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Characterization of Potassium Solubilizing Bacteria (KSB) in Rhizospheric Soils of Apple (*Malus domestica* Borkh.) in Temperate Kashmir

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Authors' contributions

This work was carried out in collaboration between both authors. Author MSA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MYZ managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Twenty seven bacterial isolates capable of solubilizing potassium were isolated from rhizospheric soils of apple var. delicious collected from sixty different orchards of Kashmir valley. Out of them, 15 isolates were isolated from soil samples collected from Shopian district and 12 were isolated from Baramulla district. Twenty three isolates formed spores and four could not form spores. All isolates had shown positive results for catalase, urease, casein hydrolysis, acid production tests and negative results for Voges-proskauer and H_2S tests. While as all isolates showed positive results for Starch hydrolysis and Methyl red tests and negative results for Denitrification and gas production tests except KSB6, KSB22, KSB26 and KSB34 which showed *vice versa* results. So, twenty three isolates were gram positive rods belonging to *Bacillus* genera and four were gram negative rods belonging to *Pseudomonas*.

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1. INTRODUCTION

Potassium, one of the essential macronutrients and the most abundantly absorbed cation (K^+) in higher plants, plays an important role in the growth and development of plants. It activates enzymes, synthesizes proteins, maintains cell turgidity, enhances photosynthesis, reduces respiration, helps in transport of sugars and in nitrogen uptake. It also improves crop quality as it helps in grain filling and kernel weight, strengthens straw, increases disease resistance and helps the plant to withstand stress [1]. The temperate region soils like soils of Kashmir exhibit a slow releasing pattern of potassium and dominated in 2:1 type clay minerals [2]. Available potassium in major soils of Kashmir is low to medium [3]. In general, black soils are high, red soils medium, and laterite soils low in available potassium. Although K deficiency is not as wide spread as that of nitrogen and phosphorus, many soils which were initially rich in K become deficit in due course due to heavy utilization by crops and inadequate K application, runoff, leaching and soil erosion [4]. Potassium is the 4th most abundant nutrient constituting about 2.5% of the lithosphere. There are three forms of potassium found in the soil *viz.,* soil minerals (>90 to 98%); non-exchangeable (1 to 10%); and available form (1 to 2%). The available form is found either in the solution or as part of the exchangeable cation on clay mineral. Its availability is dependent upon the K dynamics as well as on total K content [5].

According to Brown [6], apples are the most produced temperate fruits and they are widely grown throughout almost the entire temperate climate region in the Northern and Southern hemispheres. It is grown at an altitude ranging from 1350 to 2600 meters above mean sea level with an annual rainfall of 125 to 175 cm. The average temperature for its optimum growth should be around 21-24℃ with chilling temperature below 7℃ to break rest period. Deep, well drained loamy soils with pH range from 5.5 to 7.8 are suitable for its cultivation. Recently apple cropping has been expanding into subtropical and tropical zones [7]. Apple grown in Jammu and Kashmir which is located in extreme north of India between 32°.17′ to 37°.50′ N latitude 72°.14′ to 80°.30′ E longitude is world famous for its taste, aroma and colour. Jammu and Kashmir occupies a significant position in the horticultural map of India with an area of 347223 ha under different fruit crops and annual production of 1742142 metric tons of fruit [8].

The rhizospheric part of a plant is as important for the plant health as the phylospheric part which contains harmonious friendly microorganisms to normalize the soil health and put pathogenic organisms to stress conditions, thus extend the life span of a soil [9]. Interactions among the rhizobacteria which are present in rhizospheric soils and colonize roots of the plants have been studied intensively [10,11]. Some of the rhizobacteria play a key role in the natural nutrient cycles. Some species of rhizobacteria are capable of mobilizing potassium in accessible form in soils. Silicate bacteria were found to dissolve potassium, silicon and aluminum from insoluble minerals [12]. The main focus on potassium solubilizers is because potassium is required by all crops as a macro-nutrient.

To achieve intensive fruit production large agricultural inputs are required for achieving high productivity and fruit quality. However, the excessive use of chemical fertilizers which involves high production costs leads to environmental pollution also. The use of more sustainable technologies like biofertilizers and organic farming, is inevitable for the mitigation of environmental damage [7,13]. Keeping in view the adverse effects of agro-chemicals on the soil health of apple orchards and low availability of soil potassium an attempt through the present study was made to assess the potassium solubilizing bacterial wealth status of the rhizospheric soils of apple in Shopian and Baramulla districts of Kashmir valley. The present investigation was carried out with the following objectives.

1. Isolation of potassium solubilizing bacteria from rhizosphere soils of apple.
2. Characterization of potassium solubilizing bacteria (KSB)

2. MATERIALS AND METHODS

The investigation was carried out at Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Wadura, Sopore during 2014-15. Sixty composite rhizospheric soil samples of apple trees (var. delicious) were collected from sixty representative orchards of twenty (10+10) selected villages of

Shopian and Baramulla districts of Jammu & Kashmir and brought in sterilized zip-locked polythene bags to the laboratory. Each representative composite sample was comprised of five samples collected from different locations of each orchard. The samples were analyzed after due processing. The bacterial cultures isolated from the rhizospheric soil samples were tentatively identified up to genus level based on their morphological and biochemical characteristics.

Potassium solubilizing bacteria were isolated from the collected rhizospheric soil samples by serial dilution plate count method using Aleksandrov selective medium. The composition of the Aleksandrov medium is 1% glucose; 0.05% $MgSO_4.7H_2O$; 0.0005% $FeCl_3$; 0.01% $CaCO_3$; 0.2% $CaPO_4$ and 0.5% potassium aluminium silicate (usually mica as a source of insoluble form of potassium); agar 3% in 100 ml Distilled water with pH 6.5 [14]. Three replications of 10^{-6} and 10^{-7} dilutions were plated from each sample and the plates were allowed to dry. The plates were incubated upside down at 30±1℃ for 3 days and the colonies exhibiting clear zones were selected. The selected colonies were purified by four way streak plate method. Agar medium containing insoluble potassium bearing mineral, mica powder, were used for preservation of cultures in slants. All the selected bacterial isolates were examined for the colony morphology, cell shape, gram reaction and spore formation ability as per the standard procedures given by Anonymous [15]. Colony morphology were studied with the help of magnifying lens and cell shape of the isolates under microscope. Gram reaction studied by Gram Staining Technique [16], spore formation ability by Endospore Staining technique [17]. The bacterial isolates were biochemically characterized by catalase test, urease test, oxidase test, nitrate reduction test, Methyl red test, Voges – Proskauer test, Starch hydrolysis, Casein hydrolysis, Acid production, gas production, Hydrogen sulphide production, Gelatin liquefaction, Growth at 7% NaCl [18].

3. RESULTS AND DISCUSSION

The location wise details of soil samples collected and the KSBs isolated are furnished in Table 1. Perusal of the data presented in Table 1 revealed that out of the total 27 isolates obtained, 15 were

Table 1. Survey and isolation of potassium solubilizing bacteria from apple orchards of various selected locations of District Shopian and Baramulla

S. no.	District	Location	Number of representative samples collected	Isolates obtained
1.	Shopian	Saidpora Bala,	03	KSB13, KSB21
2.		Zawoora	03	KSB26
3.		Zainpora	03	KSB4, KSB6, KSB33
4.		ImamSahib	03	KSB18
5.		Trenz	03	KSB9
6.		Kachdoora	03	KSB1, KSB3
7.		Boonshan	03	KSB2
8.		Kiloora	03	KSB14
9.		Pinjora	03	KSB27
10.		Memendar	03	KSB22, KSB23
11.	Baramulla	Pattan	03	KSB40
12.		Delina	03	KSB44
13.		Sangrama	03	KSB8, KSB11
14.		Patukha	03	KSB34
15.		Dangerpora	03	KSB37
16.		Bomie	03	KSB50
17.		Janbazpora	03	KSB45
18.		Hadipora	03	KSB48
19.		Wadoora	03	KSB51, KSB55
20.		Watergam	03	KSB58

isolated from soil samples collected from Shopian district and 12 were isolated from Baramulla district. These findings are in agreement with the results which suggest that the chances of isolating mineral solubilizing microorganisms from rhizosphere soils of many crops are more, although they could be isolated invariably from all soils [19]. It was interesting to note that bacterial isolates from rhizospheric soils of apple were able to grow and solubilize the medium containing fixed or insoluble form of nutrients. The results are in agreement with the findings of Osman [20] who also isolated two strains of *Bacillus* sp. and *Pseudomonas* from rhizosphere soil of various crop plants as mineral potassium solubilizers.

These isolates of KSB obtained were identified upto the genus level based on their morphological and biochemical characteristics and the results are presented in Tables 2, 3, and 4. Among 27 isolates identified and characterized, 23 were gram positive rods belonging to genus *Bacillus* and 4 were gram negative rods belonging to genus *Pseudomonas*. The results of this study strongly support the work of Christophe et al. (2013) who isolated many of the root associated bacteria contributing to mineral weathering. He demonstrated that *Burkholderia glathei* PMLI (12) significantly increased biotite weathering [21]. Different groups of microorganisms like *Bacillus mucilaginosus* and *Thiobacillus thioxidans* were isolated capable of solubilizing silicates [22]. Similarly Archana et al. [23] also isolated and characterized bacteria from rhizospheric soils. The probable mechanism of action for solubilization of potassium bearing minerals was by the action of organic acids like oxalic acid and capsular polysaccharides [24].

Table 2. Morphological/colony, cell shape, gram reaction and Spore formation characteristics of various isolates of Potassium Solubilizing Bacteria

S. no.	Isolates	Colony characters	Cell shape	Gram reaction	Spore formation
1.	KSB1	White, smooth, slimy, large	Rod	+ve	+
2.	KSB2	Grayish white, smooth, widely spreading	Rod	+ve	+
3.	KSB3	White, smooth, slimy, widely spreading	Rod	+ve	+
4.	KSB4	White, raised circular	Rod	+ ve	+
5.	KSB6	Creamy white, smooth, small, Raised	Rod	-ve	-
6.	KSB8	Whitish, rough, transparent	Rod	+ve	+
7.	KSB9	Creamy white, small, slimy	Rod	+ve	+
8.	KSB11	White, rough surface, round	Rod	+ve	+
9.	KSB13	White, smooth, widely spreading	Rod	+ve	+
10.	KSB14	Creamy white, small	Rod	+ve	+
11.	KSB18	Creamy white, large, circular	Rod	+ve	+
12.	KSB21	Lava smooth, opaque, creamy white, flat surface	Rod	+ve	+
13.	KSB22	Creamy white, smooth, small, raised	Rod	-ve	-
14.	KSB23	White, smooth, circular, opaque	Rod	+ve	+
15.	KSB26	Creamy white, irregular, opaque	Rod	-ve	-
16.	KSB27	Creamy white, smooth, large	Rod	+ve	+
17.	KSB33	Creamy white, smooth, large size, widely spreading	Rod	+ve	+
18.	KSB34	Creamy, smooth, raised, large	Rod	-ve	-
19.	KSB37	White, raised, slimy, large	Rod	+ve	+
20.	KSB40	Creamy white, small	Rod	+ve	+
21.	KSB44	Grayish white, smooth, widely spreading	Rod	+ve	+
22.	KSB45	White circular, slimy	Rod	+ve	+
23.	KSB48	White, raised, slimy	Rod	+ve	+
24.	KSB50	Smooth, slimy, white, large	Rod	+ve	+
25.	KSB51	Smooth, creamy white, opaque, large	Rod	+ve	+
26.	KSB55	White, circular, raised	Rod	+ve	+
27.	KSB58	White, slimy, circular, round	Rod	+ve	+

Positive: (+) ; Negative : (-)

Table 3. Biochemical characteristics of various isolates of potassium solubilizing bacteria (KSB)

S. no.	Isolates	Catalase test	Urease test	Oxidase test	Denitrification test	Methyl red test	Voges-Proskauer test	Starch hydrolysis	Casein hydrolysis	Acid production	Gas production	H_2S production	Gelatin liquifaction	Growth at 7% Na Cl
1.	KSB1	+	+	-	-	+	-	+	+	+	-	-	+	-
2.	KSB2	+	+	-	-	+	-	+	+	+	-	-	-	-
3.	KSB3	+	+	+	-	+	-	+	+	+	-	-	+	+
4.	KSB4	+	+	-	-	+	-	+	+	+	-	-	+	-
5.	KSB6	+	+	+	+	-	-	-	+	+	+	-	+	-
6.	KSB8	+	+	-	-	+	-	+	+	+	-	-	+	-
7.	KSB9	+	+	-	-	+	-	+	+	+	-	-	+	-
8.	KSB11	+	+	+	-	+	-	+	+	+	-	-	-	-
9.	KSB13	+	+	-	-	+	-	+	+	+	-	-	-	+
10.	KSB14	+	+	-	-	+	-	+	+	+	-	-	-	-
11.	KSB18	+	+	-	-	+	-	+	+	+	-	-	-	-
12.	KSB21	+	+	-	-	+	-	+	+	+	-	-	+	-
13.	KSB22	+	+	+	+	-	-	-	+	+	+	-	+	-
14.	KSB23	+	+	-	-	+	-	+	+	+	-	-	+	-
15.	KSB26	+	+	+	+	-	-	-	+	+	+	-	-	-
16.	KSB27	+	+	-	-	+	-	+	+	+	-	-	+	-
17.	KSB33	+	+	-	-	+	-	+	+	+	-	-	+	+
18.	KSB34	+	+	+	+	-	-	-	+	+	+	-	+	-
19.	KSB37	+	+	+	-	+	-	+	+	+	-	-	+	-
20.	KSB40	+	+	-	-	+	-	+	+	+	-	-	+	+
21.	KSB44	+	+	-	-	+	-	+	+	+	-	-	+	-
22.	KSB45	+	+	-	-	+	-	+	+	+	-	-	+	-
23.	KSB48	+	+	-	-	+	-	+	+	+	-	-	+	+
24.	KSB50	+	+	-	-	+	-	+	+	+	-	-	+	-
25.	KSB51	+	+	+	-	+	-	+	+	+	-	-	+	+
26.	KSB55	+	+	-	-	+	-	+	+	+	-	-	-	-
27.	KSB58	+	+	-	-	+	-	+	+	+	-	-	+	-

Positive: (+) ; Negative : (-)

Table 4. Probable genus of potassium solubilizing bacteria (KSB) isolates

S. no.	Isolates	Probable Genus
1.	KSB1	*Bacillus*
2.	KSB2	*Bacillus*
3.	KSB3	*Bacillus*
4.	KSB4	*Bacillus*
5.	KSB6	*Pseudomonas*
6.	KSB8	*Bacillus*
7.	KSB9	*Bacillus*
8.	KSB11	*Bacillus*
9.	KSB13	*Bacillus*
10.	KSB14	*Bacillus*
11.	KSB18	*Bacillus*
12.	KSB21	*Bacillus*
13.	KSB22	*Pseudomonas*
14.	KSB23	*Bacillus*
15.	KSB26	*Pseudomonas*
16.	KSB27	*Bacillus*
17.	KSB33	*Bacillus*
18.	KSB34	*Pseudomonas*
19.	KSB37	*Bacillus*
20.	KSB40	*Bacillus*
21.	KSB44	*Bacillus*
22.	KSB45	*Bacillus*
23.	KSB48	*Bacillus*
24.	KSB50	*Bacillus*
25.	KSB51	*Bacillus*
26.	KSB55	*Bacillus*
27.	KSB58	*Bacillus*

4. CONCLUSION

The present study resulted in isolation and identification of potassium solubilizing bacteria of genera *Bacillus* and *Pseudomonas.* The range of morphological and biochemical variability seen amongst isolates indicates that it is prudent and necessary to keep a continuous programme on the isolation and characterization of beneficial bacteria like KSB. Potassium availability to crop plants in soil is generally low since nearly 98 per cent of total K in soil is in mineral forms. In this context, attempts were made to isolate different K solubilizing bacteria from rhizospheric soils of apple trees. A total of 27 KSB isolates were isolated on media supplemented with mica as a potassium source on the basis of morpho-biochemical characteristics, all the bacterial cultures were identified upto genus level and were found to belong to the genera *Bacillus* and *Pseudomonas*. All the isolates were able to solubilize mica, a potassic mineral, under *In- vitro* condition.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mc Afee J. Potassium, a key nutrient for plant growth. Department of Soil and Crop Sciences; 2008.
   Available:http://jimmcafee.tamu.edu/files/potassium
2. Subash C, Tahir A. Potassium releasing capacity in some soils of Anantnag District of Kashmir. Universal Journal of Environmental Research and Technology. 2011;1(3):373-375.
3. Rehanul H. Potassium status of soils in India. Better Crops International. 2002; 16(2):3-5.
4. Shanware AS, Kalkar SA, Trivedi MM. Potassium solublisers: Occurrence, mechanism and their role as competent biofertilizers. International Journal of Current Microbiology and Applied Science. 2014;3(9):622-629.
5. Goldstein AH. Involvement of the quino protein glucose dehydrogenase in the solubilization of exogeneous mineral phosphates by gram negative bacteria. In phosphate in micro-organisms: Cellular and molecular biology. Cell. Mol. Biol. 1994;197-203.
6. Brown S. Apple. In: Fruit Breeding, Hand Book of Plant Breeding (Eds. Badenes, M.L. and Byrne, D.H.) Springer Science Business Media, Philadelphia, PA. 2012;329-367.
7. Karakurt H, Aslantas R. Effects of some plant growth promoting rhizobacteria (PGPR) strains on plant growth and leaf nutrient content of apple. Journal of Fruit Ornamental Plant Research. 2013;18:101-110.
8. Anonymous. Area of production. Department of Horticulture, Jammu and Kashmir, Government of India; 2011.

9. Dar GH. Soil microbiology-origin, history and diversification. In: Soil Microbiology and Biochemistry. New India Publishing Agency, New Delhi, India. 2010;17-18.
10. Ambrosini A, Beneduzi A, Slefanski T, Pinheiro FG, Vargas LK, Passaglia LMP. Screening of plant growth promoting rhizobacteria isolated from sunflower (*Helianthus annuus* L.) Plant Soil. 2012;356:245-264.
11. Souza R, Beneduzi A, Ambrosini A, Costa PB, Meyer J, Vargas LK, Scheonfeld R, Rassaglia LMP. The effect of plant-growth promoting rhizobacteria on the growth of rice (*Oryza sativa* L.) cropped in Southern Brazillian fields. Plant Soil. 2013;366:585-603.
12. Aleksandrov VG, Blagodyr RN, Iiiev IP. Liberation of phosphoric acid from apatite by silicate bacteria. Mikrobiyol Zh. (Kiev). 1967;29:111-114.
13. Verma P, Shahi SK. Role of plant growth promoting rhizobacteria in plant development. Journal of International Academic Research for Multidisciplinary. 2015;3(5):282-299.
14. Zhang C, Kong F. Isolation and identification of potassium solubilizing bacteria from tobacco Rhizospheric soil and their effect on tobacco plants. Applied Soil Ecology. 2014;82:18-25.
15. Anonymous. Manual of microbiological method. McGraw Hill Book Company Inc., New York. 1957;127.
16. Gram HCJ. Method for staining bacteria. Jounal Fortschritte der Medizin. 1884;11: 25-29.
17. Dorner W. Un procede simple pour la colouration des spores. Le Lait. 1922;6:8-12.
18. Zaved HK, Rahman MM, Rahman A, Arafat SMY, Rahman MS. Isolation and characterization of effective bacteria for solid waste degradation for organic manure KMITL. Science Technology Journal. 2008;8:44-55.
19. Adesemoye AO, Torbert HA, Kloepper JW. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Canadian Journal of Microbiology. 2008;54:876-886.
20. Osman AG. Study of some characteristics of silicate bacteria. Journal of Science and Technology. 2009;10(3):27-35.
21. Christrophe C, Marie-Pairre T, Pascale FK. Increase of apatite dissolution rate by Scots pine roots associated or not with *Burkholderia glethei* PML1(12) Rp in open-system flow microcosms. Geochem Cosmochem Acta. 2013;106:287-306.
22. Friedrich S, Platonova NP, Karavaiko GI, Stichel E, Glombitza F. Chemical and microbiological solubilization of silicates. Acta Biotech. 2004;11(3):187-196.
23. Archana DS, Nandish MS, Savalagi VP, Alagawadi AR. Characterization of potassium solubilizing bacteria (KSB) from rhizosphere soil. Bioinfolet. 2013;10:248-257.
24. Meena VS, Maurya BR, Bahadur Indra. Potassium solubilization by bacterial strain in waste mica. Bangladesh Journal of Botany. 2014;43(2):235-237.

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