Towards Biological Control of Pistachio Dieback

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ABSTRACT

Xanthomonas translucens (*Xtp*) causes dieback disease of pistachio in Australia. The bacterium infects the vascular tissues of the trees, causing discolouration of the xylem, lesions on the trunk and major limbs, decline and, in some cases, death. Although hygiene and application of quaternary ammonium disinfectant to pruning wounds have been recommended to limit the spread of the disease, effective control methods are lacking. Biological control offers potential in managing this disease. The aims of this research were to assess the ability of selected bacteria to antagonise *Xtp* and to evaluate the ability of the synthetic peptide BP100 to suppress growth of *Xtp* in liquid medium.

Isolate KI of *X. translucens* (DAR75532), obtained from a commercial pistachio orchard in Kyalite (NSW) was used. The potential antagonists comprised one isolate of *Bacillus subtilis* and several bacteria isolated from pistachio wood and stored following indications of ability to inhibit *Xtp*.

Preliminary screening of the potential antagonists was conducted by means of an agar diffusion assay, in sucrose peptone agar (SPA) and nutrient agar (NA). Inhibition of growth of DAR75532 varied among bacterial isolates and with the culture medium used. Generally, the isolates produced larger inhibition zones on SPA than on NA and appeared to be bacteriostatic. When diffusible compounds were extracted from liquid cultures through centrifugation and filtration, there was no evidence of antibiotic activity. Further experiments demonstrated that two antagonists produced antibacterial metabolites in liquid medium. In contrast, culture filtrate of isolate 64161-7 grown in nutrient broth supplemented with yeast extract and glucose inhibited DAR75532, and filtrate from isolate PC397 grown in the same medium, or in nutrient broth supplemented with yeast extract and nutrient broth alone, was inhibitory. However, the antibiotic effect of PC397 was lost as the cell free culture filtrate was diluted. Competition was also identified as a possible mechanism, as DAR75532 was not recovered on SPA when mixed with isolates 64161-17, SUPP, *B. subtilis*, PC397, PC506 or PC507.

An *in vitro* assay was developed to evaluate the ability of the potential antagonists to reduce colonisation of pistachio wood by DAR75532. The pathogen, antagonists 64161L and PC397, or pathogen plus antagonists were vacuum-infiltrated into non-autoclaved excised pistachio twigs, before incubating for 10 days. The

pathogen and antagonists were recovered from the middle section of the wood, following soaking in saline and plating suspensions on SPA and NA supplemented with cephalexin, ampicillin and gentomicin (NA+ab). The antagonists were recovered and grew well on SPA but not NA+ab, indicating that they survived in pistachio wood. The pathogen was recovered on NA+ab and SPA, although indigenous wood-inhibiting bacteria also grew on SPA. Only PC397 was recovered from twigs inoculated with PC397 plus DAR75532. This suggested that PC397 inhibited colonisation of pistachio wood by the pathogen.

Peptide BP100 was obtained from the University of Girona, Spain. A turbidimetric-based system was first used to monitor the effect of BP100 on growth of DAR75532 over time. Multiplication of DAR75532 in sucrose peptone broth (SPB) was delayed or reduced in the presence of BP100. At low concentration, the peptide was bacteriostatic and DAR75532 colonies were subsequently recovered on SPA. Higher concentrations were bactericidal. To verify bactericidal activity, suspensions of DAR75532 treated with peptide were sampled over time and the colony forming units enumerated on SPA and compared with untreated controls. The minimum inhibitory (bacteriostatic) or bactericidal concentration of BP100 was influenced by the initial concentration of DAR75532 and by incubation time. Peptide at 2.5 μM was sufficient to inhibit growth of DAR75532 in SPB when the initial concentration was 10^6 CFU/ml, but a minimum of 5 μM was required to kill the cells. The mortality of DAR75532 three hours after treatment was 77.35% when 5 μM of peptide BP100 was applied.

Preliminary screening had identified isolate 64161-7 as having potential to inhibit DAR75532. The isolate was tentatively identified as *Pseudomonas tolaasii, P. fluorescens* or *P. putida* by the National Collection of Plant Pathogenic Bacteria (UK). Although this isolate survived well in excised twigs of pistachio, it did not prevent colonisation of the wood by DAR75532. However, PC397, likely to be a *Bacillus* sp., reduced colonisation by DAR75532. The other six potential antagonists remain to be tested on pistachio wood. The effect of medium composition, such as sugar content, on antibiotic production should also be investigated. In addition, peptide BP100 offers promise as a means of controlling pistachio dieback. The ability of the bacterial isolates and the peptide to reduce colonisation of DAR75532 in pistachio trees should be assessed in a natural system where the influence of other factors can be evaluated.

DECLARATION

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