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Cloning and Expression of Genes Encoding Fructosyltransferases from Higher Plants in Food Technology

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Recently, we have investigated enzymic production of non-digestible oligosaccharides which have several functional activities as “tertiary functional ingredients” of foods. Fructo-oligosaccharides synthesized from sucrose by *Eurotium repense* fructosyltransferase had the effect of no elevation of blood glucose or insulin concentrations in rats. Fructosylxyloside formed from sucrose and xylose with fructosyltransferase action of *Scopulariopsis brevicaulis* cells suppressed serum glucose and insulin responses and / or promoted the absorption of calcium and magnesium ions in rats administered with sucrose. On the other hand, we studied purification and characterization of several fructosyltransferases; sucrose: sucrose 1-fructosyltransferase (1-SST), fructan: fructan 1-fructosyltransferase (1-FFT) and a new enzyme, fructan: fructan 6^G-fructosyltransferase (6G-FFT) from asparagus roots. Previously, we reported that the asparagus 1-FFT synthesized new functional oligosaccharides elongated with one or two additional fructose units by fructosyltransfer from 1-kestose to 4^G-β-D-galactosylsucrose and the saccharides selectively stimulated growth for *Bifidobacteria*. In this study, we have tried the isolation and expression of cDNAs encoding 6G-FFT, 1-FFT and 1-SST from asparagus for industrial use. The cDNAs encoding 6G-FFT, 1-FFT and 1-SST were isolated from a cDNA library of asparagus leaves or roots. The isolated cDNAs were named AoFT 1, AoFT 2 and AoFT 3, respectively. The deduced amino acid sequences of these cDNAs showed a high homology with those of plant fructosyltransferases. Expression of these cDNAs was done using *Pichia pastoris*. The recombinant protein from *Pichia* transformed with AoFT 1 produced 1^F,6^G-di-β-D-fructofuranosylsucrose, neokestose and sucrose from 1-kestose,

while the transformant with an empty vector produced no saccharides. These results show that 6G-FFT is expressed in *P. pastoris*. In the same way, recombinant protein from *Pichia* transformed with AoFT 2 produced nystose from 1-kestose and AoFT 3 recombinant protein produced 1-kestose from sucrose. These results also show that 1-FFT and 1-SST are expressed in *P. pastoris*. Three recombinant proteins had enzymatic properties similar to those of 6G-FFT, 1-FFT and 1-SST from asparagus roots, respectively.

Key words: asparagus, fructo-oligosaccharide, fructosyltransferase, fructosyltransfer, *Pichia pastoris*

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