Using Molecular Techniques to Characterise and Quantify the Eubacteria Associated with the Cabbage Aphid (*Brevicoryne brassicae*).

Clark, E. L.[1,2]; Karley, A. J.[1]; Daniell, T. J.[1] and Hubbard, S. F. [1,2]

[1]Environment Plant Interactions, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA

[2] Division of Plant Sciences, University of Dundee at SCRI, Invergowrie, Dundee, DD2 5DA

Introduction

Aphids harbour a number of different bacteria that can have positive, negative or neutral effects on their survival and performance. The primary obligate endosymbiont of aphids is known as, *Buchnera aphidicola*, it is harboured by most aphid species and is essential for aphid survival. Four other types of secondary symbiont[1] and a number of other eubacteria including *Erwinia spp.* and *Pseudomonas spp.*[2] have been identified in pea aphid (*Acyrthosiphon pisum*) but very little is known about the diversity of eubacteria associated with other aphid species, particularly those which are important crop pests in the UK. The secondary symbionts of the pea aphid have been shown to confer various fitness traits, including resistance to parasitoid wasps[3], onto their aphid hosts and recent research suggests that the competence of aphids to vector a plant virus may depend critically on their eubacterial assemblage.

Aim

To devise molecular methods to characterise the eubacteria associated with a Scottish arable pest, the cabbage aphid (*Brevicoryne brassicae*) in order to test their impact on aphid fitness and aphid-parasitoid interactions.

Study System

The cabbage aphid *Brevicoryne brassicae* parasitised by the hymenopteran wasp *Diaeretiella rapae* on brussel sprout *Brassica oleracea* is the chosen study system (Figure 1). *B. brassicae* and *D. rapae* populations are collected in the summer of each year from a range of different field locations and *Brassica* types and clonal aphid lines with their associated parasitoid wasps reared in culture for study.

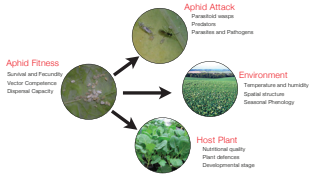

Figure 1: Aphid fitness and consequently vector competence will depend critically on the eubacterial assemblage and the abiotic and biotic conditions of the environment.

Methods

Eubacterial diversity in the selected aphid lines was analysed in the first instance using PCR and cloning and sequencing with a view to developing T-RFLP and Real-time quantitative PCR to screen the eubacterial complement of aphid lines.

DNA Extraction and PCR

DNA extracted from aphid samples from the different aphid lines, using a DNesay Blood and Tissue kit, was used as template for PCR amplification of a 2½ kb product incorporating the IGS (Inter-Genic Spacer) region between the 16S and 23S subunits (Figure 2). For most eubacteria other than *Buchnera* (the primary endosymbiont in aphids) the 16S gene is linked to the 23S gene by a region of IGS[5]. Amplifying this region using universal eubacterial primers specific to the two genes excludes the primary symbiont *Buchnera* and can be used as means of detecting the other eubacteria harboured by aphids. PCR screening revealed variation in the presence of a eubacterial PCR product in aphid lines suggesting interclonal variation in eubacterial complement (Figure 3).

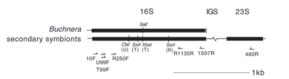

Figure 2: 16S-23S ribosomal subunits and the Inter Genic Spacer (IGS) region with the positions of the 10F and 480R general eubacterial primers used in this study.

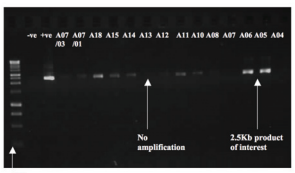

Figure 3: Gel image showing results from screening 15 aphid lines with the 16S-23S primers. In two aphid lines no PCR product amplified suggesting interclonal variation.

Cloning, Sequencing and Phylogenetic Analysis

PCR product from five different aphid lines was cloned using a Strataclone PCR cloning kit. Preliminary sequencing of clones to generate partial 16S rDNA sequences, using the 10F forward primer, indicated there were 15 different sequence types based on groupings on a maximum likelihood phylogenetic tree of the sequences. Consensus sequence was then generated for each sequence type by splitting the 16S subunit using internal sequencing primers (Figure 4). Another maximum likelihood tree was drawn which included with the consensus sequences examples, collated by BLAST searching the NCBI database, of other eubacterial types and insect secondary symbionts (Figure 5). The grouping of sequences on the tree suggests that cabbage aphid does not harbour the same secondary symbionts as the pea aphid but it does have a wide diversity of other eubacteria associated with it.

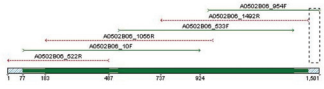

Figure 4: The entire 16S subunit was sequenced using six internal sequencing primers to generate a consensus strand for each sequence type.

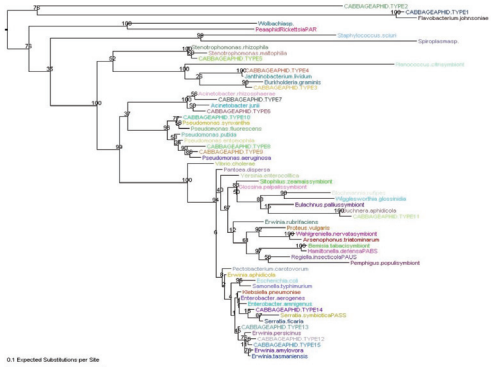

Figure 5: Maximum Likelihood Phylogenetic tree (RaxML+Cat Model) showing relative position of cabbage aphid sequence types in relation to eubacterial 16S sequences from the NCBI database.

Project Outcomes

Once the T-RFLP and Real-time PCR procedures have been designed and optimised they will provide a diagnostic tool for screening experimental aphid lines. Performance experiments and parasitism assays, to test the influence of the eubacterial complement on aphid fitness and resistance to parasitism, using aphid lines with known eubacterial complements will then be possible. By using a combination of molecular techniques and insect behavioural studies the project will shed light on a number of aspects of aphid biology that determine their impact as crop pests as well as providing diagnostic tools that can be applied to other aphid species.

References

[1] Haynes *et al.* (2003) *Appl. Environ. Microbiol.* 69, 7216-7223

[2] Nakabachi *et al.* (2003), *J. Invert. Pathol.* 82, 152-161

[3]Ferrari *et al.* (2004) *Ecol. Entomol.* 29, 60-65

[5]Sandstrom *et al.* (2001) *Mol. Ecol.* 10, 217-228