Design and Development of Novel Biopesticides

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Aim:

To design and develop novel biopesticidal molecules through binding of the Segestritoxins (SFl) from the spider *Segestria florentina* (right) to Snowdrop lectin (Galanthus nivalis agglutinin: GNA).

Image sourced from: http://en.wikipedia.org/wiki/Segestria_florentina

Introduction:

To meet the demands of the growing population, food production must at least double by 2050 in order to feed an additional 2.3 billion people (FAO 2013). This can be achieved through two methods: increasing our agricultural footprint or increase the yield of existing crops.

As a result of insect activity 14% of crops by mass are lost (Ferry and Gatehouse 2010). Thus the use of pesticides could potentially increase the yield of our crops. However many of the major synthetic pesticides that are in use today are set to be banned in the European Union within the next few years, due to adverse effects on health and pollinator insects. Further many transgenic crops, such as Bt cotton do not target aphids which are a major pest insect. This means that new pesticides must be developed.

Biopesticides might be one solution to this problem. A biopesticide is an insecticidal molecule that is produced within a biological system, such as the yeast *Pichia pastoris*. The use of SFl/GNA fusion proteins (Fitches et al. 2004) have been shown to be effective against the larvae of *Lacanobia oleracea,* as GNA functions to carry the toxin to the haemolymph of the insect where it has a paralysing effect. The SFl family are highly specific and highly toxic to pest insects, yet have no effect on mammals (Fitches et al. 2004). Further the Hv1a/GNA fusion protein has been shown to have no significant effect on survivability of pollinator insects (Nakasu et al. 2014; Figure 1). This is relevant as both SFl and the Hv1a toxins target the calcium channels which are highly variable across the Insecta class.

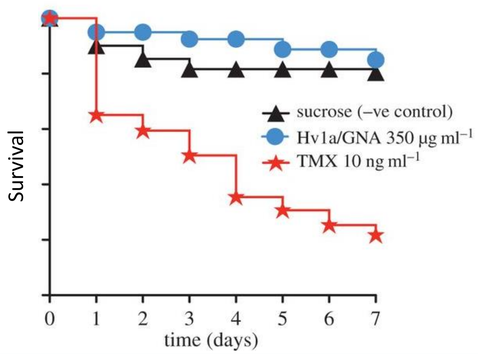


Figure 1. A feeding assay of honeybees. Honeybee survival was unaffected by daily dose of 21.7ug of Hv1a/GNA per bee. But a daily does of 0.727ng per bee of thiamethoxam (TMX) increased mortality. (n=40 bees per treatment. (Nakasu et al. 2014)

Thus the project was aimed at developing more fusion proteins based on the segestritoxin family, as they are highly conserved (Lipkin et al. 2002; Figure 2). Further we were to attempt to design a novel toxin, which swapped the first half of SFl 1 and SFL2 through the use of templateless PCR.

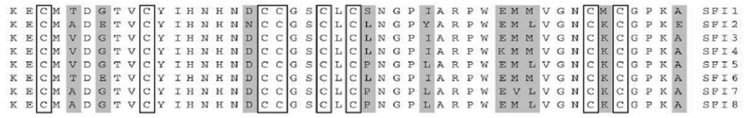


Figure 2. The amino acid sequences of the eight segestritoxins that were to be cloned into *Escherichia coli.* The variable regions are shaded to highlight the differences. (Lipikn et al. 2002)

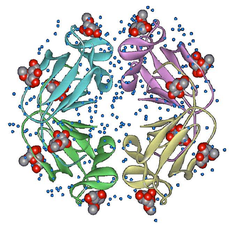
Methods:

Project A:

1. Design PCR primers for segestritoxin DNA sequences
2. Amplify segestritoxin DNA sequences using PCR.
3. Transform the sequences into *E. coli* in the PCR 2.1 plasmid vector. And grow over night, select positive clones and then grow overnight again.
4. Extract the plasmid vector from the *E. coli* and sequence to ensure correct transformation
5. Restrict out the sequence and ligate it into pGAPzα, which contains GNA.
6. Grow overnight and select the positive clones.
7. Extract the pGAPzα plasmid and transform it into *Pichia pastoris*
8. Grow for 120 hours, sampling and centrifuging 1ml every 24 hours
9. Run out the samples on SDS-PAGE gels to confirm protein expression.

Project B:

1. Design 14 short DNA sequences that would code for the whole synthetic sequence including GNA and the primers for amplification
2. Run the templateless PCR using the short sequences to produce the synthetic gene
3. Run a normal PCR to amplify the synthetic gene
4. Project B then follows the same methodology as project A. Starting at step 3.



The 3-D structure of the GNA molecule with all the sugar (Grey and Red circles) binding sites filled.

Image provided by Prof. Angharad Gatehouse

Results:

By the end of the project three fusion proteins, two of which were novel, were expressed in *P. pastoris.* The toxins that have been produced successfully were, SFl1/GNA, SFl3/GNA, SFl6/GNA. These can be seen on the SDS-PAGE gels, as bands at approximately 17kDa (Figure 3). However further testing is required to confirm the findings of the project. Further the gene assembly failed in project B thus no protein was produced.

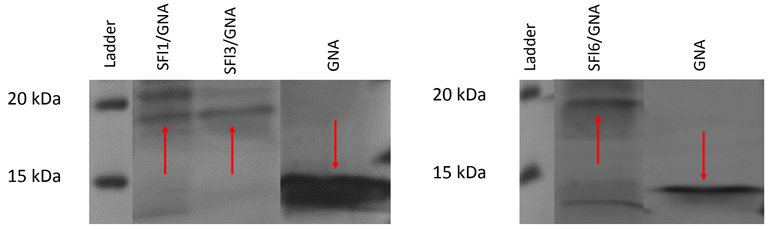


Figure 3. SDS-PAGE gel images, of the samples taken from during the yeast growth. The red arrows indicate the bands that correspond to the protein above the lane.

Discussion:

Whilst the project did not progress to the point where toxicity assays could take place, Fitches et al. 2004 looked at the toxicity of the SFl1/GNA fusion protein when incorporated into the diet of the *L. oleracea* larvae. Their study showed that GNA by itself had an insecticidal function but the SFl1/GNA fusion protein has a higher insecticidal activity (Figure 4). Due to the similarity between all of the toxins in the SFl family you would expect to see similar levels of toxicity as there is only very little variation between the different toxins (Figure 2). Further if there is a lack of toxicity when the fusion proteins are tested in injection and feeding assays, the differences could be attributed to the difference in amino acid sequences.

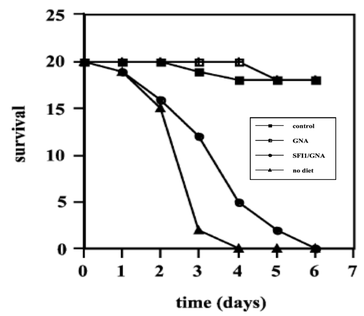


Figure 4. The toxicity of the different diet regimes given to *L. oleracea* larvae. The treatments were: No diet control; Control diet; diet containing GNA (5mg/g); and diet containing SFl1/GNA (2.5mg/g). N=20 larvae per treatment. (Fitches et al. 2004)

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