Janwit Phuttikul 2012: Expression and Characterization of Keratinase from Recombinant *Escherichia* and *Pichia* Strains. Master of Science (Biotechnology), Major Field: Biotechnology, Department of Biotechnology. Thesis Advisor: Assistant Professor Suttipun Keawsompong, Ph.D. 108 pages.

Bacillus licheniformis KUB-K0006 is an effective source of keratinase. This enzyme can be used in feather meal preparation as protein source in feed. However, the yield of enzyme production was decreased because the large amount of mucus was produced during bacterial growth. Cloning and expression of keratinase gene from *B. licheniformis* KUB-K0006 were done to construct the recombinant strains that could produce and secrete high level of keratinolytic enzyme. To express the keratinase gene, the primer pairs were designed to amplify the gene fragment. The keratinase gene was ligated into pFLAG-CTS and pPICZα B expression vector and transformed into *Escherichia coli* Rosetta and TOP10 and *Pichia pastoris* Y11430, respectively. Keratinase was successfully expressed in the *E. coli* and *P. pastoris* expression system. The recombinant keratinase from recombinant *E. coli*, using SDS-PAGE, was 54 kDa protein. However, protease and keratinase activity were not detected. The recombinant *P. pastoris* could express 47 kDa protein in the culture medium. The protease activity of 10.46 u/ml was observed but keratinase activity using feather meal as substrate was not detected.

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