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Research Paper

# Rock inhabiting potassium solubilizing bacteria from Kerala, India: characterization and possibility in chemical K fertilizer substitution

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The role of rock inhabiting bacteria in potassium (K) solubilization from feldspar and their application in crop nutrition through substitution of fertilizer K was explored through the isolation of 36 different bacteria from rocks of a major hill station at Ponmudi in Thiruvananthapuram, Kerala, India. A comprehensive characterization of K solubilization from feldspar was achieved with these isolates which indicated that the K solubilizing efficiency increases with decrease in pH and increase in viscosity and viable cell count. Based on the level of K solubilization, two potent isolates were selected and identified as *Bacillus subtilis* ANctcri3 and *Bacillus megaterium* ANctcri7. Exopolysaccharide production, scanning electron microscopic and fourier transform infrared spectroscopic studies with these efficient strains conclusively depicted the role of low pH, increase in viscosity, and bacterial attachment in K solubilization. They were also found to be efficient in phosphorus (P) solubilization, indole acetic acid production as well as tolerant to wide range of physiological conditions. Moreover, the applicability of K containing rock powder as a carrier for K solubilizing bacteria was demonstrated. A field level evaluation on the yield of a high K demanding tuberous vegetable crop, elephant foot yam (*Amorphophallus paeoniifolius* (dennst.) nicolson) established the possibility of substituting chemical K fertilizer with these biofertilizer candidates successfully.

**Abbreviations:** K – potassium; KSB – K solubilizing bacteria; EPS – exopolysaccharides; N – nitrogen P – phosphorus

**Keywords:** KSB / Rock powder bioformulation / Bacillus / Elephant foot yam

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## Introduction

Potassium (K) is one of the major nutrients required for the growth and productivity of crop plants. The availability of K in soil for plant uptake is dependent on a number of factors including the level of other K, viz., solution, exchangeable and nonexchangeable K, and degree of weathering of K bearing minerals like feldspar and micas [1]. Soils of India at many places are deficient in K both in terms of available and non-available forms. Since K being one of the important nutrients for both yield and quality of the produce, K-deficient soil has become one of the main yield limiting factors in agriculture and is usually met by the application of potassic fertilizers. As regard to world-wide consumption of K fertilizers, India ranks fourth after USA, China, and Brazil [2]. Taking into account the escalating cost of K fertilizers and its diminishing raw material resources, it is imperative to find out some alternative ecofriendly approaches to reduce the dependence on imported or costly commercial K fertilizers. Here comes the significance of K solubilizers which can act on fixed forms of K such as clay minerals

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to make it available for plant uptake. Among the soil bacterial communities, bacterial strain such as *Bacillus mucilaginosus* [3] and *Bacillus edaphicus* NBT [4] have been described as effective K solubilizers. Sheng [4] reported significant increase in shoot and root dry yield as well as greater uptake of K by cotton and rape due to application of K bearing mineral (illite) inoculated with K solubilizing strain *Bacillus edaphicus* NBT. Sugumaran and Janarthanam [5] could observe increase in the dry matter and oil content of groundnut seeds and improvement in soil available P and K due to inoculation of *Bacillus mucilaginosus.* Research work on K solubilizing microorganisms is gaining importance globally because of the ever increasing price of potassic fertilizers coupled with its higher requirement especially for high yielding varieties of crops. Moreover, there is a growing interest for exploring the mechanism and characterization of mineral solubilizing microorganisms especially K solubilizers. Studies conducted by Lin et al. [6] and Sheng and Huang [7] reported the production of carboxylic acids and capsular polysaccharides associated with solubilization of feldspar by *Bacillus mucilaginosus* and *Bacillus edaphicus* suggested their possible role in K solubilization. The association of various organic ligands produced during metabolism was also found to contribute the solubilzation process [8]. With this view, the present study was aimed at isolating, characterizing, and utilizing the rock inhabiting potassium solubilizing bacteria for crop nutrition. For this bacteria were isolated from rock samples collected from a hill station, viz., Ponmudi in Kerala, India which were analyzed for released K, pH, viscosity, and viable cells status during K solubilization. Further, the potent isolates were characterized and the mechanism of solubilization were in turn studied through exopolysaccharides analysis, scanning electron microscopy, and Fourier transform infrared spectroscopy. The survival ability and efficiency of potent KSB in rock powder on storage was also studied. Further, the efficacy of the KSB to substitute K fertilizer was determined by field experiment with elephant foot yam (*Amorphophallus paeoniifolius* (dennst.) nicolson), a high nutrient demanding (NPK@100:50:150 kg $ha^{-1}$) crop. Elephant foot yam is a popular tuberous vegetable offering unprecedented scope as a cash crop due to its high production and export potential [9].

## Materials and methods

### Mineral preparation for the experiment

The K solubilization efficacy of the bacteria was identified using feldspar as the unavailable K source in the Sucrose Minimal Salts K Limited medium (SSKM) [10] which is the specific medium for isolating K solubilizers. The feldspar purchased from the JAI MICA Company, Rajasthan, India was powdered using rock crushing machine to get a particle range of 100–250 μm. The powdered feldspar after rinsing with 0.1 mol $L^{-1}$ HCl to remove free ions was washed several times with double distilled water. The chemical composition of the feldspar was determined using X-ray fluorescence spectroscopy (XRF Bruker model S4) (Table 1) and the mineralogical composition through X-ray powder diffraction (XRD Bruker model AXS D8). This purified feldspar was used as insoluble K source in the subsequent experiments.

### Isolation of KSB

A total of ten rock samples were collected in sterile plastic containers from the collection site (Ponmudi). Rock pieces were ground and rock inhabiting bacteria were isolated in SSKM medium by dilution plate technique. After an incubation period of 48–72 h at a temperature of 28 °C, the colonies of bacteria obtained were sub-cultured in to SSKM agar slants.

### Estimation of K releasing capacity and related attributes

Twenty bacterial colonies which showed affluent growth compared to other isolates after the incubation period were selected for the characterization of K solubilizing attributes such as K releasing capacity, number of viable cells, viscosity, and pH in the SSKM broth. K solubilizing capacity was estimated by growing the bacteria in SSKM broth at an incubation period of 8 days at 28 °C in a rotary shaker at 150 rpm in the presence of 1% feldspar. The broth was digested with 30% of $H_2O_2$ for the organic matter to get oxidized. The released K in the broth was measured using flame photometer 128 (Systronics, India). Samples were also monitored for analyzing pH (pH meter (Mettler Toledo, India), viscosity (Rapid Visco Analyser, Perten Instruments, Sweden), and number of viable cells formed during the K solubilization process).

**Table 1.** Elemental composition of feldspar.

| Minerals | $SiO_2$ | $TiO_2$ | $Al_2O_3$ | $Fe_2O_3$ | CaO | $K_2O$ | MgO | $Na_2O$ | $P_2O_5$ | BaO |
|---|---|---|---|---|---|---|---|---|---|---|
| % composition | 69.19 | 0.059 | 15.06 | 0.759 | 0.523 | 11.69 | 0.144 | 1.86 | 0.432 | 0.235 |

The viable cells (cfu $ml^{-1}$) of the bacteria were determined by plating the broth on SSKM agar media. SSKM broth without the inoculation of bacteria processed under similar conditions as described above served as control and all the data generated were analyzed statistically by Analysis of Variance (ANOVA) using SAS 9.3 [11]. The Duncan's multiple range test (DMRT) was used for comparison of means at 5% level of significance. Pearson correlation analysis was also done for finding out the relationship between soluble K, pH, viscosity, and number of viable cells. Two bacterias that showed highest K solubilization capacity were selected for subsequent studies.

### Characterization of potent KSB

*Morphological and molecular characterization.* The two potent KSB were streaked on fresh SSKM plate and incubated at 28 °C for 48 h. After incubation, their colony morphological characters were recorded. Cell morphologies were studied by gram staining method with 24 h broth culture using Nikon Eclipse 80i microscope (Nikon Corporation, Japan). Identification of these isolates by 16S rDNA sequencing was carried out following the method described by Anjanadevi et al. [12]. Phylogenetic analyses of the 16S rRNA gene sequences were conducted with closely and distantly related species and genera of *Bacillus* sp. The trees were generated using MEGA 5.2 by neighbor joining method. The robustness of the branches was inferred by bootstrap replication (2000 replicates).

*P solubilizing capacity.* The potent KSB were inoculated individually in 100 ml of Pikovskayas broth medium [13] and incubated in a rotary shaker at 150 rpm for 8 days at 28 °C. The biomass was then removed by centrifugation at 8000 rpm for 10 min. Dissolved phosphate concentration in the supernatant was determined by vanado-molybdate method as described in APHA [14].

*Indole acetic acid producing efficiency.* Indole acetic acid production by the bacteria was detected as described by Ahmad et al. [15] with some modifications. Bacterial cultures were grown for 4 days in their respective media with 5 mM L-tryptophan at 28 °C. Fully grown cultures were centrifuged at 4000 rpm for 15 min. The supernatant (2 ml) was mixed with two drops of ortho phosphoric acid and 4 ml of the Salkowski reagent (mixture of 50 ml of 35% of perchloric acid and 1 ml of 0.5 M $FeCl_3$ solution) and development of pink color in the mixture indicated indole acetic acid production and its concentration was determined using a spectrophotometer at 540 nm wavelength against a standard curve.

### Effect of physiological conditions on the growth of potent KSB

Effect of various growth conditions such as temperature, salt concentration, and pH on the growth of KSB were tested in SSKM broth. For studying the effect of temperature, potent bacteria were incubated at temperatures viz., 15, 20, 25, 35, 45, 55, 65, 75 °C and 80 °C for 24 h at 150 rpm. Broth medium supplemented with different concentrations of NaCl ranging from 0 to 15% was used for salt tolerance studies and the hydrogen ion concentration (pH) in the range of 3–14 was selected for pH studies. These flasks were incubated at room temperature for 24 h in a rotary shaker at 150 rpm. The growth of KSB in the given growth conditions were observed by taking the optical density of the medium using spectrophotometer.

### Exopolysaccharide production

The two potent KSB were tested for their capacity to produce EPS during the course of K and P solubilization. For this, the potent KSB were grown separately at 28 °C for 8 days in 500 ml SSKM and Pikovskayas broth. After 8 days of incubation, EPS produced in the media was estimated by following the method described by Anju et al. [16] with slight modification. The broths were centrifuged at 8000 rpm for 10 min. The EPS was then precipitated from the supernatant by addition of three volumes of chilled 95% (v/v) ethanol and incubating overnight at 4 °C. The precipitate was collected by centrifugation at 14,000*g* for 20 min at 4 °C and dialyzed for 48 h against distilled water at 4 °C. After dialysis, the EPS were freeze dried (T25 basic, IKA labor technique, India) and weighed.

### Scanning electron microscopic study

Scanning electron microscopic analysis was conducted for evaluating the mineral dissolution of feldspar due to the action of these potent bacteria. For that, two potent KSB were grown separately in 500 ml SSKM broth with 1% feldspar as K source for an incubation period of 30 days at 100 rpm in a rotary shaker. Control flask with feldspar powder was also kept without inoculating bacteria. The feldspar grains from both the control and test flasks were taken and dried at room temperature for 1 h. The dried samples underwent fixation and gold coating as described by Sreenivasulu et al. [17]. Thereafter it was viewed under scanning electron microscope (Hitachi S-2400, CA).

### Fourier transform infrared spectroscopic analysis

The feldspar grains obtained from the above-mentioned experiment were also subjected to Fourier transform

infrared spectroscopic analysis analysis for revealing the presence of functional groups on the feldspar grains which may be associated with bacteria or its secretory products. Using the Perkin Elmer Spectrum RX-1 (USA), the infrared spectra of each bacteria treated feldspar and control samples were recorded in the region 4000–400 $cm^{-1}$.

### Rock powder bioformulation

Economic and easily available K containing pure white rock powder having a K content of 3.9% was selected for making the carrier-based inoculum for the effective storage of two potent KSB. The bacterial cultures after 48 h of incubation in SSKM broth were taken and centrifuged at 6000 rpm for 10 min. The obtained pellets were suspended in sterile double distilled water to make a final concentration of $10^9$ cfu $ml^{-1}$ and 100 ml of this suspension was mixed with 250 g rock powder containing 10 g carboxy methyl cellulose and the moisture content was adjusted to 20%. These were kept at room temperature after storing in sterile plastic bags. The viable cell population of rock powder formulation was analyzed by serial dilution technique and efficiency of K solubilization capacity of these bacteria was checked by previously described methods at an interval of 2–10 months storage.

### Effect of KSB application on tuber yield of elephant foot yam

Field trial was undertaken in block IV of ICAR-Central Tuber Crops Research Institute (CTCRI) farm, Thiruvananthapuram, Kerala, India to study the effect of KSB independently and along with different levels of chemical fertilizer K (muriate of potash containing 60% $K_2O$) as K @ 0, 50, 100, and 150 kg $ha^{-1}$ on the yield of elephant foot yam and was compared with the Package of Practice (POP) recommendation for elephant foot yam (N, P, and K @ 100:50:150 kg $ha^{-1}$, respectively) and absolute control treatments. Experiment site was thoroughly ploughed, leveled, and divided into individual plots of area 4.5 × 4.5 m to accommodate 25 plants per plot. The available K status of experimental soil was low (140 kg $ha^{-1}$). The experiment was laid out in randomized block design (RBD) with 12 treatments replicated twice. N (urea (46% N)) and P (Mussoorie phosphate (20% $P_2O_5$)) were applied based on soil test-based fertilizer recommendations (STBF) except in the KSB alone and absolute control treatments. Rock powder-based bioformulations of KSB (10 g per plant) were applied in each plot as per the treatment combinations after 1 month of planting of seed corms. An interval of 15 days was given between the biofertilizer and chemical fertilizer application. Irrigation was given to the crop as and when necessary.

## Results

### Isolation and enumeration of KSB

A total of 36 different bacteria were isolated from the rock samples in SSKM agar plate. Twenty KSB (KSB1–KSB20) that showed affluent growth compared to other isolates in SSKM medium were tested for the quantification of released K and for the estimation of viscosity, pH, and number of viable populations in the SSKM broth after the incubation period. The results of the above experiments are shown in Table 2. The quantity of released K and viscosity in the media by the action of K solubilizing bacteria at an incubation period of 8 days ranged from 309 to 522 ppm and 33.43 to 57.32 mPa s, respectively, with variations among different isolates. Among these, significantly high K solubilization was recorded by KSB2 (522 ppm) followed by KSB13 (475 ppm). It was observed that K solubilization in the liquid medium by different bacteria was accompanied by a significant drop in pH ranging from 6.93 to 5.18. On the other hand, the viscosity and the number of viable cells in the medium were increased. Statistically a positive correlation between the viscosity ($r = 0.875$), cell densities ($r = 0.821$), and soluble K concentration were observed (Table 3). In addition, the pH and the released K was negatively correlated ($r = -0.811$). These findings suggest that the increase in viscosity and number of viable cells with decrease in pH is related to the released K concentration in the media. For detailed study, the high K solubilizing activity showing isolates such as KSB2 and KSB13 were selected.

### Characterization of potent KSB

*Morphological and molecular characterization.* Colonies of KSB13 were smooth, convex, slimy, and large colorless on SSKM plate. KSB13 was smooth, convex, light white color, and small circular on SSKM plate. Microscopic examination revealed that the isolates were Gram-positive and the cells appeared as rods.

Molecular characterization of both the bacteria indicated that the partial sequences of 16S rDNA of KSB2 and KSB13 showed 99% similarity towards *Bacillus subtilis* and *Bacillus megaterium*, respectively. Phylogenetic tree based on comparative analysis of the 16S rRNA gene sequence showing the relationship among strains, KSB2, KSB13, and other related species and genera of *Bacillus* sp. are presented in Fig. 1. The obtained sequences were deposited in the Gen Bank with accession numbers viz.,

**Table 2.** K solubilization attributes of different KSB.

| Bacteria | K (ppm) | Viscosity (mPa s) | Number of cells (log cfu ml$^{-1}$) | pH |
|---|---|---|---|---|
| KSB 1 | 409.00$^{C}$ | 43.53$^{DEF}$ | 8.53$^{H}$ | 5.23$^{GH}$ |
| KSB 2 | 522.33$^{A}$ | 57.32$^{A}$ | 8.66$^{E}$ | 5.60$^{EF}$ |
| KSB 3 | 370.5$^{DE}$ | 47.33$^{CDE}$ | 8.41$^{M}$ | 5.90$^{BCDE}$ |
| KSB 4 | 370.33$^{DE}$ | 42.83$^{DEF}$ | 8.33$^{P}$ | 5.70$^{CDEF}$ |
| KSB 5 | 380.33$^{D}$ | 48.45$^{BCDE}$ | 8.44$^{L}$ | 5.83$^{BCDE}$ |
| KSB 6 | 309.63$^{I}$ | 39.07$^{FG}$ | 8.37$^{O}$ | 5.97$^{BC}$ |
| KSB 7 | 320.33$^{HI}$ | 43.88$^{DEF}$ | 8.25$^{Q}$ | 5.88$^{BCDE}$ |
| KSB 8 | 344.33$^{FGH}$ | 45.41$^{DEF}$ | 8.51$^{I}$ | 6.12$^{B}$ |
| KSB 9 | 320.42$^{HI}$ | 46.67$^{CDE}$ | 8.25$^{Q}$ | 5.97$^{BC}$ |
| KSB 10 | 453.00$^{B}$ | 55.07$^{AB}$ | 8.89$^{A}$ | 5.18$^{H}$ |
| KSB 11 | 334.33$^{GHI}$ | 42.36$^{DEF}$ | 8.49$^{J}$ | 6.07$^{B}$ |
| KSB 12 | 414.33$^{C}$ | 45.54$^{DEF}$ | 8.77$^{C}$ | 5.85$^{BCDE}$ |
| KSB 13 | 475.33$^{B}$ | 53.05$^{ABC}$ | 8.63$^{F}$ | 5.50$^{FG}$ |
| KSB 14 | 414.33$^{C}$ | 48.70$^{BCD}$ | 8.69$^{D}$ | 5.62$^{DEF}$ |
| KSB 15 | 424.00$^{C}$ | 46.51$^{CDE}$ | 8.80$^{B}$ | 5.52$^{FG}$ |
| KSB 16 | 314.33$^{I}$ | 33.43$^{G}$ | 8.39$^{N}$ | 6.08$^{B}$ |
| KSB 17 | 349.50$^{EFG}$ | 46.54$^{CDE}$ | 8.61$^{G}$ | 5.85$^{BCDE}$ |
| KSB 18 | 364.33$^{DEF}$ | 47.20$^{CDE}$ | 8.61$^{G}$ | 5.92$^{BCD}$ |
| KSB 19 | 324.32$^{GHI}$ | 41.43$^{EF}$ | 8.45$^{K}$ | 6.10$^{B}$ |
| KSB 20 | 349.33$^{EFG}$ | 46.54$^{CDE}$ | 8.61$^{G}$ | 5.85$^{BCDE}$ |
| Control | 54.00$^{J}$ | 0.00$^{H}$ | 0.00$^{R}$ | 6.93$^{A}$ |
| General mean | 361.46 | 43.85 | 8.13 | 5.84 |
| CV(%) | 4.20 | 9.88 | 0.04 | 3.17 |

Data following alphabets indicate significant difference ($p < 0.0001$) among treatments. (mPa s)-dynamic viscosity in Pascal-second; ppm-parts per million.

HQ286641 and JN005782 under the name *B. subtilis* ANctcri3 and *B. megaterium* ANctcri7, respectively.

*P solubilizing and indole acetic acid producing activities.* Both the potent KSB showed P solubilizing and indole acetic acid producing activities. KSB13 showed high P solubilizing and indole acetic acid producing capacity. It was observed that the solubilization of P from tricalcium phosphate by the potent KSB were also accompanied by a drop in pH. Furthermore, it was seen that the viscosity produced by these bacteria in Pikovskayas medium was low compared to K solubilization medium. This low viscosity in P solubilization media might be due to the decreased EPS production compared to the K solubilization media by the potent KSB (Table 4).

**Table 3.** Pearson correlation matrix between released K, pH, viscosity, and number of viable cells of SSKM broth by different KSB.

| | Pearson correlation coefficients, $N = 63$ | | | |
|---|---|---|---|---|
| | Prob > \|r\| under H0: Rho = 0 | | | |
| | Viscosity | Cell count | Released K | pH |
| Viscosity | 1.000 | 0.859 <0.0001 | 0.875 <0.0001 | −0.676 <0.0001 |
| Cell count | 0.859 <0.0001 | 1.000 | 0.821 <0.0001 | −0.661 <0.0001 |
| Released k | 0.875 <0.0001 | 0.821 <0.0001 | 1.000 | −0.811 <0.0001 |
| pH | −0.676 <0.0001 | −0.661 <0.0001 | −0.811 <0.0001 | 1.000 |

## EPS production

The yield of EPS in K solubilizing medium were 370 and 335 mg L$^{-1}$ and in P solubilizing medium were 320 and 340 mg L$^{-1}$ by KSB2 and KSB13, respectively.

## Scanning electron microscopic analysis

Scanning electron micrographs of the surfaces of feldspar showed that the surfaces of potent strains treated feldspar exhibited great variation in surface topography, but no variation displayed in control (Fig. 2). Hence, it displayed evidence of mineral dissolution including small etch pits and dissolution craters or cracks. The surfaces of bacterial-treated feldspar were sparsely covered with small particles with numerous bacteria. These particles could be mineralogical, microbial extracellular polymers, or cell debris produced by microbial dissolution.

## Fourier transform infrared spectroscopic analysis

New bands and shifting of bands were detected in the bacteria-treated feldspar compared to the control feldspar (Fig. 3). The OH bands in the region 3200–4000 cm$^{-1}$, methyl group stretch in the 3000–2500 cm$^{-1}$, and 1350 cm$^{-1}$, C═O bands at 1800–1900, 1700–1750, 1700–1650 cm$^{-1}$, N—H and C—N bands between 1500 and 1400 cm$^{-1}$, assymmetric CH3 (carbohydrate) at 1460 cm$^{-1}$, and hydrogen bond shifting also seen in 1000–1050 cm$^{-1}$ region. This spectra show functional groups related to the presence of exopolysaccharides, carboxylic acids, proteins, nucleic acid, etc. [18–20].

## Effect of physiological conditions on the growth of potent KSB

The two potent KSB were able to grow over a wide range of temperature from 20 to 75 °C, but maximum growth was found at 45 °C. There were no growth at 15 and 80 °C (Fig. 4a). KSB2 and KSB13 appeared to grow reasonably well at pH ranging from 5 to 12 and 4 to 10 with maximum growth at pH 9 and pH 7, respectively (Fig. 4b). It can be seen that both the KSB were able to survive without the addition of NaCl and had high tolerance for salt concentration up to 8% (Fig. 4c). The optical density of KSB2 was highest at salt concentration between 0.5 and 5% and peak growth at 1% NaCl. The

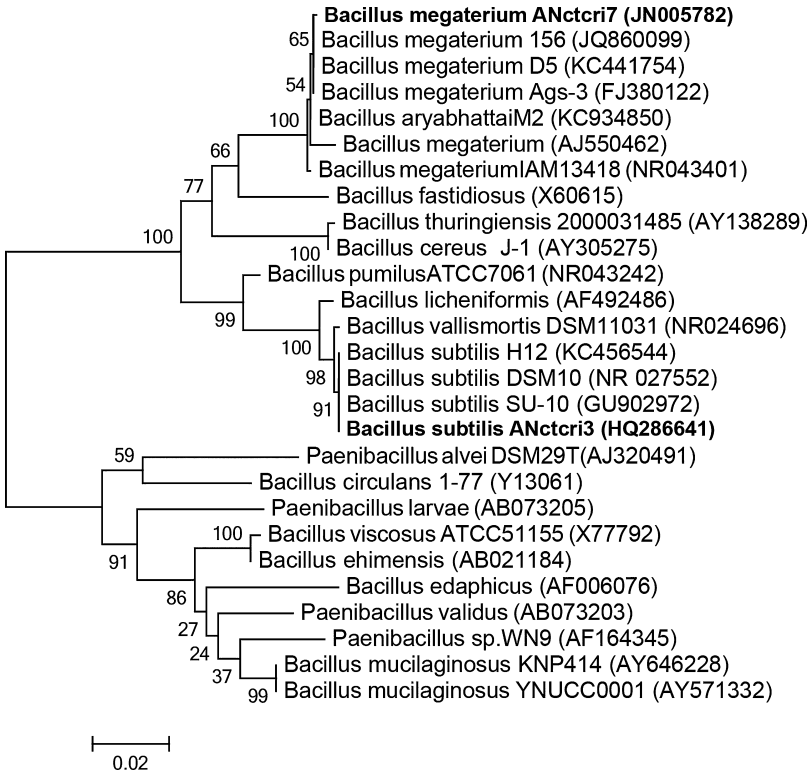


**Figure 1.** Phylogenetic analysis of the KSB2 (HQ286641) and KSB13 (JN005782) using Mega5 by maximum likely hood method. Test of phylogeny by bootstrap method with 2000 replications. The GenBank sequence accession numbers were indicated in brackets after the strain names.

KSB13 showed good growth between 0 and 6% NaCl, with the highest growth at 0.5%.

### Rock powder bioformulation

The formulation of potent KSB using K containing rock powder as carrier material was found to be very effective. The viable cell count (log cfu $ml^{-1}$) and efficiency of K solubilizing capacity (log ppm of K) of KSB during storage are presented in Fig. 5. The results of this study showed that the initial population was maintained by both the bacteria up to 4 months of storage period, without any decrease in population. Moreover, high viability of $10^7$ cfu $ml^{-1}$ was noted up to 10 months of storage. It was also observed that the K solubilizing capacity of both KSB could be retained without large variation and slight increase in efficiency after 2 months of shelf life in rock powder was also noted.

### Effect of KSB application on tuber yield of elephant foot yam

The tuber yield of elephant foot yam was influenced significantly by the two KSB and is presented in Table 5. Among the different treatments, POP (T12) recorded significantly the highest tuber yield of 37.55 t $ha^{-1}$ which was on par with the application of KSB2 and

**Table 4.** Plant growth promoting attributes of potent KSB.

| | P solubilization attributes | | | | | |
|---|---|---|---|---|---|---|
| Bacteria | Soluble P (ppm) | pH | Viscosity (mPa s) | EPS (mg $L^{-1}$) | Number of cells (log cfu $ml^{-1}$) | Production of indole acetic acid (µg $ml^{-1}$) |
| KSB2 | 600 | 5.2 | 38.8 | 320 | 8.11 | 14.5 |
| KSB13 | 625 | 4.6 | 39.5 | 340 | 8.38 | 19.7 |

mPa s, dynamic viscosity in Pascal-second.

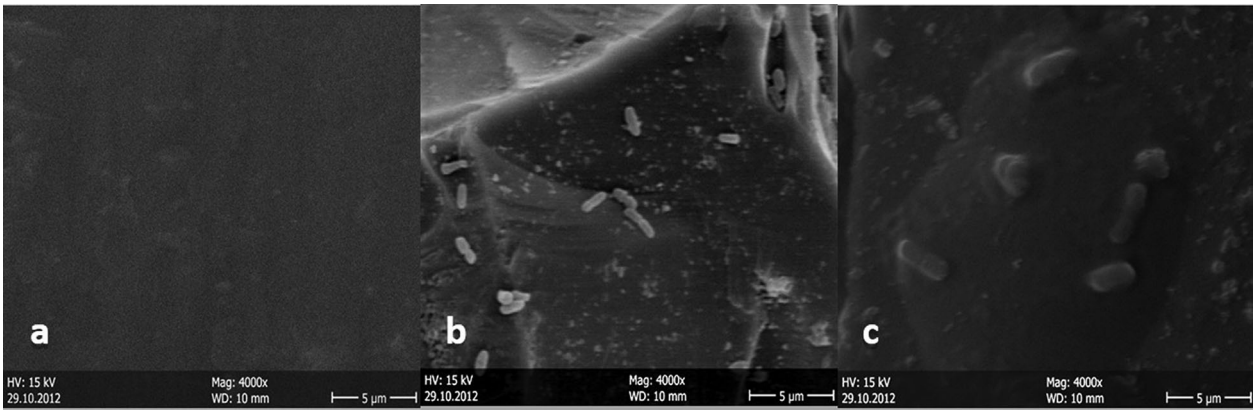


**Figure 2.** Scanning electron microscopic image of control feldspar without bacteria (a), feldspar surface with potent bacteria KSB2 (b) and KSB13 (c).

KSB13 along with K @100 and 150 kg ha$^{-1}$ (T5, T6, T9, and T10) and was followed by T3 and T8. Application of KSB2 (T1) and KSB13 (T2) alone without any chemical fertilizers also showed higher yield over absolute control treatment (T11).

## Discussion

The microbial interaction and mineral dissolution in geological system is one of the significant features in ecology with most attention being focused on the application of this principle in plant growth promotion. This concept directed us toward the detailed characterization of KSB from rocky area with plenty of vegetation. From the present study, it was found that the rock inhabiting bacteria have high capacity to solubilize K from feldspar. The hilly sample site, Ponmudi (site famous for biodiversity), provided a good number of KSB. Moreover, the bacterial diversity could be noted by the isolation of more than 30 morphologically different bacteria from this area. Among these, 55% bacteria were considered as efficient K solubilizers because of their prominent growth on SSKM agar plates compared to the other bacteria in the presence of unavailable K source viz., feldspar. Upon quantification, it is seen that they have the ability to release significant amount of K in the range of 309–522 ppm with variations among different isolates. The isolates viz., KSB2 and KSB13 exhibited higher capacity to release K from feldspar with 10.86–68.85% and 1.62–53.66% increased release, respectively, compared to other KSB under study. Badr et al. [21] also reported similar K releasing capacity of silicate solubilizing bacteria in pure culture isolated from different feldspar samples. It was also seen that the final pH of broth after the incubation period was

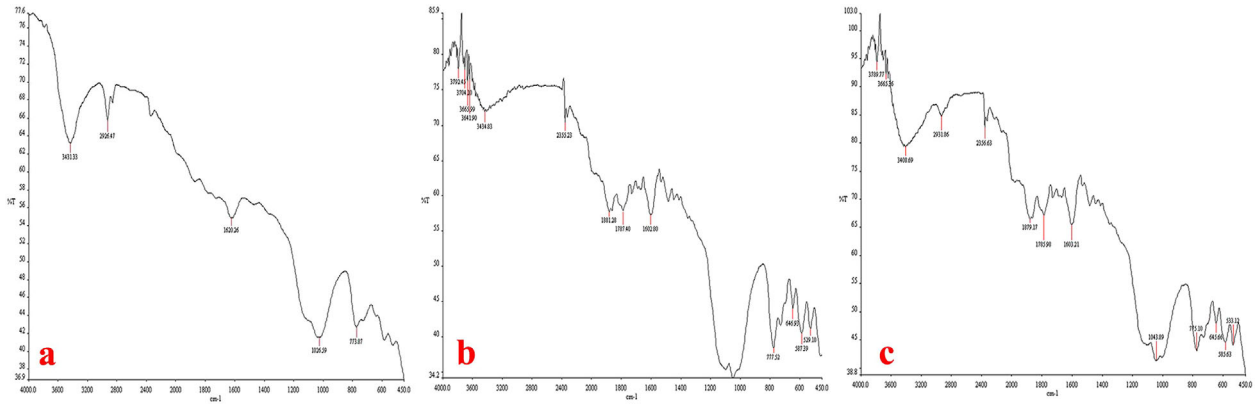


**Figure 3.** Fourier transform infrared spectroscopic analysis spectra of control feldspar (a), feldspar treated with KSB2 (b) and KSB13(c).

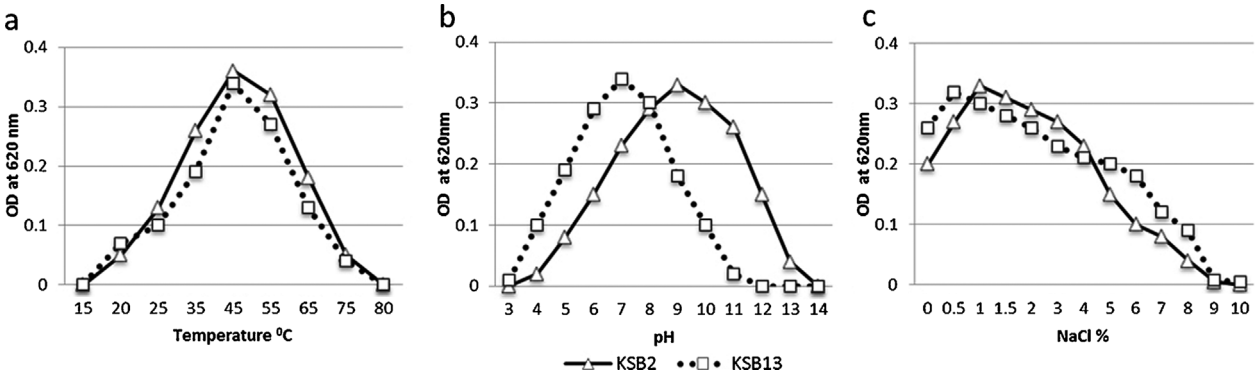


**Figure 4.** Effect of various physiological conditions on growth of KSB2 and KSB13 in SSKM broth. Growth was determined by OD at 620 nm after 24 h of incubation.

decreased. Previous experiments indicated that the pH decrease during the initial phase of mineral dissolution was due to the production of low-molecular weight organic acids [22]. The present results prove the fact that the pH of the medium supplemented with feldspar was decreased by the action of KSB which indicates the production of acids. Earlier reports suggested that the K solubilizers are capable of producing organic acids like citric, oxalic, fumaric, and tartaric acids [23, 24] and can increase mineral dissolution rates in laboratory experiments [25, 26]. However, the decrease in pH due to acidity was not the only direct reason for the release of K from feldspar.

Another important aspect visualized was the increased colony forming units and viscosity in the medium during K solubilization. As suggested by Girgis et al. [23], increases in viable cells might be attributed to the utilization of K by the organism which is followed by effective metabolic activity on the substrate. Also, the bacteria with significantly high K solubilizing capacity also produced high viscosity. However, there was also no clear correlation between the amount of K released and viscosity of the culture broth. It was suggested that the viscosity of the culture media is associated with the presence of EPS and quantification of the EPS produced by the potent strains revealed its highest EPS production potential. Warren [27] reported that microbe surface interactions play a significant role in weathering processes and the EPS produced by bacteria is able to protect bacteria against environmental stress by forming biofilm and this microenvironment facilitates the extraction of inorganic nutrients from mineral surfaces. The extracellular matrix which holds together the constituent cells of the biofilm is composed of polysaccharides, proteins, and nucleic acids, [28, 29]. Biofilm formation by these exopolymeric substances is only

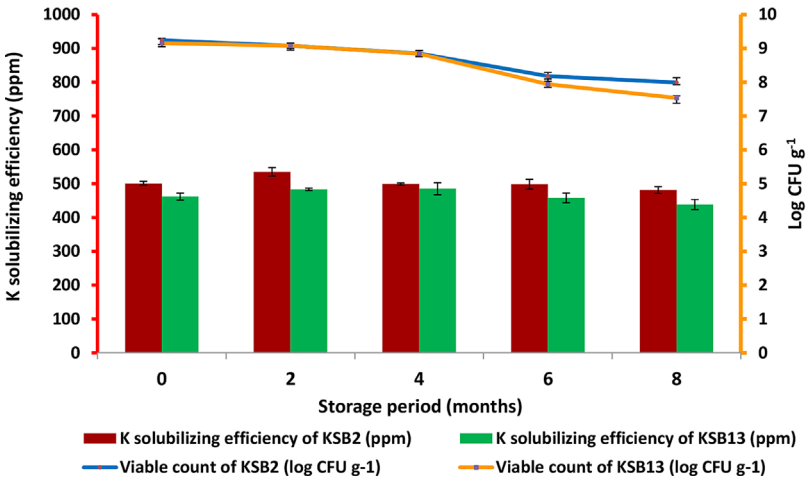


**Figure 5.** Survival and efficiency of potent KSB in rock powder formulation.

**Table 5.** Effect of KSB application on tuber yield of elephant foot yam.

| Treatment no. | Treatment description | Tuber yield (t ha$^{-1}$) |
|---|---|---|
| 1 | KSB2 alone | 31.12$^{CD}$ |
| 2 | KSB13 alone | 29.57$^{D}$ |
| 3 | K0+KSB2+ NP as per STBF | 32.81$^{BC}$ |
| 4 | K50+ KSB2+ NP as per STBF | 33.67$^{B}$ |
| 5 | K100+ KSB2+ NP as per STBF | 36.81$^{A}$ |
| 6 | K150+ KSB2+ NP as per STBF | 37.21$^{A}$ |
| 7 | K0+KSB13+ NP as per STBF | 31.09$^{CD}$ |
| 8 | K50+ KSB13+ NP as per STBF | 32.78$^{BC}$ |
| 9 | K100+ KSB13+ NP as per STBF | 36.70$^{A}$ |
| 10 | K150+ KSB13+ NP as per STBF | 37.04$^{A}$ |
| 11 | Absolute control | 24.29$^{E}$ |
| 12 | Package of practice (POP) | 37.55$^{A}$ |
| General mean | | 33.38 |
| *p*-Value | | <0.0001 |
| CV (%) | | 3.44 |

POP, N@100, P@50, and K@150 kg ha$^{-1}$; STBF, soil test based fertilizer.

partially characterized in *B. subtilis*, a model organism for biofilm study [30]. Thus, this study indicated that lower pH, increase in number of cells, and the consequent increase in viscosity due to EPS are allied factors affecting K solubilization from feldspar. Fourier transform infrared spectroscopic spectra also showed the functional groups related to them which in turn indicated the presence of EPS, organic acids, proteins [18–20]. It was also observed that several functionalities were involved in bacterial feldspar dissolution, bacteria to mineral adhesion, and/or metal complexation. There are previous reports indicating the production of organic acids and capsular polysaccharides as associated with the solubilization of feldspar by application of K solubilizing microorganisms [23, 31]. Furthermore, scanning electron microscopic study also showed small etch pits and cracks with covering of small particles on feldspar surface which may be resulted from bacterial attachment and/or their secretory products and is in agreement with the findings of Styriakova et al. [32]. Gehrke [33] also described the solubilization of minerals by a contact leaching mechanism in which exopolymeric substance that can form biofilm that in turn also aids in solubilization. It is being reaffirmed that the K solubilization by different KSB is mainly correlated with lowering of pH, increase in viscosity due to EPS and bacterial attachment.

Apart from the K solubilizing activity, potent isolates showed P solubilizing and indole acetic acid producing capacities *in vitro*. This is possibly an indication to act as a good plant growth promoting bacteria. Similar to the K solubilization process, P solubilization was also accompanied by decrease in pH and increase in viscosity and number of cells. However, solubilization of P from tricalcium phosphate resulted in low pH and decreased EPS production than in K solubilization from feldspar by KSB2 and KSB13. The observed viscosity in P solubilization was lowered by 32.31 and 26.39% compared to the viscosity formed in K solubilization by KSB2 and KSB13, respectively. This indicates that organic acids may be superior to EPS for P solubilization process than for K solubilization in feldspar by these bacteria. Previous experimental studies reported the P and K solubilization properties of *Bacillus* sp. with the change in pH and EPS production [23].

Based on the phylogenetic analysis of the 16S rDNA obtained by PCR-amplification, the KSB2 and KSB13 isolates were assigned to species as *B. subtilis* and *B. megaterium*, respectively. The sequences were submitted in the GenBank of NCBI under the definition *B. subtilis* ANctcri3 and *B. megaterium* ANctcri7, respectively. Phylogenetic tree revealed the relationship of these bacteria with different *Bacillus* sp. by forming a major cluster and also showed that it was divided from the cluster involving *Peanibacillus* group. Numerous studies have shown that *Bacillus sp.* can promote the release of K from silicate minerals [21, 34] and can produce indole acetic acid [35]. It was observed that KSB2 and KSB13 could tolerate wide range of temperatures, pH, and salinity and were found showing the growth characteristic feature of moderate thermophilic facultatively alkaliphilic halotolerent bacteria. Similar kind of results have also been found for the K solubilizing *Bacillus* sp. from mica cores of Andra Pradesh, India [36]. Several moderately thermophylic bacteria have been identified from ore deposits [37], coal spoil tips [38], coal slag pile [39], and some of them were found as iron, sulfur, and gold ore oxidizers.

The study on the shelf life of potent KSB with rock powder-based bioformulation indicated that longer shelf life can be achieved with the use of K containing rock powder. They could also retain the K solubilizing capacity in rock powder during storage. Furthermore, it was found from the field experiment that about 33% of the K fertilizer could be replaced with the application of our potent KSB in rock powder-based formulation in the cultivation of one of the high yielding and high K demanding crop, elephant foot yam under a very low soil available K condition. This in turn meant that under a

very low soil available K, the application of chemical fertilizer K could be reduced to 100 kg ha$^{-1}$ (67%) by using the KSB rock powder bioformulation. Even without any fertilizer, these KSB could yield 21.7–28.11% increase over control and this might be attributed to their high ability in K and P solubilization and production of plant growth promoting substances. The result is in conformity to the findings of other researchers [40, 41, 21]. These potent KSB can thus be used as a substitute to the chemical K fertilizer to sustain productivity in an economically feasible and ecofriendly manner.

## Conclusion

The present study indicated that out of the total isolates from Ponmudi hills, 55% of bacteria had the *in vitro* ability to extract K from feldspar. The potent bacteria *B. subtilis* ANctcri3 and *B. megaterium* ANctcri7 were efficient in K solubilization with higher capacity to produce exopolysaccharides. It was understood that lower pH, EPS, and number of viable cells are allied factors in K solubilization by both bacteria. P solubilization by the above strains resulted in low pH and EPS production was less compared to K solubilization. The present research work could also highlight the P solubilization, indole acetic acid production potential, and moderate thermophilic facultatively alkaliphilic halotolerant nature of the potent KSB. Moreover, this study clearly demonstrated the significance of bioformulation using rock powder as a good carrier material and field experiment with this formulation explored the possibility of substituting fertilizer K with these potent strains to some extent so that crop production practices can be more cost effective, ecofriendly, sustainable, and agriculturally profitable. Therefore, the findings in this study will ultimately guide in the modeling and development of mineral solubilizers as biofertilizers for application in crops as well as for other industrial uses.

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