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Creation of a Novel Hydrolase by Site-directed Mutagenesis of Malto-oligosyltrehalose Synthase

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Malto-oligosyltrehalose synthase (EC 5.4.99.15, MTSase) catalyzes the conversion of α-1,4-glucan to glycosyltrehalose by forming an α,α-1,1-glucosidic linkage on the reducing side of the α-1,4-glucan. This enzyme also slightly hydrolyses the glucan and releases glucose from the reducing end of the glucan. We mutated the gene of MTSase from *Sulfolobus acidocaldarius* ATCC 33909 and expressed the mutated gene in *E. coli*. The mutants of Asp228, Glu255 or Asp443 corresponding to the catalytic residues of the α-amylase family enzymes showed no enzymatic activity. The transglycosylation activity of the mutants of Lys390 or Lys445 decreased, but the hydrolytic activity of the mutants increased in comparison to the wild-type enzyme. The substitution of Lys390 or Lys445 for a bulky residue, tryptophan, caused the loss of the transglycosylation activity of the enzyme, and provided a novel hydrolase reacting on the reducing side of the α-1,4-glucan.

Key words: trehalose, malto-oligosyltrehalose synthase, site-directed mutagenesis, transglycosylation, hydrolysis

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