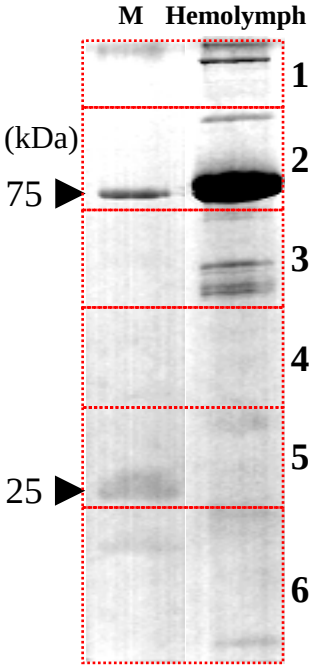


**1-DGE profile coupled with MS analysis.** Extraction of total protein was performed by precipitating the total proteins TCA/acetone extraction buffer, followed by solubilization of proteins in LB-TT to give the total soluble protein. The total protein was separated by 12.5% SDS-PAGE and visualized colloidal-CBB G-250. The gel sliced into 6 fractions. The tryptic peptides were analyzed by LC-MS/MS. Proteins were identified using SwissProt/NCBI non-redundant protein database and MASCOT search engine. The identified proteins are shown in Table I.

## Table I

A list of hemolymph proteins separated by 1-DGE and identified by nESI-LC-MS/MS

Identified proteins from hemolymph of *M. sexta* were classified into functional classes. The table lisits Peptide, Score, Accession No, Protein names, Fraction No, Theoritical *pI* , Theoritical mass (kDa), Species.

| Peptide | Score | Acc.No | Protein Name | Fraction | *pI* | MW | Species |
|---|---|---|---|---|---|---|---|
| | | | ***Defense (20)*** | | | | |
| | | | **Serine Protease Inhibitor (6)** | | | | |
| 17 | 1204 | gi\|431347 | Serine protease inhibitor* | 3 | 5.56 | 43683.28 | *Manduca sexta* |
| 16 | 1145 | gi\|1378128 | Serpin 1* | 3 | 5.25 | 43457.91 | *M. sexta* |
| 2 | 116 | gi\|27733417 | Serpin 3b* | 3 | 5.26 | 51128.25 | *M. sexta* |
| 10 | 692 | gi\|134436 | Alaserpin precursor (Serpin 1)* | 4 | 5.07 | 43510.13 | *M. sexta* |
| 1 | 67 | gi\|134436 | Alaserpin precursor (Serpin 1) | 5 | 5.07 | 43510.13 | *M. sexta* |
| 1 | 72 | gi\|134436 | Alaserpin precursor (Serpin 1) | 6 | 5.07 | 43510.13 | *M. sexta* |
| | | | **Pro-phenol oxidase (5)** | | | | |
| 3 | 196 | gi\|857370 | Prophenoloxidase* | 2 | 5.68 | 80060.7 | *M. sexta* |
| 3 | 166 | gi\|2654518 | Pro-phenol oxidase subunit 1; proPO-p1* | 2 | 7.01 | 78965.66 | *M. sexta* |
| 1 | 65 | gi\|2654518 | Pro-phenol oxidase subunit 1; proPO-p1 | 3 | 7.01 | 78965.66 | *M. sexta* |
| 2 | 137 | gi\|21630233 | Serine proteinase-like protein 2* | 3 | 5.83 | 43488.52 | *M. sexta* |
| 1 | 46 | gi\|21630231 | Serine proteinase-like protein 1* | 3 | 5.16 | 45322.22 | *M. sexta* |
| | | | **Pattern Recognition (4)** | | | | |
| 3 | 171 | gi\|7262927 | Beta-1,3-glucan recognition protein* | 3 | 5.27 | 54567.17 | *M. sexta* |
| 2 | 155 | gi\|511297 | Hemolin* | 3 | 7.91 | 45665.21 | *M. sexta* |
| 3 | 183 | gi\|9651929 | Immulectin-2* | 4 | 6.1 | 37423.55 | *M. sexta* |
| 2 | 120 | gi\|27733409 | Peptidoglycan recognition protein 1B* | 6 | 6.29 | 21530.35 | *M. sexta* |
| | | | **Lysozyme (1)** | | | | |
| 6 | 366 | gi\|84831 | Lysozyme (EC 3.2.1.17) * | 6 | 8.94 | 13983.8 | *M. sexta* |
| | | | **Other defense proteins (4)** | | | | |
| 1 | 51 | gi\|4262357 | Scolexin A* | 4 | 6.98 | 30372.7 | *M. sexta* |
| 5 | 257 | gi\|27733411 | Leureptin* | 3 | 6.99 | 46537.05 | *M. sexta* |
| 1 | 96 | gi\|5326830 | Plasmatocyte-spreading peptide precursor* | 5 | 8.41 | 14004.78 | *M. sexta* |
| 2 | 160 | gi\|29469967 | Antimicrobial peptide gloverin * | 6 | 9.39 | 18444.55 | *M. sexta* |
| | | | ***Transport and Metabolism (19)*** | | | | |
| | | | **Protein (Amino acid) Transport and Metabolism (9)** | | | | |
| 3 | 201 | gi\|136429 | Trypsin precursor* | 1 | 7 | 24409.48 | *Sus scrofa* |
| 3 | 199 | gi\|136429 | Trypsin precursor | 2 | 7 | 24409.48 | *S. scrofa* |
| 4 | 262 | gi\|136429 | Trypsin precursor | 5 | 7 | 24409.48 | *S. scrofa* |
| 4 | 268 | gi\|136429 | Trypsin precursor | 6 | 7 | 24409.48 | *S. scrofa* |
| 3 | 185 | gi\|3318722 | Chain E, Leech-Derived Tryptase InhibitorTRYPSIN COMPLEX* | 3 | 8.26 | 23472.78 | *S. scrofa* |
| 4 | 236 | gi\|3318722 | Chain E, Leech-Derived Tryptase InhibitorTRYPSIN COMPLEX | 4 | 8.26 | 23472.78 | *S. scrofa* |
| 2 | 95 | gi\|400673 | Ommochrome-binding protein precursor * | 4 | 5.89 | 30614.72 | *M. sexta* |
| 6 | 398 | gi\|400673 | Ommochrome-binding protein precursor | 5 | 5.89 | 30614.72 | *M. sexta* |
| 1 | 51 | gi\|400673 | Ommochrome-binding protein precursor | 6 | 5.89 | 30614.72 | *M. sexta* |
| | | | **Lipid Transport and Metabolism (6)** | | | | |
| 60 | 4180 | gi\|7511788 | Apolipophorin precursor protein * | 1 | 8.66 | 366946.5 | *M. sexta* |
| 13 | 830 | gi\|7511788 | Apolipophorin precursor protein | 2 | 8.66 | 366946.5 | *M. sexta* |
| 2 | 101 | gi\|7511788 | Apolipophorin precursor protein | 3 | 8.66 | 366946.5 | *M. sexta* |
| 2 | 198 | gi\|84819 | Apolipophorin III precursor * | 6 | 6.98 | 20793.37 | *M. sexta* |
| 11 | 728 | gi\|9718 | Insecticyanin B form precursor (Blue biliprotein) (INS-b)* | 5 | 6.29 | 23182.27 | *M. sexta* |
| 3 | 173 | gi\|9718 | Insecticyanin B form precursor (Blue biliprotein) (INS-b) | 6 | 6.29 | 23182.27 | *M. sexta* |
| | | | **Iron Transport and Metabolism (4)** | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2 | 121 | gi\|136206 | Transferrin precursor* | 1 | 6.98 | 75222.87 | *M. sexta* |
| 16 | 1038 | gi\|136206 | Transferrin precursor | 2 | 6.98 | 75222.87 | *M. sexta* |
| 3 | 162 | gi\|136206 | Transferrin precursor | 3 | 6.98 | 75222.87 | *M. sexta* |
| 1 | 62 | gi\|7272336 | Ferritin * | 5 | 5.44 | 26507.66 | *M. sexta* |
| | | | ***Storage (18)*** | | | | |
| 9 | 494 | gi\|1168527 | Arylphorin beta chain precursor * | 1 | 5.95 | 83848.83 | *M. sexta* |
| 39 | 2717 | gi\|1168527 | Arylphorin beta chain precursor | 2 | 5.95 | 83848.83 | *M. sexta* |
| 17 | 1077 | gi\|1168527 | Arylphorin beta chain precursor | 3 | 5.95 | 83848.83 | *M. sexta* |
| 6 | 394 | gi\|1168527 | Arylphorin beta chain precursor | 4 | 5.95 | 83848.83 | *M. sexta* |
| 4 | 236 | gi\|1168527 | Arylphorin beta chain precursor | 5 | 5.95 | 83848.83 | *M. sexta* |
| 2 | 115 | gi\|1168527 | Arylphorin beta chain precursor | 6 | 5.95 | 83848.83 | *M. sexta* |
| 6 | 412 | gi\|114240 | Arylphorin alpha chain precursor* | 1 | 6.1 | 83866.31 | *M. sexta* |
| 31 | 2082 | gi\|114240 | Arylphorin alpha chain precursor | 2 | 6.1 | 83866.31 | *M. sexta* |
| 10 | 550 | gi\|114240 | Arylphorin alpha chain precursor | 3 | 6.1 | 83866.31 | *M. sexta* |
| 4 | 248 | gi\|114240 | Arylphorin alpha chain precursor | 4 | 6.1 | 83866.31 | *M. sexta* |
| 1 | 53 | gi\|114240 | Arylphorin alpha chain precursor | 6 | 6.1 | 83866.31 | *M. sexta* |
| 4 | 220 | gi\|228382 | Met-rich storage protein SP1A* | 1 | 9.13 | 29547.46 | *M. sexta* |
| 11 | 755 | gi\|228382 | Met-rich storage protein SP1A | 2 | 9.13 | 29547.46 | *M. sexta* |
| 1 | 59 | gi\|228382 | Met-rich storage protein SP1A | 3 | 9.13 | 29547.46 | *M. sexta* |
| 3 | 168 | gi\|159526 | Methionine-rich storage protein 1* | 1 | 8.71 | 88377.23 | *M. sexta* |
| 24 | 1605 | gi\|159526 | Methionine-rich storage protein 1 | 2 | 8.71 | 88377.23 | *M. sexta* |
| 7 | 432 | gi\|159526 | Methionine-rich storage protein 1 | 3 | 8.71 | 88377.23 | *M. sexta* |
| 2 | 127 | gi\|159526 | Methionine-rich storage protein 1 | 6 | 8.71 | 88377.23 | *M. sexta* |
| | | | ***Metamorphosis (3)*** | | | | |
| 3 | 222 | gi\|47607477 | Imaginal disc growth factor* | 3 | 8.1 | 48086.76 | *Pieris rapae* |
| 2 | 147 | gi\|10801564 | IDGF like protein* | 3 | 7.64 | 48144.25 | *Bombyx mori* |
| 2 | 134 | gi\|400079 | Juvenile hormone-binding protein precursor* | 4 | 5.39 | 25410.99 | *M. sexta* |
| | | | ***Protein-protein interaction (2)*** | | | | |
| 1 | 49 | gi\|25092696 | Proteasome 26S subunit, ATPase 3, interacting protein* | 1 | 6.97 | 24748.09 | *Mus musculus* |
| 1 | 62 | gi\|19921964 | CG12214-PA, isoform A* | 3 | 5.31 | 53142.67 | *Drosophila melanogaster* |
| | | | ***Cell structure (1)*** | | | | |
| 2 | 108 | gi\|32423714 | Actin* | 3 | 5.3 | 41824.76 | *Haemaphysalis longicornis* |
| | | | ***Unclassified (4)*** | | | | |
| 3 | 164 | gi\|25090512 | Hemolymph glycoprotein precursor* | 5 | 5.88 | 25941.08 | *M. sexta* |
| 1 | 49 | gi\|54640661 | GA10602-PA* | 3 | 7.87 | 37588.38 | *D. melanogaster* |
| 1 | 48 | gi\|24585053 | CG10600-PA* | 2 | 8.99 | 150553 | *D. melanogaster* |
| 1 | 56 | gi\|7511167 | Hypothetical protein ZK484.4* | 5 | 8.93 | 98458.7 | *Caenorhabditis elegans* |

*; Non-redundant