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Solubilization of potassium containing mineral by bacteria from Kashmir iris (*Iris kashmiriana*) rhizosphere

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Abstract
Potassium is absolutely essential component of plant nutrition package limiting crop yield and Quality that performs a multitude of important biological functions to maintain plant growth. Indiscreet application of chemical fertilizers have considerably negative impact on environmental sustainability.There is a growing need to turn back to nature or sustainable agents that promote evergreen agriculture. Among such natural bio-agents, the KSB which solubilize fixed forms of K by various mechanisms including acidolysis, exchange reactions, complexolysis and production of organic acids and make K available to plants. In this study bacterial isolates were obtained on modified Aleksandrov medium containing mica powder as potassium source. From the 20 bacterial isolates, isolated from rhizospheric region of Kashmir iris (*Iris kashmiriana*), 7 bacterial strains (KS4, KS7, KS8, KS14, KS17, KS19 and KS20) 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, Bio-agents, Kashmir iris, Aleksandrov medium

Introduction

K is available in four forms in the soil which are K ions (K^+) in the soil solution, as an exchangeable cation, tightly held on the surfaces of clay minerals and organic matter. Potassium though present in as abundant element in soil or is applied to fields as natural or synthetic fertilizers, only 1 to 2 per cent of this is available to plants, the rest being bound with other minerals and therefore becomes unavailable to plants. Global crop production has Intensive cultivation practices like the use of pesticides and mineral fertilizers have improved crop yields, but also contaminated food and the environment, thus leading to a global food crisis (Challinor *et al*., 2014; Liu *et al*., 2015) [5, 9]. K can be more easily leached than N or P (Neirynck *et al*., 1998, Altamare *et al*., 1999, Meena *et al*., 2015) [13, 1, 11]. 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) [8]. Potassium (k), an element essential for plant growth, plays an essential role for enzyme activation, protein synthesis and photosynthesis (Basak and Biswas, 2009) [3].

K is one of the three main nutrients (N, P and K), which are needed for crop growth and crop production increase (Sugumaran and Janarthanam, 2007., Chen *et al*., 2008) [17, 6]. Plants can uptake potassium 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) [12]. KSM 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*., 2013, Zorb *et al*., 2014) [10, 18]. Soil microorganisms influence the soil mineral availability, plays a central role in ion cycling and soil fertility (Bin Lian *et al*., 2010) [4]. Certain bacteria are capable of decomposing alumina silicate minerals and releasing a portion of the potassium contained therein (Biswas and Basak, 2009) [3]. Mechanism of K-solubilization could be mainly attributed to excrete organic acids which either directly dissolve rock K or chelate silicon ions to bring K in to solution (Prajapati *et al*., 2013) [14]. 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 Kashmir iris (*Iris kashmiriana*).

Methodology

Sampling

The soil samples were taken from the root attached soils of Kashmir iris (*Iris kashmiriana*)**.** Some top surface soil was removed before the collection of soil samples from Kashmir iris (*Iris kashmiriana*) fields. The surface soil was digged to 10 cm soil layer, were roots of Kashmir iris (*Iris kashmiriana*) 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 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^{-5}) samples we plated on modified Aleksandrov medium which contain, 5g glucose,0.005MgSO4.7H2O, 0.1g FeCl3, 2.0g CaCO3, 3g mica powder, as a sole source of K, 2g Ca3PO4,and 20 g Solidifying agent agar (Hu *et al*., 2006) [7]. The plates were then incubated at 28 ± 2^{0}C. 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) [17]. 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.

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 (Hu *et al*., 2006) [6].

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 Anonymous (1957) [2].

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). Catalase test, Urea Hydrolysis, oxidase, Denitrification test, Methyl red test, V.P test, Starch Hydrolysis test, Casein test, Acid production, Gas production, H_2S production test, Gelatine hydrolysis, citrate test, sucrose, Mannital, glucose, 70 % NaCl, were performed.

Result and Discussion

Nitrogen, phosphorus and potassium are major essential macronutrients for plant growth and development. To enhance 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, S.S.S. *et al*., 1996) [15]. However, potassium nutrients are released slowly from the rock materials and their use as fertilizer often causes insignificant increases in the yield of crops (S.S. Sindhu, 2012) [16]. 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 Kashmir iris (*Iris kashmiriana*) grown in central 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 bacterial isolates were obtained from soil samples collected from rhizosphere of Kashmir iris (*Iris kashmiriana*), collected from central Kashmir.All the twenty isolates were tested on Aleksandrov medium for K solubilization, only seven isolates (KS4,KS7, KS8, KS14, K17, KS19, and KS20) 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 and 3.

Fig 1: K Solubilization on Aleksandrov medium

Table 1: Morphological Characters of 7 KSB isolates

Isolate	Pigmentation	Margin	Gram reaction	Slightly raised	Highly raised	Transparent	Opaque	Cell shape	Spore
KS4(Pseudomonas)	Creamy	Entire	G - IVE	+	-	-	+	Short rod	-
KS7(Bacillus)	Whitish	Elevated	G +IVE	+	-	-	+	Short rod	+
KS8(Pseudomonas)	White	Entire	G +IVE	-	+	-	+	Long Rod	+
KS14(Pseudomonas)	Creamy	Elevated	G -IVE	-	+	-	+	Long Rod	-
KS17(Pseudomonas)	Creamy	Elevated	G -IVE	+	-	-	+	short Rod	-
KS19(Pseudomonas)	whitish	Elevated	G -IVE	+	-	-	+	Long Rod	-
KS20 (Pseudomonas)	yellow	Elevated	G -IVE	--- +	-	-	+	Long rod	-

Table 2: Zone of clearance (Solubilization index)

KSB Isolates	Diameter of zone of Clearance (cm)	Diameter of Colony (cm)	Solubilization Index (si)
KS4	1.40	0.40	4.50
KS7	1.60	0.60	2.67
KS8	1.50	0.80	2.88
KS14	1.50	0.40	4.75
KS17	1.00	0.70	2.43
KS19	1.00	0.60	2.67
KS20	1.50	0.80	2.88

Table 3: Biochemical Characterization

Isolates	C	U	O	Dn	MR	VP	ST	Ca	A.P	G.P	H_2S	Gel	Cit	SU	Ml	G	NaCl
KS4	+	+	-	-	+	-	+	+	+	-	-	+	-	+	+	-	-
KS7	+	-	-	-	+	-	+	+	+	-	-	+	-	+	-	-	-
KS8	+	+	+	-	+	-	+	+	+	-	-	-	-	+	+	+	-
KS14	+	+	-	-	+	-	+	+	+	-	-	+	-	+	+	-	-
KS17	+	-	-	-	+	-	+	+	+	-	-	+	+	+	-	-	-
KS19	+	+	+	-	+	-	+	+	+	-	-	+	-	+	+	+	-
KS20	+	+	-	-	+	-	+	+	+	-	-	+	+	+	+	-	-

C = Catalase test, U = Urea Hydrolysis, O = oxidase, Dn= Denitrification test, MR = Methyl red test, V.P = V.P test, St = Starch Hydrolysis test, Ca = Casein test, A.P = Acid production, G.P = Gas production, H2S = H_2S production test, Gel = Gelatine hydrolysis, Cit = citrate test, SU = sucrose, Ml = Mannital, G = glucose, NaCl = 70 % NaCl.

Conclusion

Potassium availability to crop plants in soil is generally low since nearly 90 to 98 per cent of total potassium in the soil is in unavailable mineral forms. Moreover, fixation of added nutrients/fertilizers in soil reduces the efficiency of applied K fertilizers and thus, a large quantity of added fertilizers (K) become 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 Kashmir iris rhizosphere. Among 20 isolates /strains tested, 7 strains were found to solubilize potassium which belongs to Genus *Bacillus* (KS7) and *Pseudomonas (*KS4,8,14,17,19,20*) sp* as per morphological and biochemical characterization. It was found that maximum solubilization occurred with glucose as carbon source and at 25℃ temperature of incubation, and at medium pH 7.0. These results suggested that efficient potassium solubilizing bacterial strains could be further exploited for plant growth improvement under field conditions.

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