RESEARCH ARTICLE



Exploring waste mica as an alternative potassium source using a novel potassium solubilizing bacterium and rice residue in K deficient Alfisol

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Abstract

Background and aims Low-grade potassium (K) bearing minerals like waste mica and K-rich crop residues can be explored as an alternative K source. However, waste mica's low available K limits its efficacy. This study aims to use waste mica with a native K solubilizing bacteria (KSB) isolated from Alfisols near mica mines along with rice residue to enhance K availability in a K deficient Alfisol.

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Methods A novel KSB (JHKSB4), identified as Acinetobacter sp was isolated from soils of mica mining areas in Jharkhand, India. An incubation and pot experiment were conducted using JHKSB4, waste mica and rice residue in a K-deficient Alfisol to assess the release of K fractions and K recovery percentage. Results Incubation study revealed that waste mica with JHKSB4 and rice residue significantly increased the water-soluble K and exchangeable K contents in soil over mica alone. Pot experiment revealed that combination of mica, JHKSB4 and rice residue could increase 43-61% K uptake by wheat over control but could not exceed the impact of muriate of potash (MOP). Residual impact of this treatment was also

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observed on K recovery in rice crop. The changes in surface morphology of mica through scanning electron microscopy indicated dissolution of waste mica due to action of JHKSB4 and rice residue.

Conclusions This study established the potential of waste mica treated with JHKSB4 and rice residue as a supplementary K-source for crops. It recorded a K recovery of about 33% of that achieved with MOP. Thus, use of biologically treated waste mica could be a gamechanger in the realm of sustainable K management by partially replacing MOP.

Keywords Isolation · Potassium solubilizing bacteria · Waste mica · Crop residue · Potassium recovery · Potassium uptake · Wheat

Introduction

Potassium (K) is the third most important essential nutrient required for plant growth and development. Soil solution K, exchangeable K or surface adsorbed K, interlayer K, and mineral/structural K represents the four major pools of K (Barber 1995; Bell et al. 2021). Soil is amply supplied with soil mineral K (90–98%). However, at any point of time, K supply to crops depends mainly upon K present in soil solution K (0.1–0.2% of total K) and surface adsorbed K (1-2% of total K) (Bell et al. 2021). Hence, external application of K fertilizer is required as only a small fraction of total K is present in available form to meet the immediate demand of crops especially in K deficient soils. It is estimated that the world demand for K based fertilizers is growing steadily at 3.0–3.5% per annum (Manning 2010; Jena et al. 2019). The developing countries, particularly Southeast Asia, Africa and South America depend solely on import of K-fertilizer from foreign countries. The annual consumption of K-fertilizers in India has also witnessed an increase from the past four decades and the current consumption is about 2.52 million tonnes (Mt) (FAI 2022). Due to lack of commercial grade K-ore deposits, the entire amounts of K-fertilizer used in Indian agriculture are imported from other countries, which leads to the expense of huge amount of foreign exchange (Nishanth and Biswas 2008; Basak 2019). Further, due to increasing demand in the international market, the cost of commercial K-fertilizer is increasing day-by-day. As a result, small and marginal farmers are not able to afford commercial K-fertilizer for agricultural application (Manning 2010; Biswas 2011; Swoboda et al. 2022). Thus, there is an urgent need to find out suitable alternative K sources to minimize the brevity of this existing problem.

In this regard, India has high deposits of lowgrade K-bearing minerals like mica, glauconite, feldspar etc. According to the United States Geological Survey report 2018 (Mineral Commodity Summaries 2018), India occupies 8th position globally in mica production while Finland ranks first. Abundant mica deposits are reported in states like Andhra Pradesh, Maharashtra, Odisha, Rajasthan, Telangana, Bihar and Jharkhand. It is also reported to be available in small pockets in Gujarat, Haryana, Kerala, Tamil Nadu, and West Bengal. According to the database of the National Mineral Inventory-Indian Bureau of Mines, a total of 635,302 metric tonne (MT) of mica was estimated in India in 2015 (IBM 2019; Rani and Das 2022). The total mica deposits present in India and their percent share in different states is presented in Fig. 1. Within the different mines, mica mines have grabbed special attention due to large volume of mica waste generated (Ibeh et al. 2021). It has been reported that about 50-75% of raw mica is discarded as waste during the cleaning and processing of mica which does not have any practical utilization as of now (Pramanik and Kalita 2019). This mica waste can be converted to wealth by exploring its potential as an alternate source of K. Some recent studies showed that plants are able to access K from low-grade silicate minerals (e.g., feldspar and mica) resulting in higher yield and K uptake (Mohammed et al. 2014; Manning et al. 2017; Basak 2019). However, these silicate minerals have been found to be less effective as compared to commercial K-fertilizer due to low available K content (Basak et al. 2017; Pramanik and Kalita 2019; Ahmad et al. 2020). The K supplying capacity of silicate minerals can be improved through microbial interventions particularly with the use of K solubilizing bacteria (KSB). The main mechanisms of action of KSB include direct solubilization of K minerals through release of organic acids like oxalic acid, tartaric acid, citric acids, etc. produced by KSBs which in turn reduce the pH of the surrounding medium and bring cations such as K, magnesium (Mg) and iron (Fe) in soil solution which is then taken up by the plants (Velázquez et al. 2016). Other mechanisms include



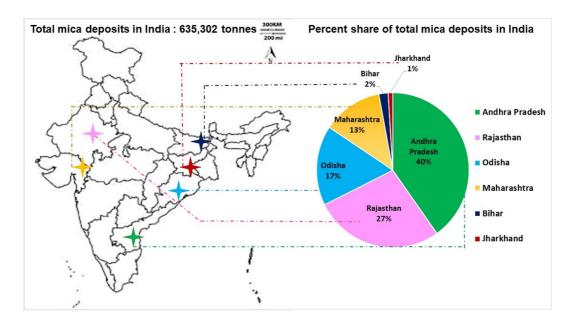


Fig. 1 Total mica deposits present in India and its distribution in different states of India. Source: Database from Indian Minerals Year Book (IBM 2019)

polysaccharide secretion, biofilm productions and indirect solubilization (supplementary Fig. S1). Many KSB strains (Bacillus edaphicus, B. circulans, B. mucilaginosus, Paenibacillus sp., Pseudomonas sp., Burkholderia sp., etc.) have been reported to solubilize K from silicate minerals (Sheng 2005; Badr 2006: Basak and Biswas 2009: Pramanik et al. 2021). However, native strain isolated from soils of mica mining areas have not been attempted yet. It is hypothesized that mica mineral enriched soils of mining areas would be inhabited by certain groups of native bacteria which may have the potential of extracting K from mineral K fraction to meet their metabolic needs. This KSB is expected to have better adaptability when inoculated in the soils from similar region. So, K solubilization potential of these bacterial strains may be explored for sustainable K management in agriculture especially in K deficient soils. In addition to the above facts, incorporation of cereal crop residues which are rich in K content (about 1-5% K) in soil may also be a promising approach for improving soil K (Liao et al. 2018). Besides their inherent K content, these crop residues may also additionally produce organic acids during decomposition and may have a positive impact on K dissolution from applied K minerals (Kumari et al. 2008; Yan et al. 2019). Crop residues can be recycled back to soil as source of K besides adding organic carbon, which in turn may further help in augmenting and maintaining the population of the added K-solubilizers.

Weathered tropical soils like Alfisols are invariably low in K and organic carbon (Rao and Srinivas 2017; Das et al. 2020, 2023). Moreover, the use efficiency of soluble chemical fertilizer (e.g., potash salts) in weathered tropical soil is very less due to low cation exchange capacity (CEC) (Theodoro and Leonardos 2006). Owing to the above soil conditions, use of waste mica treated with native KSB along with rice residue could be an opportunity to improve K availability in soil and plants. Use of treated waste mica may also help to reduce commercial K-fertilizer consumption if found suitable for soil application. However, rare information is available on waste mica treated with native KSB and rice residue as alternative K source in weathered tropical Alfisol. The current study tested a hypothesis that native KSB isolated from soils of mica mining area would improve K solubilization from waste mica in presence of rice residue in K deficient Alfisol. The present study was, therefore, undertaken with the objectives namely, (i) to isolate native KSB from the soils of mica mining area of Jharkhand, India; (ii) to investigate K solubilizing capacity of native KSB from waste mica under



laboratory conditions; and iii) to evaluate the effectiveness of waste mica treated with KSB and rice residue as alternate K source in wheat and rice crop under pot experiment grown in sequence.

Materials and Methods

Collection of soil sample and isolation of potassium solubilizing bacteria

Soil samples were collected from adjacent areas of mica mines for isolation of native KSB strain. The mica mining area comes under Jhumri Telaiya village (24°41 N and 85°55 E) situated in Koderma district, Jharkhand, India.. The collected soil samples were brought to the laboratory and stored at 4°C in sealed polythene bags. Screening of prominent bacteria was carried out using serial dilution technique. Samples were diluted up to 10^{-5} dilutions. 50 µL aliquots from dilutions of 10⁻⁴ and 10⁻⁵ was placed on nutrient broth medium by the spread plate technique and incubated at 28±2 °C for 48 h. The most prominent colonies were isolated, based on macro-morphological characteristics. All colonies growing on the plates were screened again on modified Aleksandrov agar medium containing 5 g dextrose, 1 g (NH₄)₂SO₄, 0.5 g MgSO₄.7H₂O, 0.1 g CaCO₃, 0.0005 g FeCl₃, 1.0 g Ca₃PO₄, 0.5 g yeast, 2.0 g insoluble mica powder as K source and 18.0 g agar in 1 L of deionized water, and incubated at 28±2 °C for seven days for identifying their K-solubilizing ability (Sugumaran and Janarthanam 2007). One standard K-solubilizer (Bacillus sp.) was also obtained from the Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi, India and used as standard KSB. Colonies marked by clear zone of K solubilization were selected as K-solubilizers using 10⁻⁴ and 10⁻⁵ dilutions plates (Supplementary Fig. S2) and all the strains were quantified for their K solubilizing ability by inoculating them on Aleksandrov broth for 7 days and quantifying the solubilized K (µg mL⁻¹) through flame photometer (Sarikhani et al. 2018). The corresponding changes in the pH of the broth was also analyzed. The colonies of bacterial isolates were also characterized based on their morphology (pigmentation, margin, elevation and optical density) as well as their ability to survive under salinity stress and varied temperature and pH conditions by plating them on Aleksandrov agar medium. The strain with highest K solubilizing ability was then screened and mass multiplied in nutrient broth for further use in incubation and pot culture experiments. The final population of the isolated KSB and standard KSB culture maintained in nutrient broth for conducting incubation and pot experiment were $1.0-1.5\times10^7$ CFU mL⁻¹. All operations of isolation were carried out under aseptic conditions.

Phylogenetic analysis of isolated KSB strains

The DNA isolation was carried out using MagGenome Xpress DNA bacterial mini kit following the instruction given in the manual provided with the kit. Molecular identification of the isolated bacterial strains was carried out by application of the colony-polymerase chain reaction (PCR) method using universal primer set pA (Forward primer) (5'-AgAgTTTgATCCTggCTCAg-3') and pH (Reverse primer) (5'-AAggAggTgATCCAgCCgCA-3') (Stackebrandt and Goebel 1994). The PCR conditions involved an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 58 °C for 1.5 min, and 72 °C for 2 min, and a final extension at 72 °C for 10 min. Bands of approximately 1500 bp were obtained from the amplification of 16S rDNA gene sequence (Supplementary Fig. S3). The PCR products were then sent for sequencing and molecular identification to ABA biotech, New Delhi. The 16S rDNA gene sequences were analyzed with the gapped BLASTn (http://www. ncbi.nlm.nih.gov) search algorithm and aligned to their nearest neighbours. The phylogenetic tree was generated using a neighbour-joining algorithm with nucleotide p-distances and 1000 bootstrap replicates in the Molecular Evolutionary Genetics Analysis program (MEGA, version 6.0, USA).

Collection of waste mica and rice residue

Waste mica sample was collected from mica dumping areas located near mica mining area in the Koderma district of Jharkhand, India. It was ground in a Willey mill and passed through 2-mm sieve followed by washing with distilled water to make it free from dust and dirt and then air-dried for 3 days prior to use. Total K content in mica was determined as per the standard procedure (Page et al. 1982). The ground waste mica contained 4.5% total K. Mineralogy content of the waste mica sample was studied through



X-ray diffractogram. The sample was found to contain 44.4% mica, 65.1% quartz and 1.65% pyrophyllite. Rice residue (straw) was collected from the experimental farm of ICAR-Indian Agricultural Research Institute, New Delhi, India. After proper drying, it was chopped into small pieces (≤1 cm) for further use. The total nitrogen (N), phosphorus (P) and K content of rice residue used in the present experiment were 0.50%, 0.15% and 0.89%, respectively.

Collection and processing of bulk soil sample

For incubation and pot experiments, a weathered soil deficient in K was selected. The bulk soil sample (0–15 cm) was collected from an uncultivated plot at Birsa Agricultural University, Ranchi, Jharkhand, India located at 23°44′ N latitude and 85°32′ E longitude. The soil belonged to Alfisols and classified as fine loamy, mixed, hyperthermic typic haplustalfs. The soil was selected due to its low available K status as well as similarity with the soil from where the potential K-solubilizers were isolated with respect to soil type (typic haplustalfs). The collected soil sample was air-dried, ground, passed through 2 mm sieve and used for incubation and pot culture experiments as well as for characterization of initial physicochemical properties. The initial soil was nearly neutral in reaction with pH (1:2) 6.68 and low in organic carbon content (0.38%). The soil had medium available N and P status and low available K status (45.1 mg kg⁻¹). Initial physicochemical properties of the experimental soil are presented in Table 1.

Incubation study

Changes in different pools of K in soil treated with waste mica under the influence of KSB and rice residue were monitored under laboratory condition through an incubation experiment. The incubation experiment was carried out with various combinations of waste mica, rice residue and KSB strain along with absolute control. Total seven treatments were evaluated in the incubation experiment viz., T1: Control; T2: Mica @ 50 mg K kg⁻¹ soil; T3: Mica @ 50 mg K kg⁻¹ soil+rice residue (~1 cm in size) @ 2 g kg⁻¹ soil; T4: Mica @ 50 mg K kg⁻¹ soil+JHKSB4; T5: Mica @ 50 mg K kg⁻¹ soil+Bacillus sp.; T6: Mica @ 50 mg K kg⁻¹ soil+JHKSB4+Rice residue @ 2 g kg⁻¹ soil; T7: Mica @ 50 mg K kg $^{-1}$ soil + Bacillus sp. + Rice residue @ 2 g kg⁻¹ soil. Incubation experiment was carried out at 28±1 °C for a period of 120 days. Soil sample (250 g) was taken in clean plastic bottle. Water was added frequently to maintain field capacity moisture regime. Soil was mixed as per the treatment combinations mentioned above. Soil samplings were done at 15, 30, 60, 90 and 120 days after incubation (DAI) for analysis of water soluble, exchangeable,

Table 1 Initial physicochemical properties of the experimental soil

Characteristics	Values	Method / Reference		
pH (soil: water:: 1:2)	6.68	Richards (1969)		
EC (soil: water::1:2) (dS m ⁻¹)	0.11	Richards (1969)		
Mechanical analysis		Bouyoucos (1962)		
Sand (%)	40.0			
Silt (%)	32.4			
Clay (%)	27.6			
Textural class	Clay loam			
Organic carbon (g kg ⁻¹)	3.80	Walkley and Black (1934)		
CEC [$cmol(p^+) kg^{-1} soil$]	8.84	Jackson (1973)		
Available N (mg kg ⁻¹)	180	Subbiah and Asija (1956)		
Available P (mg kg ⁻¹)	6.13	Olsen et al. (1954)		
Available K (mg kg ⁻¹)	44.9	Hanway and Heidel (1952)		
Water soluble K (mg kg ⁻¹)	5.98	Page et al. (1982)		
Exchangeable K (mg kg ⁻¹)	39	Hanway and Heidel (1952)		
Non-exchangeable K (mg kg ⁻¹)	330	Page et al. (1982)		
Total K (%)	1.65	Page et al. (1982)		



and non-exchangeable K status in soil after extraction with distilled water, 1 N neutral ammonium acetate and boiling 1 N HNO₃, respectively as per the standard procedure mentioned in Table 1.

Pot culture experiment

The K-solubilizer tested under incubation study was further used along with crop residue and compared with a standard K-fertilizer for their effectiveness under a pot culture experiment in a wheat-rice sequence. The pot experiment was carried out in the net house of the Division of Soil Science and Agricultural Chemistry, ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India. The treatment combinations used in incubation study were also followed for pot culture experiment with an additional standard K-fertilizer treatment (T8) where K was added as muriate of potash (MOP)@ 50 mg K kg⁻¹ soil. Processed soil sample (4.5 kg) was filled in each pot. A uniform dose of N as urea @ 50 mg N kg⁻¹ soil and P as NaH₂PO₄ @ 15 mg P kg⁻¹ soil was also applied to the pot in solution form. The rates of N and P have been decided in accordance with the recommended dose of N and P for wheat crop. Here, 50 mg kg⁻¹ of N, equivalent to 112 kg/ ha of N and 15 mg/kg soil, equivalent to 33.6 kg of P ha⁻¹ were applied. The dose of waste mica was taken as 50 mg kg⁻¹ soil which was on a higher side as compared to the recommended dose of K for wheat as K present in mica waste is not easily soluble and therefore slightly higher amount of K is expected to enhance higher K supply. The dose of MOP was taken same as that of waste mica for ease of comparison between the two K sources. Solution of bacterial culture was added @ 20 mL of broth culture with KSB population of $1.0-1.5\times10^7$ CFU mL⁻¹ per pot along with waste mica and rice residue as per the treatments. All the treatments were mixed thoroughly with the soil and placed in pots.

Wheat (Triticum aestivum) var HD-2967 was selected as the test crop for pot culture experiment. After wheat, rice (Oryza sativa) var Pusa Basmati 1718 was selected and grown to observe the residual effect of treated waste mica without addition of fertilizer treatments except the recommended doses of N and P. Wheat seeds were sown in the month of October while rice seedlings were transplanted in the month of July in the following year. Thinning was done 10 days after sowing (DAS) so as to maintain five healthy plants per pot and harvesting was done after maturity. Irrigation was given as and when necessary. After harvesting of both the crops, shoot biomass was recorded after drying the plant samples under sun for 2-3 days. Thereafter, the samples were oven dried at 65 ± 2 °C in hot air oven for 72 h and then ground in Willey mill using 20-mesh sieve. The samples were stored in paper bag and digested further for analysis of K content to compute the uptake by wheat and rice. Total K uptake by wheat and rice was calculated by multiplying dry shoot biomass yield (mg pot⁻¹) and K content (%) and expressed as mg K pot⁻¹. The K recovery per cent was computed using the equation given below:

K recovery (%) =
$$\frac{\text{Total K uptake in treated pot } - \text{Total K uptake in control pot}}{\text{Total K applied}} \times 100$$

Structural changes in treated waste mica after plant intervention

Changes in surface morphology and mineralogical composition of waste mica due to plant intervention were studied through scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis. A separate set of treatments were taken under pot experiment with similar above mentioned treatments except that the waste mica (10 g pot⁻¹) passed through 2-mm sieve was put inside a fine nylon mesh and placed in

pots containing soils at a depth of 10 cm. Wheat and rice crops were grown in sequence without disturbing soil after wheat crop harvest. After harvesting of rice crop, waste mica samples were recovered from nylon bag carefully, processed and analyzed through SEM and XRD. For XRD analysis, automated powder diffraction (APD) software with Cu K α radiation (λ =1.5418 Å) source was used using a power of 40 kV. Powdered mica samples were placed in the sample holder and the sample was scanned at a diffraction angle of °20 from 0° to 30° with the scan



speed of 1 min⁻¹. Full width at half maximum (°2θ) (FWHM) was determined from the X-ray diffractogram. For SEM study, the samples were completely dried, powdered and mounted on double sided carbon tape covered with copper stab with industrial glue and coated with 20 nm thick palladium layer in a vacuum of 10⁻³ torr prior to analysis in EVO/MA10 SEM of CARL Zeiss instrument equipped with back scattered electron imaging (BSE), and secondary electron imaging (SEI) detectors. The micrographs were recorded after suitable magnification and accelerating voltage.

Statistical analysis

The influence of different treatments on soil and plant parameters were statistically analyzed through one-way analysis of variance (ANOVA) using a completely randomized design with three replications. All statistical analyses were conducted using R software version 4.2.1 (R Core Team 2018). The "lm" function was used to determine the effects of different treatment combinations on measurable parameters, and ANOVA was performed using the "anova" function in R. When significant differences were found, contrast analysis was conducted using the "lsm" function from the "emmeans" package (Russell 2019). Post hoc Tukey's tests with honest significant difference (HSD) at 95% confidence level (P = 0.05) were performed for multiple comparisons using the "cld" function in the "multcompView" package (Graves et al. 2019). For data calculation and graphical presentation, the Microsoft Excel (2016) (Microsoft Corporation, USA) program was used.

Results

Isolation, characterization and identification of potassium solubilizing bacteria

A total of eight different types of bacterial strains were grown on the Aleksandrov medium and isolated on the basis of their ability to form zone of solubilization and morphological differences (Table 2). These strains were named JHKSB1, JHKSB2, JHKSB3, JHKSB4, JHKSB5, JHKSB6, JHKSB7 and JHKSB8, respectively (KSB species were isolated from the soil adjacent to mica mines of Jharkhand, therefore, JHKSB was chosen for nomenclature). The quantity of K solubilized by these isolates after 7 days of incubation period and the corresponding changes in pH of the medium are represented in Fig. 2. Results showed that the K solubilizing ability of the isolates varied from the lowest value of 7 μ g mL⁻¹ (control) to the highest value of 21 µg mL⁻¹ (JHKSB4) followed by isolate JHKSB1 (18 µg K mL⁻¹). All other isolates had significantly lower K solubilizing values than the JHKSB4 and JHKSB1 isolates (Fig. 2). The corresponding changes in the pH of the growth media revealed that the maximum drop in the pH value was observed in case of isolates JHKSB1 and JHKSB4. The pH in these two isolates dropped from 7.0 at the beginning of incubation to 4.9 after 7 days of incubation. All the 8 bacterial isolates were also characterized based on their morphology and their ability to survive under increasing levels of salinity stress, temperature and pH (Table 2).

Table 2 Colony characterization of different K-solubilizers maintained on Aleksandrov agar medium (- No growth, + Slight growth, + + Moderate growth, + + Vigorous growth)

Isolates	Pigmentation	Margin	Elevation	Optical density	Tolerance to NaCl		Tolerance to NaCl Rai of I				_	Range of temperature			
					2.5%	5%	10%	5	9	4 °C	15 °C	30 °C	45 °C		
JHKSB1	Creamy	Smooth	Slightly raised	Opaque	+++	+	+	+	+	-	+	++	-		
JHKSB2	Creamy	Rough	Slightly raised	Opaque	++	+	-	+	-	-	+	+	-		
JHKSB3	Creamy	Smooth	Flat	Translucent	++	+	-	+	-	-	+	+	-		
JHKSB4	Creamy	Smooth	Slightly raised	Opaque	+++	+	+	+	+	-	+	++	-		
JHKSB5	Yellow	Smooth	Flat	Opaque	+++	+	-	+	+	-	+	+	-		
JHKSB6	White	Rough	Slightly raised	Translucent	++	+	-	+	-	-	+	++	-		
JHKSB7	Cream	Smooth	Highly raised	Translucent	+++	+	-	+	-	-	+	+	-		
JHKSB8	Cream	Smooth	Slightly raised	Opaque	+++	+	+	+	+	-	+	+	-		



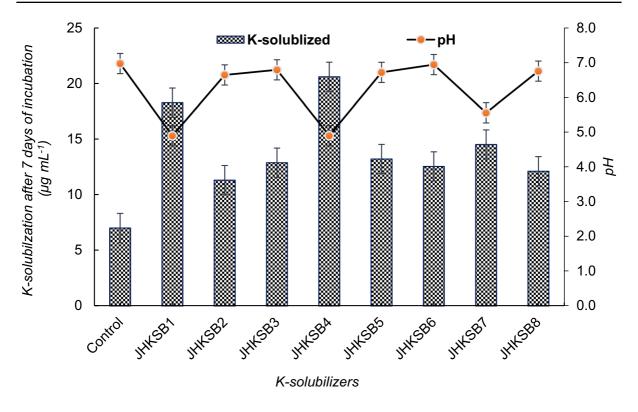


Fig. 2 Amount of K solubilized and changes in pH of Aleksandrov broth after 7 days of incubation as affected by K-solubilizers. Error bars represent \pm SE (n=3). JHKSB1 to JHKSB8

represent the different K-solubilizers isolated from soils of mica mines of Jharkhand, India

Out of 8 isolates, JHKSB4 was selected for further study as it showed the highest K solubilizing ability amongst all the isolated KSB strains. The strain JHKSB4 was found to be creamy, smooth, slightly round colony (Supplementary Fig. S4) with tolerance up to 5% of salinity stress. It belonged to mesophilic group and was able to proliferate well in pH 7 and 5 but not in pH 9.

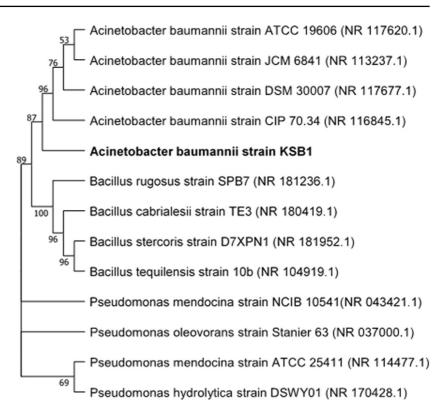
The 16S rDNA sequences of the selected KSB strains were compared with that of known 16S rDNA sequences with the help of BLAST and the Genbank database. The results showed that the isolated strain JHKSB4 had a very close similarity with *Acinetobacter* sp. and the closest relative was *Acinetobacter baumanii*. The phylogenetic tree showcasing the relationship of JHKSB4 with other representative species of related taxa is presented in Fig. 3. Previously *Acinetobacter* sp. has not been reported as a K-solubilizing bacteria and therefore, this is the first instance of report of K solubilization by this bacterium.

Water soluble K content in incubated soil

Water soluble K contents in soil clearly indicated the positive influence of the combined application of waste mica and KSB along with rice residue (Fig. 4). After 15 DAI, the value of water-soluble K content in soil sharply increased as compared to initial value (5.98 mg kg⁻¹). However, water soluble K content was not increased significantly in soil treated with mica alone (T2) and mica in presence of rice residue without KSB (T3). Further, application of both the KSBs (JHKSB4 and *Bacillus* sp.) either alone or in combination with rice residue significantly improved the value of water-soluble K. The highest water-soluble K was observed in treatments (T6 and T7) where combination of rice residue and KSB was used. However, T7 and T6 treatments did not show any significant difference to each other which indicates that both JHKSB4 and Bacillus sp. were equally active to perform its function in presence of rice residue. At 30 DAI, slight



Fig. 3 Phylogenetic tree showing the relative position of KSB isolate and other related taxa using the neighbour joining method



decrease in the water-soluble K was observed in all the treatments. However, T6 and T7 were still able to maintain significantly (p < 0.05) higher water-soluble K than the rest of the treatments. Thereafter, there was consistent increase in water soluble K with the advancement of incubation period irrespective of treatments. The highest water-soluble K value was observed in T6 and T7 throughout the period of incubation. After 120 DAI, the water-soluble K in T6 and T7 treatments were found to increase by 100 and 116%, respectively over the treatment T2 receiving only waste mica. The results indicated that application of waste mica and KSB along with rice residue was effective in improving water soluble K in soil.

Exchangeable K content in incubated soil

Exchangeable K in the treatments receiving waste mica and KSB alone or in combination with rice residue was significantly (P < 0.05) higher than the control treatment (Fig. 5). The highest exchangeable K was observed in T7 (113 mg kg⁻¹ soil) followed by T6 (106 mg kg⁻¹ soil) at 120 days of incubation period.

However, there was no significant difference between treatment T5 and T4. In general, the exchangeable K content increased after 15 DAI as compared to the initial exchangeable K content (39 mg kg⁻¹ soil) irrespective of treatments. However, at 30 DAI, there was a slight decrease in exchangeable K. After 30 DAI, there was consistent increase in exchangeable K contents with advancement of incubation periods. After 120 DAI, exchangeable K content in T6 and T7 treatments were found to increase by 102 and 91%, respectively over the treatment (T2) receiving only waste mica. Overall, the exchangeable K found in various treatments were in the following order: T6> T7> T5 = T4> T3> T2> T1.

Non-exchangeable K content in incubated soil

Non-exchangeable K content in various treatments over 120 days of incubation period (Fig. 6) showed that like water soluble K and exchangeable K, non-exchangeable K was also found to be higher at 15 DAI than the initial value (330 mg kg⁻¹). However, no noticeable changes were observed among the treatments at the end of 15 DAI. With the advancement



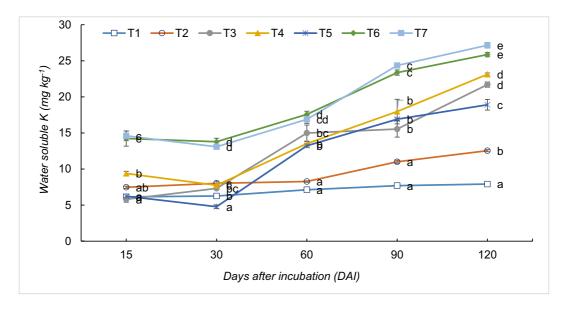


Fig. 4 Water soluble K contents at different days after incubation in soil. Values differ significantly within each column at P < 0.05 when not followed by the same small letter according to Tukey's post hoc test. Error bars represent \pm SE (n=3). T1: Control; T2: Mica @ 50 mg K kg⁻¹ soil; T3:

Mica @ 50 mg K kg⁻¹ soil+Rice residue @ 2 g kg⁻¹ soil; T4: Mica @ 50 mg K kg⁻¹ soil+JHKSB4; T5: Mica @ 50 mg K kg⁻¹ soil+Bacillus sp.; T6: Mica @ 50 mg K kg⁻¹ soil+JHKSB4+Rice residue @ 2 g kg⁻¹ soil; T7: Mica @ 50 mg K kg⁻¹ soil+Bacillus sp.+Rice residue @ 2 g kg⁻¹ soil

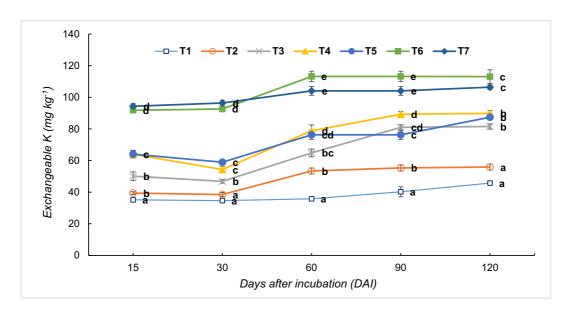


Fig. 5 Exchangeable K contents at different days after incubation in soil. Values differ significantly within each column at P < 0.05 when not followed by the same small letter according to Tukey's post hoc test. Error bars represent \pm SE (n=3). T1: Control; T2: Mica @ 50 mg K kg⁻¹ soil; T3:

Mica @ 50 mg K kg⁻¹ soil+Rice residue @ 2 g kg⁻¹ soil; T4: Mica @ 50 mg K kg⁻¹ soil+JHKSB4; T5: Mica @ 50 mg K kg⁻¹ soil+Bacillus sp.; T6: Mica @ 50 mg K kg⁻¹ soil+JHKSB4+Rice residue @ 2 g kg⁻¹ soil; T7: Mica @ 50 mg K kg⁻¹ soil+Bacillus sp.+Rice residue @ 2 g kg⁻¹ soil



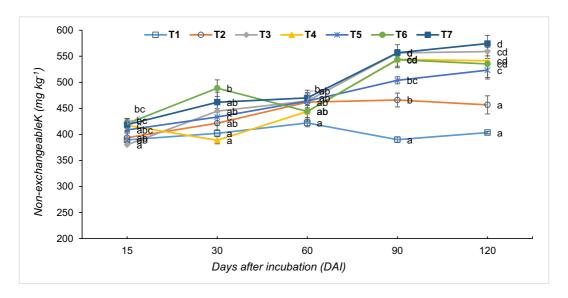


Fig. 6 Non-exchangeable K contents at different days after incubation in soil. Values differ significantly within each column at P < 0.05 when not followed by the same small letter according to Tukey's post hoc test. Error bars represent \pm SE (n=3). T1: Control; T2: Mica @ 50 mg K kg⁻¹

soil; T3: Mica @ 50 mg K kg $^{-1}$ soil+Rice residue @ 2 g kg $^{-1}$ soil; T4: Mica @ 50 mg K kg $^{-1}$ soil+JHKSB4; T5: Mica @ 50 mg K kg $^{-1}$ soil+Bacillus sp.; T6: Mica @ 50 mg K kg $^{-1}$ soil+JHKSB4+Rice residue @ 2 g kg $^{-1}$ soil; T7: Mica @ 50 mg K kg $^{-1}$ soil+Bacillus sp.+Rice residue @ 2 g kg $^{-1}$ soil

of incubation period, there was slight increase in non-exchangeable K but no significant differences were observed among the treatments. At 90 and 120 DAI, treatments like T3, T4, T5, T6 and T7 showed significantly higher non-exchangeable K than T1 and T2. These results suggest that the slowly available pool of K like non-exchangeable K is slowly influenced by KSB and rice residue than the readily available pools of K (water soluble K and exchangeable K).

Absolute changes in available K content in incubated soil

The absolute changes in available K (water soluble K+exchangeable K) presented in Fig. 7 was calculated by subtracting the available K content obtained at 15 DAI from the amount present in the initial soil (Table 1). While, the values of changes in available K under different treatments at 30 DAI represent the differences between the available K at 30 DAI and the values at 15 DAI, respectively and so on. The results showed that the values in available K content in soil was found to increase with the advancement of incubation period irrespective of treatments (Fig. 7). These values clearly indicated that the maximum absolute increase was obtained after 15 DAI and the

maximum increase was found in T6 (61.0 mg kg⁻¹) and T7 (63.9 mg kg⁻¹) and the least in case of control (-3.7 mg kg⁻¹). At 30 DAI, a decline in the values of available K was observed in all the treatments. Subsequently at 60, 90 and 120 DAI, positive values of available K were observed. However, the increase in available K in absolute terms kept on decreasing towards the end of incubation period. The values give a fair estimate of the potential of KSB and crop residues on release of available K from waste mica.

Potassium uptake and recovery by wheat and rice

Potassium uptake was calculated by multiplying dry shoot biomass yields (above ground) and K content in biomass. Results clearly indicated that treatment receiving standard K-fertilizer (MOP) (T8) outperformed all other treatments suggesting that none of the treatments are able to replace MOP in terms of K uptake. Except MOP treated soil, the K uptake under T6 (Mica @ 50 mg K kg $^{-1}$ soil+JHKSB4+Rice residue @ 2 g kg $^{-1}$ soil) and T7 (Mica @ 50 mg K kg $^{-1}$ soil+Bacillus sp.+Rice residue @ 2 g kg $^{-1}$ soil) were found to be significantly (p < 0.05) higher than others, and the lowest uptake was recorded under control (T1). However, the treatments T2, T3, T4 and



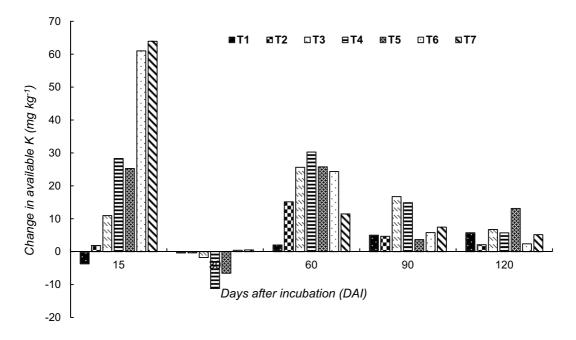


Fig. 7 Absolute increase/decrease in available K contents at each sampling day during incubation study. *T1: Control;* $T2: Mica @ 50 mg K kg^{-1} soil; T3: Mica @ 50 mg K kg^{-1} soil + Rice residue @ 2 g kg^{-1} soil; T4: Mica @ 50 mg K kg^{-1}$

soil+JHKSB4; T5: Mica @ 50 mg K kg⁻¹ soil+Bacillus sp.; T6: Mica @ 50 mg K kg⁻¹ soil+JHKSB4+Rice residue @ 2 g kg⁻¹ soil; T7: Mica @ 50 mg K kg⁻¹ soil+Bacillus sp. +Rice residue @ 2 g kg⁻¹ soil

T5 were found to be comparable in terms of their K uptake.

The residual effects of these treatments were examined by taking rice crop after harvesting of wheat crop without addition of K-fertilizers. The highest K uptake by rice shoot biomass was recorded in the treatment receiving standard K-fertilizer (T8: muriate of potash @ 50 mg K kg⁻¹ soil), while control (T1: untreated mica) recorded the lowest K uptake by rice. The treatment receiving waste mica along with KSB (T4) showed significantly higher K uptake than treatment receiving only waste mica (T2) but it was at par with treatment receiving waste mica along with KSB and rice residue (T6 and T7). This result indicates that improvement in K uptake by incorporation of rice residue is restricted to first season only but KSB continues to have some impacts in the second crop also.

The K recovery (%) were significantly influenced by application of waste mica alone or in combination with KSB and rice residue (Table 3). The lowest K recovery (%) was recorded in the treatment receiving only waste mica. Combined application of waste mica along with KSB and rice residue was able to recover

13.60% and 12.84% of the applied K in case of wheat crop and 10.25% and 12.81% in case of succeeding rice crop in treatment T7 and T6, respectively. However, the K recovery by this treatment was less than the K recovery recorded in MOP treatment (39.30% and 23.36% under wheat and rice crop, respectively). Thus, combined application of waste mica along with rice residue and KSB can enhance K up to certain limit but could not completely replace MOP. Result also indicated decrease in K recovery from first crop (wheat) to succeeding crop (rice). It was also observed that the K recovery from MOP in the second crop reduced to almost half of the first crop, while K recovery remained almost same in succeeding crop under the treatment receiving waste mica along with KSB and rice residue. However, the K recovery value was quite less as compared to MOP in succeeding crop.

Changes in surface morphology of waste mica

Mineral composition of waste mica did not change by the treatments as inferred from similar peak (Pk) position (°2θ) displayed in treated and untreated



Table 3 Potassium uptake and recovery by rice and wheat as affected by waste mica, K-solubilizers and rice residue

Treatments description	Total K up	K recovery (%)		
	Wheat	Rice	Wheat	Rice
T1: Control	80a	67.4a	-	-
T2: Mica	101b	72.8b	4.23	2.41
T3: Mica + Rice residue	108b	76.6b	7.14	4.11
T4: Mica+JHKSB4	104b	88.1c	5.48	9.19
T5: Mica+Bacillus sp.	111b	97.2c	8.51	13.20
T6: Mica+JHKSB4+Rice residue	125c	96.3c	12.84	12.81
T7: Mica + Bacillus sp. + Rice residue	129c	90.5c	13.60	10.25
T8: Muriate of potash (MOP)	180d	120d	39.30	23.36

^{*} Values differ significantly at P < 0.05 when not followed by the same small letter according to Tukey's post hoc test

samples (Table 4) in the X-ray diffractograms (Supplementary Fig. S5). However, significant changes in waste mica were observed when full width at half maxima (FWHM) was calculated (Table 4). The X-ray diffraction pattern revealed that treated waste mica resulted in higher FWHM values as compared to untreated waste mica. Inoculation with both the strain of KSBs recorded higher FWHM values as compared to untreated mica which recorded the lowest FWHM value (0.17°2θ). The highest value of FWHM was observed in case of T7 (0.28°2θ) followed by T4 and T6 (0.25°2θ). Results indicated that the waste mica had undergone some dissolution by the combined action of KSB and rice residue during the growth period of wheat and rice crops.

The SEM images have been taken at two magnifications. The images at lower magnification i.e.

Table 4 Structural changes in treated and untreated mica (control) particles under the influence of K-solubilizers and rice residue

Treatments	^a Pk (°2θ)	^b FWHM (°2θ)
T1: Control	ND*	ND
T2: Mica	8.80	0.17
T3: Mica + Rice residue	8.80	0.20
T4: Mica+JHKSB4	8.89	0.25
T5: Mica+Bacillus sp.	8.85	0.20
T6: Mica+JHKSB4+Rice residue	8.80	0.25
T7: Mica + <i>Bacillus</i> sp. + Rice residue	8.81	0.28
T8: Muriate of potash (MOP)	ND	ND

^aPk: peak position (°2θ); ^bFWHM: full width at half maximum; and *ND: not detected

100 µm did not present any clear variations in the treated samples (Supplementary Fig. S6). However, the SEM images (at 10 µm magnification) showed almost well-defined edges with sharp and distinct surfaces (Fig. 8a) in untreated waste mica. The layers of mica particles appeared to be more compact and it did not show observable morphological changes in untreated waste mica. In treated waste mica samples (Fig. 8b, c, d, e and f), it was observed that the edge surfaces were slightly transformed, the edges appeared to be more irregular and flakier as well as more rounded and smoothened. In comparison to untreated mica (control), the treated waste mica had more weathered appearance with the layers slightly opened up and separated. The higher disruption of the edges was observed (Fig. 8e and f) in the treatment receiving combined application of KSB and rice residue. Thus, the SEM images further corroborate with the results obtained in the incubation and pot culture experiments and provides visible evidence that indicated more release of K from waste mica treated with KSB and rice residue.

Discussions

In the present study, KSB strains were isolated on the basis of zone of solubilization in Aleksandrov medium. Several studies have clearly highlighted the ability of KSB to solubilize insoluble K in Aleksandrov medium (Keshavarz Zarjani et al. 2013; Maurya et al. 2014; Saha et al. 2016). It has been documented earlier that solubilization occurs on account



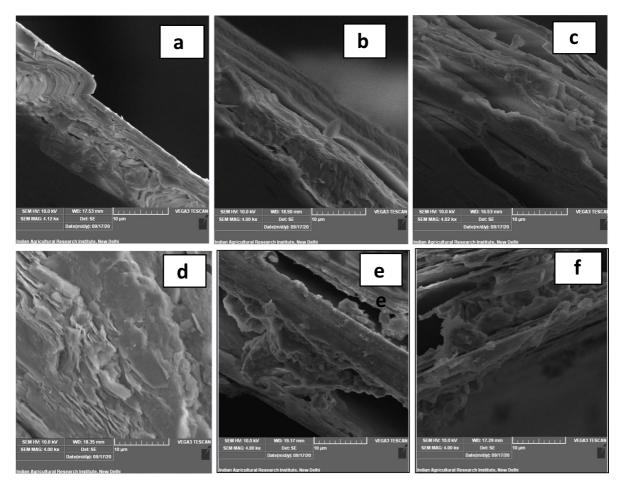


Fig. 8 Scanning electron microscopic (SEM) images of mica particles (10 μm magnification) in untreated (control) versus treated soils under pot experiments at the end of wheatrice crop sequence. 8a: Untreated mica; 8b: Mica+Rice

residue; 8c: Mica+JHKSB4; 8d: Mica+Bacillus sp.; 8e: Mica+JHKSB4+Rice residue; 8f: Mica+Bacillus sp.+Rice residue

of acidic conditions resulting from metabolic process of the microbes (Liu et al. 2006). Prevalence of acidic condition on account of production of organic acids is considered to be the main mechanism of K solubilization. Recent investigations have indicated that KSB played an important role in releasing K from insoluble sources like mica (Bagyalakshmi et al. 2012; Zorb et al. 2014; Saha et al. 2016; Rani et al. 2022a). Further, these organic acids influence mica dissolution either directly by decreasing the pH and forming framework destabilizing surface complexes or indirectly by complexing metal ions in solution (Zhang et al. 2013; Khanghahi et al. 2018). The inverse relation of K solubilization potential of the KSB strain isolated in this study with pH of solution

also confirms this hypothesis. Most common organic acids produced by KSBs are oxalic acid, citric acid, propionic acid, and gluconic acid (Biswas and Basak 2014; Basak et al. 2022). Polysaccharides produced by KSB absorb these organic acids and further aids in increasing the concentration and action of organic acids (Sattar et al. 2019; Rani et al. 2022a). The ability of KSB to cope up with abiotic stress conditions (salt stress, pH conditions, temperature stress, etc.) is also important criteria for its wider adaptability (Khanghahi et al. 2018). Characterization of the KSB isolated in this study at different induced salt stress and temperature stress showed that only JHKSB1, JHKSB4 and JHKSB8 strains were able to survive in up to 10% saline conditions and pH 5–9.



The potential KSB (JHKSB4) strain selected in this study was identified as Acinetobacter sp. It has been earlier reported that Acinetobacter sp. could survive in 7% NaCl (Ashfaq et al. 2020) to as high as 25% NaCl concentrations, between pH 5-9 (Chaiharn and Lumyong 2009), which corroborates with our findings. Identification of this species as potential KSB opens a new area of research targeting more efficient K solubilization from insoluble K-minerals like mica. Very few researches have highlighted the K solubilizing potential of this strain of bacteria from K-bearing minerals like feldspar (Bhattacharya et al. 2016) but none in case of mica and so it is a novel finding in that aspect. Further, there is a scope of exploring this microbe as an important plant growth promoting rhizobacteria specially towards designing K management strategies.

Incubation experiment was carried out to quantify the changes in different fractions of K in soil. The results indicated that addition of both the JHKSB4 and Bacillus sp. in combination with rice residue was able to increase the release of K from waste mica in a 120-days incubation study. In this experiment, there were three possible sources of K such as waste mica, rice residue and soil itself. The waste mica and rice residue contained around 4.5% and 0.89% of total K, respectively. The initial increase in water soluble and exchangeable K after 15 DAI might be due to release of K from soil minerals through various mechanisms, which have been reported earlier (Biswas and Basak 2013; Etesami et al. 2017). The most accepted mechanisms of K solubilization via KSB are (i) solubilization directly from K minerals; (ii) indirect solubilization of K minerals; (iii) polysaccharide secretion; and (iv) production of biofilm (Rani et al. 2022b). There are reports, which indicate K release from waste mica without K solubilizers. However, there was significant increase in K release when waste mica was treated with KSB (Basak and Biswas 2009). Moreover, the incorporation of rice residue might have boosted K availability from waste mica due to action of organic acids produced during organic matter (rice residue) decomposition (Basak 2018). The mineral dissolution may increase due to CO₂ production as a result of residue decomposition as carbonic acid participates directly in the silicate weathering reaction (Beerling et al. 2018). Similar result was also observed in the previous study (Basak et al. 2021) where K release from silicate mineral powder increased when incubated with cow manures and legume residue. Moreover, K content in the residue may be released as the residue's decomposition proceeds with time (Lupwayi et al. 2005). The KSB population remain more active during initial period of incubation which might have contributed to higher increase in K availability at 15 DAI (Malusa et al. 2016). The slight decrease in K availability at 30 DAI may be due to K-fixation triggered by sudden increase in soluble K contents in soil. However, this is a reversible process and fixation of K has been reported to reduce as the pH declines over time possibly due to presence of H₃O⁺ ions which might compete with K⁺ ions for inter layer exchange sites because of their similar ionic radii (Jha et al. 2016). In case of non-exchangeable K, the release was slow and the increase was observed more prominently towards the end of incubation period especially in treatments containing combination of KSB and rice residue. Ahmad et al. (2020) also reported an increase in non-exchangeable K levels in the soil by co-inoculation of KSB1 (Fraturia aurantia) and KSB2 (Bacillus edaphicus) in treatments that received recommended dose of N and P along with 50% recommended dose of K. They also reported that treatments with recommended dose of N and P along with 50% K + 50% waste mica treated with KSB2 also exhibited higher non-exchangeable K as compared to control. The difference in concentration gradient between the mineral K and other K pools present in the soil might have caused the release of some of the interlayer K. However, the process of release of non-exchangeable K is highly complex as K is found in the interstices of the Si-Al-O framework of the crystal lattice and held rigidly by the covalent bonds (Pal 2014). Bell et al. (2021) presented a modified conceptual diagram of K pools that differ with the traditional four pooled model. They have considered the K in mica and partially weathered mica as a different pool and excluded them from structural K in feldspar. According to Bell et al. (2021) release of K from interlayers of mica can be bidirectional and exchange can occur both to and from soil solution pool, exchangeable pool (surface adsorbed K) as well as non-exchangeable pool, whereas the release from structural K is only unidirectional and an increase in soil solution K cannot increase the structural K. As mica dissolution begin, the adsorption sites are slowly differentiated into edge and interlayer positions from where adsorption/desorption occurs slowly and



contribute to other K pools. Mengel and Rahmatullah (1994) also reported that soils rich in mica, exchangeable K alone is poor indicator of K availability and the increased proportion of non-exchangeable pool due to presence of mica may play a greater and important role in K supply to crops than exchangeable K alone. In the present study also, the non-exchangeable K pool was slightly improved towards the end of the end of incubation study due to release of K from mica. Increase in K pools has been previously reported due to addition of silicate mineral powder by Basak et al. (2023) silicate mineral powder (SMP). The effect of KSBs along with rice residue on K pools was observed to be more because initially K is released by water and then by weak acids produced by KSBs at a rapid rate. However, with the progress of weathering, a Si-Al-O residue envelope is formed surrounding the un-weathered core. This layer reduces the rate of K loss from the mineral and hence protects K from further degradation (Ahmad et al. 2020).

The highest K uptake by wheat and rice crop was recorded in the treatment receiving MOP. This indicates that sources having K higher in soluble form can supply K to plants in adequate amount, which reflected in K uptake in the present study. It is also clear from the study that MOP cannot be replaced entirely with the alternative sources of K like waste mica treated with KSB and rice residue. However, application of waste mica along with KSB and rice residue has been able to produce better effects than untreated waste mica (control) (T1) as well as waste mica alone (T2). Similar observation was reported by Basak and Biswas (2010) where application of waste mica alone could increase biomass yield of Sudan grass by 16.5% over control within a period of 150 days of crop growth, while treatment receiving mineral K (waste mica) and K solubilizer exhibited 70% higher biomass yield than control. Beneficial effect of KSB inoculation on yield and K uptake was also reported in crops like rice (Khanghahi et al. 2018), maize (Ahmad et al. 2020), wheat (Madar et al. 2020; Gandhi et al. 2023), tea (Pramanik et al. 2021) and cotton and rape (Sheng 2005). In our study, the effects of rice residue in enhancing biomass yield were found to prevail in first crop (wheat) only. The probable reason might be release of most of the K in the rice residue within one crop cycle. It is reported that most of the K in crop residue released over a period of one month and about 90% of the K is released over a period of 52 weeks (Lupwayi et al. 2005). Data on K uptake and recovery indicated MOP as the best performing treatment. However, combined application of waste mica along with crop residue and KSB were able to meet about 33% K requirement of the crops. So, by utilizing the potential of this indigenous KSB along with waste mica and rice residue, 33% recommended dose of costly K-fertilizer could be saved.

FWHM values obtained through X-ray diffraction (XRD) studies also indicate K release from waste mica treated with KSB and rice residue. There exists an inverse relationship of crystallinity with FWHM values following the Debye Scherrer equation $D = K\lambda$ / β Cos θ , which is used to calculate the crystalline size of the mineral particles, where D is the particle crystalline size, K represents the Scherrer constant (0.98), λ denotes the wavelength (1.54184 Å for CuK α) and β denotes the full width at half maximum (FWHM) (Pandian and Datta 2017; Sivagami and Asharani 2022). Lower the FWHM value, sharper is the peak and more is the crystallinity or the average crystallite size of the related particle (dos Santos et al. 2017). Higher FWHM shows disorder and may be considered as an indicative of dissolution of minerals. Ogasawara, et al. (2017) also concluded through the XRD analyses that 13 out of 15 of the biotite samples treated under relatively milder extraction conditions showed considerable broadening of the peak without any change in the peak position. They also presented a Pearson's correlation analysis which showed that the FWHM of the mica peak was positively correlated with the amount of K extracted. As a rule, the Kübler index (abbreviated as KI) decreases with decrease in FWHM and an increase in crystallinity. Arkai et al. (2004) presented an increase in KI in phlogopites treated with different dissolution agents (acids and bases) showing that FWHM increases as dissolution occurs. In this study also, the highest value of the FWHM in the control sample indicates maximum possibility of K release. The lower values for FWHM in the treated samples corroborate with the theory and may be considered as an indication of dissolution due to the presence of organic acids released by KSB and residue decomposition. Dissolution of edges and surfaces of waste mica might have occurred due to the action of organic acids produced by KSB and rice residue (Basak and Biswas 2009). The images of waste mica as observed in SEM analysis supported



the above result. The surface of waste mica treated with KSB and rice residue exhibited a clear difference from untreated mica. These changes might have occurred due to mineral dissolution through the action of organic acids or exopolymeric substances produced (Sheng and He 2006). The present study is in corroboration with previous report (Basak 2019) where SEM images clearly showed the microscopic changes occurred in the structure of waste mica with and without plant intervention.

Conclusions

A novel indigenous potassium solubilizing bacterial (JHKSB4) was isolated from soils of mica mining area of Jharkhand, India and identified as Acinetobacter sp. This native KSB has the potential to solubilize K and was able to increase available K content from waste mica in presence of rice residue under incubation study over a period of 120 days. Waste mica treated with both Acinetobacter sp and rice residue was able to increase the water soluble and exchangeable K approximately 2 times higher than the treatment of waste mica alone. Further, application of waste mica in combination with Acinetobacter sp and rice residue was able to partially meet the K requirement of wheat and rice crop under pot experiments. X-ray diffraction and SEM studies further depicted the solubilization of K from waste mica in presence of KSB (Acinetobacter sp and Bacillus sp.) and rice residue. So, it can be concluded that waste mica has potential as an alternative K source when applied in combination of KSB and rice residue. However, waste mica along with Acinetobacter sp and rice residue showed the potential to substitute MOP by approximately 33% in both the crops. Further study is needed to standardize the extent of MOP substitution that could be made through combined application of waste mica, KSB, and crop residue as an integrated K management strategy under field conditions.

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Author contributions Khushboo Rani and Dipak Ranjan Biswas conceptualized the study. Material collection, data

collection and analysis were done Khushboo Rani. Supervision and data curation was done by Dipak Ranjan Biswas. Microbial identification was done by Rajeev Kaushik and Jyoti Kumar Thakur. All authors participated in the design of the experiment. The first draft of the manuscript was written by Khushboo Rani and all authors commented and edited on previous versions of the manuscript. All authors have read and gave final approval for publication.

Data availability Data will be made available on request.

Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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