCl and alkali stress treatments at 21 and 45 DAS, respectively.

The activated stock solution (A_{600}~1.0) was diluted with sterile distilled water at a ratio of 1:9 and 10 mL of each diluted bacterial suspension was evenly added to each pot. The first treatment was commenced at 15 DAS. After 40 DAS, the plants were treated with the second set of bacterial suspensions, which were applied at half the concentration of the first treatment. After 42 DAS, the pots were irrigated with 100 mL per pot of Hoagland nutrient solution (without phosphate; P deficiency). Four well-grown homogeneous *S. salsa* plants were placed in each pot. The plant height, stem diameter, and above- and below-ground biomass of *S. salsa* was measured at 60 DAS.

2.8. Statistical Analysis

The statistical analyses in our paper were carried out using R Studio (R Studio, Inc., Boston, MA, USA) Version 1.1.463-© 2009–2018 [42], and performed with analysis of variance at $p < 0.05$. The variables were tested for normal or lognormal distributions initially. Figures were prepared using the "ggplot2" package. The effects of NaCl and alkali treatments (ten levels), strain treatments (two levels) and their interactions on the growth of *S. salsa* were analyzed using the R Studio linear mixed-effects package "lme4" (two-way ANOVA), and the determined index among saline and alkali treatments and strain treatments were compared after Tukey's pair-wise comparison tests using one-way ANOVA.

3. Results

3.1. Strain Identification with Morphology, Biochemistry, and Genetic Sequence

The PSB strain colonies were pale yellow, moist, and round, with a smooth surface. Transmission electron microscopy showed opaque aflagellate bacterium with oval regular borders, 0.3–1.5 µm long and 0.6–0.8 µm wide (Figure 1). Biochemical assessment revealed it was gram-positive; positive for gelatin, maltose, lipase (corn oil), mannitol, salicin, and heptagaloside hydrolysis; and negative for phenylalaninase, Simmons citrate, tryptophan broth, ornithine decarboxylase broth, urease, and xylose. Sequencing (GenBank accession number: OQ683874) showed homology with *Exiguobacterium enclense* and *Exiguobacterium indium* (Figure 2). The isolated strain was named as *Exiguobacterium* sp. DYS212.

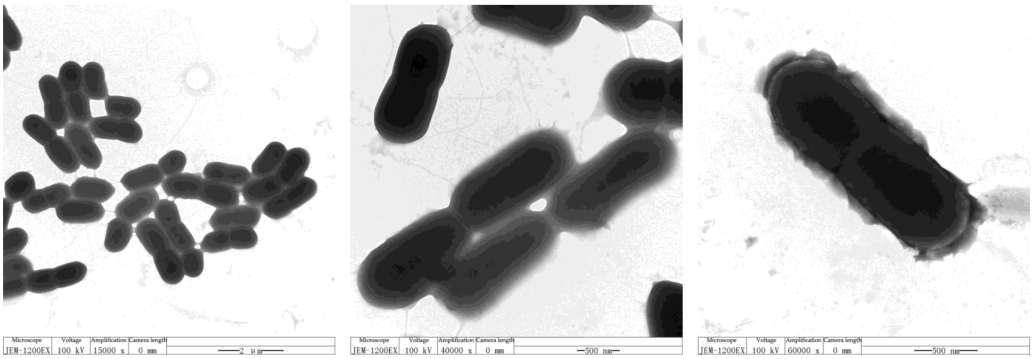

Figure 1. Transmission electron microscope image of *Exiguobacterium* sp. DYS212.

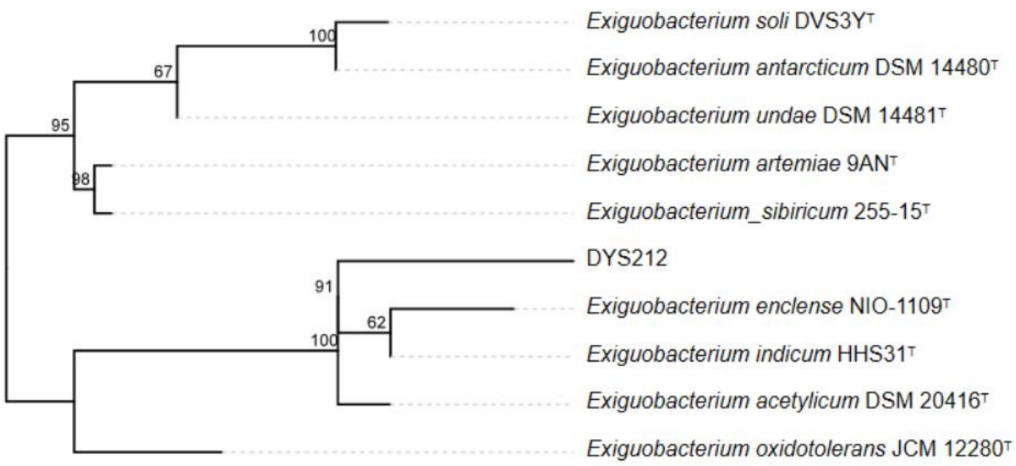

Figure 2. Phylogenetic evolutionary tree using GTR + GAMMA model based on 16S rRNA sequences.

3.2. Effect of Media on P-Solubilizing Performance

PSB had no P-solubilizing ability in the organic P solid medium. The P-solubilizing coefficient on the inorganic P solid medium was 1.375 (Figure 3). The growth of the strain in liquid inorganic P medium gradually increased with time, was rapid for the first 12 h, then slowed. The P solubilizing ability of the strain increased first and then decreased with time, reaching the maximum of 470.01 mg L^{-1} at 60 h (Figure 4). However, no solubilized P was observed in the liquid organic P medium.

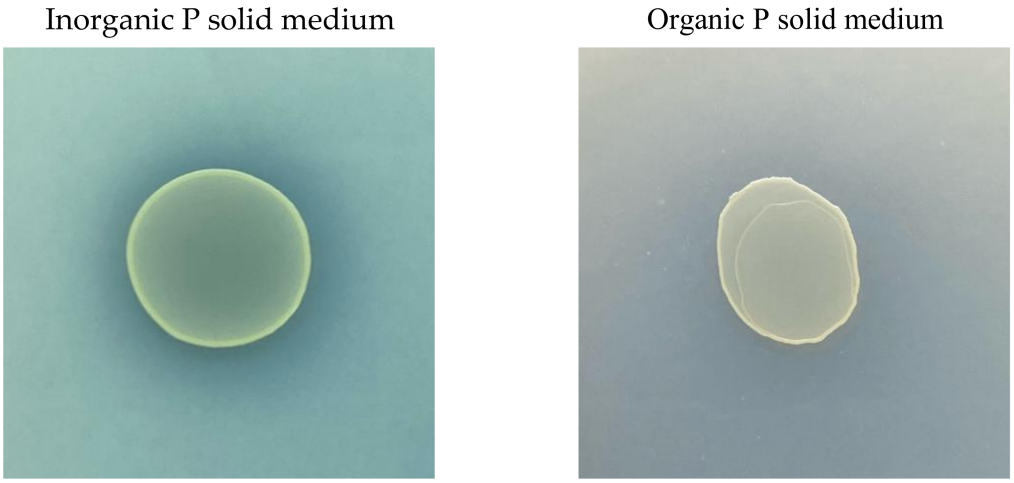

Figure 3. P-solubilizing ability of the strain in organic and inorganic P solid medium.

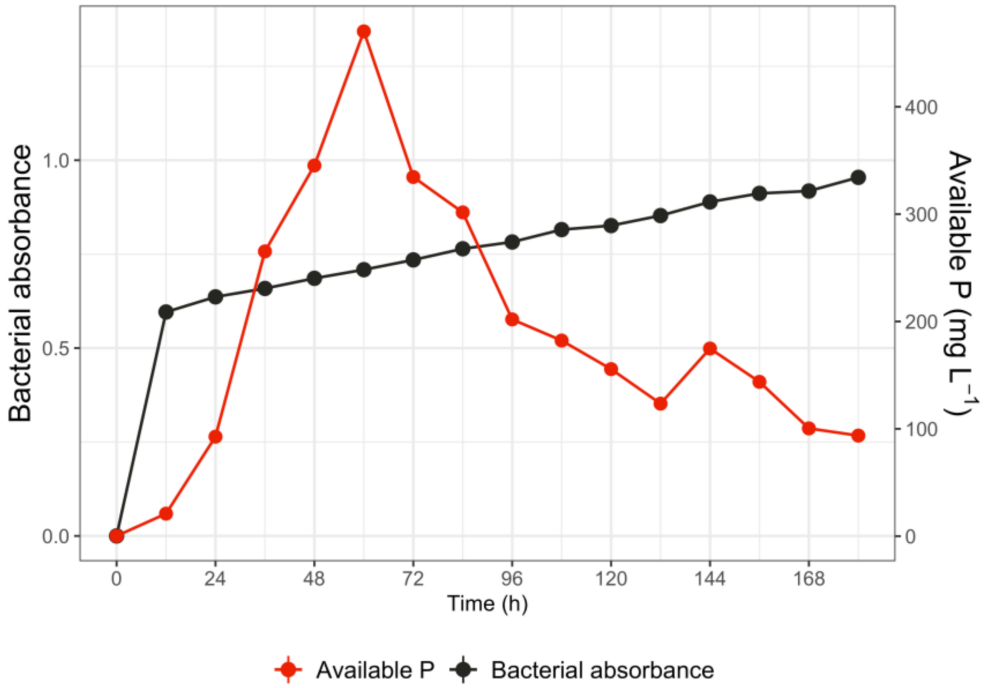

Figure 4. Kinetic curve of phosphorus dissolution of the P-solubilizing bacterium.

3.3. Effect of Salinity and pH on P-Solubilizing Performance

With an increase in NaCl concentration, the P-solubilizing ability of the strain first increased and then decreased, whereas the opposite trend was observed with an increase in pH. In the range of 1–5.5% salinity, the solubilized P content exceeded 200 mg L^{-1}. At 2.5% NaCl concentration, the available P content reached a maximum of 410.73 mg L^{-1}, and the terminal pH was 4.62 (Figure 5a).

With increasing pH, the solubilizing ability of the PSB decreased gradually. When a pH of 7, PSB exhibited maximum P-solubilizing ability, with an available P content of 406.09 mg L^{-1}, and a terminal pH of 4.92. When pH was greater than 9, the available P solubilized by PSB was less than 100 mg L^{-1} (Figure 5b).

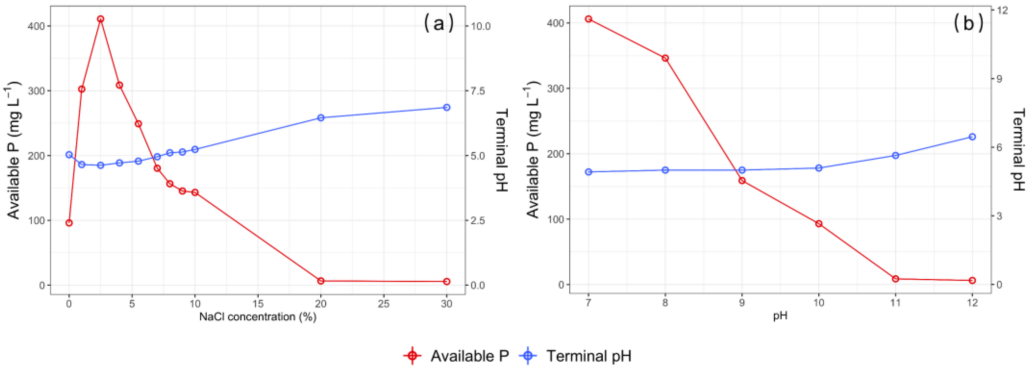

Figure 5. Effect of different NaCl concentrations and pH value on the P-solubilizing ability of the strain. (**a**) under different NaCl concentration; (**b**) under different pH.

3.4. Effect of C and N Sources on P-Solubilizing Performance

When glucose was used as the carbon source, the strain DYS212 had the best P-solubilizing ability, with the available P reaching 447.15 mg L^{-1}. The P solubilizing activity of the strain in sucrose was 92.39 mg L^{-1}. The P-solubilizing ability was poor nearly undetectable with lactose and starch (Figure 6a). When ammonium sulfate was used as the nitrogen source, the P-solubilizing ability of the strain was optimal, with available P reaching 381.69 mg L^{-1}, followed by yeast extract powder (344.57 mg L^{-1}). The P-solubilizing ability of the strain was poor in the presence of ammonium nitrate, potash nitrate, sodium nitrate, and urea (Figure 6b). The differences in the terminal pH values with different carbon and nitrogen sources were not significant.

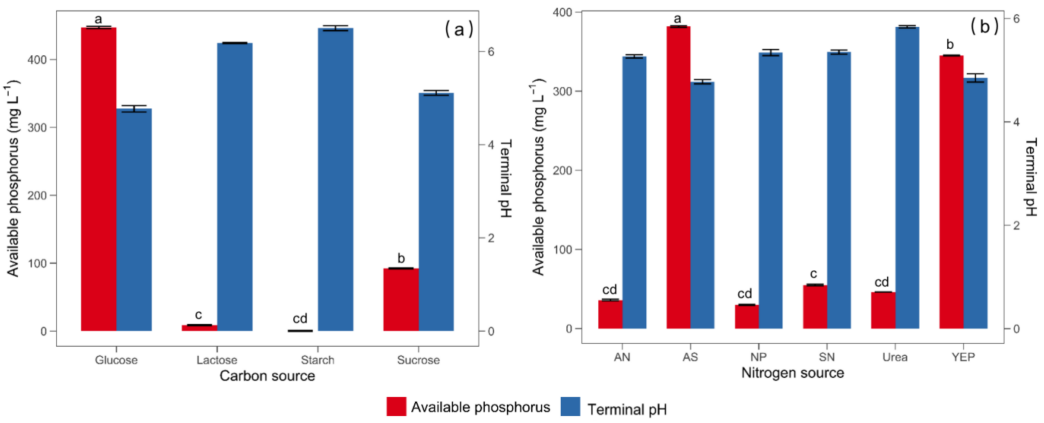

Figure 6. Changes of P-solubilizing ability of the strain under different C and N sources. AN: Ammonium nitrate; AS: Ammonium sulfate; NP: Nitrate of potash; SN: Sodium nitrate; YEP: Yeast extract powder. (**a**) under C sources; (**b**) under N sources. Error bars represent standard error of the mean, values are means ± SE (n = 3). Different lowercase letters represent significant differences in P-solubilizing ability under different C or N sources.

3.5. Effect of the PSB on S. salsa Seed Germination

Under NaCl and alkali stress conditions, the germination rate of *S. salsa* seeds decreased within 5 d with no PSB addition (Control 2). Under the N3, A3, and M3 treatments, the inhibition rates of seed germination were 15.29%, 10.59%, and 16.47%, respectively. In 5 d, the addition of PSB increased the seed germination rate, germination energy, and growth promotion rate compared with those of Control 2 seedlings without inoculation; moreover, the inhibition rate decreased. Under high-alkali conditions (A3), the growth-promoting rate of the PSB reached 5.26%. However, on day 1, the PSB treatment showed a decrease in seed germination and germination index compared with Control 2 under alkaline conditions (Table 2).

Table 2. Effect of P-solubilizing bacterium on seed germination rate of *Suaeda salsa*.

Treatment	Germination Rate on 1st Day		Germination Rate in 5 Days		Inhibition Rate in 5 Days		Germination Energy		Germination Index		Growth Promoting Rate	
	Control 2	PSB	Control 2	PSB	Control 2	PSB	Control 2	PSB	Control 2	PSB	Control 2	PSB
Control 1	41.11%	45.56%	94.44%	96.67%	0	0	92.22%	96.67%	131.25	140.15	0	2.35%
N1	40.00%	47.78%	95.56%	96.67%	0	0	92.22%	96.67%	131.12	143.65	0	1.16%
N2	41.11%	45.56%	92.22%	95.56%	2.35%	1.15%	92.22%	94.44%	127.52	137.78	0	3.61%
N3	28.89%	34.44%	80.00%	83.33%	15.29%	13.79%	77.78%	83.33%	100.40	119.75	0	4.17%
A1	41.11%	35.56%	90.00%	93.33%	4.71%	3.45%	87.78%	93.33%	128.12	127.63	0	3.70%
A2	45.56%	27.78%	88.89%	91.11%	5.88%	5.75%	86.67%	91.11%	129.83	112.90	0	4.50%
A3	32.22%	24.44%	84.44%	88.89%	10.59%	8.05%	81.11%	86.67%	111.28	106.83	0	5.26%
M1	36.67%	55.56%	90.00%	93.33%	4.71%	3.45%	90.00%	93.33%	118.12	144.13	0	3.70%
M2	37.78%	42.56%	87.78%	87.78%	7.06%	6.20%	84.44%	86.44%	112.63	115.13	0	4.00%
M3	18.89%	31.11%	78.89%	82.22%	16.47%	14.94%	76.67%	82.22%	88.28	110.47	0	4.23%

3.6. Effect of PSB on S. salsa Growth

Treatment with PSB resulted in increased plant height, stem diameter, and above- and below-ground biomass. PSB addition had the greatest contributions to plant total biomass (F = 87,640.89, Table 3), increasing by nearly 2 times under alkali conditions (A1, A2), 2.2 times under NaCl-alkali stress (M2), and 2.5 times under high NaCl stress (N3). Under low NaCl (N1), alkali (A2), and NaCl-alkali (M2, M3) conditions, after PSB addition, the above-ground/below-ground biomass ratio of *S. salsa* decreased compared with that of Control 2 (Table 4).

Table 3. Analysis of variance of the effects of saline-alkali treatment and strain on plant growth.

	df	Plant Height	Stem Diameter	Total Biomass	Above-Ground Biomass	Underground Biomass	Above-Ground Biomass/Underground Biomass
Treatment	9	60.47 ***	18.35 ***	41,359.16 ***	10,428.18 ***	12,105.07 ***	141.47 ***
Strain	1	47.32 ***	8.32 **	87,640.89 ***	26,526.72 ***	19,246.12 ***	16.34 ***
Treatment × Strain	9	5.58 ***	6.05 ***	5036.81 ***	1683.88 ***	1297.75 ***	58.37 ***

Note: F values are reported with p values indicated as follows: ** $p < 0.01$; *** $p < 0.001$.

Table 4. Effect of P-solubilizing bacterium on the growth of *Suaeda salsa*.

Treatment	Strain	Plant Height/cm	Stem Diameter/mm	Biomass/g	Above-Ground Biomass/g	Underground Biomass/g	Above-Ground Biomass/Underground Biomass
Control 1	Control 2	13.57 ± 0.27 cde	1.18 ± 0.04 abc	0.76 ± 0 j	0.57 ± 0 h	0.19 ± 0 k	3.07 ± 0.05 cd
	PSB	13.84 ± 0.29 bcd	1.28 ± 0.07 a	0.66 ± 0 k	0.55 ± 0 i	0.12 ± 0 m	4.75 ± 0.09 b
N1	Control 2	12.63 ± 0.44 efgh	1.03 ± 0.02 de	0.65 ± 0 l	0.45 ± 0 m	0.20 ± 0 j	2.20 ± 0.01 ef
	PSB	13.08 ± 0.46 def	1.05 ± 0.02 cd	1.07 ± 0 f	0.59 ± 0 g	0.48 ± 0 e	1.23 ± 0.01 jk
N2	Control 2	11.83 ± 0.22 ghi	0.83 ± 0.08 f	1.31 ± 0 d	0.67 ± 0 f	0.63 ± 0 c	1.06 ± 0.01 k
	PSB	14.67 ± 0.22 bc	1.10 ± 0.06 bcd	1.82 ± 0 a	0.94 ± 0 b	0.88 ± 0 a	1.08 ± 0 jk
N3	Control 2	11.75 ± 0.29 hi	0.91 ± 0.08 ef	0.42 ± 0 n	0.33 ± 0 o	0.09 ± 0 n	3.48 ± 0.05 c
	PSB	13.67 ± 0.22 cde	1.12 ± 0.02 bcd	1.04 ± 0 g	0.66 ± 0 f	0.38 ± 0 h	1.76 ± 0.02 gh
A1	Control 2	13.00 ± 0.38 defg	1.20 ± 0.02 ab	0.92 ± 0 h	0.48 ± 0 k	0.44 ± 0 g	1.07 ± 0.01 jk
	PSB	16.33 ± 0.30 a	1.20 ± 0.03 ab	1.74 ± 0 b	0.98 ± 0 a	0.75 ± 0 b	1.31 ± 0.01 ijk
A2	Control 2	11.92 ± 0.22 fgh	0.85 ± 0.04 f	0.76 ± 0 j	0.51 ± 0 j	0.25 ± 0 i	1.98 ± 0.01 fg
	PSB	13.58 ± 0.17 cde	1.14 ± 0.01 bcd	1.31 ± 0.01 d	0.71 ± 0 e	0.60 ± 0 d	1.17 ± 0.01 jk
A3	Control 2	10.67 ± 0.46 ij	0.81 ± 0.03 f	0.22 ± 0 p	0.16 ± 0 p	0.06 ± 0 o	2.47 ± 0.04 e
	PSB	10.08 ± 0.51 j	0.80 ± 0.03 f	0.29 ± 0 o	0.24 ± 0 q	0.05 ± 0 p	5.02 ± 0.1 b
M1	Control 2	15.00 ± 0.14 b	1.29 ± 0.05 a	0.90 ± 0 i	0.47 ± 0 l	0.44 ± 0 g	1.07 ± 0.01 jk
	PSB	14.67 ± 0.46 bc	1.00 ± 0.04 de	1.55 ± 0.01 c	0.92 ± 0 c	0.63 ± 0 c	1.48 ± 0 hij
M2	Control 2	9.67 ± 1.08 j	1.14 ± 0.04 bcd	0.57 ± 0 m	0.43 ± 0 n	0.14 ± 0 l	3.16 ± 0.05 cd
	PSB	12.50 ± 0.29 efgh	1.19 ± 0.01 abc	1.23 ± 0 e	0.77 ± 0 d	0.46 ± 0 f	1.66 ± 0.02 ghi
M3	Control 2	6.75 ± 0.52 k	0.83 ± 0.11 f	0.10 ± 0 q	0.08 ± 0 r	0.02 ± 0 q	5.47 ± 0.59 a
	PSB	7.33 ± 0.46 k	0.81 ± 0.03 f	0.10 ± 0 q	0.08 ± 0 r	0.02 ± 0 q	3.01 ± 0.18 d

Note: Error bars represent standard error of the mean (SE), values are means ± SE (n = 3). Different lowercase letters represent the significant differences of the index under different saline and alkali treatments and strain treatments. The HSD values of the significant differences of indicators in each column were 2.26, 0.26, 0.017, 0.017, 0.017, 0.77, respectively from left to right.

4. Discussion

4.1. Characteristics of PSB

After identification, the strain DYS212 was found to belong to the genus *Exiguobacterium* with the two most similar strains being *E. enclense* and *E. indicum*. The strain *E. enclense* was recently isolated from a marine sediment sample taken from Chorao Island, Goa, India [43], but no other related research has been carried out on it. *E. indicum* was isolated from the rhizosphere of the sedge *Cyperus laevigatus*. This strain was shown to exhibit strong inhibitory effects on the following: microbial motility, the production of virulence factors, and biofilm formation [44]; moreover, it achieves maximal protease production levels when treated with 3% organic municipal solid wastes [45]. In general, the *Exiguobacterium* genus has a positive effect on plant growth and the living environment.

Although the PSB strain DYS212 did not dissolve organic P, it exhibited a strong ability to dissolve inorganic P. Within the solid inorganic P medium, P-solubilizing ability was not prominent, but within the liquid inorganic P medium, the available P made by DYS212 reached 470.01 mg L^{-1}, which is a stronger P-solubilizing ability than that of previously researched PSB [46]. In the range of 2–4% salinity, the P-solubilizing ability of the strain was strong with available P above 400 mg L^{-1}; in the range of 1–5.5% salinity, the available P content was above 200 mg L^{-1}. With its salinity and alkali resistance, this strain is a viable PSB candidate for coastal re-vegetation applications.

To be biofertilizers for ecological remediation and environment sustainability of saline-alkali region, large-scale techniques for the preparation and production of high-value strains are critical. In our study, the available P content increased rapidly within 12 h of PSB addition, i.e., the strain played a crucial role in initial plant development stages. In the first 60 h, PSB maintained rapid growth and gradually reached its peak. That's all important information for efficient production. Additionally, it demonstrated effective utilization of glucose, ammonium sulfate, and yeast extract powder, which could be used as a reference in future production and culture processes.

4.2. PSB Influence on S. salsa Development

The inhibition rate of seed germination was higher with salinity and alkalinity; under salt-alkali mixed stress (M3), the inhibition rate of seed germination was as high as 16.47%, with the next largest values of 15.29% and 10.59% being noted under high salinity stress (N3) and high alkali stress (A3), respectively. In general, an alkaline environment inhibited seed germination more strongly than a saline environment, which is concordant with results of previous studies [47,48]. PSB addition can reduce the inhibition of saline-alkaline stress on seed germination, for example, by producing organic acids and decreasing the pH [26,49].

Seed germination rate of the PSB treatment was lower under alkaline stress compared with that of Control 2 on the first day of germination. In the final results, the seed growth rate-promoting effect of PSB under alkali stress was generally higher than that under salt stress; the same promoting effect was also noted under high salinity and high saline-alkaline stress. A previous study showed that the final germination of *Salsola ferganica* seeds was mainly influenced by the salinity concentration in salt-alkali mixed stress treatments [50]. This indicates that salinity controls seed germination. In a previous study, the PSB strain DQ-N exhibited no effect on soybean seed germination rates but promoted hypocotyl elongation [51]. It has been speculated that the effect of PSB on seed germination is not direct, and instead, some molecules or enzymes, such as superoxide dismutase and peroxidase, mediate this effect after germination [52].

The addition of strain DYS212 under salinity and alkali stress, had significant effects on the biomass, plant height, and stem diameter of *S. salsa* (Table 3), among which the contribution to biomass was the greatest (F = 87,640.89). The addition of PSB increased the above-ground, below-ground, and total biomass of S. salsa, but it had no effect on those under high saline-alkali conditions (M3). PSB play a positive role under salinity or alkali stress, as well as under non-stress conditions. However, under high salinity and alkali

stress, the function of PSB could be inhibited because of reciprocal enhancement [49,53], regardless of the plant or bacterial strain. The soil environment under multi-factor mixed stress is more complex, aggravating the dilemma regarding PSB and plant growth. In addition, we found that high saline-alkali conditions led to considerable water seepage from the soil, which may have led to the loss of nutrients and PSB from the soil.

DYS212 inoculation increased the above-ground/below-ground biomass ratio of *S. salsa* under high alkaline stress (A3) and no stress (Control 1), but this ratio decreased under N1, N3, A2, M2, and M3 stress, indicating that PSB promotes plant resource allocation more to the below-ground biomass of the plant under these stresses. Roots play an important role in this process and are more sensitive to soil nutrients than the above-ground organs [54].

Our strain increased plant height but had no effect on plant height under low salinity (N1), high alkalinity (A3), low saline-alkali stress (M1), or no stress (Control 1). This suggests that PSB are halophilic and adapt to alkaline conditions. PSB increased stem diameters only under moderate (N2) and high salinity (N3) and moderate alkali (A2) stress, whereas stem diameters decreased under low saline-alkali stress (M1), with other stresses having no effect. This finding also verified the halophilism of PSB and their intolerance to strong alkalinity, in addition to suggesting that the promoting effect of PSB on plants was stronger in vertical growth (plant height) than in lateral growth (stem diameter). In general, the PSB strain DYS212 has good P-solubilizing ability under common mixed saline-alkaline conditions and has good application prospects.

5. Conclusions

In order to further screen saline-tolerant PSB strains and understand the effect of PSB strain inoculation on the seed germination and plant growth, one PSB strain was isolated from bird droppings in saline and alkaline regions and named *Exiguobacterium* sp. DYS212. It has a strong ability to dissolve inorganic P and is halophilic, with a better P-solubilizing effect at 1–5.5% salinity (available P > 200 mg L^{-1}). The addition of DYS212 promoted seed germination of *S. salsa*, especially under high alkali stress, where the growth-promoting rate of PSB reached 5.26%. DYS212 inoculation also improved the growth of *S. salsa*, in terms of plant height, stem diameter, and biomass (up to 2.5 times), under saline and alkaline conditions. The PSB strain showed great growth-promoting ability under salt and alkali stress and has good application prospects. Our study has important reference significance for PSB application and vegetation restoration in saline-alkaline soils. More PSB strains are expected to undergo stress tolerance tests to assess their application potential as microbial fertilizers in the future contributing to environmental sustainability

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