E333 Cathepsin H, Cysteine Protease from *Flammulina velutipes*

Won-Young Kim, Hyun-Hee Shin and Hye-Seon Choi*
Department of Microbiology, University of Ulsan, Ulsan 680-749, Korea

Cathepsin H-like protease activity has decreased significantly during fruitbody formation of *Flammulina velutipes*. The specific activities were 73000 and 12586 Intensity/mg for mycelial culture and fruit body, respectively. The enzyme was purified through hydrophobic phenyl Sepharose, anion exchanger DEAE-Sephadex A-50, chromatofocusing, and gel filtration chromatographies. It was homogeneous electrophoretically. The M.W. of enzyme was determined to be 103,000 dalton by gel filtration and 50,000 dalton by SDS-PAGE, indicating that it is a dimer. The enzyme's pI was ~8.2 by chromatofocusing. This enzyme could provide arginine, a substrate for nitric oxide synthase which is putatively related to differentiation of *Flammulina velutipes*.

E334 Molecular cloning of ANPC which encodes a dibasic processing Endoprotease from *Aspergillus nidulans*

Kap-hoon Han, Bong-kyu Kwon, Kyu-Yong Han, Sung-Min Ju, Dong-Min Han and Won-Sin Kim
Division of Life Science, College of Natural Sciences, Wonkwang University, Iksan 570-749, Korea

We cloned and sequenced a gene from a genomic library of *Aspergillus nidulans* which encoded a 806-residue protein, named ANPC(*Aspergillus nidulans* protein convertase) that represented a member of kex2-like endoprotease involved in the processing of precursor protein. The ANPC contain the 301-residue sequence of the subtilisin-like catalitic domain(residues 132-433). Within this domain 53% of the amino acids are identical to those in yeast kex2p. The sequences around the proposed active site Asp, His, and Ser residues of ANPC are closely similar to those of other their family. The regions NH2-and COOH-terminal to the catalitic domains, previously refer to homo A and B domains, respectively, are moderately conserved between ANPC and other kex2 family members. Southern blot analysis indicated that the gene for ANPC was located on chromosome 2. These data suggest that ANPC represents a prime candidate for a precursor-processing endoprotease in the *Aspergillus nidulans*.