

SCREENING OF POTASH SOLUBILIZING BACTERIA (KSB) FROM COW DUNG

P Maheswari* and M Pavitra

Department of Microbiology, Ayya Nadar Janaki Ammal College (Autonomous),
Sivakasi - 626123, Tamil Nadu

Plant growth is enhanced by the fertilizers which provide nutrients that are responsible for about 55% of the yield (Booze-Daniels *et al.*, 2003). Organic fertilizers improve the soil structure, enhance aeration, and facilitate the release of nutrients by microbial degradation and increase soil fertility. One of the main advantages of organic fertilizers is that they boost microbial activity in the soil. Microbes help in degradation of the complex nutrients present in the organic fertilizers into readily utilizable form (Veluchamy, 2010).

After nitrogen (N) and phosphorus (P), potassium (K) is the third essential macronutrient required for plant growth and plays significant roles in activation of several metabolic process including protein synthesis, photosynthesis, enzyme activation, as well as in resistance to diseases and insects, etc. (Rehm and Schmitt, 2002). It is involved in the adjustment of plant cellular osmotic pressure and the transportation of compounds in plants (Sparks, 1987). Moreover, potassium also promotes root growth, stronger stem and increases resistance to cold and water stress. It directly improves the crop quality and reduces pest and disease incidence by enhancing crop resistance (Cakmak, 2005).

Majority of the soil potassium exists in the form of silicate. The most common forms of potassium in soil are feldspar and mica (90 to 98%) (McAfee, 2008) but little amount of this K is available for plant use. This potassium is made available to plants when the minerals are slowly weathered or solubilized (Bertsch *et al.*, 1985). Soil microorganisms influence the availability of soil minerals by playing a central role in ion cycling and soil fertility (Lian *et al.*, 2010).

The potassium solubilizing bacteria (KSB) were found to dissolve potassium, silicon and aluminium from insoluble K-bearing minerals such as micas and orthoclases by excreting organic acids (Bennett, 1998) or production of capsular polysaccharide (Han, 2006). Fertilizer production using potassium-bearing minerals (like feldspars and some mica) is not viable because the majority of them are insoluble and their dissolution-process requires strong chemical and thermal treatments (Nascimento *et al.*, 2008). India

does not have commercially viable sources of potash, India is totally depended on import of potassic fertilizers (Bahadur *et al.*, 2014)

The silicate solubilizing bacteria exert beneficial effects on plant growth and their application as biofertilizers or biocontrol agents for agriculture improvement and environmental protection have been a focus of recent research (Glick, 1995). Several strains of potassium solubilizing bacteria, such as *Pseudomonas*, *Bacillus ferroxidans*, *B. mucilogenosus*, *B. edaphicus*, *B. circulans* and *Paenibacillus sp.* (Liu *et al.*, 2012) can be used as biofertilizer. Thus, mineral potassium solubilization by microbes enhances crop growth and yield when applied with a cheaper source of rock potassium which might be agronomically more useful and environmentally more friendly than soluble K (Rajan *et al.*, 1996).

Cow dung sample was collected from Srivilliputtur, Virudhunagar district, Tamil Nadu. The cow dung was serially diluted and spread onto the Aleksandrov agar medium. After 72 hours of incubation, the appearance of clear zone around the colonies on the Aleksandrov agar indicated the presence of potassium solubilizing bacteria

Identification of bacteria by 16S rRNA gene sequencing analysis

Amplification of 16S rRNA gene sequence

Bacterial 16S rRNA was amplified from the extracted genomic DNA using the following universal eubacterial 16S rRNA primers: forward primer 5' AGAGTTTGATCCTGGCYCAG 3' and reverse primer 5' ACGGCTACCTTGTACGACTT3'. Polymerase chain reaction was performed in a typical reaction mixture involving - 2µl of template DNA, 1.5µl of reverse primer, 10µl of 2X PCR master mixes and 5µl of nuclease free water for 20µl reaction. The reaction was performed with an initial denaturation at 94°C for 45 seconds, annealing at 56°C for 1 minute, extension at 72°C for 1 minute 30 seconds followed by final extension at 72°C for 5 minute and hold at 4°C. The amplification of 16S rRNA gene was confirmed by running the amplification product in 1% agarose gel electrophoresis.

*Corresponding author : mahes02@gmail.com
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Morphological characterization

Gram staining

The gram staining was performed using the Smear of culture in the glass slide, air dried and heat fixed. Firstly, the smear was treated with crystal violet for 30 seconds followed by washing with distilled water, then Grams iodine was added and kept at 30 seconds. After washing it was decolourized with 95% ethyl alcohol for 30 seconds and washed, afterwards it was treated with counter stain (safranin) for 1minute, washed, blot dried and observed under microscope.

Biochemical characterization

Indole production test

Indole production test is used to determine whether test organism can oxidize tryptophan for the synthesis of Indole compounds. The isolates were inoculated into test tubes containing tryptophan broth and one tube without inoculums kept as control followed by incubation for 24 hours at 37°C. Culture producing a cherry red colour following addition of Kovac's reagent is indole positive. The absence of red colour indicates a negative reaction.

Methyl red test

The methyl red test is employed to detect the ability of microorganism to oxidize glucose with the production of high concentration of acid products. The isolates were inoculated into test tubes containing MR-VP broth and one tube served as control. Tubes were incubated for 24 hours at 37°C. Following incubation, 5-6 drops of methyl red solution were added. Colour change in medium was examined and recorded.

Voges-proskauer test

This test is used to differentiate the capacity of the microorganisms to produce some non-acidic (or) neutral end product such as acetyl methyl carbinol (or) 2,3, butanol. The isolate were inoculated into test tube containing MR- VP broth and one tube served as control. The tubes were incubated for 24 hours at 37°C. Development of deep rose color after 15 minutes following addition of Baritts's reagent was recorded.

Citrate utilization test

The culture was inoculated in the Simmons citrate agar slants and incubated at 37°C for 24-48 hours. This medium contains bromothymol blue (green at acidic pH around 6.0 and changes to blue at alkaline pH around 7.6).Growth of bacteria in the media leads to the development of a prussian blue colour.

Triple sugar iron test

Triple sugar Iron (TSI) Agar test differentiated the genera based on carbohydrate fermentation patterns and hydrogen sulphide production by various groups of intestinal organisms. The TSI medium was prepared and sterilized at 121°C for 15 minutes at 15 lbs pressure. The medium was dispersed into sterilized tubes and slants were prepared. The butt was stabbed and then streaking was done on the slant. Then the slants were incubated at 37°C for 24hours. After incubation results were observed and recorded.

Catalase test

Catalase test was used to check the production of enzyme catalase. The isolated culture was transferred to the center of the slide and 1-2 drops of hydrogen peroxide were added. Catalase positive organisms were indicated by strong bubble formation by breaking OH. Whereas no bubble formation indicated catalase negative.

Oxidase test

Oxidase enzymes play a vital role in the operation of the electron transport system during aerobic respiration. A drop of culture suspension was placed on clean glass slide. To the culture, few drops of p-aminodimethylaniline oxalate were added. Oxidase positive microorganisms were indicated by blue color formation.

Quantitative estimation of potassium release

A loopful of 48 hour old grown bacterial culture was inoculated into 25 ml Aleksandrov medium broth in 50 ml capacity flask containing either of different sugars; glucose, galactose, xylose or arabinose. All the inoculated flasks were incubated at 28±2°C for 10 days. The growth suspension was centrifuged at 7,000 g for 10 minutes in the Hettick Mikro Rapid centrifuge (Tuttlingen) to separate the supernatant from the cell growth and insoluble potassium. One ml of the supernatant was taken in a 50 ml volumetric flask and the volume was made to 50 ml with distilled water and mixed thoroughly. The solution was fed to atomic absorption spectrometer to determine K content. Standard curve was prepared using various concentrations of 10 ppm KCl solution i.e., 0.5, 1.0 and 1.5 ppm. The amount of potassium solubilized by the bacterial isolates was calculated from the standard curve.

Selected KSB colonies were placed on Aleksandrov agar medium and the plates were incubated for 72 hours at 37° C. After incubation clear zone formed around the colonies. Appearance of zone (Table 1) indicates the microorganisms were able to solubilize the insoluble potassium into soluble form.

Table 1. Measurement of zone

Isolate	Zone diameter (mm)
PB1	10mm
PB2	12mm
PB3	8mm
PB4	6mm
PB5	5mm

The amount of potassium solubilized by the bacterial isolate was calculated from the standard curve via atomic absorption spectroscopy (Fig. 1)

The bacterial isolates released potassium at a rate of 0.8613 ppm. The selected strain was biochemically and molecularly identified. This strain exhibited Gram negative, rod shaped and motile bacteria (Table 2).

Table 2. Morphological and biochemical characteristics of *Comamonas kerstersii* PB2

S.No.	Characterization method	Result
A)	Colony morphology	Observation
1.	Gram staining	Gram negative, cocci
2.	Motility test	Motile
B)	Biochemical characters	Result
1.	Indole production test	Negative
2.	Methyl red test	Positive
3.	Vogesproskauer test	Negative
4.	Citrate utilization test	Negative
5.	Oxidase test	Positive
6.	Catalase test	Positive
7.	Triple sugar iron test	Positive

Identification of KSB by 16SrRNA sequencing provided the following results:

CATGCAGTCGAGCGGAGCACGGACTTC
GGTCTGTTGTCGAGCGGCGAACGGGTGAGTA
ATGCATCGGAATCTGCCTGTTAGTGGGGATA
ACTTCTCGAAAGGGTGGCTAATACCGCATGAGA
CCTACGGGTGAAAGCAGGGGATCTCCGGACCTT
GTGCTAATAGAGGGGCCTAAGGCGGATTAGG
TAGTTGGTGGGATAAAGGCTTACCAAGGCGAC
GATCTGTATCTGGTCTGAGAGGATGATCCGCCA
CACTGGGACTGAGACACGGCCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATTTTGGGA
CAATGGGGAAAACCCTGATCCAGCCTGCTGCGT
GTGAAAAAAGCCCTTTTGGTTGTAACTGCTTTT
GTCCAGGAGGAGGAGCTCTAGGCTAATATCCTG
GATGCGTGGACGTACCGCAAGAAATGCACCC
GCTAAACTCTGTGTCCAGCCGCCGTAATATA
CAAAGGGGGCGCGGTTATTTCGAATTTTGGGG
TAAAAGCGCGCGTAGGTGGCTGTAAGAC
CAAATGAAACCCCGACTCTTCCTTGGAAT
GCCTTTGTGACTGGGTTATATAGTAATGGGAGA
GGATATGGAAATTCCGTGGTGCAGTGAATGC
GCATATATATCTGAGAAAAACCCATGGGAAAGC
GCTCCCCTGTGGCCTGTACTGACACTCATACTG
GAGAACGTGTGGAGAGCACACGAGAATATATAC
CGTGAGTGCACATGCCATACAATATCACTTCGAT
GTGGGGTCTTCATTGACTCTTTAACGACGCAAC
TAACAGAAATAGACCACCTGCCGGGTAGGCC
GCCGGGATGACTACTACTCAGAATTGATTGGAG
GACCGCACGCAGCAGCGGATGAGTTGTTGATT
TAATTCGCATGCCACGACAAACATTCTCACCTGT
CATATGACTAGAATCCACTTACACATAGATGGATTG
GTGGACTGAAGAACTGCAACATAAGGGTGTGCGT
GTGTGTGTCCTCATCTCGTTGCT (Fig. 2).

Maximum solubilisation of potassium was observed in isolate, *Comamonas kerstersii*. Cheng sheng Zhang (2014) isolated KSB strains from tobacco rhizosphere. Potassium feldspar powder agar plate was used in the

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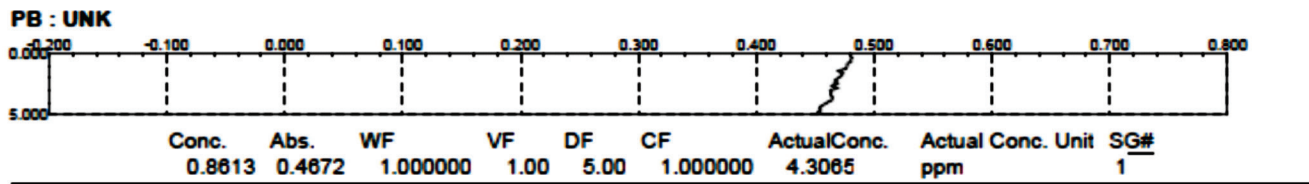


Fig 1. AAS standard curve for potassium

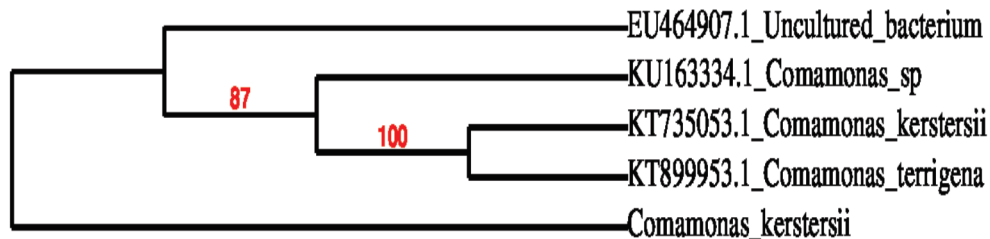


Fig 2. Phylogenetic tree of *Comamonas kerstersii* PB2

quantitative measurement of the K solubilization ability. All the bacteria were found capable of solubilizing K, and the solubilization zone ranged from 0.11 to 0.30cm. The phylogenetic relationship was obtained using neighbour joining by pair wise comparison among the 16S rRNA gene sequence of selected isolates with species. The dendrogram was constructed for their phylogenetic relationship and it revealed that the isolate *Comamonas kerstersii* was distinctly placed under separate clusters. The 16S rRNA gene sequences of the isolates had been submitted to the NCBI Genbank. Liu (2016) also investigated phylogenetic affiliation of this strain; the complete 16S rRNA gene was amplified, sequenced and deposited. Comparison of the obtained sequence with other sequences available at NCBI database revealed the greatest similarity to the corresponding sequences of *Streptomyces*.

Authors' contribution

Conceptualization of research work and designing of experiments (PM,MP); Execution of field/lab experiments and data collection (PM,MP); Analysis of data and interpretation (PM,MP); Preparation of manuscript (PM,MP)

LITERATURE CITED

Aleksandrov V G, Blagodyr R N and Illev I P 1967. Liberation of phosphoric acid from apatite by silicate bacteria. *Mikrobiyolzh (Kiev)* **29**:111-14.

Bahadur I, Meena V S and Kumar S 2014. Importance and Application of potassic Biofertilizer in Indian Agriculture. *Int Res J Sci* **3**(12):80-85.

Bennett P C, Choi W J and Rogera J R 1998. Microbial destruction of feldspars. *Mineral Manag* **8** (62A):149–50.

Bertsch P M and Thomas G W 1985. Potassium status of temperature region soils. In Munson R D (Ed.) *Potassium in agriculture ASA, CSSA and SSSP, Madison WI*. pp.131-62.

Booze-Daniels, J N and Schmidt R E 2003. *The use of slow release nitrogen fertilizers on the roadside: A literature review*. Virginia Transportation Research Council, Charlottesville, VA.

Cakmak I 2005. The role of potassium in alleviating detrimental effect of abiotic stresses in plants. *J Plant Nutr Soil Sci* **168**:521-30.

Glick B R 1995. The enhancement of plant growth by free-living bacteria. *Canadian J Microbiol* **41**:109-17.

Han S, Jung, H S, Lee K D (2006). Rock potassium and phosphate rock solubilizing bacteria as alternative sustainable fertilizers. *Agronomy and Sustainable Development*. **26**:233-40.

Lian B, Wang B, Pan M, Li C and Teng H H 2010. Microbial release of potassium from K-bearing minerals by thermophilic fungus *Aspergillus fumigatus*. *Geochim Cosmochim Acta* **72**(1): 87–98.

Liu D F, Ljan B, Wang B and Heidelberg S V B 2016. Potassium containing minerals by high temperature resistant *Streptomyces sp.* isolated from earthworms gut. *Acta Geochim* **35**(3): 262-70.

McAfee J 2008. Potassium, A Key Nutrient for Plant Growth. Department of Soil and Crop Sciences. <https://www.mdpi.com/2077-0472/10/9/401/http://jimmcafee.tamu.edu/files/potassium>

Nascimento M, Monte M B M and Loureiro F E L 2008. Agrominerals potassio In Rochase minerais industriais: usos e especificacoes. *CETEM, Riode Janeiro Brazil*. pp: 175–203.

Rehm G and Schmitt M 2002. Potassium for crop production. Retrieved February 2, 2011, from Regents of the University of Minnesota.

Sheng X F and He L Y 2006. Potassium bearing minerals by a wild type strain of *Bacillus edaphicus* and its mutants and increased potassium solubilization of uptake by wheat. *Canadian J Microbiol* **52**:66-72.

Sparks D L, Huang P M and Munson R D 1987. Physical chemistry of soil potassium. In *Potassium in agriculture. Agronomy J*: 201-76.