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Production of Functional Biosubstances from Inulin by Cell-Surface Engineered Yeast

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Yeast-based whole-cell biocatalysts displaying *Pseudomonas mucidolens* endoinulinase gene (*inu1*) or *Paenibacillus polymyxa* cyclo-inulooligosaccharide fructanotransferase (CFTase) gene (*cft*) on the yeast cell-surface were developed to degrade inulin or to produce inulooligosaccharides (IOSs) or cyclofructans (CFs) from inulin. The *inu1* and *cft* were expressed on the cell surface of *Saccharomyces cerevisiae* by fusing with Aga2p linked to the membrane anchored protein, Aga1p. 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 in the yeast transformants. The surface display of endoinulinase and CFTase were confirmed by immunofluorescence microscopy and its enzymatic ability to produce IOSs and CFs from inulin. The culture conditions of surface-engineered yeast were optimized for the maximization of enzyme production on the cell surface. In addition, to produce functional biosubstances (IOSs and CFs) from inulin, various reaction conditions such as substrate type, pH, temperature were examined and would be reported.