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PHOSPHATE SOLUBILIZING CAPACITY AND SIDEROPHORE PRODUCTION BY *ARTHRODERMA CUNICULI* DAWSON 1963 ISOLATED FROM RHIZOSPHERIC SOIL

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ABSTRACT: This is the first report of phosphate solubilizing activity and siderophore production of *Arthroderma cuniculi*.The results of phosphate solubilizing activity revealed that, *Arthroderma cuniculi* efficient phosphate solubilizer on Pikovskaya's agar medium with SI = 2.05. The tested fungus showed positive reaction for the hydroxamates nature of siderophores. *Arthroderma cuniculi* was neither showed carboxylate nature of siderophore activity nor the catecholates nature of siderophore activity.

KEYWORDS: Phosphate solubilizing, siderophores, *Arthroderma cuniculi,*Pikovskaya's agar medium

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1. INTRODUCTION

Soil harbours various micro organism among them fungi are one of the members of mixed community categorized by complex interactions. Fungi play pivotal role in the rhizospheric soil which is beneficial for plants [1]. Soil microorganisms secret low molecular weight siderophores which help in acquisition of iron from insoluble forms by the process of mineralization and sequestration [2]. Siderophores are also help in chelating other ions and effects their specificity and avidity for its most consistent feature [3]. Siderophores not only improve rhizosphere colonization of producer strain but also play significant role in iron nutrition of plant and antagonism against

phytopathogens [4]. Phosphorus (P) is one of the essential macronutrients next to nitrogen which help in growth and development of plants [5].Approximately 95– 99% soil phosphorus is present in insoluble form complexed with cations like iron, aluminum and calcium that cannot be utilized by the plants [6]. The most cheaper and expedient alternative strategy is to reclamation of exhausted soil through use of P-solubilizing microorganisms and these type of microbial-based approaches have been proposed to improve the agronomic value [7]. Use of microbial-based approaches could also be a nalternate strategy to reduce the environment pollution created by the use of conventional chemical process. Plant-microbes interactions in the rhizosphere are one of the key determinants of plant health and soil fertility [8]. Literature survey revealed that among the rhizospheric fungi belonging to the genera *Penicillium* and *Aspergillus* are the dominant and efficient P-solubilizing filamentous fungi [9].Similarly among the siderophore producing fungi *Aspergillus flavus, Rhizopus rhizopodiformis, Rhizopus oryzae,* and *Mucor mucedo* proclaimed as exclusive siderophore producer [10].The present study aim to assess the phosphate solubilizing and siderophore activity of *Arthroderma cuniculi* isolated from rhizospheric soil of forest.

2. MATERIALS AND METHODS

Study site:

Soil samples were collected from the rhizospheric forest soil located at Kamalghat area, Agartala.

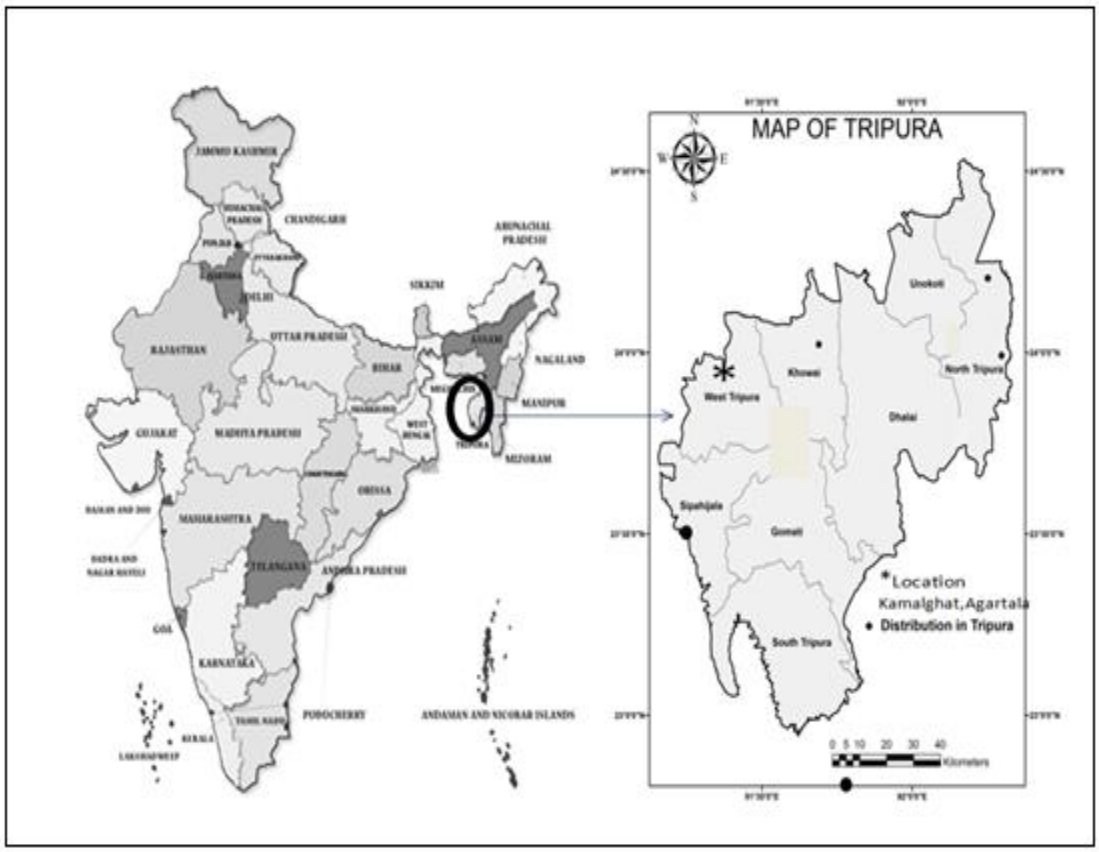

Fig 1: Map showing the study site

Isolation and identification of *Arthroderma cuniculi*:

Enumeration of soil microfungi was done by serial dilution methods [11]. Make microbial suspensions (10^{-3} - 10^{-5}).Each concentration microbial suspension of was added to sterile Petri dishes (triplicate of dilution) containing Malt extract Agar media (Himedia) and Rose Bengal Agar (Himedia). The Petri dishes were then incubated at28^0C± 2^0 C until the colony grows. Fungal morphology were studied macroscopically by observing colony features (Colour and Texture) and observed under compound microscope (Olympus, CX-21i) for the conidia, conidiophores and arrangement of spores. The fungi were identified with the help of laboratory manuals [12, 13, and 14].

Evaluation of Fungi for Phosphate Solubilization activity:

The fungal isolates *Arthroderma cuniculi* obtained from rhizospheric soils was evaluated on Pikovskaya's agar plates and liquid culture media containing insoluble sources (Tricalcium phosphates-SRL) for their activity.

Assay of Solubilization Index on Solid Medium:

Phosphate solubilization activity was done following the method described by Iman [15] on Pikovskaya's agar medium. 6mm disc of the fungal isolates place on Pikovskaya's agar medium in triplicate under aseptic condition and incubated at 25–28^0C for 7 days. Uninoculated PKV agar plate served as control. Comparative solubilization index measurement was carried out on day seven of incubation by measuring clear zone and colony diameters in centimeter. Phosphate solubilization index was determined by using the following formula: ratio of the total diameter (colony + halo zone) and the colony diameter [16].

$$\text{Solubilization Index (SI)} = \frac{\text{colony diameter + halo zone diameter}}{\text{Colony diameter}}$$

Screening of isolates for siderophore production:

The fungal isolate *A. cuniculi* was screened for their ability to produce siderophores in Modified M9 medium [17]. The glass wares were made iron free by washed with 6 (N) HCl. To ensure complete removal of iron complexes from media followed the method [18]. 50 ml medium was then dispensed in 250 mL conical flasks. These flasks were disc (0.8cm) inoculated with young culture of fungal isolate and placed on an environmental shaker (Labtech, Model-LSI 4018R) at 30 °C for 5-7 days. After 7 days of growth, the media were filtered through Whatman filter paper No.1. Culture filtrates were examined for extracellular siderophore production.

1. Presence of siderophore was detected by FeCl3 test [19]:

1 ml of culture filtrate was mixed with 1mL of 2% aqueous solution of FeCl3. Presence of siderophore was detected by formation of a red-purple colored complex.

2.Hydroxymate nature of siderophore was detected by Tetrazolium salt test[20]:

A pinch of tetrazolium salt added to 1 ml of of 7 days old culture and then 1 to 2 drops of 2 N NaOH was added. Instant appearance of a deep red colour indicated hydroxamate nature of the siderophore.

3. Catecholates nature of siderophores was detected by Arnow [21]

In 1 ml of culture filtrates 1 ml 0.5 M HCI and nitrite molybdate reagent [10 g each of NaN02 and Na2MoO4in 50 ml distilled water] were added (yellow colour appeared). Then 1 ml of 1 N NaOH was added (red colour appeared). At last volume was made upto 5.0 ml with distilled water. Absorbance was noted at 500 nm.

4. Carboxoylate nature of siderophores was detected by Vogel chemical test [22]:

To 3 drops of 2N NaOH was added 1 drop of phenolphthalein. Water was added until light pink colour developed. On addition of the test sample, disappearance of the colour indicated presence of a carboxylate siderophore.

3. RESULTS AND DISCUSSION

Table: 1: Identifying character of the isolate *Arthroderma cuniculi* (MPL-P2).

Lab. accesion no.	Micro-habitat	Identifying character
MPL-P2	Forest soil	Colonies are slow growing, grew 30 mm after 7 days of incubation, colonies are white and reverse to yellow, septate hyphae, unicinately branched, conidia borne in short, undifferentiate ,one celled or occasionally 2 or 3 celled, clavate, smooth walled.

The fungal isolate was characterized morphologically and identified as *Arthroderma cuniculi*(Table.1). The solubilization index (SI) of the isolated phosphate solubilizing fungi was found to be 2.05 at seven days of incubation at 25–28^{0}C (Fig.2).Results revealed that *Arthroderma cuniculi* was the efficient phosphate solubilizer on PVK plates with SI = 2.05.Previously there is no such report of this fungus as a phosphate solubilizer. Extensive literatures survey revealed that *Arthroderma cuniculi* act as keratinophlic fungi [23,24] but there is no report regarding the phosphate solubilizing and siderophore activity of *Arthroderma cuniculi* till today. The results shown in Fig.3 suggest that the test fungus not showed positive reaction in Vogel chemical test and Amow's test, ruling out carboxylate and catecholates nature of siderophore.

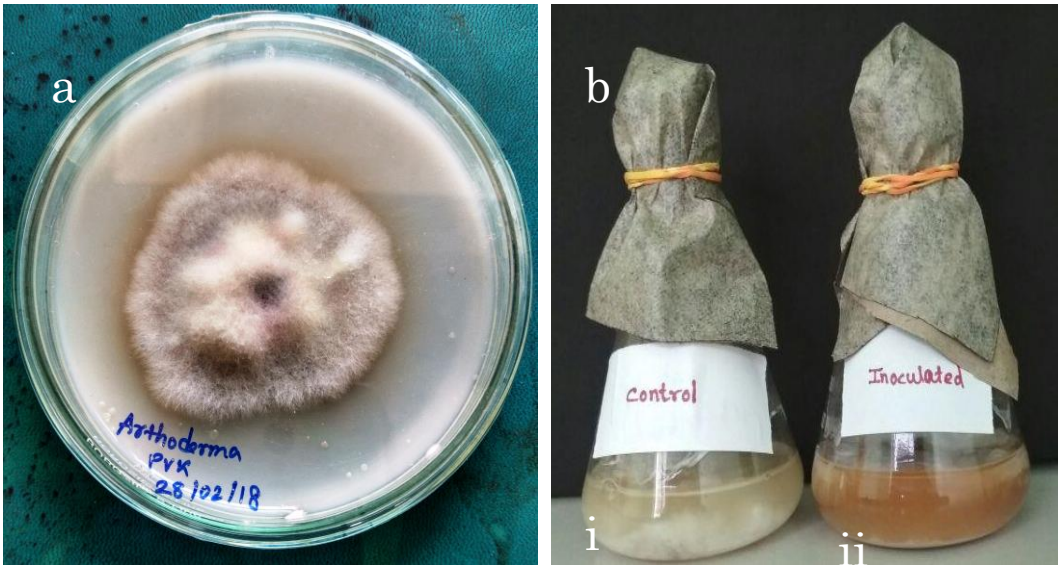

Figure 2: Phosphate solubilizing activity of *Arthroderma cunculi, a*. Pikovskaya's agar medium, b. Liquid culture media.

Figure 3: Siderophore activity of *Arthroderma cuniculi* in different biochemical test.

The results presented in Fig.3 indicate a positive reaction for hydroxymate siderophores. Previously there is no such report of this fungus as a siderophore activity. Literatures revealed *Arthroderma cuniculi*act as a keratinophlic fungi but many other soil fungi such as *Aspergillus flavus, Rhizopus rhizopodiformis, Rhizopus oryzae,* and *Mucor Mucedo* producing exclusively siderophore [25].Vala [10] has reported carboxylate siderophores produced by *Rhizopus sp., Syncephalastrum sp. and Syncephalastrum racemosum*) isolated from marine habitats.

4. CONCLUSION

The soil borne *Arthroderma cuniculi* has the ability to produce siderophore and have phosphate solubilization capacity. Better acquisition of available iron and phosphate enrichment of the plants will promote better growth by the application of this fungus.

5.ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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