Identification of Biopesticide Targets in the Pea Aphid using RNA Interference

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Introduction

The pea aphid *Acythosiphon pisum* (Fig. 1) is an ecologically important crop pest (Fig. 2). It causes direct feeding damage but is also a vector of more than 30 plant diseases including viruses of beans, peas and clover[1]. The Bean leaf roll virus (BLRV) and the Pea enation mosaic virus (PEMV) are among some significant diseases.

Figure 1. *A. pisum* feeding on a host plant.

A. pisum has an obligate mutualistic association with *Buchnera aphidicola* bacteria. The genome of *Buchnera* contains genes involved in the biosynthesis of essential amino acids required by the host (Fig. 3).

The aim of this project was to knock down (reduce activity) the genes *ilvC* and *ilvD* from the *Buchnera* genome (Fig. 2) using RNA interference to prevent biosynthesis of the amino acids Valine, Leucine and Isoleucine (Fig. 2), and to cause aphid fatality.

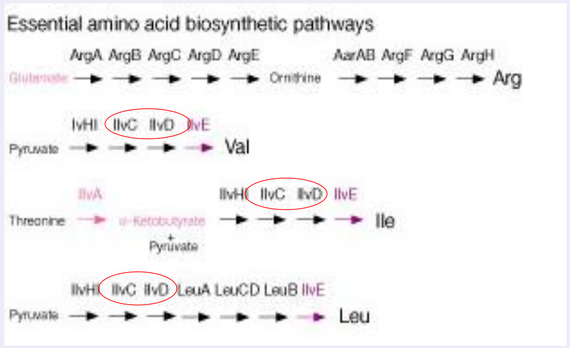

Figure 3. Essential amino acid biosynthetic pathways in *Buchnera*[2]. Arrows indicate steps catalysed by the enzyme named and the genes *ilvC* and *ilvD* of interest are circled in red.

Methods

RNA was extracted from freshly killed aphids, converted into cDNA and run in a PCR with ilvC and ilvD forward and reverse primers.

ilvC and ilvD PCR products were transformed into plasmid vectors and then grown up in *Escherichia coli* using blue/ white selection.

Clones were grown overnight in agar broth and the genes were re-isolated using a Miniprep and EcoR1 digests.

Genes were sequenced and then amplified in PCR with T7 primers, purified and converted into dsRNA using a MEGAscript kit.

The aphid diet was made up with one batch missing corresponding amino acids to the genes of interest.

Bioassays (Fig. 4) were run for 5 days with two doses (10ng/µl and 100ng/µL) of each dsRNA sample (ilvC and ilvD), plus 200 µl diet and a positive and negative control.

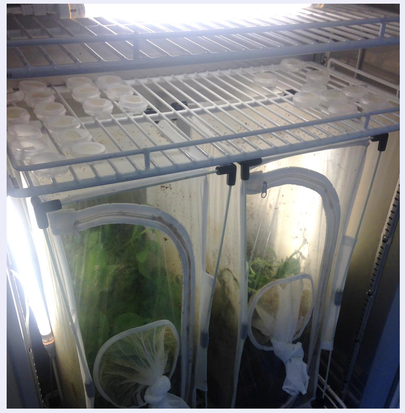

Figure 4. Bioassay trials of aphid nymphs in an incubator.

Results

- Nymph aphid survival differed significantly between the positive control (aphid diet) and treatments ($p<0.001$) (Fig. 5).
- Nymph aphid survival differed significantly between the negative control (water) and treatments ($p<0.001$) with both doses of ilvC dsRNA (Fig. 5).
- Nymph aphid survival did not differ significantly between the negative control (water) and treatments with dose 10ng ($p=0.350$) and dose 100ng ($p= 0.0913$) of ilvD dsRNA (Fig. 5).
- There was a significant difference in aphid nymph survival between the two doses of ilvC dsRNA ($p<0.001$), but not between the dose 10ng and dose 100ng ($p=0.242$) of ilvD dsRNA (Fig. 5).

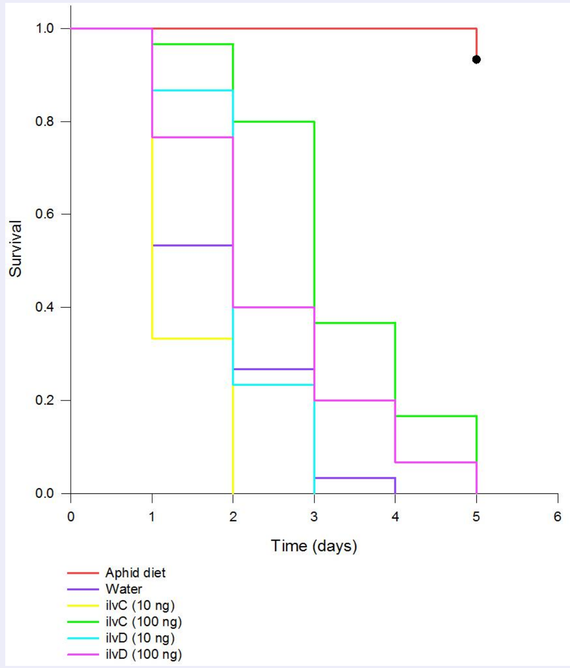

Figure 5. Survival rate of *A. pisum* under given treatments over 5 days.

Discussion

Both gene treatments (ilvC and ilvD dsRNA) resulted in increased fatality in aphids when compared with the positive control.

Neither dose (10ng or 100ng) of dsRNA seemed to have a greater significant effect across the two genes. Therefore, further investigation with different doses of dsRNA is suggested.

qPCR studies on surviving aphids are required to quantify transcription of the genes of interest following bioassays. This will give a better understanding of the effect the dsRNA treatments is having on amino acid biosynthesis in *Buchnera*.

Conclusions

Following further dosing investigations and qPCR analysis, there is potential for the use of ilvC and ilvD RNA interference in biopesticides.

Figure 2. *A. pisum* developing on a bean plant.

Bibliography

1. Emden HFV, Harrington R (2007) Aphids as crop pests.
2. Shigenobu S, Watanabe H, Hattori M, Sakaki Y, Ishikawa H (2000) Genome sequence of the endocellular bacterial symbiont of aphids Buchnera sp. APS. Nature 407:81-86.

Acknowledgments

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