Verification of Biopesticide Targets using RNA Interference and Fluorescence Microscopy with *Myzus persicae*

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1 Introduction

Recombinant fusion proteins containing toxins produced by insect predators (e.g. spiders) are being developed as biopesticides, an example of which is the omega-toxin, ω-ACTX-Hv1a. The toxin is fused to a carrier protein (GNA) which transports it across the insect gut, giving it oral toxicity.

It is hoped elucidation into the mode of action of the fusion protein can lead to its development as an environmentally sustainable and commercially viable product in the fight against crop losses to insect pests. At present, it is believed the toxin works by targeting the calcium ion (Ca^{++}) channels of the insect pests, interrupting normal neuronal signaling.

2 Aims

Verify the mode of action of the spider toxin fusion protein ω-ACTX-Hv1a. This will be achieved through the use of feeding experiments to:

- Demonstrate that the toxin requires a carrier protein to give it oral toxicity
- Evaluate the optimal dosage of toxin fusion protein
- Show, through fluorescence microscopy, localisation of the fusion protein in the gut of the insect pest *Myzus persicae* (aphids) using fluorescent labelling

3 Methods

The aphids were housed in small Petri dishes with access to the diet. Each treatment (BSA, GNA and FP5), had their own Petri dish. The aphids were fed on the fluorescently labelled protein diets for 24 hours before microscopy of a selected few from each of the three treatments. The remaining aphids were then fed on normal aphid diet, without the labelled proteins but under the same physical conditions, in chase feeding experiments for a further 24 hours before imaging.

Feeding experiments and microscopy

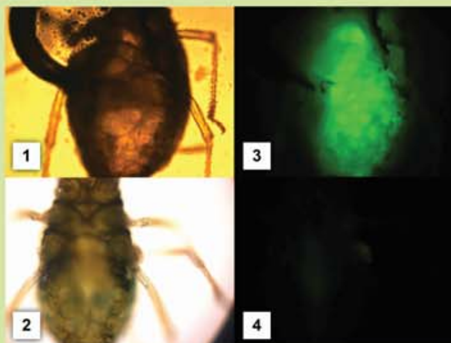
Images 1-12 are of aphids fed on fluorescent protein at 0.5 mg/ml concentration in the protein diet. Microscopy conditions of x100 magnification and x100 exposure for all images. Exposure on the bright-field images varied accordingly.

Images 1-4 were fed on BSA for 24 hours. 1 and 2 were imaged after 24 hours, 3 and 4 were imaged after a further 24 hours of chase feeding on normal aphid diet.

Images 5-8 were fed on GNA for 24 hours. 5 and 6 were imaged after 24 hours, 7 and 8 were imaged after a further 24 hours of chase feeding on normal aphid diet.

Images 9-12 were fed on FP5 for 24 hours. 9 and 10 were imaged after 24 hours, 11 and 12 were imaged after a further 24 hours of chase feeding on normal aphid diet.

4 Results

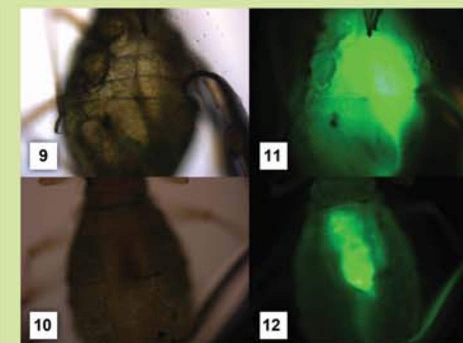


BSA made its way through the gut.

As expected, the BSA makes its way through the gut of the aphid and passes out. There is no evidence of localisation of BSA in the gut as it does not cross the gut wall. This is reflected in the reduction of fluorescence on the chase fed image 4, in comparison to the 24 feed image 2.

Some GNA crosses the gut wall

As the structure of GNA allows it to cross the gut wall, there is some localisation seen on the chased images as seen by the remaining fluorescence on image 8. If no GNA had crossed the gut wall, it would have instead been flushed through on chase feeding leaving little trace of fluorescence.



FP5 (omega-toxin) crossed the gut wall and irreversibly binds to Ca^{++} channels

There is a high level of fluorescence in image 12 meaning that a high concentration of the FP5 has crossed the gut wall and is localising. This is apparent upon comparison of images 10 and 12, where there is little reduction in fluorescence in the latter, compared with the former.

5 Brief Discussion

BSA does not cross the gut wall of aphids but instead passes through the gastrointestinal (GI) tract and out. This is observed through the chase feeding experiments whereby the BSA is washed through the GI tract.

GNA is the carrier molecule used to transport the omega-toxin across the gut wall. As such, this protein can cross the gut wall and does so upon feeding. It does not irreversibly bind to gut structures however, meaning some GNA is washed through on chase feeding.

FP5 can cross the gut wall because it is fused to its carrier protein GNA. Once across the gut wall, some localisation is observed. There is little washed through on chase feeding because it is believed that FP5 binds irreversibly to the Ca^{++} ion channels of the nerve cells in insects. There is a subsequent small reduction in fluorescence.

6 Future Research

For my 3rd year project, the potential "target" of the toxin will be investigated through an approach based on RNAi (gene silencing). Delivery of dsRNA by injection will be used to knock down the expression of one or more genes encoding a neuronal ion channels. The phenotype produced by gene knockdown will be assessed.

Acknowledgements

Many thanks to Professor Angharad Gatehouse and Doctor Martin Edwards for providing me with the opportunity to work on this project, and for the help during it. Thanks also to Newcastle University for providing the funding.

Newcastle University