

## Research

# Selecting Phosphorus-Solubilizing Strains of Purple Nonsulfur Bacteria Isolated From Pineapple Cultivated Acid Sulfate Soils

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## ABSTRACT

The presence of acid sulfate soils is such an obstacle for pineapple cultivation in Vietnam due to their low pH, high toxicity and poor nutrient availability, especially phosphorus (P), which is immobilized by cations in the soils. Therefore, the study occurred to select purple nonsulfur bacteria (PNSB) strains that can solubilize P under toxic and acidic conditions. There were 33 strains that can tolerate the acidic condition, and they were selected and tested for viability and P solubilization under conditions containing  $Al^{3+}$ ,  $Fe^{2+}$ , and  $Mn^{2+}$  toxins. Four strains, including W15, W39, W42 and W48 suffered from growth inhibition by  $Al^{3+}$ ,  $Fe^{2+}$  and  $Mn^{2+}$  less than the other strains under both microaerobic light and aerobic dark conditions (ML and AD conditions). In addition, there were four strains (W15, W25, W42 and W48) solubilizing Al-P well ( $21.4\text{--}25.2\text{ mg L}^{-1}$ ), two strains (W23 and W42) solubilizing Fe-P well ( $15.9\text{--}17.3\text{ mg L}^{-1}$ ), and two strains (W17 and W42) solubilizing Ca-P well ( $23.0\text{--}36.4\text{ mg L}^{-1}$ ) under both ML and AD conditions. Ultimately, there were five strains selected (W17, W23, W25, W42 and W48) and identified as *Rhodopseudomonas palustris* strain W17 and W23, *Cereibacter sphaeroides* strain W23, W42 and W48 based on the 16S rRNA technique. The selected strains also produced ALA, EPS and siderophores at  $1.31\text{--}2.19\text{ mg L}^{-1}$ ,  $0.78\text{--}1.89\text{ mg L}^{-1}$ , and  $16.2\text{--}55.6\%$ , respectively. Therefore, these strains were promising in providing nutrients for pineapples in the form of biofertilizer.

**Key words:** Acid sulfate soil, phosphorus, pineapple, purple nonsulfur bacteria

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## INTRODUCTION

Nowadays, the productivity of crops around the world has been severely affected by the acid sulfate soils, which are mainly found in coastal areas (Lee & Dang, 2019; Hulisz *et al.*, 2020; Loc *et al.*, 2021). This soil type accounts for 30% of the total soil area and over 50% of farming soil in the world (Fanning, 2010; Hulisz *et al.*, 2020). In particular, the acid sulfate soil is mainly located in Southeast Asia, West Africa, South America, East Australia, and Europe (Andriessse & van Mensvoort, 2002). In Vietnam, the total area of acid sulfate soil is approximately 1.6 million ha (Tri & van Mensvoort, 2004), and is found mainly in the Mekong Delta. According to Kar *et al.* (2021),  $H^+$  is toxic in acid-sulfate soil by lowering soil pH and reducing the availability of soil nutrients (Barrow & Hartemink, 2023). At pH 5.0-7.0, microbes mineralize N and P, but at pH 3.5-4.0, plants are damaged by  $H^+$  and more importantly by  $Al^{3+}$  and  $Fe^{2+}$ , because when pH is below 4.0, Fe and Al present under dissolved forms, and low pH inhibits processes of hydration, sulfurization, ammoniation (Agegnehu *et al.*, 2021; Gondal *et al.*, 2021; Lopes *et al.*, 2021).

In addition, the inorganic P source in acid sulfate soils is usually immobilized by Al, Ca, and Fe and formed into  $AlPO_4 \cdot 2H_2O$  (Al-P),  $Ca_3(PO_4)_2$  (Ca-P), and  $FePO_4 \cdot 2H_2O$  (Fe-P) compounds, these three forms cannot be absorbed by plants (Loc *et al.*, 2021; Gerónimo & Aparicio, 2022), while

P in the forms of  $\text{KH}_2\text{PO}_4$ ,  $\text{Na}_2\text{HPO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{Ca}(\text{HPO}_4)_2$  and  $\text{Mg}(\text{HPO}_4)_2$  is more soluble, and can be taken up by plants (Rose & Wissuwa, 2012; Chen *et al.*, 2022). The interaction between Fe and P generates unavailable soil nutrients on the root surface because Fe gathers there and prevents the movement of P into roots (Peng *et al.*, 2021; Chen *et al.*, 2022). Lime fertilization is a well-known approach to increasing pH in acid-sulfate soils (Sadiq & Babagana, 2012; Fu *et al.*, 2022).

Lime precipitates active Al and Fe in soils, and forms insoluble compounds such as aluminum hydroxide and iron hydroxide, leading to a reduction in their toxicity in soils (Holland *et al.*, 2018; Fu *et al.*, 2022). However, Sade *et al.* (2016) claimed that applying lime is uncommon in some countries where the lime source is insufficient; thereby, the economic efficiency is low. In addition,  $\text{Mn}^{2+}$  has been proven to reduce plant biomass when applied at  $1,000 \text{ mg kg}^{-1}$  (Dziwornu *et al.*, 2018). At the same time, the activity of microorganisms may change ions in the soil solution, which accelerates the insoluble P metabolism, and creates orthophosphate, or inorganic P metabolism (Siddique *et al.*, 2021; Alyousif, 2022). By metabolism, microorganisms directly affect the solubilizing and mineralizing processes of organic and inorganic P compounds (Dhuldhaj & Malik, 2022). Plants use organic P because of the P hydrolysis of extracellular phosphatase produced by their own or by microorganisms (Wan *et al.*, 2021; Alyousif, 2022).

Among beneficial microorganisms, purple nonsulfur bacteria (PNSB) grow well under different types of environments, e.g., anaerobic or aerobic conditions (Khuong *et al.*, 2022; Kang *et al.*, 2022), or even in conditions containing toxins, such as  $\text{Al}^{3+}$ ,  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  (Nguyen *et al.*, 2018; Khuong *et al.*, 2020a; 2023a). In the PNSB group, some strains can tolerate high concentrations of  $\text{Al}^{3+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Mn}^{2+}$  (Khuong *et al.*, 2017; 2020), and have been applied to reduce  $\text{Al}^{3+}$ ,  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  accumulation in rice grains (Khuong *et al.*, 2018; 2020a, 2020b). Moreover, the PNSB can solubilize P by producing organic acids, acid phosphatase, and phytase (Lo *et al.*, 2018; Khuong *et al.*, 2018; Wei *et al.*, 2023). Therefore, the study was conducted to determine which PNSB strains can solubilize P under toxic and acidic conditions.

## MATERIALS AND METHODS

### Materials

The Basic Isolation Medium (BIM) was used to isolate PNSB and was made of:  $1.0 \text{ g } (\text{NH}_4)_2\text{SO}_4$ ,  $0.5 \text{ g } \text{K}_2\text{HPO}_4$ ,  $2.0 \text{ g } \text{NaCl}$ ,  $5.0 \text{ g } \text{NaHCO}_3$ ,  $0.2 \text{ g } \text{MgSO}_4$ ,  $1.5 \text{ g } \text{yeast extract}$ ,  $1.5 \text{ g } \text{glycerol}$ , and  $0.03 \text{ g } \text{L-cysteine}$  and distilled water added until  $1.0 \text{ L}$  in total (Brown, 2013). The pH was adjusted to 6.8 (For the solid BIM,  $15.0 \text{ g } \text{agar/L}$  was added and autoclaved at  $121^\circ\text{C}$  for 30 min with  $1.0 \text{ atm}$  pressure).

Bacteria source: All 120 PNSB strains were isolated from 40 soil samples, 40 ditch slurry samples, and 40 ditch water samples in different pineapple farms (Huu *et al.*, 2024).

### Methods for determining PNSB strains that can solubilize insoluble P compounds

Selecting bacteria that adapt to conditions contaminated with  $\text{Al}^{3+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Mn}^{2+}$  ions:

Under acidic conditions contaminated with  $\text{Al}^{3+}$ : Bacteria were assessed in BIM (pH 4.0), with 10% of bacteria culture ( $\text{OD}_{660} = 0.5$ ) and 90% of the medium in a  $20.0 \text{ mL}$  tube for the ML condition ( $3,000 \text{ lux}$ ), and in a  $50.0 \text{ mL}$  tube under AD condition ( $150 \text{ rpm}$ ,  $30^\circ\text{C}$ ). After 48 hr of culture,  $\text{Al}^{3+}$  was supplied at the concentration of  $60.0 \text{ mg } \text{Al}^{3+} \text{ L}^{-1}$  from an  $\text{Al}^{3+} 2,500 \text{ mg } \text{L}^{-1}$  stock solution prepared from the  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  compound (Khuong *et al.*, 2017). Bacteria were raised in both ML and AD conditions as the above.

Under acidic conditions contaminated with  $\text{Fe}^{2+}$ : The procedure was similar to that for the  $\text{Al}^{3+}$  test. However,  $\text{Al}^{3+}$  was replaced by  $2.500 \text{ mg } \text{L}^{-1} \text{Fe}^{2+}$  prepared from the  $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$  compound. Bacteria were cultured as the above, but the final concentration of the bacteria and BIM was  $250.0 \text{ mg } \text{Fe}^{2+} \text{ L}^{-1}$  (Khuong *et al.*, 2017).

Under acidic conditions contaminated with  $\text{Mn}^{2+}$ : Similar to the two conditions contaminated with  $\text{Al}^{3+}$  and  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$  was applied at the concentration of  $1,100 \text{ mg } \text{L}^{-1}$  from the prepared  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  ( $10,000 \text{ mg } \text{L}^{-1}$ ) compound. The bacteria were then cultured as the above (Khuong *et al.*, 2022).

In these cases, bacteria were grown for 48 hr to measure  $\text{OD}_{660}$ , and any strains obtaining an OD over 0.5 were selected for further evaluation.

### Selecting bacteria that solubilize P

The P source in the BIM ( $\text{K}_2\text{HPO}_4$ ) was replaced by insoluble P compounds, such as  $\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$ , and  $\text{Ca}_3(\text{PO}_4)_2$  whose concentrations were 0.3, 1.0, and  $1.0 \text{ g } \text{L}^{-1}$ , respectively. The bacteria and BIM were mixed with a ratio of 10%-to-90% under pH 4.5. Then, the mixture was incubated under

ML condition illuminated at 3,000 lux. and under AD condition shaken at 150 rpm at 30°C. After 72 hr of culture, 1.0 mL of bacteria culture was centrifuged at 3,000 rpm for 15 min, and the solubilized P content was measured by the ascorbic acid method on the spectrophotometer at the 880.0 nm wavelength (Murphy & Riley, 1962).

### Methods for selecting bacteria that produce plant growth-promoting substances (PGPS)

#### *Selecting bacteria that produce ALA ( $\delta$ -aminolevulinic acid):*

First of all, 10% of bacteria culture that had been cultured in BIM for 48 hr and adjusted to  $OD_{660} = 0.5$  was mixed with 90% of BIM (pH 4.0) in a 20.0 mL tube under ML condition (3,000 lux) and in a 50.0 mL tube under AD condition (150 rpm, 30°C). After 72 hr of culture, the bacteria culture was centrifuged at 3,000 rpm for 15 min. Then, 1.0 mL of the centrifuged culture was mixed with 2.0 mL of sodium acetate 1.0 M buffer and 50.0 mL of acetylacetone. The mixture was boiled for 15 min and finally added with 3.5 mL of Ehrlich indicator, steadily shaken. The ALA content was determined by the colorimetric method at the 553.0 nm wavelength (Burnham, 1970).

#### *Selecting bacteria that produce EPS (exopolymeric substances):*

The bacteria culture was prepared as in the ALA test. After 72 hr of culture, the culture was centrifuged at 3,000 rpm for 15 min and collected for the supernatant, which was mixed with cold alcohol (4°C) at the ratio of 1-to-2.2, and incubated at -20°C to precipitate EPS. Subsequently, after 24 hr of incubation, the solution was centrifuged at 3,000 rpm for 15 min to collect the precipitated EPS. The weight of the EPS was determined according to Ferreira *et al.* (2017).

#### *Selecting bacteria that produce siderophores:*

Bacteria were cultured as the above. However, the medium was supplied with succinate (1.0 g L<sup>-1</sup>) and FeCl<sub>3</sub>·6H<sub>2</sub>O (0.5 μM) known as the precursor to produce siderophores. After 96 of culture, 2.0 mL of culture was centrifuged at 10,000 for 5 min. 0.5 mL of the centrifuge solution was mixed with 0.5 mL of indicators, and measured at the 630 nm wavelength by a spectrophotometer. Uninoculated medium broth with CAS was served as a reference. The siderophores production was detected via a reduction in blue color that was considered as percent siderophores units (SU);

$$SU (\%) = \frac{Ar - As}{Ar} * 100$$

Ar: Absorbance of reference at 630 nm, As: Absorbance of the supernatant at 630 nm. The indicators consisted of 6 mL of HDTMA 10 mM, 1.5 mL of FeCl<sub>3</sub> 1 mM (in HCl 10 mM), and 7.5 mL of CAS 2 mM (gently pour and shake). 4.307 g of Anhydrous piperazine was added to the mixture and adjusted to pH = 5.6. Then, distilled water was added, shaken, and added with 0.1017 g of 5-sulfosalicylic acid, then added with distilled water until 100 mL in total.

### Methods of identifying the selected PNSB

Selected bacteria were cultured for 48 hr in BIM. Subsequently, 2.0 mL of the colony was centrifuged at 10,000 rpm for 5 min to collect cells to extract DNA by the Genomic DNA Prep Kit (BioFACT™), according to the manufacturer's instructions. The DNA concentration and integrity were checked on 1.0% w/v agarose gel electrophoresis. DNA products were amplified at the 16S rRNA coding genes by the Polymerase Chain Reaction (PCR) techniques with the forward primer 8 F (5'-AGA GTT TGA TCC TGG CTC AG-3') and the 16S Reverse Primer - 1492 R (5'- GGT TAC CTT GTT ACG ACT T-3') (Suzuki *et al.*, 2003). The PCR followed the description of iProof™ High-Fidelity PCR Kit - Bio-Rad (BioRad, Hercules, CA) by T100™ thermocycler (BioRad). The PCR was purified by the TIANquick Midi Purification Kit (Tiangen Biotech Ltd., Beijing, China) according to the manufacturer's instructions. The amplicons were checked for integrity on 1.0% w/v agarose gel electrophoresis. The purified PCR products were sequenced by the automatic sequencing machine at MacroGen DNA Sequencing Service (MacroGen, Seoul, Korea). The sequencing results were analyzed by the BioEdit 7.0.5.3 software, and the ChromasPro version 1.7 software (<http://technelysium.com.au/wp/chromaspro>). The sequences were compared to the available ones in the Genbank by the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI).

**Statistical analysis**

All experiments were conducted in three replications. The SPSS 13.0 software was used for variance analysis (ANOVA), and the Duncan test was used to compare different strains.

**RESULTS**

**Selection for purple nonsulfur bacteria under conditions contaminated with Al<sup>3+</sup>, Fe<sup>2+</sup>, and Mn<sup>2+</sup>**

*Selection for bacteria that were viable under the Al<sup>3+</sup> condition*

All 33 strains were able to survive under Al<sup>3+</sup> toxicity at 80 mg L<sup>-1</sup>. However, the inhibition by the Al<sup>3+</sup> varied among strains. Under the ML condition, there were 4 strains greatly inhibited by Al<sup>3+</sup> (above 80%), and 7 less inhibited by Al<sup>3+</sup> (below 30%). The Al<sup>3+</sup> toxicity reduced the growth of W15, W39, W42, and W48 strains the least at 20.0%, 20.6%, 23.2%, and 22.7% under ML conditions, while under the AD condition, the inhibition values were 13.6%, 18.7%, 22.1%, and 19.2%, respectively (Table 1).

**Table 1.** The toxicity of Al<sup>3+</sup>, Fe<sup>2+</sup>, and Mn<sup>2+</sup> restricts the growth of purple nonsulfur bacteria under microaerobic light and aerobic dark conditions

Strain	% inhibition by Al <sup>3+</sup>		% inhibition by Fe <sup>2+</sup>		% inhibition by Mn <sup>2+</sup>	
	Microaerobic light	Aerobic dark	Microaerobic light	Aerobic dark	Microaerobic light	Aerobic dark
W01	79.1 <sup>b</sup>	60.1 <sup>ab</sup>	32.4 <sup>f-h</sup>	43.3 <sup>i-l</sup>	32.2 <sup>gh</sup>	26.1 <sup>gh</sup>
W03	41.1 <sup>e-i</sup>	31.3 <sup>h-m</sup>	81.9 <sup>a</sup>	59.4 <sup>d-g</sup>	36.7 <sup>b-f</sup>	33.8 <sup>bc</sup>
W04	26.0 <sup>h-l</sup>	39.8 <sup>e-j</sup>	79.1 <sup>a</sup>	60.8 <sup>c-f</sup>	32.7 <sup>gh</sup>	17.7 <sup>lm</sup>
W07	41.6 <sup>e-i</sup>	26.4 <sup>k-n</sup>	57.3 <sup>bc</sup>	30.9 <sup>n-p</sup>	35.9 <sup>b-g</sup>	31.2 <sup>b-f</sup>
W09	46.1 <sup>de</sup>	56.5 <sup>a-c</sup>	81.8 <sup>a</sup>	82.1 <sup>a</sup>	33.9 <sup>d-g</sup>	20.5 <sup>hl</sup>
W10	38.7 <sup>h-i</sup>	49.4 <sup>b-e</sup>	33.8 <sup>c-g</sup>	70.1 <sup>bc</sup>	43.6 <sup>a</sup>	37.2 <sup>a</sup>
W11	83.3 <sup>ab</sup>	46.8 <sup>c-f</sup>	81.8 <sup>a</sup>	77.9 <sup>ab</sup>	39.6 <sup>b</sup>	27.8 <sup>efg</sup>
W13	27.1 <sup>k</sup>	60.7 <sup>ab</sup>	31.9 <sup>h</sup>	46.4 <sup>h-k</sup>	38.6 <sup>bc</sup>	33.0 <sup>b</sup>
W15	20.0 <sup>m</sup>	13.6 <sup>o</sup>	13.5 <sup>j</sup>	16.0 <sup>q</sup>	21.9 <sup>kl</sup>	10.8 <sup>o</sup>
W16	41.8 <sup>e-i</sup>	42.9 <sup>d-h</sup>	45.6 <sup>c-e</sup>	37.1 <sup>k-n</sup>	37.9 <sup>bcd</sup>	32.3 <sup>b</sup>
W17	57.3 <sup>c</sup>	52.2 <sup>b-d</sup>	61.9 <sup>b</sup>	7.02 <sup>r</sup>	33.7 <sup>efg</sup>	27.6 <sup>fg</sup>
W18	43.7 <sup>d-i</sup>	42.5 <sup>d-h</sup>	18.2 <sup>j</sup>	21.9 <sup>pq</sup>	35.7 <sup>b-g</sup>	25.9 <sup>gh</sup>
W19	41.6 <sup>e-i</sup>	66.6 <sup>a</sup>	47.2 <sup>cd</sup>	57.9 <sup>e-g</sup>	32.6 <sup>fgh</sup>	31.4 <sup>b-e</sup>
W20	42.8 <sup>d-i</sup>	29.6 <sup>i-n</sup>	79.1 <sup>a</sup>	69.4 <sup>bc</sup>	32.4 <sup>gh</sup>	21.8 <sup>ijk</sup>
W22	41.0 <sup>e-i</sup>	28.6 <sup>i-n</sup>	50.0 <sup>bc</sup>	54.7 <sup>h</sup>	34.0 <sup>d-g</sup>	27.0 <sup>g</sup>
W23	48.1 <sup>d</sup>	40.0 <sup>e-i</sup>	85.1 <sup>a</sup>	65.4 <sup>c-e</sup>	37.3 <sup>b-e</sup>	31.6 <sup>bcd</sup>
W24	58.7 <sup>c</sup>	28.5 <sup>i-n</sup>	22.4 <sup>ij</sup>	69.6 <sup>bc</sup>	34.9 <sup>c-g</sup>	23.2 <sup>hi</sup>
W25	45.4 <sup>d-g</sup>	28.3 <sup>i-n</sup>	30.4 <sup>fi</sup>	52.5 <sup>fi</sup>	36.7 <sup>b-f</sup>	28.0 <sup>d-g</sup>
W26	44.9 <sup>d-h</sup>	24.5 <sup>k-o</sup>	53.3 <sup>bc</sup>	49.9 <sup>ij</sup>	34.3 <sup>d-g</sup>	30.8 <sup>b-f</sup>
W27	39.1 <sup>g-i</sup>	32.4 <sup>h-l</sup>	59.5 <sup>b</sup>	67.5 <sup>c-e</sup>	24.1 <sup>d-g</sup>	28.3 <sup>c-g</sup>
W28	29.6 <sup>j</sup>	28.1 <sup>i-n</sup>	28.5 <sup>fi</sup>	50.5 <sup>gj</sup>	33.0 <sup>fg</sup>	19.2 <sup>ijk</sup>
W29	78.2 <sup>b</sup>	46.9 <sup>c-f</sup>	30.3 <sup>fi</sup>	25.8 <sup>o-q</sup>	24.1 <sup>jk</sup>	22.3 <sup>ij</sup>
W30	45.6 <sup>d-f</sup>	27.7 <sup>j-n</sup>	37.6 <sup>d-f</sup>	37.2 <sup>k-n</sup>	28.9 <sup>hi</sup>	18.3 <sup>kl</sup>
W33	37.5 <sup>i</sup>	65.4 <sup>a</sup>	30.9 <sup>fi</sup>	33.4 <sup>m-o</sup>	31.8 <sup>gh</sup>	22.6 <sup>hij</sup>
W34	85.3 <sup>a</sup>	29.9 <sup>i-n</sup>	32.1 <sup>h</sup>	68.2 <sup>cd</sup>	27.4 <sup>ij</sup>	18.9 <sup>kl</sup>
W35	81.8 <sup>ab</sup>	56.0 <sup>a-c</sup>	36.5 <sup>d-f</sup>	42.6 <sup>i-m</sup>	24.4 <sup>kl</sup>	14.3 <sup>mno</sup>
W36	39.5 <sup>fi</sup>	31.7 <sup>h-l</sup>	34.2 <sup>e-g</sup>	35.2 <sup>o</sup>	35.1 <sup>c-g</sup>	18.9 <sup>kl</sup>
W39	20.6 <sup>lm</sup>	18.7 <sup>n-o</sup>	13.5 <sup>j</sup>	22.0 <sup>pq</sup>	15.8 <sup>m</sup>	11.8 <sup>no</sup>
W41	79.7 <sup>ab</sup>	33.7 <sup>g-l</sup>	37.7 <sup>d-f</sup>	33.0 <sup>m-o</sup>	22.4 <sup>kl</sup>	14.7 <sup>mn</sup>
W42	23.2 <sup>k-m</sup>	22.1 <sup>l-o</sup>	13.0 <sup>j</sup>	22.4 <sup>pq</sup>	15.9 <sup>m</sup>	13.9 <sup>no</sup>
W48	22.7 <sup>k-m</sup>	19.2 <sup>m-o</sup>	19.7 <sup>h-j</sup>	22.1 <sup>pq</sup>	11.1 <sup>n</sup>	11.5 <sup>no</sup>
W55	30.5 <sup>j</sup>	35.2 <sup>f-k</sup>	28.3 <sup>fi</sup>	31.1 <sup>n-p</sup>	21.2 <sup>kl</sup>	13.8 <sup>no</sup>
W58	84.0 <sup>ab</sup>	45.2 <sup>c-g</sup>	73.3 <sup>a</sup>	67.1 <sup>c-e</sup>	19.9 <sup>j</sup>	14.8 <sup>mn</sup>
Level of significance	*	*	*	*	*	*
CV (%)	7.02	16.3	15.2	11.5	6.98	8.74

Note: W: Water. Different letters following numbers show significant differences via the Duncan test at P<0.05.

*Selection for bacteria that were viable under Fe<sup>2+</sup> condition*

Under the condition contaminated with Fe<sup>2+</sup>, the 33 strains survived under Fe<sup>2+</sup> content at 250 mg L<sup>-1</sup>. Under ML condition, there were 4 PNSB strains with their growth limited by Fe<sup>2+</sup> greater than 80%,

and 4 strains (W15, W39, W42 & W48) less affected below 20% (13.5%, 13.5%, 13.0% & 19.7%, respectively). On the other hand, under the AD condition, the Fe<sup>2+</sup> toxicity put a growth limitation on these four strains at 16.0%, 22.0%, 22.4%, and 22.1%, respectively (Table 1).

#### Selection for bacteria that were viable under Mn<sup>2+</sup> condition

All 33 strains can live under the presence of 1.100 mg L<sup>-1</sup> Mn<sup>2+</sup> under both ML and AD conditions. The Mn<sup>2+</sup> toxicity restricted the growth of bacteria under the ML condition (11.1-43.6%) and under the AD condition (10.8-37.2%). Therein, the Mn<sup>2+</sup> toxicity slightly inhibited the growth of W39, W42, and W48 (below 20%) under both conditions, with 11.1-15.9 and 11.5-13.9%, respectively (Table 1).

After the viability test under toxic conditions, out of 33 strains, 25 strains with low inhibition values were selected for further experiment and were listed in Table 2.

**Table 2.** Al-P, Fe-P, and Ca-P solubilizing capacity of purple nonsulfur bacteria under microaerobic light and aerobic dark conditions

Strain	Al-P (mg L <sup>-1</sup> )		Fe-P (mg L <sup>-1</sup> )		Ca-P (mg L <sup>-1</sup> )	
	Microaerobic light	Aerobic dark	Microaerobic light	Aerobic dark	Microaerobic light	Aerobic dark
W01	14.5 <sup>c</sup>	22.5 <sup>d-g</sup>	9.51 <sup>c</sup>	16.3 <sup>ab</sup>	20.6 <sup>cde</sup>	19.2 <sup>k</sup>
W03	14.1 <sup>c</sup>	21.1 <sup>ikl</sup>	8.56 <sup>cde</sup>	9.51 <sup>c</sup>	18.2 <sup>gh</sup>	29.0 <sup>d-h</sup>
W04	14.3 <sup>c</sup>	21.5 <sup>g-k</sup>	8.22 <sup>de</sup>	14.9 <sup>ab</sup>	18.5 <sup>gh</sup>	22.0 <sup>ik</sup>
W07	14.3 <sup>c</sup>	21.1 <sup>kl</sup>	9.43 <sup>c</sup>	16.0 <sup>ab</sup>	21.7 <sup>bc</sup>	32.0 <sup>a-f</sup>
W09	14.4 <sup>c</sup>	22.8 <sup>b-f</sup>	8.27 <sup>de</sup>	14.7 <sup>b</sup>	17.8 <sup>ghi</sup>	30.3 <sup>b-f</sup>
W10	14.8 <sup>c</sup>	23.8 <sup>b</sup>	9.00 <sup>cde</sup>	17.2 <sup>a</sup>	22.1 <sup>ab</sup>	27.8 <sup>e-h</sup>
W11	14.0 <sup>c</sup>	21.4 <sup>h-l</sup>	8.95 <sup>cde</sup>	15.9 <sup>ab</sup>	20.4 <sup>c-f</sup>	31.2 <sup>a-f</sup>
W13	13.6 <sup>c</sup>	21.0 <sup>ijkl</sup>	9.68 <sup>c</sup>	16.0 <sup>ab</sup>	16.5 <sup>k</sup>	23.7 <sup>h-k</sup>
W15	22.4 <sup>a</sup>	22.7 <sup>b-f</sup>	8.61 <sup>cde</sup>	16.5 <sup>ab</sup>	20.4 <sup>c-f</sup>	30.6 <sup>b-f</sup>
W16	16.0 <sup>bc</sup>	22.9 <sup>b-f</sup>	9.43 <sup>c</sup>	16.0 <sup>ab</sup>	19.9 <sup>ef</sup>	36.4 <sup>a</sup>
W17	14.2 <sup>c</sup>	23.4 <sup>bcd</sup>	8.85 <sup>cde</sup>	16.8 <sup>ab</sup>	23.0 <sup>a</sup>	30.2 <sup>b-f</sup>
W18	14.4 <sup>c</sup>	23.4 <sup>bcd</sup>	9.14 <sup>cde</sup>	15.9 <sup>ab</sup>	13.3 <sup>l</sup>	33.5 <sup>a-e</sup>
W19	14.3 <sup>c</sup>	22.8 <sup>b-f</sup>	9.00 <sup>cde</sup>	15.9 <sup>ab</sup>	18.3 <sup>gh</sup>	27.0 <sup>fi</sup>
W20	22.4 <sup>a</sup>	22.1 <sup>fi</sup>	9.29 <sup>cd</sup>	15.1 <sup>ab</sup>	21.3 <sup>bcd</sup>	34.1 <sup>a-d</sup>
W22	14.4 <sup>c</sup>	19.2 <sup>m</sup>	9.53 <sup>c</sup>	16.8 <sup>ab</sup>	22.1 <sup>ab</sup>	35.6 <sup>ab</sup>
W23	17.8 <sup>b</sup>	20.3 <sup>l</sup>	15.9 <sup>a</sup>	16.7 <sup>ab</sup>	18.1 <sup>gh</sup>	33.7 <sup>a-d</sup>
W24	16.2 <sup>bc</sup>	22.6 <sup>cf</sup>	8.76 <sup>cde</sup>	15.0 <sup>ab</sup>	19.1 <sup>fg</sup>	24.3 <sup>g-k</sup>
W25	15.0 <sup>c</sup>	25.2 <sup>a</sup>	8.71 <sup>cde</sup>	16.5 <sup>ab</sup>	18.2 <sup>gh</sup>	27.8 <sup>e-h</sup>
W26	14.6 <sup>c</sup>	23.6 <sup>bc</sup>	11.3 <sup>b</sup>	15.9 <sup>ab</sup>	18.2 <sup>gh</sup>	31.7 <sup>a-f</sup>
W27	14.6 <sup>c</sup>	21.0 <sup>ijkl</sup>	8.71 <sup>cde</sup>	15.7 <sup>ab</sup>	17.7 <sup>hi</sup>	35.0 <sup>abc</sup>
W28	14.5 <sup>c</sup>	21.4 <sup>g-l</sup>	8.13 <sup>e</sup>	17.1 <sup>ab</sup>	20.1 <sup>def</sup>	30.6 <sup>b-f</sup>
W29	15.0 <sup>c</sup>	22.3 <sup>e-h</sup>	9.22 <sup>cde</sup>	16.7 <sup>ab</sup>	18.1 <sup>gh</sup>	29.7 <sup>c-g</sup>
W30	-	-	-	-	-	-
W33	-	-	-	-	-	-
W34	-	-	-	-	-	-
W35	-	-	-	-	-	-
W36	-	-	-	-	-	-
W39	17.8 <sup>b</sup>	20.8 <sup>ikl</sup>	9.29 <sup>cd</sup>	16.3 <sup>ab</sup>	16.3 <sup>k</sup>	28.7 <sup>d-h</sup>
W41	-	-	-	-	-	-
W42	21.4 <sup>a</sup>	22.9 <sup>b-f</sup>	8.90 <sup>cde</sup>	17.3 <sup>a</sup>	18.2 <sup>gh</sup>	36.4 <sup>a</sup>
W48	21.6 <sup>a</sup>	23.3 <sup>b-e</sup>	9.00 <sup>cde</sup>	16.0 <sup>ab</sup>	17.2 <sup>hik</sup>	35.6 <sup>ab</sup>
W55	-	-	-	-	-	-
W58	-	-	-	-	-	-
Level of significance	*	*	*	*	*	*
CV (%)	9.07	2.60	6.07	7.86	3.86	9.79

Note: W: Water. Different letters following numbers show significant differences via the Duncan test at P<0.05.

### Selection for purple nonsulfur bacteria that can solubilize P

#### Al-P solubilizing capacity

The ability to solubilize P from the Al-P compound among the studied 25 PNSB strains differed significantly at 5%. Under ML conditions, the P content solubilized from Al-P fluctuated from 13.6 to 22.4 mg L<sup>-1</sup>. Particularly, the W15, W42, and W48 strains reached the greatest Al-P solubilized content



under the ML. Under the AD condition, the result was 19.2 - 25.2 mg L<sup>-1</sup>, in which the W25 strain had the greatest Al-P solubilized content (Table 2).

#### Fe-P solubilizing capacity

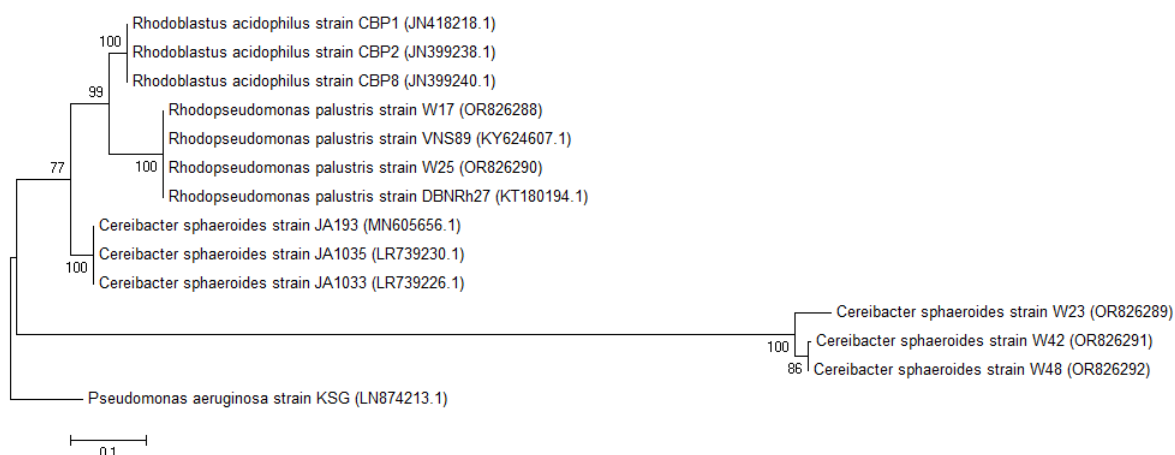
Under ML conditions, solubilized P from Fe-P form was from 8.22 to 15,9 mg L<sup>-1</sup>. Under AD condition, the result was in a range of 9.51 - 17,3 mg L<sup>-1</sup>. The greatest solubilized Fe-P under the ML condition belonged to the W15 strain, 15.9 mg L<sup>-1</sup>. However, under the AD condition, the W10 and W42 strains showed a superior result (17.2-17.3 mg L<sup>-1</sup>) than the others (Table 2).

#### Ca-P solubilizing capacity

All 25 PNSB strains can solubilize Ca-P substance at significantly different content. Under the ML condition, the P content solubilized from Ca-P ranged from 13.3 to 23,0 mg L<sup>-1</sup>. Under the AD condition, the solubilized Ca-P fluctuated from 22.0 to 36.4 mg L<sup>-1</sup>. The W10, W17, and W22 strains performed well under the ML condition, while the W07, W11, W16, W18, W20, W22, W23, W26, W27, W42, and W48 strains showed great potential under the AD condition (Table 2).

#### Identification of the selected purple nonsulfur bacteria

The selected strains consisted of W10, W15, W16, W17, W23, W25, W42, and W48 due to their ability to well solubilize one of the insoluble P compounds under one of the two conditions. Therein, the W17, W23, W25, W42 and W48 strains were identified as *Rhodopseudomonas palustris* strains W17 (OR826288) and W25 (OR826290), and *Cereibacter sphaeroides* strains W23 (OR826289), W42 (OR826291) and W48 (OR826292) at 99% similarity (Figure 1).



**Fig. 1.** Neighbor-joining phylogenetic trees based on 16S rDNA sequences of four selected PNSB strains compared to the closely related strains in the GenBank database including the reference strain *Rhodoblastus acidophilus*. The percentage levels of bootstrap analysis of 1,000 replicates are indicated at each node. Bar, 0.1 substitutions per nucleotide position. *Pseudomonas aeruginosa* strain KSG was used as the outgroup strain. Access numbers of GenBank sequences are implied in brackets.

#### The ability to produce plant growth-promoting substances of the selected P-solubilizing purple nonsulfur bacteria strains

The five PNSB strains can produce PGPS under both ML and Ad conditions. They produced ALA at 1.31-2.19 mg L<sup>-1</sup> and 1.53-1.92 mg L<sup>-1</sup>, EPS at 0.78-1.60 mg L<sup>-1</sup> and 0.91-1.89 mg L<sup>-1</sup>, and siderophores at 16.2-28.8% and 42.5-55.6%, as corresponding to the two conditions. Notably, the W42 and W48 strains produced the greatest amount of PGPS, with ALA of 1.74-2.19 mg L<sup>-1</sup>, EPS of 1.57-1.89 mg L<sup>-1</sup>, and siderophores of 27.9-55.6% (Table 3). Therefore, the W42 and W48 strains have exhibited great potential in pineapple cultivation and they were used to evaluate for further experiments.

#### DISCUSSION

The four W15, W39, W42 and W48 strains were selected under conditions contaminated with Al<sup>3+</sup>, Fe<sup>2+</sup> and Mn<sup>2+</sup> (Table 1). This result is matched with the studies by Khuong *et al.* (2017, 2022), where strains of *Rhodopseudomonas palustris* TLS12, VNS19, VNS32, VNS62, VNW95, *R. harwoodiae* TLW42, *Rhodopseudomonas* spp. TLS06, TLW99, VNW64, VNW02, VNS89 and *Rhodobacter* sp. VNW29 can also survive under conditions with the above metal toxicities. This is because strains of PNSB can

produce EPS, which has functional groups, such as -OH and -COOH to immobilize  $Al^{3+}$ ,  $Fe^{2+}$ , and  $Mn^{2+}$  in acid sulfate soils (Khuong *et al.*, 2017, 2020a; Nguyen *et al.*, 2018). However, unlike previous studies that selected PNSB from submerged acidic soils, the current study performed the PNSB selection in acid-sulfate soils for upland crops.

As reported by Perez *et al.* (2007) and Batool and Rehman (2017), most bacteria cannot completely solubilize all immobilized P or can only solubilize a portion of it for plants (Sulbarán *et al.*, 2009). However, in the current study, 25 PNSB strains can produce a great amount of available P from P minerals. P was solubilized from  $AlPO_4$  greater than that from  $FePO_4$  under both ML and AD conditions (Table 2). According to Sharma *et al.* (2013), P exists in soils under the forms of inorganic and organic compounds, but under those forms, P is one of the main limiting factors to plant development because these P forms are unavailable for plants to use. Inorganic P presents in insoluble mineral complexes in soils, P-solubilizing microorganisms such as *Pseudomonas* and *Bacillus* carry out the process by producing organic acids to reduce pH, or to chelate with metal ions (Al, Ca, and Fe) to release free P (Sharma *et al.*, 2013). Organic acids participating in P solubilization by bacteria consisted of gluconic acid, propanedioic acid, and 2,3-dimethyl fumaric acid (Elhaissoufi *et al.*, 2022). In addition, organic P accounts for 50% of farming soils (Khan *et al.*, 2009). P-solubilizing mechanisms by microorganisms included producing enzymes, such as phosphatases, phytase, and C-P lyases, to reduce P from phosphodiester or phosphoanhydride linkage of organic compounds, or to cut the C-P linkage of organophosphates (Rodriguez *et al.*, 2006; Nannipieri *et al.*, 2011; Richardson & Simpson, 2011).

**Table 3.** Content of plant growth-promoting substances produced by purple nonsulfur bacteria under microaerobic light and aerobic dark conditions

Strain	ALA (mg L <sup>-1</sup> )		EPS (mg L <sup>-1</sup> )		Siderophores (mg L <sup>-1</sup> )	
	Microaerobic light	Aerobic dark	Microaerobic light	Aerobic dark	Microaerobic light	Aerobic dark
W17	1.31 <sup>e</sup>	1.53 <sup>b</sup>	0.78 <sup>b</sup>	0.91 <sup>c</sup>	16.6 <sup>c</sup>	42.5 <sup>b</sup>
W23	1.52 <sup>d</sup>	1.72 <sup>ab</sup>	0.86 <sup>b</sup>	1.01 <sup>c</sup>	16.2 <sup>c</sup>	44.9 <sup>b</sup>
W25	1.78 <sup>c</sup>	1.72 <sup>ab</sup>	0.92 <sup>b</sup>	1.01 <sup>c</sup>	23.6 <sup>b</sup>	43.5 <sup>b</sup>
W42	1.96 <sup>b</sup>	1.74 <sup>ab</sup>	1.57 <sup>a</sup>	.72 <sup>b</sup>	28.8 <sup>a</sup>	54.7 <sup>a</sup>
W48	2.19 <sup>a</sup>	1.92 <sup>a</sup>	1.60 <sup>a</sup>	1.89 <sup>a</sup>	27.9 <sup>a</sup>	55.6 <sup>a</sup>
Level of significance	*	*	*	*	*	*
CV (%)	8.76	7.06	5.88	9.03	7.44	4.20

Note: W: Water. Different letters following numbers show significant differences via the Duncan test at  $P < 0.05$ .

Moreover, the W17, W23, W25, W42, and W48 strains can produce siderophores in ranges of 16.2-28.8 mg L<sup>-1</sup> and 42.5-55.6 mg L<sup>-1</sup> respectively under ML and AD conditions (Table 3). Likewise, Nookongbut *et al.* (2019) and Khuong *et al.* (2020b) also reveal that PNSB produces siderophores as a mechanism to solubilize P, because siderophores perform greater affinity to Fe, and are widely produced by soil microbes. Therefore, siderophores play a role in solubilizing Fe-P or P from Fe minerals to provide available P for plants (Hamdali *et al.*, 2008; Sharma *et al.*, 2013). Moreover, EPS produced by bacteria also solubilizes Ca-P (Yi *et al.*, 2008). In addition, *P. cepacia* has been detected to carry the *gabY* gene responsible for P solubilization (Babu-Khan *et al.*, 1995; Timofeeva *et al.*, 2022). Strains of *Pseudomonas*, *Bacillus*, *Rhizobium*, *Aspergillus*, and *Penicillium* are known as efficient P-solubilizers to increase P availability in soils, and PNSB can promote plant growth by producing available P for plants (Basu and Phale, 2006; Khuong *et al.*, 2021; Timofeeva *et al.*, 2022).

Supplying two strains of *L. sphaeroides* W03 and W11 increased available P content in saline soils in Hong Dan-Bac Lieu and Thanh Phu-Ben Tre, Vietnam, leading to greater accumulation of P in rice plants and reduced 50% of chemical P fertilizers in comparison with the local recommendation fertilizer dose for rice in salt-contaminated acid sulfate soils (Khuong *et al.*, 2021). Previous studies have also revealed that PNSB strains, such as *R. palustris* KKSSR66 and KTSSG55 solubilized P from different P sources, such as the Pikovskaya agar medium, with the amount of 11.7-289.3, 96.0-511.7 and 10.7-602.7 mg L<sup>-1</sup> under ML conditions, and 24.7-512.3, 61.0-438.7 and 26.3-557.67 mg L<sup>-1</sup> under AD condition, corresponding to Al-P, Ca-P and Fe-P compounds (Nookongbut *et al.*, 2019). Moreover, the application of solid PNSB biofertilizers reduced the content of insoluble P in alluvial soils in dykes in comparison with the treatment without PNSB (Khuong *et al.*, 2023b). According to Khuong *et al.* (2023c), at the same volume of chemical fertilizers, in the treatment with PNSB, contents of Al-P, Fe-P, and Ca-P were lower than the treatment with only chemical fertilizers under salt-contaminated conditions.

Furthermore, PNSB is also capable of fixing N and solubilizing K for plants (Khuong *et al.*, 2020b; 2023d; 2024), as well as providing plant growth-promoting substances for plants such as IAA, ALA, siderophores and EPS to assist plants to overcome adverse conditions and to improve their productivity (Ghosh *et al.*, 2022; Sundar and Chao, 2022; Khuong *et al.*, 2023a).

## CONCLUSION

Five PNSB strains W17, W23, W25, W42, and W48 were selected for their capability of tolerating the toxicity from Al<sup>3+</sup>, Fe<sup>2+</sup> and Mn<sup>2+</sup>, and solubilizing Al-P, Fe-P, and Ca-P, and were identified as identified to belong to *Rhodopseudomonas palustris* strain W17 and W25, *Cereibacter sphaeroides* strain W23, W42, and W48. In particular, the selected strain solubilized Al-P at 14.2-25.2 mg L<sup>-1</sup>, Fe-P at 8.71-17.3 mg L<sup>-1</sup>, and Ca-P at 17.2-36.4 mg L<sup>-1</sup>. In addition, these five bacteria strains can also produce PGPS such as ALA, EPS, and siderophores at 1.31-2.19 mg L<sup>-1</sup>, 0.78-1.89 mg L<sup>-1</sup>, and 16.2-55.6%, respectively under both conditions. Therefore, the five strains can be applied to perform the P solubilization in acid sulfate soil, and plant growth promotion as well. Thus, they should be tested under greenhouse conditions, and later on under field conditions.

## ETHICAL STATEMENT

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## REFERENCES

- Agegnehu, G., Amede, T., Erkossa, T., Yirga, C., Henry, C., Tyler, R. & Sileshi, G.W. 2021. Extent and management of acid soils for sustainable crop production system in the tropical agroecosystems: A review. *Acta Agriculturae Scandinavica, Section B Soil and Plant Science*, 71(9): 852-869. <https://doi.org/10.1080/09064710.2021.1954239>
- Alyousif, N.A. 2022. Review of genetic analysis and mechanisms of phosphate solubilization by phosphate solubilizing bacteria. *Marsh Bulletin*, 17(1): 8-21.
- Andriessse, W. & Van Mensvoort, M.E.F. 2002. Acid sulfate soils, distribution and extent. In: *Encyclopedia of Soil Science*. R. Lal (Eds.). CRC Press, Boca Raton. pp. 6.
- Babu-Khan, S., Yeo, T.C., Martin, W.L., Duron, M.R., Rogers, R.D. & Goldstein, A.H. 1995. Cloning of a mineral phosphate-solubilizing gene from *Pseudomonas cepacia*. *Applied and Environmental Microbiology*, 61(3): 972-978. <https://doi.org/10.1128/aem.61.3.972-978.1995>
- Barrow, N.J. & Hartemink, A.E. 2023. The effects of pH on nutrient availability depend on both soils and plants. *Plant and Soil*, 487: 21-37. <https://doi.org/10.1007/s11104-023-05960-5>
- Basu, A. & Phale, P.S. 2006. Inducible uptake and metabolism of glucose by the phosphorylative pathway in *Pseudomonas putida* CSV86. *FEMS Microbiology Letters*, 259(2): 311-316. <https://doi.org/10.1111/j.1574-6968.2006.00285.x>
- Batool, K. & Rehman, Y. 2017. Arsenic-redox transformation and plant growth promotion by purple nonsulfur bacteria *Rhodopseudomonas palustris* CS2 and *Rhodopseudomonas faecalis* SS5. *BioMed Research International*, 2017: 6250327. <https://doi.org/10.1155/2017/6250327>
- Brown, J.W. 2013. Enrichment and isolation of purple non-sulfur bacteria. Department of Biological Sciences, College of Sciences, North Carolina State University. [http://www.mbio.ncsu.edu/mb452/purple\\_nonsulfurs/purples.html](http://www.mbio.ncsu.edu/mb452/purple_nonsulfurs/purples.html)
- Burnham, B. F. 1970. [14] δ-Aminolevulinic acid synthase (*Rhodopseudomonas spheroides*). In: *Methods in Enzymology*. H. Tabor & C.W Tabor (Eds.). Academic Press. 17: 195-200. [https://doi.org/10.1016/0076-6879\(71\)17179-0](https://doi.org/10.1016/0076-6879(71)17179-0)
- Chen, X., Yan, X., Wang, M., Cai, Y., Weng, X., Su, D. & Zhang, F. 2022. Long-term excessive phosphorus fertilization alters soil phosphorus fractions in the acidic soil of pomelo orchards. *Soil and Tillage Research*, 215: 105214. <https://doi.org/10.1016/j.still.2021.105214>
- Dhuldhaj, U.P. & Malik, N. 2022. Global perspective of phosphate solubilizing microbes and phosphatase for improvement of soil, food and human health. *Cellular, Molecular and Biomedical Reports*, 2(3):



- 173-186. <https://doi.org/10.55705/cmbr.2022.347523.1048>
- Dziwornu, A.K., Shrestha, A., Matthus, E., Ali, B., Wu, L. B. & Frei, M. 2018. Responses of contrasting rice genotypes to excess manganese and their implications for lignin synthesis. *Plant Physiology and Biochemistry*, 123: 252-259. <https://doi.org/10.1016/j.plaphy.2017.12.018>
- Elhassoufi, W., Ghoulam, C., Barakat, A., Zeroual, Y. & Bargaz, A. 2022. Phosphate bacterial solubilization: A key rhizosphere driving force enabling higher P use efficiency and crop productivity. *Journal of Advanced Research*, 38: 13-28. <https://doi.org/10.1016/j.jare.2021.08.014>
- Fanning, D.S., Rabenhorst, M.C., Balduff, D.M., Wagner, D.P., Orr, R.S. & Zurheide, P.K. 2010. An acid sulfate perspective on landscape/seascape soil mineralogy in the U.S. Mid-Atlantic region. *Geoderma*, 154(3-4): 457-464. <https://doi.org/10.1016/j.geoderma.2009.04.015>
- Ferreira, M.L., Casabuono, A.C., Stacchiotti, S.T., Couto, A.S., Ramirez, S.A. & Vullo, D.L. 2017. Chemical characterization of *Pseudomonas veronii* 2E soluble exopolymer as Cd (II) ligand for the biotreatment of electroplating wastes. *International Biodeterioration and Biodegradation*, 119: 605-613. <https://doi.org/10.1016/j.ibiod.2016.10.013>
- Fu, J., Leo, C.P. & Show, P.L. 2022. Recent advances in the synthesis and applications of pH-responsive  $\text{CaCO}_3$ . *Biochemical Engineering Journal*, 187: 108446. <https://doi.org/10.1016/j.bej.2022.108446>
- Gerónimo, E. & Aparicio, V.C. 2022. Changes in soil pH and addition of inorganic phosphate affect glyphosate adsorption in agricultural soil. *European Journal of Soil Science*, 73(1): e13188. <https://doi.org/10.1111/ejss.13188>
- Ghosh, S., Bhattacharya, J., Nitnavare, R. & Webster, T.J. 2022. Microbial remediation of metals by marine bacteria. In: *Development in Wastewater Treatment Research and Processes*. S. Rodriguez-Couto & M.P. Shah (Eds.). Elsevier. pp. 131-158. <https://doi.org/10.1016/B978-0-323-85839-7.00011-6>
- Gondal, A.H., Hussain, I., Ijaz, A.B., Zafar, A., Ch, B.I., Zafar, H. & Tariq, M. 2021. Influence of soil pH and microbes on mineral solubility and plant nutrition: A review. *International Journal of Agriculture and Biological Sciences*, 5(1): 71-81.
- Hamdali, H., Bouizgarne, B., Hafidi, M., Lebrihi, A., Viroille, M.J. & Ouhdouch, Y. 2008. Screening for rock phosphate solubilizing Actinomycetes from Moroccan phosphate mines. *Applied Soil Ecology*, 38(1): 12-19. <https://doi.org/10.1016/j.apsoil.2007.08.007>
- Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S. & Hayes, R.C. 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. *Science of the Total Environment*, 610: 316-332. <https://doi.org/10.1016/j.scitotenv.2017.08.020>
- Hulisz, P., Michalski, A., Boman, A., Dąbrowski, M. & Kwasowski, W. 2020. Identification of potential acid sulfate soils at the Reda River mouth (northern Poland) using pH measurements. *Soil Science Annual*, 71(2): 53-61. <https://doi.org/10.37501/soilsa/122410>
- Huu, T.N., Nhan, T.C., Xuan, L.N.T., Thu, L.T.M., Trong, N.D., Thu, H.N., Ngan, N.T., Quang, L.T., Khuong, N.Q. 2024. Selection for nitrogen-providing purple nonsulfur bacteria from acid sulfate soil that grows pineapple. *Malaysian Journal of Microbiology*. Under review
- Kang, S.M., Imran, M., Shaffique, S., Kwon, E.H., Park, Y.S. & Lee, I.J. 2022. Growth and photosynthetic characteristics of sesame seedlings with gibberellin-producing *Rhodobacter sphaeroides* SIR03 and biochar. *International Journal of Plant Biology*, 13(3): 257-269. <https://doi.org/10.3390/ijpb13030022>
- Kar, D., Pradhan, A.A. & Datta, S. 2021. The role of solute transporters in aluminum toxicity and tolerance. *Physiologia Plantarum*, 171(4): 638-652. <https://doi.org/10.1111/ppl.13214>
- Khan, M.S., Zaidi, A. & Wani, P.A. 2009. Role of phosphate solubilizing microorganisms in sustainable agriculture-a review. In: *Sustainable agriculture*. E. Lichtfouse, M. Navarrete, P. Debaeke, S. Véronique & C. Alberola (Eds). Springer, Dordrecht. pp. 551-570. [https://doi.org/10.1007/978-90-481-2666-8\\_34](https://doi.org/10.1007/978-90-481-2666-8_34)
- Khuong, N.Q., Kantachote, D., Onthong, J. & Sukhoom, A. 2017. The potential of acid-resistant purple nonsulfur bacteria isolated from acid sulfate soils for reducing toxicity of  $\text{Al}^{3+}$  and  $\text{Fe}^{2+}$  using biosorption for agricultural application. *Biocatalysis and Agricultural Biotechnology*, 12: 329-340. <https://doi.org/10.1016/j.bcab.2017.10.022>
- Khuong, N.Q., Kantachote, D., Onthong, J., Xuan, L.N.T. & Sukhoom, A. 2018. Enhancement of rice growth and yield in actual acid sulfate soils by potent acid-resistant *Rhodopseudomonas palustris* strains for producing safe rice. *Plant and Soil*, 429: 483-501. <https://doi.org/10.1007/s11104-018-3705-7>
- Khuong, N.Q., Kantachote, D., Thuc, L.V., Nookongbut, P., Xuan, L.N.T., Nhan, T.C., Xuan, N.T.T. & Tantirungkij, M. 2020a. Potential of  $\text{Mn}^{2+}$ -resistant purple nonsulfur bacteria isolated from acid sulfate soils to act as bioremediators and plant growth promoters via mechanisms of resistance. *Journal of*

- Soil Science and Plant Nutrition, 20: 2364-2378. <https://doi.org/10.1007/s42729-020-00303-0>
- Khuong, N.Q., Kantachote, D., Nookongbut, P., Onthong, J., Xuan, L.N.T. & Sukhoom, A. 2020b. Mechanisms of acid-resistant *Rhodopseudomonas palustris* strains to ameliorate acidic stress and promote plant growth. Biocatalysis and Agricultural Biotechnology, 24: 101520. <https://doi.org/10.1016/j.bcab.2020.101520>
- Khuong, N.Q., Huu, T.N., Thuc, L.V., Thu, L.T.M., Xuan, D.T., Quang, L.T., Nhan, T.C., Tran, H.N., Tien, P.D., Xuan, L.N.T. & Kantachote, D. 2021. Two strains of *Luteovulum sphaeroides* (purple nonsulfur bacteria) promote rice cultivation in saline soils by increasing available phosphorus. Rhizosphere, 20: 100456. <https://doi.org/10.1016/j.rhisph.2021.100456>
- Khuong, N.Q., Kantachote, D., Thuc, L.V., Huu, T.N., Nhan, T.C., Nguyen, P.C., Thu, L.T. M., Van, T.T.B., Xuan, N.T.T., Xuan, L.N.T. & Xuan, D.T. 2022. Use of potent acid resistant strains of *Rhodopseudomonas* spp. in Mn-contaminated acidic paddies to produce safer rice and improve soil fertility. Soil and Tillage Research, 221: 105393. <https://doi.org/10.1016/j.still.2022.105393>
- Khuong, N.Q., Kantachote, D., Dung, N.T.T., Huu, T.N., Thuc, L.V., Thu, L.T.M., Quang, L.T., Xuan, D.T., Nhan, T.C., Tien, P.D. & Xuan, L.N.T. 2023a. Potential of potent purple nonsulfur bacteria isolated from rice-shrimp systems to ameliorate rice (*Oryza sativa* L.) growth and yield in saline acid sulfate soil. Journal of Plant Nutrition, 46(3): 473-494. <https://doi.org/10.1080/01904167.2022.2087089>
- Khuong, N.Q., Thuc, L.V., Giang, C.T., Xuan, L.N.T., Thu, L.T.M., Isao, A. & Jun-Ichi, S. 2023b. Improvement of nutrient uptake, yield of black sesame (*Sesamum indicum* L.), and alluvial soil fertility in dyke with spent rice straw from mushroom cultivation as biofertilizer containing potent strains of *Rhodopseudomonas palustris*. The Scientific World Journal, 2023: 1954632. <https://doi.org/10.1155/2023/1954632>
- Khuong, N.Q., Thuc, L.V., Quang, L.T., Huu, T.N., Xuan, D.T., Dac, H.H., Xuan, L.N.T. & Thu, L.T.M. 2023c. Effects of biofertilizer supplementation, *Rhodopseudomonas* spp., on nitrogen and phosphorus uptakes, growth, and yield of sesame (*Sesamum indicum* L.) on salt-affected soil. Journal of Plant Nutrition, 41(1): 1-17. <https://doi.org/10.1080/01904167.2023.2278646>
- Khuong, N.Q., Sakpirom, J., Oanh, T.O., Thuc, L.V., Thu, L.T.M., Xuan, D.T., Quang, L.T. & Xuan, L.N.T. 2023d. Isolation and characterization of novel potassium-solubilizing purple nonsulfur bacteria from acidic paddy soils using culture-dependent and culture-independent techniques. Brazilian Journal of Microbiology, 54: 2333-2348. <https://doi.org/10.1007/s42770-023-01069-0>
- Khuong, N.Q., Trong, N.D., Quang, L.T., Xuan, L.N.T., & Phong, N.T. 2024. The potency of a liquid biofertilizer containing bacterial strains of *Rhodopseudomonas* spp. on recovery of soil properties damaged by Al<sup>3+</sup> and Fe<sup>2+</sup> toxins and enhancement of rice yield in acid sulfate soil. International Journal of Phytoremediation, 1-11. <https://doi.org/10.1080/15226514.2023.2253913>
- Lee, S.K. & Dang, T.A. 2019. Spatio-temporal variations in meteorology drought over the Mekong River Delta of Vietnam in the recent decades. Paddy and Water Environment, 17(1): 35-44. <https://doi.org/10.1007/s10333-018-0681-8>
- Lo, K.J., Lin, S.S., Lu, C.W., Kuo, C.H. & Liu, C.T. 2018. Whole-genome sequencing and comparative analysis of two plant-associated strains of *Rhodopseudomonas palustris* (PS3 and YSC3). Scientific Reports, 8(1): 12769. <https://doi.org/10.1038/s41598-018-31128-8>
- Loc, H.H., Van Binh, D., Park, E., Shrestha, S., Dung, T.D., Son, V.H., Truc, N.H.T., Mai, N.P. & Seijger, C. 2021. Intensifying saline water intrusion and drought in the Mekong Delta: From physical evidence to policy outlooks. Science of the Total Environment, 757: 143919. <https://doi.org/10.1016/j.scitotenv.2020.143919>
- Lopes, L.D., Hao, J. & Schachtman, D.P. 2021. Alkaline soil pH affects bulk soil, rhizosphere and root endosphere microbiomes of plants growing in a Sandhills ecosystem. FEMS Microbiology Ecology, 97(4): 1028. <https://doi.org/10.1093/femsec/fiab028>
- Murphy, J. & Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta, 27: 31-36. [https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5)
- Nannipieri, P., Giagnoni, L., Landi, L. & Renella, G. 2011. Role of phosphatase enzymes in soil. In: Phosphorus in Action. E. Bünemann, A. Oberson, E. Frossard (Eds.). Springer, Berlin, Heidelberg. pp. 215-243. [https://doi.org/10.1007/978-3-642-15271-9\\_9](https://doi.org/10.1007/978-3-642-15271-9_9)
- Nguyen, K.Q., Kantachote, D., Onthong, J. & Sukhoom, A. 2018. Al<sup>3+</sup> and Fe<sup>2+</sup> toxicity reduction potential by acid-resistant strains of *Rhodopseudomonas palustris* isolated from acid sulfate soils under acidic conditions. Annals of Microbiology, 68(4): 217-228. <https://doi.org/10.1007/s13213-018-1332-4>
- Nookongbut, P., Kantachote, D., Khuong, N.Q., Sukhoom, A., Tantirungkij, M. & Limtong, S. 2019.

- Selection of acid-resistant purple nonsulfur bacteria from peat swamp forests to apply as biofertilizers and biocontrol agents. *Journal of Soil Science and Plant Nutrition*, 19: 488-500. <https://doi.org/10.1007/s42729-019-00044-9>
- Peng, Y., Sun, Y., Fan, B., Zhang, S., Bolan, N. S., Chen, Q. & Tsang, D.C. 2021. Fe/Al (hydr)oxides engineered biochar for reducing phosphorus leaching from a fertile calcareous soil. *Journal of Cleaner Production*, 279: 123877. <https://doi.org/10.1016/j.jclepro.2020.123877>
- Perez, E., Sulbaran, M., Ball, M.M. & Yarzabal, L.A. 2007. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biology and Biochemistry*, 39(11): 2905-2914. <https://doi.org/10.1016/j.jclepro.2020.123877>
- Richardson, A.E. & Simpson, R.J. 2011. Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiology*, 156(3): 989-996. <https://doi.org/10.1104/pp.111.175448>
- Rodriguez, H., Fraga, R., Gonzalez, T. & Bashan, Y. 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil*, 287: 15-21. <https://doi.org/10.1007/s11104-006-9056-9>
- Rose, T.J. & Wissuwa, M. 2012. Rethinking internal phosphorus utilization efficiency: A new approach is needed to improve PUE in grain crops. *Advances in Agronomy*, 116: 185-217. <https://doi.org/10.1016/B978-0-12-394277-7.00005-1>
- Sade, H., Meriga, B., Surapu, V., Gadi, J., Sunita, M.S.L., Suravajhala, P. & Kishor, P.K. 2016. Toxicity and tolerance of aluminum in plants: Tailoring plants to suit to acid soils. *Biometals*, 29(2): 187-210. <https://doi.org/10.1007/s10534-016-9910-z>
- Sadiq, A.A. & Babagana, U. 2012. Influence of lime materials to ameliorate acidity on irrigated paddy fields: A review. *Academic Research International* 3(1): 413-420.
- Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. & Gobi, T.A. 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, 2: 587. <https://doi.org/10.1186/2193-1801-2-587>
- Siddique, R., Gul, A., Ozturk, M. & Altay, V. 2021. Phosphate solubilizing bacteria for soil sustainability. In: *Handbook of assisted and amendment: enhanced sustainable remediation technology*. M.N.V Prasad (Eds.). John Wiley & Sons. pp. 423-435. <https://doi.org/10.1002/9781119670391.ch21>
- Sulbarán, M., Pérez, E., Ball, M.M., Bahsas, A. & Yarzabal, L.A. 2009. Characterization of the mineral phosphate-solubilizing activity of *Pantoea agglomerans* MMB051 isolated from an iron-rich soil in southeastern Venezuela (Bolívar state). *Current Microbiology*, 58: 378-383. <https://doi.org/10.1007/s00284-008-9327-1>
- Sundar, L.S. & Chao, Y.Y. 2022. Potential of purple non-sulfur bacteria in sustainably enhancing the agronomic and physiological performances of rice. *Agronomy*, 12(10): 2347. <https://doi.org/10.3390/agronomy12102347>
- Suzuki, Y., Kelly, S.D., Kemner, K.M. & Banfield, J.F. 2003. Microbial populations stimulated for hexavalent uranium reduction in uranium mine sediment. *Applied and Environmental Microbiology*, 69(3): 1337-1346. <https://doi.org/10.1128/AEM.69.3.1337-1346.2003>
- Timofeeva, A., Galyamova, M. & Sedykh, S. 2022. Prospects for using phosphate-solubilizing microorganisms as natural fertilizers in agriculture. *Plants*, 11(16): 2119. <https://doi.org/10.3390/plants11162119>
- Tri, L.V. & van Mensvoort, M.E.F. 2004. Decision trees for farm management on acid sulfate soils, Mekong Delta, Viet Nam. *Soil Research*, 42(6): 671-684. <https://doi.org/10.1071/SR03083>
- Wan, W., Hao, X., Xing, Y., Liu, S., Zhang, X., Li, X. & Huang, Q. 2021. Spatial differences in soil microbial diversity caused by pH-driven organic phosphorus mineralization. *Land Degradation and Development*, 32(2): 766-776. <https://doi.org/10.1002/ldr.3734>
- Wei, T.T., Fan, X.B. & Quan, Z.X. 2023. *Abyssibius alkaniclasticus* gen. nov., sp. nov., a novel member of the family *Rhodobacteraceae*, isolated from the Mariana Trench. *International Journal of Systematic and Evolutionary Microbiology*, 73(2): 005715. <https://doi.org/10.1099/ijsem.0.005715>
- Yi, Y., Huang, W. & Ge, Y. 2008. Exopolysaccharide: a novel important factor in the microbial dissolution of tricalcium phosphate. *World Journal of Microbiology and Biotechnology*, 24: 1059-1065. <https://doi.org/10.1007/s11274-007-9575-4>