A comparative profiling of protein expression in the deltamethrin-sensitive and resistant strains of the diamondback moth (*Plutella xylostella*)

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Abstract: A major challenge in pest management is insecticide resistance. To effectively prevent and manage resistance, it is essential to understand the biochemical and physiological changes induced by insecticides. Some molecular mechanisms that are responsible for insecticide resistance were well known whereas others are to be discovered. So the comparative proteomic approach was employed to investigate the proteomic differences between the deltamethrin-sensitive strain (DS) and the deltamethrin-resistant strain (DR) of the diamondback moth (*Plutella xylostella* L.). In this study, about 300 differentially expressed protein spots were detected on each SDS-PAGE gel, among which 23 proteins had a 2. 5-fold expression difference between the two strains. Eight proteins were successfully identified by MALDI-TOF-MS, including chemosensory protein CSP2, Cu/Zn superoxide dismutase (SOD1), peroxiredoxin-like protein, *etc*. Expression of the five proteins at the mRNA level was analyzed by real-time quantitative PCR, and the results showed that the mRNA expression level did not fully reflect the protein expression level. Western blotting validated the proteomic result of SOD1. This study provides strong evidence that deltamethrin can induce the proteomic change in adult diamondback moth, and is helpful to understanding the resistance development and selecting the action targets of deltamethrin.

Key words: *Plutella xylostella*; deltamethrin; proteomics; selection pressure; insecticide resistance

1 INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.) is one of the most difficult management pests because of its propensity to develop resistance to most of insecticides. It is the first crop pest to develop resistance to dichloro-diphenyl-trichloroethane (DDT), and also the first insect to become resistant to the *Bacillus thuringiensis* (BT) toxin in the field (Shelton *et al*., 1993). Insecticide resistance limits the effectiveness of pest management and it is a major challenge faced by current resistance research. Therefore, the study of resistance-associated molecular targets may represent new opportunities for managing pest resistance.

Previous researches of the diamondback moth mainly focused on its genomics (Eum *et al*., 2007; Yuan *et al*., 2010), biochemical and physiological characters (Eziah *et al*., 2009). A large number of differentially expressed genes have been identified (Huang *et al*., 1998; Cheng *et al*., 2009). The mRNA-based approaches were used in the majority of studies. However, it is not sure how changes at the mRNA level relate with the protein abundance (Gygi *et al*., 1999). Therefore, a combination of genomic and proteomic approaches will be necessary to elucidate the molecular and biochemical mechanisms of *P. xylostella* under the pressure of chemical insecticide. Proteomics is a large-scale study of gene expression at the protein level, and it ultimately provides direct measurement of protein expression levels and insights into the activity of all relevant proteins (Anderson and Anderson, 1998; Blackstock and Weir, 1999). This approach has been used in many researches of insects. Pedra *et al*. (2005) studied the profiling of abundant proteins associated with dichlorodiphenyltrichloroethane in

Drosophila melanogaster. Thornton *et al.* (2010) identified nine proteins associated with detoxification and response to oxidative stress when they used proteomic method to analyze the atrazine exposure in *D. melanogaster*.

In the present study, a comparative proteomic approach was employed to investigate the proteomic differences in deltamethrin-sensitive and resistant strains of the diamondback moth. This is the first report on the utilization of a proteomic approach to study the protein changes in the diamondback moth under the long-term selection pressure of deltamethrin.

2 MATERIALS AND METHODS

2.1 Insect strains

The deltamethrin-sensitive strain was originally obtained from Huaxi, Guiyang City, China. In comparison with the Wuhan deltamethrin-sensitive strain (LD_{50}: 1.50 μg per adult), the Guiyang strain was a deltamethrin-sensitive strain (DS) (LD_{50}: 0.55 μg per adult). The deltamethrin-resistant strain (DR) was cultured from its deltamethrin-sensitive ancestor on turnip sprouts in the laboratory with chronic exposure to deltamethrin (Liu *et al.*, 1995). After 207 generations of selection, the resistance level of the DR strain to deltamethrin increased over 5 000 times when compared with that of the DS strain. The insectary was maintained at 25 ± 1℃, with a photoperiod of 16 h light and 8 h dark.

2.2 Preparation of protein samples

For the sample of each strain, 20 adult insects were placed in liquid nitrogen and ground thoroughly with a mortar and pestle. After suspending for 1 h at 20℃ in 1 mL extraction buffer (10% TCA in acetone, 40 mmol/L DTT), the samples were centrifuged at 15 000 r/min for 30 min at 4℃, then the supernatant was removed, precipitates were resuspended three times in 3 volumes of iced-acetone for 1 h at − 20℃, followed by centrifugation at 12 000 r/min for 10 min. The protein was then resuspended in 84 500 μL of lysis buffer [7 mol/L urea, 2 mol/L thiourea, 4% (v/v) CHAPS, 2% (v/v) Bio-lyte ampholytes (pH 3 − 10) and 40 mmol/L DTT] for 2 h at 4℃, followed by centrifugation at 15 000 r/min for 30 min. The supernatant was stored at − 70℃ and the protein concentration was determined by Bradford's method (Bradford, 1976) using Bradford Protein Assay Kit (Generay, China) according to the manufacturer's instructions.

2.3 Two-dimensional gel electrophoresis of proteins

The extracted proteins (350 μg each sample) were loaded on 17 cm immobiline dry strips (pH 3 − 10, NL, Bio-Rad) with rehydration buffer [7 mol/L urea, 2 mol/L thiourea, 2% (v/v) CHAPS, 2% (v/v) Bio-lyte ampholytes (pH 3 − 10), 40 mmol/L DTT, 0.002% bromophenol blue]. Following 15 h of passive rehydration, the DryStrip gels were transferred to an Ettan IPGPhor Ⅲ system (GE Healthcare). The isoelectric focusing was performed by applying 500 V for 3 h, 1 000 V for 2 h, and 10 000 V for 3 h until 60 000 Vh had been achieved. After isoelectric focusing, strips were equilibrated in SDS equilibration buffer [75 mmol/L Tris-HCl, pH 8.8, 6 mol/L urea, 29.3% (v/v) glycerol, 2% (w/v) SDS, 0.002% (w/v) bromphenol blue, 1% (w/v) DTT] for 15 min followed by a further incubation in fresh SDS equilibration buffer [containing 2.5% (w/v) iodoacetamide] for 15 min at room temperature. Strips were then applied to 12% (w/v) SDS-PAGE gels and sealed with 0.5% agarose. SDS-PAGE was performed at 1 W/gel for 45 min, 13 W/gel until the bromophenol blue front reached the bottom of the gel using an Ettan DALT-6 separation unit (GE Healthcare).

2.4 Gel imaging and image analysis

After two dimensional gel electrophoresis, the gels were visualized by silver staining with the methods described by Amersham Biosciemces (GE Healthcare), and then the two gels were scanned at 300 dpi using Imagemaster Scanner Ⅲ. Spot detection, spot matching and quantitative intensity analysis were performed automatically with Imagemaster 2D software (version 6.0) according to the manufacturer's protocols. The identified protein spots were manually rechecked. Three replications were performed for each sample. The expression levels of corresponding protein spots which changed more than 2.5-fold were submitted to the Student's *t*-test. A *P*-value of 0.05 or less was considered to be statistically significant.

2.5 In-gel digestion, protein identification and database searching

The protein spots with prominent ratio were extracted from the silver-stained gels, and washed twice in milli-Q water and destained with 50 μL of 25 mmol/L ammonium bicarbonate in 50% acetonitrile water for 30 min at 37℃. Then dehydrated by acetonitrile and dried in a vacuum drier for 30 min. About 5 μL of digestion buffer [50 mmol/L ammonium bicarbonate, pH 7.8; 0.02 μg/

μL trypsin (Promega)] was added. The samples were kept on ice for 45 min. Then 20 μL of digestion buffer was added. After overnight incubation (37℃), the supernatant was recovered. The remaining peptides were extracted from the gel piece using 67% acetonitrile/0. 1% formic acid in water and pooled with the supernatant. For mass spectrometric analysis, the pooled samples were dried and redissolved in 12 μL of 0. 1% formic acid. The above prepared peptide mixtures (0. 4 μL) were mixed with an equal volume of matrix solution R-cyano-4-hydroxy-cinnamic acid (CHCA, Sigma, St. Louis, MO) in 0. 1% TFA and 50% ACN and spotted on the target plate. Samples were allowed to air-dry and be analyzed by 4800 MALDI-TOF-MS Proteomics Analyzer (Applied Biosystems). GPS Explorer software Version 3. 0 (Applied Biosystems) was used to create and search files in the MASCOT search engine (Matrix Science, London, U. K.) for peptide and protein identification with the following parameters: NCBInr database or EST _ others database, taxonomy of all entries, trypsin digest with one missing cleavage, fixed modifications of carbamidomethyl, variable modifications of oxidation, peptide mass tolerance of ± 50 mg/kg. The searching results of the proteins and the individual MS/MS spectra with statistically significant difference (confidence level > 95%) were accepted.

2. 6   Real-time quantitative RT-PCR

Total RNA was extracted from ten adults for each strain with RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The adults used here were sampled from the same generation with those used for 2-DE. The purity and concentration of total RNAs were determined by spectrophotometry. The RNA integrity was observed on a 1% agarose gel. cDNA was synthesized from 1 μg of DNase-treated total RNA with PrimeScript™ RT Enzyme Mix I (TaKaRa, Japan). Specific primers for corresponding genes were listed in Table 1. Real-time PCR was performed via SYBR Premix EX Taq™ (TaKaRa, Japan) in 20 μL reactions containing gene specific primers, 2 μL cDNA and 10 μL SYBR Premix EX Taq (TaKaRa, Japan). Real-time RT-PCR was done by ABI 7000 Sequence Detection System (Applied Biosystems) with each sample running in triplicate. Experiments were performed three times using *P. xylostella* GAPDH gene (GenBank accession no.: AJ489521) as the reference gene. The relative gene expression level was calculated with $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001).

Table 1   Primer sequences used for real-time quantitative RT-PCR

Protein spot no.	Primer sequences (5′ –3′)
Spot 23	F: CAGCGGCAGCATTTTCTT
	R: CCCCGAACTCGTGGATGT
Spot 21	F: CCCCAACGACTCCCACTA
	R: CCAGCACGCACTTCACAT
Spot 9	F: CCCTGGTGGCTGTTGCT
	R: GGTGATGGTGCGGTAGTT
Spot 17	F: TCCGTAGGATCTATCATCAGGT
	R: AGGTAGTGCGGGTGGGT
Spot 16	F: CAGTCACACTCGCTGCCCCG
	R: GCCGGTGTGGGGAGACAACG
gapdh	F: TGGAAGGTGGTGCCAAGAA
	R: AAGGGGAGCGAGGCAGTTAG

2. 7   Western blotting of protein

Proteins were extracted from DR and DS as described above. An exact equal amount (30 μg) of protein samples were separated on 15% SDS-PAGE and then transferred onto nitrocellulose (NC) membrane. The membrane was blocked for 2 h in TBST (10 mmol/L Tris-HCl, pH 7. 4; 150 mmol/L NaCl, 0. 05% Tween-20) containing 5% w/v nonfat dry milk and subsequently incubated with a rabbit anti-SOD1 antibody (Abcam, UK) at 1 : 5 000 dilution for 8 h at 4℃. After incubation for 2 h with fluorescence-labelled goat anti-rabbit secondary antibody (Rockland, USA), the immunoblot signals were detected by the infrared imaging system (Odyssey, Lincoln, NE, USA) according to the manufacturer's instructions.

2. 8   Statistical analysis

Data are expressed as mean ± standard error (*SE*) and were analyzed by *t*-test using SPSS PASW Statistics version 18. 0. $P < 0. 05$ was considered statistically significant.

3   RESULTS

3. 1   Difference in gene expression at the protein level between the DS and DR strains of *P. xylostella*

In order to understand the changes of proteins under the selective pressure of deltamethrin, the two-dimensional electrophoresis (2-DE) gels of proteins from DS and DR strains of *P. xylostella* were compared (Fig. 1). There were about 300 differentially expressed proteins on each gel. Among them, 23 proteins had a 2. 5-fold difference between

DR and DS. Eight proteins listed in Table 2 were identified successfully by mass spectroscopy from the resistant strain. The expression of five proteins (spots 5, 17, 18, 21 and 22) increased and that of 3 proteins (spots 9, 16 and 23) decreased in DR compared to that in DS (Fig. 1, Table 2).
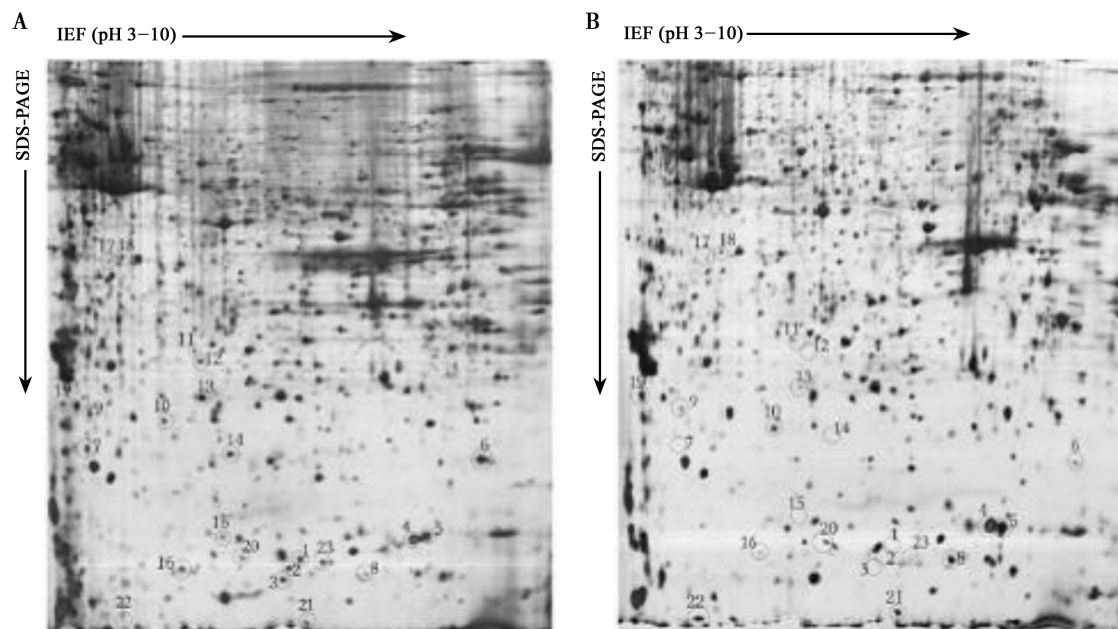

Fig. 1　Expression profiles of proteins from the deltamethrin-sensitive strain (A) and the resistant strain (B) of
Plutella xylostella detected on 2-DE gels

Equal amounts (350 μg) of proteins were separated on the 17 cm, pH 3 – 10 NL immobiline dry strips. SDS-PAGE was performed with 12% gels, and protein spots were visualized by silver staining. DS: Deltamethrin-sensitive strain; DR: Deltamethrin-resistant strain. The same for the following figures.

Table 2　Differentially expressed proteins identified by 2-DE and MS/MS in the deltamethrin-sensitive and resistant strains of *Plutella xylostella*

Protein spot no.	Protein name	Gi number	Species	Database	Matched peptides	Mascot score
Spot 5	Peroxiredoxin-like protein	gil55233150	*Aedes aegypti*	NCBInr	K. VVLFAVPGAFTPGCSK. T	92
Spot 9	Pximaginal disk growth factor	gil117970190	*Plutella xylostella*	NCBInr	K. IVVGIATYAR. T K. EADYTAPIYAPQNR. H R. NAGAPPTKIVVGIATYAR. T	257
Spot 16	Thrombin inhibitor infestin precursor	gil53883964	*Plutella xylostella*	EST_others	R. YCIDKDRPYLR. S R. CLLECAQSKDPSVHEAHPR. Y	173
Spot 17	Chymotrypsin	gil53884010	*Plutella xylostella*	EST_others	R. ATVGTNVWNQGGASYAIAR. N	166
Spot 18	Pyruvate dehydrogenase E1 component beta subunit	gil114052426	*Bombyx mori*	NCBInr	K. THHLITVEQGWPQSGIGAEICAR. V	97
Spot 21	Chemosensory protein CSP2	gil122894082	*Plutella xylostella*	NCBInr	R. RLLVPYVK. C K. ELKEHIQEALENNCGK. C K. MIGHLINHEQEFWDQLIAK. Y	304
Spot 22	Cuticle protein	gil188250349	*Antheraea assama*	EST_others	R. AAEAAIQYNTEAVR. Q	57
Spot 23	Cu/Zn superoxide dismutase	gil53148457	*Plutella xylostella*	NCBInr	R. IACGVIGLAK. A R. TLVVHADPDDLGAGGHELSK. T	253

3.2　Differences in gene expression at mRNA level between the DS and DR strains of *P. xylostella*

To investigate the mRNA expression of these proteins, five proteins (spots 9, 16, 23, 17 and 21) were selected for mRNA expression analysis using real-time RT-PCR. The expression level of Cu/Zn superoxide dismutase in DR was down-regulated compared to that in DS, whereas that of pximaginal disk growth factor and chemosensory protein CSP2

were up-regulated, but that of thrombin inhibitor infestin precursor and chymotrypsin had no significant difference between DS and DR (Fig. 2: B). The expression patterns of spot 23 and spot 21 at mRNA level in DS and DR were in accordance with those at the protein level; the mRNA expression pattern of spot 9 between DS and DR was opposite to protein expression pattern; the expression level of spot 17 and spot 16 between DS and DR had no significant difference at the mRNA level, though had obvious difference at the protein level (Fig. 2).
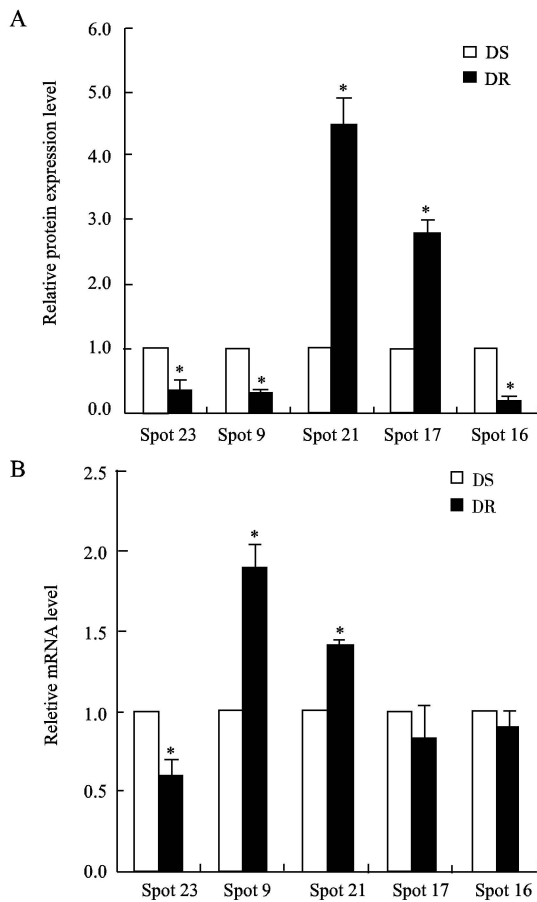

Fig. 2   Expression of five genes at the protein and mRNA levels between the deltamethrin-sensitive and resistant strains of *Plutella xylostella*

A: Protein expression identified by 2-DE and mass spectrometry; B: mRNA expression identified by qPCR. Spot 23: Cu/Zn superoxide dismutase; Spot 9: Disk growth factor; Spot 21: Chemosensory protein; Spot 17: Chymotrypsin; Spot 16: Thrombin inhibitor infestin precusor. The expression level of protein and mRNA in DS was set as 1. The asterisk indicates significant difference exits tween DS and DR at the 0.05 level (*t* test).

3.3   Differences in the expression of SOD1 between the DS and DR strains of *P. xylostella* detected by Western blotting

In order to validate the proteomic results, SOD1 was further examined by Western blotting analysis using specific antibody. The result showed that the protein band was located in the expected size with 18

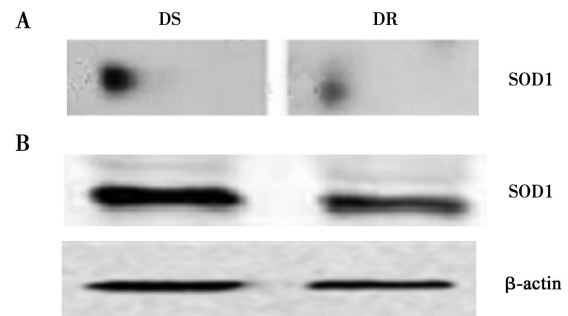kDa and the expression of SOD1 was significantly higher in DS than in DR (Fig. 3).

Fig. 3   SOD1 expression detected by 2-DE (A) and Western blotting (B) between the deltamethrin-sensitive and resistant strains of *Plutella xylostella*

SOD1 was detected by anti SOD antibody and goat anti rabbit antibody labeled with FITC. β-actin was used as an internal control.

4   DISCUSSION AND CONCLUSION

Proteomic technique has been used to investigate the regulation mechanisms of plasma of parasitized *P. xylostella* larvae (Song *et al.*, 2008), but there is no report on proteomic analysis of *P. xylostella* in response to chemical insecticides. Two-dimensional gel electrophoresis followed by mass spectroscopy was first used successfully to identify proteins which were differentially expressed between DS and DR in this study. About 23 differently expressed proteins were selected. Eight proteins were identified successfully. These results showed that deltamethrin has an effect on the gene expression in the diamondback moth. It may provide useful information to unveil the responsive mechanisms of *P. xylostella* to chemical insecticides.

4.1   Antioxidant enzyme

Peroxiredoxin-like protein and Cu/Zn superoxide dismutase (SOD1) belong to antioxidant enzymes. SOD1, one of the SOD enzymes, is a key superoxide scavenging enzyme found primarily in the cytoplasm and plays a protective role in all aerobic organisms against oxidative toxicity (Crapo *et al.*, 1992). Goulielmos *et al.* (2003) found that Cu/Zn superoxide dismutase of *Drosophila* can strengthen the paraquat resistance. The activities of peroxiredoxin and Cu, Zn-SOD were related to the resistance of *B. cinerea* to the dicarboximide fungicides (Choi *et al.*, 1997). Most of the studies focused on transcript levels (Aguilar *et al.*, 2005) or the activities of the enzymes, but there was no report on revealing the abundance difference of these two proteins. In our study, we found that their expression levels were different between DS and DR. Peroxiredoxin-like protein was up-regulated in DR, SOD1 is down-regulated in DR compared to that in

DS. The Western blotting analysis of SOD1 further validated the proteomic investigation.

4.2　Chemosensory proteins

Chemosensory proteins, a group of small, water soluble, and extracellular binding proteins, are essential in the transport of chemical signals. Its functions are to solubilize and transport messenger molecules to the olfactory receptors. A chemosensory protein (CSP2, spot 21) was found to be up-regulated in the DR of the diamondback moth. In insects, chemical signals are detected by one of two major chemosensory mechanisms: olfaction and taste. The main events include the uptake of signal molecules from the external environment, transport through the sensory hair and interaction with the chemoreceptor, which in turn activates the cascade of events leading to spike activity in sensory neurons (Sánchez-Gracia *et al.*, 2009). It is involved in chemical communication (Pelosi *et al.*, 2005). Aguilar *et al.* (2005) reported that CSP2 was up-regulated during the response to microbial infection. However, the expression difference of CSP2 between resistant strain and sensitive strain of insects is first reported, it is worthy of our further study.

4.3　Cuticle proteins

The insect cuticle is located in the outer layer of the body surface. It supports the insect, provides its shape, and protects its body from physical injury and dehydration (Vincent and Wegst, 2004). The mechanical properties of the cuticle depend on the relative amounts of chitin and proteins, the kind and degree of sclerotization, and the sequences of the proteins (He *et al.*, 2007). The cuticular epithelial cells of *Hyalophora cecropia* have been shown to actively participate in defense (Brey *et al.*, 1993). A global gene expression analysis of whole mosquitoes in response to microbial challenge showed that cuticle domain-containing proteins are participated in the mosquito defense response (Aguilar *et al.*, 2005). Moreover, slow penetration of chemical insecticides increases chances for the detoxification enzymes to degrade pesticides. Cuticle proteins play an important role in this process. So the increased expression of cuticle gene found in the resistant strain of this study may play a role in insecticide resistance of the diamondback moth.

4.4　Other proteins

4.4.1　Disk growth factor:

Spot 9 was identified as imaginal disk growth factor (IDGF), which contains a putative chitin-binding lectin domain (Renkema *et al.*, 1998). It is a kind of recognition molecule in the diamondback moth (Eum *et al.*, 2007). It was also reported that this protein is a system regulator to respond to environmental changes in the silkworm (Wang *et al.*, 2009). Though we found the differential expression of the IDGF gene between DR and DS, we are not sure whether it is associated with the resistance.

4.4.2　Pyruvate dehydrogenase:

the beta subunit of pyruvate dehydrogenase E1 catalyzes the overall conversion of pyruvate to acetyl-CoA and CO_2. It was reported that pyruvate dehydrogenase E1 component beta subunit was expressed differently in human cells infected by the avian H9N2 virus (Liu *et al.*, 2008) and decreased during *Brucella melitensis* response to heat shock (Teixeira-Gomes *et al.*, 2000). This may indicate that when the environment changes, the expression of this protein also fluctuates. Whether the differential expression of E1 gene is linked with selection of insecticide still should be further studied.

This study showed that the proteome of adult *P. xylostella* was altered under the long-term selection pressure of deltamethrin, but we did not find cytochrome P450s and sodium channel proteins previously shown to be responsive to pyrethroid. Various factors, such as different cell types, different experimental techniques or the samples derived from different developmental phases and so on, can affect the experimental findings. Another reason may be that the expression difference of these proteins was masked by other abundant proteins with similar molecular weight and pI. Finally, these proteins may be expressed in specific tissues such as fat body or midgut, so we should further analyze the gene expression in specific tissues of DR and DS. To the best of our knowledge, this is the first report on the proteome research of diamondback moth.

In addition, we investigated the expression difference of five proteins at the mRNA level between DS and DR by using real-time quantitative PCR. We observed that the expression patterns at the mRNA and protein levels were not all correlated with each other. Disparity of the expression levels between mRNA and their corresponding protein has also been shown elsewhere (Yan *et al.*, 2006). The lack of correspondence between mRNA and protein levels might be due to the fact that mRNA levels usually reach the peak before protein increments. Post-transcriptional and post-translational modifications as well as differential mRNA and protein degradation rates may also contribute to these discrepancies (Kolkman *et al.*, 2006; Torabi *et al.*, 2009).

In summary, using 2-DE coupled with mass spectrometry, we provided evidence that deltamethrin induces alternation in the proteome of

adult *P. xylostella*. This study can provide some proteome-wide information that helps to understand the physiological and biochemical changes under the long-term selection pressure of deltamethrin on the diamondback moth and lays a foundation for further selection of insecticide targets.

References

Aguilar R, Jedlicka AE, Mintz M, Mahairaki V, Scott AL, Dimopoulos G, 2005. Global gene expression analysis of *Anopheles gambiae* responses to microbial challenge. *Insect Biochemistry and Molecular Biology*, 35: 709 – 719.

Anderson NL, Anderson NG, 1998. Proteome and proteomics: new technologies, new concepts, and new words. *Electrophoresis*, 19: 1853 – 1861.

Blackstock WP, Weir MP, 1999. Proteomics: quantitative and physical mapping of cellular proteins. *Trends Biotechnol.*, 17: 121 – 127.

Bradford MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248 – 254.

Brey PT, Lee WJ, Yamakawa M, Koizumi Y, Perrot S, Franois M, Ashida M, 1993. Role of the integument in insect immunity: epicuticular abrasion and induction of cecropin synthesis in cuticular epithelial cells. *Proc. Natl. Acad. Sci. USA*, 90: 6275 – 6279.

Cheng LG, Xu JN, Xue J, Wang Y, Li ZY, 2009. Downregulation of ubiquitin gene expression in the deltamethrin-resistant diamondback moth (*Plutella xylostella* L.). *J. Appl. Entomol.*, 133: 533 – 538.

Choi GJ, Lee HJ, Cho KY, 1997. Involvement of catalase and superoxide dismutase in resistance of *Botrytis cinerea* to dicarboximide fungicide vinclozolin. *Pestic. Biochem. Physiol.*, 59: 1 – 10.

Crapo JD, Oury T, Rabouille C, Slot JW, Chang LY, 1992. Copper, zinc superoxide dismutase is primarily a cytosolic protein in human cells. *Proc. Natl. Acad. Sci. USA*, 89: 10405 – 10409.

Eum JH, Seo YR, Yoe SM, Kang SW, Han SS, 2007. Analysis of the immune-inducible genes of *Plutella xylostella* using expressed sequence tags and cDNA microarray. *Dev. Comp. Immunol.*, 31: 1107 – 1120.

Eziah VY, Rose HA, Wilkes M, Clift AD, 2009. Biochemical mechanisms of insecticide resistance in the diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Yponomeutidae), in the Sydney region, Australia. *Aust. J. Entomol.*, 48: 321 – 327.

Goulielmos GN, Arhontaki K, Eliopoulos E, Tserpistali K, Tsakas S, Loukas M, 2003. *Drosophila* Cu, Zn superoxide dismutase gene confers resistance to paraquat in *Escherichia coli*. *Biochemical and Biophysical Research Communications*, 308: 433 – 438.

Gygi SP, Rochon Y, Franza BR, Aebersold R, 1999. Correlation between protein and mRNA abundance in yeast. *Molecular and Cell Biology*, 19: 1720 – 1730.

He N, Botelho JMC, McNall RJ, Belozerov V, Augustine DW, Mize T, Orlando R, Willis JH, 2007. Proteomic analysis of cast cuticles from *Anopheles gambiae* by tandem mass spectrometry. *Insect Biochemistry and Molecular Biology*, 37: 135 – 146.

Huang HS, Hu NT, Yao YE, Wu CY, Chiang SW, Sun CN, 1998. Molecular cloning and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the diamondback moth, *Plutella xylostella*. *Insect Biochemistry and Molecular Biology*, 28: 651 – 658.

Kolkman A, Daran-Lapujade P, Fullaondo A, Olsthoorn MMA, Pronk JT, Slijper M, Heck AJR, 2006. Proteome analysis of yeast response to various nutrient limitations. *Molecular Systems Biology*, 2: 1 – 16.

Liu CX, Li FL, Han ZJ, Chen ZH, 1995. Studies on deltamethrin resistance breeding and its resistant mechanism of diamondback moth. *Acta Phytophylacica Sinica*, 22: 367 – 372. [刘传秀, 李凤良, 韩招久, 陈之浩, 1995. 小菜蛾对溴氰菊酯抗性选育及其机理. 植物保护学报, 22(4): 367 – 372]

Liu N, Song W, Wang P, Lee K, Chan W, Chen H, Cai Z, 2008. Proteomics analysis of differential expression of cellular proteins in response to avian H9N2 virus infection in human cells. *Proteomics*, 8: 1851 – 1858.

Livak KJ, Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods*, 25(4): 402 – 408.

Pedra JHF, Festucci-Buselli RA, Sun W, Muir WM, Scharf ME, Pittendrigh BR, 2005. Profiling of abundant proteins associated with dichlorodiphenyltrichloroethane resistance in *Drosophila melanogaster*. *Proteomics*, 5: 258 – 269.

Pelosi P, Calvello M, Ban L, 2005. Diversity of odorant-binding proteins and chemosensory proteins in insects. *Chemical Senses*, 30: 291 – 292.

Renkema GH, Boot RG, Au FL, Donker-Koopman WE, Strijland A, Muijsers AO, Hrebicek M, Aerts JMFG, 1998. Chitotriosidase, a chitinase, and the 39-kDa human cartilage glycoprotein, a chitin-binding lectin, are homologues of family 18 glycosyl hydrolases secreted by human macrophages. *European Journal of Biochemistry*, 251: 504 – 509.

Sánchez-Gracia A, Vieira FG, Rozas J, 2009. Molecular evolution of the major chemosensory gene families in insects. *Heredity*, 103: 208 – 216.

Shelton AM, Wyman JA, Cushing NL, Apfelbeck K, Dennehy TJ, Mahr SER, Eigenbrode SD, 1993. Insecticide resistance of diamondback moth (Lepidoptera: Plutellidae) in North America. *Journal of Economic Entomology*, 86: 11 – 19.

Song KH, Jung MK, Eum JH, Hwang IC, Han SS, 2008. Proteomic analysis of parasitized *Plutella xylostella* larvae plasma. *Journal of Insect Physiology*, 54: 1271 – 1280.

Teixeira-Gomes AP, Cloeckaert A, Zygmunt MS, 2000. Characterization of heat, oxidative, and acid stress responses in *Brucella melitensis*. *Infection and Immunity*, 68: 2954 – 2961.

Thornton BJ, Elthon TE, Cerny RL, Siegfried BD, 2010. Proteomic analysis of atrazine exposure in *Drosophila melanogaster* (Diptera: Drosophilidae). *Chemosphere*, 81: 235 – 241.

Torabi S, Wissuwa M, Heidari M, Naghavi MR, Gilany K, Hajirezaei MR, Omidi M, Yazdi-Samadi B, Ismail AM, Salekdeh GH, 2009. A comparative proteome approach to decipher the mechanism of rice adaptation to phosphorous deficiency. *Proteomics*, 9: 159 – 170.

Vincent JFV, Wegst UGK, 2004. Design and mechanical properties of insect cuticle. *Arthropod Structure & Development*, 33: 187 – 199.

Wang HB, Sakudoh T, Kawasaki H, Iwanaga M, Araki K, Fujimoto H, Takada N, Iwano H, Tsuchida K, 2009. Purification and expression analysis of imaginal disc growth factor in the silkworm, *Bombyx mori*. *Journal of Insect Physiology*, 55: 1065 – 1071.

Yan SP, Zhang QY, Tang ZC, Su WA, Sun WN, 2006. Comparative proteomic analysis provides new insights into chilling stress responses in rice. *Molecular & Cellular Proteomics*, 5: 484 – 496.

Yuan G, Gao W, Yang Y, Wu Y, 2010. Molecular cloning, genomic structure, and genetic mapping of two Rdl-orthologous genes of GABA receptors in the diamondback moth, *Plutella xylostella*. *Archives of Insect Biochemistry and Physiology*, 74: 81 – 90.

小菜蛾溴氰菊酯敏感和抗性品系
蛋白质表达谱的比较分析

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摘要：杀虫剂抗性是害虫防治中的一个主要挑战。为有效治理抗性，我们必须了解杀虫剂诱导的害虫生理生化变化。目前害虫抗药性的一些机理已经清楚，但更多的相关机制还有待探究。本研究通过蛋白质组学方法检测了小菜蛾 *Plutella xylostella* 溴氰菊酯敏感和抗性品系间蛋白质组的表达差异。结果显示：SDS-PAGE 胶上有大约 300 个蛋白差异点，其中 23 个蛋白点具 2.5 倍以上的表达差异，通过 MALDI-TOF-MS，我们成功鉴定出 8 个蛋白，其中包括化感蛋白 CSP2、铜锌超氧化物歧化酶和 peroxiredoxin 样蛋白。通过实时定量 PCR（real-time quantitative PCR，qPCR）分析了其中 5 个蛋白的 mRNA 表达水平，结果表明 mRNA 表达水平不能真实反映蛋白的表达水平。免疫印迹验证了双向电泳中 SOD1 的表达差异。本研究有力地证明溴氰菊酯诱导小菜蛾成虫蛋白质组表达变化，这为进一步筛选抗性靶标提供很大的帮助。

关键词：小菜蛾；溴氰菊酯；蛋白质组；选择压力；杀虫剂抗性

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