

Characterization of a Cellulose and xylan Degrading Bacterial Strain *Bacillus* sp. MGM7

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ABSTRACT

In 21st century, energy sources can be absent due to overuse of oil. Thus, Biological replacement should be developed. In all biological energy, biodiesel and bioethanol are two most expected bio-energy sources. Bioethanol is generated by microbial-fermented starch or cellulose and given glucose for ethanol production. The degradation of starch for bioethanol production mainly utilizes crops that can be used as food, therefore these processes will cause food shortening. In contrast, cellulose is the most abundant organic carbon source in the world; it is composed of polyglucose that linked by β -glycan bond and stored as crystal form. The structure of cellulose is extremely stable and is difficult to digest by normal animal; however, it can be degraded by microbes. In this thesis, *Bacillus* sp. MGM7 was screened from soil samples by cellulolytic activity. Zymogram analysis showed that the cellulose degrading activity protein was a 100~110 kDa protein. Sau3AI partial digestion of genomic DNA was proceeded after genomic DNA extraction and the fragments were ligated into phagemid vector pBCKS+. The ligates were then transformed into *E. coli* DH5 α , and positive clones were screened by cellulolytic activities.

Keywords : Biological energy、Bioethanol、Cellulose、Zymogram

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REFERENCES

1. Aita, G. A., Salvi, D. A., and Walker, M. S. 2011. Enzyme hydrolysis and ethanol fermentation of dilute ammonia pretreated energy cane. *Bioresour Technol* 102 (6):4444-4448.
2. Bajpai, P. 1997. Microbial xylanolytic enzyme system: properties and applications. *Advances in applied microbiology* 43:141-194.
3. Beguin, P., Millet, J., and Aubert, J. P. 1987. The cloned cel (cellulose degradation) genes of *Clostridium thermocellum* and their products. *Microbiological sciences* 4 (9):277-280.
4. Biely, P., Markovic, O., and Mislovicova, D. 1985. Sensitive detection of endo-1,4-beta-glucanases and endo-1,4-beta-xylanases in gels. *Analytical biochemistry* 144 (1):147-151.
5. Blanco, A., Diaz, P., Martinez, J., Vidal, T., Torres, A. L., and Pastor, F. I. 1998. Cloning of a new endoglucanase gene from *Bacillus* sp. BP-23 and characterisation of the enzyme. Performance in paper manufacture from cereal straw. *Appl Microbiol Biotechnol* 50 (1):48-54.
6. Chen, M., Tang, H., Ma, H., Holland, T. C., Ng, K. Y., and Salley, S. O. 2010. Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. *Bioresour Technol* 102 (2):1649-1655.
7. Chi, Z., Zheng, Y., Jiang, A., and Chen, S. 2011. Lipid Production by Culturing Oleaginous Yeast and Algae with Food Waste and Municipal Wastewater in an Integrated Process. *Appl Biochem Biotechnol*.
8. Chuang, S. E., Chen, A. L., and Chao, C. C. 1995. Growth of *E. coli* at low temperature dramatically increases the transformation frequency by electroporation. *Nucleic acids research* 23 (9):1641.
9. Jeihanipour,

A., and Taherzadeh, M. J. 2009. Ethanol production from cotton-based waste textiles. *Bioresour Technol* 100 (2):1007-1010. 10. Merad, T., Archibald, A. R., Hancock, I. C., Harwood, C. R., and Hobot, J. A. 1989. Cell wall assembly in *Bacillus subtilis*: visualization of old and new wall material by electron microscopic examination of samples stained selectively for teichoic acid and teichuronic acid. *Journal of general microbiology* 135 (3):645-655. 11. Prasetyo, J., Zhu, J., Kato, T., and Park, E. Y. 2011. Efficient production of cellulase in the culture of *Acremonium cellulolyticum* using untreated waste paper sludge. *Biotechnol Prog* 27 (1):104-110. 12. Selvendran, R. R. 1985. Developments in the chemistry and biochemistry of pectic and hemicellulosic polymers. *Journal of cell science* 2:51-88. 13. Shi, S., Valle-Rodriguez, J. O., Siewers, V., and Nielsen, J. 2011. Prospects for microbial biodiesel production. *Biotechnol J* 6 (3):277-285. 14. Subramaniyan, S., and Prema, P. 2002. Biotechnology of microbial xylanases: enzymology, molecular biology, and application. *Critical reviews in biotechnology* 22 (1):33-64. 15. Warnecke, F., Luginbuhl, P., Ivanova, N., Ghassemian, M., Richardson, T. H., Stege, J. T., Cayouette, M., McHardy, A. C., Djordjevic, G., Aboushadi, N., Sorek, R., Tringe, S. G., Podar, M., Martin, H. G., Kunin, V., Dalevi, D., Madejska, J., Kirton, E., Platt, D., Szeto, E., Salamov, A., Barry, K., Mikhailova, N., Kyrpides, N. C., Matson, E. G., Ottesen, E. A., Zhang, X., Hernandez, M., Murillo, C., Acosta, L. G., Rigoutsos, I., Tamayo, G., Green, B. D., Chang, C., Rubin, E. M., Mathur, E. J., Robertson, D. E., Hugenholtz, P., and Leadbetter, J. R. 2007. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature* 450 (7169):560-565. 16. Winterhalter, C., and Liebl, W. 1995. Two Extremely Thermostable Xylanases of the Hyperthermophilic Bacterium *Thermotoga maritima* MSB8. *Applied and environmental microbiology* 61 (5):1810-1815. 17. Wu, L., and Birch, R. G. 2007. Doubled sugar content in sugarcane plants modified to produce a sucrose isomer. *Plant Biotechnol J* 5 (1):109-117. 18. Zhang, J., Shao, X., and Lynd, L. R. 2009. Simultaneous saccharification and co-fermentation of paper sludge to ethanol by *Saccharomyces cerevisiae* RWB222. Part II: investigation of discrepancies between predicted and observed performance at high solids concentration. *Biotechnol Bioeng* 104 (5):932-938.