Study of Two Acidic Proteinases, Probably Involved in the Dimorphism and Pathogenicity of *Ustilago maydis*, Basidiomycete of the Corn Smut Disease

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Ustilago maydis is a dimorphic phythopathogenic fungus and the causal agent of the corn smut disease. In this work, the purification and biochemical characterization of the acid proteinases pumAe (extracellular) and pumAi (intracellular) of *U. maydis* were performed. Also, identity of the gene that encodes for pumAi (*PRAum*) was explored in the genome of the fungus. The proteases were purified and biochemically characterized. The molecular masses of pumAe and pumAi were 72 and 35.3 kDa respectively. The optimal pH of activity of proteinases was 4.0. The pumAe K_m value was of 3.5 μM and a V_{max} of 11430 μmol h^{-1} mg^{-1} when Suc-R-P-F-H-L-L-V-Y-MCA was used as substrate. The protease pumAi was inhibited by pepstatine A. Yeast-to-mycelium transition was inhibited by Pepstatine A in the culture medium. The hypothetical gene that encodes for protease pumAi (*PRAum* gene) was located in the *U. maydis* genome project and was amplified by PCR and cloned into TOPO-TA 2.1 plasmid and pNMT-1, a *Schizosaccharomyces pombe* expression vector. In the *U. maydis* genome one copy of the gene by Southern blot analyses was detected. In brief, the expression of this gene (*PRAum*), performed by RT-PCR assays, was regulated by the source of nitrogen. The heterologous expression experiments in *S. pombe* allowed a fast purification and confirmed that pumAi enzymatic activity was encoded by *PRAum* gene.

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