Novel Insect Control Molecules for Development of Biopesticides

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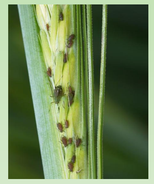
Aim

To investigate whether fusion proteins, comprising a spider toxin and a "carrier" molecule, cause aphid mortality.

Introduction

- The venom of the Australian funnel web spider *Hadronyche versuta* contains a neurotoxin (ω-hexatoxin-Hv1a) which specifically targets insect voltage-gated calcium channels, causing paralysis and death[2].
- Hv1a has limited oral toxicity as it cannot cross the gut epithelium, and therefore cannot reach its site of action which is the CNS.
- To overcome this, Hv1a is linked to snowdrop lectin (GNA) to form a fusion protein (Figure 1). GNA carries Hv1a across the gut epithelium to the CNS[1].
- The fusion protein is toxic towards many insect pests, including the grain aphid *Sitobion avenae*.
- The fusion protein has been modified (GNA/ModHv1a, GNA/Hv1a, ModHv1a/GNA) to improve yield.

https://en.wikipedia.org/wiki/Australian_funnel-web_spider



http://aphid.aphidnet.org/Sitobion_avenae.php

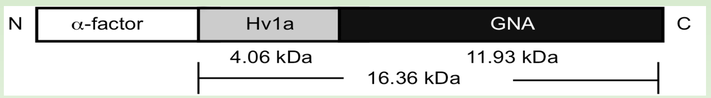


Figure 1. Original fusion protein construct showing the molecular masses of Hv1a and GNA[1].

Methods

Grow *Pichia pastoris* in YPD medium for 48 hours at 30 °C with constant shaking

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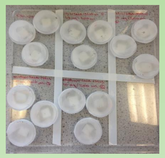
Centrifuge at 2000*g* for 30 minutes



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Analyse supernatant by SDS-PAGE and western blotting

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Purify protein using hydrophobic interaction chromatography

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Small-scale fermentation using *P. pastoris* clones

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Dialyse and freeze-dry purified protein

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Perform feeding bioassay on three day old *S. avenae* nymphs

Results

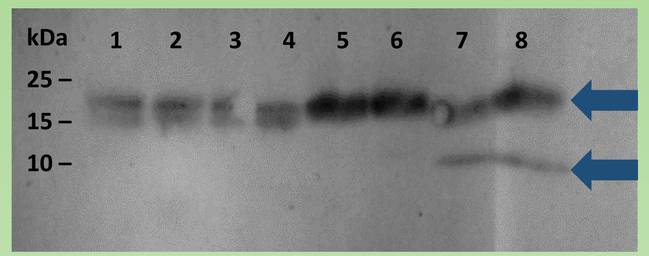

Figure 2. Western blot (using anti-GNA antibodies) showing fusion proteins expressed by *P. pastoris* after incubation at 30 °C for 48 hours. Lanes 1 and 2: His/GNA/ModHv1a, lanes 3 and 4: His/GNA/Hv1a, lanes 5 and 6: ModHv1a/GNA/His, lanes 7 and 8: Hv1a/GNA/His.

- Figure 2 confirms that the four fusion proteins were among the many proteins secreted by *P. pastoris*.

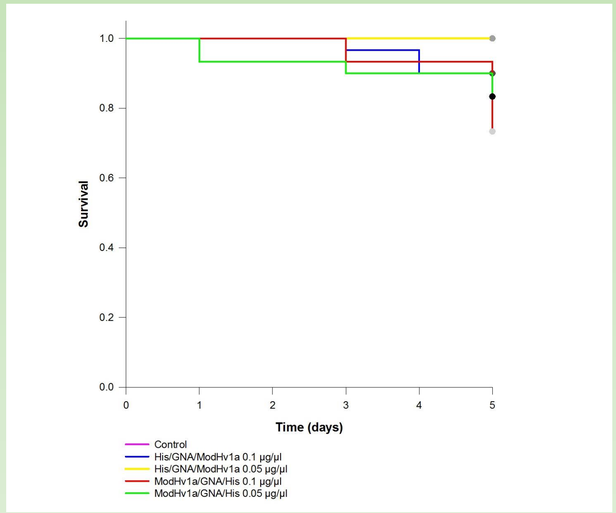

Figure 3. Survival analysis of three day old *S. avenae* nymphs fed an artificial diet every two days containing either His/GNA/ModHv1a or ModHv1a/GNA/His at two concentrations (0.1 and 0.05 µg/µl) over a five day period (n=30). Control nymphs were fed artificial diet only.

- Figure 3 shows that ModHv1a/GNA/His at a concentration of 0.1 µg/µl was the most successful at causing mortality.

Discussion and Conclusions

- SDS-PAGE and western blot analysis showed that the fusion proteins were successfully expressed and secreted by *P. pastoris.*
- However, the original fusion protein (Hv1a/GNA/His) showed signs of cleavage (Figure 2). Pyati et al. (2014) found that Hv1a/GNA was subject to proteolysis when it was being produced in *P. pastoris* as there is a cleavage site between Hv1a and GNA.
- The modified fusion proteins (His/GNA/ModHv1a, His/GNA/Hv1a and ModHv1a/GNA/His) remained intact as the cleavage site had been removed by altering the amino acid sequence.
- Of the two modified fusion proteins that were used in the bioassay, ModHv1a/GNA/His was more successful at causing mortality. However, there was not a significant difference between either ModHv1a/GNA/His or His/GNA/ModHv1a and the control.
- In conclusion, ModHv1a/GNA/His was more successful at causing mortality than His/GNA/ModHv1a at both the higher and lower dose.

Further Study

- Carry out the bioassay over a longer time period and increase the concentration of the toxin.
- Measure growth (weight or length) and fecundity.
- Perform the bioassay on other insect pest species such as the pea aphid *Acyrthosiphon pisum.*
- Look into using toxins from other sources such as the cone snail.

https://en.wikipedia.org/wiki/Conus

References

1. Fitches E, Pyati P, King G F, Gatehouse J A (2012) Fusion to snowdrop lectin magnifies the oral activity of insecticidal ω-Hexatoxin-Hv1a peptide by enabling its delivery to the central nervous system. PLoS One. DOI:10.1371/journal.pone.0039389

2. Pyati P, Fitches E, Gatehouse J A (2014) Optimising expression of the recombinant fusion protein biopesticide ω-hexatoxin-Hv1a/GNA in Pichia pastoris: sequence modifications and a simple method for the generation of multi-copy strains. Journal of Industrial Microbiology Biotechnology 41:1237-47

E.T. acknowledges support from Newcastle University Vacation Scholarship.