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Isolation, screening and characterization of potassium solubilizing bacteria from rice rhizosphere

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ABSTRACT

As the third crucial nutrient, potassium (K) is essential for plant growth and development. However, 98% of K in soil is insoluble, with only 2% being soluble. The ability of microorganisms to solubilize K from bound minerals and convert it into forms accessible to plants is called potassium-solubilizing bacteria (KSB). This study aimed to isolate and characterize KSB from rice rhizospheric soil samples collected from Durg, Chhattisgarh, India, ultimately selecting three highly efficient isolates (R3-6, R3-8, R4-10) based on their significant K-solubilization zones, with R3-8 demonstrating the highest efficiency (D/d ratio 2.3 cm). Morphological and biochemical analyses revealed all isolates to be Gram-positive with similar general colony characteristics and consistent positive results for indole, MR, VP, and citrate, but differentiated by traits like R3-8's motility, spore formation, and raised colony elevation, R4-10's rod shape, and R3-6's unique ability to hydrolyze gelatin. Molecular identification confirmed R3-8 as Bacillus aerius, underscoring the potential of these diverse KSB to enhance plant nutrition and contribute to sustainable agricultural practices.

Key Words: Solubilization, Potassium, Agriculture, Soil Fertility, Nutrition

INTRODUCTION

K-ions are readily available in mineral-rich soils with a high capacity to adsorb and retain nutrients (Prajapati and Modi, 2012). Potassium (K) alongside nitrogen (N) and phosphorus (P), is a vital macronutrient for plants. It plays a crucial role in numerous biological processes, including protein synthesis, photosynthesis, enzyme activation, and enhancing the plant's resistance to diseases, drought, and cold stress (Das and Pradhan, 2016, Dotaniya *et al.,* 2016). However, only a small fraction, approximately 1-2%, of the total potassium in the soil is readily accessible to plants. The remaining 98% is tightly bound to mineral structures like mica, orthoclase, and lignite, making it unavailable for plant uptake (Meena *et al.,* 2016). Certain beneficial microorganisms residing in the rhizosphere, the soil region surrounding plant roots, play a crucial role in solubilizing and releasing nutrients, such as K, bound to mineral particles, making them accessible to plants (Priydarshani and Muthukumar, 2016). Bacteria like *Burkholderia, Pseudomonas*, and *Bacillus* have been identified as K-solubilizers due to their mineralization capabilities, earning them the designation of KSB (Sharma *et al.,* 2016). Soil K levels are declining due to erosion, water runoff, leaching, and nutrient depletion. Soil infertility caused by unbalanced chemical fertilizer application can lead to insufficient K-availability, negatively impacting plant growth, yield, and crop development. This necessitates using alternative sources to mitigate these losses (Teotia *et al.,* 2016). KSB can rapidly solubilize potassium-bearing minerals, making the bound potassium accessible to plants. This microbial intervention helps conserve existing mineral resources but reduces the environmental risks of excessive chemical fertilizers (Zahedi, 2016). These bacteria produce organic acids, polysaccharides, siderophores and complexolysis to solubilize K-bearing minerals and ensure their survival in soil (Etesami *et al.,* 2017; Sattar *et al.,* 2018). A higher concentration of KSB in the rhizosphere can significantly enhance crop quality and productivity by increasing potassium availability to plants, leading to improved plant growth, nutrient uptake, and overall yield (Rajavat *et al.,* 2019). Leveraging KSB as biofertilizers can significantly boost crop growth and development (Pandey *et al.,* 2020). Therefore, a comprehensive understanding of KSB is crucial in addressing the pressing challenges facing sustainable agriculture, including soil degradation, nutrient deficiency, and the need to reduce reliance on chemical fertilizers (Sun *et al.,* 2020). *Bacillus cereus* when applied as a

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potassium biofertilizer, demonstrated the most significant positive impact on potato growth in field trials, as reported by (Ali *et al.,* 2020). *Paenibacillus mucilaginosus* as KSB was isolated from an apple orchard and subsequently studied for its impact on the growth of apple seedlings, as reported by (Hui *et al.,* 2020). The significant effect of *Klebsiella oxytoca* on maize plant growth was investigated by (Imran *et al.,* 2020). The growth-promoting effects of *Bacillus mucilaginosus* on garlic were evaluated over two growing seasons, as reported by (Mounir *et al.,* 2020). KSB reduces dependence on chemical fertilizers by sustaining soil fertility in an environmentally friendly manner, a feature not possessed by chemical fertilizers. Therefore, KSB offers a promising solution for enhancing plant growth and increasing crop productivity (Pandey *et al.,* 2020).

The primary aim of this study was to isolate and characterize a potassium-solubilizing bacterial strain from rice rhizosphere soil and assess its potential to enhance potassium availability for crop growth. This study underscores the potential of Bacillus aerius R3-8 as a biofertilizer that can improve potassium availability in soil, thereby enhancing crop growth and yield. This study is the first to report the isolation and characterization of Bacillus aerius R3-8 from rice rhizosphere soil with significant potassium-solubilizing potential.

MATERIAL AND METHODS

Sample Collection: Rhizospheric soil samples were collected from rice rhizosphere from four cropland areas of Patan (Latitude: 21.0358˚N and Longitude: 81.5456˚), Umarpoti (Latitude: 20.8625˚N and Longitude: 81.4451˚E), Utai (Latitude: 21.1186˚N and Longitude: 81.3803˚E) and Purai (Latitude: 21.1123˚N and Longitude: 81.3468˚E) Durg, Chhattisgarh, India. During the kharif season, around twenty rice rhizospheric soil samples (0-9” depth) were collected in sterilized plastic zip lock bags and brought to the laboratory of St. Thomas College, Bhilai, Chhattisgarh.

Adaptation and Enrichment: One gram of soil from each sample was inoculated in 10 ml Aleksandrow broth containing potassium aluminum silicate, which gives the insoluble potassium source, and kept for one week at room temperature. KSB needs enrichment for improving or enhancing the quality of solubilization and making it more fit for existence or adaptation- the method followed by Prajapati and Modi (2012).

Isolation and Screening: After enrichment, 1ml of enriched soil sample was diluted up to 10^{-5} suspension; each dilution was inoculated on Aleksandrow agar medium, constituted 0.50g $MgSO_4$, 0.10g $CaCO_3$, 2.00g Potassium aluminium silicate, 5.00g glucose, 0.005g $FeCl_3$, 2.00g Ca_3 $(PO_4)_2$, 20g Agar, pH= 7.2, suspended in 1L distilled water and incubated at 30°c for up to 7 days. After the incubation period, potent KSB exhibited zone around the colony indicated solubilization was measured by the formula of D/d ratio: Diameter of zone of clearance (D) / diameter of the growth of the organism (d) were calculated followed by Kammar *et al.* (2016); Prajapati and Modi (2012).

Characterization of Potent KSB: The isolates were characterized using different morphological, biochemical, physiological and other activity tests. Methods followed by Aneja KR, 2017, 5e.

Morphological characterization: The selected isolates of KSB were cultured on Aleksandrow agar medium. Colony characteristics such as shape, forms, elevation, margin, color, density and consistency were examined, gram-staining; motility test and endospore staining were carried out for the isolates.

Biochemical characterization: The potent KSB was characterized by some biochemical parameters, such as the Indole test, methyl red test, Voges-Proskauer test, Citrate utilization test, catalase test, Nitrate reduction test, Starch hydrolysis test, Urease test and Gelatin hydrolysis test.

Molecular Identification and Phylogenetic Analysis: The pure culture of the selected isolate (R3-8) was sent to the National Collection of Industrial Microorganisms (NCIM) in Pune, Maharashtra, for sequencing. 16S rRNA gene sequencing was compared with the database at the Gene Bank using the BLAST-N search program in the National Center Biotechnology

Information (NCBI). The phylogenetic analysis was done using MEGA11 software for an evolutionary tree generated by neighbour-joining method.

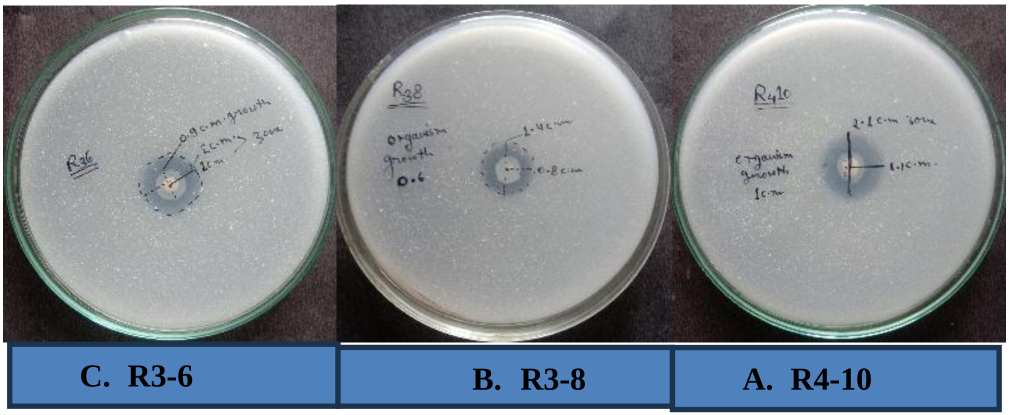
OBSERVATION AND RESULTS

Isolation and Screening: A total of 99 isolates were selected from the twenty rice rhizospheric soil samples based on light zones and clear zones that became visible around the colonies. Following the initial selection, a secondary screening was performed to evaluate the efficacy of the bacterial isolates. In this phase, ten isolates were selected, and the final selection was the rapidity of visible zone formation within approximately seven days of incubation and a total of three isolates were selected (R3-6, R3-8 and R4-10).

Table 1: Zone of solubilization

S.No.	Isolates	Diameter of zone of the clearance (D), average value in cm	Diameter of Growth of the organism (d), average value in cm	(D/d) Ratio
		Rice Rhizospheric isolates		
2.	R3-6	2±0.5	0.9±0.03	2.2
3.	R3-8	1.4±0.05	0.6±0.03	2.3
4.	R4-10	2.1±0.03	1±0	2.1

Description: From that isolates, R3-8 exhibited the highest D/d ratio with 2.3 centimeter, as highest solubilization



C. R3-6 B. R3-8 A. R4-10

Figure 1: (A, B, C) Solubilization by KSB strain on Aleksandrow medium

Morphological and Biochemical Characterization: Three efficient isolates R3-6, R3-8, and R4-10 were characterized based on their colony morphology and cellular properties. All three isolates were Gram-positive and produced circular, white, opaque, mucoid colonies with entire margins. However, they exhibited distinct differences: R3-8 was unique among the three for being motile and capable of spore formation, and it also had a "raised" colony elevation. Most notably, while R3-6 and R3-8 were round-shaped (cocci), R4-10 was distinctly rod-shaped (bacilli). All three isolates demonstrated positive results for Indole, Methyl Red (MR), Voges-Proskauer (VP), and Citrate Utilization tests, while consistently testing negative for Catalase and Starch Hydrolysis, and showing variable results for Nitrate Reduction. Key distinctions emerged in the Urease and Gelatin Hydrolysis tests: R3-6 and R3-8 were positive for urease, whereas R4-10 showed a variable urease reaction, and most significantly, only R3-6 exhibited positive gelatin hydrolysis, indicating its ability to break down gelatin, unlike R3-8 and R4-10.

Table 2: Colony and morphological characteristics of five efficient isolates

S.N.	Isolates	R3-6	R3-8	R4-10
1.	Forms	Circular	Circular	Circular
2.	Elevation	Convex	Raised	Convex
3.	Margin	Entire	Entire	Entire
4.	Colour	White	White	White
5.	Density	Opaque	Opaque	Opaque
6.	Consistency	Mucoid	Mucoid	Mucoid
7.	Motility	-	+	-
8.	Type	Gm+ve	Gm+ve	Gm+ve
9.	Shape	Round	Round	Rods
10.	Spore Formation	-	+	-

Description: All the isolates are positive in gram staining (+ as positive and – as negative)

Table 03: Biochemical tests

S.N.	Isolates	R3-6	R3-8	R4-10
1.	Indole	+	+	+
2.	MR	+	+	+
3.	VP	+	+	+
4.	Citrate Utilization	+	+	+
5.	Catalase	-	-	-
6.	Nitrate reduction	+/-	+/-	+/-
7.	Starch hydrolysis	-	-	-
8.	Urease test	+	+	+/-
9.	Gelatin hydrolysis	+	-	-

Description: positive reaction (+), negative reaction (-), weakly positive (+/-)

Molecular Identification and Phylogenetic Analysis: As a result of their prominent potassium solubilization activity, isolate R3-8 was selected and sent to the National Collection of Industrial Microorganisms (NCIM), Pune, for amplification and 16S rRNA gene sequenced. The obtained sequences were then aligned and compared with those deposited in the Gene Bank database. Using BLAST-N, the maximum aligned sequences were utilized for identification. The analysis demonstrated that R3-8 exhibited 100% homology with *Bacillus aerius* and submitted to the NCBI gene bank under the accession number.

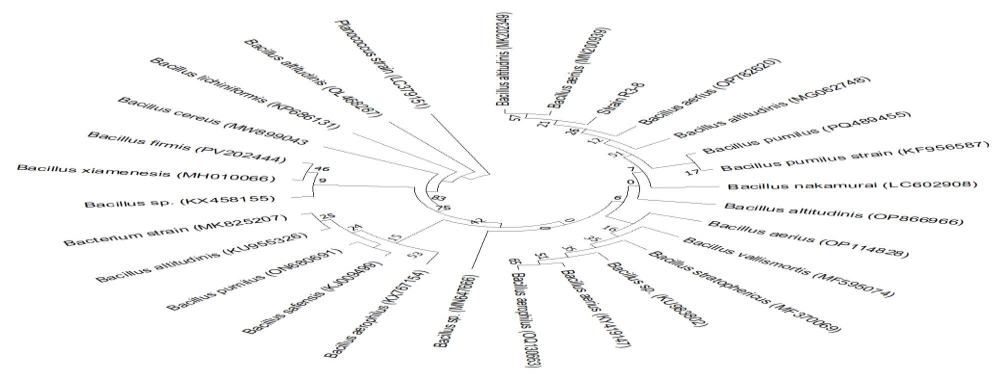


Fig. 2: Evolutionary tree analysis

The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates represents the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 27 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option Evolutionary analyses were conducted in MEGA11.

DISCUSSION

Existing research in the field of potassium-solubilizing bacteria supports the screening process using Aleksandrow agar and the selection of isolates based on the D/d ratio. The selection of the three rice KSB isolates (R3-6, R3-8, and R4-10) with the highest D/d ratios. This approach is supported by Prajapati and Modi (2012), who also used Khandeparker's selection ratio to identify efficient KSB isolates. The comprehensive cultural characterization revealed consistent circular morphology, entire margins, opaque density, and mucoid consistency across all bacterial isolates, suggesting variations in underlying physiological characteristics like cell adhesion, metabolic pathways, or pigment synthesis, as similarly reported in studies differentiating bacterial species Sagar *et al.* (2017) and Fatharani and Rahayu (2018). The motility test revealed that isolate R3-8 was motile, while the other were non-motile, in accordance with Zarjani (2013) and Madigan *et al.* (2018). The Gram staining and cell morphology results provided crucial initial classification: isolate all isolates are Gram-positive. Similar findings are also reported by Beveridge (1999), Verma *et al.* (2017). The endospore test uniquely identified isolate R3-8 as an endospore-former, distinguishable by its bright green spores. This trait, characteristic of genera like *Bacillus* and *Clostridium* (Setlow, 1995), findings are in conformity to Saha *et al.* 2016 and Bagyalakshmi *et al.* (2017). The biochemical tests collectively reveal distinct metabolic profiles among all isolates. All isolates universally perform Indole production and Methyl Red (MR) positive reactions, indicating tryptophan metabolism and mixed acid fermentation. R3-6, R3-8, and R4-10 are Voges-Proskauer (VP) and Citrate positive, and Catalase negative, signifying butanediol fermentation and inability to detoxify hydrogen, Urease activity (R3-6, R3-8 positive), and Gelatin hydrolysis (only R3-6 positive) further differentiate these isolates, providing crucial clues for their specific taxonomic identification and ecological roles. The results conform with Hu *et al.* (2006), Kammar *et al.* (2016) and *Kuan* (2016). Molecular identification is supported by other research. The genus *Bacillus* is widely recognized for its ability to solubilize potassium. Several studies have identified various *Bacillus* species as effective KSB. For instance, *Bacillus mucilaginosus* and *Bacillus edaphicus* are mentioned as KSB that can be used as biofertilizers according to Norkina and Pumpyanskaya (1956) and Han *et al.* (2006). One study by Bagyalakshmi *et al.* (2017) isolated *Bacillus subtilis* from wheat rhizosphere and observed its potassium solubilization capabilities. These findings suggest that *Bacillus aerius* isolate (R3-8) likely contributes to plant growth, potentially through potassium solubilization or other mechanisms.

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