Nawapan Pongsapipatana 2014: Cloning, Expression and Characterization of Mannanase from *Klebsiella oxytoca* CW2-3. Master of Science (Biotechnology), Major Field: Biotechnology, Department of Biotechnology. Thesis Advisor: Associate Professor Sunee Nitisinprasert, D.Sc. 148 pages.

A gene encoding a mannanase was cloned from the genomic library of *Klebsiella oxytoca* CW2-3. A 3,314 bp DNA fragment containing a mannanase gene was sequenced. An open reading frame (ORF) of 1,164 bp encoded a protein of 387 amino acids named KMAN-2 which exhibited 50-71% identity to β-mannanase produced by other microbial sources. It belonged to glycosyl hydrolase family 26. The mannanase gene *kman-2* was subcloned into pFlag-CTS and overexpressed in *Escherichia coli* TOP10 resulting in an active recombinant clone *E. coli* KMAN-2. Its molecular weight was approximately 43.2 kDa on SDS-PAGE. The optimum temperature and pH of KMAN were 30-50 °C and 4-6, respectively. It was stable at low temperature and wide pH range of 4-10. The enzyme also has wide range of substrate specificities of konjac glucomannan (KGM) (100%), locust bean gum (LBG) (92.63%), CMC (30.56%), Ivory nut mannan (5.98%) Avicel (4.98%) and copra meal in both non-defatted (5.28%) and defatted (DCM) (7.02%). The hydrolysate of KGM, LBG and DCM analyzed by TLC were difference in molecular weight indicating an endo action of mannanase. Moreover, the hydrolysates of LBG and DCM enhanced the growth of lactic acid bacteria with specific growth rate of 0.36-0.83 h^{-1} while the one of KGM did not. Considering to their effects to pathogenic bacteria, it was found that all pathogenic bacteria could grow in the medium containing each three hydrolysates at the specific growth rate of 0.21-0.58 h^{-1} which were lower than specific growth rate of bacteria in glucose condition.

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