

Zespri Report Milestone 1 – October 2011

Objective 1 – A number of virulent Psa-V phage samples supplied to ZESPRI

Summary

Environmental samples of soil and leaf litter have been used for the isolation of bacteriophages that infect *Pseudomonas syringae* pv. *actinidiae* (Psa). To date 51 bacteriophages have been isolated and lysate of each bacteriophage prepared and titrated. Based on the plaque morphology greater than 5 clearly distinct bacteriophages have been isolated. A more thorough characterisation of the bacteriophage isolates is likely to increase this number. Preliminary host-range analysis suggests that the phages may be useful as a typing / diagnostic toolkit.

Isolation of Psa-V bacteriophage from environmental samples

Samples of soil, leaf litter and infected vine material were collected from a Psa-V infected orchard near Te Puke. Soil samples were also collected from several areas around Dunedin. Waste water inflow from the Tahuna (Dunedin) waste water treatment plant was also used.

Samples were either incorporated directly into the overlay plaque assay or an enrichment step was performed first. Enrichment involved incubation of the sample in medium with gentle agitation for a couple of hours prior to inoculation with a Psa-V strain. This culture was incubated overnight, followed by centrifugation to remove bacterial cells and debris. The resulting supernatant was then incorporated into the overlay plaque assay.

Samples were screened against *Pseudomonas syringae* pv. *actinidiae* ICMP 9853 (Psa, Japan), ICMP 18708 (Psa-V, NZ), ICMP 18744 (Psa, Italy), ICMP 18800 (Psa-V, NZ), ICMP 18804 (Psa-LV/Psd, NZ), and ICMP 18806 (Psa-LV/Psd, NZ). This has indicated that panel of phage that we have has some discriminatory power to “type” the different strains. There could be a possibility of using a panel of phage as a strain typing / diagnostic procedure. This area could be worth pursuing further.

Nutrient agar was used for the overlay plaque assay and the concentration of the top agar was 0.35%. The *Pseudomonas* strains were grown in nutrient broth. Plates were incubated at 25°C overnight, and then plaques were picked and re-plated until a pure stock of the bacteriophage was obtained. A range of plaques were picked in each experiment and a wide variety of plaques morphologies were chosen.

From this point bacteriophage lysates (about 2-3 ml) were prepared and the titre determined. The titres of the bacteriophage lysates are all $10^8 - 10^{10}$ plaque forming units/ml. These lysates will be used for future work to determine host range and to further characterise the phage.

Using this method, 51 bacteriophage lysates have been prepared and the titres determined. Of the bacteriophage isolated so far, at least 5 can be said to be different based on plaque morphology alone. There are likely to be more within the bacteriophage isolated so far, however further characterisation is required to identify them. For example, we are investigating genome sequencing of key candidate phages to confirm they are non-lysogenic and carrying no virulence genes. The sequence information will also aid in future tracking experiments and for charactering the different phage groups.

We have received a selection of *Pseudomonas* strains from Dr Andrew Pitman, they include *P. syringae* pv. *syringae*, *P. syringae* pv. *tomato*, *P. syringae* pv. *phaseolicola*, *P. syringae* pv. *morsprunorum*, *P. syringae* pv. *atrofaciens*, *Pseudomonas corrugata* and *Pseudomonas fluorescens*.

Future work to be performed before next report due (15/01/12):

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To increase the variation of bacteriophage in the bank another set of samples should be screened. The change in season and conditions in an orchard could provide more variation in phage isolates.

Appointment of Technician:

The technician position is currently being advertised. Applications close 4/11/11.

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

Work will begin on characterising the phages isolated so far. The host range will be tested against a range of Psa strains and other *Pseudomonas* strains isolated from kiwifruit plants. This information will help determine how many different phages have been isolated and which bacteriophage are the best to continue with. The ability to integrate into the bacterial chromosome will also be tested (lysogeny).

Trials for the scale-up of bacteriophage lysates will be started. The stability of bacteriophage in different buffers and at different temperatures will be tested in order to determine the optimal formulation for trials. However, it may be preferred to partner with a company for formulation of bacteriophage.