

Article

Effectiveness of Nitrogen-Fixing Bacteria *Rhodobacter sphaeroides* in Soil–Plant Nitrogen and Rice Performance in Extremely Saline Acid Sulfate Soil over Two Consecutive Seasons

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Abstract: The overuse of chemical fertilizers under adverse conditions endangers the sustainability of agriculture. A biological approach should be investigated to address this issue. Therefore, this study aimed to detect the potency of purple non-sulfur bacteria that can fix nitrogen (N) (PNSB-fN) *Rhodobacter sphaeroides* in soil N fertility, plant N uptake, growth, and rice yield. In brief, an experiment was conducted to check whether the biofertilizer containing PNSB-fN strains can improve rice yield and soil fertility under a highly saline acidic condition. A randomized complete block design was used with four replicates on saline soil in An Bien-Kien Giang, Vietnam. The first factor was the N fertilizer level, i.e., (i) 100%, (ii) 75%, (iii) 50%, and (iv) 0%; the second factor was the PNSB-fN (*R. sphaeroides*), i.e., (i) the control, (ii) S01, (iii) S06, and (iv) combined S01–S06. In the results, supplying PNSB-fN increased NH_4^+ compared with the control, i.e., 104.7–112.0 mg NH_4^+ kg^{-1} compared with 94.0 mg NH_4^+ kg^{-1} in season 1 and 35.9–38.0 mg NH_4^+ kg^{-1} compared with 34.2 mg NH_4^+ kg^{-1} in season 2. Additionally, by supplying each PNSB-fN strain, the soil Na^+ and plant Na in culm leaf and grain were decreased in comparison with those in treatments without PNSB-fN. The total N uptake was also enhanced by the PNSB-fN compared with the control. Moreover, supplying PNSB-fN improved the crop height, panicle length, panicle quantity pot^{-1} , grain quantity panicle^{−1}, filled spikelet rate, and grain yield compared with the control. Ultimately, in extremely saline soil, the mixture of PNSB-fN not only improved soil fertility and reduced soil salinity but also replaced 25% of chemical N fertilizer to ensure sustainable agriculture. This newly developed biofertilizer was potent in not only improving the rice and soil health in the locality but also performing the same under similar conditions around the globe.

Keywords: nitrogen fixation; purple non-sulfur bacteria; rice; saline soil; yield



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1. Introduction

In Vietnam and many regions around the world, saline soil is the main environmental factor limiting the efficiency of agricultural cultivation [1,2]. Agricultural rice production faces many difficulties. For example, rice productivity decreases due to salinity, acidity, diseases, drought, lack of fresh water, and dyke erosion, which are occurring at an increasingly high and widespread level [3]. Salinity adversely affects the growth, physiological characteristics, and biochemical processes of crops due to the disruption of the soil's ability to provide nutrients [4,5]. Saline soils contain high levels of Na^+ , causing an imbalance in the uptake of water and nutrients for crops [6].

Rice is particularly sensitive to high Na concentration because Na affects the processes of photosynthesis, respiration, assimilation, wilting, and drying, eventually leading to the death of all parts of the plant [7]. In the Mekong Delta, farmers often provide N to rice plants by applying chemical N fertilizers. However, the use of chemical fertilizer harms the environment, endangering agricultural sustainability [8]. Under flooded rice cultivation conditions, 60–70% of N is lost in the form of N_2O and N_2 from chemical fertilizers [9], and the efficiency of N fertilizer use is only about 30–35% due to high losses [10,11]. Fertilizers leach from the soil into the groundwater system, contributing to the eutrophication of water bodies [12]. NO_3^- pollution of public water resources increases significantly with the use of N fertilizers and poses serious health challenges to the general population [13]. The interaction between plant roots and the microbial-rich soil environment is crucial for plant health. Therefore, many approaches have been used to address these issues, such as using nanofertilizers to improve N use efficiency [14], using lime to reduce acid sulfate effects [15], developing salt-tolerant rice varieties [16], etc. However, the cost of these methods is not suitable for the agriculture of developing countries.

Recently, safe agricultural production has become a global trend, to reduce the use of chemical fertilizers and pesticides and to change soil biodiversity and beneficial soil microorganisms, under the forms of biofertilizers and biopesticides [17,18]. Some well-known N fixers include *Azotobacter* spp. [19] and *Rhizobium* spp. [20]. However, these bacteria are aerobic [19,21], which is not suitable under submerged conditions under paddy fields for rice. Moreover, these bacteria have not been tested under conditions of both high salinity and acidity. Thus, a group of bacteria named purple non-sulfur bacteria (PNSB) appears as a potent alternative. This is because PNSB belong to photoautotrophic bacteria that use light and carbon dioxide in the environment to produce energy through photosynthesis and can also live photoheterotrophically, chemoheterotrophically, or chemoautotrophically depending on environmental conditions [22]. PNSB belong to a group that can fix N at different growth rates [23,24]. PNSB strains are capable of producing ALA, IAA, siderophores, and NH_4^+ , significantly improving the germination ability of rice grains [25], solubilizing unavailable P forms, and increasing available P [26]. The use of PNSB as a biofertilizer in rice fields has been studied to improve the adverse effects of soil environments such as acidity or salinity [27,28] to support growth and increase rice yield [28–31]. N plays an important role in enhancing rice growth and yield. Specifically, NH_4^+ produced by *R. palustris* contributes to reducing the need for chemical N fertilizer and increasing rice grain yield [22,32].

Therefore, this study was conducted to evaluate the effect of nitrogen-fixing purple non-sulfur bacteria (PNSB-fN) on soil fertility, N uptake, growth, and the yield of rice grown on saline soil. Thus, this study offers an alternative for chemical N fertilizer for rice cultivation in highly saline soil, sustaining soil health and crop yield.

2. Materials and Methods

2.1. Materials

Time: the experiment was conducted from February 2023 to May 2024.

Location: the pot experiment was arranged in the greenhouse of the College of Agriculture, Can Tho University.

Rice variety: OM5451 variety was used in the experiment.

C10 black plastic pots had a mouth diameter of 25 cm, a bottom diameter of 21 cm, and a height of 21 cm.

The fertilizers used were Urea (46% N), superphosphate (16% P_2O_5), and potassium chloride (60% K_2O).

The biofertilizer containing PNSB-fN *Rhodobacter sphaeroides* S01 and S06 was isolated and selected from saline soil and stored at the Faculty of Crop Science, the College of Agriculture, Can Tho University [33].

2.2. Methods

Soil treatment: The soil was collected from a paddy field An Bien, Kien Giang, Vietnam [9°52'56.6" N 105°01'45.4" E]. The soil was dried after the plant residue was removed and mixed well before use. Each pot contained 8 kg of soil, soaked with water, and muddled before sowing. Each pot was sowed with 10 grains, and 8 plants were selected 5 days after sowing (DAS). Harvest time was 90 DAS. After harvesting season 1, water was added to just the ground level, the stubble continued to be removed, and the soil was soaked until soft and mixed well before sowing season 2.

Experimental arrangement: The two-factor experiment was arranged in a randomized complete block design, at the greenhouse of the College of Agriculture, Can Tho University. Factor A was the urea fertilization levels (100, 75, 50, and 0%, as recommended) and factor B was PNSB-fN (the control, supplementing a single strain of *R. sphaeroides* S01, a single strain of *R. sphaeroides* S06, and a mixture of 2 bacterial strains *R. sphaeroides* combined S01–S06), with 4 replications, each of which was 1 pot of 8 plants, i.e., a total of 64 pots. The experiment was arranged similarly in season 1 and season 2. The temperature was 37.8 °C, the humidity was 60.3%, and the light and dark hours per day were 11 and 13 in season 1. In season 2, the temperature, humidity, and the light and dark hours per day were 34.5 °C, 65.1%, and 11.5 and 12.5, respectively.

Inoculating grains with bacteria: Grains were cleaned before inoculation with bacteria, by soaking for 10 min with 70% ethanol and 1% sodium hypochlorite, and then cleaned with sterilized deionized water. Next, the rice grains were incubated in dark conditions for 24 h to help the grains germinate. Then, the rice grains were divided into 4 groups to be treated with 4 different solutions with 4 beakers containing bacteria suspensions with a density of 10^{10} CFU mL^{-1} consisting of (1) sterilized deionized water, (2) a bacterial strain solution *R. sphaeroides* S01, (3) bacterial strain solution *R. sphaeroides* S06, (4) a mixture of bacterial strains *R. sphaeroides* S01 and S06. The density of the PNSB-fN was ensured by spectrophotometry at 660 nm with a relative optical density of 1.0 [34]. The beakers were placed in a shaker at 60 rpm for 1 h and allowed to stabilize at room temperature for 1 h. After that, the grains were dried with laminar air flow before sowing. Bacterial biofertilizer was added 6 times to rice plants with 4 $mL\ time^{-1}\ pot^{-1}$ for 8 rice grains, equivalent to 5.4×10^4 CFU g^{-1} dry soil. The irrigation was according to each treatment at 7, 14, 21, 28, 35, and 42 DAS.

Fertilizer: The fertilizer formula for rice applied was according to the formula of De [35]: 100N-60 P_2O_5 -30 K_2O (217.4 kg of urea, 375 kg of superphosphate, and 50 kg of potassium chloride) for 2000 t of soil per ha, which was calculated as an amount of fertilizer for 8 kg of soil in a pot: 0.87 g of urea, 1.5 g of superphosphate, and 0.4 g of potassium

chloride. The superphosphate was applied 100% at the beginning. The urea was applied at a rate of 30, 40, and 30%, respectively, at 10, 20, and 45 DAS. The potassium chloride was applied two times, and 50% at 10 and 45 DAS was used at each time.

Evaluating criteria:

Growth indicators: Crop height and panicle length were determined at 90 DAS. Crop height was measured from the ground to the tip of the highest leaf of the rice plant, measuring 3 plants in each pot. Panicle length was determined from the neck of the panicle to the tip of the panicle, measuring 8 panicles for each pot [36].

Chlorophyll content:

For the SPAD index, in each pot, 10 random mature leaves at the $2/3$ position from the leaf stem to the leaf tip were measured by the Chlorophyll Meter SPAD [37]. For the chlorophyll (Chl) content in leaves, mature leaves were collected at 45 DAS and measured [38]. Particularly, 1 cm² of leaf flesh was reacted with 3 mL N, N-Dimethyl formamide, and incubated for 24 h in the dark in a test tube. The extract was collected and measured by a spectrophotometer at 664 nm (A664) and 647 nm (A647) wavelengths. The Chl content was calculated according to the following formulas: $\text{Chl } a = 12.64 \times A664 - 2.99 \times A647$; $\text{Chl } b = 23.26 \times A647 - 5.60 \times A664$; and $\text{Chl } (a + b) = 7.04 \times A664 + 20.27 \times A647$.

Indicators of yield and yield components: Panicle quantity pot⁻¹ was counted as the total number of rice panicles in each pot. Grain quantity panicle⁻¹ was counted as the total number of grains (filled and unfilled) on 8 rice panicles in each pot. The filled spikelet rate was calculated as (total number of filled spikelets/total number of spikelets) $\times$ 100%. The weight of 1000 spikelets was the same as the weight of 1000 filled spikelets in each experimental pot. Actual yield was weighed as the grain weight at harvest time of each pot and converted to 14% moisture.

The soil analysis methods were summarized according to Sparks et al. [39] as follows:

pH_{H2O}, pH_{KCl}, and EC: Soil samples were extracted with distilled water or KCl solution (1.0 M) at a ratio of 1:5, measured with a pH meter. The EC was measured from the extract after pH_{H2O} measurement using an EC meter.

Total nitrogen: the soil was inorganicized with a mixture of H₂SO₄ saturated-CuSO₄-Se, with a ratio of 100-10-1, determined by Kjeldahl distillation, and titrated with H₂SO₄ 0.01 N.

Available N (NH₄⁺): soil was extracted with 2.0 M KCl and measured with a spectrophotometer at a 650 nm wavelength.

Total P: the soil sample was inorganicized with H₂SO₄ saturated-HClO₄, developed with a color of phosphomolybdate with ascorbic acid as a reducing agent, and measured on a spectrophotometer at a wavelength of 880 nm.

Soluble phosphorus (PO₄³⁻) was determined by the Bray II method. Therein, the soil was extracted with 0.1 N HCl and 0.03 NH₄F with a soil/water ratio of 1:7. Color was detected as phosphomolybdate with the reducing agent of ascorbic acid and measured on a spectrophotometer at a wavelength of 880 nm.

Unavailable phosphorus: the Al-P, Fe-P, and Ca-P contents from soil samples were extracted with NH₄F 0.5 M (pH = 8.2), NaOH 0.1 M, and H₂SO₄ 2.5 M, respectively, washed twice with saturated NaCl, and measured with a spectrophotometer at 880 nm.

Cation exchange capacity (CEC): soil was extracted with MgSO₄ 0.02 M and titrated with 0.01 M EDTA.

K⁺, Na⁺, Ca²⁺, and Mg²⁺ were determined by soil extraction with BaCl₂ 0.1 M and measured on an atomic uptake spectrophotometer at wavelengths of 766, 589, 422.7, and 285.5 nm, respectively.

Method of processing and analyzing plant samples through dry biomass: Culms, leaves, and grains were dried at 70 °C for 72 h and weighed for dry biomass. A plant crusher was used to crush culms, leaves, and grains. The sample was inorganicized with

H₂SO₄ and H₂O₂ until it turned into a clear solution and diluted with distilled water to a volume of 50 mL. This solution was used to determine N and Na content [40].

Statistical analysis: Data were processed using Microsoft Excel software. SPSS software version 13.0 was used to compare the mean differences between the treatments with Duncan's test at the 5% significance level. The interaction between two factors for yield and uptake was recorded.

3. Results

3.1. Characteristics of Saline Soil in an Bien-Kien Giang

Soil characteristics at the beginning of the season in An Bien-Kien Giang had a pH_{H₂O} of 3.07, pH_{KCl} of 2.92, EC of 30.0 mS cm^{−1}, NH₄⁺ of 102.7 mg kg^{−1}, and soluble P of 17.5 mg kg^{−1}. Total N and P contents were 0.126% and 0.025%, respectively. The soil also had an Al-P of 161.0 mg kg^{−1}, Fe-P of 58.7 mg kg^{−1}, and Ca-P of 42.8 mg kg^{−1}. In addition, other characteristics of soil at the beginning of the season are presented in Table 1.

Table 1. Characteristics of paddy saline soil collected in An Bien-Kien Giang for greenhouse experiment.

Indicator	Unit	Value
pH _{H₂O}	-	3.07
pH _{KCl}	-	2.92
Electrical conductivity	mS cm ^{−1}	20.0
N _{total}	%	0.126
NH ₄ ⁺	mg kg ^{−1}	102.7
P _{total}	%	0.025
Fe-P	mg kg ^{−1}	58.7
Al-P	mg kg ^{−1}	161.0
Ca-P	mg kg ^{−1}	42.8
P _{soluble}	mg kg ^{−1}	17.5
Cation exchange capacity	meq 100 g ^{−1}	11.6
K ⁺	meq 100 g ^{−1}	0.145
Na ⁺	meq 100 g ^{−1}	3.67
Ca ²⁺	meq 100 g ^{−1}	0.394
Mg ²⁺	meq 100 g ^{−1}	23.2

3.2. Effects of Nitrogen-Fixing Purple Non-Sulfur Bacteria on Biochemical Characteristics of Rice Plants Grown on Saline Soils in an Bien-Kien Giang

Chlorophyll content in leaves was significantly affected by PNSB-fN supplementation at 21, 28, 35, and 42 DAS, with a SPAD index of 31.9–41.3 in both seasons. Compared with the control, the single-strain addition of *R. sphaeroides* or combined S01–S06 increased the SPAD index in both seasons. Fertilization levels of 0, 50, 75, and 100% N changed the SPAD index at 21, 28, 35, and 42 DAS in both seasons. There were interactions between the urea fertilization and PNSB-fN supplementation in season 1 and season 2 (Table 2).

The amount of Chl *a*, *b*, and (*a* + *b*) in leaves at different urea fertilization levels had a statistically significant difference of 5% in both seasons. In particular, the Chl *a* increased in the treatment using single or combined S01–S06 strains in both seasons. PNSB-fN supplementation reduced the Chl *b* compared with the control in both seasons. The Chl (*a* + *b*) content of the treatment with single-strain supplement S06 was equivalent to the treatment with single-strain supplement S01. While those of the treatment with combined supplement S01–S06 were equivalent to the treatment with single-strain supplement S01 in season 1. However, supplementing PNSB-fN did not change the Chl (*a* + *b*) content in season 2. The interaction had a statistically significant difference of 5% between the urea

fertilization and PNSB-fN supplementation in Chl *a*, *b*, and (*a* + *b*) in season 1 and season 2. However, the Chl (*a* + *b*) interaction was not statistically significant in season 2 (Table 2).

Table 2. Effect of nitrogen-fixing purple non-sulfur bacteria *Rhodobacter sphaeroides* on chlorophyll content and proline content in rice cultivated in salinized soil under greenhouse conditions.

Factor		SPAD				Chlorophyll ($\mu\text{g g}^{-1}$ Fresh Mass)			Proline ($\mu\text{mol g}^{-1}$ DW)
		21 Days	28 Days	35 Days	42 Days	<i>a</i>	<i>b</i>	(<i>a</i> + <i>b</i>)	
Season 1									
Urea level (A) (%)	100	34.1 ^a	39.4 ^a	41.1 ^a	42.1 ^a	5.24 ^a	1.58 ^a	6.82 ^a	13.3 ^a
	75	33.7 ^a	39.0 ^b	40.3 ^b	41.1 ^b	5.00 ^b	1.60 ^a	6.60 ^b	12.8 ^a
	50	32.9 ^b	38.5 ^c	39.8 ^c	40.3 ^c	5.10 ^b	1.60 ^a	6.68 ^b	10.8 ^b
	0	31.7 ^c	37.7 ^d	38.2 ^d	39.2 ^d	4.95 ^b	1.48 ^b	6.44 ^c	10.3 ^c
Nitrogen-fixing PNSB (B)	No bacteria	31.9 ^b	38.1 ^c	39.4 ^c	39.6 ^b	4.72 ^c	1.65 ^a	6.37 ^c	15.3 ^a
	S01	34.2 ^a	39.1 ^a	40.2 ^a	40.8 ^a	5.25 ^a	1.47 ^c	6.72 ^{ab}	13.0 ^b
	S06	32.3 ^b	38.7 ^b	40.1 ^a	41.3 ^a	5.23 ^a	1.59 ^b	6.81 ^a	9.30 ^c
	S01 + S06	34.0 ^a	38.8 ^b	39.8 ^b	41.0 ^a	5.07 ^b	1.56 ^b	6.63 ^b	9.50 ^c
Significance level (A)		*	*	*	*	*	*	*	*
Significance level (B)		*	*	*	*	*	*	*	*
Significance level (A $\times$ B)		ns	*	*	*	*	*	*	*
CV (%)		3.13	0.86	0.65	2.75	3.67	2.78	2.80	5.64
Season 2									
Urea level (A) (%)	100	35.4 ^a	39.5 ^a	38.9 ^a	38.0 ^a	4.45 ^a	2.21 ^a	6.67 ^a	10.7 ^a
	75	35.2 ^{ab}	39.4 ^a	38.5 ^a	36.7 ^b	4.36 ^a	2.15 ^a	6.52 ^a	9.90 ^b
	50	35.0 ^b	39.1 ^a	37.1 ^b	33.3 ^c	3.57 ^b	1.97 ^b	5.54 ^b	9.02 ^c
	0	30.3 ^c	31.9 ^b	30.7 ^c	29.4 ^d	2.58 ^c	1.69 ^c	4.27 ^c	9.99 ^b
Nitrogen-fixing PNSB (B)	No bacteria	33.5 ^c	36.1 ^c	35.1 ^c	33.6 ^b	3.24 ^b	2.37 ^a	5.61	11.2 ^a
	S01	33.9 ^{bc}	37.1 ^b	35.7 ^b	33.2 ^b	3.76 ^a	1.97 ^b	5.73	9.39 ^b
	S06	34.0 ^b	38.3 ^a	37.3 ^a	35.4 ^a	3.95 ^a	1.97 ^b	5.92	11.2 ^a
	S01 + S06	34.4 ^a	38.3 ^a	37.1 ^a	35.4 ^a	4.03 ^a	1.71 ^c	5.75	7.83 ^c
Significance level (A)		*	*	*	*	*	*	*	*
Significance level (B)		*	*	*	*	*	*	ns	*
Significance level (A $\times$ B)		*	*	*	*	ns	*	ns	*
CV (%)		1.77	1.99	1.95	2.31	12.3	6.49	8.69	8.38

Note: In the same column, numbers followed by different letters are statistically different at the 5% level (*); ns: no statistically significant difference; S01: *R. sphaeroides* S01; S06: *R. sphaeroides* S06; S01 + S06: *R. sphaeroides* S01 and S06.

Supplementing PNSB-fN helped reduce proline content in culm leaf, with an average content of 10.6 $\mu\text{mol g}^{-1}$ DW and 9.47 $\mu\text{mol g}^{-1}$ DW in both seasons, compared with the control, 15.3 and 11.2 $\mu\text{mol g}^{-1}$ DW, respectively. However, the treatment with N fertilizer levels with proline content in season 1 helped increase proline content, but in season 2, the treatment with the control was equivalent to the treatment with 75% N fertilizer and higher than the treatment with 50% N fertilizer. The interaction of proline content between the urea fertilization and PNSB-fN supplementation had a statistically significant difference of 5% in both seasons (Table 2).

3.3. Effects of Nitrogen-Fixing Purple Non-Sulfur Bacteria on Growth and Yield of Rice Grown on Saline Soils in an Bien-Kien Giang

Supplementing PNSB-fN helped the improvement in crop height, panicle length, panicle pot number⁻¹, grain quantity panicle⁻¹, filled spikelet rate, and yield compared with the control. In particular, the treatment with a mixture of two strains of bacteria or the single strain of *R. sphaeroides* S06 had the same crop height, followed by the strain *R. sphaeroides* S01 in season 1. The crop height was the highest in the treatment with the mixed addition of two PNSB-fN strains, followed by *R. sphaeroides* S06 and *R. sphaeroides* S01 in season 2. Panicle length ranked as follows: S01–S06~S06 > S01 in season 1 and S01 + S06 > S01 > S06 in season 2. Panicle quantity pot⁻¹ in season 1 of the treatment

with combined S01–S06 was equivalent to that of the treatment with a single strain of *R. sphaeroides* S06 or *R. sphaeroides* S01 (26.4–28.2 panicles pot^{-1}) and higher than the control (25.4 panicles pot^{-1}). Meanwhile, the treatment with combined S01–S06 had the number of panicles reaching 18.5 panicles pot^{-1} , as high as the treatment with bacterial strain S06, with 18.6 panicles pot^{-1} , followed by the treatment with a single strain of S01, with 16.4 panicles pot^{-1} in season 2. The grain quantity panicle $^{-1}$ in the treatment with S06 had the highest result with 128.3 grains panicle $^{-1}$, followed by the combined S01–S06 and S01 with 122.1 and 115.8 grains panicle $^{-1}$, respectively. In season 1, the treatment with combined S01–S06 had the highest number of 86.4 grains panicle $^{-1}$, followed by the treatment with the addition of each equivalent S06 or S01 single strain, with 79.2 and 79.0 grains panicle $^{-1}$ in season 2. The treatments with PNSB-fN had a higher filled spikelet rate compared with the control. The yield of rice supplemented with a single bacterial strain of S01 had the highest yield, followed by the treatment with a mixture of two strains PNSB-fN and the treatment with a single strain of S06, with a yield increase of 6.57–18.6% in season 1 and 6.32–13.0% in season 2 compared with the control. Moreover, in season 2, the treatment with a mixture of two bacterial strains had the next highest yield, followed by S01 and S06 (Table 3).

The treatments with urea fertilization combined with PNSB-fN supplementation had a statistically significant difference in rice yield at 5% in both seasons (Figure 1). In season 1, the treatments using single strains of S01, S06, or combined S01–S06 achieved grain yields that gradually increased with increasing fertilization levels of 0%, 50%, 75%, and 100% N. Additionally, adding the mixture of two PNSB-fN strains, a single strain of S01 combined with 50% urea fertilization, had yields equivalent to the treatment with only 100% urea fertilization without bacteria. The treatments applying different N fertilizer levels without bacteria achieved lower yields than those using combined S01–S06 or a single bacterial strain of S01 or S06 combined with N fertilizer levels, respectively, in season 2.

Table 3. Effect of nitrogen-fixing purple non-sulfur bacteria *Rhodobacter sphaeroides* on growth and yield of rice cultivated in salinized soil under greenhouse conditions.

Factor		Crop Height (cm)	Panicle Length	Panicle Quantity Pot $^{-1}$ (Panicles)	Grain Quantity Panicle $^{-1}$ (Grains)	1000-Spikelet Weight (g)	Filled Spikelet Rate (%)	Yield (g pot $^{-1}$)
Season 1								
Urea level (A) (%)	100	109.3 ^a	23.2 ^a	31.0 ^a	125.7 ^a	23.6	78.5 ^a	45.5 ^a
	75	107.9 ^{ab}	22.5 ^b	29.3 ^b	122.1 ^a	23.3	75.2 ^b	41.2 ^b
	50	105.7 ^b	21.7 ^c	26.7 ^c	115.4 ^b	23.6	74.4 ^b	39.3 ^c
	0	97.9 ^c	20.7 ^d	20.4 ^d	97.2 ^c	23.9	73.5 ^b	28.1 ^d
Nitrogen-fixing PNSB (B)	No bacteria	92.6 ^c	20.7 ^c	25.4 ^c	94.4 ^d	23.2	71.5 ^c	35.0 ^d
	S01	106.4 ^b	22.1 ^b	28.2 ^a	115.8 ^c	23.6	77.8 ^a	41.5 ^a
	S06	109.8 ^a	22.6 ^a	26.4 ^{bc}	128.3 ^a	23.5	76.9 ^{ab}	37.3 ^c
	S01 + S06	112.0 ^a	22.7 ^a	27.3 ^{ab}	122.1 ^b	24.1	75.5 ^b	40.2 ^b
Significance level (F_A)		*	*	*	*	ns	*	*
Significance level (F_B)		*	*	*	*	ns	*	*
Significance level ($F_{A \times B}$)		ns	*	ns	ns	ns	ns	*
CV (%)		3.04	2.94	5.93	7.16	5.55	3.87	4.10
Season 2								
Urea level (A) (%)	100	101.9 ^a	19.2 ^a	25.3 ^a	96.2 ^a	22.2	88.8 ^a	51.5 ^a
	75	98.6 ^b	18.6 ^b	20.7 ^b	91.9 ^b	22.0	87.4 ^b	44.8 ^b
	50	91.9 ^c	17.8 ^c	17.7 ^c	82.4 ^c	22.0	85.5 ^c	35.7 ^c
	0	75.2 ^d	16.6 ^d	7.06 ^d	48.7 ^d	22.0	81.8 ^d	9.61 ^d

Table 3. Cont.

Factor		Crop Height (cm)	Panicle Length	Panicle Quantity Pot ⁻¹ (Panicles)	Grain Quantity Panicle ⁻¹ (Grains)	1000-Spikelet Weight (g)	Filled Spikelet Rate (%)	Yield (g pot ⁻¹)
Nitrogen-fixing PNSB (B)	No bacteria	89.9 ^c	17.4 ^d	16.4 ^c	74.6 ^c	22.0	82.8 ^c	33.2 ^c
	S01	91.5 ^b	18.2 ^b	17.3 ^b	79.0 ^b	21.7	84.9 ^b	35.6 ^b
	S06	92.1 ^b	18.0 ^c	18.6 ^a	79.2 ^b	21.9	88.2 ^a	35.3 ^b
	S01 + S06	94.1 ^a	18.7 ^a	18.5 ^a	86.4 ^a	22.3	87.4 ^a	37.5 ^a
Significance level (F _A)		*	*	*	*	ns	*	*
Significance level (F _B)		*	*	*	*	ns	*	*
Significance level (F _{A×B})		*	*	*	*	ns	ns	*
CV (%)		2.17	2.13	6.34	6.15	3.38	2.05	2.96

Note: In the same column, numbers followed by different letters are statistically different at the 5% level (*); ns: no statistically significant difference; S01: *R. sphaeroides* S01; S06: *R. sphaeroides* S06; S01 + S06: *R. sphaeroides* S01 and S06.

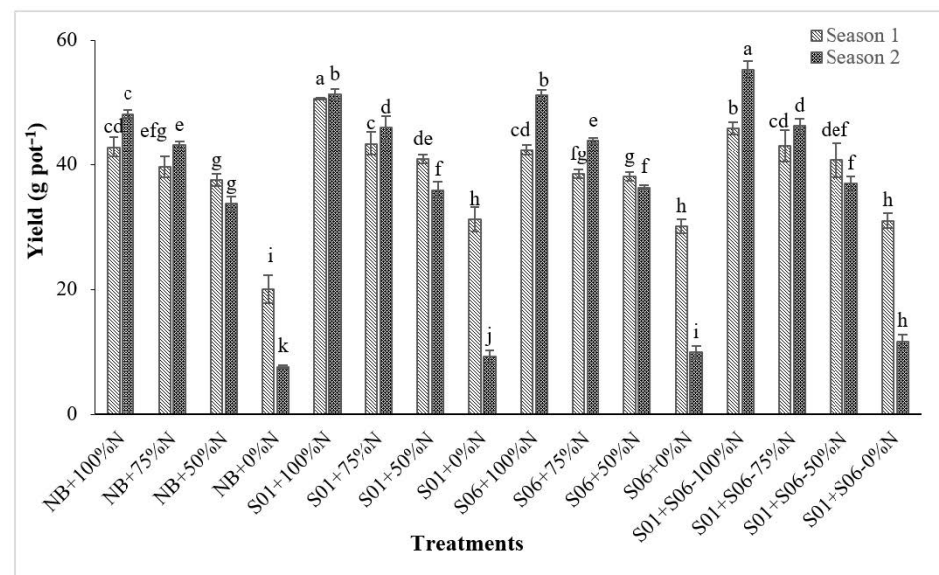


Figure 1. Effects of N fertilizer level and nitrogen-fixing purple non-sulfur bacteria *Rhodobacter sphaeroides* on rice yield grown on saline soil in An Bien-Kien Giang under greenhouse conditions. Note: Different lowercase letters indicate a 5% statistically significant difference between treatments; NB: no bacteria, S01: *R. sphaeroides* S01; S06: *R. sphaeroides* S06; S01 + S06: *R. sphaeroides* S01 and S06.

3.4. Effects of Nitrogen-Fixing Purple Non-Sulfur Bacteria on the Fertility of an Bien-Kien Giang Saline Soil for Rice Cultivation

pH_{H2O}, pH_{KCl}, and EC changed significantly in the treatment with supplementation of single strains or a mixture of two strains, S01 and S06, in both seasons, except for pH_{H2O} in season 1 and pH_{KCl} in season 2. Specifically, pH_{H2O} in the control was equivalent to the treatment with a single S06 (4.16 and 4.23) in season 2. Additionally, the pH_{KCl} increased compared with the control (3.71–3.77 compared with 3.60) in season 1. On the contrary, the treatment with supplementation of a single strain or a mixture of two PNSB-fN strains had reduced EC compared with the control in two seasons (12.0–12.6 mS cm⁻¹ and 8.17–8.67 mS cm⁻¹, compared with 13.7 and 9.16 mS cm⁻¹, respectively). Urea fertilization levels affect the pH_{H2O}, statistically different at 5% compared with 0% N treatment in both seasons. pH_{H2O} under the fertilization levels of 50 and 0% N was higher than the fertilization levels of 75 and 100% N (3.38 and 3.41 compared with 3.30 and 3.32, respectively) in season 1. The treatment with 50% N had a pH_{H2O} of 4.36, equivalent to the

treatment with 0% N with a $\text{pH}_{\text{H}_2\text{O}}$ of 4.32. In the meantime, the treatment with 100% N fertilizer had $\text{pH}_{\text{H}_2\text{O}}$ reaching 4.22, equivalent to the treatment with no urea fertilization and the treatment with 75% urea fertilization, with $\text{pH}_{\text{H}_2\text{O}}$ of 4.21 in season 2. There were interactions between the two factors of urea fertilization and PNSB-fN supplementation, with a statistically significant difference at 5% for $\text{pH}_{\text{H}_2\text{O}}$, and pH_{KCl} in season 1 and EC in two seasons (Table 4).

Table 4. Effect of nitrogen-fixing purple non-sulfur bacteria *Rhodobacter sphaeroides* on the fertility of salinized paddy soil under greenhouse conditions.

Factor		pH _{H2O}	pH _{KCl}	EC	N total	P total	NH ₄ ⁺	P soluble	Fe-P	Ca-P	Al-P	CEC	K ⁺	Mg ²⁺	Ca ²⁺	Na ⁺
		-	-	mS cm ⁻¹	%				mg kg ⁻¹					meq 100 g ⁻¹		
Season 1																
Urea level (A) (%)	100	3.32 ^b	3.72	12.65	0.172	0.032	109.3 ^a	29.9	40.9	77.4	166.3	15.7	1.27 ^b	13.1	0.442	2.76
	75	3.30 ^b	3.69	12.64	0.174	0.032	107.0 ^a	32.2	40.0	77.7	167.3	15.6	1.29 ^b	12.4	0.438	2.73
	50	3.38 ^a	3.72	12.86	0.173	0.031	102.7 ^b	31.1	40.4	76.6	165.9	15.7	1.32 ^{ab}	12.8	0.445	2.59
	0	3.41 ^a	3.72	12.63	0.169	0.032	100.3 ^b	30.7	40.5	75.9	163.3	16.1	1.36 ^a	12.7	0.456	2.68
Nitrogen-fixing PNSB (B)	No bacteria	3.35	3.60 ^c	13.7 ^a	0.170	0.032	94.0 ^d	29.9 ^b	47.0 ^a	83.7 ^a	179.5 ^a	14.9 ^c	1.21 ^c	12.4 ^b	0.461 ^b	2.92 ^a
	S01	3.37	3.77 ^a	12.6 ^b	0.172	0.031	104.7 ^c	30.3 ^{ab}	41.2 ^b	73.8 ^c	144.6 ^d	15.5 ^b	1.33 ^b	13.0 ^{ab}	0.422 ^c	2.81 ^a
	S06	3.33	3.71 ^b	12.5 ^b	0.175	0.032	108.5 ^b	31.6 ^{ab}	35.2 ^d	71.6 ^c	172.3 ^b	16.3 ^a	1.31 ^b	12.5 ^{ab}	0.377 ^d	2.48 ^b
	S01 + S06	3.35	3.77 ^a	12.0 ^c	0.171	0.032	112.0 ^a	32.1 ^a	38.4 ^c	78.4 ^b	166.5 ^c	16.5 ^a	1.39 ^a	13.2 ^a	0.521 ^a	2.51 ^b
Significance level (F _A)		*	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns
Significance level (F _B)		ns	*	ns	ns	ns	*	*	*	*	*	*	*	*	*	*
Significance level (F _{A×B})		*	*	*	*	ns	*	ns	ns	*	*	ns	*	*	*	*
CV (%)		1.87	1.46	2.33	6.18	5.46	4.15	8.03	7.30	4.54	4.88	3.33	5.52	7.46	4.48	6.68
Season 2																
Urea level (A) (%)	100	4.22 ^{bc}	3.71	8.85	0.231	0.029	36.8	13.1 ^b	133.6	44.6	17.7	14.0	0.243 ^d	5.10 ^a	0.148	10.7
	75	4.21 ^c	3.76	8.65	0.227	0.029	36.1	13.2 ^b	130.0	46.0	16.9	14.7	0.278 ^c	4.71 ^b	0.151	11.0
	50	4.36 ^a	3.87	8.70	0.229	0.028	36.6	14.3 ^a	130.4	45.7	17.4	14.7	0.327 ^b	4.78 ^b	0.151	11.2
	0	4.32 ^{ab}	3.84	8.65	0.228	0.028	36.4	13.9 ^a	128.6	45.6	17.6	14.4	0.430 ^a	4.53 ^b	0.148	11.1
Nitrogen-fixing PNSB (B)	No bacteria	4.16 ^b	3.82	9.16 ^a	0.231	0.026	34.2 ^c	10.2 ^c	137.7 ^a	49.4 ^a	24.7 ^a	14.6	0.270 ^c	5.31 ^a	0.151	12.1 ^a
	S01	4.34 ^a	3.87	8.85 ^b	0.233	0.029	37.8 ^a	15.0 ^a	127.4 ^b	44.5 ^b	14.5 ^c	14.7	0.345 ^a	4.38 ^b	0.149	11.0 ^b
	S06	4.23 ^b	3.71	8.67 ^b	0.222	0.031	35.9 ^b	13.9 ^b	134.3 ^a	45.0 ^b	16.5 ^b	14.0	0.313 ^b	5.11 ^a	0.151	11.2 ^b
	S01 + S06	4.38 ^a	3.78	8.17 ^c	0.229	0.028	38.0 ^a	15.4 ^a	123.0 ^c	43.1 ^b	14.0 ^c	14.4	0.350 ^a	4.34 ^b	0.147	9.78 ^c
Significance level (F _A)		*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	*	*	ns	ns
Significance level (F _B)		*	ns	*	ns	ns	*	*	*	*	*	ns	*	*	ns	*
Significance level (F _{A×B})		ns	ns	*	ns	ns	ns	*	*	*	*	ns	*	ns	*	*
CV (%)		3.15	4.86	3.82	5.66	18.0	4.33	6.89	4.08	6.32	10.7	7.26	0.048	7.51	6.50	1.50

Note: In the same column, numbers followed by different letters are statistically different at the 5% level (*); ns: no statistically significant difference; S01: *R. sphaeroides* S01; S06: *R. sphaeroides* S06; S01 + S06: *R. sphaeroides* S01 and S06.

The treatments with urea fertilization and bacterial addition did not affect N_{total} and P_{total} , with an average content of 0.688% N and 0.032% P_2O_5 in season 1 and 0.229% N and 0.029% P_2O_5 in season 2. The NH_4^+ content and soluble P in the treatments with PNSB-fN increased compared with the control, with 104.7–112.0 $\text{mg NH}_4^+ \text{ kg}^{-1}$ compared with 94.0 $\text{mg NH}_4^+ \text{ kg}^{-1}$ and 30.3–32.1 mg P kg^{-1} compared with 29.9 mg P kg^{-1} in season 1 and 35.9–38.0 $\text{mg NH}_4^+ \text{ kg}^{-1}$ compared with 34.2 $\text{mg NH}_4^+ \text{ kg}^{-1}$ and 13.9–15.4 mg P kg^{-1} compared with 10.2 mg P kg^{-1} in season 2. The soluble P was improved in the treatment with PNSB-fN supplement because bacteria dissolved the unavailable P from Fe-P, Ca-P, and Al-P, leading those compounds to decrease in both seasons, except for the treatments with the single-strain PNSB-fN to dissolve unavailable P forms from Fe-P in season 2. However, in the urea fertilization factor, there was no change in unavailable P contents at fertilization levels of 0, 50, 75, and 100% N. There were interactions between the urea fertilization level and PNSB-fN with a statistically considerable difference at 5% in the amount of NH_4^+ in season 1, soluble P and Fe-P in season 2, and Al-P and Ca-P in two seasons (Table 4).

PNSB-fN contributed to changes in CEC and Ca^{2+} in season 1 and K^+ , Mg^{2+} , and Na^+ in two seasons. Specifically, the treatment with the PNSB-fN supplements had increased CEC in season 1 and K^+ in both seasons compared with the control. However, the Mg^{2+} in the control was equivalent to the PNSB-fN treatments in season 1 and the single strain S06 treatment in season 2. Ca^{2+} in the control was higher than in single-strain treatments and lower than in the combined treatments. In contrast, the Na^+ in the treatment with the addition of a single strain of S06 or combined S01–S06 was lower than the treatment with a single strain of S01 and the control in season 1. In season 2, the lowest was the mixture of two strains of S01–S06, followed by each strain of S01 and S06 compared with the control (Table 4).

3.5. Effects of Nitrogen-Fixing Purple Non-Sulfur Bacteria on N and Na Content and Uptake of Rice Grown on Saline Soil in Bien-Kien Giang

Culm-leaf and dry grain biomass increased in the case of supplementing urea or PNSB-fN over both seasons. Specifically, for the urea fertilization factor, culm-leaf biomass and dry grains were the lowest in the treatment with 0% N and gradually increased at higher urea fertilization levels in the order of 0%N < 50%N < 75% N < 100%N in both seasons. For the PNSB-fN factor, dry culm-leaf biomass increased on average by 16.4% and 9.91% in season 1 and 11.0% and 7.71% in season 2, respectively. The interaction between the two factors of N and PNSB-fN urea fertilization levels on culm-leaf and dry grain biomass had a statistically considerable difference, at 5% across both seasons (Table 5).

Table 5. Effect of nitrogen-fixing purple non-sulfur bacteria *Rhodobacter sphaeroides* on contents and uptake of Na and N in rice cultivated in salinized soil under greenhouse conditions.

Factor		Biomass		Na Content		Na Uptake		Total Na Uptake	N Content		N Uptake		Total N Uptake
		Culm Leaf	Grain	Culm Leaf	Grain	Culm Leaf	Grain		Culm Leaf	Grain			
		g pot ^{−1}		%		mg Na pot ^{−1}		%		mg N pot ^{−1}			
Season 1													
Urea level (A) (%)	100	52.8 ^a	41.7 ^a	1.63 ^a	0.513 ^a	842.5 ^a	209.7 ^a	1052.2 ^a	0.920 ^a	1.45 ^a	604.2 ^a	486.5 ^a	1090.7 ^a
	75	49.5 ^b	41.4 ^a	1.37 ^c	0.411 ^b	678.4 ^b	168.1 ^b	846.5 ^b	0.826 ^b	1.33 ^b	549.2 ^b	409.9 ^b	959.2 ^b
	50	47.0 ^c	38.8 ^b	1.45 ^b	0.394 ^b	678.4 ^b	152.2 ^c	830.6 ^b	0.754 ^c	1.24 ^c	482.8 ^c	355.4 ^c	838.1 ^c
	0	39.2 ^d	25.7 ^c	1.38 ^{bc}	0.394 ^b	540.4 ^c	101.7 ^d	642.0 ^c	0.700 ^d	1.17 ^d	301.1 ^d	275.4 ^d	576.5 ^d
Nitrogen-fixing PNSB (B)	No bacteria	42.0 ^d	34.3 ^c	1.83 ^a	0.579 ^a	783.2 ^a	204.5 ^a	987.7 ^a	0.753 ^c	2.23 ^d	424.5 ^c	318.2 ^d	742.8 ^d
	S01	50.2 ^b	38.8 ^a	1.49 ^b	0.362 ^c	743.6 ^b	141.7 ^b	885.3 ^b	0.793 ^b	1.26 ^b	498.0 ^b	401.4 ^b	899.4 ^b
	S06	44.8 ^c	35.4 ^b	1.24 ^c	0.412 ^b	552.4 ^d	145.3 ^b	697.8 ^d	0.828 ^a	1.39 ^a	496.0 ^b	374.7 ^c	870.7 ^c
	S01 + S06	51.6 ^a	39.1 ^a	1.28 ^c	0.359 ^c	660.4 ^c	140.2 ^b	800.7 ^c	0.825 ^a	1.31 ^b	518.6 ^a	432.8 ^a	915.6 ^a
Significance level (F _A)		*	*	*	*	*	*	*	*	*	*	*	*
Significance level (F _B)		*	*	*	*	*	*	*	*	*	*	*	*
Significance level (F _{A×B})		*	*	*	*	*	*	*	*	*	*	*	*
CV (%)		2.93	2.85	6.94	9.83	7.16	10.75	6.53	3.28	3.43	4.34	4.91	3.61
Season 2													
Urea level (A) (%)	100	41.5 ^a	38.0 ^a	0.539 ^d	0.177 ^a	221.6 ^a	66.9 ^a	288.5 ^a	0.592 ^a	1.38 ^a	246.3 ^a	525.1 ^a	771.4 ^a
	75	33.6 ^b	31.5 ^b	0.624 ^c	0.176 ^a	209.0 ^a	55.5 ^b	264.5 ^b	0.500 ^b	1.28 ^b	168.3 ^b	402.6 ^b	570.9 ^b
	50	26.8 ^c	25.6 ^c	0.718 ^b	0.161 ^b	191.5 ^b	41.2 ^c	232.7 ^c	0.441 ^c	1.18 ^c	118.3 ^c	301.8 ^c	420.1 ^c
	0	7.21 ^d	6.88 ^d	0.868 ^a	0.151 ^c	62.3 ^c	10.4 ^d	72.7 ^d	0.434 ^c	1.19 ^c	31.4 ^d	82.5 ^d	113.9 ^d
Nitrogen-fixing PNSB (B)	No bacteria	25.2 ^c	24.1 ^c	0.834 ^a	0.224 ^a	195.7 ^a	58.7 ^a	254.4 ^a	0.441 ^d	1.17 ^d	115.2 ^d	285.4 ^c	400.7 ^c
	S01	27.5 ^b	25.1 ^b	0.611 ^b	0.125 ^c	155.6 ^c	30.8 ^c	186.4 ^c	0.480 ^c	1.27 ^b	137.0 ^c	326.6 ^b	463.5 ^b
	S06	28.0 ^{ab}	25.4 ^b	0.654 ^b	0.131 ^c	161.8 ^{bc}	33.0 ^c	194.8 ^c	0.499 ^b	1.23 ^c	145.5 ^b	319.3 ^b	464.8 ^b
	S01 + S06	28.4 ^a	27.4 ^a	0.651 ^b	0.186 ^b	171.3 ^b	51.2 ^b	222.7 ^b	0.546 ^a	1.36 ^a	166.7 ^a	380.6 ^a	547.2 ^a
Significance level (F _A)		*	*	*	*	*	*	*	*	*	*	*	*
Significance level (F _B)		*	*	*	*	*	*	*	*	*	*	*	*
Significance level (F _{A×B})		*	*	*	*	*	*	*	*	*	*	*	*
CV (%)		2.76	1.75	9.07	8.29	11.0	10.9	8.58	4.02	2.69	4.08	4.14	2.65

Note: In the same column, numbers followed by different letters are statistically different at the 5% level (*); S01: *R. sphaeroides* S01; S06: *R. sphaeroides* S06; S01 + S06: *R. sphaeroides* S01 and S06.

Table 5 shows significantly different N and Na content in the culm leaf and grain, influenced by the levels of urea fertilization and PNSB-fN supplementation. N content in culm leaves and grains decreased for the treatments with reduced urea fertilization in the order of 100%N > 75%N > 50%N > 0%N in season 1 and 100%N > 75%N > 50%N ~ 0%N in season 2. Na content in culm leaves and grains in the treatments with 100% N fertilizer was higher compared with the control. The treatments with 50 and 75% N fertilizer levels had equivalent Na content in culm leaf and grain to no urea fertilization in season 1. However, when urea fertilization levels decreased, the Na in the culm leaf increased and the Na content in the grain decreased in season 2. In particular, the Na content in the culm and leaves was 0.539–0.718%, lower than 0.868% in the treatment with no urea fertilization. The Na content in grain was 0.161–0.177% higher than 0.151% in the treatment with no urea fertilization.

N content in culm leaves and grains increased in the treatments with PNSB-fN supplements compared with the control. On the contrary, the Na content in culm leaves and grains in the treatments with PNSB-fN decreased compared with the control in both seasons.

Additionally, total N uptake increased at different levels of urea fertilization or bacterial supplement compared with the control in both seasons, corresponding to 838.1–1090.7 mg pot⁻¹ and 899.4–915.6 mg pot⁻¹ compared with 576.5 and 742.8 mg pot⁻¹ in season 1 and 420.1–771.4 mg pot⁻¹ and 463.5–547.2 mg pot⁻¹ compared with 113.9 and 400.7 mg pot⁻¹ in season 2. The interaction between urea fertilization and PNSB-fN supplementation had a statistically meaningful difference at 5% in content, Na, N uptake, total N uptake, and total Na uptake.

The Na uptake in culm leaf and grain had a statistically significant difference at 5%, and the total Na uptake in the treatments with urea fertilization increased by an average of 188.6–410.2 mg per pot⁻¹ in season 1 and 160.0–215.8 mg pot⁻¹ in season 2. The total Na uptake decreased in the treatment with the addition of a single strain or a mixture of two bacterial strains compared with the control in two seasons, with 697.8–885.3 mg pot⁻¹ and 186.4–222.7 mg pot⁻¹ compared with 987.7 and 254.4 mg pot⁻¹.

Regarding Table 5 and Figure 2, the total N uptake of rice gradually increases through urea fertilization levels, i.e., 0% > 50% > 75% > 100%. Additionally, the use of the single strain of PNSB-fN S01 or S06 or a mixture of both bacterial strains increased the total N uptake by rice. The treatment with 100% N combined with S01–S06 had the highest total N uptake in both seasons. Moreover, the treatments with 75% N fertilizer added with single strains or combined S01–S06 had a higher total N uptake in the plant or were equivalent to the treatment with 100% N fertilizer in season 1. In season 2, the treatment with 75% N combined with S01–S06 had a total N uptake equivalent to the treatment fertilizing 100% N. Furthermore, the treatments with no urea fertilization supplemented with PNSB-fN had a higher total N uptake than the treatment with no urea fertilization and no bacteria.

Table 5 and Figure 3 show that total Na uptake gradually increased with urea fertilization levels of 0, 50, 75, and 100% N in both seasons. Additionally, PNSB-fN supplementation had a lower total Na uptake than the treatment with the control in season 1. In season 2, the levels of N fertilizer and bacterial supplement reached a total Na uptake statistically significant difference at 5% between the treatments. The bacterial strains contributed to reducing the total Na uptake in rice plants. Specifically, the treatment with only 100% N had the highest total Na uptake compared with the treatments with PNSB-fN supplementation combined with different urea fertilization levels.

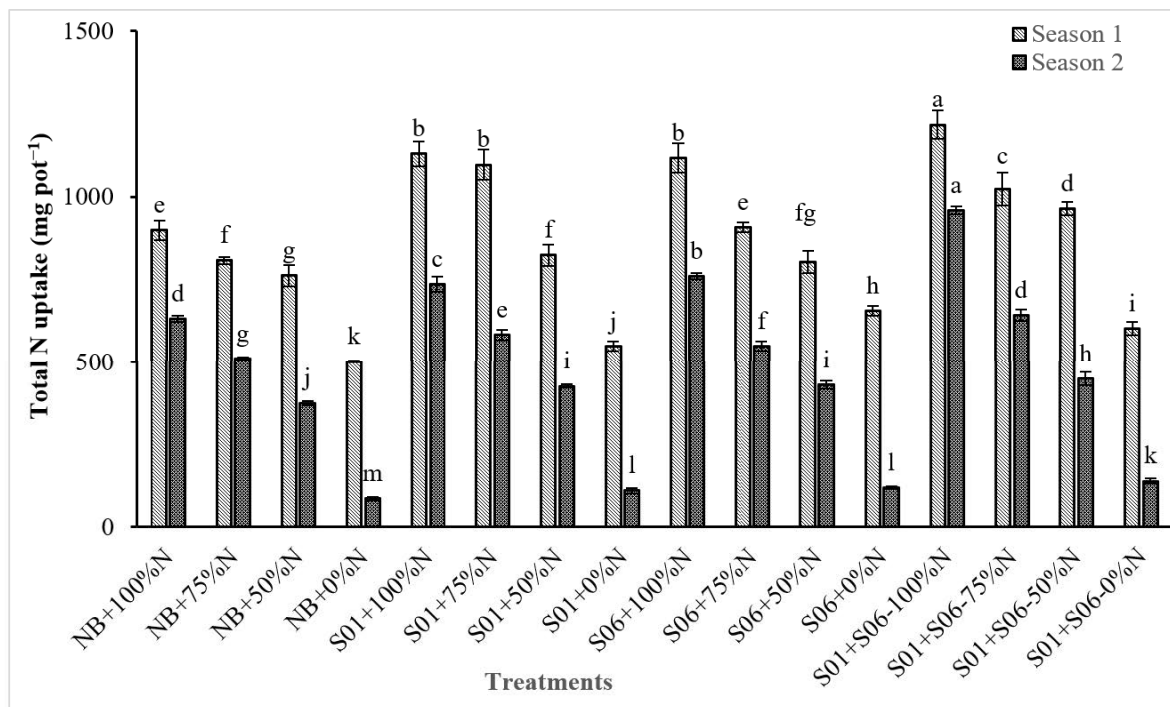


Figure 2. Effects of N fertilizer level and nitrogen-fixing purple non-sulfur bacteria *Rhodobacter sphaeroides* on total N uptake in rice grown on saline soil in An Bien-Kien Giang under greenhouse conditions. Note: Different lowercase letters indicate a 5% statistically significant difference between treatments; NB: no bacteria, S01: *R. sphaeroides* S01; S06: *R. sphaeroides* S06; S01 + S06: *R. sphaeroides* S01 and S06.

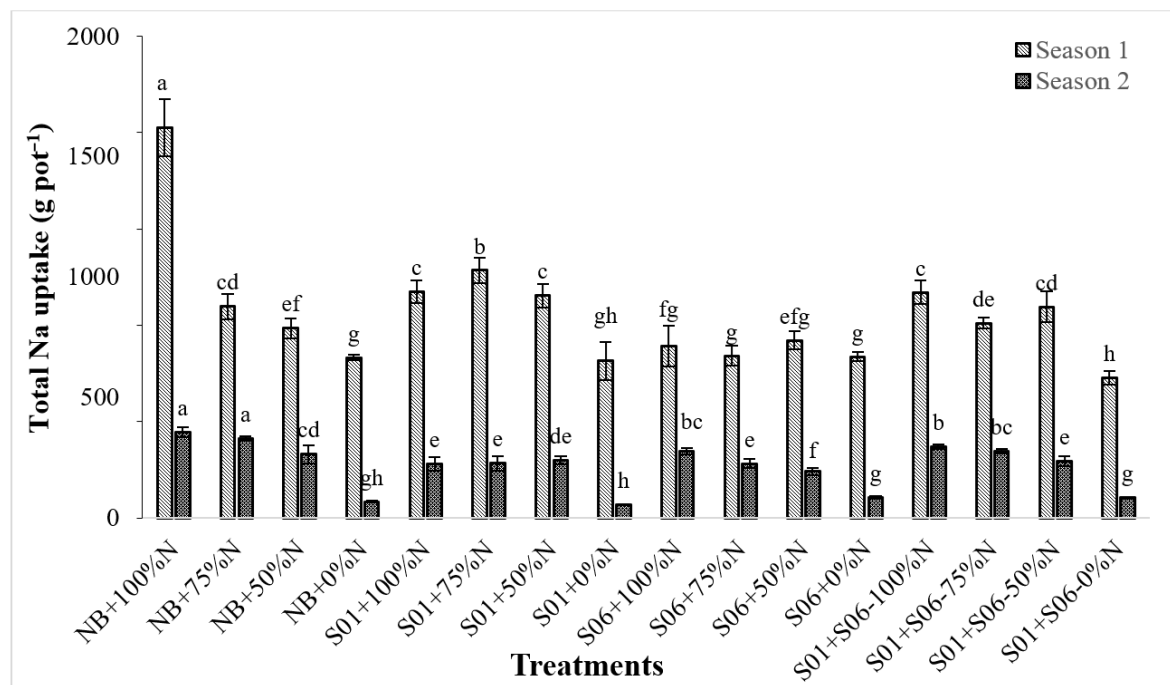


Figure 3. Effects of N fertilizer level and nitrogen-fixing purple non-sulfur bacteria *Rhodobacter sphaeroides* on total Na uptake in rice grown on saline soil in An Bien-Kien Giang under greenhouse conditions. Note: Different lowercase letters indicate a 5% statistically significant difference between treatments; NB: no bacteria, S01: *R. sphaeroides* S01; S06: *R. sphaeroides* S06; S01 + S06: *R. sphaeroides* S01 and S06.

4. Discussion

4.1. Soil Characteristics at the Beginning of the Autumn Season in an Bien-Kien Giang Used for Experiments in the Greenhouse

Results in Table 1 show that the soil characteristics in An Bien-Kien Giang had a $\text{pH}_{\text{H}_2\text{O}}$ value of 3.07 and pH_{KCl} of 2.92. According to Horneck et al. [41], a pH less than 5.10 is considered very acidic. Therefore, the pH in the study area was considered to be very acidic. The EC had a value of 20.0 mS cm^{-1} . As per Horneck et al. [41], with this EC value, only a few crops can adapt. The total P content (0.025%) was assessed at a poor level according to the assessment of Cu et al. [42]. According to Metson [43], the total N content (0.126%) was low. NH_4^+ had a value of 102.7 mg kg^{-1} , while soluble P was 17.5 mg kg^{-1} , which is rated as low according to the rating scale of Horneck et al. [41]. The Al-P, Fe-P, and Ca-P contents were 161.0 mg kg^{-1} , 58.7 mg kg^{-1} , and 42.8 mg kg^{-1} , respectively. As reported by Landon [44], a CEC of $11.6 \text{ meq } 100 \text{ g}^{-1}$ is low. As per Maff [45], the K^+ ($0.145 \text{ meq K}^+ 100 \text{ g}^{-1}$) was assessed at a low threshold. According to Marx et al. [46], Ca^{2+} ($0.394 \text{ meq Ca}^{2+} 100 \text{ g}^{-1}$) was rated at a low level. Mg^{2+} ($23.2 \text{ meq Mg}^{2+} 100 \text{ g}^{-1}$) was rated at a very high level [41]. Na^+ was $3.67 \text{ meq Na}^+ 100 \text{ g}^{-1}$. In general, the soil fertility in the study area was assessed at an average level. Despite the adverse condition of the current soil, the current bacteria still performed properly at improving rice yield and soil health because these bacteria were isolated from a highly saline acidic condition [33]; i.e., the saline acidic environment barely affected the performance of the PNSB-fN strains.

4.2. Effects of Nitrogen-Fixing Purple Non-Sulfur Bacteria *R. sphaeroides* on Biochemical Properties of Rice Plants

The SPAD index correlated closely with the chlorophyll content in the plant. The level of urea fertilization affected the chlorophyll content in the leaves, representing the N content in the leaves, as well as the photosynthetic ability of the plant, and factors relating to crop productivity [47,48]. Table 2 shows that, in season 1 and season 2, the SPAD index gradually decreased with the level of reduced urea fertilization at 21, 28, 35, and 42 DAS. Similarly, Chl *a*, *b*, and (*a* + *b*) contents decreased at 42 DAS.

Single or combined S01–S06 strains improved the SPAD index. The Chl *b* and (*a* + *b*) contents in both seasons of the treatment with combined S01–S06 were enhanced compared with no additional PNSB-fN (Table 2). This proves that PNSB-fN helped increase chlorophyll content in leaves through the ability to fix N. According to Kafi et al. [49], the treatment with the use of biofertilizer contributed to increasing chlorophyll content and nutritional content in the leaves of plants. Photosynthetic activities, metabolism, and protein synthesis increased, leading to increased productivity [22,50]. In addition, supplementing PNSB contributed to increasing chlorophyll content in plant leaves by 15% compared with the control [51].

As per Shiade et al. [52], proline content is synthesized to maintain cell osmosis and protect enzymes, proteins, membranes, and cell structures in water-deficient conditions, thereby increasing plant tolerance under stress conditions. Proline content increases as salinity increases. This is consistent with the study by Raza et al. [53], where proline content increased when plants were exposed to salt stress. Supplementing a single strain or mixture of four bacterial strains of *L. sphaeroides*, W01, W14, W22, and W32, contributed to reducing proline content in rice leaves cultivated in saline soils [28]. The proline content decreased in the treatment with PNSB-fN supplementation because EPS secreted from PNSB-fN reduced Na content, leading to low proline content (Table 2).

4.3. Effects of Nitrogen-Fixing Purple Non-Sulfur Bacteria *R. sphaeroides* Rice Growth and Yield

Growth, yield components, and yield in the treatment with PNSB-fN supplementation were higher than in the control (Table 3, Figure 1). This result is consistent with Yen et al. [51], where PNSB improved the growth, biomass, and yield of rice. Apart from rice, other crops also benefit from the PNSB inoculations. For instance, grain plants, such as dajulis [54], Komak beans [55], and sesame plants [56], had their growth and yield improved and when combining with chemical fertilizers; at least 25% of the fertilizer can be replaced by the PNSB inoculants. Fruits, such as pineapple [57] and canary melon [58], had their recommended fertilizer rate reduced by 25% by the PNSB. A herb, lemon balm [59], also benefits from the PNSB. In addition, previous studies reported that PNSB improved growth such as crop height and yield components such as grain quantity panicle⁻¹, panicle quantity pot⁻¹, the filled spikelet rate, and the yield of rice grown on saline soil [34,60–63]. PNSB possess these functions as N fixation [23,64], P solubilization [30,34], and K solubilization [65] and secrete ALA, IAA, and EPS compounds to reduce salt stress to promote rice growth [34,61,62]. This is consistent with some other N-fixers, such as *Azotobacter chroococcum*, *Azospirillum brasilense*, and *Bacillus megaterium*, which reduced 30% of chemical fertilizers for sugar beet [66]. *A. brasilense* Sp245 is also proven to be able to promote wheat growth [67].

4.4. Effects of Nitrogen-Fixing Purple Non-Sulfur Bacteria *R. sphaeroides* on Soil Fertility

The combined S01–S06 increased pH_{H2O}, NH₄⁺, P_{soluble}, K⁺, Mg²⁺, and Ca²⁺ and reduced Na⁺ and EC (Table 4). This is consistent with the study by Sundar and Chao [23], where some PNSB can secrete several growth stimulants and ALA metabolites that contribute to improving soil pH. According to Khuong et al. [61], the treatment with the addition of a single strain or a mixture of two strains of bacteria, *L. sphaeroides* W03 and *L. sphaeroides* W11, increased pH_{H2O} compared with the control. Therefore, using PNSB minimizes the adverse effects of chemical fertilizers on the microbial community in the soil to improve soil fertility [65]. Additionally, the treatment with supplements *Rh. palustris* KKSSR66 can provide significant amounts of available N for plants [68]. PNSB biofertilizer containing bacteria *Rh. palustris* can fix N, providing available N soil fertility [69]. Therefore, PNSB-fN supplementation contributed to improving the performance of rice cultivation soil.

4.5. Effects of Nitrogen-Fixing Purple Non-Sulfur Bacteria *R. sphaeroides* on N and Na Content and Uptake of Rice

As shown in Table 5 and Figure 3, the PNSB-fN increased N uptake compared with the control. This is in accordance with the study by Sundar and Chao [23], where PNSB strains can fix N under acidic conditions and provide biological N for rice. Additionally, PNSB also produce ALA, IAA, and siderophores to resist abiotic stress by increasing pH and fixing Na⁺ by EPS [61,62]. Therefore, PNSB supplementation leads to significant cost savings due to reduced fertilizer use, required dosage, and associated production costs for PNSB biomass.

Results in Table 5 and Figure 2 reveal that Na content in rice in the treatment with PNSB-fN decreased compared with the treatment with the control. The total N uptake of rice in the treatment with single-strain PNSB-fN S01 or S06 and the combination of them in both seasons was reduced compared with the control. Because the vulnerability of rice to NaCl is very high, Na⁺ uptake cannot be controlled from saline soil into root hairs, leading to toxic Na accumulation in culms and leaves [70]. In addition, H⁺ in acidic soil is the main obstacle to rice productivity [71]. PNSB supports rice growth in saline acidic soil conditions by reducing Na⁺, leading to a decrease in rice proline [29]. PNSB improve the concentration of Na⁺ and H⁺ in rice soil because PNSB secrete more EPS under stress conditions to bind

these cations, leading to lower Na^+ and H^+ [72]. According to Nunkaew et al. [73], *L. sphaeroides* and *Rh. palustris* provide EPS bound to Na^+ to resist NaCl. Khuong et al. [61] also showed that Na uptake in culm leaf and rice grains on saline acidic soil decreased in the treatment using PNSB. Specifically, Khuong et al. [61] showed that the treatments with the addition of a single strain or a mixture of two strains of W03 and W11 had lower Na uptake in leaves and grains compared with the control without adding PNSB, with 139–152 and 4.66–5.67 mg pot^{−1} compared with 162 and 6.80 mg pot^{−1} on saline acidic soil in Hong Dan district, Bac Lieu province. Furthermore, Sabki et al. [62] also stated that the *R. palustris* strains can be considered as a promising biofertilizer for agricultural production.

However, the current biofertilizer has not been tested under field conditions yet. Thus, a carrier should be chosen to reduce the impacts of the environment on the biofertilizer [74]. A carrier can promote the growth and support the viability of the bacteria under adverse conditions [75]. There are several carriers for PNSB that can be considered, such as rubber wood ash, decanter cake, rice husk ash, and spent coffee grounds [30]. Thereby, further research can be conducted under field conditions with similar soil features, and the number of treatments can be shortened by eliminating the single use of PNSB-fN. Although there have been several successful field studies investigating the effects of PNSB on rice [26,51,54], the combination of chemical fertilizer and PNSB on rice has not been focused on yet, while biofertilizers cannot entirely replace N and P chemical fertilizers due to their main effects on crop growth [76]. Thus, a further field trial is necessary after the current pot experiment. Moreover, unfortunately, the current study measured only the effects of the biofertilizer on the rice plants and soil health, while the pathways were still predicted according to previous studies [26,77,78]. Deeper research should be conducted on the molecular principles, mechanisms, and pathways regarding how PNSB can fix N, solubilize P and K, reduce metal toxicities, improve soil pH, enhance plant growth traits, etc.

5. Conclusions

Supplementing single-strain bacteria *R. sphaeroides* S01, S06, or combined S01–S06 increases NH_4^+ in two seasons. Additionally, supplementing single-strain bacteria *R. sphaeroides* reduced soil salinity and Na content in the culm leaf and grain compared with the treatments with no addition of PNSB-fN in saline soil in An Bien-Kien Giang. Furthermore, supplementing PNSB-fN helped the improvements in crop height, panicle length, panicle pot number^{−1}, grain quantity panicle^{−1}, filled spikelet rate, and grain yield compared with the control. PNSB-fN addition improved soil–plant N nutrient dynamics. According to the results of this study, the new biofertilizer containing PNSB-fN strains was promising as a replacement for a portion of chemical P fertilizer and should be further investigated in a field trial to maintain sustainable rice cultivation in extremely saline soil. The current study has demonstrated a potent candidate to alter the use of chemical fertilizers for rice under adverse conditions.

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Abbreviations

The following abbreviations are used in this manuscript:

ALA	5-aminolevulinic acid
Al-P	Aluminum phosphate
Ca-P	Calcium phosphate
CFU	Colony forming unit
DAS	Days after sowing
EC	Electrical conductivity
EPS	Exopolymeric substances
Fe-P	Ferrous phosphate
IAA	Indole-3-acetic acid
N	Nitrogen
Na	Sodium
P	Phosphorus
pH _{H2O}	Actual acidity
pH _{KCl}	Potential acidity
PNSB	Purple non-sulfur bacteria
PNSB-fN	Nitrogen-fixing purple non-sulfur bacteria
SPAD	The Soil–Plant Analysis Development

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