Whole-cell biocatalyst for inulin degradation through cell-surface display of endoinulinase in *Saccharomyces cerevisiae*

Hyun-Chul Kim[1], Hyun-Jin Kim, and Soo-Wan Nam*

*Dept. of Biotechnology and Bioengineering, [1]Dept. of Biomaterial Control,
Dong-Eui University, Busan 614-714, Korea

E-mail : swnam@deu.ac.kr TEL : +82-51-890-2276 FAX : +82-52-890-1619

We constructed a yeast-based whole-cell biocatalyst displaying *Pseudomonas mucidolens* endoinulinase on the yeast cell-surface and endowed the yeast-cells with the ability to degrade inulin. The endoinulinase gene (*inuI*) from *P. mucidolens* was expressed on the cell surface of *Saccharomyces cerevisiae* by fusing with Aga2p linked to the membrane anchored protein, Aga1p. The *inuI* gene of *P. mucidolens* was subcloned into the surface display vector, pCTcon (*GAL1* promoter). The constructed plasmid, pCTENIU (8.5 kb) was introduced to *S. cerevisiae* EBY100 cell and then yeast transformants were selected on the synthetic defined media lacking uracil and on the inulin-containing media. The *inuI* gene under the control of *GAL1* promoter was successfully expressed in the yeast transformant. The surface display of endoinulinase was confirmed by immunofluorescence microscopy and its enzymatic ability to produce inulooligosaccharides (IOSs) from inulin. The total activity of the enzyme reached about 2.31 unit/ml by cultivation of yeast transformant on YPDG medium. To hydrolyze inulin efficiently, various reaction conditions such as substrate type, pH, temperature were examined. The optimized conditions determined for the hydrolysis of inulin were as follows; substrate type, Jerusalem artichokes; pH, 7; temperature, 50℃. Under the optimized condition, IOSs were started to be produced after 10 min of enzymatic reaction. The maximum yield of IOSs (71.2%) was achieved at 30 hr without any significant loss of initial enzyme activity. By the reaction with inulin, the IOSs consisting of inulobiose (F2), inulotriose (F3), inulotetraose (F4) and inulopentaose (F5) were produced and among them F4 was the major product.