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ORIGINAL PAPER

# Overexpression and characterization of a thermostable trehalose synthase from *Meiothermus ruber*

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**Abstract** A thermostable trehalose synthase (TreS) gene from *Meiothermus ruber* CBS-01 was cloned and overexpressed in *Escherichia coli*. The purified recombinant TreS could utilize maltose to produce trehalose, and showed an optimum pH and temperature of 6.5 and 50°C, respectively. Kinetic analysis showed that the enzyme had a twofold higher catalytic efficiency ($k_{cat}/K_m$) for maltose than for trehalose, indicating maltose as the preferred substrate. The TreS also had a weak hydrolytic property with glucose as the byproduct, and glucose was a strong competitive inhibitor of the enzyme. The maximum production of trehalose by the enzyme reached 65% at 20°C. The most importantly the enzyme could maintain very high activity (above 90%) at pH 4.0–8.0 and 60°C 5 h. These results provided that the stable TreS was suitable for the industrial production of trehalose from maltose in a one-step reaction.

**Keywords** Trehalose · Trehalose synthase (TreS) · *Meiothermus ruber* · *Escherichia coli*

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## Introduction

Trehalose is a non-reducing disaccharide that has two glucose units linked by a α,α-1,1-glycosidic linkage. This disaccharide is widespread throughout bacteria, archaea, yeast, fungi, insects and a number of invertebrates (Elbein et al. 2003) and plays very important roles, such as a carbon and energy reserve (Thevelein 1984), a compatible solute under stress conditions (Giæver et al. 1988; Reinders et al. 1997; Silva et al. 2003) and a structural component of the cell wall (Lederer 1976). Trehalose can protect biological macromolecules from the adverse effects of stresses, such as heat, cold, desiccation and oxygen radicals (Crowe et al. 1984). Therefore, the disaccharide has been widely used as a protectant of enzymes, proteins, biomasses, pharmaceutical preparations and blood cells during cryopreservation and desiccation (Satpathya et al. 2004; Schiraldi et al. 2002).

There are five pathways for trehalose biosynthesis: (1) TPS/TPP pathway; (2) TreY/TreZ pathway; (3) TreS pathway; (4) TreT pathway; (5) TreP pathway (Avonce et al. 2006; Kouril et al. 2008). Trehalose synthase catalyzes the reversible interconversion of trehalose and maltose, and the enzyme is suitable for trehalose industrial production, because of its simple one-step reaction and using low-cost raw material. Hence, various TreS genes have been isolated and cloned from different bacterial strains, and the enzymes have been purified and/or expressed heterologously. The gene cloning of TreS from *Pimelobacter* sp. R48 (Tsusaki et al. 1996) and *Thermus aquaticus* ATCC33923 (Tsusaki et al. 1997) was first reported. Then, TreS genes of *Pseudomonas stutzeri* CJ38 (Lee et al. 2005), *Picrophilus torridus* (Chen et al. 2006) and *Thermobifida fusca* (Wei et al. 2004) were cloned and expressed in *E. coli*, and proved to be suitable for the

production of trehalose. Nowadays, the relationship between structure and function of TreS has also been well studied (Koh et al. 2003; Pan et al. 2004), so that the activity and stability of the enzyme could be increased. Moreover, the latest research reported the TreS from *Mycobacterium smegmatis* could convert glycogen to trehalose with maltose as an intermediate (Pan et al. 2008).

In our previous research, a thermophilic *Meiothermus ruber* strain CBS-01 producing TreS was isolated from geothermal water and the TreS gene was obtained (Zhu et al. 2008). Here, the properties of the TreS were characterized in detail. From the results, we are sure that the stable enzyme is suitable for industrial production of trehalose.

## Materials and methods

### Bacterial strains, plasmids and media

*Meiothermus ruber* strain CBS-01 was grown at 50°C for 24 h in the Thermus medium containing 1 g of tryptone, 1 g of yeast extract, 100 mg of nitrilotriacetic acid, 60 mg of $CaSO_4 \cdot 2H_2O$, 100 mg of $Mg_2SO_4 \cdot 7H_2O$, 8 mg of NaCl, 103 mg of $KNO_3$, 689 mg of $NaNO_3$, 140 mg of $Na_2HPO_4 \cdot 2H_2O$, 0.47 mg of $FeCl_3 \cdot 6H_2O$, 2.2 mg of $MnSO_4 \cdot H_2O$, 0.5 mg of $ZnSO_4 \cdot 7H_2O$, 0.5 mg of $H_3BO_3$, 25 μg of $CuSO_4 \cdot 5H_2O$, 25 μg of $Na_2MoO_4 \cdot 2H_2O$, 46 μg of $CoCl_2 \cdot 6H_2O$ per liter, pH 7.8.

The expression vector pET-21a (+) (Novagen, Madison, WI) was used and the *E. coli* strains DH5α and Rosetta gami (DE3) (Novagen, Madison, WI) were used for cloning and expression, respectively.

*E. coli* strains were cultured in LB broth and on LB agar supplemented with 100 μg/ml ampicillin for cloning host or with 200 μg/ml ampicillin, 15 μg/ml kanamycin and 34 μg/ml chloramphenicol (LB-AKC medium) for expression host.

### Plasmid constructions and molecular modification

DNA sequence encoding the TreS gene was obtained in our previous works and submitted to Genbank (accession no. EU443098). The genomic DNA of *Meiothermus ruber* strain CBS-01 was purified with bacteria genome DNA extracting kit (Tiangen Biotech, Beijing, China) and used as a template for PCR amplification with the forward primer pTreSF (5′-GGAATTCCAT**ATG**GGTGTGGATC CTCTTTGG-3′, *Nde*I cleavage site underlined, start codon in bold face) and the reverse primer pTreSR (5′-CGGAA TTCGCGGGCCCGTTCCTTCCAC-3′, *Eco*RI cleavage site underlined). The stop codon of TreS was eliminated to in-frame read a His-tag on the C-terminus of the protein for one-step purification.

To enhance the expression amount of the heterologous protein, the nucleotides at +6 to +12 and +51 to +58 of the TreS gene were modified according to the *E. coli* codon usage database without changing the amino acid sequence, so that they could not reversely complement with the SD sequence in the vector. The modified TreS gene was amplified with a long forward primer pMtreSF (5′- GGA ATTCCAT**ATG**GA*CCCGCTG*TGGTACAAGGACGCA GTCATCTACCAGCTCCACGTCCG*TAGCTTTT*ACGAT GC-3′, *Nde*I cleavage site underlined, start codon in bold face, the modified nucleotides in italics face) and the reverse primer pTreSR.

The amplified fragments were digested with *Nde*I and *Eco*RI, and then ligated into pET-21a (+), which had been treated with the same restriction enzymes. The constructed plasmids were named pET-21TreS and pET-21TreSM, and used to transform *E. coli* Rosetta gami (DE3). General molecular techniques were performed as described by (Sambrook et al. 1989).

### Expression and purification of the recombinant TreS

The strains harboring the plasmids were cultivated overnight in LB-AKC medium and refreshed in a ratio of 1:100 with the same medium. When the absorbance at 600 nm of the culture reached 0.6, isopropyl β-D-1-thiogalactopyranoside (IPTG, final concentration 0.5 mmol/l) was added for induction, and the cultivation was continued for additional 12 h at 20°C. The culture broth was centrifuged at 5000×*g* for 10 min at 4°C and the supernatant decanted. The resulting cell pellet was suspended in 10 mmol/l potassium phosphate buffer (pH 7.0) and cells were disrupted using a sonicator (dr. hielscher UP 200s) set to a power of 90% for ten times of 20 s bursts, with 20 s intermissions. After centrifugation at 10000×*g* for 10 min and filtration through 0.22 μm filter, the crude enzyme was collected and then purified using an NTA nickel-ion column according to the following procedures: the crude extract was loaded onto the NTA–Ni column equilibrated with 20 mmol/l sodium phosphate buffer (pH 7.4) containing 0.5 mol/l NaCl and 5 mmol/l imidazole on a fast protein liquid chromatography system (ÄKTAprimer, Amersham, GE). After the column was washed, the TreS was eluted with a linear gradient of 5–500 mmol/l imidazole in the same buffer. Active fractions were pooled, concentrated and desalted using Amicon Ultra-4 centrifugal filter (Millipore, USA). The purified enzymes were analyzed on 10% SDS–PAGE.

### Enzyme characterization

The activity of TreS was assayed by measuring the amount of trehalose produced from maltose. The standard reaction was performed by adding 5 μg of purified enzyme into 500 μl of reaction solution containing 10 mmol/l potassium phosphate (pH 6.5) and 60 mmol/l maltose and incubating at 50°C for 1 h. The reaction was terminated by heating the mixture in boiling water for 10 min. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the formation of 1 μmol of trehalose per minute.

Kinetic analysis was performed under conditions of pH 6.5 and 50°C for 30 min in 10 mmol/l potassium phosphate buffer containing substrate (maltose, trehalose or maltose plus constant 10 mmol/l glucose) at various concentrations. The values of $K_m$, $V_{max}$ and $k_{cat}$ were obtained by using Lineweaver–Burk plot. The value of $K_i$ for glucose was determined by Dixon plot and Lineweaver–Burk plot.

The effects of temperature on the enzyme activity of TreS were determined at various temperatures ranging from 4 to 90°C. To determine the stability against thermal denaturation, the enzymes were incubated at various temperatures for 1 h and the residual activities were examined.

The effects of pH on the enzyme activity of TreS were determined at various pH ranging from 2.5 to 10.0 using 10 mmol/l glycine–HCl buffer (pH 2.0–3.5), acetate buffer (pH 3.5–5.5), sodium phosphate buffer (pH 6.0–8.5) and glycine–NaOH buffer (pH 9.0–10.0). To examine the pH stability of TreS, the enzyme was incubated at various pH values and 50°C for 1 h, and the residual activity was examined at pH 6.5.

The effects of temperature on glucose formation catalyzed by TreS were performed by incubating the enzyme reactions for 24 h at various temperatures ranging from 20 to 50°C in an assay buffer containing 60 mmol/l maltose.

The effects of metal ions on the activity of TreS were determined in an assay buffer containing 2 and 10 mmol/l metal ions under the standard reaction conditions.

All experiments used to determine the properties of the recombinant TreS were carried out at least in duplicate.

### Expression, purification and activity examination of the truncated TreS protein

To examine the effect of the C-terminal domain on the TreS activities, a truncated TreS gene containing 1602 nucleotide residues was amplified with the forward primer pMtreSF and the reverse primer pTreS1.6R (5′-CGGAA TTCAGGGCGAAAAGGGTGAAG-3′, *Eco*RI cleavage site underlined). The construction of the plasmid, expression and purification of the TreSΔC protein were the same as those of TreS. The activity of the TreSΔC protein was examined with maltose and trehalose as substrates, respectively. The reactions were performed at 20, 30, 40 and 50°C.

### Carbohydrates analysis

The quantity of sugars after each enzymatic reaction was measured by a high-performance liquid chromatography (HPLC) system equipped with a differential refractometer detector. The conditions of HPLC were as follows: Ø4.6×250 mm Hypersil-NH2 column; acetonitrile–water eluent (80:20, *v/v*); 1.0 ml/min of flow velocity; 30°C column temperature.

## Results

### Cloning, overexpression and purification of recombinant enzymes

*Escherichia coli* Rosetta gami bearing the vector pET-21aTreS was induced, but the recombinant protein was rarely expressed by detecting with SDS–PAGE. Because the nucleotides at +6 to +12 (5′-TCCTCTT-3′) and +51 to +58 (5′-CTCCTTCT-3′) of TreS gene could reversely complement with the SD sequence (5′-AGAAGGAG-3′) in the pET-21a partially or completely, the translation of the *treS* mRNA could not be initiated effectively. Therefore, these nucleotides were modified and another expression vector pET-21aTreSM was constructed. After induced by IPTG, the TreS was expressed abundantly and showed a clear protein band with SDS–PAGE.

The recombinant TreS was purified by NTA–Ni column; most of the activity was eluted in 100 mmol/l imidazole. SDS–PAGE analysis of the purified enzymes showed a single protein band around 110 kDa (not shown).

### Kinetics analysis of TreS

The kinetics analysis of TreS was showed in Table 1. The $K_m$ value for maltose was close to that for trehalose, which indicated that this enzyme had the same affinity for maltose and trehalose. However, TreS had twofold higher enzyme efficiency ($k_{cat}/K_m$) toward maltose than trehalose, which indicated the trend of reaction equilibrium was producing trehalose. Moreover, it was found that the addition of glucose into the reaction mixture would retard trehalose formation. In the presence of 10 mmol/l glucose, TreS showed a 3.6-fold increase in $K_m$ and a nearly unchanged $V_{max}$ for maltose, implying that glucose is a competitive inhibitor of TreS; and the $K_i$ value of glucose was 3.76 mmol/l, indicating the inhibition of glucose to the enzyme was strong.

**Table 1** Kinetic parameters of TreS

| Substrate | $K_m$ (mmol/l) | $V_{max}$ (mmol/min/mg) | $k_{cat}$ ($s^{-1}$) | $k_{cat}/K_m$ (mol/l/s) |
|---|---|---|---|---|
| Maltose | 126.2 ± 2.6 | 0.808 ± 0.02 | 147.0 ± 1.5 | 1,164.5 ± 12.0 |
| Trehalose | 98.9 ± 1.8 | 0.379 ± 0.01 | 68.9 ± 3.0 | 696.6 ± 7.8 |
| Maltose + glucose | 461.9 ± 5.2 | 0.838 ± 0.03 | 152.4 ± 2.0 | 330.1 ± 6.4 |

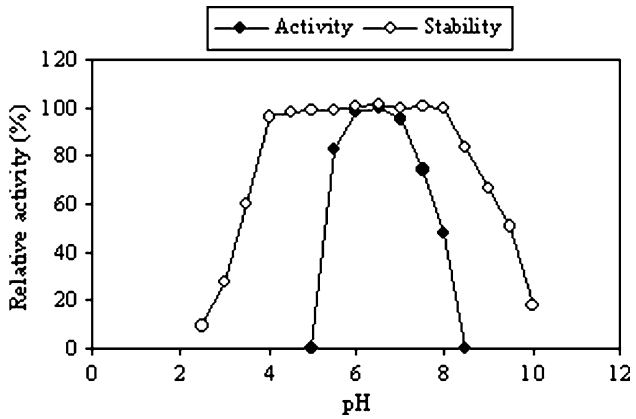


**Fig. 1** Effects of pH on the activity and stability of TreS. The pH stability of enzyme was examined by measuring the residual activity of enzyme. The *solid circle* and *open circle* indicate the enzyme activity and pH stability, respectively

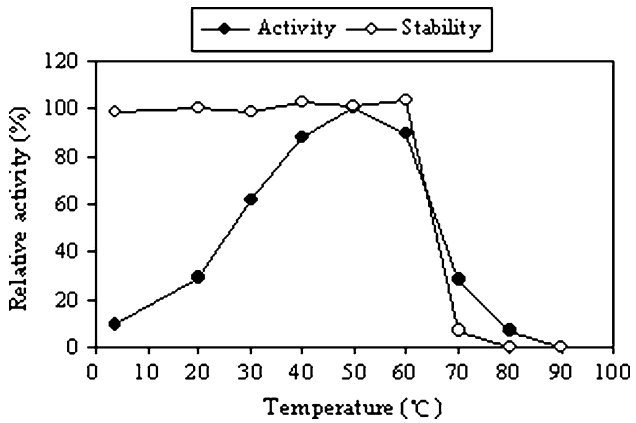


**Fig. 2** Effects of temperature on the activity and stability of TreS. The temperature stability of enzyme was examined by measuring the residual activity of enzyme. The *solid circle* and *open circle* denote the enzyme activity and temperature stability, respectively

## Effects of pH and temperature on the activity and stability of recombinant TreS

The optimum pH for TreS was 6.5, and the enzyme maintained high activity at pH 6.0–7.0, but below pH 5.0 the enzyme showed no activity. However, TreS showed a highly residual activity within a pH range of 4.0–8.0 (Fig. 1).

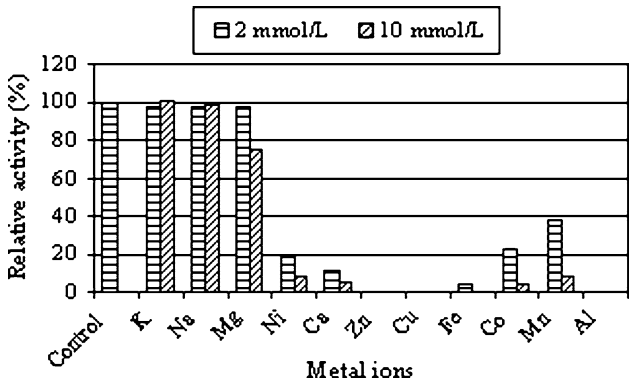


**Fig. 3** Effects of metal ions on the activity of TreS

The effects of temperature on TreS activity and stability are depicted in Fig. 2. The optimum temperature was 50°C. Furthermore, the TreS could maintain high activity (90% relative activity) after incubating at 60°C for 5 h (not shown).

## Effects of metal ions on the activity of recombinant TreS

The effects of metal ions on TreS activity and stability are depicted in Fig. 3. The results indicated that the enzyme activity was inhibited strongly by $Zn^{2+}$, $Fe^{2+}$, $Cu^{2+}$ and $Al^{3+}$ and moderately by $Ni^{2+}$, $Ca^{2+}$, $Co^{2+}$ and $Mn^{2+}$. However, we were unable to find some metal ions increasing the enzyme activity.

## Effects of temperature on the maximum yield of TreS

For the effects of temperature, reaction mixtures containing 60 mmol/l maltose were incubated under pH 6.5 at 20, 30, 40 and 50°C for 24 h, and the maximum production of trehalose were 63.9, 61.4, 56.4 and 47.2% with glucose contents of 2.3, 4.5, 9.2 and 18.4%, respectively (Fig. 4). Although a faster catalytic rate can be achieved at higher temperature, a slightly lower conversion was displayed due to more glucose generated.

Effects of cold storage on the stability of recombinant TreS

The TreS was stored at 4°C, and the activities were examined after 1, 5, 15, 30 and 60 days with the standard reaction conditions. The activities of TreS stored for various days were constant. It is indicated that the recombinant TreS was very stable at cold storage.

## Discussion

Trehalose synthase catalyzes the reversible reaction of maltose and trehalose, and several TreS have been isolated from various bacteria. In our study, a TreS was found in *Meiothermus ruber*, a thermophilic bacterium. The TreS gene had a length of 2889 bp and encoded 962 amino acids. According to BLAST result, the N-terminal domain of the TreS amino acid sequence belonged to the α-amylase family. According to the reaction mechanism and crystal structures of some enzymes belonging to the α-amylase family (MacGregor et al. 2001), the catalytic residues, Asp and Glu, and substrate-binding residues, His, have been deduced and verified in TreS of *Thermus caldophilus* (Koh et al. 2003) and *Picrophilus torridus* (Chen et al. 2006). These residues are also conserved in *M. ruber* TreS. Therefore, it was suggested that Asp-199, Asp-308 and Glu-242 in *M. ruber* TreS are involved in catalysis, and His-103 and His-307 in substrate binding (Fig. 5). Moreover, the amino acid sequence of the N-terminal domain (at position 1–540) shared much higher identity (88.3%) with that of *T. thermophilus*. However, the identity of the TreS C-terminal domain between *Thermus* and *Meinthermus* genus was not so high (55.7%, Fig. 6). It was reported that the existence of the C-terminal domain increased the thermostability of the TreS from *T. thermophilus* (Wang et al. 2007). However, in our study, it was found that the C-terminal domain of TreS from *M. ruber* played a very important role to the activity of the enzyme. The TreSΔC protein without the C-terminal domain was constructed, but showed no activities whatever maltose or trehalose was used as the substrate at various temperatures. The results of native-PAGE indicated the natural TreS was a tetramer, while the non-active TreSΔC protein was just a dimer. It was suggested that the C-terminal domain was required for tetramer formation, which might be crucial for activity.

The purified recombinant enzyme had an optimal pH of 6.5 and optimal temperature of 50°C. In comparison with other previously reported trehalose synthases, the TreS is more acid tolerant (Table 2). It could maintain 90% of its activity after incubating at pH 4.0–8.0, while the other well-studied TreSes showed dramatic decrease in enzyme activity after incubating at pH below 5.0. Furthermore, the recombinant TreS was a thermostable one, which could maintain 90% of its activity after incubating at 60°C for 5 h. Although the optimal temperature for enzymatic activity was not as high as that of *Thermus* genus, the optimal temperature for trehalose production using the TreS is close to room temperature (Fig. 4), so the energy consumption in temperature control could be decreased. Hence, the TreS reported here is very suitable for industrial production of trehalose. The comparison of some properties of TreS from different bacteria was showed in Table 2.

The TreS from *M. ruber* also had a weak hydrolytic property by itself, which could hydrolyze trehalose and/or maltose to glucose. Hence, decreasing the yield of glucose was crucial to increase the efficiency of the enzyme. As reported, lower reaction temperature could decrease the yield of glucose (Koh et al. 2003) and for the TreS isolated by us, glucose was hardly produced at 20°C. It is possible that higher temperature would increase the flexibility of the

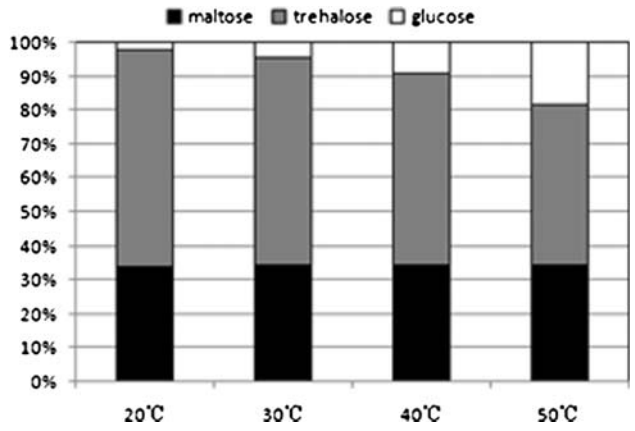


**Fig. 4** Effects of temperature on the maximum yield of TreS

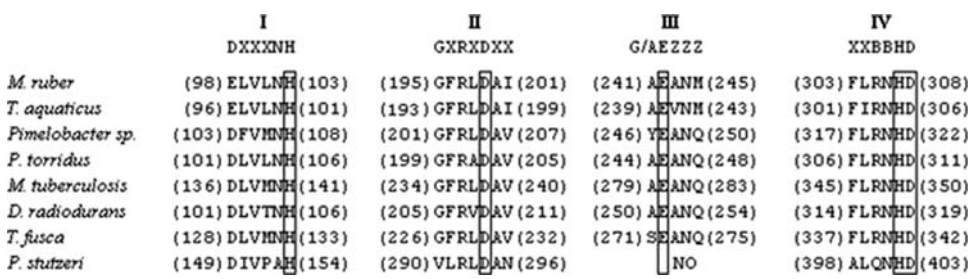


**Fig. 5** Four conserved amino acid sequences of trehalose synthase and their putative catalytic and substrate-binding residues. The catalytic and substrate-binding residues are in *box*. The *I*, *II*, *III* and *IV* regions show the four conserved sequence of α-amylase family

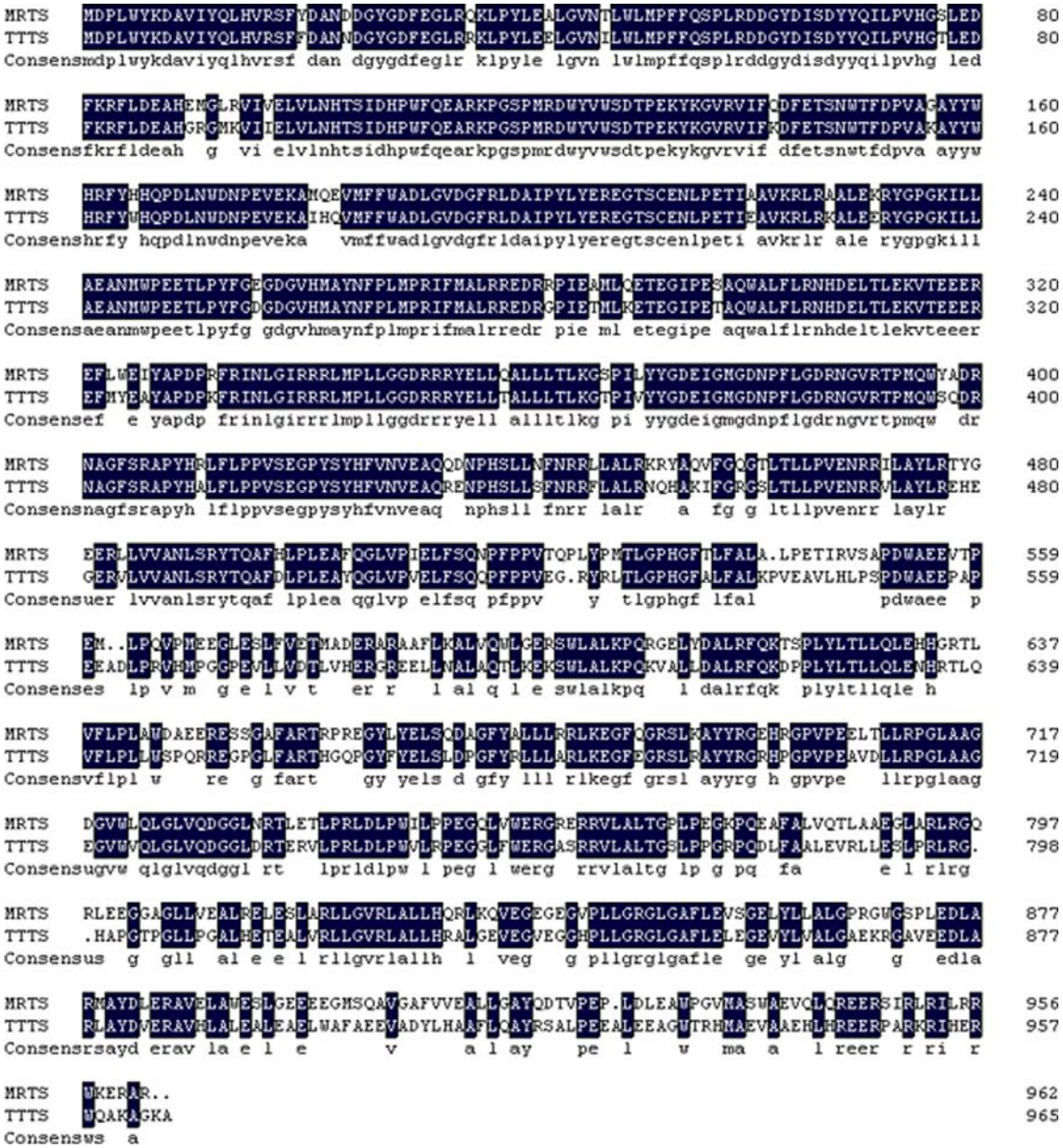

**Fig. 6** Alignment of the amino acid sequence of trehalose synthases from *M. ruber* and *T. thermophilus*. MRTS, *M. ruber* TreS (GenBank accession number ACA35051); TTTS, *T. thermophilus* TreS (GenBank accession number AAQ16097)

**Table 2** Comparison of the molecular and enzymatic properties of trehalose synthase from different sources

| | *Meiothermus ruber* | *Thermus aquaticus* ATCC 33923 | *Pimelobacter* sp. R48 | *Pseudomonas stutzeri* CJ38 | *Picrophilus torridus* |
|---|---|---|---|---|---|
| Amino acid no. | 962 | 963 | 573 | 689 | 558 |
| Optimum pH | 6.5 | 6.5 | 7.5 | 8.5–9.0 | 6.0 |
| Optimum temperature (°C) | 50 | 65 | 35 | 20 | 45 |
| pH stability | 4.0–8.0 | 5.5–9.5 | 6.0–9.0 | 6.0–9.0 | 5.0–7.5 |
| Thermal stability (°C) | 60 (5 h) | 80 (1 h) | 30 (1 h) | 30 (1 h) | 60 (1 h) |
| Ref. | This study | Nishimoto et al. 1996b; Tsusaki et al. 1997 | Nishimoto et al. 1996a; Tsusaki et al. 1996 | Lee et al. 2005 | Chen et al. 2006 |

protein structure, making the active site more accessible to water molecules to attack the split glucose before the formation of the α,α-1,1-glycosidic bond. Moreover, the TreS from *P. stutzeri* CJ38 did not has the hydrolytic property (Lee et al. 2005), and it was found that the amino acid sequence of the TreS was a little different from the others by sequence alignment (Fig. 5). Hence, it is possible that the structure of the protein was tighter than the others, so that water molecule could not access to the activity centre.

The equilibrium of TreS reaction lay far in the direction of the synthesis of trehalose. However, the role of this enzyme in vivo is not trehalose synthesis, but trehalose degradation, according to the previous reports (Cardoso et al. 2007; Makihara et al. 2005; Silva et al. 2003). The genes of TPS and TPP were also found in *M. ruber*, indicating that there are two pathways for trehalose synthesis in *M. ruber*. Kinetics analysis showed that the recombinant TreS had a little greater affinity (1.3-fold) for trehalose than maltose. Moreover, this TreS showed threefold higher $K_m$ value for maltose than that of *P. torridusfrom* (Chen et al. 2006). Therefore, we supposed that the biosynthesis of trehalose in *M. ruber* was due to TPS/TPP pathway, but the role of the TreS in vivo remains unknown. It was found that the mutant with the TPS/TPP pathway knocked out could not survive in the medium containing 1% NaCl, while the wild-type strain could survive. The knockout of TreS pathway is now in progress and the detail results on the two pathways will be reported soon.

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## References

Avonce N, Mendoza-Vargas A, Morett E, Iturriaga G (2006) Insights on the evolution of trehalose biosynthesis. BMC Evol Biol 6:109

Cardoso FS, Castro RF, Borges N, Santos H (2007) Biochemical and genetic characterization of the pathways for trehalose metabolism in *Propionibacterium freudenreichii*, and their role in stress response. Microbiology 153:270–280

Chen YS, Lee GC, Shaw JF (2006) Gene cloning, expression, and biochemical characterization of a recombinant trehalose synthase from *Picrophilus torridus* in *Escherichia coli*. J Agric Food Chem 54:7098–8104

Crowe JH, Crowe LM, Chapman D (1984) Preservation of membranes in anhydrobiotic organism: the role of trehalose. Science 223:701–703

Elbein AD, Pan YT, Pastuazak I, Carroll D (2003) New insights on trehalose: a multifunctional molecule. Glycobiology 13:17–27

Giæver HM, Styrvold OB, Kaasen I, Strøm AR (1988) Biochemical and genetic characterization of osmoregulatory trehalose synthesis in *Escherichia coli*. J Bacteriol 170:2841–2849

Koh S, Kim J, Shin HJ, Lee D, Bae J, Kim D, Lee DS (2003) Mechanistic study of the intramolecular conversion of maltose to trehalose by *Thermus caldophilus* GK24 trehalose synthase. Carbohydr Res 338:1339–1343

Kouril T, Zaparty M, Marrero J, Brinkmann H, Siebers B (2008) A novel trehalose synthesizing pathway in the hyperthermophilic Crenarchaeon *Thermoproteus tenax*: the unidirectional TreT pathway. Arch Microbiol 190:355–369

Lederer E (1976) Cord factor and related trehalose esters. Chem Phys Lipids 16:92–106

Lee JH, Lee KH, Kim CG, Lee SY, Kim GJ, Park YH, Chung SO (2005) Cloning and expression of a trehalose synthase from *Pseudomonas stutzeri* CJ38 in *Escherichia coli* for the production of trehalose. Appl Microbiol Biotechnol 68:213–219

MacGregor EA, Janecek S, Svensson B (2001) Relationship of sequence and structure to specificity in the α-amylase family of enzymes. Biochim Biophys Acta 1546:1–20

Makihara F, Tsuzuki M, Sato K, Masuda S, Nagashima KV, Abo M, Okubo A (2005) Role of trehalose synthesis pathways in salt tolerance mechanism of *Rhodobacter sphaeroides* f. sp. denitrificans IL106. Arch Microbiol 184:56–65

Nishimoto T, Nakano M, Nakada T, Chaen H, Fukuda S, Sugimoto T, Kurimoto M, Tsujisaka Y (1996a) Purification and properties of a novel enzyme, trehalose synthase, from *Pimelobacter* sp. R48. Biosci Biotechnol Biochem 60:640–644

Nishimoto T, Nakada T, Chaen H, Fukuda S, Sugimoto T, Kurimoto M, Tsujisaka Y (1996b) Purification and characterization of a thermostable trehalose synthase from *Thermus aquaticus*. Biosci Biotechnol Biochem 60:853–859

Pan YT, Edavana VK, Jourdian WJ, Edmondson R, Carroll JD, Pastuszak I, Elbein AD (2004) Trehalose synthase of *Mycobacterium smegmatis*: purification, cloning, expression, and properties of the enzyme. Eur J Biochem 271:4259–4269

Pan YT, Carroll JD, Asano N, Pastuszak I, Edavana VK, Elbein AD (2008) Trehalose synthase converts glycogen to trehalose. FEBS J 275:3408–3420

Reinders A, Bürckert N, Hohmamm S, Thevelein JM, Boller T, Wiemken A, de Virgilio C (1997) Structural analysis of the subunits of the trehalose-6-phosphate synthase/phosphatase complex in *Saccharomyces cerevisiae* and their function during heat shock. Mol Microbiol 24:687–695

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor, NY, USA

Satpathya GR, Töröka Z, Balia R, Dwyrea DM, Littlea E, Walkera NJ, Tablina F, Crowea JH, Tsvetkovaa NM (2004) Loading red blood cells with trehalose: a step towards biostabilization. Cryobiology 49:123–136

Schiraldi C, di Lernia I, de Rose M (2002) Trehalose production: exploiting novel approaches. Trends biotechnol 20:421–425

Silva Z, Alarico S, Nobre A, Horlacher R, Marrugg J, Boos W, Mingote AI, da Costa MS (2003) Osmotic adaptation of *Thermus thermophilus* RQ-1: a lesson from a mutant deficient in the synthesis of trehalose. J Bacteriol 185:5943–5952

Thevelein JM (1984) Regulation of trehalose mobilization in fungi. Microbiol Rev 48:42–59

Tsusaki K, Nishimoto T, Nakada T, Kubota M, Chaen H, Sugimoto T, Kurimoto M (1996) Cloning and sequencing of trehalose synthase gene from *Pimelobacter* sp. R48. Biochim Biophys Acta 1290:1–3

Tsusaki K, Nishimoto T, Nakada T, Kubota M, Chaen H, Fukuda S, Sugimoto T, Kurimoto M (1997) Cloning and sequencing of trehalose synthase gene from *Thermus aquaticus* ATCC 33923. Biochim Biophys Acta 1334:28–32

Wang JH, Tsai MY, Chen JJ, Lee GC, Shaw JF (2007) Role of the C-terminal domain of *Thermus thermophilus* trehalose synthase in the thermophilicity, thermostability, and efficient production of trehalose. J Agric Food Chem 55:3435–3443

Wei YT, Zhu QX, Luo ZF, Lu FS, Chen FZ, Wang QY, Huang K, Meng JZ, Wang R, Huang RB (2004) Cloning, expression and identification of a new trehalose synthase gene from *Thermobifida fusca* genome. Acta Biochim Biophys Sin 36:477–484

Zhu Y, Zhang J, Wei D, Wang Y, Chen X, Xing L, Li M (2008) Isolation and identification of a thermophilic strain producing trehalose synthase from geothermal water in China. Biosci Biotechnol Biochem 72:2019–2024