Grower Summary

CP 082

Discovery and Development of New Phylloplane Bio-control Agents to Control Insect Pests

Final 2013

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GROWER SUMMARY

Headline

Key message: Novel aphid-killing biocontrol agents have been discovered that may have use for control of a range of aphids and for plant-growth promotion.

Background

Aphid and thrips pests cause major problems in horticulture, through physical damage of crops, deposition of sticky honeydew and the spread of viruses. With a reduction in available pesticides, predatory wasps and *Bacillus thuringiensis* (Bt) bacteria biocontrol agents are becoming increasingly important for control, although Bt resistance manifests rapidly and broadly within pest populations. There is therefore an urgent need for alternative control measures. The aim of this project is to use bioprospecting to identify novel biocontrol bacteria that can kill aphids and thrips and to characterize their efficacy and mode of action. We aim to understand the population dynamics of the bacteria during plant colonization to determine whether certain plants and /or growing conditions would help to proliferate or maintain the biocontrol bacteria. We are also aiming to identify the underlying cause of aphid killing. Ultimately, it is hoped that the novel biocontrol bacteria can be commercially developed.

Summary

We have adopted a semi-targeted approach to isolating and identifying candidate biocontrol bacteria. We hypothesized that plants that do not suffer from aphid pests, or have an ability to deter them, might not suffer from these pests due to a component of their microbial microflora. For example, bacteria may occupy the plant surfaces or exist as endophytes living within the plant tissue. We therefore isolated a range of bacteria (140 colony types) from eleven different plant species and then inoculated them into our novel aphid-feeding assay to identify bacteria that can kill aphids. From 140 strains tested, nine were found to be effective against six different aphid species: *Myzus persicae* (peach-potato aphid), *Aphis fabae* (pea aphid), *Brevicoryne brassicae* (brassica aphid), *Macrosiphum albifrons* (lupin aphid), *Nasonovia ribsnigri* (lettuice aphid) and *Aulacorthum solani* (glasshouse-potato aphid).

DNA sequence analysis was used to identify the nine bacteria isolated. Although some bacteria (eg *Escherichia fergusonii* and *E. albertii*) were undesirable due to them being related to opportunistic human pathogens, most of the bacteria were discovered to be

related to other apparently harmless environmental bacteria. To focus on the most effective bacteria to use in further experiments, a series of tests were done to discover which strains could be genetically manipulated and exhibited antibiotic sensitivity – these are key requirements for identification of toxin and virulence factors. The bacteria were also tested for their ability to kill other insects, an important test of host range as we do not wish to work on bacteria that might kill beneficial insects. Finally, we also tested whether bacteria applied to a plant surface would be ingested by an aphid – this test is important in the context of foliar application.

By assessing dose response and timing of killing, the most potent bacteria that killed the aphids are *Pseudomonas poae*, *Pseudomonas fluorescens* and *Citrobacter werkmanii*. All the bacteria were taken up by aphids from surfaces as well as from liquids, indicating they may be useful for foliar application. These bacteria also demonstrate antibiotic resistance, acceptance of plasmids and the ability to be mutated, which means all would be suitable for genetic manipulation to find the mode of action of aphid killing. This technique is intended to determine the mechanism behind the bacterial virulence, and is not intended to modify the bacterium for further use. To facilitate our studies, we have had the genomes sequenced *i.e.* to read the entire genetic blueprint of the bacteria of these three bacteria. We visited an insect control group in Spain led by Prof. Primitivo Caballero and in collaboration with them we discovered a range of insecticidal toxin genes in each of the genome sequences. The three strains were also tested in feeding assays with Lepidoptera caterpillars, but none of the strains were able to kill the insects, providing some hope that the toxicity is highly specific.

We had intended to carry out testing in thrips last year, but we were unable to do this due to logistical issues with ADAS. However, at the recent AAB meeting at EMR in November 2013, Dr Jean Fitzgerald informed us that she has a thrips colony and will happily collaborate with us – this is particularly important because of our dual interests in strawberry control. We have also contacted Prof. Ameur Cherif in Tunisia who has kindly agreed to collaborate with us such that we can gain access to their bee larvae pathogenicity test. This test allows us to culture our aphid-killing bacteria in the presence of bee larvae to determine if they can kill the larvae – this is a key test for determining safety and host specificity.

We have made a very exciting discovery in a recent experiment. In a previous small scale experiment, we had observed that plants treated with *P. poae* may deter aphids from colonizing. This has been upscaled to 3 plants each treated with water or the bacterial suspension and then placing the plants into an insect tent containing 10 aphids in growth sachet. The plants were monitored over 4 weeks and there appears to have been a preferential colonization, and subsequent replication, of aphids on plants treated with water. This effect lasted for three weeks. This suggests the aphids are deterred from colonizing the

plants treated with the bacterium when given a choice of an untreated control. We clearly need to repeat this experiment again with more plants, but if this proves to be a realistic result, then the bacterium may be useful as a deterrent agent, allowing growers to apply the bacterium at an early stage of plant growth and then reapplying.

In the last few weeks, we have also discovered that there may be a plant growth promotion effect provided by *P. poae*. Cells of this bacterium were inoculated into potting mix (containing peat) and pepper seeds sown into the soil. Over a period of 6 weeks, the plants were monitored for total plant weight, root length, and shoot length. Compared to a water control, the plants exhibited a 20-35% increase in each parameter, suggesting the bacterium may provide some beneficial property to the plant. This might show promise for using the bacterium as a biofertiliser.

Financial Benefits

Since this project holds more strategic value to gauge the potential for developing novel biocontrol products against aphid and thrips pests, the project remains at fairly early stage of fundamental science discovery. There is still much work to do in understanding the nature of the aphid-control, but several bacteria show great promise for development. We have recently been contacted by an SME who are interested in acquiring the IP on *P. poae* and developing it commercially. They have the equipment to allow them to grow the bacterium in bulk and produce different formulations. The University of Reading research and knowledge exchange office are involved for examining all the legal ramifications. With our industry contacts we hope to make connections that would allow us to test the aphid-killing bacteria within an industry setting. If the trials prove successful, then the bacteria could provide growers with a significant financial benefit in reducing losses due to aphids.

Action Points

In the last 8 months of her PhD project Amanda Livermore will carry out the following experiments:

1. Travel to East Malling Research to carry out thrips tests
2. Travel to Tunisia to carry out bee tests
3. Repeat plant growth promotion experiment on a larger scale
4. Repeat aphid deterrent experiment on a larger scale
5. Test the application of *P. poae* on aphids established on leaves for killing effects
6. Knockout the toxin genes in *P. poae* to elucidate their effect on killing