Molecular Analysis of Aminopeptidase PumAPE from *Ustilago maydis* Encoded by *APEum* Gene: Enzyme Purification and Differential Expression

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Heterobasidiomycete *Ustilago maydis* is a dimorphic phytopathogenic fungus, causal agent of corn smut, a widespread disease. Recently, proteolytic system of this fungus was described and an aminopeptidase activity, probably involved in pathogenicity, was detected. The aminopeptidase pumAPE was purified from the haploid phase of *U. maydis* FB1 strain. The purification procedure consisted of ammonium sulphate fractionation and three chromatographic steps, resulting in a 23% recovery. The molecular mass of the dimeric enzyme was estimated to be 110 kDa and 58 kDa by gel filtration chromatography and SDS-PAGE respectively. Enzymatic activity was optimal at pH 7.0 and 35 ºC toward Lys-*pNA* and the p*I* was determined to be 5.1. The enzyme was inhibited by EDTA-Na_2, 1,10-phenanthroline, bestantin, PMSF and several divalent cations (Cu^{2+}, Hg^{2+} and Zn^{2+}). The aminopeptidase exhibited a higher specificity for substrates with lysine and arginine in the N-position. The K_m value was 54.4 μM and the V_{max} value was 408 μmol min^{-1} mg^{-1} for Lys-*pNA*. A pair of primers was designed in order to amplify the gene *APEum* encoding this activity. In order to determine the number of copies in the genome, a *APEum* gene fragment was used as probe in a Southern blot. Only one copy of the gene by genome was detected. Also, differential expression of *APEum* was assessed under different physiological conditions. In brief, high expression levels were detected on media supplemented with corn infusion, proline, and ammonium.

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