Morphological and Biochemical Characterization of Potassium Solubilizing Bacteria from Rhizospheric Soils of Pinus (*Pinus wallichiana*).

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Abstract

Among natural bio-agents, the potassium solubilizing bacteria (KSB) which solubilize fixed forms of K by various mechanisms including acidolysis, exchange reactions, complexolysis and production of organic acids and make potassium (K) available to plants. In this study bacterial isolates were obtained on modified Aleksandrov medium containing mica powder as potassium source. From the 25 bacterial isolates, isolated from rhizospheric region of Pinus (*Pinus wallichiana*) south kashmir, india , 7 bacterial strains (KSB3, KSB8, KSB9, KSB13, KSB18, KSB24 and KSB25) were selected which exhibiting highest potassium solubilization on solid medium and characterized on the basis of cultural, morphological and biochemical characteristics.

Keywords: Potassium solubilizing bacteria (KSB), mica powder, *Pinus wallichiana*, aleksandrov medium

Introduction

Global crop production has intensive cultivation practices like the use of pesticides and mineral fertilizers have improved crop yields, but also contaminated the environment, thus leading to a global food crisis (Challinor *et al.,* 2014; Liu *et al.,* 2015). Potassium can be more easily leached than N or P (Neirynck *et al.,* 1998; Altamare *et al.,* 1999 and Meena *et al.,* 2015). Therefore crops need to be supplied with soluble K fertilization, the demand of which is expected to increase, particularly in developing countries (kato *et al.,* 2015). Potassium (k), an element essential for plant growth, plays an essential role for protein synthesis, enzyme activation, and photosynthesis (Basak and Biswas, 2009). K is among the three essential nutrients (N, P and K), which is necessary for crop growth and crop production (Sugumaran and Janarthanam, 2007 and Chen *et al.*, 2008).

Plants can uptake K through the soil minerals, organic minerals, and synthetic fertilizers. K deficiency in the rhizosphere of economically important crops has become an important limiting factor responsible for sustainable development of evergreen agriculture in India (Naidu *et al.,* 2010). Potassium solubilizing microorganisms can help in enhancing the availability of nutrients playing an essential role in dynamic soil environment by contributing release of key nutrients from primary minerals and ores. In addition to release of plant growth regulating substances, antibiotics, biodegradation of organic matter and nutrients cycling in the soil by KSM can also be benefited for crop productivity and ecological sustainability (Meena *et al.,* 2014, Zorb *et al.,* 2014). Soil microorganisms influence the soil mineral availability, plays a central role in ion cycling and soil fertility (Lian *et al.,* 2010). Certain bacteria are capable of decomposing alumina silicate minerals and releasing a portion of the potassium contained therein (Biswas and Basak, 2009). Mechanism of K-solubilization could be mainly attributed to excrete organic acids like oxalic acid ,malic acid etc which either directly dissolve rock K or chelate silicon ions to bring K in to solution (Prajapati *et al.,* 2013). In light of above facts, it can be observed that application of K can contribute to sustainable high yield and high K efficiency. Therefore the objective of this research is to isolate and Characterize Potassium solubilizing bacteria (KSB) from rhizospheric soils of Pinus (*Pinus wallichiana*).

Methodology

Sampling

The soil samples were taken from the root attached soils of Pinus (*Pinus wallichiana*). Some top surface soil was removed before the collection of soil samples from Pinus. The surface soil was digged to 10 cm soil layer, where roots of Pinus 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 10 samples from 5 sites were taken randomly, the samples were sealed in a zip lock bags, stored in fridges and were used in 10-20 hours.

Isolation of K solubilizing rhizobacteria

The serially diluted soil (up to 10^{-6}) samples we plated on modified Aleksandrov medium which contain, 5g glucose,0.005$MgSO_4.7H_2O$, 0.1g $FeCl_3$, 2.0g $CaCO_3$, 3g mica powder, as a sole source of K, 2g Ca_3PO_4,and 20 g Solidifying agent agar (Hu *et al.,* 2006) .

The plates were then incubated. After three days the colonies showing formation of clear zones around, were considered to be KSB and selected for further studies (Sugumaram and Janartham, 2007).

Screened isolates were gram stained for presumptive identification and pure colonies were transferred to nutrient agar slants. Bacterial isolates were Characterized using different cultural, microscopical, and biochemical Characteristics.

Potassium solubilizers screening 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 (Hu *et al.*, 2006) (**Table 2**).

Morphological Characterization of Potassium solubilizers

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 Zhang and Fanyu (2014).

Biochemical Characterization of potassium solubilizers

The Characterization of the isolates was carried out as per the procedures outlined by Bergey's manual of systematic Bacteriology 9th Edition (1993). Catalase test, Urea Hydrolysis, oxidase, Denitrification test, Methyl red test, V.P test, Starch Hydrolysis test, Casein test, Acid production, Gas production, H2S production test, Gelatine hydrolysis, citrate test, sucrose, Mannital, glucose, 70 % NaCl, were performed.

Result and discussion.

Potassium is a major essential macronutrient for plant growth and development. To boost crop yields, nitrogenous and phosphatic fertilizers are applied at high rates which cause environmental and economic problems. Therefore, direct application of rock phosphate and rock potassium materials may be agronomically more useful and environmentally safer than soluble P and K fertilizers (Rajan, *et al.*, 1996). However, potassium nutrients are released slowly from the rock materials and their use as fertilizer often causes insignificant increases in the yield of crops (Sindhu, 2012).

Therefore, concerted efforts are made to understand the combined effects of rock material addition and inoculation of KSB on nutrient availability in soils and growth of different crops. In this study, potassium solubilizing bacteria were isolated from rhizosphere soil of Pinus south Kashmir, India. Bacterial isolates were examined for their ability to solubilize insoluble K in solid and liquid media and mica powder was used as K source.

Isolation and screening of K solubilizing bacteria from the Rhizospheric Soil

Twenty five bacterial isolates were obtained from soil samples collected from rhizosphere of Pinus, south Kashmir.All the twenty five isolates were tested on Aleksandrov medium for K solubilization, only seven isolates (KSB3, KSB8, KSB9, KSB13, KSB18, KS24, and KSB25) Showed zone of clearance on mica powder containing medium plates.

Morphological and biochemical characterization

From the twenty isolates seven showed the zone of clearance, were morphologically and biochemically characterized, **Table 1 & 3.**

Table 1: Morphological characterization of KSB isolates

Isolate	Pigmentation	Margin	Gram reaction	Slightly raised	Highly raised	Opaque	Spore	Probable genus
KSB3	Yellowish rods	undulating	-	-	raised	-	-	*Pseudomonas sp*
KSB8	red	circular	+	+	flat	-	-	*Micrococcus sp*
KSB9	White	Entire	-	-	slightly	-	-	*Azotobacter sp*
KSB13	Whitish rods	Entire	+	+	slight	+	+	*Bacillus sp*
KSB18	Whitish rods	Entire	+	+	slight	+	+	*Bacillus sp*
KSB24	Yellowish rods	undulating	-	-	raised	-	-	*Pseudomonas sp*
KSB25	Yellowish rods	undulating	-	-	raised	-	-	*Pseudomonas sp*

Table 2: Zone of clearance (Solubilization index)

KSB Isolates	Diameter of zone of clearance (cm)	Diameter of Colony (cm)	Solubilization Index (si)
KSB3	2.30	0.80	3.88
KSB8	2.30	0.80	3.88
KSB9	1.50	0.50	4.00
KSB13	1.50	0.60	3.50
KSB18	1.20	0.60	3.00
KSB24	1.90	0.70	3.71
KSB25	2.30	0.80	3.88

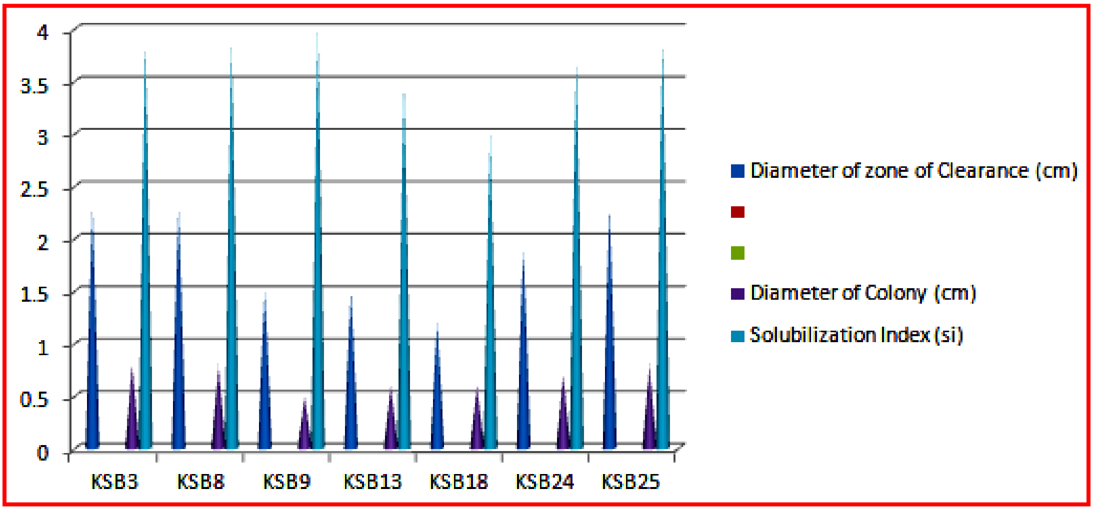

Figure 1: Graphical representation of solubilization index

Table 3: Biochemical Characterization

Isolates	C	O	VP	MR	UH	GH	SH	CH	H2S	G	CIT	Probable genus
KSB3	+	+	-	+	+	+	-	+	-	+	+	*Pseudomonas sp*
KSB8	+	+	+	-	+	-	+	+	-	+	+	*Micrococcus sp*
KSB9	+	+	+	-	+	-	+	+	+	+	+	*Azotobacter sp*
KSB13	+	-	-	+	-	+	-	+	-	+	+	*Bacillus sp*
KSB18	+	-	-	+	-	+	-	+	-	+	+	*Bacillus sp*
KSB24	+	+	-	+	+	+	-	+	-	+	+	*Pseudomonas sp*
KSB25	+	+	-	+	+	+	-	+	-	+	+	*Pseudomonas sp*

C = Catalase test, O=oxidase, MR = Methyl red test, V.P = V.P test, UH= urea hydrolysis, St = Starch Hydrolysis test, CH = Casein Hydrolysis, H2S = H2S production test, GH = Gelatine hydrolysis, Cit = citrate test, G = Glucose

Conclusion

Fixation of added nutrients/fertilizers in soil reduces the efficiency of applied K fertilizers and thus, a large quantity of added fertilizers (K) becomes unavailable to plants. Rhizosphere microorganisms contribute significantly in solubilization of bound form of soil minerals in the soil. In this study, different K solubilizing bacteria were isolated from rhizosphere soil samples collected from Pinus rhizosphere. Among 25 isolates /strains tested, 7 strains were found to solubilize potassium (KSB3, 8, 9, 13, 18, 24 and 25) which belongs to Genus *Bacillus* and *Pseudomonas sp, Azotobacter sp ,Micrococcus sp* as per morphological and biochemical characterization.

Acknowledgement

Authors are thankful to the Division of Plant Pathology, Biocontrol Lab, SKUAST-Kashmir for providing facilities for this research.

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