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Research Paper

Characterization of Potassium Solubilizing Bacteria from Rhizospheric Soils of Cherry (*Prunus avium*)

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ABSTRACT

The soluble potassium (K) concentration in the soil is usually very low and more than 90 percent of K exists in insoluble form. The rhizospheric microflora has the only ability to dissolve potassium from insoluble minerals. Rhizosphere bacteria of cherry have been found to dissolve K from insoluble K- bearing minerals. In this study bacterial isolates from rhizospheric soil were obtained on modified Aleksandrov medium containing mica powder as potassium source. From the 22 isolates 9 bacterial strains (KSB2, KSB5, KSB7, KSB9, KSB10, KSB11, KSB12, KSB19 and KSB22) were selected which showed highest zone of clearance on Aleksandrov medium and were characterized on the basis of morphological and biochemical characteristics.

***Key words*:** Potassium, Rhizospheric bacteria, Morphology, Biochemical characterization, Aleksandrov medium

Chemical fertilizers have improved crop yield, but contaminated the environment thus leading to global food crisis [1-2]. Potassium (K) is one of the essential nutrients which plays important role in growth and development of plants. In addition to disease resistance, K is required to activate over 80 different enzymes responsible for plant and animal processes. e.g. such as energy metabolism, nitrate reduction, photosynthesis, and sugar degradation [3-7]. Only 1 to 2 percent of potassium is available to plants in the soil [8] and rest is in the locked condition. Since cost of K-fertilizers (the price of potash $470 per ton since 2011) is increasing every year [9] and these chemical fertilizers have harmful effect on environment, so it is necessary to find the way for maintaining availability of K in soil for sustainable plant growth. Potassium solubilizing microorganisms (KSM) can help in making the availability of nutrients playing an essential role in dynamic soil environment by contributing release of key nutrients from primary minerals and ores. These key macronutrients are central for nutrition of microbial population present in the soil and in turn also benefit to plant nutritional status [10-12]. In addition to release of plant growth promoting substance like IAA, organic matter biodegradation, antibiotics and nutrient cycling (P, N_2, and K) in the soil by KSM can also be benefited for crop productivity and ecological sustainability [13]. Among all the microorganisms, potassium solubilizing microorganisms have attracted attention of agriculturalist as inoculum to promote the yield and growth of the plants. The potassium solubilizing bacteria (KSB) are effective in releasing potassium (K) from inorganic sources of K by solubilization [14-16]. Inoculation with theses KSB strains has been reported to produce beneficial effect on growth of various different plants [17-18]. Mechanism of K-solubilization could be mainly attributed to excrete organic acids like malic acid, oxalic acid etc. which either directly dissolves rock K or chelate silicon ions to bring K in to solution [19]. Therefore, the objective of this research was to isolate, Characterize KSB from rhizospheric soils of cherry (*prunus avium*).

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MATERIALS AND METHODS

Soil sampling

The soil samples were taken from the rhizospheric soil of Cherry. Some top surface soil was removed before the collection of soil samples from cherry fields. The surface soil was digged to 10 cm soil layer, were roots of cherry trees are concentrated. From about 0 to 2.5 mm away from the root surface, a zone of soil is located that is significantly influenced by living roots and is referred to as the rhizosphere. Rhizosphere soil and roots were separated from the bulk of the soil by hand. The 20 samples from 10 locations were taken randomly in every block. All the samples were sealed in a zip lock bags stored in fridges and were used in 10-20 hours. All the samples were collected from May to July 2016.

Isolation of K solubilizing rhizobacteria

Modified Aleksandrov medium, with waste mica powder as a sole source of K, was used to screen K - solubilizing bacteria. The serially diluted soil samples were plated on modified Aleksandrov medium which contain, 5g glucose, 0.005g $MgSO_4.7H_2O$, 0.1g $FeCl_3$, 2.0g $CaCO_3$, 3g mica powder as a sole source of K, 2g Ca_3PO_4 and 20 g agar [20]. The plates were then incubated at 28±2°C. After three days the colonies showing formation of Clear Zones around them were considered to be KSM and selected for further studies [21]. Screened isolates were gram stained for presumptive identification and pure colonies were transferred to sterile slants on nutrient agar medium. Further in another set of experiments, Zone of solubilization of purified KSB isolates were measured, using a scale after 7 days of plating on modified Aleksandrov medium. The diameter of the solubilization zone was measured in centimeter.

Characterization of the potassium solubilizing bacteria

The identification of the different bacteria, isolated from rhizospheric soils of cherry was made based on its morphological and biochemical characteristic studies.

Screening of potassium solubilizers on the basis of zone ratio

Screening of potassium solubilizing bacteria were done on Aleksandrov medium on the basis of zone ratio (Zone diameter /colony diameter) and solubilization index [22].

Morphological characterization

All the selected isolates were examined for the colony morphology, cell shape, gram reaction and ability to form spores as per the Standard procedures given by [23].

Biochemical characterization

The characterization of the isolates was carried out as per the procedures outlined by Bergey's manual of systematic Bacteriology 9th Edition (1993). MR-VP test, Urea hydrolysis, Gelatine hydrolysis, Starch hydrolysis, Casein Hydrolysis, Hydrogen sulphide production, citrate was performed etc.

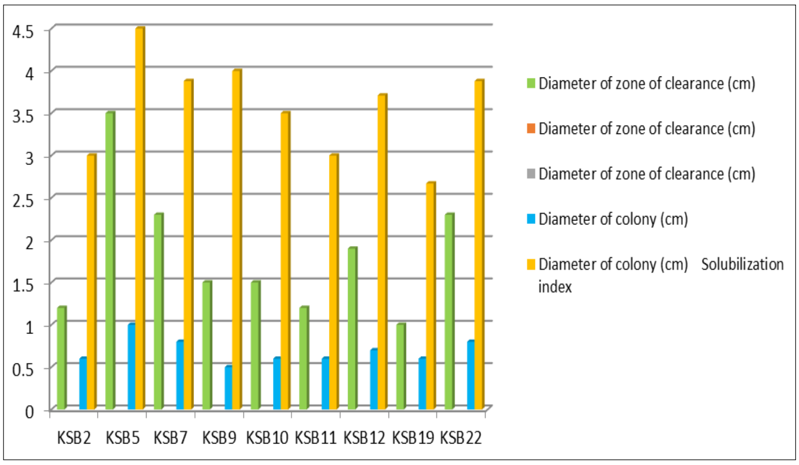
RESULTS AND DISCUSSION

Isolation and screening

Colonies exhibiting zone of clearance indicating potassium solubilization were selected, colonies were selected which are morphologically different. A total of 22 different type colonies were isolated on different media like Nutrient agar, kings B, YEMA. The isolated colonies were streaked on media and then pure isolated colonies were tested for K solubilization on modified Aleksandrov medium which contains Mica powder as K source. Out of 22 colonies 9 showed the highest zone of clearance.

Table 1 Zone of clearance shown by eight ksb isolates

KSB Isolates	Diameter of zone of clearance (cm)	Diameter of colony (cm)	Solubilization index
KSB2	1.20	0.60	3.00
KSB5	3.50	1.00	4.50
KSB7	2.30	0.80	3.88
KSB9	1.50	0.50	4.00
KSB10	1.50	0.60	3.50
KSB11	1.20	0.60	3.00
KSB12	1.90	0.70	3.71
KSB19	1.00	0.60	2.67
KSB22	2.30	0.80	3.88



Graphical representation of solubilization index of KSB

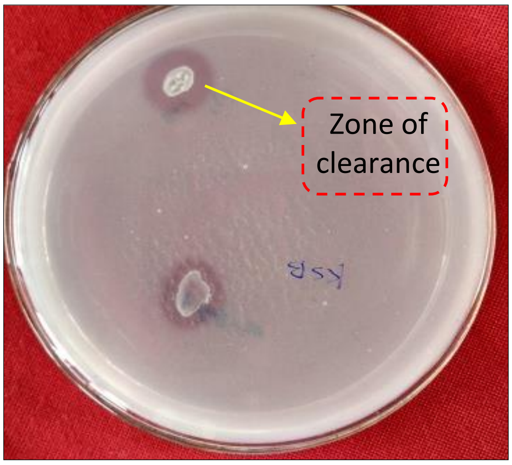


Fig 1 Zone of clearance on Aleksandrov medium

Table 2 Colonical and morphological characteristics of isolates

KSB isolates	Pigmentation	Margin	Spore formation	Gram reaction	Raised	Transparent	Opaque	Probable genus
KSB2	Creamy rods	Entire	+	+	Slight	+	-	*Bacillus sp*
KSB5	Yellowish rods	Undulating	-	-	Raised	-	+	*Pseudomonas sp*
KSB7	White	Entire	-	-	Slightly	-	+	*Azotobacter sp*
KSB9	Whitish rods	Entire	+	+	Slight	+	-	*Bacillus sp*
KSB10	Yellowish rods	Undulating	-	-	Raised	-	+	*Pseudomonas sp*
KSB11	Red	Circular	-	+	Flat	-	+	*Micrococcus sp*
KSB12	Whitish spherical	Circular	-	+	Flat	-	+	*Micrococcus sp*
KSB19	Whitish rods	Entire	+	+	Slight	+	-	*Bacillus sp*
KSB22	Creamy rods	Entire	+	+	Slight	+	-	*Bacillus sp*

CONCLUSIONS

Because of fixation 90 to 98 percent of potassium becomes unavailable to plants. Rhizospheric microorganisms contribute significantly in solubilization of locked form of soil minerals in the soil.in this study 22 bacterial strains were isolated from Rhizospheric soil of cherry out of them only 9 isolates (KSB1, KSB2, KSB3, KSB9, KSB10, KSB11, KSB12, KSB13 and KSB22) were found to Solubilize Potassium on Aleksandrov medium supplemented with mica. All the 9 isolates were Characterized morphologically, and Biochemically. These nine strains can be used as best biofertilizers.

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Table 3 Biochemical characterization

KSB isolates	C	O	VP	MR	UH	GH	SH	CH	H_2S	G	C	Probable genus
KSB2	+	-	-	+	-	+	-	+	-	+	+	*Bacillus sp*
KSB5	+	+	-	+	+	+	-	+	-	+	+	*Pseudomonas sp*
KSB7	+	+	+	-	+	-	+	+	+	+	+	*Azotobacter sp*
KSB9	+	-	-	+	-	+	-	+	-	+	+	*Bacillus sp*
KSB10	+	+	-	-	+	+	-	+	-	+	+	*Pseudomonas sp*
KSB11	+	+	-	+	+	-	+	+	-	+	+	*Micrococcus sp*
KSB12	+	+	+	-	+	-	+	+	-	+	+	*Micrococcus sp*
KSB19	+	-	-	+	-	+	-	+	-	+	+	*Bacillus sp*
KSB22	+	-	-	+	-	+	-	+	-	+	+	*Bacillus sp*

Catalase: C, oxidase: O, VP = V.P test, MR = Methyl red test, UH = urea hydrolysis, GH = gelatin hydrolysis, SH = starch hydrolysis, CH = Casein Hydrolysis, H_2S = Hydrogen sulphide production, G =Glucose, C = Citrate.

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