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Comparative proteomic profiling within each developmental stage of the solanum fruit fly, *Bactrocera latifrons* Hendel

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ABSTRACT

Fruit fly development has been widely studied in details, understanding of "what", "when", "where", "why", and "how" many hundred thousand proteins exist in an insect cell interact and express during development at molecular level largely remained to be clarified. We conducted proteome mapping in all developmental stages of the solanum fruit fly, *Bactrocera latifrons* (Hendel), by comparing all ages within a stage to their 1-d-old, using 2-D gel electrophoresis and mass spectrometry. Samples of designated ages of each stage of *B. latifrons* were collected, analyzed, and described. A custom peptide database, derived from a publically available de novo *B. latifrons* transcriptome assembly was adopted for peptide identification. Identified differentially expressed proteins (DEPs) and their putative protein functions were annotated in representative SDS gel images, charts, and tables. Based on our proteomic data, we constructed a preliminary and descriptive reference proteome maps which not only provide valuable information toward a comprehensive understanding of fruit fly development, but also build a foundation for development of novel advanced fruit fly control techniques or further studies related to sterilization insect technique (SIT) and genome deletion. Any epigenetic impacts due to abiotic or biotic environmental factors will be easier to be identified, manipulated, and further led to gene editing research.

Introduction

Fruit fly control technologies, none of which were as efficient as needed, include chemical pesticides, sterile insect releases, and area wide pest management programs. Multiple innovative genetic based programs such as RNAi based Sterilization Insect Technique (SIT) have recently been developed (Ali et al., 2017; Dong et al., 2016). To develop and implement these techniques, identification of the specific target genes is the initial step. By taking a proteomic profiling approach within the developmental stages of this insect, target genes/proteins for potential use in developing novel SIT methodologies can be identified, and this data compliments other functional genomic data types, such as transcriptome reconstruction and RNA-seq (Shen et al., 2011). Protein analysis allows us to understand what these proteins are, where, when, and why they express, how they interact with each other and how they respond to abiotic or biotic environmental impacts.

The solanum (Malaysian) fruit fly, *B. latifrons*, native to South and Southeast Asia is distributed through parts of China, Taiwan, Malaysia, Thailand, Laos, India, Pakistan, Tanzania, Kenya, and Hawaii (Vargas and Nishida, 1985a; Vargas and Nishida, 1985b). It infests Solanancease and Cucurbitaceae crops and has the potential to permanently establish itself and compete and/or coexist with other tephritid fruit fly species in new areas (Liquido et al., 1994). The morphological and ecological characteristics of this species on development have been described (Liquido et al., 1994; McQuate and Liquido, 2013; Vargas et al., 1997). There are four stages (egg, larva, pupa, adult) in its life cycle (~48 days), 21 d of which are from egg to adult; 2–3 days for eggs to hatch, 8–9 days for larval development to pupae stage, adult emergence on day 10 followed by a 10–11 days pre-oviposition period.

We recently developed a fruit fly birth control diet using lufenuron (LFN) and identified two differentially expressed proteins after feeding LFN diet to adults of Solanum fruit fly for a period of 7 days. Odorant binding protein encoded OBP56d was upregulated and endocuticular glycoprotein encoded ABD_4 was downregulated (Chang, 2017a). While much biological information is available about this species, information on protein expression – which may point to key target genes useful for genetic control programs – is lacking. In order to further manipulate these two proteins we identified and understand their pathways and network, we profiled the whole proteome of Solanum fruit fly. Here, we report on protein changes during each distinct developmental stages of fruit fly in respect to their 1-d-old from egg to adult using proteomics approach.

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Materials and methods

In this study, we utilized two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization time of flight/time of flight mass spectrometry to identify proteins and by comparing differentially expressed proteins (DEPs) between each distinct developmental age within a stage to their 1-d-old to establish protein profiling.

Insects

The *B. latifrons* colony maintained at 24.5 °C, 65% RH (relative humidity), 12D-12 L photoperiod in the USDA-ARS rearing facility in Hilo, Hawaii was used in this study (Vargas and Nishida, 1985b). Eggs (1–3 d), larvae (1–10 d), pupae (1–12 d), females (1–9 d), and males (1–9 d) were collected and stored at −80 °C before processing for protein analysis.

Protein extraction

For each sample, we followed established protocols for sample preparation, 2-D electrophoresis, and MS/MS (mass spectrometry) analysis (Chang, 2017b). Samples from all stage samples (0.25 g adults or pupae, 0.7 g eggs or larvae/ml buffer,) were homogenized in 1 ml 10 mM Tris-HCl (pH 7.0) containing protease inhibitors (final dilution = 1:100; Sigma, P8340, St. Louis) for 15 s repeated three consecutive times with 30 s intervals using a Fast Prep-24 Instrument (MP Biomedicals, Solon, OH). Homogenates were centrifuged twice at 15,294*g* for 15 min at 4 °C. The resulting infra-natants were transferred to new vials on ice for immediate use.

Protein quantification

Protein levels were determined using the Pierce Micro BCA (Bicinchoninic acid) Protein Assay Kit (Pierce, Prod #23227 and lot #HJ107762), using bovine serum albumin (BSA) as a quantitative standard (Rockford, IL). Five μg/μl of total protein were calibrated and used. Three independent biological replicates from the same generation were processed for each treatment (Chang, 2017b).

2D-Electrophoresis

Two dimensional gel electrophoresis was performed in this study as follow:

IEF (isoelectric focusing) – separating different proteins based on pH value

Five μl of 2D gel protein standards (Bio-Rad, Hercules, CA, #161–0320) were added to each sample tube containing 5 μg/μl of total protein. IPG strips (pH 3–10; 11 cm; Bio-Rad, #163–2014) were rehydrated overnight with these sample solutions. Isoelectric focusing was performed with a Protean IEF cell system (Bio-Rad, Hercules, CA) using the standard protocol and a preset linear volt ramp program (8000 V and 50 μA/strip max., 35,000 vH). The focused strips were stored at −80 °C for later use.

SDS gel electrophoresis – based on molecular weight (MW)

Before proceeding to separation on the second dimension, the IPG strips were equilibrated (15 min/buffer: 6 M urea, 2% SDS, 20% glycerol, 130 mM DTT, 0.375 M Tris-HCl, pH 8.7 [Buffer I], followed by 6 M urea, 2% SDS, 20% glycerol, 135 mM iodoacetamide, 0.375 M Tris-HCl, pH 8.7 [Buffer II]). Before running the sample, molecular weight standards (10 μl/lane, Bio-Rad #161–0363) were applied to each gel (precast gels, 8–16% Tris-HCl, Bio-Rad #345–0105), and proteins were separated on SDS-PAGE using the Criterion Cell system (Bio-Rad, Hercules, CA, #165–6001). Gels were stained with Coomassie Blue G-250 (BioSafe Stain, Bio-Rad, Hercules) and scanned using a BioRad GS-900 calibrated densitometer. Three independent biological replicates were performed.

Images acquisition and data analysis

After discoloration (destaining), images were scanned with a GS-900 scanner and trimmed, optimized, and analyzed using PDQuest advanced 2D gel analysis version 8.0.1 software (Bio-Rad Laboratories, Inc). At least 3 scanned images for each condition were analyzed using Delta 2D software (Decodon GmbH, Greifswald, Germany) to identify spots on gel and match orthologous spots between gels. Protein spots with ratios (either equal to 2 or 0.5 or larger than 2 or smaller than 0.5) significantly different between treatments at 95% or above the level of significance as determined by a student's *t*-test, were cut from the gels using a 1.5 mm spot picker (The Gel Company, San Francisco, CA) and stored at −80 °C for future trypsin digestion and MS/MS analysis.

MS/MS analysis- protein identification

Proteins were digested with trypsin, extracted, then lyophilized and reconstituted with water in preparation for MS/MS analysis (Stanley et al., 2008). A portion of each protein was mixed with alpha-cyano-4-hydroxycinnamic acid matrix and applied to the Applied Biosystems 4700 MALDI TOF/TOF target plate and analyzed (AB Sciex). The resulting sequence data, combined with observed MW and pI values, were used to establish protein identities (Stanley et al., 2008). Spot ID was labelled as IDXXX (e.g. ID1145).

Protein quantitative analysis

Differentially expressed proteins (DEPs) were determined based on the ratios of two means comparisons provided by Delta 2D software. Fold change was calculated as the average comparison pairs among 3 biological replicates. Only the proteins with expression fold changes ≥2.0 or ≤0.5 fold between all the comparisons of biological replicates, as well as *P* value of all the differences between protein abundance comparisons < 0.05 were identified as DEPS. Each fold change (ratio), ratio standard errors, and the corresponding *p*-value were plotted in the daily charts and images (Tables 1, 2, 3 and figures in (Chang and Geib, 2018)). Black spot label stands for ratios equal or < 0.5 (ratio ≤ 0.5) and red labels are for ratios equal to or larger than 2.0 (ratio ≥ 2.0) while green labels represent ratios sit between 0.5 and 2 (0.5 < ratio < 2.0). Ratio standard errors were calculated from the equation of [(mean ratio of day X/mean ratio of day 1)*(coefficient value of day X/coefficient value of day1)]/sqare root of # of biological replicates (Chang and Geib, 2018).

Data search and analysis

Database searches were performed with Matrix Science's Mascot search engine v. 2.4 (www.matrixscience.com) on an in-house server against a concatenation of NCBInr Insecta database, combined with a *B. latifrons* species specific peptide database, derived from NCBI TSA BioProject: PRJNA281765. Differentially expressed proteins (DEPs) were identified in each stage, using the first day of the stage as the

Table 1
Differentially expressed larval proteins between 2 and 10 days old and 1 day old larvae of *Bactrocera latifrons*.

No.	Protein names (abbreviations)	Spot ID #
1	Actin-2, muscle-specific	289
2	Actin-3, muscle-specific	303
3	Uncharacterized protein LOC108972624)	598, 615
4	Calreticulin (CALR)	709
5	Larval cuticle protein 5 (Lcp-5)	716
6	Endocuticle structural protein SgAbd-6 like (CUD-6)	776, 792