bacterial canker | kiwifruit | Pseudomonas syringae pv. actinidiae | phage biocontrol

Phages for the Biocontrol of Bacterial Canker of Kiwifruit

Rute Rego [1], Diana Priscila Pires [2], Joana Azeredo [2], Conceição Santos [3], Luísa Moura [1,4*]

1. Instituto Politécnico de Viana do Castelo, Escola Superior Agrária, 4990-706 Ponte de Lima, Portugal.
2. CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.
3. Department of Biology, Faculty of Sciences of University of Porto (FCUP), LAQV-REQUIMTE, FCUP, Porto, Portugal.
4. CISAS, Instituto Politécnico de Viana do Castelo, 4900-347, Viana do Castelo, Portugal.

Correspondence:
luisamoura@esa.ipvc.pt

Bacterial canker of kiwifruit (*Actinidia* spp.) caused by *Pseudomonas syringae* pv. *actinidiae* (Psa), causes significant yield and financial losses. The use of copper-based products and antibiotics are the current techniques for Psa control. These compounds are phytotoxic and also promote copper and antibiotic resistance. The isolation and characterization of (bacterio)phages for the control of Psa was the focus of this research, motivated by the demand for safe and effective biocontrol techniques against this disease. A Portuguese collection of Psa strains was characterized by molecular and phenotypic tests. Phages were isolated from branches, buds, leaves and flowers of kiwifruit plants in the North of Portugal. Phages were isolated by the enrichment procedure with Psa strains CFBP 7286 and P84 as possible hosts, and the lytic spectra of 6 selected one's were tested against the Psa collection. The two phages displaying broader host ranges (between 71% and 84% of efficacy among Psa strains) were stable between -20ºC and 50ºC, pH range of 3 to 11 and UV light at 366 nm. Transmission Electron Microscopy was used to characterize phages morphology. In vitro efficacy studies revealed that, with MOI=1, phage 177T decreased the number of CFUs after 4 hours of inoculation while maintaining a low bacterial load for up to 24 hours. Over 24 hours, phage VC3 maintained the bacterial growth stable. Preliminary *ex vivo* and *in vivo* assays on kiwifruit leaf discs and directly applied to the plant, showed differences between the phage application and the control after 12 days of inoculation. One phage has been sequenced and confirmed to be lysogenic, data that corresponded to the *ex vivo* results. Even so, this lysogenic phage showed potential to be used as a biological control agent against Psa.

This work was supported by the project GesPSAKiwi - Ferramenta Operacional para gestão sustentável do cancro bacteriano (Psa) da Actinídea. NORTE-01-0247-FEDER-033647- (NORTE2020).