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Short communication

Cloning of a trehalose-6-phosphate synthase gene from *Exopalaemon carinicauda* and its expression response to bacteria challenge

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ABSTRACT

Trehalose, a nonreducing disaccharide, is present in a wide variety of organisms and plays a key role in many organisms under different stress conditions. In the study, the full-length cDNA sequence encoding trehalose-6-phosphate synthase (EcTPS) was obtained from *Exopalaemon carinicauda*. The complete nucleotide sequence of *EcTPS* contained a 2532 bp open reading frame (ORF) encoding a putative protein of 843 amino acids. The domain architecture of the deduced EcTPS contained a glycol_transf_20 domain and a trehalose_PPase domain. *EcTPS* mRNA was predominantly expressed in the hepatopancreas. The expression of *EcTPS* in the prawns challenged with *Vibrio parahaemolyticus* and *Aeromonas hydrophila* changed in a time-dependent manner. The function of *EcTPS* was also studied by double-strand RNA interference. The results showed that the knock-down of *EcTPS* increased the mortality of the *Vibrio*-challenged group and *Aeromonas*-challenged group compared with the control group. The present study provides some new insight into the immune function of the trehalose-6-phosphate synthase in prawns.

1. Introduction

Trehalose is a nonreducing disaccharide in which two glucose molecules are linked together in a 1,1-glycosidic linkage [1]. This sugar is present in a wide variety of organisms, including bacteria, yeast, fungi, insects, invertebrates, and lower and higher plants [1,2]. Trehalose synthesis in insects and other invertebrates is thought to occur via the trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) pathways [3]. The most widely distributed pathway of trehalose synthesis in the organism involves the transfer of glucose from UDP-glucose to glucose 6-phosphate, and then form trehalose-6-phosphate and UDP via TPS [1]. It is well documented in decapod crustaceans that they usually utilize glucose for energy metabolism and employ an immediate shift of energy metabolism during initial adaptation in response to changes in environmental conditions, exercise or migration [4]. Trehalose protects bioactive substances and cell structures, such as proteins, nucleic acids, and biological membranes, under adverse environmental stresses, such as drought, freezing, oxidation, high salt, high temperature and low temperature [3,5,6]. To better clarify the structure and function of TPS, a lot of TPS genes have been cloned from animals, plants, and microorganisms [3,7–13]. In animals, the TPS genes were mainly focused on the species of arthropods, especially in insects [14,15]. At present, there are only two TPS genes reported in Crustacean, including *Callinectes sapidus* [2,4] and Chinese shrimp, *Fenneropenaeus chinensis* [16].

In our previous research [16], the full-length cDNA of TPS (*FcTPS*) was cloned from Chinese shrimp *F. chinensis* and its expression profiles were also obtained when the shrimp were challenged with WSSV or *Vibrio*. Recently, Zhang et al. [14] cloned TPS gene (*MdTPS*) from *Musca domestica* and confirmed that *MdTPS* acted as an inducible anti-stress gene taking part in immune defense in *M. domestica*.

As we know, there is no model animal in Crustacean to be used in basic research [17]. In our laboratory, *E. carincauda* was used as an experimental animals in basic research and it could be maintained with reproductive capacity all the year round in the laboratory environment with an about 60-day reproduction cycle [18]. In our previous research, we succeeded in developing a high efficient microinjection method in *E. carinicauda* and deleting the interest gene using CRISPR/Cas9 technology [19–21]. In addition, Yuan et al. [22] performed the low-coverage sequencing and *de novo* assembly of the *E. carinicauda* genome which exhibited potential for the genomic and experimental research of decapods.

In this research, the full-length cDNA of TPS (*EcTPS*) was cloned from *E. carinicauda* and its expression profiles were also obtained when the prawns were challenged with *Vibrio parahaemolyticus* and *Aeromonas hydrophila*.

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2. Materials and methods

2.1. Experimental animals and immune challenge

E. carinicauda with body length of 5.5 ± 0.5 cm were bred in tanks filled with aerated fresh seawater at 24–26 °C, 30 ppt salinity, and fed twice per day with fresh clam meat. Fifteen healthy adult *E. carinicauda* were dissected to epidermis, eyestalk, gill, hepatopancreas, heart, intestine, muscle, nerve, and stomach. Then, the samples were preserved in liquid nitrogen for RNA extraction [23].

According to our previous report [17], the prawns with the same size were challenged with *V. parahaemolyticus* or *A. hydrophila*. Experimental groups and the control group were set up for each sampling point (0, 12, 24, 48, 72, 96, 120 h) and 200 prawns were sampled from each group. For the bacterial challenge experiment, the experimental group was injected individually with 10 μL phosphate buffer saline (PBS) containing *V. parahaemolyticus* or *A. hydrophila* (10^7 CFU mL^{-1}). Each prawn was injected intramuscularly into the last abdominal segment. At the same time, the prawns injected with 10 μL sterile PBS were maintained as the control. The hepatopancreas of five prawns from each group were collected at 0, 12, 24, 48, 72, 96, and 120 h. All the samples were preserved in liquid nitrogen for RNA extraction.

2.2. RNA isolation, cDNA synthesis and bioinformatic analysis

Total RNA was extracted from the collected samples with Trizol® reagent (Thermo, USA). Then, the extracted RNA was treated with RQI RNase-Free DNase (Promega, USA). Two micrograms of total RNA and 0.2 μM random hexamer primers were used to synthesize cDNA by M-MLV reverse transcriptase (Promega, USA).

Based on the transcriptomic and genomic data of *E. carinicauda* [22], the full-length *TPS* sequence of *E. carinicauda* (*EcTPS*) was confirmed by reverse transcription-polymerase chain reaction (RT-PCR). The nucleotide sequence and deduced amino acid sequence of EcTPS were analyzed by BLAST on-line (http://www.ncbi.nlm.nih.gov/BLAST/). The characteristic structure of deduced EcTPS was predicted by the simple modular architecture research tool (SMART) program (http://smart.embl-heidelberg.de/). Genomic organization was clarified by the Genewise tool (http://www.ebi.ac.uk/Wise2/index.html). The multiple sequence alignments and phylogenetic analysis were performed using CLUSTAL W and MEGA 7.0 [24].

2.3. Quantitative real-time PCR (qRT-PCR) analysis of EcTPS mRNA expression

Quantitative real-time PCR (qRT-PCR) [25] was used to analyze *EcTPS* distribution in different tissues of *E. carinicauda* and its expression profiles at different sampling time in the hepatopancreas using Mastercycler ep realplex (Eppendorf). 18S rRNA was used as the internal control. Primers are shown in Table 1. The expected size of *EcTPS* and 18S rRNA was 143 bp and 147 bp in length, respectively. The PCR products were firstly sequenced to confirm the specificity and effectiveness of primers for qRT-PCR. The qRT-PCR for *EcTPS* and 18S rRNA was performed according to the program of 40 cycles of 95 °C for 15 s, 55 °C for 20 s and 72 °C for 20 s, following by an extension of 72 °C for 10 min. The data were analyzed using the comparative C_T method and then subjected to one-way ANOVA using SPSS 19.0. The p values less than 0.05 were considered statistically significant.

2.4. Synthesis of double strand RNA (dsRNA) and gene silencing of EcTPS

According to information of *EcTPS* cDNA full-length sequence, primers (shown in Table 1) with T7 promoter sequence to amplify cDNA fragment of about 458 bp were designed. Meanwhile, primers (shown in Table 1) with T7 promoter sequence to amplify cDNA fragment of 289 bp for enhanced green fluorescent protein (*EGFP*) were also designed [26]. Then, the corresponding dsRNA for *EcTPS* and *EGFP* was synthesized using TranscriptAid T7 High Yield Transcription Kit (Thermo Fisher Scientific, USA). Double strand RNAs were purified by phenol-chloroform method and assessed by electrophoresis on 1% agarose gel.

In order to optimize the silencing efficiency of dsRNA, healthy prawns were randomly divided into four groups (three experiment groups and one control group). *EcTPS* and *EGFP* dsRNAs were injected into muscle of each prawn, respectively. Three concentration gradients including 1 μg, 2 μg, and 4 μg for each individual were set to detect the efficiency. Results of *EcTPS* transcriptional level in hepatopancreas at 48 h after dsRNA injection showed that all the 3 concentration gradients had significant silencing effects. Dosage of 2 μg was chosen to inject into each prawn for further RNAi experiment.

After optimization for the dsRNA dosage, 2 μg *dsEcTPS* dissolved in 10 μL PBS were injected into each healthy prawn. At 24 h after *dsEcTPS* injection, the prawns were divided into three groups including *dsEcTPS/Vibrio* group, *dsEcTPS/Aeromonas* group, and *dsEcTPS/*PBS group. The prawns of *dsEcTPS/Vibrio* and *dsEcTPS/Aeromonas* group were injected individually with 10 μL phosphate buffer saline (PBS) containing *V. parahaemolyticus* or *A. hydrophila* (10^7 CFU mL^{-1}). At the same time, the prawns of *dsEcTPS/*PBS group were injected with 10 μL sterile PBS as the control. The residual prawns were calculated at 0, 12, 24, 48, 72, 96, and 120 h.

3. Results

3.1. Characterization of EcTPS

Based on the transcriptomic and genomic data of *E. carinicauda* [22], the full-length cDNA sequence of *EcTPS* was obtained with 3338 bp (GenBank accession no. MK896805). The nucleotide sequence of *EcTPS* contains a 2532 bp open reading frame (ORF) encoding a putative protein of 843 amino acids (Fig. 1 A). The deduced protein has a predicted molecular weight (MW) about 95367.93 Da and theoretical isoelectric point (pI) of 6.35. The deduced EcTPS contains a

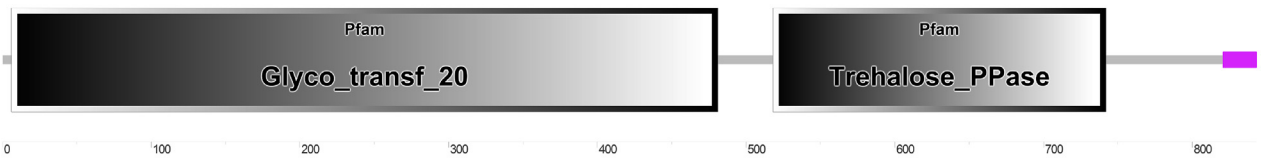
Table 1
Primers mentioned in the paper.

Primers	Sequences (5′- 3′)	Sequence information
EcTPSF	GGTACTTCCGGTATGTCTT	Real-time PCR
EcTPSR	TGACTTGTCTGTATGCCTC	Real-time PCR
18S-F	TATACGCTAGTGGAGCTGGAA	Real-time PCR
18S-R	GGGGAGGTAGTGACGAAAAAT	Real-time PCR
EcTPST7F	TAATACGACTCACTATAGGGCTGTACGAAGCCCTTCACGC	*In vitro* transcription
EcTPST7R	TAATACGACTCACTATAGGGGTCTAATGTGTATTGTGCCT	*In vitro* transcription
EGFP-F	TAATACGACTCACTATAGGGGTTGCCCCAGTGGTCGTG	*In vitro* transcription
EGFP-R	TAATACGACTCACTATAGGGAATGGAATGTGAAGGAAGAATG	*In vitro* transcription

Note: F and R stand for forward primers and reverse ones, respectively.
The underline sequences showed a T7 RNA polymerase promotor.

```
1    CGGGGGGGAACATTGTGAGTTGCTATCAAGAACCGTGAGAAAGAAAGAGAGAGGAGATCTAGAAAGGCACTTCTACAAATCTAATCGCTAGAAACTGCAGCAAATCTAATCACAAGAGTG 120
121  GAAGCAAGTTACTAATAGCAAAAATGGTGTTGTCAACATCACCTATGGTTGTCGTAGCCAACCGGTTACCATTCATCCTGGCTAAGGACAGCAAAGGCCAACTAGTTAGAAAACAATGCG 240
                          M  V  L  S  T  S  P  M  V  V  V  A  N  R  L  P  F  I  L  A  K  D  S  K  G  Q  L  V  R  K  Q  C
241  CTGGTGGACTGGTCACAGCCGTTGCCCCAGTGGTCGTGGAAACCGAGGGACTCTGGGTTGGCTGGTCAGGGCTACACGAAGAGGACAGCACAGGAGAAATTCCTGAAGCCGATCCTAACG 360
     A  G  G  L  V  T  A  V  A  P  V  V  V  E  T  E  G  L  W  V  G  W  S  G  L  H  E  E  D  S  T  G  E  I  P  E  A  D  P  N
361  ACCAGTCTCCCACTGCAGGACTCAAGAGTAAACAGGTACTTCCGGTATGTCTTCCAAAGAAAAAGTTTGATGATTATTACAATGGTTGTTGCAATGCAACATTTTGGCCCCTTTTCCACT 480
     D  Q  S  P  T  A  G  L  K  S  K  Q  V  L  P  V  C  L  P  K  K  K  F  D  D  Y  Y  N  G  C  C  N  A  T  F  W  P  L  F  H
481  CCATGCCTGATCGAGCTGTATTCCAAGCTGATAAGTGGGAGGCATACAGACAAGTCAATGAAGAGTTCGCCATGCTGACTGTTGAAGCCGTGAAGAAACTGGTAAACAGTAACCCCGAGT 600
     S  M  P  D  R  A  V  F  Q  A  D  K  W  E  A  Y  R  Q  V  N  E  E  F  A  M  L  T  V  E  A  V  K  K  L  V  N  S  N  P  E
601  GCATTCCACTAGTCTGGCTTCATGATTACCACCTGATGATGGCTGCCAACACCATCAGAGAACGCTGTGATGAGCTCGGATTACCCATAAAGATGGCATTCTTCCTTCACATTCCATTCC 720
     C  I  P  L  V  W  L  H  D  Y  H  L  M  M  A  A  N  T  I  R  E  R  C  D  E  L  G  L  P  I  K  M  A  F  F  L  H  I  P  F
721  CCTCTTGGGACATCATGCGCCTCTTCCCCTGGGATGACGAGCTGCTACAAGGCATCCTCGGCTGCGACTCAGTTGGTTTCCATGTGGAAGACTACTGCTTGAACTTCATCGACTGCTGTC 840
     P  S  W  D  I  M  R  L  F  P  W  D  D  E  L  L  Q  G  I  L  G  C  D  S  V  G  F  H  V  E  D  Y  C  L  N  F  I  D  C  C
841  AGCGACGCCTTGGATGTCGAGTGGATCGTCAACAGATGCTGGTCGAGCATAACAATCGAAGTGTGTCGGTGCATCCTCTCCCAATCAGTATACCTTATGAGAGGTTCGTAAATTTGGCAG 960
     Q  R  R  L  G  C  R  V  D  R  Q  Q  M  L  V  E  H  N  N  R  S  V  S  V  H  P  L  P  I  S  I  P  Y  E  R  F  V  N  L  A
961  AAAAGGCTCCTCAGGTGGTGAAAAATCACGATCAAGAGCAACTTCTCCTGGGAGTTGACAGACTAGATTACACTAAGGGCCTGGTTCATAGAATCAAAGCCTTTGAAACTCTACTTCAAA 1080
     E  K  A  P  Q  V  V  K  N  H  D  Q  E  Q  L  L  L  G  V  D  R  L  D  Y  T  K  G  L  V  H  R  I  K  A  F  E  T  L  L  Q
1081 AGCATCCAGAGCACATTGAACATGTTACTTTCTTGCAAGTGGCTGTCCCATCTCGTACTGATGTGAAAGAATACCAAGAACTAAAAGAAGAACTGGATCAACTTATCGGTCGTATTAATG 1200
     K  H  P  E  H  I  E  H  V  T  F  L  Q  V  A  V  P  S  R  T  D  V  K  E  Y  Q  E  L  K  E  E  L  D  Q  L  I  G  R  I  N
1201 GACAATTCTCCACTCCAAACTGGTCTCCCATTCGTTACATCTATGGTTGTGTATCCCAAGATCAACTGGCAGCTTTTTATCGTGACTCATCTGTAGCTGTTGTGACCCCACTCAGGGATG 1320
     G  Q  F  S  T  P  N  W  S  P  I  R  Y  I  Y  G  C  V  S  Q  D  Q  L  A  A  F  Y  R  D  S  S  V  A  V  V  T  P  L  R  D
1321 GCATGAATCTTGTTGCCAAGGAATTCGTCGCTTGCCAGACTGGAGAACCAGGAGTACTAATTCTATCTCCGTTTGCTGGTGCTGGAACTACAATGCATGAAGCTCTTCTAGTTAACCCTT 1440
     G  M  N  L  V  A  K  E  F  V  A  C  Q  T  G  E  P  G  V  L  I  L  S  P  F  A  G  A  G  T  T  M  H  E  A  L  L  V  N  P
1441 ATGAAACTAATGAATTTGCTGAAGTAATCCATCGTGCATTAACAATGCCTAAGGATGAGCGAGAGTTGCGAATGAAACAGCTGAGACGCAGAGAACGCGAGAGGGATGTCAATTTCTGGC 1560
     Y  E  T  N  E  F  A  E  V  I  H  R  A  L  T  M  P  K  D  E  R  E  L  R  M  K  Q  L  R  R  R  E  R  E  R  D  V  N  F  W
1561 TGCGTTCCTTCCTCAAAACTGTGGATTGTCTATCGGATGATTCTGTAACTCAGGGACGTCTACAACCTTTGACAGAAGAAGATTTCAGTCAGTTCTTATCTTCATATGTCACAGAATCAT 1680
     L  R  S  F  L  K  T  V  D  C  L  S  D  D  S  V  T  Q  G  R  L  Q  P  L  T  E  E  D  F  S  Q  F  L  S  S  Y  V  T  E  S
1681 CTCGTCTGGCTCTTCTTCTCGATTACGATGGTACATTGGCTCCCATTGCCCCTCATCCTGACCTTGCTAGAATGCCTAGTGAAACGCGTCACGTCTTGGAAAAGCTAGCTCATATGCCTG 1800
     S  R  L  A  L  L  L  D  Y  D  G  T  L  A  P  I  A  P  H  P  D  L  A  R  M  P  S  E  T  R  H  V  L  E  K  L  A  H  M  P
1801 ATGTTAATGTTGCTATTATTTCTGGACGTTCTCTTCAAAATGTTAAATCGATGGTTGGTATTGAAGGCATCACATATGCTGGTAGCCATGGTTTTGAAATCCTTCATCCAGATGGCACAC 1920
     D  V  N  V  A  I  I  S  G  R  S  L  Q  N  V  K  S  M  V  G  I  E  G  I  T  Y  A  G  S  H  G  F  E  I  L  H  P  D  G  T
1921 TATTCATGCATCCTATACCCCATGAATACGAGGTCCAGTTAGAAACCCTAAAACAACGTCTTCAGGAAGTGAGCACTGACGGGGCATGGGTTGAGGTCAAGCAAACAGGAATTACCTTTC 2040
     L  F  M  H  P  I  P  H  E  Y  E  V  Q  L  E  T  L  K  Q  R  L  Q  E  V  S  T  D  G  A  W  V  E  V  K  Q  T  G  I  T  F
2041 ACTACCGTGAAGTTACCGCAGCAAAATATTCATCCATTACCTCGCGTGCTCAAGAAATTTTCAGTGAAGTCGGCATAAAGATCCACCAGTCCCACAAAGCTTACGAAGCGCGTCCACCAG 2160
     H  Y  R  E  V  T  A  A  K  Y  S  S  I  T  S  R  A  Q  E  I  F  S  E  V  G  I  K  I  H  Q  S  H  K  A  Y  E  A  R  P  P
2161 TGACGTGGGATAAGGGCCGCGCTGCCATCTACATACTTCGCAGTCTTTTTGGTCTTGACTGGTGTGATCGGGTATCAACTATATACGCGGGAGATGACAAAACCGATGAAGATGCTATGC 2280
     V  T  W  D  K  G  R  A  A  I  Y  I  L  R  S  L  F  G  L  D  W  C  D  R  V  S  T  I  Y  A  G  D  D  K  T  D  E  D  A  M
2281 GAGCTCTACAGGGAATGGCCGTTACCTTCAGAGTGACCACTTCACAGATTCTGCGTACAGCTGCCAGTCATCGCCTTCCAAATACTGATGCTGTGTTGACTATGCTCAAGTGGGTTGAAA 2400
     R  A  L  Q  G  M  A  V  T  F  R  V  T  T  S  Q  I  L  R  T  A  A  S  H  R  L  P  N  T  D  A  V  L  T  M  L  K  W  V  E
2401 GAAGACTTGGGGCTAGATTACCAAATAACTCTAACGGTTTCAGACAAAGAACATTCAGTGCTTCAAGCCATGGTAACCATTCACCCCCAATTCACCCTTCGACTCCACAAAATCGTTCTC 2520
     R  R  L  G  A  R  L  P  N  N  S  N  G  F  R  Q  R  T  F  S  A  S  S  H  G  N  H  S  P  P  I  H  P  S  T  P  Q  N  R  S
2521 GAACAAACTCTATGTCGAAGCCAATGGGGGAACCAACTAAACAGATGATGGTCATCACTGATAAAGTCTACCATGACCTCATCTCCAGGAAATCGTCTCCTCCCAGATGTTCCAGTACCT 2640
     R  T  N  S  M  S  K  P  M  G  E  P  T  K  Q  M  M  V  I  T  D  K  V  Y  H  D  L  I  S  R  K  S  S  P  P  R  C  S  S  T
2641 CTCCTCCACACTCTCCAACCAGATCTACAGTTTGAACTCGTTTTAACTGTGCTGAAGATTGGTTTTGCAATTCGCGTGCCTTAGCAGTTGGTTCTTTCTAAGGCATTGAGAGTTCTTAGG 2760
     S  P  P  H  S  P  T  R  S  T  V  *
2761 TGTGATTGGCCTTAGTTTCGTGGTTCCCCCCATGGTGCAGAGGTTAGCCCCTTTTCACTCCGGAGGACATGAAACAGTCCTCTCATCTAGGGACGTTTTCATGTACAAATTTCTAGAACG 2880
2881 TTTTATATCATCATATTATTTATGAGGCTTGACGCAAAGCCTTGCTCATTAGGTAAATGTACATAGACAATATTTTATGATCCAAAGAGTGATGCTTGCTCAGAGTTTTCAAATAAATCA 3000
3001 TAAATTTATTTATATATTTAGAATTAATGGGGTATTAAATATAATCATTAATATCTTTTTATCTCGAGGAGATTTCACTTTGTATACTCTCAGATGTGTTATTTGTAGATTTATCAAATT 3120
3121 TCTGAAATATAATTTTGCCTAGTTTATGAGAAAGTACAATTCTGGTGAATGAATCTTTATACTGTGTTGAAAGTGATAAATAAAAATCAGTAAATTTATAATGTTAGTTAAAGAGTCGTG 3240
3241 CAACCAAATTTTGTATTTTTGTCTTTTTAAATATTTCGCTGAGTAAAAGGATTTACTGTGAATGACTGTGATAATTTTTTTTTATTATTAAATGGCAG 3338
```

(A)



(B)

Fig. 1. Nucleotide and deduced amino acid sequences of *EcTPS* gene (A) and the schematic representation of functional domain of deduced EcTPS (B). Nucleotides are numbered on the both sides of the sequence. The letters marked with single underline and double underline represented the Glyco_transf_20 and Trehalose_PPase domains respectively.

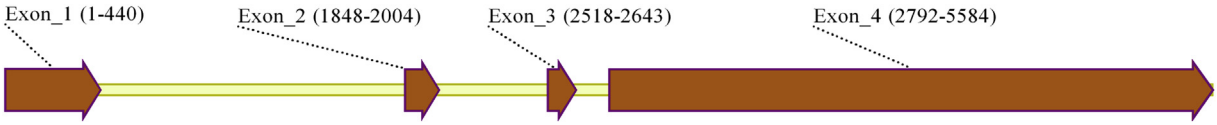


Fig. 2. Schematic representation of *EcTPS* gene structure.

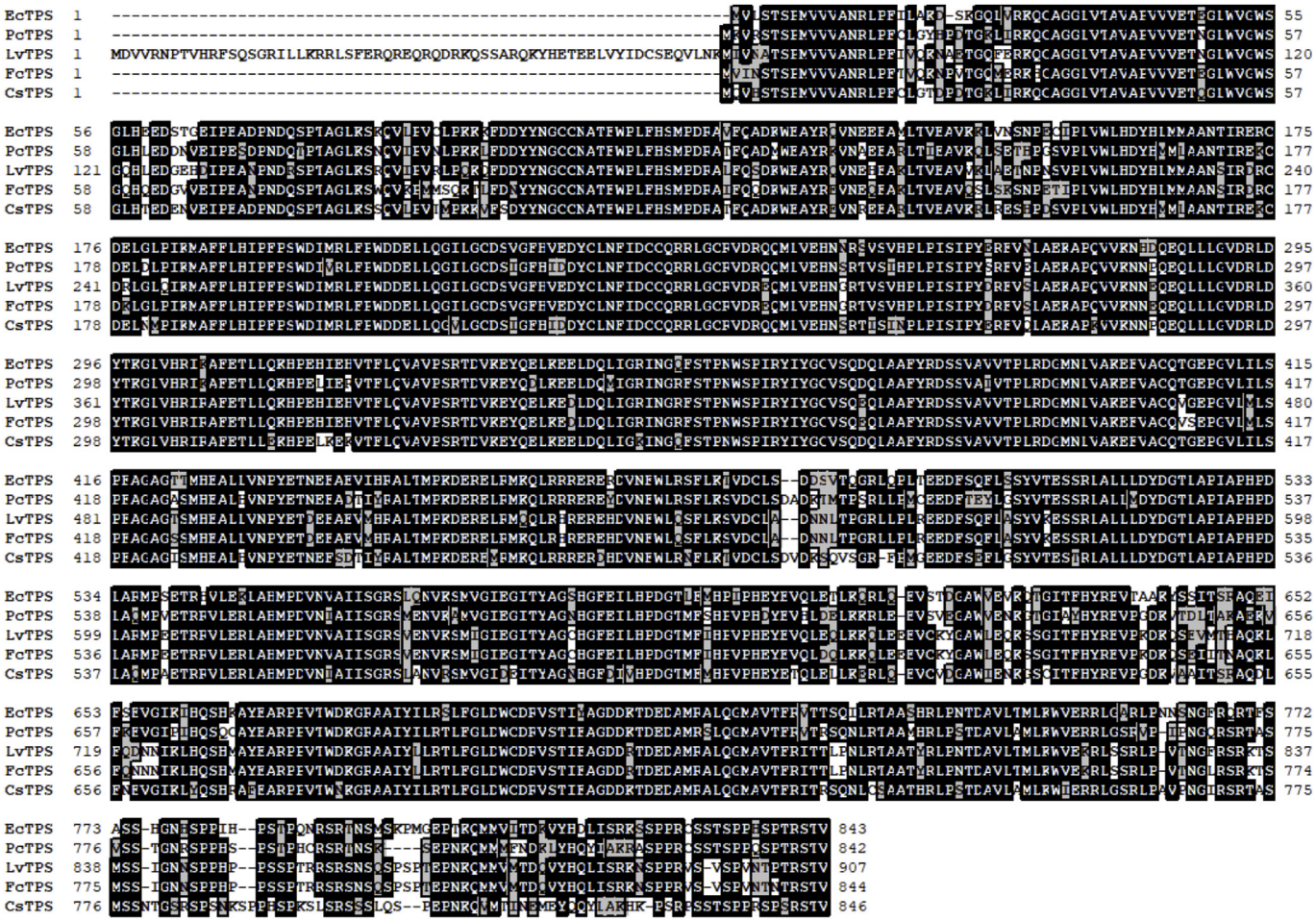


Fig. 3. Alignments of the amino acid sequences of EcTPS with known Decapoda TPSs. The identical residues are shown in solid boxes. Sequences start at the first methionine residue. *Procambarus clarkia* (PcTPS, GenBank accession no. ASW35095.1); *Litopenaeus vannamei* (LvTPS, ROT75322.1); *Fenneropenaeus chinensis* (FcTPS, ACD74843.1); *Callinectes sapidus* (CsTPS1, ACL00655.1); *Exopalaemon carinicauda* (EcTPS, MK896805, in this research).

glyco_transf_20 domain (residues 6–481) and a trehalose_PPase domain (residues 518–742) (Fig. 2A). No putative signal peptide is found. In addition, the genomic DNA fragment of *EcTPS* with the corresponding cDNA sequence was obtained, which showed that it is composed of four exons and three introns (Fig. 2). All intron-exon boundaries are consistent with the consensus splicing junctions at both the 5′ splice donor site (GT) and the 3' splice acceptor sites (AG) of each intron.

A multiple sequence alignment showed that EcTPS displayed high identities with that of *Litoenaeus vannamei* (LvTPS, 82%), *Procambarus clarkii* (PcTPS, 81%), *F. chinensis* (FcTPS, 81%), *C. sapidus* (CsTPS, 79%) (Fig. 3).

Amino acid sequences of TPS from different species were collected from the NCBI database and a phylogenetic tree was constructed using Neighbor-joining method (Fig. 4). The phylogenetic analysis showed that Arthropoda TPS could be divided into two groups, Malacostraca TPS and Insecta TPS. EcTPS was divided into the Malacostraca TPS branch (Fig. 4).

3.2. Tissue distribution of EcTPS

Expression profile of *EcTPS* in different tissues of *E. carinicauda* was examined by qRT-PCR (Fig. 5). It was predominantly expressed in hepatopancreas. Therefore, hepatopancreas were selected as the tissue to study the expression profile after the prawns were challenged with bacteria.

3.3. Time course of EcTPS expression after V. parahaemolyticus or A. hydrophila challenge

It had been reported that *TPS* from *F. chinensis* exhibited putative immune function against *Vibrio* [16]. Herein, we tried to study the immune function of *EcTPS* against different bacteria in the prawns and the expression of *EcTPS* in hepatopancreas of *E. carinicauda* was measured through the qRT-PCR method. The results showed that the expression of *EcTPS* in the prawns challenged with *V. parahaemolyticus* or *A. hydrophila* changed in a time-dependent manner (Fig. 6). Compared with the expression of *EcTPS* in the control group, the expression of *EcTPS* in the prawns challenged with *V. parahaemolyticus* was

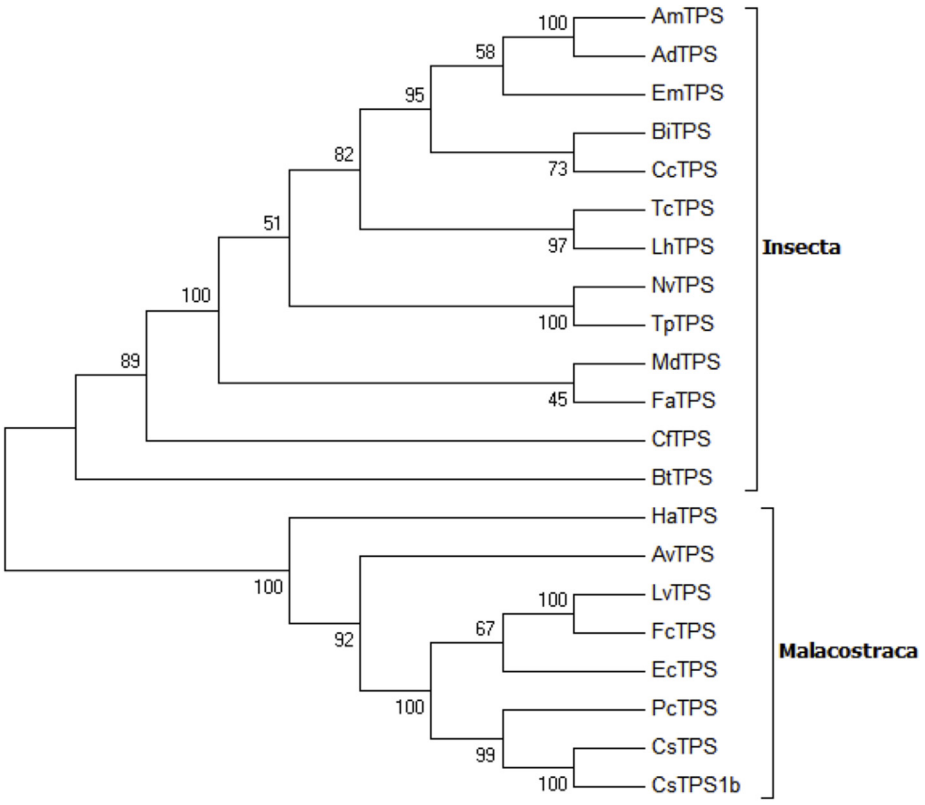


Fig. 4. Phylogenetic analysis of EcTPS showing relationship with other known TPSs. Alignment of amino acid sequences with CLUSTALW, consensus Neighbor-Joining tree with MEGA7.0. *Armadillidium vulgare* (AvTPS, RXG53447.1); *Callinectes sapidus* (CsTPS1b, ACL00657.1); *Hyalella azteca* (HaTPS, XP_018007795.1); *Bemisia tabaci* (BtTPS, XP_018915964.1); *Bombus impatiens* (BiTPS, XP_003490305.2); *Nasonia vitripennis* (NvTPS, XP_016837588.1); *Microplitis demolitor* (MdTPS, XP_008544498.1); *Trichogramma pretiosum* (TpTPS, XP_014221069.1); *Apis mellifera* (AmTPS, XP_026297280.1); *Linepithema humile* (LhTPS, XP_012234592.1); *Temnothorax curvispinosus* (TcTPS, XP_024871503.1); *Apis dorsata* (AdTPS, XP_006618859.1); *Fopius arisanus* (FaTPS, XP_011311569.1); *Eufriesea Mexicana* (EmTPS, XP_017752467.1); *Ceratina calcarata* (CcTPS, XP_017889688.1); *Ctenocephalides felis* (CfTPS, XP_026465719.1). Values on the line are bootstrap values showing percentage confidence of relatedness.

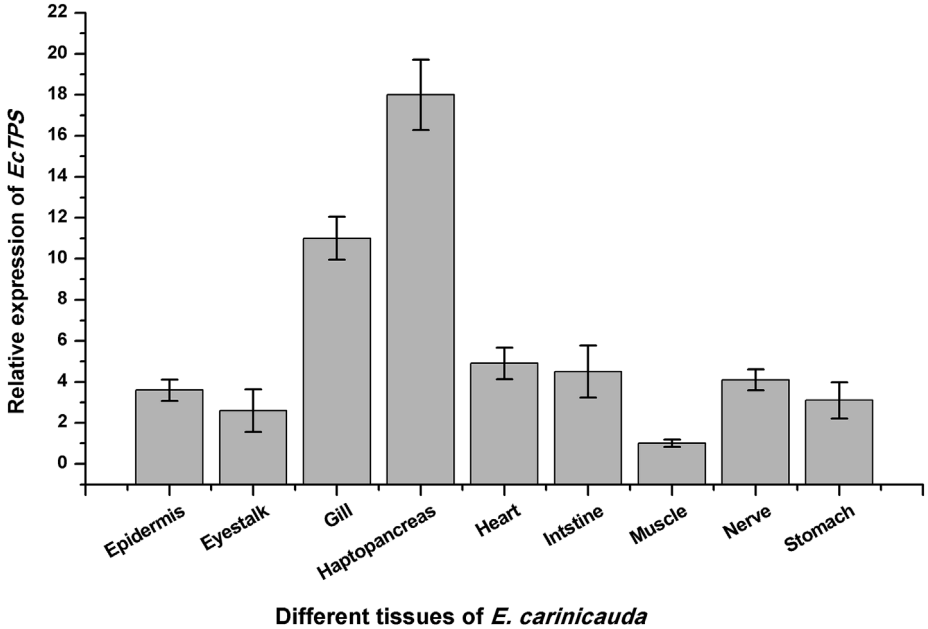


Fig. 5. Detection of *EcTPS* transcripts in the different tissues from healthy *E. carinicauda*. Tissues were shown in the abscissa. The amount of *EcTPS* mRNA was normalized to the 18S rRNA transcript level. Data are shown as means ± SD (n = 3).

significantly up-regulated at 12, 24 and 48 h post-injection ($p < 0.01$), at 72 h post-injection ($p < 0.05$), and then returned to the normal level at 96 h post-injection ($p > 0.05$). At the same time, the expression in *Aeromonas*-challenged group was significantly up-regulated at 12 h post-injection ($p < 0.01$), at 24 and 48 h ($p < 0.05$) and returned to the normal level at 72 h post-challenge ($p > 0.05$).

3.4. Effects of EcTPS interference on the survival of challenged prawns

Three concentration gradients including 1 μg, 2 μg and 4 μg were set to detect the silencing efficiency of ds*EcTPS*. The result showed that all the 3 concentration gradients had significant silencing effects and the dosage of 2 μg was chosen to inject into each prawn for further RNAi experiment (Fig. 7A). At 24 h after dsEcTPS injection, the prawns were challenged with *V. parahaemolyticus* or *A. hydrophila* to detect the effects of *EcTPS* interference on the mortality of pathogen-challenged

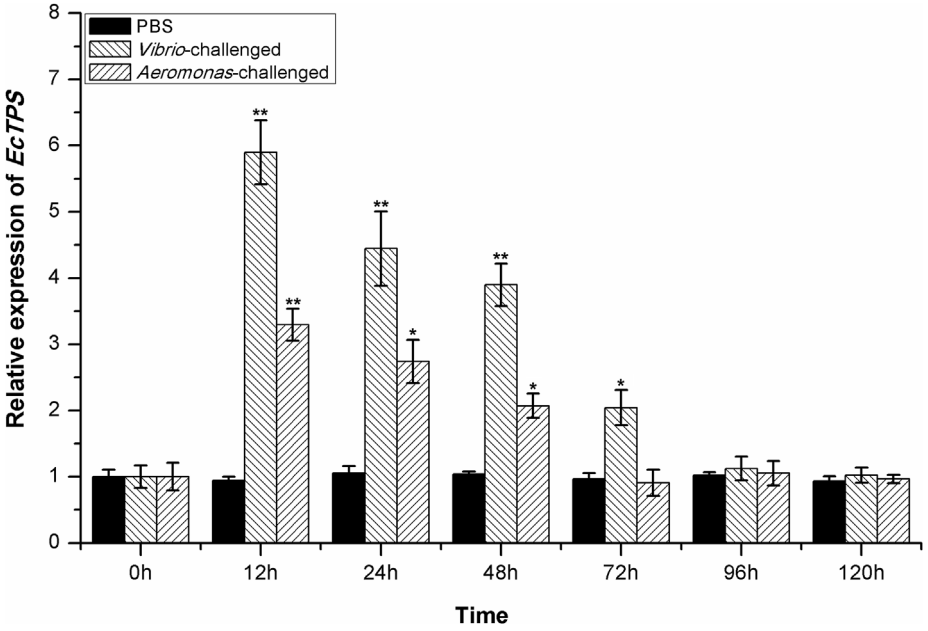


Fig. 6. Expression profiles of *EcTPS* in the hepatopancreas after the prawns were challenged with *V. parahaemolyticus* or *A. hydrophila* and equal volume of PBS at 0, 12, 24, 48, 72, 96, and 120 h. The amount of *EcTPS* mRNA was normalized to the 18S rRNA transcript level. Data are shown as means ± SD (n = 3).

prawns (Fig. 7B). The results of continuous 120 h observation showed the mortality of the *Vibrio*-challenged group and *Aeromonas*-challenged group reached 55% and 50% at 48 h post-challenge, respectively. However, the dsEcTPS/PBS group showed a cumulative mortality of 10%. Overall, these results indicated that the interference of EcTPS in *E. carinicauda* increased prawns' mortality following *V. parahaemolyticus* or *A. hydrophila* challenge.

4. Discussion

Trehalose is a common disaccharide of bacteria, fungi and invertebrates that appears to play a major role in desiccation tolerance [1]. In insects, trehalose is the major hemolymph sugar that is exclusively synthesized in the fat body in which hypertrehalosemic hormone (HTH) positively regulates its production [2]. In this research, the full-length cDNA and genomic DNA of Trehalose-6-phosphate synthase gene (*EcTPS*) was obtained from *E. carinicauda*. There are two functional domains (one Glyco_transf_20 domain and one Trehalose_Ppase domain) in the deduced EcTPS amino acid sequence. From the bioinformatic analysis, it is showed that EcTPS has the activity of glycosyltransferase and trehalose-phosphatases. Glycosyltransferase acts the biosynthesis of disaccharides, oligosaccharides and polysaccharides. Trehalose-phosphatase catalyzes the de-phosphorylation of trehalose-6-phosphate to trehalose and orthophosphate. Therefore, TPS in arthropods appears to be a fused protein of a homolog of Ost A and Ost B in *E.coli* [16].

In our previous researcher, one TPS gene *(FcTPS*) was firstly cloned from *F. chinensis* and its expression profiles were confirmed. In this research, *EcTPS* expressed in all over the tissues of *E. carinicauda*, which is consistent with what had found in *C. sapidus* and *F. chinensis* [2,16]. In addition, the result showed that the expression level of *EcTPS* was highest in hepatopancreas of *E. carinicauda* analyzed by qRT-PCR. It was reported that trehalose was exclusively synthesized in the fat body of insects [3]. As we know, the hepatopancreas in shrimp plays a similar function with the fat body of insects [16]. Therefore, the expression pattern of *TPSs* from different arthropod might be similar.

Recently, Zhang et al. [14] reported that TPS from *M. domestica* (*MdTPS*) was up-regulated following bacterial challenge by *Escherichia coli* or *Staphylococcus aureus* and speculated that *MdTPS* took part in immune defense in *M. domestica via* synthesizing its product trehalose. In shrimp, Wang et al. [27] used microarray technology to study differentially expressed genes in WSSV-infected Chinese shrimp and found a lot of immunity-related genes including *FcTPS*. The result of semi-quantitative PCR showed that the expression of *FcTPS* in shrimp tissues 6 h post-injection of WSSV increased about 15 times over that of the control [27]. In our previous research, we found that the expression of *FcTPS* in hepatopancreas was down-regulated significantly in response to the challenge of *Vibrio* at the early of 5 h post-challenge and then up-regulated significantly at 14 h [16].

As we know, bacteria are also key pathogen in fisheries. In this research, two kinds of gram-negative bacteria *V. parahaemolyticus* and *A. hydrophila* were used to challenge the prawns and the immune function of EcTPS against bacteria. The expression of *EcTPS* in hepatopancreas was significantly upregulated after *V. parahaemolyticus* or *A. hydrophila* challenge by qRT-PCR, which indicated that *EcTPS* might play a key role in immune defense against bacteria.

To furtherly clarify the immune function of EcTPS, the double-strand RNA of *EcTPS* was synthesized by *in vivo* transcription and injected into the prawns. Knock-down of *EcTPS* led to much higher mortality of prawns after *V. parahaemolyticus* or *A. hydrophila* challenge than the control group. The result of knock-down indicated that *EcTPS* might play a key role against bacteria.

Conflicts of interest

There is no conflict of interest.

Acknowledgements

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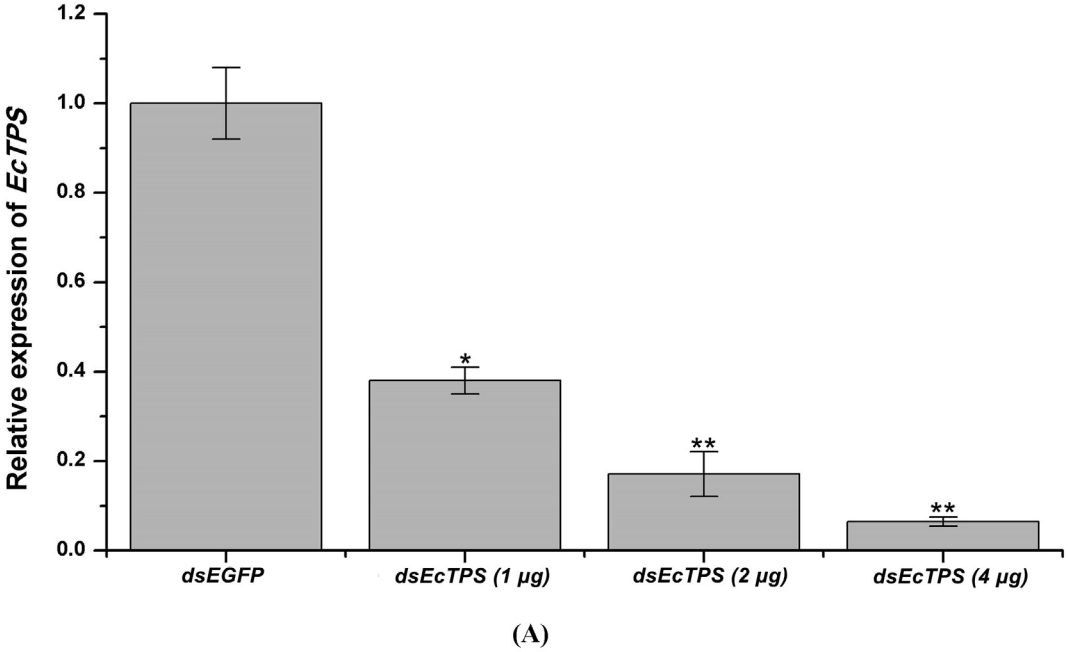
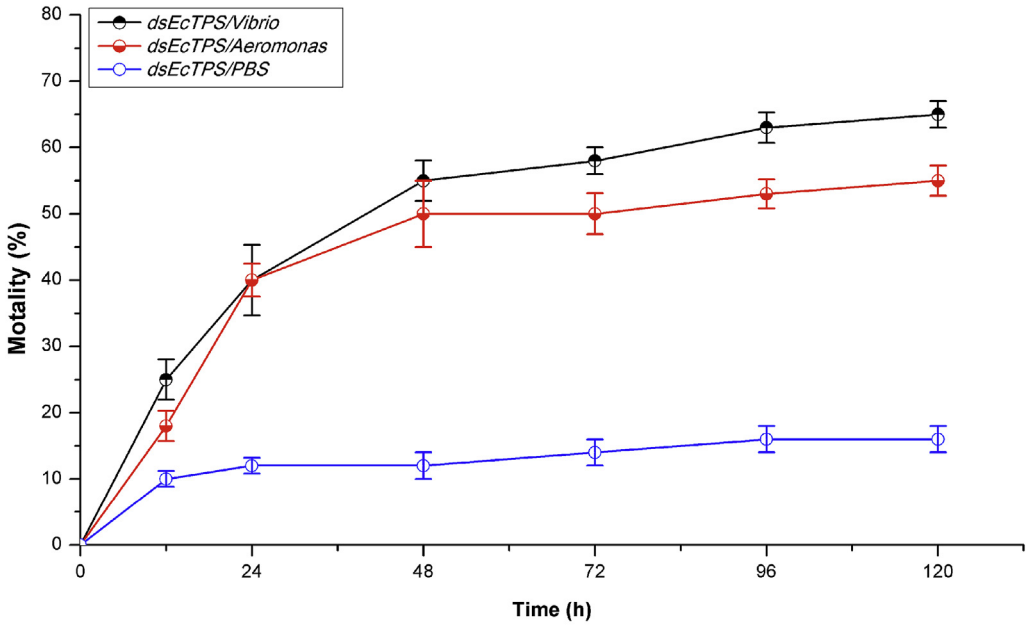


(A)



(B)

Fig. 7. The silencing efficiency of *EcTPS* (A) and the mortality of the *EcTPS*-silenced prawns after the prawns were challenged with *V. parahaemolyticus*, *A. hydrophila* and equal volume of PBS (B) at 0, 12, 24, 48, 72, 96, and 120 h.

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