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Protein Profiles of the Midgut of *Spodoptera litura* Larvae at the Sixth Instar Feeding Stage by Shotgun ESI−MS Approach

Jisheng Liu, Sichun Zheng, Lin Liu, Ling Li,* and Qili Feng*

Guangdong Provincial Key Lab of Biotechnology for Plant Development, School of Life Sciences, South China Normal University, Guangzhou, China

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By using shotgun HPLC−ESI−MS proteomics approach, 2043 peptides were identified from the midgut of *Spodoptera litura* larvae at the sixth instar feeding stage, out of which 1489 (72.9%) were found to have their homologues in the public protein databases and 842 had identities of molecular functions. Seven-hundred forty-one peptides were annotated according to Gene Ontology Annotation in terms of molecular function, biological process, and cellular localization, with 336 and 251 peptides being related to catalytic activity and binding activity, respectively. Most of the catalytic proteins had activity of hydrolases, oxidoreductases and transferases and most of the binding proteins were involved in protein-binding activity. Among the annotated peptides, 487 were classified into different cellular processes and 490 were classified to locate in the cytoplasm. Nonredundant enzymes associated with the metabolisms of carbohydrates, lipids and fatty acids, amino acids and proteins, translation, transport, and stress resistance were identified. Presence and expression at high levels of numerous enzymes of glycolysis pathway, synthesis of proteins, and absorption and transport of fatty acids and lipids indicate that active metabolism processes of carbohydrates, proteins, and lipids occurred in the midgut of sixth instar feeding larvae of *S. litura*. The protein profile provides a basis for further study of the physiological events in the midgut of *S. litura*.

Keywords: ESI−MS • shotgun • midgut • metabolism • food digestion I *Spodoptera litura*

1. Introduction

Many methods, such as two-dimensional gel electrophoresis/mass spectrometer (2-DE/MS), matrix-assisted laser desorption/ionization time-of-flight/mass spectrometer (MALDI-TOF-MS), surface-enhanced laser desorption/ionization time-of-flight/mass spectrometry (SELDI-TOF-MS), and liquid chromatography mass spectrometry (LC−MS/MS), have been used to study protein expression profiles in insect tissues. The 2-DE/MS method is currently the main approach for separation and comparison of complex protein mixtures and is advantageous because of its high resolution and sensitivity. However, it has a few restrictions, such as limited loading capacity, dependence on physicochemical properties of proteins, and problems of transferring proteins from the first-dimensional gel onto the second one. The 2-DE/MS method is often unable to detect those proteins that have extremes in molecular mass and isoelectric points and that are hydrophobic or unsolvable, such as the integral membrane proteins.[1] During the past several years, shotgun proteomics has been proven to be an alternative technology capable of identifying hundreds of proteins from single samples and to be complementary to 2-DE-based analysis.[2−4] Shotgun proteomics relies on protein separation after the proteolytic digestion and takes advantage of MS/MS to infer the amino acid sequences of individual peptides. Compared to the 2-DE/MS proteomics approach, the shotgun method possesses the virtues of high efficiency and time and labor saving.

Insect gut is a main organ for food digestion, nutrient absorption and protection from pathogen invasion and, therefore, is an important target for novel biological and chemical controlling strategies. Studies on expression profiles of genes and/or proteins in the midgut can facilitate identification of molecular targets that can be used for developing novel and environmentally benign controlling strategies. Four-hundred fifty individual proteins were detected in the brush border membrane vesicles (BBMV) of the midgut of *Manduca sexta* fifth instar larvae by using a 2-DE/MS approach.[5] Changes of gut proteins in fourth instar larvae of the Indianmeal moth *Plodia interpunctella* exhibiting resistance to *Bacillus thuringiensis* toxins were examined, and approximately 300 individual proteins with molecular sizes ranging from 15 to 150 kDa and isoelectric points between 4 and 10 in 2-DE gels were identified.[6] In the posterior midgut of fifth instar larvae of *Bombyx mori*, 1100 individual protein spots were identified by using 2-DE gels.[7] Protein profiles of midguts of the trypanosome-susceptible and wild type tsetse flies, *Glossina morsitans morsitans*, were compared using isotope coded affinity tag (ICAT) technique, and a total of 207 proteins were identified in the trypanosome-susceptible flies, including 17 up-regulated proteins and 9 down-regulated proteins.[8] Most of the up-

* To whom correspondence should be addressed. School of Life Sciences, South China Normal University, Guangzhou, China, 510631. Dr. Qili Feng, e-mail qlfeng@scnu.edu.cn; Fax +86-20-85215291. Dr. Ling Li, e-mail liling@scnu.edu.cn; Fax +86-20-85211372.

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regulated proteins were associated with midgut immunity. Nine proteins that were differentially expressed in response to dietary Bowman-Birk inhibitor in the *Drosophila melanogaster* midgut were identified.[9] Using 2-DE/MS/MS *de novo* peptide sequencing major proteins persisting in the midgut lumen were examined in the cotton bollworm *Helicoverpa armigera*.[10]

Spodoptera litura is one of the most damaging insect pests in the tropical and subtropical areas around the world. In the present study, midgut protein profile of *S. litura* sixth instar larvae at the feeding stage was analyzed for the first time by using the shotgun high performance liquid chromatography electrospray ionization mass spectrometry (HPLC−ESI−MS) proteomics approach. A detailed list of midgut proteins involved in different metabolisms are given and discussed.

2. Materials and Methods

2.1. Preparation of Protein Extracts from the *S. litura* Midgut. *Spodoptera litura* (Lepidoptera:Noctuidae) larvae were provided by the Entomology Institute of SUN YAT-SEN University, Guangzhou, China, and reared at 25 °C on artificial diet after egg hatching. Midguts were dissected carefully from the larvae at day 3 after ecdysis into sixth instar stage, when the larvae were actively feeding. Protein extraction procedure was conducted as described in Feng et al.[11] with modification. The midguts were homogenized in homogenization buffer (5 mL/g tissue; 50 mM Tris, 10 mM EDTA, 15% glycerol, 0.005% phenylthiourea, pH 7.8) using a motor-driven Teflon pestle in a 1.5 mL polypropylene microcentrifuge tube. The homogenate was centrifuged at 10 000× *g* for 5 min. The supernatant was collected and recentrifuged again under the same conditions. A mixture of 0.07% β-mercaptoethanol and 10% trichloracetic acid in cold acetone were added to the supernatant, which was then kept at 4 °C for 10 min. The protein extract was then centrifuged at 12 000× *g* and 4 °C for 10 min. The pellet was washed in cold acetone with 0.07% β-mercaptoethanol and centrifuged at 12 000× *g* and 4 °C for 10 min. This washing step was repeated twice. The resultant pellet was resuspended in 10 mL lysis buffer consisting of 7 M urea, 2 M thiourea, 4% (w/v) CHAPS and 40 mM Tris, 1% (w/v) dithiothreitol[12] and then centrifuged at 12 000× *g* and 4 °C for 10 min. The supernatant was stored at −80 °C and total protein concentration was determined using Bradford's method[13] according to the manufacturer's instruction (Invitrogen, Carlsbad, CA).

2.2. SDS-PAGE Separation of the Proteins. One-hundred micrograms of the midgut proteins was denatured at 100 °C for 5 min in an equal volume of 2× protein loading buffer (0.1 M Tris buffer, pH 6.8, 4% SDS, 0.2% β-mercaptoethanol, 40% glycerol, and 0.002% brompenol blue) and subjected to SDS-PAGE in 12.5% acrylamide gels and Tris-glycine-SDS buffer (10 mM Tris, 50 mM glycine, 0.1% SDS, pH 8.0) at 15 mA for 20 min and then 30 mA for 1.5 h in a mini-vertical electrophoresis system. The gels were then stained with Coomassie Brilliant Blue G250 (Invitrogen, Carlsbad, CA).

2.3. In-Gel Trypsin Digestion. The in-gel trypsin digestion of proteins was conducted according to Shevchenko et al.[14] The protein lane of the stained gel was cut into four pieces in equal size (Figure 1), which were destained with 0.2 mL of 100 mM NH_4HCO_3 in 50% acetonitrile for 45 min at 37 °C, followed by excision and dehydration in acetonitrile for 5 min. The gel pieces were then dried in a vacuum centrifuge. A volume of 10 mM dithiotreitol in 100 mM NH_4HCO_3 sufficient to cover the gel pieces was added to treat the proteins at 56 °C for 1 h. After cooling to room temperature, the dithiotreitol solution was

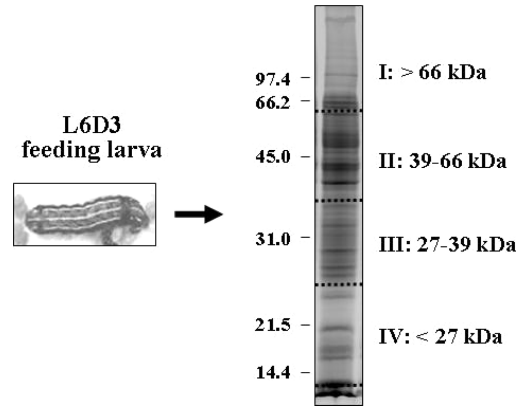

Figure 1. Separation of the midgut proteins by SDS-PAGE. One hundred micrograms of the midgut proteins isolated from 3-day-old 6th instar feeding larvae of *S. litura* were separated on a 12.5% acrylamide SDS-PAGE gel. Four equally spaced sections were excised and subsequently used for shotgun ESI−MS analysis. The portion I contained the proteins larger than 66 kDa; the portion II contained the proteins between 39 and 66 kDa; the portion III contained the proteins between 27 and 39 kDa and the portion IV contained the proteins smaller than 27 kDa.

replaced with the same volume of 55 mM iodoacetamide in 100 mM NH_4HCO_3. After 45 min incubation at room temperature in the dark with occasional vortexing, the gel pieces were washed with 100 μL of 100 mM NH_4HCO_3 for 10 min, dehydrated in 100 μL of acetonitrile, swollen by rehydration in 100 μL of 100 mM NH_4HCO_3, and shrunk again by adding the same volume of acetonitrile. The liquid phase was removed and the gel pieces were dried in a vacuum centrifuge. The gel pieces were swollen in 10 μL of digestion buffer containing 50 mM NH_4HCO_3, 5 mM $CaCl_2$, and 12.5 ng/μL of trypsin in an ice-cold bath. After 45 min, the supernatant was removed and replaced with 10 μL of the same buffer, but without trypsin, to keep the gel pieces wet during enzymic cleavage at 37 °C for overnight. Peptides were extracted by one change of 20 mM NH_4HCO_3 and three changes of 5% formic acid in 50% acetonitrile (20 min for each change) at room temperature.

2.4. HPLC−ESI−MS/MS Shotgun Analysis. Chromatography was performed using a surveyor LC system (Thermo Finnigan, San Jose, CA) on a C18 reverse phase column (RP, 180 μm × 150 mm, BioBasic C18, 5 μm, Thermo Hypersil-Keystone). The pump flow rate was split 1:100 for a column flow rate of 1.5 μL/min. The mobile phase A was 0.1% formic acid in water, and the mobile phase B was 0.1% formic acid in acetonitrile. Separation of the peptides obtained by enzymatic digestion was achieved with a gradient of 2−80% solution B over 60 min. The effluent from the reverse phase column was analyzed by an ESI mass spectrometer (LCQ Deca XP; Thermo Finnigan, San Jose, CA). The microelectrospray interface used a 30 μM metal needle, which was orthogonal to the inlet of the LCQ. The mass spectrometer was set so that one full MS scan was followed by three MS/MS scans on the three most intense ions from the MS spectrum with the following Dynamic Exclusion settings: repeat count, 2; repeat duration, 0.5 min; exclusion duration, 3.0 min.

2.5. Protein Identification and Annotation. Protein identification results were extracted from the SEQUEST out.file with in-house software (BuildSummary). The protein identification and annotation criteria were based on Delta CN (≥0.1) and Xcorr (one charge ≥1.9, two charges ≥2.2, three charges ≥3.75).[15] Insect protein databases in the National Center for

Table 1. Numbers of the Peptides Identified from the Midgut of the Sixth Instar Feeding Larvae of *S. litura* by Shotgun ESI−MS Analysis

	no. of proteins	percentage (%)
Total	2043	100
Unannotated	1201	58.8
No homologue	554	27.1
Hypothetical/putative/predicted	647	31.7
Annotated	842	41.2
Mitochondrial proteins	17	0.8
Ribosomal proteins	84	4.1
Others	741	36.3

Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) were searched for protein identification and annotation. Classifications were performed using Gene Ontology Annotation (GOA; http://www.ebi.ac.uk/goa/) according to the protein accession numbers.

3. Results and Discussion

3.1. Identification and Annotation of the Midgut Proteins. Protein extracts from the midgut of sixth instar actively feeding larvae was separated by SDS-PAGE and the gel was cut into four equal pieces in size for shotgun ESI−MS analysis (Figure 1). The portion I contained the proteins larger than 66 kDa; the portion II contained the proteins between 39 and 66 kDa; the portion III contained the proteins between 27 and 39 kDa and the portion IV contained the proteins smaller than 27 kDa. A total of 2043 peptides were identified by the shotgun ESI−MS analysis, of which 842 (41.2%) peptides were annotated by Blast search in the NCBI insect protein databases and the remaining 1201 (58.8%) peptides could not be annotated (Table 1). Among these unknown (or unannotated) proteins, 647 were either hypothetical, putative or predicted proteins, while the remaining 554 had no any homologues in these databases. Among the 842 annotated proteins, 741 had identities of molecular functions, while 84 were ribosomal proteins and 17 were mitochondrial proteins. Overall, 1489 (72.9% of 2,043) proteins, including hypothetical, putative, predicted and annotated proteins, were found to have their homologues in these insect protein data sets.

3.2. Characterization of the Midgut Protein Profile. Distribution of molecular mass and isoelectric points (p*I*) of the identified proteins was analyzed. Molecular mass ranged between 1.38 kDa (a homologue of vespid chemotactic peptide L from *Vespula lewisii*[16] and 2353 kDa [a predicted protein similar to the isoform C of *B. mori* BmKettin (CG1915-PC)[17]] with 1541 proteins (75.4%) smaller than 100 kDa (Figure 2A). For the annotated proteins, most of them were between 10 and 60 kDa in size (Figure 2B). p*I* of the proteins ranged between 3.8 (a homologue of peritrophic matrix insect intestinal mucin from *Plutella xylostella*[18] and 11.99 (a predicted protein similar to that in *Anopheles gambiae* str. PEST) with the most p*I*s between 5 and 10 (Figure 3).

Classification of the 741 annotated peptides (except mitochondrial and ribosomal proteins) in terms of molecular function, biological process and cellular localization was performed according to the Gene Ontology Annotation (http://www.ebi.ac.uk/goa/). Twelve catalogues of molecular function were clustered (Figure 4A) and the catalytic activity (336 proteins, 45.3% of 741 annotated peptides) and binding activity (251, 33.9%) groups were the most majority. The proteins in the catalytic activity group were further classified into nine subgroups based on their specific functions (Figure 4B). Most proteins are related to hydrolase activity (122 out of 336, 36.3%), oxidoreductase activity (81, 24.1%) and transferase activity (70, 20.8%). High levels of hydrolases, oxidoreductase and transferases indicated that hydrolysis, oxidoreduction and transformation of carbohydrates, lipids and proteins were extremely active in the feeding midgut. More than a half of the binding

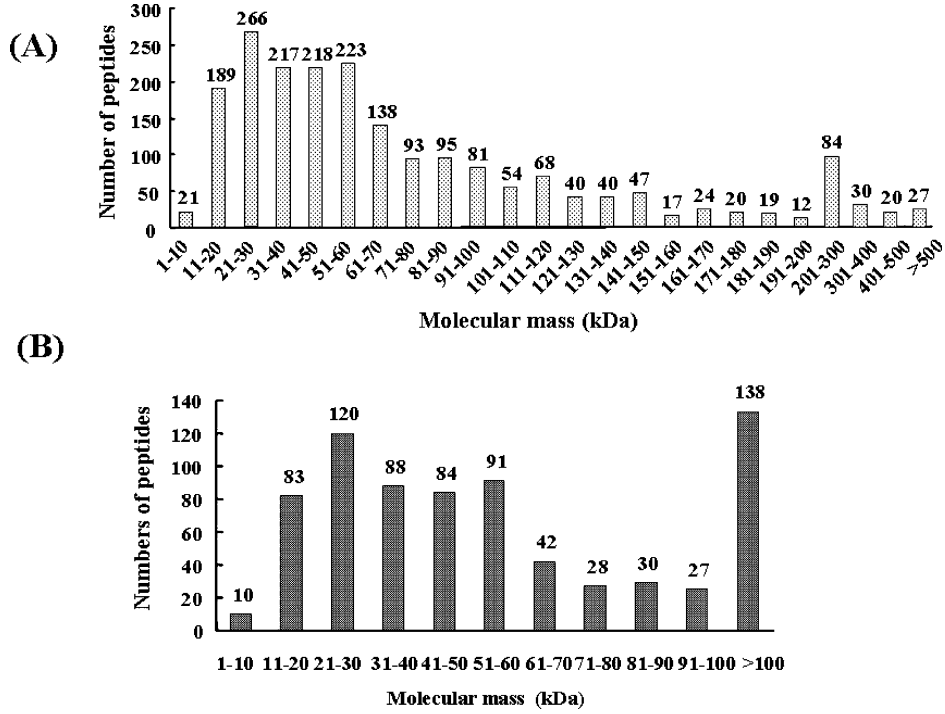

Figure 2. Distributions of molecular mass for all of the proteins (A) and for the annotated proteins (B) identified by the shotgun ESI−MS approach.

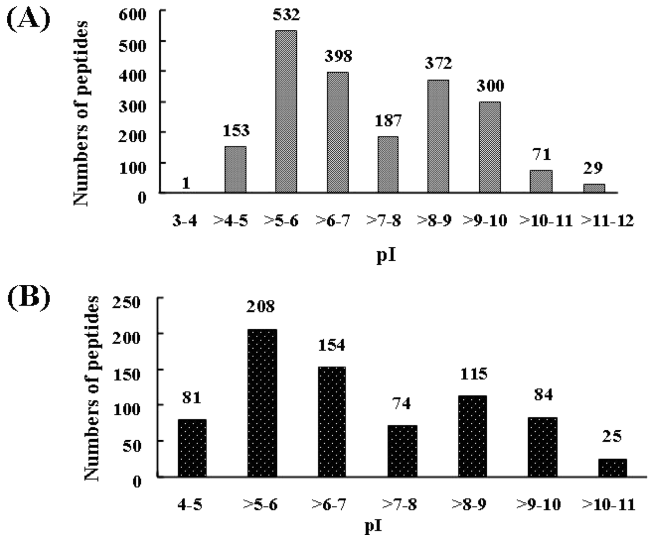

Figure 3. Distribution of isoelectric points (p*I*) for all of the proteins (A) and for the annotated proteins (B) identified by the shotgun ESI−MS approach.

proteins (142 out of 251, 56.6%) were associated with the protein-binding (Figure 4C).

Most (487 out of 741, 65.7%) of the annotated proteins were involved in different cellular processes, particularly in various metabolism processes (320 out of 487 peptides, 65.7%) and cellular component organization and biogenesis (114, 23.4%). Less than 3% (19 proteins) were directly involved in developmental events. This suggests that at the feeding stage, most of physiological and biochemical events occurring in the midgut are involved in the metabolic processes associated with the feeding functions such as food digestion and nutrient absorption, but not associated with tissue formation or degeneration.

Most (490, 66.1%) of the annotated proteins were localized in the cytoplasm, while only 130 (17.5%) and 93 (12.6%) proteins were localized in cell membranes and nuclei, respectively. Although the extraction of the membrane and nuclear proteins is usually more difficult than soluble cytoplasmic proteins and the method of protein isolation in this study may be favor to soluble proteins, a high ration of cytoplasmic proteins to membrane and nuclear proteins indicates that at the feeding stage, the epithelium cells of the *S. litura* midgut was undertaking the active metabolic activities.

3.3. Most Abundant Proteins in the Midgut of Sixth Instar Feeding Larvae. The top 20 most abundant proteins in the midgut of sixth instar feeding larvae are listed in Table 2. Most were the products of the so-called house-keeping genes, such as vascular ATP synthase subunit, tubulin, actin, arginine kinase and heat shock proteins. As these house-keeping proteins are constitutively and highly expressed in cells and provide the basic but essential functions for cell survival and growth, it is therefore not surprised to find that these proteins are the most majority in the epithelium cells of the midgut.[19] This result simply proves that this shotgun strategy is an unbiased approach for protein profile analysis.

The top 20 most abundant nonredundant proteins are listed in Table 3 after excluding the house-keeping proteins listed in Table 2. Three proteins, diazepam-binding inhibitor, apolipophorin, and sterol carrier protein 2/3-oxoacyl-CoA thiolase, were found to be highly expressed in the feeding midgut. Some proteins involved in the transport and metabolisms of proteins and carbohydrates, such as aminopeptidase N (EC3.4.11.2), methionine-rich storage protein, glucose-3-phosphate dehydrogenase (EC1.1.1.49), enoyl-CoA hydratase precursor 1, glucosidase, glutamate dehydrogenase (EC1.4.1.3), fructose 1,6-bisphosphate aldolase (EC4.1.2.13) and triosephosphate isomerase (EC5.3.1.1) also had high expression levels.

3.4. Proteins that are Involved in Different Metabolism Processes. 3.4.1. Carbohydrate Metabolism. Several key enzymes in the carbohydrate metabolism, particularly glycolysis pathway, were identified at relatively high levels, including phosphopyruvate dehydratase (EC4.2.1.11), glucose-3-phosphate dehydrogenase (EC1.1.1.49), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC1.2.1.12), glycerol-3-phosphate dehydrogenase (GPDH, EC1.1.1.8), fructose 1,6-bisphosphate aldolase (FBPA, EC4.1.2.13), isocitrate dehydrogenase (IDH, EC1.1.1.42), acyl-CoA dehydrogenase (EC1.3.99), alcohol dehydrogenase (ADH, EC1.1.1.1), aldehyde dehydrogenase (EC1.2.1.3), and triosephosphate isomerase (TIM, EC5.3.1.1) (Table 4). Phosphopyruvate dehydratase (also called enolase or 2-phospho-D-glycerate hydrolase) is a key glycolytic enzyme responsible for catalyzing the interconversion of 2-phospho-D-glycerate (2-PG) and phosphoenolpyruvate (PEP) between the glycolysis and gluconeogenesis pathways (Figure 5).[20] In vertebrates, three tissue-specific isoenzymes (designated alpha, beta and gamma) of enolase were found and the functional enzyme exists as a dimer of any 2 isoforms. The existence of this essential glycolytic enzyme at high abundance in the midgut of feeding larvae indicates that the glycolysis pathway is very active for the carbohydrate conversion during the feeding stage.

For the glycolysis pathway, three key enzymes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC1.2.1.12), fructose-bisphosphate aldolase (FBPA, EC4.1.2.13), and triosephosphate isomerase (TIM, EC5.3.1.1), were identified (Figure 5). FBPA catalyzes conversion of D-fructose 1,6-bisphosphate into D-glyceraldehyde 3-phosphate, which can subsequently be conversed by GAPDH into 3-phospho-D-glyceroyl phosphate, which is used for gluconeogenesis. TIM catalyzes interconversion between D-glyceraldehyde 3-phosphate and glycerone phosphate. High levels of expression of these enzymes indicate that the pentose phosphate pathway might be essential for carbohydrate utilization in the midgut of *S. litura* feeding larvae.

For the further phosphoenolpyruvate metabolism, several key enzymes were identified (Figure 5), including pyruvate kinase (PK, EC2.7.1.40), D-lactate dehydrogenase (LDH, EC1.1.1.27), pyruvate dehydrogenase (PDH, EC1.2.4.1), dihydrolipoyl dehydrogenase (DLD, EC1.8.1.4), alcohol dehydrogenase (EC1.1.1.1), and aldehyde dehydrogenase (EC1.2.1.3). LDH catalyzes pyruvate synthesis from D-lactate, while PK converses phosphoenolpyruvate into pyruvate, which then enters either the citrate cycle through acetyl-CoA or the amino acids metabolism pathway. PDH catalyzes the pyruvate conversion into dihydroxyethyl-ThPP, 6-*S*-acetyl-dihydrolipoamide and finally acetyl-CoA. DLD catalyzes the interconversion between dihydrolipoamide and lipoamide in the formation of acetyl-CoA. Alcohol dehydrogenase and aldehyde dehydrogenase catalyze the reactions of ethanol to aldehyde and of aldehyde to acetate, respectively. The existence of these enzymes for formation of phosphoenolpyruvate, pyruvate, acetyl-CoA and acetate implies that pyruvate metabolism, therefore glycolysis pathway, is very active in the feeding midgut.

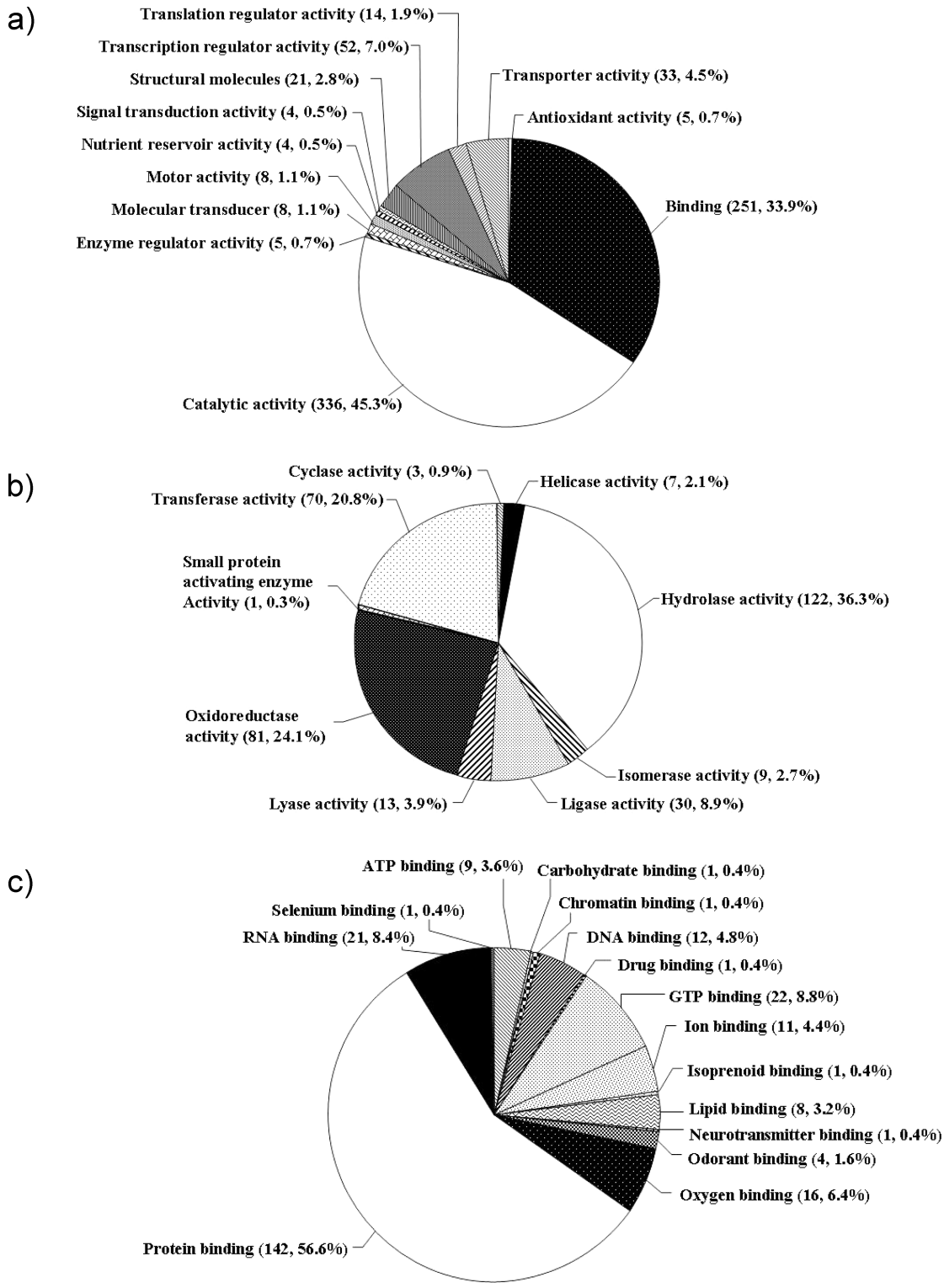

Figure 4. Classification of molecular functions of the annotated midgut proteins of *S. litura* 3-day-old 6th instar feeding larvae (L6D3). (A) Numbers and percentages of the annotated proteins in 12 groups of molecular functions; (B) numbers and percentages of the annotated proteins with catalytic activity; (C) numbers and percentages of the annotated proteins with binding activity.

Other enzymes, including trehalase (EC3.2.1.28), which is involved in the hydrolysis of ingested trehalose, and UTP-glucose-1-phosphate uridylyltransferase (EC2.7.7.9), which catalyzes the formation of UDP-glucose and plays a central role as a glucosyl donor in the glycogen biosynthetic process, were also found in the midgut, implying that different types of carbohydrates such as trehalose, glucose and fructose can be used for sugar sources for carbohydrate metabolism in the *S. litura* midgut. On the other hand, α-amylase, which preferentially hydrolyzes long α-1,4-glucan chains of native starch or glycogens, had a relatively low abundance in the feeding midgut of the insect.

3.4.2. Lipid and Fatty Acid Metabolisms. For lipid and fatty acid metabolisms, most of lipid- and fatty acid-related proteins

Table 2. Top 20 Most Abundant Proteins Identified in the Midgut of Sixth Instar Feeding Larvae of *S. lituraby* Shotgun ESI−MS Analysis

no.	GAN[a] of homologues	protein description	MW	p*I*	species	no. of peptide sequences	no. of unique peptides	cover percent (%)	sequences
1	P31409	Vacuolar ATP synthase subunit B	54549.54	5.25	*D. melanogaster*	134	21	60.41	K.AVVGEEALTPDDLLYLEFLTK.F; K.AVVQVFEGTSGIDAK.N; K.GPPILAEDFLDIQGQPINPWSR.I; K.HVLVILTDM*SSYAEALR.E; K.HVLVILTDMSSYAEALR.E; K.IPIFSAAGLPHNEIAAQICR.Q; K.NFISQGNYENR.T; K.NTLCEFTGDILR.T; K.RIPASILAEFYPR.D; K.TVSGVNGPLVILDEVK.F; K.TVSGVNGPLVILDEVKFPK.F; R.DFISQPR.L; R.GFPGYM*YTDLATIYER.A; R.IPASILAEFYPR.D; R.IYPEEMIQTGISAIDVMNSIAR.G; R.LALTAAEFLAYQCEK.H; R.NGSITQIPILTM*PNDDITHPIPDLTGYITEGQIYVDR.Q; R.QIYPPVNVLPSLSR.L; R.TPVSEDM*LGR.V; R.TVFESLDIGWQLLR.I; R.VFNGSGKPIDK.G
2	P31410	Vacuolar ATP synthase subunit B	54894.03	5.26	*H. virescens*	129	21	59.92	K.AVVGEEALTPDDLLYLEFLTK.F; K.AVVQVFEGTSGIDAK.N; K.FSEIVQLR.L; K.GPPILAEDFLDIQGQPINPWSR.I; K.HVLVILTDM*SSYAEALR.E; K.HVLVILTDMSSYAEALR.E; K.IPIFSAAGLPHNEIAAQICR.Q; K.NFISQGNYENR.T; K.NTLCEFTGDILR.T; K.RIPASILAEFYPR.D; R.DFISQPR.L; R.GFPGYM*YTDLATIYER.A; R.IPASILAEFYPR.D; R.IYPEEMIQTGISAIDVMNSIAR.G; R.LALTAAEFLAYQCEK.H; R.NGSITQIPILTM*PNDDITHPIPDLTGYITEGQIYVDR.Q; R.QIYPPVNVLPSLSR.L; R.SGQVLEVSGTK.A; R.TPVSEDM*LGR.V; R.TVFESLDIGWQLLR.I; R.VFNGSGKPIDK.G
3	P06605	Tubulin alpha-3	49890.44	5.00	*D. melanogaster*	121	11	40.67	K.DVNAAIATIK.T; K.EIVDIVLDR.I; .LADQCTGLQGFLIFHSFGGGTGSGFTSLLM*ER.L; K.TVGGGDDSFNTFFSETGAGK.H; K.VGINYQPPTVVPGGDLAK.V; R.AVCM*LSNTTAIAEAWAR.L; R.FDGALNVDLTEFQTNLVPYPR.I; R.IHFPLVTYAPVISAEK.A; R.LIGQIVSSITASLR.F; R.NLDIERPTYTNLNR.L; R.QLFHPEQLITGK.E
4	AAY81972	Actin 5	41821.9	5.30	*A. aegypti*	119	19	62.77	-.VAIQAVLSLYASGR.T; K.AGFAGDDAPR.A; K.DLYANTVLSGGTTM*YPGIADR.M; K.DSYVGDEAQSK.R; K.DSYVGDEAQSKR.G; K.EITALAPSTM*K.I; K.EITAXAPSTM*K.I K.IKIIAPPER.K; K.IKIXAPPER.K; K.IWHHTFYNELR.V; K.LCYVALDFEQEMATAASSSSLEK.S; K.QEYDESGPSIVHR.K; K.SYELPDGQVITIGNER.F; K.YPIEHGIITNWDDM*EK.I; R.AVFPSIVGR.P; R.AVFPSIVGRPR.H; R.GYSFTTTAER.E; R.HQGVMVGMGQK.D; R.TTGIVLDSGDGVSHTVPIYEGYALPH.-; R.TTGIVLDSGDGVSHTVPIYEGYALPHAILR.L; R.VAPEEHPVLLTEAPLNPK.A
5	XP_966415	Actin-87E isoform 1	41828.9	5.29	*T. castaneum*	107	16	50.27	-.VAIQAVLSLYASGR.T; K.AGFAGDDAPR.A; K.DLYANTVLSGGTTM*YPGIADR.M; K.DSYVGDEAQSK.R; K.DSYVGDEAQSKR.G; K.IKIIAPPER.K; K.IWHHTFYNELR.V K.SYELPDGQVITIGNER.F; K.YPIEHGIITNWDDM*EK.I; R.AVFPSIVGR.P; R.AVFPSIVGRPR.H; R.GYSFTTTAER.E; R.HQGVMVGMGQK.D; R.TTGIVLDSGDGVSHTVPIYEGYALPH.-; R.TTGIVLDSGDGVSHTVPIYEGYALPHAILR.L; R.VAPEEHPILLTEAPLNPK.S
6	P31400	Vacuolar ATP synthase catalytic subunit A	68165.8	5.14	*M. sexta*	103	23	50.73	K.ADFDQLLEDMSAAFR.N; K.ASLAETDKITLEVAK.L; K.DDFLQQNSYSSYDR.F; K.DINELTQSIYIPK.G; K.EILQEEEDLSEIVQLVGK.A; K.LLKDDFLQQNSYSSYDR.F; K.LPANHPLLTGQR.V; K.VGSHITGGDLYGIVHENTLVK.H; K.VKEILQEEEDLSEIVQLVGK.A; K.VTDVVLETEFDGEK.A; K.YSNSDVIIYVGCGER.G; R.DAM*GNVLYQLSSM*K.F; R.DM*GYNVSM*M*ADSTSR.W; R.EASIYTGITISEYFR.D; R.EGSVSIVGAVSPPGGDFSDPVTAATLGIVQVFWGLDK.K; R.GNEM*SEVLR.D; R.HAVESTAQSDNK.I; R.LAEM*PADSGYPAYLGAR.L; R.M*SGSAM*YELVR.V; R.MSGSAMYELVR.V; R.TALVANTSNM*PVAAR.E; R.TALVANTSNMPVAAR.E; R.TGKPLSVELGPGILGSIFDGIQRPLK.D

Table 2. Continued

no.	GAN[a] of homologues	protein description	MW	p*I*	species	no. of peptide sequences	no. of unique peptides	cover percent (%)	sequences
7	EAT47188	Actin	41776.85	5.22	*A. aegypti*	101	17	53.72	-.VAIQAVLSLYASGR.T; K.AGFAGDDAPR.A; K.DLYANTVMSGGTTMYPGIADR.M; K.DSYVGDEAQSK.R; K.DSYVGDEAQSKR.G; K.EEYDESGPGIVHR.K; K.IKIIAPPER.K K.IWHHTFYNELR.V; K.SYELPDGQVITIGNER.F; K.YPIEHGIITNWDDM*EK.I; R.AVFPSIVGR.P; R.AVFPSIVGRPR.H; R.GYSFTTTAER.E; R.HQGVMVGMGQK.D; R.TTGIVLDSGDGVSHTVPIYEGYALPH.-; .TTGIVLDSGDGVSHTVPIYEGYALPHAILR.L; R.VAPEEHPVLLTEAPLNPK.A
8	ABF18092	Actin	41791.86	5.30	*A. aegypti*	98	16	52.13	-.VAIQAVLSLYASGR.T; K.AGFAGDDAPR.A; K.DLYANTVMSGGTTMYPGIADR.M; K.DSYVGDEAQSK.R; K.DSYVGDEAQSKR.G; K.IKIIAPPER.K; K.IWHHTFYNELR.V; K.LCYVALDFEQEMATAASSSSLEK.S; K.SYELPDGQVITIGNER.F; R.AVFPSIVGR.P R.AVFPSIVGRPR.H; R.GYSFTTTAER.E; R.HQGVMVGMGQK.D; R.TTGIVLDSGDGVSHTVPIYEGYALPH.-; R.TTGIVLDSGDGVSHTVPIYEGYALPHAILR.L R.VAPEEHPVLLTEAPLNPK.A
9	XP_625015	Actin	41796.87	5.29	*A. mellifera*	93	15	43.88	-.VAIQAVLSLYASGR.T; K.AGFAGDDAPR.A; K.DSYVGDEAQSK.R; K.DSYVGDEAQSKR.G; K.EITALAPSTM*K.I; K.IKIIAPPER.K; K.IKIXAPPER.K; K.IWHHTFYNELR.V; K.QEYDESGPSIVHR.K; K.SYELPDGQVITIGNER.F; R.AVFPSIVGR.P; R.AVFPSIVGRPR.H; R.GYSFTTTAER.E; R.IAPEEHPVLLTEAPLNPK.A; R.TTGIVLDSGDGVSHTVPIYEGYALPH.-; R.TTGIVLDSGDGVSHTVPIYEGYALPHAILR.L
10	AAS92314	Arginine kinase	28993.77	6.23	*Epicephala* sp. E97AT	88	16	65.10	K.ETQQKLIDDHFLFK.E; K.GTFYPLTGM*SK.E; K.NWGDVETLGNLDPAGEFVVSTR.V; K.TFLVWCNEEDHLR.I; K.VASTLSGLEGELK.G; R.FLQAANACR.F; R.GEHTEAEGGVYDISNK.R; R.GIYHNENK.T; R.GTRGEHTEAEGGVYDISNK.R; R.GTRGEHTEAEGGVYDISNKR.R; R.LGFLTFCPTNLGTTVR.A; R.LISM*QM*GGDLK.Q; R.LVTAVNDIEK.R; R.LVTAVNDIEKR.I; R.RLVTAVNDIEKR.I; R.SM*EGYPFNPCLTEAQYK.E
11	XP_624112	H(+)-transporting ATPase	55144.19	5.41	*A. mellifera*	88	15	43.84	K.AVVQVFEGTSGIDAK.N; K.GPPILAEDFLDIEGQPINPWSR.I; K.HVLVILTDM*SSYAEALR.E; K.HVLVILTDMSSYAEALR.E; K.IPIFSAAGLPHNEIAAQICR.Q; .PIDKGPPILAEDFLDIEGQPINPWSR.I; K.TVSGVNGPLVILDEVK.F; K.TVSGVNGPLVILDEVKFPK.F; R.DFISQPR.L; R.GFPGYM*YTDLATIYER.A; R.LALTAAEFLAYQCEK.H; R.NGSITQIPILTM*PNDDITHPIPDLTGYITEGQIYVDR.Q; R.QIYPPVNVLPSLSR.L; R.TPVSEDM*LGR.V; R.TVFESLDIGWQLLR.I; R.VFNGSGKPIDK.G
12	AAB06239	Heat shock protein 70	71863.22	5.50	*T. ni*	73	20	35.38	K.DAGTISGLNVLR.I; K.FELTGIPPAPR.G; K.ISDSDKQTILDK.C; K.LLQDFFNGK.E; K.M*KETAEAYLGK.T; K.MKETAEAYLGK.T; K.NQVAM*NPNNTIFDAK.R; K.QTQTFTTYSDNQPGVLIQVFEGER.A; K.SQIHDIVLVGGSTR.I; K.TFFPEEVSSM*VLTK.M; K.TFFPEEVSSMVLTK.M; K.TVQNAVITVPAYFNDSQR.Q; K.VEIIANDQGNR.T; R.ARFEELNADLFR.S; R.IINEPTAAAIAYGLDK.K; R.IINEPTAAAIAYGLDKK.G; R.KFEDATVQADM*K.H; R.M*VNHFVQEFK.R; R.MVNHFVQEFK.R; R.STAGDTHLGGEDFDNR.L; R.TTPSYVAFTDTER.L
13	O16109	Vacuolar ATP synthase catalytic subunit A	68599.51	5.44	*A. aegypti*	73	13	25.20	K.ASLAETDKITLEVAK.L; K.EILQEEEDLSEIVQLVGK.A; K.LPANHPLLTGQR.V; K.NFQEFVPTAYKVK.E; K.VKEILQEEEDLSEIVQLVGK.A; K.YSNSDVIIYVGCGER.G; R.DM*GYNVSM*M*ADSTSR.W; R.EASIYTGITISEYFR.D; R.GNEM*SEVLR.D; R.LAEM*PADSGYPAYLGAR.L; R.M*SGSAM*YELVR.V; R.MSGSAMYELVR.V; R.TALVANTSNM*PVAAR.E; R.TALVANTSNMPVAAR.E

Table 2. Continued

no.	GAN[a] of homologues	protein description	MW	p*I*	species	no. of peptide sequences	no. of unique peptides	cover percent (%)	sequences
14	Q9U639	Heat shock 70 kDa protein cognate 4	71431.58	5.33	*M. sexta*	72	20	36.96	K.DAGTISGLNVLR.I; K.FELTGIPPAPR.G; K.ISDSDKQTILDK.C K.LLQDFFNGK.E; K.M*KETAEAYLGK.T; K.MKETAEAYLGK.T K.NQVAM*NPNNTIFDAK.R; K.QTQTFTTYSDNQPGVLIQVFEGER.A; K.SINPDEAVAYGAAVQAAILHGDK.S; K.SQIHDIVLVGGSTR.I; K.TFFPEEVSSM*VLTK.M; K.TFFPEEVSSMVLTK.M; K.TVQNAVITVPAYFNDSQR.Q; K.VEIIANDQGNR.T; R.ARFEELNADLFR.S; R.IINEPTAAAIAYGLDK.K; R.IINEPTAAAIAYGLDKK.G; R.KFEDATVQADM*K.H; R.M*VNHFVQEFK.R; R.MVNHFVQEFK.R; R.STAGDTHLGGEDFDNR.L
15	BAB92074	Heat shock cognate protein	71175.3	5.33	*B. mori*	70	20	36.98	K.FELTGIPPAPR.G; K.ISDSDKQTILDK.C; K.LLQDFFNGK.E; K.M*KETAEAYLGK.T; K.MKETAEAYLGK.T; K.NQVAM*NPNNTIFDAK.R; K.QTQTFTTYSDNQPGVLIQVFEGER.A; K.SINPDEAVAYGAAVQAAILHGDK.S; K.TFFPEEVSSM*VLTK.M; K.TFFPEEVSSMVLTK.M; K.TVQNAVITVPAYFNDSQR.Q; K.VEIIANDQGNR.T; R.ARFEELNADLFR.S; R.IINEPTAAAIAYGLDK.K; R.IINEPTAAAIAYGLDKK.G; R.KFEDATVQADM*K.H; R.M*VNHFVQEFK.R; R.MVNHFVQEFK.R; R.STAGDTHLGGEDFDNR.L; R.TTPSYVAFTDTER.L
16	AAV91465	Heat shock protein 4	71602.76	5.33	*L. obliqua*	68	19	35.47	K.DAGTISGLNVLR.I; K.FELTGIPPAPR.G; K.LLQDFFNGK.E; K.M*KETAEAYLGK.T; K.MKETAEAYLGK.T; K.NQVAM*NPNNTIFDAK.R; K.QTQTFTTYSDNQPGVLIQVFEGER.A; K.SINPDEAVAYGAAVQAAILHGDK.S; K.SQIHDIVLVGGSTR.I; K.TFFPEEVSSM*VLTK.M; K.TFFPEEVSSMVLTK.M; K.TVQNAVITVPAYFNDSQR.Q; K.VEIIANDQGNR.T; R.ARFEELNADLFR.S; R.IINEPTAAAIAYGLDK.K; R.IINEPTAAAIAYGLDKK.G; R.KFEDATVQADM*K.H; R.STAGDTHLGGEDFDNR.L; R.TTPSYVAFTDTER.L
17	BAB86853	Beta-tubulin	50214.47	4.75	*B. mori*	67	14	43.40	K.EVDEQM*LNIQNK.N; K.EVDEQMLNIQNK.N; K.FWEIISDEHGIDPTGAYHGDSDLQLER.I; K.GHYTEGAELVDSVLDVVR.K; K.IAVNM*VPFPR.L; K.IAVNMVPFPR.L; K.MAATFIGNSTAIQELFK.R; R.AILVDLEPGTM*DSVR.S; R.ALTVPELTQQM*FDAK.N; R.FPGQLNADLR.K; R.IM*NTYSVVPSPK.V; R.IMNTYSVVPSPK.V; R.INVYYNEASGGK.Y; R.ISEQFTAM*FR.R; R.ISEQFTAMFR.R; R.KIAVNM*VPFPR.L; R.SGPFGQIFRPDNFVFGQSGAGNNWAK.G; R.YLTVAAIFR.G
18	AAS92300	Arginine kinase	28863.63	6.08	*Epicephala* sp. E90AT	65	14	57.25	K.ETQQKLIDDHFLFK.E; K.GTFYPLTGM*SK.E; K.NWGDVETLGNLDPAGEFVVSTR.V; K.TFLVWCNEEDHLR.I; K.VASTLSGLEAELK.G; R.FLQAANACR.F; R.GIYHNENK.T; R.LGFLTFCPTNLGTTVR.A; R.LISM*QM*GGDLK.Q; R.LVTAVNDIEK.R; R.LVTAVNDIEKR.I; R.RLVTAVNDIEKR.I; R.SM*EGYPFNPCLTEAQYK.E
19	Q24560	Tubulin beta-1 chain	50147.37	4.76	*D. melanogaster*	65	12	33.56	K.EVDEQM*LNIQNK.N; K.EVDEQMLNIQNK.N; K.GHYTEGAELVDSVLDVVR.K; K.IAVNM*VPFPR.L; K.IAVNMVPFPR.L; R.ALTVPELTQQM*FDAK.N; R.AVLVDLEPGTM*DSVR.S; R.AVLVDLEPGTMDSVR.S; R.FPGQLNADLR.K; R.IM*NTYSVVPSPK.V; R.IMNTYSVVPSPK.V; R.INVYYNEASGGK.Y; R.ISEQFTAM*FR.R; R.ISEQFTAMFR.R; R.KIAVNM*VPFPR.L; R.SGPFGQIFRPDNFVFGQSGAGNNWAK.G; R.YLTVAAIFR.G
20	Q95PM9	Arginine kinase	39879.37	6.24	*P. interpunctella*	59	13	42.54	K.EM*YDGIAELIK.I; K.ETQQKLIDDHFLFK.E; K.NWGDVETLGNLDPAGEFVVSTR.V; K.TFLVWCNEEDHLR.I; R.FLQAANACR.F; R.GEHTEAEGGVYDISNK.R; R.GIYHNENK.T; R.GTRGEHTEAEGGVYDISNK.R; R.GTRGEHTEAEGGVYDISNKR.R; R.LGFLTFCPTNLGTTVR.A; R.LISM*QM*GGDLK.Q; R.M*GLTEYEAVK.E; R.SM*EGYPFNPCLTEAQYK.E

[a] GAN stands for GenBank Accession Numers in all of the tables.

Table 3. Top 20 Most Abundant Nonredundant Proteins Identified in the Midgut of Sixth Instar Feeding Larvae of *S. lituraby* Shotgun ESI−MS Analysis

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
1	AAR37334	Diazepam-binding inhibitor	9823.28	8.86	*H. armigera*	45	6	60.00	K.AKFEAWSK.Q; K.APGFLDLK.G; K.FEAWSK.Q; K.QATVGDSDPSKAPGFLDLK.G; K.SLPSDADLLELYALFK.Q; K.VEKLIASIGLQ.-
2	O77248	Apolipophorin-3 precursor	20649.27	7.03	*S. litura*	41	9	53.19	K.AIKDGSDSVLQQLSALSSSLQSAM*TDANAK.A; K.DGSDSVLQQLSALSSSLQSAM*TDANAK.A; K.DGSDSVLQQLSALSSSLQSAMTDANAK.A; K.EVASNVEETNEK.L; K.HVEEVQK.K; K.LKEAYENFSK.H; K.LQAAVQNTAQEVQK.L; K.TFSEQLNSIANSK.N; R.DAPPANTLLQDIEK.H
3	AAO32817	ADP/ATP translocase	32891.13	9.88	*B. mori*	28	11	36.67	K.DFLAGGISAAVSK.T; K.EQGLLSFWR.G; K.GIVDAFVR.I; K.LLLQVQHVSK.Q; K.SDGIIGLYR.G; R.ASYFGFYDTAR.G; R.GNFANVIR.Y; R.GTGGAFVLVLYDEIK.K; R.LAADVGKGDGQR.E; R.YFPTQALNFAFK.D; R.YKGIVDAFVR.I
4	AAS79891	GST1	23939.4	6.20	*S. litura*	26	6	48.39	K.EQTDKLNSAYEILDK.F; K.LTAWFNTIQQEDWYK.K; K.NPQHTVPLLEDGDFYVADSHAINTYLASK.Y; K.YGGAQSAQLYPTDLQVR.A; R.GDITSPTKEQTDK.L; R.LYFDISAIAGNSGAIVSALLR.G
5	CAB55605	Arylphorin subunit	84112.88	6.70	*S. litura*	21	10	17.05	K.DLHQYSYEIIAR.H; K.DYDIEANIQNYSNK.Q; K.FYELDWFVQK.L; K.QAVEEFLLLYR.T; K.SDVASDAVFK.I; K.TFFQFLQK.A; K.VPYDM*SVQPDNM*PR.R; K.YTFM*PSALDFYQTSLR.D; R.DEAIALFHVLYYAK.D; R.SNDYNLHNEK.N
6	ABB90022	Glucose-3-phosphate dehydrogenase	33604.26	7.17	*C. meadii*	21	6	31.33	K.ASAHIEGGAK.K; K.GAKVVAINDPFIGLDYM*VYLFK.Y; K.GAKVVAINDPFIGLDYMVYLFK.Y; K.VIHDNFEIVEGLM*TTVHATTATQK.T; K.VIHDNFEIVEGLMTTVHATTATQK.T; K.VISNASCTTNCLAPLAK.V; R.GAQQNIIPAATGAAK.A; R.LGKPASYDAIK.Q
7	ABD36107	Enoyl-CoA hydratase precursor 1	31853.56	8.44	*B. mori*	19	5	17.91	K.AFAAGADIK.E; K.FGQPEINIGTIPGAGGTQR.L; K.LLEETIK.L; K.NVGLIQLNRPK.A; K.SGLQFEK.S
8	AAT72922	Sterol carrier protein 2/ 3-oxoacyl-coa thiolase (lipid)	57450.85	8.21	*S. littoralis*	18	8	19.25	K.EAVLAALADAR.I; K.FIDAGDNTYGGR.V; K.GHPLGATGLAQCAELVWQLR.G; K.ILEEAM*ANDTDNLIEK.V; K.ILEEAMANDTDNLIEK.V; K.KYGTTELHLAK.I; R.EYTVEEVLNSR.R; R.LYQNTGVSPK.Q; R.VVVNPSGGLIAK.G
9	Q9V3P0	Peroxiredoxin 1	21737.93	5.52	*D. melanogaster*	18	4	22.16	R.DYGVLDEETGIPFR.G; R.GLFIIDDK.Q; R.LVQAFQYTDK.H; R.QITVNDLPVGR.S

Table 3. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
10	AAR15420	Thiol peroxiredoxin	21916.03	6.09	*B. mori*	17	6	32.31	K.QGGLGPM*NIPLISDK.S; R.DYGVLDEETGIPFR.G; R.GLFIIDDK.Q; R.KIGCEVLGASTDSHFTHLAWINTPR.K; R.KQGGLGPM*NIPLISDK.S; R.KQGGLGPMNIPLISDK.S
11	EAT36327	Chaperonin-60kd	60793.61	5.47	*A. aegypti*	15	4	10.28	K.IGLQVAAVK.A; K.LVQDVANNTNEEAGDGTTTATVLAR.A; K.VEFQDALVLFSEK.K; R.NVIIEQSWGSPK.I;
12	AAP13852	Glucosidase	55596.07	4.80	*B. mori*	14	4	13.03	K.DTGSITSLEVGGDSASEWLR.V; K.SENVWDR.L; K.SSYNDPPIYITENGFSDRGTLQDYGR.I; R.LEQFDDYWIQR.I
13	ABD36303	Glutamate dehydrogenase	61397.42	8.36	*B. mori*	13	7	16.79	K.AYEGENM*LYEK.C; K.CACVDVPFGGAK.A; K.FNLGLDLR.T; K.GFIGPGVDVPAPDM*GTGER.E; K.IIAEAANGPTTPAADK.I; R.ESNYHLLESVQESLER.R; R.IPVTPSESFQK.R
14	ABF51427	NADP-dependent oxidoreductase	36784.2	6.90	*B. mori*	13	1	6.87	K.AGETVVVTGAAGAVGSLVGQIAK.I
15	BAD12426	Fructose 1,6-bisphosphate aldolase	39708.37	7.59	*A. yamamai*	12	6	21.15	K.GILAADESTGTMGK.R; K.VTEVVLAAVYK.A; R.IVPIVEPEVLPDGEHDLDR.A; R.KIAEAIVAPGK.G; R.LQDIGVENTEENR.R; R.YASICQSQR.I
16	AAC24317	Cellular retinoic acid binding protein	14770.83	5.65	*M. sexta*	10	4	25.00	K.AANAVTPTVELR.K; K.AIGVGLITR.K; K.SVCTFEGNTLK.Q; R.KAANAVTPTVELR.K
17	CAB55604	Methionine-rich storage protein	88965.34	9.12	*S. litura*	9	7	13.45	K.AANDPVLM*NYYGIK.V; K.AANDPVLMNYYGIK.V; K.DNM*VNFDIK.M; K.LLNHILQPTIYDDVR.E; R.GEVFVHTNELHIIQAVK.V; R.LGGFPLQM*YVIISPVK.T; R.M*VLGGM*GLVSDDAK.F; R.WSVCFDTM*PLGFPFDR.K
18	AAK69605	Aminopeptidase N	108505.87	5.53	*S. litura*	9	6	10.29	K.AIAEDHTFLSDFPNINFGNVFDSWVQNR.G; K.TLGFEVLDFLR.S; K.VNLENIDLEGAR.F; R.AQIVNDVLHFIR.S; R.EAYLLYDPANTNLVNK.I; R.SETDYYVWNGALTQLDWIR.R
19	ABD36156	Triosephosphate isomerase	26874.64	4.98	*B. mori*	9	4	20.58	K.AIGSGSEGAQQSLK.E; R.GVNTFSPEGR.L; R.LFQVEYAIEAIK.L; R.PFGVAVMFAGIDEK.G
20	ABD36319	Proteasome zeta subunit	35462.92	6.85	*B. mori*	9	4	16.01	K.VLVVGNPANTNALICSK.Y; R.IFKEQGQALDK.V; R.KDLLAANVR.I; R.WVSM*GVVSDGSYGTPR.D

Table 4. Proteins Involved in Carbohydrate Transport and Metabolism in the Midgut of Sixth Instar Feeding Larvae of *S. lituraby* Shotgun ESI−MS Analysis

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
1	EAT43123	2-hydroxyacid dehydrogenase	46960.83	6.68	*A. aegypti*	1	1	2.52	R.IGSGVDNIDVK.A
2	ABD36222	2-hydroxyphytanoyl-CoA lyase	64844.86	7.11	*B. mori*	2	1	1.69	K.AERPLIIVGK.G
3	AAF70499	3-dehydroecdysone 3 alpha-reductase	26079.02	5.82	*S. littoralis*	4	3	14.86	K.EGASVAFVGR.N; K.GSIVNVSSILSTIVR.I; K.LDVLVNNAGILR.F
4	ABF51362	3-hydroxyacyl-CoA dehydrogenase	26931.12	8.55	*B. mori*	1	1	4.71	K.AGVVGM*TLPLAR.D
5	ABF71566	Acyl-coenzyme A dehydrogenase	47080.19	7.07	*B. mori*	13	4	14.69	K.CIDFMGGVGFTR.D; K.IGTIYEGTSNM*QLQTIAK.L; K.IGTIYEGTSNMQLQTIAK.L; K.YAAGFLNEGR.I; R.VDPAVAAYVDIHNTLVNSLFM*K.L; R.VDPAVAAYVDIHNTLVNSLFMK.L
6	ABF51211	Alcohol dehydrogenase	40033.17	6.47	*B. mori*	7	2	6.65	K.FGVNEFVNPK.D; R.IIGVDINPDKFEVAK.K;
7	ABF51508	Alcohol dehydrogenase	33423.56	9.41	*B. mori*	1	1	5.94	K.NMFLPFFLGFGGPIGDGK.Q
8	AAR09963	Alcohol dehydrogenase	11741.98	4.44	*D. yakuba*	1	1	12.50	K.NGFISHEEFSGPK.H
9	XP_396487	Aldehyde dehydrogenase	414742.62	5.80	*A. mellifera*	1	1	0.41	K.M*EFSITEQQVPMLLR.L
10	NP_609285	Aldehyde dehydrogenase	57018.95	6.37	*D. melanogaster*	2	2	5.38	K.RVTLELGGK.S; R.ANNSEYGLAAAVFTKDLDK.A
11	EAT33638	Aldehyde dehydrogenase	53053.04	5.47	*A. aegypti*	7	3	5.32	K.EEIFGPVQSIIK.F; K.VAFTGSVETGR.L; R.IAREEIFGPVQSILK.F
12	EAT45278	Alpha-amylase	68470.4	5.27	*A. aegypti*	1	1	3.50	K.DSDGDGIGDLNGITSKLSYLK.E
13	AAV84202	Alpha-L-fucosidase	32281.04	5.56	*C. sonorensis*	1	1	5.51	K.SSIEVNPNVIFLPTK.N
14	AAC06038	Beta-glucosidase precursor	58137.54	4.79	*S. frugiperda*	3	2	4.32	K.M*FITFNEPR.E; K.MFITFNEPR.E; R.FGLYEVDFSDPAR.T
15	EAT42323	Brain Chitinase and chia	44804.59	5.78	*A. aegypti*	1	1	4.80	K.LLRTINNAIVAAEDEIHNS.-
16	EAT38418	Carboxylase	130633.97	6.43	*A. aegypti*	1	1	0.93	R.EANLLLGDIIK.V
17	ABD36319	Cytosolic malate dehydrogenase	35462.92	6.85	*B. mori*	9	4	16.01	K.VLVVGNPANTNALICSK.Y; R.IFKEQGQALDK.V; R.KDLLAANVR.I; R.WVSM*GVVSDGSYGTPR.D
18	O18480	Dihydrolipoamide dehydrogenase	53082.37	8.86	*M. sexta*	2	2	10.26	K.ATDVILGTHIIGPGGGELINEAVLAQEYGAAAEDVAR.V; R.EANLAAYCGKPINF.-
19	AAU95200	Enolase	46692.18	5.92	*O. nigricans*	29	3	9.01	R.GNPTVEVDLVTELGLFR.A; R.SGETEDTFIADLVVGLSTGQIK.T
20	EAL33448	Enolase	116718.76	7.83	*D. pseudoobscura*	1	1	1.40	R.AEDRLM*YSILIYLSR.A
21	ABD36107	Enoyl-CoA hydratase precursor 1	31853.56	8.44	*B. mori*	19	5	17.91	K.AFAAGADIK.E; K.FGQPEINIGTIPGAGGTQR.L; K.LLEETIK.L; K.NVGLIQLNRPK.A; K.SGLQFEK.S
22	BAD12426	Fructose 1,6-bisphosphate aldolase	39708.37	7.59	*A. yamamai*	12	6	21.15	K.GILAADESTGTMGK.R; K.VTEVVLAAVYK.A; R.IVPIVEPEVLPDGEHDLDR.A; R.KIAEAIVAPGK.G; R.LQDIGVENTEENR.R; R.YASICQSQR.I
23	EAT42704	Fructose-bisphosphate aldolase	39119.67	8.02	*A. aegypti*	3	1	2.75	R.IAKAIVAPGK.G
24	EAT44642	Glucose dehydrogenase	68893.4	9.27	*A. aegypti*	2	1	1.61	K.ILM*LSGIGPK.K
25	EAL29916	Glucose dehydrogenase	16746.81	4.57	*D. pseudoobscura*	1	1	7.84	R.ATMEELHKSQQK.Q
26	NP_524684	Glucose dehydrogenase	18456.27	4.68	*D. melanogaster*	1	1	8.98	K.FTCDM*FHPNIFADGR.V
27	ABB90022	Glucose-3-phosphate dehydrogenase	33604.26	7.17	*C. meadii*	21	6	31.33	K.ASAHIEGGAK.K; K.GAKVVAINDPFIGLDYM*VYLFK.Y; K.GAKVVAINDPFIGLDYMVYLFK.Y; .VIHDNFEIVEGLM*TTVHATTATQK.T; K.VIHDNFEIVEGLMTTVHATTATQK.T; K.VISNASCTTNCLAPLAK.V; R.GAQQNIIPAATGAAK.A; R.LGKPASYDAIK.Q
28	AAP13852	Glucosidase	55596.07	4.80	*B. mori*	14	4	13.03	K.DTGSITSLEVGGDSASEWLR.V; K.SENVWDR.L; K.SSYNDPPIYITENGFSDRGTLQDYGR.I; R.LEQFDDYWIQR.I
29	EAT35441	Glucosidase II beta subunit	63039.28	4.52	*A. aegypti*	1	1	2.17	K.M*GNQLRTEMSQR.G
30	EAT47339	Glucosyl/glucuronosyl transferases	58560.18	9.12	*A. aegypti*	1	1	2.86	R.PYLPNIVEVGGLQIK.A
31	ABD36303	Glutamate dehydrogenase	61397.42	8.36	*B. mori*	13	8	16.79	K.AYEGENM*LYEK.C; K.CACVDVPFGGAK.A; K.FNLGLDLR.T; K.GFIGPGVDVPAPDM*GTGER.E; K.IIAEAANGPTTPAADK.I; R.ESNYHLLESVQESLER.R; R.IPVTPSESFQK.R
32	AAT01075	Glyceraldehyde 3-phosphate dehydrogenase	35476.5	8.30	*H. coagulata*	15	4	21.69	K.VIDILK.A; K.VIHDNFEIVEGLM*TTVHATTATQK.T; K.VIHDNFEIVEGLMTTVHATTATQK.T; K.VIISAPSADAPM*FVVGVNLDAYDPSYK.V; R.GAQQNIIPAATGAAK.A
33	BAD38675	Glycerol-3-phosphate dehydrogenase-2	38684.94	5.62	*B. mori*	12	4	15.01	K.DADLLIFVVPHQFVR.T; K.FPLFTAVFR.I; K.FVDVFYPGSK.L; K.GFDIAEGGGIDLISHIITR.C
34	BAE96011	Glycerol-3-phosphate dehydrogenase	35428.41	7.7	*B. mori*	34	7	28.01	K.ASAHIEGGAK.K; K.GAKVVAINDPFIGLDYM*VYLFK.Y; K.GAKVVAINDPFIGLDYMVYLFK.Y; K.VIDILK.A; K.VIHDNFEIVEGLM*TTVHATTATQK.T; K.VISNASCTTNCLAPLAK.V; R.VPVANVSVVDLTVR.L

Table 4. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
35	AAY41178	Glycerol-3-phosphate dehydrogenase	35662.69	6.96	*G. morsitans morsitans*	11	3	13.21	K.LISWYDNEFGYSNR.V; K.VIDILK.A; K.VIHDNFEIVEGLM*TTVHATTATQK.T; K.VIHDNFEIVEGLMTTVHATTATQK.T
36	CAD33827	Glycerol-3-phosphate dehydrogenase	35468.4	6.54	*P. xylostella*	18	5	24.10	K.ASAHIEGGAK.K; K.LISWYDNEYGYSNR.V; K.VIHDNFEIVEGLM*TTVHATTATQK.T; K.VIHDNFEIVEGLMTTVHATTATQK.T; K.VISNASCTTNCLAPLAK.V; R.GAQQNIIPAATGAAK.A;
37	EAT32291	Hydroxyacyl dehydrogenase	38416.14	8.66	*A. aegypti*	1	1	3.80	R.TVSINLIGSFNMIR.L
38	ABD36131	Hydroxyacyl-coenzyme A dehydrogenase	82141.33	9.38	*B. mori*	1	1	1.57	R.FVNEAVLSLEEK.I
39	ABD36133	Hydroxymethylglutaryl-CoA lyase isoform 2	30625.28	8.86	*B. mori*	2	2	10.79	K.TVDSSISGLGGCPYAR.G; R.VPGVNYPVLVPNLK.G
40	Q9W2Q7	Inositol-pentakisphosphate 2-kinase	67446.89	9.16	*D. melanogaster*	1	1	2.58	R.AAAATATTQRYTKVAR.V
41	NP_788476	Isocitrate dehydrogenase	50431.75	7.59	*D. melanogaster*	14	6	13.56	K.AGPVVDVLGDEM*TR.I; K.ETSTNPIASIFAWTR.G; K.SEGGFVWACK.N; K.TVEAEAAHGTVTR.H; R.NILGGTVFR.E
42	ABD36136	Isocitrate dehydrogenase	46176.05	6.24	*B. mori*	13	6	13.24	K.ETSTNPIASIFAWTR.G; K.SEGGFVWACK.N; K.TVEAEAAHGTVTR.H; R.IIWDLIK.E; R.NILGGTVFR.E
43	EAT35353	Mannosyl-oligosaccharide glucosidase	84443.63	8.87	*A. aegypti*	1	1	1.24	K.LDLVVESIR.F
44	EAT35463	O-linked N-acetylglucosamine transferase	32004.34	5.71	*A. aegypti*	2	1	7.02	K.AVALDPNFLDAYINLGNVLK.E
45	EAT43202	Phosphoglycerate dehydrogenase	35344.88	8.08	*A. aegypti*	1	1	2.71	K.TLAILGLGR.I
46	AAO32154	Phosphoenolpyruvate carboxykinase	23082.2	5.46	*C. grandidieri*	1	1	6.70	K.M*XETKTPAAHPNSR.F
47	XP_562547	Phosphoenolpyruvate carboxykinase	27425.8	10.03	*A. gambiae* str. PEST	1	1	6.67	K.M*YDALKEVVEKLPSHK.V
48	AAF32488	Phosphoenolpyruvate carboxykinase	19898.54	5.53	*G. fabri*	2	1	6.63	R.SEATAAAEHAGK.V
49	AAL58080	Phosphoglycerate kinase	44057.63	8.21	*A. aegypti*	1	1	5.30	K.VLNNM*EIGRSLFDEEGSKIVQK.L
50	EAT43251	Pyruvate dehydrogenase	45901.15	8.61	*A. aegypti*	2	1	3.32	R.YSGHSM*SDPGTSYR.S
51	BAD01636	Pyruvate kinase	43580.99	5.34	*B. mori*	3	2	7.20	K.NITNVVKPGNR.I; R.KGVNLPGIPVDLPAVSEK.D
52	ABD36166	Short-chain dehydrogenease/ reductase 2	27639.57	7.63	*B. mori*	3	2	5.79	K.GNIVNVSSVTGLR.S; K.TKGNIVNVSSVTGLR.S
53	AAD05302	SN-glycerol-3-phosphate dehydrogenase isoform	38165.62	6.67	*L. migratoria*	1	1	4.29	K.VCIIGSGNWGSAIAK.I
54	EAT37543	Sugar transporter	52181.00	5.59	*A. aegypti*	2	1	2.50	R.KNDAIQSLQFLR.G
55	EAT38745	Transaldolase	36991.64	6.18	*A. aegypti*	1	1	3.60	K.LASTWEGIQAAR.V
56	ABD36172	Transketolase	67384.01	6.40	*B. mori*	4	3	6.11	K.LDNLVVIFDVNR.L; R.AVELAANTR.G; R.TSRPNTAILYPNDEVFK.V
57	ABE27189	Trehalase	67210.2	4.72	*S. frugiperda*	3	3	4.77	K.AATYGQLAQQWR.D; R.EFAKNINDIWPLLAR.K; R.KAATYGQLAQQWR.D
58	CAA40804	Triosephosphate isomerase	26579.47	6.00	*D. melanogaster*	7	4	21.86	K.DIGADWVILGHSER.R; K.TATPDQAQEVHASLR.Q; K.VIACIGETLEER.E; R.IQYGGSVTAANAK.-

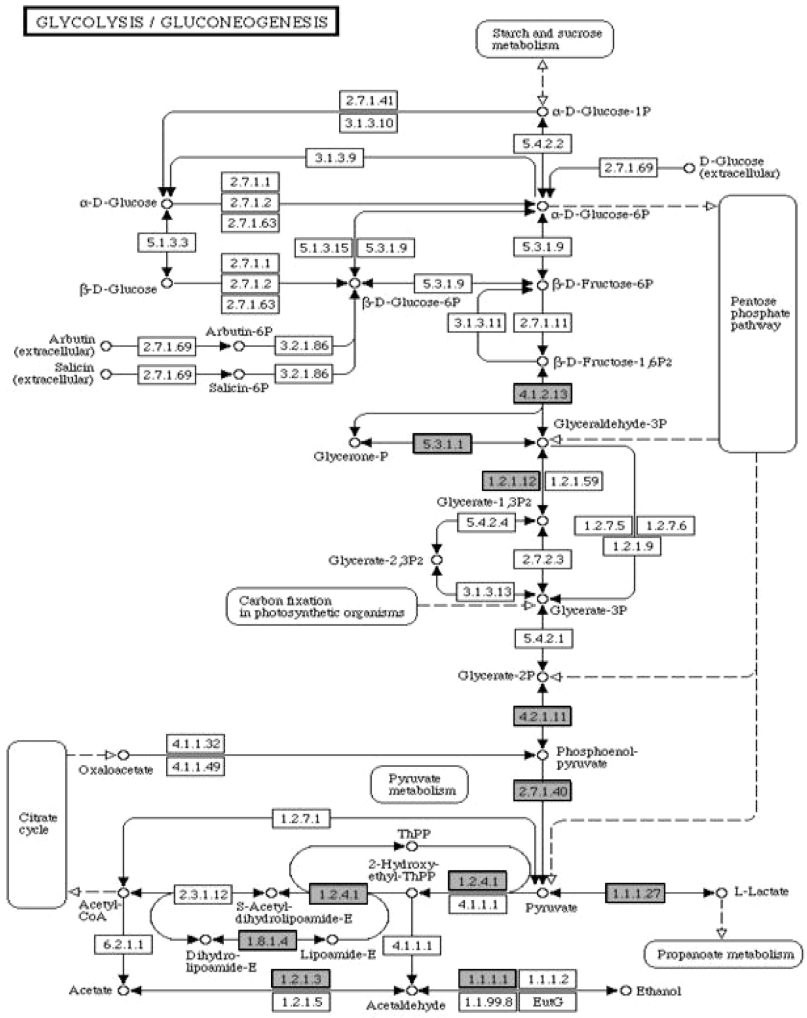

Figure 5. Glycolysis and gluconeogenesis pathways constructed by KEGG bioinformatics resource developed by the Kanehisa Laboratories in the Bioinformatics Center of Kyoto University and the Human Genome Center of the University of Tokyo (http://www.kegg.jp/). The enzymes that were identified in this study are labeled with gray background. EC4.2.1.11, phosphopyruvate dehydratase; EC1.2.1.12, glyceraldehyde-3-phosphate dehydrogenase; EC4.1.2.13, fructose-bisphosphate aldolase; EC5.3.1.1, triose-phosphate isomerase; EC2.7.1.40, pyruvate kinase; EC1.2.4.1, pyruvate dehydrogenase; EC1.8.1.4, dihydrolipoyl dehydrogenase; EC1.1.1.1, alcohol dehydrogenase; EC1.2.1.3, aldehyde dehydrogenase; EC1.1.1.27, L-lactate dehydrogenase.

were generally in low abundance, while the expression levels of diazepam-binding inhibitor, apolipophorin-3 precursor, and sterol carrier protein 2/3-oxoacyl-CoA thiolase were much higher than the others (Table 5). Diazepam-binding inhibitor (DBI) or acyl-CoA-binding protein (ACBP) is a small and highly conserved multifunctional protein involved in regulation of gamma-aminobutyric acid (GABA) receptor activity, synthesis and transport of medium-chain acyl-CoA-ester, synthesis of steroid hormones, and secretion of glucose-induced insulin.[21] In *H. armigera* this protein is found to regulate biosynthesis of ecdysteroids in the prothoracic gland.[22] This protein is also found to express predominantly in the midgut columnar cells of *H. armigera* and to be stimulated by a high juvenile hormone titer and increased along with feeding at 12 h postecdysis, suggesting that it is probably associated with nutrition absorption.[23] This protein also plays a significant role in the production of sex pheromones regulated by the pheromone biosynthesis activating neuropeptide (PBAN) in *B. mori*.[24] In this study, DBI/ACBP was found to express at the highest abundance in the lipid metabolism catalog, implicating that this protein may play an unidentified role in the feeding process.

Apolipophorin 3 is a lipid carrier protein in the hemolymph of insects. It helps loading diacylglycerol onto the hemolymph lipoprotein, lipophorin, increasing its lipid carrying capacity and therefore plays a critical role in the transport of lipids.[25] In *Galleria mellonella*, the level of apolipophorin-3 reached a maximum in the hemolymph at the end of the feeding phase of the seventh instar larvae and declined to a background level in the pupal and the adult stages.[26] This is consistent with the result observed in this study.

Sterol carrier protein 2/3-oxoacyl-CoA thiolase (SCPx) belongs to a well-characterized SCP-2 gene family. SCP-2 is present in both vertebrates and invertebrates and involved in intracellular sterol/lipid transfer, synthesis and metabolism of steroids and fatty acids.[27] In insects, cholesterol is required for cellular membranes and ecdysteroid biosynthesis.[28] Cholesterol, which is converted from phytosterols, is a precursor of ecdysteroids, which are synthesized in the prothoracic glands.[28] However, insects can not synthesize cholesterol via *de novo* biosynthesis pathway using simple molecules because they lack at least two key enzymes, squalene monooxygenase and lanosterol synthase, therefore insects must uptake cholesterol

Table 5. Proteins Involved in Lipid and Fatty Acid Transport and Metabolism in the Midgut of Sixth Instar Feeding Larvae of *S. lituraby* Shotgun ESI−MS Analysis

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
1	EAT43442	Alpha-esterase	63689.21	5.96	*A. aegypti*	1	1	4.26	K.APIDVMKTPNAIPNIPVM*M*GVNNR.E
2	AAR26516	Antennal esterase	61583.58	7.6	*M. brassicae*	1	1	2.56	K.YSDPTPASSSISPK.W
3	AAQ17038	Apolipophorin-3	20727.37	9.04	*B. mori*	2	1	7.53	K.LQAAVQNTVQESQK.L
4	O77248	Apolipophorin-3 precursor	20649.27	7.03	*D. melanogaster*	41	9	53.19	K.AIKDGSDSVLQQLSALSSSLQSAM*TDANAK.A; K.DGSDSVLQQLSALSSSLQSAM*TDANAK.A; K.DGSDSVLQQLSALSSSLQSAMTDANAK.A; K.EVASNVEETNEK.L; K.HVEEVQK.K; K.LKEAYENFSK.H; K.LQAAVQNTAQEVQK.L; K.TFSEQLNSIANSK.N; R.DAPPANTLLQDIEK.H
5	XP_624415	Apolipophorin-3 precursor	33262.91	4.83	*A. mellifera*	1	1	5.48	K.LQNNLAEDM*ESLVRER.D
6	XP_967513	Apolipophorin-3 precursor	66866.71	5.79	*T. castaneum*	1	1	2.26	K.EVAKALLKEIAVK.R;
7	Q25490	Apolipophorins precursor	366946.45	8.66	*M. sexta*	4	2	0.76	R.AVVVNGQHIFTFDGR.H; R.LEFHATNDNK.N
8	EAT34967	Calcium-independent phospholipase	90190.19	6.13	*A. aegypti*	1	1	1.33	K.IMVTGVM*ADRK.P
9	CAD29634	Calcium-independent phospholipase	64764.97	8.11	*A. gambiae*	1	1	1.57	K.INDAADQIR.K
10	ABE01157	Carboxylesterase	60622.21	5.69	*S. litura*	7	5	12.66	K.AGTAPDAEETQFWDK.L; K.YGNPTPDDSLGVK.W; R.GGLEINFTVVNEK.K; R.SLGFYSEDDKELYEFFK.N; R.VNEGLLEGER.V
11	EAT48423	Carboxylesterase	67912.26	5.79	*A. aegypti*	1	1	2.19	R.NESLAEEPVVISR.L
12	XP_395028	Carboxylesterase	45057.75	6.76	*A. mellifera*	1	1	4.81	-.MMESLCQQRLAVLSFDQVK.R
13	NP_648696	Carboxylase:pyruvate/ acetyl-coa/ propionyl-CoA	96196.43	5.39	*D. melanogaster*	1	1	2.40	R.EARFLGLKMFIENLM*TAPSR.V
14	NP_609857	Carbamoylphosphate synthetase	456825.96	6.14	*D. melanogaster*	1	1	0.42	K.KGGTSGEVNANRQVCVR.M
15	EAT36562	Carnitine O-acetyltransferase	68537.51	8.76	*A. aegypti*	1	1	2.93	K.VAIEKFMAPGGDGAKLQK.L
16	AAR37334	Diazepam-binding inhibitor	9823.28	8.86	*H. armigera*	45	7	60.00	K.AKFEAWSK.Q
17	P18167	Esterase P precursor	61054.31	6.33	*D. melanogaster*	1	1	3.86	R.RAFELGRIVGCGHTNVSAELK.D
18	NP_524261	Esterase-7	65435.93	6.09	*D. melanogaster*	1	1	2.10	K.DKDVLEFLQNVK.A
19	P54398	Fat body protein 2	28998.5	6.16	*D. melanogaster*	1	1	3.91	K.DIKM*M*GIMHR.M
20	EAT47728	Fatty acid synthase	265056.27	6.21	*A. aegypti*	4	1	0.70	R.ELYLPTRIEKIVINPAR.H
21	P31417	Fatty acid-binding protein 2	14211.75	5.10	*M. sexta*	1	1	15.91	K.EYNGDDLVVTITSSNWDGVAR.R
22	ABF51385	Hydroxysteroid dehydrogenase	44073.93	8.51	*B. mori*	6	1	4.43	K.LPGTIYTAAEEIEALGGK.A
23	Q9W2Q7	Inositol-pentakisphosphate 2-kinase	67446.89	9.16	*D. melanogaster*	1	1	2.58	R.AAAATATTQRYTKVAR.V
24	ABF51345	Leukotriene A4 hydrolase	69314.08	5.34	*B. mori*	2	2	5.12	K.FTYDAEVTAPEEFTVLMSALR.G; K.VWSEKEEIER.S
25	EAT41283	Lipase	36443.94	5.12	*A. aegypti*	1	1	5.62	R.WDDASSISNSNFNPANPTR.F
26	EAT40623	Low-density lipoprotein receptor	205372.14	5.21	*A. aegypti*	1	1	0.70	R.TINALDYEGKKLR.T

Table 5. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
27	ABD36151	Phytanoyl-CoA dioxygenase peroxisomal precursor	34364.48	8.27	*B. mori*	2	1	4.00	K.ELIDFTSLYSYK.Q
28	ABA12145	Salivary lipase-like protein	46629.78	8.86	*P. argentipes*	1	1	4.10	K.TGKFSLFDYGSSENM*VK.Y
29	XP_967659	Salivary lipase-like protein SP14	17109.25	10.18	*T. castaneum*	1	1	9.74	K.TGVGLIGDKVLVAIK.G
30	Q9VS60	Sphingomyelin synthase	67063.08	6.19	*D. melanogaster*	1	1	1.50	R.DASVDPFSR.T
31	AAT72922	Sterol carrier protein 2/3-oxoacyl-CoA thiolase	57450.85	8.21	*S. littoralis*	18	8	19.25	K.EAVLAALADAR.I; K.FIDAGDNTYGGR.V; K.GHPLGATGLAQCAELVWQLR.G; K.ILEEAM*ANDTDNLIEK.V; K.ILEEAMANDTDNLIEK.V; K.KYGTTELHLAK.I; R.EYTVEEVLNSR.R; R.LYQNTGVSPK.Q; R.VVVNPSGGLIAK.G
32	ABA53824	Sterol carrier protein x	57879.03	6.54	*B. mori*	5	3	7.09	K.KYGTTELHLAK.I; R.NGPDGAEGYWVINAK.E; R.VVVNPSGGLIAK.G

or sterols from diet to fulfill the requirements for their normal growth, development and reproduction.[29,30] In the lepidopteran insects *B. mori* and *S. littoralis*, a single SCPx gene encodes a fusion protein containing 3-oxoacyl-CoA thiolase (SCPx-t) and SCPx-2 domains, which are post-translationally cleaved into two separate proteins.[31,32] A *S. litura* SCPx gene was cloned and characterized.[33] High levels of *S. litura* SCPx expression in the midgut of sixth instar feeding larvae were detected and overexpression of this gene increased cholesterol absorption in the cells *in vitro* cultured.

A low-density lipoprotein (LDL) receptor was found in the feeding midgut. LDL receptor binds LDL, the major cholesterol-carrying lipoprotein of plasma, and transports it into cells by endocytosis, an important mechanism for cholesterol uptake in mammals.[34] But in insects, it seems this receptor has a different mechanism from the mammal system for cholesterol shuttle.[35] Another protein that was highly expressed in the feeding midgut and involved in steroidogenesis is hydroxysteroid dehydrogenase, or 3-beta-hydroxy-delta (5)-steroid dehydrogenase (EC1.1.1.145). This protein is a bifunctional enzyme that catalyzes the oxidative conversion of delta (5)-ene-3-beta-hydroxy steroid, and the oxidative conversion of ketosteroids. The 3-beta-steroid dehydrogenase enzymatic system plays a crucial role in the biosynthesis of all classes of hormonal steroids. It would be interesting to know the physiological roles of this enzyme in the midgut of the feeding larvae.

The discovery of LDL receptor, hydroxysteroid dehydrogenase and SCPx in the midgut indicates that active absorption and conversion of steroids take place during the feeding stage. Other enzymes and proteins involved in lipid and fatty acid absorption, transport and metabolism included esterases, lipases, transferases, fatty acid synthase, which catalyzes the formation of long-chain fatty acids from acetyl-CoA, malonyl-CoA and NADPH, and fatty acid-binding proteins (Table 5), but transcripts of these enzymes were relatively low abundant.

3.4.3. Protein and Amino Acid Metabolism. Numerous proteins that are involved in transport and metabolism of proteins and amino acids were found in the feeding midgut (Table 6). Among these identified proteins, as expected, proteasome-related proteins were relatively abundant. Many uniquitin and uniquitin-related proteins are found, including ubiquitin, ubiquitin-like protein, E3 ubiquitin ligase, ubiquitin-conjugating enzyme and ubiquitin specific proteases. Proteasomes are large protein complexes and their main function is to degrade proteins by proteolysis into small polypeptides. Proteins to be degraded are tagged by ubiquitin through ubiquitin ligases. This ubiquitin-proteasome system is essential for many cellular processes, including the cell cycle, the regulation of gene expression, and responses to oxidative stress.[36] The presence of numerous enzymes of this system indicates that active protein degradation was in processing in the epithelial cells of the midgut during the feeding stage.

Another group of abundant enzymes for degradation of proteins include aminopeptidase N (EC 3.4.11.2), prolyl endopeptidase (EC 3.4.21.26), tripeptidyl peptidase II (EC 3.4.14.10), chymotrypsin (EC 3.4.21.1), trypsin (EC 3.4.21.4), cysteine proteinase (EC 3.4.22.1) and other serine proteases. Unlike the ubiquitin-proteasome system, this group of protein-degradation enzymes usually are involved in degradation of secreted, intercellular or exogenous proteins, such as hemolymphic, pathogenic and food proteins. These proteases usually digest the peptides internally or from terminal ends of the protein peptides in the processes of protein activation and/or degrada-

Table 6. Proteins Involved in Amino Acid and Protein Transport and Metabolism of in the Midgut of Sixth Instar Feeding Larvae of *S. lituraby* Shotgun ESI−MS Analysis

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
1	AAP76306	Amino acid transporter protein	62321.25	4.96	*A. aegypti*	1	1	2.31	R.VDLQLSNPLAKDK.L
2	AAK69605	Aminopeptidase N	108505.87	5.53	*S. litura*	9	6	10.29	K.AIAEDHTFLSDFPNINFGNVFDSWVQNR.G; K.TLGFEVLDFLR.S; K.VNLENIDLEGAR.F; .AQIVNDVLHFIR.S; .EAYLLYDPANTNLVNK.I; R.SETDYYVWNGALTQLDWIR.R
3	CAB55605	Arylphorin subunit	84112.88	6.70	*S. litura*	21	10	17.05	K.DLHQYSYEIIAR.H; K.DYDIEANIQNYSNK.Q; K.FYELDWFVQK.L; K.QAVEEFLLLYR.T; K.SDVASDAVFK.I; K.TFFQFLQK.A; K.VPYDM*SVQPDNM*PR.R; K.YTFM*PSALDFYQTSLR.D; R.DEAIALFHVLYYAK.D; R.SNDYNLHNEK.N
4	ABC69171	Asparagine synthetase	62857.93	6.98	*B. mori*	1	1	2.18	R.LLSDIYLYDGLR.A
5	EAT40065	Aspartate ammonia lyase	46719.75	7.11	*A. aegypti*	1	1	4.14	K.PAIQILHDALKAKSNEFK.D
6	EAT44761	Calcium-dependent protein kinase	48778.2	6.07	*A. aegypti*	1	1	4.07	R.GRVM*LDTPEWKHVSSTGK.D
7	EAT41045	cAMP-dependent protein kinase catalytic subunit	40667.72	8.78	*A. aegypti*	1	1	4.53	K.VRFPSHFGSELKDLLR.N
8	AAF35867	Cathepsin b-like cysteine proteinase	37580.2	5.95	*H. armigera*	4	1	5.33	K.NGPVEGAFTVYSDLLNYK.T
9	P32023	cGMP-dependent protein kinase	105907.04	8.42	*D. melanogaster*	1	1	0.86	R.EPPPEPPK.R
10	AAO75039	Chymotrypsin precursor	30734.69	7.62	*S. frugiperda*	8	4	19.19	K.NINVEDAIDLEDITAYGYLAK.I; R.FTVVLGSIR.L; R.GCQVGSPAAFAR.V; R.STCQGDSGGPLVVTR.S
11	CAA72958	Chymotrypsin-like protease	29254.83	8.63	*H. armigera*	1	1	3.99	R.VTSYISWINQR.L
12	EAT33817	D-alanyl-D-alanine carboxypeptidase	53256.98	6.13	*A. aegypti*	1	1	2.73	R.RLVDDLTATIDEK.R
13	ABD36207	DNA-damage inducible protein	43501.65	5.20	*B. mori*	1	1	3.34	R.M*M*NSDPFDTEAQR.M
14	Q9VVI3	E3 ubiquitin-protein ligase	114875.1	6.13	*D. melanogaster*	1	1	1.19	R.IISSVTKTDLLK.T
15	EAT39376	Ecotropic viral integration site	60572.45	7.67	*A. aegypti*	1	1	2.47	K.YAM*HGLFIEGFPK.L
16	EAT37261	Glutaminyl-peptide cyclotransferase	39944.59	6.01	*A. aegypti*	1	1	4.62	K.LNFANIIGTLNPNAER.F
17	EAT40581	Hect E3 ubiquitin ligase	311338.74	5.34	*A. aegypti*	1	1	0.49	K.VVSVCDDM*GKAAAK.E
18	EAT43483	Heterogeneous nuclear ribonucleoprotein	30833.82	8.44	*A. aegypti*	1	1	5.23	R.NHFGQYGEIESVNVK.T
19	EAT43970	Histone deacetylase	39991.88	5.89	*A. aegypti*	1	1	3.35	R.DGKRPEDGQNLR.S
20	ABF51218	Karyopherin alpha 3	56500.85	4.85	*B. mori*	2	1	3.29	K.DTQVINVVLDGLSNMLK.M
21	EAT47540	Mannose-1-phosphate guanyltransferase	40055.78	6.22	*A. aegypti*	1	1	3.06	R.ALILVGGYGTR.L

Table 6. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
22	CAB55604	Methionine-rich storage protein	88965.34	9.12	*S. litura*	9	7	13.45	K.AANDPVLM*NYYGIK.V; K.AANDPVLMNYYGIK.V; K.DNM*VNFDIK.M; K.LLNHILQPTIYDDVR.E; R.GEVFVHTNELHIIQAVK.V; R.LGGFPLQM*YVIISPVK.T; R.M*VLGGM*GLVSDDAK.F; R.WSVCFDTM*PLGFPFDR.K
23	AAP44964	Midgut class 1 aminopeptidase N	114922.27	5.11	*S. exigua*	5	3	3.53	K.ATFDITLVR.D; R.LGFIEGTNGNFMDDLLR.M; R.YLDENLSNEK.V
24	AAP44965	Midgut class 2 aminopeptidase N	108106.6	4.89	*S. exigua*	3	2	3.75	K.AAVPDFAAGAM*ENWGLVIYR.E; R.VQGLTGTTNILNAFAR.R
25	AAP44966	Midgut class 3 aminopeptidase N	114146.63	5.38	*S. exigua*	4	2	2.19	K.AQIVNDVFSFAR.A; K.FINENLYTEK.I
26	AAP44967	Midgut class 4 aminopeptidase N	108491.28	5.65	*S. exigua*	5	4	5.57	K.AIASYLNSNNR.E; K.TLGFEVLDFLR.S; R.AQIVNDVLHFIR.S; R.SETDYYVWNGALTQLDWIR.R
27	CAB55603	Moderately methionine rich storage protein	90345.37	8.96	*S. litura*	7	4	5.57	K.AIASYLNSNNR.E
28	ABF51260	Nascent polypeptide associated complex protein alpha subunit	22673.24	4.65	*B. mori*	2	1	6.16	K.NILFVINSPDVYK.N
29	EAT35463	O-linked N-acetylglucosamine transferase	32004.34	5.71	*A. aegypti*	2	1	7.02	K.AVALDPNFLDAYINLGNVLK.E
30	ABD98771	Peptidyl-prolyl cis−trans isomerase	17954.25	8.99	*G. atropunctata*	4	1	16.36	K.HTGPGVMSMANAGPNTNGSQFFITTVK.T
31	AAN38751	Prolyl endopeptidase	6002.65	5.66	*S. frugiperda*	1	1	18.52	K.FIAALQHAAR.D
32	DAA04179	Prolyl endopeptidase	18101.83	10.25	*D. melanogaster*	1	1	10.26	K.YLPDCIRKSGIFAVVR.V
33	ABF51224	Proteasome 25 kda subunit	25824.45	5.88	*B. mori*	2	1	4.70	K.SILYDEHSVNK.V
34	ABD36244	Proteasome 26s non-ATPase subunit 4	39297.00	4.76	*B. mori*	1	1	4.42	K.MRIVVFVGSPVNTDEK.E
35	ABF51478	Proteasome 26s non-ATPase subunit 7	37494.59	5.92	*B. mori*	5	3	9.70	K.ITNQLLGLK.G; R.DIKDTTVGSLSQR.I; R.VVGWYHTGPK.L
36	ABF51297	Proteasome alpha 3 subunit	28258.04	5.27	*B. mori*	4	2	9.02	K.AVENSGTVIGLR.G; R.GKDGVVFAVEK.L
37	BAD52258	Proteasome alpha 4 subunit	28550.43	5.87	*P. xylostella*	3	2	12.06	K.ATCIGNNSAAAVSSLK.Q; K.ENETTLAEAQALAIK.V
38	ABF51431	Proteasome beta 3 subunit	23060.58	5.03	*B. mori*	6	3	20.49	K.TFSAM*LSNLLYER.R; K.TFSAMLSNLLYER.R; R.DAISGWGAVVYIIEK.D; R.FGIQAQTVSTNFPK.V
39	ABD36245	Proteasome beta-subunit	27871.9	8.29	*B. mori*	2	1	6.02	K.NYTADEVATENGAVK.L
40	EAT34701	Proteasome subunit alpha type	28322.08	7.61	*A. aegypti*	4	2	10.67	R.LHQVEYAM*EAVK.L; R.LHQVEYAMEAVK.L; R.NQYDSDVTVWSPQGR.L
41	ABF51430	Proteasome subunit alpha type 6-A	27143	6.44	*B. mori*	3	1	5.28	K.AINQGGLTSVALR.G

Table 6. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
42	EAT39423	Proteasome subunit beta type	25623.3	6.31	*A. aegypti*	5	1	7.79	R.FFPYYVSNVLAGLDADGK.G
43	ABD36156	Proteasome zeta subunit	26874.64	4.98	*B. mori*	9	4	20.58	K.AIGSGSEGAQQSLK.E; R.GVNTFSPEGR.L; R.LFQVEYAIEAIK.L; R.PFGVAVMFAGIDEK.G
44	EAT48286	Protein disulfide isomerase	55953.62	4.95	*A. aegypti*	6	3	6.88	K.M*DATANELEHTK.I; K.VLVEFYAPWCGHCK.Q; R.ILEFFGMK.K
45	AAG45936	Protein disulfide isomerase	55588.87	4.60	*B. mori*	6	4	10.53	K.QHLLSEDLPADWAAK.P; K.TGPPAVEVTSAEQAK.E; K.VLVEFYAPWCGHCK.Q; R.ILEFFGMK.K
46	BAA36352	Protein disulfide isomerase like protein	26205.49	5.68	*A. pernyi*	1	1	6.06	K.SQFDVSGFPTIYWK.P
47	EAT48086	Protein kinase C	85062.8	6.31	*A. aegypti*	1	1	1.73	K.DETRPTPEDAAQR.F
48	XP_966807	Protein kinase c	106794.75	9.15	*T. castaneum*	1	1	2.16	K.DGFKVLMEAIKHSNALPSGR.D
49	P18431	Protein kinase shaggy	114440.44	5.58	*D. melanogaster*	1	1	1.87	K.RNTTVASNLAASGAVVDDTK.D
50	EAT39320	Protein regulator of cytokinesis 1	84531.36	9.30	*A. aegypti*	1	1	1.78	R.KEMWEKMMSLEQK.S
51	CAA05747	Protein tyrosine kinase	5775.77	9.43	*D. melanogaster*	1	1	15.69	K.IGGKMPIK.W
52	BAD02367	Protein tyrosine phosphatase	24582.63	9.68	*B. mori*	1	1	7.08	R.WIKYNACGRVIEGTR.I
53	EAT40776	Serine protease	28104.97	5.55	*A. aegypti*	2	1	5.98	K.DSCQGDSGGPLVAER.P
54	ABC25080	Serine protease inhibitor	48740.79	4.49	*G. morsitans morsitans*	1	1	4.00	R.TAEMTDLQAKVIEM*PFK.N
55	XP_971070	Serine protease inhibitor 3	47626.35	5.64	*T. castaneum*	1	1	2.78	K.KNKLIPVSTMYK.I
56	ABD36244	Serine protease inhibitor 5	39297.00	4.76	*B. mor*	1	1	4.42	K.MRIVVFVGSPVNTDEK.E
57	O61366	Serine-enriched protein	144770.04	8.87	*D. melanogaster*	1	1	1.15	K.KVDSSPEALCPERHR.S
58	AAD34349	Serine/threonine-protein kinase Ial	38252.86	8.94	*D. melanogaster*	1	1	2.74	R.ELQLQKRDR.G
59	BAD52261	Serpin 1	43786.28	5.11	*P. xylostella*	1	1	4.56	R.IYLPLTAELADDFAAASR.D
60	AAV91429	Serpin 2	43764.36	5.22	*L. obliqua*	1	1	3.80	K.FKIETEIDLMEILPK.I
61	AAB67169	Small GTP-binding protein	22364.31	5.44	*B. mori*	3	2	13.86	K.IIILGDSGVGK.T; K.NSTNVEQAFMTMAAEIK.A
62	ABF85698	Transport protein Sec61 beta subunit	10121.47	11.8	*B. mori*	1	1	10.31	R.FYTDDSPGVK.V
63	EAT41156	Tripeptidyl peptidase II	91002.31	6.23	*A. aegypti*	1	1	1.43	R.LGSM*ETGTALVR.A
64	EAT42004	Trypsin	27919.14	8.54	*A. aegypti*	1	1	5.22	R.LADSGSSYYVGMK.C
65	P35042	Trypsin CFT-1 precursor	27332.8	8.76	*C. fumiferana*	4	1	3.91	R.TLDNDIAILR.S
66	CAA72962	Trypsin-like protease	26948.07	9.35	*H. armigera*	2	1	4.33	R.IVGGSVTTIDR.Y
67	EAT36899	Tyrosine-protein kinase	145472.11	6.29	*A. aegypti*	1	1	1.23	R.KNSSSSGTVSNKGGSK.S
68	EAT48092	Ubiquitin	34319.34	6.94	*A. aegypti*	22	4	15.41	K.ESTLHLVLR.L; K.IQDKEGIPPDQQR.L; K.TITLEVEPSDTIENVK.A; R.TLSDYNIQK.E
69	EAT38992	Ubiquitin ligase E3 alpha	288338.99	6.85	*A. aegypti*	1	1	0.71	K.NFMTSNAELFENNSDDEK.E
70	EAT36643	Ubiquitin specific protease	113312.4	7.24	*A. aegypti*	1	1	1.64	K.STSNGGGGSSGATTLPK.A
71	EAT37186	Ubiquitin-conjugating enzyme	19189.92	5.27	*A. aegypti*	2	1	11.38	R.WEVLIIGPPDTLYEGGFFK.A

Table 6. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
72	ABD36355	Ubiquitin-like protein	10308.64	5.29	*B. mori*	1	1	13.19	K.VLGQDNAIVQFK.I
73	EAT42894	Utp-glucose-1-phosphate uridylyltransferase 2	57970.61	6.69	*A. aegypti*	4	2	5.26	R.LLEIAQVPK.E; R.NDLTFLDLTVQQIEHLNK.T
74	EAT38904	Zinc metalloprotease	98225.75	5.46	*A. aegypti*	1	1	1.29	K.HVKFNPTEKAK.V

tion. Aminopeptidases N from several insect species have been shown to be putative receptors for *Bacillus thuringiensis* (Bt) toxins in the midgut epithelial cells of susceptible insects.[37−39] Homologues of all four midgut class of aminopeptidase N that were found in Bt Cry1Ca-resistant *S. exigua*[40] were present in the midgut of *S. litura* at relatively high levels as compared to other proteases. Interestingly, not many carboxypeptidases, which hydrolyze single amino acids from the *C*-terminus of the protein peptide chains, were identified in the midgut of *S. litura* feeding larvae.

Serine proteases are one of the most important groups of digestive enzymes in the larval gut and account for about 95% of digestive activity.[40−42] Insects produce and release serine proteases into the lumen of the gut to digest food proteins for being absorbed by the insects.[43] In addition to protein digestion, serine proteases may be involved in other physiological processes in the gut. For example, a *M. sexta* chymotrypsin is involved in activation of chitin synthase, which is necessary for peritrophic matrix formation.[44] In this study, both trypsin and chymotrypsin were identified at similar abundance in the feeding midgut (Table 6). Interestingly, several serine protease inhibitors including serpin 1 and serpin 2 were also found in the same sample, suggesting that serine proteases and serpins can simultaneously exist in an organ at the same stage, although it is not clear whether or not the identified serpins act at these serine proteases.

Another major group of the identified proteins includes protein kinases, such as calcium-dependent protein kinase (EC2.7.11.1), cAMP-dependent protein kinase (EC2.7.11.11), cGMP-dependent protein kinase (EC2.7.11.12), Serine/threonine-protein kinase Ial (EC2.7.11.1), protein kinase C, protein kinase shaggy (EC2.7.11.1) and protein tyrosine kinase (EC2.7.10.2). Protein kinases are a superfamily of enzymes that catalyze phosphorylation of proteins involved in different signal transduction pathways. Phosphorylation usually results in a functional change of the target proteins by changing enzyme activity, cellular location, or association with other proteins. Ca^{2+}-dependent and cAMP-dependent protein kinases and protein kinase C were found in this study, indicating that active phosphorylation of proteins is necessary for functions of the midgut proteins.

High level (21 peptides) of arylphorin protein was found in the feeding midgut (Table 6). Arylphorin is a larval storage protein and used primarily as a source of aromatic amino acids for protein synthesis.[45] It is also involved in the sclerotizing system of the cuticle and serves as a carrier for ecdysteroid hormone and usually secreted into the hemolymph and binds with its substrates such as ecdysteroid hormones.[46]

3.4.4. Nucleotide Metabolism. Abundance of proteins related to nucleotide transport and metabolism was relatively lower as compared to the proteins for other metabolisms (Table 7). A high level of ADP/ATP translocase protein was found. This enzyme catalyzes the exchange of ADP and ATP across the mitochondrial inner membrane.[47] The presence of this enzyme at a high level in the feeding midgut indicated that active exchange of ADP and ATP between the mitochondria and cytoplasm and energy supply were required for the feeding midgut.

Several homologues of DEAD box DNA/RNA helicases were found in the protein profile, including DNA repair helicase rad3/xp-d, ATP-dependent RNA helicase (EC 3.6.1.), Dead-box protein 3 and DEAD/DEAH RNA helicase 1 (Table 7). DNA/RNA helicases unwind double-stranded DNA/RNA in a 3′ to 5′

Table 7. Proteins Involved in Nucleotide Metabolism of in the Midgut of Sixth Instar Feeding Larvae of *S. lituraby* Shotgun ESI−MS Analysis

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
1	NP_788898	Adenine nucleotide translocase	33744.24	9.60	*D. melanogaster*	4	2	9.12	-.MGDEGGGGGHGKGDLK.S; R.YFPTQALNFAFK.D
2	NP_523836	Adenylate kinase-2	26542.54	7.69	*D. melanogaster*	4	3	15.42	K.FCVCHLSTGDM*LR.A; K.KPM*TDDVTGEPLIR.R; K.NGFLLDGFPR.T
3	NP_001014745	Adenylyl cyclase	186869.89	6.29	*D. melanogaster*	1	1	1.01	R.FGAENETEGPPTLATPR.Y
4	AAO32817	ADP/ATP translocase	32891.13	9.88	*B. mori*	28	12	36.67	K.DFLAGGISAAVSK.T; K.EQGLLSFWR.G; K.GIVDAFVR.I; K.LLLQVQHVSK.Q; K.SDGIIGLYR.G; R.ASYFGFYDTAR.G; R.GNFANVIR.Y; R.GTGGAFVLVLYDEIK.K; R.GTGGAFVLVLYDEIKK.V; R.LAADVGKGDGQR.E; R.YFPTQALNFAFK.D; R.YKGIVDAFVR.I
5	P21894	Alanyl-tRNA synthetase	108177.97	5.68	*B. mori*	1	1	1.76	K.IVDLTNEISQAQISYWK.K
6	EAT41170	ATP-dependent RNA helicase	133463.25	8.13	*A. aegypti*	1	1	1.69	R.DPEWVCFQEAYETVEGGNAK.M
7	EAT42685	Bifunctional aminoacyl-tRNA synthetase	190226.62	8.72	*A. aegypti*	1	1	0.65	R.IELGFKDLKNK.Q
8	EAT46335	DEAD box ATP-dependent RNA helicase	81149.95	6.47	*A. aegypti*	1	1	1.65	R.FFVLDEADGLLK.Q
9	BAA20268	Dead box protein 3	12445.39	6.92	*B. mori*	1	1	15.04	K.TLAYILPAIVHIINQPR.L
10	NP_524019	DEAD/DEAH RNA helicase 1	116508.25	6.70	*D. melanogaster*	1	1	1.95	R.NNFVTPTKIQAAAIPMALAK.M
11	ABD36142	DNA polymerase accessory subunit	37136.99	8.49	*B. mori*	1	1	5.03	K.NIHVNWLRFTLGKTQK.H
12	NP_525106	DNA polymerase interacting tpr containing protein	46906.4	5.76	*D. melanogaster*	1	1	3.03	K.KDVLSEELLYPK.F
13	EAT48594	DNA repair helicase rad3/xp-d	86903.57	7.20	*A. aegypti*	2	1	1.71	R.VSEGVDFDHHLGR.A
14	EAT3738	DNA-directed RNA polymerase II largest subunit	17855.51	5.24	*A. aegypti*	3	3	3.24	K.MWTGKQIFSLILK.P; K.QSEILLISDVIAK.H; R.TTRGQLEAFLTQVRNK.Y
15	NP_608519	Equilibrative nucleoside transporter 1	53143.16	5.30	*D. melanogaster*	1	1	3.78	R.NASINNTDLEEELTPLQK.S;
16	AAR18459	FK506-binding nuclear protein	18225.79	6.95	*C. quinquefasciatus*	1	1	9.62	K.IKLDTLDEELYESDK.N
17	EAT42765	Glycyl-tRNA synthetase	85754.76	6.98	*A. aegypti*	1	1	1.70	K.LPFAAAQIGNSFR.N
18	EAT43236	Guanine nucleotide exchange factor	60981.58	9.56	*A. aegypti*	1	1	2.07	K.TASPIKSPKAR.S
19	P25157	Guanine nucleotide-binding protein subunit alpha homologue	52753.21	8.78	*D. melanogaster*	1	1	3.72	K.GISIILFLNKTDLLEQK.V

Table 7. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
20	NP_788565	Isoleucyl-tRNA synthetase	141099.33	7.3	*D. melanogaster*	1	1	1.46	R.NDIERSVALM*QSVVELGR.V
21	Q07152	Inosine-5-monophosphate dehydrogenase	57829.23	7.1	*D. melanogaster*	1	1	2.98	K.YRGM*GSLEAMERGDAK.G
22	Q03427	Lamin-C	69859.85	6.42	*D. melanogaster*	1	1	2.09	R.QIEISEIDGRLSR.Q
23	EAT42009	Leucyl-tRNA synthetase	134875.19	6.72	*A. aegypti*	1	1	0.93	K.FKVQSQNDRDK.L
24	BAB86288	Mariner transposase	39876.79	9.34	*A. cerana*	1	1	3.27	R.KGVVFHHDNAK.A
25	EAT44749	Methionine-tRNA synthetase	63352.61	7.00	*A. aegypti*	1	1	3.07	R.PLADTDAVLFRRIIPPK.V
26	EAT42010	Mitosis protein dim1	16782.31	5.53	*A. aegypti*	1	1	10.56	R.NKHIMIDLGTGNNNK.I
27	EAT36079	Nonsense-mediated mRNA decay protein 1	125193.26	6.70	*A. aegypti*	1	1	0.98	R.NVFVLGFIPAK.A
28	EAT46256	Nuclear matrix protein	77939.92	5.00	*A. aegypti*	1	1	1.46	K.LIEESPPNGK.K
29	EAT43446	Nuclear RNA export factor 1	27318.54	10.79	*A. aegypti*	3	2	8.43	R.QGGGGSSGAPR.G; R.SLGTADVVFER.R
30	EAT34275	Nucleolar protein 10	77826.29	6.69	*A. aegypti*	1	1	1.62	K.FERCFDSEVVK.F
31	ABF51275	Phosphoribosylaminoimidazole carboxylase	47344.42	8.08	*B. mori*	1	1	3.53	R.NLTTVTAADLDTVKR.N
32	EAT37710	Polyadenylate-binding protein	69723.44	9.48	*A. aegypti*	2	1	2.71	R.SLGYAYVNFQQPADAER.A
33	EAT40603	Prokaryotic DNA topoisomerase	113359.86	9.02	*A. aegypti*	1	1	1.18	R.GGGGGAISTGSR.T
34	EAT39227	Regulator of telomere elongation helicase 1	113780.29	8.39	*A. aegypti*	1	1	1.68	R.KKLGGDGGSGM*EELLDK.L
35	EAT34982	Replication factor a 1, rfa1	67743.26	7.09	*A. aegypti*	1	1	1.78	R.VLIILDLHVVK.P
36	EAT40967	Retinal guanylate cyclase	115001.6	8.23	*A. aegypti*	1	1	1.26	R.GSLQDVLIMDEIK.L
37	AAZ15235	Reverse transcriptase	41430.51	9.46	*A. aegypti*	1	1	5.03	K.LRNFGIDLVFSSRDNQLR.T
38	ABD72698	Reverse transcriptase	21226.52	6.17	*A.pisum*	1	1	9.42	K.NVFDEAILAALEPPEPVK.K
39	Q9V727	Reverse transcriptase	179841.76	5.89	*D. melanogaster*	1	1	1.08	K.LSASIKSEPKPPATSQQK.P
40	P56721	Reverse transcriptase	62494.54	8.68	*D. melanogaster*	1	1	1.74	K.LYPSAVSGPR.S
41	EAT34801	Rho guanine dissociation factor	23089.3	5.08	*A. aegypti*	1	1	4.50	K.IRIDFIVQR.E
42	EAT44565	RNA binding motif protein	97225.42	8.90	*A. aegypti*	1	1	1.86	K.LFFRNLAYSVKEDDLK.Q
43	EAT43532	Seryl-tRNA synthetase	55968.86	5.99	*A. aegypti*	1	1	3.00	R.VVNIVSGALNHAASK.K
44	EAT47530	Small nuclear ribonucleoprotein	10737.38	6.05	*A. aegypti*	1	1	20.00	K.NDLSICGTLHSVDQYLNIK.L
45	Q9VEU5	Soluble guanylate cyclase 89Db	75882.25	5.57	*D. melanogaster*	1	1	1.79	K.TFIGSHILERFK.C
46	AAC28260	Transposase	17870.68	10.26	*D.nikananu*	2	1	13.25	K.SWVNPGQPSTSTAKPDRFGK.K
47	ABC16633	Transposase	100974.43	9.15	*A. gambiae*	1	1	1.82	R.SCAAAFCKNNAENVKK.R
48	BAA23532	Transposase	39206.58	9.30	*B. mori*	1	1	4.44	R.FRSGNFDLQNQPRGR.P
49	EAT36273	Tyrosyl-DNA phosphodiesterase	59864.54	9.32	*A. aegypti*	1	1	2.61	K.PEAQPSTSGSQAPK.Q

Table 7. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
50	EAT37451	Tyrosyl-tRNA synthetase	51511.58	9.15	*A. aegypti*	1	1	2.81	R.IIGAGGFTINLNK.A
51	EAT40421	Zinc finger protein	56998.64	8.35	*A. aegypti*	1	1	3.02	K.CDKCYEVYFDEAKLK.E
52	XP_966821	Zinc finger protein	40843.63	5.26	*T. castaneum*	1	1	5.18	K.CSLKIEPRLAFGGVGLPPK.I

direction and influence the interactions between the proteins and their target DNA or RNA molecules. Therefore, DNA/RNA helicases usually function as a transcriptional activator and are involved in mRNA splicing. Several proteins that are involved in RNA modification post transcription were found, such as nonsense-mediated decay protein, which is involved in decay of mRNAs containing premature stop codons,[48] Ser/Arg repetitive matrix protein 2, poly(A)-binding protein 1, RNA binding motif protein (mRNA 3′-end-processing protein RNA15), mitosis protein DIM1 and small nuclear ribonucleoprotein, which is required for splicing of pre-mRNA.[49] At least three proteins involved in RNA transport between nucleus and cytosol were identified, such as equilibrative nucleoside transporter 1, importin subunit alpha-3 and nuclear RNA export factor 1.

Numerous amino acid-tRNA synthetases (or amino acid-tRNA ligases) were identified, including alanyl-tRNA synthetase (EC6.1.1.7), glycyl-tRNA synthetase (EC6.1.1.14), isoleucyl-tRNA synthetase (EC6.1.1.5), leucyl-tRNA synthetase (EC6.1.1.4), methionine-tRNA synthetase (EC6.1.1.10), seryl-tRNA synthetase ((EC6.1.1.11), tyrosyl-tRNA synthetase (EC6.1.1.1) and bifunctional aminoacyl-tRNA synthetase. Abundance of helicases and proteins for RNA modification, RNA transport and amino acid-tRNA synthesis indicated that active RNA and protein synthesis were taking place in the epithelial cells of the feeding midgut.

3.4.5. Protein Translation. A large number of proteins that are associated to protein translation initiation and elongation processes were found in the midgut (Table 8). Different eukaryotic initiation factor (eIF) proteins were found, including eIF-1, eIF-2, eIF-3, eIF-4 and eIF-6. These factors are required for the formation of initiation complexes with 5′ mRNA, the binding mRNA-eIF to Met-tRNA, and the scanning mRNA for the initiator codon AUG. Several different eukaryotic elongation factors (eEFs) were detected in high abundance. Totally, 619 peptides of eEFs were identified and these include α, β and γ subunits of eEF-1 and eEF-2. The α and β subunits and the γ subunit of eEF-1 act as the prokaryotic counterparts EF-Tu and EF-Ts, respectively. eEF-2 is homologous to the prokaryotic EF-G. High levels of eIFs and eEFs presenting in the feeding midgut indicate again that very active protein synthesis was occurring during the feeding stage.

3.4.6. Transporters. Midgut maintains balance of ions and water between the midgut epithelium and the lumen by transmembrane transporters. Many transporters and membrane-bound receptor proteins were detected in the midgut but at a relatively low abundance, such as ATP-binding cassette transporter (ABC transporter), adaptin and voltage-dependent ion channels (Table 9). ABC transporters are members of a transmembrane protein superfamily[50] and they utilize energy generated by ATP hydrolysis to transport a wide variety of substrates across extra- and intracellular membranes, including metabolic products, lipids, sterols and exogenous drugs. Transferrin is a blood plasma protein for iron ion delivery in insects. When a transferrin protein loaded with iron reaches a transferrin receptor on the surface of a cell, it binds to its receptor and is transported into the cell in a vesicle. Vacuole sorting proteins are transmembrane proteins that associate with different functions such as autophagy, cell adhesion and membrane traffic.[51] Many proteins associated with voltage gradient and ion channels such as calcium, potassium and sodium channels were also identified. Although the abundance of these proteins was relatively low, they play important roles in regulation of the ion balance in the midgut of the insect.

Table 8. Proteins Involved in Transcription and Translation in the Midgut of Sixth Instar Feeding Larvae of *S. lituraby* Shotgun ESI−MS Analysis

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
1	AAV66691 AAO16241	Elongation factor 1 alpha	37730.16	7.80	*B. polystictus*	42	10	21.16	-.GSFRYAWVLDK.L; -.VTIIDAPGHR.D; K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.MDSTEPPYSESR.F; K.QLXVGVNK.M; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q
2	AAG45065	Elongation factor 1 alpha	28787.94	6.36	*C. asperatus*	27	8	23.64	-.VTIIDAPGHR.D; K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.MDSTEPPYSESR.F; K.QLXVGVNK.M; K.YYVTIIDAPGHR.D; R.GITIDIXLWKFETAK.Y
3	AAW71508	Elongation factor 1 alpha	32101.89	7.14	*N. aoede*	30	8	23.29	-.VTIIDAPGHR.D; K.CPIEALDAILPPARPTDK.A; K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.QLXVGVNK.M; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q
4	BAD26687	Elongation factor 1 beta	24473.6	4.59	*P. xylostella*	6	2	14.35	K.IQEFEDFVQSVDIAAFNKI.-; R.TIEM*DGLLWGASK.L; R.TIEMDGLLWGASK.L
5	BAB21108	Elongation factor 1 gamma	48388.00	5.84	*B. mori*	3	2	6.15	K.FDPENYSIWYAEYK.Y; K.VAPNFVFGETNK.S
6	P84322	Elongation factor 1-alpha	45119.71	8.67	*A. infecta*	44	13	31.48	-.VTIIDAPGHR.D; .EVSSYIK.K; .IGGIGTVPVGR.V; .MDSTEPPYSESR.F; .QLXVGVNK.M;. SGDAAIVNIVPSKPLCVESFQEFPPLGR.F; K.STTTGHLIYK.C; K.STTTGHLIYKCGGIDK.R; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q; R.VETGILKPGTIVVFAPANITTEVK.S
7	ABB90869	Elongation factor 1-alpha	45000.55	7.72	*M. menophilus*	27	8	18.40	-.VTIIDAPGHR.D; K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.MDSTEPPYSESR.F; K.QLXVGVNK.M; K.STTTGHLIYECGGIDK.R; R.EHALLAFTLGVK.Q
8	AAK54633	Elongation factor 1-alpha	20351.35	7.11	*P. compertus*	17	6	32.61	-.VTIIDAPGHR.D; K.EGKAEGKTLIDALDAILQPSR.P; K.EVSSYIK.K; K.QLXVGVNK.M; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q
9	AAV31943	Elongation factor 1-alpha	13700.75	9.16	*P. brevibarbis*	21	6	25.25	K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.QLXVGVNK.M; K.YYVTIIDAPGHR.D; R.XHALLAFTLGVK.Q
10	AAY18606	Elongation factor 1-alpha	14349.58	6.83	*T. hematodes*	13	3	21.88	K.QLXVGVNK.M; R.EHALLAFTLGVK.Q; R.LPLEDVYK.I
11	P_975086	Elongation factor Ts	199251.13	8.76	*T. castaneum*	1	1	1.05	K.LTSSQDIPPLPEPEGTILK.N
12	ABD36111	Elongation factor Ts	51025.15	8.47	*B. mori*	4	3	8.17	K.GYADIDNAPEEK.A; K.QIGIQHVVVFINK.V; R.LGDITLGTGVITK.I
13	ABA19107	Elongation factor-1 alpha	38123.84	8.23	*A. rubricornis*	15	4	12.50	K.IGGIGTVPVGR.V; K.MDSTEPPYSESR.F; K.TTEENPKAIKSGDAAIIILVP.-
14	AAO50241	Elongation factor-1 alpha	15109.39	7.03	*A. bicolor*	11	3	20.25	K.M*DSTEPPXXEXR.F; K.QLXVGVNK.M; R.EHALLAFTLGVK.Q

Table 8. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
15	AAM13778	Elongation factor-1 alpha	38623.31	8.19	*A. paphia*	35	8	25.07	-.VTIIDAPGHR.D; K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.QLXVGVNK.M; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q; R.GITIDIALWKFETNK.Y; R.VETGILKPGTIVVFAPANITTEVK.S
16	AAC47898	Elongation factor-1 alpha	44824.47	8.67	*C. hercules*	44	13	32.93	-.TIEKFEKEAQEMGXGSFK.Y; -.VTIIDAPGHR.D; K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.QLXVGVNK.M; K.SGDAAIVNIVPSKPLCVESFQEFPPLGR.F; K.STTTGHLIYK.C; K.STTTGHLIYKCGGIDK.R; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q; R.VETGILKPGTIVVFAPANITTEVK.S
17	ABA19133	Elongation factor-1 alpha	38015.62	7.23	*D. stigma*	30	7	20.29	-.VTIIDAPGHR.D; K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.QLXVGVNK.M; K.TTEENPKAIKSGDAAIIILVP.-; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q
18	AAL34067	Elongation factor-1 alpha	39490.44	8.62	*D. distinctus*	46	8	23.35	-.GSFRYAWVLDK.L; -.VTIIDAPGHR.D; K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.QLXVGVNK.M; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q; R.VETGILKPGTIVVFAPANITTEVK.S
19	AAK93885	Elongation factor-1 alpha	33775.8	6.93	*H. nevadensis*	31	8	25.81	-.TIEKFEKEAQEMGXGSFK.Y; -.VTIIDAPGHR.D; K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.MDSTEPPYSESR.F; K.QLXVGVNK.M; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q
20	AAZ76698	Elongation factor-1 alpha	27915.17	7.21	*I. excavata*	34	7	25.29	-.VTIIDAPGHR.D; K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.QLXVGVNK.M; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q; R.GITIDIXLWKFETAK.Y
21	AAF89848	Elongation factor-1 alpha	36293.72	8.73	*O. nubilali*	14	6	18.13	-.VTIIDAPGHR.D; K.EVSSYIK.K; K.IGGIGTVPVXR.V; K.MDSTEPPYSESR.F; K.QLXVGVNK.M; R.EHALLAFTLGVK.Q
22	AAF31080	Elongation factor-1 alpha	45073.71	8.63	*S. reptans*	37	8	17.19	-.VTIIDAPGHR.D; K.IGGIGTVPVGR.V; K.M*DSTEPPFCESR.F; K.QLXVGVNK.M; K.STTTGHLIYK.C; K.STTTGHLIYKCGGIDK.R; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q
23	AAM13781	Elongation factor-1 alpha	38119.57	6.79	*V. egista*	30	7	17.95	-.VTIIDAPGHR.D; K.EVSSYIK.K; K.IGGIGTVPVGR.V; K.QLXVGVNK.M; K.YYVTIIDAPGHR.D; R.EHALLAFTLGVK.Q; R.GYVAGDSENNPPK.G
24	AR01306	Elongation factor-2	81605.3	6.11	*N. meinerti*	12	5	8.13	K.GVQYLNEIK.D; K.STLTDSLVSK.A; R.ALLELQLEQEELYQTFQR.I; R.GGGQIIPTTR.R; R.NM*SVIAHVDHGK.S
25	AAZ15319	Eukaryotic initiation factor 5A	17524.78	5.16	*B. mori*	6	3	17.50	K.VHLVGIDIFNGK.K; K.VHLVGIDIFNGKK.Y; R.EDLKIPDGDLGTQLR.S

Table 8. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
26	EAT44263	Eukaryotic translation initiation factor 1A	17079.13	5.01	*A. aegypti*	3	1	9.46	K.VWINQGDIILIGLR.D
27	XP_623849	Eukaryotic translation initiation factor 2 alpha subunit	127884.99	6.40	*A. mellifera*	1	1	1.33	K.WFPSPEERVLHFLTR.T
28	EAT4818	Eukaryotic translation initiation factor 3 subunit	161576.88	6.44	*A. aegypti*	6	1	1.25	R.KLHGDLM*YLYVVTMEDKR.F
29	XP_971369	Eukaryotic translation initiation factor 3 subunit 5	144665.97	5.36	*T. castaneum*	1	1	1.27	K.DLPPSYQKYASAFKSK.T
30	ABD36299	Eukaryotic translation initiation factor 3 subunit 6	52114.33	5.54	*B. mori*	3	3	8.76	K.LASEILVQNWDGALDDLTK.L; R.SEALTSLVER.K; R.YLATAVIINR.G
31	EAT41266	Eukaryotic translation initiation factor 3, theta subunit	133297.75	8.81	*A. aegypti*	2	1	0.88	R.ALDTLQEVFR.I
32	ABF51379	Eukaryotic translation initiation factor 4A	47540.21	5.10	*B. mori*	14	7	23.33	K.LFVLDEADEM*LSR.G; K.TATFSISILQQIDTSIR.E; K.VVIALGDHLNAK.C; R.DFTVSAM*HGDM*DQR.E; R.GFKDQIHDVFK.M;. GIYAYGFEKPSAIQQR.A; R.QLESGVHVVVGTPGR.V
33	ABF51282	Eukaryotic translation initiation factor 6	26345.77	4.70	*B. mori*	1	1	5.71	R.VQFENNNEVGVFSK.L
34	AAL83698	Translation elongation factor 2	94621.05	6.14	*S. exigua*	44	18	22.04	K.AYLPVNESFGFTADLR.S; K.DLVFITNPDQR.E; K.DSVVAGFQWAAK.E; K.GSVGFGSGLHGWAFTLK.Q; K.GVQYLNEIK.D; K.GVQYLNEIKDSVVAGFQWAAK.E; K.PYTIVQDTR.K; K.SDPVVSYR.E; K.STAISM*FFELEEK.D; K.STLTDSLVSK.A; K.VFDAIM*NFK.K; R.ALLELQLEAEELYQTFQR.I; R.GGGQIIPTTR.R; R.IM*GPNFTPGK.K; R.LWGENFFNAK.T; R.NM*SVIAHVDHGK.S; R.SFCM*YVLDPIYK.V
35	EAT34087	Translation elongation factor g	73992.76	5.46	*A. aegypti*	1	1	1.79	R.NIGILAHIDGGK.T
36	CAG29675	Translation initiation factor 2 gamma subunit	50500.73	9.12	*S. libatrix*	2	1	3.85	R.LVGQVLGAVGALPGIFVK.L

Table 9. Proteins Involved in Transporter and Signaling Pathway of in the Midgut of Sixth Instar Feeding Larvae of *S. lituraby* Shotgun ESI−MS Analysis

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
1	EAT39576	ABC transporter	66288.38	6.22	*A. aegypti*	1	1	3.20	R.MKTAAIPNGYNNHSNGNPK.P
2	EAT40111	ABC transporter	96606.27	8.40	*A. aegypti*	2	1	2.48	R.LLFRFYDVESGSISIDGQNIK.T
3	NP_523415	Adaptin	101179.74	5.00	*D. melanogaster*	1	1	1.95	R.ITPRLAHANAAVVLSAVK.V
4	EAT45339	Ion channel nompc	74491.26	9.24	*A. aegypti*	1	1	2.42	K.SLPPNLKELMAQILYR.L
5	BAA92811	Ion channel nompc	35748.67	5.00	*B. mori*	1	1	7.43	K.SELTGNLENVIVALM*TPLPHFYAK.E
6	ABF51479	Phosphate transport protein	39210.79	9.01	*B. mori*	2	1	3.35	K.M*VKNEGYGTFYK.G
7	EAT37543	Sugar transporter	52181.00	5.59	*A. aegypti*	2	1	2.50	R.KNDAIQSLQFLR.G
8	O01666	Synaptotagmin	32871.19	9.29	*D. melanogaster*	1	1	7.07	K.IANEVLQTGYDYTEGKIVYNR.F
9	EAT35928	Transferrin	88986.77	6.24	*A. aegypti*	1	1	2.04	R.TALKAQLIKPEMQCFK.A
10	EAT36265	Transferrin	85597.31	4.98	*A. aegypti*	1	1	2.19	R.HPFNLTAAM*FQETEKEK.Y
11	ABF85698	Transport protein Sec61 beta subunit	10121.47	11.80	*B. mori*	1	1	10.31	R.FYTDDSPGVK.V
12	Q24314	Vacuolar protein sorting 18	115334.42	5.52	*D. melanogaster*	1	1	1.70	R.NWLVCLLGTPERTTLLR.F
13	XP_392829	Vacuolar sorting protein	45613.67	5.96	*A. mellifera*	1	1	2.76	K.LEDPNIKKLEK.E
14	DAA03342	Vacuole membrane protein	26566.35	11.01	*D. melanogaster*	1	1	6.69	-.MPNLQSSIPHTKKAPK.A
15	EAT43838	Vesicle docking protein	95057.62	4.91	*A. aegypti*	1	1	1.76	R.IGLETFSAKLGEVSK.H
16	EAT36648	Voltage-dependent p/q type calcium channel	206218.09	6.11	*A. aegypti*	1	1	0.66	R.LVKLLARGEGIR.T
17	EAL28531	Voltage-gated potassium channel	75765.4	5.80	*D. pseudoobscura*	1	1	2.25	-.M*YGMLYESVQHYIQK.E
18	EAT45488	Voltage-gated sodium channel	360473.38	6.25	*A. aegypti*	1	1	0.44	K.SNSRCSLLNTSDHR.Q

3.4.7. Stress Resistance. Three major groups of proteins related to stress resistance were identified in the midgut (Table 10). The first group is cytochrome P450 (CYP, EC 1.14.14.1), which is a family of dedox enzymes involved in metabolism of an extremely large number of endogenous and exogenous compounds.[52] Fifteen peptides of CYPs were identified in the midgut; it would be interesting to know the functions of these CYPs in the midgut involved in exogenous compounds and undertakes remodeling during molting and metamorphosis.

The second group consists of glutathione peroxidase (EC1.11.1.9) and glutathione *S*-transferase (GST, EC 2.5.1.18). Glutathione peroxidase is the general name of an enzyme family with peroxidase activity. Glutathione peroxidase reduces lipid hydroperoxides to their corresponding alcohols and reduces free hydrogen peroxide to water, protecting the organism from oxidative damage. GSTs are involved in detoxification of xenobiotics. In insects, GSTs have been classified into six classes: delta, epsilon, omega, sigma, theta and zeta.[53,54] Several GSTs including delta, epsilon, omega, sigma and theta were found in the feeding midgut of *S. litura*.[55]

Heat shock proteins (HSPs) is the third group. A large number (452) of different HSP peptides were identified in the feeding midgut (Table 10). High levels of HSPs can be triggered by exposure to elevated temperatures and different kinds of environmental stress conditions, such as infection, inflammation, exercise, toxins, starvation, hypoxia, nitrogen deficiency or water deprivation.[56] HSPs, particularly Hsp70, are involved in binding antigens and presenting them to the immune system.[57] Of all the HSPs identified in this study, Hsp70 was most highly expressed, though its role in the feeding midgut is not clear.

3.5. Shotgun Analysis and Protein Expression Profile. Shotgun protein sequencing strategy has been proven to be a powerful proteomics analysis approach.[2,58,59] The prominent advantage of this method is avoiding complicated sample preparation and high efficiency to identify hundreds of proteins in a single run. In this study, 2043 peptides were identified by using shotgun approach in the midgut of the sixth instar feeding larvae. However, only approximately 600 protein spots were identified in the same sample using 2-DE/MS approach (Jisheng Liu and Qili Feng, unpublished data). McNall and Adang identified 450 individual proteins from BBMV of the midgut of *M. sexta* fifth instar larvae by using 2-DE/MS technique.[5] Approximately 300 individual protein spots were identified in the gut of fourth instar larvae of *Plodia interpunctella* by 2-DE/MS analysis.[6] Zhang et al. identified 1100 individual protein spots from the posterior midgut of *B. mori* fifth instar larvae by using 2-DE/MS method.[7] Haddow et al. used isotope coded affinity tag (ICAT) technique to identify a total of 207 proteins from the midgut of *G. morsitans morsitans*.[8] It appears that the shotgun approach has a higher efficiency to identify proteins than 2-DE/MS analysis, as indicated by identification of 2,043 peptides in the midgut of the sixth instar feeding larvae. Among the proteins identified in *M. sexta*,[5] *P. interpunctella*,[6] *B. mori*,[7] and *G. morsitans morsitans*,[8] at least 56 proteins were detected in the midgut of *S. litura* in this study.

In addition, the identified proteins from this study had broad ranges of molecular mass and *pI* (Figure 2 and 3). Molecular mass of the identified proteins from the midgut ranged between 2353 and 1.38 kDa, with the most (74.4% of the total proteins) being 10 to 100 kDa and p*I*s ranged between 3.8 and 11.99. 2-DE/MS method can efficiently separate proteins between 7

Table 10. Proteins Involved in Stress Resistance in the Midgut of Sixth Instar Feeding Larvae of *S. lituraby* Shotgun ESI−MS Analysis

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
1	EAT39564	Cytochrome P450	61222.92	8.87	*A. aegypti*	1	1	1.87	R.RYHVEYNYEK.L
2	EAT38452	Cytochrome P450	58046.46	8.28	*A. aegypti*	1	1	2.94	R.M*GEKVLAHDLITSLR.L
3	EAT39028	Cytochrome P450	56819.02	8.60	*A. aegypti*	1	1	2.40	R.KVFQLQGLGFLK.L
4	EAT40473	Cytochrome P450	57164.51	8.99	*A. aegypti*	1	1	2.62	R.KCNGNITHVSIKR.M
5	NP_610418	Cytochrome P450	59433.09	5.19	*D. melanogaster*	1	1	2.01	K.YAVGAAATILK.V
6	AAC23917	Cytochrome P450	58428.89	5.61	*D. melanogaster*	1	1	3.26	K.YNLPFLM*VVGGGGYTIR.K
7	ABC72318	Cytochrome P450	17296.81	4.98	*S. litura*	2	2	17.69	K.DTSVILNIYQIQR.Q; K.NPFSFLAFSAGPR.N
8	ABF51323	Cytochrome P450	25756.35	5.97	*B. mori*	1	1	3.88	K.DVFISAAER.D
9	P13527	Cytochrome P450	58730.88	8.63	*M. domestica*	2	1	4.32	R.FSPEM*VKQRDSVDWLGFGDGPR.N
10	AAT52056	Cytochrome P450	82969.69	6.22	*D. buzzatii*	1	1	2.32	K.AQLEQLLAKPAAHVVSK.P
11	ABD18735	Cytochrome P450	58237.44	9.29	*M. sexta*	1	1	3.49	R.FVELELHLLLAKIM*QRWR.V
12	ABB58823	Cytochrome P450	58132.7	8.48	*B. mandarina*	1	1	3.37	R.FELAKNTPRNLDIDPTR.L
13	AAY85606	Cytochrome P450	28912.27	5.47	*A. funestus*	1	1	4.80	R.LDM*IHLLMQANK.G
14	NP_725339	Glutathione peroxidase	989550.2	5.50	*D. melanogaster*	1	1	0.10	K.ADPAVRDIK.A
15	ABB29466	Glutathione S-transferase (epsilon)	24475.96	8.55	*C. cephalonica*	2	2	12.96	R.FADYFYPQVFGGAPADK.D; R.LYFDIGTLYQR.F
16	EAT41000	Glutathione S-transferase (sigma)	228499.66	5.50	*A. aegypti*	1	1	0.59	K.ELAQETEMDVLK.A
17	EAL25669	Glutathione S-transferase 2 (sigma)	36025.77	6.31	*D. pseudoobscura*	1	1	4.33	K.KYDLLTLLTFVSQR.V
18	ABC79690	Glutathione S-transferase 3 (omega)	24661.49	5.15	*B. mori*	3	1	5.00	R.LDFDIGTLYPR.F
19	P13276	Glutathione-s-transferase (theta)	20793.33	6.98	*M. sexta*	1	1	7.41	K.LQAAVQTTVQESQK.L
20	AAS79891	Glutathione S-transferase (delta)	23939.4	6.20	*S. litura*	26	6	48.39	K.EQTDKLNSAYEILDK.F; K.LTAWFNTIQQEDWYK.K; K.NPQHTVPLLEDGDFYVADSHAINTYLASK.Y; K.YGGAQSAQLYPTDLQVR.A; R.GDITSPTKEQTDK.L; R.LYFDISAIAGNSGAIVSALLR.G
21	Q9U639	Heat shock 70 kda protein cognate 4 (Hsc 70−4)	71431.58	5.33	*M. sexta*	72	20	36.96	K.DAGTISGLNVLR.I; K.FELTGIPPAPR.G; K.ISDSDKQTILDK.C; K.LLQDFFNGK.E; K.M*KETAEAYLGK.T; K.MKETAEAYLGK.T; K.NQVAM*NPNNTIFDAK.R; K.QTQTFTTYSDNQPGVLIQVFEGER.A; K.SINPDEAVAYGAAVQAAILHGDK.S; K.SQIHDIVLVGGSTR.I; K.TFFPEEVSSM*VLTK.M; K.TFFPEEVSSMVLTK.M; K.TVQNAVITVPAYFNDSQR.Q; K.VEIIANDQGNR.T; R.ARFEELNADLFR.S; R.IINEPTAAAIAYGLDK.K; R.IINEPTAAAIAYGLDKK.G; R.KFEDATVQADM*K.H; R.M*VNHFVQEFK.R; R.MVNHFVQEFK.R; R.STAGDTHLGGEDFDNR.L

Table 10. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
22	P29845	Heat shock 70 kda protein cognate 5	74065.99	6.02	*D. melanogaster*	7	5	9.62	K.DSGIDIRKDNIAMQR.L; K.EQQIVIQSSGGLSK.D; R.RFDDPEVKK.D; R.SKLESLVGDLIK.R; R.VINEPTAAALAYGM*DK.T
23	ABF18258	Heat shock cognate 70	72286.68	5.06	*A. aegypti*	40	13	24.27	K.DVDEIVLVGGSTR.I; K.EFFNGKEPSR.G; K.EKIVITNDQNR.L; K.IVITNDQNR.L; K.M*KETAEAYLGK.T; K.MKETAEAYLGK.T; K.NQLTTNPENTVFDAK.R K.SQIFSTASDNQHTVTIQVYEGERPM*TK.D; .VEIIANDQGNR.T; .AKFEELNM*DLFR.S; R.IEIESFYEGDDFSETLTR.A; R.IINEPTAAAIAYGLDK.K; R.IINEPTAAAIAYGLDKK.G; R.ITPSYVAFTADGER.L
24	AAN86047	Heat shock cognate 70 protein	73108.71	5.20	*S. frugiperda*	52	13	23.82	K.DVDEIVLVGGSTR.I; K.EFFNGKEPSR.G; K.EKIVITNDQNR.L; K.IVITNDQNR.L; K.M*KETAEAYLGK.T; K.MKETAEAYLGK.T; K.NQLTTNPENTVFDAK.R; K.SLEDVVQPIIAK.L; K.SQIFSTASDNQHTVTIQVYEGERPM*TK.D; K.VEIIANDQGNR.T; R.AKFEELNM*DLFR.S; R.IEIESFFEGEDFSETLTR.A; R.IINEPTAAAIAYGLDK.K; R.IINEPTAAAIAYGLDKK.G
25	BAB92074	Heat shock cognate protein	71175.3	5.33	*B. mori*	70	20	36.98	K.DAGTISGLNVLR.I; K.FELTGIPPAPR.G; K.ISDSDKQTILDK.C; K.LLQDFFNGK.E; K.M*KETAEAYLGK.T; K.MKETAEAYLGK.T; K.NQVAM*NPNNTIFDAK.R; K.QTQTFTTYSDNQPGVLIQVFEGER.A; K.SINPDEAVAYGAAVQAAILHGDK.S; K.TFFPEEVSSM*VLTK.M; K.TFFPEEVSSMVLTK.M; K.TVQNAVITVPAYFNDSQR.Q; K.VEIIANDQGNR.T; R.ARFEELNADLFR.S; R.IINEPTAAAIAYGLDK.K; R.IINEPTAAAIAYGLDKK.G; R.KFEDATVQADM*K.H; R.M*VNHFVQEFK.R; R.MVNHFVQEFK.R; R.STAGDTHLGGEDFDNR.L; R.TTPSYVAFTDTER.L
26	EAT48688	Heat shock protein	79782.09	6.66	*A. aegypti*	9	2	3.70	K.GVVDSEDIPLNLSR.E; R.ELISNASDALEK.F
27	EAT36187	Heat shock protein	81534.47	4.94	*A. aegypti*	17	9	14.55	K.ADLVNNLGTIAK.S; K.EGLELPEDEEEK.K; K.FENLCKVMKSVLDNK.V; K.GVVDSEDIPLNLSR.E; K.HSQFIGYPIK.L; K.SLTNDWEDHLAVK.H; R.ELISNSSDALDK.I; R.ELISNSSDALDKIR.Y; R.YESLTDPSKLDSGK.E

Table 10. Continued

no.	GAN of homologues	protein description	MW	p*I*	species	no. of peptides	no. of unique peptides	cover percent (%)	sequences
28	AAV91465	Heat shock protein 4 heat shock cognate 70 protein	71602.76	5.33	*L. obliqua*	68	19	35.47	K.DAGTISGLNVLR.I; .FELTGIPPAPR.G; .LLQDFFNGK.E; .M*KETAEAYLGK.T; .MKETAEAYLGK.T; .NQVAM*NPNNTIFDAK.R; K.QTQTFTTYSDNQPGVLIQVFEGER.A;. SINPDEAVAYGAAVQAAILHGDK.S; K.SQIHDIVLVGGSTR.I; K.TFFPEEVSSM*VLTK.M; K.TFFPEEVSSMVLTK.M; K.TVQNAVITVPAYFNDSQR.Q; K.VEIIANDQGNR.T; R.ARFEELNADLFR.S; .IINEPTAAAIAYGLDK.K; .IINEPTAAAIAYGLDKK.G;. KFEDATVQADM*K.H; R.STAGDTHLGGEDFDNR.L; R.TTPSYVAFTDTER.L
29	AAB94640	Heat shock protein 60	61850.13	6.39.	*C. variipennis*	6	4	9.98	K.IGLQVAAVK.A; .LVQDVANNTNEEAGDGTTTATVLAR.A; .DQIKETTSQYEK.E; R.NVIIEQSWGSPK.I
30	BAD42358	Heat shock protein 70	71205.46	5.76	*C. yoshimatsui*	7	3	7.75	K.VCGPIM*AKM*HGGAGAGQK.A; R.IINEPTAAAIAYGLDK.K; .STAGDTHLGGEDFDNR.L
31	O02192	Heat shock protein 83 (HSP 82)	81760.34	4.89	*D. auraria*	19	6	10.89	K.GVVDSEDIPLNLSR.E; K.HFSVEGQLEFR.A; K.HSQFIGYPIK.L; K.SLTNDWEDHLAVK.H; R.MKENQKHVYFITGESK.D; R.YESLTDPSKLDSGK.E
32	BAE44307	Heat shock protein 90	82472.45	4.99	*C. suppressalis*	36	13	22.73	K.ADLVNNLGTIAK.S; K.DQVANSSFVER.V; K.EGLELPEDEEEK.K; K.GVVDSEDIPLNLSR.E; K.HFSVEGQLEFR.A; K.HIYYITGENR.D; K.HLEINPDHSIVETLR.Q; K.HSQFIGYPIK.L; K.SEGTLTIIDTGIGM*TK.A; K.SEGTLTIIDTGIGMTK.A; K.SLTNDWEDHLAVK.H; R.NADDITQEEYGEFYK.S; R.RAPFDLFENK.K; R.YESLTDPSKLDSGK.E
33	AAG44630	Heat shock protein HSP83	82573.6	4.98	*S. frugiperda*	47	17	27.62	K.ADLVNNLGTIAK.S; K.DQVANSSFVER.V; K.EGLELPEDEEEK.K; K.GVVDSEDIPLNLSR.E; K.HFSVEGQLEFR.A; K.HIYYITGENR.D; K.HLEINPDHSIVETLR.Q; K.HSQFIGYPIK.L; .SEGTLTIIDTGIGM*TK.A; K.SEGTLTIIDTGIGMTK.A; K.SLTNDWEDHLAVK.H; K.YYEQFSK.N; R.ELISNSSDALDK.I; R.ELISNSSDALDKIR.Y; .NADDITQEEYGDFYK.S; R.RAPFDLFENK.K; R.YESLTDPSKLDSGK.E; R.YHTSASGDEACSLK.D
34	ABE57140	Heat shock protein Hsp19.5	19467.89	5.56	*L. sativae*	2	1	9.94	R.QDDGSTVNIDKDKFEVK.L

and 200 kDa with p*I*s between 4 and 9.6.[60] Although there are some shortcomings with the shotgun approach, such as lower resolution of protein isoforms as compared to the 2-DE/MS method,[61] this technique holds great promises to be a more valid and high-throughput strategy for insect proteomics, particularly, given that the midgut lumens of Lipidoptera usually have a broad and alkaline pH range.[62]

4. Conclusion

In summary, 2043 peptides were identified, out of which 842 were annotated, from the midgut of *S. litura* sixth instar feeding larvae by the shotgun RP-HPLC−ESI−MS approach. Numerous nonredundant proteins, particularly associated with the processes of carbohydrate conversion, protein synthesis and degradation, lipid and sterol absorption and metabolism are identified, with many being found to be highly expressed, such as diazepam-binding inhibitor, sterol carrier protein 2/3-oxoacyl-CoA, glucose-3-phosphate dehydrogenase, enoyl-CoA hydratase, glutamate dehydrogenase, fructose 1,6-bisphosphate aldolase and triosephosphate isomerase in the feeding midgut. The results suggest that these processes of carbohydrate, protein and lipid metabolisms are extremely active in the midgut during the feeding stage. This protein profile provides for the first time comprehensive information on midgut protein expression of this tropical and subtropical lepidopteran species, which feeds on large numbers of plant species, and will benefit the comparative analysis of feeding habits and digestive enzyme systems with other insects, such as polyphagous *D. melanogaster*, blood-feeding *A. gambiae* and monophagous *B. mori*, and further study of the relationships between the physiological events and functions of the related proteins in the midgut and development of novel controlling strategies against *S. litura*, as well as other Lepidoptera.

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