

Research Article

Molecular Characterization of Indigenous Potassium-Solubilizing Bacteria from Rice Rhizosphere in Lima Puluh Kota Regency

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ABSTRACT

Potassium (K) is one of the macronutrients essential for plant growth; however, most soil K exists in insoluble mineral forms that are not readily available for plant uptake. The use of potassium-solubilizing bacteria (KSB) offers a sustainable approach to enhance K bioavailability in agricultural systems. This study aimed to isolate, characterize, and identify KSB from the rhizosphere of seven local rice varieties cultivated in Lima Puluh Kota Regency, West Sumatra, Indonesia. Total culturable heterotrophic bacterial populations were enumerated using nutrient agar, and one morphologically distinct colony from each sample was purified for further analysis. K-solubilizing activity was evaluated on Aleksandrov agar by measuring the dissolution index (DI) and in liquid Aleksandrov medium supplemented with K-feldspar powder. All seven isolates formed clear halo zones on solid medium. The highest DI value was observed in isolate KSP (2.2), followed by KKP and KJG, while KKK exhibited the lowest DI. In liquid medium, soluble K release ranged from 0.95 to 1.95 mg L⁻¹, with KSP demonstrating the highest solubilization capacity. Statistical analysis revealed significant differences among isolates ($p < 0.05$). Molecular identification based on 16S rRNA gene sequencing indicated that the isolates belonged to *Lysinibacillus xylanilyticus*, *Bacillus velezensis*, *Acinetobacter johnsonii*, *Pseudarthrobacter phenanthrenivorans*, *Klebsiella aerogenes*, *Paenarthrobacter nicotinovorans*, and *Enterobacter cloacae*. These findings demonstrate the diversity and functional potential of indigenous KSB associated with rice rhizosphere soils and support their prospective application as biofertilizer candidates for sustainable rice production.

Key words: 16S rRNA gene, Aleksandrov medium, dissolution index, potassium-solubilizing bacteria, rice rhizosphere

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INTRODUCTION

Potassium (K) has received less interest compared to nitrogen and phosphorus due to the common theory that agricultural soils contain sufficient K reserves. However, intensive and continuous cropping systems, as well as low application rates of K-fertilizer, may result in an increasing depletion of available K, specifically in rhizosphere soils (Soumare *et al.*, 2023). K is an essential macronutrient required in large quantities after nitrogen and phosphorus (Salam *et al.*, 2023), and plays a critical role in enzyme activation, preservation of cell turgor, photosynthesis process, metabolism of carbohydrates, and plant resistance to biotic and abiotic stresses (Wang *et al.*, 2013; Pathak *et al.*, 2020). In rice-based production

systems, insufficient K may directly affect grain filling, plant vigor, and lodging resistance.

While total soil K may reach up to 98%, less than 2% is readily accessible for plant uptake. The majority exists in insoluble mineral forms such as mica, feldspar, and other aluminosilicates, making it unavailable to plants (Bahadur *et al.*, 2019). The utilization of these insoluble K reserves through biological processes corresponds to a sustainable option to the application of chemical fertilizer. K-solubilizing microorganisms are able to enhance K-availability and improve nutrient uptake efficacy (Sardans & Peñuelas, 2015; Sun *et al.*, 2020). These microorganisms significantly impact nutrient cycling by dissolving insoluble minerals (Hu *et al.*, 2018), and various

groups, including fungi, bacteria, and actinomycetes, are known to solubilize mineral K through the production of organic acids and related metabolites (Sarikhani *et al.*, 2018). KSB release K⁺ from feldspar and aluminosilicate minerals through acidolysis, chelation, exchange reactions, and complexation mechanisms (Meena *et al.*, 2018; Sattar *et al.*, 2019), while also contributing to the decomposition of organic residues (Etesami *et al.*, 2017).

KSB are commonly regarded as plant growth-promoting rhizobacteria (PGPR), which improve plant growth through nutrient solubilization, phytohormone production, and improved nutrient acquisition (Numan *et al.*, 2018). Several rhizobacterial genera such as *Niallia circulans*, *Paenibacillus edaphicus*, *Paenibacillus mucilaginosus*, and nitrogen-fixing rhizobacteria (NFR) have been reported to exhibit K-solubilizing activity (Hu *et al.*, 2018). The utilization of KSB as biofertilizer agents has been proposed as an environmentally friendly strategy to enhance soil fertility and support sustainable agricultural systems. The commercialization of K-solubilizing microorganisms may reduce dependence on synthetic fertilizers by improving the mobilization of insoluble-K reserves in soil, thereby increasing the availability of exchangeable K for plant uptake and improving crop productivity (Olaniyan *et al.*, 2022; Elita *et al.*, 2024).

Rice is a major staple crop developed extensively in tropical agroecosystems, where soil nutrient management remains a critical challenge. Indigenous rhizosphere microorganisms adapted to local soil and climatic conditions may possess distinct functional capacities compared to strains reported from other regions. Earlier studies have shown that KSB isolated from rice rhizosphere demonstrate valuable traits such as K solubilization and indole-3-acetic acid (IAA) production (Khanghahi *et al.*, 2018). However, exploration of KSB diversity associated with different local rice varieties in Lima Pulu Kota Regency has not been comprehensively reported. Understanding the rhizobacterial diversity may provide valuable insights into the microbial resource potential that can be further developed as a location-specific biofertilizer.

Therefore, this study aimed to isolate and characterize KSB from the rhizosphere of several rice varieties in Lima Pulu Kota District, and to determine their morphological characteristics, K-solubilizing activity, as well as molecular identity based on 16S rRNA gene analysis. The findings are expected to contribute to the identification of indigenous bacterial strains with potential application in sustainable rice production systems.

MATERIALS AND METHODS

Sampling

Soil samples were collected from the rhizosphere of seven rice varieties cultivated in different districts of Lima Pulu Kota Regency, West Sumatra, Indonesia. The Bujang Marantau (KBM) variety was sampled in Taeh Baruah Village, Payakumbuh District, while the Mundam (KMM) variety was collected from Sipingai Village, Guguak District. The Cilalek (KCK) variety was obtained from Anding Village, Suliki District, and the Randah Kuning (KRK) variety from Piobang Village, Payakumbuh District. Soil from the Saganggam Panuah (KSP) variety was collected in Tujuh Koto Talago Village, Guguak District, whereas the Keriting Putih (KKP) variety was sampled in Guguak 8 Koto Village, Mungka District. The Junjuang (KJG) variety was collected from Andaleh Baliak Bukit Village, Luak District. The corresponding rhizosphere soil samples were designated as S-KBM, S-KMM, S-KCK, S-KRK, S-KSP, S-KKP, and S-KJG, respectively. Soil samples were randomly collected from the rhizosphere of healthy rice plants using a soil auger to a depth of approximately 10 cm. Approximately 3 kg of rhizosphere soil was collected from each sampling site. The soil samples were placed in plastic bags and stored in a refrigerator until use. All samples were air-dried before further analysis. Approximately 100 g of

each air-dried soil sample was then randomly subsampled for subsequent microbiological analysis. The experiments were conducted at the Microbiology Laboratory of the Politeknik Pertanian Negeri Payakumbuh.

Enumeration of total culturable heterotrophic bacterial populations

Ten grams of dried soil from each sample were suspended in 90 mL of 0.85% physiological saline and homogenized using a shaker. Serial dilutions were prepared up to 10⁻⁶. A 0.1 mL aliquot from the appropriate dilution was spread onto nutrient agar plates and incubated at 30°C for 24–48 hr. Colony-forming units (CFU) were counted and expressed as CFU per gram of soil. Following enumeration, one morphologically distinct colony from each sample was randomly selected and subcultured repeatedly to obtain a pure isolate for further characterization. The bacterial isolates obtained from rhizosphere soil samples S-KBM to S-KJG were designated as KBM to KJG, respectively. Pure isolates were maintained at 4°C for further analysis.

Morphological characterization of bacterial isolates

Morphological characterization was performed on pure colonies obtained. Colony morphology was observed macroscopically and microscopically based on colony shape, surface, texture, pigmentation, and margin, as well as Gram staining.

Molecular identification of K-solubilizing bacteria

The seven bacterial isolates were subjected to 16S rRNA gene sequencing at the Biotech Center Laboratory of IPB for molecular identification via polymerase chain reaction (PCR) technique (Capuccino & Sherman 2002; Tamura *et al.*, 2021). PCR amplification was performed using universal primers 27F (5'-AGAGTTTGATCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The 50 µL PCR reaction mixture contained 1.0 µL genomic DNA (20 ng µL⁻¹), 5.0 µL 10× buffer, 1.0 µL Taq polymerase (5 U µL⁻¹), 1.0 µL dNTP mix (10 mM), 1.5 µL of each primer (10 µM), and sterile distilled water. The PCR amplification protocol involved initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min 30 sec, and a final extension at 72°C for 7 min. After the reaction, 3 µL of PCR product was analyzed by 1% agarose gel electrophoresis to verify the amplified fragments. PCR products were then purified using the AxyPrep DNA Gel Recovery Kit (Axygen Biosciences, Union City, CA, USA). Purified PCR products from each strain were sequenced using an ABI3730-XL sequencer. Sequence alignment was performed using the NCBI BLAST tool to compare the sequences with the NCBI 16S database, identifying species with the highest sequence similarity to the test specimens. A phylogenetic tree was constructed using MEGA 11 software (Tamura *et al.*, 2021).

K-solubilizing activity on solid medium

A pure colony of each bacterial isolate was spotted onto Aleksandrov agar medium containing (per liter): 5 g glucose, 0.005 g MgSO₄, 0.005 g FeCl₃, 0.1 g CaCO₃, 2 g K source (K-feldspar), 2 g calcium phosphate, and 20 g agar (Meerna *et al.*, 2015). Plates were incubated at 30°C for 2–3 days, and their growth as well as the presence of a clear halo was observed. The dissolution index (DI) was calculated using Equation 1.

$$DI = \frac{\text{Diameter of the clear zone (a)}}{\text{Diameter of the colony (b)}}$$

Equation 1

K-solubilizing activity in liquid medium

Liquid Aleksandrov medium supplemented with K-feldspar powder was used to assess K release capacity. Fifty mL of medium was dispensed into 150 mL flasks and sterilized at 121°C for 30 min. Bacterial cultures were grown in nutrient broth to approximately 10⁸ CFU mL⁻¹ (OD₆₀₀ ≈ 0.5) by incubating overnight at 30°C with shaking at 150 rpm. Each flask was inoculated with 1 mL of bacterial

Table 1. Phenotypic traits, K solubilization efficiency, and molecular identification of the rhizosphere bacterial isolates. Values in parentheses indicate the percentage identity of the 16S rRNA gene sequence to the closest reference sequence in the GenBank database.

Isolate ID	Colony Morphology (Surface, Shape, Texture, Edge, Color)	Gram Stain	K-solubilizing activity	Diameter of clear zone (mm)	Diameter of bacterial colony (mm)	Dissolution Index (DI)	Identified species & Similarity (%)	GenBank accession number
KBM	Rough, circular, Butyrous, entire, white	+	+	3.5 ± 0.21	2.1 ± 0.06	1.7	<i>Lysinibacillus xylanilyticus</i> (100%)	PZ054079
KMM	Smooth, circular, mucoid, entire, cream	+	+	4.0 ± 0.21	2.3 ± 0.06	1.7	<i>Bacillus velezensis</i> (100%)	PZ054078
KCK	Smooth, circular, butyrous, entire, white	–	+	3.8 ± 0.10	2.2 ± 0.15	1.7	<i>Acinetobacter johnsonii</i> (100%)	PZ054080
KRK	Smooth, circular, butyrous, entire, cream	+	+	3.7 ± 0.12	2.8 ± 0.12	1.3	<i>Pseudarthrobacter phenanthrenivorans</i> (100%)	PZ054082
KSP	Smooth, circular, mucoid, entire, cream	–	+	3.7 ± 0.15	1.7 ± 0.15	2.2	<i>Klebsiella aerogenes</i> (100%)	PZ054084
KKP	Smooth, circular, mucoid, entire, cream	+	+	4.0 ± 0.21	2.1 ± 0.15	1.9	<i>Paenarthrobacter nicotinovorans</i> (100%)	PZ054083
KJG	Smooth, circular, butyrous, entire, cream	–	+	3.5 ± 0.12	1.9 ± 0.15	1.8	<i>Enterobacter cloacae</i> (100%)	PZ054081

suspension. Five replicates per isolate were incubated at 28 ± 1°C with shaking at 150 rpm for 7 days. Cultures were centrifuged at 5000 rpm for 10 min. Soluble K in the supernatant was quantified using flame spectrophotometry. Potassium chloride was used to prepare the standard calibration curve of 0.5 mg – 5.0 mg/L (Martin & Sparks, 1985).

Statistical analysis

Data were expressed as mean ± standard deviation and were analyzed using SPSS (Statistical Package for the Social Sciences) version 26.0. One-way ANOVA was carried out, and the significant difference was indicated by a probability value of less than 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

Total culturable heterotrophic bacterial population in the rice rhizosphere

Total culturable heterotrophic bacterial populations for each rhizosphere were determined using nutrient agar. The colony counts varied among samples, with KRK exhibiting the greatest number significantly, while KBM samples showed the lowest abundance ($p < 0.05$) (Figure 1). From each sample, only one morphologically distinct colony was randomly selected and subcultured to obtain pure isolates. The pure isolates were further phenotypically characterized based on colony morphology.

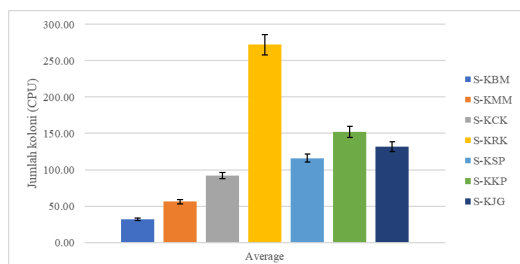


Fig. 1. Enumeration of total culturable heterotrophic bacterial populations from rhizosphere samples plated on nutrient agar. Error bars represent standard deviation. Significant differences were observed among samples ($p < 0.05$).

The CFU calculations obtained from nutrient agar generally reflect the abundance of microorganisms within the rhizosphere but do not specifically indicate KSB populations. A single distinct colony was initially chosen at random from each sample to allow more appropriate functional screening and identification (Etesami *et al.*, 2017; Saheewala *et al.*, 2023). In cases where the primary selected colony did not exhibit K-solubilizing potential, alternative morphologically distinct colonies from the same sample were screened as replacements, although this approach may still not fully represent the complete diversity of KSB present in the rhizosphere sample.

Characterization and taxonomic identification of K-solubilizing bacterial isolates

A comprehensive assessment encompassing the colonial morphology, Gram staining reactions, qualitative K solubilization performance, and molecular taxonomic identities of the seven isolates was conducted, with the consolidated data summarized in Table 1.

A total of seven presumptive KSB isolates were successfully isolated from the rhizosphere of different rice varieties developed in Lima Pulu Kota District, labelled as KBM, KMM, KCK, KJG, KRK, KKP and KSP, respectively, following the origin of rhizosphere sampling. The morphological characterization among isolates was summarized in Table 1. Colonies were butyrous and mucoid in texture and exhibited white to cream pigmentation. Phenotypic analysis through Gram staining reactions revealed that the seven KSB isolates consisted of four Gram-positive and three Gram-negative bacteria, which is fully consistent with their molecular taxonomic identities (Table 1). Specifically, isolates KBM (*L. xylanilyticus*), KMM (*B. velezensis*), KRK (*Ps. phenanthrenivorans*), and KKP (*Pa. nicotinovorans*) were confirmed as Gram-positive (+). Conversely, isolates KCK (*A. johnsonii*), KSP (*K. aerogenes*), and KJG (*E. cloacae*) exhibited true Gram-negative (–) characteristics.

Preliminary characterization of bacterial isolates generally began with observation of colony morphology, which provides data on phenotypic traits such as colony shape, surface, texture, margin, and pigmentation (Saheewala *et al.*, 2023). However, the macroscopic observations may vary depending on growth condition parameters, age of pure cultures, and other strain-specific differences,

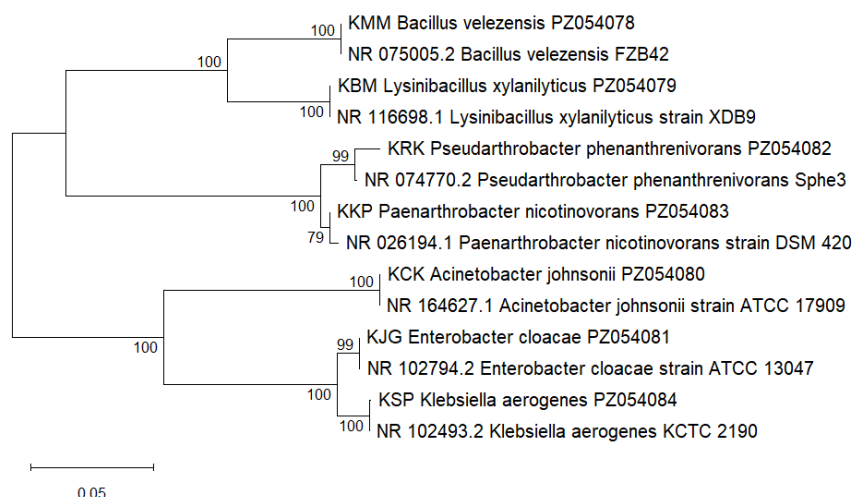


Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the seven bacterial isolates and their closely related reference sequences retrieved from GenBank.



Fig. 3. Representative clear zone formation of K-solubilizing bacteria on Aleksandrov agar (KKP isolate shown).

and may not regularly reflect taxonomic identity (Pérez-Pérez *et al.*, 2021). Common investigations on the isolation of KSB typically use colony morphology as initial descriptive data, followed by additional biochemical and molecular analysis, particularly 16S rRNA sequencing, for more accurate taxonomic classification (Etesami *et al.*, 2017).

Molecular identification of K-solubilizing bacteria

The bacterial isolates were genotypically identified through 16S rRNA gene sequencing. Sequence analysis revealed that the isolates matched the reference sequences available in the GenBank database with 100% sequence identity, supporting species-level identification. Their taxonomic placement was further validated by conducting phylogenetic analysis using the aligned 16S rRNA gene sequences of the isolates together with closely related reference sequences retrieved from GenBank. The results confirmed that each isolate clustered with its respective reference strains, forming distinct clades within the corresponding genera (Figure 2). All verified 16S rRNA sequences were deposited into the GenBank database, with their respective accession numbers listed in Table 1.

K-solubilizing activity on solid medium

The K-dissolving ability of the isolates was qualitatively evaluated by the formation of clear halos surrounding the colonies on Aleksandrov agar. Visible dissolution halos were observed on all isolates on Aleksandrov agar, confirming their ability to solubilize insoluble potassium sources. All seven isolates showed positive K solubilization activity, as indicated by the formation of clear zones (Table 1).

The diameter of the solubilization zone (a) and colony diameter (b) were measured to determine the dissolution index (DI). The highest DI value was recorded for the KSP isolate, followed by KKP and KJG, indicating a stronger K-solubilizing efficiency. In contrast, KRK showed the lowest DI value. A representative clear zone is shown in Figure 3 (KKP isolate).

Several studies have reported that rhizosphere soils shelter diverse KSB, including *Klebsiella variicola* and *Enterobacter cloacae* isolated from the tobacco rhizosphere (Zhang & Kong, 2024), as well as efficient strains of *Pseudomonas* spp., *Bacillus* spp., and *Burkholderia* spp. from tea plantation soils (Bagyalakshmi *et al.*, 2017). These bacteria can dissolve K from insoluble sources such as K-feldspar and montmorillonite in both solid and liquid media. Similarly, all isolates obtained in this study demonstrated visible halo formation on Aleksandrov agar and were capable of solubilizing K in broth culture. The presence of KSB within the rice rhizosphere highlights its probable responsibility in K and nutrient cycling within the ecosystem. Previous studies have also documented that soil inoculation with KSB enhances K release and availability (Khanghahi *et al.*, 2018; Sarikhani *et al.*, 2018).

K-solubilizing activity in liquid medium

The potassium-solubilizing activity of the seven KSB strains in liquid Aleksandrov medium supplemented with K-feldspar powder was determined. All isolates were capable of releasing soluble potassium into the medium, with values ranging from 0.95 mg L⁻¹

to 1.95 mg L⁻¹. The KSP isolate displayed the highest potassium release (1.95 mg L⁻¹), followed by KKP (1.55 mg L⁻¹) and KJG (1.35 mg L⁻¹). Slightly lower solubilization activity was observed in KCK, KMM, and KBM (1.25 mg L⁻¹), whereas KRK showed the lowest potassium release (0.95 mg L⁻¹). One-way ANOVA revealed that all isolates were significantly different from each other ($p < 0.05$), except for KCK, KMM, and KBM, which were not significantly different among themselves. This observation is consistent with the earlier statement that total culturable counts do not necessarily represent functional KSB populations, emphasizing that microbial abundance does not directly correlate with potassium-solubilizing efficiency.

A number of studies on plant growth-promoting rhizobacteria have generally focused more on nitrogen and phosphorus, which may be due to the more clearly revealed success of nitrogen-fixing and phosphate-solubilizing microorganisms (Rodríguez-Caballero *et al.*, 2017; Stefanowicz *et al.*, 2017). However, K is the most abundant cation in plant cells and the second most abundant nutrient in leaves after nitrogen. In the present study, KSB isolated from the rice rhizosphere exhibited measurable solubilization activity in liquid medium, with the KSP isolate showing the highest K release (1.95 mg L⁻¹). This value is comparable to or slightly higher than values reported in previous studies (Sun *et al.*, 2020), indicating the strong solubilization potential of the selected isolate.

In rice cultivation, K fertilization is often restricted due to its relatively high cost and limited affordability for farmers. Yet, K plays a crucial role in plant development, growth, and metabolic regulation, particularly during the generative phase, contributing to grain development and filling (Sindhu *et al.*, 2016). K deficiency may lead to weakened stems and increased susceptibility to lodging under strong wind conditions, which can significantly reduce yield. Therefore, the utilization of KSB represents a promising alternative strategy to enhance K availability sustainably and cost-effectively.

In this study, seven KSB isolates were successfully obtained from the rhizosphere of rice plants cultivated in Lima Puluh Kota Regency. To our knowledge, this is amongst the pioneer reports relating to the isolation of KSB from rice rhizosphere soils in this locality, highlighting the region as a potential reservoir of agriculturally beneficial microorganisms. Species-level identification through 16S rRNA sequencing confirmed their taxonomic affiliation with previously reported K-solubilizing genera. The combination of phenotypic characterization, functional screening, and molecular identification strengthens the reliability of the findings. The identified isolates comprised both Gram-positive (*Lysinibacillus*, *Bacillus*, *Pseudarthrobacter*, *Paenarthrobacter*) and Gram-negative genera (*Acinetobacter*, *Klebsiella*, *Enterobacter*), suggesting taxonomic diversity of KSB in the rice rhizosphere. The isolation of phylogenetically distinct species across sampling sites suggests spatial heterogeneity in rhizosphere microbial communities. This observation suggests that local soil conditions and plant-microbe interactions play important roles in the diversity of KSB.

Consistent with previous work, isolates affiliated with *Bacillus*, *Enterobacter*, and other genera have demonstrated the ability to contribute to K solubilization and enhanced nutrient availability in soil systems (Etesami *et al.*, 2017; Saheewala *et al.*, 2023). Members of the genus *Bacillus* have also been reported to solubilize K-bearing minerals and improve plant uptake of K via the production of organic acids and other metabolic activities (Sheng & He, 2006). Besides that, rhizospheric KSB have been documented for their role in raising K availability and improving plant nutrition under agricultural conditions (Meena *et al.*, 2014). Interestingly, in this study, isolates affiliated with the *Bacillus* and *Enterobacter* genera demonstrated higher K release compared to other taxa, suggesting possible genus-specific differences in solubilization efficiency.

A key novelty of this study lies in the identification of less commonly reported KSB species. While the Gram-negative isolate KSP (*K. aerogenes*) exhibited the highest dissolution index (DI), the Gram-positive isolate KKP (*Pa. nicotinovorans*) demonstrated the second-highest K-solubilization efficiency (Table 1). In existing literature, *Ps. phenanthrenivorans* and *Pa. nicotinovorans* are almost exclusively cited for their specialized metabolic roles in degrading polycyclic aromatic hydrocarbons or nicotine, with virtually no documentation regarding their mineral-weathering capabilities. The finding that *Ps. phenanthrenivorans* and *Pa. nicotinovorans* exhibited K-solubilization efficiencies comparable to those of the fast-growing enterobacterial isolate extends the current knowledge of potassium-solubilizing bacteria. Although several strains of *Pa. nicotinovorans* have previously been reported to possess plant growth-promoting traits (Jiang *et al.*, 2022), published evidence specifically describing the potassium-solubilizing activity of *Ps. phenanthrenivorans* and *Pa. nicotinovorans* remains very limited.

The ultimate agricultural application of introducing K-solubilizing bacteria (KSB) into the rhizosphere is to enhance crop growth and yield by overcoming K deficiencies. The KSB strains identified in this study—spanning the genera *Bacillus*, *Lysinibacillus*, *Pseudarthrobacter*, *Paenarthrobacter*, *Enterobacter*, *Klebsiella*, and *Acinetobacter*—have been widely documented as potent plant growth-promoting bacteria (PGPB) in various staple crops, particularly paddy rice (*Oryza sativa*). For instance, *Bacillus velezensis* (isolate KMM) and *Lysinibacillus* species (isolate KBM) are well-known for their robust spore-forming nature and multifunctional PGPR traits, including the secretion of phytohormones like indole-3-acetic acid (IAA) and siderophores, which collectively trigger root elongation and enhance nutrient uptake efficiency (Ahmad *et al.*, 2021; Shah *et al.*, 2022). Furthermore, *Pseudarthrobacter* and *Paenarthrobacter* species (isolates KRK & KKP) contribute significantly to soil health restoration and phosphorus-potassium mobilization, leading to enhanced chlorophyll content and grain yield under intensive cultivation systems (Singh *et al.*, 2020). Although the Gram-negative isolates such as *Enterobacter cloacae* (KJG), *K. aerogenes* (KSP), and *A. johnsonii* (KCK) require stringent biosafety assessments before open-field application due to their opportunistic nature, their literature-proven capacity to drastically accelerate mineral dissolution and vegetative growth in cereal crops underlines their high agronomic value as biofertilizer candidates (Nadeem *et al.*, 2014; Suman *et al.*, 2022). Consequently, the inoculation of these multi-trait PGPB strains shows promise for sustainable rice production by reducing reliance on chemical K fertilizers.

The highly efficient K release by this *Paenarthrobacter* strain is primarily driven by the active production of low-molecular-weight organic acids, such as citric and oxalic acids. These acids simultaneously lower the micro-environmental pH and chelate structural cations (Al³⁺ and Fe³⁺) from insoluble potassium aluminosilicates, effectively liberating free K⁺ ions into the solution (Meena *et al.*, 2016). Given that *Paenarthrobacter* species are ubiquitous, hardy soil actinobacteria capable of surviving fluctuating environmental stresses, this strain represents a far more sustainable and biosafe biofertilizer candidate for agricultural fields than the opportunistic *Klebsiella* strain. Overall, this study contributes to a broader understanding of K solubilization traits among rhizosphere bacteria and provides a theoretical foundation for the future development and application of these isolates as potential biofertilizers.

CONCLUSION

Recent research has shown that the rhizosphere of rice plants has the potential to become a breeding ground for K-solubilizing bacteria that can be utilized for the decomposition of K minerals, thereby increasing their bioavailability in rice fields. The identified KSB strains exhibited measurable capacity for K mobilization in

both solid and liquid media. Seven strains of KSB were identified through phenotypic characterization and 16S rRNA gene sequencing, confirming their taxonomic affiliation at the species level. The KKP isolate of the genus *Pa. nicotinovorans* exhibited the second-highest K solubilization index, as observed through the formation of clear zones on Aleksandrov agar, representing a Gram-positive genus. *Ps. phenanthrenivorans* and *Pa. nicotinovorans* have been almost exclusively cited for their specialized metabolic roles in degrading polycyclic aromatic hydrocarbons or nicotine, with virtually no documentation regarding their mineral weathering capabilities. This finding suggests that *Ps. phenanthrenivorans* and *Pa. nicotinovorans* may possess promising K-solubilizing potential comparable to that of commonly reported KSB species. Further studies are essential to determine the optimal environmental conditions influencing KSB activity, such as soil pH and content of organic matter. Additionally, field evaluations are necessary to assess their effectiveness and stability as biofertilizer candidates for sustainable rice cultivation.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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