Kwankanit Intaratrakul 2008: Cloning and Expression of Keratinase Gene from *Bacillus licheniformis* KUB-K0006 in *Bacillus subtilis*. Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor : Assistant Professor Suttipun Keawsompong, Ph.D. 87 pages.

Keratinase enzyme from *Bacillus licheniformis* KUB-K0006 was applied to increase the digestibility of feather meal. However, the yield of enzyme production was decreased because large amount of mucus was produced during bacterial growth. Cloning and expression of keratinase gene from *B. licheniformis* KUB-K0006 were done to construct a recombinant cell that can produce high level of keratinolytic enzyme. To clone the keratinase gene, genomic library was constructed by partial digestion of the chromosomal DNA from *B. licheniformis* KUB-K0006 with 0.1 U/µl of *Bsp*143I at 37 °C, 30 minutes. The cut DNA sized 2 to 6 Kb was ligated with *Bam*HI digested pHT43. Transformation of recombinant DNA into *Escherichia coli* DH5α was done and 5.73 U/ml of the keratinase activity was detected from clone named e101. However, the cloned keratinase gene could not be analyzed because of its plasmid instability. The 1,140 bp keratinase gene was successfully cloned by PCR. This gene was predicted the protein structure and compared with the model of keratinase gene from *B. licheniformis* MKU3, *B. licheniformis* RPk and *B. licheniformis* PWD-1 by Homology modeling. Model construction and identification of active site were based on template 1cseE and 1bh6A, respectively. The results of homology modeling showed that the model of keratinase gene from *B. licheniformis* KUB-K0006 had the same structure with other models even there were differences of amino acid at Lue15, Arg249, Ala327 and Ala376. In addition, the amino acids in active site were also located in the same position; Asp137, His168 and Ser325, on the model. Based on the similarity of the model, the keratinase gene from *B. licheniformis* KUB-K0006 could be translated to mature protein and form the active keratinase same as reported *B. licheniformis* keratinase sequences in database. Expression of this keratinase gene was done in *B. subtilis* 1A751 using pHT43 as a vector. Keratinase gene was detected in all clones by PCR but keratinase activity was not detected in these clones.

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