International Journal of Science, Environment and Technology, Vol. 5, No 5, 2016, 3030 – 3037
ISSN 2278-3687 (O)
2277-663X (P)

ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF POTASSIUM SOLUBILIZING BACTERIA FROM RHIZOSPHERE SOIL OF MAIZE (*Zea mays*).

Parmar K.B., Mehta B.P. and Kunt M.D.
Department of Plant Pathology
N.M. College of Agriculture, Navsari

Abstract: Potassium is a vital component of plant nutrition package limiting crop yield and quality that performs a maltitude of important biological functions to maintain plant growth. Isolation of potassium solubilizers were carried out using mica from the soil samples of maize rhizosphere, on Aleksandrov medium. From the 25 isolates, 5 bacterial isolates were selected exhibiting highest potassium solubilization and characterized on the basis of colony, morphological and biochemical characteristics. The highest solubilization (46.52 µg/ml) was observed in isolate KSB-1 and was identified as *Bacillus licheniformis* by Biolog system and was followed by KSB-3 (42.37 µg/ml) which was identified as *Bacillus subtilis*.
Keywords: Solubilizing, Mica, Maize.

Introduction

Potassium (K) is seventh most common element in the earth's crust. It constitutes about 2.5 per cent of the lithosphere. However, actual soil concentrations of this nutrient vary widely ranging from 0.04-3.00 per cent (Sparks and Huang, 1985). Highest proportions of potassium in soils are in insoluble rocks and minerals (Goldstein, 1994) such as micas, illite, feldspar and orthoclases. Potassium plays a foremost role in translocation of carbohydrates, photosynthesis, water relations, resistance against insects and diseases and sustains balance between monovalent and divalent cations (Brar and Tiwari, 2004). Deficiency of K is not as wide spread as that of nitrogen and phosphorus but with the introduction of high yielding varieties and hybrids during green revolution and with the progressive intensification of agriculture, the soils are getting depleted in potassium reserve at a faster rate, extensive use of chemical fertilizers is proven to destroy soil structures as well as aggravate environmental pollution by contaminating underground water. Many types of microorganisms are known to inhabit soil, especially rhizosphere and play an important role in plant growth and development. K-solubilizing bacteria are able to release potassium from insoluble minerals. The considerable populations of KSMs are present in rhizospheric soils which promote the plant growth (Sperberg, 1958) and they can convert the insoluble or mineral structural

*Received Aug 24, 2016 * Published Oct 2, 2016 * www.ijset.net*

potassium compounds into soluble forms in soil as a soil solution and make them available to the plants (Zeng *et al*., 2012). A wide range of KSMs *viz. Bacillus mucilaginosus*, *Bacillus edaphicus*, *Bacillus circulans*, *Paenibacillus spp*., *Acidothiobacillus ferrooxidans*, *Pseudomonas*, *Burkholderia* (Sheng *et al*. 2008; Lian *et al*. 2002; Rajawat *et al*. 2012; Liu *et al.* 2012; Basak and Biswas, 2012; Singh *et al*. 2010) have been reported to release potassium in accessible form from K-bearing minerals in soils. There uses as biofertilizers or biocontrol agents for agriculture improvement and environmental protection have been a focus of recent research.

MATERIALS AND METHODS

Collection of soil samples

Samples of soil and root system from healthy rhizosphere of maize were collected in sterile plastic bags from ten plants each at random from the field. Each sample consisted of 100 g soil and 10 g roots.

Isolation of potassium soluiblizing bacteria

One gram of rhizosphere soil was mixed thoroughly in 100 ml sterile water and was processed following serial dilution agar plate technique (Aneja, 2002). Suitable dilutions (10^{-5} and 10^{-6}) of both rhizosphere and rhizoplane solutions were plated on Aleksandrov medium (Hu *et al.*, 2006). Aleksandrov medium is a selective medium for the isolation of potassium solubilizers, containing insoluble potassium bearing mineral (mica). The plates were incubated at room temperature (30±1°C) for 3 days and the colonies exhibiting clear zones were selected, purified by four way streak plate method.

Characterization of the potassium solubilizing bacteria

The identification of the different bacteria, isolated from maize plants rhizosphere were made based on its morphological and biochemical characteristic studies.

Screening of potassium solubilizers on the basis of zone ratio

Screening of potential mineral potash solubilizing bacteria were done on Aleksandrov medium on the basis of zone ratio (zone diameter/colony diameter) and solubilization index (Hu *et al.*, 2006).

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).

Biochemical characterization

The biochemical characterizations of the isolates were carried out as per the procedures outlined by Bergey's Manual of Systematic Bacteriology 9th Edition (1993). Sugar utilization, Methyl red test, Voges-Proskauer (VP) test, Urea hydrolysis, Nitrate reduction test, Gelatine hydrolysis test, catalase test, starch hydrolysis, Casein hydrolysis and H_2S production test were performed.

Identification of different isolates using Biolog system

Biolog carbon substrates utilization patterns Biolog GP2 MicroPlates (Biolog, Inc., Hayward, CA, USA) were inoculated in duplicate using the standard procedures (Garland and Mills, 1991) and were incubated at 30 -35 ^{O}C for 24-36 h. The optical density at 590nm produced from the reduction of tetrazolium violet in each well was read after 12, 24, 36 and 48 h using a Biolog Microplate reader in conjunction with the MicroLog software (Release version 4.0). Similarity index (SIM), correlated with genetic distance (DIS) was calculated among each strain and the 10 most closely related species present in the database.

RESULTS AND DISCUSSION

Isolation and screening of potassium

Colonies exhibiting zone of clearance indicating potassium solubilization were selected. Colonies were selected which were morphologically distinct. Total 25 bacterial isolates were selected as potassium solubilizers and named as KSB-1 to KSB-25 (Table-1).

Table 1: Details of solubilization index of KSB isolates

Sr Number	KSB Isolates	Solubilization Index(SI)
1	KSB-1	5.20
2	KSB-2	1.20
3	KSB-3	5.00
4	KSB-4	1.30
5	KSB-5	0.50
6	KSB-6	1.00
7	KSB-7	2.60
8	KSB-8	3.24
9	KSB-9	4.60
10	KSB-10	1.35

11	KSB-11	2.64
12	KSB-12	3.25
13	KSB-13	3.68
14	KSB-14	2.70
15	KSB-15	2.80
16	KSB-16	3.17
17	KSB-17	4.56
18	KSB-18	1.20
19	KSB-19	2.30
20	KSB-20	3.78
21	KSB-21	1.26
22	KSB-22	1.90
23	KSB-23	2.40
24	KSB-24	2.72
25	KSB-25	3.28

Morphological and Biochemical characterization

From the twenty five isolates, five bacterial isolates KSB-1, KSB-3, KSB-9, KSB-17 and KSB-20 exhibiting higher solubilization index for potassium solubilization were selected.

The morphological and biochemical characteristics of five good potassium solubilizers are presented in Table-2 and Table-3 respectively. From these isolates three are Gram positive while two are Gram negative.

Table 2: Colony and morphological charecterization of the KSB isolates

KSB isolate	Colony charecteristics								Morphological charecteristics			
	Size	Shape	Margin	Eleva-tion	Texture	Consis-tency	Opacity	Pigmen-tation	Cell shape	Aggan-gement	Gram reaction	Spo-re
KSB-1	Medium	Round	Entire	Convex	Smooth/ Mucoid	viscous	Translucent	White	Long Rod	Chain	+ve	+
KSB-3	Small	Round	Entire	Convex	Smooth	viscous	Translucent	White	Long Rod	Chain	+ve	+
KSB-9	Medium	Round	Entire	Raised	Rough	viscous	Translucent	White	Long Rod	Chain	+ve	-
KSB-17	Small	Round	Entire	Convex	Smooth	viscous	opaque	Green	Short Rod	Singly	-ve	-
KSB-20	Small	Round	Entire	Convex	Rough	viscous	opaque	White	Short Rod	Pair	-ve	-

Table 3: Biochemical characterization* and identification of the KSB

KSB Isolate	Biochemical charecteristics									Sugar utilization			Tentative assigned sp.
	MR*	VP*	UH*	NR*	GH*	CT*	SH*	Ca*	HP*	Mn*	Su*	Ml*	
KSB-1	+	-	+	+	+	+	+	+	-	+	+	+	*Bacillus sp.*
KSB-2	+	-	+	-	+	+	+	+	-	+	+	+	*Bacillus sp.*
KSB-9	-	-	+	+	-	+	-	-	-	+	-	+	*Bacillus sp.*
KSB-17	-	-	+	+	+	+	+	-	-	+	+	+	*Pseudomonas* sp.
KSB-20	-	+	+	-	+	--	-	+	-	+	-	+	*Pseudomonas* sp.

*MR.:Methyl red test , VP- V.P. Test, UH-Urea hydrolysis, NR-Nitrate reduction test, GH-Gelatine hydrolysis test, CT-Catalase test, SH-Starch hydrolysis test, Ca-Casein test, HP-H_2S production test, Mn- Mannitol, Su-Sucrose, Ml- Maltose
\+ Positive - Negative

Quantitative estimation of K Solubilization:

The isolates showing zone of solubilization on Aleksandrov agar medium were further examined for their ability to release K from broth media.

The amount of K released from muscovite mica in the broth by the isolates were studied at 7, 15 and 20 days after incubation (DAI) in lab condition and found in the range of 1.89 to 46.52µg/ml (Table-4). The results indicated that the amount of released K increased as the days of incubation increases and the highest amount of K present at 20 DAI. The maximum solubilization was observed in case of KSB-1 *i.e.* 46.52 µg/ml which is followed by KSB-3 *i.e.* 42.37 µg/ml. From all the screened isolates, 15 isolates showed more than 20 µg/ml of potassium solubilization from muscovite mica.

Table 4: Solubilization of muscovite mica by the KSB isolates

Sr No	KSB Isolates	7DAI*(µg/ml)	15DAI(µg/ml)	20DAI(µg/ml)
1	KSB-1	21.32	43.29	46.52
2	KSB-3	25.76	38.56	40.58
3	KSB-9	6.48	19.65	30.21
4	KSB-17	18.66	26.32	39.76
5	KSB-20	12.58	22.78	34.75

Identification of potent isolates using Biolog system

From the five isolates two best isolates showing highest solubilization of potassium mineral (mica) in liquid medium were further identified by Biolog system. The plate contains 26 sugars, two hexose phosphate, nine amino acids, nine hexose acids, 18 carboxilic acids, testers and fatty acids, two acidic pH (5&6), three level of NaCl (1%, 4% and 8 %), lactic acid, reducing agent (Tetrazolium blue and Tetrazolium violet) and antibiotics. Software assisted computation identified KSB-1 isolate as *Bacillus licheniformis*. The isolate KSB-3 was identified as *Bacillus subtilis*.

Among the K bearing silicate minerals mica was found to solubilize readily than other minerals (Sugumaran and Janarthanam, 2007; Mikhailouskaya and Tehernysh, 2005). Similar results to this study were also reported by Archana *et al.* (2008) on KSB, who found that many species of *Bacillus* and *Pseudomonas* were able to solubilize mica and gave zone of solubilization in solid media. Some potassium solubilizing rhizobia (KSR) *Agrobacterium tumefaciens* OPVS11 (*Zea mays*) and *Rhizobium pusense* OPVS6 (*Saccharum officinarum*) were found to dissolve waste mica (Meena *et. al*., 2015).

The results obtained from this study are in agreement with other investigators who reported that *Bacillus megaterium* (Hu and Boyer, 1996) and *B. mucilaginosus* (Biswas and Basak, 2014) were capable of solubilizing mica in appreciable amounts. Archana *et al.* (2008) reported that KSB *Bacillus sp*. solubilize 44.49 µg/ml mica in liquid medium.

Conclusion

Potassium solubilising bacterial isolates were isolated from the rhizosphere of maize from various areas of Navsari district. After secondary screening five bacterial isolates were selected from 25 isolates able to solubilize higher amount of potassium mineral. Among them KSB-1, KSB-3 and KSB-9 were Gram positive long rods, identified as *Bacillus sp.* while KSB-17 and KSB-20 were Gram negative short rods identified as *Pseudomonas Sp*. The amount of K released from muscovite mica in the broth by the isolates was found in the range of 1.89 to 46.52µg/ml. The maximum solubilization was observed in case of KSB-1 *i.e.* 46.52 µg/ml which is followed by KSB-3 *i.e.* 42.37 µg/ml. These two best bacterial strains were identified by Biolog system as *Bacillus licheniformis* and *Bacillus subtilis*.

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