The use of *Pseudomonas fluorescens* A506 and *Agrobacterium radiobacter* K1026 for management of crown gall in herbaceous perennials

Melodie L. Putnam and M. L. Miller
Oregon State University, Botany and Plant Pathology, Corvallis, OR 97331

Introduction

Crown gall (Figure 1), caused by the bacterium *Agrobacterium tumefaciens* (and often mistaken for callus tissue), can be a persistent problem in nurseries producing herbaceous perennials. Economic losses from crown gall have been extensive since there is no control other than destruction of affected plants and strict sanitation to prevent re-introduction of the pathogen.

The biological control agent *Agrobacterium radiobacter* strain K84 and the genetically nonconjugative strain K1026 have been widely used to prevent crown gall in woody plants. BlightBan A506, a biocontrol agent registered for use in management of fire blight, consists of the antagonistic bacterium *Pseudomonas fluorescens* strain A506. When combined with chelated iron, *P. fluorescens* A506 produces an antibiotic inhibitory to other bacteria.

The objectives of this study were to:
1) test pathogenic *A. tumefaciens* strains isolated from herbaceous perennials for *in vitro* sensitivity to *A. radiobacter* K1026 and *P. fluorescens* A506; and
2) test *A. radiobacter* K1026 and *P. fluorescens* A506 *in planta* to determine efficacy in preventing crown gall

Figure 1. Hibiscus cutting with crown gall at the stem base.

Materials and Methods

In vitro Assays

A. radiobacter K1026

- 23 and 26 pathogenic *A. tumefaciens* isolates originating from herbaceous perennials were screened against K1026 and A506, respectively
- 100 µl (1 x 10^8 cfu/ml) of *A. radiobacter* K1026, *P. fluorescens* A506 alone, or *P. fluorescens* A506 plus chelated iron were applied to the centers of agar plates (one biocontrol treatment/plate)
- After 48 h, all plates were exposed to chloroform vapor to kill the biocontrol bacteria
- Individual *A. tumefaciens* isolates in molten agar were applied to the plates
- Controls used were *A. tumefaciens* C58 (sensitive to K1026) and B49C (insensitive to K1026); and *Erwinia carotovora* 273 (sensitive to A506) and *Erwinia herbicola* 26-SR-6-2 (insensitive to A506)
- Zones of inhibition were observed after 48 h.

Results

A. radiobacter K1026

Only 3 of 23 pathogenic *A. tumefaciens* isolates screened were sensitive to *A. radiobacter* K1026 (Figure 2). These 3 isolates were as sensitive as the known susceptible comparison strain C58.

P. fluorescens A506

We screened 26 isolates of *A. tumefaciens* for sensitivity to *P. fluorescens* A506 and *P. fluorescens* A506 plus iron. None of the isolates were inhibited by either treatment.

Figure 2. An isolate of *Agrobacterium tumefaciens* sensitive to the antibiotic (agrocin 84) produced by *A. radiobacter* K1026. Note the zone of inhibition.

Materials and Methods

In planta Assays

A. radiobacter K1026

- We used chrysanthemum 'Kory' (first experiment) and chrysanthemum 'Kory' plus *Bryophyllum daigremontianum* (second experiment)
- 3 leaves/plant were wounded and inoculated with a suspension of *A. radiobacter* K1026 (1 x 10^8 cfu/ml)
- 2 h later 10 µl of a suspension (1 x 10^7 cfu/ml) of four pathogenic *A. tumefaciens* isolates (combined) was applied to the wounds
- Controls were wounded, untreated leaves; wounded leaves treated with a mixture of 4 pathogenic isolates of *A. tumefaciens* (1 x 10^7 cfu/ml); wounded leaves treated with *A. radiobacter* K1026 (1 x 10^8 cfu/ml)
- Plants were rated after 60 d
- The experiment was performed twice

P. fluorescens A506

- Host used: *Bryophyllum daigremontianum*
- 4 leaves/plant were wounded and inoculated with 10 µl a 1 x 10^8 cfu/ml suspension of the following (applied separately): *P. fluorescens* A506, *P. fluorescens* A506 + Fe, or *A. radiobacter* K1026 used as a comparator.
- 2 h later 10 µl of a suspension (1 x 107 cfu/ml) of four pathogenic *A. tumefaciens* isolates (combined) was applied to the wounds
- Controls: were wounded leaves applied with *P. fluorescens* A506 (1 x 10^8 cfu/ml) in broth with and without iron; 4 pathogenic isolates of *A. tumefaciens* (1 x 10^7 cfu/ml) in broth with and without iron; *A. radiobacter* K1026 (1 x 10^8 cfu/ml) in broth, bacterial broth alone; bacterial broth + iron; and unwounded, untreated leaves
- Plants were rated after 60 d
- The experiment was performed twice

Results

A. radiobacter K1026

- Results for experiments 1 and 2 were similar; only results for experiment 2 are shown (Table 1).
- 63% of inoculated wounds on chrysanthemum 'Kory' and 45% of inoculated wounds of *Bryophyllum daigremontianum* treated with *A. radiobacter* K1026 developed galls when challenged with a mixture of four pathogenic isolates.
- Galls on plants treated with *A. radiobacter* K1026 and then challenged with pathogenic *A. tumefaciens* isolates were smaller in size and weight than those produced by the pathogen alone (Figure 3).

Table 1. Response of chrysanthemum 'Kory' and *Bryophyllum daigremontianum* to inoculation with the biocontrol agent *Agrobacterium radiobacter* strain K1026 and pathogenic *Agrobacterium tumefaciens* strains*.

	No. of plants inoc.	Total # of wounds	% of wounds w/galls		Ave. wt of galls/plant (g)	
			'Kory'	*B. diag.*	'Kory'	*B. diag.*
Positive control[1]	5	15	100	100	3.26	6.60
Negative control (K1026)[2]	5	15	0	0	0	0
K1026 + *A. tum.*[3]	20	60	63	45	0.66	0.5
Wounded untreated controls	5	15	0	0	0	0

*Plants were wounded at three sites per plant. Tumors were cut off and weighed 60 d post inoculation.
[1] inoculated with a mixture of 4 pathogenic *A. tumefaciens* strains at a concentration of 1 x 10^7 cfu/ml.
[2] inoculated with *A. radiobacter* K1026 at a concentration of 1 x 10^8 cfu/ml.
[3] inoculated first with *A. radiobacter* K1026 (1 x 10^8 cfu/ml) then challenged with the pathogen mix (1x10^7 cfu/ml) 2 hr later.

Figure 3. Response of *B. daigremontianum* leaves to treatment with *A. tumefaciens* (A), K1026 followed by *A. tumefaciens* (B), and K1026 alone (C). D shows leaves wounded and untreated.

P. fluorescens A506

- Results are summarized in Table 2
- In experiment 1, there was no significant difference in mean gall weight between plants treated with *A. radiobacter* K1026 or *P. fluorescens* A506 without iron and then challenged with *A. tumefaciens.*
- In experiment 2, mean gall weight was significantly less when plants were treated with *A. radiobacter* K1026 than with *P. fluorescens* A506 with or without iron prior to inoculation with *A. tumefaciens.*
- In both experiments, *P. fluorescens* A506 without iron reduced crown gall incidence when compared to all other treatments.
- In experiment 1 no galls formed where pathogens were not applied. In experiment 2 some negative control plants formed small galls in the absence of the pathogen. We attribute this to contamination occurring during watering and handling of the plants.

Table 2. Crown gall formation in *Bryophyllum daigremontianum* after biocontrol treatments and inoculation with pathogenic isolates of *Agrobacterium tumefaciens.*

	Experiment 1		Experiment 2	
Treatment*	**Mean gall wt (g)****	**Gall incidence⁺**	**Mean gall wt (g)****	**Gall incidence⁺**
K1024 + pathogen	0.27[a]	87	0.54[a]	95
A506 + pathogen	0.27[a]	66	1.21[b]	79
A506 + Fe + pathogen	0.53[b]	91	1.63[bc]	96
Broth + pathogen	1.04[c]	99	1.79[c]	89
Broth + Fe + pathogen	1.36[d]	97	1.58[bc]	92
K1026 + broth	0	0	0	0
A506 + broth	0	0	0.22	4
A506 + Fe + broth	0	0	0.33	1
Broth	0	0	0.18	5
Unwounded, untreated	0	0	0	0

* Each treatment consisted of 20 plants with 4 inoculations/plant. Leaves were wounded (except where specified) prior to inoculation with the biocontrol agent.
** Values followed by the same letter are not significantly different using the least significant difference multiple range test.
⁺ Values represent the percentage of wounded sites that formed galls.

Discussion

A. radiobacter 1026 produces a highly specific antibiotic active against certain pathogenic strains of *Agrobacterium*. Unlike in woody plants, *A. radiobacter* K1026 was not inhibitory, *in vitro*, to the majority of *A. tumefaciens* isolates obtained from herbaceous perennials. *In planta*, leaves treated with *A. radiobacter* K1026 formed fewer and smaller galls than leaves treated with *A. tumefaciens* alone.

The isolates of *A. tumefaciens* we recovered from herbaceous perennials and used in these experiments were biovar 1; those present in woody plants are predominately biovar 2. Biovar 1 isolates from herbaceous perennials may be less susceptible than biovar 2 isolates to the antibiotic produced by *A. radiobacter* K1026.

Pseudomonas fluorescens A506 suppresses growth of the fire blight pathogen through preemptive competitive exclusion. A506 also is known to produce an antibiotic when grown on a minimal medium amended with iron.

We found no *in vitro* inhibition of *A. tumefaciens* by *P. fluorescens* A506, with or without iron. *In planta*, *P. fluorescens* A506 was not better at reducing mean gall weight than *A. radiobacter* K1026. K1026 reduced the mean gall weight significantly, but the number of galls produced was unacceptably high (Tables 1 and 2).

Crown gall is a disease for which partial control is undesirable, since even small galls render plants unsuitable for propagation or sale. Galls must be completely inhibited or severely reduced in incidence in order to provide an acceptable level of control. Neither *P. fluorescens* A506 nor *A. radiobacter* K1026 offered this level of control and hence have limited usefulness for management of crown gall.

Funding for this study was received from the Oregon State University Agricultural Research Foundation and the Oregon Department of Agriculture.