Cloning, expression and characterization of the maltooligosyl trehalose synthase from the archaeon *Sulfolobus tokodaii*

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Abstract: The maltooligosyl trehalose synthase gene from the hyperthermophilic archaeon *Sulfolobus tokodaii* strain 7 was cloned and the recombinant peotein was expressed in *E. coli*. The protein was purified to homogeneity by nickel column chromatography. The archaeal enzyme could catalyze an intramolecular transglycosylation reaction and convert the glycosidic bond at the reducing end of dextrins from α-1, 4 (reducing end) into α-1, 1 (non-reducing end). The most suitable temperature was 75°C and the optimal pH was 5. Substrate specificity investigation revealed that maltodextrin and maltooligosaccharide were used as substrates by the enzyme but maltose, chitooligosaccharide, sucrose and β-cyclodextrin weren't used.

Keywords: Maltooligosyl trehalose synthase, *Sulfolobus tokodaii*, trehalose, archaeon.

INTRODUCTION

Maltooligosyl trehalose synthase (MTSase) could process an intramolecular transglycosylation reaction to produce a non-reducing end by changing the α-1,4-glucosidic bond at the reducing end to α-1,1-glucosidic bond (Ahn *et al.*, 2004). It was reported that the hyperthermophilic archaeon *Sulfolobus solfataricus* could produce trehalose from starch (Lama *et al*., 1991). Subsequently, the trehalose forming enzymes, maltooligosyl trehalose synthase and maltooligosyl trehalose trehalohydrolase (MTHase) have been characterized from many species such as *S.acidocaldarius* and *S. shibatae*. Moreover, two genes encoding MTSase and MTHase respectively could be fused together and expressed in *E. coli* to produce a bifunctional fusion enzyme (Ju-Seok *et al*., 2008). On the other hand, it was reported that the trehalose synthase from *T. caldophilus* could be used for trehalose production from maltose (Koh *et al*., 1998). We recently reported the enzymatic properties of a trehalose-6-phosphate synthase from the thermoacidophilic archaeon *Thermoplasma acidophilum* (Gao *et al*., 2014).

In this paper, we cloned the gene encoding MTSase from the archaeon *Sulfolobus tokodaii* strain 7, expressed the recombinant protein and characterized the enzymatic properties.

MATERIALS AND METHODS

Chemicals, bacterial strains and plasmid

The *Escherichia coli* strains DH5α and BL21-codonPlus(DE3)-RIL and plasmid pET15b were from Novagen. The substrate maltopentaose was from Tokyo Chemical Industry co., Ltd. (Tokyo, Japan). Maltodextrin was purchased from Xiwang group (Binzhou, China). Ni Sepharose™ High Performance was from GE Healthcare. Unless stated, all other chemicals were analytical grade and obtained from Sangon Biotech (Shanghai, China).

Cloning, expression and purification the two MTSases

The nucleotide sequence of the gene ST0929 (GenBank: BAB65941) were synthesized by the Taihe Biotechnology Co., Ltd (Beijing, China). Two primers [sense (5'-ATTGAACTCGAGATGAAGTTACTTTCAACCTATAG-3')] and antisense (5'- GCGAGGATCCTATTTAAC AAGAATTAAAGGTAATTTATC-3'), underlined nucleotides indicated the XhoI and BamHI restriction enzyme sites respectively] were used to amplify the gene by PCR. The PCR product was subjected to double restriction enzyme digestion (*Xho*I and *BamH*I), and the product was cloned into vector pET-15b. The procedure of the expression and purification of the recombinant proteins was as reported (Gao *et al*., 2014). The purified enzymes were analyzed by SDS-PAGE.

Enzyme activity assay

The standard assay for the recombinant MTSase activity was performed at 75°C for 10 min in the reaction mixture (1 ml) containing 50mM citrate-phosphate (pH 5), 0.1% maltopentaose and an appropriate amount of enzyme. The reducing sugars released were examined by the dinitrisalicylic acid (DNS) method, with maltopentaose as standard. One unit activity of the enzyme which was the amount of enzyme required to convert 1 μmol of reducing sugars per minute was defined (Gueguen *et al*., 2001). All enzyme assays were performed at least in triplicate and the data were averaged. Maltopentaose was replaced by maltodextrin, maltose, oligochitosan, sucrose and β-cyclodextrin respectively under the standard enzyme

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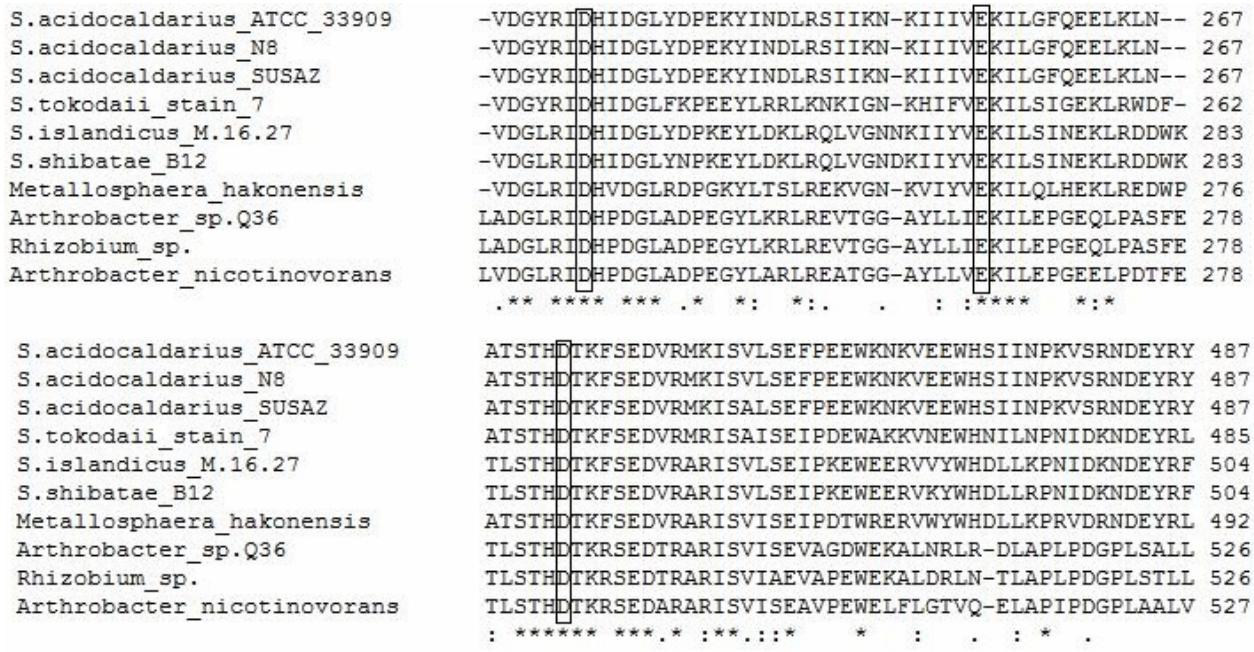

Fig. 1: Sequence alignment and comparison of the active site amino acids of the MTSase from *Sulfolobus tokodaii* strain 7 with its homologous proteins from other species. The names of species are listed on the left. Asterisks indicate identical residues among proteins, and single and double dots indicate residues with semiconservative and conservative characteristics. The three proposed catalytic amino acids Asp, Glu and Asp according to the crystal strcture of *S. tokodaii* MTSase are boxed.

assay conditions to study the substrate specificity of the enzyme.

Kinetic properties

Kinetic properties were studied using twelve different substrate concentrations (0.8-3mg/ml, 0.2mg/ml interval for maltodextrin) according to the standard enzyme assay except that the initial reaction mixture was incubated at 75°C for 4 min. Kinetic parameters were calculated with the Lineweaver-Burk plots.

RESULTS

Sequence analysis of the MTSase from Sulfolobus tokodaii

Sulfolobus tokodaii MTSase showed high sequence identity to its homologous proteins in the Genbank databse as revealed by blast search (NCBI), and some of these proteins have been functionally characterized. For example, Kobayashi *et al* revealed the crystal structure of MTSase from *sufolobus acidocaldarius* and proposed its probable catalysis mechanism (Kobayashi *et al*., 2003). Notably, three carboxyl groups of D228, E225 and D443 in *S. acidocaldaricus* MTSase were confirmed to be essential for its catalysis. These amino acids are conserved in the *S. tokodaii* MTSase as shown in the multiple sequence alignment analysis (fig. 1), suggesting the similar catalytic mechanism in the MTSases.

Expression and purification of the recombinant enzyme

The MTSase from hyperthermophilic archaeon *Sulfolobus tokodaii* was expressed and purified electrophoretically homogeneous from the cell extract of *sulfolobus tokodaii* strain 7. SDS-PAGE showed that the purified enzyme possessed a molecular mass of 83.6 kDa (data not shown). These results showed that the protein was expressed in *E. coli* and purified to homogeneity successfully.

Enzymatic properties

The MTSase from *Sulfolobus tokodaii* could utilize maltodextrin and maltopentaose as substrate. However, no activity of the enzyme was found towards maltose, chitooligosaccharide, sucrose and β-cyclodextrin. The catalytic efficiency towards two different substrates maltopentaose and maltodextrin were shown in table 1 and the *Km* and *Vmax* values from the Lineweaver-Burk plots method were 2.96 mg/ml and 0.38 mg $ml^{-1}min^{-1}$ respectively.

The MTSase from *Sulfolobus tokodaii* was optimal active at 75°C and pH 5, respectively. Moreover, the activity of the purified enzyme was almostly unchanged after incubation at 75°C and 85°C for 10 hours. Even if the enzyme was incubated at 95°C for 8h, more than 60% of the original activity was retained. These results suggested that the recombinant enzyme was well folded and it was an extremely thermostable protein. Furthermore, the activity of the purified enzyme was almostly unchanged

when incubated up to 10h at pH values 10 and 11. In addition, we found that residual activity of the enzyme was beyond 65% and 90% under pH 3 and 4 for 10 hours, respectively. These results demonstrated that the enzyme could be resistant to high basic and acid conditions.

DISCUSSION

In this study, we cloned, expressed and characterized the MTSase from the archaeon *sulfolous tokodaii* strain 7. The archaeal enzyme showed notable thermo stability and acid/basic stability. Considering the biotechnological advantages of extremozymes such as improvement of reaction rate, contamination avoidance, the enzyme could be exploited in biotechnological applications to produce maltooligosyltrehalose from starch at high temperatures.

Table 1: Comparison of the MTSase activity towards maltopentaose and maltodextrin

Substrate	Enzyme activity (U/ml)
Maltopentaose	0.56±0.01
Maltodextrin	0.51±0.04

MTSase is a key enzyme involved in trehalose synthesis (Tetsuya *et al*., 1995). Some key catalytic residues have been revealed based on the solved structure of its two homologous enzymes (Tseng *et al*., 2014). The detailed enzymatic properties reported in this study from the hyperthermophilic archaeon *Sulfolobus tokodaii* strain 7 could be a meaningful basis to study the structure and function relationship of this thermophilic trehalose forming enzyme.

CONCLUSION

The maltooligosyl trehalose synthase (MTSase) was successfully expressed in *E. coli*, and the enzymatic properties of the recombinant protein were characterized. The optimal temperature and pH of the enzyme were 75°C and 5, respectively. In addition, the enzyme could efficiently catalyze maltopentaose and maltodextrin, suggesting its potential application in the fields of biotechnology.

ACKNOWLEDGEMENTS

We acknowledge the experimental support for this study from Dr. Liu Yang in the Department of Food Science and Engineering, Qilu University of Technology.

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