Original Article

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Molecular Characterization of Juvenile Hormone Esterase like (JHE-like) in *Apis cerana*

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Abstract

The Juvenile Hormone Esterase (JHE) is major protein, when during Juvenile Hormone (JH) metamorphosis and reproduction in a honey bee life cycle. It was enzymes involved in JH metabolism. We cloned a full-length cDNA encoding JHE-like in *Apis cerana* (Acjhe-like) by RT-PCR (Reverse transcriptase Polymerase Chain Reaction). Acjhe was transcribed all tissue in *A. cerana* (Head, Fat body, Midgut, Epidermis, Muscle). Here we report the characteristics of JHE-like gene and comparison of gene expression in different tissues. We were analysed the Acjhe-like in different tissues that was expressed in head more than other tissue by quantitative real time PCR (qPCR). Furthermore, Acjhe-like was found to consist of 1,695-bp and deduced 565-amino acid mature protein that included a consensus GLSAG motif that is compulsory for the enzymatic activity of JHE-like protein.

Key words: Honeybee, *Apis cerana*, Juvenile Hormone Esterase like (jhe-like), Expression, Quantitative Real Time-PCR (qPCR)

INTRODUCTION

Honeybee is a social insect spending the entire life as each of the entities serves as a distinct role in their life cycles. Among various types of genes expressed in honeybees, representative proteins that derive formative and physiological changes such as metamorphosis are Juvenile Hormone Esterase (JHE), Juvenile Hormone (JH), Vitellogenin, and Ecdysteroid. Their interaction serves as an important role in changes in appearance including ecdysis (Riddiford *et al*., 2003). Juvenile hormone (JH) is known to be a hormone that derives changes in appearance, mating actions of male bees, and behavioral characteristics of work bees (Hartfelder and Engels, 1998). Honeybees have divided roles in each class. What plays an important role in such role division in each class is how much Juvenile Hormone (JH) is expressed. As they are involved in collection of honeybee and pollen including changes in appearance of honeybees and control of behavioral characteristics (cleaning behaviors and communication, etc.) in the bee colony, the number of spawn and larva is high depending on situations in bee colony. If there are enough feeds, a number of nurse bee is required for raising young bees and in the bee colony. Therefore, forager that serves as a role of collecting bees and pollens by controlling the release of hormone such as

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Juvenile Hormone (JH) can serve as a role of nurse bee (Huang *et al*., 1994; Huang and Robinson, 1996; Jassim *et al*., 2000).

Hereupon, as for honeybees living in a group play a role of dividing roles of each one and controlling behavioral characteristics. However, those hymenopterans that individually live such as bumber bee tend to involve in the control of hormone for breeding. They also artificially express juvenile hormone (JH) and, hence, suppress the expression of vitellogenin of young work bees (Bloch *et al*., 2000; Pinto *et al*., 2000). Such juvenile hormone (JH) serves as a role of suppressing the expression of vitellogenin of work bees from hymenopterans including the honeybees (*Apis mellifera*) while deriving the division of roles among classes of social insects and seamlessly maintaining the bee colony (Guidugli *et al*., 2005; Amdam *et al*., 2007).

Therefore, JHE protein serves as an important role for controlling the expression of JH or vitellogenin proteins. In this study, genes that express such JHE-like proteins has been identified the sequence and characteristics of expression of genes.

MATERIALS AND METHODS

Samples

Samples of honeybees used in order to analyze genetic characteristics of Juvenile Hormone Esterase like (Acjhe-like) genes in this study were *Apis cerana* that was raised in experiment apiary in National Institute of Agricultural Sciences in Rural Development Administration. Nursing bee of the samples was immediately used after they were collected.

cDNA Synthesis and PCR (Polymerase Chain Reaction)

Apis cerana worker bees, pupa, and larva that were collected were separated with total RNA by using Trizol (Sigma, CA). Using the liquid nitrogen, collected samples were pulverized by liquid nitrogen on mortar. The pulverized sample was mixed with 200ul of chloroform and 1ml of Trizol mixture, for phase separation. After the reaction, samples were centrifuged at 12,000 x g in 4°C for 15 minutes while separating the supernatant and mixing with 500μl isopropanol to react in the room temperature for 10 minutes. Then, they were centrifuged in the conditions of 12,000 x g in 4°C for 15 minutes followed by washing the pellet with 80% EtOH. The final pellet has been used for synthesis of cDNA by melting it in DEPC water. Synthesis of cDNA has used oligo dT primer with extracted total RNA as a template reacting with PrimeScript II 1st strand cDNA Synthesis kit in 65°C and for 5 minutes. Then, they were cooled in ice and synthesized with cDNA in 42°C and for 60 minutes.

Synthesized cDNA has been used as a mold to amplify the Juvenile Hormone Esterase like (Acjhe-like) gene, and Acjhe-like gene has been repeated in pre-denature 94°C for 5 minutes, denature 94°C for 1 minute, annealing 50°C for 1 minute, extension 72°C for 1 minute, and in 33 cycles by using Forward primer 5'-ATGAAGCTACTATTCCT AGTACTTC-3', Reverse primer 5'-TTATAATTCT TCTTT CAATGTGTCAC-3'while performing final extension in 72°C for 5 minutes. The primer set was designed by *A. mellifera* JHE DNA sequence. PCR outcome has been confirmed with UV-light by electrophoresis with the 1% agarose gel that contained 0.5ug/ml ethidium bromide.

Quantitative Real Time-PCR (qPCR)

Quantitative real time PCR for quantitative analysis of particular Acjhe-like gene uses JHE-reF-5'-CTGTTTC ACCATGCGTCCAG-3' as a forward primer and JHE-reR-5'-ATTGGCGCCCTCTCGATTC-3' as a reverse primer. As for control gene, β-actin gene was used, and qPCR was reacted by mixing primer in 10 pmol, 1× SYBR Green(Applied Biosystems), and 1st strand cDNA 1μl so that total volume becomes 25μl. With the analysis on the Ct value of final results, the amount of expressed gene has been compared (Applied Biosystems, user bulletin #2).

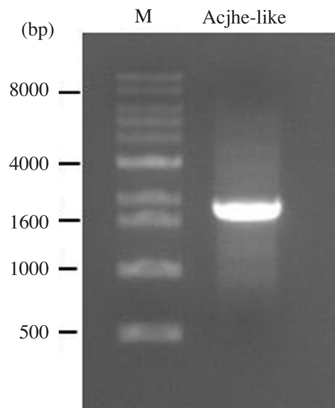

Fig. 1. Gel eletroporation of Juvenile Hormone Esterase like (Acjhe-like) by RT-PCR in *A. cerana*. Total RNA was isolated from whole body respectively. The cDNA was synthesised by RT-PCR. M is 1kb ladder molecular weight marker. The amplification products were electrophoresed on 1% agarose gel, stained with ethidium bromide, and visualized under UV light.

Northern blotting

Northern blotting has been crossed linked with UV after separating the total RNA by using Trizol, proceeding electrophoresis on Formamid gel, and transferring to nitrocellulose membrane for 18 hours. Membrane has been pre-hybridized in hybridization solution for an hour followed by hybridization for 18 hours. Probe has labeled the radiation isotope, p^{32}, on PCR outcome of Acjhe-like gene and exposed to X-ray film for 24 hours and printed by using the development solution and fixer.

RESULTS AND DISCUSSION

PCR (Polymerase Chain Reaction)

In order to acquire Juvenile Hormone Esterase (JHE) gene expressed in *Apis cerana*, gene specific primer was used proceeding PCR with cDNA as a mold. As a result, PCR outcome in the size of 1,695 was amplified (Fig. 1). This PCR outcome is in the similar size of JHE expressed in *Apis mellifera*.

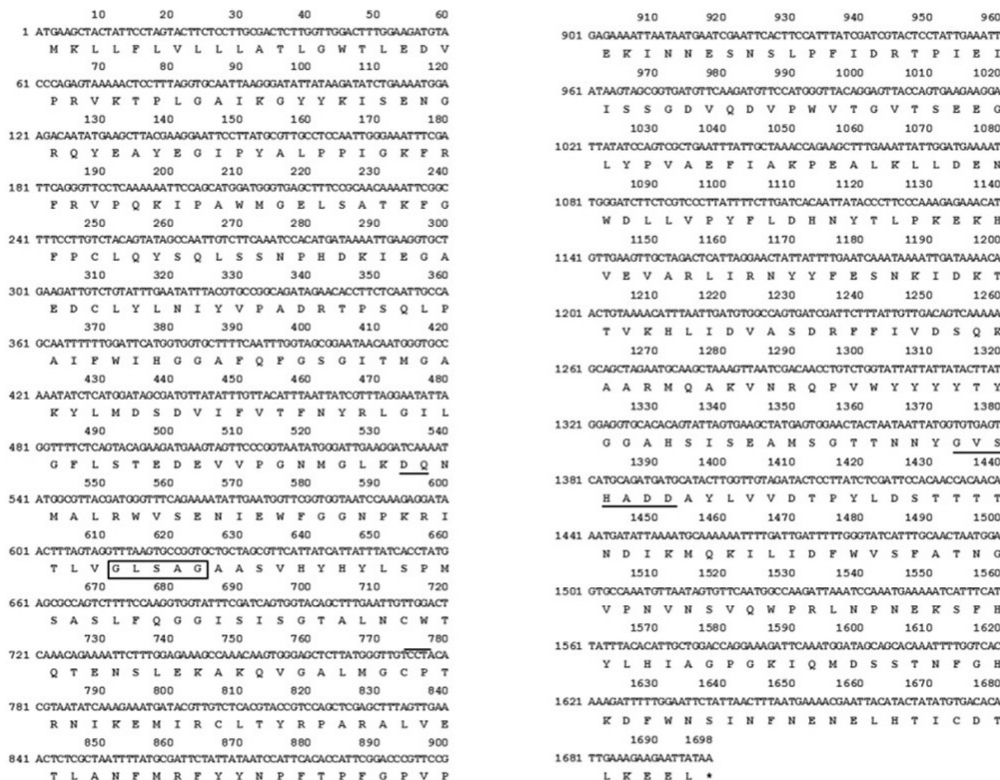

Fig. 2. Nucleotide and deduced amino acid sequence of Juvenile Hormone Esterase like (Acjhe-like). The putative signal peptide is indicated by the thin line under the amino acid sequence. The stop codon is indicated by an star. Catalytic domains, including the GLSAG motif within which the catalytic serine is contained are indicated by solid box the amino acid sequence. The catalytic motifs which are conserved in honeybee JHEs are indicated by thick underlines.

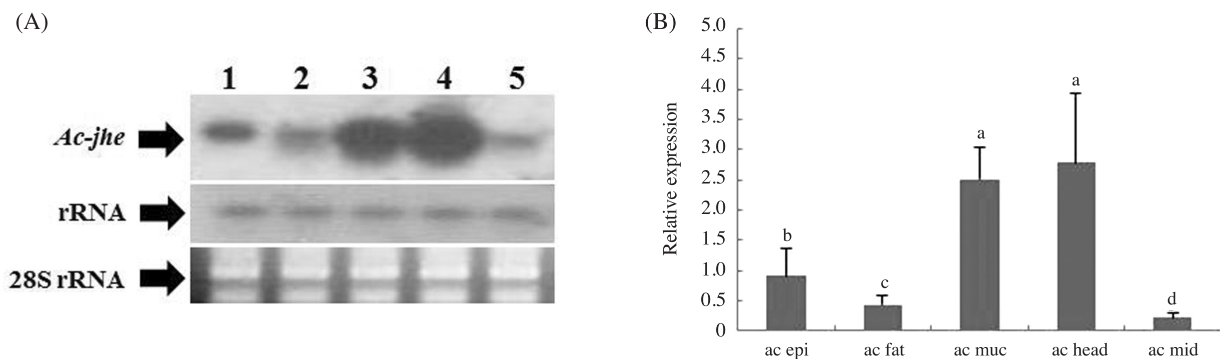

Fig. 3. Transcriptional expression of the Juvenile Hormone Esterase like (Acjhe-like) gene in different tissue of *A. cerana*. (A) Northern blot analysis of the Acjhe-like in *A. cerana*. Total RNAs were isolated from the epidermis (lane 1), fat body (lane 2), muscle (lane 3), head (lane 4), midgut (lane 5). The RNAs were separated by 1.0% formaldehyde agarose gel electrophoresis, transferred onto a nylon membrane and hybridized with radio-labled 1,695-bp Juvenile Hormone Esterase like(Acjhe-like) gene. (B) Transcript levels of Acjhe-like genes in different tissue by qPCR (t-test, $P<0.001$).

Sequence analysis

The outcome secured with PCR has been refined by using PCR clean up kit (Qiazen) litigating on pGEMT easy vector (Promega) and transforming them on Topo 10F' (Stratagen). After separating the plasmid, ABI 3100 sequence listing analyzer was used to analyze sequence listing. As a result, gene that produced proteins consisting of 565 amino acids in 1,695 bp coding areas was confirmed.

According to the result of NCBI blast, this gene turned out to code Juvenile Hormone Esterase like(Acjhe-like) proteins that genes from the 612 bp to 625bp represented the G(Glycine) L(leucine) S(Serine) A (Alanine) G(Glycine) amino-acid listing as an active site of JHE gene and G(Glycine) V(Valin) S(Serine) H(Histidine) A(Alanine) D(Aspartic acid) D(Aspartic acid) listing in characteristics (Aline Mackert *et al*., 2008) (Fig. 2). The predicted gene has hence been termed Acjhe-like to denote its similarity to insect jhe genes and at the same time calling attention to this difference in the canonical GQSAG motif. Therefore, this gene was confirmed to produce Juvenile Hormone Esterase like (Acjhe-like) proteins.

Northern blotting & Quantitative Real time PCR (qPCR)

In order to check how Juvenile Hormone Esterase like (Acjhe-like) protein was expressed in *Apis cerana*, Northern blotting and quantitative real time PCR (qPCR) were conducted. As a result, Acjhe-like protein was confirmed to be evenly expressed in skin, fat body, head muscle, head, and midgut. It was also confirmed that the most amount of protein was expressed in muscle and head by result of both northern blotting and qPCR (Fig. 3). It is believed that protein was expressed in each organization as shown in this result as ecdysis was expressed on the entire organizations in consideration of how Juvenile Hormone Esterase (JHE) was expressed in relation with expression and mode of actions with Juvenile Hormone (JH) and Vitellogenin proteins.

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