Cloning and expression of xylose reductase from *Candida* sp. HY200

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Xylitol is a major product formed during anaerobic and oxygen limited fermentation of xylose by many natural xylose-fermenting yeasts[1)]. The use of xylose-utilizing yeasts is disadvantageous to produce a xylitol from xylose because a fraction of xylose, which is an expensive substrate, has to be further utilized for endogenous metabolism and cellular maintenance[2)]. Enzymatic conversion of xylose to xylitol by xylose reductase (XR) can be obtained a yield of 100 % without any by-product formation. Therefore, this study was carried out to be cloning a XR gene and characterize a XR enzyme in detail. We first cloned the XR gene from chromosomal DNA of *Candida* sp. HY200 by PCR with synthetic primers designed for simple purification. The PCR product was cloned into pMAL-c2X expression vector. The enzyme was easily purified to homogeneity by using a amylose resin affinity chromatography as a maltose binding protein(MBP), and thus resulted in a pure form of protein from crude cell extract in a single step. XR gene from *Candida* sp. HY200 was 975 bp. The molecular mass of the xylose reductase was estimated to be about 36 kDa on SDS-PAGE, and an identical result was also shown in gel filtration chromatography. Expressed protein by pMAL-XR was made to divide XR(about 35kDa) and MBP(about 45kDa). Specific activity (U/mg-prtotein) for pMAL-XR and pQE30-XR were 0.045 and 0.037, respectively. We obtained unsatisfactory results that were to form inclusion body and to bind linker protein incompletely. We supposed that target protein (XR) did not express because XR of *Candida* sp. HY200 was dissimilar in characters to gene of other eukaryotes.

References

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