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cDNA Cloning and mRNA Expression of A Cuticle Protein Gene Homologue from *Protaetia brevitarsis*

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A cuticle protein gene, *PbLCP12.1*, from the white-spotted flower chafer, *Protaetia brevitarsis*, was isolated and characterized. The gene contains an ORF of 336 nucleotides capable of encoding a 113 amino acid polypeptide with a predicted molecular mass of 12,138 Da and pI of 4.15. The *PbLCP12.1* protein contained a type-specific consensus sequence identifiable in other insect cuticle proteins. The deduced amino acid sequence of the *PbLCP12.1* cDNA is most similar to *Bombyx mori* cuticle protein BmLCP18 (37% protein sequence identity). Northern blot analysis revealed that *PbLCP12.1* showed the epidermis-specific expression.

Key words: cDNA cloning, Cuticle protein, Insect, *Protaetia brevitarsis*

Introduction

The insect cuticle is a complex extracellular structure composed mainly of chitin and proteins that are synthesized and secreted by epidermal cells. Cuticle proteins, the major components of insect integument, are being studied for the mechanisms of gene regulation during molting and metamorphosis (Shofuda *et al.*, 1999; Togawa *et al.*, 2001).

A number of cuticle proteins have been identified and characterized in various insect species. In the silkworm, *Bombyx mori*, larval cuticle proteins (LCPs) designated as LCP17, LCP18, LCP22 and LCP30 are well characterized. Biosynthesis of cuticle proteins is controlled stage-dependently and is regulated by the hormones ecdysteroid and juvenile hormone (Nakato *et al.*, 1994, 1997; Braquart *et al.*, 1996; Hiruma *et al.*, 1997; Kramer and Wolbert, 1998; Shofuda *et al.*, 1999). In addition, the cuticle protein genes from the wild silkmoths, *Antheraea yamamai* and *A. pernyi*, have been reported (Kim *et al.*, 2005a, b). In the mulberry longicorn beetle, *Apriona germari*, larval cuticle proteins are expressed differentially after ecdysis (Kim *et al.*, 2003). Although the insect cuticle protein genes have been intensively studied, the molecular information on the cuticle protein genes is still limited in some insect species.

In order to obtain further genetic information on the cuticle protein in insects, we have cloned a cDNA encoding larval cuticle protein homologue from the white-spotted flower chafer, *Protaetia brevitarsis*. In this paper, the cloning, sequencing and characterization of the *P. brevitarsis* larval cuticle protein cDNA are described.

Materials and Methods

Insects

The larvae of the white-spotted flower chafer, *P. brevitarsis*, were maintained at 28°C, 70% relative humidity, and photoperiod of 16 L : 8 D (Kim *et al.*, 2002) and final instar larvae were used for the experiment.

cDNA library screening, nucleotide sequencing and data analysis

Total RNAs were isolated from the whole body of *P. brevitarsis* larvae following the procedure of Total RNA extraction kit (Promega). Poly(A) + mRNA was purified using oligo(dT) columns of Quick mRNA isolation kit (Stratagene). A cDNA library was constructed from poly (A) + mRNA isolated from the whole body of *P. brevitarsis* larvae by Uni-ZAP XR vector and Gigapack III

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Gold Packing Extract (Stratagene). The cDNA was ligated into *Eco*RI-*Xho*I sites of Uni-ZAP XR vector. Ligated library was transformed into *E. coli* XL1-Blue MRF' strain. *Escherichia coli* XL1-Blue MRF' strain was infected by the Uni-ZAP XR library harboring *P. brevitarsis* cDNAs and cultured on the NZY (NZ Amine yeast extract) agar medium. Each plaque was suspended in SM buffer [5.8 g NaCl, 2 g $MgSO_4 \cdot 7H_2O$, 0.05 M TrisHCl (pH 7.5) and 0.01% gelatin solution] containing 0.02% (v/v) chloroform and stored at 4°C for 1 day. The phages were eluted into SM buffer. The pBluescript phagemids were *in vivo* excised from the Uni-ZAP XR vector using an ExAssist helper phage. *E. coli* strain, SOLR cell (Stratagene), was infected by the excised phagemids and plated on LB-Amp medium (50 μg ampicillin/ml). Plasmid DNA from the overnight culture was isolated. The size of inserted cDNA was estimated with a 1% agarose gel electrophoresis after treatment with restriction enzymes (*Eco*RI and *Xho*I). For DNA sequencing, plasmid DNA was extracted by Wizard mini-preparation kit (Promega). Sequence of the 5' end of each cDNA clone was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). The sequences were translated into 6 reading frames and compared using the DNASIS and BLAST programs provided by the NCBI. GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program (www.ncbi.nlm.nih.gov/BLAST). MacVector (ver. 6.5, Oxford Molecular Ltd.) was used to align the amino acid sequences of cuticle protein gene.

RNA isolation and Northern blot analysis

The larvae of *P. brevitarsis* were dissected under a stereomicroscope, individual samples such as fat body, midgut, and epidermis were harvested, and washed twice with PBS (140 mM NaCl, 27 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4). Total RNA was isolated from the fat body, midgut, and epidermis of the *P. brevitarsis* larvae by using the Total RNA Extraction Kit (Promega). Total RNA (10 μg/lane) from the *P. brevitarsis* larvae was denatured by glyoxalation (McMaster and Carmichael, 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell, Dassel, Germany) and hybridized at 42°C with a probe in a hybridization buffer containing 5 × SSC, 5 × Denhardt's solution, 0.5% SDS, and 100 μg denatured salmon sperm DNA/ml. The *P. brevitarsis* cuticle protein cDNA clone was labeled with [α-^{32}P]dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA) for use as a probe for hybridization. After hybridization, the membrane filter was washed three times for 30 min each in 0.1% SDS and 0.2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) at 65°C and exposed to autoradiography film.

Results and Discussion

Cloning, sequencing, and alignment of *PbLCP12.1* cDNA

A cDNA library was constructed using whole bodies of *P. brevitarsis* larvae. Sequencing of randomly selected clones harboring cDNA inserts was performed to generate *P. brevitarsis* ESTs. One clone of 537 bp had a full-length coding sequence similar to that of previously reported cuticle proteins. The cDNA clone including the full-length open reading frame (ORF) was sequenced and characterized. The nucleotide sequence and its deduced amino acid sequence of the cDNA encoding larval cuticle protein are presented in Fig. 1. The *P. brevitarsis* cuticle protein cDNA encoding a putative member of the insect cuticle protein family contains an open reading frame (ORF) of 339 bp encoding 113 amino acid residues. The ORF had both a start codon (ATG) and stop codon (TAA), indicating that the sequences contain the complete coding region. A putative polyadenylation signal, AATAAA, is located at nucleotide position 487 - 492. The predicted molecular mass for the *P. brevitarsis* cuticle protein was approximately 12,138 Da and pI of 4.15, and we designated *PbLCP12.1*.

Multiple sequence alignment of the deduced protein sequence of *PbLCP12.1* with other cuticle protein sequences

```
-13                                                    tttgagacaagta
  1 ATGGAATCAAGTCACGACGAAGAGGGTCAACTGCAGAACGCCGGTTCGGAGAACGAACAG
  1 M  E  S  S  H  D  E  E  G  Q  L  Q  N  A  G  S  E  N  E  Q
 61 ATGCAAGCTCACGGTAGTTTCAAATTATCCACTATAGGGCTCGAGCGGCCGCCCGGGCAG
 21 M  Q  A  H  G  S  F  K  L  S  T  I  G  L  E  R  P  P  G  Q
121 GTTAATGATAATATCGGAGTCGACGGTTACAAATTCGGCTTCGAGACAAGTAATGGAATC
 41 V  N  D  N  I  G  V  D  G  Y  K  F  G  F  E  T  S  N  G  I
181 AGTCACGACGAAGAGGGTCAACTGCAGAACGCCGGTTCGGAGAACGAACAGATGCAAGCT
 61 S  H  D  E  E  G  Q  L  Q  N  A  G  S  E  N  E  Q  M  Q  A
241 CACGGTAGTTTCAAATTCACCGATCCCGCTAGCGGTAAAACGATTACCGTCACTTACACT
 81 H  G  S  F  K  F  T  D  P  A  S  G  K  T  I  T  V  T  Y  T
301 GCCGACGAAAACGGTTACAGACCGTCGGTCTCAATCGCCTAAaattattaattaaatgat
101 A  D  E  N  G  Y  R  P  S  V  S  I  A  *
361 taaatgtaaataagaaatattaaatctcgcgccattaggccaaaatcgttatactgtttt
421 tgtaccctgacttgtgtggaatcgtgaaaaataaaaaatataccttacgataaataaaat
481 gtacataataaacgccttttttacgtaaaaaaaaaaaaaaaaaa
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Fig. 1. The nucleotide and deduced amino acid sequences of the *PbLCP12.1* cDNA. The start codon of ATG is boxed and the termination codon is shown by asterisk. In the cDNA sequence, the polyadenylation sequence AATAAA is underlined. This cDNA sequence has been deposited in GenBank under accession number DQ178132.

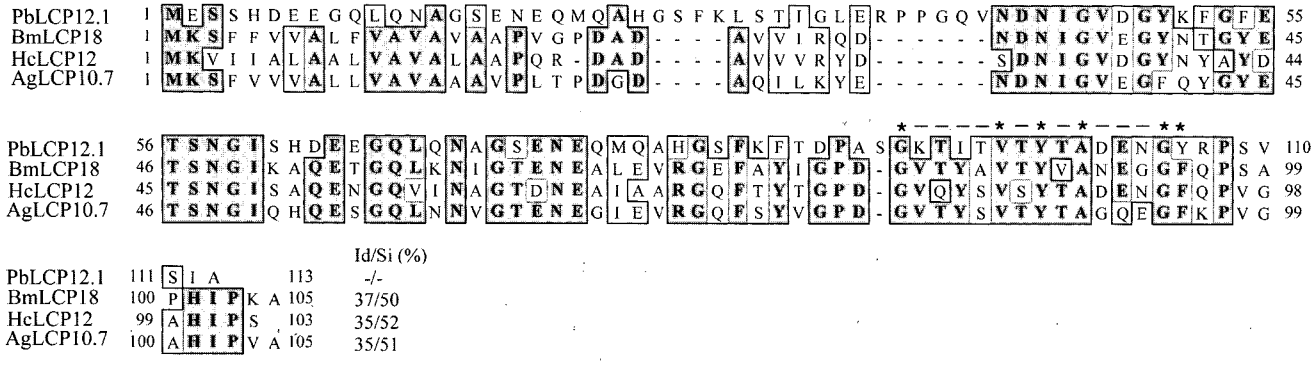
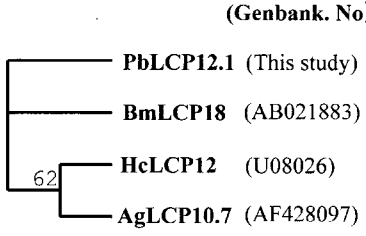


Fig. 2. Multiple sequence alignment of the deduced protein sequence of *PbLCP12.1* cDNA with related insect cuticle protein sequences. The identical residues are shown in solid boxes. Asterisks indicate the cuticle protein consensus sequence of G-------(D/E)G----(V/L/I)-(Y/F)-A---G(Y/F). The abbreviation and GenBank accession number for the cuticle protein sequences analyzed are: *PbLCP12.1*, *P. brevitarsis* larval cuticle protein (XXX; this study); BmLCP18, *Bombyx mori* larval cuticle protein (AB012081); *HcLCP12*, *Hyalophora cecropia* cuticle protein (U08026); and *AgLCP10.7*, *Apriona germari* larval cuticle protein (AF428097).

is shown in Fig. 2. Alignment of the *PbLCP12.1* sequences with those of cuticle proteins from several insect species indicates the extent of the identity that exists. The *PbLCP12.1* contained cuticle protein consensus sequence of G-------(D/E)G----(V/L/I)-(Y/F)-A---G(Y/F), present in a hydrophilic region near the C-terminal end. This consensus sequence was conserved among 13 cuticle protein sequences from dipteran, lepidopteran, coleopteran and orthopteran species (Andersen *et al.*, 1995), suggesting that the *PbLCP12.1* is a putative member of the insect cuticle protein family. The *PbLCP12.1* showed 37% protein sequence identity to *B. mori* silk moth cuticle protein *BmLCP18*, but no clear phylogenetic relationship of the protein to other insect cuticle proteins was detected (Fig. 3). This may have caused possibly due to distant taxonomic relationships of the *PbLCP12.1* to other included proteins and small number of the included protein sequences.



	GenBank No.	1	2	3	4
		Percent similarity			
1. PbLCP12.1	This study		50	52	51
2. BmLCP18	AB021883	37		73	82
3. HcLCP12	U08026	35	60		74
4. AgLCP10.7	AF428097	35	65	59	
		Percent identity			

Fig. 3. Phylogenetic relationships and pairwise identities and similarities of the deduced amino acid sequence of *P. brevitasis* cuticle protein among insect cuticle protein sequences. The insect cuticle protein sequences were taken from the following sources: *PbLCP12.1*, *P. brevitarsis* larval cuticle protein (XXX; this study); BmLCP18, *Bombyx mori* larval cuticle protein (AB012081); *HcLCP12*, *Hyalophora cecropia* cuticle protein (U08026); and *AgLCP10.7*, *Apriona germari* larval cuticle protein (AF428097).

Expression of *PbLCP12.1* mRNA

To confirm the tissue-specific expression of the *PbLCP12.1* at transcriptional level, Northern blot analysis was performed using mRNA prepared from epidermis, midgut and fat body (Fig. 4). A hybridization signal was detected

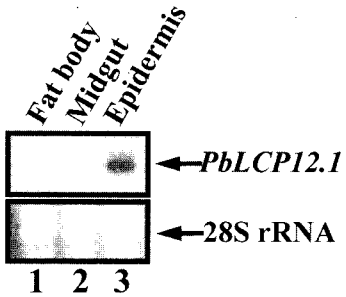


Fig. 4. Tissue-specific expression of *PbLCP12.1* mRNA. Total RNA was isolated from the fat body (lane 1), midgut (lane 2), and epidermis (lane 3) of *P. brevitarsis* larva, respectively. The RNAs were separated by 1.0% formaldehyde agarose gel electrophoresis (lower panel), transferred on to a nylon membrane, and hybridized with the radiolabelled *PbLCP12.1* cDNA (upper panel). Transcripts of *PbLCP12.1* are indicated on the right of the panel by arrow.

as a single band of mRNA from the epidermis, indicating that the *PbLCP12.1* is expressed only in the epidermis. The result is consistent with the previous reports in that the cuticle protein gene is tissue-specifically expressed in epidermis (Nakato *et al.*, 1997; Rebers *et al.*, 1997; Shofuda *et al.*, 1999; Togawa *et al.*, 2001; Kim *et al.*, 2003; Sawada *et al.*, 2003; Kim *et al.*, 2005a, b).

In conclusion, we have cloned a novel cuticle protein gene from *P. brevitarsis*. We hope that the molecular characterization of cuticle protein in a beetle in this study will expand the understanding of insect cuticle proteins.

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