# Effect of Cry3Aa toxin on gut peptidases expression levels in *Tenebrio molitor larvae*

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Worldwide agriculture has to deal with the everyday problem of conserving crops and their stocks from insect pests. Most of synthetic insecticides are not safe for the environment as well as human health, and with regular use can lead to adverse effects. Safe bioinsecticides are highly advantageous compared to their chemical analogues. One of the most successful methods of safe protection (~ 2% of the market share of insecticides) are delta endotoxins originating from *Bacillus thuringiensis* bacteria (Bt). They are completely harmless to human and highly specific to target insects from orders Lepidoptera, Diptera and Coleoptera.

High efficiency of Bt-transgenic plants has led to the rapid growth of their popularity, especially in developing countries where they represent a large share of the crops. Such a large scale of cultivation has led to a significant increase in the selective pressure on populations of the target pests, leading to appearance of resistant strains. In most cases, toxin resistance was achieved due to changes in one of the steps of the protoxin activation in insect gut – variations in gut peptidases set, changes of gut pH, as well as with the mutations of toxin-binding receptors, which led to a decrease in their affinity [1 2, 3, 4]. Larvae of *T. molitor*, a common stored grains pest and widely-used laboratory insect, are known to be тавьsusceptible to Bt-toxin Cry3Aa.

This study is devoted to the analysis of the effect of Bt Cry3Aa toxin on the

expression of gut mRNA in the larvae of *Tenebrio molitor* (Coleoptera: Tenebrionidae). Preparation of biological material, cDNA sequencing and contigs assembly was performed as described in [5]. We received 3 parallel control cDNA sequence reads arrays synthesized on the *T. molitor* larvae gut mRNA (mRNA Seq, Illumina HiSeq 2000), 3 parallel arrays of reads from the guts of larvae treated with the control Bt toxin Cry1Ac, shown to be highly toxic to representatives of Lepidoptera order, and 3 gut read arrays from the larvae intoxicated with Cry3Aa for 12 h. Using the BLAST algorithm (blastn - http://blast.ncbi.nlm.nih.gov/Blast.cgi), custom script and modified blast2sam.pl script (from the package samtools - http://samtools.sourceforge.net/) were used to refine and build consensus sequences of cysteine peptidases form the C1 papain family and serine peptidases from S1 chymotrypsin family [6]. Expression levels of the obtained sequences were evaluated by calculating RPKM (reads per kilobase per million mapped reads) [7]. The data obtained for 12 h intoxication period were compared with a small set of data obtained previously for 24 h intoxicaton of *T.molitor* larvae with Cry3Aa [8].

The analysis of changes in expression levels of *T.molitor* larvae gut peptidases has led to the following results:

1. 13 out of the 29 *T. molitor* larvae gut cysteine peptidases after 12 h of exposure to Cry3Aa toxin showed a statistically significant increase in mRNA expression levels. mRNA expression levels of 2 low expressed peptidases changed drastically (33-fold), while the remaining increased only 2-4-fold. The mRNA expression level of the main digestive cathepsin L1 did not change, whereas the expression level of digestive cathepsin B1 increased 2.8-fold. After 24 h of intoxication the mRNA expression levels of the majority of cathepsins decreased.
2. 32 out of 87 active serine peptidases' mRNA expression levels showed a statistically significant changes after 12 h exposure to Cry3Aa toxin. Most of highly expressed peptidases' expression levels decreased, whereas medium- and low-expressed mRNAs showed increased levels. The expression levels of the major digestive trypsin and chymotrypsin decreased 4 and 2.7-fold, respectively. The increased and decreased expression levels showed the same tendency after 24 hours of intoxication.

3. Among inactive serine peptidases homologs, in 74 cases out of 104 the 12 h exposure to Cry3Aa toxin has led to statistically significant changes in levels of mRNA expression. The expression level of the majority of homologs (64 of 74) decreased, and the impact of intoxication is significantly stronger on homologs than on active peptidases (up to 91-fold decrease).

4. The control Cry1Ac toxin did not cause any significant changes in the expression levels o*f T. molitor* larvae peptidases mRNA and is suitable for the use in the control experiments in studies of the effect of toxins on the larvae of *T. molitor*.

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1. Oppert B., Kramer K.J., Beeman R.W., Johnson D.E., McGaughey W.H., 1997. Proteinase-mediated insect resistance to *Bacillus thuringiensis* toxins. J Biol Chem.; 272(38):23473-6.

2. Karumbaiah L., Oppert B., Jurat-Fuentes J.L., Adang M.J., 2007. Analysis of midgut proteinases from *Bacillus thuringiensis*-susceptible and -resistant *Heliothis virescens* (Lepidoptera: Noctuidae). Comp Biochem Physiol B Biochem Mol Biol; 146(1):139-46.

3. Ferré J., Van Rie J., 2002. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. Annu Rev Entomol.; 47:501-533.

4. Xie R., Zhuang M., Ross L.S., Gomez I., Oltean D.I., Bravo A., Soberon M., Gill S.S., 2005. Single amino acid mutations in the cadherin receptor from *Heliothis virescens* affect its toxin binding ability to Cry1A toxins. J Biol Chem.; 280(9):8416-8425.

5. Martynov A.G., Elpidina E.N., Perkin L., Oppert B., 2015. Functional analysis of C1 family cysteine peptidases in the larval gut of *Tenebrio molitor* and *Tribolium castaneum*. BMC Genomics, 16:75.

6. Rawlings ND, Walle M, Barrett AJ, Bateman A. MEROPS: the database of proteolytic enzymes, their substrates and inhibitors. Nucleic Acids Res. 2014;42:D503–9. doi:

10.1093/nar/gkt953

7. Mortazavi A., Williams B.A., McCue K., Schaeffer L., Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods. 2008 Jul;5(7):621-8.

8. Oppert B., Martynov A.G., Elpidina E.N., 2012. *Bacillus thuringiensis* Cry3Aa protoxin intoxication of *Tenebrio molitor* induces widespread changes in the expression of serine peptidase transcripts. Comp. Biochem. Physiol., 7D: 233–242.