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CHARACTERIZATION OF THE CDNA ENCODING MEMBRANE-BOUND TREHALASE, ITS EXPRESSION AND ENZYME ACTIVITY IN *BACTROCERA DORSALIS* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Trehalase plays a critical role in metabolic processes by catalyzing the hydrolysis of trehalose to glucose. In this study, we cloned and characterized a full-length cDNA encoding membrane-bound trehalase in the oriental fruit fly, *Bactrocera dorsali*s (Hendel) (Diptera: Tephritidae) and named it as *BdTre2*. *BdTre2* contained an open reading frame (ORF) of 1842 bp,which encoded a putative 613 amino acids. Alignment analysis revealed that the deduced protein sequence of BdTre2 had about a 70% identity to *Tre2* of most dipteran insects, suggesting that this gene was conserved in the evolution of the Diptera. Phylogenetic analysis showed that the deduced protein of *BdTre2* was more homologous to the trehalase2 gene than insect trehalase1 gene. The expression pattern in different developmental stages and tissues of *BdTre2* in *B. dorsalis* was measured by quantitative real-time PCR (qPCR), and the results showed that *BdTre2* was strongly expressed in the adult stage, particularly in the midgut. Concurrently, the enzyme activity assay showed that trehalase 2 was more active in the adult stage and Malpighian tubules than in other developmental stages or tissues. Our results provide evidence that *BdTre2* is involved in activity of the midgut, possibly in chitin synthesis and energy metabolism, and it is important for *B. dorsalis* adults.

Key Words: oriental fruit fly, membrane-bound trehalase, cloning, quantitative PCR, enzyme activity assay

RESUMEN

La trehalasa juega un papel crítico en los procesos metabólicos por catalizar la hidrólisis de trehalosa a glucosa. En este estudio, clonamos y caracterizamos la trehalasa destinada para la membrana por medio codificar el cADN de la mosca oriental de la fruta, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) que fue nombrado como *BdTre2*. El *BdTre2* contiene un marco de lectura abierto (MLA) de 1842 pb y codifica 613 aminoácidos putativos. El análisis de alineación reveló que la secuencia deducida de la proteína de *BdTre2* tiene un 70% de identidad conla *Tre2* de la mayoría de los insectos dípteros, lo que sugiere que este gen fue conservado en la evolución de los Diptera. El análisis filogenético mostró que la proteína deducida de *BdTre2* fue más homóloga con el gen de trehalasa 2 que al gen 1 de la trehalasa de insectos. Se midió el patrón de expresión en diferentes etapas de desarrollo y los tejidos de *BdTre2* en *B. dorsalis* por PCR cuantitativa en tiempo real (qPCR) y los resultados mostraron que *BdTre2* se expresó fuertemente en el estadio adulto y en particular en intestino medio. Al mismo tiempo, el ensayo de actividad de la enzima trehalasa 2 mostró que fue más activa en el estadio del adulto y en los túbulos de Malpighi que en los otros estadios de desarrollo y tejidos. Nuestros resultados proveen evidencia de que la *BdTre2* está implicada en la actividad del intestino medio, posiblemente en la síntesis de la quitina y el metabolismo de la energía y es importante para los adultos de *B. dorsalis*.

Palabras Clave: mosca oriental de la fruta, trehalasa destinado para la membrana, clonación, PCR cuantitativa, ensayo de actividad enzimática

Trehalose, a disaccharide,is a major carbohydrate in insect hemolymph. It plays a critical role in energy metabolism, and coping with stress. Trehalose is abundant in larvae, pupae and adult insects (Elbein et al. 2003). Trehalase (alpha, alpha-trehalose glucohydrolase, EC 3.2.1.28) is the main enzyme that catalyzes the hydrolysis of trehalose into 2 molecules of glucose. In insects, trehalase exists in many tissues where it serves to supply energy. There are 2 different forms of trehalase in insects, i.e., a soluble and a membrane-bound form, and the latter was characterized by gene cloning, purification of protein and enzyme assay(Tang et al. 2008). Presence/absence of the

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transmembrane domain is the judgment standard for membrane-bound or soluble form of trehalase (Tatun et al. 2008b). The soluble form mainly exists in the cytoplasm, while the membrane-bound form sticks to the cytomembrane. Both forms are important for energy metabolism and participate in the activation of the chitin synthesis pathway in insects (Tang et al. 2008). Thus, trehalase has become a new potential target of insecticide development. Trehalase obligate inhibitors, such as trehazolin (Ando et al. 1991), salbostatin (Temesvari & Cotter 1997), and validoxylamine A (Zheng et al. 2004), can specifically inhibit the activity of trehalase. Injection of these inhibitors into an immature insect can cause lethal metamorphosis into the adult (Wegener et al. 2010), and unsuccessful pupation of the larva(Asano et al. 1990). However, other methods of delivery did not work as well as injection to effectively inhibit trehalase, because the inhibitory compounds cannot penetrate the epidermis (Kono 2002).

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is a destructive insect pest distributed in Asia, North America, and Oceania. This insect can damage the fruits of more than 250 plant species (Chen & Ye 2007; Clarke et al. 2005) and most of them are economically important fruit and vegetable crops. Currently, the control of this pest mainly depends on chemical insecticides. However in recent yr, the pest has developed high levels of resistance to organophosphates, pyrethroids, and avermectin (Jin et al. 2011). To effectively control this pest, it is urgent to develop new insecticides based on still unexploited physiological or biochemical targets. Since the energy supply of vertebrates is provided by the metabolic pathway of trehalose, and because this disacharide is critically important in insect metabolism, trehalase may be developed as a specific target of new biopesticides. Therefore, it is necessary to discover the specific function of trehalase in *B. dorsalis*.

Here, we report the cloning and characterization of the membrane-bound trehalase cDNA *BdTre2* in *B. dorsalis*. In addition, expression profiles of *BdTre2* and the activities of trehalase 2 in the various developmental stages and tissues were investigated using the methods of quantitative real-time PCR and enzyme assays, respectively. The current study provides the first insights into the *B. dorsalis* trehalase genes at the molecular level.

Materials and Methods

Insects

The laboratory stock of *B. dorsalis* was originally collected in Fujian province, People's Republic of China, in autumn of 2010, and cultured in the laboratory at 27 ± 1 °C,70 ± 5% RH, and 14:10 h L:D photoperiod. The insects were fed using artificial diets (Cong et al. 2012). The various developmental stages of *B. dorsalis* including egg, larva, pupa, and adult were collected and stored at -80 °C for RNA isolation. The midgut, Malpighian tubules, and fat body of *B. dorsalis* adults were dissected in PBS with 0.75% NaCl under a stereomicroscope and were used immediately for enzyme activity assay and RNA isolation.

Total RNA Isolation and cDNA Synthesis

Total RNA was extracted from *B. dorsalis* adults using RNeasy® Plus Mini Kit (Qiagen, Hilden, Germany).The samples were dissolved in 30 mL DEPC-treated water, and the total RNA was tested by the ratio of OD_{260}/OD_{280} and electrophoresis to detect its purity and quality. High quality RNA was stored at -80 °C. Twoμg of RNA were used to synthesize cDNAs by means of the PrimeScript® 1st Strand cDNA Synthesis Kit following the manufacturer's instructions (TaKaRa, Dalian, China). The cDNAs were stored at -20 °C. The template for rapid amplification of cDNA ends (RACE) was synthesized by the ClontechSMARTer™ RACE cDNA Amplification Kit (TaKaRa, Dalian, China).

Cloning *BdTre2*c DNA

The specific primers (Table 1) were designed according to the 3 fragments of *BdTre2*, and synthesized by Invitrogen (Invitrogen Life Technologies, Shanghai, China) based on the transcriptome data of our previous study (Shen et al. 2011). First-strand cDNA was used as a template in PCR under the following conditions: initial denaturation at 95 °C for 3 min, followed by 34 cycles of denaturation at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min with final extension at 72 °C for 10 min. The total volume was 25 mL, contained: 15 mL ddH_2O, 2.5 mL 10 × PCR Buffer (Mg^{2+} free), 2.5 mL Mg^{2+} (2.5 mM), 2 mL dNTP (2.5 mM), 1 mL of each primer (10 mM), 1 mL cDNA, and 0.25 mL rTaq™ polymerase (TaKaRa, Dalian, China). The PCR products were detected in 1.0% agarose gel mixed with GoodView™ (SBS Genetech, Beijing, China). After excised, the target band of cDNAs was recovered by the Gel Extraction Mini Kit (Watson Biotechnologies, Shanghai, China). Purified cDNA fragments were cloned into pGEM®-T Easy vector (Promega, Madison, Wisconsin) and transformed into DH5α competent cells (Transgen, Beijing, China). After the selection of successful clones, cDNAs were sequenced by BGI (Beijing Genomics Institute, Beijing, China). A long length of fragment was obtained by the amplification of *BdTre2* SA-F/R. Based on the fragment, 3'- and 5'- RACE were executed using specific primers to acquire full length of cDNA sequence.

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TABLE 1. PRIMERS USED FOR CLONING THE 3 FRAGMENTS OF *BDTRE2*.

Experiments	Primers and sequences	
	Sense	Anti-sense
Specific amplification	BdTre2 SA-F: CACCGACGAGGATAAGGAACAA	BdTre2 SA-R: GCCCAAGTTCTCCAAACCTCTA
5' RACE	BdTre2-5R1: CCGTATCTCCGCCTCGGTTGG	NUP: AAGCAGTGGTATCAACGCAGAGT
	BdTre2-5R2: CTTTACCCCGCTCCTCGAAAT	UPM: CTAATACGACTCACTATAGGGC
3' RACE	BdTre2-3R1: GGGGACATCGCTGGGTGAAAT	NUP: AAGCAGTGGTATCAACGCAGAGT
	BdTre2-3R2: TAGAGGTTTGGAGAACTTGGGC	UPM: CTAATACGACTCACTATAGGGC
Full-length confirmation	BdTre2 Full F: GAACCACGTTCTACTACATTGC	BdTre2 Full R: TGCCTTTAAAATGTGTCAGATG
qPCR	Tre-DLL: AGAATCTCGGCATCTGTGCT	Tre-DLR: GTCCCCATCGTTCTGACAGT

Sequence Analysis and Phylogenetic Tree Construction

The sequence of *Tre2*c DNA was compared with other sequences downloaded from the GenBank by "nucleotide-blast" tool at the NCBI website (http://www.ncbi.nlm.nih.gov/). The homological analysis and construction of the phylogenetic tree were completed by Clustal X (Aiyar 2000) and MEGA 4.1 (Kumar et al. 2008), respectively. The transmembrane domain of protein and *N*-linked glycosylation sites were analyzed using TMHMM Server v.2.0 (http://www.cbs.dtu.dk/services/TMHMM) and NetNGlyc 1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc/), respectively. The DNAMAN 5.2.2 (Lynnon BioSoft, Quebec, Canada) was applied to predict molecular weight and isoelectric point. For use as outgroups, a total of 36 insect trehalase genes were obtained from GenBank, including 16 *Tre2*, 14 *Tre1* and another 6 trehalase genes from microbes and mammals (Table 2). All these genes were aligned by Clustal X 1.81 and assembled to a neighbor-joining tree by MEGA 4.1.

Quantitative Real-Time PCR (qPCR)

Quantitative real-time PCR (qPCR) was used to analyze the stage- and tissue-specific expression of *BdTre2*. RNAs from different tissues (fat body, midgut, and Malpighian tubules of adults), as well as 1st- and 2nd-instar larvae, were isolated by using RNeasy® Plus Micro Kit with Qiageng DNA Eliminator spin column (Qiagen, Hilden, Germany). Total RNA for 3rd-instar larva, pupa and adult was extracted using Trizol (Transgen, Beijing, China), and treated with RQ1 DNAase (TaKaRa, Dalian, China) to exclude gDNA. RNA isolation was repeated 3 times in 3 independent experiments. First-strand cDNAs were obtained by PrimeScript® RT reagent Kit (TaKaRa, Dalian, China)and the specific primers (Tre-DLL&Tre-DLR, Table 1) were designed by Primer 3v0.4.0 (http://frodo.wi.mit.edu/primer3/). The reaction conditions of RT-PCR were the same as mentioned above.

Based on the results of RT-PCR, the corresponding qPCR was executed to advance the investigation of changes in *BdTre2* expression in the various developmental stages. The whole reaction was performed using a Mx3000P thermal cycler (Stratagene, La Jolla, California) in 25 µL reactions containing 2 µL of template cDNA, 12.5 µL iQ™ SYBR® Green Supermix (BIO-RAD, Hercules, USA) and 0.2 mM of the primers(Tre-DLL&Tre-DLR). Amplification conditions were as follows: initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s. The α-tubulin gene was used as a stable reference (Shen et al. 2011; Shen et al. 2012). PCR efficiencies of *BdTre2* and α-tubulin gene were calculated by the Mxpro-Mx3000P version 4.01 (Stratagene, La Jolla, California). The data for both development- and tissue-specific expression patterns were subjected to analysis of variance and means were separated by least significant difference test (LSD) (SPSS 12.0 for Windows).

Measurement of Trehalase Activity

The samples in 6 developmental stages (including egg, 1st-, 2nd-, and 3rd- instar larva, pupa, and adult), and 3 kinds of tissue in the adult (fat body, midgut, and Malpighian tubule) were collected. The experiments were repeated 3 times in 3 independent experiments. The samples (80-

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TABLE 2. THE SPECIES AND GENBANK ACCESSION NUMBERS USED IN THE PHYLOGENETIC ANALYSIS.

Gene	GenBank Accession Number	Species
AmTre1	XM_393963	*Apis mellifera*
AmTre2	NM_001112671	*Apis mellifera*
ApTre1	XP_001950264	*Aphis glycines*
AaTre2	XM_001660243	*Aedes aegypti*
AgTre	XM_320471	*Anopheles gambiae*
BmTre1	D86212	*Bombyx mori*
BmTre2	NM_001043445	*Bombyx mori*
CqTre2	XP_001847934	*Culex quinquefasciatus*
DpTre1	EHJ77355	*Danaus plexippus*
DpTre2	EHJ67296	*Danaus plexippus*
DmTre2	DQ864060	*Drosophila melanogaster*
DsTre2	DQ864075	*Drosophila simulans*
LsTre1	AFL03409	*Laodelphax striatella*
LsTre2	AFL03410	*Laodelphax striatella*
LmTre1	ACP28173	*Locustamigratoria*
NvTre2	XM_001602129	*Nasonia vitripennis*
OfTre1	EF426724	*Ostrinia furnacalis*
OfTre2	EF426723	*Ostrinia furnacalis*
PhTre1	AJ459958	*Pimpla hypochondriaca*
SeTre1	EU427311	*Spodoptera exigua*
SeTre2	EU106080	*Spodoptera exigua*
TmTre1	D11338	*Tenebrio molitor*
SfTre1	DQ447188	*Spodoptera frugiperda*
SfTre2	EU872435	*Spodoptera frugiperda*
SlTre1	ADA63846	*Spodoptera litura*
SlTre2	ADA63845	*Spodoptera litura*
TcTre1	XM_967517	*Tribolium castaneum*
TcTre2	XM_968826	*Tribolium castaneum*
NlTre1	ACN85420	*Nilaparvata lugensand*
NlTre2	EJ790319	*Nilaparvata lugensand*
CkTre	YP_001452785	*Citrobacter koseri*
EcTre	ZP_06653159	*Escherichia coli*
StTre	ZP_09722139	*Salmonella typhimurium*
PaTre	NP_251106	*Pseudomonas aeruginosa*
MmTre	NM_021481	*Mus musculus*
HsTre	NM_007180	*Homo sapiens*

90 mg) were homogenized in liquid nitrogen with mortar and pestle, and after removal of liquid nitrogen, 2 mLs phosphate buffer (0.02 mol/L, pH 5.8) was added. The sample solution was centrifuged at 10,000 × g for 1 h at 4 °C and the supernatant was discharged. The sediment was re-suspended and homogenized in phosphate buffer, incubated with 30 mM CHAPS with gentle stirring at room temperature for 30 min. The supernatant was centrifuged at 10,000 × g for 1 h at 4 °C, and it contained membrane-bound trehalase.

The trehalase activity was measured by the method of Lindsay (1973) using 3,5-dinitrosalicylic acid (DNS). The color development reagent contained 1g DNS, 1g NaOH, 0.2g phenol and 0.05g Na_2SO_3 and was dissolved in 100 mL ddH_2O. Fifty µL supernatant was added into the 1.5 mL tube, compounded with 100 µL trehalose solution (40 mmol/L) (Sigma-Aldrich, Shanghai, China). The mixture was incubated in a water bath at 37 °C for 30 min. The reaction was ended in boiling water for 2-3 min and cooled on ice. Then 150 mL color development reagent was added into the solution and incubated in a 90 °C water bath for 5 min. After the tube was cooled in ice, 50 µL Rochelle salt solutions (40%) was added. The absorbance was measured at 550 nm by a Microplate Spectrophotometer XMark™ (BIO-RAD, Hercules, USA). The amount of glucose catalyzed by trehalase was then determined from the standard curve. The protein content was determined using Coomassie brilliant blue G-250 and bovine

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serum albumin as a standard. Trehalase activity was expressed asμmol.mg^{-1}protein.min^{-1}.

Results

Sequence Analysis

A full-length cDNA(*BdTre2*) of trehalase from *B. dorsalis* was obtained by cDNA amplification (Fig. 1). The *BdTre2* transcript contains an UTR of 808 bp and ORF of 1842 bp, which encodes 613 amino acids. The molecular weight and p*I* of BdTre2 were predicted to be approximately 70.1 kDa and 4.97, respectively. A transmembrane helix region existed in the 7-29 amino acid residue region. Residues 282, 349, and 447 were detected to be *N*-linked glycosylation sites. Multiple alignment showed that there were 2 signature motifs, "PGGRFIEFYYWDSY(185-199)" and "QWDFPNVWPP(483-493)", and 5 other conserved sequences, "DSKTFVDMK (residues70-79)", "IPNGGRVYY (230-239)", "RSQPPFL (241-248)", "GPRPESYREDI (300-311)", and "ELKAGAESGMDFSSRWFV (328-346)". Residues 547-553("GGGGEY") were detected to be a glycine-rich region. In *BdTre2*, there is no signal peptide site, which is different from other Dipteran insects, like *Drosophila melanogaster* Meigen and *D. simulans* Sturteveant, (Drosophilidae).

Phylogenetic Analysis

Using the protein sequences, we analyzed the phylogenetic relationships among *BdTre2* and other *Tre2* and *Tre1* genes across insect species based on the neighbor-joining method (Fig. 2). *Tre2* genes of Diptera were clustered separately from those of other insects. In the dipteran part of the phylogenetic tree, *BdTre2* clustered with fruitflies, but was separated from mosquitoes (Culicidae). In addition, BLAST-P in NCBI showed that *BdTre2* shared 65% identity with *Tre2* of *D. melanogaster* (DQ864060), 65% identity with *D. simulans* (ABH06710), 57% identity with *Aedes aegypti* (XM_001660243), and 54% identity with *Anopheles gambiae* (XM_320471), all of which suggested that *BdTre2* was most closely related to the *Tre2* of Dipteran species.

Expression Patterns in Different Developmental Stages and Tissues

The expression patterns of *BdTre2* were analyzed using qPCR of samples taken from various developmental stages and tissues (Fig. 3). The results showed that the expression of *BdTre2* was detectable in all tested developmental stages. The highest and lowest mRNA levels were found in adult and pupa stages, respectively. The expression levels in the adult were 3-, 6-, 2-,4-,49-fold higher than in the egg, 1st, 2nd, and 3rd- instar larva, and pupa, respectively (Fig. 3A). Moreover, statistical analysis showed that the expression in adult was significantly higher than those in other stages ($P < 0.05$).

The relative expression patterns of *BdTre2* in different tissues were shown in Fig. 3B. *BdTre2* was detectable in the midgut, Malpighian tubules, and fat body. Expression of *BdTre2* was significantly greater in the midgut than in the other 2 tissues ($P < 0.05$). More specifically, the expression level in midgut was 8- and 4- fold higher than that in Malpighian tubules and fat body, respectively.

Trehalase Activity Measurements

In the enzyme activity assay, membrane-bound trehalase of *B. dorsalis* presented activity in all of the developmental stages. More specifically, enzyme activity in adults was higher than in the other stages, especially significantly higher than in the egg ($P < 0.05$). The enzyme activities in the egg, 1st, 2nd and 3rd- instar larvae, and pupae were 2-, 1.2-, 4-, 1.6- and 4-fold lower than in the adult (Fig. 4A; $P < 0.05$).

Trehalase2 activity found in the midgut, Malpighian tubules and fat body was 0.28, 0.34 and 0.25 μmol mg^{-1}protein min^{-1}, respectively. Trehalase activity in Malpighian tubules was significantly higher than in the midgut and fat body (Fig. 4B, $P < 0.05$).

Discussion

The trehalase gene has been cloned from many insect species. The soluble trehalase gene was first reported from *Tenebrio molitor* (Takiguchi et al. 1992) and subsequently from other species, including *Bombyx mori* (Su et al. 1993), *Pimpla hypochondriaca* (Parkinson et al. 2003), and *Locusta migratoria* (Liu et al. 2012). However, the membrane-bound isoform was first detected in *Bombyx mori* in 2005 (Mitsumasu et al. 2005). These 2 types of trehalase were found to exist in many insects including *Apis mellifera* (Lee et al. 2007), *Omphisa fuscidentalis* (Tatun et al. 2008a), *Spodoptera exigua* (Chen et al. 2010), and *Laodelphax striatellus* (Zhang et al. 2012). In this study, BdTre2 has signature motifs of trehalase in the trans-membrane helix region. The phylogenetic analysis found that *BdTre2* has higher identity with other *Tre2* genes in the Diptera. This confirmed that *BdTre2* was the membrane-bound trehalase gene of *B. dorsalis*.

Trehalases are important enzymes in insect physiological activities, as they can catalyze one molecule of trehalose into 2 molecules of glucose (Azuma & Yamashita 1985; Su et al. 1993). The

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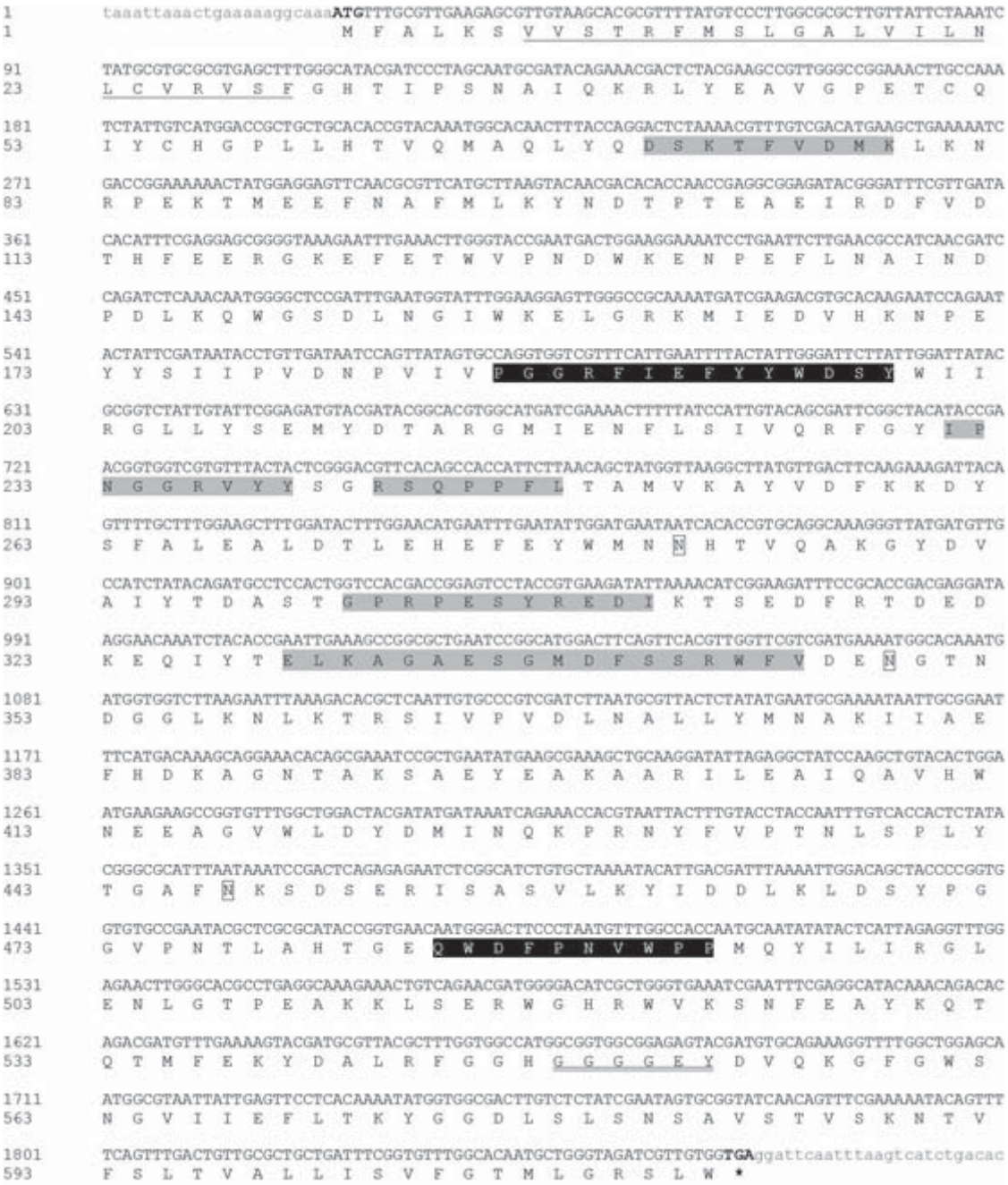


Fig. 1. Nucleotide and amino acid sequence of *B. dorsalis* membrane-bound trehalase gene (GenBank accession number: JQ228448). The start and stop codon are indicated with bold, bold and asterisk, respectively. Black boxes: trehalase signature motifs (residues 185-199 and 483-493); white boxes: N-linked glycosylation sites (residues 282, 349 and 447); gray boxes: conserved regions (residues 70-79, 230-239, 241-248, 300-311 and 328-346). The transmembrane helix region is marked by single underlining (residues 7-29). The glycine-rich region is marked by double underlining (residues 547-553).

main function of trehalase 2 is to hydrolyze sugar in muscle and the midgut in order to provide energy for activities. Through RNA interference technology (RNAi), it was reported that the feeding of ds*Tre2* can cause weight loss and death in *Laodelphax striatellus* (Zhang et al. 2012). The membrane-bound trehalase presents high activity in *Locusta migratoria* flight muscle, whereas

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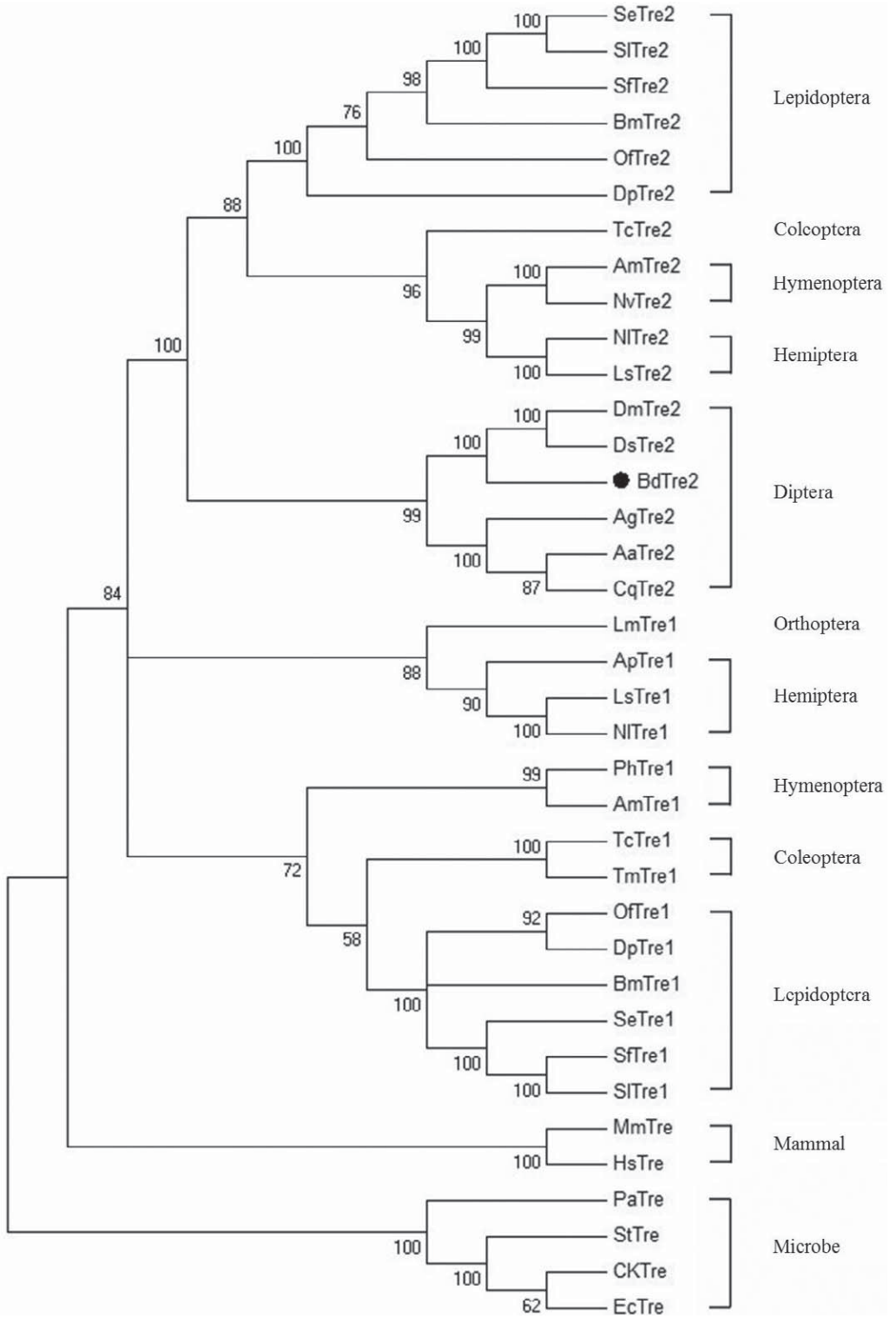


Fig. 2. Phylogenetic tree of trehalases based on the deduced amino acid sequences. These sequences from different organisms were aligned and analyzed by using MEGA 4.1. The topology was tested by the Neighbor-joining algorithm and a bootstrap of 1,000 replications. The species and GenBank accession numbers are presented in Table 2.

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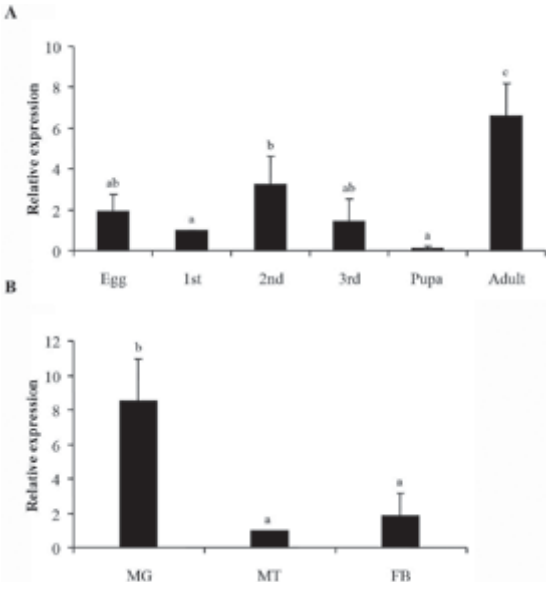


Fig. 3. Expression patterns of the *BdTre2* in different developmental stages (A) and tissues (B) of *B. dorsalis* (each bar presents the mean ± SD; different letters above each bar indicated a significant difference, $P < 0.05$, LSD in ANOVA).1st, 2nd, and 3rd means the 1st-, 2nd-, and 3rd- instar. MG, MT, and FB represent the midgut, Malpighian tubules, and fat body, respectively, dissected from adults.

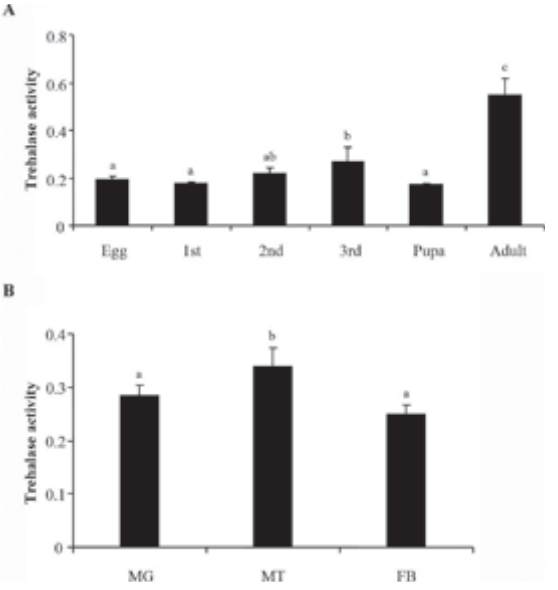


Fig. 4. Enzyme activity assay of membrane-bound trehalase in different developmental stages (A) and tissues (B) of *B. dorsalis*. Samples were incubated for 15 min in the presence of 40 mM trehalose. Results are mean ± SD for 3 independent determinations. Different letters above each bar indicated a significant difference ($P < 0.05$, LSD in ANOVA). 1st, 2nd, and 3rd means the 1st-, 2nd-, and 3rd- instar larva. MG, MT, and FB represent midgut, Malpighian tubule, and fat body, respectively, dissected from adults. Trehalase activity is expressed as $\mu mol.mg^{-1}protein.min^{-1}$.

the soluble trehalase does not appear to have obvious activity in flight muscle (Wegener et al. 2010). Moreover, since trehalase 2 participated in transporting sugar into ovarian cells (Su et al. 1994), the gene expression and enzyme activity of trehalase 2 were enhanced in the ovary at day 7 after a blood meal of *Rhodnius prolixus* (Santos et al. 2012). *Tre2* expression and enzyme activity assay throughout the whole life cycle of *B. dorsalis* showed that this gene and its corresponding enzyme mainly played a role in the adult. Both the enzyme activity and gene expression level were significantly enhanced in the adult compared to the pupa. It is possible that *Tre2* expression and enzyme activity are involved in a major way in the frequent flight and reproduction behaviors of adults.

In *S. exigua* larvae, *Tre2* was found highly expressed in the midgut, Malpighian tubules and fat body, but much less so in the brain, cuticle, trachea and testes (Tang et al. 2008). After *R. prolixus* had been fed, the trehalase activity in midgut was higher than in the fat body and Malpighian tubules(Mariano et al. 2009). Both types of trehalase existed in midgut of *B. mori* (Mitsumasu et al. 2005); trehalase 2 was associated with supporting peristaltic movement of the midgut by providing energy to visceral muscles (Azuma & Yamashita 1985; Mitsumasu et al. 2005). Our results suggested that this gene was expressed in these metabolic tissues both in larvae and adults. In addition, *Tre2* was strongly expressed in the midgut not only because of the energy requirements of this organ, but also to support the chitin synthesis pathway in the midgut (Chen et al. 2010; Tang et al. 2008). After the injection of ds*Tre2*, the expression of chitin synthase gene B was also suppressed (Chen et al. 2010). In our study, the gene expression level in the midgut was significantly higher than in other tissues and this suggested that trehalase 2 may be involved in providing energy to the chitin synthesis process in the midgut.

In insects, trehalase 2 is involved in many physiological processes, like flight (Wegener et al. 2010), reproduction, development (Santos et al. 2012) and digestion in the midgut (Mitsumasu et al. 2005). In fight muscle, the concentration of trehalose decreased in the initial period of flight and remains low during long durations of flight (van der Horst et al. 1978). Prolonged flight can cause glycolysis in flight muscle (Wegener 1996). Trehalose must be catabolized by trehalase. Therefore, we assume that *Bdtre2* may be involved in the flight of *B. dorsalis*. In addition, in the metabolic pathway of trehalose, trehalase transport fac-

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tor and trehalase synthase are as important as trehalase (Kikawada et al. 2007; Kunieda et al. 2006; Xu et al. 2009). The relative functions of these genes, plus their importance in the physiology of *B. dorsalis* suggest that they present a possible target for RNAi strategies to manage this pest.

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