PAPER TITLE: ISOLATION AND IDENTIFICATION OF PHOSPHATE SOLUBILIZING FUNGI FROM RHIZOSPHEREC SOIL OF CHICKPEA PLANT

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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. In this study total of 23 fungi were isolated from rhizospheric soil of chickpea plants. Out of 23, thirteen fungi were showed as phosphate solubilizers on the Pikovskaya's Agar medium. Fungi that showed halo zones around the colonies are good phosphate solubilizers and belong mainly to the genera of Aspergillus, Penicillium. The present study would help in utilizing the indigenous phosphate solubilizing fungal strains as an effective biofertilizers by the farmers.

Keywords: Aspergillus, Biofertilizer, Penicillium , Phosphate solubilizing fungi & Solubilization

I.INTRODUCTION

Phosphorus is one the most essential elements for plant growth after nitrogen. However, the availability of this nutrient for plants is limited by different chemical reactions especially in arid and semi-arid soils. Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, and transporting of the genetic traits.

Phosphate solubilizing microorganisms refer to a group of soil microorganisms that as components of phosphorus cycle, can release it from insoluble sources by different mechanisms [1]. Phosphate solubilizing fungi and bacteria are known as effective organisms in this process [2]. Fungi are the important components of soil microbes typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. Fungi have been reported to have greater ability to solubilize insoluble phosphate than bacteria [3]. A wide range of soil fungi are reported to solubilize insoluble phosphorous such as *Aspergillus niger* and *Penicillium sp.* which are the most common fungi capable of phosphate solubilization [4]. Exploration of phosphate solubilizing microorganisms has been conducted by many researchers from soils, mangrove and rhizosphere (5). Since large population of Chhattisgarh state is dependent on agriculture the present investigation is aimed to isolate some fungal strains that may have high efficiency for phosphate solubilization.

II.MATERIAL AND METHOD

The present investigation was carried out in the SoS Life Sciences, Pt. Ravishankar Shukla University, Raipur. Chhattisgarh.

2.1Study Site

Raipur is situated in East Central part of Chhattisgarh at latitude of $21^{0}16^{\circ}$ N, longitude $81^{0}36^{\circ}$ E and altitude 289.5 m above mean sea level. The climate of Raipur is falling under sub-humid with mean annual rainfall of about 1489 mm out of which 90 per cent (1348 mm) is received during monsoon (June to September). During *rabi*, (December to February) only 8 mm of rainfall is being received on an average. The maximum temperature ranged from 24.4 to 42.6 $^{\circ}$ C and minimum temperature ranged between 10.0 to 27.5 C respectively.

2.2Collection of soil samples

Soil samples were collected from rhizosphere of Chickpea plantation from 3 different villages of Raipur city of Chhattisgarh state. Samples were collected in polythene bags, transported to laboratory and stored in refrigerator for further processing. Soil samples were separated from roots, air dried at room temperature, crushed, sieved and collected in separate polythene bags. pH of the samples was recorded using pH meter (Elico made).

2.3Culture media for isolation

Pikovskaya's (6) agar medium (HIMEDIA) was used for the isolation and maintenance of phosphate solubilizing fungi. It contained (g litre⁻¹) Dextrose 10; Calcium phosphate 5; Ammonium sulphate 0.5; Potasium chloride 0.2; Magnesium sulphate 0.1; Manganease suplhate 0.0001; Yeast extract 0.5; Ferrous sulphate 0.0001, Agar 15. The pH of medium was 7.0 (\pm 0.2).

Potato dextrose agar (PDA, HIMEDIA) was used for the isolation maintenance of fungal cultures. It contained (g.litre⁻¹) potato infusion 200; Dextrose 20; Agar 15 and the pH of medium was 5.6 (\pm 0.2) The pH of culture media was adjusted using 1N NaOH or 1N HCl. Media were sterilized by autoclaving at 121°C for 15 min.

2.4Isolation

Suitable dilutions prepared in 0.85% saline were spread on plates containing Potato Dextrose Agar (PDA) medium. The plates were kept for incubation at 28±2°C for 5-7days. Fungal colonies were subcultured several times on PDA plates till the appearance of pure cultures. The isolates were stored in refrigerator on PDA slants for further studies.

2.5 Screening

The isolates were screened by inoculating on plates containing Pikovskaya's Agar (PKA) medium (6) amended with 0.5% tricalcium phosphate (TCP) as insoluble phosphate source and were incubated at 28±2°C for 5 days. Fungal colonies with clear halozone around them were screened as phosphate solubilizers.

2.6 Identification

The fungal cultures were identified on the basis of colony characteristics and microscopic examination (7, 8, and 9). Some of the fungal isolates have been sent and deposited to NFCCI for identification

III.RESULTS AND DISCUSSIONS

Total 23 fungi were isolated from rhizospheric soils of chickpea plantation and 13 were screened as phosphate solubilizers based on appearance of clear halozone on Pikovskaya's agar medium (**Table**). Fungi that showed halo zones around the colony are good phosphate solubilizers and belong mainly to the genera of *Aspergillus*, and *Penicillium. Aspergillus niger* was found to be the dominant group followed by Penicillium sp. and other species of Aspergillus. The similar results were highlighted by Mahamuni et al. (10) and Deepa et al. (11). The higher number of the species of the fungus can be attributed to its ability to grow in diverse conditions (12). Soil fungi make a very important part of the ecosystem along with other microbes in turnover of the biomass (13). 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 (14 and 15).

IV.PHOTOGRAPH OF HALOZONE FORMATION & ASPERGILLUS NIGER



V.TABLE

S. No	Village Name	Soil p ^H	Isolated Fungi name	Formation of
	(Raipur)			Halozone
01	Jora Village	7.70	Aspergillus sp.J1	Yes
			Aspergillus niger	Yes
			Aspergillus fumigatus	Yes
			Alternaria alternata	Yes
			Penicillium sp.J1	No
			Aspcergillus sp.J2	Yes
			Aspergillus sp. J3	Yes
			Rhizopus sp.	No
			Fusarium sp.	No
			Penicillium sp. J2	No
02	Tekari	6.8	Curvularia sp.	No
			Aspergillus sp.	Yes
			Fusarium sp	No
			Aspergillus niger	Yes
			Penicillium sp.	Yes
			Rhizopus sp.	No
03	Nakti	7.2	Aspergillus niger	Yes
			Aspergillus sp.	Yes
			Penicillium sp.	Yes
			Curvularia sp.	No
			Mucor sp.	No
			Rhizopus sp.	No
			Alternaria sp	Yes

Table: Screening of the phosphate solublizing properties by the isolated fungi on the basis of halo zone formation.

VI.CONCLUSION

It is concluded that phosphate solubilizing fungi were present in the rhizosphere of chickpea Further research should be continued with these efficient strains especially *Aspergillus niger Penicillium* sp. These isolates may be used as bioinoculant in agriculture for increasing crop productivity.

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REFERENCES

- (1). Salehrastin, N., 1999. *Biological Fertilizers. Scientific Journal of Soil and Water*. Soil and Water Research Institute of Iran, *Vol. 12. No. 3*.
- [2.] (2). Reyes, I., L. Brnir, R. Simard and H. Antoun, 1999. Characteristics of phosphate solubilization by an isolate of a tropical *Penicillium regulusum* and UV induced mutants. *FEMS Microbiology Ecology*, 23: 291-295.
- [3.] (3). Nahas E (1996) Factors determining rock phosphate solubilization by microorganisms isolated from soil. *World J Microbiol Biotech 12: 567-572.*
- [4.] (4). Whitelaw MA, Harden JT, Helyar RT(1999) Phosphate solubilization in solution culture by the soil fungus *Penicillum radicum*. Soil Biol Biochemi 31: 655-665.
- [5.] (5). Gupta M, Kiran S, Gulati A, Singh B, Tewari R (2012). Isolation and identification of phosphates olubilizing bacteria able to enhance the growth and aloin-A biosynthesis of Aloe barbadensis miller. *Microbiological Research 167: 358- 363.*
- [6.] (6). Pikovskaya, R.I. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiologiya*, 17: 362-370.
- [7.] (7). Ellis, M.B. (1971). Dematiceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.
- [8.] (8). Barnett, H.L., and Hunter, B.B. (1998).Illustrated Genera of Imperfect Fungi.4th ed., St. Paul Minnesota, APS Press.
- [9.] (9). Gilman, J.C. (2008). A manual of soil fungi. Biotech books, Delhi, India.
- [10.] (10). Mahamuni, S.V., Wani, P.V., and Patil, A.S. (2012). Isolation of phosphate solubilizing fungi from rhizosphere of sugarcane and sugar beet using Tcp and Rpsolubilization. *Asian Journal of Biochemical and Pharmaceutical Research*, 2: 237-244.
- [11.] (11). Deepa, V., Prasanna, A., Murthy, B.P., and Sridhar, R. (2010). Efficient phosphate solubilization by fungal strains isolated from rice-rhizosphere soils for the phosphorus release. *ResearchJournal of Agriculture and Biological Sciences*, 6: 487-492.

- [12.] (12). Saikia, R., Das K., Deka, S. and Azad P. (2004). Status and Prospects of Soil Microbial Diversity of Dibru- Saikhowa Biosphere Reserve. *Himalayan Biosphere Reserves* 6(1-2) 61-63.
- [13.] (13). James, E.B.G., and Hyde, K.D. (1998). Methods for the study of Mangrove Fungi, In: Mangrove Microbiology. Role of Microorganisms in Nutrient Cycling of Mangrove Soils and Waters.Ed by A.D. Agate, C.V. Subramanian, H. Vannuccie.UNDP 9-27.
- [14.] (14). Illmer, P. andSchinner, F. (1992). Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biol Biochem.*24: 89-395.
- [15.] (15). Vazquez, P., Holguin, G., Puente, M.E. Lopez Cortes, A. and Bashan, Y. (2000). Phosphate solubilizing Microorganisms Associated with the Rhizosphere of Mangroves in a Semi-arid coastal Lagoon. *Biol. Fertil. Soils (30): 460-468*.