RNA interference in *Manduca sexta* induced by double-stranded RNA feeding

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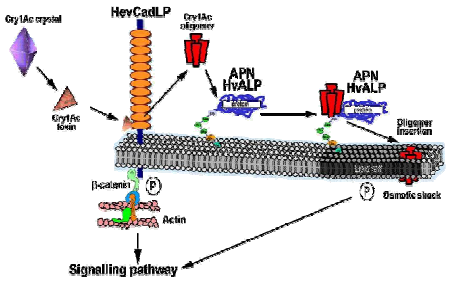
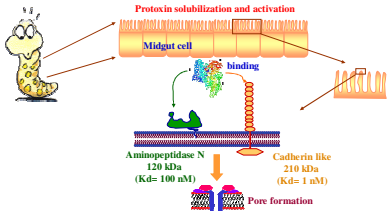
***Bacillus thuringiensis*, is a Gram positive bacterium, produces various insecticidal proteins called Cry toxins. These bacteria are used as microbial insecticides and for the genetic development of insect-resistant plants, because they are specific to their target insects. The mode of action of Cry toxins is a multi-step process that involves the interaction of several receptor molecules leading to membrane insertion and lysis of cells. Binding molecules include cadherins, aminopeptidases, alkaline phosphatases, and glycosphingolipids. The characterization of receptor molecules in susceptible insects pests will be vital in order to fully understand the mode of action of Cry toxins, and for the development of novel toxins with unique specificities and to increase the use of these toxins.**

RNA interference represents a breakthrough technology for conducting functional genomics research. This study investigated RNAi via voluntary feeding in the lepidopteran *Manduca sexta*, our insect model. We used double-stranded (ds) RNA feeding approach to silence cadherin gene, that is a key receptor, and serve as the initial contact site for toxin. Contrary to results from previous studies that examined injection-based RNAi, silencing of cadherin gene through dsRNA feeding led to significantly reduced expression and mortality. Cadherin silencing led to resistance of the larvae to Cry1Ab toxin effect. These results validate *in vivo* the roll of cadherin in the mechanism of action.

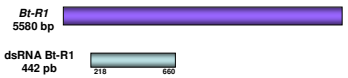
Mode of action of Cry1A toxins from *Bacillus thuringiensis*



After activation the Cry toxins bind to specific receptors



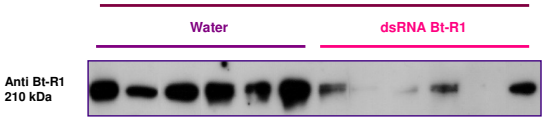
dsRNA fragment



Experiment 1: dsRNA injection

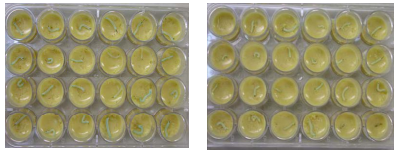
Experiment 2: dsRNA feeding

Injection

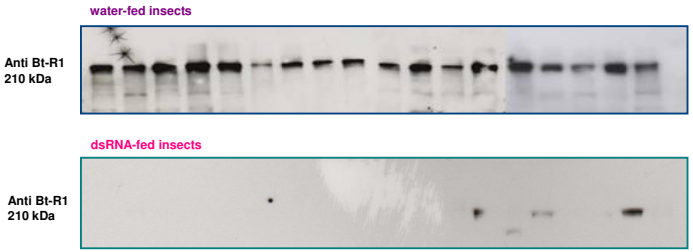


Effect of dsRNA injection on cadherin protein expression in *M. sexta* larvae. Western blott was tested for the *M. sexta* cadherin protein (Bt-R_1) that binds Cry1Ab toxin using anti Bt-R_1 antibody. Lanes 1-6: larvae injected with water only. Lanes 7-12: larvae injected with 100 ng dsRNA Bt-R1.

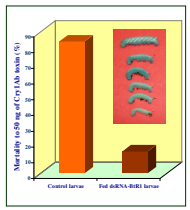
Diet without toxin **Diet with 20 ng of Cry1Ab toxin**



Effect of dsRNA injection dsRNA Bt-R1 on susceptibility of *M. sexta* larvae against Cry1Ab toxin. Larvae were resistant to the intoxication effect.



Effect of fed dsRNA on cadherin protein expression in *M. sexta* larvae. Western blott was tested for the *M. sexta* cadherin protein (Bt-R_1) that binds Cry1Ab toxin using anti Bt-R_1 antibody.



Effect of feed dsRNA Bt-R1 on susceptibility of *M. sexta* larvae against Cry1Ab toxin. Larvae were resistant to the intoxication effect with 50 ng of Cry1Ab toxin.

Conclusions:

Cadherin silencing induced by injection of dsRNA-BtR1 was not efficiently and is an invasive methodology.

We show that feeding of dsRNA to *M. sexta* larvae can knockdown the expression of genes expressed in the larval midgut that are involved as receptors of Cry toxins.