Zespri Report Milestone 4 – 15 January 2011

Objective 2 – Psa-V phage library screened against endo/epiphytic kiwifruit bacterial strains and phage formulated for trials

Host range analysis and search for a potential carrier bacterium / phage combination

Our bacteriophage collection now contains roughly 100 bacteriophage. Redundancy (multiple isolations of the same phage) in the collection remains likely and furthermore we do not know which bacteriophage are likely to be the most effective as biocontrol agents. To establish the level of redundancy we are performing host range analysis, which will allow us to group the phage. Given that the bacterial hosts we are testing include potential beneficial microbes and biocontrol agents, this will also allow us to identify any phage that can infect and be amplified on carrier bacteria.

We have ~60 *Pseudomonas* strains, many isolated from kiwifruit, and some other commercial biocontrol strains. These strains have been used in host range screens against the first 40 phages in the collection. A method for testing the host range using a plate replicator has been developed and allows us to screen 40 bacteriophage at a time against a large number of bacterial strains. Any potential hosts identified in the first screen must then be re-assessed using dilution series and full agar plates to confirm the ability of phage to infect and produce single plaques rather than a non-replicative lysis phenotype, which is not useful for application. Full dilutions have been performed for the Psa strains and demonstrates a range of infection efficiencies and host ranges. The differential host range of these phages will allow us to target Psa-V alone or other combinations of Psa depending on the needs of the grower. By using a barcoding host-range system we can show that there is less redundancy in the collection than expected with probably greater than 20 different phages of the 40 tested.

Unfortunately, to date, no phages of the 40 tested from our collection infect both a *Pseudomonas fluorescens* strain and a Psa-V strain. *P. fluorescens* is a good candidate as a carrier bacterium as they are non-pathogenic and ubiquitous in the environment. Thus, we are continuing to search for phages that infect this combination. With this in mind, colleagues have obtained a further 70 uncharacterised bacterial isolates from kiwifruit, that may include beneficial strains that we can use as carrier bacteria. These are presently being characterised and will be tested in our infection assays. Our immediate focus is to identify phages that co-infect Psa and a suitable carrier. This will allow us to screen a larger number of phage and will improve the chances of finding a broad host range bacteriophage.