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Recombinant thermophilic enzymes for trehalose and trehalosyl dextrins production

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Abstract

Two thermophilic and thermostable enzymes, trehalosyl dextrins forming enzyme (TDFE) and trehalose forming enzyme (TFE), able to convert starch and dextrins to α,α-trehalose were recently purified and characterized from *Sulfolobales* [I. Di Lernia, A. Morana, A. Ottombrino, S. Fusco, M. Rossi, M. De Rosa, Extremophiles, 2 (1998) 409; T. Nakada, S. Ikegami, H. Chaen, M. Kubota, S. Fukuda, T. Sugimoto, M. Kurimoto, Y. Tsujisaka, Biosci., Biotechnol., Biochem., 60 (1996) 267; T. Nakada, S. Ikegami, H. Chaen, M. Kubota, S. Fukuda, T. Sugimoto, M. Kurimoto, Y. Tsujisaka, Biosci., Biotechnol., Biochem., 60 (1996) 263; M. Kato, Y. Miura, M. Kettoku, K. Shindo, A. Iwamatsu, K. Kobayashi, Biosci., Biotechnol., Biochem., 60 (1996) 921; M. Kato, Y. Miura, M. Kettoku, K. Shindo, A. Iwamatsu, K. Kobayashi, Biosci., Biotechnol., Biochem., 60 (1996) 925]. The first enzyme transforms starch and dextrins to the corresponding trehalosyl derivatives, with an intramolecular transglycosylation process, which converts the glucosidic linkage at the reducing end from α-1,4 to α-1,1. The second, hydrolyzes the α-1,4 linkage adjacent to the α-1,1 bond of trehalosyl dextrins, forming trehalose and lower molecular weight dextrins. Herein, we report the cloning and high level expression of the two enzymes of *Sulfolobus solfataricus* strain MT4 in *Escherichia coli* using pTrc expression vector. The yield of TDFE and TFE obtained in this expression system was of 180 U/l and of 3630 U/l of medium, respectively.

Keywords: Extremophiles; Starch; Trehalose; Trehalose producing enzymes; *E. coli*

1. Introduction

Trehalose is widely present in yeasts, mushrooms, and insects. It is an unusual sugar as compared with other disaccharides, and empirical evidences indicate that its high concentration in the tissues of certain insects and desert plants allows them to survive in a state of "suspended life" under water deficiency. Trehalose, is a non-reducing disaccharide consisting of two α-1,1 linked glucose molecules and has about half the sweetness of sucrose. It can be used as a component of sweeteners, seasonings, preserved and

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frozen foods and soft drinks, and as a moisture retainer in cosmetics and preservatives in pharmaceuticals [1]. Since its extraction from baker's yeast is too expensive, various methods for the industrial production of trehalose have been developed [2–4]. We have found that the hyperthermophilic microrganism *Sulfolobus solfataricus* strain MT4 was able to produce trehalose from starch and dextrins. In an attempt to obtain high yield of trehalose production at high temperature, we have started a project aimed at the over-production of trehalose forming enzymes (TFEs) in *Escherichia coli*. Herein, we report the cloning and characterization of trehalosyl dextrins forming enzyme (TDFE) and TFE of *S. solfataricus* strain MT4.

2. Experimental

2.1. Materials

Molecular mass standards for SDS-PAGE were obtained from Gibco BRL, Pharmacia Biotech International and Sigma. pTrc99A and pUC18 *Sma*/BAP plasmids were from Pharmacia Biotech International. Radioactive materials were obtained from Amersham International. Deoxynucleotides and enzymes for DNA restriction and modification were purchased from Boehringer. *E. coli* strain Rb791 was kindly provided by Prof. G. Sannia (Dipartimento di Chimica Organica e Biologica, Università di Napoli, Italy). Gene amplification experiments were carried out with Expand High Fidelity TAQ system supplied by Roche. All synthetic oligonucleotides were purchased from PRIMM (Milano, Italy). Other chemicals were from Sigma-Aldrich. Trehalosylglucose and a series of trehalosylmaltodextrins: trehalosylmaltose, trehalosylmalto-triose, trehalosylmaltotetraose and trehalosylmaltopentaose were prepared in our laboratory using TFE enzyme from *S. solfataricus*.

2.2. Analytical methods for DNA

DNA electrophoresis on 1% (w/v) agarose gel was performed in TBE buffer (90 mM Tris/borate/20 mM EDTA). Plasmid transformation of *E. coli* cells was carried out by electroporation using a Gene Pulser purchased from Biorad.

DNA sequencing was carried out by the dideoxy chain termination method with [^{35}–S]dATP, using the Sequenase version 2.0 sequencing kit (Amersham) on alkali-denatured double-stranded templates and the universal primer or specific synthetic oligonucleotides. More than 90% of the DNA sequences were determined in duplicate on both strands. Gene sequence analysis was performed using PC-GENE software (Intelligenetics, Mountain View, CA, USA).

2.3. Construction of S. solfataricus strain MT4 gene bank

Isolation of chromosomal DNA from *S. solfataricus* was performed as described by Sambrook et al. [5].

High molecular weight DNA of *S. solfataricus* strain MT4 was partially digested with *Sau3AI* (1 h of incubation with 0.15 unit of enzyme per μg of genomic DNA), and fragments of 2–4 kb were isolated by electroelution from 3.5% polyacrylammide gel. DNA fragments were then subjected to partial filling in with the *E. coli* DNA polymerase (Klenow fragment), dGTP, and dATP and inserted into the vector pGEM7zf(+), previously made end-compatible by linearization with *XhoI* and partial filling in with the Klenow fragment, dCTP, and dTTP.

E. coli BO3310-competent cells were transformed with the ligation mixture and grown in Luria–Bertani medium containing 100 μg/mg ampicillin for 4 h [5] for propagation and amplification of the gene bank.

2.4. Cloning of the TDFE gene

The genomic bank was screened using as a probe a fragment of the TDFE gene, spanning nucleotides 853 to 1479. The probe was obtained by PCR amplification using, as a template, 200 ng of genomic DNA from *S. solfataricus* strain MT4 and the oligo-

nucleotides intN and C2 as primers, designed on the basis of TDFE gene sequences of *S. solfataricus* KM1 and *S. acidocaldarius* ATCC33909.

Oligonucleotide intN: 5′-GATGGGACTACTGG-ATATGAT/CT-3′ corresponding amino acids 285–291 of TDFE from *S. solfataricus* KM1.

Oligonucleotide C2: 5′-CGCTGCAGTCACC-CAATNAA/GCTCAC/T/GTNNN-3′ complementary to the sequence corresponding amino acids 711–716 of TDFE from *S. solfataricus* KM1. This oligonucleotide includes a translation stop codon and the recognition site for *PstI* endonuclease.

The PCR reaction was performed at 94°C for 15 s, at 48°C for 30 s and at 68°C for 1 min and 30 s, extension 20 s/cycle for 30 cycles.

The amplified product was digested with *HaeIII* endonuclease to eliminate the sequence encoding the C-Terminus of the protein. The resulting fragment, corresponding to the sequence encoding amino acids 285–493, was purified by electroelution from 1.2% agarose gel.

S. solfataricus MT4 genomic bank was screened by colony hybridization in stringent conditions. Replica filters were used as a control of signal specificity. A 25-ng of the probe were labelled with [α-32P]dATP by random priming and freed of unincorporated dNTPs by G-50 gel filtration. The labelled probe (10^6 cpm/pmol) was incubated with the nylon membranes for 16 h at 68°C the hybridization mixture ($5 \times$ SSC, 0.1% SDS, $5 \times$ Denhardt's solution, 10 mM NaP pH 7, 100 μg/ml denatured salmon sperm DNA) according to Sambrook et al. [5]. Filters were washed with $0.1 \times$ SSC, 0.1% SDS at 58°C. Twenty-five positive clones out of 5×10^4 were identified and subsequently analyzed by Southern blot using the hybridization conditions described above. This analysis revealed inserts of three different sizes.

A clone designed p5bIIb, containing the largest insert of about 3 kb, was purified and selected for further analysis.

2.5. Costruction of the expression vector pTrcTDFE

In order to clone the entire TDFE sequence into pTrc99A expression vector, we created an *NcoI* site at the translation start codon of the gene, following this procedure.

The 1200 bp N-terminus fragment of the TDFE gene, starting from the ATG, was amplified by PCR using p5bIIb as template and the following oligonucleotides as primers.

Oligonucleotide N: 5′-GGCCCATGGTAATAG-GCACATATAGG-3′ containing the recognition site for *NcoI* endonuclease and sequence corresponding to the N-terminus of TDFE.

Oligonucleotide int C: 5′-ATTGCTGGCATG/ATATTGTTGN-3′ complementary to the sequence encoding amino acids 398–403.

The reaction mixture was heated for 5 min at 94°C, then subjected to the following thermal profile: 15 s at 94°C; 30 s at 45°C; 1 min and 30 s at 68°C, extension 20 s/cycle, for 30 cycles.

The amplified fragment was digested with *NcoI* and *SpeI* endonucleases and the fragment of 210 bp, encoding amino acids 1–70, was purified as described above.

The remaining portion of the TDFE gene ORF was obtained from p5bIIb genomic clone digested with *SpeI* and *BamHI* endonucleases. This fragment spans *SpeI* site at 210–215 and about 350 bp of 3′ flanking region.

The two fragments were ligated and inserted into pTrc99A plasmid, previously digested with *NcoI* and *BamHI* restriction endonucleases.

The recombinant clone, designed pTrcTDFE, was checked by restriction analysis and DNA sequencing. The pTrcTDFE was subsequently digested with *BlgII* and *BamHI* restriction enzymes, made blunt end, and ligated again to reduce 3′ flanking region to 40 bp.

2.6. Cloning of the TFE gene

The complete coding sequence of the TFE gene was amplified, using as template 200 ng of genomic DNA from *S. solfataricus* strain MT4.

The following oligonucleotides derived from the nucleotide sequence of TFE from *S. solfataricus* KM1 were used: Oligonucleotide AAA-N: 5′-GCGT-TCATGACGTTTGCTTATAAAATAG-3′ containing the recognition site for *RcaI* endonuclease that encode a first Met residue, followed by sequence

encoding the N-terminus of TFE. Oligonucleotide AAA-C: 5′-CCCGGATCCTAAAGTTTA-TATAAAGCA-3′ containing the recognition site for *BamHI* endonuclease and sequence complementary to the one encoding C-terminus of TFE.

The thermal profile was the same utilized in the construction of pTrcTDFE expression vector. Amplified DNA (TFE) was made blunt by filling in with the Klenow fragment, in the presence of dNTPs. TFE gene was digested with *RcaI* and *BamHI*, purified from preparative 1% agarose gel and cloned in pTrc99A, previously digested with *NcoI* and *BamHI* enzymes. The recombinant expression vector was designed pTrcTFE.

2.7. Microorganism and cultivation

E. coli Rb791 competent cells were transformed with pTrcTDFE and pTrcTFE expression vectors and grown at 37°C to different cells densities in 500 ml of Luria–Bertani medium. Isopropyl-β-D-thiogalactoside (IPTG) was added at 1 mM final concentration and the induction time was varied from 8 to 18 h.

2.8. Enzyme assays

The TDFE and TFE were assayed under the same standard conditions at 75°C, in 50 mM citrate phosphate buffer pH 5.5 on different substrates at a concentration of 0.67 mM. TDFE activity was determined by incubating maltohexaose in the standard mixture with 1–10 μg of enzyme for 30 min. The reaction, linear for at least 2 h, was stopped in an ice–water bath and the amount of product formed, trehalosylmaltotetraose, was determined by high-pressure liquid chromatography (HPLC). One unit was defined as the amount of enzyme, which produces 1 μmol/min of trehalosylmaltotetraose.

TFE activity was determined by incubating trehalosylmaltotetraose in the standard mixture with 0.1–1 μg of enzyme for 30 min. The reaction, linear for at least 2 h, was stopped in an ice–water bath and the products formed, maltotetraose and trehalose, were determined by HPLC. One unit was defined as the amount of enzyme, which produces 1 μmol/min of maltotetraose.

2.9. Protein assay

Protein concentration was determined using the Bradford's method [6], with bovine serum albumin (BSA) as the standard. Absorbance at 280 nm was used to monitor protein concentration in column eluates.

2.10. Electrophoresis

The molecular mass of the enzyme was estimated at 10% SDS-PAGE by the method of Laemmli [7]. Carbonic anhydrase (bovine erythrocyte, 29 kDa), egg albumin (hen egg, 45 kDa), BSA (66 kDa), phosphorylase b (rabbit muscle, 97.4 kDa), β-galactosidase (*E. coli*, 116 kDa) and myosin (porcine muscle, 205 kDa) were used as molecular weight standard.

2.11. HPLC

The quantitative determinations of the substrate and products in the reaction mixtures were performed by a Dionex Chromatograph, equipped with an electrochemical detector, using a Carbopac PA 1 column. The elution was carried out with the following gradient: NaOH 160 mM (Buffer A) and, sodium acetate 300 mM (Buffer B), ($t = 0$ min 0% Buffer B; $t = 6$ min 0% Buffer B; $t = 36$ min 60% Buffer B).

2.12. Purification of the enzymes

Crude extracts were submitted to progressive and selective thermoprecipitations in order to separate the recombinant enzymes from *E. coli* proteins.

2.13. EcTDFE purification

2.13.1. Step 1: extraction

Wet cells (5 g) were suspended in 15 ml of 50 mM citrate phosphate buffer pH 5.5 and treated in a French Press disruptor (2000 psi). Cell debris were removed by centrifugation at $10\,000 \times g$ for 1 h.

2.13.2. Step 2: heat treatment

The cell-free extract (10 ml, 29 mg/ml, 18 U/ml) was heated at 65°C for 10 min in a water bath and

centrifuged at 10 000 × *g* at 4°C for 30 min. The supernatant (8 ml, 5 mg/ml, 11.5 U/ml) was heated for 15 min at 70°C. The enzyme preparation was then centrifuged at 10 000 × *g* at 4°C for 30 min. The supernatant (6 ml, 2.5 mg/ml, 13 U/ml) concentrated twice on a UF module (PM-10; Amicon) was heated again for 15 min at 70°C. The sample was then centrifuged at 10 000 × *g* at 4°C for 30 min.

2.13.3. Step 3: Sephacryl S-200 column chromatography

The supernatant (1.5 ml, 4 mg/ml, 40 U/ml) was dialyzed against 200 ml of 10 mM Tris–HCl buffer pH 8 and filtered through a Sephacryl S-200 column (1.5 × 56 cm) with 0.2 M NaCl in the same buffer. Active fractions contained about 2 mg (57 U) of purified *Ec*TDFE.

2.14. EcTFE purification

2.14.1. Step 1: extraction

Wet cells (5 g) were suspended in 15 ml of 50 mM citrate phosphate buffer pH 5.5 and treated in a French Press disruptor (2000 psi). Cell debris were removed by centrifugation at 10 000 × *g* for 1 h.

2.14.2. Step 2: heat treatment

The cell-free extract (16 ml, 9 mg/ml, 227 U/ml) was heated at 65°C for 10 min in a water bath and centrifuged at 10 000 × *g* at 4°C for 30 min. The supernatant (15 ml, 4.5 mg/ml, 240 U/ml) was heated for 15 min at 70°C. The enzyme preparation was then centrifuged at 10 000 × *g* at 4°C for 30 min. The supernatant (14 ml, 2.5 mg/ml, 255 U/ml) concentrated twice on a UF module (PM-10; Amicon) was heated again for 15 min at 70°C. The sample was then centrifuged at 10 000 × *g* at 4°C for 30 min.

2.14.3. Step 3: Sephacryl S-200 column chromatography

The supernatant (5 ml, 5 mg/ml, 710 U/ml) concentrated twice on a UF module (PM-10; Amicon) was dialyzed against 200 ml of 10 mM Tris–HCl buffer pH 8 and filtered through a Sephacryl S-200 column (1.5 × 56 cm) with 0.2 M NaCl in the same buffer. Active fractions contained about 11 mg (3510 U_{tot}) of purified *Ec*TFE.

3. Results and discussion

In recent years, a number of hyperthermophilic microorganisms of *Sulfolobales* have been found to produce trehalose from starch and dextrins and more recently, the genes encoding the TFE from *S. solfataricus* KM1 and *S. acidocaldarius* ATCC33909 have been cloned [8,9]. Amino acid sequences of *S. acidocaldarius* and *S. solfataricus* show approximately 50% similarity [10]. Southern analysis suggests that homologous genes are widely present in *Sulfolobales*. Herein, we report the cloning and expression of *S. solfataricus* MT4 genes encoding the TDFE and TFE; moreover, we describe a simple and efficient purification procedure of the two recombinant proteins.

A genome library of *S. solfataricus* MT4 was constructed by directional cloning of genomic DNA into plasmid pGEM 7zf(+) and screened for TDFE gene. This procedure was chosen since there was high efficient for the insertion of one single DNA fragment from *S. solfataricus* genome per vector molecule.

The isolated clone insert of about 3.0 kb contained the TDFE encoding sequence with an ORF of 2.199 bp and about 350 bp of both 5′ and 3′ flanking regions.

The TFE coding sequence of 1.686 bp was amplified from genomic *S. solfataricus* DNA by PCR.

Nucleotide sequences encoding TDFE and TFE are shown in Figs. 1 and 2, respectively. The deduced amino acid sequences of these enzymes indicate proteins with a length of 732 and of 561 amino acids, respectively. The calculated molecular mass, i.e., 86.5 kDa for TDFE and 64.4 kDa for TFE agree with those determined by SDS-PAGE for the purified natural enzymes (data not shown).

Analyzing the amino acid sequences of *S. solfataricus* MT4 trehalose producing enzymes can recognize sequences highly conserved of the α-amylase family and a different percentage of identity with the gene of the some enzymes isolated from other *Sulfolobales*. *S. solfataricus* MT4 TDFE shares 95% of

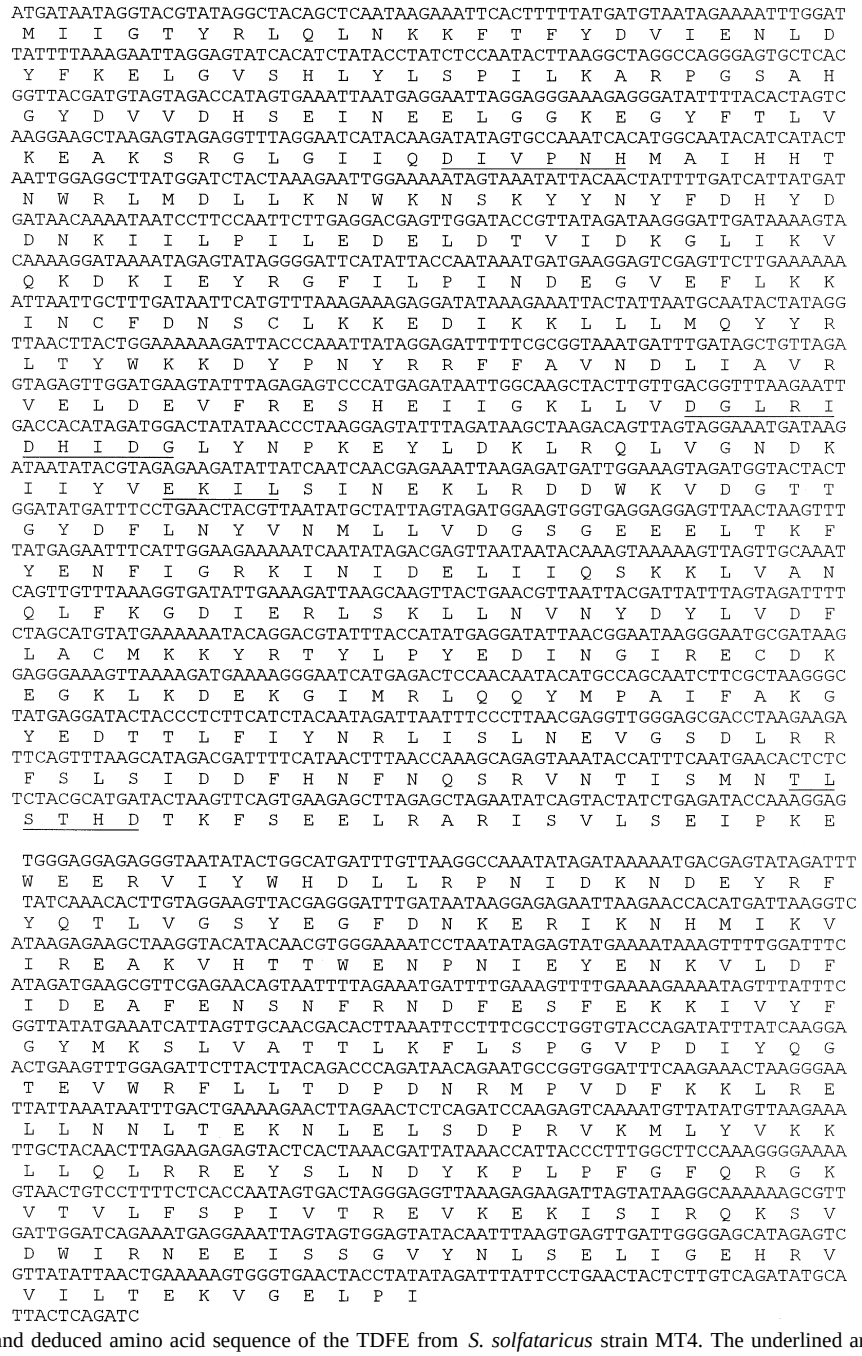

Fig. 1. Nucleotide and deduced amino acid sequence of the TDFE from *S. solfataricus* strain MT4. The underlined amino acids represent the highly conserved regions in the α-amylase family.

identity with the corresponding enzyme from *S. solfataricus* KM1 while 50% of identity is found toward *S. acidocaldarius* enzyme. The TFE sequence of *S. solfataricus* MT4 shares 80% and 60%

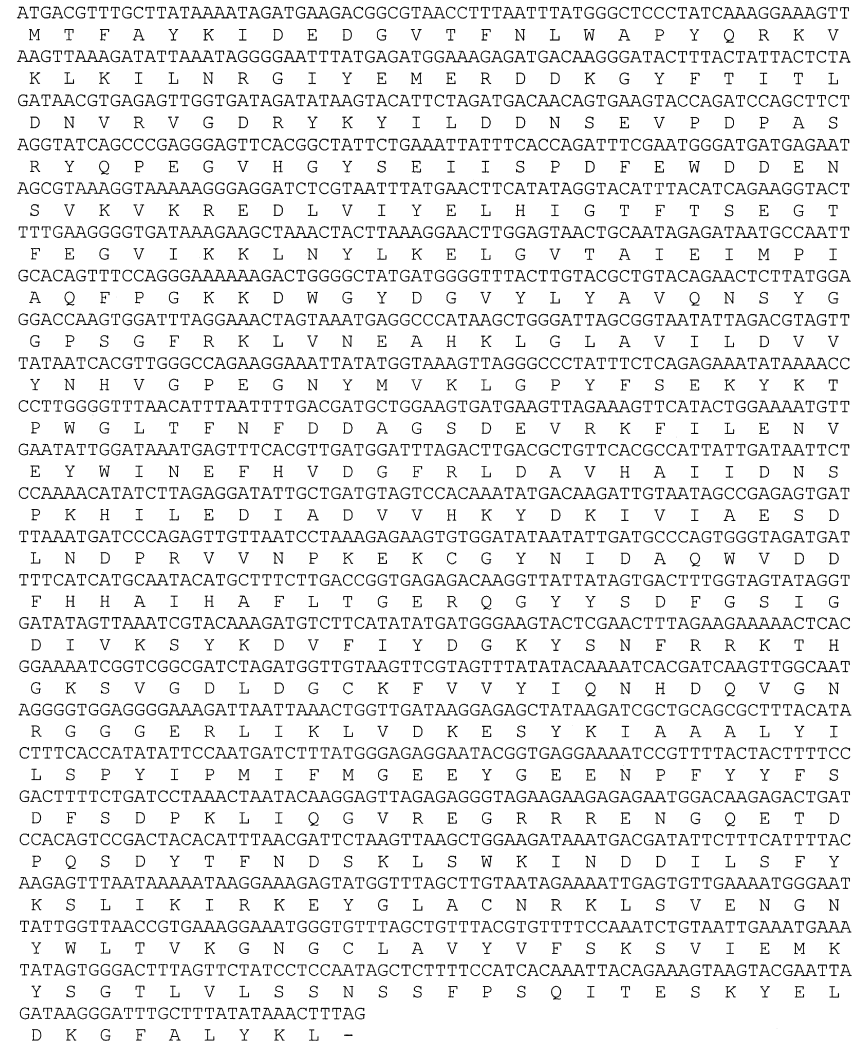

Fig. 2. Nucleotide and deduced amino acid sequence of the TFE from *S. solfataricus* strain MT4. The underlined amino acids represent the highly conserved regions in the α-amylase family.

of identity with the corresponding *S. solfataricus* KM1 and *S. acidocaldarius* enzymes, respectively.

3.1. Heterologous expression of the TFE and TDFE

The plasmid pTrcTFE showed high level expression of the protein in *E. coli*, after induction with IPTG indicating that they were not sequences interfering with the codon usage.

The yield of recombinant TFE was 3630 U/l, while the yield of the natural enzyme produced from *S. solfataricus* MT4 was about 3 U/l. The recombinant enzyme represented more than 50% of the soluble proteins after the first heat treatment of the cell-free extract and the removal of the denatured *E. coli* proteins.

IPTG treatment of the *E. coli* strain RB791 transformed with pTrcTFE yielded more than two-fold increase of enzyme content. On the contrary, TDFE protein was not expressed at high level and the IPTG induction of *E. coli* transformed with pTrcTDFE failed to increase the synthesis of the recombinant

Table 1
Purification procedure of TDFE from *E. coli* Rb791

Step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification factor	Yield (%)
Crude extract	180	290	0.620	1	100
Heat treatment	60	6	10	16	34
S-200 gel filtration	57	2	28.5	46	32

enzyme. A number of other different expression systems, in which the synthesis of the TDFE exogenous protein was under the control of a strong promoter, such as T7 and Pλ gave poor results. The highest level of protein expression, however, was obtained with pTrc99A vector in the absence of IPTG induction. The yield of TDFE produced in *E. coli* was 180 U/l, while *S. solfataricus* strain MT4 yielded 1 U/l.

3.2. Purification of TDFE and TFE

TDFE and TFE recombinant proteins were purified to homogeneity from the cell-free extract of *E. coli* as described in Materials and Methods. Tables 1 and 2 summarize the results of the purification procedures. Both proteins were purified by heat treatments followed by a gel filtration chromatography (Sephacryl S-200). The purification factor was of 46.5-fold for TDFE and of 12-fold for TFE, with a recovery of 31% for TDFE and of 96% for TFE.

The two enzyme preparations were homogenous since both gave a single protein band on SDS-PAGE (not shown). Specific activity of pure enzyme was 28.5 units/mg of protein for TDFE and 319 units/mg of protein for TFE.

3.3. Characterization and properties of TDFE

The effects of pH and temperature on TDFE activity and stability are shown in Fig. 3. The enzyme showed a pH optimum of 5.0, and it remained stable in the range of pH between 4.5 and 10.5 (Fig. 3a), with an apparent optimal activity temperature at 75°C. The enzyme was quite stable at temperatures up to 85°C (Fig. 3b).

These properties are quite similar to those determined for the purified enzymes from natural sources.

3.4. Characterization and properties of TFE

The pH and temperature effects on TFE activity and stability are shown in Fig. 4. The enzyme showed a pH optimum of 5.0, was stable in the range of pH between 4.5 and 10.5 (Fig. 4a), had an apparent optimal activity temperature at 85°C and was quite stable at temperatures up to 85°C (Fig. 4b). These properties are quite similar to those determined for the purified enzymes from natural sources.

Table 2
Purification procedure of TFE from *E. coli* Rb791

Step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification factor	Yield (%)
Crude extract	3632	144	25	1	100
Heat treatment	3550	25	142	5.6	98
S-200 gel filtration	3510	11	319	13	96

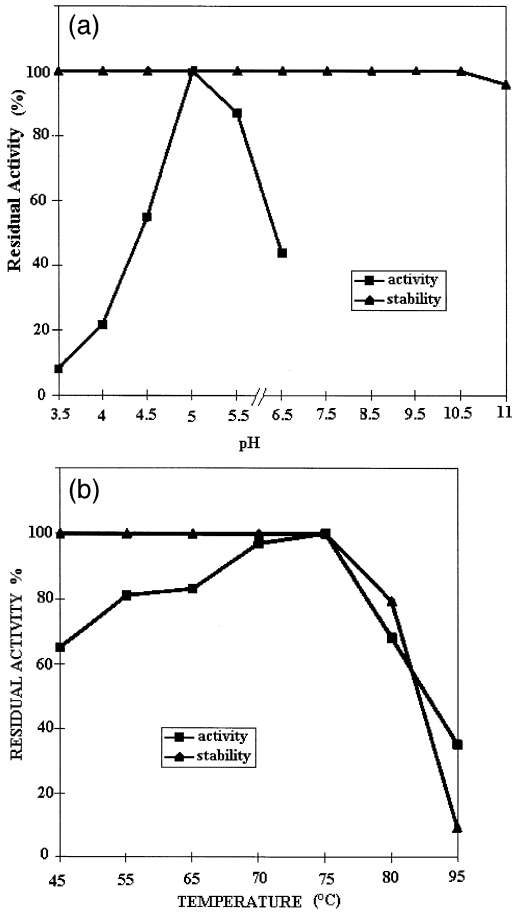

Fig. 3. Effects of pH and temperature on the activity and stability of TDFE. (a) Effects of pH. The enzyme (0.6 U/ml) was assayed under standard conditions using the following buffers: 50 mM citrate–phosphate buffer (pH 3.5–6.5), 50 mM Tris–HCl buffer (pH 7.5–8.5), and 50 mM $NaHCO_3$–Na_2CO_3 buffer (pH 9.5–11). To determine pH stability, the enzyme (2 U/ml) was incubated in the appropriate buffer at 4°C for 24 h and the residual activity was measured under standard conditions. (b) Effects of temperature. The enzyme (0.6 U/ml) was incubated at different temperatures under standard conditions. Thermostability was determined by incubating the enzyme (1 U/ml) at pH 5.0 for 120 min at different temperatures and measuring the residual activity at 75°C.

3.5. Combined action of TDFE and TFE on maltodextrins

Since the two enzymes showed similar physicochemical parameters, their combined use for trehalose production was possible. The concerted action of the TDFE and TFE enzymes, using maltodextrins as substrate allowed a cyclic process, in which the terminal trehalose molecules were removed from the non-reducing end, converting the whole glycosidic chains into trehalose. For maltodextrins having an odd number of glucose residues, the end product was a maltotriose, whereas maltose was obtained from maltodextrins with an even number of glucose residues.

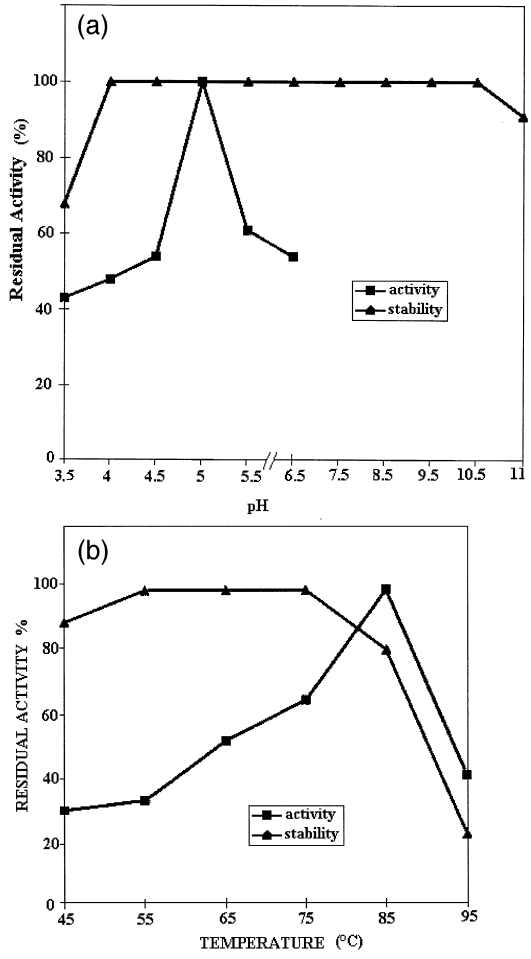

Fig. 4. Effects of pH and temperature on the activity and stability of TFE. (a) Effects of pH. The enzyme (0.3 U/ml) was assayed under standard conditions using the following buffers: 50 mM citrate–phosphate buffer (pH 3.5–6.5), 50 mM Tris–HCl buffer (pH 7.5–8.5), and 50 mM $NaHCO_3$–Na_2CO_3 buffer (pH 9.5–11). To determine pH stability, the enzyme (0.5 U/ml) was incubated in the appropriate buffer at 4°C for 24 h and the residual activity was measured under standard conditions. (b) Effects of temperature. The enzyme (0.3 U/ml) was incubated at different temperatures under standard conditions. Thermostability was determined by incubating the enzyme (0.5 U/ml) at pH 5.0 for 120 min at different temperatures and measuring the residual activity at 75°C.

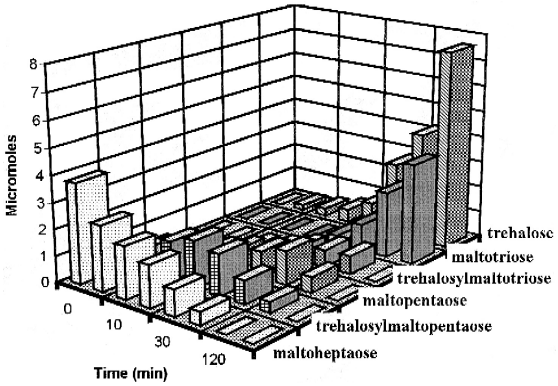

Fig. 5. Kinetic analysis of reaction using TDFE and TFE. A reaction mixture (0.750 ml) containing 5 mg of maltoheptaose and 0.036 U of each enzyme was incubated at 75°C, pH 5.5, for 3 h. Samples were withdrawn at different times and reaction products were analyzed by HPLC.

Analysis of the reaction kinetics was performed using maltoheptaose as substrate (Fig. 5) of the two enzymes. After 20 min of the reaction trehalosylmaltopentaose, the first product, was the most abundant compound. After 3 h, the final products of the enzymatic process, maltotriose and trehalose were present, as expected, in a 1:2 molar ratio.

From the results discussed above, it is clear that these two proteins are of great interest for the production of trehalose and non-reducing maltodextrins.

4. Conclusions

Two thermophilic and thermostable enzymes that convert starch and dextrins into trehalose have been cloned and expressed in *E. coli* with high yields. Furthermore, the two recombinant proteins have been purified and characterized. For their properties, the two enzymes could be used for the production of trehalosyl dextrins and trehalose at high temperatures at high yields.

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