Bioprospection and genetic characterization of phytate-utilizing fungi community from Cerrado soil

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Introduction

Phosphorus is one of the major plant nutrients that is least available in the "cerrado" soil. This type of soil is frequently found in the Brazilian tropical region and it is characterized by low soil fertility and a quite diverse climatological condition, mainly related to extend and intensity of the dry and wet seasons. In most soil, phytic acid constitutes a majority of the organic P (Harrison, 1987) and it is considered to be available at low levels. To release phosphate, organics forms of soil P must first be mineralized by phosphatases enzymes. Several studies have shown that phytate appears to be only poorly utilized by plants (Hayes et al., 2000). However, a large range of microorganisms, including bacteria, yeasts, and filamentous fungi produce phytases which could increase the availability of phosphorus to the plants (Berka, 1998). Microbial phytase activity is frequently detected in fungi, particularly in *Aspergillus* sp. (Volfova et al 1994). Isolation and characterization of phytase producing microorganism is an important tool in studies of managing phosphate levels in soil. Considering this aspects, the aim of this work was to isolate and characterize fungi from brazilian cerrado soil with potential to degrade phytate. The strategy used was to screen in a specific media phytase producing fungi and classify them by comparing their rDNA region (NS) sequence to Genebank database.

Metodology

A total of 55 fungal strains isolated from "cerrado" area and storaged at the Microbiology and Biochemistry laboratory at Embrapa Maize and Sorghum were plated in PDA medium and checked for purity. Phytase producing fungi was screened by PSM phytase medium (Howson & Davis, 1983) using sodium phytate as a phosphorus source. The pH was adjusted to 5.0 before autoclavation at 120°C for 20 min. Each isolate was inoculated into three replicate in 30 mL PSM media and incubated for 17 days. Then the cultures were filtrated and phosphorus determined based on the phospho - molybdate method and measured photometrically at 400 nm (Parsons et al., 1984). The classification of the most effective P-phytate solubilizer isolate was based on the comparison of the 18S rDNA sequence using primers NS1 and NS4 (White et al. 1990) to the Genebank database.

Results and Discussion

Due to the low information about phytase producing microorganisms in "cerrado" soil and consequently their impact on the availability of phosphorus (Richardson, 2000), the objective of this work was to isolate and characterize fungi strains that mineralize phytate *in vitro* to increase the amount of information about this topic. Microbiol analysis had shown that there is high variability for mineralizated P content. The estimated frequency showed that 80% of the fungi analyzed had phytate-degrading activity. There was high variability with inorganic P values varied from 0 to 487 mg/L in filtered culture medium. The best performances were observed with the fungi strains phylogenetically identified as *Paecilomyces* sp. (487 mg/L), *Aspergillus* sp. (204 mg/L) and *Penicillium* sp. (198 mg/L Pi). Sequencing analysis of the 18 most efficient phytase producing fungi isolates has shown that eight had similarity with Penicillium, one Aspergillus, two

Eupenicillium, three Paecilomyces, one Trichoderma and three had no match with known sequences. *Paecilomyces* sp. is a cosmopolitan filamentous fungus which inhabits the soil, decomposing plants and food products. According to the literature, high microbial phytase activity is more frequently detected in fungi, particularly in *Aspergillus* and *Penicillium* species (Volfova et al 1994, Gargova et al 1997; Tseng et al 2000). These results show the possibility of success in the selection process of microorganisms for future use as inoculants to improve the acquisition of organic phosphorus by plants in "cerrado" soil.

Conclusions

The variability observed show the potential to a selection process of fungi for future use as inoculant to improve the acquisition of organic phosphorus by plants in "cerrado" soil. The fungi most efficient falling within *Paecilomyces*, *Aspergillus* and *Penicillium.*

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