

# Characterization of a Novel Membrane Bound Xylanase from *Bacillus thermoamylovorans* MG73

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## ABSTRACT

In this thesis, a novel thermostable xylanase was isolated from a xylan-utilizing bacterial strain *Bacillus thermoamylovorans* MG73 and characterized. Unlike other bacillus strain that secrete the xylanase out to the living environment, the xylanase found in this thesis is a membrane-bound xylanase. In order to understand the approximate molecular weight of the xylanase, zymogram analysis was applied and the molecular weight of this novel xylanase was found to be around 60 Kda. To further characterize the novel xylanase, this thesis also tried to determine the influences of temperature, pH, thermostability and ions concentration on the enzyme activity. The optimal temperature of the enzyme is 100 ° C, where the optimal working pH is pH10. For enzyme stability, the enzyme activity can remain over 80% after two hours in 100 ° C. Ions are very important for the novel xylanase, especially Mn<sup>2+</sup> and Ni<sup>2+</sup>, however, higher ion concentrations will reduce the xylanase activity. In conclusion, this thesis revealed a novel membrane bound thermostable xylanase, as the first Gram positive membrane bound xylanase ever reported.

Keywords : xylanase, *Bacillus thermoamylovorans*, membrane bound, zymogram

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## REFERENCES

- Bailey, M.J., Biely, P. and Poutanen, K. 1992. Interlaboratory testing of methods for assay of xylanase activity. *Journal of Biotechnology*, 23, 257-270.
- Bajaj, B.K. and Abbass, M. 2011. Studies on an alkali-thermostable xylanase from *Aspergillus fumigatus* MA28. *Biotech*, 1, 161-171.
- Bajpai, P. 1997. Microbial xylanolytic enzyme system: properties and applications. *Adv Appl Microbiol*, 43, 141-94.
- Beg, Q.K., Kapoor, M., Mahajan, L. and Hoondal, G.S. 2001. Microbial xylanases and their industrial applications: a review. *Appl Microbiol Biotechnol*, 56, 326-38.
- Bhat, M.K. 2000. Cellulases and related enzymes in biotechnology. *Biotechnology Advances*, 18, 355-383.
- Biely, P., Markovic, O. and Mislovicova, D. 1985. Sensitive detection of endo-1,4-beta-glucanases and endo-1,4-beta-xylanases in gels. *Anal Biochem*, 144, 147-51.
- Biely, P., Vrsanska, M., Tenkanen, M. and Kluepfel, D. 1997. Endo-beta-1,4-xylanase families: differences in catalytic properties. *J Biotechnol*, 57, 151-66.
- Campenhout, L.V., Somers, I., Van de Craen, S. and Adams, C. 2003. In vitro test to evaluate protein degradation by feed enzymes. in: C.M. Courtin, W.S. Veraverbeke, J.A. Delcour (Eds.), *Recent Advances in Enzymes in Grain Processing*. Kat. Univ. Leuven, Leuven, pp. 387-390.
- Christakopoulos, P., Katapodis, P., Kalogeris, E., Kekos, D., Macris, B.J., Stamatis, H. and Skaltsa, H. 2003. Antimicrobial activity of acidic xylo-oligosaccharides produced by family 10 and 11 endoxylanases. *Int J Biol Macromol*, 31, 171-5.
- Collins, T., Gerday, C. and Feller, G. 2005. Xylanases, xylanase families and extremophilic xylanases. *FEMS Microbiol Rev*, 29, 3-23.
- Combet-Blanc, Y., Ollivier, B., Streicher, C., Patel, B.K., Dwivedi, P.P., Pot, B., Prensier, G. and Garcia, J.L. 1995. *Bacillus thermoamylovorans* sp. nov., a moderately thermophilic and amyolytic bacterium. *Int J Syst Bacteriol*, 45, 9-16.
- Dheeran, P., Nandhagopal, N., Kumar, S., Jaiswal, Y.K. and Adhikari, D.K. 2012. A novel thermostable xylanase of *Paenibacillus macerans* IIPSP3 isolated from the termite gut. *J Ind Microbiol Biotechnol*, 39, 851-60.
- Fawzi, E.M. 2011. Highly thermostable xylanase purified from *Rhizomucor miehei* NRL 3169. *Acta Biol Hung*, 62, 85-94.
- Ghaffar, A., Khan, S.A., Mukhtar, Z., Rajoka, M.I. and Latif, F. 2011. Heterologous expression of a gene for thermostable xylanase from *Chaetomium thermophilum* in *Pichia pastoris* GS115. *Mol Biol Rep*, 38, 3227-33.
- Gilkes, N.R., Claeysens, M., Aebersold, R., Henrissat, B., Meinke, A., Morrison, H.D., Kilburn, D.G., Warren, R.A., Miller, R.C. and Jr. 1991. Structural and functional relationships in two families of beta-1,4-glycanases. *Eur J Biochem*, 202, 367-77.
- Huber, R., Langworthy, T., Koenig, H., Thomm, M., Woese, C., Sleytr, U. and Stetter, K. 1986. *Thermotoga maritima* sp. nov. represents a new genus of unique extremely thermophilic eubacteria growing up to 90 ° C. *Archives of Microbiology*, 144, 324-333.
- Imanaka, T. and Sakurai, S. 1992. Method of washing super precision devices, semiconductors, with enzymes. Nikko Bio Technica Co., Ltd., United States patent 5,078,802.
- Kamal Kumar, B., Balakrishnan, H. and Rele, M.V. 2004. Compatibility of alkaline xylanases from an alkaliphilic *Bacillus* NCL (87-6-10) with commercial detergents and proteases. *J Ind Microbiol Biotechnol*, 31, 83-7.
- Katapodis, P., Vardakou, M., Kalogeris, E., Kekos, D., Macris, B.J. and Christakopoulos, P. 2003. Enzymic production of a feruloylated oligosaccharide with antioxidant activity from wheat flour arabinoxylan. *European Journal of Nutrition*, 42, 55-60.
- Liu, B., Zhang, N., Zhao, C., Lin, B., Xie, L. and Huang, Y. 2012. Characterization of a recombinant thermostable xylanase from hot spring thermophilic *Geobacillus* sp. TC-W7. *J Microbiol Biotechnol*, 22, 1388-94.
- Masui, D.C., Zimbardi, A.L., Souza, F.H., Guimaraes, L.H., Furriel, R.P. and Jorge, J.A. 2012. Production of a xylose-stimulated beta-glucosidase and a cellulase-free thermostable xylanase by the thermophilic fungus *Humicola brevis* var. *thermoidea* under solid state fermentation. *World J Microbiol Biotechnol*, 28, 2689-701.
- Mathlouthi, N., Lalles, J.P., Lepercq, P., Juste, C. and Larbier, M. 2002. Xylanase and beta-glucanase supplementation improve conjugated bile acid fraction in intestinal contents and increase villus size of small intestine wall in broiler chickens fed a rye-based diet. *J Anim Sci*, 80, 2773-9.
- Mielenz, J.R. 2001. Ethanol production from biomass: technology and commercialization status. *Current Opinion in Microbiology*, 4, 324-329.
- Okazaki, F., Nakashima, N., Ogino, C., Tamaru, Y. and Kondo, A. 2012. Biochemical characterization of a thermostable beta-1,3-xylanase from the hyperthermophilic eubacterium, *Thermotoga neapolitana* strain DSM 4359. *Appl Microbiol Biotechnol*.
- Pastor, F.I.J., Gallardo, O., Sanz-Aparicio, J. and Diaz, P. 2007. Xylanases: molecular properties and applications. in: J. Polaina, A.P. MacCabe (Eds.), *Industrial Enzymes*. Springer, pp. 65-82.
- Prakash, P., Jayalakshmi, S.K., Prakash, B., Rubul, M. and Sreeramulu, K. 2012. Production of alkaliphilic, halotolerant, thermostable cellulase free xylanase by *Bacillus halodurans* PPKS-2 using agro waste: single step purification and characterization. *World J Microbiol Biotechnol*, 28, 183-92.
- Saha, B.C. 2003. Hemicellulose bioconversion. *J Ind Microbiol Biotechnol*, 30, 279-91.
- Saleem, M., Aslam, F., Akhtar, M.S., Tariq, M. and Rajoka, M.I. 2012. Characterization of a thermostable and alkaline xylanase from *Bacillus* sp. and its bleaching impact on wheat straw pulp. *World J Microbiol Biotechnol*, 28, 513-22.
- Selvendran, R.R. 1985. Developments in the chemistry and biochemistry of pectic and hemicellulosic polymers. *J Cell Sci Suppl*, 2, 51-88.
- Shallom, D. and Shoham, Y. 2003. Microbial hemicellulases. *Curr Opin Microbiol*, 6, 219-28.
- Taibi, Z., Saoudi, B., Boudelaa, M., Trigui, H., Belghith, H., Gargouri, A. and Ladjama, A. 2012. Purification and biochemical characterization of a highly thermostable xylanase from *Actinomadura* sp. strain Cpt20 isolated from poultry compost. *Appl Biochem Biotechnol*, 166, 663-79.
- Viikari, L., Kantelinen, A., Sundquist, J. and Linko, M. 1994. Xylanases in bleaching: From an idea to the industry. *FEMS Microbiology Reviews*, 13, 335-350.
- Wang, Z., Jin, Y., Wu, H., Tian, Z., Wu, Y. and Xie, X. 2012. A novel, alkali-tolerant thermostable xylanase from *Saccharomonospora viridis*: direct gene

cloning, expression and enzyme characterization. *World J Microbiol Biotechnol*, 28, 2741-8. 34. Winterhalter, C., Heinrich, P., Candussio, A., Wich, G. and Liebl, W. 1995. Identification of a novel cellulose-binding domain within the multidomain 120 kDa xylanase XynA of the hyperthermophilic bacterium *Thermotoga maritima*. *Mol Microbiol*, 15, 431-44. 35. Winterhalter, C. and Liebl, W. 1995. Two extremely thermostable xylanases of the hyperthermophilic bacterium *Thermotoga maritima* MSB8, pp. 1810-5. 36. Wong, K.K., Tan, L.U. and Saddler, J.N. 1988. Multiplicity of beta-1,4-xylanase in microorganisms: functions and applications. *Microbiol Rev*, 52, 305-17. 37. Xin, F. and He, J. 2012. Characterization of a thermostable xylanase from a newly isolated *Kluyvera* species and its application for biobutanol production. *Bioresour Technol*. 38. Zhang, F., Chen, J.J., Ren, W.Z., Lin, L.B., Zhou, Y., Zhi, X.Y., Tang, S.K. and Li, W.J. 2012. Cloning, expression, and characterization of an alkaline thermostable GH11 xylanase from *Thermobifida halotolerans* YIM 90462T. *J Ind Microbiol Biotechnol*, 39, 1109-16. 39. Zhang, G., Mao, L., Zhao, Y., Xue, Y. and Ma, Y. 2010. Characterization of a thermostable xylanase from an alkaliphilic *Bacillus* sp. *Biotechnol Lett*, 32, 1915-20. 40. Zheng, H., Liu, Y., Liu, X., Wang, J., Han, Y. and Lu, F. 2012. Isolation, purification, and characterization of a thermostable xylanase from a novel strain, *Paenibacillus campinasensis* G1-1. *J Microbiol Biotechnol*, 22, 930-8. 41. Zhengqiang, J., Kobayashi, A., Ahsan, M.M., Lite, L., Kitaoka, M. and Hayashi, K. 2001. Characterization of a thermostable family 10 endo-xylanase (XynB) from *Thermotoga maritima* that cleaves p-nitrophenyl-beta-d-xyloside. *Journal of Bioscience and Bioengineering*, 92, 423-428. 42. Zhu, Y., Li, X., Sun, B., Song, H., Li, E. and Song, H. 2012. Properties of an Alkaline-tolerant, thermostable xylanase from *Streptomyces chartreusis* L1105, suitable for xylooligosaccharide production. *J Food Sci*, 77, C506-11.