DEVELOPMENT OF A QUANTITATIVE BIOASSAY TO STUDY THE INTERACTION BETWEEN BIOLOGICAL CONTROL AGENTS AND SOFT ROT *ERWINIA*

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Erwinia carotovora subsp. *carotovora*, *Erwinia carotovora* subsp. *atroseptica* and *Erwinia chrysanthemi* are responsible for bacterial soft rot on a wide range of horticultural crops such as potato and ornamental crops such as calla lily. We are developing a biological control strategy for this economically important disease for which no control is currently available. We found previously that *Erwinia herbicola* Eh252, an epiphytic bacterium originally selected for its ability to control fire blight in apple and Asian pear orchards (Beer *et al.* 1984; Vanneste and Yu 1990, 1993; Vanneste *et al.* 1995), is also able to control soft rot on potato slices (Vanneste *et al.* 1994). The assay on potato slices used in that report has several limitations; it is a qualitative assay only and results, though consistent, are variable. Since quantitative assays to measure the susceptibility of different carrot cultivars to bacterial soft rot have recently been developed (Michalik *et al.* 1992), we investigated the possibility of modifying one of these assays to study the interaction between biological control agents and the soft rot pathogen in the laboratory.

Carrots were washed, surface sterilised with 10% Clorox, rinsed with de-ionised water and allowed to air dry before being sliced and arranged on wet paper towels in a large tray used as a humid chamber. Twenty µl of a biological control agent suspension, containing between 1×10^6 to 1×10^8 cfu/ml, or of MgSO₄ 10 mM, used as a negative control, were pipetted onto the surface of the carrot slice. At least five, but usually 10, carrot slices were used per treatment. Inoculation was done by placing a disc of filter paper (Whatman 3M) previously dipped in a suspension of *E. carotovora* subsp. *carotovora* Ecc11523 on the area previously treated. Trays were incubated in plastic bags at 28°C for 4 days before the percentage area rotted was assessed.

To verify that the percentage area rotted of carrot slices was proportional to the level of inoculum, the first experiments were done without any biological control agents. In all the experiments, the higher the concentration of the inoculum, the higher was the percentage area rotted of the carrot slices (Fig. 1).

When carrot slices were treated with Eh252 before inoculation the development of rot was inhibited. The degree of inhibition was dependent on the amount of Eh252 applied, but was complete when the concentration of Eh252 was about 10 times higher than that of the pathogen. This is in agreement with results found previously on potato (Vanneste *et al.* 1994).

Eh252 produces a peptide that inhibits all the strains of *E. carotovora*, *E. atroseptica* and *E. chrysanthemi* that were examined (Vanneste *et al.* 1992, 1994). A derivative of Eh252, 10:12, which lost the ability to produce this inhibitory compound does not protect potato against soft rot as well as Eh252 (Vanneste *et al.* 1994). We verified this result by comparing the ability of Eh252 and 10:12 to limit infection by *E. carotovora* on carrot slices. The number of carrot slices with 5% or more of their surface rotten was higher when carrots were treated with 10:12 than when they were

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FIGURE 1: Number of carrot slices showing rot after inoculation with different concentrations of *E. carotovora* subsp. *carotovora* Ecc11523 (mean of five independent experiments).

treated with Eh252, while these two strains were applied at the same concentration (2.5×10^8 cfu/ml) (Fig. 2). This confirms that the inhibitory peptide produced by Eh252 is one of the mechanisms involved in the control soft rot.

We now have a quantitative bioassay that will allow the study of the interactions on plant tissue between the soft rot pathogens and potential biological control agents.

FIGURE 2: Number of carrot slices showing rot after treatment with Eh252 or 10:12 (mean of three independent experiments).

REFERENCES

- Beer, S.V., Rundle, J.R. and Norelli, J.L., 1984. Recent progress in the development of biological control for fire blight - A review. *Acta Hort.* 151: 195-201.
- Michalik, B., Simon, P.W., and Gabelman, W.H., 1992. Assessing susceptibility of carrot roots to bacterial soft rot. *HortScience* 27: 1020-1022.
- Vanneste, J. L. and Yu, J., 1990. Control of fire blight by *Erwinia herbicola* Eh 252 in an experimental orchard in Dax (Southwest of France). *Acta Hort.* 273: 409-410.
- Vanneste, J. L. and Yu, J., 1993. Control of fire blight on apple blossoms using *E. herbicola* and *P. fluorescens* separately or together. *Biological and Cultural Tests for Control of Plant Diseases* 9:10.
- Vanneste, J. L., Yu, J. and Beer, S. V., 1992. Role of antibiotic production by *Erwinia herbicola* Eh252 in biological control of *Erwinia amylovora*. *J. Bacteriol.* 174: 2785-2796.
- Vanneste, J. L., Perry, J. H., Perry-Meyer, L. J. and Bedford, R.J., 1994. *Erwinia herbicola* Eh252 as a biological control agent of bacterial soft rot on potatoes. *Proc. 47th N.Z. Plant Protection Conf.*: 198-200.
- Vanneste, J.L., Yu, J., and Ede, F.J., 1995. Evaluation of two biological control agents used separately or together to reduce fire blight infection on Asian pear. *Biological and Cultural Tests for Control of Plant Diseases* 10: in press.