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Molecular Characterization and Sequence Analysis of Trehalose Biosynthesis Genes in *Escherichia coli*

SREEVIDHYA Lawrance ANBU RAJAN* Yeragudipati RAJALAKSHMI Vetrivel UMASHANKAR
School of Biosciences, Department of Bioinformatics, SRM University, Ramapuram, Chennai, Tamil Nadu, INDIA

*Corresponding Author
e-mail: anburajanl@yahoo.co.in

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Abstract

Trehalose, a non-reducing disaccharide is a major compatible solute, maintains fluidity of membranes and protects the biological structure of the organisms under stress. In this study, trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) genes encoding for trehalose biosynthesis were PCR amplified from genomic DNA of Escherichia coli isolated from Rohu (Labeo rohita) collected from local fish market of Cochin, Kerala, India. The amplified genes were cloned and nucleotide sequences were determined. The sequencing results showed that (TPS) and (TPP) genes contain1,424 bp and 801 bp long ORF encoding 474 and 266 amino acids respectively. In silico sequence analysis revealed that the (TPS) and (TPP) sequences of E. coli were conserved in many eubacteria.

Key words: trehalose-6-phosphate synthase, trehalose-6-phosphate phosphatase, compatible solutes, salt stress

INTRODUCTION

Trehalose is a nonreducing disaccharide, having two glucose units are linked in a, α-1, 1-glycosidic linkage. Present in a wide variety of organisms, including bacteria, yeast, fungi, insects, invertebrates, lower and higher plants, where it serves as a source of energy and carbon [1]. Trehalose has the remarkable ability to protect plants from damage [2]. In yeast and plants, it serves as a signaling molecule to direct or control certain metabolic pathways [3]. In addition, trehalose also protects the proteins and cellular membranes from inactivation or denaturation caused by variety of stress conditions including desiccation, heat, cold, and oxidation [4]. In E. coli, trehalose biosynthesis reactions are catalyzed by trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP), encoded by the otsA and otsB gene respectively [3, 4]. E. coli synthesize trehalose only when exposed to high osmolarity [5-8]. This is different from Saccharomyces cerevisiae, in which trehalose is synthesized by a large multisubunit complex with the catalytic activities of both otsA and otsB [9-11]. In mycobacteria and corynebacteria, trehalose is an integral component of various glycolipids that are important cell wall structures. Trehalose stabilizes enzymes, foods, cosmetics, and pharmaceuticals at high temperatures [12-14]. Due to its desirable physical and chemical characteristics, commercial production of trehalose is anticipated. In this study, for the first instant we report the molecular characterization and in silico sequence analysis of TPP and TPS genes in E. coli isolated from fish. Moreover, the sequence analysis of trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase from our isolate shows several base substitutions with that of reported sequences in GenBank, resulting in the altered amino acid sequences of the translated protein structures.

MATERIAL AND METHODS

Bacterial strain, growth conditions and DNA isolation

E. coli TCJAR023 was isolated from a finfish; Rohu (Labeo rohita) procured from local fish markets in Cochin, India. Microbial identification and biochemical characterization of E. coli was carried out as per [U.S. Food and Drug Administration (USFDA)] methods. E. coli was grown aerobically in Luria-Bertani (LB) broth medium containing 1% tryptone, 0.5% yeast extract, 0.5% NaCl and incubated at 37°C. Genomic DNA of E. coli was prepared as described by [15].

Polymerase chain reaction

The trehalose biosynthesis genes were amplified by using gene specific primers. The PCR reaction was done in a final volume of 50 µl that contained 0.5 µM each of forward and reverse primers; 1.0 µl of crude genomic DNA; 200 µM of dNTPs; 1X Pfu buffer; 2.5 mM MgSO4; 1.25U Pfu DNA polymerase (MBI Fermentas, USA) and autoclaved Millipore water. The PCR was performed using a PTC-150 Mini cycler (MJ Research, USA) with the following conditions; initial denaturation at 94°C for 3 min, followed by 30 repeated cycles of 94°C for 30 sec, 50°C for 1 min and 72°C for 2 min and final extension at 72°C for 5 min. The PCR amplified product was analyzed on 1.5% agarose gel along with DNA ladder (MBI Fermentas) and documented using a gel documentation system (Alpha Imager 1220, Alpha Innotech Corporation, San Leandro, CA, USA).

Cloning of PCR product

The amplified gene fragments were purified with MinElute Gel purification Kit (Qiagen, Hilden, Germany) and cloned into pDrive (Qiagen), according to the manufacture's instructions. The cloned inserts were transformed into the E. coli JM109 and plated on LB agar containing ampicillin (100 µg/ml), IPTG (50

μM) and X-gal (80 μg/ml). The plates were incubated at 37°C and the transformants were selected by blue white selection. The white recombinant colonies were selected and inoculated in 5 ml LB broth containing ampicillin. The plasmids were isolated from the overnight culture by alkaline lysis method [16].

Characterization of the recombinant plasmid

The recombinant plasmid was double digested with restriction enzymes, EcoRI & HindIII for (TPS) and SmaI & HindIII for (TPP). The reaction mixture contained recombinant plasmid 2 μl, enzyme buffer (10X) 2 μl, each restriction enzyme (10U/μl) 0.5 μl and volume up to 20 μl with autoclaved Millipore water. The reaction mixture was incubated overnight at 37°C in a water bath. The digested products were analyzed on 1.5% agarose gel. The clone with the correct insert as judged by size was sequenced on an ABI PRISM 377 genetic analyzer (Applied Biosystems, Perkin Elmer Co., Foster City, CA, USA).

In silico sequence analysis

The nucleotide sequences obtained were compared against database sequences using BLAST provided by NCBI (http://www.ncbi.nlm.nih.gov) and were aligned and clustered using CLUSTAL-X version 1.81 program [17]. The output alignments were imported into the GeneDoc program (http://www.psc.edu/biomed/genedoc/) and BioEdit version 7.05 program (www.mbio.ncsu.edu/BioEdit/) to calculate the percent identities among the nucleotide and amino acid sequences. The molecular masses and the theoretical pI values of the polypeptides were predicted using the ProtParam tool (http://www.expasy.org/tools/protparam.html).

RESULTS

The TPS and TPP genes encode the trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase respectively. Together these proteins constitute the trehalose biosynthetic pathway. The TPS and TPP genes were PCR amplified and is encoded by polynucleotides of 1,424 bp and 801 bp (Figure 1). The TPS and TPP genes encodes proteins of 474 and 266 amino acids with the calculated molecular masses of 53,611 and 29,177 Da., based on the in silico estimates. After PCR amplification, the products were purified from the agarose gel and cloned into pDrive cloning vector. The white colonies were selected and screened for the presence of insert, which also exhibited the specific product by PCR amplification. The recombinant transformants with TPS and TPP genes were also confirmed by double digestion with restriction enzymes. The nucleotide sequence of inserts TPS and TPP genes were submitted to Genbank and have been given accession nos. EU070413 and EU070412.

Homology search of the genes and deduced amino acid sequence were performed using BLAST. The nucleotide sequence of TPS and TPP genes matches significantly with the trehalose biosynthesis genes from other organisms. The TPS and TPP sequences from E. coli TCJAR023 were analyzed with reported nucleotide and amino acid sequences of other eubacteria viz. Shigella sonnei (GenBank accession no. CP000038), Pseudomonas savastanoi (AY308799), Salmonella enterica (AE017220) using Clustal W software. In silico analysis of the nucleotide sequences of TPS and TPP genes showed a high degree of similarity with other eubacteria. The amino acids analysis revealed that the TPS gene encoded protein belongs to the trehalose-6-phosphate synthase family. It had partial homology with phosphate synthase family from other bacteria: S. enterica, 86% identity; P. savastanoi, 99% identity and S. sonnei with 100% identity. In silico sequence analysis also revealed an amino acid substitution at position 153 in P. savastanoi; resulted in the substitution of phenylalanine (F) with leucine (L) residue. In S. enterica, various amino acid substitutions were observed in N-terminal, middle and C-terminal regions (Figure 2). The amino acid analysis of TPP gene suggests that the encoded protein belongs to the trehalose-6-phosphatase family. The TPP of E. coli TCJAR023 had highly conserved regions with phosphate phosphatase from other bacteria. S. enterica sequence has 73% identity; 98% identity and S. sonnei with 99% identity. The sequence analysis of otsB revealed various amino acid substitutions with that of other eubacteria. Upon analysis a total of two amino acid substitutions were found in S. sonnei at positions 124 and 208, resulted in the amino acid substitution of threonine (T) with alanine (A). In P. savastanoi, a total of four amino acid substitutions were observed at positions 8, 116, 124 and 208 resulted in the substitution of threonine (T) with proline (P); glutamine (Q) with arginine (R); threonine (T) with alanine (A) and alanine (A) with valine (V) residue respectively. In S. enterica, various amino acid substitutions were observed in N-terminal, middle and C-terminal regions as predicted in TPS (Figure 3).

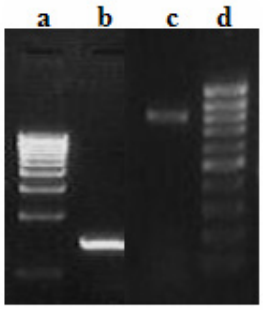

Figure 1.

Figure 1: Agarose gel electrophoresis of PCR products of TPS and TPP. Lane a, 1 kb DNA marker; Lane b, TPS amplicon 1,425 bp; Lane c, TPP amplicon 801 bp; Lane d, 100 bp DNA marker.

Phylogenetic tree based on evolutionary distances was constructed from nucleotide and amino acid sequences of TPS and TPP genes using MEGA software

(Molecular Evolutionary Genetics Analysis, version 3.1) [18] using the neighbor-joining method. The tree at nucleotide and amino acid level of TPS and TPP (data not shown) reveals the phylogenetic similarity of TPS and TPP genes from E. coli TCJAR023 with other organisms. The bacterial species switched to different clusters for TPS and TPP genes at nucleotide and amino acid level indicating divergence among the organisms and the degree of divergence between the two genes.

DISCUSSION

Trehalose (1-α-D-glucopyranosyl-1-α-D-glucopyranoside), a non-reducing disaccharide is a major compatible solute, which maintains fluidity of membranes and protects the biological structure of organisms under stress. Trehalose represents the predominant class of osmolytes in aerobic chemoheterotrophic eubacteria [7]. Trehalose has many potential applications in biotechnology including increased stress tolerance of

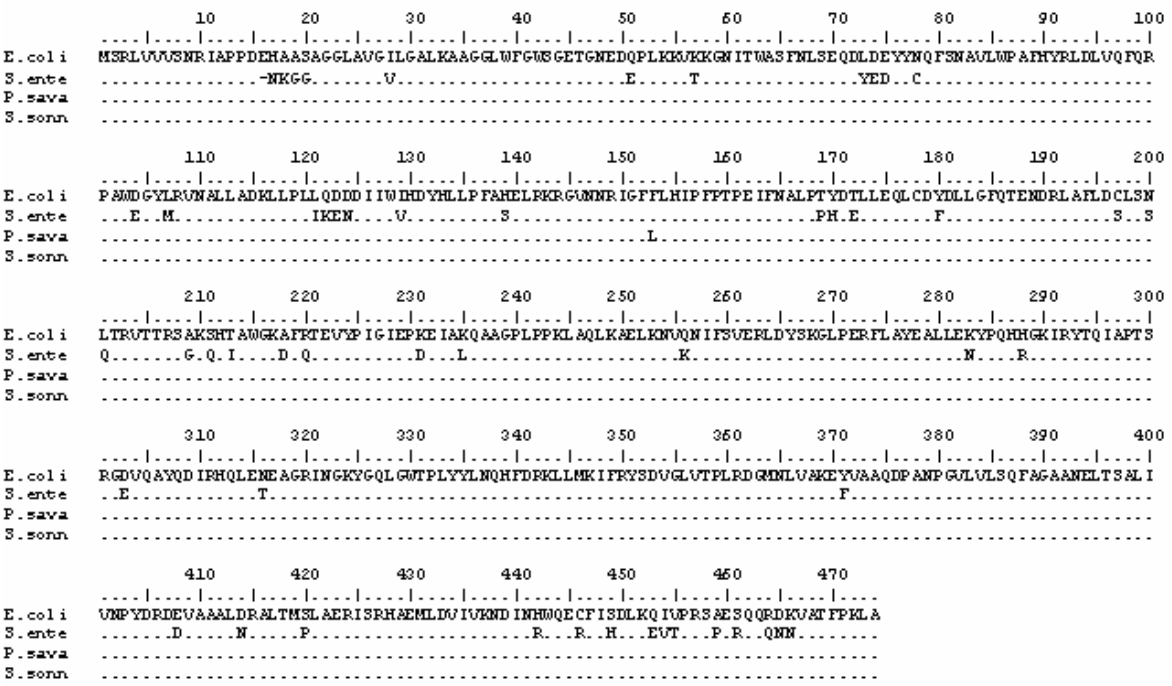

Figure 2: Alignment of the amino acid sequence of TPS gene from E. coli TCJAR023 with sequences from Salmonella enterica, Pseudomonas savastanoi and Shigella sonnei. Dots indicate amino acid identity and letters represents amino acid substitutions.

important crops, stability of recombinant proteins, etc., as well as in the food industry [19]. The biosynthetic pathway for trehalose comprises two steps, the first being the conversion of glucose from UDP-glucose to glucose 6-phosphate to form trehalose-6-phosphate and UDP. This is followed by de-phosphorylation of trehalose-6-phosphate to trehalose and orthophosphate. The trehalose biosynthesis involved two genes TPS and TPP. The presence of these two genes was confirmed by PCR amplification of genomic DNA from E. coli TCJAR023. The fragment sizes of 1,424 bp and 801 bp were obtained in amplification. Restriction analysis also showed the similar pattern. Finally, the sequences of the genes were confirmed by sequencing. The translated protein of TPS and TPP genes has 474 and 266 amino acids, with the isoelectric points of 6.37 and 5.38 respectively. The predicted molecular weights were 53 KDa and 29 KDa respectively. The amino acids analysis of TPS gene reveals that the encoded protein belongs to the trehalose-6-phosphate synthase family. It had minimum conserved regions with phosphate synthase from other eubacteria. The amino acid analysis also showed that the amino acid sequence of S. sonnei and P. savastanoi has the maximum identity of 100% and 99% respectively with that of E. coli TCJAR023. The sequence analysis of TPS revealed that amino acid residues of E. coli TCJAR023 were well conserved with S. sonnei amino acids. These results revealed that, the catalytic activity of trehalose-6-phosphate synthase was similar in both organisms. The amino acid analysis of TPP gene suggests that the encoded protein belongs to the trehalose-6-phosphatase family. It had highly conserved regions with phosphate phosphatase from other bacteria. The amino acid analysis suggests that S. sonnei has the maximum identity of 99% with the amino acid sequence of E. coli TCJAR023. The other organism's also accounted considerable similarity; P. savastanoi with 98% and S. enterica with 73% identity. The sequence analysis revealed that, the amino acid residues of TPP in E. coli TCJAR023 were partially conserved with S. sonnei, S. enterica and P. savastanoi sequences.

Based on phylogenetic analysis, it was found that

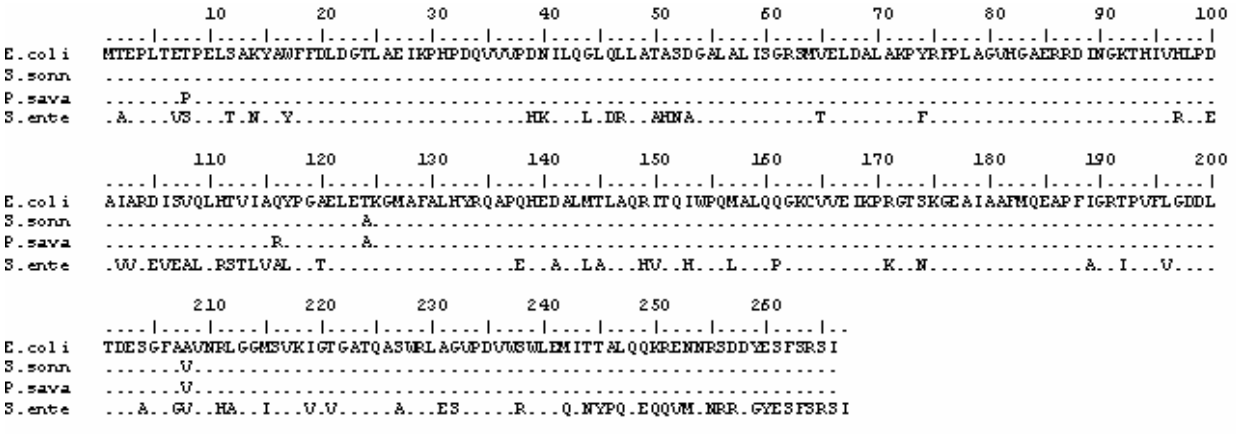

Figure 3.

Figure 3: Alignment of the amino acid sequence of TPP gene from E. coli TCJAR023 with sequences from Salmonella enterica, Pseudomonas savastanoi, Shigella flexneri and Shigella sonnei. Dots indicate amino acid identity and letters represents amino acid substitutions.

the E. coli TCJAR023 and S. sonnei were clustered together whereas, P. savastanoi and S. enterica form separate clusters for both in nucleotide and amino acid sequences of TPS gene. The phylogenetic tree analysis of amino acid sequences from TPP gene showed that S. sonnei and P. savastanoi were clustered together while, E. coli TCJAR023 and S. enterica form separate clusters in nucleotide and amino acid level. From the phylogenetic grouping of nucleotide and amino acid sequence between the different organisms, it was inferred that E. coli TCJAR023 had comparatively higher conserved sequences both at nucleotide and amino acid level for the genes involved in trehalose biosynthesis.

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