Production, Purification and Characterization of a Thermophilic Serine-Protease from *Myceliophtora sp.*

Zanphorlin, L.M.[1], Gomes, E.[1], Cabral, H.[2], Juliano, L.[3], Bonilla-Rodriguez, G.O.[1]

[1]IBILCE-UNESP, São José do Rio Preto, SP, [2]FCFRP-USP, Ribeirão Preto, SP, [3]UNIFESP, São Paulo, Brazil.

Proteases represent a class of enzymes with important roles in physiological processes and they are one of the three largest groups of industrial enzymes, accounting for about 60% of the total world-wide sale of enzymes. The inability of plant and animal proteases to meet the global demand for enzymes has led to an increasing interest by microbial proteases. Among these enzymes, significantly increased the interest for those from thermophilic microorganisms since thermal resistance has become a desired property for applications in biotechnology and industrial processes. Thus, the objective of this work was to produce, purify and characterize a protease from a thermophilic fungus *Myceliophtora sp.* The production was in solid state fermentation and in the time of 72 hours of fermentation the fungus *Myceliophtora sp* had the highest proteolytic output. The enzyme extract was purified by precipitation with ethanol followed by gel filtration and ion exchange chromatography. Biochemical and functional characterizations of the purified enzyme were performed with the fluorogenic substrate Abz-KLRFSKQ-EDDnp. The enzyme was significantly inhibited by PMSF indicating to be a serine protease. Optimum temperature and pH were 45ºC and 9.0, respectively. The presence of high ionic strength and magnesium ions increased catalytic activity, while some organic solvents and metal ions decreased it. Based on these results, we conclude that this enzyme display relevant features for biotechnological and industrial applications. Further studies will be carried out to obtain detailed information about the functional and structural properties of this enzyme.

Keys words: *fluorogenic substrate, serine-protease, thermophilic fungi.*
Supported by: CAPES (LMZ), FAPESP and CNPq (GOBR and EG).