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PHOSPHATE SOLUBILIZING ACTIVITY OF SOME FUNGAL STRAINS ISOLATED FROM RHIZOSPHERE OF BT COTTON *(GOSSYPIUM SPP.)* PLANT.

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ABSTRACT Phosphorus is one of the most vital macronutrients required for the growth and development of plants. The most important function of phosphorus in the plant system is energy storage. Phosphate solubilizing microorganisms solubilize insoluble forms of inorganic phosphorus and improve the availability of phosphorus to the plants. Six (06) rhizospheric sample of BT cotton plant of Mehsana district of North Gujarat region were selected for isolation and screening of phosphate solubilizing fungi. Out of nineteen (19) isolates, nine (09) fungal isolates showed zones of solubilization on pikovskaya's agar medium. These nine (09) fungal isolates were selected for the further study such as qualitative as well as quantitative screening. Among these nine (09) isolates, PSF-2 (*Aspergillus awamori*) showed the maximum phosphate solubilization index 1.53 ± 0.026 on PVK agar plates along with phosphate solubilizing activity 561 ± 7.47 µg mL^{-1} in PVK broth and pH of the medium decreased up to 2.59 ± 0.015 . These strains were used for further study as effect of various culture conditions on efficiency of phosphate solubilization. *Aspergillus awamori* showed good phosphate solubilizing ability and thus proved to be potential candidate for using as biofertilizers.

KEYWORDS : Phosphate Solubilizing Fungi, *Aspergillus awamori*, BT Cotton, Rhizosphere

INTRODUCTION:

Phosphorus is a major essential macro element required for plant of growth and development. The bioavailability of soil inorganic phosphorus in the rhizosphere varies considerably with plant species, nutritional status of soil and ambient soil condition. It is mostly deficient in soil as it is fixed as water insoluble iron and aluminium phosphates in acidic soils or calcium phosphate in alkaline soils (Singh and Kapoor, 1994). A greater part of soil phosphorus, approximately 95– 99%, is present in insoluble form complexed with cations like iron, aluminum, and calcium that cannot be utilized by the plants (Son *et al.,* 2006). Chemical phosphate fertilizers are only meagerly soluble under the condition in which they are applied to the soil. However, under such condition microorganisms offer a biological rescue capability of solubilizing the insoluble inorganic phosphorus of soil (Rashid *et al.,* 2004). Many bacteria living in the rhizosphere are useful bacteria for plant growth, also known as plant growth promoting rhizobacteria. These microorganisms have nitrogen-fixing, phosphate solubilizing and potassium solubilizing abilities, which facilitate the plant absorption and utilization of mineral nutrition, leading to the promotion of plant growth (Lu and Huang, 2010). High proportion of phosphate solubilizing microorganisms is concentrated in rhizosphere and they are metabolically more active then microorganisms from other sources. Filamentous fungi are frequently used in laboratories to solubilize insoluble phosphate salts, *Penicillin* and *Aspergillus* are two important genera frequently used for phosphate solubilization. Ability of solubilization of inorganic insoluble phosphate salts by different microorganisms depends on their ability to produce and release organic acids to their respective environments (Nath *et al.,* 2012). Several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by phosphate solubilizing microorganisms (Cunningham and Kuiack, 1992). The aim of our investigation was to isolate phosphate solubilizing fungi from rhizosphere of BT Cotton plant.

MATERIALS AND METHODS:

Collection of soil sample

Soil samples were collected from rhizosphere BT cotton plant (*Gossypium spp*.). Collected soil samples were stored in polythene bags and maintained in the laboratory for further study.

Isolation of phosphate solubilizing fungi

From each soil sample, 1.0g of soil was suspended in 9.0ml sterile distilled water and serially diluted up to 10^{-6}. The dilutions were plated on pikovskaya's (PVK) agar medium in order to isolate the phosphate solubilizing fungi. Those colonies surround with a halo zone (zone of phosphate solubilization) were retransferred to PVK agar medium to maintain the purity of the culture. All isolated fungi were stored at 4^0C and regularly transferred on potato dextrose agar (PDA) slants during study.

Analysis of phosphate solubilizing activity

Phosphate solubilizing activity of the nine (09) fungal isolates was conducted by qualitative and quantitative screening, respectively.

Qualitative measurement of phosphate solubilization

Fungal isolates were screened for their tri-calcium phosphate (TCP) solubilizing activity on PVK plates. Isolates were spot inoculated on the center of agar plate aseptically. All the plates were incubated at 28^0C for 5 days. A clear zone around a growing colony indicated phosphate solubilization and was measured as phosphate solubilization index (SI). All the observations were recorded in triplicate. Solubilization index (SI) was measured using following formula:

$$SI = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

Quantitative measurement of phosphate solubilization

Phosphate solubilizing ability of fungal strains was tested in PVK broth media with 0.5% tri calcium phosphate. Flasks were inoculated with 8 % (v/v) spore suspension and incubated on shaker at 28^0C for 6 days 100 rpm. After incubation the broth were centrifuged at 10,000 rpm for 15 min, the pH of the supernatant was measured with pH meter and dissolved phosphate concentration in supernatant was determined by vanado- molybdate method as described in APHA (1995).

Identification of phosphate solubilizing fungi

The isolated fungal culture was sent to Labreq bioscientific, Ahmadabad, Gujarat for 18s rRNA sequencing where sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied Bio Systems, USA). The fungal isolate PSF-2 was identified as *Aspergillus awamori* on the basis of 18s rRNA sequencing.

RESULTS AND DISCUSSION:

Table-1. Qualitative and Quantitative Screening of Phosphate Solubilizing Fungi

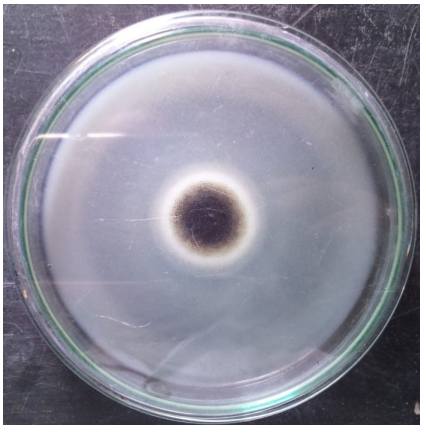
Isolates	Solubilization index (SI)	Soluble P concentration (µg mL-1)	End pH
PSF-1	1.36 ± 0.032	308 ± 3.45	3.35 ± 0.032

PSF-2	1.53 ± 0.026	561 ± 7.47	2.59 ± 0.015
PSF-3	1.45 ± 0.020	391 ± 6.09	2.99 ± 0.060
PSF-4	1.38 ± 0.017	464 ± 4.27	2.74 ± 0.031
PSF-5	1.21 ± 0.015	260 ± 4.78	3.94 ± 0.032
PSF-6	1.44 ± 0.056	182 ± 3.70	4.15 ± 0.025
PSF-7	1.30 ± 0.006	316 ± 9.48	3.18 ± 0.035
PSF-8	1.41 ± 0.012	428 ± 4.59	2.95 ± 0.021
PSF-9	1.23 ± 0.006	379 ± 4.57	3.90 ± 0.025

Nineteen (19) fungal isolates were isolated from the total of six (06) rhizospheric samples collected from BT cotton plant grown in Mehsana district of North Gujarat. From these, nine (09) fungal isolates were selected for the further study such as qualitative as well as quantitative screening. Table-1 summarizes the solubilization index of phosphate solubilizing activity in PVK agar plates after five days of incubation and also shows values of phosphate solubilization in liquid culture and the pH of the corresponding media after six days of incubation. Microorganisms capable of producing a clear zone due to P solubilization in the surrounding medium were selected as potential phosphate solubilizer (Singal *et al.,* 1991). It clearly appears that in media with tri calcium phosphate, the values of solubilized phosphate obtained with all the isolates were significantly different from those of control, showing that the tested isolates have effectively converted the insoluble phosphate into soluble form. Also, a decrease in pH values was observed in the tested isolates as compared to control. Fungal isolates showed the development of solubilization zone, ranging from 1.21 ± 0.015 to 1.53 ± 0.026 (Table-1). According to their efficiency, the most efficient phosphate solubilizing strain was PSF-2 (561 ± 7.47 µg mL^{-1}) while strain PSF- 6 was the least solubilizing (182 ± 3.70 µg mL^{-1}). Solubilization of tri calcium phosphate in the liquid medium by different strains was accompanied by a significant drop in pH upto 2.59 ± 0.015 and 4.15 ± 0.025 from an initial pH of 7.0 after six days of incubation by PSF-2 and PSF-6.

Chakraborty *et al.,* (2010) stated that *Aspergillus sp.* showed high levels of phosphate solubilizing activity. Yadav *et al.,* (2011) reported that *Aspergillus niger* was found to solubilize tri calcium phosphate with maximum activity of 512 µg mL^{-1} and minimum activity of 348 µg mL^{-1}. Das *et al.,* (2013) reported that *Aspergillus niger* and *Aspergillus flavus* were solubilized 340.5 µg mL^{-1} and 272.1 µg mL^{-1} tri calcium phosphate and SI of phosphate solubilizing fungi ranging from 1.41 to 1.87. Alam *et al.,* (2002) reported SI of phosphate solubilizing fungi ranging from 1.53 to 1.80, these fungal strains isolated from maize rhizosphere. Ruangsanka, (2014) reported that *Penicillium oxalicum* has the highest phosphate solubilizing ability (556 µg mL^{-1}).

Our data also indicate that *Aspergillus awamori* has a greater potential to solubilize the inorganic phosphates (TCP) as compared to other fungal species.



Aspergillus awamori

CONCLUSION:

From the above study, it can be concluded that *Aspergillus awamori* was most efficient phosphorus solubilizing strain. The decrease in pH of the culture medium there by solubilizing the insoluble tri-calcium phosphate indicated the production of multiple organic acids. So, further studies are required to understand the significance and mechanism used by an unknown acid in phosphate solubilization. *Aspergillus awamori* showing more phosphate solubilizing activity can be used in the field as efficient biofertilizer for increasing crop productivity.

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