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RESEARCH ARTICLE

SCREENING OF ALKALINE PHOSPHATASE ACTIVITY OF PHOSPHATE SOLUBILIZING FUNGI FROM RHIZOSPHERE SOIL.

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Abstract

Soil is a mixture of organic nutrients, which provides nutrients for plant growth. Plants require N, P & K for their growth and vegetative propagation. Among vital nutrients, Phosphorus is less available to plants and microorganisms aid in phosphate solubilization. The present study is an ecofriendly method to induce phosphate solubilization in Rhizosphere soil. Totally 25 isolates were isolated from rhizosphere using Pikovskaya medium and screened for phosphate solubilization using Tricalcium phosphate supplemented minimal medium. Of these, Aspergillus sp and *Penicillium* sp were found to produce remarkable solubilization index. Hence these two isolates were used for further assays. Enzyme assay was carried out to determine the enzyme activity using cell free extracts and the activity was analyzed by spectrophotometry. In addition, HPLC also performed to determine the end products of phosphate solubilization. The results of HPLC studies revealed that tricalcium phosphate was solubilized into organic acids like citric acid, gluconic acid and malic acid. The results of present study are an evidence for the production of organic acids by *Aspergillus* sp and phosphate solubilization which induce crop improvement as well as sustainable agriculture.

Introduction:-

Phosphate is an important nutrient for plant growth and development. All plants require phosphate for metabolic activities like nucleic acid synthesis, respiration, energy production, energy storage and transfer, cell division and cell growth. Phosphorus fertilizers when applied during the early stages of plant growth promotes early root formation, and is important for development of primordia for reproductive parts of plants. Seed formation requires phosphorus and its content is higher in seeds than in any other part of the plant. It improves survival of plants in winter climates. Phosphorus is available in rocks primarily as minerals like oxypatite, hydroxypatitite and apatite. These are highly insoluble and constitute about 40% of total phosphorus in Indian soils. Indigenous microorganisms convert insoluble phosphates into soluble phosphates and aid in the bioavailability of phosphorus. Phosphate solubilizing microorganisms assimilate phosphorus and release them to plants. Phosphate solubilization is a complex phenomenon which highly depends on many factors such as nutritional, physiological and growth

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conditions1. *Bacteria such as Pseudomonas, Alcaligens, Rhizobium, Seratia, Erwinia* are capable of phosphate solubilization 2.

Mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids3, which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms. There is experimental evidence to support the role of organic acids in mineral phosphate solubilization4.

Phosphate solubilizers possess enzymes that convert insoluble phosphate into soluble phosphate. Phosphate solubilizers like soil fungi are capable to produce extracellular enzyme, i.e. group of phosphatase enzyme which are able to mineralize organic P into inorganic P so that high P is available for plant. There are several soil phosphatases and the most commonly determined are: phosphomonoesterases, phosphodiesterases and phytases. Phosphomonoesterases act on phosphate monoesters and according to their optimum pH are divided in acid and alkaline phosphomonoesterases[5]. Both are adaptive enzymes: acid phosphomonoesterase predominates in acid soils while alkaline phosphomonoesterase predominates in neutral and basic soils 5.

An ecofriendly approach towards converting insoluble phosphates by potential phosphate solubilizing fungi from the soil is inevitable. The present study was designed to determine alkaline phosphatase activity of indigenous fungi from the natural rhizosphere soil.

Materials and methods:-

Isolation of phosphate solubilizing microorganisms:-

The serially diluted soil samples were placed on standard agar medium (pH 6.8-7.0) containing tricalcium phosphate (TCP) as sole phosphorus source for selectively screening the bacteria which have the ability to release inorganic phosphate from tricalcium phosphate. After 3 days of incubation at 25◦C, phosphate solubilizing fungi developed clear zones around colonies. Colonies with clear zones were further purified by replating .The isolation of phosphate solubilizing fungi by serially diluting the soil sample and plating method using Pikosvikyos agar medium supplemented with Tricalcium Phosphate.

Identification of phosphate solubilizing Microorganisms fungal identification:-

The morphological identification of fungal isolates were done by the methods described in experiments in Microbiology, Plant Pathology and Biotechnology.

Phosphate Solubilization activity:-

(Ngugen *et al.*, 1992)

The bacterial and fungal isolates were screened for inorganic phosphate solubilization. A loopfull of fresh bacterial and fungal cultures were streaked on to Pikosvikyos agar medium inorganic phosphate and plates were incubated at 28 ± 2 C for 3 days. After 3 days, the colonies showing the clear halo zone around them indicated solubilization of mineral phosphate. Phosphate solubilization activities were screened by measuring the clearing zone surrounding the developed bacterial colony via calculation of phosphate solubilization
index (Nautiyal, 1999).

Phosphate solubilization Index = A/B×100.

A= total diameter (colony + halo zone).

B =diameter of colony.

Identification and Characterization of Phosphate Solubilizing fungi.

PDA was used to accelerate the growth rate and the production of enough conidia as reported by Diba et al.The characteristics of fresh cultures were compared with mycological identification keys and taxonomic description to identify the isolated fungi to the genus level. Identification was based on colony characteristics and microscopic features, among the colonial characteristics such as surface appearance, texture, and colour of the colonies both from upper and lower side. In addition, conidia, conidiophores, arrangement of spores, and vegetative structures were determined with microscopy.

The identified fungi were maintained on Potato Dextrose Agar (PDA) slant at (4◦C) for further investigation. Slide culture was prepared in order to identify spores and mycelia of pure fungal isolates and identified by lacto phenol cotton blue staining using microscope and identified after growing them on slide according to Stevens et al

Results and discussion:-

Fungal isolates were identified by observing colony characteristics on RBCA plates. Growth pattern of the isolates were identified as *Aspergillus* sp and *Penicillium* sp(Fig 1,2). Further it was confirmed by microscopic analysis of colony using lacto phenol cotton blue staining method.

Solubilization index for the 24 isolates was in the range from 0.5cm-0.10cm. solubilization index for *Pseudomonas* sp was observed as 0.9cm10,11 and present study also revealed that fungal isolates was 0.6cm for *Aspergillus* sp and 0.5cm for *Penicillium* sp.

In liquid media, fungal isolates produced acid in high amount which is evident from decolourization of broth. Phosphate solubilization by these isolates was observed for 28 days. Quantitative estimation revealed that solubilization initiated after 3 days and was highest on the 7^{th} day. Decrease in pH was observed in all the isolates. Phosphate solubilization was observed in the range of 100µg $ml^{-1}$to 250µg ml^{-1}. Maximum phosphate was solubilized by *Aspergillus* sp(190µg ml^{-1}), and also it produced significant amount of citric acid, gluconic acid and moderate amount of oxalic acid. Organic acids were also produced including succinic, glycolic and malic acid in small amounts. *Penicillium* sp solublized phosphate(150µg ml^{-1})and also produced low levels of glycolic, citric, succinic, gluconic and oxalic acids.

Phosphatase activity:-

Phosphatase activity of fungi (*Aspergilus* sp and *Penicillium* sp.) were higher than bacteria10.Phosphatase activity of fungi (*Aspergillus* sp and *Penicillium* sp.) were higher than bacteria (*Pseudomonas mallei, Bacillus subtilis*) as reported earlier11**.** *Aspergillus* sp solubilised phosphste into organic acid and accumulated organic acids having retention times of 5.5 (approximating the retention time of citric acid), and 7.1 min. *Aspergillus* sp isolate also accumulated a small amount of organic acids having retention time of 7.8 min (approximating the retention time of malic acid), and 8.3 min. *Penicillium* sp accumulated malic acid. The results of the present study revealed that *Aspergillus* sp proved to be an efficient strain for phosphate solubilization and *Penicillium* sp needs small genetic manipulation. In addition, no significant amounts of organic acid production was observed from a phosphate solubilizer fungus, *Penicillium* sp12'.Tricalcium phosphate induced extracellular phosphatase production(119U/ml) compared to other phosphate sources14,15,similar result was observed when tricalcium phosphate was added (10g/l) in the medium. Phosphatase activity by fungal isolates was maximum in the pH4.0-6. 5 , which was similar to the results obtained during optimization studies13 for *Trichoderma* sp

The physico chemical characteristics of soil such as pH was neutral, the nitrogen content of the rhizosphere soil was found to be low ,while the amount of Potassium in soil was quite high and phosphorus content was also low14,16,17. The results of the present study on soil properties were in agreement to the results obtained by15which reported that the fertility of soil was moderate.

Conclusion:-

Two phosphate solubilizing fungi were isolated and identified as *Aspergillus* sp and *Penicillium* sp. Phosphorus solubilization activity of PSM is associated with the release of organic acid and a drop in the pH of the medium. In the present study, there is a decrease in the pH values of the Pikosvikyos media because of the release of organic acids in the cultural medium. Furthermore, the results of HPLC studies revealed that fungi solubilizes Phosphate into organic acid viz., Citric acid, Gluconic acid, Glycolic acid, Malic acid, Succinic acid and oxalic acid, which is evident from their retention time. Consequently it was proved that the isolated *Aspergillus* sp and *Penicillium* sp have the ability to solubilize phosphate which was confirmed by its phosphatase activity. Further molecular characterization of the isolates will provide their genetic relationship with other phosphate solubilizers.

Fig 1:- Isolation of fungi on RBCA plates

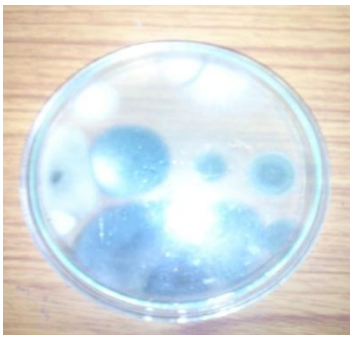

Fig 2:- Blackcoloured colonies

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