PRODUCTION, PURIFICATION AND CHARACTERIZATION OF A KERATINOLYTIC PROTEASE BY *MYROTHECIUM VERRUCARIA.*

Moreira, F.G., Souza, C.G.M., Peralta, R.M.
Universidade Estadual de Maringá, Departamento de Bioquímica, 87020900, Maringá, PR, Brazil. E-mail: rmperalta@wnet.com.br

The proteolytic enzymes which are able to hydrolyze insoluble keratins are called keratinases (EC 3.4.9.11). The object of this study was to produce and purify an unusual keratinolytic protease by the plant pathogenic fungus, *Myrothecium verrucaria.* In submerged and solid state cultures using feather meal as substrate, maximal proteolytic activities (150 and 180 U/ml, respectively) were produced after 4 days of cultivation. The crude protease hydrolyzed keratinous substrates in the following order: poultry feather keratin>sheep wool keratin>human nail keratin>human hair keratin. This enzyme was purified to apparent electrophoretic homogeneity using a gel filtration column (Sephadex G-100) with a high yield of 62%. The enzyme was a monomeric protein with molecular masses of 22 and 23 kDa by SDS-PAGE and gel filtration, respectively. The enzyme was stable in a broad range of pH (5.0-12.0) and temperature up to 45^{o} C, being optimally active at 40^{o}C and pH 8.0-9.0. Protease activity was highly sensitive to PMSF indicating that the enzyme belonged to the serine protease family. The purified enzyme hydrolyzed poultry feather meal liberating free aminogroups quantified by the ninhydrin method. The efficiency of hydrolysis was improved when the keratin was previously treated with reducing agents. This study may help to understand the role of this type plant pathogenic fungus in the degradation of complex keratinous substrates in nature.

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