Panisara Haemin 2008: Cloning and Expression of Mannanase Gene from *Bacillus amyloliquefaciens* NT 6.3 and *Bacillus circulans* NT 6.7. Master of Science (Biotechnology), Major Field: Biotechnology, Department of Biotechnology. Thesis Advisor: Assistant Professor Suttipun Keawsompong, Ph.D. 92 pages.

Mannanase can be applied in the production of Manno-oligosaccharides, which are prebiotics utilized selectivity by probiotics. Mannanase has been produced from various organisms, especially bacterial sources which are preferred when large amounts of enzyme are required. In this research, mannanase gene from *Bacillus amyloliquefaciens* NT 6.3 and *Bacillus circulans* NT 6.7 were cloned and expression by using *Escherichia coli* DH5α as a host cell. The result revealed that three recombinant cells had mannanase activity were found, named 6.3-379, 6.7-33 and 6.7-780, These recombinants had specific activity against locust bean gum 0.080, 0.132 and 0.304 unit/mg protein, respectively. Diameter of clear zone from recombinant cells 6.3-379, 6.7-33 and 6.7-780 were 1.3, 1.4 and 1.7 cm. All recombinant enzyme exerted high specific for glucomannan and galactomannan substrate. Zymogram of the recombinant enzyme and native enzyme showed active-band against locust bean gum in the same position. However, when recombinant cells were grown in the next generation to detect nucleotide sequences of mannanase gene by restriction analysis and polymerase chain reaction. The result indicated that DNA fragment of recombinant plasmid and chromosomal DNA of all recombinant cells were not detected. This might be caused by the unstability of plasmid pHT43.

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