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ISOLATION AND SCREENING OF POTASSIUM SOLUBILISING MICROORGANISMS

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Abstract:
Potassium (K) is an essential macronutrient that plays an important role in the growth and development of plants. In plants potassium deficiency results into poor development of roots, decrease in the growth rate, production of small seeds and lower crop yields. In soil potassium exists in three forms as unavailable, slowly available or fixed and readily available or exchangeable. The soluble form of potassium is present in very low concentration in soil and it is only utilised by the plants. Many soil microorganisms have ability to solubilise unavailable and fixed form of potassium and use of such microorganisms as bioinoculant is economical as well as ecofriendly over chemical fertilizers. In this study twenty bacterial strains having ability of potassium solubilisation were isolated from the soil samples collected from different areas of Maharashtra. Aleksandrov's agar medium containing potassium aluminoisilicate as insoluble potassium source was used for study. All strains were further screened for their potassium solubilisation ability so as to identify the potent strain.
Keywords: Potassium solubilisation; Insoluble potassium; Bioinoculant.

Introduction

Potassium, an essential nutrient required for the growth of the plants, is present mostly in insoluble form in the soil which is not utilisable by plants. Again small amount of soluble potassium present is found to be removed from soil due to various processes such as leaching, runoff or erosion. At present the demand of plants for potassium is fulfilled by application of potassium in the form of chemical fertilizers. But overuse of chemical fertilizers causes number of problems such as pollution of water resources, reduction in soil fertility, and increase in soil salinity, development of acid rain and health problems. Thus it is necessary to investigate an alternative indigenous source of K for plant uptake. Many soil microorganisms have ability to solubilise unavailable and fixed form of potassium and called as potassium solubilising microorganisms. Thus the application of such microorganisms in the soil as bioinoculant can help to increase crop production as well as can conserve our existing resources.

Materials and Method

Soil samples were collected from different areas of Maharashtra such as Radhanagari, Rajputwadi, Malkapur, Jotiba, Nandani, Balinga, Gargoti, Hatkangale, Fulewadi, Solapur as well as soil was collected from cement factories. Soil samples were inoculated in Aleksandrov's broth containing glucose, yeast extract and insoluble potassium such as potassium aluminosilicate and incubated on shaker for 7 days. Gram staining of enriched samples showed mixed micro flora such as Gram positive long rods, short rods arranged singly as well as in chains and Gram positive cocci arranged in bunches. Enriched samples were inoculated on normal Aleksandrov's agar medium and Aleksandrov's agar medium containing bromothymol blue (BTB) as indicator. Plates were incubated at RT. On normal Aleksandrov's agar medium potassium solubilizing bacteria were identified on the basis of clear zone obtained around the growth and on BTB medium on the basis of yellow zone appeared around the growth. The twenty well isolated colonies on Aleksandrov's agar media were studied for Gram characteristics so as to check the purity of isolates. Impure isolates were purified by serial transfers. The all purified cultures further confirmed for their potassium solubilization ability on Aleksandrov's agar medium by spot inoculation and then positive strains were coded as C6, Y8, C1, Z, V, C4, C3, MHB, C2, C5, X and AL. The potassium solubilization ability of all positive strains were tested qualitatively for identification of potent strain. The equal cell numbers ($3x10^8$ cells/ml) of all positive strains (10 µl) were spot inoculated with the help of micropipette on Aleksandrov"s agar medium and after incubation for 48 hrs. diameter of colony and diameter of clear zone obtained were measured and on the basis of Khande Parkar's ratio, potent strain was screened. The primary identification of potent strain was carried out by studying morphological characteristics.

Result and Discussion

Out of twenty well isolated selected bacterial strains, twelve bacterial strains obtained in pure culture form were used for screening of potent potassium solubilizer.

Table 1: Results of potassium solubilisation by bacteria isolated on Aleksandrov's agar medium from soil samples collected from various areas of Maharashtra

Soil Sample	Results of K solubilisation	
	On normal Aleksandrov's medium	On BTB medium
Radhanagri	-	-
Malkapur	+ After 72 hrs.	+ After 48 hrs.
Balinga	+ After 72 hrs.	+ After 48 hrs.
Fulewadi	+ After 72 hrs.	+ After 48 hrs.
Gargoti	-	-
Rajputwadi	+ After 72 hrs.	+ After 48 hrs.
Solapur (soil)	-	-
Solapur (compost)	-	+ After 48 hrs
Hatkanagle	-	-
Nandani	-	-
Jotiba	-	-
Cement factory	+ After 72 hrs.	+ After 48 hrs.

- :No growth; + :Growth

Table 2: Results of qualitative assay of isolates after 48 hrs. incubation on normal Aleksandrov's agar medium

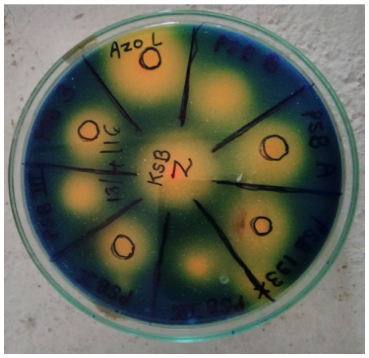
Isolate	d in mm	D in mm	D/d
C6	0.5	1.3	2.6
Y8	-	-	-
C1	0.7	1.2	1.7
Z	0.7	1.2	1.7
V	0.9	1.2	1.3
C4	**0.6**	**1.6**	**2.7**

Isolate	d in mm	D in mm	D/d
C3	0.7	-	-
MHB	0.8	1.3	1.6
C2	0.7	1.2	1.7
C5	0.8	1.4	1.8
X	0.9	1.4	1.55
AL	0.7	1	1.4

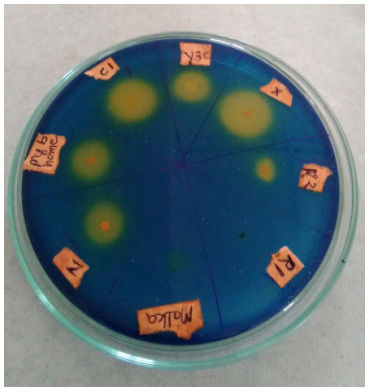
d : Diameter of colony, D : Diameter of zone

Table 3: Results of morphological characteristics of potent strain C4

Gram Nature	Gram negative, short rods arranged singly
Motility	Nonmotile
Spore formation	Non spore forming
Capsule production	Noncapsulated



(a)



(b)

Figure 1: (a) and (b) KSB isolates showing yellow zones of potassium solubilisation around the growth on Aleksandrov's agar medium containing bromothymol blue.

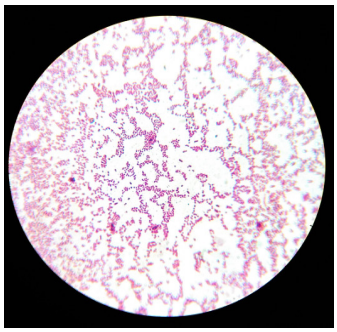


Figure 2: (c) Gram characteristics of isolate C4

Conclusion

A number of bacterial strains having ability of potassium solubilisation were isolated from the soil samples collected from various areas of Maharashtra. All positive strains when analyased for their potassium solubilisation ability by qualitative assay method, the C4 strain was found to be the most potent strain on the basis of Khande Parkar's ratio. The morphological study of C4 strain showed that it is a Gram negative, rod shaped, nonmotile, nonspore forming and non capsulated bacterium.

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