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Mobilization of organic and poorly soluble phosphates by *Chaetomium globosum*

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Abstract

A phosphatase and phytase releasing fungus *Chaetomium globosum* was isolated and tested under green house conditions (wheat as test crop) and in the field (pearl millet as test crop) in a loamy sand soil. The population build up and efficiency were compared under both sterilized and non-sterilized soil conditions. The 68% organic phosphorus (Po) in the experimental soil was present as phytin; less than 1% of P was present in a plant available form. Exploitation of plant unavailable (poorly soluble) P was higher in sterilized soil mainly due to increased population of *C. globosum*. A gradual increase in microbial build up, between 7.5 and 16 times the inoculated population, occurred over a 4-week period. The test plants influenced acid phosphatase and phytase activity but resulted in no significant increase in alkaline phosphatase activity in the inoculated soil. The depletion of organic P was much higher than mineral P. The microbial contribution was significantly higher than the plant contribution to the hydrolysis of the different P fractions. The maximum effect of inoculation on different enzyme activities (acid phosphatase, alkaline phosphatase, phytase, dehydrogenase) was observed between 5 and 8 weeks of plant age. A significant improvement in plant biomass, root length, plant P concentration, seed and straw yield and seed P content resulted from inoculation. The results suggested that *C. globosum* produces phosphatases and phytases, which mobilize P and enhance the production of wheat and pearl millet crops.

Keywords: P mobilization; Loamy sand soil; *Chaetomium globosum*; Acid and alkaline phosphatase; Phytase; Wheat; Pearl millet; Yield

1. Introduction

The development of sustainable agricultural systems will require new techniques that help to minimize fertilizer application rates while maintaining adequate crop yields. The application of biological resources to exploit nutrients present in the soil may hold promise for the future (Jeffries and Barea, 1994).

Soil phosphorus exists predominantly in either an insoluble inorganic form or an organic form, both of which are not directly available to plants. The rate of phosphorus (P) mineralization depends on microbial activity (Tarafdar and Claassen, 1988) and on the activity of free phosphatase and phytase enzymes (Tarafdar et al., 2002), which is controlled by the solution P concentration (McGill and Cole, 1981).

The ability of soil microorganisms to solubilize various forms of insoluble P fractions is well documented (Richardson, 1994; Whitelaw et al., 1999). However, the potential of soil fungi to mediate the availability to plants of P from otherwise poorly available sources under field condition is less clear. The importance of soil microorganisms in increasing the available P of phytate and glycerophosphate to plant roots has been suggested by Tarafdar and Marschner (1995). They showed that the P nutrition of wheat grown in soil supplied with phytate was increased when

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the plants were co-inoculated with the mycorrhizal fungus *Glomus mosseae* and *Aspergillus fumigatus*, a fungus with known phytase activity (Wyss et al., 1999). Yadav and Tarafdar (2003) indicated that fungal isolates differed in their abilities to hydrolyze different organic P compounds.

An examination of the breakdown of unavailable P compounds suggested the importance of identifying potential phosphatase and phytase-releasing organisms, which can exploit the substantial amounts of less available soil P. In addition, little information is available on the partitioning of plant and microbial contributions to P hydrolysis. We therefore isolated a phosphatase and phytase releasing fungi, *Chaetomium globosum*, and examined the efficiency of its enzymes in releasing available phosphorus from unavailable P sources for plant nutrition under both green house and field experimental conditions.

2. Materials and methods

2.1. Isolation, identification and selection of fungi

Fungi were isolated from 27 diverse Indian soils, using a dilution plate technique (Tarafdar and Chhonkar, 1979) with Martin's Rose Bengal agar containing streptomycin sulphate (Allen, 1959). Twenty-one phosphatase and phytase-producing organisms were isolated, purified from the single spore in slants, identified by Agharkar Research Institute, Pune, India. The pure cultures were maintained on a potato dextrose agar (PDA) medium. Based on intra- and extracellular acid phosphatase, alkaline phosphatase and phytase activity the best fungus, *C. globosum*, was selected.

2.2. Intra- and extracellular enzyme activity

To determine enzyme activity, fungi were grown in 125 mL Czapek-Dox broth in 250 mL Erlenmeyer flasks. The medium was incubated with 8 mm discs of 4-day-old fungal growth (on PDA medium) and the flasks were incubated at 30 ± 1 °C for 14 days. Three flasks of each fungal culture were chilled in ice and the contents were filtered through Whatman no. 1 filter paper into another flask kept in ice. The final volume of each filtrate was made using sterilized cold distilled water. The filtrate was used to assay the extracellular acid phosphatase, alkaline phosphatase and phytase activity.

To determinate intracellular activity, fungal mats were ground with acid-washed quartz sand in a mortar. Ice-cold sterilized distilled water was added to obtain a fine suspension. The extract obtained was centrifuged at 12,000 rpm for 20 min. A clear supernatant containing the intracellular enzymes was obtained and made up to a known volume.

2.3. Efficiency of fungi

To test the efficiency of the fungi in the hydrolysis of different organic P compounds, 1 g of fungal mat, in triplicate, was crushed in acid washed quartz sand in a mortar with 30 mL of ice-cold sterilized water. The extract was centrifuged as described earlier. Five hundred milligram per litre as P of Na-glycerophosphate or phytin was added to the clear supernatant and incubated at 30 °C for 24 h. The release of P was estimated colorimetrically as described by Jackson (1967) and expressed as microgram P release per minute per gram of fungal mat.

2.4. Pot experiment

Pots, 30 cm high and 11 cm diameter, were used for this study. The soil used was a loamy sand (<30 cm depth) collected from five different soil pits at the Central Research Farm, CAZRI, Jodhpur, where the field experiment were conducted. The physico-chemical characteristics of the soil are presented in Table 1. The soil was grind and sieved through a 20-mesh (0.9 mm) sieve before use. The experiment was carried out using four treatments; sterilized soil with plants, sterilized soil

Table 1
Characteristics of the soil used in the study

Parameter	Characteristics[a]
pH (soil:water 1:2)	7.9 ± 0.05
EC (dS m^{-1})	0.2 ± 0.01
Organic matter (%)	0.2 ± 0.02
Sand (%)	85.1 ± 0.1
Silt (%)	5.5 ± 0.1
Clay (%)	7.9 ± 0.05
Total P (mg kg^{-1})	1260.9 ± 13.4
Mineral P (mg kg^{-1})	884.3 ± 8.5
Organic P (mg kg^{-1})	366.6 ± 5.4
Olsen's P (mg kg^{-1})	10.5 ± 1.1
Water soluble Pi (mg kg^{-1})	1.6 ± 0.1
Water soluble Po (mg kg^{-1})	4.4 ± 0.3
Phytin P (mg kg^{-1})	250.2 ± 7.6
Acid phosphatase activity (EU $\times 10^{-4}$)	0.08 ± 0.01
Alkaline phosphatase activity (EU $\times 10^{-4}$)	0.03 ± 0.01
Phytase activity (EU $\times 10^{-4}$)	1.52 ± 0.18
Dehydrogenase activity (pkat g^{-1})	1.11 ± 0.20
Fungi ($\times 10^4$)	12 ± 1.1
Bacteria ($\times 10^4$)	144 ± 8.8
Actinomycetes ($\times 10^4$)	108 ± 9.7

[a] Mean value, ± indicate the standard error of mean.