

Thesis Title Expression of Recombinant Chitinase Genes from *Aeromonas hydrophila* and *Pseudomonas maltophilia* in *Escherichia coli*

Name Apinan Sirirattanathon

Degree Master of Science (Pharmacy)

Thesis Supervisory Committee

 Saichandana Subhanka , Ph. D.

 Chanpen Wiwat , Ph. D.

 Amaret Bhumiratana , Ph. D.

Date of Graduation 15 May B.E. 2540 (1997)

ABSTRACT

The chitinase genes from *Pseudomonas maltophilia* (pJP971) and *Aeromonas hydrophila* (pJP2514) were constructed in *Escherichia coli* to define the construction of genes that could effectively express the genes. Eight recombinant plasmids containing different fragments of chitinase gene from *P. maltophilia*, pPHK, pPHS, pPHKf, pPHKs, pPPK, pPPS, pPPKf and pPPKs were constructed but none of them possessed chitinolytic activity. That probably resulted from the frame-shifting incorporation. The correctness of the gene was also suspected to be a cause of the inability.

For chitinase encoding gene from *A. hydrophila*, 4 recombinant plasmids, pAES, pAEK, pACS and pACK, were constructed. All of them showed chitinolytic activity but in relatively low level. The highest activities in each clone were 40.19 mU/mg protein in pACS, 14.55 mU/mg protein in pACK, 31.75 mU/mg protein in pAES and 13.35 mU/mg protein in pAEK. Interestingly, pACS No.6 expressed

chitinase activity (40.19 mU/mg protein) higher than pACS No.2 and No. 5 (7.41 and 10.26 mU/mg protein respectively).

Under the non-selective condition the pKK223-3-based constructs, pAEK and pACK, were stable for more than 4 weeks. This time was longer than for the pBluescript SK/KS-based constructs, pAES and pACS, of which the stabilities were just 1-2 weeks.