INTRODUCTION: *Trichoderma harzianum* is a saprophytic fungus able to produce hydrolytic enzymes, such as chitinases, glucanases and phosphatases. The acid phosphatases hydrolyze phosphate esters, releasing soluble phosphorus, which is a limiting nutrient for microorganism’s growth. These enzymes act in several cellular events and have been applied in clinical diagnostic and remediation processes. Here we present the purification and biochemical characterization of an acid phosphatase produced by *T. harzianum*. MATERIAL AND METHODS: The crude proteic extract was obtained from culture of spores previously inoculated in minimum medium 1.5% glucose and 20-fold concentrated by ultrafiltration. The protein of 95 kDa was purified by molecular exclusion chromatography on a Superdex 200 column using FPLC facility (GE Healthcare) in 0.15 M sodium acetate pH 4.8. The identification and classification of the enzyme was carried out based on amino acid sequence of peptides, obtained from the protein hydrolyzed with trypsin, using MALDI-TOF/TOF spectrometry. Seven peptides were sequenced and compared to those sequences from GenBank. RESULTS AND DISCUSSION: The amino acid sequences were similar to phytase from *T. Harzianum*, histidine acid phytase from *Trichoderma pleuroticola* and hypothetical protein from *Trichoderma virens*. Additionally, a broad substrate specificity was observed for this enzyme, specifically for glucose 1-phosphate, phenylphosphate and phytic acid. The optimum pH and temperature were estimated in 3.8 and 65°C, respectively, after 15 min of reaction using the pNP-P substrate. The enzyme retained 50% of its activity after 60 min at 55°C and was partially and strongly inhibited by inorganic phosphate and sodium tungstate, respectively. CONCLUSIONS: Taken together, the sequence analysis of the seven peptides and biochemical features allow to conclude that the purified protein is a bifunctional enzyme belonging to the acid phosphatase and phytase families.

**Keywords:** *Trichoderma harzianum*, acid phosphatase, phytase, bifunctional enzymes.

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