FINAL PROJECT REPORT

Project Title: Identification of Bt toxin targets in codling moth larvae

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Total Project Funding:

Budget History:

Item	**Year 1: 2007**	**Year 2: 2008**	**Year 3:**
Salaries			
Benefits			
Wages	6,240		
Benefits	150		
Equipment			
Supplies	28,110		
Travel	500		
Miscellaneous	5000		
Total	$40,000	0	

ORIGINAL OBJECTIVES

The specific objectives of this proposal include:

1) Determine the potencies of 10 Bt toxins against codling moth larvae and cell line.
2) Determine the mode of action of the most potent Bt toxins.
3) Identify key molecules affected by the bioactive Bt toxins
4) Clone transcripts encoding the key molecules affected by Bt toxins
5) Develop a cell-based assay system to search for novel insecticides that alter key molecules affected by Bt toxins

Revised Objectives converting original objectives from a 3 year to 2 year time line as requested by Jim McFerson. Additionally, year 2 funds were not requested due to unexpected

Year 1

1) Determine the toxicity of Bt toxins against codling moth larvae and a codling moth cell line
2) Using the codling moth cell line as a model, determine Bt toxin effects on signal transduction pathways using established assays that monitor chemical signals and cell response.
3) Determine Bt toxin membrane receptors in codling moth larvae and cell line.

Year 2

1) Determine the effects of Bt toxins on signal transduction pathways in codling moth larvae.
2) Identify the key molecules affected by the bioactive Bt toxins.
3) Clone genes encoding key signal transduction proteins affected by the Bt toxins.

SIGNIFICANT FINDINGS (ACCOMPLISHMENTS)

- Growth conditions for bacteria expressing Bt toxins were optimized.
- Toxin purification methods were developed.
- Toxicity of 7 Bt toxins against codling moth was obtained.
- Effects of Bt resistance on sex pheromone perception in *Helicoverpa zea* was determined.
- A G-protein that potentially mediates signal transduction of odorant receptors in codling moth was cloned and sequenced. This G-protein is a potential Bt toxin target.

RESULTS AND DISCUSSION

The crystal (Cry) protein toxins produced by the bacterium *Bacillus thuringiensis* (Bt) are used to control pest insect species in the Orders Diptera (flies and mosquitoes), Coleoptera (beetles), and Lepidoptera (moths). The Bt toxins produced with lepidopteran activity mainly belong to the Cry1 family. Cry1 toxins have been used for years to control moth larvae either in formulations or in transgenic crops. Because of the life cycle of codling moth, Bt formulations have not been an effective means of control in the orchard. However, Cry1 proteins toxic to codling moth can be used as tools to discover new targets for insecticide development.

Cry1 toxin growth, purification and potency toward codling moth larvae

An initial hurdle facing this project was the preparation of Cry1 toxins. Bacterial strains expressing Cry1 proteins were obtained from the Bacillus stock center (Columbus, OH). In our hands, these bacterial strains did not produce the vast quantity of toxin needed for this study. Eventually, we determined optimal media and growth conditions to produce enough toxin for our studies (data not shown). Additionally, to obtain pure toxin required advanced chromatography conditions, which were also optimized for purification of large amounts of Cry1 proteins. Once these

hurdles were overcome, experiments determining Cry1 potency toward codling moth were able to proceed. Table 1 summarized the potency of Cry1 toxins toward codling moth larvae. The rank order of potency was Cry1Da > Cry1Ac > Cry1Aa > Cry1Ab > Cry1Fa > Cry1Ba >>>>Cry1Ca (see Table 1). Currently, we are determining Cry1 toxicity toward two insect derived cell lines, one from codling moth, and the other from *Trichoplusia ni*. Our goal is to use the cell lines as a model system to study Bt toxin mode of action.

Table1. Toxicity of Various Bt toxins against neonate Codling moth larvae

Toxin	***LC_{50} (95% fiducial limits)[a]***
Cry1Aa	23 (14–33)
Cry1Ab	25 (14–38)
Cry1Ac	20 (8–34)
Cry1Ba	35 (25–46)
Cry1Ca	>5000
Cry1Da	6 (4–8)
Cry1Fa	34 (4–74)

[a]Toxicity is indicated as LC_{50} in nanograms of toxin per gram of diet.

Resistance to Bt Affects Sex Pheromone Attraction to Males

A colleague who has a lab colony of *Helicoverpa zea* (corn earworm/cotton bollworm) that is resistant to the affects of Bt toxin observed that the males did not readily mate with females. Because our hypothesis is that Bt toxins exert their effects on signal transduction pathways, we started a collaborative effort explore the resistance mechanism of the Bt resistant *H. zea* colony. Males of the Bt resistant *H. zea* line do not recognize females. We have verified this observation using synthetic pheromones in the flight tunnel (Table 2). For control insects (a Bt susceptible lab colony), 41 of 50 males flew to and made contact with a synthetic sex pheromone lure in a flight tunnel (Table 2). However, only 9 of 34 males flew to and made contact with the same lure in the flight tunnel (Table 2). Because of our previously WTFRC funded project on pheromone receptors in codling moth, we were able to examine the chemosensory system in *H. zea* males. We have so far determined that a member of the pheromone receptor family expressed in Bt susceptible *H. zea* has not yet been detected in the Bt resistant colony (data not shown). Because a potential target of Bt toxins has been identified as a G-protein, we also tried to determine if it was being expressed in *H. zea*. The G-protein that potentially mediates signal transduction of odorant receptors in the antenna has not yet been detected in the Bt resistant colony of *H. zea*.

Table 2. Effects of Bt Resistance on Sex Pheromone Perception in *Helicoverpa zea* males

Colony	***# Flown***	***Wing Fan***	***Take off***	***Upwind***	***Midway***	***Close***	***Contact***
Bt Susc.	50	50	50	47	47	47	41
Bt res lab	34	31	26	25	25	18	9
Bt res field	19	15	13	11	11	11	9

Based on published results from *B. mori*, BmOR1 response to Bombykol and BmOR3 response to Bombykal are dependent on co-expression of BmOR2 (the ubiquitous receptor) and the olfactory specific G protein, BmGαq. Because of the potential importance of the G-protein in Bt toxin mode of action, I cloned the codling moth ortholog of the olfactory specific G protein, BmGαq. The deduced amino acid sequence from the cloned cDNA encoding the putative olfactory specific G

protein, CpGαq is shown in Fig. 1. The deduced amino acid sequence of the putative codling moth olfactory specific G protein is highly similar to those previously reported for *B. mori* and *Mamestra brassicae* with CpGαq sharing 97.3% identity and 98.2% similarity, and 96.4% identity and 97.6% similarity, respectively. A mammalian cell line expressing CpGαq has been generated and will be screened with Bt toxins to determine the effects.

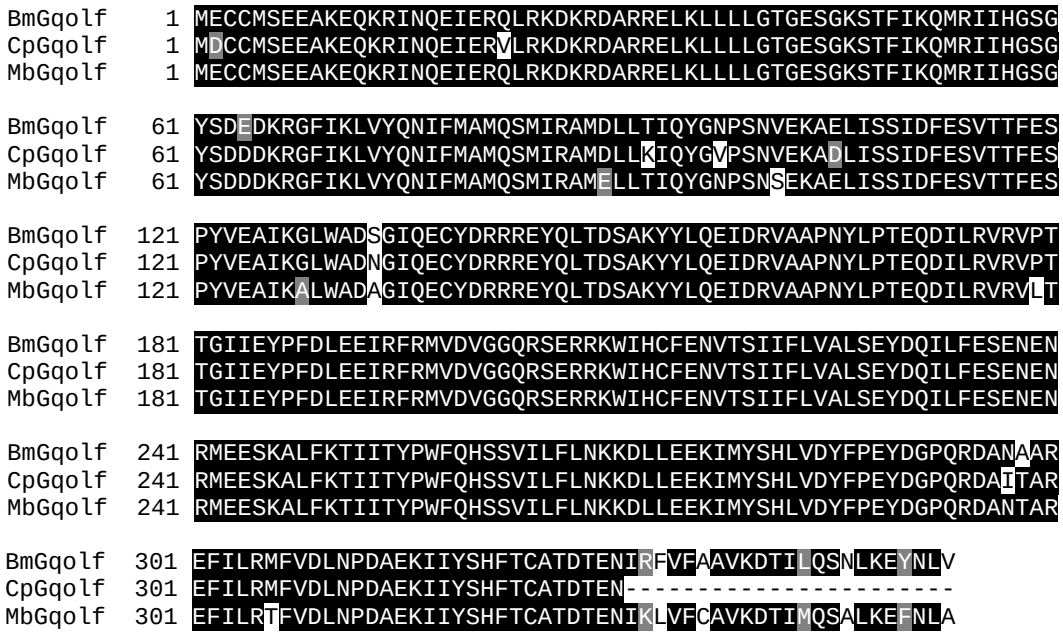

Fig. 1. Boxshade alignment of deduced amino acid sequences of putative olfactory specific Gq-like protein alpha subunits.

Future Work

Examining Bt toxin mode of action with insect cell lines and codling moth is still an active component of research in my laboratory. As such, this final report is still a work in progress as I believe that a further understanding of how Cry1 toxins kill codling moth will potentially provide other targets for insect control.

EXECUTIVE SUMMARY

Commercial formulations containing *Bacillus thuringiensis* Cry proteins have been used for more than 40 years to control lepidopteran larvae and most recently, transgenic plants expressing these protein toxins are being used in insect control programs. Codling moth larvae are susceptible to Cry proteins, but these toxins must be ingested to be effective making their use in the orchard difficult because larvae rapidly bore into and are protected by the apple. However, if we take advantage of the fact that codling moth larvae are susceptible to Cry proteins, a full understanding of the mode of action of these toxins may yield targets for the development of novel insecticides for use in the orchard.

Progress was made in the development of procedures to grow and purify toxins for use in mode of action studies in cell line assays and with codling moth larvae. Based on studies with a Bt resistant colony of *Helicoverpa zea*, Cry toxins may affect the signal transduction pathway involved in the detection of sex pheromones. An olfactory specific G-protein was cloned from codling moth antennae and is now expressed in a mammalian cell line so that further examination of the effects of Bt toxins can be monitored.

Future directions for this project include the development of the cell based assay system to explore the mode of action of Bt toxins in further detail. Because there are over 50 different Bt toxins that kill lepidopteran larvae, there is the possibility that many new proteins can be identified as potential targets for novel insecticide development. We hope that potential advances in this line of investigation will help make codling moth a recognized model organism for the development of biorational means of pest control.