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Potential of nitrogen-fixing purple non-sulfur bacteria isolated from acid sulfate soil in improvements of soil property, nutrient uptake, and yield of pineapple (*Ananas comosus* L. Merrill) under acidic stress

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Abstract

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The study was aimed to assess the potency of nitrogen (N)-fixing purple non-sulfur bacteria (NF-PNSB) on ameliorating soil quality, N uptake, and yield of pineapple in acid sulfate soil. The experiment, following a completely randomized block design, had two factors: (i) supplementation of biofertilizers containing the NF-PNSB (No applied bacteria, *Rhodobacter sphaeroides* W15, W39, and a mixture of both W15 and W39) and (ii) levels of N fertilizer [0, 50, 75, and 100% N of recommended fertilizer formula (RFF)]. Results demonstrated that supplying biofertilizers containing the mixture of both W15 and W39 increased the values of pH_{H_2O} and NH_4^+, and decreased the values of EC, Al^{3+}, and Fe^{2+} at a soil depth of 0–20 cm, in comparison with the no supplied biofertilizers, by 15.8, 39.9, 26.9, 17.8, and 52.9%, respectively. Applying biofertilizers enhanced the total N uptake by 28.9%, resulting in greater growth and a 22.9% increase in pineapple yield, in comparison with the no biofertilizers supplied treatments. Supplying the two strains W15 and W39 not only contributed to reducing 25% N of RFF but also preserved the pineapple yield, compared to the fertilizing 100% N of RFF. Both W15 and W39 are potent for use as crop yield enhancers.

Keywords: acid sulfate soil; pineapple; purple nonsulfur bacteria; *Rhodobacter sphaeroides*

Introduction

Pineapple is the third most important tropical fruit, after banana and citrus fruits. A lot of healthy nutrients can be found in pineapple (Ali et al., 2020). Simultaneously, nutrient uptakes in pineapple fruits are relatively high with 175 kg nitrogen (N) ha^{-1}, 27 kg phosphorus (P) ha^{-1}, 336 kg potassium (K) ha^{-1}, 47 kg calcium (Ca) ha^{-1}, and 27 kg magnesium (Mg) ha^{-1}. Therefore, pineapple requires a high amount of N and K nutrients to obtain high yield. In particular, N fertilizer volumes applied from 577 to 600 kg ha^{-1} correspond to a plant density of 54,400 to 75,000 plant ha^{-1} (Lobo and Paull, 2017; Sanya et al., 2020). However, highly fertilizing inorganic N to agricultural cultivation causes risks, including environmental pollution and greenhouse gas emissions (Chai et al., 2019; Woo et al., 2021). Thus, chemical N fertilizers need to be changed to other sources, in which bacteria are considered to be promising (Khuong et al., 2022a; 2022b). Nowadays, the use of N-fixing microbes on plants that are not legumes is aimed to reduce chemical fertilizers volume and improve agricultural soil properties, which is necessary and considered to be a nutrient management approach that is least likely to harm the environment (Malusà et al., 2016; Rosenblueth et al., 2018; Singh et al., 2019).

To be more specific, numerous studies isolated strains of N-fixing bacteria and applied them to non-legume crops, such as rice (Khuong et al., 2018; 2021; 2022c; Okamoto et al., 2021; Ikhwani et al., 2022), sugarcane (Li et al., 2017; Singh et al., 2020; Saranraj et al., 2021), wheat, barley (Barros et al., 2020; Vijayalakshmi et al., 2020; Plaza et al., 2022) and maize (Wen et al., 2021; Khuong et al., 2022a). Nevertheless, in acid sulfate soil (ASS), pH is low, and concentrations of aluminium (Al^{3+}) and ferrous (Fe^{2+}) are high, causing a reduction in the availability of P and other nutrients, such as Ca, K, and Mg (Hidayat & Fahmi, 2020). Strains of purple non-sulfur bacteria (PNSB) adapt well in various conditions, including ASS (Khuong et al., 2022c). These bacterial strains have the ability to immobilize toxins in ASS, including Fe^{2+}, Al^{3+}, and Mn^{2+} (Nguyen et al., 2018; Khuong et al., 2020a; 2022d). Thus, they have been widely utilized in crops under anaerobic conditions, such as submergence in rice (Khuong et al., 2017; 2018; 2022c) and under aerobic conditions, such as sesame (Kang et al., 2022), because these bacteria are capable of living well under both conditions (Nunkaew et al., 2012; Nguyen et al., 2018; Khuong et al., 2022c; 2022d). Moreover, PNSB can also fix N in ASS (Khuong et al., 2017; 2018; 2022c; Nookongbut et al., 2019) and solubilize P in saline soil (Khuong et al., 2021).

Furthermore, applications of indigenous bacteria have been proven to be highly efficient, in comparison with bacteria from other ecosystems (Pérez-Rodriguez et al., 2020), indicating the potential of applying selected PNSB strains in the form of biofertilizers on upland soil for pineapple cultivation. Therefore, the study was designed to determine the effects of N-fixing purple non-sulfur bacteria isolated from acid sulfate soil for pineapple on soil properties as well as N uptake, growth, yield, and quality of the plant.

Materials and Methods

Materials

Location

The experiment was performed in Vi Thanh city, Hau Giang province, Vietnam, from January 2021 to May 2022. Soil properties at the beginning of the crop are presented in Table 1. The N-fixing purple non-sulfur bacteria (NF-PNSB) strains utilized in the present study were *R. sphaeroides* W15 and *R. sphaeroides* W39 isolated from ASS for pineapple (our preliminary work). The fertilizers used in this study were urea (46% N), DAP (18% N, 46% P_2O_5), and potassium chloride (60% K_2O).

Soil Preparation and Plantation

Upland soil was dug at 30 cm depth. The ditch: bed ratio was 6: 4. "Queen" pineapple was gathered from slips of the previous crop. The pineapple field followed single lines whose length and width were 0.55 × 0.40 m, and the area of each plot was 5.0 × 5.0 m.

Table 1. Properties of initial soil for pineapple cultivation with nitrogen fixing bacteria

Soil property	Unit	Layer, cm	
		0 – 20	20 – 40
pH_{H2O}	–	2.60	2.49
pH_{KCl}	–	2.54	2.03
EC	mS cm^{-1}	0.64	0.98
CEC	meq 100 g^{-1}	13.2	14.6
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}	2.60	2.17
Ca^{2+}	meq Ca^{2+} 100 g^{-1}	1.44	1.11
OM	%	3.56	3.75
Total N	%	0.22	0.16
NH_4^+	mg NH_4^+ kg^{-1}	78.0	61.3
NO_3^-	mg NO_3^- kg^{-1}	21.6	38.1
Total P	%	0.015	0.021
Available P	mg kg^{-1}	5.21	3.67
Total acidity	meq H^+ 100 g^{-1}	18.2	12.4
Al^{3+}	meq Al^{3+} 100 g^{-1}	13.2	8.46
Fe^{2+}	mg kg^{-1}	153.5	118.6
Fe_2O_3	%	0.93	1.14
Fe-P	mg kg^{-1}	205.6	166.8
Al-P	mg kg^{-1}	37.5	35.9
Ca-P	mg kg^{-1}	35.3	23.9

EC: Electrical conductivity; CEC: Cation exchange capacity; OM: Organic matter; N: Nitrogen; P: Phosphorus

Experimental Design

A two-factor experiment was carried out in a completely randomized block design, with 4 replications. The first factor was the supplementation of biofertilizers, including (1) not supplying NF-PNSB, (2) supplying *R. sphaeroides* W15, (3) supplying *R. sphaeroides* W39, and (4) supplying both *R. sphaeroides* W15 and *R. sphaeroides* W39. The second factor was the application of N fertilizer levels, including (i) 0, (ii) 50, (iii) 75, and (iv) 100% N of recommended fertilizer formula (RFF). Combining the two factors together resulted in 16 treatments as follows: (i) 0% N, (ii) 50% N, (iii) 75% N, (iv) 100% N, (v) *R. sphaeroides* W15 + 0% N, (vi) *R. sphaeroides* W15 + 50% N, (vii) *R. sphaeroides* W15 + 75% N, (viii) *R. sphaeroides* W15 + 100% N, (ix) *R. sphaeroides* W39 + 0% N, (x) *R. sphaeroides* W39 + 50% N, (xi) *R. sphaeroides* W39 + 75% N, (xii) *R. sphaeroides* W39 + 100% N, (xiii) the mixture of *R. sphaeroides* W15 and W39 + 0% N, (xiv) the mixture of *R. sphaeroides* W15 and W39 + 50% N, (xv) the mixture of *R. sphaeroides* W15 and W39 + 75% N, and (xvi) the mixture of *R. sphaeroides* W15 and W39 + 100% N. The RFF was designed as 12 g N, 9 g P_2O_5, and 8 g K_2O (g plant^{-1}). Liquid biofertilizers from strains of *R. sphaeroides* were utilized at a dose of 30.0 mL per plant, with an initial density of 10^8 cells mL^{-1}) at each stage of 30, 60, 120, 180, and 240 days after planting (DAP).

Analysis of Soil and Plant Samples

Soil Analysis

Crop soil at the beginning was collected at depths of 0–20 cm and 20–40 cm following a diagonal cross at the middle and four corners by sampling probe and mixed into one sample. Samples were dried on a plastic tray and then ground by pedal and pestle before being sieved via a 2.0 mm sieve to detect soil properties, including pH_{H2O}, pH_{KCl}, EC, total N, NH_4^+, NO_3^-, total P, soluble P, and insoluble P compounds (Al-P, Fe-P, and Ca-P) to be detected. All of these characteristics were analyzed according to Sparks et al. (1996), who briefly described the following: soil pH_{H2O} and EC were measured with a soil: distilled water ratio of 1: 2.5 using a pH meter and an EC meter, while pH_{KCl} was extracted with 1.0 M KCl with a ratio of 1: 2.5 using a pH meter, and total N was determined according to the Kjeldahl method. NH_4^+ extracted with 2.0 M KCl was determined by a mixture of sodium nitroprusside, sodium salicylate, trisodium citrate dehydrate, sodium tartrate dehydrate, sodium hydroxide, and sodium hypochlorite, and measurement was at 650 nm wavelength. NO_3^- was also extracted with 2.0 M KCl, read in colors with a mixture of 0.5 M HCl, vanadium (III) chloride, sulfanilamide, and N- (1-naphthyl) ethylenediamine dihydrochloride, and measured at 540 nm wavelength. Total P concentration was analyzed by the ascorbic acid method after having been digested by a mixture of perchloric acid and nitric acid. The Bray II method was utilized for measuring the concentration of soluble P. Insoluble compounds of P were extracted with 0.5 M NH_4F, 0.1 M NaOH, and 0.25 M H_2SO_4 for each of the Al-P, Fe-P, and Ca-P compounds, respectively. All P concentrations were measured by a spectrophotometer at 880 nm wavelength.

Pineapple Growth

Plant height (cm) was measured from the ground to the tip of the highest leaf and D-leaf length (cm) from the joint with stem to its tip. The number of leaves (leaves plant^{-1}) was counted as the total number of leaves on a plant. These growth parameters were measured on 20 plants in each plot and averaged at 420 DAP.

Pineapple Fruit Yield

As regards pineapple yield (t ha^{-1}), the weight of pineapple fruit was measured in 12 m^2 of each plot and converted into tons per hectare.

Fruit Agronomic Parameters

Peduncle height (cm) was measured from the peak of the stem to the fruit, and peduncle diameter (cm) was measured at the bottom, the middle, and the top of a peduncle and averaged. Crown height (cm) was measured from the peak of the fruit to the highest tip, and crown diameter (cm) was measured in the middle of the crown. Fruit height (cm) was measured from the top to the bottom of a fruit, and fruit diameter (cm) was measured at the bottom, the middle, and the top of a fruit and averaged. These agronomic parameters were measured on 20 plants in each plot and averaged at 420 DAP.

Fruit Chemical Properties

Water content (mL fruit^{-1}) was the juice completely squeezed from the fruit and measured by a measuring cylinder. A Brix meter was utilized for measuring the degree Brix at the top, the middle, and the bottom of the fruit and averaged. Titratable acidity (TA) was measured by titration with 0.1 N NaOH until pH 8.1 and was calculated as grams of acid citric per 100 g of fresh pulp weight. The content of ascorbic acid was estimated by titration with 2,6-dichlorophenol indophenol (Horwitz, 2010). As regards colors, those at the top, the middle, and the bottom of the fruit were measured by a CR-200 color reader to detect values of L*, a*, and b*. Pulp to core ratio per shell was the weight of the pulp and core from the peeled part (1.5 cm from the shell surface) and the shell peeled at 1.5 cm.

N and P in the crown, slip, peduncle, stem removed from leaves (butt), leaves, pulp, core, and shell were digested by a method containing salicylic acid and sulfuric acid. Total N content was determined by the Kjeldahl method. The total P content was measured by the color method at 880 nm wavelength.

Regarding nutrient uptakes, determining N and P uptakes was based on dry biomass and nutrient concentrations in parts of a plant. As regards dry biomass (kg ha^{-1}) in the crown, slips, peduncle, butt, leaves, pulp, core, and shell, the samples were dried up at 70°C for 72 hours by a vacuum drying cabinet, and dry weight was measured by an electronic weight. Total N uptakes (kg ha^{-1}) were measured by dry biomass (kg ha^{-1}) x N concentrations (%) in plant parts and summed up. Total P uptakes (kg ha^{-1}) were measured by dry biomass (kg ha^{-1}) x P concentrations (%) in plant parts and summed up.

Statistical Analysis

Numeric data were processed by the Microsoft Excel software, version 2017, and ANOVA was carried out using the SPSS software, version 13.0. The Duncan post-test was utilized for comparing differences between means of treatments.

Results

Effects of Biofertilizers Containing Nitrogen-Fixing Purple Non-Sulfur Bacteria on Characteristics of Acid Sulfate Soil Cultivated Pineapple

In Table 2, the analytic result of the end-crop soil for pineapple demonstrated that, at a depth of 0–20 cm, the treatment supplied with the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 had higher values of pH_{H2O} and NH_4^+ with 2.84 and 55.1 mg NH_4^+ kg^{-1}, respectively, than those in the treatment without biofertilizers supplied, with 2.39 and 33.1 mg NH_4^+ kg^{-1}, respectively. Concentrations of Al^{3+} and Fe^{2+} in the treatment supplied with the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 were 13.4 and 75.6 mg kg^{-1}, respectively, lower than those in the treatment without biofertilizers supplied, with 16.3 and 160.4 mg kg^{-1}, respectively. Values of pH_{KCl} and total N content between treatments with and without biofertilizers supplied differed insignificantly and ranged from 2.44 to 2.55 and from 0.18 to 0.20%, respectively. Moreover, fertilizing 50, 75, and 100% N of RFF had higher values of pH_{H2O} and NH_4^+ content than those with no N fertilizer applied (Table 2).

In the treatment supplied with the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39, an insignificant difference was observed in the values of pH_{H_2O}, pH_{KCl}, total N, and NH_4^+ at a depth of 20–40 cm compared to those in the treatments supplied with the biofertilizers containing only one of the strains of *R. sphaeroides* W15 and *R. sphaeroides* W39 and in the one without biofertilizers supplied. Meanwhile, lower concentrations of EC, Al^{3+}, and Fe^{2+} in the treatment supplied with the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 were noted in comparison with those in the treatment with no biofertilizers supplied (Table S1, Supplementary Data).

Effects of Biofertilizers Containing Nitrogen-Fixing Purple Non-Sulfur Bacteria on Dry Biomass, Nitrogen Concentrations, and Uptakes in Pineapple Planted in Acid Sulfate Soil

Dry Biomass

In Table 3, biofertilizers containing only one strain of either *R. sphaeroides* W15 or *R. sphaeroides* W39 are associated with an increase in dry biomass in the crown, pulp, core, and shell of pineapple. Concurrently, supplying the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 led to higher dry biomass in the crown, pulp, core, and shell (341.0, 1432.1, 365.4, and 1235.6 kg ha^{-1}, respectively) in comparison with not supplying the biofertilizers, with 306.3, 1180.6, 275.2, and 921.4 kg ha^{-1}, respectively.

Dry biomass in stems and leaves in treatments supplied with the biofertilizers containing either *R. sphaeroides*

Table 2. Effects of added biofertilizers of N_2 fixing purple non-sulfur bacteria and nitrogen fertilizer levels on characteristics of top acid sulfate soil (0–20 cm) cultivated pineapple

Factors		pH_{H_2O}	pH_{KCl}	EC	N_{total}	NH_4^+	Al^{3+}	Fe^{2+}
			mS cm^{-1}	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	
(A) Biofertilizers (Initial cell density 1×10^8 cells mL^{-1})	No	2.39^c	2.29	1.60^a	0.19	33.1^d	16.3^a	160.4^a
	W15	2.65^b	2.35	1.35^b	0.20	44.9^c	15.8^b	71.5^b
	W39	2.76^{ab}	2.34	1.78^a	0.19	46.9^b	13.3^c	149.7^a
	W15 + W39	2.84^a	2.35	1.17^b	0.19	55.1^a	13.4^c	75.6^b
(B) Nitrogen fertilizer levels, %	0	2.49^b	2.28	1.52	0.20	37.1^d	15.1^a	132.2
	50	2.71^a	2.31	1.45	0.18	43.6^c	14.1^b	100.6
	75	2.72^a	2.35	1.43	0.19	48.2^b	14.8^a	108.8
	100	2.73^a	2.29	1.52	0.20	50.9^a	14.7^a	115.6
F (A)		*	ns	*	ns	*	*	*
F (B)		*	ns	ns	ns	*	*	ns
F (A × B)		ns	ns	*	ns	*	*	*
C.V, %		7.13	5.90	22.9	21.1	3.86	4.50	4.10

Note: Numbers in each column with the same following letters are insignificantly different from each other; ns: Not significant difference; *: Significant difference at 5%; No: No added biofertilizers; W15: Biofertilizers from *Rhodobacter sphaeroides* W15; W39: Biofertilizers from *R. sphaeroides* W39; W15 + W39: Biofertilizers from a mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39; EC: Electrical conductivity

W15 or *R. sphaeroides* W39 and in the one supplied with the mixture of both *R. sphaeroides* W15 and *R. sphaeroides* W39 was statistically the same. In detail, dry biomass in stems and leaves in the treatment supplied with the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 was 830.9 and 7382.9 kg ha^{-1}, respectively, higher than that in the treatment with no biofertilizers supplied, with 708.1 and 6583.4 kg ha^{-1}, respectively. Furthermore, the treatment supplied with the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 also presented the highest values of dry biomass in slip, sucker, and peduncle, while the treatment without biofertilizers supplied had the lowest ones (Table 3).

Table S1. Effects of added biofertilizers of N_2 fixing purple non-sulfur bacteria and N fertilizer levels on characteristics of acid sulfate subsoil (20–40 cm) planted pineapple

Factors		pH_{H_2O}	pH_{KCl}	EC	N_{total}	NH_4^+	Al^{3+}	Fe^{2+}
		–	mS cm^{-1}	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	
(A) Biofertilizers (Initial cell density 1×10^{8} cells mL^{-1})	No	2.63	2.37	2.61^{a}	0.21	52.8	161.2^{a}	178.6^{a}
	W15	2.78	2.49	1.63^{b}	0.23	52.4	71.5^{c}	129.8^{b}
	W39	2.68	2.44	2.41^{a}	0.20	52.4	143.4^{b}	109.1^{c}
	W15 + W39	2.69	2.36	1.78^{b}	0.20	52.9	71.4^{c}	101.3^{c}
(B) Nitrogen fertilizer levels, %	0	2.58^{b}	2.38	1.88^{b}	0.21	52.8	132.2^{a}	112.4^{c}
	50	2.78^{a}	2.44	1.98^{b}	0.23	52.8	97.2^{b}	133.8^{b}
	75	2.81^{a}	2.45	2.28^{a}	0.20	53.2	108.8^{b}	119.4^{c}
	100	2.61^{ab}	2.39	2.28^{a}	0.21	51.7	109.3^{b}	153.0^{a}
F (A)		ns	ns	*	ns	ns	*	*
F (B)		*	ns	*	ns	ns	*	*
F (A × B)		*	ns	*	ns	*	*	*
C.V, %		10.0	10.3	17.7	23.6	4.8	19.5	11.2

Note: Numbers in each column with the same following letter are insignificantly different from each other; ns: Not significant difference; *: Significant difference at 5%; No: No added biofertilizers; W15: Biofertilizers from *Rhodobacter sphaeroides* W15; W39: Biofertilizers from *R. sphaeroides* W39; W15 + W39: Biofertilizers from a mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39; EC: Electrical conductivity.

Table 3. Effects of added biofertilizers of N_2 fixing purple non-sulfur bacteria and nitrogen fertilizer levels on the dry biomass of parts of pineapple planting in acid sulfate soil

Factors		Crown	Pulp	Core	Shell	Slip, stem shoot and sucker	Peduncle, stem	Butt (Stem with leaves stripped off)	Leaf
	kg ha^{-1}								
(A) Biofertilizers (Initial cell density 1×10^{8} cells mL^{-1})	No	306.3^{c}	1180.6^{d}	275.2^{d}	921.4^{d}	163.0^{d}	215.7^{d}	708.1^{b}	6583.4^{b}
	W15	353.3^{a}	1370.3^{b}	333.4^{c}	1022.0^{c}	247.4^{b}	264.8^{b}	847.8^{a}	7313.6^{a}
	W39	331.5^{b}	1333.1^{c}	344.2^{b}	1157.1^{b}	237.9^{c}	250.2^{c}	833.6^{a}	7138.9^{a}
	W15 + W39	341.0^{b}	1432.1^{a}	365.4^{a}	1235.6^{a}	274.3^{a}	273.7^{a}	830.9^{a}	7382.9^{a}
(B) Nitrogen fertilizer levels (%)	0	275.9^{d}	1164.5^{d}	238.8^{d}	935.3^{d}	187.6^{d}	203.9^{c}	608.2^{d}	6264.6^{d}
	50	328.7^{c}	1297.2^{c}	328.5^{c}	1038.7^{c}	228.9^{c}	242.4^{b}	760.6^{c}	6949.4^{c}
	75	357.1^{b}	1371.2^{b}	364.4^{b}	1164.1^{b}	246.2^{b}	277.2^{a}	881.9^{b}	7395.5^{b}
	100	370.3^{a}	1483.3^{a}	386.5^{a}	1198.1^{a}	259.9^{a}	281.0^{a}	969.8^{a}	7809.3^{a}
F (A)		*	*	*	*	*	*	*	*
F (B)		*	*	*	*	*	*	*	*
F (A × B)		*	*	*	*	ns	*	*	ns
C.V, %		4.06	1.87	3.22	3.45	5.60	4.08	3.66	6.69

Note: Numbers in each column with the same following letters are insignificantly different from each other; ns: Not significant difference; *: Significant difference at 5%; No: No added biofertilizers; W15: Biofertilizers from *Rhodobacter sphaeroides* W15; W39: Biofertilizers from *R. sphaeroides* W39; W15 + W39: Biofertilizers from a mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39.

Not fertilizing N reduced dry biomass in the crown, pulp, core, shell, slips, peduncle, butt, and leaves, in comparison with fertilizing 50, 75, and 100% N of RFF. In the treatment fertilized without N fertilizers, the values of dry biomass in the crown, pulp, core, shell, slips, peduncle, butt, and leaves were 275.9, 1164.5, 238.8, 935.3, 187.6, 203.9, 608.2, and 6264.6 kg ha^{-1}, respectively, while in the treatment fertilized with 100% N of RFF, these values were 370.3, 1483.3, 386.5, 1198.1, 259.9, 281.0, 969.8, and 7809.3 kg ha^{-1}, respectively (Table 3).

N Concentrations and N Uptakes

The treatment not supplied with biofertilizers containing the PNSB had the smallest N contents in the crown, pulp, core, shell, slips, peduncle, butt, and leaves, with 1.24, 1.18, 1.02, 1.17, 1.72, 1.51, 2.14, and 2.17%, respectively, and was different at 5% significance from the treatment supplied with the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39, whereas N concentrations in plant parts were higher, with 1.38, 1.30, 1.19, 1.26, 2.12, 1.95, 2.33, and 2.41%, respectively. In treatments supplied with biofertilizers containing either *R. sphaeroides* W15 or *R. sphaeroides* W39 and with the biofertilizer containing the mixture of both *R. sphaeroides* W15 and *R. sphaeroides* W39, N concentrations in the crown, core, shell, peduncle, and butt were statistically the same, except for N contents in leaves in the two treatments supplied with biofertilizers containing either *R. sphaeroides* W15 or *R. sphaeroides* W39, which were 2.25 and 2.27%, lower than those in the treatment supplied with the biofertilizers containing the mixture of both the PNSB, with 2.41% (Table 4).

Not fertilizing N resulted in reducing N concentrations in the crown, pulp, core, shell, slips, stem shoot, peduncle, butt, and leaves, in comparison with fertilizing 50, 75, and 100% N of RFF. Fertilizing 75% N of RFF led to equivalent N concentrations in the crown, core, shell, slips, peduncle, and butt with 1.41, 1.23, 1.33, 2.01, 1.93, and 2.42% in comparison with those in the treatment fertilized with 100% N of RFF (Table 4).

The results in Figure 1 and Tables S2 and S3 (Supplementary Data) demonstrated that supplying the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 combined with fertilizing 100% N of RFF had the highest N uptake, with 311.0 kg ha^{-1}. Supplying the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 plus fertilizing 75% N of RFF presented the total N at 275.8 kg ha^{-1}, which is higher by 50.9 and 46.7 kg ha^{-1} than fertilizing 75 and 100% N not combining with the biofertilizers supplementation. Not fertilizing chemical N fertilizer but supplying biofertilizers containing either only a strain of *R. sphaeroides* W15 and *R. sphaeroides* W39 or the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 improved total N uptake by 23.3, 34.8, and 44.0 kg ha^{-1}, in comparison with the control treatment without N application.

Table 4. Effects of added biofertilizers of N_2 fixing purple non-sulfur bacteria and nitrogen fertilizer levels on the nitrogen content in parts of pineapple planting in acid sulfate soil

Factors		Crown	Flesh	Core	Shell	Slip, stem shoot and sucker	Peduncle, stem	Butt (Stem with leaves stripped off)	Leaf
	%								
(A) Biofertilizers (Initial cell density 1×10^{8} cells mL^{-1})	No	1.24^{b}	1.18^{c}	1.02^{b}	1.17^{b}	1.72^{c}	1.51^{b}	2.14^{b}	2.17^{b}
	W15	1.36^{a}	1.41^{a}	1.15^{a}	1.31^{a}	1.98^{b}	1.93^{a}	2.45^{a}	2.25^{b}
	W39	1.40^{a}	1.46^{a}	1.21^{a}	1.32^{a}	2.08^{ab}	2.00^{a}	2.54^{a}	2.27^{b}
	W15 + W39	1.38^{a}	1.30^{b}	1.19^{a}	1.26^{a}	2.12^{a}	1.95^{a}	2.33^{ab}	2.41^{a}
(B) Nitrogen fertilizer levels, %	0	1.24^{b}	1.11^{c}	0.89^{b}	1.09^{b}	1.75^{b}	1.57^{b}	2.02^{b}	1.92^{c}
	50	1.36^{a}	1.33^{b}	1.19^{a}	1.29^{a}	2.05^{a}	1.94^{a}	2.55^{a}	2.29^{b}
	75	1.41^{a}	1.39^{b}	1.23^{a}	1.33^{a}	2.01^{a}	1.93^{a}	2.42^{a}	2.41^{a}
	100	1.37^{a}	1.52^{a}	1.27^{a}	1.35^{a}	2.10^{a}	1.94^{a}	2.48^{a}	2.49^{a}
F (A)		*	*	*	*	*	*	*	*
F (B)		*	*	*	*	*	*	*	*
F (A × B)		ns	ns	ns	*	*	*	ns	ns
C.V, %		7.42	10.9	11.7	7.38	9.00	6.27	11.6	6.53

Note: Numbers in each column with the same following letters are insignificantly different from each other; ns: Not significant difference; *: Significant difference at 5%; No: No added biofertilizers; W15: Biofertilizers from *Rhodobacter sphaeroides* W15; W39: Biofertilizers from *R. sphaeroides* W39; W15 + W39: Biofertilizers from a mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39.

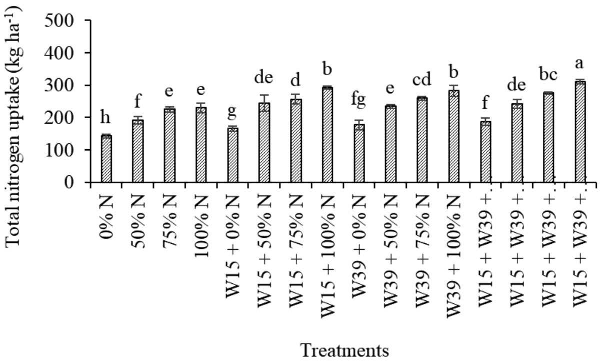

Fig. 1. Effects of added biofertilizers of N_2 fixing purple non-sulfur bacteria and nitrogen fertilizer levels on the total nitrogen uptake of pineapple planted in acid sulfate soil

Note: Columns with the same lower letters were insignificantly different. Treatments were statistically different at 5%. W15: Biofertilizers from *Rhodobacter sphaeroides* W15; W39: Biofertilizers from *R. sphaeroides* W39; W15 + W39: Biofertilizers from a mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39; N: Nitrogen.

Effects of Biofertilizers Containing Nitrogen-Fixing Purple Non-Sulfur Bacteria on Growth, Yield Components, and Yield of Pineapple Planted in Acid Sulfate Soil

Pineapple Growth

The treatments supplied with the biofertilizers containing only the strain of *R. sphaeroides* W39 and the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 had equivalent plant height, leaves number, D-leaf length, and slips number. In the treatment supplied with the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39, plant height, leaves number, D-leaf length, and slips number had higher values of 75.1 cm, 50.8 leaves, 51.3 cm, and 6.06 slips, respectively, in comparison with those in the treatment without biofertilizers supplied with 72.3 cm, 46.9 leaves, 47.4 cm, and 4.83 slips, respectively (Table 5).

Table S3. Interactions of added biofertilizers of N_2 fixing purple non-sulfur bacteria and nitrogen fertilizer levels on pineapple yield, total nitrogen uptake, total phosphorus uptake in pineapple planted in acid sulfate soil

Treatments	Pineapple fruit yield, $\mathrm{t\ ha^{-1}}$	Total nitrogen uptake, $\mathrm{kg\ ha^{-1}}$	Total phosphorus uptake, $\mathrm{kg\ ha^{-1}}$
0% N	13.1^{e}	142.2^{h}	14.5^{i}
50% N	15.5^{d}	191.2^{f}	16.3^{i}
75% N	18.2^{bc}	224.9^{e}	25.4^{ef}
100% N	18.7^{bc}	229.1^{e}	24.8^{fg}
W15 + 0% N	14.9^{d}	165.5^{g}	22.1^{gh}
W15 + 50% N	19.2^{bc}	243.7^{de}	30.8^{bcd}
W15 + 75% N	22.1^{a}	256.0^{d}	28.7^{cde}
W15 + 100% N	22.9^{a}	291.8^{b}	30.9^{bc}
W39 + 0% N	14.7^{d}	177.0^{fg}	19.5^{h}
W39 + 50% N	17.5^{c}	233.5^{e}	27.4^{def}
W39 + 75% N	21.3^{a}	259.4^{cd}	27.6^{cdef}
W39 + 100% N	21.6^{a}	282.6^{b}	35.1^{a}
W15 + W39 + 0% N	15.8^{d}	186.2^{f}	25.7^{ef}
W15 + W39 + 50% N	19.7^{b}	241.8^{de}	27.6^{cdef}
W15 + W39 + 75% N	21.6^{a}	275.8^{bc}	32.9^{ab}
W15 + W39 + 100% N	23.0^{a}	311.0^{a}	35.0^{a}
F	*	*	*
C.V, %	6.0	5.2	8.19

Note: Numbers in each column with the same following letters are insignificantly different from each other; ns: Not significant difference; *: Significant difference at 5%; No: No added biofertilizers; W15: Biofertilizers from *Rhodobacter sphaeroides* W15; W39: Biofertilizers from *R. sphaeroides* W39; W15 + W39: Biofertilizers from a mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39

Table S2. Effects of added biofertilizers of N_2 fixing purple non-sulfur bacteria and nitrogen fertilizer levels on the total N uptake of pineapple planting in acid sulfate soil

(A) Biofertilizers (Initial cell density 1×10^{8} cells $\mathrm{mL^{-1}}$)	(B) Nitrogen fertilizer levels, %				Mean (B)
	0	50	75	100	
No	142.2	191.2	224.9	229.1	196.8^{c}
W15	177.0	233.5	259.4	282.6	238.1^{b}
W39	165.5	243.7	256.0	291.8	239.2^{b}
W15 + W39	186.2	241.8	275.8	311.0	253.7^{a}
Mean (A)	167.7^{d}	227.5^{c}	254.0^{b}	278.6^{a}	
F (A)	*				
F (B)	*				
F (A*B)	*				

Note: Numbers in each column with the same following letters are insignificantly different from each other; ns: Not significant difference; *: Significant difference at 5%; No: No added biofertilizers; W15: Biofertilizers from *Rhodobacter sphaeroides* W15; W39: Biofertilizers from *R. sphaeroides* W39; W15 + W39: Biofertilizers from a mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39

Table 5. Effects of added biofertilizers of N_2 fixing purple non-sulfur bacteria and nitrogen fertilizer levels on growth and agronomic traits of pineapple planting in acid sulfate soil

Factors cm		Plant height,	Number of leaves	D-leaf length,	Number of slips	Peduncle		Fruit		Crown	
						Height	Diameter	Height	Diameter	Height	Diameter
		leaf	cm	slip							
(A) Biofertilizers (Initial cell density 1×10^{8} cells mL^{-1})	No	72.3^{b}	46.9^{c}	47.4^{b}	4.83^{b}	22.1^{b}	1.98^{c}	14.1^{d}	8.39^{c}	15.3^{b}	4.58^{b}
	W15	74.3^{a}	48.9^{b}	49.5^{a}	5.34^{b}	23.3^{a}	2.08^{bc}	14.7^{c}	8.70^{a}	15.3^{b}	4.79^{ab}
	W39	74.7^{a}	50.3^{ab}	50.0^{a}	6.00^{a}	24.0^{a}	2.14^{ab}	15.4^{b}	8.58^{b}	16.3^{a}	4.72^{b}
	W15 + W39	75.1^{a}	50.8^{a}	51.3^{a}	6.06^{a}	24.0^{a}	2.23^{a}	16.1^{a}	8.71^{a}	16.6^{a}	4.94^{a}
(B) Nitrogen fertilizer levels, %	0	71.2^{b}	47.0^{c}	47.3^{b}	4.19^{c}	22.4^{b}	1.92^{c}	14.6^{c}	8.28^{d}	14.9^{c}	4.54^{b}
	50	74.3^{a}	48.3^{bc}	49.6^{a}	5.31^{b}	23.7^{a}	2.07^{b}	14.8^{bc}	8.54^{c}	15.7^{b}	4.71^{ab}
	75	75.2^{a}	49.7^{b}	50.5^{a}	6.21^{a}	24.0^{a}	2.20^{a}	15.3^{ab}	8.69^{b}	16.3^{a}	4.91^{a}
	100	75.6^{a}	51.9^{a}	51.0^{a}	6.53^{a}	23.3^{a}	2.25^{a}	15.5^{a}	8.88^{a}	16.6^{a}	4.87^{a}
F (A)		*	*	*	*	*	*	*	*	*	*
F (B)		*	*	*	*	*	*	*	*	*	*
F (A × B)		ns	ns	ns	ns	ns	ns	ns	*	ns	ns
C.V, %		3.28	4.18	5.87	13.4	5.58	6.26	5.57	1.66	5.56	6.00

Note: Numbers in each column with the same following letters are insignificantly different from each other; ns: Not significant difference; *: Significant difference at 5%; No: No added biofertilizers; W15: Biofertilizers from *Rhodobacter sphaeroides* W15; W39: Biofertilizers from *R. sphaeroides* W39; W15 + W39: Biofertilizers from a mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39

Fertilizing 50 and 75% N of RFF presented the same plant height and D-leaf length as fertilizing 100% N of RFF. To be more specific, fertilizing 75% N of RFF resulted in higher plant height, D-leaf length, and slips number (75.2 cm, 50.5 cm, and 6.21 slips, respectively) compared to the treatment fertilized without N (71.2 cm, 47.3 cm, and 4.19 slips, respectively) (Table 5).

Yield Components

In the treatment supplied with the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39, the height and diameter of the peduncle, fruit, and crown were the highest with 24.0, 2.23, 16.1, 8.71, 16.6, and 4.94 cm, respectively, while in the treatment without biofertilizers supplied, these values were 22.1, 1.98, 14.1, 8.39, 15.3, and 4.58 cm, respectively (Table 5).

Fertilizing 75% N of RFF provided the same height and diameter of peduncle, fruit, and crown as fertilizing 100% N of RFF. At the same time, both treatments were superior to the treatment without N fertilizer applied in the height and diameter of the peduncle, fruit, and crown (Table 5).

Pineapple Fruit Yield

Fertilizing 75% N of RFF plus supplying biofertilizers containing only *R. sphaeroides* W15, only *R. sphaeroides* W39, or their mixture had pineapple yield of 22.1, 21.3, and 21.6 kg ha^{-1}, respectively, which were higher than those in the treatment fertilized with one 100% N of RFF without biofertilizers supplementations, with 3.4, 2.6, and 2.9 kg ha^{-1}, respectively (Figure 2; Table S3 (Supplementary Data)). Moreover, not fertilizing N in treatments supplied with biofertilizers containing only *R. sphaeroides* W15, only *R. sphaeroides* W39, or their mixture reached a higher yield, in comparison with the control treatment where N fertilizer and biofertilizers were not utilized, with 1.8, 1.6, and 2.7 kg ha^{-1}, respectively. This indicated that supplying biofertilizers containing only *R. sphaeroides* W15, only *R. sphaeroides* W39,

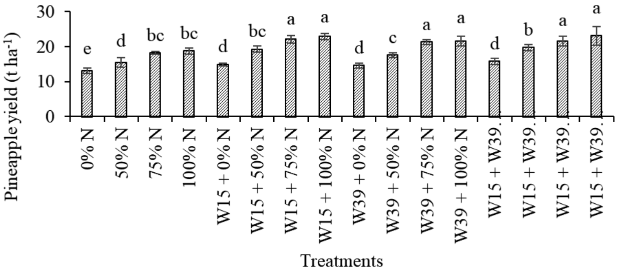

Figure 2. Effects of added biofertilizers of N_2 fixing purple non-sulfur bacteria and nitrogen fertilizer levels on the pineapple yield planted in acid sulfate soil

Note: Columns with the same lower letters were insignificantly different. Treatments were statistically different at 5%. W15: Biofertilizers from *Rhodobacter sphaeroides* W15; W39: Biofertilizers from *R. sphaeroides* W39; W15 + W39: Biofertilizers from a mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39; N: Nitrogen or their mixture helped in reducing 25% N of RFF, but still maintained pineapple yield. Moreover, both the P contents in parts of pineapple plant and the total P uptake were also improved by the addition of NF-PNSB (Figure S2; Table S4 (Supplementary Data)). The result demonstrated that biofertilizers containing the two bacterial strains of *R. sphaeroides* W15 and *R. sphaeroides* W39 did alter chemical N fertilizer and keep up pineapple yield in ASS.

Effects of Biofertilizers Containing Nitrogen-Fixing Purple Non-Sulfur Bacteria on Chemical Properties of Pineapple Planted in Acid Sulfate Soil

Supplying the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 raised water content in fruit, vitamin C content, and values of L^*, a^*, and b^* in shell colors, in comparison with no biofertilizers supplementations. In particular, supplying the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 resulted in water content of 221.2 mL fruit^{-1}, vitamin C content of 16.8 mg 100 g^{-1}, and L^*, a^*, and b^* values of 41.7, 12.0, and 16.9, respectively, while in the treatment without biofertilizers supplied, the results were 183.5 mL fruit^{-1}, 13.8 mg 100 g^{-1}, 39.3, 11.1, and 15.7, respectively. TA content, degree Brix, pH, and pulp on shell ratio were insignificantly different among treatments, with an average of 0.18 $g_{\text{citric acid}}$ 100 $g^{-1}_{\text{pulp weight}}$, 10.4, 3.64, and 1.35, respectively (Table 6).

Between levels of N fertilizer, water content and pulp on shell ratio varied significantly at 5%, while TA content, vitamin C, degree Brix, pH, and fruit colors were equivalent among treatments. In detail, the treatment fertilized with 100% N of RFF presented the highest water content and pulp on shell ratio, with 240.8 mL fruit^{-1} and 1.47, respectively, in comparison with those in the treatment without N fertilizer with 176.9 mL fruit^{-1} and 1.2 (Table 6).

Discussion

Ameliorating Soil Properties by Biofertilizers Containing Nitrogen-Fixing Purple Non-Sulfur Bacteria

Soil pH correlates with nutrients availability and diversity of soil microbial communities (Zhalnina et al., 2015; Miller, 2016; Cui et al., 2021; Wongkiew et al., 2022). According to Farhana et al. (2017), soil pH lower than 3.50 results in reduced availability of soil nutrients, including soil N and P contents. Numerous N-fixing strains denote poor acid tolerance. However, the strains of *Acetobacter pasteurianus*, *Candida glabrata*, *Rhodopseudomonas palustris* KTSSR54, *Pseudomonas bereopolis* CP-18.2, and *Pseudomonas bereopolis* CP-18.2 can tolerate low pH (Wu et al., 2018; Zheng et al., 2018; Nookongbut et al., 2019; Xa et al., 2022). Similarly, applying the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 led to an increase

Table 6. Effects of added biofertilizers of N_2 fixing purple non-sulfur bacteria and nitrogen fertilizer levels on the chemical properties of pineapple planting in acid sulfate soil

Factors		Water content	TA	Vitamin C	°Brix	pH	Flesh and core per shell	Fruit shell colours		
		mL fruit^{-1}	$g_{\text{citric acid}}$ 100 $g^{-1}_{\text{fresh weight}}$	mg 100 g^{-1}	–	–	–	L^*	a^*	b^*
(A) Biofertilizers (Initial cell density 1×10^{8} cells mL^{-1})	No	183.5^{c}	0.19	13.8^{b}	10.2	3.64	1.30	39.3^{b}	11.1^{c}	15.7^{b}
	W15	224.4^{a}	0.16	15.8^{ab}	10.6	3.64	1.31	40.5^{ab}	11.9^{ab}	17.4^{a}
	W39	212.7^{b}	0.19	15.1^{ab}	10.5	3.61	1.47	40.4^{ab}	11.2^{bc}	16.9^{a}
	W15 + W39	221.2^{ab}	0.17	16.8^{a}	10.2	3.66	1.31	41.7^{a}	12.0^{a}	16.9^{a}
(B) Nitrogen fertilizer levels, %	0	176.9^{d}	0.17	13.8	10.5	3.63	1.20^{b}	40.2	11.2	16.1
	50	195.9^{c}	0.18	15.5	10.1	3.65	1.2^{8}b	40.3	11.8	16.7
	75	228.2^{b}	0.18	15.9	10.4	3.64	1.44^{a}	40.4	11.5	16.8
	100	240.8^{a}	0.17	16.4	10.5	3.64	1.47^{a}	41.0	11.8	17.4
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	*
C.V, %		7.14	19.0	17.9	7.80	1.82	15.5	5.58	8.27	9.34

Note: Numbers in each column with the same following letters are insignificantly different from each other; ns: Not significant difference; *: Significant difference at 5%; No: No added biofertilizers; W15: Biofertilizers from *Rhodobacter sphaeroides* W15; W39: Biofertilizers from *R. sphaeroides* W39; W15 + W39: Biofertilizers from a mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39.

TA: Titrable acidity = gram of citric acid 100 g^{-1} of flesh weight, Vitamin C: Milligram of ascorbic acid kg^{-1} of flesh, °Brix: Total soluble solids in the values of pH_{H2O} in soil for pineapple, in comparison with the case not supplied with biofertilizers, resulting in an increase in NH_4^+ concentrations in soil (Table 2). This could be explained that PNSB strains have been proved the ability to enhance soil pH by releasing plant growth-promoting substances, such as siderophores, IAA and ALA (Khuong et al., 2020b). However, the soil pH was noticeably increased by the application of N fertilizers, though different levels of N fertilizers did not change the soil pH. This could have been the interaction between the bacteria and the N fertilizer, in which the bacteria produce exopolymeric substances (EPS) to form biofilm to increase the soil pH (Nookongbut et al., 2016). To be more specific, when the N fertilizer was applied to the soil, the acidity increases as in the study by Han et al. (2015), which exhibited a stress condition to the PNSB, the bacteria tended to secrete a higher amount of EPS to increase the soil pH as a way of adaptability. Therefore, the amount of EPS and other substances produced by the PNSB at different pH levels should be further investigated.

Ameliorating Dry Biomass, N Concentrations, and N Uptakes in Pineapple by Biofertilizers Containing Nitrogen-Fixing Purple Non-Sulfur Bacteria

Plants absorb N and P from various sources, such as chemical fertilizers, but N is fixed from the atmosphere, or biological N fixation, and P is immobilized by cations (Leghari et al., 2016; Huu et al., 2022). Supplying biofertilizers containing the two bacterial strains of *R. sphaeroides* W15 and *R. sphaeroides* W39 was efficient in increasing concentration of available N (NH_4^+) and available P (PO_4^{3-}) in soil, while N and P are vital nutrients contributing to increased crop yield (Sainju et al., 2019). Biofertilizers containing the PNSB can also produce siderophores, indole-3-acetic acid (IAA), and 5-aminolevulinic acid (ALA) for plants (Khuong et al., 2020b). Among them, IAA plays a major role in developing root cells, leading to improvement in minerals in plants (Sakpirom et al., 2017). In addition, Aasfar et al. (2021) have reported that N-fixing bacterial strains are able to not only produce N, but also increase P, K, and Zn availability, which contributed to an increase in N concentrations in plant parts. Because of the development of the root and the nutrients provided by the PNSB, the nutrient uptakes in plants increased in the current study. As demonstrated by Angulo et al. (2020), supplying strains of *Acidovorax facilis, Bacillus licheniformis, B. subtilis, B. oleronius, B. marinus, B. megatherium,* and *Rhodococcus rhodochrous* with chemical fertilizers increases N and P uptakes in lettuce. Based on the increases in nutrient uptakes, dry biomass in leaves, butt, peduncle, slips, shell, core, and pulp was also raised while supplying the biofertilizers containing the mixture of *R. sphaeroides* W15 and *R. sphaeroides* W39 (Table 3). Moreover, supplying the biofertilizers containing the mixed PNSB also resulted in increased N concentrations in leaves, slips, and stem shoots by 2.41, 2.12, and 1.38%, respectively, in comparison with those in the treatment without biofertilizers supplied (Table 4). A similar result was also reported by Wang et al. (2020); that is, bacterial supplements reduced 25% of chemical fertilizers and increased N, P, and K concentrations in wheat.

Ameliorating Growth, Agronomic Parameters, and Yield of Pineapple by Biofertilizers Containing Nitrogen-Fixing Purple Non-Sulfur Bacteria

Fertilizing from 50 to 100% N of RFF ameliorated plant height, D-leaf length, leaves number, slips number, height, and diameter of fruit, peduncle, and crown of pineapple, in comparison with the treatment without N fertilizer applied (Table 5) because N is one of the 17 essential elements for growth of plants (Osman, 2013; Leghari et al., 2016). Nevertheless, ASS possesses low pH and high concentrations of Al^{3+} and Fe^{2+}, posing a negative effect on the availability of P, Ca, K, and Mg (Hidayat & Fahmi, 2020). However, biofertilizers containing the two bacterial strains of *R. sphaeroides* W15 and *R. sphaeroides* W39 still improved pineapple development in ASS via the results in plant height, D-leaf length, leaves number, fruit height, and fruit diameter (Table 5), which indicated that the PNSB can perform well in low pH soil. This was because the PNSB can secrete EPS reducing Fe^{2+}, Al^{3+}, and Mn^{2+} toxins (Nguyen et al., 2018; Khuong et al., 2022c). In addition, PNSB is also able to provide ALA in low pH conditions (Khuong et al., 2020b), which is a precursor to synthesize chlorophyll, vitamin B_{12}, antioxidants, and other metabolites, helping pineapple in overcoming biological and nonbiological stresses during its development and increasing yield (Wongkantrakorn et al., 2009; Nunkaew et al., 2014; Sakpirom et al., 2017). Moreover, N-fixing bacterial strains can also synthesize plant growth-promoting substances, enhancing the availability of P, K, and Zn for plants (Aasfar et al., 2021). Because of this and the enhancement in dry biomass and nutrient uptakes mentioned above, the parameters of growth elevated. For instance, supplying biofertilizers containing the mixed PNSB increased all of the agronomic traits, including plant height, number of leaves, D-leaf length, Number of slips, and size of peduncle, fruit and crown (Table 5). Furthermore, according to Timmusk et al. (2017) and Trivedi et al. (2017), using microorganisms and their metabolites increased nutrient uptakes and pest control and reduced the effects of stress on plants, thus, increasing crop yield. Consequently, the growth and yield of pineapple was remarkably improved by the supplementation of the two bacterial strains of *R. sphaeroides* W15 and *R. sphaeroides* W39 (Figure 2).

Ameliorating Pineapple Fruit Quality by Biofertilizers Containing Nitrogen-Fixing Purple Non-Sulfur Bacteria (Table S4, Figure S1)

In accordance to Py et al. (1987) and Bonomo et al. (2020), where increasing N fertilizer does not remarkably change fruit carbohydrate contents and pH in pineapple pulp, in the current study, an insignificant difference in Brix index and fruit pH was observed among treatments fertilized with 0, 50, 75, and 100% N of RFF (Table 6). However, the supplied PNSB did not change these parameters either. Moreover, the PNSB mixture increased the vitamin C content and water content in pineapple fruits. This can be explained that PNSB released the plant growth promoting substances to stimulate the growth parameters, resulting in higher volume of water content. This was in accordance with the study by Huu et al. (2022). Results in Table 6 demonstrated that supplying biofertilizers containing the two bacterial strains of *R. sphaeroides* W15 and *R. sphaeroides* W39 resulted in higher values of a^* and b^* (12.0 and 16.9, respectively) compared to the treatment without biofertilizers supplied. In the meantime, as reported by Itle & Kabelka (2009), a^* and b^* values denote mature levels of fruits; that is, low a^* and b^* represent green fruits, and high a^* and b^* indicate changes in colors of fruit shell due to chlorophyll reduction and synthesis of carotenoid (Guzman et al., 2010; Christ & Hörtensteiner, 2014). This could reveal that both *R. sphaeroides* W15 and *R. sphaeroides* W39 stimulated the fruit ripening. This was one of the most novel findings of the study.

Conclusions

The application of biofertilizers containing the two bacterial strains of *R. sphaeroides* W15 and *R. sphaeroides* W39 increased pH_{H2O} and NH_4^+, decreased EC, Al^{3+}, and Fe^{2+} in the soil at a depth of 0–20 cm, and enhanced N content, biomass, and pineapple yield by 22.9% in comparison with the no biofertilizers supplied case.

Fertilizing 75% N of RFF plus supplying biofertilizers containing the two bacterial strains of *R. sphaeroides* W15 and *R. sphaeroides* W39 provided equivalent plant height, slips number, fruit height, and pineapple yield to the treatment fertilized with 100% N of RFF. Both *R. sphaeroides* W15 and *R. sphaeroides* W39 strains have been proven to be able to not only alter 25% chemical N fertilizer but also preserve the yield and fruit quality of pineapple.

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