

B2. (Hunter) HLB-psyllid interactions - genomics approach. The goal of this project is to obtain information on the molecular genetics of the disease and psyllid, information which will be used to examine the genetic basis for disease acquisition and transmission. Genes associated with the disease will be scrutinized as targets for the development of novel disease management strategies. (Wayne Hunter) **Progress as of September 30, 2008 –**

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Pathogen-Vector Interactions

Genetic sequencing- from *D. citri* has successfully produced three gene expression libraries: Midgut tissues (6,200 sequences), Testes (8,650), and a current library from Mycetosome tissues which contain endosymbiotic bacteria of the psyllid which is just starting sequencing, the library will be ~10,000 sequences. Other accomplishments include the 1) International *Diaphorina* Genome Consortium was established (Hunter); 2) the *Diaphorina citri* Metagenome database; and the *Diaphorina Wolbachia* database webpages have been set up (Hunter) online- NCBI. 3) Identification of eight species of bacteria which includes primary and secondary psyllid endosymbionts, from these four of these bacteria species may be a first report discovery from psyllids. Data are being prepared for public database. Currently ~17,106 sequences from *Diaphorina citri* have been made publicly available in the NCBI database, with ~5,200 Testes EST submitted this month, with another ~4,000 being prepared for submission in October 2008. Just over 90 proteins have been annotated and published in-NCBI database, along with ~354 *Wolbachia* sequences for submission to public databases. A library targeted to bacteria produced ~75% of the *Wolbachia* genome, these sequences are being processed, annotated and release. Plans are to finish the gaps and complete this genome in 2009 but this will depend on continued funding to keep currently trained personnel. Efforts for additional funding have been made to hire a second technician which will be trained to assist with annotation and sequencing on these projects. **Examination of Digestive enzymes-** isolated a set of 17 cathepsins from *D. citri*, forming five predicted phylogenetic groups which contain: four F-like proteases, three B-like protease and procathepsin, four B-like cysteine proteases, two B-S cysteine proteases, and a L-like cysteine protease, and D-cathepsin. Whereas mammalian cathepsins are well studied, emerging studies for arthropods on cathepsins have only started to characterize these enzymes. Cathepsins in insects function in digestion and some are involved in embryonic vitellin degradation (egg yolk proteins and hormones), taking part in changes during metamorphosis (body formation). Function of these enzymes includes results which showed that the lysosomal cathepsins, especially cathepsin D and sometimes cathepsin L, are responsible for the degradation of muscle protein during stresses such as metamorphosis, maturation and starvation. So cathepsins are important to psyllid survival, development, and reproduction making them key targets for further studies which will target their expression and more importantly what happens when they are 'down-regulated' or silenced by RNAi experiments to determine their potential in psyllid management. **Identification of FK506-binding protein (FKBP).** The FKBP's represent a large gene family in plants and insects that are involved in growth and development [pfam00254]. They are highly conserved and ubiquitous group of chaperones that bind immunosuppressive proteins. This is the first *D. citri*-FK506BP member identified for any psyllid, molecular weight of 11.7 kDa, and 109 amino acids in length. These proteins also regulate the function of the Calcium ion channels within intracellular membrane systems which is associated with certain pathological states. This means that the movement of salts and water within cell fluids are regulated such that disruption of these channels causes or stimulates cell death. Disruption of genes encoding FKBP's in plants and animals has underlined the importance of this family of proteins in the regulation of cell division and differentiation. **Psyllid Serine proteases:** Serine proteases play critical roles in a variety of invertebrate immune processes. Examples of serine protease mediated defense responses include hemolymph (blood) coagulation, activation of antimicrobial peptide synthesis, and melanin synthesis which is used to surround and isolate pathogens in insects. The majority of insect serine protease genes have been cloned primarily through genome projects, Fruit flies, Honey Bee, and others. Understanding how Serine Proteases interact with Liberibacter within psyllids, or how Liberibacter can avoid this insect defense system may provide another angle by which altered psyllids may become unable to transmit Liberibacter.