Isolation of a thermally stable 24 kDa endo-1,4-beta-xylanase gene from an isolate *Leuconostock mesenteroides* BM825, its overexpression in *Bacillus subtilis* WB700 and biochemical characterization of the enzyme

Jong Mi Park[1], Jong Kook Rho[1], Mun Hwan Choi[2], and Sung Chul Yoon*,[1,2,3]
Biomaterials Science Laboratory, [1]Division of Applied Life Sciences (BK21), Graduate School [2]Environmental Biotechnology National Core Research Center; [3]Division of Life Science, College of Natural Sciences, Gyeongsang National University, Chinju 660-701, Korea
TEL : +82-55-751-5942, FAX : +82-55-753-0765

A thermo-tolerant extracellular xylanase was produced from *Leuconostock mesenteroides*[2)] BM825 strain that isolated from a sewage treatment plant in Chinju, Korea. The optimum growth temperature of the strain was 37℃ and its optimum growth pH was 7.0. The xylanase gene was identified from a genomic 1.2 kbp BamH I restriction fragment. Nucleotide sequence analysis of this fragment revealed an open reading frame (ORF) of 684 base pairs encoding a polypeptide of 228 amino acids. This ORF encoding endo-1,4-beta-xylanase exhibited a high homology (57% amino acid identity) with the corresponding proteins of *Bacillus subtilis* (P18429) and *Bacillus circulans* (P09850) endo-1,4-beta-xylanase. A strong promoter, BJ27[1)], was applied to overexpress the *Leuconostock mesenteroides* BM825 xylanase gene in *Bacillus subtilis*. The shuttle (*E. coli-Bacillus subtilis*) expression vector, pJH27-xyl, was designed to contain the open reading frame of the xylanase. The recombinant plasmid DNA pJH27-xyl was transformed into *Bacillus subtilis* WB700[3)]. The expressed xylanase was partially purified. Its optimum temperature for activity was 70℃ and optimum pH was between pH 6.0 and pH 7.0. The enzyme was fairly stable even at pH 4.0 and 10.0. The activity of the partially purified

enzyme was largely inhibited by acetonitrile and propanol, while phenylmethylsulfonyl fluoride (PMSF), dithiothreitol (DTT), toluene and methanol didn't affect its activity, where the concentration of the compounds for stability study was equally 30(wt/v)%.

Reference

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