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Cloning and characterization of ubiquitin ribosome fusion gene *RpS27a*, a deltamethrin-resistance–associated gene from diamondback moth (*Plutella xylostella* L.)

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Abstract: The ubiquitin ribosome fusion gene *RpS27a*'s coding sequence was cloned from diamondback moth, *Plutella xylostella* (L.). An open reading frame of 468 bp was found to encode a putative 155 amino acid precursor protein, which shares over 90% similarity with other insects' RpS27a protein. Real-time quantitative PCR was carried out to determine the relative expression level in the fourth instar larvae of deltamethrin-resistant and deltamethrin-susceptible strains. The results showed that the expression of *RpS27a* was significantly higher in the deltamethrin-resistant strain than in the deltamethrin-susceptible strain. There are reports indicating that upregulation expression of some ribosomal proteins confers some insecticide resistance. For the first time, we have predicted that the increased expression of the *RpS27a* gene may have some association with pesticide resistance in *Plutella xylostella*.

Key words: *Plutella xylostella*, ubiquitin ribosome fusion gene, insecticide resistance, cDNA cloning, real-time quantitative PCR

1. Introduction

In recent years, the diamondback moth, *Plutella xylostella* (L.), has been the most destructive pest of Cruciferae crops all over the world; the annual cost from it is prodigious (Talekar and Shelton, 1993). Chemical control is now the main method for managing insect pests in agriculture. The synthetic pyrethroid-class pesticides, including deltamethrin, are the most effective insecticides and are commonly used in agriculture as well as in the household, because they have demonstrated limited pesticide persistence in soil, high efficiency in killing pests, and low toxicity to humans and other mammals (Soderlund et al., 2002; Sayeed et al., 2003; Yang et al., 2008). Unfortunately, resistance to insecticides has appeared after their long-term use and massive application, which is now a major obstruction and problem in pest control (Widawsky et al., 1998; Moulton et al., 2000).

Resistance to pesticides is a polygenic inheritance phenomenon. Traditionally, types of resistance have been mainly divided into 3 classes: reduced cuticular penetration (Noppun et al., 1989), target site resistance (mutations in the target genes) (Soderlund and Knipple, 2003), and metabolic resistance. The voltage-sensitive sodium channel of the nerve membrane is considered to be the primary target of pyrethroid insecticides and DDT, and nerve insensitivity is one of the major mechanisms responsible for resistance to these compounds. This type of resistance is termed knockdown resistance and was first observed in *Musca domestica* (Busvine, 1951), then later found in *P. xylostella* (Hama et al., 1987; Schuler et al., 1990). Many studies have indicated that metabolic resistance is closely related to enhanced activity of P450 enzymes (Shen et al., 2003), nonspecific esterases (Hemingway et al., 2000; Paton et al., 2000), and glutathione S-transferases (Vontas et al., 2001). In addition, more genes may be involved in insecticide resistance, and the candidate genes must be identified. To investigate insecticide resistance in the diamondback moth, we previously have separated several genes using cDNA representational difference analysis (Cheng et al., 2005). One of the differentially expressed genes in *Plutella xylostella* is of great homology with the *ubiquitin* gene.

Ubiquitins, whose fundamental role is in mediating intracellular selective protein degradation by the ubiquitin proteasome system, are highly conserved in all eukaryotes investigated (Ozkaynak et al., 1987; Binet et al., 1991). They are encoded by 2 classes of genes: polyubiquitin and ubiquitin ribosome precursor protein genes, whose primary translation products are fusion proteins, either fused to ribosomal protein or to themselves (Baker and Board, 1991; Mezquita et al., 1997). According to the different types of ribosome proteins, ubiquitin ribosome

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precursor protein genes also can be divided into 2 classes: *RpL40* (*Uba52*) and *RpS27a* (*Uba80*). Both types of translation products are then processed by specific enzymes (Amerik and Hochstrasser, 2004).

To obtain the deltamethrin-resistance–associated genes, we isolated and characterized the differentially expressed *ubiquitin* gene by cDNA representational difference analysis and found that the ubiquitin protein is differentially expressed in the deltamethrin-resistant and -susceptible strains of *P. xylostella* (Cheng et al., 2005; Cheng et al., 2009). Because there are 3 types of *ubiquitin* gene, we isolated and sequenced the complete coding sequence of 1 type of *ubiquitin* gene (ubiquitin ribosome fusion gene *RpS27a*) from diamondback moth. The expression pattern of *RpS27a* in the fourth instar larvae of the 2 diamondback moth strains was characterized, and its possible correlation with pesticide resistance was also discussed.

2. Materials and methods

2.1. *Plutella xylostella*

P. xylostella was obtained from Huaxi cabbage fields (Guiyang, China). Toxicity evaluation showed that it was a deltamethrin-susceptible strain (LD_{50} = 0.55 μg/larva). It was reared in our laboratory as the standard susceptible strain in this study.

The deltamethrin-resistant strain of *P. xylostella* was established and maintained by our laboratory selection (Liu et al., 1995), with continuous exposure to deltamethrin (Roussel-Uclaf, France) of the susceptible strain generation by generation as previously described (FAO, 1979). After more than 300 generations of laboratory selection, the resistance level was increased over 5000 times compared with the susceptible strain. Larvae of both strains were kept at 26 ± 1 °C with a constant photoperiod of 16 h light and 8 h dark and 75% relative humidity.

2.2. Total RNA extraction and cDNA synthesis

Total RNA of 10 whole larvae was extracted from fourth instar larvae of both the deltamethrin-resistant and -susceptible strains using the RNeasy Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. To avoid potential DNA contamination, total RNA was treated with DNase I (TaKaRa, Japan). Total RNA was subjected to gel electrophoresis after extraction. RNA concentration and purity were measured with a NanoDrop 1000 spectrophotometer (Thermo, USA). The first strand cDNA was synthesized with the PrimeScript RT Reagent Kit (TaKaRa) following the manufacturer's protocol.

2.3. Cloning and sequencing of *RpS27a*

The first strand cDNA was used as the template to amplify the full-length coding sequence of *RpS27a* using a pair of degenerate primers: the forward primer 5'-ATGCAGATYTTCGTNAARACC-3' and the reverse primer 5'-YYATTTGTCGTCRTCYTTGAAG-3'. PCR conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 s, 45 °C for 30 s, and 72 °C for 1 min, with a final 72 °C extension for 10 min. cDNA fragments were separated by electrophoresis on a 1% agarose gel, and PCR products were purified using a Nucleic Acid Purification Kit (Axygen, USA). The purified products were ligated into a pMD 19-T vector (TaKaRa) at 16 °C for 30 min, and then the ligation mixture was transformed into *E. coli* DH5α competent cells and cultured on LB plates containing ampicillin (100 mg/mL), IPTG (24 mg/mL), and X-Gal (20 mg/mL). White colonies were picked out and subjected to colony PCR to confirm the presence of the expected band. Plasmid DNA was extracted using the AxyPrep Plasmid Miniprep Kit (Axygen) and sequenced by an automated DNA sequencer (Applied Biosystems, USA).

2.4. Sequence analysis of *RpS27a* gene

The amino acid sequence, protein molecular mass, and isoelectric point (pI) of *RpS27a* were predicted with the Compute pI/Mw tool (http://www.expasy.org/). The potential subcellular localization was predicted on the online server (http://psort.hgc.jp/form2.html). Homology search was performed with the BLAST program at the National Center for Biotechnology Information. Multiple sequence alignment was performed with the Clustal X program (Jeanmougin, 1998). A phylogenetic tree was constructed using MEGA 4.0 programs (Tamura et al., 2007) using the neighbor-joining method. Bootstrap values were calculated on 1000 replications.

2.5. Quantitative real-time PCR analysis

The relative transcription levels of *RpS27a* in the fourth instar larvae of *P. xylostella* from the deltamethrin-resistant and -susceptible strains were examined by quantitative real-time PCR (qRT-PCR). Total RNAs were extracted from both strains using the RNeasy Mini Kit (QIAGEN). The purity and concentration of total RNAs were determined by spectrophotometry. The RNA integrity was observed on a 1.5% agarose gel. RNA (1 μg) was synthesized into cDNA. Real-time PCR was conducted using the LightCycler real-time PCR detection system (Roche, Switzerland) with the housekeeping gene GAPDH as the internal control. Two pairs of qRT-PCR specific primers were designed using Primer Premier 5 software (Lalitha, 2000): *RpS27a* gene (forward: 5'-TCGCACGCTCTCCGACTACAAC-3'; reverse: 5'-CAACCTTGTAGAACCTGAGCACGG-3') and GAPDH (forward: 5'-TGGAAGGTGGTGCCAAGAA-3'; reverse: 5'-AAGGGGAGCGAGGCAGTTAG-3'). The amplification was carried out in 20 μL reactions containing 10 μL of 2X SYBR Premix Ex Taq (TaKaRa), 2 μL of cDNA,

0.4 µL of 10 µM forward and reverse primers, and 7.2 µL of ddH_2O. The real-time PCR program was 95 °C for 30 s, followed by 45 cycles of 95 °C for 5 s, 56 °C for 10 s, and 72 °C for 15 s. The dissociation curve analysis program was 95 °C for 5 s, 65 °C for 60 s, and 95 °C for continuous. All analyses were based on the Ct value automatically normalized by the software. The $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to analyze the relative expression level of the *RpS27a* from the 2 strains. Each sample was run in triplicate and Ct values were averaged along with the internal control. The experiment was performed 3 times with independent RNA samples to assure repeatability of the results.

2.6. Statistics

Data were shown as mean ± standard deviation (SD). Significance levels were analyzed with Student's t-test. A P-value of 0.05 or less between groups was considered to be significant.

3. Results and discussion

RT-PCR was adopted to amplify the full-length coding sequence of the ubiquitin ribosome fusion gene *RpS27a*. The encoding region sequence has a 468 bp open reading frame sequence (Figure 1) encoding a putative precursor protein of 155 amino acid residues with a predicted molecular weight of 17.918 kDa and a theoretical isoelectric point of 9.81. The precursor protein sequence from 1 to 76 amino acids belongs to the ubiquitin superfamily, and that from 77 to 155 belongs to the ribosomal protein S27 superfamily. Sequence analysis showed that there were only 4 different nucleotides between the deltamethrin-resistant and -susceptible strains, and only 1 amino acid difference between the 2 strains (Figures 2 and 3). A number of studies have demonstrated that deltamethrin could induce genotoxic and immunotoxic effects (Ismail and Mohamed, 2012). In this study, we found one amino acid mutation in the RpS27a protein after the diamondback moth's continuous exposure to deltamethrin. Whether the mutation confers knockdown resistance still needs further research.

The sequence KKRKKKNYSTPKKIHKKKKA at the 78 position of the RpS27a protein is the nuclear location signal (Figure 1, shadowed area), which means that the RpS27a protein can be imported into the nucleus and function within the nucleus. The precursor protein has high basic amino acids with 23.2% basic residues (lysine and arginine), and only 12.9% acidic amino acids. This highly basic feature suggests it may be a nucleic acid binding protein. *RpS27a* encodes a protein of ubiquitin monomer fused to ribosomal protein S27a (Redman and Rechsteiner, 1989). Studies have shown that the ubiquitin moiety probably acts as a molecular chaperone of the C-terminal ribosomal protein tail (Finley et al., 1989). The attachment of ubiquitin to the ribosomal protein

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ATGCAGATCTTCGTCAAAACCCTAACGGGTAAGACCATCACCCTTGAGGTCGAACCCTCG   60
 M  Q  I  F  V  K  T  L  T  G  K  T  I  T  L  E  V  E  P  S    20
GATACTATCGAGAATGTCAAAGCCAAGATCCAGGACAAGGAGGGAATCCCCCCAGATCAG  120
 D  T  I  E  N  V  K  A  K  I  Q  D  K  E  G  I  P  P  D  Q    40
CAGCGTCTGATCTTCGCCGGCAAGCAGCTGGAGGATGGTCGCACGCTCTCCGACTACAAC  180
 Q  R  L  I  F  A  G  K  Q  L  E  D  G  R  T  L  S  D  Y  N    60
ATCCAGAAGGAGTCGACCCTGCACCTCGTGCTGCGCCTGCGCGGAGGCGCCAAGAAGCGC  240
 I  Q  K  E  S  T  L  H  L  V  L  R  L  R  G  G  A  K  K  R    80
AAGAAGAAGAACTACTCCACGCCCAAGAAGATCAAGCACAAGAAGAAGAAGGCCAAGCTC  300
 K  K  K  N  Y  S  T  P  K  K  I  K  H  K  K  K  K  A  K  L   100
GCCGTGCTCAGGTTCTACAAGGTTGACGAGAATGGCAAGATCCACCGTCTGCGTCGCGAG  360
 A  V  L  R  F  Y  K  V  D  E  N  G  K  I  H  R  L  R  R  E   120
TGCACCAGCGAGCAGTGCGGCGCGGGCGTGTTCATGGCCGTGATGGAGGACCGCCACTAC  420
 C  T  S  E  Q  C  G  A  G  V  F  M  A  V  M  E  D  R  H  Y   140
TGCGGCAAGTGCCATCGCACCATGGTCTTCAAGGACGACGACAAATAA              468
 C  G  K  C  H  R  T  M  V  F  K  D  D  D  K  *               155
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Figure 1. The nucleotide and deduced amino acid sequences of the *P. xylostella RpS27a* gene coding region. The deduced amino acid sequence is presented below the nucleotide sequence in a single letter. The nuclear localization signal sequence is shaded. The initial and termination codon are underlined. The stop codon is denoted with an asterisk.

```
R    ATGCAGATCTTCGTCAAAACCCTAACGGGTAAGACCATCACCCTTGAGGTCGAACCCTCGGATACTATCGAGAATGTCAAAGCCAAGATCCAGGACAAGG [100]
S    ATGCAGATCTTCGTCAAAACCCTAACGGGTAAGACCATCACCCTTGAGGTCGAACCCTCGGATACTATCGAAAATGTCAAGGCCAAGATCCAGGACAAGG [100]
     ************************************************************************ ******** ******************

R    AGGGAATCCCCCCAGATCAGCAGCGTCTGATCTTCGCCGGCAAGCAGCTGGAGGATGGTCGCACGCTCTCCGACTACAACATCCAGAAGGAGTCGACCCT [200]
S    AGGGAATCCCCCCGGATCAGCAGCGTCTGATCTTCGCCGGCAAGCAGCTGGAGGATGGTCGCACGCTCTCCGACTACAACATCCAGAAGGAGTCGACCCT [200]
     *********** ***************************************************************************************

R    GCACCTCGTGCTGCGCCTGCGCGGAGGCGCCAAGAAGCGCAAGAAGAAGAACTACTCCACGCCCAAGAAGATCAAGCACAAGAAGAAGAAGGCCAAGCTC [300]
S    GCACCTCGTGCTGCGCCTGCGCGGAGGCGCCAAGAAGCGCAAGAAGAAGAACTACTCCACGCCCAAGAAGATCAAGCACAAGAAGAAGAAGGTCAAGCTC [300]
     ************************************************************************************************ *******

R    GCCGTGCTCAGGTTCTACAAGGTTGACGAGAATGGCAAGATCCACCGTCTGCGTCGCGAGTGCACCAGCGAGCAGTGCGGCGCGGGCGTGTTCATGGCCG [400]
S    GCCGTGCTCAGGTTCTACAAGGTTGACGAGAATGGCAAGATCCACCGTCTGCGTCGCGAGTGCACCAGCGAGCAGTGCGGCGCGGGCGTGTTCATGGCCG [400]
     ***************************************************************************************************

R    TGATGGAGGACCGCCACTACTGCGGCAAGTGCCATCGCACCATGGTCTTCAAGGACGACGACAAATAA [468]
S    TGATGGAGGACCGCCACTACTGCGGCAAGTGCCATCGCACCATGGTCTTCAAGGACGACGACAAATAA [468]
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Figure 2. Alignment of the *RpS27a* gene coding sequences between the deltamethrin-resistant and -susceptible strains of *Plutella xylostella*. R: deltamethrin-resistant strain; S: deltamethrin-susceptible strain.

```
R        MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
S        MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
         ********************************************************************************

R        KKKNYSTPKKIKHKKKKAKLAVLRFYKVDENGKIHRLRRECTSEQCGAGVFMAVMEDRHYCGKCHRTMVFKDDDK [155]
S        KKKNYSTPKKIKHKKKKVKLAVLRFYKVDENGKIHRLRRECTSEQCGAGVFMAVMEDRHYCGKCHRTMVFKDDDK [155]
         ******************. ************************************************************
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Figure 3. Alignment of the *RpS27a* amino acid sequences between the deltamethrin-resistant and -susceptible strains of *Plutella xylostella*. R: deltamethrin-resistant strain; S: deltamethrin-susceptible strain.

S27a could increase the tail's stabilization and facilitate the ribosomal protein to incorporate into the ribosome. After cotranslation of ubiquitin moiety and ribosome protein, RpS27a protein would be cleaved by deubiquitinating enzymes (Love et al., 2007).

The putative protein sequence is conserved among different species (Figure 4). Amino acid sequence alignment among different species showed that *P. xylostella* protein RpS27a shares 98% identity with *M. sexta*; 97% identity with *Antheraea yamamai*, *Papilio dardanus*, *Bombyx mori*, and *Danaus plexippus*; and 93% identity with *Aedes aegypti*. All evidence strongly suggests that this gene is the ubiquitin ribosome fusion gene *RpS27a* from *P. xylostella*. Figure 5 shows the phylogenetic relationships between *P. xylostella RpS27a* and some other species' *RpS27a*. Results indicate that *P. xylostella RpS27a* is more closely related to *A. yamamai RpS27a*, and that there is common ancestry among insects of the order Lepidoptera.

The expression of ubiquitin ribosome fusion gene *RpS27a* in the fourth instar larvae developmental phase in both deltamethrin-resistant and -susceptible strains was analyzed by performing qRT-PCR. As shown in Figure 6, *RpS27a* is expressed highly at a significant level in the deltamethrin-resistant strain compared with the susceptible strain. The results suggest that *RpS27a* gene expression was upregulated in the resistant strain.

Insecticide resistance has been a serious agricultural and public health problem, which has limited the management of pest control. Insecticide resistance, to a large extent, depends on the increasing ability of antioxidant enzyme activity. Genome transcription profile data suggest that a broader range of genes may be involved in insecticide resistance (Pedra et al., 2004). This indicates that the insecticide resistance mechanism is more complicated than we had previously considered. In the previous study, we found that ubiquitin protein was differentially expressed in the deltamethrin-resistant strain compared with the susceptible strain. *Ubiquitin* will express excessively in the organism in various adverse environments (O'Mahony and Oliver, 1999). Furthermore, followed by Keap 1 and IkB ubiquitination and degradation by 26S proteasome, transcription factors Nrf2 and NF-kB will translocate to the nucleus and activate the detoxicity enzyme genes, respectively (Magnani et al., 2000; Singh et al., 2010). Ubiquitin protein also participated in a number of cellular processes, such as selective protein degradation, cell cycle progress, signal transduction, programmed cell death, and DNA repair (Hoege et al., 2002).

Ribosomal protein, whose basic role is to assemble the ribosome subunit and participate in protein synthesis, was recently found to have extra-ribosome functions. Studies have shown that some ribosomal proteins are differentially

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Manduca sexta                MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Papilio polytes              MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Papilio xuthus               MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Papilio dardanus             MQIFVETLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Bombyx mori                  MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPNQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Spodoptera frugiperda        MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Danaus plexippus             MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Antheraea yamamai            MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Plutella xylostella          MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Aedes aegypti                MQIFAKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Culex quinquefasciatus       MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Drosophila melanogaster      MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
Triatoma rubida              MQIFVKTLTGKTITLEVESSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDNRTLSDYNIQKESTLHLVLRLRGGAKKR [80]
                             ****. :***********. ********************:**********. ***************************

Manduca sexta                KKKNYSTPKKIKHKKKKVKLAVLRFYKVDENGKIHRLRRECTGEQCGAGVFMAVMEDRHYCGKCHSTMVFKDDDK- [156]
Papilio polytes              KKKNYSTPKKIKHKKKKVKLAVLRFYKVDENGKIHRLRRECTGEQCGAGVFMAVMEDRHYCGKCHSTMVFKDDDK- [156]
Papilio xuthus               KKKNYSTPKKIKHKKKKVKLAVLRFYKVDENGKIHRLRRECTGEQCGAGVFMAVMEDRHYCGKCHSTMVFKDDDK- [156]
Papilio dardanus             KKKNYSTPKKIKHKKKKVKLAVLRFYKVDENGKIHRLRRECTGEQCGAGVFMAVMEDRHYCGKCHSTMVFKDDDK- [156]
Bombyx mori                  KKKNYSTPKKIKHKKKKVKLAVLRFYKVDENGKIHRLRRECTGEQCGAGVFMAVMEDRHYCGKCHSTMVFKDDDK- [156]
Spodoptera frugiperda        KKKNYSTPKKIKHKKKKVKLAVLRFYKVDENGKIHRLRRECTGEQCGAGVFMAVMEDRHYCGKCHSTMVFKDDDRP [156]
Danaus plexippus             KKKNYSTPKKIKHKKKKVKLAVLRFYKVDENGKIHRLRRECTGEQCGAGVFMAVMDDRHYCGKCHSTMVFKDDEK- [156]
Antheraea yamamai            KKKNYSTPKKIKHKKKKVKLAVLRYYKVDENGKIHRLRRECTGEQCGAGVFMAVMEDRHYCGKCHSTMVFKDDDK- [156]
Plutella xylostella          KKKNYSTPKKIKHKKKKVKLAVLRFYKVDENGKIHRLRRECTSEQCGAGVFMAVMEDRHYCGKCHRTMVFKDDDK- [156]
Aedes aegypti                KKKKYSTPKKIKHKRKKVKLAVLKYYKVDENGKIHRLRRECTSETCGAGVFMAAHEDRHYCGKCHLTLVYSKQEEK [156]
Culex quinquefasciatus       KKKNYSTPKKIKHKRKKVKLAVLKYYKVDENGKIHRLRRECTSETCGAGVFMAAHEDRHYCGKCHLTLVYSKQEEK [156]
Drosophila melanogaster      KKKNYSTPKKIKHKRKKVKLAVLKYYKVDENGKIHRLRRECPGENCGAGVFMAAHEDRHYCGKCNLTFVFSKPEEK [156]
Triatoma rubida              KKKNYSTPKKIKHKKKKIKLAVLKYYKVDENGKISRLRRECPSDQCGAGVFMAAMEDRHYCGKCSYTLVFKQPEEK [156]
                             ***:**********:** *****::********* ******..: ********. :******** *:*:.. :.
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Figure 4. Multiple alignment of the amino acid sequence of *RpS27a* among different species. Asterisks, double dots, and single dots signify identical, strongly conserved, and minimally conserved amino acids, respectively.

expressed in tumor cells compared with normal cells (Kondoh et al., 2001; Wang et al., 2006; Han et al., 2012). Ribosomal proteins related to antibiotic resistance were also reported (Yonath, 2005). Ribosomal protein genes *S29* and *L39* were found overexpressed in a deltamethrin-resistant strain from *Culex pipiens pallens* and may confer some role in deltamethrin resistance (Tan et al., 2007; Sun et al., 2011). This means that some ribosomal protein genes are candidate genes in insecticide metabolism, and their roles must be fully elucidated.

One factor in drug resistance is an increased ability to repair DNA damage (Kim et al., 2000). Many studies have demonstrated that some ribosomal proteins are correlated to DNA damage repair; some of them are DNA damage repair enzymes (Yacoub et al., 1996; Sandigursky et al., 1997; Boiteux and Radicella, 1999). It is possible that through the elevation of ribosomal protein's ability to repair DNA, the diamondback moth acquired deltamethrin resistance to a certain degree. Some ribosomal proteins are also reported to function as gene expression regulators. Through removing inhibition of p53 inhibitors such as MDM2 by ribosomal proteins, ribosomal proteins activated transcription factor p53 (Jin et al., 2004; Takagi et al., 2005; Horn and Vousden, 2008). Sun et al. (2011) found that RpS27a interacts with MDM2 and suppresses MDM2-mediated p53 ubiquitination, and finally leads to p53 activation. p53 can either prevent the cell cycle of the damaged cells until repair has ended or is directly involved in damage repair (Smith et al., 2000). We thus postulate that the *RpS27a* gene may be an upstream factor in insecticide resistance, and its increased expression may induce an increase of downstream proteins, which can be directly involved in pesticide resistance.

Here we characterized a new ribosome protein gene fused to *ubiquitin*; its expression was determined in deltamethrin-resistant and deltamethrin-susceptible strains from *Plutella xylostella.* The transcript is consistent with the expected role of *RpS27a*. The cytochrome *P450* family is constitutively overexpressed in insecticide-resistant strains of all kinds of insects and constitutes the enzyme systems that are essential for insecticide detoxification. A close relationship between ribosomal protein L22 and CYP450 has also been reported (He et al., 2009), and *RpS27a* gene overexpression may be dose-dependent. It is possible that the effect is positively related with resistance below a threshold level, and above a threshold level, the effect may be reversed.

In conclusion, we have reported the cloning and

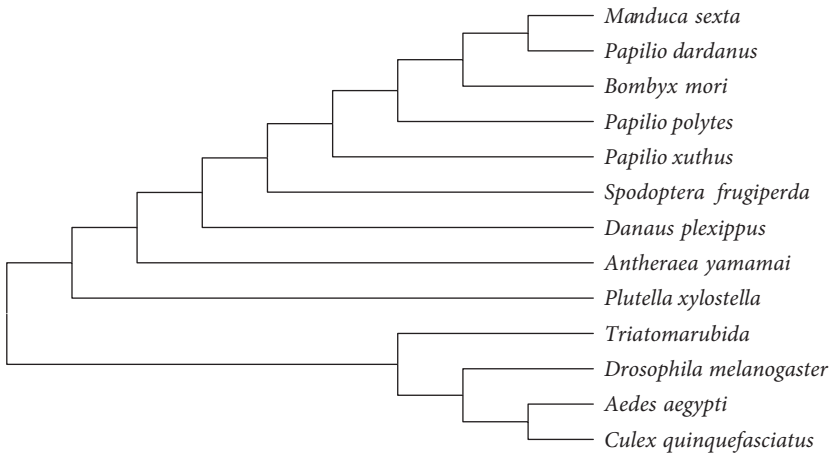

Figure 5. Phylogenetic relationship between *P. xylostella RpS27a* and some other species. Corresponding GenBank accession numbers are: *M. sexta*: ACY95367.1; *P. dardanus*: CAH04128.1; *Bombyx mori*: NP_001091826.1; *P. polytes*: BAM18943.1; *P. xuthus*: BAM17728.1; *S. frugiperda*: AAL62473.1; *D. plexippus*: EHJ77179.1; *A. yamamai*: BAD05031.1; *P. xylostella*: JX437934; *T. rubida*: AER92457.1; *D. melanogaster*: NP_476778.1; *A. aegypti*: AAS79344.1; *C. quinquefasciatus*: XP_001844485.1.

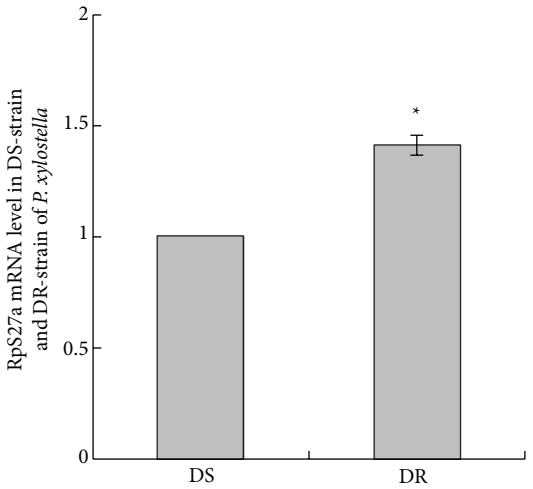

Figure 6. mRNA level of *RpS27a* in DS-strain and DR-strain of *P. xylostella*. All values are expressed as means ± SD. DS-strain: deltamethrin-susceptible strain; DR-strain: deltamethrin-resistant strain. *P < 0.01.

identification of a novel gene, *RpS27a*, from *Plutella xylostella*. Phylogenetic tree analysis suggested that the relationship of *RpS27a* between *Plutella xylostella* and other Lepidoptera insects was the closest, similar to classical systematics. In the fourth instar larvae, *RpS27a* is overexpressed in the deltamethrin-resistant strain compared to the susceptible strain. Though some ribosomal proteins were reported to be associated with deltamethrin resistance, there has been no report showing that *RpS27a* is correlated with insecticide resistance. The relationship between *RpS27a* and deltamethrin resistance still needs further investigation.

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