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In vitro Potassium Solubilization by *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11 and *Rhodospeudomonas palustris* M-So-14 Isolated from Alluvial Soils of An Giang, Vietnam

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ABSTRACT

Background: Potassium (K) is a key limiting nutrient in most soils due to soil characteristics. Hence, biological K solubilization may be a potential tool for enhancing the availability of native K and thereby plant growth. The present study evaluated the potassium-solubilizing (K-sol) capacity from insoluble K minerals by strains of K-solublizing purple non-sulfur bacteria (PNSB) *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11 and *Rhodospeudomonas palustris* M-So-14 isolated from alluvial soil.

Methods: The experiment was conducted in a completely randomized design in the laboratory with six treatments: (i) no soil and no PNSB, (ii) soil but no PNSB (iii) soil with a single strain of M-Sl-09, (iv) soil with a single strain of M-So-11, (v) soil with a single strain of M-So-14 and (vi) soil with mixture of K solubilizer.

Result: The results revealed that the exchangeable K content (exK) in M-Sl-09, M-So-14, or mixed treatments were greater than those ofM-So-11 (2.35-2.42>2.19 meq 100 g^{-1}) and bottomed in the treatment without soil nor PNSB (0.093 meq 100 g^{-1}) at 10 days after PNSB inoculation. Furthermore, the cation exchange capacity of treatments with PNSB was 11.0-15.4% greater than those without PNSB. Likewise, optical density of PNSB in treatments with PNSB were greater than those without PNSB with turbidity of 2.14-2.44>1.02, respectively.

Key words: Insoluble potassium minerals, Potassium solubilizing capacity, Purple non-sulfur bacteria.

INTRODUCTION

Potassium (K) is an essential mineral nutrient for plants (Vasileva *et al.*, 2022; Thriveni *et al.*, 2024). It participates in activating enzymes, osmosis, protein synthesis, cation-anion balance and supporting plants to overcome abiotic stresses (Marschner, 2012). In particular, K^+ can activate more than 60 enzymes during glycolysis and Krebs cycle within plants (Nieves-Cordones *et al.*, 2016). Long-term intensive agricultural soil results in low K availability in soil (Das *et al.*, 2021). Furthermore, sustainable agriculture trends reduce chemical fertilizer use, leading to lower and lower soil exchangeable K (exK) (Mouhamad *et al.*, 2016). Approximately 98% of soil K exists in mineral forms, including muscovite, biotite and feldspar (Mouhamad *et al.*, 2016). Alluvial soil is usually deposited with alluvial materials from rivers, leading to great nutrient content in soil (Tong, 2017). However, in some regions, dykes were constructed to prevent floods and protect agricultural land, leading to restriction of alluvial deposition into the soil and eventually lower nutrient content compared with regions without dykes (Khuong *et al.*, 2023a; Tuan *et al.*, 2007).

K-solubilizing (K-sol) bacteria are potent in exploiting insoluble K minerals in soil (Etesami *et al.*, 2017). Because of low exK and high cost of chemical fertilizer, the role of K-sol bacteria becomes increasingly important (Singh *et al.*, 2023). Normally, these K-sol bacteria produce organic acids, such as oxalic acid, gluconic acid, malic acid, lactic acid, and succinic acid (Naraian and Kumari, 2017). These

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acids release H^+ to directly solubilize K into soluble forms (Reddy *et al.*, 2023). Among K-sol bacteria, purple non-sulfur bacteria (PNSB) belonging to α and β-proteobacteria (Imhoff *et al.*, 2005) feature flexible metabolism, *i.e.* they can perform as a photoautotroph, photoheterotroph, chemoautotroph or chemoheterotroph (Madigan and Jung, 2009). Thus, PNSB are highly potent in solubilizing K from soil. Thus, the current study was conducted to assess the K-sol. capacity of K-sol PNSB *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11 and *R. palustris* M-So-14 in undeposited alluvial soil under *in vitro* conditions.

MATERIALS AND METHODS

Materials

The origin of bacteria

The K-sol PNSB used in the study included *Cereibacter sphaeroides* M-Sl-09 (PP814768), *Rhodopseudomonas thermotolerans* M-So-11 (PP814769) and *R. palustris* M-So-14 (PP814770). These K-sol. PNSB strains were isolated from un-deposited alluvial soil for maize cultivation and kept in the Faculty of Crop Science, College of Agriculture, Can Tho University (Thu *et al.*, 2024).

Un-deposited alluvial soil belonged to the dyke system for maize cultivation in An Phu district, An Giang province.

The basic isolation medium (BIM) included 1.0 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, 0.2 g $MgSO_4.H_2O$, 2.0 g NaCl, 5.0 g $NaHCO_3$, 1.5 g yeast extract, 1.5 g glycerol and 0.03 g L-cysteine and distilled water making up to 1 L of solution.

Methods

Bacteria preparation: The K-sol PNSB strains were propagated in BIM under a light intensity of 3,000 lux for 72 h. The PNSB culture was diluted to reach an OD_{660} of 0.5 before being used.

The experiment was conducted in a completely randomized with 6 treatments and 3 replications in the Faculty of Crop Science, College of Agriculture, Can Tho University from September 2025 to December 2025. Each replication was a centrifugal tube containing 25 g of dry soil, 0.25 g of insoluble K minerals ($K_nNa_{12-n}[(AlO_2)_{12}(SiO_2)_{12}]\bullet xH_2O$), 2.0 mL of bacteria culture, 1.0 mL of sterilized distilled water as described in Table 1.

The amount of exK and cation exchange capacity (CEC) were determined on days 2, 4, 6, 8 and 10 after PNSB inoculation. The exK was measured by extracting soil with $BaCl_2$ 0.1 M and using an atomic absorption spectrometer at the 766.5 nm wavelength. The CEC in soil was determined by extracting soil with $MgSO_4$ 0.02 M and titrating extracted solution with EDTA 0.01 M (Sparks *et al.*, 2020). The PNSB density was measured 10 days after PNSB inoculation. In brief, 1 g of soil of each treatment was added into BIM, illuminated at 3,000 lux for 5 days and finally measured for optical destiny (OD_{660}) to determine the relative density.

Statistical analysis

The SPSS version 13.0 was used to examine the differences among treatments according to Duncan's test at 5% significance.

RESULTS AND DISCUSSION

Changes in exchangeable K content in soil by the potassium-solubilizing PNSB

Table 2 shows that at day 2 after PNSB inoculation, the exK in treatment with mixed PNSB strains was greatest (3.31 meq 100 g^{-1}) and lowest was under the treatment without soil nor PNSB (0.085 meq 100 g^{-1}). Likewise, on days 4, 6, 8 and 10, treatments with single or mixture of K-sol PNSB out-performed the ones without bacteria, with 2.48-2.78>0.085-2.26 meq 100 g^{-1} (day 4), 2.29-2.62>0.088-1.42 meq 100 g^{-1} (day 6), 2.37-2.47>0.093-1.55 meq 100 g^{-1} (day 8) and 2.19-2.42>0.093-1.67 meq 100 g^{-1} (day 10).

Changes in soil CEC by potassium-solubilizing PNSB

On days 2, 4, 8 and 10 days after PNSB inoculation, treatments with had equivalent CEC to each other and greater than those of treatments without PNSB, with 13.2-14.1>11.9 meq 100 g^{-1}, 13.4-14.6>12.1 meq 100 g^{-1}, 13.2-13.5>12.2 meq 100 g^{-1} and 13.6-14.3>12.1 meq 100 g^{-1}. Furthermore, supplying single M-Sl-09 strain had greater CEC on day 6 than supplying single M-So-14 strain or the mixed PNSB, with 14.6>13.8-13.9 meq 100 g^{-1}, respectively (Table 3).

Changes in soil bacteria density by potassium-solubilizing PNSB

As per Fig 1, on 10 after PNSB inoculation, PNSB density in soil varied significantly at 5%. In particular, supplying single M-Sl-09 strain had an equivalent OD_{660} mixed PNSB,

Table 1: Treatment details to evaluate potassium solubilizing capacity under bare soil conditions.

Soil	$K_nNa_{12-n}[(AlO_2)_{12}(SiO_2)_{12}]\bullet xH_2O$	PNSB	Water
-	+	-	+
+	+	-	+
+	+	M-Sl-09	+
+	+	M-So-11	+
+	+	M-So-14	+
+	+	M-Sl-09, M-So-11 and M-So-14	+

Note: "+": addition. "-": no addition; PNSB: Purple nonsulfur bacteria.

roughly 2.32-2.44. Moreover, treatments with a single strain of M-So-11 or M-So-14 had equivalent OD_{660} to each other and were greater than those without bacteria, with 2.14-2.27>1.02, respectively.

Supplying K-sol. PNSB strains *C. sphaeroides* M-Sl-09, *R. thermotolerans* M-So-11 and *R. palustris* M-So-14 solubilized insoluble K minerals ($K_nNa_{12-}n$ [(A $IO_2)_{12}$ $(SiO_2)_{12}$]•xH_2O) into available forms (Table 2). Bangun *et al.* (2023) have successfully selected strains of *Burkholderia paludis* KSB.TB5, *B. cepacia* KSB.TB6 and *Paraburkholderia phymatum* KSB.PB1 can solubilize insoluble K from weathered materials of mountains in Bahorok, Langkat province, Indonesia. In addition, K-sol bacteria KSB-40, KSB-17, KSB-31, KSB-94 and KSB-105 can release K^+ from biotite minerals at the amounts of 3.50-5.00, 4.40-6.30 and 5.30-7.20 µg mL^{-1} after 7, 14 and 21 days of incubation, respectively (Nath *et al.*, 2017). Khuong *et al.* (2023b) found that PNSB *Rhodopseudomonas pentothenatexigens* TT07.4, AN05.1 and AC04.1 isolated from acid sulfate soil in paddy fields can solubilize K at 11.8-17.7 mg L^{-1} under microaerobic light and 16.4-24.7 mg L^{-1} aerobic dark conditions. Furthermore, strains of *Pantoea agglomerans* P5, *Pseudomonas putida* P13, *P. fluorescens* Chao, *P. putida* Tabriz, *P. fluorescens* Tabriz, *Azotobacter* sp. and *Bacillus megaterium* JK3 can perform K solubilization from minerals of muscovite at 3.50-5.38 mg g^{-1} and biotite at 5.44-7.25 mg g^{-1} (Sarikhani, 2016). K appears in soil as components of minerals such as muscovite, biotite and feldspar (Paramisparam *et al.*, 2021). Therefore, the strains of *C. sphaeroides* M-Sl-09, *R. thermotolerans* M-So-11 and *R. palustris* M-So-14 successfully performed K solubilization from minerals, which is potent to reduce the use of chemical fertilizers for sustainable agriculture.

CEC is an important soil feature indicating the nutrient availability for plants (Manrique *et al.*, 1991). The increase

Table 2: Influences of *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11 and *Rhodospeudomonas palustris* M-So-14 on soil exchangeable potassium.

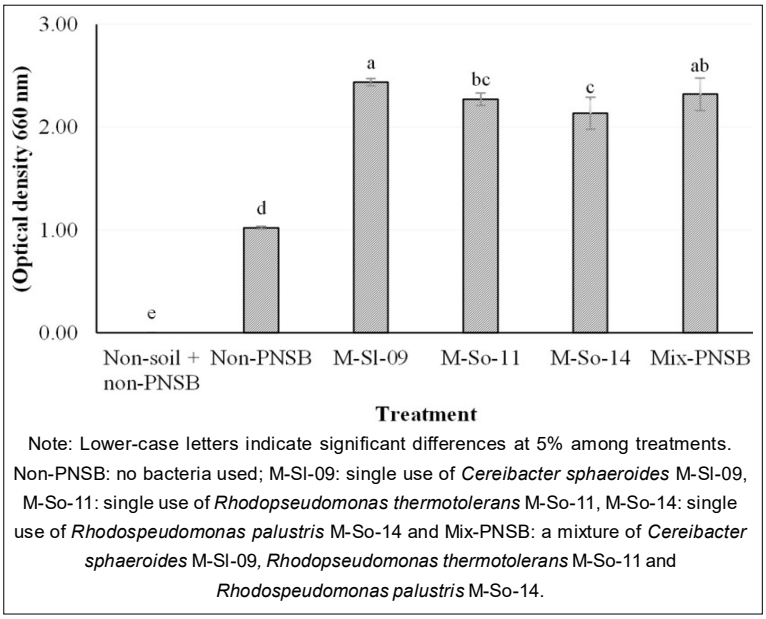
Treatment	Exchangeable potassium (meq 100 g-1)				
	Day 2	Day 4	Day 6	Day 8	Day 10
Non-soil + non-PNSB	0.085^{d}	0.088^{d}	0.088	0.093^{c}	0.093^{d}
Non-PNSB	1.91^{c}	2.26^{c}	1.42^{d}	1.55^{b}	1.67^{c}
M-Sl-09	2.41^{b}	2.78^{a}	2.29^{c}	2.47^{a}	2.42^{a}
M-So-11	2.30^{b}	2.61^{b}	2.62^{a}	2.45^{a}	2.19^{b}
M-So-14	2.59^{b}	2.48^{b}	2.35^{bc}	2.37^{a}	2.41^{a}
Mix-PNSB	3.31^{a}	2.75^{a}	2.53^{ab}	2.40^{a}	2.35^{a}
Significance	*	*	*	*	*
CV (%)	11.0	6.68	7.68	6.22	4.52

Note: In the same column, numbers with identical following letters are insignificant from each other according to Duncan's test.*: 5% significant different; M-Sl-09: *Cereibacter sphaeroides* M-Sl-09, M-So-11*: Rhodopseudomonas thermotolerans* M-So-11, M-So-14: *Rhodospeudomonas palustris* M-So-14, Mix-PNSB: a mixture of *Cereibacter sphaeroides* M-Sl-09*, Rhodopseudomonas thermotolerans* M-So-11 and *Rhodospeudomonas palustris* M-So-14.

Table 3: Influences of *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11 and *Rhodospeudomonas palustris* M-So-14 on cation exchange capacity of soil.

Treatment	Cation exchange capacity (meq 100 g^{-1})				
	Day 2	Day 4	Day 6	Day 8	Day 10
Non-soil + non-PNSB	-	-	-	-	-
Non-PNSB	11.9^{b}	12.1^{b}	12.6^{c}	12.2^{b}	12.1^{b}
M-Sl-09	14.1^{a}	14.5^{a}	14.6^{a}	13.5^{a}	13.6^{a}
M-So-11	13.2^{a}	14.5^{a}	14.4^{ab}	13.5^{a}	13.6^{a}
M-So-14	14.1^{a}	13.4^{a}	13.8^{b}	13.2^{a}	14.2^{a}
Mix-PNSB	13.6^{a}	14.6^{a}	13.9^{b}	13.2^{a}	14.3^{a}
Significance	*	*	*	*	*
CV (%)	5.14	5.93	3.09	3.37	5.08

Note: In the same column, numbers with identical following letters are insignificant from each other according to Duncan's test.*: 5% significant different; M-Sl-09: *Cereibacter sphaeroides* M-Sl-09, M-So-11: *Rhodopseudomonas thermotolerans* M-So-11, M-So-14: *Rhodospeudomonas palustris M*-So-14, Mix-PNSB: a mixture of *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11 and *Rhodospeudomonas palustris* M-So-14.



Note: Lower-case letters indicate significant differences at 5% among treatments. Non-PNSB: no bacteria used; M-Sl-09: single use of *Cereibacter sphaeroides* M-Sl-09, M-So-11: single use of *Rhodopseudomonas thermotolerans* M-So-11, M-So-14: single use of *Rhodospeudomonas palustris* M-So-14 and Mix-PNSB: a mixture of *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11 and *Rhodospeudomonas palustris* M-So-14.

Fig 1: Influences of *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11 and *Rhodospeudomonas palustris* M-So-14 on bacteria density in soil.

in exK when supplying *C. sphaeroides* M-Sl-09, *R. thermotolerans* M-So-11 and *R. palustris* M-So-14 enhanced soil CEC (Table 3). Cations hold onto anions on the surface of organic matter or shale. These exchange positions are the main sources of exchangeable cations for plants. Cations loosely attached to these surfaces are taken by plants and resupplied to soil solution. Thus, current study focused on facilitating plants to take these cations more easily, especially K.

CONCLUSION

Supplying K-sol. PNSB *C. sphaeroides* M-Sl-09, *R. thermotolerans* M-So-11 and *R. palustris* M-So-14 increased exK and CEC in soil compared with no bacteria supplementation. Furthermore, PNSB density in with PNSB was greater than ones without PNSB. Further research should be carried to evaluate the potency of current K-sol PNSB strains under greenhouse conditions and field trials.

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Conflict of interest

The authors declare no conflicts of interest.

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