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Full Length Article

Molecular Cloning Expression Profile and 5'Regulatory Region Analysis of Ribosomal Protein S16 Gene from the Diamondback Moth *Plutella xylostella*

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ABSTRACT

The cDNA (GeneBank accession NO: EU818707) encoding ribosomal protein S16 of diamondback moth, *Plutella xylostella* was cloned, sequenced and analyzed. The cDNA is 587 bp with an open reading frame of 465 bp that encodes 154 putative amino acids. The amino acid sequence shares high similarity with RpS16 of other organisms. The RpS16 of diamondback moth genomic sequence consists of three exons and two introns having approximately 2kb in size. The 5' regulatory region of this gene has also been isolated using the method of Tail-PCR, which contains the putative promoter sequence. Semi-quantitative RT-PCR analysis showed that the RpS16 of diamondback moth gene was expressed in all examined stages whereas female adults were found to have highest expression level, suggesting the functional importance for the female adult development. Phylogenetic analysis indicated that the RpS16 of diamondback moth was more closely related to their equivalents in *Heliconius melpomene*. Prediction of secondary structure showed that 37.01% of the amino acid sequence of the protein forms Alpha helix, 21.43% is in extended strand and 41.56% is in random coil. The RpS16 is strongly conserved among different orders of insects. © 2010 Friends Science Publishers

Key Words: *Plutella xylostella*; Ribosomal protein S16; cDNA cloning; Sequence analysis; Genomic organization; Expression pattern

Abbreviations: BR-C Z, Broad-Complex Z; HSF, heat shock transcription factor; Dfd, Deformed; Hb, Hunchback; ORF, open reading frames; Pxly, *Plutella xylostella*; RpS16, Ribosomal Protein S16; RACE, rapid amplification of cDNA ends; UTR, untranslated region.

INTRODUCTION

The diamondback moth, *Plutella xylostella*, is a major pest of cruciferous crops all over the world. Owing to serious damage to crucifer production and high resistance to a wide range of insecticides, *P. xylostella* has received much attention throughout the world and the annual cost for its management is estimated to be U.S. $1billion (Talekar & Shelton, 1993; Capinera, 2001; Songa *et al.*, 2008). *P. xylostella* also has been found to be the first insect to develop a resistance against *Bacillus thuringiensis* (Bt), which is a widely used biological insecticide and has important role in reducing insect damage, as well as chemical pesticides pollution (Yu & Zhan, 1999; Sarfraz & Keddie, 2005). So, the behavior physiology and evolution of *P. xylostella* should be deeply studied.

Ribosome is the organelle for protein synthesis in all living cells. It is a complex assemblage of ribosomal RNAs and ribosomal proteins. The eukaryotic ribosomes are composed of large (60S) and small (40S) subunits (Doudna & Rath, 2002). The large subunits catalyses the peptide bond formation and is responsible for channeling the nascent proteins through their exit tunnel, whereas the small ribosomal subunits bind mRNA and are responsible for the fidelity of translation (Zarivach *et al.*, 2004; Manchado *et al.*, 2007). Ribosomal protein S16 (RpS16) is one of the ribosomal 40S components, which has been studied extensively in mammalians, rice and bacteria (Chan *et al.*, 1990; Zhao *et al.*, 1995; Vgren & Wikstro, 2001; Parakhnevitch *et al.*, 2005), but little work has been reported on ribosomal protein S16 in insects.

The ribosomal protein families of prokaryotes and eukaryotes have about 55 and 80 proteins, respectively. They are generally highly conserved and small molecular weight proteins (Doudna & Rath, 2002). Each of them is very necessary for the building of the ribosome, but some proteins of this family are multifunctional. In *Drosophila*, low expression of some proteins of this family lead to embryonic death or developmental disorders (Warner & Nierras, 1998). In insect, some genes of ribosomal protein

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families also have been cloned. Tan *et al*. (2007) has cloned the ribosomal protein L39 gene of *Culex pipiens pallens* and showed that this gene was associated with resistance of deltamethrin in this insect. The ribosomal protein S7 gene of *Anopheles stephensi* has also been obtained and its sequence is conserved having relation to nuclear localization signal (NLS) region of human rpS7 (Dixit *et al.,* 2007). But in *P. xylostella* only the ribosomal protein S13 have been studied (Wang *et al.,* 2005), so this family has a great potential of being discovered and worked out. This kind of research can be helpful in determining the cause of emerging resistance in the insects, especially in this species.

In this study, the method of rapid amplification of cDNA ends (RACE) was followed to obtain the full-length cDNA coding for ribosomal protein S16 in *P. xylostella* (*Pxyl-RpS16*) by using degenerate primers. This study was also used to get full genomic organization information to understand the biological functions and regulation expression of RpS16 in *P. xylostella*.

MATERIALS AND METHODS

Biological materials: *P. xylostella* pupae were collected from insecticide-free cabbage and brought to laboratory for rearing. Larvae were allowed to feed on cabbage leaves in a rearing room with conditions set at 25±1°C, 16:8 L: D and 70%–85% relative humidity.

RNA preparation and cloning of RpS16: Total RNA was extracted from adults of *P. xylostella* using the Trizol Reagent (Invitrogen, USA) according to the protocol provided by the manufacturer. First-strand cDNA was synthesized from the total RNA with reverse transcriptase AMV and oligod $(T)_{18}$. 5'-and 3'-RACE-ready cDNA were prepared according to the instructions of Gene RacerTM Kit's protocol (Catalog nos: L1500-01, invitrogen, USA).

A pair of degenerate primers was determined by alignment of published RpS16-like transcripts from distantly related species.F1:5-ATMCTATDGHTTVGAAAGGA-3 and R1: 3-GGCAATBAKAGCCTYGGADA-5. The PCR reaction was performed with the following conditions: one cycle (94℃, 3 min); 35 cycles (94℃, 30 s; 52℃, 45 s; 72℃, 1 min) and a last cycle 72℃, 10 min. The PCR product was then cloned into a pMD-20-T vector (TaKaRa, Dalian, China) and Positive clones were sequenced.

According to the RpS16-like transcript fragment amplified from *P. xylostella* by degenerate primers, two sequence specific primers were designed and used to amplify the full length. Primers are as follows: 5'RACE primer: 5'-CCTGTCGTATTGGACCAGGATGTCC-3', 3'RACE primer: 5' -TCAGGAACCCATCCTATTGCTTGGA -3'. A full-length cDNA of Pxyl-RpS16 gene was obtained by overlapping the three cDNA fragments.

Genomic DNA isolation and DNA sequence amplification: Genomic DNA from *P. xylostella* was extracted according to instruction of TIANamp Genomic DNA kit's protocol (TIANGEN, China), genomic DNA was further precipitated with ddH_2O and agarose gel electrophoresis to determine its quality and it was shown on a single band. The specific primers were designed to amplify the genomic DNA corresponding to the cDNA code region of *RpS16*. The sense primer was 5'-AAAACCGCTACAGCCGTTGCTTACT- 3' and the antisense primer was 5' –ACGGTAAGACTTCTGGTAGCGAGCG-3'. The PCR reaction was performed with the following conditions: one cycle (94℃, 3 min); 35 cycles (94℃, 30 s; 60℃, 45 s; 72℃, 1 min) and a last cycle 72℃, 10 min. The amplified DNA was sequenced.

Isolation of genomic 5'-upstream regulatory region of Pxyl-RpS16: Genomic DNA of *P. xylostella* was prepared as above. In order to obtain 5'–upstream regulatory sequence of *Pxyl-RpS16*, genome working approach was performed according to the introductions of genome walking kit (TaKaRa, Dalian, China). The PCR principle of the genome walking approach is thermal asymmetric interlaced PCR (Tail-PCR). The specific reverse primers were designed according to 5'-terminal nucleotide sequence of Pxyl-RpS16 by following: SP1: 5-GCCAAGAGTACTCAGGAAGTCAATG-3; SP2: 5-CAGTAGGGTGTTGCAAACCAAATCG-3; SP3: 5-GGGTTCCTGAAGTTTGTACTGAAGG-3 and the forward primers were supported by the kit. The conditions for PCR response were set according to the kit's introductions. The PCR fragments obtained through the genome walking approach were detected using 1.5% agarose gel electrophoresis and the positive clones were sequenced.

Semi-quantitative RT-PCR analysis: Semi-quantitative RT-PCR was performed to measure gene expression at different developmental stages. The cDNA samples were prepared from male and female adults, all larval stages and pupae, using plant RNA kit (Catalog nos: R6827, OMEGA, USA) and reverse transcriptase AMV (TaKaRa, Dalian, China).

The gene specific primer was designed from cDNA sequence of Pxyl-RpS16 the forward and reverse primers were 5-ATGGCGGCCGTGCAGGAGATGTTAC-3 and 5-TTAACGGTAAGACTTCTGGTAGCGA -3. The18S rRNA gene of *P. xylostella* was used as reference the primers as follows: 18S-F: 5'-CCGATTGAATGATTTAGTGAGGTCTT-3'; 18S-R: 5'-TCCCCTACGGAAACCTTGTTACGACTT-3'. One to two μL of the cDNA was used to amplify and final volume of reaction mixture was 50 μL. The PCR amplification were performed using the following thermal cycle conditions: one cycle(94℃, 3 min); 27 cycles (94℃, 30 s; 60℃, 45 s; 72℃,1 min) and a last cycle 72℃, 10 min. PCR products

were detected by 1.5% agarose gel electrophoresis.

Each sample was repeated three times. Quantification of the bands was performed by SinsiAnsys1.0.3 software, grey value of bands was expressed as relative quantity units. Calibration (Vol. uL) of cDNA samples was done with the reference to amplified concentrations of actin gene. SPSS 13.0 was used to estimate significance of variance between different expression levels at stages and $P < 0.05$ was considered significant.

Bioinformatics analysis: 15 amino acid sequence of RpS16 were retrieved from BLAST search using the NCBI BLAST analysis programs blastP and use the 'nr' databases. Molecular mass and isoelectric point of Pxyl-RpS16 was predicted using the software of ExPASy (http://www.expasy.ch/). Promoter prediction and characterization were carried out using the Neural Network Promoter Prediction (NNPP) server (http://www.fruitfly.org/seq_tools/promoter.html) (Reese *et al*., 2001). Sequence analysis seeking for transcriptional regulation response elements was carried out with TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) (Heinemeyer *et al.*, 1998). Multiple sequence alignment was carried out with ClustalW2 program and the online service was http://www.ebi.ac.uk/Tools/clustalw2/index.html (Higgins *et al.*, 1994). Secondary structure of Pxyl-RpS16 was analyzed using on-line tools: http://npsa-pbil.ibcp.fr. Phylogenetic tree was constructed using MEGA 3.0 software (Kumar *et al.*, 2004), using the neighbor joining method and reconstructed with 1000 replicate bootstrap analysis.

RESULTS

Gene cloning of Pxyl-RpS16: A 587 bp cDNA of Pxyl-RpS16 was obtained by RACE-PCR using the degenerate primers. BLAST results revealed that the cDNA was highly homologous to RpS16 of other insects, which included a 14 bp 5' untranslated region (UTR), a 108 bp3'UTR with a 33 bp poly (A) tail and an 465 bp open reading frame (ORF) encoding 154 amino acids. The deduced protein has a computed molecular mass of 17.4 kDa and a predicted isoelectric point of 10.07.

Genomic characterization of Pxly-RpS16: PCR amplification of genomic DNA with primers, designed corresponding to the cDNA of Pxyl-RpS16, resulted (Fig. 1) in a product having a size of about 2000 bp. By comparing the genomic sequence and cDNA sequence of *Pxyl-RpS16*, it was found that Pxyl-RpS16 gene is composed of two introns. The first and the second intron are about 390 bp and 1118 bp, respectively. The sequence of the exon/intron-splicing junction of Pxyl-RpS16 is shown in Fig. 2.

5' upstream regulatory region analysis of Pxyl- RpS16: In order to achieve a better understanding of the regulatory mechanisms of ribosomal protein S16 in *P. xylostella*, we succeeded in isolating the 5 regulatory region of *Pxyl-RpS16* having the size of 467 (Fig. 7) by performing genome working experiments. Using the online server of TFSEARCH, the putative regulatory elements were obtained. The results showed that the 5'upstream region of *Pxyl-RISP*, include not only the promoter sequences, but also having some transcriptional elements (BR-C Z, Hb, Dfd, HSF etc.).

Fig. 1. Schematic models of genomic DNA region of *Pxly-RpS16*. Line indicate 5' and 3'URTs, exons and introns are illustrated by black and white boxes, respectively, the upper and lower PCR primers for genomic sequence are indicated by small arrows, 5'upstream region expressed by argentite rectangle and the specific primers for this experiment also shown

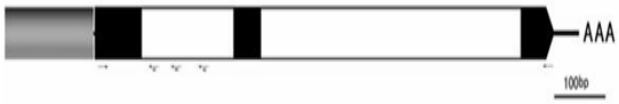

Fig. 2. Complete cDNA and deduced amino acid sequences of Pxyl-RpS16, the locations of two introns are shown by boldfaced minuscule letters in bracket, the stop codon is indicated by an asterisk

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1   CTTTCTCTGTCAAA
15 ATG GCG GCC GTG CAG GAG ATG TTA CAG GCG AGG CGC GAA CCT
     M   A   A   V   Q   E   M   L   Q   A   R   R   E   P
57 ATC CAA GCT GTG CAG GTC TTC GGC CGC AAA AAA ACC GCT ACA
     I   Q   A   V   Q   V   F   G   R   K   K   T   A   T
99 GCC GTT GCT TAC TGC AAG CGT GGT AAT GGA GTG CTG AGG GTG
     A   V   A   Y   C   K   R   G   N   G   V   L   R   V
141 AAC GGA CGC CCG CTG GAG CTG GTG GAG CCC CGT CTC CTT CAG
     N   G   R   P   L   E   L   V   E   P   R   L   L   Q
183 TAC AAG CTT CAG GAA CCC ATC CTA TTG CTT GGA(aaggt...aactt)AAG
     Y   K   L   Q   E   P   I   L   L   L   G               K
219 GAG AAG TTC TCC GGAGTG GAC ATC AGA GTG ACG GTG AAG GGT
     E   K   F   S   G   V   D   I   R   V   T   V   K   G
261 GGA GGT CAC GTT GCG CAG GTG TAC GCT ATC AGA CAA GCC ATC
     G   G   H   V   A   Q   V   Y   A   I   R   Q   A   I
303 TCC AAG GCT CTC ATT GCC TAC TAC CAA AAA T(gtaag...tccag)AT GTT
     S   K   A   L   I   A   Y   Y   Q   K   Y               V
339 GAC GAG GCC TCC AAG AAG GAG ATC AAG GAC ATC CTG GTC CAA
     D   E   A   S   K   E   K   I   K   D   I   L   V   Q
381 TAC GAC AGG AGC CTG CTA GTG GCC GAC CCC AGG CGC TGC GAG
     Y   D   R   S   L   L   V   A   D   P   R   R   C   E
423 CCC AAG AAG TTC GGA GGT CCG GGC GCG CGC GCT CGC TAC CAG
     P   K   K   F   G   G   P   G   A   R   A   R   Y   Q
465AAG TCT TAC CGT TAA

     K   S   Y   R   *

480GGATCCCGGAGGCGTGCACGGTGTGGTATTGCAGTGGTGTGTTTTTGG
528TCAGTGCAATATCATATGGTGTTTTATAAAAAAAAAAAAAAAAAAAAAA
577AAAAAAAAAAA
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Expression study of Pxyl-RpS16. The method of semi-quantitative RT-PCR was performed to investigate the expression at different developmental stages. The results (Fig. 3) showed that *RpS16* was expressed in all examined stages. Expression levels were low in first instar, third instar and male adults, higher in second instar, fifth instar and pupae, but the highest expression level of Pxyl-RpS16 was found in female adult, which had significantly ($p<0.05$) higher expression level than all the other stages except the fourth instar larvae.

Homology and phylogenetic analysis. Sequence alignment of amino acids of Pxyl-RpS16 with other known RpS16 suggested that it has the greatest homology to the others with a 94% identity to the RpS16 of *Spodoptera frugiperda*,

Fig. 3: Semi-quantitative RT-PCR analysis showing the expression of *Pxly-RpS16* in seven developmental stages, B: Vertical scales show the relative grey value of *Pxly-RpS16* relative to 18S rRNA, Horizontal data 1–7 represents the seven development stages of *Plutella xylostella*, first instar, second instar, third instar, fourth instar larvae, pupae, female and male adults, respectively

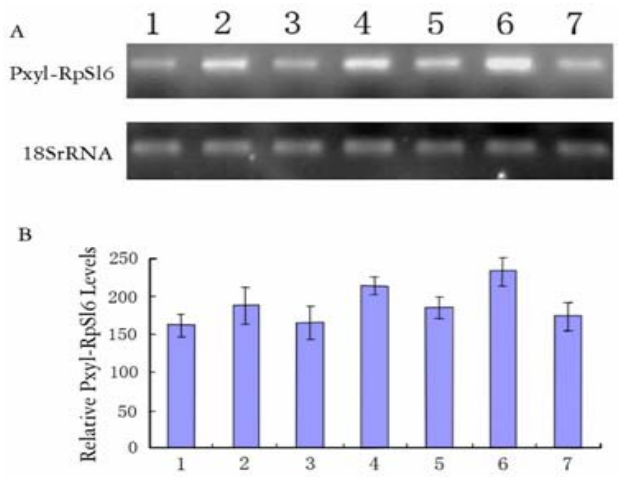
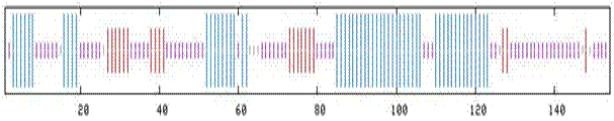


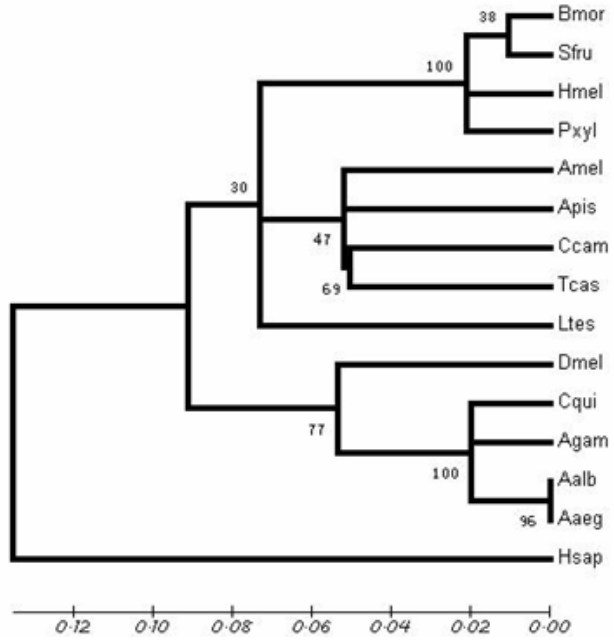
Fig. 4: Secondary structure prediction of Pxly-RpS16, Alpha helices are shown in blue, β-sheets in red and random coils in magenta



92% to RpS16 of *Bombyx mori*, 88% to RpS16 of *Apis mellifera* and 87% to RpS16 of *Drosophila melanogaster*. Even the homology of Pxyl-RpS16 to human is relatively high (79% identity). Sequence alignment of amino acids is shown in Fig. 6. Secondary structure Prediction showed that 37.01 % of the amino acid sequence of the protein forms Alpha helix, 21.43% is in extended strand and 41.56% is in Random coil (Fig. 4).

When the homologous sequence of human RpS16 was used as out group, the phylogenetic tree analysis showed that the selected RpS16 can be divided into 2 groups (Fig. 5). The first group includes three subgroups. Lepidopteran insects like *B. mori*, *S. frugiperda*, *H. melpomene* and *P. xylostella* assembled into the first subgroup; the second subgroup includes Hymenopteran insects as *A. mellifera*, Coleopteran insects *C. campestris*, *T. castaneum* and an Homopteran insect *A. pisum*; the third subgroup only comprised of one Hymenopteran insect *L. testaceipes*. All the members of the second group came from insect order Diptera including *A. albopictus*, *A. gambiae*, *C. quinquefasciatus*, *A. aegypti* and *D. melanogaster*.

Fig. 5: A phylogenetic tree of RpS16. The sequences were obtained from the GenBank database and their GenBank accession numbers are as follows: *Bombyx mori*(Bmor), No: NP_001037508, *Spodoptera frugiperda*(Sfru), No: Q95V31, *Heliconius melpomene*(Hmel), No: ABS57443. *Apis mellifera*(Amel), No: XP_001122174.*Acyrthosiphon pisum*(Apis), No: XP_001943977, *Cicindela campestris*(Ccam), No: CAJ17204, *Tribolium castaneum*(Tcas), No: XP_970777, *Lysiphlebus testaceipes*(Ltes),No: AAX62440, *Drosophila melanogaster*(Dmel), No: NP_611685, *Culex quinquefasciatus*(Cqui), No: XP_001849641, *Anopheles gambiae*(Agam), No: XP_317881, *Aedes albopictus*(Aalb), No: AAV90714, *Aedes aegypti*(Aaeg), No: AAS79339. *Homo sapiens*(Hasp), No: XP_947797



DISCUSSION

In this study, the full-length cDNA coding for Ribosomal Protein S16 of the diamondback moth was obtained by RACE-PCR. First report of insect Ribosomal Protein S16 was in *Spodoptera frugiperda* using the method of annotation of ESTs library (Landais *et al.*, 2003), which was followed by this current research about the gene code for *RpS16* in agricultural pests. The genomic sequence of *RpS16* gene has been isolated, identified and was found to have much larger genomic sequence than code region. It suggested that shearing of mRNA is necessary, when the *Pxyl-RpS16* gene was duplicated. The results from 5'upstream regulatory region analysis of *Pxyl-RpS16* showed that this sequence contained both promoter sequence and some transcriptional regulation response elements. This DNA sequence information may help to understand the feedback and regulation mechanism in some

Fig. 6: Multiple alignments of Pxyl-RpS16 with other insect RpS16, Identical amino acids are shaded in black

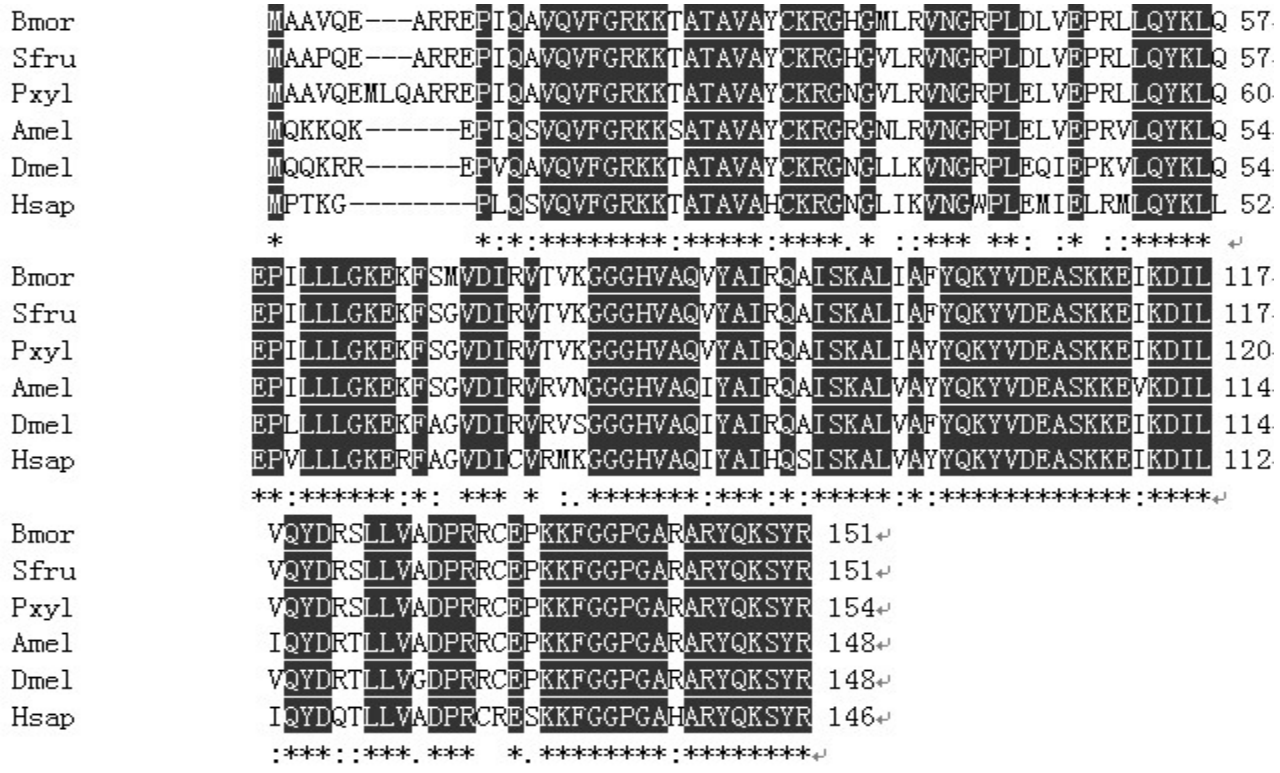
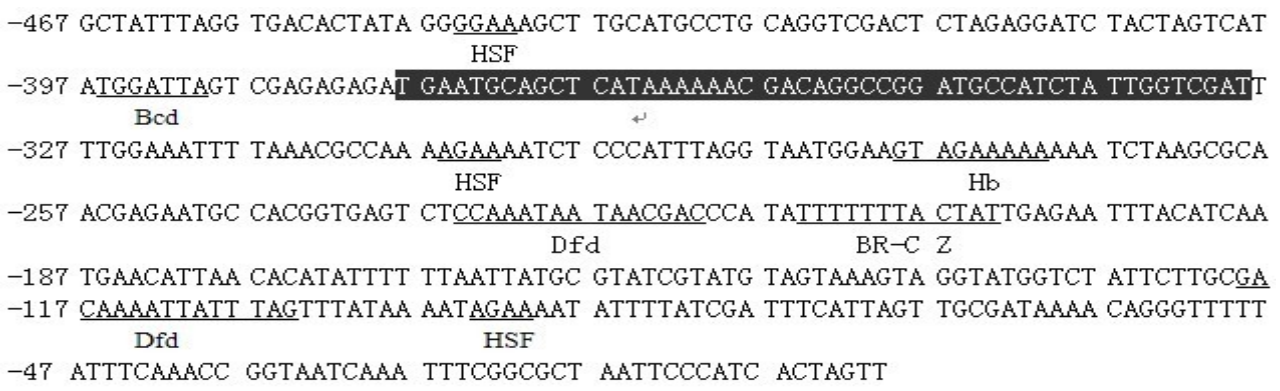


Fig. 7. Nucleotide sequence of the Pxyl-RpS16 gene 5'upstream region, Putative promoter sequences are indicated by black background, Part of transcriptional regulation response elements are underlined



physiological process of RpS16 in diamondback moth.

The RpS16 has an important role in the study of insect evolution (Edwards & Gadek, 2001; Roberts *et al*., 2008). The data from Phylogenetic tree indicated that *P. xylostella* was more closely related to *H. melpomene* than other insects. Because of the small amount of insect RpS16 sequence data availability, it was difficult to analyze the evolution and duplication of *RpS16* genes in different insects. Nevertheless our work is valuable for genetic study of diamondback moth and related insects.

A few researchers have reported the detailed function of ribosomal protein S16. Some investigations indicated that human ribosomal protein S16 binds to 18S rRNA region 1203–1236/1521–1698 (Yanshina *et al.*, 2007) and can be a clue to understand physiological role of this protein. But the data of secondary structure from human ribosomal protein S16 (21% α-helices & 24% is in β-strands) indicated that there was significant difference with Pxyl-RpS16 (Parakhnevitch *et al.*, 2005) suggesting that RpS16 has various structural characters between different species. Semi-quantitative RT-PCR was considered a sensitive method to detect minute changes in gene Transcript levels (Bustin *et al.*, 2002). The results showed that RpS16 has the highest expression in female adult of diamondback moth, suggesting that Pxyl-RpS16 is necessarily functional for the female adult development. But the biological functions of

RpS16 in insects still needs to be unrevealed to fully explain the physiological roles of RpS16 in diamondback moth and related insects.

The method of RNA interference to control pest, have been focused by many scientist, but it is difficult to select the suitable insect specific target genes or transcription factors in order to achieve successful pest management (Price & Gatehouse, 2008). Some data showed that ribosomal protein was a good choice for controlling pest through RNAi, for example, ribosomal protein S4 and S14 of *Diabrotica virgifera LeConte* have high control effect (LC_{50} values <5.2 ng/cm^2) using this method (Baum *et al.*, 2007). In this study the complete genomic sequence of *Pxyl-RpS16* may contribute to the control of diamondback moth by RNA interference.

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REFERENCES

Bustin, S.A., 2002. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): Trends and problems. *J. Mol. Endocrinol.*, 29: 23–39

Baum, J.A., T. Bogaert, W. Clinton, G.R. Heck, P. Feldmann, O. Ilagan, S. Johnson, G. Plaetinck, T. Munyikwa, M. Pleau, T. Vaughn and J. Roberts, 2007. Control of coleopteran insect pests through RNA interference. *Nat. Biotechnol.*, 5: 1322–1326

Chan, Y.L., V. Paz, J. Olvera and I.G. Wool, 1990. The primary structure of rat ribosomal protein S16. *FEBS*, 263: 88–88

Capinera, J.L., 2001. *Handbook of Vegetable Pests*, p: 729. Academic Press, San Diego, California

Doudna, J.A. and V.L. Rath, 2002. Structure and function of the eukaryotic ribosome: the next frontier. *Cell*, 109: 153–156

Dixit, R., S. Dixit, U. Roy, Y.S. Shouche and S. Gakhar, 2007. Partial genomic organization of ribosomal protein S7 gene from malaria vector Anopheles stephensi. *Insect. Sci.*, 14: 101–106

Edwards, K.J. and P.A. Gadek, 2001. Evolution and Biogeography of *Alectryon* (Sapindaceae). *Mol. Phylogenet. Evol.*, 2: 14–26

Higgins, D., J. Thompson, T. Gibson and J.D. Thompson, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.,* 22: 4673–4680

Heinemeyer, T., E. Wingender and I. Reuter, 1998. Databases on transcriptional regulation: TRANSFAC, TRRD and COMPEL. *Nucleic Acids Res.,* 26: 364–370

Kumar, S., K. Tamura and M. Nei, 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief. Bioinform.*, 5: 150–163

Landais, I., M. Ogliastro, K. Mita, J. Nohata, M. Lopez-Ferber, M. Duonor-Cerutti, T. Shimada, P. Fournier and G. Devauchelle, 2003. Annotation pattern of ESTs from Spodoptera frugiperda Sf9 cells and analysis of the ribosomal protein genes reveal insect-specific features and unexpectedly low codon usage bias. *Bioinformat.,* 19: 2343–2350

Manchado, M., C. Infante, E. Asensio, J.P. Cañavate and S.E. Douglas, 2007. Comparative sequence analysis of the complete set of 40S ribosomal proteins in the Senegalese sole (*Solea senegalensis Kaup*) and Atlantic halibut (*Hippoglossus hippoglossus L.*) (Teleostei: Pleuronectiformes): phylogeny and tissue-and development-specific expression. *BMC. Evol. Biol.,* 7: 107–118

Price Daniel, R.G. and J.A. Gatehouse, 2008. RNAi-mediated crop protection against insects. *Trends Biotechnol.*, 26: 393–400

Parakhnevitch, N.M., A.A. Malygin and G.G. Karpova, 2005. Recombinant Human Ribosomal Protein S16: Expression, Purification, Refolding and Structural Stability. *Biochem. (Moscow)*, 70: 777–781

Roberts, E., A. Sethi, J. Montoya, C.R. Woese and Z. Luthey-Schulten, 2008. Molecular signatures of ribosomal evolution. *Proc. Natl. Acad. Sci. USA,* 105: 13953–13958

Reese, M.G., 2001. Application of a time-delay neural network to promoter annotation in the Drosophila melanogaster genome. *Comput. Biol. Chem.*, 26: 51–56

Songa, K.H., M.K. Junga, J.H. Euma, I.C. Hwangb and S.S. Hana, 2008. Proteomic analysis of parasitized *Plutella xylostella* larvae plasma. *J. Insect. Physiol.,* 54: 1271–1280

Sarfraz, M. and B.A. Keddie, 2005. Conserving the efficacy of insecticides against *Plutella xylostella* (*Lepidoptera*: *Plutellidae*). *J. Appl. Entomol.,* 129: 149–157

Tan, W., L.X. Sun, D.H. Zhang and J. Sun, 2007. Cloning and overexpression of ribosomal protein L39 gene from deltamethrin-resistant *Culex pipiens pallens*. *Exp. Parasitol.,* 115: 369–378

Talekar, N.S. and A.M. Shelton, 1993. Biology, ecology and management of the diamondback Moth. *Annu. Rev. Entomol.,* 38: 275–301

Vgren, J.M. and P.M. Wikstro, 2001. Hybrid Protein between Ribosomal Protein S16 and RimM of *Escherichia coli* Retains the Ribosome Maturation Function of Both Proteins. *J. Bacteriol.,* 183: 5352–5357

Warner, J.R. and C.R. Nierras, 1998. Trapping human ribosomal protein genes. *Genome. Res.*, 8: 419–421

Wang, L.S., C.F. Sheng, C.L. Qiao and M. Tadshi, 2005. cDNA sequence analysis of ribosomal protein S13 gene in Plutella xylostella (*Lepidoptera: Plutellidae*). *Insect. Sci.*, 12: 225–229

Yanshina, D.D., A.A. Malygin and G.G. Karpova, 2007 Binding of Human Ribosomal Protein S16 with 18S rRNA Fragment 1203–1236/1521–1698. *Genome Res.*, 41: 932–938

Yu, D.Y. and Z.X. Zhan, 1999. Advances in studies on resistance to Bacillus thuringiensis toxins in Plutella xylstella and its management. *Nat. Enem. Insects*, 21: 21–27

Zhao, Y., J.C. Watson, S.D. Kung and P.J. Bottino, 1995. Characterization of a cDNA encoding ribosomal protein S16 in Rice. *Plant Physiol.,* 107: 1471–1472

Zarivach, R., A. Bashan, R. Berisio, J. Harms, T. Auerbach, F. Schluenzen, H. Bartels, D. Baram, E. Pyetan and A. Sittner, 2004. Functional aspects of ribosomal architecture: symmetry, chirality and regulation. *J. Phys. Org. Chem.,* 17: 901–912

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