Production of Inulin-Based Oligosaccharides by Surface-Displayed Yeast Biocatalyst

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In order to produce inulin-based oligosaccharides (IOSs) and cyclofructans (CFs), *Saccharomyces cerevisiae* cells displaying *Pseudomonas mucidolens* endoinulinase gene (*inu1*) or *Paenibacillus polymyxa* cyclo-inulooligosaccharide fructanotransferase (CFTase) gene (*cft*) on the yeast cell-surface were developed. After subcloning of *inu1* and *cft* into the surface display vector, pCTcon (*GAL1* promoter), the constructed plasmids, pCTENIU (8.5 kb) and pCTECFTN (9.1 kb), were 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 *inu1* and *cft* under the control of *GAL1* promoter were successfully expressed on the cell surface of *S. cerevisiae* EBY100 by fusing with Aga2p linked to the membrane anchored protein, Aga1p. The surface display of endoinulinase and CFTase were confirmed by immunofluorescence microscopy and by its enzymatic ability to produce IOSs and CFs from inulin. The culture conditions of surface-engineered yeast were optimized for the maximization of surface-displayed enzyme production. In addition, to enhance the productivity of inulin-based oligosaccharides (IOSs and CFs), various reaction conditions such as substrate type, pH, temperature were optimized.