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Research Article

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

Tran Ngoc Huu, Tran Thi Ngoc Giau, Phan Ngoc Ngan, Tran Thi Bich Van, and Nguyen Quoc Khuong

Faculty of Crop Science, College of Agriculture, Can Tho University, Can Tho 9400, Vietnam

Correspondence should be addressed to Nguyen Quoc Khuong; nqkhuong@ctu.edu.vn

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This study aimed to (i) evaluate purple nonsulfur bacteria (PNSB) strains possessing the highest phosphorus (P) solubilizing capacity in field and (ii) determine the efficacy of PNSB biofertilizers in improving soil quality, P uptake, growth, and yield of pineapple cultivated in acid sulfate soil (ASS). A field experiment was conducted in a completely randomized block design with two factors, including the first factor as P fertilizer levels (0, 50, 75, and 100% P) based on recommended fertilizer formula (RFF) and the second factor as supplementation of biofertilizers containing P solubilizing PNSB (no inoculated PNSB, *Rhodobacter sphaeroides* W48, *R. sphaeroides* W42, and a mixture of *R. sphaeroides* W48 and W42). The results indicated that the supplementation of PNSB biofertilizers led to an increase of 25.3–33.9% in soluble P concentration in soil compared to control treatment. Among the selected PNSB strains, *R. sphaeroides* W42 and a mixture of the PNSB in biofertilizers solubilized all insoluble P fractions (Fe-P, Al-P, and Ca-P) and strain W48 in biofertilizers for Fe-P and Al-P. Furthermore, the supplementation of biofertilizers from *R. sphaeroides* W48 and W42 individually and their mixture raised plant height by 3.56–4.10% and available P concentration by 25.3–33.9%. Total P uptake in pineapple treatments with biofertilizers from mixed PNSB was 42.9% higher than that in the control treatment ($p < 0.05$). Application of mixed PNSB strains can reduce 25% P of chemical fertilizer, but the pineapple yield rose over 12.1%. Both *R. sphaeroides* W48 and W42 are potent for use as crop yield enhancers to obtain the sustainable pineapple cultivation under acidic stress.

1. Introduction

Pineapple (*Ananas comosus* L. Merrill) plays a vital role in the agricultural and food industries because pineapple fruit is highly appreciated for its unique aroma and sweet taste. Besides, it has been proved that pineapple has various health benefits [1]. In Vietnam, pineapple is mainly cultivated in the Mekong Delta in acid sulfate soil (ASS) with a pH in the topsoil ranging from 3.84 to 4.23 [2], and this value continues to decrease along with intensive pineapple cultivation [3]. Although pineapple has been recorded with high adaptation to ASS [4], the soil characteristics of low pH and high aluminum (Al), iron (Fe), hydrogen sulfide, and organic acids result in low availability of P by their immobilization with Al^{3+} and Fe^{2+} to form insoluble compounds as $AlPO_4 \bullet H_2O$ and $FePO_4 \bullet H_2O$ [5–9]. Moreover, high acidity and toxicity inhibited plant growth changed the structure and diversity of microorganism [2, 10–14]. P plays a vital role in pineapple growth; this means that P is deficient, and plant growth and quality are affected adversely [15]. However, the isolated and selected bacteria strains should possess properties as biofertilizers and bioremediators.

P solubilizing bacteria can release different types of organic acids due to the microbial metabolism production.

However, their activities were not inhibited by these organic acids [16, 17] which produce soluble and available P to plants for the improvement of plant accumulation, thereby reducing the use of chemical fertilizers and negatively impacting the environment; however, plant growth and yield were still unchanged [18, 19]. For example, mango plant growth and soil health have been improved when being cultivated with P solubilizing bacteria [20], whereas the efficiency of the two bacteria *Enterobacter* sp. S16-3 and *Pseudomonas* sp. C16-2O has been proved to increase the growth and shoot dry weight of maize [21]. Furthermore, the P solubilizing bacteria have reduced soil legacy P by increasing the fertilizers use efficiency and improving the long-term efficiency of agricultural production [22].

Specifically, PNSB have a great potential for solubilizing P to support plant growth [2, 7]. PNSB also can produce metabolites that enable plants to overcome the stresses as H^+, Al^{3+}, and Fe^{2+} in ASS [6, 14]. It means that PNSB can be evolutionarily adapted in different ecological systems [23], particularly in ASS. For example, this bacterial group has been selected and applied as it has high efficacy in improving soil fertility and paddy yield, whereas cultivation in ASS does not have the same properties [6, 7, 24, 25]. This is because of the differences between wetland (rice) and upland (pineapple) crops. However, PNSB can be grown under both aerobic and anaerobic environments [2, 6, 7, 13, 14, 24, 25] to improve crop yield [23]. Besides, PNSB were effective in providing plant growth regulators such as IAA and siderophores [13]and compounds such as 5-aminolevulinic acid (ALA) and exopolymeric substances (EPS) to help pineapple development in ASS [13, 26, 27], and reducing Fe^{2+}, Al^{3+}, and Mn^{2+} contamination [2, 13, 14]. Therefore, this study aimed to (i) assess purple nonsulfur bacteria (PNSB) possessing the highest (P) solubilizing capacity and (ii) determine the efficacy of PNSB biofertilizers in the improving soil quality, P uptake, growth, and yield of pineapple cultivated in ASS.

2. Materials and Methods

The experiment was conducted on the "Queen" pineapple variety in Vi Thanh District, Hau Giang Province, Vietnam, from January 2021 to May 2022. The initial soil properties for pineapple cultivation are shown in Table 1. The P solubilizing bacteria used were *R. sphaeroides* W48 and *R. sphaeroides* W42, isolated from ASS-planted pineapple (our preliminary work). The former featured 18.9 mg NH_4^+ L^{-1}, 21.4 mg·P·L^{-1} from Al-P, 8.90 mg·P·L^{-1} from Fe-P, 18.2 mg·P·L^{-1} from Ca-P, and 11.7 mg IAA L^{-1} in microaerobic light conditions and 35.8 mg NH_4^+ L^{-1}, 22.9 mg·P·L^{-1} from Al-P, 17.3 mg·P·L^{-1} from Fe-P, 36.4 mg·P·L^{-1} from Ca-P, and 11.2 mg IAA L^{-1} aerobic dark conditions, while the latter produced 18.9 mg NH_4^+ L^{-1}, 21.6 mg·P·L^{-1} from Al-P, 9.00 mg·P·L^{-1} from Fe-P, 17.2 mg·P·L^{-1} from Ca-P, and 13.9 mg IAA L^{-1}; and 36.5 mg NH_4^+ L^{-1}, 23.3 mg·P·L^{-1} from Al-P, 16.0 mg·P·L^{-1} from Fe-P, 35.6 mg·P·L^{-1} from Ca-P, and 14.1 mg IAA L^{-1}, respectively.

The fertilizers used in this study consisted of urea (46% N), DAP (18% N, 46% P_2O_5), and potassium chloride (60% K_2O).

Table 1: Properties of initial soil for pineapple cultivation.

Soil property	Unit	Layer (cm)	
		0–20	20–40
pH_{H2O}	—	2.62	2.50
pH_{KCl}	—	2.56	2.05
EC	mS·cm^{-1}	0.64	0.99
CEC	meq 100 g^{-1}	13.3	14.7
Na^+	meq Na^+ 100 g^{-1}	0.28	0.31
K^+	meq K^+ 100 g^{-1}	0.14	0.09
Mg^{2+}	meq Mg^{2+} 100 g^{-1}	2.62	2.18
Ca^{2+}	meq Ca^{2+} 100 g^{-1}	1.45	1.12
OM	%	3.58	3.77
Total N	%	0.22	0.16
NH_4^+	mg·kg^{-1}	78.5	61.7
NO_3^-	mg·kg^{-1}	21.7	38.3
Total P	%	0.021	0.023
Available P	mg·kg^{-1}	5.24	3.69
Total acidity	meq H^+ 100 g^{-1}	18.3	12.5
Al^{3+}	meq Al^{3+} 100 g^{-1}	13.3	8.51
Fe^{2+}	mg·kg^{-1}	117.5	120.2
Fe_2O_3	%	0.94	1.15
Fe-P	mg·kg^{-1}	206.8	167.8
Al-P	mg·kg^{-1}	37.7	36.1
Ca-P	mg·kg^{-1}	35.5	24.1

EC: electrical conductivity; CEC: cation exchange capacity; OM: organic matter.

2.1. Soil Preparing and Planting. A pineapple field experiment was located in Hau Giang Province, Vietnam. The upland soil for cultivating pineapple was plowed to a minimum depth of 30 cm. The canal and banks ratio was 6 : 4. The pineapple variety was "Queen," which was taken from slips of previous season pineapple. The plantation was set up in a single row system with width and length as 0.55 × 0.40 m of each plot 5.0 × 5.0 m separated by 1.0 m.

2.2. Preparation of Liquid Biofertilizers. Liquid biofertilizers were prepared as follows. Each culture was subcultured twice in basic isolation medium (BIM) to achieve pure cultures. The purity was checked by a light microscope. A 10% inoculum of each tested PNSB was grown in a screw cap bottle blue cap (1,000 mL) under microaerobic light (3,000 lux) conditions for 48 h. Each culture broth was measured by a spectrophotometer at a wavelength of 660 nm and adjusted to an OD_{660} of 0.8 by diluting with distilled water to get a bacterial cell suspension at 10^8 cells mL^{-1}. The inoculums used were 30 mL of strain W48, 30 mL of strain W42, and a mixed culture of the two strains. To obtain equal cell counts in the mixed culture, 15 mL aliquots of each single strain were mixed well together.

2.3. Experimental Design. The two-factorial experiment was arranged in a completely randomized block design: the first factor was P fertilizer levels of (A) (1) 0, (2) 50, (3) 75, and (4) 100% P_2O_5; the second factor was PNSB biofertilizers including (B) (1) no added PNSB, (2) *R. sphaeroides* W48, (3) *R. sphaeroides* W42, and (4) a mixture of PNSB. The combination of two factors produced 16 treatments, namely, (i) 0% P, (ii) 50% P, (iii) 75% P, (iv) 100% P, (v) W48 + 0% P,

(vi) W48 + 50% P, (vii) W48 + 75% P, (viii) W48 + 100% P, (ix) W42 + 0% P, (x) W42 + 50% P, (xi) W42 + 75% P, (xii) W42 + 100% P, (xiii) mixed PNSB + 0% P, (xiv) mixed PNSB + 50% P, (xv) mixed PNSB + 75% P, and (xvi) mixed PNSB + 100% P. The recommended fertilizer formula (RFF) of P was 12 g·N, 9 g P_2O_5, and 8 g K_2O (g per plant). Liquid biofertilizers from *R. sphaeroides* was used at the volume of 30.0 mL per plant (initial cell density 10^8 cells mL^{-1}) for each stage at 30, 60, 120, 180, and 240 days after planting.

2.4. Soil Samples Analysis. Soil samples were collected from 0 to 20 cm and 20–40 cm topsoil at five sites following a diagonal line of experimental replications using a Dutch Auger and bulk to form a composite. The samples were put on plastic trays and air-dried; after that, these samples were crushed using a mortar and pestle and allowed to pass through a 2.0 mm sieve for soil pH_{H2O}, pH_{KCl}, EC, total N, NH_4^+, NO_3^-, total P, available P, and insoluble P fractions (Al-P, Fe-P, Ca-P compounds); all the elements above were analysed following Sparks et al.'s study [28]. In short, the soil pH_{H2O} and EC were measured at a soil-to-distilled water ratio of 1 : 2.5 using a pH meter, while pH_{KCl} was measured at soil-to-distilled water in a 1.0 M KCl solution ratio of 1 : 2.5 using a pH meter; total N content was measured using the Kjeldahl digestion method. Determination NH_4^+ was extracted by 2.0 M KCl, indicated in color by a mixture of sodium nitroprusside, sodium salicylate, sodium citrate, sodium tartrate, sodium hydroxide, and sodium hypochlorite, and detected at 650 nm wavelength, NO_3^+ content was also extracted by 2.0 M KCl, indicated by a mixture of 0.5 M HCl, vanadium (III) chloride, sulfanilamide, and N-(1-naphthyl) ethylenediamine dihydrochloride, and detected at 540 nm wavelength. Total P content was analyzed by the ascorbic acid method in a spectrophotometer at a wavelength of 880 nm after being digested by perchloric acid and nitric acid mixture. The Bray II method was used to determine the available P. Fractions of inorganic P were extracted by 0.5 M NH_4F, 0.1 M NaOH, and 0.25 M H_2SO_4 for Al-P, Fe-P, and Ca-P compounds at a wavelength of 880 nm, respectively. PNSB densities in the soil field experiment using liquid biofertilizers were counted by the most probable number (MPN) technique in acidic BIM broth, at pH 5.0 and 3,000 lux, under microaerobic light conditions at [29].

2.5. Plant Growth. Height plant (cm): the measurement took place at the segment from the ground to the highest top leaf. Length of D-leaf (cm): the measurement took place at the segment from the stalk that joins a leaf to a stem to the top of the D-leaf. Number of leaves (leaves $plant^{-1}$): leaves were counted in each plant. These parameters were performed in 10 plants for each plot, and the average number was calculated. All these plants were determined 420 days after planting.

2.6. Yield. Pineapple fruit yield ($t{\cdot}ha^{-1}$) was weighed as the total pineapple fruit weight per 12 square meters to calculate the fruit yield per hectare.

2.7. Agronomic Traits of Fruit. Peduncle height (cm): the measurement took place at the segment from the top of the stem to the fruit. Peduncle diameter (cm): the value was the average diameter inferred from the diameter measurement of the plant top, middle, and bottom of the peduncle. Crown height (cm): the measurement took place at the segment from the top of the fruit to the highest site of the crown. Diameter crown (cm): it was measured from the middle of the crown. Fruit height (cm): the measurement took place at the segment from the bottom to the top of the fruit, and the diameter of the fruit (cm) was measured by a caliper at the bottom, middle, and top of the fruit, and the mean value was calculated.

2.8. Chemical Properties of a Fruit. Titratable acidity (TA) was determined by titration with 0.1 N NaOH to pH 8.1, and it was expressed as grams of anhydrous citric acid per 100 g of fruit flesh weight. Ascorbic acid content was estimated by the 2,6-dichlorophenolindophenol visual titration method [30]. Color: colors at the top, middle, and bottom of fruit were measured by Colorimeter CR-200 to infer indices of L^*, a^*, and b^*. Flesh and core per shell was the mean weight from the weight of the pineapple shell (peeled 1.5 cm from the peel) and flesh and core. N and P concentrations in the crown, slip, stem, stem with leaf stripped off, leaf, flesh, core, and shell were determined by the method of digestion with salicylic acid and sulfuric acid. Total N content was determined using the Kjeldahl method. The total P was measured by a colorimetric procedure.

2.8.1. Nutrient Uptake. To determine N, P uptake, dry biomass, and their concentrations should be measured. Dry biomass ($kg{\cdot}ha^{-1}$) of the crown, slip, stem, stem with leaf stripped off, leaf, flesh, core, and shell were dried at 70°C for 72 h using the oven and dry weight was measured by digital weight. Total N uptake ($kg{\cdot}ha^{-1}$) was the dry biomass ($kg{\cdot}ha^{-1}$) × N content (%) in each part of the plant and their calculated sum. Total P uptake ($kg{\cdot}ha^{-1}$) was the dry biomass ($kg{\cdot}ha^{-1}$) × P content (%) in each part of the plant and their calculated sum.

2.9. Statistical Analysis. The data were analyzed using analysis of variance (ANOVA). The normality was also assessed before conducting the ANOVA analysis by plotting a histogram. The differences between treatment means were separated using Duncan's posthoc range test at 5% level of significance using SPSS software version 13.0.

3. Results

3.1. Influences of Adding Liquid Biofertilizers with P Solubilizing Purple Nonsulfur Bacteria on Soil Properties. At harvest, the supplementation of PNSB biofertilizers ameliorated soil properties as pH_{H2O}, NH_4^+, and soluble P concentration in soil at a depth of 0–20 cm (Table2). To be more specific, the pH value in treatments with biofertilizers application ranged from 3.08 to 3.15 and is significantly higher than that of the treatments without biofertilizers (2.68) at a 5% level. NH_4^+ concentration in treatments with biofertilizers fluctuated roughly between 74.4 and 77.1 mg·kg^{-1} and dominated the treatment without ones (60.6 mg·kg^{-1}). Besides, the soluble P also increased in treatments with biofertilizers (51.0–54.5 mg·kg^{-1}), while the concentration in the treatment with no supplemented biofertilizers was only 40.7 mg·kg^{-1}. Other soil properties, including EC, Fe-P, Al-P, and Ca-P, had a downtrend in treatments with biofertilizers. The treatment without supplementation of biofertilizers obtained Fe-P, Al-P, and Ca-P concentrations of 193.5, 39.5, and 28.8 mg·kg^{-1}, respectively, which were statistically higher than those in the treatment with both biofertilizers from *R. sphaeroides* strains W48 and W42 (152.5, 32.1, and 21.6 mg·kg^{-1}, respectively). Moreover, the pH_{KCl} and total P content values ranged from 2.78 to 2.90 and from 0.020 to 0.025%, respectively. At these parameters, differences between treatments with and without biofertilizers were insignificant. For P fertilizer levels, treatments with 0, 50, 75, and 100% P of RFF properties, including pH_{H2O}, pH_{KCl}, EC, NH_4^+, and total P content in the soil, had no noticeable difference (Table 2). Among them, the pH_{KCl} value fluctuated from approximately 2.83 to 2.88 and was less than the pH_{H2O} value (2.97–3.06). Soluble P content, Fe-P, Al-P, and Ca-P compounds were all low in the treatment with 0% P of RFF and gradually increased following P levels of 50, 75, and 100% of RFF. The available P contents corresponding to treatments with 0, 50, 75, and 100% P of RFF were 33.8, 50.0, 52.1, and 63.0 mg·kg^{-1}. The contents of P compounds, including Fe-P, Al-P, and Ca-P, in the treatment with 100% P of RFF were 194.9, 44.8, and 30.0 mg·kg^{-1}, respectively. There were interactions between inorganic P fertilization and biofertilizers supplementation in parameters that included NH_4^+, soluble P, Fe-P, and Ca-P. The PNSB density significantly differed at a 5% level between the inoculated and control treatments. For instance, in the application treatment, a mixture of *R. sphaeroides* W48 and *R. sphaeroides* W42 achieved the highest value with 6.57 MPN g^{-1} DSW, while the control treatment produced the lowest value at 2.75 MPN g^{-1} DSW. The application of four P fertilizer levels (0, 50, 75, and 100% P) as RFF had insignificant difference (Table 2).

The soil properties at a depth of 20–40 cm in pineapple cultivation were recorded in Table 1 (Supplementary data). Only NH_4^+ and Fe-P contents differed statistically among treatments at a 5% level, while the other characteristics documented insignificant differences.

3.2. Influences of Adding Liquid Biofertilizers with P Solubilizing Purple Nonsulfur Bacteria on Dry Biomass, P Concentration, and Uptake of Pineapple Cultivated in Acid Sulfate Soil (ASS)

3.2.1. Dry Biomass. Compared to the control treatment, the treatments with biofertilizers of *R. sphaeroides* W48 and *R. sphaeroides* W42 had higher biomass in pineapple crown, flesh, core, shell, slip, peduncle, butt, and leaf (Table 3). Among these parts of pineapple, the treatment with supplementation of biofertilizers from the combination of two strains, *R. sphaeroides* W48 and W42, gained the highest biomass in leaf (6990.0 kg·ha^{-1}), while the treatment without biofertilizer supplementation yielded the least value (5976.5 kg·ha^{-1}). Similar results happened in biomass of peduncle, slip, and crown. The biomass of pineapple butt in the treatments with biofertilizers fluctuated from 1492.5 kg·ha^{-1} to 1541.9 kg·ha^{-1}, remarkably higher than the control treatment, with 1230.4 kg·ha^{-1}. The mean dry biomass of pineapple shell, flesh, and core in treatments with biofertilizers was 1355.3, 1165.0, and 333.3 kg·ha^{-1}, respectively, significantly higher than the treatment without biofertilizers (1138.8, 1016.6, and 267.3 kg·ha^{-1}, respectively). Applying 100% P of RFF kept dry biomass of leaf, peduncle, slip, crown, shell, flesh, and core of pineapple higher than that of other treatments (Table 3). Treatment with no P fertilization reduced dry biomass of pineapple parts, which was significantly lower than treatments with 50, 75, and 100% P of RFF. The dry biomass of shell, flesh, and core in treatments with 75% P of RFF was recorded at 1363.3, 1207.9, and 334.9 kg·ha^{-1}, respectively, which is significantly higher than the treatment with 50% P of RFF (1261.1, 1075.8, and 296.4 kg·ha^{-1}, respectively). Dry biomass of leaf, crown, and peduncle showed a similar trend. The interaction between factors of biofertilizers supplementation and inorganic P fertilizer levels appeared at dry biomass of leaf, butt, crown, slip, shell, flesh, and core (Table 3).

3.2.2. P Concentration and Uptake. According to Table 4, the P content in parts of pineapple, including crown, core, slip, peduncle, butt, and leaf, varied statistically among treatments at 5%. P concentrations in the crown, slip, peduncle, and leaf of pineapple supplemented with biofertilizers with a bacterial mixture of *R. sphaeroides* W48 and *R. sphaeroides* W42 were 0.408, 0.345, 0.299, and 0.238%, respectively, significantly higher than those in the control treatment (0.306, 0.317, 0.191, and 0.197%). Supplementation with biofertilizers had not resulted in the difference in P concentration in pineapple shell and flesh. In the shell, P content ranged from 0.191 to 0.208%, and the number in the flesh fluctuated from 0.179 to 0.189%. Applying inorganic P fertilizer also enhanced P content in parts of pineapple cultivated in ASS. In leaf, butt, peduncle, and slip, the highest P content went to the treatment with 100% P of RFF, the second highest was the treatments with 75 and 50% P of RFF, and the lowest was recorded in the treatment not being supplied with inorganic P fertilizer. The differences among them were significant at 5%. For pineapple flesh, core, and shell, in the treatment with 100% P of RFF, P concentrations were 0.217, 0.112, and 0.228%, respectively, which were insignificantly different from the treatment with 75% P of RFF (0.207, 0.110, and 0.221%, respectively). In both treatments with 100% and 75% P of RFF, the P concentrations in the shell, flesh, and core of pineapple fruit were noticeably higher than those in the treatments with 50% P of RFF or 0% P of RFF. P concentration of pineapple parts has interactions between the factors for parts in leaf, butt, peduncle, slip, crown, and flesh.

Table 2: Effects of added liquid biofertilizers containing P solubilizing purple nonsulfur bacteria (PNSB) and phosphorus fertilizer levels on characteristics of top acid sulfate soil (0–20 cm) cultivated pineapple.

Factor		pH_{H2O}	pH_{KCl}	EC	NH_4^+	P_{total}	$P_{available}$	Fe-P	Al-P	Ca-P	Log PNSB
		—	—	$mS{\cdot}cm^{-1}$	$mg{\cdot}kg^{-1}$	%	$mg{\cdot}kg^{-1}$	$mg{\cdot}kg^{-1}$	$mg{\cdot}kg^{-1}$	$mg{\cdot}kg^{-1}$	MPN g^{-1} DSW
(A) Biofertilizers (initial cell density 10^8 cells·mL^{-1})	None	2.68^b	2.78	0.59^a	60.6^b	0.020	40.7^b	193.5^a	39.5^a	28.8^a	2.75^c
	W48	3.13^a	2.88	0.42^b	75.5^a	0.020	51.0^a	185.1^b	30.6^b	27.7^a	6.21^b
	W42	3.15^a	2.90	0.45^b	77.1^a	0.025	54.5^a	163.8^c	31.7^b	22.4^b	6.18^b
	W48 + W42	3.08^a	2.87	0.48^b	74.4^a	0.022	52.7^a	152.5^d	32.1^b	21.6^b	6.57^a
(B) Phosphorus fertilizer levels (%)	0	2.97	2.86	0.48	71.1	0.020	33.8^c	148.8^d	21.3^c	19.4^b	5.45
	50	3.00	2.83	0.52	66.3	0.022	50.0^b	172.6^c	31.7^b	22.2^b	5.42
	75	3.06	2.84	0.45	72.7	0.022	52.1^b	178.7^b	36.1^b	28.9^a	5.43
	100	3.01	2.88	0.47	77.4	0.024	63.0^a	194.9^a	44.8^a	30.0^a	5.42
F (A)		*	ns	*	*	ns	*	*	*	*	*
F (B)		ns	ns	ns	ns	ns	*	*	*	*	ns
F (A × B)		ns	ns	ns	*	ns	*	*	ns	*	ns
C.V. (%)		3.79	6.74	25.6	16.2	25.6	11.7	4.69	24.1	26.2	1.04

Numbers in each column with the same following letters are insignificantly different from each other; ns: not significant difference; *: significant difference at 5%; none: no added biofertilizers; W48: *Rhodobacter sphaeroides* W48; W42: *R. sphaeroides* W42; W48 + W42: a mixture of *R. sphaeroides* W48 and *R. sphaeroides* W42; EC: electrical conductivity; MPN: most probable number; DSW: dry soil weight.

Total P uptake of pineapple peaked at 26.8 kg·P·ha^{-1} in the treatment supplemented with biofertilizers with both strains *R. sphaeroides* W48 and W42. Using biofertilizers containing *R. sphaeroides* W42 and *R. sphaeroides* W48 individually resulted in significantly lower P uptake at 24.7 and 22.5 kg·P·ha^{-1}. The treatment without biofertilizers supplementation had the smallest P uptake (18.4 kg·P·ha^{-1}). Without inorganic fertilizers, the total P uptake of pineapple dropped compared to the treatments using 50, 75, and 100% P of RFF. Corresponding to the order from 100, 75, 50, and 0% of RFF, total P uptake was recorded at 32.8, 27.6, 21.7, and 10.4 kg·P·ha^{-1} (Table 2-Supplementary). The total P uptake of pineapple cultivated in ASS and combined 75% P of RFF with PNSB biofertilizers containing *R. sphaeroides* W48, *R. sphaeroides* W42, or both strains *R. sphaeroides* W48 and *R. sphaeroides* W42 were documented at 27.9, 28.8, and 31.5 kg·P·ha^{-1}, respectively, and did a great deal in boosting the total P uptake of pineapple, in comparison to the no-applied biofertilizers (Figure 1). In addition, total P uptake of pineapple in treatments used both strains *R. sphaeroides* W48 and *R. sphaeroides* W42. Still, no-applied P chemical fertilizer achieved 13.7 kg·P·ha^{-1}, while the total P uptake was only 9.4 kg·ha^{-1} in the treatment without biofertilizers (Figure 1, Table 2 in Supplementary data).

3.3. Influences of Adding Liquid Biofertilizers with P Solubilizing Purple Nonsulfur Bacteria on Growth, Yield Components, and Yield of Pineapple Cultivated in Acid Sulfate Soil

3.3.1. Pineapple Growth. A significant improvement in the growth of pineapple cultivated in ASS was observed under the influences of PNSB biofertilizers (Table 5). The treatments with biofertilizers increased plant height to be 75.7–76.1 cm, which was significantly different from that in the treatment without biofertilizer (73.1 cm). In the treatment with biofertilizers supplementation (*R. sphaeroides* W48 and W42), pineapple had D-leaf length, number of leaves, and number of slips higher than any other treatments. These parameters in the treatment of either one of the PNSB biofertilizers (*R. sphaeroides* W48 or *R. sphaeroides* W42) differed insignificantly, but are remarkably higher than those in the treatment without biofertilizers. The PNSB biofertilizers of *R. sphaeroides* W48 and *R. sphaeroides* W42 revealed their efficacy in enhancing plant height, height, and diameter of peduncle, crown, and fruit compared to the control treatment. For inorganic P fertilizer levels, there were significant differences in plant height, D-leaf length, number of leaves, and number of slips. In brief, these parameters in treatment without P fertilizer application were remarkably low compared to the treatments with inorganic P fertilizer levels at 50, 75, and 100% P of RFF. For plant height, D-leaf length and numbers of leaves, height peduncle, diameter peduncle, diameter crown, height fruit, and diameter fruit, the values in the treatment with 75% P of RFF were sequentially 76.6 cm, 53.1 cm, 52.3 leaves, 22.4 cm, 2.6 cm, 10.4 cm, 14.9 cm, and 8.1 cm and insignificantly different from the treatment with 100% P of RFF, whose values were 77.4 cm, 53.0 cm, 52.5 leaves, 23.2 cm, 2.7 cm, 10.7 cm, 14.9 cm, and 8.2 cm, respectively. Crown height varied slightly among treatments. Moreover, both factors interacted with plant height and crown height.

3.3.2. Pineapple Yield Components. The mean height and diameter of pineapple fruit treated with PNSB biofertilizers were 14.9 cm in height and 8.2 cm in width, significantly higher than the treatment without biofertilizers (13.4 cm and 7.6 cm, respectively). From the finest fruit size to the poorest one, it followed the decline in the percentage of recommended P fertilizer levels applied, from 100% to 0% (Table 5). More specifically, pineapple fruit's diameter in treatment with P fertilizer levels ranged from 8.1 to 8.2 cm and dominated those in the treatment without P (7.6 cm).

Table 3: Effects of added liquid biofertilizers containing P solubilizing purple nonsulfur bacteria and phosphorus fertilizer levels on the dry biomass parts of pineapple cultivated in acid sulfate soil.

Factor		Crown	Flesh	Core	Shell	Slip, stem, shoot, and sucker	Peduncle (stem)	Butt (stem with leaves stripped off)	Leaf
		kg·ha^{-1}							
(A) Biofertilizers (initial cell density 10^8 cells·mL^{-1})	None	248.3^d	1016.6^d	267.3^c	1138.8^c	115.1^c	352.2^c	1230.4^b	5976.5^c
	W48	227.9^c	1135.5^c	342.4^a	1326.7^b	183.4^{ab}	403.2^{ab}	1541.9^a	6399.9^b
	W42	286.8^b	1159.8^b	311.9^b	1323.2^b	177.9^b	379.9^{bc}	1495.3^a	7081.9^a
	W48 + W42	311.2^a	1199.7^a	345.6^a	1416.1^a	192.7^a	420.3^a	1492.5^a	6990.0^a
(B) Phosphorus fertilizer level (%)	0	247.6^d	987.0^d	255.9^d	1138.0^d	133.4^d	330.7^c	1171.1^c	5290.0^d
	50	281.9^c	1075.8^c	296.4^c	1261.1^c	157.4^c	371.5^b	1419.7^b	6244.9^c
	75	292.4^b	1207.9^b	334.9^b	1363.3^b	180.5^b	426.5^a	1569.6^a	7205.1^b
	100	302.4^a	1240.8^a	380.1^a	1442.4^a	197.8^a	427.0^a	1599.7^a	7708.2^a
F (A)		*	*	*	*	*	*	*	*
F (B)		*	*	*	*	*	*	*	*
F (A × B)		*	*	*	*	*	ns	*	*
C.V. (%)		3.89	2.72	4.46	1.79	8.23	10.2	5.06	5.70

Numbers in each column with the same following letters are insignificantly different from each other; ns: not significant difference; *: significant difference at 5%; none: no added biofertilizers; W48: *Rhodobacter sphaeroides* W48; W42: *R. sphaeroides* W42; W48 + W42: a mixture of *R. sphaeroides* W48 and *R. sphaeroides* W42.

Table 4: Effects of added biofertilizers containing P solubilizing purple nonsulfur bacteria and phosphorus fertilizer levels on the phosphorus content in parts of pineapple cultivated in acid sulfate soil.

Factor		Crown	Flesh	Core	Shell	Slip, stem, shoot, and sucker	Peduncle (stem)	Butt (stem with leaves stripped off)	Leaf
		%							
(A) Biofertilizers (initial cell density 10^8 cells·mL^{-1})	None	0.306^d	0.179	0.088^b	0.191	0.317^d	0.191^d	0.229^b	0.197^c
	W48	0.338^c	0.191	0.98^a	0.203	0.398^a	0.225^c	0.235^b	0.220^b
	W42	0.373^b	0.189	0.091^b	0.205	0.367^b	0.264^b	0.236^b	0.226^{ab}
	W48 + W42	0.408^a	0.189	0.100^a	0.208	0.345^c	0.299^a	0.274^a	0.238^a
(B) Phosphorus fertilizer levels (%)	0	0.282^d	0.140^c	0.068^c	0.152^c	0.263^c	0.109^d	0.129^d	0.122^d
	50	0.354^c	0.186^b	0.087^b	0.206^b	0.366^b	0.265^c	0.250^c	0.220^c
	75	0.384^b	0.207^a	0.110^a	0.221^a	0.375^b	0.283^b	0.275^b	0.253^b
	100	0.406^a	0.217^a	0.112^a	0.228^a	0.421^a	0.321^a	0.319^a	0.286^a
F (A)		*	ns	*	ns	*	*	*	*
F (B)		*	*	*	*	*	*	*	*
F (A × B)		*	*	ns	ns	*	*	*	*
C.V. (%)		8.88	11.9	9.63	9.72	5.76	6.50	6.61	8.21

Numbers in each column with the same following letters are insignificantly different from each other; ns: not significant difference; *: significant difference at 5%; none: no added biofertilizers; W48: *Rhodobacter sphaeroides* W48; W42: *R. sphaeroides* W42; W48 + W42: a mixture of *R. sphaeroides* W48 and *R. sphaeroides* W42.

3.3.3. Pineapple Fruit Yield. The result in Figure 2 and Table 3 in Supplementary data indicated that pineapple cultivated in ASS and applied with PNSB biofertilizers containing *R. sphaeroides* W48 and *R. sphaeroides* W42 did a great deal in boosting the yield of pineapple compared to the no-applied biofertilizers case. In detail, pineapple fruit yield in treatments of 75% P combined with biofertilizers gained a higher yield ranging from 21.0 to 22.2 t·ha^{-1}, while in the treatment of 75% P combined without biofertilizers, the yield was only 20.0 t·ha^{-1}. When supplementing PNSB biofertilizers *R. sphaeroides* W48 and *R. sphaeroides* W42 separately, the yield was statistically the same as that when applying biofertilizers with both strains. For P fertilizer levels, the pineapple yields in treatments with 100, 75, and 50% P of RFF were 19.8, 20.0, and 15.9 t·ha^{-1}, respectively, significantly different from the control treatment (13.4 t·ha^{-1}), whereas treatments with both strains *R. sphaeroides* W48 and *R. sphaeroides* W42 combined with 100, 75, 50, and 0% P of RFF yielded sequentially 21.8, 22.2, 18.5, and 15.4 t·ha^{-1}. Pineapple yield in treatment with 100 and 75% P of RFF and supplementing biofertilizers *R. sphaeroides* W48 and *R. sphaeroides* W42 was not significantly different.

3.4. Influences of Adding Liquid Biofertilizers with P Solubilizing Purple Nonsulfur Bacteria on Chemical Properties of Pineapple Fruit. In Table 6, three different trends could be divided depending on the influences of biofertilizers and P fertilizer levels on the chemical properties of pineapple fruit. Firstly, the water content was deeply affected by both factors. There was a significant increase in the amount of water inside

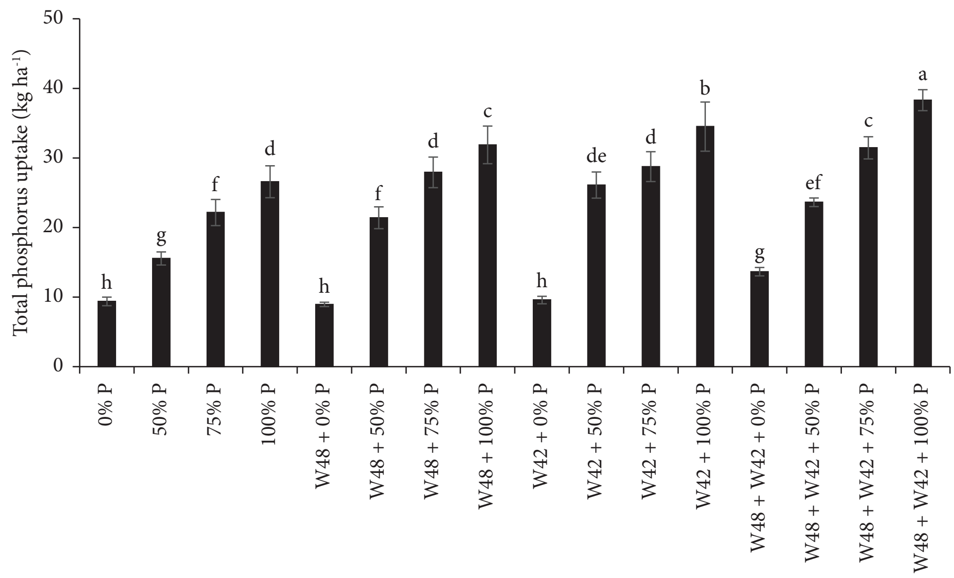

Figure 1: Effects of added liquid biofertilizers containing P solubilizing purple nonsulfur bacteria and phosphorus fertilizer levels on the total phosphorus uptake of pineapple cultivated in acid sulfate soil. Bars with the same lower letters were insignificantly different; treatments were statistically different at 5%; W48: *Rhodobacter sphaeroides* W48; W42: *R. sphaeroides* W42; W48 + W42: a mixture of *R. sphaeroides* W48 and *R. sphaeroides* W42.

Table 5: Effects of added liquid biofertilizers containing P solubilizing purple nonsulfur bacteria and phosphorus fertilizer levels on growth and agronomic traits of pineapple cultivated in acid sulfate soil.

Factor		Plant height	D-leaf length	Number of leaves	Number of slips	Peduncle		Crown		Fruit	
						Height	Diameter	Height	Diameter	Height	Diameter
		cm	cm	—	—	cm					
(A) Biofertilizers (initial cell density 10^8 cells·mL^{-1})	None	73.1^{b}	49.5^{c}	48.3^{c}	5.6^{c}	20.5^{c}	2.3^{c}	12.7^{b}	9.2^{b}	13.4^{c}	7.6^{b}
	W48	76.1^{a}	52.3^{ab}	50.4^{b}	6.6^{ab}	23.0^{a}	2.7^{a}	14.3^{a}	10.6^{a}	14.8^{a}	8.3^{a}
	W42	75.7^{a}	51.7^{b}	51.6^{ab}	6.3^{b}	21.5^{b}	2.5^{b}	14.3^{a}	10.9^{a}	14.2^{b}	8.0^{a}
	W48 + W42	75.9^{a}	53.6^{a}	52.6^{a}	6.9^{a}	22.6^{ab}	2.7^{a}	14.2^{a}	10.3^{a}	15.01^{a}	8.2^{a}
(B) Phosphorus fertilizer levels (%)	0	72.1^{c}	49.2^{b}	47.3^{b}	5.2^{d}	20.8^{b}	2.4^{c}	13.8	9.1^{b}	13.0^{b}	7.6^{b}
	50	74.7^{b}	51.9^{a}	50.8^{a}	6.0^{c}	21.2^{b}	2.5^{bc}	14.0	10.8^{a}	14.6^{a}	8.1^{a}
	75	76.6^{a}	53.1^{a}	52.3^{a}	6.6^{b}	22.4^{a}	2.6^{ab}	13.9	10.4^{a}	14.9^{a}	8.1^{a}
	100	77.4^{a}	53.0^{a}	52.5^{a}	7.5^{a}	23.2^{a}	2.7^{a}	13.8	10.7^{a}	14.9^{a}	8.2^{a}
F (A)		*	*	*	*	*	*	*	*	*	*
F (B)		*	*	*	*	*	*	ns	*	*	*
F (A × B)		*	ns	ns	ns	ns	ns	*	ns	ns	ns
C.V. (%)		1.82	3.69	4.66	8.00	6.90	8.25	7.23	12.6	5.54	4.44

Numbers in each column with the same following letters are insignificantly different from each other; ns: not significant difference; *: significant difference at 5%; none: no added biofertilizers; W48: *Rhodobacter sphaeroides* W48; W42: *R. sphaeroides* W42; W48 + W42: a mixture of *R. sphaeroides* W48 and *R. sphaeroides* W42.

pineapple fruit based on the presence of either P fertilizer levels or biofertilizers supplementation. Specifically, pineapple fruits were inoculated with PNSB strain *R. sphaeroides* W48 or a mixture of *R. sphaeroides* W48 and W42, and the water volume was 214.4 mL fruit^{-1} and 213.1 mL fruit^{-1}, considered to be the highest volume statistically. The lowest was recorded in the treatment with no-applied biofertilizers (173.1 mL fruit^{-1}), which was significantly different from the other treatments. On the other hand, the volume of water in pineapple fruits affected by P fertilizer levels decreased from 234.1 > 216.3 > 186.4 > 171.0 mL fruit^{-1} according to the decline of P fertilizer levels applied as 100, 75, 50, and 0% P of RFF, respectively. Secondly, vitamin C content and Brix index were impacted by only the biofertilizers factor. Brix index in pineapple fruits in treatments with biofertilizers ranged from 9.97 to 10.34, noticeably higher than the no-applied biofertilizers treatments (8.96). However, the vitamin C content slightly dropped under the influence of the PNSB biofertilizers. Finally, TA and pH fluctuated from 0.311 to 0.344 $g_{\text{citric acid}}$ 100 $g^{-1}{}_{\text{flesh weight}}$ and from 3.65 to 3.72 among treatments and received no noticeable effect from both factors.

The flesh and core per shell ratio differed significantly at 5% between P fertilizer levels. The treatments with 75 and 100% P of RFF yielded a higher flesh and core per shell ratio

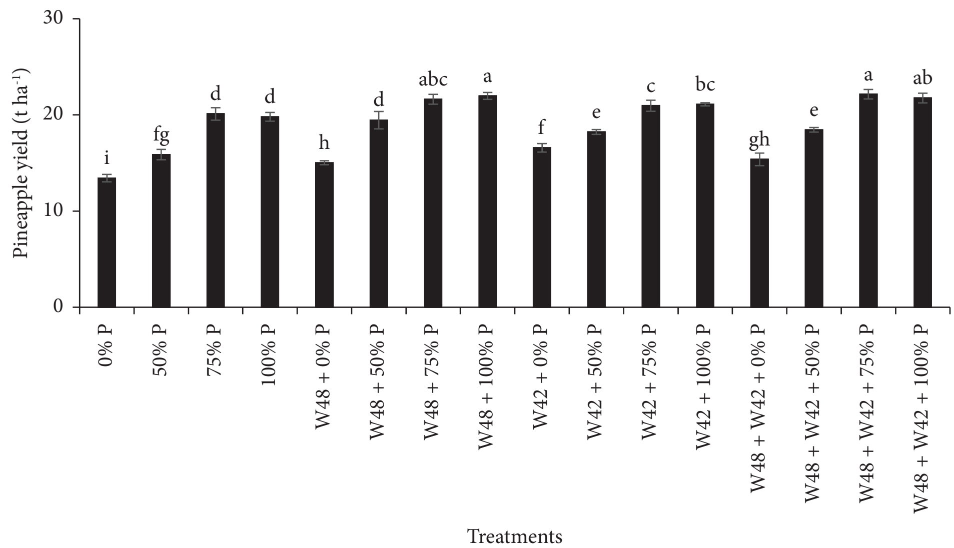

Figure 2: Effects of added liquid biofertilizers containing P solubilizing purple nonsulfur bacteria and phosphorus fertilizer levels on the pineapple yield cultivated in acid sulfate soil. Bars with the same lower letters were insignificantly different. Treatments were statistically different at 5%. W48: *Rhodobacter sphaeroides* W48; W42: *R. sphaeroides* W42; W48 + W42: a mixture of *R. sphaeroides* W48 and *R. sphaeroides* W42.

Table 6: Effects of added liquid biofertilizers containing P solubilizing purple nonsulfur bacteria and phosphorus fertilizer levels on the chemical properties of pineapple cultivated in acid sulfate soil.

Factor		Water content	TA	Vitamin C	Brix index	pH	Flesh and core per shell	Fruit shell colors		
								L^*	a^*	b^*
		mL fruit^{-1}	$g_{\text{citric acid}}$ 100 g$^{-1}_{\text{flesh weight}}$	mg·100 g^{-1}	—	—	—	—	—	—
(A) Biofertilizers (initial cell density 10^8 cells·mL^{-1})	None	173.1^c	0.344	16.5^a	8.96^b	3.72	1.48	40.7^c	14.4^b	20.8
	W48	214.4^a	0.317	13.4^b	10.34^a	3.65	1.46	41.8^b	15.4^a	20.9
	W42	207.1^b	0.311	13.7^b	10.18^a	3.67	1.48	42.1^b	15.5^a	21.2
	W48 + W42	213.1^a	0.341	15.4^a	9.97^a	3.65	1.56	42.9^a	15.2^a	21.1
(B) Phosphorus fertilizer levels (%)	0	171.0^d	0.332	14.9	9.77	3.69	1.38^b	41.8	15.2	21.1
	50	186.4^c	0.330	14.4	9.63	3.66	1.46^{ab}	41.6	15.0	20.8
	75	216.3^b	0.332	14.5	10.04	3.67	1.58^a	42.0	15.1	21.0
	100	234.1^a	0.319	15.1	10.02	3.67	1.56^a	42.0	15.2	21.0
F (A)		*	ns	*	*	ns	ns	*	*	ns
F (B)		*	ns	ns	ns	ns	*	ns	ns	ns
F (A × B)		*	ns	ns	ns	ns	ns	ns	ns	ns
C.V. (%)		3.76	13.5	13.2	3.76	3.75	12.7	1.68	2.74	4.25

Numbers in each column with the same following letters are insignificantly different from each other; ns: not significant difference; *: significant difference at 5%; none: no added biofertilizers; W48: *Rhodobacter sphaeroides* W48; W42: *R. sphaeroides* W42; W48 + W42: a mixture of *R. sphaeroides* W48 and *R. sphaeroides* W42; TA: titratable acidity = gram of citric acid 100 g^{-1} of flesh weight; Vitamin C: milligram of ascorbic acid kg^{-1} of flesh; brix index: total soluble solids.

compared to the treatment with 0% P of RFF; the ratio of flesh and core per shell of treatments of 0, 75, and 100% P of RFF was 1.38, 1.58, and 1.56, respectively. The treatments with supplementation of *R. sphaeroides* W48, W42, or a mixture of *R. sphaeroides* W48 and W42 were insignificantly different from the treatment without biofertilizers in flesh and core per shell ratio. This ratio between either PNSB biofertilizers or no-applied biofertilizers treatments fluctuated slightly from 1.46 to 1.56 (Table 6).

The L^* index was the highest in the treatment with supplementation of mixed strains *R. sphaeroides* W48 and W42 at 42.9, while treatments with supplementation of single strain W48 or W42 were insignificantly different, with L^* values of 41.8 and 42.1, respectively, which was lower than those of the treatment with supplementation of *R. sphaeroides* W48 and W42 combination but higher than the treatment without biofertilizers. The treatments with P fertilizer levels from 0 to 100% P of RFF gained the same L^* index with values between 41.6 and 42.0. The value of a^* index in treatments with biofertilizers differed significantly at the 5% level. The treatment with no biofertilizers had an a^* index of 40.7 lower than three treatments with *R. sphaeroides* W48, *R. sphaeroides* W42, and a mixture of *R. sphaeroides* W48 and W42. The values of a^* in biofertilizers factor were 40.7, 41.8, 42.1, and 42.9 for none, *R. sphaeroides* W48, *R. sphaeroides* W42, and a mixture of *R. sphaeroides* W48 and W42, respectively. The value of a^* and b^* indices of P fertilizer levels was insignificantly different and ranged from 15.0 to 15.2 and from 20.8 to 21.1, respectively. Besides, the b^* index of the biofertilizers factor differed insignificantly and fluctuated slightly from 20.8 to 21.2 (Table 6).

4. Discussion

4.1. Improvement of Soil Properties by Adding Biofertilizers with P Solubilizing Purple Nonsulfur Bacteria. ASS is widely distributed in South East Asia, especially in Vietnam, with 1.6 million hectares, including potential and actual ASS [31], where the pH value was always lower than 3.5 [12]. Metal ions, such as Al^{3+}, Fe^{2+}, and Mn^{2+}, presenting in ASS rise highly when soil pH is lower than 3.5 [24, 32, 33], while the availability of nutrients, including N and P, keeps low [34]. The application of the PNSB biofertilizers improved some soil chemical properties in the 0–20 cm layer used for pineapple cultivation (Table 2). P solubilizing bacteria were able to reduce the soil acidity, meaning that biofertilizers play key roles in raising pH as well [32, 35]; in this case, pH_{H2O} values at harvest were all higher than those at the initial crop (2.62) in every treatment (Tables 1 and 2), leading to an increase in NH_4^+ and soluble P concentrations in soil (Table 2) and increase in high available nutrients such as N, P, K, Ca, and Mg as reported by Fernández and Hoeft [11, 36]. Biofertilizers enhanced the P solubilizing PNSB density in soil (Table 2), resulting in higher available P and reduced the insoluble P compounds, including Fe-P, Al-P, and Ca-P, compared to treatments without biofertilizers (Table 2). On the other hand, using inorganic P fertilizer at different levels raised all of the P content, consisting of soluble and insoluble P compounds, compared to treatments that were not used. Moreover, NH_4^+ treatments with biofertilizers had higher content of NH_4^+ than treatment without ones (Table 2). It may originate from activities of N-fixing. N-mineralization also produce NH_4^+, but is equal in all treatments. This was because plants did not take all of P from fertilizer; the P was soon fixed in insoluble compounds, which is consistent with previous studies [2, 37, 38]. Besides, when soil pH drops below 4.0, P is present mainly in the Fe-P form [8, 32]. Moreover, both strains have the ability to solubilize the insoluble P forms, but strain W42 and W48 equally produced the soluble P. However, strain W42 showed a better P-solubilizing ability of Fe-P and Ca-P forms. That is a reason why a mixture of PNSB generated much available P from Fe-P and Ca-P forms compared to strain W42 (Table 2). It means that the mixed PNSB demonstrated synergistic impacts from each other. These results also matched with previous result, mixed PNSB cultures illustrated more influences than single strain in improvements of soil fertility and grain yield in acidic paddy field [2]. The concentration of NH_4^+ at a depth of 20–40 cm had a similar pattern to its trend at a depth of 0–20 cm (Table 2; Table 1 in Supplementary data).

4.2. Improvement of Dry Biomass, N and P Concentrations, and Uptake in Pineapple by Adding Biofertilizers with P Solubilizing Purple Nonsulfur Bacteria. Dry biomass in parts of pineapple was ameliorated in treatments with either biofertilizers or P fertilizer levels compared to treatments without supplementation (Table 3). N, P, and potassium (K) are three of 17 essential elements for plant growth [39, 40]. Therefore, plants' yield and dry biomass rise when these elements are applied. The treatment with biofertilizers from the combination of *R. sphaeroides* W48 and W42 had N concentrations in butt and fruit core of 2.365 and 0.955%, which were higher than those values in treatments with biofertilizers from the strains and the treatment without biofertilizers (Table 4 in Supplementary data). N concentration in butt, peduncle, slip, and fruit core of pineapple applied biofertilizers with *R. sphaeroides* W48 and W42 individually were insignificantly different from each other, while they were all distant from the data in treatment without any biofertilizers. PNSB biofertilizers raised the available N and soluble P concentration in soil [13, 41]. From that, plants grew strongly, and N and P content in plant parts, including crown, flesh, shell, slip, butt, and leaf increased (Table 4). According to the result of Wang et al. [42], bacteria improved concentrations of N, P, and K in plants and reduced the use of chemical fertilizers.

Although the treatment with only biofertilizers application from *R. sphaeroides* W48 achieved a lower N uptake than the treatment with both *R. sphaeroides* W48 and *R. sphaeroides* W42, it still had a better result than the treatment without biofertilizers. Reducing P fertilizer levels in the case of without biofertilizers supplementation decreased the total P uptake in pineapple. The result also showed that using biofertilizers from *R. sphaeroides* W42 either separately or in a mixture with *R. sphaeroides* W48, the total N and P uptakes still dominated those in the treatment without biofertilizers (Figure 1). Because of nutrients originated from activities of N fixation and P solubilization supported the ability to absorb N, P [43]. Therefore, the combination of biofertilizers and inorganic fertilizers increases N and P uptakes in plants [44, 45].

4.3. Improvement of Growth and Agronomic Traits and Fruit Yield of Pineapple by Adding Biofertilizers with P Solubilizing Purple Nonsulfur Bacteria. The interaction between microorganisms and plants is vital for producing nutrients in agriculture. In this study, the supplementation of PNSB biofertilizers helped promote pineapple growth as plant height, D-leaf length, and leaves number (Table 5). PNSB can produce IAA which stimulate plant growth parameters. Strain W48 produced higher IAA content resulting in better plant growth traits. This happened because PNSB biofertilizers can live in low pH conditions and provide NH_4^+ and soluble P for plants. This result matched with previous researches [2, 24, 26]. Moreover, biofertilizers containing rhizosphere bacteria can produce phytohormones, including acetic acid, auxins, and cytokinins, to support the plant development [46, 47]. Thus, PNSB can be used as plant growth promoting bacteria to supply IAA, ALA for plant

[14, 24]. N and P are elements participating in cell division, root development, root traits, anatomy modifications, and root hair density, resulting in improvement in photosynthesis and plant growth [48–51]. The study of Santoyo et al. [52] revealed that bacteria possess a mechanism to stimulate plant growth by increasing the availability of nutrients in the soil. The PNSB from biofertilizers can produce essential nutrients for plants and help them to tolerate biotic and abiotic stresses [53, 54]. PNSB are able to release NH_4^+ by nitrogen fixing, PO_4^{3-} by phosphorus solubilizing and exchangeable K^+ by potassium solubilizing. These nutrients are key factors to overcome obstacles and promote the plant growth and yield [55, 56]. Furthermore, P fertilization helped pineapples grow well and improve their height and fruit diameter (Table 5) because P supports root development, flowering, and maturity of fruit [48, 57].

Plant-associated and rhizosphere microorganisms benefit plant growth and yield [58, 59]. Thus, pineapple treated with PNSB biofertilizers had a higher yield than those treated without biofertilizers (Figure 2). This result could be attributed to the fact that the use of microorganisms and their metabolites increase nutrient uptake, pest control, and stress tolerance, leading to higher yield [60–62]. To make it clear, PNSB biofertilizers produce NH_4^+, PO_4^{3-}, siderophores, indole-3-acetic acid, and 5-aminolevulinic acid production to encourage plant growth [13]. On the other hand, treatments with inorganic P fertilizer enhanced plant yield as well, compared to treatments with no P fertilization (Figure 2). The result was in accordance with Valleser [63], where P increased by 14.2% in the "MD-2" pineapple yield when treated with $169\,\mathrm{kg{\cdot}P{\cdot}ha^{-1}}$ compared to without P. P solubilizing bacteria can interact with inorganic fertilizers to improve plant yield and fertilizer use efficiency [64, 65].

4.4. Improvement of Chemical Traits of Pineapple Fruit by Adding Biofertilizers with P Solubilizing Purple Nonsulfur Bacteria. Brix index in the pineapple fruit in the treatments with supplementation of *R. sphaeroides* W48, W42, or a mixture of W48 and W42 was 10.34, 10.18, and 9.97, respectively, significantly different from that in the treatment without biofertilizers (Table 6). Treatments with biofertilizers increased the Brix index via plant growth and anabolism stimulation from the metabolism of microorganisms. That is a signal for ethylene production [66] and accelerates enzyme synthesis, decreasing polycarbohydrate components in cell walls and increasing monocarbohydrate, leading to a Brix index rise in the fruit ripening process [67, 68]. Between four phosphorus fertilizer levels, 0, 50, 75, and 100% P of RFF, the TA content slightly fluctuated from 3.19 to $3.32\,\mathrm{g_{citric\ acid}}\ 100\,\mathrm{g^{-1}}_{\mathrm{flesh\ weight}}$, whereas vitamin C was recorded from 14.4 to $15.1\,\mathrm{mg}\ 100\,\mathrm{g^{-1}}$, which were insignificantly different for TA and vitamin C content in fruit (Table 6). A similar result was documented by Spironello et al. [69], and an insignificant difference in vitamin C between phosphorus levels was also observed.

5. Conclusions

Application of each *R. sphaeroides* P solubilizing strain enhanced soil properties, including soil pH, and soluble P content, at a depth of 0–20 cm. The biofertilizers from the *R. sphaeroides* W48 and W42 gave the greatest number of solubilized concentrations of Al-P, Fe-P, and Ca-P compounds. Both PNSB biofertilizers contributed to improvements in plant height, pineapple crown height and width, D-leaf length and leaves number, number of slips, length and diameter of the peduncle, and fruit size. Total P uptake enhanced by biofertilizers from the bacterial mixture was 42.9% higher than that by the control treatment. Thus, the yield increased by 12.1% as well. Moreover, applying either the mixture of W48 and W42 or only the W48 strain reduced 25% P of RFF while maintaining pineapple yield. Both biofertilizers application of individual and dual *R. sphaeroides* W48 and W42 strains raised the Brix index and improved the red color of the fruit shell. The combination of the PNSB biofertilizers increased fruit water and vitamin C, and decreased the dark color of the fruit shell.

Data Availability

The data presented in this article are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Table 1: Effects of added liquid biofertilizers containing P solubilizing purple nonsulfur bacteria and phosphorus fertilizer levels on characteristics of acid sulfate subsoil (20–40 cm) cultivated pineapple. Table 2: Effects of added liquid biofertilizers containing P solubilizing purple nonsulfur bacteria and phosphorus fertilizer levels on the total P uptake of pineapple cultivated in acid sulfate soil. Table 3: Interactions of added liquid biofertilizers containing P solubilizing purple nonsulfur bacteria and phosphorus fertilizer levels on pineapple yield, total nitrogen uptake, total phosphorus uptake in pineapple cultivated in acid sulfate soil. Table 4: Effects of added liquid biofertilizers containing P solubilizing purple nonsulfur bacteria and phosphorus fertilizer levels on the nitrogen content in parts of pineapple cultivated in acid sulfate soil. Figure 1: Effects of added liquid biofertilizers containing P solubilizing purple nonsulfur bacteria and phosphorus fertilizer levels on the total nitrogen uptake of pineapple cultivated in acid sulfate soil. (*Supplementary Materials*)

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