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Full Length Research Paper

Expression of *Deinococcus geothermalis* trehalose synthase gene in *Escherichia coli* and its enzymatic properties

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A novel trehalose synthase gene from *Deinococcus geothermalis* (DSMZ 11300) containing 1692 bp reading-frame encoding 564 amino acids was amplified using polymerase chain reaction (PCR). The gene was ligated into pET30Ek/LIC vector and expressed after isopropyl β-D-thiogalactopyranoside induction in *Escherichia coli* BL21(DE3)p*Lys*S. The recombinant trehalose synthase (*Dgeo*TreS) containing a His_6 tag at the C-terminus was purified by metal affinity chromatography and characterized. The expressed enzyme is a homodimer with deduced molecular mass of 64.69 kDa for each subunit, and exhibits the highest activity at pH and temperature of 7.6 and 40°C, respectively. The activity of *Dgeo*TreS was almost unchanged after 8 h preincubation at 40°C and pH 7.6, and retained about 57% of maximal value after 8 h of incubation at 55°C. The *Dgeo*TreS was highly inhibited by Cu^{2+}, Hg^{2+} and 10 mM Tris as well as by EDTA when its concentration exceeded 1 mM, but slightly activated by 1 mM dithiotreitol. The K_m and k_{cat} values of maltose conversion were 254 mM and 31.86 s^{-1}, respectively.

Key words: Trehalose synthase, *Deinococcus geothermalis*, transglucosylation, gene expression, *Escherichia coli*.

INTRODUCTION

Deinococcus geothermalis, a red pigmented, Gram-positive, non-pathogenic moderate thermophile is extremely resistant to ionizing radiation, ultraviolet light and desiccation. Presented results show that *D. geothermalis* is to be a potential source of enzyme producing trehalose (α-D-glucopyranosyl α-D-glucopyranoside). This stable, non-reducing disaccharide contains α,α-1,1-glucoside linkage between the α-glucose moieties (Elbain et al., 2003). Trehalose can be used in the food, cosmetics, medical and biotechnological industries, as well as for the stabilization of vaccines, enzymes, antibodies, hormones, pharmaceutical preparations and organs for transplantation (Richards et al., 2002; Higashiyama, 2002). The account for trehalose applications is the creation of hydrogen bonds between trehalose and protein or lipids, which participate in stabilization of proteins and biological membranes and formation of amorphous glass which protects biological molecules without essential changes to their native structure during desiccation, freezing, heating or oxidation (Crove and Crove, 2002; Roser, 1991; Benaroudi et al., 2001). Furthermore, the mild sweetness of trehalose, its low cariogenicity, good solubility in water, high water retention capabilities, stability, reduction of water activity, depression of freezing point and protein protection properties make it a valuable food ingredient (Richards et al., 2002). Trehalose is resistant to hydrolysis at low pH values, even at elevated

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temperatures and does not caramelize, and does not undergo Maillard reactions. What is more, this compound has been accepted as a food additive under the generally recognized as safe and effective (GRAS) terms in the U.S. and the EU.

Enzymes involved in different pathways of trehalose biosynthesis were extensively studied (Schiraldi et al., 2002; Woo et al., 2010). Most of the microorganisms have only a single pathway, but some have two and even three pathways. The most common biosynthesis involves the successive action of trehalose-6-phosphate synthase (TPS) which catalyses the transfer of glucose from uridine-diphosphate-glucose to glucose-6-phosphate. Afterwards, a trehalose-phosphate phosphatase (TPP) liberates phosphate forming trehalose. Another pathway, which involves two enzymes, was found in some hyperthermophilic archaeons, for example, *Sulfolobus solfataricus*. The first enzyme, maltooligosyltrehalose synthase (MTSase) catalyses the conversion of α-1,4-glycosidic linkage at the reducing end of maltooligosaccharide or glycogen to an α-1,1- linkage. Then, maltooligosyltrehalose trehalohydrolase (MTHase) cleaves the α-1,4-glucosidic linkage next to the α-1,1-linkage of trehalosyl-oligosaccharide to produce trehalose and oligosaccharide with lower molecular mass (Kato, 1999). Recently, Kim et al. (2011) reported two steps enzymatic production of trehalose from sucrose using amylosucrose from *D. geothermalis* and maltooligosyltrehalose synthase from *Bacillus helvolum*. The third pathway, catalyzed by trehalose glycosyltransferring synthase (TreT), involves the conversion of nucleoside diphosphate glucose and glucose, instead of glucose-6-phosphate, into trehalose (Ryu et al., 2005; Empadinhas and da Costa, 2006). On the other hand, production of trehalose in fungi involves glucose-1-phosphate and glucose as substrates and trehalose phosphorylase (TreP) (Schwarz et al., 2007).

However, for industrial applications, the preference is to use maltose-α-D-glucosyl transferase, EC 5.4.99.16 (trehalose synthase, TreS). This enzyme converts maltose into α,α-trehalose and releases a small amount of glucose and α,β-trehalose as by-products (Nishimoto et al., 1996a; Nishimoto et al., 1996b; Koh et al., 2003). This process has the advantage of a one-step reaction, and employs an inexpensive substrate derived from starch. Currently, trehalose synthases have been identified from a variety of bacterial sources, for example, *Pseudomonas putida*, *Thermus thermophilus*, *Meiothermus ruber*, *Arthrobacter aurescens* and *Deinococcus radiodurans* (Ma et al., 2006; Zdzieblo and Synowiecki, 2006; Zhu et al., 2008, Xiuli et al.,2009, Wang et al., 2007). In the present work, the gene encoding trehalose synthase from *D. geothermalis* was cloned and expressed in *E. coli*. To the best of present knowledge, this is the first report on the possible use of *D. geothermalis* as a source of trehalose synthase and constitutes an important step in extending the knowledge of TreS diversity.

MATERIALS AND METHODS

Bacterial strains, plasmids and culture conditions

D. geothermalis (DSMZ 11300, Braunschweig, Germany) was used as a source of trehalose synthase gene. Bacterial cells were cultivated aerobically on a rotary shaker (Forma Orbital, Thermo Fisher Scientific Inc, Marietta, OH, US) at 45°C (pH 7.2) in a modified medium recommended by DSMZ as described previously (Ferreira et al., 1997). The *E. coli* TOP10F' (resistant to tetracycline) (Invitrogen, Carlsbad, CA, US) and BL21(DE3)p*Lys*S (resistant to chloramphenicol) (Promega, Madison, WI, US) strains were used as a cloning host and expression host, respectively. The *E. coli* cells with plasmids were cultured aerobically at 37°C to OD_{600} of 0.6 in LB medium supplemented with appropriate antibiotics. The cells were harvested by centrifugation at 3000 × *g* for 10 min. The pJET (resistant to ampicillin) (Fermentas UAB, Vilnius, Lithuania) and pET30Ek/LIC (resistant to kanamycin) (Novagen, Darmstadt, Germany) plasmids were used for construction of expression system. Restriction enzymes were purchased from Fermentas.

Amplification of trehalose synthase (DgeoTreS) gene

The DNA from *D. geothermalis* isolated using a genomic DNA preparation kit (A&A Biotechnology, Gdynia, Poland) was used for polymerase chain reaction (PCR) amplification with two primers: 5' aaaacatATGACGCAAACCTCCACCTCCGAGT 3' and 5' aaaCTCGAGCCGCACCCCCGACAGCTTC 3' containing underlined recognition sites for restriction endonucleases NdeI and XhoI (Fermentas). The reaction was performed using 250 ng of DNA, 10 pmoles of each primer, 12 μmoles of dNTPs, 12.5 μl 2 × PCR buffer (5 mM $MgCl_2$,100 mM Tris, pH 9.0, 40 mM $(NH_4)_2SO_4$, 10 mM DMSO) and 0.5 μl Marathon DNA polymerase (*Pwo* and *Taq* polymerase mixture). After 1 min of preliminary heating at 95°C in a thermal cycler (EpGradient S, Eppendorf, Hamburg, Germany), each of the 30 cycles was conducted 5 times at 95°C for 1 min, 59°C for 1 min, and 72°C for 2 min, then 25 times at 95°C for 1 min, 64°C for 1 min and 72°C for 1.5 min with a final step of 5 min at 72°C. The DNA fragment encoding *Dgeo*TreS was obtained and cloned into pJET vector with the following steps described by producer of CloneJet kit (Fermentas). After sequencing and confirming of the proper sequence, the DNA construct was digested with NdeI and XhoI restriction endonucleases and resubcloned into a pET30Ek/LIC vector. The digestion product approximately below 1700 bp was isolated from an agarose gel bands using Gel-Out kit (A&A Biotechnology).

Construction of expression vector

The competent cells *E. coli* TOP10F' suspended in 1 ml 100 mM $CaCl_2$ were transformed by the ligation mixture for 1 h at 8°C and after heat shock (1.5 min at 42°C, then 1.5 min at 4°C), incubated for 1 h at 37°C in 400 μl SOC medium (Invitrogen). The transformed *E. coli* cells plated on LB-agar with appropriate antibiotics plates were incubated at 37°C. After 16 to 18 h of growth, the obtained colonies were examined for the presence of trehalose synthase gene of *D. geothermalis* by PCR amplification and restriction analysis. The authenticity of the clone was ascertained by sequencing of the complete DNA fragment. The obtained construction designated as pET30Ek/LIC-*Dgeo*TreS was isolated and selected by electrophoresis on a 1% agarose gel, and then

transformed into *E. coli* BL21(DE3)p*Lys*S cells.

Expression of the His_6-tagged trehalose synthase

A single colony of transformed *E. coli* from LB-agar plates was inoculated in a 50 ml liquid LB medium supplemented with kanamycin/chloramphenicol and cultivated at 37°C. When OD_{600} reached the value of about 0.6, the cell suspension was transferred to 2 L of LB medium containing kanamycin/chloramphenicol. The cultures were grown up to OD_{600} of 0.6 at conditions described above, then D*geo*TreS expression in *E. coli* BL21(DE3)p*Lys*S was induced at 37°C with isopropyl β-D-thiogalactopyranoside (IPTG) at a final concentration of 1 mM. The cells were harvested 4 h after induction by centrifugation, and the pellet washed with distilled water was centrifuged and stored at -20°C until use.

Isolation and purification of recombinant trehalose synthase

Transformed *E. coli* cells, harvested from 2 L of induced culture, were sonicated at 20 kHz (three times for 10 s with 30 s intervals) in 80 ml 10 mM phosphate buffer (pH 7.6) containing 1 M EDTA, 100 mM $CaCl_2$, 0.1 mg lysozyme and 1 mg RNase, using Brenson Ultrasonic Sonifier II W250D (Geneve, Switzerland). The resulting suspension was centrifuged (4°C) at 12,000 × *g* for 40 min, incubated at 56°C for 10 min and precipitated host proteins were removed by centrifugation at 9,000 × *g* for 40 min. The clear lysate was then applied to Co^{2+}-IDA-agarose column (His· Bind®Resin, Novagen) equilibrated with 50 ml of phosphate buffer (pH 7.6) containing NaCl and imidazole at concentrations 0.5 M and 5 mM, respectively (buffer A). After loading, the column was washed three times with 50 ml of the same buffer. The recombinant *Dgeo*TreS was then washed twice with 20 ml of buffers A, B, C, D containing 25, 50, 80, 100 mM imidazole, respectively. The protein of interest was eluted with two portions of 20 ml elution buffer E containing imidazole at concentration of 0.5 M. The eluted fraction was purified, concentrated on a centrifugal filter device (Amicon®Ultra-15 30,000 MWCO Carrigtwohill, Cork, Ireland), and after changing the buffer to physiological salt, was used as a final preparation of the enzyme.

Enzyme assay

The activity of *Dgeo*TreS was determined by measuring the trehalose produced from maltose. The assays were initiated by the addition of 0.5 ml of *Dgeo*TreS solution to 1.0 ml of 0.3 M maltose solution in a 50 mM Britton-Robinson buffer (pH 7.6). The reaction at 40°C was terminated after sample heating at 100°C for 10 min. The samples purified by centrifugation (10,000 × *g* for 10 min) and filtration on a 0.2 μm Chromafil® PEF 20/25 filter (Machery-Nagel GmbH&Company, Düren, Germany) were passed through a APS-2 HYPERSIL column (Thermo Electron Corporation, Dreieich, Germany) using acetonitrile/methanol/water (78:11:11, v/v/v) as mobile phase, at a flow rate of 1.8 ml/min. The column temperature was 30°C. The amounts of the products formed during conversion of maltose were calculated from the area of the peaks obtained after sample separation by high-performance liquid chromatography (HPLC) using a refractive index detector (La Chrom-7490, Merck, Hitachi, Tokyo, Japan). Trehalose, maltose and glucose were used as standards at concentrations of 10 mg/ml. *Dgeo*TreS activity was expressed as the amount of enzyme that produces 1 μmol of trehalose per minute under described conditions. The relative enzyme activity (%) was defined as the percentage of enzyme activity in the control. The temperature dependence of enzyme activity was assayed in the range 5 to 70°C. The pH stability of *Dgeo*TreS was determined by measuring the residual activities after 1 h incubation (40°C) in 50 mM Britton-Robinson buffers at various pH values. The thermal stability of trehalose synthase was investigated by incubation of enzyme solution in 50 mM Britton-Robinson buffer (pH 7.6) at 40, 55, and 60°C for different periods (up to 8 h). Protein concentrations (mg/ml) were determined by measurement of absorbance at 280, using a Nano-Drop spectro-photometer (Thermo Fisher Scientific Inc. Wilmington, DE, US). The effects of metal ions and chemicals were determined in assay buffer containing a final concentration of cations or chemicals given in Table 1, and after pH adjustment to the optimal value, the *Dgeo*TreS activity was determined according to standard procedure.

Protein sequence analysis

The amino-acid sequence of *Dgeo*TreS was analysed using standard protein-protein BLAST and RPS-BLAST. Multiple sequence alignment was generated by using the program ClustalX. The results were prepared using the editor program Gendoc (copyright Karl Nicholas).

Molecular mass determination of recombinant DgeoTreS

The purified *Dgeo*TreS was subjected on a Superdex 200 HR 10/30 column (Amersham Bioscences AB, Uppsala, Sweden) equilibrated with 150 mM NaCl and 10 mM EDTA in a phosphate buffer (pH 7.5) and then was eluted with the same buffer. Absorbance at 280 nm was recorded to monitor the elution profile. The elution patterns of recombinant *Dgeo*TreS proteins were then compared with those of standard proteins: carbonic anhydrase (29 kDa), ovoalbumin (43 kDa), monomer of bovine serum albumin (66 kDa), alcohol dehydrogenase (150 kDa), β-amylase (200 kDa), apoferritin (443 kDa) and thyroglobulin (669 kDa). The molecular mass of the enzyme subunit was determined by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 12% (w/v) polyacrylamide gel in a Tris-glycine buffer (pH 8.3) (Laemmli, 1970). The samples (20 μl) denatured by β-mercaptoethanol as a reducing agent and SDS were layered on the gel and separated using a voltage gradient of 15 V/cm. Protein bands were located by staining with Coomassie Brillant Blue R250. The molecular mass was determined using a molecular mass marker kit (Fermentas).

RESULTS AND DISCUSSION

Molecular cloning and characterization of DgeoTreS

This study merits the assigning of a function to a previously uncharacterized recombinant trehalose synthase from *D. geothermalis*. The cloning experiments were conducted using the sequence annotated as a putative trehalose synthase in GenBank (locus Dgeo-0537), as well as in the CAZy database which show that trehalose synthase share common structural features with the members of the glucosidases family (GH13). Our experiments indicate that the region of *D. geothermalis* gene amplified by PCR had a sequence consisting of 1668 nucleotides encoding 556 amino acid residues (Figure 1). Theoretical values of molecular mass (M_r) and isoelectric point of the native enzyme were calculated to be 63.62 kDa and pI 4.92, respectively. However, recombinant

Table 1. Effect of some cations and chemicals on activity of trehalose synthase from *D. geothermalis* expressed in *E. coli.*

Cation/chemical	Relative activity (%) at reagent concentration of		
	1.0 mM	**5.0 mM**	**10.0 mM**
none	100.0	100.0	100.0
Ca^{2+}	102.3±1.3	73.5±0.3	69.1±0.8
Mg^{2+}	74.9±0.4	50.9±0.6	47.0±0.2
Zn^{2+}	29.4±0.5	17.5±1.5	6.4±0.3
Al^{3+}	81.8±2.1	0.0	0.0
Ni^{2+}	90.1±0.2	18.0±0.0	12.9±0.3
Co^{2+}	55.1±0.4	19.7±0.2	10.6±0.3
Fe^{3+}	60.4±0.7	0.0	0.0
Cu^{2+}	5.4±0.3	0.0	0.0
Hg^{2+}	14.7±0.7	2.0±0.6	0.8±0.1
Mn^{2+}	68.5±1.1	27.5±0.3	17.8±0.6
EDTA	99.2±1.2	30.6±0.3	1.4±0.1
Dithiotreitol	110.5±0.9	82.1±0.6	82.7±1.1
SDS	74.0±0.3	69.9±0.1	68.3±1.1
Tris	10 mM	15 mM	20 mM
	9.1±0.2	4.4±0.1	3.3±0.1

Results are mean values of three determinations ± standard deviation.

protein contains the His_6 tag at the C-terminus preceded by two residues: Leu (L) and Glu (E). It ensured that recombinant protein encoded by 1692 nucleotides has 564 amino-acids residues with theoretically estimated molecular mass (M_r) equal to 64.69 kDa. The M_r value deduced from the amino acid sequence of the enzyme was confirmed by SDS-PAGE (Figure 2). To determine the oligomeric state of this protein, it was analyzed by gel filtration chromatography, which showed that native *Dgeo*TreS has the molecular mass of 132.3 kDa (Figure 3). This confirmed that *Dgeo*TreS exists as homodimer.

Similar M_r values were reported for subunits of trehalose synthases from *Pseudomonas* sp. F1 (Ohguchi et al., 1997), *D. radiodurans* (Wang et al., 2007), and *Pimelobacter* sp. R48 (Nishimoto et al., 1996a). In comparison with enzymes from these sources, higher M_r value was detected for *T. aquaticus* and *T. thermophilus* (Nishimoto, 1996b; Zdziebło and Synowiecki, 2006). The monomer of native *Dgeo*TreS contains 556 amino-acid residues compared to 552 amino-acids in the bacterial TreS polypeptide from *D. radiodurans* (Figure 1). The analysis of the primary structures revealed the presence of several regions common to previously reported proteins of the Deinococcus/Thermus phylum (Wang et al., 2007). The comparison of *Dgeo*TreS sequences and those for trehalose synthases from *D. radiodurans, Meiothermus silvanus* and *Mycobacterium avium* shows that even among Deinococcus phylum, there are significant differences. The sequence of *Dgeo*TreS shares 83% identity and 89% similarity with *D. radiodurans* R1 TreS, 63% identity and 76% similarity to *M. silvanus* TreS, 54% identity and 68.2% similarity to *M. avium* TreS. More similar fragments are located on the N-terminus of the protein molecules (Figure 1).

Activity and kinetic of maltose conversion

Single step purification of the enzyme by metal affinity chromatography allowed 16.14% of protein recovery. To identify the function of recombinant protein, we incubated the purified enzyme with a maltose solution, and the reaction mixture was analyzed by HPLC. Obtained results show that the enzyme could convert maltose to trehalose accompanied by glucose as by-product (Figure 4). The small amount of glucose usually released in most of the reactions catalyzed by trehalose synthases is generated under entry of water molecules into the catalytic site prior to isomerization of the glycosidic linkage (Koh et al., 2003). This weak hydrolytic reaction catalysed by trehalose synthases depends on the enzyme origin and increases with temperature of the reaction (Wei et al., 2004). The temperature optimum for maltose conversion was found to be 40°C (Figure 5) and this value was higher than that of the trehalose synthase of *D. radiodurans* but close to that of the enzyme from *Pseudomonas* sp. F1 (Ohguchi et al., 1997). Although the thermostability of *Dgeo*TreS was not as high as that of the Thermus genus, it is more thermostable in comparison with the enzyme from *D. radiodurans*. The half-life of *Dgeo*TreS activity at 40°C (pH 7.6) was 42 h, while trehalose synthase from *D. radiodurans* showed dramatic decrease of the activity after incubating at this temperature (40°C) for 30 min (Wang et al., 2007). When

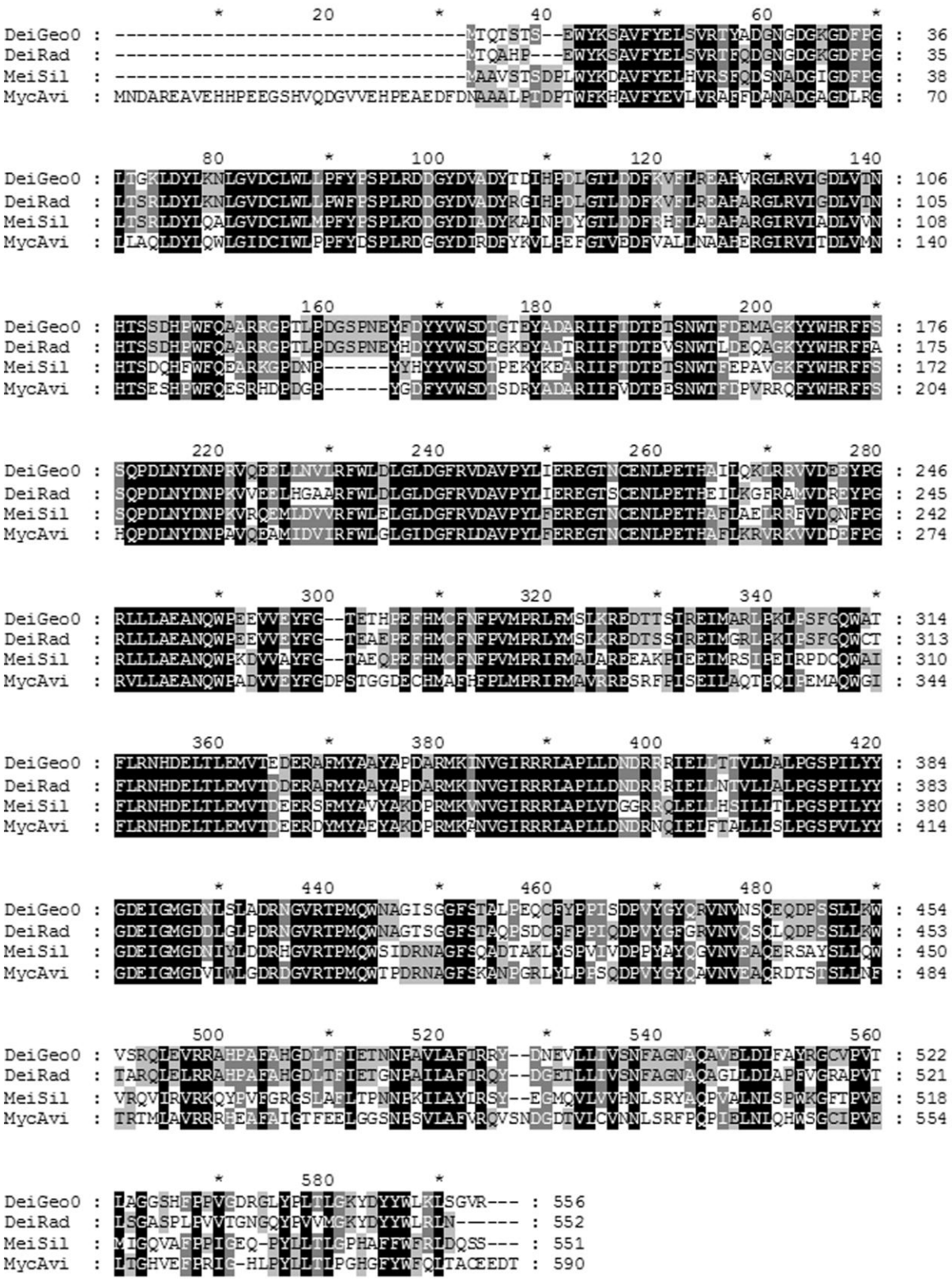

Figure 1. Multiple amino acid sequence alignment of TreS proteins. Alignment was performed by dividing amino acids into six similarity groups. Group 1: V, L, I and M; group 2: W, F and Y; group 3: E and D; group 4: K and R; group 5: Q and D; group 6: S and T. Description of similarity: white fonts on black boxes 100% identity; white fonts on grey boxes similarity < 80%; black fonts on grey boxes similarity < 60%. DeiGeo0 - *D. geothermalis* DSMZ 11300, DeiRad - *D. radiodurans* R1, MeiSilv - *Meiothermus silvanus*, MycAvi - *Mycobacterium avium*.

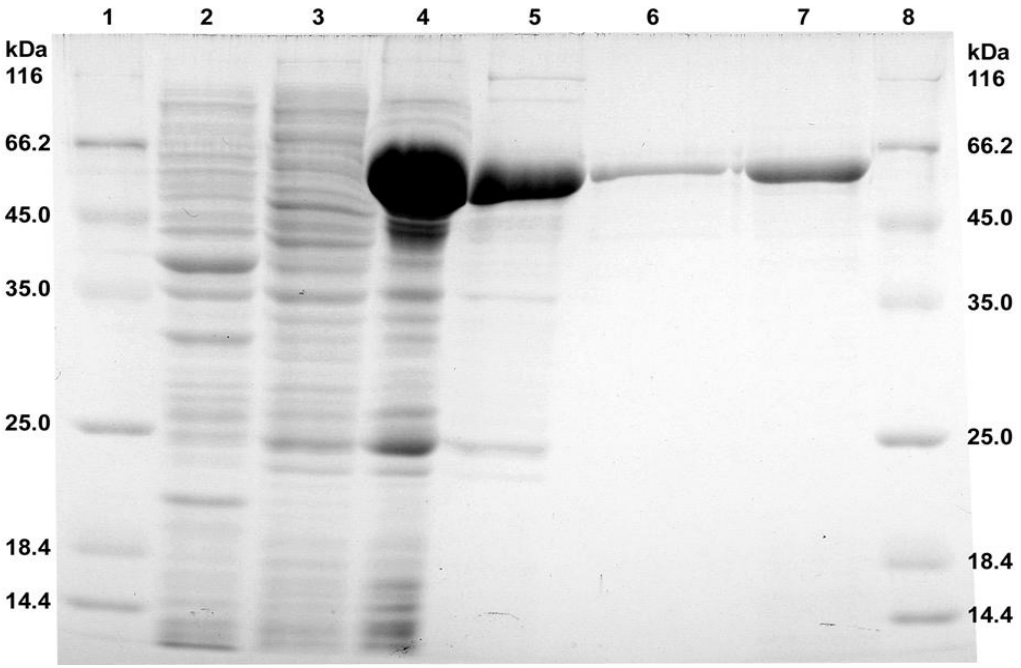

Figure 2. SDS/PAGE in 12% polyacrylamide gel of the fractions obtained by expression and purification of the recombinant *Dgeo*TreS. Lane 1 and 8 protein marker (Fermentas SM0431); lane 2, control: *E. coli* strain with vector before induction; lane 3, control: *E. coli* strain with vector after 18 h of IPTG induction; lane 4, cell extract after 18 h of protein expression induced by IPTG; lane 5, heat treatment at 65°C for 10 min; lane 6, *Dgeo*TreS purified by Co^{2+} -affinity chromatography; lane 7, purified *Dgeo*TreS concentrated in physiological salt solution.

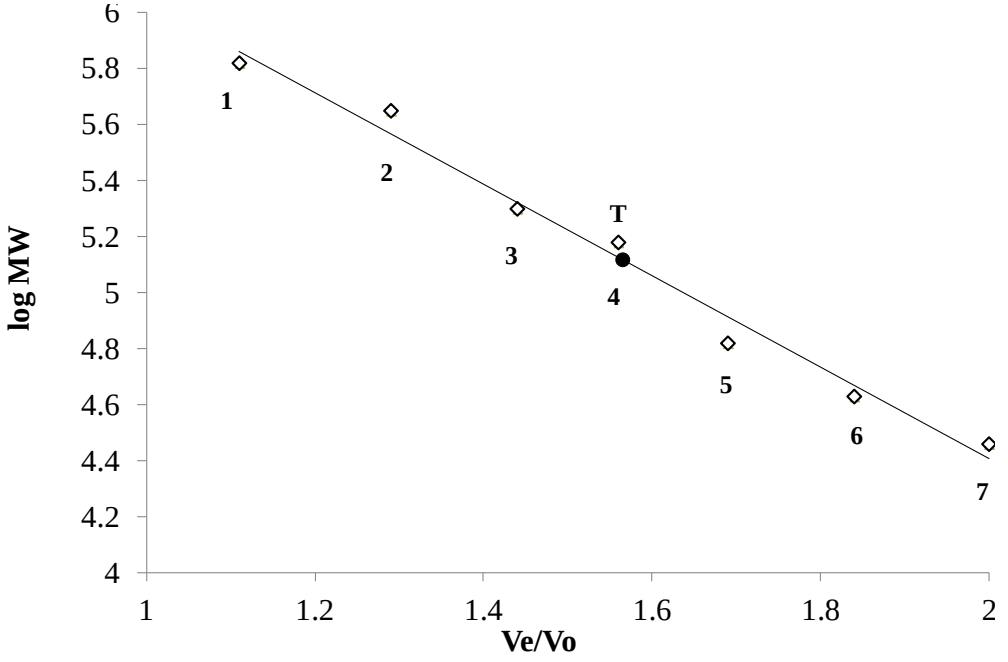

Figure 3. Native molecular mass of *Dgeo*TreS (●) determined with Superdex 200 HR 10/30 column. The molecular mass standards (◊) are represented by a number from 1 to 7, respectively: thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), ovoalbumin (43 kDa) and carbonic anhydrase (29 kDa). Retention rate is V_e/V_0, where, V_e elution volume of each protein, V_0 void volume of the column determined with blue dextran.

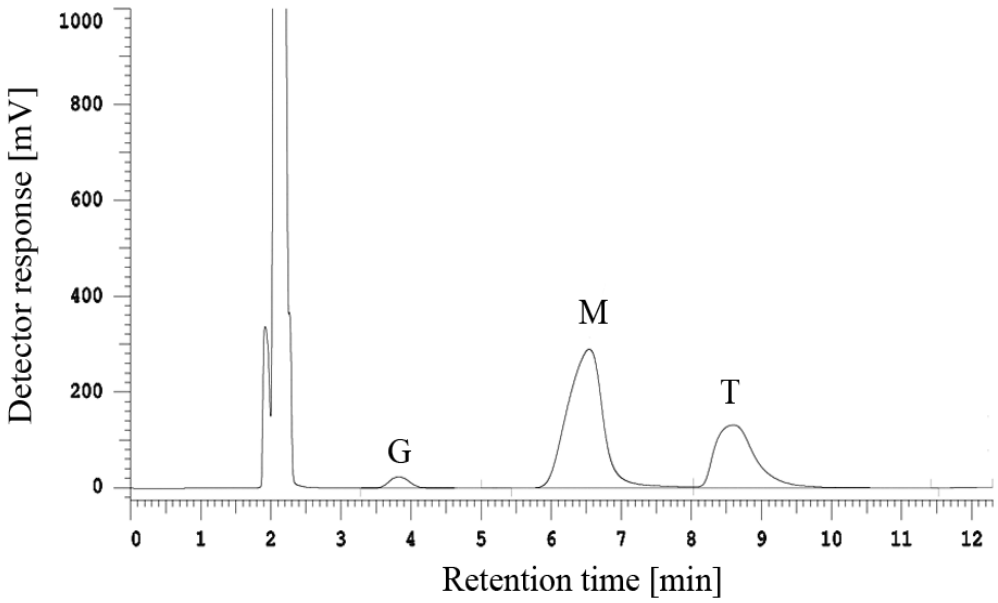

Figure 4. Analysis of the products of the *Dgeo*TreS-catalyzed reaction by HPLC. G, glucose; M, maltose; T, trehalose.

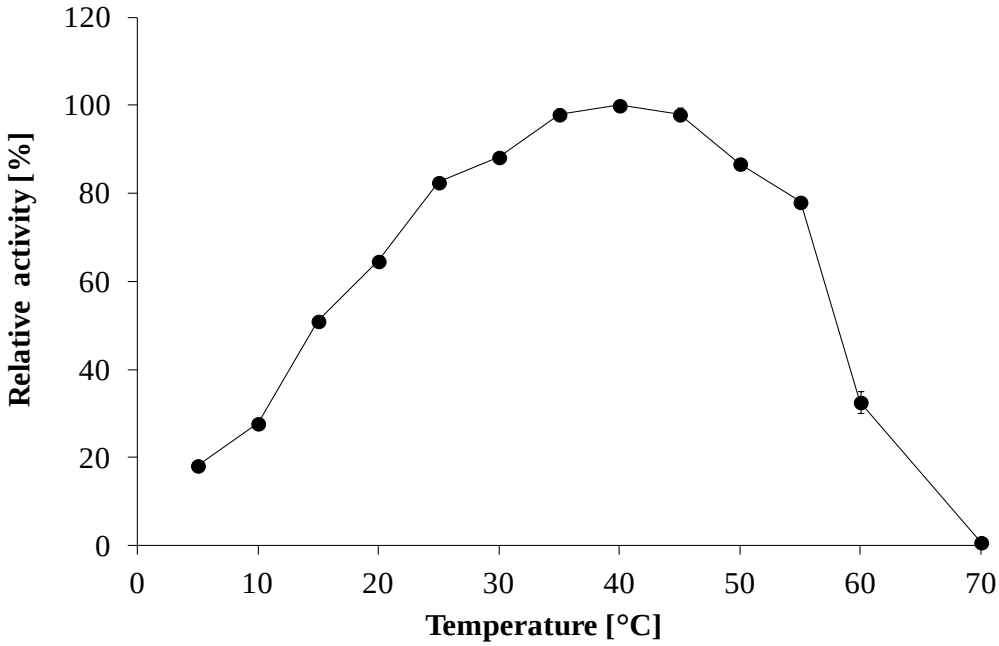

Figure 5. Effect of temperature at pH 7.6 on activity of *Dgeo*TreS. The results are mean values of three replicates.

the thermostability of *Dgeo*TreS was examined at 55 and 60°C, the enzyme retained about 57 and 20% of its maximal activity after 8 and 2 h of heating, respectively.

The optimal pH for maltose conversion was 7.6 in a 50 mM Britton-Robinson buffer as shown in Figure 6. This pH value is similar to that reported for trehalose synthases from *Pimelobacter* sp. R48 (Nishimoto et al., 1996a) and *T. aquaticus* (Nishimoto et al., 1996b). The retention of about 50% of maximal activity of *Dgeo*TreS was observed at a wide pH range of 6.0 to 9.0 (Figure 6). The K_m values for recombinant *Dgeo*TreS calculated using Lineweaver-Burk plot (Lineveaver and Burk, 1934) were 256 mM for maltose and 529 mM for trehalose. With regards to this result, the recombinant *Dgeo*TreS had a

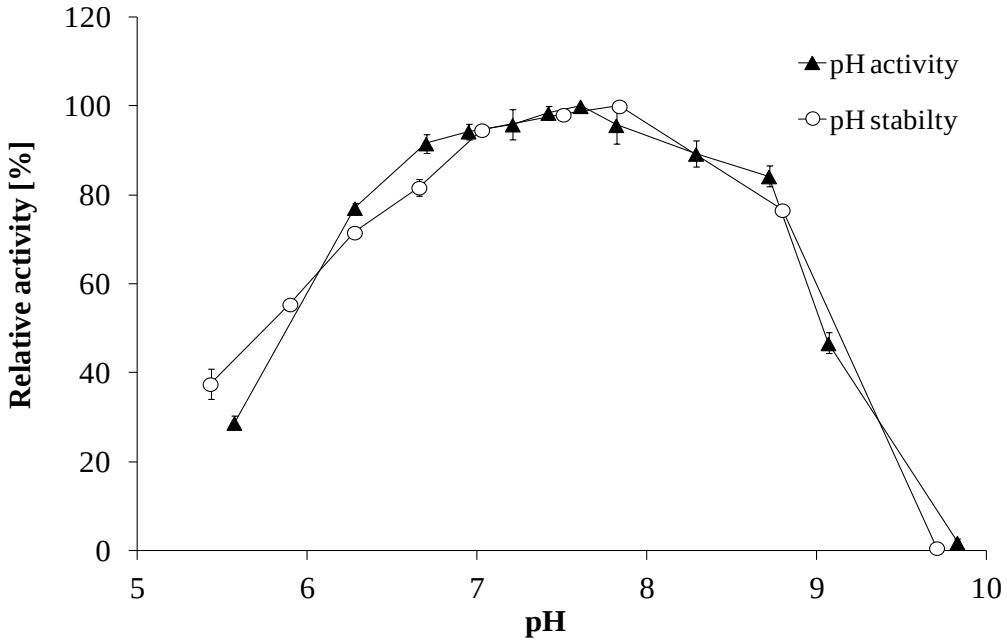

Figure 6. Effect of pH on *Dgeo*TreS activity (▲) assayed using 50 mM Britton Robinson buffers with various pH values. The pH stability (○) was determined by measuring residual activity after 1 h incubation at 40°C in the same buffer as those used for the optimal pH determination.

lower affinity to maltose than enzymes from *T. aquaticus* (K_m 34.5 mM) (Nishimoto et al., 1997) and *Enterobacter hormaechei* (K_m 25 mM) (Yue et al., 2009). Furthermore, the K_m value of trehalose-synthesizing enzyme from *Pseudomonas* sp. F1 was 1.1 mM (Ohguchi et al., 1997). The turnover number (k_{cat}) values of recombinant *Dgeo*TreS for maltose and trehalose were 31.86 and 30.25 s^{-1}, respectively. What is more, *Dgeo*TreS had over two-fold higher enzyme efficiency (k_{cat}/K_m) toward maltose (0.124 mM^{-1} s^{-1}) than trehalose (0.057 mM^{-1} s^{-1}) indicating maltose as the preferred substrate. The effect of reaction time on the yield or trehalose by *Dgeo*TreS was determined at 40°C using a 0.3 M solution of maltose in a 50 mM Britton-Robinson buffer (pH 7.6). The conversion of maltose was almost terminated after 16 h, and the reaction mixture contained trehalose (56.79%), glucose (7.29%) and 35.90% of maltose. A further increase of reaction time up to 24 h enhanced the trehalose and glucose contents to 60.40 and 8.61%, respectively.

Effect of some cations and chemicals on activity of DgeoTreS

The results shown in Table 1 indicate that the enzyme was highly sensitive to Hg^{2+} and Cu^{2+} and to a lesser extent to Zn^{2+} at concentrations of 1 mM. However, at a concentration of 5 mM, almost all examined cations strongly decreased *Dgeo*TreS activity. Unexpectedly, EDTA causes *Dgeo*TreS inactivation when concentration of this chelator exceeded 1 mM (Table 1). It indicates that some cations are required for enzyme activity analogical to other members of GH13 hydrolases, which have binding sites with different affinity to metal ions. The results presented in Table 1 indicating strong inhibition of *Dgeo*TreS by 10 mM Tris was also reported for trehalose synthases originated from *Pimelobacter* sp.48, *A. aurescens*, *T. thermophilus* and *Mycobacterium smegmatis* (Nishimoto et al.,1996a; Xiuli et al., 2009; Wang et al., 2007; Pan et al., 2004; Wei et al., 2004). The *Dgeo*TreS activity was also diminished in the presence of SDS whereas, 1 mM dithiotreitol, which acts as a suppressor of thiol groups oxidation, caused a minor enhancing effect on *Dgeo*TreS activity.

Conclusion

Our results indicate the potential for the production of *D. geothermalis* strain trehalose synthase by an *E. coli* expression system. This study adds a new member of available enzymes and *Dgeo*TreS might be a useful biocatalyst for trehalose production. This is the first report on the trehalose synthase from *D. geothermalis*, which allowed broadening our knowledge about biodiversity microbial sources of this enzyme.

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