PROTEIN MAP OF GUT IN ADULT SUNN PEST, *EURYGASTER INTEGRICEPS* PUT. (HEM.: SCUTELLERIDAE): TWO-DIMENSIONAL ELECTROPHORESIS TECHNIQUE

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ABSTRACT: Gut is main part of digestive system in sunn pest. Digestion and absorption of food materials is the main role of gut and proteins are key molecules for such a function. In this study protein pattern of gut in new adult insects was investigated by Two-dimensional gel electrophoresis in which 100 protein spots could identified by CBB staining. Seventy one protein spots were tagged by molecular weight and isoelectric point. These proteins were divided into different functional category based to their physiological role. These proteins were belong to musculature proteins; carbohydrate, lipid and protein metabolism; energy metabolism; immune related proteins and nutrition proteins. This is the first report of 2-DE pattern of gut in sunn pest and with these ongoing studies, our aim will be to develop organ-related proteome map in sunn pest.

KEY WORD: Gut, protein, proteomics, sunn pest, digestion.

Sunn pest, *Eurygaster integriceps* is a key pest against wheat and barley in Middle east countries such a Iran, Iraq, Turkey and Syria (Javaheri et al., 2009). This insect is monovoltine and has two different phases in life cycle. Sunn spend two to three months as active form and feed from wheat. Then, immigration phase occurs in which they immigrate to wintering sites and diapauses take place. This insect has five insatr nymphs that 1st instars cannot feed and feeding startfrom end of 2nd instars (Critchley, 1998). The most of nutrition performed in adult insects and 5th insatrs. In iran, chemical control against this insect performed in adult stage and sometimes in 5th instars.

Digestive system of hemiptera consisted of two organs, salivary gland and gut (Saxena, 1963). These insects have extra oral digestion in which salivary gland injects some of saliva to tissue plant and after liquefy substrates, pumped them to gut. Main digestion of food materials performed in gut to prepare for absorption (Habibi et al., 2008). Gut of sunn pest like the other hemipterous bugs consisted of three parts, foregut, midgut and hindgut in which midgut divided to four parts. The largest part of gut in hemiptera is midgut.

Gut is a barrier to enthomopathogenic agents that entered from oral (Pauchet et al., 2008). For example Bt toxins act in gut of insects and disturbed ion balances in epitheliums cells (Hakim et al., 2010). Also some of digestive insecticides and plant toxins like plant protease inhibitors acts in gut and interrupt feeding process (Jouanian et al., 1998; Alborn et al., 1997). Using of oral toxins that posses selectivity effects only against of target insects are new strategies for pest management.

Efficiency of gut in digestion and immunity system is related to expressed proteins in it (Pauchet et al., 2008). Proteins are product of genes activity which

causes an action cell. The study of protein is a critical step in understanding physiological role of a selected organ (Liu et al., 2010; Alborn et al., 2007). Proteomics is a new technique for protein researchers. In this technology at first, total proteins separated by two dimensional electrophoresis and then some (Proteomics analysis) or all of them (Protein mapping) were identified by mass spectrometry.

Protein mapping of gut were studied in several insects such a *Helicoverpa armigera* (Pauchet et al., 2008), *Bombyx mori* (Zhang et al., 2011), *Aedes aegypti* (Popva-butler and Dean, 2008), *Tribolium castaneum* (Moris et al., 2009) and *Spodoptera littoralis* (Liu et al., 2009).

In this study, we separated gut proteins from new adult of sunn pest and identified some of them according to molecular weight and isoelectric point. To our knowledge this is the first report of sunn pest proteome analysis.

MATERIAL AND METHODS

Insects

Adult insect collected from wheat farm around Tabriz area in summer 2010 and transferred to insectary room for rearing. Insects was reared on wheat var. Alvand in 27°C±1 and humidty 40% with 16:8 (L:D) photoperiod regime. Gut of adults dissected under stereomicroscope and washed with PBS (pH= 6.9). After dissection, guts were transferred to micro tube contains ice PBS and cocktail of protease inhibitors and kept in -80°C until use.

Protein extraction

A portion including three guts in one ml PBS was homogenized and centrifuged at 30000 g, 30 min, 4°C to remove insoluble materials. Gut proteins were precipitated by 10% trichloroacetic acid and then washed by 100% acton three times and pellets were solubilized in lysis buffer (7 M Urea, 2 M thiourea, 2% CHAPS, 60 mM DDT and 1% ampholyte (pH:3-10)). Insoluble material was removed after two times centrifugation (20000g, 20min, 25°C). Total protein was determined according to Bradford method using protein dye reagent and bovine serum albumin as standard.

Two- dimensional polyacrylamaide gel electrophoresis (2-DE)

A total of 600 µg of extracted proteins were separated in the first dimension by isoelectric focusing (IEF) tube gels and in the second dimension by SDS-PAGE. An IEF tube gel of 11 cm length and 3 mm diameter was prepared. IEF gel solution consisted of 8 M urea, 3.5% polyacrylamide, 2% NP-40, 2% ampholines (pH 3.5–10.0 and pH 5.0–8.0), ammonium persulfate and TEMED. Electrophoresis was carried out at 200 V for 30 min, followed by 400 V for 17 h and 600 V for 1 h. After IEF, SDS–PAGE in the second dimension was performed using 15% polyacrylamide gels with 5% stacking gels. The gels were stained with Coomassie brilliant blue (CBB), and image analysis was performed. The position of individual proteins on gel was evaluated automatically with Melanie 7 software. The pI and Mr of each protein were determined using 2D-PAGE markers (Bio-Rad, Hercules, CA, USA).

The information of gut proteins from other insects available in data banks were used for identifiing of gut proteins in sunn pest.

RESULTS

Proteome pattern of gut in new adult insects were analysed with 2-DE technique. A total of 100 clear protein spots were marked as showed in figure 1.

Vertical margin of the gel showing molecular weight determined experimentally using a protein ladder. Upper side of gel indicating range of pI of protein spots calculated according to gradient of pH in first dimension. Out of marked protein spots, 71 proteins were identified and 29 spots reported as not identified (ni) proteins (Table 1). Identified proteins based on their physiological roles classified into 10 functional groups containing musculature proteins (spots 1, 2, 3, 4, 20, 21, 71, 72 and 83), energy metabolism (spots 5, 6, 7, 35, 36, 45, 50, 68 and 69), protein metabolism (spots 8, 9, 10, 15, 16, 22, 23, 24, 54, 61, 95 and 97), lipid metabolism (spots 11, 12, 13, 14, 39, 57, 62, 66, 67, 77, 80, 85 and 94), carbohydrate metabolism (spots 17, 18, 25, 26, 27, 37, 53, 55 and 56), nutrition storage (spots 19, 63, 92 and 93); cell growth (spots 33); immune related (spots 51, 52, 58, 59 and 60); epithelium (spots 47 and 48) and other proteins (spots 38, 64, 65, 81 and 84) as indicated in table 2.

DISCUSSION

Gut of insects contains various proteins that are necessary for keeping of homeostasis in normal condition. Efficiency of gut is related to proteins existing in this organ and proteomics is suitable approach for investigation proteome (Yao et al., 2009). Main role of gut is food digestion which performed in two ways, mechanical and chemical digestion. Mechanical digestion was performed by muscular action which is effected with muscle contraction (Zhang et al., 2011). Muscle cells components from myofibrils themselves constituted from thick and thin filaments. Sliding between thick and thin filament is due to muscle contraction. Myosin is the abundant protein in thick filament in invertebrates world (Parry et al., 1973). Also myosin is hexamer protein which possesses two heavy chain, two alkali light chain and two regulatory light chain (Yao et al., 2009). In this study six protein spots was identified as myosin heavy chain and myosin tail, three each, , respectively. . The thin filament is composed actin and tropomyosin. Argnine kinase is an effective protein that bound to actin to prepare energy for muscle contraction (Pauchet et al., 2008; Yao et al., 2009). Our result proved that these three proteins in thin filaments are expressed in gut. Mechanical digestion has a key role in preliminary digestion of food particle and it seems that all effective proteins in this process was expressed in gut. Chemical digestion performs by digestive enzyme in which divided into two main groups, ectoenzyme and endoenzyme (Morris et al., 2009; Boyd, 2003; Barbehenn, 2002). Ectoenzymes secreted to lumen of gut and performed preliminary hydrolysis and then produced metabolites hydrolyzed with endoenzymes which can be absorbed from gut cells.

Our result showed that trypsin is ectoenzyme exists in lumen of gut in sunn pest. Carboxy peptidase Cpep-1 identified in present study, before was reported as ectoenzyme that secreted in lumen of *H.armigera* (Pauchet et al., 2008). Aspartate aminotransferase is an important enzyme in amino acid metabolism which transfer a amino group between aspartate and glutamate. Glyoxylate reductase belongs to oxidoreductases which act on the CH-OH group of donor with NAD+ or NADP+ as acceptor (Zhang et al., 2011). Peptidylprolyl isomerase B catalyse the *cis–trans* isomerisation of peptide bonds N-terminal to proline residues in polypeptide chains in which this function is very important in immunity reactions in organism. Also this protein is very important in folding of new synthesized proteins (Liu et al., 2009). Protein disulfide isomerase or PDI is an important enzyme in the endoplasmic reticulum that is effective in hydrolyzing of disulfide bounds between cysteine residue and protein folding (Male and

Storey, 1983). Dihydrolipomide dehydrogenase is a part of mitochondrial glycine cleavage system. Ribosomal proteins related to rRNA and are effective in protein synthesis. Overall, eight different proteins related to protein metabolism were identified in protein map of gut in sunn pest.

α-amylase is essential enzyme in carbohydrate metabolism which hydrolyze α-bound in starch (Zeng and Cohen, 2000). Glycosyl hydrolase also called glycosidaes catalyzes glycosidic bound between two or more carbohydrates (Kunieda et al., 2006). β-galactosidase is effective enzyme in converting β-galactosides to monosaccharide (Mattiaci et al., 1995). Triosephosphate isomerase is an enzyme (EC 5.3.1.1) that catalyzes the reversible interconversion of the triose phosphate isomers dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate in glycolysis process. Glyceraldehide 3-phosphate dehydrogenase and GADPH breaks down glucose for energy production (Kunieda et al. 2006). These proteins expressed in gut of sunn pest are related to carbohydrate metabolism.

Lipase is enzyme that act on the glycerol backbone and catalyzes triglyceride to monoglyceride and fatty acids (Pauchet et al., 2007). Lipases are a subclass of esterases. Fatty acid binding protein are a family of protein carriers that transfer fatty acids and other hydrophilic substrate a cross membrane (Hou et al., 2010). Acyl- Co A dehydrogenase is a key enzyme in lipid metabolism and beta oxidation (Chandra et al., 2006). Lysophospholipase is belong to family of hydrolysis and catalyzed on the carboxylic ester bounds (Zhang et al., 2011). Apolipophorin III precursor is carrier protein that transfer hydrophilic substrate. Enol- CoA acyltransferase catalyzed the final step of beta oxidation. This enzyme belongs to thiolase family of proteins (Yao et al., 2009). Acetoacetyl Co A thiolase also called β-ketothiolase has two physiological role, at first it breakdown acetoacety Co A generated from beta oxidation and secondly catalyze the final step in metabolism of isoleucine. Expression of these proteins in gut of sunn pest indicate dynamic role of gut in lipid metabolism.

Six proteins were identified in gut of sunn pest were effective in energy metabolism. ATP synthase is a complex enzyme that has proton canal in creating of energy with ATP (Chandra et al., 2006). Arginine kinase is a specific enzyme in insect organs. This enzyme has a critical role in producing of ATP that needed in muscle contraction. Cytochrome C and ubiquinol- cytochrome C reductase are important enzymes in electron transport chain in mitochondrial (Zhang et al., 2011).

Immune related Hdd 13, Cyclophilin A and Prophenol oxidase belong to immunity system of gut. This result suggest the contribution of gut in defense mechanisms.

In conclusion, organ specific proteome map generated valuable information in functional genomics. So far, genome studies was important but, nowadays proteome investigation is more interesting for researchers, since proteins are key responsible macromolecule for most of physiological functions. This is the first proteome study of gut in sunn pest reported here and we hope to complete the total proteome map of different organs in this insect in near future.

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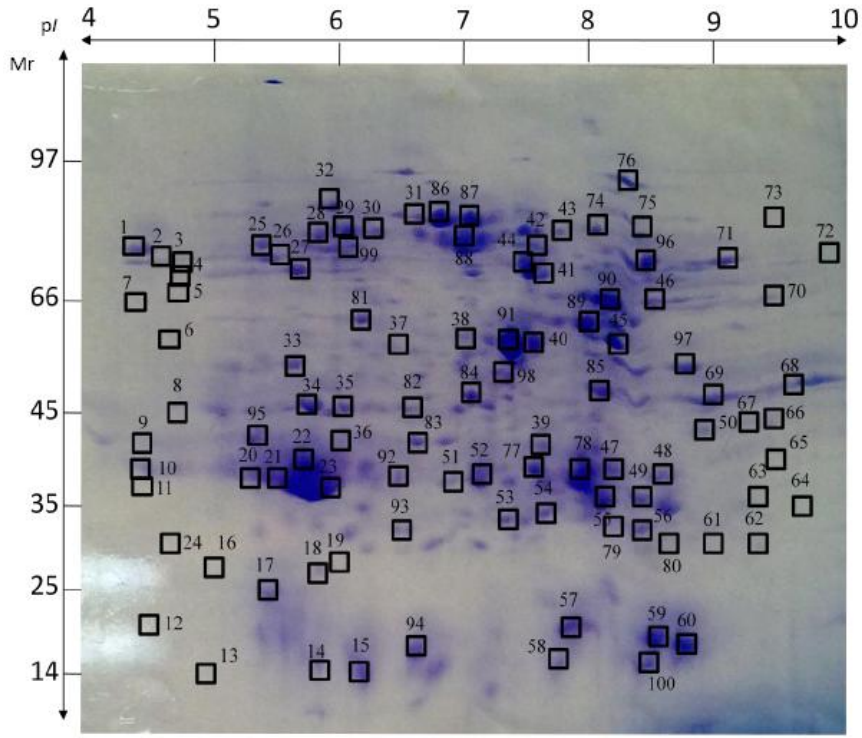

Figure 1. Protein patterns in new adult sunn pest, *E. integriceps*. Proteins (600 µg) were extracted from the adult sunn, separated by 2D-PAGE and stained by CBB. Following scanning, the gel patterns were analyzed using the Melanie software, and the relative abundance ratio of proteins was analyzed of three times experiment.

Table 1. List of adult sunn pest proteins identified by molecular weigt (Mr) and isoelectric point(pI).

SSP	Mr	pI	protein	*Species*	Reference
1	94	4.2	Myosin heavy chain	*Bombyx mori*	Zhang et al., 2011
2	92	4.5	Myosin heavy chain	*B.mori*	Zhang et al., 2011
3	88	4.7	Myosin heavy chain	*B. mori*	Zhang et al., 2011
4	77	4.8	Tropomyosin(A)	*B. mori*	Yao et al., 2009
5	69	4.9	ATP synthase subunit A	*B. mori*	Zhang et al., 2011
6	57	4.8	ATP synthase subunit B	*B. mori*	Zhang et al., 2011
7	69	4.6	ATP synthase	*B. mori*	Yao et al., 2009
8	47	4.6	Carboxy peptidase Cpep-1	*Helicovepa armigera*	Pauchet et al., 2008
9	44	4.4	Carboxy peptidase Cpep-1	*H.armigera*	Pauchet et al., 2008
10	42	4.4	Carboxy peptidase Cpep-1	*H.armigera*	Pauchet et al., 2008
11	37	4.4	Lipase	*Aedes aegypti*	Pauchet et al., 2008
12	25	4.5	Phosphatidyl ethanolamine binding protein	*B. mori*	Zhang et al., 2011
13	14	4.8	Fatty acid binding protein	*B. mori*	Zhang et al., 2011
14	23	6.2	Phosphatidyl ethanolamine binding protein	*B. mori*	Zhang et al., 2011
15	21	6.4	Ribosomal protein	*B. mori*	Zhang et al., 2011
16	28	4.9	Trypsin	*Ostrinia nubilialis*	Pauchet et al., 2008
17	29	5.3	Triosephosphate isomerase	*B. mori*	Zhang et al., 2011
18	29	5.5	Triosephosphate isomerase	*B. mori*	Zhang et al., 2011
19	28	5.8	30 kDa protein	*B. mori*	Zhang et al., 2011
20	37	5.2	Actin A3	*B. mori*	Zhang et al., 2011
21	37	5.5	Actin A3	*B. mori*	Zhang et al., 2011
22	38	5.7	Aspartate aminotransferase	*B. mori*	Zhang et al., 2011
23	37	5.8	Aspartate aminotransferase	*B. mori*	Zhang et al., 2011
24	35	4.7	trypsin	*H.armigera*	Pauchet et al., 2008
25	87	5.6	glycosyl hydrolase	*Tribolium castaneum*	Morris et al., 2009
26	88	5.8	glycosyl hydrolase	*T. castaneum*	Morris et al., 2009
27	85	5.9	B-galactosidase	*Tribolium castaneum*	Morris et al., 2009
28	88	6.2	ni		
29	85	6.3	ni		
30	85	6.4	ni		
31	84	6.6	ni		
32		5.8	ni		
33	66	5.4	Transferrin	*B. mori*	Yao et al., 2009
34	55	5.7	isomerase	*B. mori*	Zhang et al., 2011
35	53	5.9	ATP synthase subunit B	*B. mori*	Zhang et al., 2011
36	42	6.1	Arginin kinase	*Poecilus intepunctella*	Pauchet et al., 2008
37	60	6.3	α-amylase	*Spodoptera frugiperda*	Pauchet et al., 2008
38	70	6.9	Heat shock protein 90	*B. mori*	Yao et al., 2009
39	45	7.4	Acyl- Co A dehydrogenase	*B. mori*	Zhang et al., 2011
40	69	7.4	ni		
41	79	7.4	ni		
42	86	7.5	ni		
43	96	7.8	ni		
44	76	7.3	ni		
45	61	8.2	cytochrome C	*B. mori*	Yao et al., 2009
46	76	8.6	ni		
47	45	8.3	3-hydroxy isobutyrate dehydrogenase	*B. mori*	Yao et al., 2009

Table 1. continued

48	45	8.8	3-hydroxy isobutyrate dehydrogenase	*B. mori*	Yao et al., 2009
49	42	8.6	Lipase	*Aedes aegypti*	Pauchet et al., 2008
50	43	9	ubiquinol- cytochrome C reductase	*B. mori*	Zhang et al., 2011
51	38	6.7	Immune related Hdd 13	*B. mori*	Zhang et al., 2011
52	35	6.9	Immune related Hdd 13	*B. mori*	Zhang et al., 2011
53	33	7.5	GAPDH	*B. mori*	Yao et al., 2009
54	35	7.8	Glyoxylate reductase	*B. mori*	Zhang et al., 2011
55	33	7.9	GAPDH	*B. mori*	Yao et al., 2009
56	35	8.3	Glyceraldehide 3-phosphate dehydrogenase	*B. mori*	Zhang et al., 2011
57	25	7.3	Lysophospholipase	*B. mori*	Zhang et al., 2011
58	14	7.3	Cyclophilin A	*B. mori*	Zhang et al., 2011
59	16	7.8	Prophenol oxidase	*B. mori*	Yao et al., 2009
60	14	8	Cyclophilin A	*B. mori*	Zhang et al., 2011
61	27	8.9	Peptidylprolyl isomerase B	*B. mori*	Zhang et al., 2011
62	25	9.3	Apolipophorin III precursor	*B. mori*	Zhang et al., 2011
63	32	9.5	Low molecular mass 30 Kda lipophorin	*B. mori*	Zhang et al., 2011
64	34	9.4	NADPH oxidae	*B. mori*	Zhang et al., 2011
65	38	9.5	NADPH oxidae	*B. mori*	Zhang et al., 2011
66	38	9.6	Enol- CoA acyltransferase	*B. mori*	Yao et al., 2009
67	39	9.3	Enol- CoA acyltransferase	*B. mori*	Yao et al., 2009
68	50	9.3	ATP synthase	*B. mori*	Yao et al., 2009
69	59	9.1	ATP synthase	*B. mori*	Yao et al., 2009
70	69	9.7	ni		
71	84	9.6	myosin tail	*B. mori*	Yao et al., 2009
72	83	10	myosin tail	*B. mori*	Yao et al., 2009
73	90	9.5	myosin tail	*B. mori*	Yao et al., 2009
74	86	8.3	ni		
75	93	8.1	ni		
76	93	8.4	ni		
77	40	7.7	Acetoacetyl Co A thiolase	*B. mori*	Zhang et al., 2011
78	39	8.1	ni		
79	37	8.2	ni		
80	35	8.6	Enol- CoA hydrotase precursor 1	*B. mori*	Zhang et al., 2011
81	60	6.1	Heat shock protein 70	*B. mori*	Zhang et al., 2011
82	48	6.4	ni		
83	42	6.3	Actin	*B. mori*	Zhang et al., 2011
84	50	7	Mitochondrial aldehyde deydrogenase	*B. mori*	Zhang et al., 2011
85	48	8	Acyl- Co A dehydrogenase	*B. mori*	Zhang et al., 2011
86	90	6.8	ni		
87	90	7	ni		
88	88	7	ni		
89	64	7.9	ni		
90	66	8.1	ni		
91	66	7.4	ni		
92	38	6.5	Low molecular mass 30 Kda lipophorin	*B. mori*	Zhang et al., 2011
93	32	6.5	Low molecular mass 30 Kda lipophorin	*B. mori*	Zhang et al., 2011
94	17	6.6	ni		
95	43	5.2	protein disulfide isomerase	*B. mori*	Zhang et al., 2011
96	73	8.3	ni		
97	51	8.6	Dihydrolipomide dehydrogenase	*B. mori*	Zhang et al., 2011
98	50	7.1	ni		
99	73	6	ni		
100	16	8.2	ni		

Table 2. Functional classification of proteins in adult sunn pest. A total of 100 unique proteins identified in Fig. 1 were grouped into 10 functional categories and unknowns.

Protein classification	SSP	Description
Musculature	1, 2, 3	Myosin heavy chain
	4	Tropomyosin(A)
	20, 21, 83	Actin A3
	71, 72 , 73	myosin tail
Energy metabolism	5	ATP synthase subunit A
	6, 35	ATP synthase subunit B
	7, 68, 69	ATP synthase
	36	Arginin kinase
	45	Cytochrome C
	50	ubiquinol- cytochrome C reductase
Protein metabolism	8, 9, 10	Carboxy peptidase Cpep-1
	16, 24	Trypsin
	22, 23	Aspartate aminotransferase
	15	Ribosomal protein
	54	Glyoxylate reductase
	61	Peptidylprolyl isomerase B
	95	Protein disulfide isomerase

Table 2. Continued

Protein classification	SSP	Description
	97	Dihydrolipomide dehydrogenase
Lipid metabolism	11, 94	Lipase
	12, 14	Phosphatidyl ethanolaminebinding protein
	13	Fatty acid binding protein
	39, 85	Acyl- Co A dehydrogenase
	57	Lysophospholipase
	62	Apolipophorin III precursor
	66, 67	Enol- CoA acyltransferase
	77	Acetoacetyl Co A thiolase
	80	Enol- CoA hydrotase precursor 1
Carbohydrate metabolism	17, 18	Triosephosphate isomerase
	25, 26	glycosyl hydrolase
	27	β-galactosidase
	37	α-amylase
	56	Glyceraldehide 3-phosphate dehydrogenase
	53, 55	GAPDH
Nutrition storage	19	30 kDa protein
	63,92,93	Low molecular mass 30 Kda lipophorin
Transport protein/cell growth	33	Transferrin
Immune related	51, 52	Immune related Hdd 13
	58, 60	Cyclophilin A
	59	Prophenol oxidase
Epithelium	47, 48	3-hydroxy isobutyrate dehydrogenase
Other proteins	38	Heat shock protein 90
	81	Heat shock protein 70
	84	Mitochondrial aldehyde deydrogenase
	64, 65	NADPH oxidae