Cloning and Nucleotide Sequence of the Acid Protease-encoding Gene (pepA) from Aspergillus oryzae

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We have cloned a genomic DNA sequence encoding the acid protease (PEPA) from aspergillus oryzae using a 0.6-kb fragment as a probe. This fragment was amplified by the polymerase chain reaction (PCR) using oligonucleotide primers designed from the partial amino acid sequences of peptide fragments of the purified protein. Nucleotide sequencing analysis has shown that the cloned gene (designated pepA) encodes 404 amino acid residues and contains 3 putative introns ranging in length from 50 to 61 nucleotides. The deduced amino acid sequence of the A. oryzae PEPA has a high degree of homology (67%) to the A. awamori PEPA. Comparison with the amino acid sequence of A. awamori PEPA suggests that the A. oryzae PEPA may consist of a 78 amino acid prepro-peptide and 326 amino acid mature protein. The amino acid composition of the mature protein was almost consistent with that of the acid protease purified from A. oryzae reported previously. Southern hybridization analyses showed that the pepA gene exists as a single copy in the A. oryzae chromosome. The cloned gene was found to be functional, since transformants of A. oryzae containing multiple copies of the pepA gene showed a 2-6 fold increase in acid protease activity compared with the recipient strain.