ORIGINAL RESEARCH PAPER

Microbiology

ISOLATION OF POTASSIUM SOLUBILIZING ACTINOMYCETES FROM CERAMIC INDUSTRY SOILS

KEYWORD: Solubilization, Actinomycetes, Potassium, Fertilizer

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ABSTRACT

Soil fertility and plant nutrition require an adequate management of essential macronutrients such as potassium (K) is mandatory for plant development. Potassium replenishment, particularly in smallholder agriculture, remains a challenge as it is mainly fertilizer dependent. While the user of soluble mineral potassium fertilizers is the obvious best means to combat potassium deficiency in soil, they were limited by high cost of fertilizers and availability at farmer's level. This research is to isolate and select potassium solubilizing soil Actinomycetes from ceramic industry soils. 22 Actinomycetes isolates were inoculated on Aleksandrov agar supplemented with 0.5% potassium aluminium silicate, isolates which gave the high ratio of clear zone were selected. Out of 22 Actinomycetes Isolates two (KSA 09 and KSA 16) were selected which gave highest solubilization in liquid medium by solubilizing insoluble potassium source i.e. feldspar. This two strain selected for further studies for future prospectus.

INTRODUCTION

Potassium (K) has been recognized as an essential element in intensive agricultural production systems and deficiency of K usually results in a decrease in crop yields. Potassium (K) is the major essential macronutrient for biological growth and development. Plants acquire potassium from soil as potassium ions. However, the concentrations of soluble K in soil are usually very low, and the biggest proportions of K in soil are insoluble rocks, minerals and other deposits (Goldstein, 1986 &1994). In spite of that, these sources constitute the biggest reservoirs of K in soil under appropriate conditions, they can be solubilized and become available for plants. Microorganisms play a central role in the natural K cycle. There are considerable populations of K-solubilizing microorganisms in soil and in plant rhizosphere (Sperberg, 1958). It is generally accepted that the major mechanism of mineral potassium solubilization is the action of organic acids synthesized by soil microorganism. Production of organic acids results in acidification of the microbial cell and its surroundings. Silicate bacteria were found to resolve potassium, silicon and aluminum from insoluble minerals (Aleksandrov *et al.* 1985).

Actinomycetes synthesize valuable bioactive compounds such as enzymes, antibiotics, neutraceuticals, antitumor agents, plant growth regulators and vitamins and also produce enzymes industrially (proteases, amylases, etc) (Prakash *et al.* 2013; Kamjam *et al.* 2017: Wietzorrek and Bibb 1997; Shigeri *et al.* 2009). Actinomycetes are predominantly present in various natural habitats such as plant tissues as well as soil, thereby, have been isolated from various niches and production of extracellular hydrolytic enzymes by theses Actinomycetes confer them the properties of disease control and plant growth promotion. However, the applications of Actinomycetes as enzyme producers in agriculture field are relatively less explored (Rani.K, 2021). Actinobactes are able to thrive in extremely different soils, play important ecological roles in soil nutrient cycling and are recently being regarded as plant growth promoting rhizobacteria (Pathom-Aree et al., 2006; Franco-Correa et al., 2010, Sun F., *et al.* 2020).

MATERIALS AND METHODS

Sample Collection

Majority of the ceramic industries are using insoluble source of potassium i.e. feldspar as their raw material so samples were collected from the various ceramic industries. Total 15 samples were collected from different ceramic industries of Gujarat nearby Morbi, Meshana and Kadi region. The samples labelled as S1 to S15.

Adaptation And Enrichment:

Collected soil samples were mixed with insoluble potassium (Feldspar) and $CaCo_3$ (for Actinomycetes growth) and incubated for 1 week at room temperature. After adaptation 1 gm of soil was inoculated in 100 ml liquid medium containing 1% glucose, 0.05%yeast extract and 0.5% feldspar and incubated at 28 + 2° C on 120 rpm for 1 week.

Isolation and Screening of Potassium Solubilizing Actinomycetes

Enriched samples were inoculated after serial dilution from 10^{-1} to 10^{-6} on Aleksandrov's agar medium constituted 1% glucose, 0.05% $MgSo_4.7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCO_3$, 0.2% $CaPo_4$ and 0.5% potassium aluminium silicate, agar 3 % pH-6.5 (Sugumaran and Janartham, 2007) and incubated at 28 + 2° C for 1 week. Colonies exhibiting clear zone of potassium solubilization were selected.

Secondary Screening was carried out on the basis of study of zone diameter of the different isolates by using Khandeparkar's selection ratio on same Aleksandrov's agar medium.

Ratio = D/d = Diameter of zone of clearance / Diameter of growth

To study the mechanism of potassium solubilization, selected Actinomycetes isolates were also inoculated on the same Aleksandrov's medium with pH indicator dye (0.025% Bromothymol blue). (K B Prajapati, 2012)

Macroscopic/colonical And Morphologicak Characterization

Colony characteristics of the selected Actinomycetes strains were studied on Glycerol Asparagine Agar (GAA) medium. Cell morphologies of the isolates were observed using a compound microscope after performing Gram staining (H. C. J .Gram, 1884).

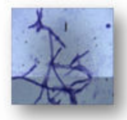
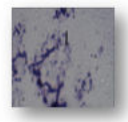
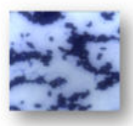
RESULTS AND DISCUSSION

Colonies exhibiting zone of clearance on Aleksandrov's agar medium were selected as potassium solubilizers. Total 22 Morphologically distinct actinomycetes colonies were selected and labeled as KSA1 to KSA22 (Table 1). Isolates shows higher ratio of potassium solubilization by Khandeparkar's selection ratio were selected, i.e. KSA9, KSA10, KSA12, KSA16 and KSA17 (table 2).

Table-1. Potassium solubilization values of Actinomycetes isolates by Khandeparkar's selection ratio mm

Isolates	Diameter of zone of clearance (D) mm	Diameter of growth (d)	D/d (ratio)	Isolates	Diameter of zone of clearance (D) mm	Diameter of growth (d)	D/d (ratio)
KSA01	09	09	1	**KSA12**	**11**	**08**	**1.37**
KSA02	10	10	1	KSA13	10	09	1.11
KSA03	11	10	1.1	KSA14	10	10	1
KSA04	12	12	1	KSA15	10	09	1.11
KSA05	09	08	1.13	**KSA16**	**13**	**08**	**1.62**
KSA06	10	10	1	**KSA17**	**10**	**08**	**1.25**
KSA07	11	11	1	KSA18	11	11	1
KSA08	09	08	1.13	KSA19	10	10	1
KSA09	**14**	**09**	**1.56**	KSA20	11	10	1.1
KSA10	**11**	**08**	**1.37**	KSA21	12	12	1
KSA11	10	10	1	KSA22	09	08	1.13

Table-2. Potassium solubilizing Actinomycetes Isolates Description (colony morphology, microscopic features)

Isolate no.	Description (colony morphology, microscopic features.)	Growth at GAA medium	Microscopic view
KSA09	Aerial mycelium cream, smooth, powdery, circular, colony reverse off white Filaments branched.		
KSA10	Aerial mycelium gray, rough, powdery, circular, colony reverse gray Filaments branched.		
KSA12	Aerial mycelium brown, rough, irregular, colony reverse brownish pigment Filaments branched.		
KSA16	Aerial mycelium dark yellow, smooth, circular, reverse light yellowish pigment producer Filaments branched,		
KSA17	Aerial mycelium creamy, smooth, irregular, colony reverse off white, Filaments branched.		

No reports found for Potassium solubilizing Actinomycetes, but the research suggested that microbixal solubilization of mineral phosphate might be either due to the excretion of organic acids causing acidification of the external medium or to the excretion of chelating substances (such as sidero-pshores) that form stable complexes with phosphate adsorbents (aluminium, iron and calcium) (Whitelaw, 1999, Welch *et al.* 2002; Hamdali *et al.* 2008)

Five selected Actinomycetes isolates showed low pH associated with yellow colour formation on the Aleksandrov's agar medium supplemented with Bromothymol blue after 72 hrs of incubation indicates that K solubilization was through acidification mechanism. The zone of Potassium solubilization with Bromothymol blue containing medium by KSA09 &KSA16 (Fig-1and 2).

Figure 1. Zone Of Potassium Solubilization Aleksandrov + Btb Agar Medium.

Figure-2. Zone Of Potassium Solubilization By Actinomycetes Isolates On Aleksandrov's Agar Medium By Ksa9 And Ksa16 Isolates.

Many microorganisms have been found to possess this K-releasing ability, e.g. *Pseudomonas, Burkholderia, Acidithiobacillus, Bacillus mucilaginosus, B. edaphicus and B. circulans, etc*. (Lian *et al*. 2002; Sheng 2005b; Zhao *et al*. 2008, Sugumaran and Janartham, 2007). Actinomycetes are widely distributed in many natural habitats including various soil, freshwater, marine, organic matter. Generally actinomycetes are neutrophiles, a few actinomycetes such as Streptomyces acidiphilus and members of the genus Streptacidiphilus5 have been reported to require acidic conditions (pH 2.6–5.5) for growth(Jenson 1928, Kim et al 2003).

CONCLUSION

Total 22 Actinomycetes strains were isolated from various K rich ceramic industries soil samples. All the isolates were characterized for morphological and cultural characters.Among them 5 Actinomycetes isolates were selected for further study due to their higher potassium solubilization capabilities. All the isolates were able to solubilize (feldspar) insoluble potassium mineral under in vitro condition. Further two Actinomycetes strains i.e.KSA 9 and KSA 16 were selected which showed the highest zone of potassium solubilization on Aleksandrov's agar medium. Selected actinomycetes isolates showed low pH associated with yellow colour formation on the Aleksandrov's Agar medium supplemented with bromothymol blue indicates that potassium solubilization is due to acid production. Actinomecetes species play a major role in biogeochemical cycles so our selected potassium solubilizing actinomycetes could be used for development and formulation of new technology for non polluting farming practices and Biofertilizer.

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