Yotthachai Piwpankaew 2014: Cloning and Expression of Mannanase from *Bacillus circulans* NT 6.7. Doctor of Philosophy (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Suttipun Keawsompong, Ph.D. 106 pages.

The full-length mannanase gene of *B. circulans* NT 6.7 including its native signal sequence was cloned and expressed in *Escerichia coli and Lactobacillus plantarum* expression systems. The *B. circulans* NT 6.7 mannanase gene consisted of 1,083 nucleotides encoding 360 amino acid residue long polypeptide which belong to glycosyl hydrolase family 26. In *E. coli* system, the gene was cloned into pET21d and expressed in *E. coli* BL21* (DE3). The recombinant β-mannanase was successfully produced and also secreted. β-Mannanase activities in the culture supernatant and crude cell extract were 37.1 and 515 U/ml, respectively. The mannanase gene was cloned into pSIP403 and expressed in *Lactobacillus plantarum* WCFS1 Δ*alr*. β-Mannanase activity was detected in cell, 0.82 u/ml. Therefore this *E. coli* expression system was very efficient for the secretory production of recombinant β-mannanase from *B. circulans* NT 6.7. The optimum temperature of recombinant β-mannanase activity was 50°C and the optimum pH was 6.0 with high stability at this condition. The enzyme was very specific for β-mannan substrates with a preference for galactomannan. The recombinant β-mannanase showed the random manner with required at least 4 mannose monomers for degradation. It hydrolyzed mannan substrates consisted of locust bean gum, konjac glucomannan and defatted copra meal into various mannooligosaccharides including mannohexaose, mannopentaose, mannotetraose, mannotriose and mannobiose, while mannose could not be detected. The recombinant β-mannanase from *B. circulans* NT 6.7 showed the good enzyme characteristic including optimum temperature and pH with high specificity that can be used for several applications.

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