Expressed sequences in Three Leafhoppers Vectors of Pierce’s Disease


ABSTRACT-Leafhoppers are considered the second most important vector of agricultural diseases. We examined the gene expression across three leafhopper leaffeaters, Homalodisca vitripennis, Graphocephala atropunctata, and Oncometopia nigricans, which are vectors of the plant-infecting bacterium, Xylella fastidiosa, which causes Pierce’s disease of grapes. The use of genomic data is providing new information on the biology and relatedness of these and other leafhoppers. Using a genomics approach has also advanced the understanding of leafhopper immunity, pathology, and development. As new developments in genomics and RNAi methodologies emerge, researchers will be able to use this genetic information to design highly specific and effective management tools to reduce other leafhopper populations, and/or leaffeather-transmitted diseases. The importance of these leaffeaters as the vectors of Pierce’s disease, the abundance of ESTs produced for each, and their differences in host plant preferences, provide an excellent opportunity to conduct comparative examination of these leaffeaters’ several CDNA libraries which had been made from adult GWSS, BGGS, and BWSS, plus nymphs, and tissues, provided a resource totaling ~50,000 ESTs. When assembled we obtained ~5,000 specific transcripts for each species for comparison. This is approximately one-third of all predicted active genes available, as other insect genomes have demonstrated ~15,000 total genes. These were used for analyses between these species as well as for larger analysis to known genomes. Further analyses were conducted in silico using software programs available online Internet Resources, NCBI, EXPASY, and others to compare assembled data, predict proteins and compare them to the broader scope of insect genomes. Many other genes of interest which have various functions in leaffeather biology and physiology have also been identified but are not reported herein. The EST sequences reported in this study have been deposited in GenBank’s dbEST. (see references: Hunter 2005, 2006, 2007).

Fig. 1. Composite figure showing distribution of Homalodisca vitripennis transcripts across other species (along left), with the top 6 species homologies being in these insects whose genomes have been completed: Drosophila melanogaster, Aedes aegypti, Tribolium castaneum, Anopheles gambiae, Nasonia vitripennis, and Apis mellifera.

Digestive Enzymes: Amino peptidase, several cathepsins L-like cysteine proteases, and other proteases have been identified in these leaffeaters which are also in other piercing-sucking feeding insects (Foissac et al., 2002, Wright et al., 2006, Zhu et al., 2003). In aphids, a cathepsin B protease has been shown to be constitutively expressed in all aphid individuals, suggesting gene duplication and evolution of a novel biological function of cathepsin B in the aphid lineage (Houseman and Downe 1983). Cathepsin B proteases were also identified in these leaffeaters and may show similar duplication. Cleavage of food proteins into peptides and amino acids is an important process for which an array of proteases of different substrate specificity and enzymatic activities are produced in the alimentary tract and are involved in protein digestion. Annotation of these data advances current understanding of leaffeather biological pathways while providing clues to the genetic basis of such processes in insect/pathogen, and insect-plant interactions (Fig. 2, 3). The availability of genomic and EST data for these leaffeaters continues to increase, thus uses of the current data provides a solid foundation for future studies in leaffeather functional genomics.

CONCLUSIONS - The information gained from this study provides the first investigation using comparative genomics of the transcriptome from three leaffeather vectors of Pierce’s disease of grapes: H. vitripennis, G. atropunctata, and O. nigricans. Amino acid sequence comparisons BLASTX, BLASTP with other known proteins relies on conserved motifs of specific domain(s), NCBI GenBank. In silico comparative analyses continues to be a valuable tool to identify proteins and their functional domains as widely accepted method to address many biological questions.

Fig. 2. Sequence Distribution: Molecular Functions. Categories had to have at least 50 members. Represents EST’s from three CDNA libraries, Adults, 5th instar, and Midgut. Homalodisca vitripennis, (BLAST2GO analysis). Highest Categories in descending order: Ribosome structure, 296, Calcium ion binding, 219, ATPase activity, 154, Actin binding, 134, Microfilament motor activity, 109, Protein Kinase activity, 107.

Fig. 3. Sequence Distribution: Cellular Component. Categories had to have at least 20 members. Represents EST’s from three CDNA libraries, Adults, 5th instar, and Midgut. Homalodisca vitripennis, (BLAST2GO analysis).

Many of the discoveries made in other insects, such as Drosophila, and Honey Bee, can be applied when the same generic transcripts can be identified. For example we increased our understanding of the roles and pathways of heat shock proteins in leaffeaters by examining the data completed in Locusts, Flies, and Nematodes. The same is true for digestive enzymes. The increasing application of transcriptional data is leading the way in the development of new strategies to reduce plant diseases and their insect vectors. Application of RNAi against a wide range of insect species from spruce budworm to whiteflies are viewed as the future in insect pest control, and many new methods which incorporate the use of native endophytic bacteria and/or viruses as the mechanism for delivery or expression of dsRNA within plants are being widely evaluated. The main advantages of applying genomic data in this manner to solve agricultural problems is that the plants are not ‘transformed’, thus the quality of the crop is not altered, saving time, money, and reducing the effort needed to find solutions to many emerging devastating agricultural problems.

