INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN 2278 – 0211 (Online)

Occurrence of the Phosphate Solubilizing Fungi Isolated from the Rhizosphere Soil of Different Ginger Growing Sites of Churachandpur District, Manipur, India

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Abstract:
The present investigation was carried out for understanding and exploring the indigenous phosphate solubilizing fungi and their efficient utilization as a potential biofertilizer for the improvement of soil fertility. For our study, the rhizospheric soils were collected from 21 different ginger fields of Rengkai, Churachandpur District,Manipur. Fungi were isolated from the rhizospheric soil samples. The dominant species were selected and screened for phosphate solubilizing activities using rock phosphate and tri-calcium phosphate in NBRIP Growth Medium. Fungi that showed halo zones around the colonies are good phosphate solubilizers and belong mainly to the genera of Aspergillus, Penicillium and Trichoderma. The present study would help in utilizing the indigenous phosphate solubilizing fungal strains as an effective biofertilizers by the farmers.

Keywords: *Biofertilizer, solubilization, Aspergillus, Penicillium and Trichoderma*

1. Introduction

Soil microorganisms play a role in maintaining the ecological balance by active participation in carbon, nitrogen, sulphur and phosphorous cycles in nature.Phosphate solubilizing microbes play an important role in plant nutrition through increase in phosphate uptake by plants and used as biofertilizers for agricultural crops. Phosphate is one of the most vital macronutrient required for the growth and development of plants. A large number of microorganisms present in the rhizosphere are known to solubilize and make available the insoluble phosphorus in the available form to the plants. So, it is necessary to look out for an alternative renewable biological resource to improve soil condition on environment friendly agriculture through biological phosphate biofertilizer. Thus, with the specific objective of surveying the occurrence of phosphate solubilizing fungi isolated from the rhizosphere soil of different ginger growing sites of Churachandpur District.

2. Materials and Methods

2.1. Study Site

Churachandpur District is situated in the South western corner of Manipur and it covers an area of 4570 sq. km. It lies between 24.0^0N and 24.3^0North latitudes and between $93^{\cdot}15^0$E to 94.0^0East longitudes. The town has an altitude of 914.4 metres.The maximum temperature recorded is 41^0C and the minimum recorded is 0^0C. The maximum humidity is 89% and the minimum is 20%.

2.2. Collection of Samples

The rhizospheric soils were collected from different 21 ginger fields of Churachandpur District. The soil samples collected were air-dried in shade, gently crushed to powder in aceramic mortar using wooden mallet and sieved through a 2mm sieve to remove stones, roots, and large organic residues, passed through a 20 mesh sieve to obtain very fine particles which is then stored in clean polyethylene containers before conducting analysis for chemical and physical characteristics.

2.3. Isolation of the Microorganisms (fungi) and their Identification

10g of soil were taken in a 250 ml. conical flask containing sterile distilled water (100ml.). This stock solution was thoroughly hand shaken for about 10-15minutes. An initial 1:10 dilution was prepared and the subsequent dilutions was prepared by transferring 10ml. of this suspension into 90 ml. sterile distilled water until the desired final dilutions were obtained i.e. 1/10000 and 1/30000 dilution were prepared for isolation of fungi and bacteria, respectively. 1.0ml of inoculum from the above mentioned dilutions were transferred aseptically into petridishes in triplicate and to that appropriate agar medium was added. The dishes were rotated gently by hand, so that the inoculum spread uniformly. In each case separate pipettes were used for transferring the inoculum. The plates were incubated upside down at a temperature of 25+1°C for 5-7 days for fungi.

The most dominant species of fungi were isolated and the pure culture was maintained in culture tubes containing agar slants of Potato Dextrose Agar Medium. The fungal cultures were identified with the help of literature available (Gilman, 1956 and Barnett *et al*., 1972). The dominant fungi were selected for screening phosphate solubilizing activity using National Botanical Research Institute's phosphate growth medium, NBRIP (Nautiyal, 1999).

2.4. Composition of Culture Media

- Potato Dextrose Agar (for Fungi)

Peeled Potato: 300g; Dextrose: 20g; Agar: 15g and Distilled water: 1liter Adjust pH: 6.0 to 6.5

- National Botanical Research Institute's phosphate growth medium, NBRIP (Nautiyal, 1999):

Glucose: 10g; $Ca_3(PO_4)$:2: 5g; $(NH_4)2SO_4$: 0.1g; NaCl:0.2g; $Mg.SO_4.7H_2O$: 0.0025g; KCl :0.2g; Mn SO_4. H_2O: 0.002g; $FeSO_4.7H_2O$: 0.002g; BPB (Bromophenol blue): 0.25g. Adjust pH: 6.8 to 7.2

3. Results and Discussions

Screening of fungal isolates for phosphate solubilization revealed variations among different species of fungi. 31 fungal isolates were screened for phosphate solubilization. 15 fungi were found to be solubilized tri-calcium phosphate and rock phosphate in the solid state of the medium. The selected fungal strains were grown in cultural conditions and evaluated for both tri-calcium phosphate and rock phosphate solubilization. Fungi that showed halo zones around the colony are good phosphate solubilizers and belong mainly to the genera of *Aspergillus, Penicillium* and *Trichoderma.*

In the present investigation of isolating fungi from the rhizospheric soil from ginger fields of ChurachandpurDistrict,a vast microbial diversity has been recorded. A total of 31 species have been isolated and identified. The root system of higher plants influences the abundance and distribution of fungi. If not total, but specific fungal genera are stimulated with the plant species, plant age and soil types and environmental factors (Upadhyay and Rai, 1982). The qualitative and quantitative composition of the soil mycoflora depends upon the nature of soil and its chemical constituents (Kamal and Bhargava, 1973). The higher number of the species of the fungus can be attributed to its ability to grow in diverse conditions (Saikia *et al.,* 2004). Soil fungi make a very important part of the ecosystem along with other microbes in turnover of the biomass (James and Hyde, 1998). It is known that the species of *Aspergillus*, *Trichoderma*, *Penicillium*, *Fusarium*, *Mucor* etc. are the dominantly occurring fungi isolated from different rhizospheric soils and screened for phosphate solubilization by many workers (Asea *et al.,* 1988; Illmer and Schinner, 1992 and Vazquez *et al.,*2000).

In the present study, the occurrence of phosphate solubilizing fungi useful for both tricalcium phosphate and rock phosphate solubilization has been screened. Hence, an understanding of the distributions of phosphate solubilizing fungi is important for refining agricultural management practices and assessing their effects in agriculture for increasing crop productivity.

Sl. No.	Fungi	Tricalcium phosphate	Rock phosphate
1	*Aspergillus awamori* Nakaz	+	+
2	*Aspcergillus candidus* Link	–	+
3	*Aspergillus flavus* Link	+	+
4	*Aspergillus fumigatus* Fresenius	+	+
5	*Aspergillus melleus* Yukawa	–	–
6	*Aspergillus nidulans* (Eidam) Wint.	–	–
7	*Aspergillus niger* Van Tieghem	+	+
8	*Aspergillus ochraceous* Wilhelm	+	–
9	*Aspergillus oryzae* (Ahlburg) Cohn	+	+
10	*Aspergillus terreus* Thom	–	–
11	*Cladosporium herbarum* (Persoon) Link	–	–
12	*Curvularia geniculata* (Tracy and Earle) Boedijn	–	–
13	*Curvularia lunata* (Walker) Boedijn	+	–
14	*Fusarium oxysporium*	+	–
15	*Mucor hiemalis* Wehmer	+	+
16	*Penicillium chrysogenum* Thom	+	+
18	*Penicillium citrinum* Thom	–	–
19	*Penicillium expensum (*Link)Thom	–	+
20	*Penicillium frequentans* Westling	–	–
21	*Penicillium oxalicum*	+	+
22	*Penicillium rubrum* Stoll	+	+
23	*Rhizopus nigricans* Ehrenberg	–	–
24	*Humicola sp.*	–	–
25	*Trichoderma atroviride* Karsten	–	–
26	*Trichoderma citrinoviride* Bisset	+	+
27	*Trichoderma lignorum*	–	+
28	*Trichoderma glaucum* Abbott	–	–
29	*Trichoderma harzianum* Rifai	+	+
30	*Trichodermavirens (*Miller, Giddens and Foster*)* Von Arx	–	–
31	*Trichoderma viride* Pers.ex.S.F.Gray	+	+

Table 1: Screening of the phosphate solublizing properties by the isolated fungi using Tricalcium phosphate and rock phosphate Abbreviations: '+' = Halozone formation, '-'= no halozone.

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