Biochemical Characterization of a Conidial Alkaline Phosphatase from the Thermophilic Fungus *Humicola insolens*

Muys, B. R., Guimarães, L. H. S., Polizeli, M. L. T. M., Terenzi, H. F. and Jorge, J. A.

Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil.

Humicola insolens is one of the few thermophilic eukaryotic organisms, which show minimal temperature of growth above 20ºC whereas the maximal reaches up to 50ºC. Particularly, the optimal growth temperature of this thermophilic fungus is 46ºC. Thermophilic molds usually produce enzymes with very high thermal stability, recommended for industrial processes. Moreover, fungal conidia are resistance cells, adapted to inhospitable conditions. Phosphatases are largely employed in kits for several biological analyses, and may be classified as acid, neutral and alkaline according to their pH of action. The aim of this work was the biochemical characterization of a crude conidial alkaline phosphatase from *H. insolens*. Analyses in PAGE showed that *H. insolens* conidia produce a unique alkaline phosphatase. Optima of temperature and pH were 9.5 and 70ºC, respectively. Magnesium sulfate (2.0 mM) increased enzyme activity up to 3.5-fold. In contrast, inorganic phosphate (K_i= 0.47mM) and EDTA inhibited the enzyme. Curiously, zinc chloride inhibited phosphatase activity by 45% in Tris-HCl buffer, but increased it about 13-fold when glicine was the buffer. Further, in glicine buffer magnesium and zinc synergistically stimulated the enzyme. The crude enzyme was stable at 70ºC up to 1 hour and exhibited a half-life of 40 min at 80ºC. The apparent K_M and Vmax for p-nitrophenylphosphate were 0.43 mM and 218 U/mg protein, respectively. The thermal stability exhibited by the conidial *H. insolens* alkaline phosphatase suggests that this enzyme has potential for biotechnological applications.

Keywords: alkaline phosphatase, thermophilie, Humicola.
Supported by CNPq and FAPESP.