Improving fruit fly nutrition and performance through proteomics

Chiou Ling Chang[1] and Thomas A. Coudron[2]
[1] USDA-ARS-PBARC-TPRU-Hawaii, USA
[2] USDA-ARS-BCIRL-Missouri, USA

Abstract
Samples of eggs, larvae, pupae, and adults from fruit flies that were reared as larvae in a liquid diet with or without wheat germ oil (WGO) were analyzed using 2-dimensional (2-D) differential in gel electrophoresis (DIGE) and protein sequencing to determine the protein changes associated with dietary ingredients and to correlate those changes with performance. The results of this study demonstrated that dietary WGO during the larval stage altered larval and adult protein patterns which affected the vitellogenic adult stage. Further research to understand the functional interactions between diet nutrients, protein, and performance is underway. Thus far, we have demonstrated that wheat germ oil influences the stage-specific quantity of several fruit fly proteins, including stress proteins, detoxification proteins and glutathione-related proteins. This work is significant at the levels of basic and applied insect nutritional science. At the basic level, we will produce linkages between specific diet components and regulation of specific insect genes and proteins, from which we will be able to understand the mechanisms of how diets influence performance parameters. At the practical level, we are identifying key biomarkers that can be used to accelerate the development of optimal diets.

Introduction

The causes of colony fluctuations when rearing insects are often unknown. Nutrition, rearing methods and environmental conditions are three common causes of poor performance. Environmental conditions and rearing methods have been well studied and reported. However, the functional relationships between diet components and performance have been less studied (Fuchs et al., 2005; Coudron et al., 2006).

Nutritional proteomics is the application of proteomics methodology to nutrition-related research but also represents the interaction of bioactive food ingredients with proteins. Nutrients can interact with proteins in two specific ways: the effect of nutrients on protein expression and the interaction of nutrients with proteins by post-translational modifications or small molecule protein interactions. The latter interactions can cause reduced enzymatic activity or alter molecular interactions. The characterization of such modifications together with functional data from established biochemical and physiological methods will result in a better understanding of the interplay between bioactive dietary components and diet related performance (Yocum et al., 2006; Coudron et al., 2007; Schweigert, 2007). Therefore, proteomics can be defined as the qualitative and quantitative comparison of proteome under different conditions to further unravel the biological process.

We have been rearing tephritid fruit flies to support a control program based on the sterile insect technique. Recently we developed a cost effective yeast-based liquid diet to rear fruit fly larvae. We screened available yeast preparations for dietary quality and we were able to identify changes in insect performance associated with different diet components. These results highlight our curiosity to understand the functional relationships between diet components and insect performance. This study is part of an effort to use proteomic analyses

to reveal the influence of diet components on the profile of fruit fly proteins in relation to their performance.

Materials and Methods

Insects

One hour old eggs of Oriental fruit fly, *Bactrocera dorsalis* (Hendel), used as parental generation were provided by Tropical Pest Research Unit of Agricultural Research Service of United Stated Department of Agriculture (USDA-ARS-TPRU) in Hawaii. Liquid diets and bioassays were prepared and performed as described by Chang et al. (2004, 2006, 2007) and Chang and Vargas (2007). Sample resources were identified and collected through the bioassay during the performance of fruit fly liquid diet development. The third instar larvae, 5-d-old pupae, less than 24 h old eggs and adults (both less than 24 h old and 11-d-old) both treated and controls were collected into a 5 ml cryogenic vial (Nalgene, Rochester, NY). All these samples were stored in an -80°C freezer for later use.

Procedure for tissue preparation

Tested samples were weighed and placed in lysis solution with protease inhibitor tablet. Samples were homogenized using Tissue Terror on ice bath or liquid nitrogen. The samples were then centrifuged at 4°C and 15,000xg for 15 minutes and the center phase (aqueous) was removed and placed in a 1.5 ml microtube and stored at -80°C until analyzed by 2-D DIGE.

2-D DIGE procedure

Proteins were extracted in a 7 M urea, 2 M thiourea, 4% CHAPS, 25 mM Tris-Cl pH 8.5, 0.25% SDS and protease inhibitor buffer. Samples were split into multiple aliquots and stored at -80°C. Protein concentration was estimated using the EZQ method (Molecular Probes). An aliquot equal to 50μg was brought to 20 μL volume using DIGE buffer, and labeled with the appropriate CyDye using 400 pmol of dye per 50 μg of protein. After 30 minutes incubation on ice, lysine was added to stop the reaction followed by an additional incubation on ice for 30 min. Cy3 and Cy5 samples had 30 mM DTT volumes added (separately), then were mixed together and brought to the final 450 μL rehydration volume with final concentrations: DTT (60 mM), glycerol (5%), Triton x-100 (2%), 4-7 IPG buffer (0.5%) and 2-hydroxyethyl disulfide (2.2%). To each run, 400 μg per sample of unlabeled protein was added to the rehydration volume for a total protein load of 900 μg. Rehydration solutions were incubated at 4°C for several hours and centrifuged at 16000xg for 20 min at 12°C. Strips were rehydrated on the bench top overnight. IEF run settings: Program "24cm long". 250 V, 500 Vhrs, fast ramp. 1000 V, 500 Vhrs, fast ramp. 8000 V, 3 hr., gradient ramp. 8000 V, 80,000 Vhrs, fast ramp. 500 V hold for 5 hours. Stored at 120°C. IEF Reramping settings: Program "24cm Short". 250 V, 5 min, fast ramp. 1000 V, 5 min, fast ramp. 8000 V, 30 min, gradient ramp. 8000 V, 30 min, fast ramp. Strips were equilibrated with a urea based buffer containing 5% SDS and 2% DTT for 20 min, then equilibrated in the same with 2.5% iodoacetamide. Strips were then loaded onto second dimension, sealed with a 1% agarose solution in SDS-PAGE buffer (0.2% SDS) with Sigma Wide Range MW marker used on anode end. Run parameters: 3 Watt/gel for 1 hr and 2 Watt/gel for overnight (approximately 15 hrs). Immediately following the run, gels were rinsed in 20% methanol for at least one hour then laser scanned with a Fuji FLA5000 laser imager using 2 channel PMT and simultaneous 2 laser excitation/emission (Cy3/Cy5). Immediately following the scans, gels were fixed in 5:4:1

(methanol:water:acetic acid) for 1 hr, rinsed briefly in distilled water and stained with Coomassie G-250 for several days. After 2 days, gels were destained with distilled water and imaged using a flatbed scanner in transmissive, 256 grayscale mode. 16. Images were loaded into DeCyder for analysis.

Results and Discussion

A mixture of hydrolyzed yeast (FNI200) and GSH (200G) was considered as one of the most cost effective yeasts (Lallemand BioIngredients, Montreal, Canada) for use as a dietary ingredient (Chang 2007, in review). However, diets based on 200G yielded adults with low egg production (2.51 eggs/female/day) and/or low egg hatch rates (32%) compared to adults from our conventional diet (30 eggs/female/day and 76% egg hatch). Supplementing 200G based diet with wheat germ oil (WGO) (200GW) significantly improved these parameters (20 eggs/female/d and 76% egg hatch) (Table 1). Frozen samples of the third instar larvae, 5-d-old pupae, less than 24 h old eggs and adults (either less than 24 h old or 11-d-old) from larvae reared on an artificial diet with, or without WGO and (control) were analyzed through 2-D DIGE.

Table 1. The comparison of egg production and percent egg hatch from adults that were reared as larvae on liquid diets (200G, 200GW, and control)

	Eggs/female/day	Egg hatch (%)
200G	2.51±0.48b	32.19±4.51b
200GW	20.26±11.81a	75.94±5.38a
Control	29.78±4.59a	76.24±1.66a
F value	23.37	37.08
df	2,8	2,8
P	0.0015	0.0004

Differences were observed in the protein expression levels between the larvae control (L7: conventional diet) and larvae treatment diet without WGO (L1) (200G) and between larvae control (L7: conventional diet) and larvae treatment diet with WGO (L2) (200G+WGO). This means that the treatment diets caused physiological changes that are, in part, responsible for the differences in protein expression levels. There appeared to be more protein level expression differences between L7 and L1 than between L7 and L2, which if real, would suggest that the insect response to L2 is closer to L7 than is the insect response to L1. That is, nutrients in a liquid diet with WGO are closer to the conventional control diet than those without WGO. Sequence data and subsequent BLAST search comparisons are needed to identify the proteins that manifested different expression levels. Subsequently, that protein identification will allow us to make some type of projection of the physiological meaning of the differences.

Only marginal protein expression level differences were detected between newly emerged adult control (A7) and adult treatment diet without WGO (A1) and treatment diet with WGO (A2). This implies that the physiological state of the insect in the newly emerged adult stage is similar regardless of what diet was provided. If there are meaningful differences

as a result of the diet fed during the immature stage, then we would expect the resulting adults to manifest some difference. Therefore, we have also run 2-D gels on the following four comparisons: L1 [larvae, diet 200G] compared to L2 [larvae, diet 200G+WGO]; VA1 [vitellogenic adult, diet 200G] compared to VA2 [vitellogenic adult, diet 200G+WGO]; VA7 [vitellogenic adult, control diet] compared to VA1; and VA7 compared to VA2 using DeCyber image software. There were no "obvious" differences between L1 and L2 nor between VA7 and VA2. However, protein differences were found between VA1 and VA2 and between VA7 and VA1. These results not only demonstrated that dietary WGO causes changes in protein patterns but they also showed that WGO may interact with proteins in the vitellogenic adult stage in addition to the immature stage. Further research to understand the functional interactions between diet nutrients and protein is underway.

References

Chang, C. L., Caceres, C., Ekesi, S. 2007. Life history parameters of *Ceratitis capitata* (Diptera: Tephritidae) reared on liquid diets. Ann. Entomol. Soc. Am., in press.

Chang, C. L., Caceres, C., Jang, E. B. 2004. A novel liquid larval diet and its rearing system for Melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). Ann. Entomol. Soc. Am., 97: 524-528.

Chang, C. L., Vargas, R. I. 2007. Wheat germ oil and its effects on a liquid larval rearing diet for oriental fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 100: 322-326.

Chang, C. L., Vargas, R. I., Jang, E. B., Caceres, C., Cho, I. K. 2006. Development and assessment of a liquid larval diet for *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Ann. Entomol. Soc. Am., 99: 1191-1198.

Coudron, T. A., Brandt, S. L., Hunter, W. B. 2007. Molecular profiling of digestive genes in *Homalodisca coagulata* (Hemiptera: Auchenorrhyncha: Cicadellidae) feeding on sunflower and cowpea. Arch. Insect Biochem. Physiol., in press.

Coudron, T. A., Yocum, G. D., Brandt, S. L. 2006. Nutrigenomics: A case study in the measurement of insect response to nutritional quality. Entomol. Exper. Appl. 121: 1-14.

Fuchs D., Winkelmann I., Johnson I. T., Mariman E., Wenzel U., Daniel H. 2005. Proteomics in nutrition research: principles, technologies and applications. Br. J. Nutr. 94: 302–314.

Schweigert, F. J. 2007. Nutritional proteomics: Methods and concepts for research in nutritional Science. Ann. Nutr. Metab. 51: 99-107.

Yocum, G. D., Coudron, T. A., Brandt, S. L. 2006. Differential gene expression in *Perillus bioculatus* nymphs fed a suboptimal artificial diet. J. Insect Physiol. 52: 586-592.