

Zespri Report Milestone 5 – 15 April 2012

Objective 1 – Isolation of a range of virulent Psa-V bacteriophages

The library of virulent Psa-V bacteriophages was expanded by screening further samples from orchards in the Te Puke region and screening soil and water samples from the wider Dunedin area. Individual plaques were picked from screening plates directly into 96-well plates. Purified bacteriophage lysate preparations have been made for a smaller set of bacteriophage. The library now contains 288 bacteriophage isolates that infect Psa-V.

Objective 2 – Psa-V bacteriophage library screened against endo/epiphytic kiwifruit bacterial strains

In addition to 60 *Pseudomonas* strains obtained earlier, a further 51 uncharacterised bacterial isolates from the kiwifruit phyllosphere were received. These are being identified by Andrew Pitman at Plant and Food Research, Lincoln. Preliminary 16S rDNA sequencing indicates some might be Psa-V but there are also several others that are likely to be non-pathogenic.

A high-throughput screen was used to examine the entire Psa-V phage library (288) for the ability to infect these kiwifruit isolates. Plaques for various phages were observed against 13 of the kiwifruit bacterial isolates, including the potential Psa-V strains. The bacteriophages were grouped based on their infection profile and representatives from each group were chosen for further characterisation. This has resulted in a short-list of 24 bacteriophages for further analysis.

The 16S rDNA data indicates that some of these strains are closely related to *Pseudomonas fluorescens*, *Pseudomonas putida* and *Pantoea agglomerans*, all of which could be useful for biocontrol or as potential carrier strains. The entire phage library was also screened against 5 additional *Pseudomonas fluorescens* strains and 5 other strains isolated from commercial biocontrol products to determine if any of those would act as non-pathogenic hosts. To date, only one of these potential non-pathogenic strains reproducibly supported replication of one Psa-V phage. This could provide a possible phage-carrier bacterium system for further development. Overall, the results suggest the majority of Psa-V phages isolated have a relatively narrow host range. There are some with a broader host range, but these are quite rare.

Objective 3 – Characterisation of phage

Pure small scale master stocks and additional working stocks of all 24 phages of interest have been prepared. The positive results from the high-throughput host-range analyses have been double-checked for most of these phages using full dilution series using these phage lysates. The stability of these phages is also being assessed over time at 25°C and 4°C. These preparations will now be used for TEM analysis to classify the phage based on morphology.

Adsorption assays are underway with these phage using a variety of different conditions to determine those phage that bind the most efficiently. Phage DNA has been prepared for 12 phages and is being analysed by digestion with a number of different restriction enzymes to examine genome patterns. This will enable a more accurate classification and the removal of redundant phage with similar genomes.