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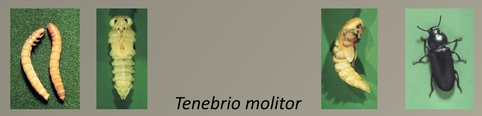
Strategies to Monitor and Reduce Resistance to *Bacillus thuringiensis* among Targeted Insects Symposium

Snapshots of Gene Expression in Cry3Aa-intoxicated *Tenebrio molitor* larvae



Tenebrio molitor
Stored Product Insect Image Database
(Flinn and Oppert)

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Why study Cry3Aa intoxication in *T. molitor?*

- One of the primary interfaces of an insect with its environment is through the gut.
- Understanding gene expression profiles in the insect gut can provide understanding of interactions with the environment as well as identify novel control methods for pests.
- The first sequence of a coleopteran genome was from the genetic model *Tribolium castaneum*, and our microarray study provided expression data for gut transcripts (Morris et al., 2009).
- However, *Tenebrio molitor* lacks a sequenced genome, so we obtained gene expression data via 454 pyrosequencing and microarray analysis of the larval gut transcriptome.

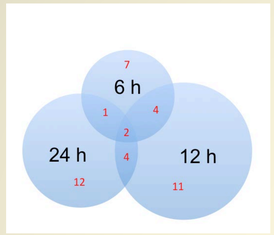
RNA-Seq Design

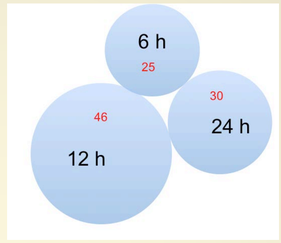
- *T. molitor* larvae (approximately 1 mo old) were placed on control diet (85% wheat germ, 10% wheat flour, 5% brewers yeast) or the same diet containing 0.1% Cry3Aa toxin (Bt *tenebrionis*). After 24 h, 23-26 larvae were sacrificed and guts were collected in RNA later.
- Gut tissues were processed to obtain total RNA (RNeasy, Invitrogen, Carlsbad, CA) and samples were shipped to 454 Life Sciences for processing. Poly+RNA was purified (Invitrogen) and fragmented. First strand cDNA was prepared with Superscript II (Invitrogen), and directional adaptors were ligated for clonal amplification and sequencing on the Genome Sequencer FLX pyrosequencing system.
- All reads from control and treated larvae were combined into individual dataets for analyses. Sequences were compared to other insect genomes; tentative functions were assigned to gene products; RNA-Seq (i.e., counting numbers of reads associated with each gene product) was used to compare gene expression in control and Cry3Aa-treated larvae.

Microarray Design

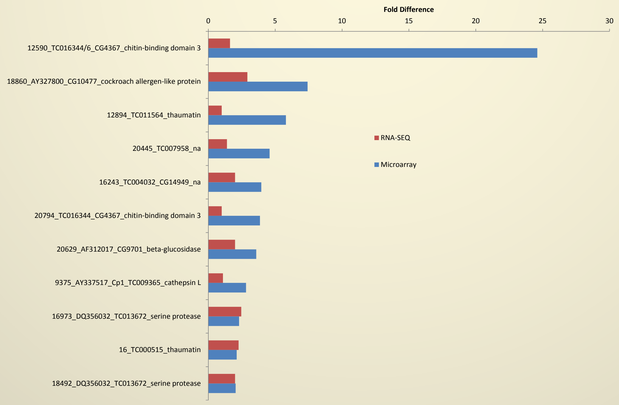
- A custom *T. molitor* microarray was developed using the uniESTs generated from the second assembly, with eArray software used for probe design (Agilent Technologies, Inc., Santa Clara, CA USA).
- Of the 25,201 uniESTs, 23,671 oligos were selected as unambiguous (with the program selecting specific oligos representative of each contig) and were arrayed in duplicate or triplicate on a custom array chip (4x44K, Agilent Technologies, Inc.), incorporating standards supplied by eArray.
- Newly molted *T. molitor* larvae (approximately 1 mo old and mean mass of 5–6 mg) were selected from a standard laboratory colony (reared on 50% rolled oats, 2.5% brewer's yeast, 47.5% flour) and were starved overnight.
- Larvae were placed on a diet of 85% stabilized wheat germ, 10% flour, and 5% brewer's yeast with a 3% dilution of a concentrated solution of FD&C Blue #1 and 97% of a mixture of 85% stabilized wheat germ, 10% flour, 5% brewer's yeast with or without 0.1% (w/w) Cry3Aa (pre-equilibrated at 28ºC, 75% R.H. over saturated sodium chloride).
- Larvae were monitored for blue guts every 30 min, and those with blue guts (containing either protoxin or not) were selected and reared further on diet containing protoxin or control diet, respectively, for 6, 12, or 24 h. Each corresponding time interval had a separate control and treatment. For each treatment, midguts from four larvae were dissected under and into RNA*later*® (Qiagen, Valencia, CA USA). A biological replicate was with larvae from a different oviposition tray.
- mRNA was obtained from each treatment and was labeled with colored dyes and hybridized to the custom array.
- Hybridized probes were measured for relative intensity, and treatment groups were compared.

Number of genes significantly enriched (left) or repressed (right) in intoxicated *T. molitor* larvae exposed to Cry3Aa for 6, 12, or 24 h.

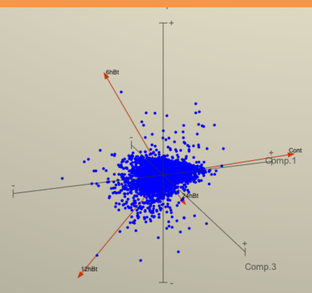




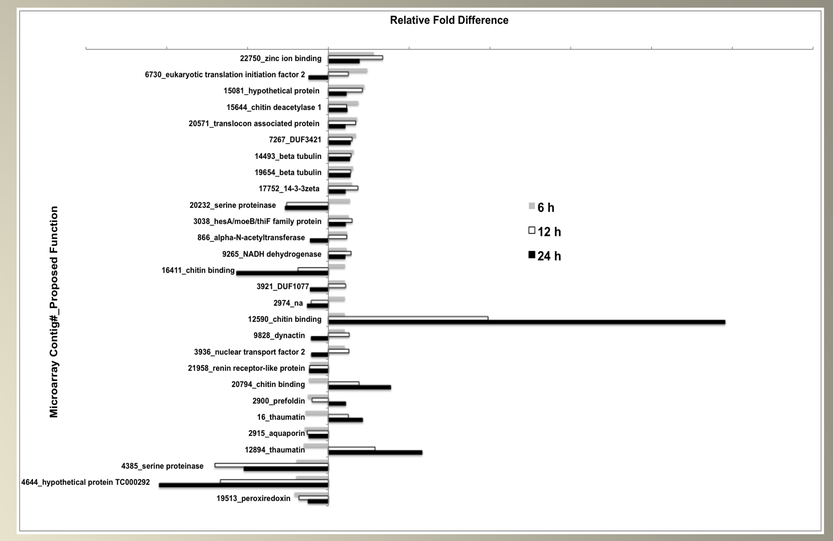
Comparison of increased gene expression in Cry3Aa-intoxicated *T. molitor* larvae, as determined by microarray and RNA-Seq (24 h Cry3Aa exposure).



Principle Component Analysis of microarray data demonstrated that gene expression in larvae exposed to Cry3Aa for 24 h was more similar to control, whereas 12 h-exposed larvae were most different from all treatments.

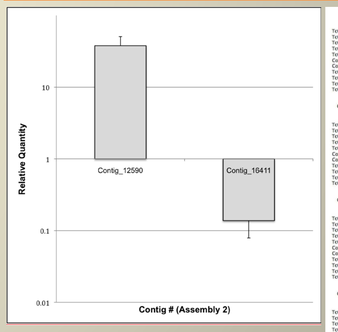


ANOVA of differential gene expression in *T. molitor* larvae exposed to Cry3Aa for 6, 12, or 24 h ($p<0.05$).



Relative expression of CBD3 transcripts in Cry3Aa-treated *T. molitor* larvae.

In microarray and RNA-Seq analyses, transcripts encoding proteins with a chitin-binding domain 3 (CBD3) were the most enriched and repressed in 24 h Cry3Aa-intoxicated larvae. Therefore, we used quantiative PCR to confirm that contigs representing these transcripts with both increased (Contig_12590) and decreased (Contig_16411) when larvae were exposed to Cry3Aa (upper left panel). CBD3 proteins are highly conserved among bacteria, plants and animals, but their function is unknown. In the related tenebrionid *Tribolium castaneum*, there are nine CBD-3 genes, seven in tandem on chromosome 7, which have homology to *T. molitor* CBD-3 transcripts at the gene (lower left) and predicted protein level (below).



Conclusions

- As transcriptome sequencing is becoming more economical, we have demonstrated that it can be combined with gene expression to address biological questions.
- RNA-Seq and microarray analysis can be used for validation.
- Cry3Aa induces multiple changes in the *T. molitor* transcriptome.

Future Directions

- Transcriptome sequencing will be used to provide additional data to understand gut physiology in coleopteran pests.
- Questions to address:
  - What are these prevalent and highly conserved CBD3 proteins in the insect gut?
  - Why are they differentially expressed in response to Cry3Aa intoxication?