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[ADVANCED](#)[TOP](#) > [Available Issues](#) > [Table of Contents](#) > [Abstract](#)

ONLINE ISSN : 1880-7291

PRINT ISSN : 1344-7882

Journal of Applied Glycoscience

Vol. 53 (2006) , No. 3 pp.199-203

[\[PDF \(2040K\)\]](#) [\[References\]](#)**Creation of a Novel Hydrolase by Site-directed Mutagenesis of Malto-oligosyltrehalose Synthase**Kazuhiko Maruta¹⁾, Michio Kubota¹⁾, Hiroshi Yamashita¹⁾, Tomoyuki Nishimoto¹⁾, Hiroto Chaen¹⁾ and Shigeharu Fukuda¹⁾

1) Glycoscience Institute, Research Center, Hayashibara Biochemical Laboratories, Inc.

(Received February 16, 2006)

(Accepted April 3, 2006)

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-glycosidic 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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To cite this article:

Kazuhiko Maruta, Michio Kubota, Hiroshi Yamashita, Tomoyuki Nishimoto, Hiroto Chaen and Shigeharu Fukuda: Creation of a Novel Hydrolase by Site-directed Mutagenesis of Malto-oligosyltrehalose Synthase . *J. Appl. Glycosci.*, **53**, 199-203 (2006) .

JOI JST.JSTAGE/jag/53.199

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