ISOLATION AND CHARACTERIZATION OF POTASSIUM SOLUBILIZING BACTERIA (KSB) FROM RHIZOSPHERE SOILS OF RICE AT VEERANAM COMMAND AREA IN CUDDALORE DISTRICT, TAMILNADU

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Abstract- **Potassium is a vital mineral and the second most important macronutrient required by the majority of crops. Potassium sources in soil are ample but most of it being insoluble is unavailable for plant uptake. Potassium deficiency results in poor plant growth and development which eventually reduces crop output. Microorganisms have the capability to release soluble potassium from potassium-bearing minerals, therefore making the unavailable potassium available to the plants in soluble form. For this investigation, rhizosphere soil samples from the Rice crop were gathered from 15 different locations at Veeranam command area in Cuddalore district, Tamil Nadu. The isolated potassium solubilizing bacteria in rice rhizosphere (KSBR) were tested for their ability to solubilize the insoluble source of potassium (Mica). Mica was used as an insoluble potassium source. The isolates were identified based on morphological and biochemical characteristics. The solubilization zone for potassium ranges from 0.23 to 1.29 cm in diameter. The isolate, KSBR-12 had the maximum solubilization zone at 72 hours after incubation, measuring 1.29 cm in diameter. Also, the strain (KSBR-12) released the highest level of potassium (40.12 µg/ml of broth) at 21 days after incubation in broth containing Mica.**

Keywords: **Potassium solubilizing bacteria, Insoluble potassium source (Mica), Zone of solubilization, Rice rhizosphere soil.**

I. INTRODUCTION

A macronutrient that is essential for plant growth and development is potassium. Potassium (K) plays a vital role in plant growth, resilience to stress, metabolism, development, and reproduction. It is the second most important nutrient required by the majority of crops and its absorption causes an unceasing process of exhaustion of different fractions of this element which is more obvious in low ammonium acetate extractable K soils [5]. The potassium reserves in soil are ample but most of it being insoluble is unavailable for plant uptake. Plants can only directly take up solution K [3]. Despite the abundance of total K in agricultural soils which could reach up to 62 tons ha^{-1} in the surface layer of the soil [18], the quantity of K that is readily available to plants during the crop cycle is frequently found to be inadequate thereby leading to potassium exhaustion and failing to achieve aspired crop yields [6].

Soil consists of four different forms of potassium such as unavailable K (mineral K), available K (soluble K), non-exchangeable K (fixed or trapped K), and exchangeable K (ionic K) [15]. The fixed K in the soil is a reserve source of potassium, while the exchangeable K (ionic K) is readily taken up by the plant's root system and substituted for potassium on the exchange sites. Most soils around the globe are K-deficient [9]. Potassium deficiency decreases the production in natural ecosystems [7] and K deficiency results in poor plant growth and development which eventually reduces crop output [14]. Almost 72 % of Indian agricultural soils were reported to be deficient in potassium [24]. Thus, the crops need to be supplied with soluble potassium fertilization, the demand of which is expected to increase significantly, particularly in developing regions of the world [12].

The process of potassium being released from rock minerals occurs quite slowly. Hence, using a few potential microbes in addition to these insoluble minerals can enhance the rate of release of K. Microorganisms can play an important role in the solubilization, transport, and deposition of metals and minerals in this environment. Among these microorganisms, there is a group of soil bacteria known as plant growth promoting rhizobacteria [4]. PGPR promotes

plant growth directly by either facilitating resource acquisition (nitrogen, phosphorus, and vital minerals) or modulating plant hormone levels and indirectly by decreasing the inhibitory effects of numerous pathogens in the forms of biocontrol agents [17]. In India, the farmers regularly use more quantity of chemical fertilizers for crop production this way Indian soils receive pollutants which lead to pollution and ultimately cause health hazards. In order to avoid environmental pollution, especially soil pollution, most scientists are recommending the use of biofertilizers to replace the use of inorganic fertilizers.

Potassium solubilizing microorganisms (KSMs) have the capability to release soluble K from K-bearing minerals [8] therefore making the unavailable K available to the plants in soluble form [22]. The potassium solubilizing bacteria (KSB) are frequently found in much higher concentrations in the rhizosphere than in the non-rhizospheric soil. Most of them belonged to the *Bacillus*, *Pseudomonas*, *Acidithiobacillus*, *Frateuria*, and *Burkholderia* genus families.

Thus, the present study aimed to isolate and identify soil microorganisms that may solubilize insoluble sources of potassium into soluble forms, which has great importance in crop growth as well as increasing the yield and quality of crops.

II. MATERIALS AND METHODS

The present study was carried out to isolate and characterize potassium solubilizing bacteria from the rhizosphere soils of rice. The screened isolates were subjected to analyse the morphological and biochemical characteristics. Laboratory studies were done to find out the efficient potassium solubilizing bacteria from the rhizosphere soils of rice.

2.1 Collection of soil samples

A total of 15 rhizosphere soil samples had been amassed from rice rhizosphere (5-15 cm depth) collected from 15 locations of Veeranam command area, Cuddalore district, Tamil Nadu. After collection, a portion of every sample was without delay transferred to the laboratory and saved at 4°C for microbial analysis at the same time as the relaxation, a part of the soil samples was shade dried, powdered, and stored to analyze the physical and chemical parameters.

2.2 Isolation of potassium solubilizing bacteria

Potassium solubilizing bacteria were enumerated from the rhizosphere soils of different rice-grown fields by serial dilution plate count technique using Aleksandrov agar medium [10] containing (5 g glucose, 0.005 g $MgSO_4.7H_2O$, 0.1 g $FeCl_3$, 2.0 g $CaCO_3$, 2.0 g calcium phosphate, and 15 g agar/L) 0.1 % mica as insoluble potassium supply which is a selective medium for isolation of potassium solubilizers. The soil samples were diluted up to 10^{-4} dilution by serial dilution technique. One ml of aliquots of the last dilution was plated in a sterile petri dish using Aleksandrov agar medium containing 0.1 % of insoluble potassium source (Mica). The plates were then incubated at room temperature (30 ± 1°C) for 7 days and the colonies exhibiting large clear halo zones were considered to be the potassium solubilizing bacteria [13] and selected. The selected strains were purified by the four-way streak plate method and were preserved on agar slants at 4°C for further study. These 15 most predominant and morphologically high-quality bacterial colonies have been designated as KSBR-1 to KSBR-15.

2.3 Identification and morphological characterization of the bacterial isolates

All the selected isolates were examined for colony morphology, cell shape, margin, elevation, optical density, motility, Gram reaction, and endospore formation as per the standard procedures given by Barthalomew & Mittewer [2] and Anonymous [1].

2.4 Biochemical characterization of the bacterial isolates

Biochemical tests like the H_2S production test, acid production test, methyl red test, starch hydrolysis test, citrate utilization test, catalase test, gelatin hydrolysis test, and Voges-Proskauer test were performed for the isolated strains. Based on the morphological and biochemical characterization, the chosen isolates have been identified up to the genus level.

2.5 Screening of isolates for potassium solubilization under invitro condition

2.5.1 Qualitative estimation for the zone of solubilization

The Aleksandrov medium was prepared by supplementing 0.1% insoluble potassium source viz., mica and autoclaved at 121°C for 20 min. Actively growing cultures of each strain were spot-inoculated (10 µL) onto the agar and plates were incubated at 30°C. The diameter of the clear halo zone was measured with the help of a scale successively after 24 hours, up to 7 days, and expressed in centimetres.

2.5.2 Quantitative estimation of K released from insoluble K-bearing compound (Mica) [11]

The isolates showing solubilization zone on Aleksandrov agar were further examined for their ability to release K from Aleksandrov broth medium (supplemented with 1 % mica). One ml of overnight culture of each isolate was inoculated to 25 ml of Aleksandrov broth [10] in nine replications. The inoculated flasks were incubated for two weeks at 28 ± 2°C. At 7, 14, and 21 days of incubation, the amount of potassium released in the broth was estimated from triplicate flasks at each stage in comparison with a set of uninoculated controls. The broth cultures were centrifuged at 10,000 rpm for 10 minutes in the REMI microcentrifuge to separate the supernatant from the cell growth and insoluble potassium.

The available K content in the supernatant was determined by flame photometry [21]. One ml of the culture supernatant obtained was taken in a 50 ml volumetric flask and using distilled water the volume was made up to 50 ml and then mixed thoroughly. After that, the solution was fed to a flame photometer and compared using standards. Simultaneously, using various concentrations from 2 ppm KCl solution, a standard curve was prepared. Thus, the amount of potassium solubilized by the isolates was determined from the standard curve.

Preparation of KCl solution standard curve

A. Reagents

i. 1N ammonium acetate (neutral in pH)

Ammonium acetate (CH_3COONH_4), 77 g was dissolved in 100 ml of distilled water and the pH adjusted to 7.0.

ii. Standard potassium solutions

1.908 g of potassium chloride was dried at 60°C and dissolved in 1 litre of distilled water. This solution contained 1000 ppm potassium. From this a series of standards 0, 10, 20 up to 100 ppm potassium was prepared and fed to a flame photometer. A standard curve was drawn and the unknowns were calculated.

B. Method

5 g of soil sample was transferred into a polyethylene shaking bottle. Then 25 ml of 1N ammonium acetate extractant was added and the contents were shaken in a mechanical shaker for 5 min. The contents were filtered through Whatman No. 1 filter paper and the filtrate was fed into a flame photometer. The available potassium content was calculated from the standard solution used for calibration.

III. RESULTS AND DISCUSSION

The entire 15 bacterial isolates showed halo zones on potassium solubilizing medium supplemented with 0.1 % mica and only the best performers were selected based on halo zone diameter and were maintained in nutrient agar slants for further utilization. After morphological and biochemical characterization, the isolates were identified up to genus level as out of 15 isolates, seven were *Bacillus* type, six were *Pseudomonas* kind and two isolates belonged to the *Frateuria* genus.

The isolates were named from KSBR-1 to KSBR-15 and were further characterized based on morphological (Table 1) and biochemical analysis (Table 2). Among the screened 15 isolates, the isolate KSBR-12 showed a maximum zone of solubilization with 1.29 cm diameter at 72 hours after incubation followed by KSBR-4 (1.25 cm) and KSBR-2 (1.22 cm). However, the isolate KSBR-6 showed the least solubilization zone of 0.23 cm diameter (Table 3). All 15 isolates significantly increased available potassium in broth containing mica at 7, 14, and 21 DAI. Highest solubilization in broth media was shown by the isolate KSBR-12 with 40.12 µg/ml of broth, followed by KSBR-4 (36.89 µg/ml of broth) and KSBR-7 (32.94 µg/ml of broth) at 21 DAI (Table 4).

Deficiency of this essential element (K) leads to retarded shoot growth, chlorosis, reduced leaf size, increased susceptibility to heat, light, and fungal infections, as well as affects grain yield, pollen formation, root development, water uptake and transport [16]. K deficiency can be observed at the beginning of internodal elongation at the early reproductive stage, which aggravates with time [26].

Similarly, Sheng and He [20] also isolated and characterized the bacteria, *Bacillus mucilaginosus* which is capable of solubilizing two potassium-bearing minerals like feldspar and illite. Hu, Chen and Guo [10] reported that both *Bacillus megatherium* and *B. mucilaginosus* were able to solubilize both rock phosphate and potassium. Thus, the ability of bacteria to release K largely depends on various parameters like the nature of the potassium compounds, carbon source, temperature, pH, and variable concentration of sodium chloride and glucose [19].

Table 1: Morphological characteristics of potassium solubilizing bacterial isolates

Isolates	Colony characters	Gram reaction	Cell shape	Margin	Elevation		Optical density		Motility	Endospore formation
					Slightly raised	Highly raised	Translucent	Opaque		
Bacillus sp.	Creamy white, viscid, small	+	Rod	Smooth elevated	-	-	-	+	Motile	+
Pseudomonas sp.	Light yellowish, viscid, dry	-	Rod	Rough flat	+	-	+	-	Motile	-
Frateuria sp.	Dark yellow to brown, small	-	Rod	Smooth elevated	+	-	-	+	Non motile	-
Bacillus sp.	Creamy whitish, viscid, large	+	Rod	Smooth elevated	-	+	-	+	Motile	+

Pseudomonas sp.	Creamy dirty yellow, irregular	-	Rod	Rough undulated	-	+	-	+	Motile	-

Note: Positive (+); Negative (-)

Table 2: Biochemical characterization of potassium solubilizing bacterial isolates obtained from the rhizosphere soils of rice

Isolate Code	H_2S production test	Acid production test	Methyl red test	Voges -Proskauer test	Starch Hydrolysis test	Urease test	Citrate Utilization	Catalase test	Gelatin Hydrolysis	Tentative Characterization of the Species
KSBR-1, KSBR-5, KSBR-6, KSBR-8, KSBR-10, KSBR-13, KSBR-14	-	++	-	+	+	+	-	+	-	*Bacillus* sp.
KSBR-2, KSBR-3, KSBR-7, KSBR-9, KSBR-11, KSBR-15	+	++	-	-	-	-	+	+	+	*Pseudomonas* sp.
KSBR-12, KSBR-4	+	++	+	+	-	+	+	-	+	*Frateuria* sp.

KSBR- Potassium solubilizing bacteria in Rice rhizosphere
(+) Showed positive response; (++) Fast reaction; (-) Showed negative response

S. No.	Strain	Zone of solubilization (cm)
1	KSBR-6	0.23
2	KSBR-2	1.22
3	KSBR-4	1.25
4	KSBR-12	1.29

Table 3: Zone solubilization by K-solubilizing bacterial isolates

Isolates	Available potassium (µg ml^{-1} broth)			
	Inorganic source-Mica			
	Days after incubation			
	7	14	21	Mean
KSBR-7	22.99	29.21	32.94	28.38
KSBR-4	26.49	31.52	36.89	31.63
KSBR-12	29.55	38.48	40.12	36.05

Table 4: Screening of potassium solubilizing bacterial isolates for their efficiency to solubilize inorganic source of potassium (Mica)

Plate:1 Isolation of potassium solubilizing bacteria from the rhizosphere soils of rice

Plate:2 Potassium solubilization by potassium solubilizing bacterial isolates

Sugumaran and Janarthanam [21] isolated potassium solubilizing bacteria from orthoclase, muscovite, and mica. Among the isolates, *B. mucilaginosus* produced slime in muscovite mica by which it solubilized more potassium. In the present study, the results of all the experiments conclude that *Pseudomonas* sp., *Bacillus* sp., and *Frateuria* sp. were potential in solubilizing the potassium mineral used in this study. The results are in agreement with Yaghoubi Khanghahi, Pirdashti, Rahimian, Nematzadeh and Ghajar Sepanlou [25] who isolated potassium solubilizing bacteria (KSB) that have the potential ability to solubilize potassium from mica viz., *Pantoea agglomerans*, *Rahnella aquatilis* and *Pseudomonas orientalis* from paddy rhizosphere soil and Won *et al*., [23] who isolated *Frateuria* sp. from greenhouse soil samples.

IV. CONCLUSION

The potassium solubilizing bacteria were isolated from the rice rhizosphere soils which are characterized and studied for their K solubilization potential. Out of all the 15 isolates, the isolates KSBR-12 and KSBR-4 showed the highest solubilization both qualitatively and quantitatively. The efficient isolates KSBR-12 and KSBR-4 were morphologically and biochemically identified as *Frateuria* sp. In future studies, these isolates were further screened for their efficiency to produce plant growth promoting substances such as IAA and GA_3, organic acid production, siderophore production, and polysaccharide production, and the effective KSB isolate was molecularly identified by 16S rRNA gene sequencing. Hence, these potassium solubilizing bacterial isolates will be applied alone or in consortium mode which aids in enhancing the potassium uptake of various crops such as cereals, vegetables, flower crops, and medicinal crops while simultaneously enhancing the growth and yield.

REFERENCES:

1. Anonymous (1957). Manual of microbiological methods. McGraw Hill Book Co., New York, U.S.A. p. 315.
2. Barthalomew, J. W. and T. Mittewer (1950). A simplified bacterial strain. *Stain Tech*, *25*, 153.
3. Berde, C. V., S. S. Gawde and V. B. Berde (2021). Potassium solubilization: Mechanism and functional impact on plant growth. *Soil Microbiomes for Sustainable Agriculture: Functional Annotation*, 133-148.
4. Bharathiraja, S. and P. Tholkappian (2011). AM fungal interaction and its beneficial effects on Crossandra infundibuliformis. *International Journal of Current Research*, *3*(6), 100-102.
5. Bhat, M. A., D. Tomar, K. S. Grewal, J. Sahoo and E. A. Dar (2023). Non-exchangeable potassium release and supplying power of soils of Haryana, Northwest India. *Journal of Plant Nutrition*, *46*(17), 4140-4155.

6. Bilias, F., S. Tsigili and N. Barbayiannis (2021). A preliminary evaluation of cation exchange resins as a soil test of potassium availability in soils of northern Greece with different K loadings. *Journal of Soil Science and Plant Nutrition*, *21*, 1004-1012.
7. Chen, Y. H., X. Z. Yang, L. I. Zhuang, X. H. An, Y. Q. Li and C. G. Cheng (2020). Efficiency of potassium-solubilizing Paenibacillus mucilaginosus for the growth of apple seedling. *Journal of Integrative Agriculture*, *19*(10), 2458-2469.
8. Das, I. and M. Pradhan (2016). Potassium-solubilizing microorganisms and their role in enhancing soil fertility and health. *Potassium solubilizing microorganisms for sustainable agriculture*, 281-291.
9. Dhillon, J. S., E. M. Eickhoff, R. W. Mullen and W. R. Raun (2019). World potassium use efficiency in cereal crops. *Agronomy Journal*, *111*(2), 889-896.
10. Hu, X., J. Chen and J. Guo (2006). Two phosphate-and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World journal of Microbiology and Biotechnology*, *22*(9), 983-990.
11. Kammar, S. C., R. C. Gundappagol, S. Shubha, G. P. Santhosh and M. V. Ravi (2016). In vitro screening of potassium solubilizing potential (efficiency) of bacteria. *Journal of Pure and Applied Microbiology*, *10*(4), 2815-2819.
12. Kato, N., N. Kihou, S. Fujimura, M. Ikeba, N. Miyazaki, Y. Saito, ... and S. Itoh (2015). Potassium fertilizer and other materials as countermeasures to reduce radiocesium levels in rice: Results of urgent experiments in 2011 responding to the Fukushima Daiichi Nuclear Power Plant accident. *Soil Science and Plant Nutrition*, *61*(2), 179-190.
13. Maurya, B. R., V. S. Meena and O. P. Meena (2014). Influence of Inceptisol and Alfisol's potassium solubilizing bacteria (KSB) isolates on release of K from waste mica. *Vegetos*, *27*(1), 181-187.
14. Nain, A., K. Chaudhary, C. Sharma and H. Sheoran (2023). Potassium solubilization in soils.
15. Olaniyan, F. T., E. T. Alori, A. O. Adekiya, B. B. Ayorinde, F. Y. Daramola, O. O. Osemwegie and O. O. Babalola (2022). The use of soil microbial potassium solubilizers in potassium nutrient availability in soil and its dynamics. *Annals of Microbiology*, *72*(1), 45.
16. Panda, A., L. Das and B. B. Mishra (2023). Zinc solubilization and potash mobilization by potent plant growth promoting bacteria isolated from Odisha. *Annals of Plant and Soil Research*, *25*(2), 285-296.
17. Ranjitha, M. and S. Bharathiraja (2021) "Studies on the screening of mycorrhization and different bio inoculant regimes on the growth and chlorophyll of aloe vera (*Aloe barbadensis*) ". International Journal of Botany Studies, Volume 6, Issue 5, Pages 1405-1411.
18. Sarikhani, M. R., S. Oustan, M. Ebrahimi and N. Aliasgharzad (2018). Isolation and identification of potassium-releasing bacteria in soil and assessment of their ability to release potassium for plants. *European journal of soil science*, *69*(6), 1078-1086.
19. Shahab, S. and N. Ahmed (2008). Effect of various parameters on the efficiency of zinc phosphate solubilization by indigenous bacterial isolates. *African Journal of Biotechnology*, *7*(10).
20. Sheng, X. F. and L. Y. He (2006). Solubilization of potassium-bearing minerals by a wild-type strain of Bacillus edaphicus and its mutants and increased potassium uptake by wheat. *Canadian journal of microbiology*, *52*(1), 66-72.
21. Sugumaran, P. and B. Janarthanam (2007). Solubilization of potassium containing minerals by bacteria and their effect on plant growth.
22. Verma, P., A. N. Yadav, K. S. Khannam, A. K. Saxena and A. Suman (2017). Potassium-solubilizing microbes: diversity, distribution, and role in plant growth promotion. *Microorganisms for green revolution: Volume 1: Microbes for sustainable crop production*, 125-149.
23. Won, M., J. Heo, H. Y. Weon, D. Lee, B. H. Han, S. B. Hong and S. W. Kwon (2023). Frateuria soli sp. nov. and Frateuria edaphi sp. nov., isolated from greenhouse soil. *International Journal of Systematic and Evolutionary Microbiology*, *73*(3), 005759.
24. Yadav, B. K. and A. S. Sidhu (2016). Dynamics of potassium and their bioavailability for plant nutrition. *Potassium solubilizing microorganisms for sustainable agriculture*, 187-201.
25. Yaghoubi Khanghahi, M., H. Pirdashti, H. Rahimian, G. Nematzadeh and M. Ghajar Sepanlou (2018). Potassium solubilising bacteria (KSB) isolated from rice paddy soil: from isolation, identification to K use efficiency. *Symbiosis*, *76*, 13-23.
26. Zhu, B., Q. Xu, Y. Zou, S. Ma, X. Zhang, X. Xie and L. Wang (2020). Effect of potassium deficiency on growth, antioxidants, ionome and metabolism in rapeseed under drought stress. *Plant growth regulation*, *90*, 455-466.