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Expressed Sequence Tag (EST) Survey of Life Stages of the Potato Psyllid, *Bactericera cockerelli*, Using 454 Pyrosequencing

D. Hail, W. B. Hunter, S. E. Dowd, and B. R. Bextine

Abstract. The potato psyllid, *Bactericera cockerelli* (Sulc.), is an insect pest of solanaceous plants and has recently become of interest because of its association with zebra chip, an immerging potato disease in the US. Genomic information on the potato psyllid is limited but could be used to develop novel management strategies for the insect and disease. To that end, cDNA libraries were prepared for the 5th instar and adult life stages. Structural and muscular, neurological and reproductive developmental processes, stress response and primary metabolism sequences were recovered. Metagenomic sequences aligning to microbial, viral and fungal symbionts or pathogens were also identified. Additionally, general housekeeping, life stage, and gender-specific targets were selected for RNAi-based knockdown experiments.

The potato psyllid, *Bactericera cockerelli* (Sulc.), is one of approximately 40 species of psyllids (Hemiptera: Psyllidae) of economic importance (Percy 2000). Potato psyllids are pests of solanaceous plants such as peppers, *Capsicum* sp.; tomatoes, *Solanum lycopersicum* L.; and potatoes, *Solanum tuberosum* L.; and have been implicated as the vectors of Candidatus *Liberibacter* sp., recently identified as the putative causal agent of zebra chip (Hansen et al. 2008; Liefting et al. 2008, 2009). Zebra chip is a potato disease characterized by above-ground tubers, yellowing and curling of leaves, and the presence of alternating light and dark bands radial to the center of a sliced tuber (Gao et al. 2009). When fried, these bands are caramelized because of their higher soluble sugar content and are rejected by processors because of their altered appearance and taste. Plants with symptoms of zebra chip represent a near total loss to producers. Since its appearance in Saltillo, Mexico, in 1994, heavy infestations of zebra chip have forced some farmers to abandon their fields (Flores et al. 2004).

Little information is available relative to psyllid genomics with a unigene set of 5,000 sequences derived from a dataset of ~20,000 expressed sequence tags, at NCBI, representing about a fifth of the predicted total genes within the Asian citrus psyllid genome (Hunter et al. 2009; NCBI – datasets). To investigate the genetic basis of the biology for potato psyllid, new cDNA libraries, collections of complementary DNA, were needed. These transcripts provide information about actively transcribed genes used to study the interactions between plant/psyllid, psyllid/pathogen, and pathogen/plant. As psyllid genomics grows, affordable sequencing along with new technologies for data analyses provides researchers with tools for the development of innovative management strategies. One such emerging approach is RNA-interference, RNAi. These strategies disrupt transcript expression, thus reducing pest and disease fitness. Genomics is now considered a first step in many difficult to manage disease systems to obtain the maximum

benefits through identification of genetic targets in the fastest time. One example is the pea aphid genome (International Aphid Genome Consortium 2010) that identified a greater number of genes than was predicted. Analyzing the genomics of potato psyllid provides researchers the ability to identify viable genes linked to critical biological processes that can be targeted through the development of methods, such as RNAi. As more is learned about psyllid genomics it may even become possible to disrupt the ability of the potato psyllid to be an active host, or vector, for Candidatus *Liberibacter* bacteria. Two cDNA libraries, one isolated from adults and another from 5th instar nymphs, were developed to advance the field of potato psyllid genomics.

Potato psyllids were initially provided by Drs. Tong-Xian Liu and Xiangbing Yang (Texas AgriLife Research) and used to start a colony maintained on potatoes (25°C; 40% humidity). Adult and 5th instar psyllids of mixed genders were collected and stored at -80°C in 1.5 ml microcentrifuge tubes. Total RNA was extracted from the psyllids using the RNeasy mini Kit and Poly-A mRNA was isolated using the Oligotex mRNA mini kit (Qiagen). Using Stratagene's ZAP-cDNA synthesis kit, the mRNA was retrotranscribed and sent to the Research and Testing Laboratory of the Medical Biofilm Research Institute in Lubbock, TX, for 454 pyrosequencing. Double-stranded cDNA was quantified, nebulized to 300-550 bp fragments, pyrosequencing library created and sequenced as per the Roche 454 pyrosequencing manufacturer's instructions. Resulting sequences were assembled using DNAstar's NGEN assembler (Madison WI) and annotated using BLASTx - W.ND BLAST (Dowd et al. 2005) and cross referenced to functional annotations using DAVID (Huang et al. 2009).

Sequences corresponding to various cellular functions and metabolic activities were recovered from the adult (Fig. 1) and 5th instar (Fig. 2) cDNA libraries. In the libraries, sequences related to ribosomal functions, organelle construction, and muscular, neurological, and reproductive developmental processes were recovered. Stress response, ion transporters, nucleic acid binding, and primary metabolism sequences were also recovered. Numerous metagenomic

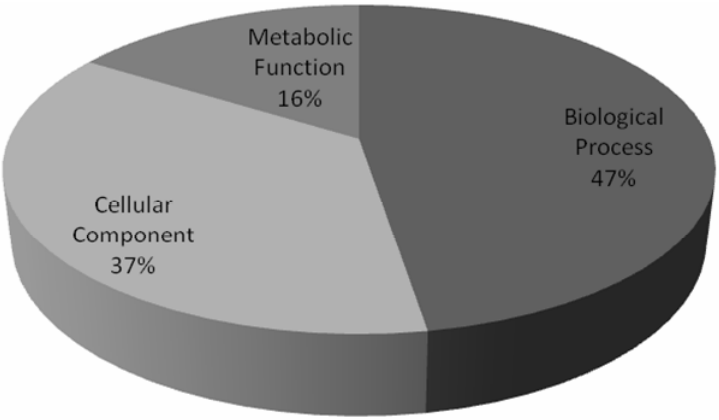

Fig. 1. Proportion of assembled sequences from the adult potato psyllid cDNA library sorted by Gene Ontology.

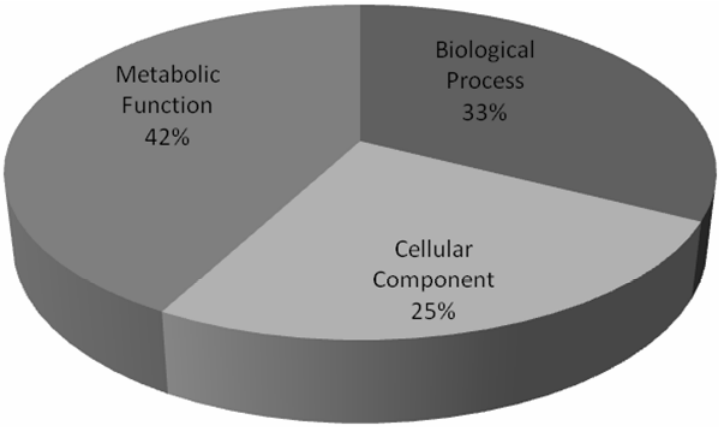

Fig. 2. Proportion of assembled sequences from the 5th instar potato psyllid cDNA library sorted by Gene Ontology.

sequences aligning to microbial, viral, and fungal symbionts or pathogens were also identified - similar results were obtained from 5th instar nymphs (Table 1). These results provide new information on psyllid genomics relating specifically to potato psyllid. The application of this information to elucidate RNAi targets provides a new direction for researchers who previously did not have this information. Some of the gender and life-stage-specific targets include vitellogenin, a yolk precursor protein

Table 1. Non-insect Metagenomic Organisms Recovered from Adult and 5th Instar Potato Psyllid cDNA Libraries

Virus	Bacteria	Fungi
Brome mosaic virus	*Agrobacterium tumefaciens*	*Aspergillus fumigatus*
Deformed wing virus*	*Bacillus* sp*	*Candida albicans*
Eggplant mosaic virus	*Buchnera aphidicola*	*Cochliobolus carbonum*
Enterobacteria phage k11	*Burkholderia* sp*	*Debaryomyces hansenii*
Enterobacteria phage t7	*Escherichia coli*	*Emericella nidulans*
Fowlpox virus	*Mycobacterium* sp	*Encephalitozoon cuniculi*
Haemophilus phage hp1	*Nitrosomonas europaea*	*Phytophthora infestans*
Impatiens necrotic spot virus	*Pseudomonas* sp	*Saccharomyces cerevisiae**
Melanoplus sanguinipes† entomopoxvirus*	*Ralstonia solanacearum*	*Schizosaccharomyces pombe**
Pepper mild mottle virus	*Wigglesworthia glossinidia*	*Ustilago maydis*
Tick-borne encephalitis virus	*Wolbachia pipientis**	*Xanthophyllomyces dendrorhous*
Zucchini yellow mosaic virus	*Xanthomonas* sp	*Yarrowia lipolytica*

†North American migratory grasshopper

*Also recovered from 5th instar nymphs

from adult females, ejaculatory bulb protein, an adult male specific reproductive protein, and actin II, a protein responsible for wing function. More general house-keeping transcripts include heat shock proteins, troponin, a general contractile protein, and transferrin, an iron delivery protein.

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