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Original article

Cloning and molecular identification of triosephosphate isomerase gene from *Apis cerana cerana* and its role in response to various stresses

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Abstract – *Apis cerana cerana* triosephosphate isomerase (*AccTPI*), a key regulatory enzyme in glycolysis and gluconeogenesis pathway, catalyzes the interconversion of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate. However, its role in *A. cerana cerana* has not been completely clarified. In this study, a TPI gene was cloned from *A. cerana cerana* and named *AccTPI*. The open reading frame (ORF) of *AccTPI* is 744 bp, which encodes 247 amino acids with a predicted molecular weight and an isoelectric point of 26.8 kDa and 8.42, respectively. The expression of *AccTPI* was up-regulated by some abiotic stresses, while it was down-regulated by $HgCl_2$, ultraviolet light (UV), and vitamin C (VC) treatment. Finally, a disc diffusion assay revealed that recombinant AccTPI proteins can protect cells from oxidative stresses. Taken together, all of the results indicate that *AccTPI* may play an important role in the metabolism, in antioxidant defense, and in many biological functions in the growth and development of *A. cerana cerana*.

triosephosphate isomerase / *Apis cerana cerana* / abiotic stress / metabolism

1. INTRODUCTION

As a pollinator, *Apis cerana cerana* is significant in keeping the balance between regional ecologies and agricultural economics (Weinstock et al. 2006). *A. cerana cerana* has been raised in Asian countries for thousands of years and has brought considerable economic benefits to the apicultural industry (Park et al. 2015). The honeybee is an important model organism for neuroethological studies; it exhibits high behavioral plasticity and a remarkable ability to learn (Zhang et al. 2015). Compared with *Apis mellifera*, *A. cerana cerana* has actually been shown to learn better in a controlled laboratory setting (Qin et al. 2012). Recent colony losses of *A. cerana cerana* have thus raised concern, and possible explanations for bee decline include nutritional deficiencies and exposures to pesticides and pathogens. Thus, we chose *A. cerana cerana* as the experimental organism for this study. *A. cerana cerana* has unique characteristics including heat hardiness and disease resistance (Li et al. 2008). However, the survival of *A. cerana cerana* is under increasing threat due to various stressors including the indiscriminate use of pesticides and global warming. Moreover, organisms are exposed to a variety of unfavorable

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environmental stressors which are believed to induce the formation of reactive oxygen species (ROS) (Kottuparambila et al. 2012).

ROS, which include superoxide anions, hydrogen peroxide, and hydroxyl radicals, can be induced by external or internal factors, such as prooxidants, heavy metals, and pesticides (Imlay 2003). Generally, there is a balance between the generation of ROS and antioxidant processes, but exogenous stressors can break this balance (Brennan et al. 2008). High ROS concentrations contribute to degenerative diseases and aging through the oxidation of nucleic acids, proteins, and lipid membranes (Valko et al. 2006). To avoid the damage caused by ROS, insects have evolved an intricate mechanism of enzymatic antioxidant systems (Felton and Summers 1995). Reduced ROS generation, increased antioxidant protection or a combination of both increases the lifespan of social insects (Keller and Jemielity 2006). Aerobic organisms have also developed sophisticated antioxidant mechanisms and evolved various enzymatic antioxidant systems to avoid oxidative damage (Jia et al. 2014).

Metabolic regulation is very important for cellular tolerance to oxidants, providing the reducing power for the antioxidant machinery (Chu et al. 2008). Previous studies have demonstrated that triosephosphate isomerase (TPI) catalyzes the isomerization of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate and plays an essential role in glycolysis, gluconeogenesis, and fatty acid synthesis (Zinsser et al. 2013). TPI plays an important function in generating energy and contributing to metabolism. Without TPI, there would be no net production of ATP to promote aerobic metabolism. Furthermore, the deficiency of TPI can result in metabolic diseases (Zhou et al. 2015).

Aerobic metabolism depends on the activities of glycosomal TPI and a mitochondrial glycerophosphate oxidase in *Trypanosoma brucei* (Helfert et al. 2001). During the life cycle of *Clonorchis sinensis*, adult worms can take in external glucose for their energy supply through the glycolysis pathway (Zhou et al. 2015). TPI deficiency in *Drosophila melanogaster* exhibits phenotypes such as susceptibility to infection, neurodegeneration, and reduced life span (Hrizo and Palladino 2010). The catalytic activity of TPI is unrestricted, and it is crucial to behavior and longevity (Roland et al. 2013). Yeast cells expressing a TPI variant are more resistant against oxidative stress and so TPI cannot tolerate all oxidative stress (Ralser et al. 2006). Furthermore, several studies demonstrate variations of TPI activity in relation to the levels of antioxidants in lymphocytes (Ralser et al. 2006). In bees, Wang et al. (2013) found that mixed-function oxidative enzymes are the most important enzymes in acaricide detoxification and resistance. Hence, we speculated that the TPI gene in bees might be associated with ROS, so we investigated the TPI antioxidant system in *A. cerana cerana* and its role in ROS defense mechanisms.

As the biochemical characterization of *AccTPI* was still unclear, this is the first study describing the cloning, overexpression, and characterization of a recombinant TPI from *A. cerana cerana*. The function of TPI in *A. cerana cerana* was studied to understand its role in cellular defense systems and metabolism. The RNA and protein expression levels of *AccTPI* were calculated. Furthermore, a disc diffusion assay was performed to evaluate the capacity of AccTPI to defend against oxidative stress.

2. MATERIALS AND METHODS

2.1. Animals and various treatments

A. cerana cerana were obtained from the experimental apiary at Shandong Agricultural University, Taian, China. Based on age, eye color, and shape, the worker honeybees were generally classified as larvae, pupae, and adults (Elias-Neto et al. 2010). The first (L1), third (L3), fifth (L5), and seventh (L7) day larvae; white-eyed (Pw), pink-eyed (Pp), brown-eyed (Pb), and dark-eyed (Pd) pupae; and newly emerged adults (A1, 1 day post-emergence), 1 week-old bees (A7, usually 7–10 days post-emergence), which are likely nurses, and 2 week-old bees (A15, older than 15 days post-emergence), which can be foragers, were gathered during the spring season from two hives maintained in the experimental apiary. For tissue-specific analyses of *AccTPI* expression levels, the antenna (AN), abdomen (AB), head (HE), hemolymph (HA), epidermis (EP), venom gland (VG), muscle (MU), thoracic (TH), and midgut (MI) of adult workers were used. These were dissected on ice, washed twice with PBS, frozen in liquid nitrogen, and stored at −70 °C.

Fifteen-day-old adult bees were collected randomly and reared under 34 °C and 60 % relative humidity and fed a diet of water and a combination of pollen and sucrose (Alaux et al. 2010). The worker honeybees were randomly separated into eight groups, and each group contained 30 individuals. Groups 1, 2, and 3 were exposed to 4, 14, and 44 °C, respectively. Group 4 was treated with ultraviolet light (UV) (254 nm, 30 mJ/cm^2). Groups 5 and 6 were exposed to two pesticides (2.0 mg/L of paraquat and pyridaben was added to the basic adult diet, respectively). The control group was fed on a normal diet containing water, 70 % powdered sugar, and 30 % honey from the source colonies. Group 7 was fed $HgCl_2$ (3 mg/mL). Group 8 was subjected to vitamin C (20,000 mg/kg). The honeybees were flash-frozen in liquid nitrogen at a suitable time and stored at −70 °C until use.

2.2. RNA extraction, cDNA synthesis, and DNA preparation

Total RNA was extracted from adult bees with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and the RNA was treated with RNase-free DNase I (Promega, Madison, WI, USA). Then, the cDNA was synthesized using a reverse transcription system (TransGen Biotech, Beijing, China). The genomic DNA was isolated from the honeybees with the EasyPure Genomic DNA Extraction Kit (TransGen Biotech, Beijing, China).

2.3. Primers and PCR procedure

The primer sequences and PCR procedure are listed in Tables I and II, respectively.

2.4. Bioinformatics analysis and phylogenetic tree construction

The conserved nucleotide sequences of *AccTPI* were analyzed using DNAman software 6.0.3 and the National Center for Biotechnology Information (NCBI) bioinformatics tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The predicted amino acid sequence was aligned with the homologous sequences of various insects, which were acquired from the NCBI Genbank database. The molecular weight and theoretical isoelectric point were predicted using the online tool ExPASy (http://web.expasy.org/compute_pi/). A phylogenetic tree was constructed using the Molecular Evolutionary Genetic Analysis (MEGA version 4.0) software.

2.5. Quantitative real-time PCR

To evaluate the expression profile of *AccTPI* in different developmental stage tissues and abiotic stresses, RT-qPCR was performed using the SYBR® PrimeScript™ RT-PCR Kit (TaKaRa, Dalian, China) with two specific primers (DLF and DLR). The reaction volume and the reaction requirements were as those of Yao et al. 2013. The PCR reactions were performed using a CFX96™ Real-Time System (Bio-Rad), and the *β-actin* gene (GenBank accession no. HM640276; *A. cerana cerana actin-related protein 1*) was used as an internal control. We also obtained experimental data in which we tested and compared additional reference genes (*β-actin*, *ribosomal protein 49*, *elongation factor 1-alpha*, *tbp-association factor*); see Supplemental Fig. 3. All of the samples were analyzed in triplicate. The relative gene expression levels of *AccTPI* were calculated using the $2^{-\Delta\Delta Ct}$ comparative CT method (Livak and Schmittgen 2001). The analysis was performed with Duncan's multiple range test using Statistical Analysis System (SAS) software version 9.1.

2.6. Protein expression and purification of recombinant AccTPI

To obtain the AccTPI protein, the recombinant plasmid pET-30a (+)-*AccTPI* was transfected into *Escherichia coli* BL21 (DE3). The bacteria were incubated in Luria-Bertani (LB) broth with kanamycin at 37 °C until the cell density at 600 nm reached 0.4–0.6. Then, the AccTPI expression was induced with 1.0 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) at 18 °C overnight. The protein was purified on a HisTrap™ FF column (GE Healthcare, Uppsala, Sweden). The expression of the target protein was analyzed by 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

2.7. Western blotting analysis

The white mice for the generation of antibodies came from China Biologic Products, Inc. (Shandong). The protocol numbers of certificates that they produced and used for animal research were SCXK (LU) 2013 0006 and SYXK (LU) 2013 0011, respectively. The Tissue

Table I. The primers in this study.

Abbreviation	Primer sequence (5′–3′)	Description
TF	GGCCATGGGTCGTAAATTTTTTG	cDNA sequence primer, forward
TR	CATCTCACTGCTTAGCGTTAACG	cDNA sequence primer, reverse
5TW	TGCAACCTTATATGTATTTTGTCCAGC	5′RACE reverse primer, outer
5TN	TGCAACCTTATATGTATTTTGTCCAGC	5′RACE reverse primer, inner
3TW	GTAGCCCATGCTTTAGATAGTGGAC	3′RACE forward primer, outer
3TN	CACACCACAACAAGCTCAAGAG	3′RACE forward primer, inner
AAP	GGCCACGCGTCGACTAGTAC$(G)_{14}$	Abridged anchor primer
AUAP	GGCCACGCGTCGACTAGTAC	Abridged universal amplification primer
B25	GACTCTAGACGACATCGA	3′RACE universal primer, outer
B26	GACTCTAGACGACATCGA$(T)_{18}$	3′RACE universal primer, inner
DLF	TGTTGAGGTTGTCGTTGGAGTAC	Real-time PCR primer, forward
DLR	GGACTTATTTCTCCAGTAAATGCTC	Real-time PCR primer, reverse
β-s	TTATATGCCAACACTGTCCTTT	Standard control primer, forward
β-x	AGAATTGATCCACCAATCCA	Standard control primer, reverse
G1	GGGGATTTCATAAAATCGGGTTGG	Genomic sequence primer, forward
G2	GGTTATGTGATTACTTATGCACTATATTTGTG	Genomic sequence primer, reverse
YHF	GATATCATGGGTCGTAAATTTTTTGTTGGTGG	Protein expression primer, forward
YHR	CGAGCTCTCACTGCTTAGCGTTAACGAT	Protein expression primer, reverse

Protein Extraction Kit (CoWin Bioscience Co., Beijing, China) was used to extract the total *A. cerana cerana* protein, and it was quantified with the BCA Protein Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Western blotting was performed according to the procedure of Chen et al. (2014). The following treatments were performed to analyze the protein expression levels: (1) 4 °C, (2) H_2O_2, (3) methomyl, (4) pyridaben, (5) UV, and (6) vitamin C (VC).

2.8. Disc diffusion assay

Disc diffusion assays were performed according to Chen et al. (2014) for the evaluation of the activity of recombinant AccTPI. *E. coli* BL21 (DE3) cells were incubated in LB liquid medium with kanamycin at 37 °C until the cell density reached 0.4–0.6 OD_{600}, then IPTG was introduced to the LB broth at a final concentration of 1 mM.

Table II. PCR amplification conditions.

Primer pair	Amplification conditions
TF/TR	10 min at 94 °C, 40 s at 94 °C, 40 s at 54 °C, 60 s at 72 °C for 35 cycles, 10 min at 72 °C
5TW/AAP	10 min at 94 °C, 40 s at 94 °C, 40 s at 54 °C, 60 s at 72 °C for 35 cycles, 10 min at 72 °C
5TN/AUAP	10 min at 94 °C, 40 s at 94 °C, 40 s at 55 °C, 60 s at 72 °C for 35 cycles, 10 min at 72 °C
3TW/B26	10 min at 94 °C, 40 s at 94 °C, 40 s at 57 °C, 60 s at 72 °C for 35 cycles, 10 min at 72 °C
3TN/B25	10 min at 94 °C, 40 s at 94 °C, 40 s at 55 °C, 60 s at 72 °C for 35 cycles, 10 min at 72 °C
G1/G2	10 min at 94 °C, 40 s at 94 °C, 40 s at 55 °C, 120 s at 72 °C for 35 cycles, 10 min at 72 °C
YHF/YHR	10 min at 94 °C, 40 s at 94 °C, 40 s at 58 °C, 60 s at 72 °C for 35 cycles, 10 min at 72 °C

They were induced using IPTG and incubated for 8 h at 18 °C with shaking at 180 rpm. Approximately 5×10^8 bacterial cells overexpressing AccTPI were seeded onto LB-kanamycin agar plates and incubated at 37 °C for 1 h. Filter discs (8-mm diameter) soaked with different concentrations of *t*-butylhydroperoxide (0, 15, 20, 30, or 40 mM) and cumene hydroperoxide (0, 30, 50, 80, or 100 mM) were placed on the surface of the top agar. Eventually, the cells were incubated at 37 °C for 24 h before the inhibition zones around the paper discs were measured as described by Burmeister et al. (2008). *E. coli* BL21 cells transformed with empty pET-30a(+) were used as the control. The greater of the bacteriostatic circle bacteria is more sensitive, rather than vise versa.

3. RESULTS

3.1. Identification of the full-length cDNA and sequence analysis

The full-length cDNA of the *AccTPI* gene (GenBank accession no. KP994676) was cloned. It comprised 1894 bp containing a 744-bp open reading frame (ORF), a 158-bp 5′-untranslated region (UTR), and a 992-bp 3′-UTR. The ORF encodes a polypeptide of 247 amino acids with a predicted molecular weight of 26.8 kDa and theoretical isoelectric point of 8.42.

Multiple sequence alignments of several TPI proteins indicates that the putative AccTPI shares high homology with *A. mellifera* AmTPI (NP 001090623.1), *Megachile rotundata* MrTPI (XP 003700534.1), *Harpegnathos saltator* HsTPI (XP 011146748.1), and *Acromyrmex echinatior* AeTPI (EGI64718.1), respectively (Figure 1a). As shown in Figure 1, we found one TPI locus and several protein kinase C phosphorylation sites in the ORF. A phylogenetic tree was built to illustrate the evolutionary relationship of TPI among insects. The exons and introns of *AccTPI* were aligned with *AmTPI* (NM 001097154.2), *MrTPI* (XM 003700486.1), and *HsTPI* (XM 011148446.1). This analysis showed that *AccTPI*, *AmTPI*, *MrTPI*, and *HsTPI* contain two introns and three exons (Supplemental Fig. 1).

3.2. Developmental and tissue-specific expression patterns of *AccTPI*

To investigate the expression patterns at different developmental stages and specific tissues, RT-qPCR assays were performed. As shown in Figure 2a, the expression levels peaked in the forager stage. The mRNA expression levels of *AccTPI* are higher in the L3 larval stages. Additionally, the expression levels in different *AccTPI* tissues were higher in the abdomen and thorax (Figure 2b).

3.3. Expression patterns of *AccTPI* under abiotic stresses

To verify whether abiotic stresses could affect *AccTPI* expression, a 15-day post-emergence adult stage was chosen and total RNA was extracted. The expression of *AccTPI* was induced by 4 °C treatment and reached the highest level at 2 h after exposure. Moreover, the expression of *AccTPI* was inhibited by 14 °C treatment and the expression level subsequently increased after 44 °C treatment (Figure 3a–c). The expression of *AccTPI* was up-regulated in response to the paraquat and pyridaben treatments (Figure 3d, e). In contrast, the expression of *AccTPI* was down-regulated after UV, $HgCl_2$, and VC treatments (Figure 3f–h).

3.4. Expression and purification of recombinant AccTPI protein

To further characterize the properties of *AccTPI* via protein levels, AccTPI was transformed into *E. coli* BL21 (DE3) with the pET-30a(+) vector. The *E. coli* cells were obtained from the LB solution after induction with IPTG overnight. SDS-PAGE analysis indicated that a recombinant protein was expressed and had a molecular mass of approximately 33 kDa (Supplemental Fig. 2, lane 2). It contained the AccTPI protein (26.8 kDa) and the His tag (7 kDa). The recombinant protein was purified with HisTrap™ FF columns. The result is shown in Supplemental Fig. 2, lane 7.

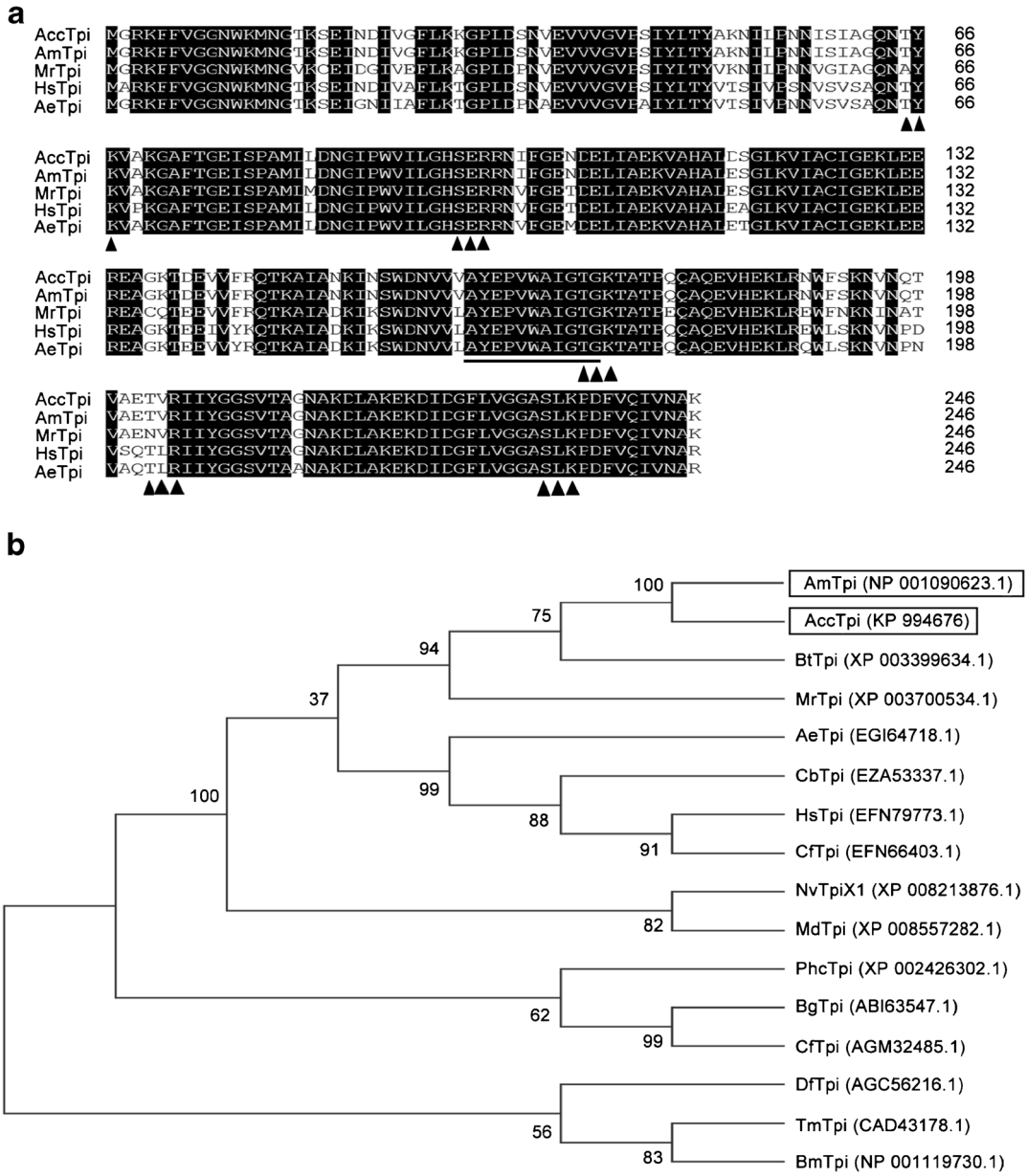

Figure 1. Multiple alignment analysis of AccTPI and a phylogenetic tree of the TPI amino acid sequences of several species. **a** Alignment of the deduced AccTPI protein sequence with other known species of TPI. The amino acid sequences of AmTPI, MrTPI, HsTPI, and AeTPI were used in the analysis. The TPI locus is marked by a horizontal line. The protein kinase C phosphorylation sites are marked by a black triangle. **b** Phylogenetic tree analysis of TPI from different species. The amino acid sequences of TPI were all obtained from NCBI.

3.5. Western blot analysis

A western blot analysis was performed to further detect the expression profiles of *AccTPI* under various adverse stresses. The results displayed that the expression of AccTPI protein levels were consistent with the transcriptional expression levels. The expression levels of AccTPI

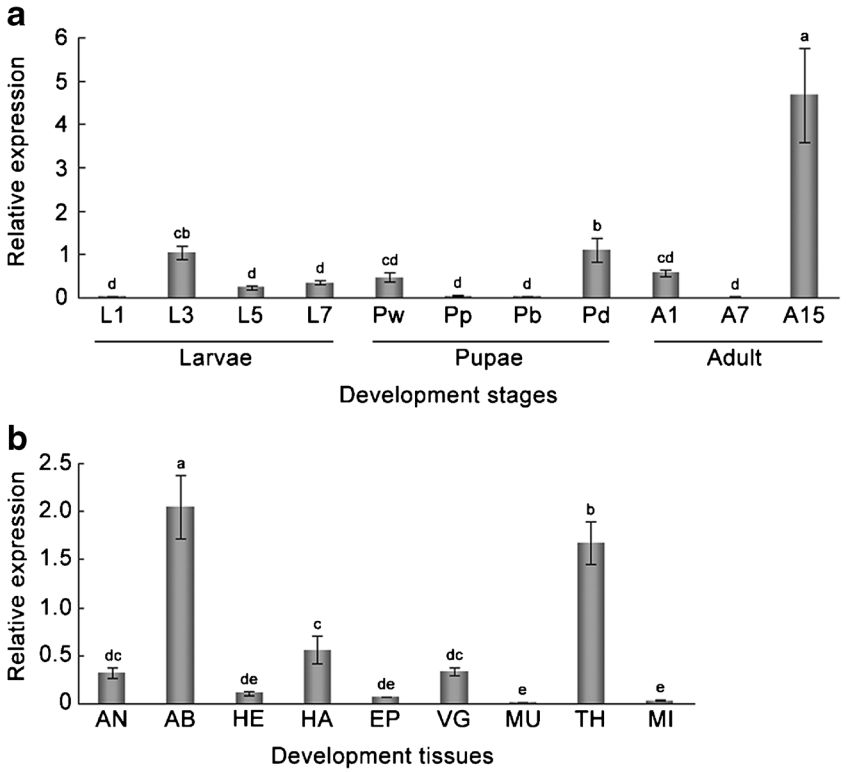

Figure 2. Expression profile of *AccTPI* in different developmental stages and tissues. **a** The expression profile of *AccTPI* was examined by RT-qPCR in different developmental stages including larvae, pupae, newly emerged adult workers, nurses, and foragers. **b** The expression profile of *AccTPI* was studied by RT-qPCR in various tissues. The data are the mean ± SEM of three independent experiments. The *letters* above the *columns* indicate significant differences ($P < 0.01$) according to Duncan's multiple range test.

were up-regulated by 4 °C, H_2O_2, and methomyl and pyridaben treatments. Furthermore, the expression levels of AccTPI were down-regulated by UV and VC treatments (Figure 4).

3.6. Disc diffusion assay under various stresses

To provide direct evidence that AccTPI is responsible for antioxidant defenses, a disc diffusion assay was performed according to Zhang et al. (2013). After overnight exposure to various stressors, the halo diameters of the death zones were significantly different for the various treatment concentrations. Compared with plates containing the control bacteria, the inhibition zones around the stressor-soaked filters were smaller in diameter containing *E. coli* overexpressing AccTPI (Figure 5).

4. DISCUSSION

Sequence alignment and the phylogenetic tree analysis showed that AccTPI has a high degree of homology with several representative TPI amino acid sequences (Figure 1). Taken together, these results indicate that the gene we isolated is a bona fide *AccTPI*.

To better understand its physiology, *AccTPI* expression at different development stages and in various tissues was analyzed. The expression of *AccTPI* reached a maximum in the 15-day post-emergence workers, which likely are foragers (Figure 2a). TPI, a key regulatory enzyme of glycolysis and gluconeogenesis, plays an

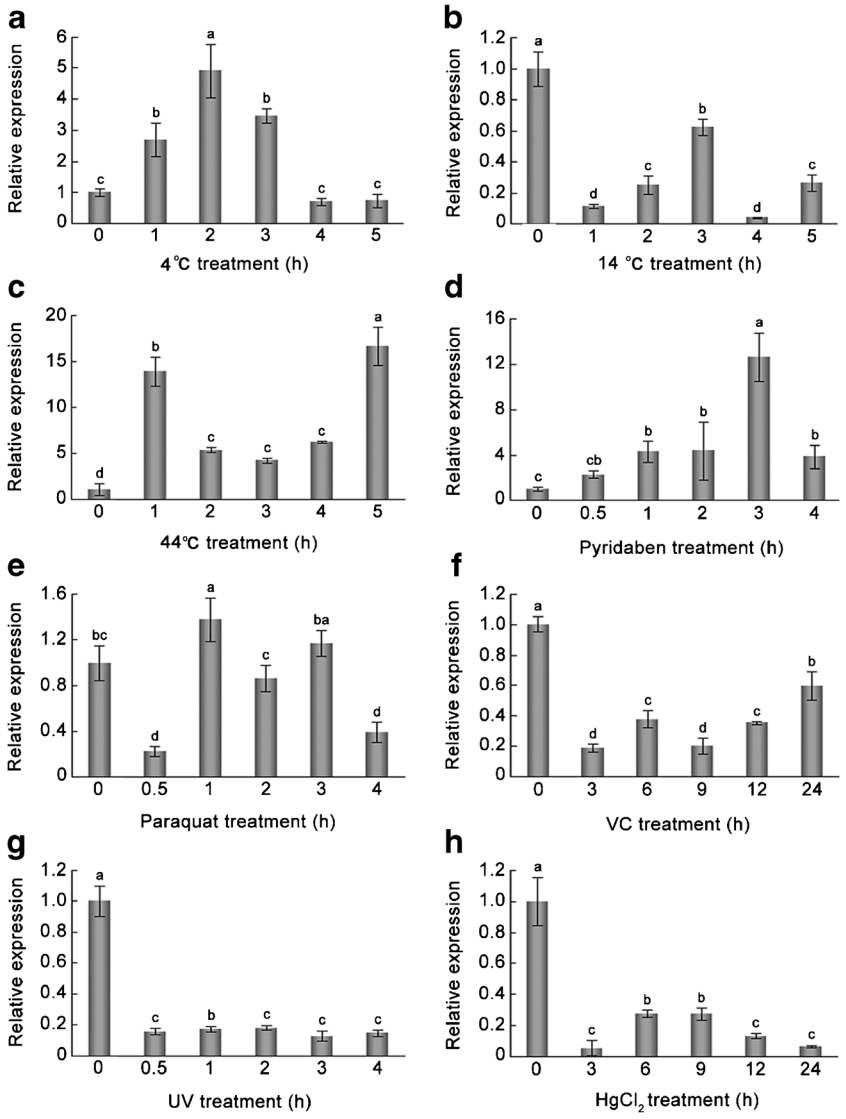

Figure 3. Expression profile of *AccTPI* under different abiotic stresses. The expression of *AccTPI* was analyzed by RT-qPCR using total RNA extracted from *A. cerana cerana*, which was treated with different stressors. For the stress treatments, 15-day-old adult bees were subjected to treatments with **a** 4 °C, **b** 14 °C, **c** 44 °C, **d** pyridaben, **e** paraquat, **f** VC, **g** UV, and **h** $HgCl_2$. CK is the abbreviation for control check. The *letters* above the *bars* indicate significant differences ($P < 0.01$) according to the SAS software version 9.1. The data are the mean ± SEM of three independent experiments. The *letters* above the *columns* indicate significant differences ($P < 0.01$) according to Duncan's multiple range test.

essential role in metabolism and development of most organisms (Zhou et al. 2015). These results revealed that *AccTPI* might be essential for the growth and development of bees. Additionally, the abdomen and thorax have higher *AccTPI* expression levels compared with other tissues (Figure 2b). Therefore, it is reasonable to deduce that *AccTPI* may be involved in differentiation or development relating to the mechanism of maturation, especially in the case of oxidation

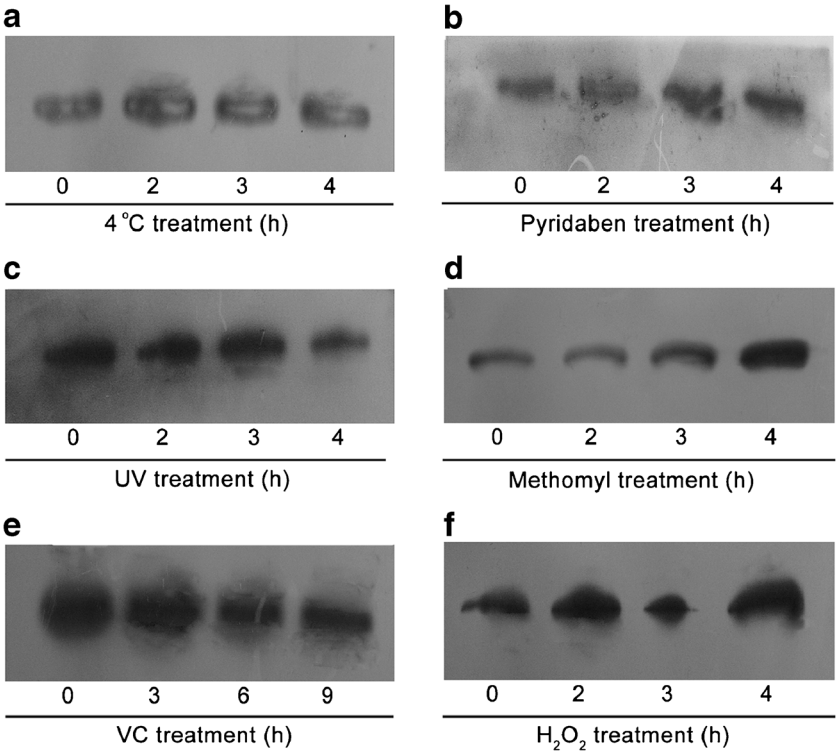

Figure 4. Western blot analysis of *AccTPI*. Western blot conditions include 15-day-old adult bees that were subjected to treatments with **a** 4 °C, **b** pyridaben, **c** UV, **d** methomyl, **e** VC and **f** H_2O_2. All *lanes* were loaded with an equivalent amount of extracted protein for each group.

resistance. Considering all of these results, we propose that *AccTPI* may be involved in organismal development and growth.

Previous studies have indicated that *A. cerana cerana* are stressed due to environmental changes and ROS (Yan et al. 2012). As the expression of *AccTPI* in *A. cerana cerana* due to exposure to oxidative stress has not yet been systematically reported, we investigated in this study the expression levels of *AccTPI* in response to various abiotic stresses. The results demonstrate that *AccTPI* may play an important role in protecting *A. cerana cerana* from oxidative stresses.

Temperature is an abiotic environmental factor that causes physiological changes in organisms and affects herbivorous insects (An and Choi 2010). In general, heat shock stress can result in polyamine oxidation to generate H_2O_2, and ROS act as key mediators of cold-induced apoptosis (Rauen et al. 1999). In the present study, the expression levels of *AccTPI* under 4 and 44 °C conditions were up-regulated following treatment. Western blot analysis of *AccTPI* under 4 °C (Figure 4a) treatments confirmed the result, which demonstrates that *AccTPI* may play a vital role in response to extreme temperature, low temperature in particular. These results suggest that *AccTPI* may function by removing intracellular superoxide anions generated under high- or low-temperature conditions to protect honeybees from ROS damage. All of these results indicated that *AccTPI* may be associated with the prevention of the oxidative damage.

Oxidative stress can be induced by insecticides, temperature, and UV radiation (Kottuparambila et al. 2012). The role of pesticides in honeybee colony losses, with their sub-lethal and synergistic effects, has recently regained consideration (Boncristiani et al. 2012). TPI also plays an important role in response to adverse environmental

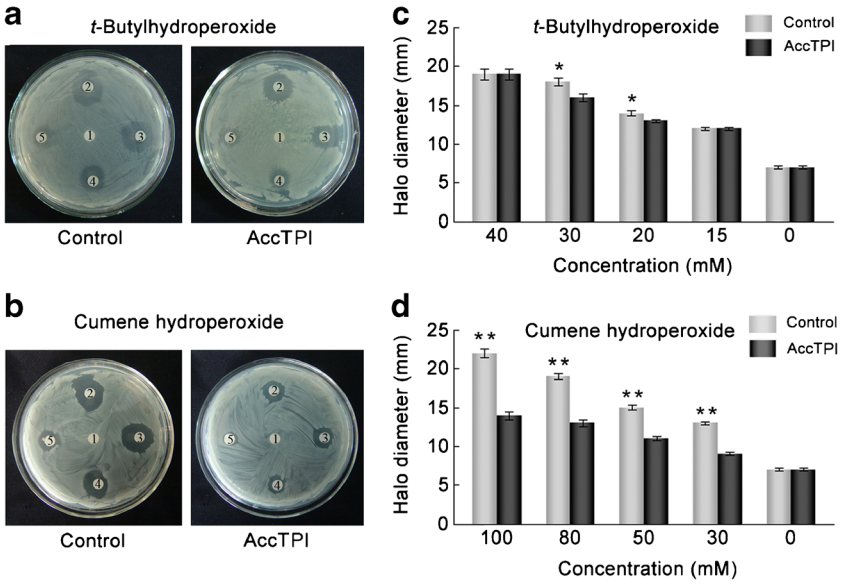

Figure 5. Disc diffusion assays for AccTPI. Filter discs soaked with different concentrations of **a**, **c** *t*-butylhydroperoxide and **b**, **d** cumene hydroperoxide were placed on agar plates. The picture on the *left* corresponds with the numbers on the *right*. The data are the mean ± SEM of three independent experiments. Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) software version 19.0. Statistical significance was determined by unpaired Student's *t* test; $*P < 0.05$, $**P < 0.01$.

conditions (Hewitson et al. 2014). TPI is highly up-regulated under stress conditions in mammalian cells (Yamaji et al. 2004). In this study, paraquat and pyridaben treatments up-regulated TPI expression levels in *A. cerana cerana* workers. The expression of *AccTPI* was up-regulated under some pesticide treatments. All of these results demonstrate that *AccTPI* expression is induced by abiotic stress and that *AccTPI* may play a crucial role in survival under adverse environmental conditions.

There is much evidence that UV is one of the most ubiquitous environmental hazards for most animals which can cause oxidative stress and in turn lead to protein damage (Schauen et al. 2007). Many heavy metals can induce the formation of endogenous ROS (Liu et al. 2009a). $HgCl_2$ treatment suppresses the expression of *Acctpx-3* (Yao et al. 2013). When honeybees were treated with UV and $HgCl_2$, expression levels of *AccTPI* were significantly down-regulated. These results indicated that *AccTPI* may play various roles in dealing with various adverse conditions. The mRNA expression of *AccTPI* is specifically induced by certain stresses and repressed by others, and these results indicate that TPI is a mediator of the stressors. With an increase in the treatment concentration or an extension of the treatment time, the antioxidant system may temporarily be ineffective due to decreased expression. All of these results suggest that *AccTPI* is involved in resisting oxidative stress of *A. cerana cerana* restrictedly.

Western blot results for AccTPI under H_2O_2 and VC treatments verified that the expression is up-regulated and down-regulated depending on the stressor. H_2O_2-induced ROS increase results in cell death caused by oxidative stress (Casini et al. 1986). H_2O_2-induced ROS increase results in cell death caused by oxidative stress (Goldshmit et al. 2001). Vitamin C, a well-

known antioxidant, induces the decomposition of lipid hydroperoxides to endogenous genotoxins and results in DNA oxidative damage (Lee et al. 2001). The expression levels of *AccTPI* in VC treatments were significantly down-regulated. A possible explanation for this was that with the increase of stress, other stress-related genes might play important roles in cellular stress responses and the role of *AccTPI* might be weakened. The expression levels of the *AccTPI* gene in *A. cerana cerana* were varied in response to the oxidative stresses. Thus, *AccTPI* may have a significant influence on the response to environmental stresses.

To understand the role of honeybee TPI in defending against oxidative stresses, disc diffusion assays (Figure 5) were used to detect the level of protection of AccTPI in *E. coli* cells from oxidative stressors (cumene hydroperoxide and *t*-butylhydroperoxide). The model external pro-oxidants cumene hydroperoxide and *t*-butylhydroperoxide were used because they are more stable than H_2O_2 under the applied incubation conditions (Zhang et al. 2013). There were variations in the diameters of the death zones between the *AccTPI*-overexpressing bacteria and the control bacteria. Hence, we can conclude that *AccTPI* can significantly contribute to cellular resistance under oxidative stresses and that the *AccTPI* gene has antioxygenic properties. The purified recombinant AccTPI protein showed a capacity to protect DNA from oxidative damage. Taken together, our data provide very useful information for further understanding the mechanisms responding to environmental pressures in *A. cerana cerana*.

In conclusion, all of the results indicate that *AccTPI* may play an important role in the metabolism and antioxidant defense of honeybees. The unique functional characteristics, expression patterns, and potential physiological roles of *AccTPI* that were demonstrated in this study offer the basic knowledge for further studies. It is an eternal theme for the survival of living organisms to protection against oxidative stress.

ACKNOWLEDGMENTS

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Clonage et identification moléculaire du gène de la triose-phosphate isomérase extrait d'*Apis cerana cerana* et son rôle dans la réponse à des stress variés

stress abiotique / métabolisme / enzyme / développement / Apidae

Klonierung und molekulare Identifizierung des Triosephosphatisomerase-Gens von Apis cerana cerana und seine Rolle in der Antwort auf verschiedene

Stressfaktoren Triosephosphatisomerase / Apis cerana cerana / abiotischer Stress / Metabolismus

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