International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (2), pp. 001-011, February, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals



*Full Length Research Paper*

# Soluble proteome analysis of male *Ericerus pela* Chavannes cuticle at the stage of the second instar larva

**Pu Yang[1], Jiaying Zhu[2], Meng Li[1], Junmin Li[3] and Xiaoming Chen[1]***

[1]Key laboratory of Cultivating and Utilization of Resources Insects of State Forestry Administration, Research Institute of Resources Insects, Chinese Academy of Forestry, Kunming, Yunnan, 650224, China. [2]Key Laboratory of Forest Disaster Warning and Control of Yunnan Province, College of Conservation Biology, Southwest Forestry University, Kunming, Yunnan, 650224, China. [3]State Key Laboratory of Rice Biology, Institute of Insect Sciences, Zhejiang University, Hangzhou, Zhejiang, 310029, China.

Accepted 07 January, 2019

**Shotgun liquid chromatography tandem mass spectrometry (LC -MS/MS) were employed to character the soluble proteome of male *Ericerus pela* cuticle at the stage of the second instar larva. A total of 278 protein groups (2584 peptides) were identified that involved in a wide spectrum of biological process and molecular function. The proteins were classified into seven categories according to their corresponding functions. Molecular weight (MW) of these identified proteins range from 11 kD to above 110 kD. (isoelectric point) pI were distributed in a range of pH 3.5 to 11.0. Gene ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed that the proteins involved in binding, catalytic, cellular process, metabolic process, biological regulation and protein folding, sorting and degradation are highest. Many important functional proteins related to cell motility and cytoskeleton, stress response, DNA transcription translation and signal transduction were identified. Most heat shock proteins were identified in the proteome of *E. pela* cuticle at this stage. Chitin deacetylase, gasp and chemosensory protein (CSP) was also identified. The results were expected to provide a better understanding of the development of *E. pela*.**

**Key words:** Shotgun liquid chromatography tandem mass spectrometry, proteome, cuticle, biological process, pathway, metabolism.

## INTRODUCTION

The Chinese white wax scale, *Ericerus pela* Chavannes (Homopetera: Coccidae) is a famous resource insect of China. The wax secreted by the scale insect was widely used in wax printing, engraving print, wax candle production and Chinese medicine about one thousand years ago, and in chemical, pharmaceutical, food and cosmetic industry now (Chen and Feng, 2009) . *E. pela* is sexual dimorphism, the female is coated with hard chitin and undergoes hemimetabolism, and the male is coated with filiform waxy secretion and undergoes holometa-bolism. The male secretes wax continuously at the early and later stage of the second instar larva, the wax form a thick wax layer that could avoid the sun burning and prevent water evaporation. The larva and pupa that coated with the wax layer does not move until eclosion. The difference between male and female is shown by the cuticle. Cuticle is important to the survival and sexual dimorphism of *E. pela* (Chen et al., 2009).

Insect cuticle consists mainly of chitin and matrix proteins but also containing lipids and phenoliac materials (Kalume et al., 2003; Andersen, 2010; Moussian, 2010; Suderman et al., 2010), which accounts for the structural and mechanical support, chemical communication and

*Corresponding author. E-mail: cafcxm@tom.com. Tel:/Fax: +86 871 3860019.

preventing water evaporation (Nguyen et al., 2009; Bogus et al., 2010; Loof et al., 2010; Moussian, 2010). The cuticle of *E. pela* play an important role not only in supporting the body, but also in protecting the insect from high temperature and environmental stresses. The male and female are different in the properties of cuticle because they take different ecological strategy response to adverse environment (Chen et al., 2007). The female is coated with hard chitin cuticle, but the male is coated with soft transparent cuticle and thick wax layer. The wax layer secreted by the male larva is important to the survival of the male *E. pela*. The properties of the cuticle of male *E. pela* not only depend on its chitin, but also on the roles of its protein components. Although insect cuticle proteins have been reported by many researchers (Yatsu and Asano, 2009; Gallot et al., 2010; Papandreou et al., 2010), there is no report on large-scale characterization of the cuticle proteome of *E. pela*. Shotgun proteomics provides an eligible tool for a global profile of the proteins of insect at a special stage (Bandeira, et al., 2007; Dong et al., 2008; Li et al., 2009a, b, 2010). In this study, shotgun liquid chromatography tandem mass spectrometry (LC -MS/MS) was employed to identify the cuticle proteome of male *E. pela* at the stage of the second instar larva. The results were expected to provide a better understanding of the development of *E. pela*. The possible roles of the proteins were also discussed finally.

## MATERIALS AND METHODS

### Insect and protein isolation

The male *E. pela* at the stage of the second instar larva were collected from one branch of *Ligustrum lucidum*. The cuticle was detached from the substances in the body and washed in phosphate buffer solution (PBS) for 3 min, repeated three times, then homogenized in lysis buffer (8 M urea, 150 mM Tris, 10 mM DL-Dithiothreitol, 2% protease inhibitor, pH 8.5), centrifuged for 45 min at 15, 000 g at 4°C, the supernate was transfered to a new 1.5 ml tube and stored at -80°C for shotgun LC-MS/MS.

### Shotgun LC-MS/MS

The sample of soluble proteins of male *E. pela* cuticle was put in a foam box with enough dry ice and sent to Shanghai Institutes for Biological Seciences. The soluble proteins were digested by trypsin, the digested protein's separation and identification was conducted by a Finnigan LTQ mass spectrometer (ThermoQuest, San Jose, CA, USA) coupled with a Surveyor high performance liquid chromatography (HPLC) system (ThermoQuest) . Protein identification using MS/MS raw data was performed with SEQUEST software (University of Washington, licensed to Thermo Finnigan). There was no genomic resources and protein sequences of *E. pela* available, therefore a localization database was constructed from the National Center for Biotechnology Informationnon-redundancy (NCBInr) protein database. The peptides and proteins are identified according to the database of proteins of insects, and accession number of these protein sequences were obtained. Protein identification results were filtered with the Xcorr (1+ 1.9, 2 + 2.2, 3+ 3.75) and DelCn ( 0.1).

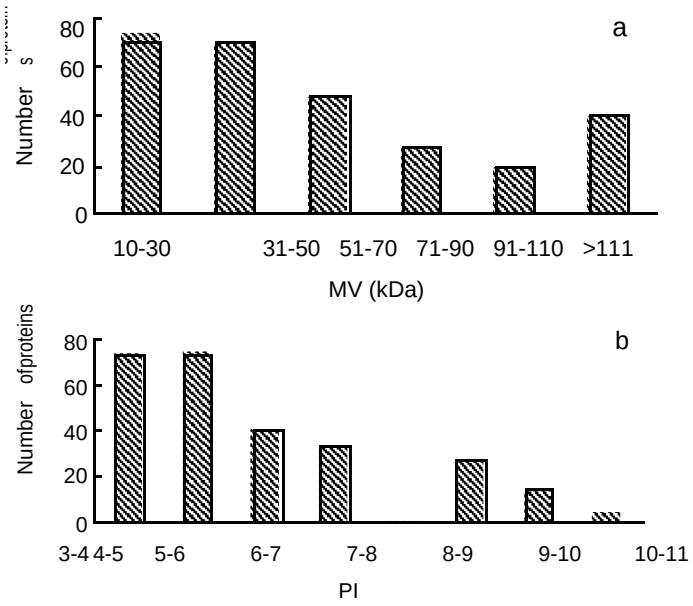


**Figure 1.** Molecular weight (MW) and theoretical isoelectric point (pI) distribution of the identified proteins. (a) distribution of MW, (b) distribution of pI.

### Bioinformatics analysis

The accession numbers of the identified proteins were submited to UniProtKB (http://www.uniprot.org) for function analysis. The proteins were classified into different categories according to their functions. Gene Ontology (GO) classification of the identified proteins was conducted as follows. Accession numbers of these protein sequences were submited to Blast2GO (http://blast2go.bioinfo.cipf.es/home) . The corresponding GO numbers were obtained. The GO annotation terms result was conducted using the online GO tool WEGO [Web Gene Ontology Annotation Plot, (http://wego.genomics.org.cn/)]. The accession numbers of the identified proteins were subjected to search against Kyoto Encyclopedia of Genes and Genomes (KEGG) reference pathway database (http://www.genome.jp/kegg). The pathways in which proteins involved were classified according to the definition of KEGG.

## RESULTS

### Identification and classification of the soluble proteins

A total of 278 protein groups (2584 peptides) were identified. Theoretical molecular weight (MW) of these proteins range from 11 kD to above 110 kD, most of them were below 70 kD (Figure 1a). The theoretical isoelectric point (pI) were distributed in a range of pH 3.5 to 11.0 (Figure 1b) . 216 of the 278 identified protein groups have UniProtKB entries, and the proteins wer classifie into seven categories according to their functions, including

stress resistance (21 proteins), cell motility and cytoskeleton (50 proteins), signal transduction (10 proteins), metabolism (47 proteins), genetic information (47 proteins), transport and protein folding (9 proteins) and others. While the largest set of proteins was assigned to the group of cell motility and cytoskeleton, metabolism and genetic information. We identified more than thirty enzymes involved with intermediary metabolism, signal transduction and muslce contraction, and a lot of proteins associated with different functions, which represented the soluble proteome of cuticle of *E. pela* Chavannes. Part of the proteins was described in Table 1.

**Bioinformatics analysis**

The identified proteins were submitted to Blast2GO for GO term annotation. Most proteins have no GO entries or annotation terms because of lack of distinctive features to the known proteins. 83 proteins could be assigned specific cellular component, molecular function or biological process categories. GO annotation identified 9 molecular function and 17 biological process categories, there was no major class dominant in the proteome. The proportions of binding, catalytic, cellular process, metabolic process and biological regulation are highest (Figure 2).

All identified proteins were also been subjected to the KEGG to predict the metabolic pathway. Among the identified proteins, most of them belong to genetic information processing (include protein folding, sorting and degradation, DNA replication and repair, transcription and translation) and metabolism (include energy metabolism, carbohydrate metabolism, amino acid metabolism, nucleotide metabolism). 122 proteins were found to be involved in 11 pathways, and some of them belonging to multiple pathways. Most proteins could not be ascribed any biological function (Figure 3).

**DISCUSSION**

Gene expression profiling or transcriptome analysis can predict the level of gene expression and provide more genetic information (Wang et al., 2010; Zhang et al., 2010), but mRNA levels may not correlate with the protein level, so proteome analysis is indispensable for entomological research (Li et al., 2009a). The shotgun proteomics strategy has been widely used in proteome research (Bandeira, et al., 2007; Dong et al., 2008; Li et al., 2009a, b, 2010), and has been employed in the research of model insects, such as *Drosophila melanogaster* (Baggerman et al., 2005), *Anopheles gambiae* (Kalume et al., 2005) and *Bombyx mori* (Li et al., 2009a, b, 2010). Although, there was no fully developed protein database for *E. pela*, genomic resources and protein database of other organisms provide much reference information. In this report, we characterized the larval cuticle proteome of male *E. pela* using shotgun proteomics approach. 278 protein groups were identified that involved in different biological process. Most pathways in which these proteins involved were housekeeping pathways that supporting cellular activity. Possible contributions of some proteins were discussed thus.

**Cell motility and cytoskeleton**

Proteins are key determinants of the insect cuticular physical properties. A lot of proteins related to cell motility and cytoskeleton were identified such as myosin, actin, tropomyosin, tubulin and spectrin. Myosin and actin are important proteins whose functions are related to muscle contraction and motility (Alvite and Esteves, 2009; Lehman et al., 2009; Li et al., 2010; Vikhoreva and Mansson, 2010). Tropomyosin, which interacts with actin in the muscle was also identified. These proteins are represented by multiple isoforms, and are highly conserved and essential within insects (Watabe and Ikeda, 2006; Kim et al., 2009; Creed et al., 2 0 1 0; Prochniewicz et al., 2010), these proteins are identified abundantly. Although the *E. pela* larva does not move until eclosion, muscle contraction and cell motility are essential for the physiological activity.

Meanwhile, tubulin multigene family was also identified in the *E. pela* cuticlar proteome. Tubulin has been described as cytoskeleton proteins, and also plays a key role in the mitotic spindle assembly (Yang et al., 2009). Tubulin is important to cell division and development of *E. pela* larva. Motility and cytoskeleton related proteins were identified abundantly because of their conventional functions.

**Stress response proteins**

The cuticle of insects not only supports the insect body, but also protects the insect from environmental stresses (Nguyen et al., 2009). White wax production in China was processed in Hunan and Sichuan province, where the climate is hot and humid. According to wax production and practice, heat stress and high humidity increase wax secretion of *E. pela*, resulting in thick wax layer. Chen et al. (1998, 2007, 2009) further studied links between wax secretion and stress resistance in the *E. pela*, and found that wax secretion is the survival strategy response to heat stress and high humidity. Stress response proteins, which is concurrent with environmental stresses, such as heat shock proteins (HSPs) will protect the cells from damage (Kang et al., 2009; Benoit et al., 2010; Heikkila, 2010; Rungrassameea et al., 2010; Walcott, Heikkila, 2010). In this work, peroxidase, thiol peroxiredoxin and five heat shock protein gene family were detected

**Table 1.** Classification of the identified proteins.

| Protein | Biological process | Molecular function |
|---|---|---|
| **Cell motility and cytoskeleton** | | |
| myo-2 | | ATP binding |
| Actin | | ATP binding |
| Tropomyosin 1 | Muscle contraction | Actin binding |
| Paramyosin, long form | | Motor activity |
| Tubulin-α-chain | Protein Polymerization | GTP binding |
| Tubulin -4 chain | Microtubule-Based Movement | GTP binding |
| α-Spectrin | Cytoskeleton organization | Actin binding |
| Collagen α-2(IV) chain | | Extracellular matrix structural constituent |
| Lachesin | Maintenance of epithelial integrity, open tracheal system | Protein binding |
| Moesin/ezrin/radixin homolog 1 | Establishment or maintenance of epithelial cell apical/basal polarity | Actin binding |
| Calbindin-32 | | Calcium ion binding |
| Lectin 4 C-type lectin | | Sugar binding |
| ***Stress response proteins*** | | |
| Heat shock protein 20.6 | Response to heat | |
| Heat shock protein 60 | Response to stress | ATP binding |
| Heat shock protein 70 | Response To Stress | ATP binding |
| kDa Heat shock protein | Response To Stress | ATP binding |
| 82 Heat shock protein 90 | Response To Stress | ATP binding |
| 1-cys peroxiredoxin | Cell redox homeostasis | antioxidant activity |
| Putative peroxidase DnaJ- | Response to oxidative stress | |
| lik protein Thiol | Response to heat | ATP binding |
| peroxiredoxin | Response to oxidative stress | |
| **Energy metabolism** | | |
| ADP, ATP carrier protein | Transmembrane transport | ATP:ADP antiporter activity |
| ATP synthase subunit α | Plasma Membrane Atp Synthesis Coupled Proton Transport | hydrogenion transporting ATP synthase activity, rotational mechanism |
| ATP synthase subunit | Plasma Membrane Atp Synthesis Coupled Proton Transport | Hydrogenion Transporting Atp Synthase activity, Rotational Mechanism |
| V-type proton ATPase catalytic subunit A | ATP synthesis coupled proton transport | Hydrogen Ion Transporting Atp Synthase activity, Rotational Mechanism |

**Table 1.** Contd.

| | | |
|---|---|---|
| lethal (2) 01289 | Cell redox homeostasis | Protein disulfide isomerase activity |
| Transitional endoplasmic reticulum ATPase TER94 | | ATP binding |
| **Carbohydrate metabolism** | | |
| 2-oxoglutarate dehydrogenase | Glycolysis | Oxoglutarate dehydrogenase (succinyl-transferring) activity |
| Aconitase, mitochondrial | Tricarboxylic acid cycle | Aconitate hydratase activity |
| ATP citrate lyase | Citrate metabolic process; | ATP citrate synthase activity |
| Pyruvate carboxylase | Pyruvate metabolic process | Pyruvate carboxylase activity |
| Chitin deacetylase 1 | Chitin metabolic process | Chitin deacetylase activity |
| Gasp | Chitin metabolic process | Chitin binding |
| Pyruvate dehydrogenase | Metabolic process | Catalytic activity |
| Pyruvate kinase, muscle, b | Glycolysis | Catalytic activity |
| Isocitrate dehydrogenase | Tricarboxylic acid cycle | Isocitrate dehydrogenase (NAD+) activity |
| Probable citrate synthase 2, mitochondrial | Tricarboxylic acid cycle | Citrate (Si)-synthase activity |
| Enolase | Glycolysis | Phosphopyruvate hydratase activity |
| Fructose-bisphosphate aldolase | Glycolysis | Fructose-bisphosphate aldolase activity |
| Glyceraldehyde-3-phosphate dehydrogenase | Glycolysis | NAD or NADH binding |
| Phosphoglycerate kinase | Glycolysis | Phosphoglycerate kinase activity |
| Phosphoglycerate mutase | Glycolysis | 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase activity |
| Triosephosphate isomerase | Metabolic process | Triose-phosphate isomerase activity |
| Malate dehydrogenase | Tricarboxylic acid cycle | Malic enzyme activity |
| **Nucleic acid metabolism** | | |
| Purine biosynthesis protein 6, pur6 | Purine nucleotide biosynthetic process | ATP binding |
| Protein catabolic process | | |

**Table 1.** Contd.

| | | |
|---|---|---|
| Tat-binding protein-1 | Protein catabolic process | Endopeptidase activity |
| Calpain | Proteolysis | Calcium-dependent cysteine-type endopeptidase activity |
| Ubiquitin carrier protein | Post-translational protein modification | Small conjugating protein ligase activity |
| **Genetic information processing** | | |
| Histone H2A | Nucleosome assembly | DNA binding |
| Histone H2B | Nucleosome assembly | DNA binding |
| Histone H3 | Nucleosome assembly | DNA binding |
| Histone H4 | Nucleosome assembly | DNA binding |
| Methyltransferase | Methylation | Methyltransferase activity |
| DNA polymerase | DNA replication | DNA binding |
| Calmodulin-binding transcription activator (Camta), drome | Regulation of transcription | Transcription regulator activity |
| Retinoblastoma binding protein 4 | Regulation of transcription | |
| DNA-binding protein smubp-2 | | DNA binding |
| 40S ribosomal protein SA | Translation | Structural constituent of ribosome |
| 60S ribosomal protein L5 | Translation | 5S rRNA binding |
| Ribosomal protein L19 | Translation | Structural constituent of ribosome |
| Elongation factor 1-$\alpha$ | Translational elongation | Translation elongation factor activity |
| Inhibitor of apoptosis protein (AGAP002651-PA) | Post-translational protein modification | Small conjugating protein ligase activity |
| **Signal transduction processing** | | |
| 1D-myo-inositol-trisphosphate 3-kinase isoform C | | Inositol trisphosphate 3-kinase activity |
| AGAP010767-PA | Regulation of Rho protein signal transduction | Rho guanyl-nucleotide exchange factor activity |
| Citron ser/thr kinase | Protein amino acid phosphorylation | Protein serine/threonine kinase activity |
| Integrin-$\alpha$-1 | Integrin-mediated signaling pathway | Receptor activity |
| GTP binding protein | | GTP binding |
| Putative cAMP-dependent protein kinase regulatory chain type I | | Kinase activity |

**Table 1.** Contd.

| | | |
|---|---|---|
| Calmodulin | | Calcium Ion Binding |
| Protein kinase C, eye isozyme Neuroglian | Adaptation of rhodopsin mediated signaling<br>Axon ensheathment | Protein binding<br>Calcium ion binding |
| Cdk5 | Protein amino acid phosphorylation | Protein serine/threonine kinase activity |
| **Chemosensory protein CSP3** | | |
| Putative accessory gland protein | Protein folding | Unfolded protein binding |
| Transport and protein folding | | |
| ATP-binding cassette sub-family A member 3, putative | ATP binding | ATP-binding cassette sub-family A member 3, putative |
| Low-density lipoprotein receptor (Ldl) | Receptor activity | Low-density lipoprotein receptor (Ldl) |
| Calreticulin | Protein folding | Calreticulin |
| Chaperonin | Protein folding | Chaperonin |
| Nascent polypeptide associated complex protein alpha subunit | Nascent polypeptide-associated complex | Nascent polypeptide associated complex protein alpha subunit |

which were known as stress resistance proteins. HSPs were induced by high temperature and humidity and abundantly identified in the cuticlar proteome suggests that *E. pela* possess self-protection mechanisms against environmental stresses though both cuticle protection enhancement and wax secretion. A lot of stress resistance proteins were revealed at the proteomic level could explain the higher tolerance of the male *E. pela* to environmental stress.

## Metabolism

Among the proteins found during this proteomic study, many proteins were involved in metabolism, while most of them were involved in carbohydrate metabolism. These proteins cover a wide spectrum of biological functions, such as oxidative phosphorilation, transport, electron carrier activity, ATP binding and catalytic activity.

Energy metabolism is necessary to provide energy for insect physiological activity. ADP, ATP carrier protein and ATP synthase were identified in the proteome of *E. pela* cuticle. ATP synthase plays a crucial role in oxidative phosphorylation (Willers and Cuezva, 2010). These housekeeping proteins were also observed in survey of proteome of *Culex pipiens quinquefasciatus* and *B. mori* (Ribeiro et al., 2004; Hou et al., 2007), wich were important to energy metabolism. We also identified a lot of proteins involved in carbohydrate metabolism (including citrate cycle, pyruvate metabolism, glycolysis), which account for the housekeeping pathways supporting cellular activity. We identified 2 proteins (Chitin deacetylase and Gasp) known to be involved in chitin metabolic process, which is important to insect cuticle (Shrestha et al., 2004; Cai et al., 2006; Nisole et al., 2010). Proteins involved in purine nucleotide biosynthetic process and protein catabolic process were identified. As a whole, these results illustrate that, abundant proteins involved in metabolism were important to cuticle physiological activity.

It is regretful that there was no identified protein similar to wax genes of plant, such as wax synthase and CER genes of *Arabidopsis thaliana*

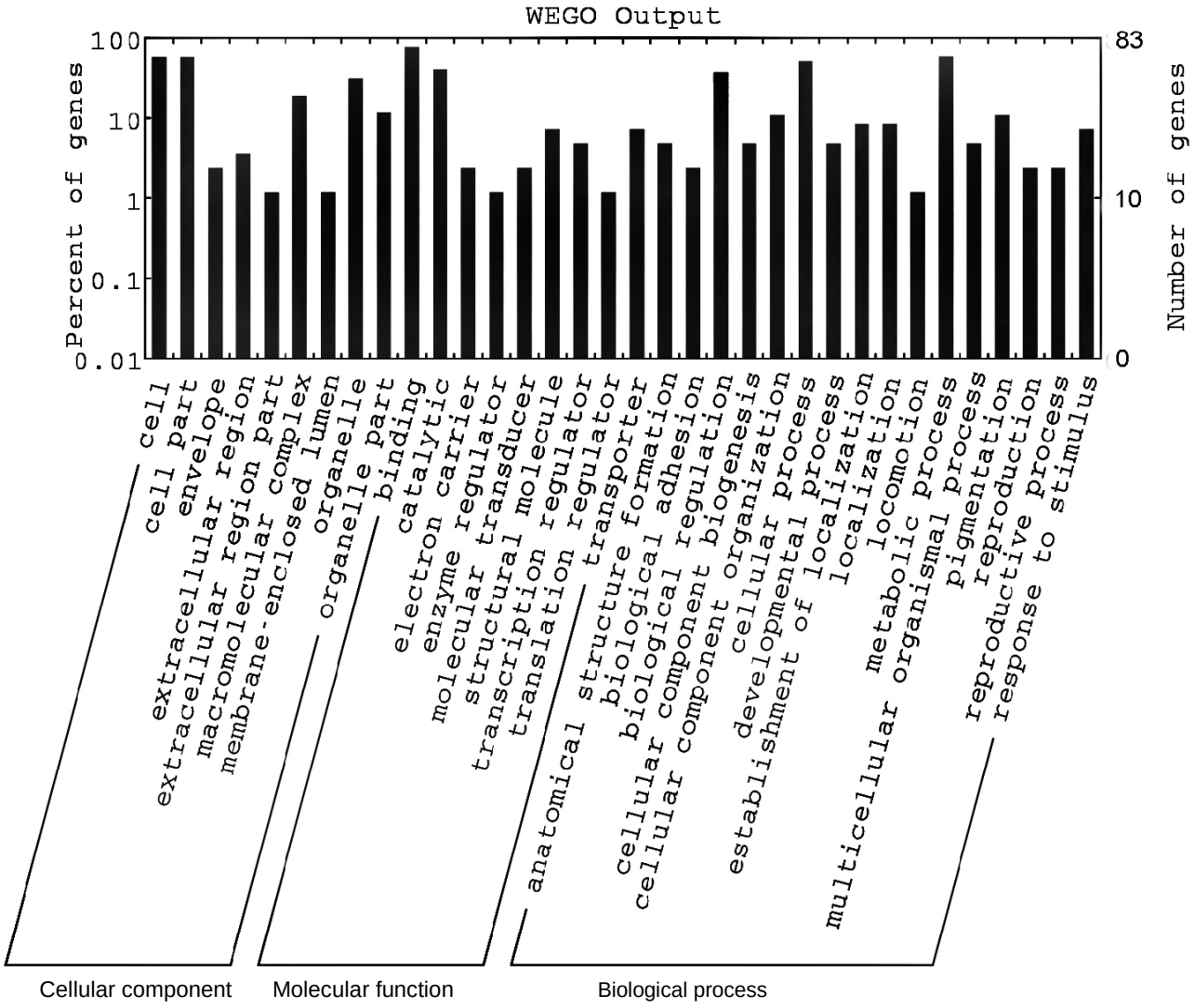


**Figure 2.** Gene ontology (GO) categories of the identified proteins.

(Rowland et al., 2007; Klypina, Hanson, 2008; Holmes, 2010) . This maybe because proteins of *E. pela* share lower homology with wax genes of plant, no homologous proteins were identified in the cuticle proteome of *E. pela.* Although, silk gland proteins from the silkworm has been studied (Zhang et al., 2006), there was no reference sequence of silkworm because of the different metabolism pathways of *E. pela*, which result in different component of wax to silk. Progress in understanding genes involved in wax metabolism was hindered by difficulty in accurately describing wax gene product functions based on their amino acid sequence homologies with known proteins (Goodwin et al., 2005). This would explain why no proteins involved in wax metabolism was identified in the proteome research of *E. pela*.

**Proteins associated with genetic information processing and signal transduction**

We identified 47 proteins known to be involved in genetic information processing. These proteins are histone, DNA polymerase, ribosomal proteins, elongation factor 1-alpha, chaperonin, methyltransferase and so on. These results confirmed high expression activities at this stage.

Ten proteins are associated with signal transduction pathways including GTP binding protein, cAMP-dependent protein kinase, protein kinase C and Cdk5. GTP binding protein have been suggested to play an important role in many signal transduction pathways that involved in receptor phosphorylation, down-regulation and pathway switching (Gomperts et al., 2009). Chemosensory protein (CSP) was identified in the cuticle

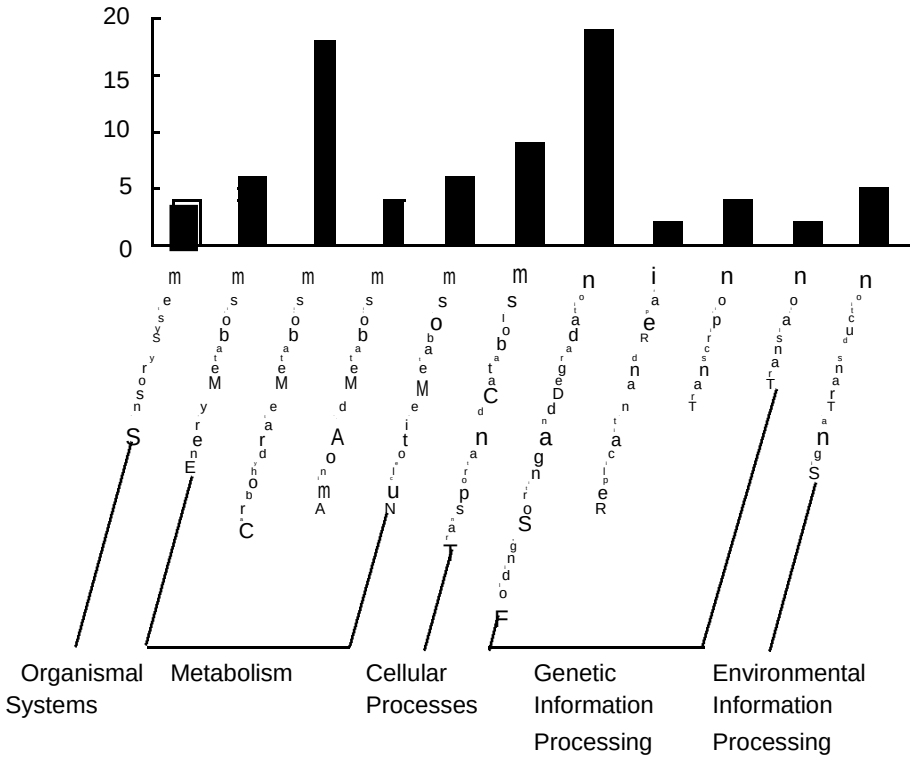


**Figure 3.** Classification of pathways according to the definition in KEGG.

proteome. CSPs has been found to play an important role in recognition of different ant colony by combining with cuticular hydrocarbons (Ozaki et al., 2005). Based on the research of *Camponotus japonicus* and *Apis mellifera ligustica* (Ozaki et al., 2005; Dani et al., 2005), CSP play a crucial role in swarming of *E. pela*.

The *E. pela* cuticle proteome is a complex mixture of proteins, in this proteomic study, we found many housekeeping proteins and insect-specific proteins. because of the bottleneck for many shotgun proteomics experiments, there were some proteins not identified, and we did not found proteins related to the interesting property of scale insect, such as wax secretion. Because the cuticle proteins were insoluble, it is difficult to isolate the cuticle proteins, they were not identified in this soluble proteome research.

The results of this study are based on large-scale characterization of the cuticlar proteome of *E. pela* , and thus can be considered as fully representative. The pathway analysis and protein function classification at the proteomic level provided many information of physiological activity of *E. pela* at the stage of the second instar larva. The function of some important proteins warrant further studies.

## ACKNOWLEDGEMENTS

This study was financially supported by the National Natural Science Foundation of China (Youth Program) (Grant No. 31000983), the Applied Basic Research Fundation of Yunnan Province (Grant No. 2010ZC235) and the National Nonprofit Institute Research Grant of RIRI-CAF (Grant No. 7-015-3).

## REFERENCES

Alvite G, Esteves A (2009). *Echinococcus granulosus* tropomyosin isoforms: From gene structure to expression analysis. Gene, 433: 40-49.

Andersen SO (2010). Insect cuticular sclerotization: A review. Insect Biochem. Mol., 40: 166-178.

Baggerman G, Boonen K, Verleyen P, Loof AD, Schoofs L (2005). Peptidomic analysis of the larval *Drosophila melanogaster* central nervous system by two-dimensional capillary liquid chromatography quadrupole time-of-flight mass spectrometry. J. Mass Spectrom, 40: 250-260.

Bandeira N, Clauser KR, Pevzner PA (2007). Shotgun protein sequencing. Mol Cell Proteomics, 6: 1123-1134.

Benoit JB, Lopez-Martinez G, Phillips ZP, Patrick KR, Denlinger DL (2010). Heat shock proteins contribute to mosquito dehydration tolerance. J. Insect Physiol., 56: 151-156.

Bogus MI, Czygier M, Gołębiowski M, K dra E, Kuci ska J, Mazgajska J, Samborski J, Wieloch W, Włóka E (2010). Effects of insect cuticular fatty acids on *in vitro* growth and pathogenicity of the entomopathogenic fungus *Conidiobolus coronatus*. Exp. Parasitol., 125: 400-408.

Cai J, Yang JH, Du YM, Fan LH, Qiu YL, Li J, Kennedy JF (2006). Purification and characterization of chitin deacetylase from *Scopulariopsis brevicaulis*. Carbohydr. Polym., 65: 211-217.

Chen XM, Chen Y, Zhou CH, Wang ZL, Ye SD, Wang SY (1998). Studies on wax secretion of the Chinese white wax scale (*Ericerus*

*pela* Chavannes) I: The comparison on wax secretion of different geographic varieties. For. Res., 11: 34-38.
Chen XM, Feng Y (2009). The Chinese white wax scale *E. pela* Chavannes. In: Chen XM, Feng Y, eds. An introduction to resource entomology. Beijing: Science Press, pp. 29-30.
Chen XM, Wang ZL, Chen Y, Ye SD, Wang SY, Feng Y (2007). The main climate factors affecting wax excretion of *Ericerus pela* Chavannes (Homopetera: Coccidae) and an analysis of its ecological adaptability. Acta Entomol. Sin., 50(2): 136-143.
Creed SJ, Desouza M, Bamburg JR, Gunning P, Stehn J (2010). Tropomyosin isoform 3 promotes the formation of filopodia by regulating the recruitment of actin-binding proteins to actin filaments. Exp. Cell Res., p. 30.
Dani FR, Jones GR, Corsi S, Beard R, Pradella D, Turillazzi S (2005). Nestmate Recognition Cues in the Honey Bee: Differential Importance of Cuticular Alkanes and Alkenes. Chem. Senses, 30: 477 – 489.
Dong MY, Ding GL, Zhou J, Wang HZ, Zhao Y, Huang HF (2008). The Effect of Trophoblasts on T Lymphocytes: Possible Regulatory Effector Molecules - A Proteomic Analysis. Cell Physiol. Biochem., 21: 463 – 472.
Gallot A, Rispe C, Leterme N, Gauthier JP, Jaubert-Possamai S, Tagu D (2010). Cuticular proteins and seasonal photoperiodism in aphids. Insect Biochem. Mol., 40: 235-240.
Gomperts BD, Kramer IJM, Tatham PER (2009). GTP-binding Proteins and Signal Transduction, Signal Transduction. 2nd ed., pp. 81-129.
Goodwin SM, Rashotte AM, Rahman M Feldmann KA, Jenks MA (2005). Wax constituents on the inflorescence stems of double eceriferum mutants in Arabidopsis reveal complex gene interactions. Phytochemistry, 66: 771-780.
Heikkila JJ (2010). Heat shock protein gene expression and function in amphibian model systems. Comp. Biochem. Physiol. A, 156: 19-33.
Holmes RS (2010). Comparative genomics and proteomics of vertebrate diacylglycerol acyltransferase (DGAT), acyl CoA wax alcohol acyltransferase (AWAT) and monoacylglycerol acyltransferase (MGAT). Comp Biochem Physiol. D, 5: 45-54.
Hou Y, Xia QY, Zhao P , Zou Y, Liu HL, Guan J, Gong J, Xiang ZH (2007). Studies on middle and posterior silk glands of silkworm (*Bombyx mori*) using two-dimensional electrophoresis and mass spectrometry. Insect Biochem. Mol., 37: 486-496.
Kalume DE, Kieffer S, Rafn K, Skou L, Andersen SO, Roepstorff P (2003). Sequence determination of three cuticular proteins and isoforms from the migratory locust, *Locusta migratoria*, using a combination of Edman degradation and mass spectrometric techniques. Biochim. Biophys. Acta, 1645(2): 152-163.
Kalume DE, Okulate M, Zhong J, Reddy R, Suresh S, Deshpande N, Kumar N, Pandey A (2005). A proteomic analysis of salivary glands of female *Anopheles gambiae* mosquito. Proteomics, 5: 3765-3777.
Kang L, Chen B, Wei JN, Liu TX (2009). Roles of thermal adaptation and chemical ecology in Liriomyza distribution and control. Annu. Rev. Entomol., 54: 127-145.
Kim BK, Kim KS, Oh CW, Mykles DL, Lee SG, Kim HJ, Kim HW (2009). Twelve actin-encoding cDNAs from the American lobster, *Homarus americanus*: Cloning and tissue expression of eight skeletal muscle, one heart, and three cytoplasmic isoforms. Comp. Biochem. Physiol. B, 153: 178-184.
Klypina N, Hanson SF (2008). *Arabidopsis thaliana* wax synthase gene homologues show diverse expression patterns that suggest a specialized role for these genes in reproductive organs. Plant Sci., 175: 312-320.
Lehman W, Gali ska-Rakoczy A, Hatch V, Tobacman LS, Craig R (2009). Structural basis for the activation of muscle contraction by troponin and tropomyosin. J. Mol. Biol., 388: 673-681.
Li JY, Chen X, Hosseini Moghaddam SH, Chen M, Wei H, Zhong BX (2009a). Shotgun proteomics approach to characterizing the embryonic proteome of the silkworm, *Bombyx mori*, at labrum appearance stage. Insect Mol. Biol., 18(5): 649-660.
Li JY, Chen X, Fan W, Moghaddam SH, Chen M, Zhou ZH, Yang HJ, Chen JE, Zhong BX (2009b). Proteomic and bioinformatic analysis on endocrine organs of domesticated silkworm, *Bombyx mori* L. for a comprehensive understanding of their roles and relations. J. Proteome Res., 8: 2620-2632.
Li JY, Moghaddam SH, Chen JE, Chen M, Zhong BX (2010). Shotgun proteomic analysis on the embryos of silkworm *Bombyx mori* at the end of organogenesis. Insect Biochem. Mol., 40: 293-302.
Li XC, Holmes KC, Lehman W, Jung H, Fischer S (2010). The shape and flexibility of tropomyosin coiled coils: implications for actin filament assembly and regulation. J. Mol. Biol., 395: 327-339.
Loof AD, Huybrechts J, Geens M, et al. Sexual differentiation in adult insects: Male-specific cuticular yellowing in *Schistocerca gregaria* as a model for reevaluating some current (neuro) endocrine concepts. J. Insect Physiol., 56: 919-925.
Moussian B (2010). Recent advances in understanding mechanisms of insect cuticle differentiation. Insect Biochem Mol., 40: 363 – 375.
Nguyen TTA, Michaud D, Cloutier C (2009). A proteomic analysis of the aphid *Macrosiphum euphorbiae* under heat and radiation stress. Insect Biochem. Mol., 39: 20-30.
Nisole A, Stewart D, Bowman S, Zhang D, Krell PJ, Doucet D, Cusson M (2010). Cloning and characterization of a Gasp homolog from the spruce budworm, *Choristoneura fumiferana*, and its putative role in cuticle formation. J. Insect Physiol., 56: 1427-1435.
Ozaki M, Wada-Katsumata A, Fujikawa K, Iwasaki M, Yokohari F, Satoji Y, Nisimura T, Yamaoka R (2005). Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. Science, 309(5732): 311-314.
Papandreou NC, Iconomidou VA, Willis JH, Hamodrakas SJ (2010). A possible structural model of members of the CPF family of cuticular proteins implicating binding to components other than chitin. J. Insect Physiol., 56: 1420-1426.
Prochniewicz E, Chin HF, Henn A, Hannemann DE, Olivares AO, Thomas DD, De La Cruz EM (2010). Myosin isoform determines the conformational dynamics and cooperativity of actin filaments in the strongly bound actomyosin complex. J. Mol. Biol., 396: 501-509.
Ribeiro JMC, Charlab R, Pham VM, Garfield M, Valenzuela JG (2004). An insight into the salivary transcriptome and proteome of the adult female mosquito *Culex pipiens quinquefasciatus.* Insect Biochem. Mol., 34: 543-563.
Rowland O, Lee R, Franke R, Schreiber L, Kunst L (2007). The CER3 wax biosynthetic gene from *Arabidopsis thaliana* is allelic to *WAX2/YRE/FLP1*. FEBS Lett., 581: 3538-3544.
Rungrassameea W, Leelatanawita R, Jiravanichpaisal P, Klinbunga S, Karoonuthaisiri N (2010). Expression and distribution of three heat shock protein genes under heat shock stress and under exposure to *Vibrio harveyi in Penaeus monodon*. Dev. Comp. Immunol., 34: 1082 – 1089.
Shrestha B, Blondeau K, Stevens WF, Hegarat FL (2004). Expression of chitin deacetylase from *Colletotrichum lindemuthianum* in *Pichia pastoris*: purification and characterization. Protein Expres Purif., 38: 196-204.
Suderman RJ, Dittmer NT, Kramer KJ, Kanost MR (2010). Model reactions for insect cuticle sclerotization: Participation of amino groups in the cross-linking of *Manduca sexta* cuticle protein MsCP36. Insect Biochem. Mol., 40: 252-258.
Vikhoreva NN, Mansson A. Regulatory light chains modulate in vitro actin motility driven by skeletal heavy meromyosin. Biochem. Biophys. Res. Co., 403: 1-6.
Walcott SE, Heikkila JJ (2010). Celastrol can inhibit proteasome activity and upregulate the expression of heat shock protein genes, hsp30 and hsp70, in *Xenopus laevis* A6 cells. Comp. Biochem. Physiol. A, 156: 285-293.
Wang H, Zhang H, Wong YH, Voolstra C, Ravasi T, Bajic VB, Qian PY (2010). Rapid transcriptome and proteome profiling of a non-model marine invertebrate, *Bugula neritina*. Proteomics, 10(16): 2972-2981.
Wang XW, Luan JB, Li JM, Bao YY, Zhang CX, Liu SS (2010). *De novo* characterization of a whitefly transcriptome and analysis of its gene expression during development. BMC Genomics, 11: 400.
Watabe S, Ikeda D (2006). Diversity of the pufferfish Takifugu rubripes fast skeletal myosin heavy chain genes. Comp. Biochem. Physiol. D, 1: 28-34.
Willers IM, Cuezva JM (2010). Post-transcriptional regulation of the mitochondrial H+-ATP synthase: A key regulator of the metabolic phenotype in cancer. BBA, p. 30.
Yang P, Zhou WW, Zhang Q, Cheng JA, Zhu ZR, Mo W (2009). Differential gene expressio n profiling in the developed ovaries

between the parthenogenetic and bisexual female rice water weevil , *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae). Chinese Sci. Bull, 54: 3822 – 3829.

Yatsu J, Asano T (2009). Cuticle laccase of the silkworm, *Bombyx mori*: Purification, gene identification and presence of its inactive precursor in the cuticle. Insect Biochem Mol., 39: 254 – 262.

Zhang FJ, Guo HY, Zheng HJ, Zhou T, Zhou YJ, Wang SY, Fang RX, Qian W, Chen XY (2010). Massively parallel pyrosequencing-based transcriptome analyses of small brown planthopper (*Laodelphax striatellus*), a vector insect transmitting rice stripe virus (RSV). BMC Genomics, 11: 303.

Zhang PB, Aso Y, Yamamoto K, Banno Y, Wang Y, Tsuchida K, Kawaguchi Y, Fujii H (2006). Proteome analysis of silk gland proteins from the silkworm, *Bombyx mori*. Proteomics, 6: 2586-2599.