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The Efficacy of 5-Aminolevulinic Acid-Producing *Luteovulum sphaeroides* Strains on Saline Soil Fertility, Nutrient Uptakes, and Yield of Rice

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Abstract: Saline soils negatively affect and cause serious problems for rice cultivation. This study aimed to evaluate the efficacy of the purple nonsulfur bacteria (PNSB) capable of secreting 5-aminolevulinic acid (ALA) to reduce soil salinity, improve soil fertility, and enhance rice growth and yield. A two-factorial experiment was conducted in a randomized complete block design with four replications. Factor one was the salinity of the irrigated water, and factor two was the supplementation of the ALA-producing PNSB. The results indicated that watering with saline water above 3‰ led to decreases in plant growth and rice yield compared to the treatments watered with tap water. Application of either an individual strain or the mixture of W01, W14, and W22 ameliorated soil properties and increased total NPK uptake, whereas treatments supplied with the mixed strains reduced total Na uptake (9.50 mg Na pot^{-1}). Supplying the W01, W14, and W22 strains individually or in a mixture enhanced the plant height by 3.51–5.45% and rice grain yield by 14.7–26.2%, compared with those of the control treatment. From the study, the combination of the *L. sphaeroides* W01, W14, and W22 strains is promising for application in saline or salt-contaminated regions to aid the damages caused by salinity on cultivars there, especially rice. Furthermore, this is a biological approach to ease an environmental problem and improve crop performance, which is supposed to be a trend in the sustainable agriculture.

Keywords: 5-aminolevulinic acid; *Luteovulum sphaeroides*; purple nonsulfur bacteria; rice; saline soil

Citation: Khuong, N.Q.; Dung, N.T.T.; Thu, L.T.M.; Quang, L.T.; Xuan, L.N.T.; Phong, N.T. The Efficacy of 5-Aminolevulinic Acid-Producing *Luteovulum sphaeroides* Strains on Saline Soil Fertility, Nutrient Uptakes, and Yield of Rice. *Agriculture* **2023**, *13*, 1761. https://doi.org/10.3390/agriculture13091761

Academic Editors: Othmane Merah, Hailin Zhang, Purushothaman Chirakkuzhyil Abhilash, Magdi T. Abdelhamid, Bachar Zebib and Guisheng Zhou

Received: 21 June 2023
Revised: 1 September 2023
Accepted: 1 September 2023
Published: 5 September 2023

1. Introduction

The Mekong Delta is considered to be one of the regions that are severely affected by climate change. Salt intrusion is a critical factor influencing agriculture in the Mekong Delta [1]. More specifically, the salt intrusion significantly impacts regions for rice–shrimp cultivation in Bac Lieu, Vietnam [2]. In 2013, the phenomenon damaged 625 ha of rice and 55 ha of rice in the rice–shrimp system [2]. When living under saline conditions, the growth and yield of plants tend to decrease. This can be found in many studies on different types of plants, such as peas [3], rice [4], potatoes [5], and Cayenne peppers [6]. This could be due to changes caused by saline stress on nutrient uptake. Nutrient uptake decreases, while sodium (Na) uptake increases during saline stresses [7,8]. Moreover, during saline stress, proline production is enhanced [9]. Thereby, to evaluate the damage caused by salinity on rice, proline is a good indicator. Proline is an amino acid that can stabilize proteins, membranes, and subcellular structures to protect them from damage [10]. A dramatically increasing proline production occurs when plants encounter abiotic stresses, including saline stress [11].

Nowadays, although there are many chemical approaches to minimize the harm caused by Na^+ in rice–shrimp soils, to obtain sustainable agriculture, it is necessary to find methods using biofertilizers containing beneficial bacteria to reduce not only the damage caused by the salinity on crops but also the amount of Na^+ existing in rice paddy fields. Some methods have used purple nonsulfur bacteria (PNSB) because they have potential with their wide-ranging adaptability, including stress conditions [12]. Moreover, PNSB belong to a group of bacteria that can provide a metabolite called 5-aminolevulinic acid (ALA) [13,14] and have been isolated from rice fields to serve as plant growth promoters producing ALA to help rice to tolerate salinity [15].

In Vietnam, PNSB are applied to provide nutrients for rice via processes of nitrogen (N) fixation and phosphorus (P) solubilization [16]. Recently, some of the PNSB strains are capable of producing ALA and have been isolated from saline soils of systems for rice–shrimp cultivation [16]. Nevertheless, the efficiency of these bacteria in improving the soil fertility and nutrient uptake of plants has not been investigated yet. ALA is assumed to hold an important role in agriculture [17] because of its role in promoting plant growth [18,19], reducing heavy metal contaminations in soils, and helping plants to overcome salt stress [20]. Furthermore, ALA has been reported to regulate the manganese (Mn) absorption from soils [21] and stimulate chrome (Cr) tolerance in crops [22]. Additionally, the two popular precursors of ALA are glycine and glutamate [23].

However, the metabolite of ALA has not been studied yet in the case that the bacteria live in a saline condition, though ALA can be considered as a mechanism to decrease soil salinity. Therefore, applying ALA-producing bacteria to saline soil to enhance rice yield and help the rice to overcome saline stress is well grounded to robust. In addition, other parameters, such as soil chemical properties and plant growth and yield, should also be considered to evaluate if the bacteria are efficient and have any undesirable effects on the environment. Ultimately, the study was conducted to determine the efficiency of ALA-producing PNSB application to decrease the saline stress on rice plants; improve rice nutrients uptake, growth, and yield; and remediate the saline condition.

2. Materials and Methods

2.1. Soil Collection

The soil was collected at a depth of 0–20 cm at the beginning of the rice crop, and at rice harvest in the rice–shrimp system in Hong Dan Commune, Bac Lieu Province, Vietnam. Its characteristics are described in Table 1.

Table 1. The characteristics of the soil used in the experiment.

Parameters	Unit	Values
pH_{water}	-	3.15 ± 0.13
pH_{KCl}	-	2.77 ± 0.09
EC	mS cm^{-1}	5.10 ± 0.36
N_{total}	%N	0.84 ± 0.03
NH_4^+	mg kg^{-1}	120 ± 2.89
P_{total}	$\%P_2O_5$	0.017 ± 0.003
$P_{soluble}$	mg P kg^{-1}	9.39 ± 1.27
Al-P	mg P kg^{-1}	13.9 ± 1.65
Fe-P	mg P kg^{-1}	53.9 ± 5.37
Ca-P	mg P kg^{-1}	22.4 ± 2.22
CEC	meq CEC 100 g^{-1}	6.86 ± 0.85
Na^+	meq Na^+ 100 g^{-1}	1.78 ± 0.08
K^+	meq K^+ 100 g^{-1}	0.56 ± 0.11
Mg^{2+}	meq Mg^{2+} 100 g^{-1}	9.00 ± 1.29

Note: Data are means ± standard deviations, with 4 replications.

2.2. Bacteria Source

The PNSB *Luteovulum sphaeroides* W01, W14, and W22 strains, capable of excreting ALA, were isolated from the saline soil of the rice–shrimp system [16]. In addition, these strains are able to fix N, solubilize P, and produce plant growth-promoting substances, including indole-3-acetic acid (IAA), exopolymeric substances (EPS), and siderophores [16], which other bacteria rarely produce. Furthermore, these bacterial strains can adapt to various types of environments. These above traits made bacteria strains in this study more potent.

2.3. Fertilizers

The fertilizers used in the study consisted of urea (46% N), Long Thanh superphosphate (16% P_2O_5), and potassium chloride (60% K_2O).

2.4. Soil Preparation

After the soil collection, the soil was left to dry naturally, cleaned from plant residues, moderately smashed, and well mixed. Next, 8 kg of soil was put into each pot, added with 5 L of water, soaked for 48 h, and turned into mud for rice cultivation.

2.5. Experimental Design

The two-factor experiment was conducted in a randomized complete block design with four replications. In detail, the first factor was watering with saline water at a concentration of 0‰, 2‰, 3‰, and 4‰. The second factor was the application of the ALA-producing PNSB, which consisted of no bacteria applied, applying the W01 strain, applying the W14 strain, applying the W22 strain, and applying the mixture of the three strains of W01, W14, and W22. The dimensions of pots were 23 cm × 17 cm × 18 cm (top × base × height).

The fertilizer formula for rice in this study was 90N-60P_2O_5-30K_2O. The N fertilization was separated into three portions at the rate of 30%, 30%, and 40% and fertilized at 10, 20, and 40 days after sowing (DAS). The P fertilization was applied as the ground three days before sowing. The potassium (K) fertilization was applied at 10 and 40 DAS, with 50% each time.

2.6. Saline Watering

The saline solution was prepared by mixing NaCl at different concentrations of 0, 2, 3, and 4‰ with water. Ten milliliters of the prepared saline water was added to each pot at 10, 30, and 45 DAS.

2.7. Bacterial Inoculation to Rice Seeds

To sterilize rice seeds, 70% ethanol and 1% sodium hypochlorite solution was applied for 3 and 10 min, respectively, and then sterilized distilled water was used to wash them. After that, the seeds were incubated for 24 h in the dark for germination. To inoculate the PNSB into the seeds, a bacterial cell suspension at 10^8 colony-forming units (CFU) mL^{-1}, as prepared previously, was used to soak the seeds in flasks. The mixture was set in a reciprocal shaker at 60 rpm for 1 h and held at room temperature for 1 h to stabilize. Then, before sowing, the seeds were dried under a laminar airflow; in addition, at this moment, the rice seeds were coated with roughly 6.3×10^6 PNSB cells per seed. The seeds used as the negative control were similarly prepared, but sterilized distilled water was used instead of the PNSB inoculants.

The bacterial solution was added to each pot with a volume of 3 mL for applying an individual strain of the PNSB. For the treatment using the combination of the three strains, the application of each strain was 1 mL. The bacteria were supplied for rice a day after saline watering, i.e., at 11, 31, and 46 DAS.

2.8. Plant Biochemical Analysis

The concentration of proline was analyzed at 35 and 50 DAS by the Ninhydrin method [24].

2.9. Growth Measurement

The growth parameters were measured at harvest and consisted of: plant height (cm), where eight plants in each pot were measured from the soil surface to the top leaf of a plant; and panicle length (cm), which was measured from the panicle neck to the end of the panicle in eight plants in each pot.

2.10. Analysis of Yield Components and Yield

The yield components were measured as follows. The number of seeds per panicle: all filled seeds and unfilled seeds were counted in eight panicles per pot; the 1000-seed weight: the weight of 1000 filled seeds of each pot was determined; the number of panicles per pot: the total number of panicles was counted in each pot; seed moisture: the humidity of seeds at harvest was measured by a seed moisture meter (Model PM410, Kett, Silicon Valley, CA, USA).

Grain yield: all of the seeds in each pot at harvest were weighed and converted into that at 14% humidity.

2.11. Soil Analysis

Soil properties, including pH_{water}, pH_{KCl}, electrical conductivity (EC), and cation exchange capacity (CEC; Ca^{2+}, K^{+}, Na^{+}, and Mg^{2+}), were analyzed according to the method of Sparks et al. [25].

2.12. Analysis of Nutrients Concentration in Straw and Seeds

Dry biomass: straw and seeds were dried at 70 °C for 72 h to achieve their dry weights.

Milled samples: a mill was used for crushing straws and seeds to analyze their components.

The plant components, including the concentration of macronutrients and Na, were analyzed according to the method of Walinga et al. [26] in the study.

2.13. Statistical Analysis

Numbers were analyzed using the Microsoft Excel software and the SPSS 13.0 software (SPSS Inc., Chicago, IL, USA), and Duncan's test was applied to compare the differences between the means of treatments.

3. Results

3.1. The Impact of the Water Salinity and the Supplementation of Luteovulum sphaeroides on the Soil Chemical Characteristics

Although pH_{water} did not differ significantly between the salinity levels of the water used, pH_{water} in the treatments with the three mixed bacterial strains (3.72) was greater than that in the treatment without bacteria (3.51). However, pH_{KCl} was not statistically different and was roughly 3.15–3.19 for both factors. The EC was different ($p < 0.05$) for the salinity factor. The treatment watered with no salt had an EC of 0.48 mS cm^{-1}, which was lower than that of the treatments watered with saline irrigation (approximately 0.53–0.57 mS cm^{-1}). On the other hand, the application of either an individual strain of W01, W14, and W22 or their mixture resulted in lower EC values (0.51 to 0.54 mS cm^{-1}) than those in the treatments without bacteria—roughly 0.58 mS cm^{-1} ($p < 0.05$) (Table 2).

Table 2. The influence of the water salinity and the supplementation of the purple nonsulfur bacteria *Luteovulum sphaeroides* capable of producing ALA on the chemical characteristics of the saline soil in the rice–shrimp system.

Factor		pH_{water}	pH_{KCl}	EC	N_{total}	NH_4^+	P_{total}	$P_{soluble}$
				mS cm^{-1}	%	mg kg^{-1}	%	mg kg^{-1}
The water salinity (A) (‰)	0	3.65 ± 0.17	3.19 ± 0.10	0.48 ± 0.05 ^{b}	0.128 ± 0.014	18.8 ± 2.8 ^{b}	0.048 ± 0.002	51.5 ± 2.1 ^{d}
	2	3.53 ± 0.17	3.18 ± 0.08	0.53 ± 0.03 ^{a}	0.133 ± 0.011	18.9 ± 2.9 ^{b}	0.048 ± 0.003	55.3 ± 2.5 ^{c}
	3	3.58 ± 0.18	3.16 ± 0.08	0.57 ± 0.04 ^{a}	0.128 ± 0.013	68.7 ± 5.6 ^{a}	0.046 ± 0.002	59.9 ± 2.5 ^{b}
	4	3.64 ± 0.21	3.18 ± 0.11	0.56 ± 0.06 ^{a}	0.133 ± 0.013	71.2 ± 2.9 ^{a}	0.047 ± 0.002	64.7 ± 2.3 ^{a}
The bacteria (B) (1.812×10^5 CFU g^{-1} dry soil)	NAB	3.51 ± 0.12	3.19 ± 0.06	0.58 ± 0.04 ^{a}	0.131 ± 0.009	39.3 ± 3.3 ^{c}	0.047 ± 0.003	53.5 ± 1.9 ^{d}
	W01	3.63 ± 0.27	3.15 ± 0.07	0.54 ± 0.04 ^{b}	0.131 ± 0.013	45.4 ± 2.5 ^{ab}	0.048 ± 0.003	56.2 ± 2.9 ^{c}
	W14	3.57 ± 0.20	3.18 ± 0.11	0.53 ± 0.05 ^{b}	0.132 ± 0.014	47.9 ± 6.2 ^{a}	0.049 ± 0.003	60.9 ± 2.3 ^{a}
	W22	3.56 ± 0.12	3.17 ± 0.13	0.53 ± 0.03 ^{b}	0.133 ± 0.016	44.2 ± 2.3 ^{b}	0.047 ± 0.002	58.5 ± 2.4 ^{b}
	MTB	3.72 ± 0.21	3.18 ± 0.08	0.51 ± 0.06 ^{b}	0.125 ± 0.011	45.2 ± 3.4 ^{ab}	0.047 ± 0.001	60.2 ± 2.3 ^{ab}
F (A)		ns	ns	*	ns	*	ns	*
F (B)		ns	ns	*	ns	*	ns	*
F (A × B)		ns	ns	*	ns	*	*	*
CV (%)		5.76	3.30	10.2	10.1	9.74	6.73	4.55

Factor		Ca-P	Al-P	Fe-P	CEC	Ca^{2+}	Mg^{2+}	Na^+	K^+
		mg kg^{-1}			meq 100 g^{-1}				
The water salinity (A) (‰)	0	13.5 ± 1.3	23.5 ± 2.7 ^{c}	106.0 ± 5.4	13.5 ± 0.9	2.57 ± 0.33	1.32 ± 0.13	0.481 ± 0.033 ^{c}	0.187 ± 0.017
	2	12.5 ± 1.0	24.6 ± 3.6 ^{c}	107.4 ± 6.2	14.1 ± 0.9	2.40 ± 0.35	1.32 ± 0.12	0.555 ± 0.044 ^{b}	0.187 ± 0.017
	3	12.8 ± 2.4	26.9 ± 3.2 ^{b}	104.0 ± 10.2	14.1 ± 1.1	2.40 ± 0.30	1.30 ± 0.39	0.608 ± 0.053 ^{a}	0.175 ± 0.012
	4	12.8 ± 1.1	29.2 ± 2.3 ^{a}	103.8 ± 4.8	14.0 ± 1.3	2.37 ± 0.29	1.25 ± 0.17	0.634 ± 0.036 ^{a}	0.183 ± 0.012
The bacteria (B) (1.812×10^5 CFU g^{-1} dry soil)	NAB	16.2 ± 1.6 ^{a}	33.5 ± 2.4 ^{a}	121.9 ± 13.4 ^{a}	13.5 ± 1.3	2.50 ± 0.29	1.31 ± 0.19	0.614 ± 0.065 ^{a}	0.169 ± 0.010 ^{b}
	W01	12.4 ± 2.1 ^{b}	22.8 ± 2.4 ^{c}	97.7 ± 3.4 ^{c}	13.9 ± 0.7	2.40 ± 0.31	1.38 ± 0.32	0.564 ± 0.027 ^{b}	0.185 ± 0.011 ^{a}
	W14	11.7 ± 1.0 ^{b}	23.5 ± 4.1 ^{bc}	106.1 ± 7.5 ^{b}	14.3 ± 1.1	2.52 ± 0.30	1.28 ± 0.18	0.563 ± 0.040 ^{b}	0.188 ± 0.020 ^{a}
	W22	11.8 ± 1.8 ^{b}	25.7 ± 3.6 ^{b}	95.6 ± 3.3 ^{c}	13.9 ± 1.1	2.30 ± 0.29	1.18 ± 0.17	0.547 ± 0.042 ^{b}	0.184 ± 0.017 ^{a}
	MTB	12.4 ± 0.9 ^{b}	24.9 ± 2.3 ^{bc}	105.1 ± 5.6 ^{b}	14.1 ± 1.0	2.45 ± 0.39	1.33 ± 0.15	0.561 ± 0.033 ^{b}	0.190 ± 0.013 ^{a}
F (A)		ns	*	ns	ns	ns	ns	*	ns
F (B)		*	*	*	ns	ns	ns	*	*
F (A × B)		ns	*	ns	ns	ns	ns	*	*
CV (%)		13.9	12.4	8.26	8.44	13.8	22.6	7.84	9.05

Note: Values with the same following lowercase letters are insignificantly different. *: different at 5% significance; ns: not significant; 0: the water at 0‰ salinity; 2: the water at 2‰ salinity; 3: the water at 3‰ salinity; 4: the water at 4‰ salinity; NAB: no applied bacteria; W01: applying the single strains of ALA-producing *Luteovulum sphaeroides* W01; W14: applying the single strains of ALA-producing *L. sphaeroides* W14; W22: applying the single strains of ALA-producing *L. sphaeroides* W22; MTB: the mixture of the three bacteria, i.e., applying the three strains of ALA-producing *L. sphaeroides* W01, W14, and W22.

Regarding the N concentration in the soil, the total content did not change significantly between treatments and was valued at 0.130% on average. In contrast, the concentration of NH_4^+ dropped gradually along with the decrease in the salinity from 4 to 0‰. Moreover, the addition of either individual strains of W01, W14, and W22 or their mixture remarkably raised the NH_4^+ concentration—roughly 44.2–47.9 mg NH_4^+ kg^{-1}—which was greater than that in the control treatment (39.3 mg NH_4^+ kg^{-1} ($p < 0.05$)). Moreover, the total P content was not affected significantly by both factors (approximately 0.047%). However, the concentration of the soluble P was reduced from 64.7 to 51.5 mg P kg^{-1} according to the reduction in the saline levels from 4 to 0‰. The supplementation of either an individual strain of W01, W14, or W22 or their mixture resulted in greater concentration of the soluble P (56.2–60.9 mg P kg^{-1}) than that in the treatment without bacteria (53.5 mg P kg^{-1}). For the insoluble P forms, the salinity of water did not significantly influence the concentration of Ca-P and Fe-P. On the other hand, the application of either individual strains or the mixture of W01, W14, and W22 decreased the amounts of Ca-P, Al-P, and Fe-P, compared with those in the control treatment (Table 2).

The results of CEC, Ca^{2+}, and Mg^{2+} were not different, and their means were 13.9 meq CEC 100 g^{-1}, 2.43 meq Ca^{2+} 100 g^{-1}, and 1.29 meq Mg^{2+} 100 g^{-1}, respectively. Moreover, the salinity of the water did not significantly affect the concentration of K^+ (0.327 meq K^+ 100 g^{-1}). However, the application of W01, W14, and W22 either individually or in a mixture resulted in a greater K^+ content than the control treatment (0.184–0.190 meq K^+ 100 g^{-1} compared with 0.169 meq K^+ 100 g^{-1}). Moreover, the concentration of Na^+ in the treatments watered at 0, 2, 3, and 4‰ salinity rose steadily from 0.481 < 0.555 <

0.608–0.634 meq Na^+ 100 g^{-1}. On the other hand, the treatments without bacteria had the greatest Na^+ concentration (0.614 meq Na^+ 100 g^{-1}). In contrast, in the treatments supplied with an individual strain of W01, W14, and W22 or their mixture, the Na^+ content was lower than the previous one and ranged from 0.547 to 0.563 meq Na^+ 100 g^{-1} (Table 2).

3.2. The Impact of the Water Salinity and Supplementation of Luteovulum sphaeroides on Proline Concentration

The concentration of proline secreted at 35 and 50 DAS was affected by both the salinity of the water and the bacterial application. The increase in the saline levels led to the rise in the proline produced at both points of time. For instance, at 35 DAS, the proline production increased from 2.57 µmol g^{-1} DW to 7.74 µmol g^{-1} DW, according to the increasing salinity. However, applying the strain of W22 or the mixture of W01, W14, and W22, the concentration of proline was lower than that in the case of no bacteria applied (4.98–5.04 µmol g^{-1} DW to 5.54 µmol g^{-1} DW at 35 DAS and 4.39–4.83 µmol g^{-1} DW to 5.32 µmol g^{-1} DW at 50 DAS) (Table 3).

Table 3. The influence of the water salinity and the supplementation of the purple nonsulfur bacteria *Luteovulum sphaeroides* capable of producing ALA on the proline concentration in rice plants cultivated on the rice–shrimp saline soil.

Factor		Proline (µmol g^{-1} Dry Weight)	
		35 DAS	**50 DAS**
The water salinity (A) (‰)	0	2.57 ± 0.26 ^{d}	3.03 ± 0.29 ^{d}
	2	3.31 ± 0.44 ^{c}	3.97 ± 0.44 ^{c}
	3	7.06 ± 0.41 ^{b}	5.15 ± 0.39 ^{b}
	4	7.74 ± 0.40 ^{a}	5.99 ± 0.50 ^{a}
The bacteria (B) (1.812 × 10^5 CFU g^{-1} dry soil)	NAB	5.54 ± 0.53 ^{a}	5.32 ± 0.25 ^{a}
	W01	5.08 ± 0.22 ^{ab}	5.05 ± 0.57 ^{ab}
	W14	5.24 ± 0.36 ^{ab}	3.08 ± 0.35 ^{d}
	W22	5.04 ± 0.56 ^{b}	4.39 ± 0.42 ^{c}
	MTB	4.98 ± 0.22 ^{b}	4.83 ± 0.43 ^{b}
F (A)		*	*
F (B)		*	*
F (A × B)		*	*
CV (%)		9.55	9.96

Note: Values with the same following lowercase letters are insignificantly different. *: different at 5% significance; 0: the water at 0‰ salinity; 2: the water at 2‰ salinity; 3: the water at 3‰ salinity; 4: the water at 4‰ salinity; NAB: no applied bacteria; W01: applying the single strains of ALA-producing *Luteovulum sphaeroides* W01; W14: applying the single strains of ALA-producing *L. sphaeroides* W14; W22: applying the single strains of ALA-producing *L. sphaeroides* W22; MTB: the mixture of the three bacteria, i.e., applying the three strains of ALA-producing *L. sphaeroides* W01, W14, and W22.

3.3. The Impact of the Water Salinity and the Supplementation of Luteovulum sphaeroides on the Dry Biomass and the Concentration, Uptake, and Total Uptake of N, P, K, and Na in Seeds and Straw

3.3.1. The Dry Biomass and the Concentration of N, P, K, and Na in Seeds and Straws

Applying water above 2‰ salinity significantly reduced the biomass of dry seeds: 14.3–18.2 g pot^{-1} compared with 19.9 g pot^{-1} at 0‰ salinity. Similarly, the dry straw biomass followed the same trend, with 16.9–19.2 g pot^{-1} compared with 20.8 g pot^{-1}, in the same order. On the other hand, the application of the strain of W14 or the mixture of W01, W14, and W22 resulted in the greatest dry seed biomass (18.7–18.9 g pot^{-1}), and the second greatest was in the treatments supplied with a single strain of either W01 or W22 (16.8–17.4 g pot^{-1}), while the poorest biomass in dry seeds was in the treatment added with no bacteria (15.4 g pot^{-1}). For the dry straw biomass, values in the treatment supplied with the strain of W01 or the mixture were the greatest, and the second greatest was in the

treatments applied with the strain of W14 or W22, while the treatment without bacteria still resulted in the lowest (Table 4).

The concentrations of N and Na in seeds in the treatments watered at 2–4‰ salinity were greater than those in the treatment applied with no saline water ($p < 0.05$): 2.116–2.468% and 0.055–0.101% compared with 1.846% and 0.045%, respectively. Simultaneously, using saline water above 2‰ remarkably reduced the concentration of P and K in seeds, with 0.313 and 0.408% at 4‰ salinity compared with 0.349 and 0.467%, respectively. On the other hand, when supplying individual strains of W01 and W14 or the mixture of W01, W14, and W22, amounts of N, P, and K in rice seeds were increased, while the concentration of Na in seeds dropped from 0.082% to 0.050–0.079%, compared with the treatment without bacteria (Table 4).

The concentration of N and P in straw did not change according to different saline levels of water applied (1.123 and 0.188%, respectively). Nevertheless, at above 2‰ saline levels, the concentration of K in straw was 0.642–0.699%, which was significantly reduced as compared with the case of no saline water applied (0.758%). Furthermore, the concentrations of Na at the saline levels of 0, 2, 3, and 4‰ were, correspondingly, 0.186%, 0.216%, 0.228%, and 0.252%. On the other hand, applying the single strains of W01, W14, and W22 or their mixture led to greater concentrations of N, P, and K in straw but lower Na contents compared with the control treatment (Table 4).

3.3.2. The N, P, K, and Na Uptake in Seeds and Straw

Applying the saline water at 3–4‰ salinity brought about statistically equal N uptake in rice seeds compared to the treatments without saline water (from 358.4 to 369.0 mg N pot^{-1}); however, in straw, the saline watering at 2–4‰ led to a reduction in the N uptake (from 237.3 to 189.3–215.5 mg N pot^{-1}). In the meantime, the supplementation of an individual strain or the mixture of W01, W14, and W22 increased the N uptake in rice seeds (from 293.9 to 393.4–418.8 mg N pot^{-1} and from 177.1 to 205.1–229.6 mg N pot^{-1}) compared with the control treatment (Table 5).

Along with the increase in the salinity of the water, the P uptake in seeds went down. In particular, in the treatments watered at 0, 2, 3, and 4‰ salinity, the P uptake decreased gradually from 69.5 > 61.3 > 57.8 > 44.7 mg P pot^{-1}, respectively. Furthermore, the P uptake in straw reduced from 36.6 to 31.2 mg P pot^{-1} when the saline levels in the water went up from 2‰ to 4‰. On the other hand, the P uptake in seeds was 58.3–63.7 mg P pot^{-1} in the treatments supplied with an individual strain or the mixture of W01, W14, and W22 and was greater than 48.3 mg P pot^{-1} in the treatment without bacteria. The P uptake in straw followed the same trend (Table 5).

The K uptake in seeds and straw declined when the salinity of the water rose from 2 to 4‰ (from 93.4 mg K pot^{-1} to 89.1–59.4 mg K pot^{-1}) compared with the control treatment. Following the same trend, the K uptake in straw dropped from 157.9 mg to 135.1–105.8 mg K pot^{-1}. In addition, the application of a single strain or the mixture of W01, W14, and W22 resulted in K uptake of 78.9–99.3 mg K pot^{-1} in rice seeds and 133.9–145.6 mg K pot^{-1} in rice straw, which was greater than in the treatments without bacteria (57.9 and 92.7 mg K pot^{-1}, respectively) (Table 5).

Applying the saline water at 3 and 4‰ resulted in Na uptake of 11.5 and 14.0 mg Na pot^{-1} in seeds and 42.4 and 41.9 mg Na pot^{-1} in straw, which was greater than 9.03 and 38.7 mg Na pot^{-1} in the treatment without the saline water, respectively. Moreover, the supplementation of the mixture of W01, W14, and W22 resulted in Na uptake of 9.24 mg Na pot^{-1} in seeds and 36.2 mg Na pot^{-1} in straw, which values were lower than those in the control treatment (11.8 and 43.1 mg Na pot^{-1}, respectively) ($p < 0.05$) (Table 5).

Table 4. The influence of the water salinity and the supplementation of the purple nonsulfur bacteria *Luteovulum sphaeroides* capable of producing ALA on the dry biomass and the concentration of N, P, K, and Na in seeds and straw of rice cultivated on the rice–shrimp saline soil.

Factor		Biomass		Concentration (%)							
		(g pot^{-1})		N		P		K		Na	
		Seeds	Straw	Seeds	Straw	Seeds	Straw	Seeds	Straw	Seeds	Straw
The water salinity (A) (‰)	0	19.9 ± 1.1 ^{a}	20.8 ± 1.0 ^{a}	1.846 ± 0.085 ^{c}	1.142 ± 0.082	0.349 ± 0.025 ^{a}	0.192 ± 0.014	0.467 ± 0.038 ^{ab}	0.758 ± 0.066 ^{a}	0.045 ± 0.008 ^{d}	0.186 ± 0.008 ^{d}
	2	18.2 ± 0.9 ^{b}	19.2 ± 0.9 ^{b}	2.126 ± 0.113 ^{b}	1.121 ± 0.044	0.337 ± 0.012 ^{ab}	0.189 ± 0.013	0.489 ± 0.034 ^{a}	0.699 ± 0.034 ^{b}	0.055 ± 0.006 ^{c}	0.216 ± 0.022 ^{c}
	3	17.3 ± 1.0 ^{c}	18.7 ± 0.7 ^{b}	2.116 ± 0.095 ^{b}	1.112 ± 0.085	0.333 ± 0.014 ^{b}	0.189 ± 0.013	0.446 ± 0.037 ^{b}	0.630 ± 0.040 ^{c}	0.067 ± 0.011 ^{b}	0.228 ± 0.017 ^{b}
	4	14.3 ± 0.8 ^{d}	16.9 ± 0.7 ^{c}	2.468 ± 0.089 ^{a}	1.118 ± 0.082	0.313 ± 0.013 ^{c}	0.184 ± 0.013	0.408 ± 0.022 ^{c}	0.624 ± 0.031 ^{c}	0.101 ± 0.008 ^{a}	0.252 ± 0.016 ^{a}
The bacteria (B) (1.812×10^5 CFU g^{-1} dry soil)	NAB	15.4 ± 0.9 ^{c}	16.8 ± 0.6 ^{c}	1.965 ± 0.101 ^{c}	1.049 ± 0.079 ^{c}	0.312 ± 0.014 ^{b}	0.163 ± 0.011 ^{c}	0.372 ± 0.016 ^{c}	0.546 ± 0.036 ^{c}	0.082 ± 0.010 ^{a}	0.259 ± 0.017 ^{a}
	W01	17.4 ± 0.7 ^{b}	20.3 ± 0.5 ^{a}	2.291 ± 0.118 ^{a}	1.136 ± 0.088 ^{ab}	0.337 ± 0.019 ^{a}	0.188 ± 0.012 ^{b}	0.456 ± 0.044 ^{b}	0.688 ± 0.054 ^{b}	0.053 ± 0.005 ^{c}	0.214 ± 0.018 ^{c}
	W14	18.7 ± 1.0 ^{a}	18.9 ± 0.7 ^{b}	2.193 ± 0.066 ^{b}	1.177 ± 0.020 ^{a}	0.339 ± 0.016 ^{a}	0.201 ± 0.016 ^{a}	0.443 ± 0.028 ^{b}	0.703 ± 0.025 ^{ab}	0.072 ± 0.009 ^{b}	0.212 ± 0.015 ^{c}
	W22	16.8 ± 0.9 ^{b}	18.5 ± 1.4 ^{b}	2.017 ± 0.092 ^{c}	1.106 ± 0.083 ^{bc}	0.344 ± 0.023 ^{a}	0.182 ± 0.013 ^{b}	0.469 ± 0.037 ^{b}	0.721 ± 0.035 ^{ab}	0.079 ± 0.009 ^{a}	0.235 ± 0.015 ^{b}
	MTB	18.9 ± 1.1 ^{a}	19.9 ± 1.0 ^{a}	2.228 ± 0.100 ^{ab}	1.148 ± 0.096 ^{ab}	0.331 ± 0.009 ^{a}	0.210 ± 0.014 ^{a}	0.523 ± 0.038 ^{a}	0.731 ± 0.064 ^{a}	0.050 ± 0.009 ^{c}	0.183 ± 0.015 ^{d}
F (A)		*	*	*	ns	*	ns	*	*	*	*
F (B)		*	*	*	*	*	*	*	*	*	*
F (A × B)		*	*	*	ns	*	*	*	*	*	*
CV (%)		1.65	5.42	4.90	7.96	5.88	7.76	6.98	8.08	13.9	8.09

Note: Values with the same following lowercase letters are insignificantly different. *: different at 5% significance; ns: not significant; 0: the water at 0‰ salinity; 2: the water at 2‰ salinity; 3: the water at 3‰ salinity; 4: the water at 4‰ salinity; NAB: no applied bacteria; W01: applying the single strains of ALA-producing *Luteovulum sphaeroides* W01; W14: applying the single strains of ALA-producing *L. sphaeroides* W14; W22: applying the single strains of ALA-producing *L. sphaeroides* W22; MTB: the mixture of the three bacteria, i.e., applying the three strains of ALA-producing *L. sphaeroides* W01, W14, and W22.

Table 5. The influence of the water salinity and the supplementation of the purple nonsulfur bacteria *Luteovulum sphaeroides* capable of producing ALA on the N, P, K, and Na in seeds and straw of rice cultivated on the rice–shrimp saline soil.

Factor		Uptake (mg pot^{-1})							
		N		P		K		Na	
		Seeds	Straw	Seeds	Straw	Seeds	Straw	Seeds	Straw
The water salinity (A) (‰)	0	369.0 ± 28.3 ^{b}	237.3 ± 17.0 ^{a}	69.5 ± 6.7 ^{a}	40.1 ± 3.8 ^{a}	93.4 ± 10.1 ^{a}	157.9 ± 17.6 ^{a}	9.03 ± 1.67 ^{c}	38.7 ± 2.5 ^{b}
	2	388.2 ± 27.6 ^{a}	215.5 ± 10.9 ^{b}	61.3 ± 4.0 ^{b}	36.6 ± 2.8 ^{b}	89.1 ± 9.0 ^{a}	135.1 ± 9.3 ^{b}	9.98 ± 1.14 ^{c}	41.1 ± 4.8 ^{ab}
	3	368.6 ± 26.6 ^{b}	208.3 ± 20.3 ^{b}	57.8 ± 3.6 ^{c}	35.5 ± 3.2 ^{b}	77.9 ± 7.3 ^{b}	118.7 ± 10.1 ^{c}	11.5 ± 1.94 ^{b}	42.4 ± 3.3 ^{a}
	4	358.4 ± 27.3 ^{b}	189.3 ± 16.4 ^{c}	44.7 ± 2.9 ^{d}	31.2 ± 2.7 ^{c}	59.4 ± 4.9 ^{c}	105.8 ± 8.3 ^{d}	14.0 ± 1.48 ^{a}	41.9 ± 3.9 ^{a}

Table 5. *Cont.*

Factor		Uptake (mg pot^{-1})							
		N		P		K		Na	
		Seeds	Straw	Seeds	Straw	Seeds	Straw	Seeds	Straw
The bacteria (B) (1.812×10^5 CFUg^{-1} dry soil)	NAB	293.9 ± 22.7 ^{d}	177.1 ± 16.6 ^{c}	48.3 ± 2.5 ^{c}	27.6 ± 2.7 ^{d}	57.9 ± 5.0 ^{c}	92.7 ± 8.2 ^{c}	11.8 ± 1.76 ^{b}	43.1 ± 3.6 ^{a}
	W01	393.4 ± 23.7 ^{b}	229.6 ± 15.5 ^{a}	58.6 ± 4.8 ^{b}	38.1 ± 2.4 ^{b}	79.6 ± 9.0 ^{b}	139.9 ± 9.9 ^{a}	8.81 ± 1.01 ^{c}	43.2 ± 3.6 ^{a}
	W14	408.4 ± 29.0 ^{b}	223.5 ± 7.7 ^{a}	63.7 ± 4.4 ^{a}	37.9 ± 3.1 ^{b}	83.9 ± 7.4 ^{b}	133.9 ± 7.5 ^{b}	13.1 ± 1.83 ^{a}	39.9 ± 3.4 ^{b}
	W22	334.3 ± 25.4 ^{c}	205.1 ± 20.5 ^{b}	58.3 ± 5.2 ^{b}	33.9 ± 4.1 ^{c}	78.9 ± 7.1 ^{b}	135.1 ± 15.3 ^{b}	12.7 ± 1.64 ^{b}	42.9 ± 4.3 ^{a}
	MTB	418.8 ± 36.3 ^{a}	227.6 ± 20.5 ^{a}	62.7 ± 4.7 ^{a}	41.7 ± 3.3 ^{a}	99.3 ± 10.7 ^{a}	145.6 ± 15.7 ^{a}	9.24 ± 1.52 ^{c}	36.2 ± 3.1 ^{c}
F(A)		*	*	*	*	*	*	*	*
F (B)		*	*	*	*	*	*	*	*
F (A × B)		*	ns	*	*	*	*	*	*
CV (%)		7.59	8.88	8.50	9.76	11.1	10.5	15.1	9.66

Note: Values with the same following lowercase letters are insignificantly different. *: different at 5% significance; ns: not significant; 0: the water at 0‰ salinity; 2: the water at 2‰ salinity; 3: the water at 3‰ salinity; 4: the water at 4‰ salinity; NAB: no applied bacteria; W01: applying the single strains of ALA-producing *Luteovulum sphaeroides* W01; W14: applying the single strains of ALA-producing *L. sphaeroides* W14; W22: applying the single strains of ALA-producing *L. sphaeroides* W22; MTB: the mixture of the three bacteria, i.e., applying the three strains of ALA-producing *L. sphaeroides* W01, W14, and W22.

3.3.3. The Total N, P, K, and Na Uptake in Rice

The total uptake of N, P, and K in rice followed the same pattern under the influences of both factors. In detail, using the water above 3‰ salinity resulted in a drop from 606.3 to 576.9–542.7 mg N pot^{-1}, from 109.6 to 93.3–75.9 mg P pot^{-1}, and from 251.4 to 196.5 to 165.2 mg K pot^{-1}. On the other hand, the application of the mixture of W01, W14, and W22 improved the total uptake of N, P, and K, reaching 646.4 mg N pot^{-1}, 104.4 mg P pot^{-1}, and 244.8 mg K pot^{-1}, which values were greater than those in the control treatment (471.1 mg N pot^{-1}, 75.9 mg P pot^{-1}, and 150.5 mg K pot^{-1}, respectively) (Figures 1–3). The interaction analysis of the factors influencing the total uptake of N, P, and K showed significant changes among the combinations of the two factors. For instance, overall, the results all followed the same trend, where the higher the salinity was, the lower the nutrient uptake became, while the bacteria improved the uptake simultaneously. The greatest results were all in the treatments with the bacteria but not saline irrigation, while the lowest ones were in the treatments with saline water at 3–4‰ but no bacteria (Tables S1–S3). However, there were differences between the performance of the three PNSB strains under different levels of saline irrigation. For example, the W014 strain resulted in better N, P, and K uptake, whereas the results of the W01 and W22 strains were poor in total P and N uptake, respectively. Moreover, both of them also resulted in poor K uptake. For the total Na uptake, at the salinity above 2‰, the results increased from 47.8 to 51.1–56.0 mg Na pot^{-1}, compared with the control treatment. Supplying a single strain of W01, W14, or W22 was insufficient to reduce Na total uptake (51.9–55.6 mg Na pot^{-1}). The bacteria could not cause statistical differences in the result from the control treatment (54.9 mg Na pot^{-1}). Nevertheless, applying their mixture resulted in the Na uptake of 45.4 mg Na pot^{-1}, which was lower than 54.9 mg Na pot^{-1} in the control treatment ($p < 0.05$) (Figure 4). Based on the interaction results, generally, the individual applications of each bacterial strain could not help reduce the total Na uptake of rice plants under saline conditions. The treatment with the bacteria mixture at 0‰ salinity was the only one with the lowest result (32.0 mg Na pot^{-1}) (Table S4). However, the total Na uptake seemed insignificantly affected by the salinity because, in the treatments without bacteria, the total Na uptake ranged from 52.8 to 57.1 mg Na pot^{-1} among 0–4‰ salinity (Table S4).

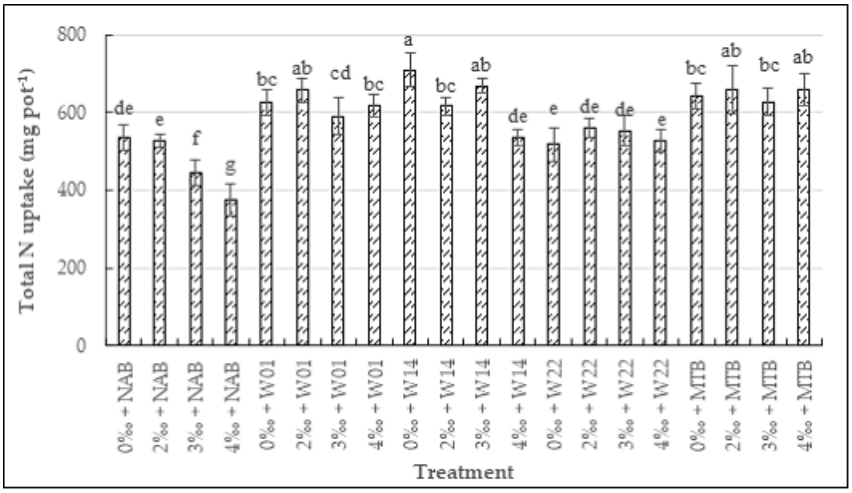

Figure 1. The influence of the water salinity and the supplementation of the purple nonsulfur bacteria *Luteovulum sphaeroides* capable of producing ALA on the total N uptake in rice cultivated on the rice–shrimp saline soil. Note: Values with the same following lowercase letters are insignificantly different. 0: the water at 0‰ salinity; 2: the water at 2‰ salinity; 3: the water at 3‰ salinity; 4: the water at 4‰ salinity; NAB: no applied bacteria; W01: applying the single strains of ALA-producing *Luteovulum sphaeroides* W01; W14: applying the single strains of ALA-producing *L. sphaeroides* W14; W22: applying the single strains of ALA-producing *L. sphaeroides* W22; MTB: the mixture of the three bacteria, i.e., applying the three strains of ALA-producing *L. sphaeroides* W01, W14, and W22.

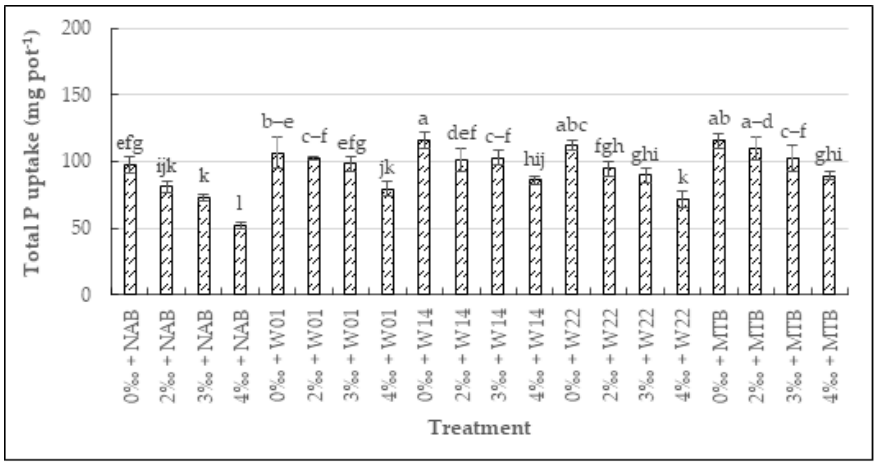

Figure 2. The influence of the water salinity and the supplementation of the purple nonsulfur bacteria *Luteovulum sphaeroides* capable of producing ALA on the total P uptake in rice cultivated on the rice–shrimp saline soil. Note: Values with the same following lowercase letters are insignificantly different. 0: the water at 0‰ salinity; 2: the water at 2‰ salinity; 3: the water at 3‰ salinity; 4: the water at 4‰ salinity; NAB: no applied bacteria; W01: applying the single strains of ALA-producing *Luteovulum sphaeroides* W01; W14: applying the single strains of ALA-producing *L. sphaeroides* W14; W22: applying the single strains of ALA-producing *L. sphaeroides* W22; MTB: the mixture of the three bacteria, i.e., applying the three strains of ALA-producing *L. sphaeroides* W01, W14, and W22.

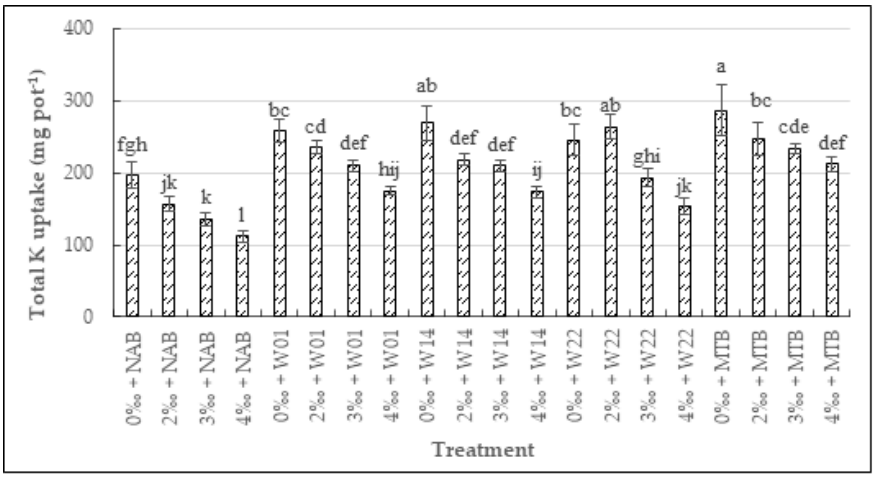

Figure 3. The influence of the water salinity and the supplementation of the purple nonsulfur bacteria *Luteovulum sphaeroides* capable of producing ALA on the total K uptake in rice cultivated on the rice–shrimp saline soil. Note: Values with the same following lowercase letters are insignificantly different. 0: the water at 0‰ salinity; 2: the water at 2‰ salinity; 3: the water at 3‰ salinity; 4: the water at 4‰ salinity; NAB: no applied bacteria; W01: applying the single strains of ALA-producing *Luteovulum sphaeroides* W01; W14: applying the single strains of ALA-producing *L. sphaeroides* W14; W22: applying the single strains of ALA-producing *L. sphaeroides* W22; MTB: the mixture of the three bacteria, i.e., applying the three strains of ALA-producing *L. sphaeroides* W01, W14, and W22.

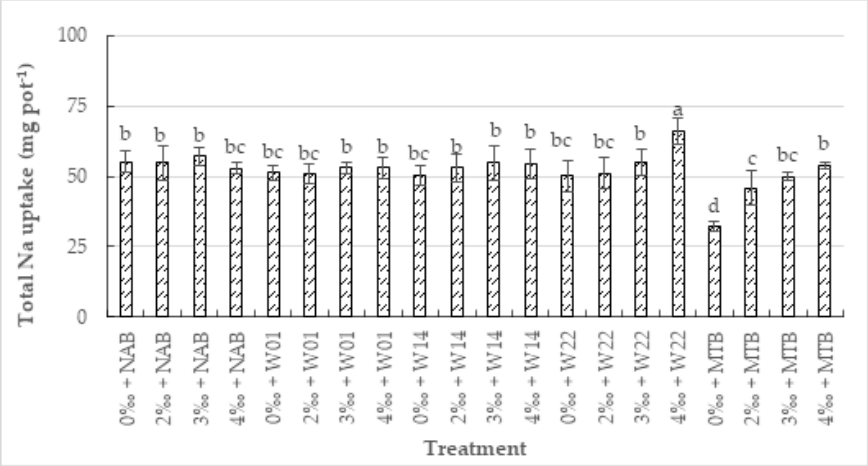

Figure 4. The influence of the water salinity and the supplementation of the purple nonsulfur bacteria *Luteovulum sphaeroides* capable of producing ALA on the total Na uptake in rice cultivated on the rice–shrimp saline soil. Note: Values with the same following lowercase letters are insignificantly different. 0: the water at 0‰ salinity; 2: the water at 2‰ salinity; 3: the water at 3‰ salinity; 4: the water at 4‰ salinity; NAB: no applied bacteria; W01: applying the single strains of ALA-producing *Luteovulum sphaeroides* W01; W14: applying the single strains of ALA-producing *L. sphaeroides* W14; W22: applying the single strains of ALA-producing *L. sphaeroides* W22; MTB: the mixture of the three bacteria, i.e., applying the three strains of ALA-producing *L. sphaeroides* W01, W14, and W22.

3.4. The Impact of the Water Salinity and the Supplementation of Luteovulum sphaeroides on the Growth, the Yield Components, and the Grain Yield of Rice

Applying the water at above 3‰ salinity remarkably reduced rice plant height. The plant height in the treatment watered with 3–4‰ salinity was 70.8–71.2 cm, which was shorter than in the treatment watered with 2 and 0‰ (72.2 and 72.6 cm). However, supplementing a single strain or the mixture of the three PNSB strains enhanced the height of rice plants in comparison with those to which bacteria were not applied (71.8–73.1 cm compared with 69.3 cm, respectively). In addition, at 4‰ salinity, the panicle length noticeably shortened to 18.4 cm, which was shorter than that in the treatment without saline water (19.3 cm). Nevertheless, supplying either a single strain or the mixture of W01, W14, and W22 resulted in an average panicle length of 18.8–19.3 cm, which was significantly longer than the control treatment (18.2 cm) (Table 6).

The rise in the salinity of the water caused the decline in the number of panicles in a pot. At 3–4‰ salinity, the number of panicles per pot was 15.7–18.7 panicles pot^{-1}, which was significantly less than in the treatments watered at 0–2‰ (18.7–20.7 panicles pot^{-1}). At 2–4‰ salinity, the filled grain percentage dropped, compared with watering at 0‰ salinity (75.7–81.0% compared with 86.2%, respectively). On the other hand, in the treatments supplied with a single strain of W01, W14, and W22 or their mixture, the number of panicles per pot was greater than that in the treatment without bacteria (19.1–20.0 panicles pot^{-1}–16.3 panicles pot^{-1}). Moreover, for the filled grain percentage, only in the treatments supplied with the strain of W01 or the mixture of the three PNSB, the result was greater than in the treatment without bacteria (82.9–83.6% compared with 76.6%, respectively). In addition, the number of seeds per panicle (67.2 seeds panicle^{-1}) and the 1000-seed weight (25.4 g) at different levels of salinity and with different bacteria supplementations were insignificantly different (Table 6).

Although using saline water at 2‰ did not cause any influences on the grain yield when the salinity reached 3 and 4‰, rice grain yield dropped significantly from 23.7 to 21.4–17.5 g pot^{-1}, compared with the treatment without saline water. On the other hand, the grain yield in the treatments without bacteria was only 18.3 g pot^{-1}, while in the treatments supplied with the single strain of W01, W14, and W22 or their mixture, it was 21.0–23.0 g pot^{-1} (Figure 5). According to the interaction analysis, from 0 to 3‰ salinity,

treatments with bacteria resulted in greater grain yield than treatments without bacteria at the same saline level, except for the treatment with the W14 strain at 2‰ salinity (Table S5). On the other hand, at 4‰ salinity, the performance of the bacteria declined, and only the treatments with the W22 strain or the PNSB mixture showed a better result than that without bacteria (19.6–19.9 g pot^{-1}). However, all the performances of the bacteria dropped according to the increase in salinity (Table S5).

Table 6. The influence of the water salinity and the supplementation of the purple nonsulfur bacteria *Luteovulum sphaeroides* capable of producing ALA on the growth and the yield components of rice cultivated on the rice–shrimp saline soil.

Factor		Plant Height (cm)	Panicle Length (cm)	Panicle Number pot^{-1} (panicles)	Seed Number panicle^{-1} (seeds)	Filled Seeds Ratio (%)	1000-Seed Weight (g)
The water salinity (A) (‰)	0	72.6 ± 1.8 ^{a}	19.3 ± 0.3 ^{a}	20.7 ± 2.3 ^{a}	65.9 ± 3.3	86.2 ± 8.8 ^{a}	25.3 ± 1.2
	2	72.2 ± 1.7 ^{ab}	18.8 ± 0.9 ^{ab}	20.6 ± 2.5 ^{a}	69.0 ± 3.9	81.0 ± 5.8 ^{b}	25.4 ± 1.3
	3	71.2 ± 1.6 ^{bc}	18.7 ± 0.5 ^{ab}	18.7 ± 2.4 ^{b}	68.2 ± 4.3	77.9 ± 4.9 ^{bc}	25.2 ± 1.1
	4	70.8 ± 1.5 ^{c}	18.4 ± 0.6 ^{b}	15.7 ± 1.9 ^{c}	65.8 ± 3.6	75.7 ± 8.5 ^{c}	25.5 ± 1.5
The bacteria (B) (1.812 × 10^{5} CFU g^{-1} dry soil)	NAB	69.3 ± 1.7 ^{b}	18.2 ± 0.6 ^{c}	16.3 ± 2.7 ^{b}	67.8 ± 1.8	76.6 ± 5.3 ^{b}	25.3 ± 1.4
	W01	71.8 ± 1.4 ^{a}	19.2 ± 0.5 ^{ab}	19.7 ± 2.7 ^{a}	68.2 ± 3.5	82.9 ± 7.7 ^{a}	25.6 ± 1.6
	W14	72.0 ± 1.9 ^{a}	18.6 ± 1.0 ^{bc}	19.1 ± 1.9 ^{a}	65.1 ± 3.1	76.7 ± 9.4 ^{b}	25.2 ± 1.3
	W22	72.2 ± 1.8 ^{a}	18.8 ± 0.5 ^{ab}	20.0 ± 2.8 ^{a}	68.5 ± 5.7	81.2 ± 6.1 ^{ab}	25.4 ± 1.3
	MTB	73.1 ± 1.4 ^{a}	19.3 ± 0.3 ^{a}	19.4 ± 1.3 ^{a}	66.4 ± 4.9	83.6 ± 6.6 ^{a}	25.3 ± 0.8
F (A)		*	*	*	ns	*	ns
F (B)		*	*	*	ns	*	ns
F (A × B)		ns	ns	ns	ns	*	ns
CV (%)		2.59	3.87	13.7	6.76	9.87	5.50

Note: Values with the same following lowercase letters are insignificantly different. *: different at 5% significance; ns: not significant; 0: the water at 0‰ salinity; 2: the water at 2‰ salinity; 3: the water at 3‰ salinity; 4: the water at 4‰ salinity; NAB: no applied bacteria; W01: applying the single strains of ALA-producing *Luteovulum sphaeroides* W01; W14: applying the single strains of ALA-producing *L. sphaeroides* W14; W22: applying the single strains of ALA-producing *L. sphaeroides* W22; MTB: the mixture of the three bacteria, i.e., applying the three strains of ALA-producing *L. sphaeroides* W01, W14, and W22.

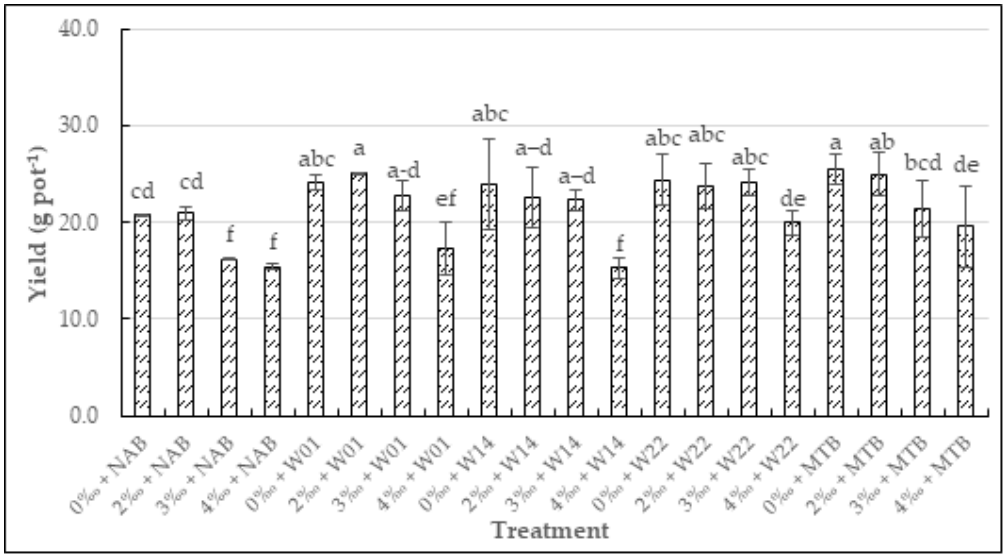

Figure 5. The influence of the water salinity and the supplementation of the purple nonsulfur bacteria *Luteovulum sphaeroides* capable of producing ALA on the yield of rice cultivated on the rice–shrimp saline soil. Note: Values with the same following lowercase letters are insignificantly different. 0: the water at 0‰ salinity; 2: the water at 2‰ salinity; 3: the water at 3‰ salinity; 4: the water at 4‰ salinity; NAB: no applied bacteria; W01: applying the single strains of ALA-producing *Luteovulum sphaeroides* W01; W14: applying the single strains of ALA-producing *L. sphaeroides* W14; W22: applying the single strains of ALA-producing *L. sphaeroides* W22; MTB: the mixture of the three bacteria, i.e., applying the three strains of ALA-producing *L. sphaeroides* W01, W14, and W22.

4. Discussion

The application of the three strains of the PNSB increased pH_{water} to 3.72 as compared with that in the control treatment (3.51) and the treatment supplied with the W22 strain (3.57) (Table 2). The results of studies conducted on acid sulfate soil reveal that the PNSB strain *Rhodospeudomonas palustris* increased pH in vitro, in a greenhouse condition, and on acid sulfate paddy fields [27–29]. This can be interpreted as showing that the PNSB can produce plant growth-promoting substances to increase the pH value [29]. In the current study, the strains of PNSB *L. sphaeroides* could excrete ALA to enhance the pH of saline soils under greenhouse conditions. Thus, the strains of *L. sphaeroides* W01, W14, and W22 were potent in improving the pH value in rice cultivation in the saline soil in the field. A study also recorded that the strains of *L. sphaeroides* could produce ALA, EPS, IAA, and siderophores [16] that contributed to the improvement in the acidity of the soil (Table 2). The biochemical production by PNSB increases under saline conditions up to 4.5% salinity [30]. This could be the mechanism of adaptation of this group of bacteria. The greater the stress, the more compounds, such as ALA, siderophores, and EPS, are produced to alleviate the stress condition.

In particular, interactions between the bacterial supplementation and the water salinity influenced the EC value, the NH_4^+ concentration, the soluble P, the insoluble P (such as Al-P), and the Na^+ concentration (Table 2). Applying the ALA-secreting PNSB remarkably reduced the EC and Na^+ contents. In detail, the EC value was 0.58 mS cm^{-1} in the treatments without bacteria, while that in the treatments supplied with the bacteria was 0.51–0.54 mS cm^{-1}. Moreover, the decrease in the soil salinity was expressed via the concentration of exchangeable Na^+ in the soil, with 0.547–0.564 meq Na^+ 100 g^{-1} in the treatments supplied with the bacteria and 0.614 meq Na^+ 100 g^{-1} in the treatments without bacteria. This could be explained by the fact that the strains of *L. sphaeroides* W01, W14, and W22 can produce EPS that immobile the exchangeable Na^+ content [16]. Furthermore, the PNSB secrete more EPS under saline conditions [31]. Previous studies also found that functional groups, including –OH, –COOH, and amines as the components of EPS, can immobilize Na^+ [16,32–34]. In addition, this was in accordance with the study by Nunkaew et al. [35], where the strains of PNSB *R. palustris* TN114 and PP803 reduced Na^+ by producing EPS, whose main component is a polysaccharide containing galacturonic acid. Surprisingly, in the current study, the soil NH_4^+ concentration and soluble P increased in the treatments with water at above 2‰ salinity, from 18.8–18.9 to 68.7–71.2 mg kg^{-1} and from 51.5 to 64.7 mg kg^{-1}, respectively. On account of this and significant interactions between both factors at these soil parameters, it can be assumed that the PNSB may have elevated the rates of N fixation and P solubilization at high salinity. This needs to be investigated in future studies.

Along with the increase in pH, there is a dynamic in increasing the availability of soil nutrients [36]. In detail, the application of a single strain or the mixture of W01, W14, and W22 boosted the available N concentration (4.87–8.63 mg NH_4^+ kg^{-1}) and soluble P concentration (2.69–7.50 mg P kg^{-1}) compared with the control treatment (Table 2). This could be inferred by the ability of *L. sphaeroides* W01, W14, and W22 strains to fix N and solubilize Al-P, Fe-P, and Ca-P [16]. Specifically, the concentrations of Al-P, Fe-P, and Ca-P in the treatments supplied with the bacteria were lower than those without bacteria (Table 2). Similarly, the strains of *R. palustris* TLS06, VNW02, VNW64, and VNS89 were found to be capable of solubilizing insoluble forms of P [28,29,37], which contributed to the growth of rice [16,38]. In addition, the application of the ALA-producing PNSB increased the exchangeable capacity of K^+, and the ability to solubilize K of the strains of *L. sphaeroides* W01, W14, and W22 has been investigated in the study by Khuong et al. [16]. In the study by Ge and Zhang [39], the PNSB were also found to be able to solubilize K. Therefore, to reduce the amount of K fertilizer supplied for rice, the ability of *L. sphaeroides* W01, W14, and W22 to solubilize K needed to be evaluated in the further studies. This indicated that the supplementation of the ALA-secreting *L. sphaeroides* W01, W14, and W22 that were able

to fix N and solubilize P reduced the amount of macronutrient fertilizer (N, P, and K) to obtain sustainable rice agriculture.

The application of the PNSB helped the rice to overcome the saline stress. This could be revealed via the concentration of proline in rice. The amount of proline changed at 35 and 50 DAS (Table 3), i.e., the higher the salinity, the greater the concentration of proline. Nevertheless, the supplementation of the W01, W14, and W22 strains decreased the concentration of Na^+ in the soil (Table 2), leading to a lower amount of proline (Table 3). In the treatments supplied with either a single strain of *L. sphaeroides* W14, W22, or the mixture of *L. sphaeroides* W01, W14, and W22, the concentration of proline was 3.08–4.83 μmol g^{-1} DW, while in the treatment without bacteria or the one supplied with the *L. sphaeroides* W01 strain, the concentration of proline was 5.05–5.32 μmol g^{-1} DW at 50 DAS (Table 3). The result illustrated that the supplementation of *L. sphaeroides* W01, W14, and W22 reduced the content of Na^+ so that the rice secreted less proline. This result is consistent with the study by Ábrahám et al. [40], where the proline concentration rose when plants were cultivated under saline stress. Additionally, the proline concentration in the treatments supplied with bacteria at 50 DAS was lower than that at 35 DAS. Moreover, as per the study by Khuong et al. [16], strains of PNSB have also been proven to decrease the proline content in rice on rice–shrimp fields. The decrease could result from the decline of Na^+ in soil due to the appearance of EPS and ALA produced by PNSB strains [41]. It could be inferred that the greater the density of the PNSB, the better the support for rice against the saline stress. Previous studies also stated that the use of ALA-producing PNSB promoted growth and reduced the saline stress of rice [42–44], but the proline concentration had not been determined in these studies.

The application of the saline water and the bacteria significantly affected the concentration of N, P, K, and Na in plant stovers. This could be due to increased soil nutrient availability and Na^+ immobilization facilitated by PNSB strains, as above. In detail, the concentration of P and K in seeds dropped at a high salinity, while the trend was opposite in the N case. Nevertheless, the amount of Na increased at the saline levels of 2, 3, and 4‰. Furthermore, the concentration of N and P in straw did not change significantly under the influence of the saline water. However, the concentration of K in straw was inversely proportional to the saline levels of the water. On the other hand, applying the ALA-secreting PNSB enhanced the concentration of N, P, and K and lowered the amount of Na (Table 4). For the total uptake of N, P, K, and Na, there were interactions between the salinity and the bacterial supplementation (Figures 1–3). Applying the saline water above 2‰ led to a reduction in the total P and K uptake, and that above 3‰ led to a reduction in the total N uptake and increased the Na uptake in comparison with the treatment without saline water (Figures 1–3). In addition, the supplementation of a single strain of W01, W14, or W22 boosted the total uptake of P and K and limited the total Na uptake in rice compared with the treatment without bacteria (Figures 2–4). This could be due to the importance of lateral roots in nutrient uptakes and the effects of PNSB on the development of the roots [45]. The lateral roots develop from the main root and play a significant role in nutrient uptake, while PNSB are found to stimulate the growth of the lateral roots [45], which might indirectly have increased the nutrient uptake in the current study. This could be observed in the studies by Huu et al. [46] and Khuong et al. [47]. Supplying the two strains of P-solubilizing PNSB increased N and P uptake and decreased Na uptake in rice cultivated on saline soils [28]. Moreover, the results of previous studies point out that the application of the mixture of *R. palustris* TLS06, VNW02, VNW64, and VNS89 increases P uptake on acid sulfate soils [19], and that of the N-fixing *R. palustris* TLS12, VNS19, VNS32, VNS62, and VNW95, and *R. harwoodiae* TLW42 improved N uptake on acid sulfate soil as well [48]. In addition, rice nutrient uptake was increased by the PNSB strains in the current study (Tables S1–S3). The W14 strain can greatly enhance N, P, and K uptake, while the other two strains can perform excellently in increasing uptake of one of these elements. This is in accordance with the review by Maeda [49], where different PNSB can have one or two nitrogenases. Although the PNSB strains in the current study exhibited different

characteristics, they can have synergetic effects [50]. Notably, the effect was shown in the total Na uptake in the treatment with the PNSB mixture at 0‰ salinity, which introduced the lowest amount of Na uptake compared with the other treatments (Table S4).

Saline stress inhibits the growth and yield of rice [51]. In the current study, plants became shorter in height when applied with the saline water at 3‰, while the panicle length dropped when the salinity was 4‰. According to Puvanitha et al. [52], NaCl caused a reduction in rice plant height, i.e., in the treatment without NaCl, the plant height was 78.9 cm, but in the treatment with NaCl, it was only 68.2 cm. On the contrary, applying PNSB strains improves the performance of rice under saline conditions [49]. In this study, the application of a single strain of W01, W14, and W22 or their mixture significantly ameliorated the plant height and the panicle length, compared with the treatment without bacteria, except for the panicle length in the treatment supplied with the strain of W14. This result was in accordance with the studies by Lee et al. [42], Kantha et al. [43], and Nunkaew et al. [44], where the plant heights of rice and potato were improved in the treatment supplied with bacteria in saline conditions. Applying the saline water above 3‰ led to a decrease in the number of panicles per pot. The panicle number per pot was reduced by 2–5 panicles compared with the treatment with no saline water. Similarly, the filled grains percentage remarkably dropped as well, compared with the treatment without bacteria. Moreover, in the treatments supplied with the PNSB, the panicle number per pot and the filled grains percentage were greater than those in the treatment without bacteria (Table 6). Moreover, underground traits of rice inoculated with PNSB have been observed in the study by Iwai et al. [45], where the underground parts were also improved by this group of bacteria. Altogether, these contributed to greater rice grain yield in treatment with the PNSB under saline stress. In the study by Shan et al. [53], rice grain yield was improved by 33%, while that in the current study was improved by up to 25.7% under saline stress, which shows that the ALA-producing PNSB strains in the current study were efficient in enhancing rice productivity under saline conditions. This could be explained by the fact that the ALA-producing PNSB strains could not only tolerate saline conditions but also promote growth and enhance chlorophyll synthesis, leading to better photosynthesis, from which the yield was improved under saline conditions. This is consistent with the results of previous studies; ALA is one of the factors participating in chlorophyll synthesis, enzymes production, antioxidants synthesis, and saline stress reduction [15,44,54]. In addition, in the study by Kantha et al. [18], ALA promoted growth and increased rice yield in saline conditions. This has also been observed in the study by Khuong et al. [55], where PNSB strains, which can synthesize ALA, improved the performance of rice even when the plants were treated with 5‰ saline water four times. Therefore, supplying the ALA-excreting PNSB was efficient in helping rice plants to overcome the saline stress and to ameliorate their yield (Figure 5).

5. Conclusions

Applying the saline water above 2‰ increased Na^+ accumulation in the soil. At 3 and 4‰ salinity, the plant height was reduced by 1.40–1.75 cm and the filled grains percentage decreased by 8.37–10.5%, leading to a 2.35–6.22% decline in the yield, compared with the treatment without saline water. Moreover, applying a single strain of either W01, W14, and W22 or their mixture improved the rice plant height and yield components, including the panicle length, the panicle number per pot, the filled grains percentage, and the grain yield, compared with the control treatment. In addition, supplying a single strain of W01, W14, and W22 or their mixture increased the pH (0.05–0.21), the available concentrations of NH_4^+ (4.87–8.63 mg NH_4^+ kg^{-1}) and soluble P (2.69–7.50 mg P kg^{-1}), and the total uptake of N (68.3–175.3 mg N pot^{-1}), P (16.3–25.6 mg P pot^{-1}), and K (63.5–94.3 mg K^+ pot^{-1}) and decreased the concentration of Na^+ in the soil (0.050–0.067 meq Na^+ 100 g^{-1}) and Na in plants (1.86–9.81 mg Na pot^{-1}), compared with the control treatment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13091761/s1, Table S1: Interactions between the two factors influencing the total N uptake; Table S2: Interactions between the two factors influencing the total P uptake; Table S3: Interactions between the two factors influencing the total K uptake; Table S4: Interactions between the two factors influencing the total Na uptake; Table S5: Interactions between the two factors influencing the rice grain yield.

Author Contributions: Conceptualization, N.Q.K. and N.T.T.D.; methodology, N.Q.K.; validation, L.T.M.T., L.T.Q. and N.T.T.D.; formal analysis, L.N.T.X.; investigation, N.Q.K., L.N.T.X., N.T.T.D., L.T.M.T. and L.T.Q.; resources, L.T.M.T.; data curation, N.Q.K.; writing—original draft preparation, N.Q.K.; writing—review and editing, N.Q.K., N.T.P. and L.T.Q.; visualization, L.N.T.X.; supervision, N.T.P.; project administration, N.Q.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Can Tho University, grant number 70T/HĐ-QLKH2020.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors sincerely appreciate the Purple Nonsulfur Bacteria Laboratory (Faculty of Crop Science, College of Agriculture, Can Tho University), which provided equipment, chemicals, and materials for this study to be completed.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Van, P.D.T.; Popescu, I.; Van Griensven, A.; Solomatine, D.P.; Trung, N.H.; Green, A. A study of the climate change impacts on fluvial flood propagation in the Vietnamese Mekong Delta. *Hydro. Earth Syst. Sci.* **2012**, *16*, 4637–4649. [CrossRef]
2. Vu, P.H.; Vu, P.T.; Tri, V.P.D. Classification of risk zones in agriculture under impacts of salt water intrusion in Bac Lieu province. *Can Tho Univ. J. Sci.* **2016**, *42*, 70–80. [CrossRef]
3. Ismail, L.M.; Soliman, M.I.; Abd El-Aziz, M.H.; Abdel-Aziz, H.M.M. Impact of silica ions and nano silica on growth and productivity of pea plants under salinity stress. *Plants* **2022**, *11*, 494. [CrossRef] [PubMed]
4. Shultana, R.; Kee Zuan, A.T.; Yusop, M.R.; Saud, H.M. Characterization of salt-tolerant plant growth-promoting rhizobacteria and the effect on growth and yield of saline-affected rice. *PLoS ONE* **2020**, *15*, e0238537. [CrossRef] [PubMed]
5. Ali, N.M.; Altaey, D.K.; Altaee, N.H. The impact of selenium, nano (SiO_2) and organic fertilization on growth and yield of potato *Solanum tuberosum* L. under salt stress conditions. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *735*, 012042. [CrossRef]
6. Abd El-Mageed, T.A.; Rady, M.M.; Taha, R.S.; Abd El Azeam, S.; Simpson, C.R.; Semida, W.M. Effects of integrated use of residual sulfur-enhanced biochar with effective microorganisms on soil properties, plant growth and short-term productivity of *Capsicum annuum* under salt stress. *Sci. Hortic.* **2020**, *261*, 108930. [CrossRef]
7. Shams, M.; Ekinci, M.; Ors, S.; Turan, M.; Agar, G.; Kul, R.; Yildirim, E. Nitric oxide mitigates salt stress effects of pepper seedlings by altering nutrient uptake, enzyme activity and osmolyte accumulation. *Physiol. Mol. Biol. Plants* **2019**, *25*, 1149–1161. [CrossRef]
8. Tolay, I. The impact of different zinc (Zn) levels on growth and nutrient uptake of basil (*Ocimum basilicum* L.) grown under salinity stress. *PLoS ONE* **2021**, *16*, e0246493. [CrossRef]
9. Hnilickova, H.; Kraus, K.; Vachova, P.; Hnilicka, F. Salinity stress affects photosynthesis, malondialdehyde formation, and proline content in *Portulaca oleracea* L. *Plants* **2021**, *10*, 845. [CrossRef]
10. Salinas, R.; Sánchez, E.; Ruíz, J.M.; Lao, M.T.; Romero, L. Proline, betaine, and choline responses to different phosphorus levels in green bean. *Commun. Soil Sci. Plant Anal.* **2013**, *44*, 465–472. [CrossRef]
11. Kaur, G.; Asthir, B. Proline: A key player in plant abiotic stress tolerance. *Biol. Plant.* **2015**, *59*, 609–619. [CrossRef]
12. Imhoff, J.F. Diversity of anaerobic anoxygenic phototrophic purple bacteria. In *Modern Topics in the Phototrophic Prokaryotes*; Hallenbeck, P., Ed.; Springer: Cham, Switzerland, 2017; pp. 47–85. [CrossRef]
13. Liu, S.; Zhang, G.; Li, X.; Zhang, J. Microbial production and applications of 5-aminolevulinic acid. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 7349–7357. [CrossRef] [PubMed]
14. Xu, X.; Gao, Z.; Wu, X.; Chen, X. Light and oxygen facilitating the directly treatment food wastewater and poly-β-hydroxybutyrate, 5-aminolevulinic acid, pigment productions by *Rubrivivax gelatinosus*. *Water Sci. Technol.* **2023**, *87*, 1367–1375. [CrossRef] [PubMed]
15. Sakpirom, J.; Kantachote, D.; Nunkaew, T.; Khan, E. Characterizations of purple non-sulfur bacteria isolated from paddy fields, and identification of strains with potential for plant growth-promotion, greenhouse gas mitigation and heavy metal bioremediation. *Res. Microbiol.* **2017**, *168*, 266–275. [CrossRef] [PubMed]

16. Khuong, N.Q.; Kantachote, D.; Dung, N.T.T.; Huu, T.N.; Thuc, L.V.; Thu, L.T.M.; Quang, L.T.; Xuan, D.T.; Nhan, T.C.; Tien, P.D.; et al. Potential of potent purple nonsulfur bacteria isolated from rice-shrimp systems to ameliorate rice (*Oryza sativa* L.) growth and yield in saline acid sulfate soil. *J. Plant Nutr.* **2023**, *46*, 473–494. [CrossRef]
17. Kang, Z.; Ding, W.; Gong, X.; Liu, Q.; Du, G.; Chen, J. Recent advances in production of 5-aminolevulinic acid using biological strategies. *World J. Microbiol. Biotechnol.* **2017**, *33*, 200. [CrossRef] [PubMed]
18. Kantha, T.; Kantachote, D.; Klongdee, N. Potential of biofertilizers from selected *Rhodopseudomonas palustris* strains to assist rice (*Oryza sativa* L. subsp. *indica*) growth under salt stress and to reduce green-house gas emissions. *Ann. Microbiol.* **2015**, *65*, 2109–2118. [CrossRef]
19. Nookongbut, P.; Kantachote, D.; Meghrai, M.; Naidu, R. Reduction in arsenic toxicity and uptake in rice (*Oryza sativa* L.) by As-resistant purple nonsulfur bacteria. *Environ. Sci. Pollut. Res.* **2018**, *23*, 1681–1690. [CrossRef]
20. Korkmaz, A.; Şirikçi, R.; Kocaçınar, F.; Değer, Ö.; Demirkırıan, A.R. Alleviation of salt-induced adverse effects in pepper seedlings by seed application of glycinebetaine. *Sci. Hortic.* **2012**, *148*, 197–205. [CrossRef]
21. Li, J.; Pan, J.; Najeeb, U.; El-Beltagi, H.S.; Huang, Q.; Lu, H.; Xu, L.; Shi, B.; Zhou, W. Promotive role of 5-aminolevulinic acid or salicylic acid combined with citric acid on sunflower growth by regulating manganese absorption. *Antioxidants* **2023**, *12*, 580. [CrossRef]
22. Kaya, C.; Ugurlar, F.; Ashraf, M.; Alyemeni, M.N.; Moustakas, M.; Ahmad, P. 5-aminolevulinic acid induces chromium [Cr (VI)] tolerance in tomatoes by alleviating oxidative damage and protecting photosystem II: A mechanistic approach. *Plants* **2023**, *12*, 502. [CrossRef] [PubMed]
23. Sasaki, K.; Watanabe, M.; Tanaka, T. Biosynthesis, biotechnological production and applications of 5-aminolevulinic acid. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 23–29. [CrossRef] [PubMed]
24. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
25. Sparks, D.L.; Page, A.L.; Helmke, P.A.; Loeppert, R.H.; Soltanpour, P.N.; Tabatabai, M.A.; Johnston, T.; Summer, M.E. *Methods of Soil Analysis. Part 3—Chemical Methods*, 1st ed.; SSSA Book Series 5.3; SSSA/ASA: Madison, WI, USA, 1996. [CrossRef]
26. Walinga, I.; van Vark, W.; Houba, V.J.G.; van der Lee, J.J. Soil and plant analysis, Part 7. In *Plant Analysis Procedures: Department of Soil Science and Plant Nutrition*; Wageningen Agricultural University: Wageningen, The Netherlands, 1997; p. 263.
27. Khuong, N.Q.; Kantachote, D.; Onthong, J.; Sukhoom, A. The potential of acid-resistant purple nonsulfur bacteria isolated from acid sulfate soils for reducing toxicity of Al^{3+} and Fe^{2+} using biosorption for agricultural application. *Biocatal. Agric. Biotechnol.* **2017**, *68*, 217–228. [CrossRef]
28. Khuong, N.Q.; Kantachote, D.; Onthong, J.; Xuan, L.N.T.; Sukhoom, A. Enhancement of rice growth and yield in actual acid sulfate soils by potent acid-resistant *Rodopseudomonas palustris* trains for producing safe rice. *Plant Soil* **2018**, *429*, 483–501. [CrossRef]
29. Khuong, N.Q.; Kantachote, D.; Nookongbut, P.; Onthong, J.; Xuan, L.N.T.; Sukhoom, A. Mechanisms of acid-resistant *Rhodopseudomonas palustris* strains to ameliorate acidic stress and promote plant growth. *Biocatal. Agric. Biotechnol.* **2020**, *24*, 101520. [CrossRef]
30. Carlozzi, P.; Di Lorenzo, T.; Ghanotakis, D.F.; Touloupakis, E. Effects of pH, temperature and salinity on P3HB synthesis culturing the marine *Rhodovulum sulfidophilum* DSM-1374. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 2007–2015. [CrossRef]
31. Xu, W.; Liu, L.; He, T.; Cao, M.; Sha, L.; Hu, Y.; Li, Q.; Li, J. Soil properties drive a negative correlation between species diversity and genetic diversity in a tropical seasonal rainforest. *Sci. Rep.* **2016**, *6*, 20652. [CrossRef]
32. Cheah, C.; Cheow, Y.L.; Ting, A.S.Y. Comparative analysis on metal removal potential of exopolymeric substances with live and dead cells of bacteria. *Int. J. Environ. Res.* **2022**, *16*, 1–12. [CrossRef]
33. Panwichian, S.; Kantachote, D.; Wittayaweerasak, B.; Mallavarapu, M. Removal of heavy metals by exopolymeric substances produced by resistant purple nonsulfur bacteria isolated from contaminated shrimp ponds. *Electron. J. Biotechnol.* **2011**, *14*, 2. [CrossRef]
34. Nguyen, K.Q.; Kantachote, D.; Onthong, J.; Sukhoom, A. Al^{3+} and Fe^{2+} toxicity reduction potential by acid-resistant strains of *Rhodopseudomonas palustris* isolated from acid sulfate soils under acidic conditions. *Ann. Microbiol.* **2018**, *68*, 217–228. [CrossRef]
35. Nunkaew, T.; Kantachote, D.; Nitoda, T.; Kanzaki, H.; Ritchie, R.J. Characterization of exopolymeric substances from selected *Rhodopseudomonas palustris* strains and their ability to adsorb sodium ions. *Carbohydr. Polym.* **2015**, *115*, 334–341. [CrossRef] [PubMed]
36. Gondal, A.H.; Hussain, I.; Ijaz, A.B.; Zafar, A.; Ch, B.I.; Zafar, H.; Sohail, M.D.; Niazi, H.; Touseef, M.; Kan, A.A.; et al. Influence of soil pH and microbes on mineral solubility and plant nutrition: A review. *Int. J. Agric. Biol. Sci.* **2021**, *5*, 71–81. [CrossRef]
37. Khuong, N.Q.; Kantachote, D.; Thuc, L.V.; Nookongbut, P.; Xuan, L.N.T.; Nhan, T.C.; Xuan, L.N.T.; Tantirungkij, M. Potential of Mn^{2+}-resistant purple nonsulfur bacteria isolated from acid sulfate soils to act as bioremediators and plant growth promoters via mechanisms of resistance. *J. Soil Sci. Plant Nutr.* **2020**, *20*, 2364–2378. [CrossRef]
38. Khuong, N.Q.; Sakpirom, J.; Oanh, T.O.; Thuc, L.V.; Thu, L.T.M.; Xuan, D.T.; Quang, L.T.; Xuan, L.N.T. Isolation and characterization of novel potassium-solubilizing purple nonsulfur bacteria from acidic paddy soils using culture-dependent and culture-independent techniques. *Braz. J. Microbiol.* **2023**. [CrossRef] [PubMed]
39. Ge, H.; Zhang, F. Growth-promoting ability of *Rhodopseudomonas palustris* G5 and its effect on induced resistance in cucumber against salt stress. *J. Plant Growth Regul.* **2019**, *38*, 180–188. [CrossRef]

40. Ábrahám, E.; Hourton-Cabassa, C.; Erdei, L.; Szabados, L. Methods for determination of proline in plants. In *Plant Stress Tolerance, Methods in Molecular Biology*; Sunkar, R., Ed.; Humana Press: Totowa, NJ, USA, 2010; Volume 639, pp. 317–331. [CrossRef]
41. Sakarika, M.; Spanoghe, J.; Sui, Y.; Wambacq, E.; Grunert, O.; Haesaert, G.; Spiller, M.; Vlaeminck, S.E. Purple non-sulphur bacteria and plant production: Benefits for fertilization, stress resistance and the environment. *Micro. Biotechnol.* **2020**, *13*, 1336–1365. [CrossRef]
42. Lee, K.H.; Koh, R.H.; Song, H.G. Enhancement of growth and yield of tomato by *Rodopseudomonas* sp. under greenhouse condition. *J. Microbiol. Biotechnol.* **2008**, *46*, 641–646.
43. Kantha, T.; Chaiyasut, C.; Kantachote, D.; Sukrong, S.; Muangprom, A. Selection of photosynthetic bacteria producing 5-aminolevulinic acid from soil of organic saline paddy fields from the Northeast region of Thailand. *Afr. J. Microbiol. Res.* **2010**, *4*, 1848–1855.
44. Nunkaew, T.; Kantachote, D.; Kanzaki, H.; Nitoda, T.; Ritchie, R.J. Effects of 5-aminolevulinic acid (ALA)-containing supernatants from selected *Rodopseudomonas palustris* trains on rice growth under NaCl stress, with mediating effects on chlorophyll, photosynthetic electron transport and antioxidative enzymes. *Electron. J. Biotechnol.* **2014**, *17*, 4. [CrossRef]
45. Iwai, R.; Uchida, S.; Yamaguchi, S.; Sonoda, F.; Tsunoda, K.; Nagata, H.; Nagata, D.; Koga, A.; Goto, M.; Maki, T.-A.; et al. Effects of seed bio-priming by purple non-sulfur bacteria (PNSB) on the root development of rice. *Microorganisms* **2022**, *10*, 2197. [CrossRef] [PubMed]
46. Huu, T.N.; Giau, T.T.N.; Ngan, P.N.; Van, T.T.B.; Khuong, N.Q. Potential of phosphorus solubilizing purple nonsulfur bacteria isolated from acid sulfate soil in improving soil property, nutrient uptake, and yield of pineapple (*Ananas comosus* L. Merrill) under acidic stress. *Appl. Environ. Soil Sci.* **2022**, *2022*, 8693479. [CrossRef]
47. Khuong, N.Q.; Thuc, L.V.; Giang, C.T.; Xuan, L.N.T.; Thu, L.T.M.; Isao, A.; Jun-Ichi, S. Improvement of nutrient uptake, yield of black sesame (*Sesamum indicum* L.), and alluvial soil fertility in dyke by spent rice straw from mushroom cultivation as biofertilizer containing potent strains of *Rhodopseudomonas palustris*. *Sci. World J.* **2023**, *2023*, 1954632. [CrossRef] [PubMed]
48. Khuong, N.Q.; Kantachote, D.; Huu, T.N.; Nhan, T.C.; Nguyen, P.C.; Thu, L.T.M.; Van, T.T.B.; Xuan, N.T.T.; Xuan, L.N.T.; Xuan, D.T. Use of potent acid resistant strains of *Rhodopseudomonas* spp. in Mn-contaminated acidic paddies to produce safer rice and improve soil fertility. *Soil Tillage Res.* **2022**, *221*, 105393. [CrossRef]
49. Maeda, I. Potential of phototrophic purple nonsulfur bacteria to fix nitrogen in rice fields. *Microorganisms* **2021**, *10*, 28. [CrossRef]
50. Sundar, L.S.; Chao, Y.Y. Potential of purple non-sulfur bacteria in sustainably enhancing the agronomic and physiological performances of rice. *Agronomy* **2022**, *12*, 2347. [CrossRef]
51. Lu, L.; Chang, M.; Han, X.; Wang, Q.; Wang, J.; Yang, H.; Guan, Q.; Dai, S. Beneficial effects of endophytic *Pantoea ananatis* with ability to promote rice growth under saline stress. *J. Appl. Microbiol.* **2021**, *131*, 1919–1931. [CrossRef]
52. Puvanitha, S.; Mahendran, S. Effect of salinity on plant height, shoot and root dry weight of selected rice cultivars. *Scholars J. Agric. Vet. Sci.* **2017**, *4*, 126–131. [CrossRef]
53. Shan, Y.K.; Sundar, L.S.; Chao, Y.Y. Foliar application of *Rhodopseudomonas palustris* enhances the rice crop growth and yield under field conditions. *Plants* **2022**, *11*, 2452. [CrossRef]
54. Wongkantrakorn, N.; Sunohara, Y.; Matsumoto, H. Mechanism of growth amelioration of NaCl—Stressed rice (*Oryza sativa* L.) by δ-aminolevulinic acid. *J. Pestic. Sci.* **2009**, *34*, 89–95. [CrossRef]
55. Khuong, N.Q.; Minh, D.P.T.; Thu, L.T.M.; Thuc, L.V. The potential of bacterial strains of *Luteovulum sphaeroides* W22 and W47 for producing δ-aminolevulinic acid to improve soil quality, growth and yield of saline-irrigated rice cultivated in salt-contaminated soil. *Agronomy* **2023**, *13*, 1409. [CrossRef]