Thesis Title Cloning and Characterization of the Alkaline Protease Gene *(alpA)* from *Aspergillus oryzae*

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Abstract

The gene *alpA* encoding *Aspergillus oryzae* alkaline protease (Alp) was isolated from a genomic library of an industrial strain *A. oryzae U212* (a high protease producer used in soy sauce production in Thailand) by using oligodeoxyribonucleotide probes based on the published cDNA sequence [Tatsumi *et al.*, Agric. Biol. Chem. 52(1988) 1887-1888]. The entire nucleotide sequence of the genomic clone obtained was determined. By comparison with the published cDNA sequence, it was found that Alp was encoded by four exons of 314, 445, 89, and 351 bp. Three introns, which interrupted the coding sequence, were 50, 59, and 56 bp in length. The gene contains a typical TATA box 103 base pairs upstream from the ATG start codon, and a consensus polyadenylation signal, AATAAA, 189 bp from the TAA stop codon.

A transformation system for *A. oryzae* was established. The plasmid pOBT conferring phleomycin resistance was used. Protoplasts were generated using Novozym 234 and regenerated on sucrose-stabilized minimal medium containing 200 µg ml^{-1} phleomycin. A frequency of 3 to 7 transformants per 10^6 viable protoplasts was obtained. All transformants tested showed increased resistance to phleomycin when subcultured onto selective medium and the mitotic stability of the transformants was 100% after growth without selective pressure.

The *alpA* gene, introduced into a protease deficient strain (*A. oryzae U1638*) by cotransformation, directed the secretion of enzymatically active Alp into the culture medium. Cotransformants of the high protease producing strain U212 contained multiple additional copies of the *alpA* gene and were able to secrete up to five times more protease than the wild type strain. Although no linear relation between copy number of the *alpA* gene and protease production could be shown for the transformants isolated in this study, it was clear that multiple copies in the genome did increase the amount of excreted enzyme substantially. This indicates a promising potential for expression and secretion of heterologous genes in substantial quantities.