**ERWINIA HERBICOLA EH252 AS A BIOLOGICAL CONTROL
AGENT OF BACTERIAL SOFT ROT ON POTATOES**

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Soft rot on potato caused by *Erwinia* sp. is responsible for significant losses during storage, and constitutes a major problem to the potato industry world wide. This problem is made worse by the absence of effective chemical control, and by the absence of cultivar of potato resistant to bacterial soft rot. *Erwinia herbicola* Eh252 is a non pathogenic epiphytic bacterium originally selected for its ability to limit, in apple orchards, the incidence of fire blight disease caused by *Erwinia amylovora* (Beer *et al.* 1984; Vanneste and Yu 1990, 1993). All strains of *E. amylovora* examined were later found to be inhibited *in vitro* by a peptide antibiotic produced by Eh252 (Vanneste *et al.* 1992). Further studies showed that this peptide antibiotic was one of the major mechanisms responsible for the biological control of fire blight (Vanneste *et al.* 1990, 1992). Therefore, using the sensitivity assay previously described (Vanneste *et al.* 1992), we first assessed whether Eh252 could also inhibit *in vitro* the different species of *Erwinia* that cause soft rot on potato. The 52 strains of *E. chrysanthemi*, *E. carotovora* pv. *carotovora*, and *E. carotovora* pv. *atroseptica* that were examined were all inhibited by Eh252 *in vitro*.

The ability of Eh252 and of its antibiotic and non-antibiotic producing derivatives to control bacterial soft rot was then assessed in the laboratory on potato tubers cultivar: Rua and Iam Hardy. The tubers were surface disinfected by immersion for 3 mins in a 3% sodium hypochlorite solution, rinsed with sterile distilled water, sliced and placed in trays on moist towel paper. The biological control agents and *E. carotovora* pv *carotovora* 8972, the pathogenic strain used in all these experiments, were freshly grown at 28°C in Luria broth to exponential phase before use. Bacterial concentration was visually estimated and the next day populations were confirmed by counting the colonies in serial dilutions plated on Luria medium. Twenty microliters of the appropriate dilution of the biological control agents were pipetted into a well bored in the potato slice with a sterile cork borer. When this bacterial suspension was absorbed, 10 µl of a suspension of the pathogenic strain was introduced into the same well. All the dilutions were made in a 10 mM MgSO₄ solution and this solution was also used, without Eh252 and 8972, as a negative control. The trays were put in a plastic bag and incubated at 25°C for 48 h. Since variation in the humidity level in the bag could influence the development of rot, only slices that were on the same tray were compared to each other. The size of the experiments was therefore limited by the size of the trays; however, we always used a minimum of four slices per treatment.

When potato slices were treated with a suspension of Eh252 before inoculation with *E. carotovora* pv. *carotovora*, development of soft rot was inhibited (Table 1). The degree of inhibition was dependent on the amount of Eh252 applied. However, the amount of Eh252 required for complete control differed with each experiment (Table 1). Some of the factors responsible for this variation were inherent in the experiments (such as the cultivar of potato, or the relative humidity present in the box in which the potatoes slices were incubated), while others were independent of the experiment (such as conditions of storage of the potato tubers before the experiment) (Vanneste *et al.*, unpublished results).

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TABLE 1: Number of potato slices treated with different populations of *E. herbicola* Eh252 which developed soft rot 48 h after inoculation.

Treatment	Experiment I		Experiment II	
	Eh252 population applied (cfu/ml)	No. rotten potato slices ¹	Eh252 population applied (cfu/ml)	No. rotten potato slices ¹
Eh252	4 x 10 ⁸	0	6 x 10 ⁶	0
	4 x 10 ⁷	2	6 x 10 ⁵	2
	4 x 10 ⁶	3	6 x 10 ⁴	3
	4 x 10 ⁵	4	6 x 10 ³	4
MgSO ₄		4		4

¹ Four potato slices were inoculated per treatment

The role of the peptide antibiotic produced by Eh252 in the inhibition of bacterial soft rot on potato was evaluated comparing the ability of Eh252 to control this disease with that of a transposon-induced non-antibiotic producing mutant called 10:12, for which a causal link between the insertion of the transposon and the loss of antibiotic production has been demonstrated (Vanneste *et al.* 1992). Since, the genes for antibiotic production have recently been cloned (Vanneste and Yu, unpublished data), we included in these tests a derivative of 10:12 complemented for antibiotic production (10:12 pANT3) and as a control the same derivative harbouring only the plasmid vector (10:12 pCPP25). In all the experiments the derivatives that lost ability to produce the peptide antibiotic also lost most of their ability to control soft rot (Table 2). Restoration of antibiotic production restored completely the ability to control the soft rot pathogens. This indicates that production of this peptide antibiotic is one of the mechanisms responsible for the biological control of soft rot on potato by Eh252. It is, however, not the sole mechanism of control since in some experiments treatment with a non-producing antibiotic strain resulted in some inhibition of bacterial soft rot.

TABLE 2: Number of potato slices treated with Eh252 or with antibiotic or non-antibiotic producing derivatives which developed soft rot 48 h after inoculation.

Treatment	Ant.	Exp. I		Exp. II		Exp. III		Exp. IV		Exp. V	
		In.	No.	In.	No.	In.	No.	In.	No.	In.	No.
Eh252	+	4 x 10 ⁵	0	1 x 10 ⁴	0	2 x 10 ⁴	0	4 x 10 ⁴	0	4 x 10 ⁶	0
10:12	-	2 x 10 ⁵	2	1 x 10 ⁴	2	1 x 10 ⁴	2	3 x 10 ⁴	4	3 x 10 ³	1
10:12 pCPP25	-	4 x 10 ⁵	3	9 x 10 ³	2	2 x 10 ⁴	3	2 x 10 ⁴	4	2 x 10 ⁶	1
10:12 pANT3	+	2 x 10 ⁵	0	1 x 10 ⁴	0	8 x 10 ³	0	4 x 10 ⁴	1	4 x 10 ⁶	0
MgSO ₄			4		4		4		4		4

In. inoculum of biological control agent in cfu/ml

No. number of slices of potatoes that developed soft rot. Four slices were inoculated per treatment

E. herbicola Eh252 can inhibit bacterial soft rot on potato slices. One of the mechanisms of this inhibition is the production of a peptide antibiotic. As is usually the case when an inhibitory compound is involved in biological control, production of this compound is not the sole mechanism of control (Vanneste *et al.* 1992). However,

the involvement of the peptide antibiotic produced by Eh252 in the control of bacterial soft rot is an important result which opens new opportunities for control of this economically important disease.

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.
- 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. In: Biological and Cultural Tests for Control of Plant Diseases Volume 9 (in press).
- Vanneste, J.L., Smart, L.B., Zumoff, C.H., Yu, J. and Beer S.V., 1990. Role of antibiotic production by *Erwinia herbicola* Eh 252 in reducing fire blight incidence. Pp 443-446 In: Z. Klement (Ed), *Proc. 7th International Conference on Plant Pathogenic Bacteria*. Akademiai Kiado, Budapest, Hungary, June 1989.
- 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.