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Agrocin-producing Pathogenic and Nonpathogenic Biotype-3 Strains of *Agrobacterium tumefaciens* Active against Biotype-3 Pathogens

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Abstract. Agrocin-producing pathogenic and nonpathogenic biotype-3 strains of *Agrobacterium tumefaciens* were isolated from grapevine gall tissue. In vitro activity of the nonpathogenic agrocin producers was restricted to biotype-3 pathogens used. Pathogenic agrocin producers were active in vitro against biotype-3 agrocin-producing nonpathogens, non-agrocin-producing pathogens, and biotype-1 strains when cultivated on a modified Stonier's medium; on a medium designated AB, two strains tested showed no activity against agrocin-producing nonpathogens, but agrocin of one of these strains was active against other agrocin-producing pathogens. In a greenhouse experiment a marked tendency toward decreased gall formation by biotype-3 pathogens on grapevines was obtained when biotype-3 pathogens and nonpathogenic biotype-3 agrocin producers were applied to wounds simultaneously. In this experiment, agrocin-producing pathogens tended to be more virulent than non-agrocin-producing pathogens.

Reports of successful biological control of crown gall in Australia with the agrocin-producing strain 84 of *Agrobacterium radiobacter* [12, 24] were confirmed in other countries [5, 7, 10, 14, 21, 22]. Evidence indicating the involvement of the nucleotide bacteriocin 84 is overwhelming although control, or lack of control, of crown gall formation by some strains of *Agrobacterium tumefaciens* could not be linked to agrocin production by strain 84 [15, 16, 21, 22]. In some of these cases blockage of infection sites by the antagonist could have been the mechanism of control [6, 18].

Recently another promising agrocin-producing strain, *A. tumefaciens* D286, which had been isolated in South Africa and which had spontaneously lost its pathogenicity, was reported [9]. Whereas the activity of strain 84 is theoretically restricted to strains harboring the nopaline Ti plasmid [8, 17], strain D286 was found capable of preventing the formation of crown gall tissue on potato slices by strains harboring either nopaline, octopine, or agropine Ti plasmids [9]. It seemed likely that this isolation of a second apparently useful strain, which could possibly complement strain 84 in a control program for biotype-1 and 2 strains, would soon lead to the detection of other useful agrocin-producing strains [15].

In contrast to the success obtained with biological control of biotypes 1 and 2 of *A. tumefaciens*, strains of a third biotype, biotype 3, which has a narrow host range and appears to be restricted to grapevines [16, 25], were all found to be insensitive to agrocin 84. Failure to control biotype-3 pathogens on plants with each of 11 avirulent biotype-1 and 2 strains that formed bacteriocins active in vitro against biotype-3 strains tested led Panagopolous et al. [25] to conclude that the isolation of a strain similar to 84 but inhibiting biotype-3 strains was unlikely; in fact they considered the prospect of biological control of crown gall on grapevines as remote.

Biotype-3 strains were first reported in South Africa in 1978 [19] and were considered to pose a threat to grapevine production in the northern summer rainfall areas of the country. This report deals with the isolation from gall tissue of grapevines of both pathogenic and nonpathogenic biotype-3 strains that showed strong in vitro agrocinogenic activity against pathogenic biotype-3 isolates. Some of these strains successfully reduced crown gall formation on grapevines in a greenhouse experiment.

Materials and Methods

Isolation of *Agrobacterium tumefaciens* from galls. Freshly collected galls were scrubbed and rinsed under tap water and dried with paper towels. Each gall was broken by hand into two or

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three pieces before ~0.5–1.0 g of watery tissue was removed from the fractured plane by means of a sterile forceps and crushed in 3 ml phosphate-buffered saline (20 m*M*, pH 7.0). The suspension was kept for 24 h at 4°–6°C before 1.0 ml of the supernatant was serially diluted. Aliquots of 0.1 ml of each dilution were spread onto surface-dried agar plates of each of three media: oxoid CM3 nutrient agar (NA), yeast extract mannitol agar (YMA) containing 0.025 g/liter Congo red [1], and nitrate lactose agar [4] supplemented with 0.1 g/liter yeast extract. To each medium were added separately sterilized $Na_2Se_2O_3 \cdot 5H_2O$ and cyclohexamide at concentrations of 50 and 250 ppm, respectively. Inoculated plates were incubated at 27°C for 7–10 days before colonies resembling *Agrobacterium* were picked and purified on unsupplemented NA and YMA media. All cultures were maintained on NA and YMA slants at room temperature.

Characterization and biotyping of isolates. Isolates consisting of Gram-negative rod-shaped cells with polar to subpolar flagella as observed by TEM, and with colony morphology characteristic of *Agrobacterium* on one of the biotype-selective media, were maintained for further study. Biotype determinations were based on the production of 3-ketolactose [2], litmus milk reaction, the production of acid from erythritol and melezitose, the production of alkali from the sodium salts of certain organic acids, maximum growth temperature, and growth on 2% NaCl [16]. Biotyping of isolates was verified by cultivation on biotype-selective media of Brisbane and Kerr [3], New and Kerr [23], and Schroth et al. [26].

Pathogenicity tests. All biotyped isolates were tested for pathogenicity on young plants of dwarf sunflower, tomato, *Datura stromonium*, and *Nicotiana glutinosa*. In addition, biotype-3 isolates were tested on grapevine rootstock seedlings (cv. Jaquez) in 12-cm-diameter pots, each containing 1 kg pasteurized soil, and on green shoots of mature grapevine cv. Sultana in 28-cm-diameter pots, each containing 25 kg soil. Grapevine seedlings were obtained by germinating seed [11]. A plant was inoculated by making a 5-mm cut along the stem of a three- to ten-week-old seedling (depending on the type of plant) or the shoot of a mature plant, through a drop of a 72-h-old aqueous cell suspension of an isolate. A shoot wound was covered with polythene tape, while a wounded seedling was maintained for 48 h at a R.H. of 100% and temperature of 27°C. Plants were then removed to a greenhouse with temperatures varying between 22°C and 28°C. All plants were examined regularly for gall production over a six-week period; the period was extended to 12 weeks in the case of grapevines. An isolate that failed to induce tumors on any of the test plants in two separate trials was regarded as nonpathogenic.

Agrocin production and sensitivity. The method of Stonier [27] was used with the following modifications: The plate medium was supplemented with 0.01 g/liter yeast extract and 200 μg/liter biotin. Each plate was spotted with a YM broth culture of the isolate to be tested for agrocin production and incubated for 72 h before the overlay agar seeded with the indicator strain from YM broth was added. Some agrocin-producing strains were also tested on medium designated AB [20] and supplemented with 5.9 g/liter Na-glutamate.

Tests for biological control. According to the method of Kerr et al. [15, 24] aqueous cell suspensions of a pathogenic and an agrocin-producing isolate were prepared from 72-h-old YMA slants and standardized to contain 2×10^9 cells/ml. Different combinations of pathogens and agrocin producers were mixed in a 1 : 1 ratio and the suspensions used to inoculate *N. glutinosa* and grapevine seedlings (cv. Jaquez) by the wounding method described for pathogenicity tests. Care was taken to deposit at least 2×10^6 cells of the mixed suspension in a wound; suspensions of single strain inoculants were diluted with equal amounts of sterile water and provided 1×10^6 cells in a wound. Tumor formation was assessed 12 weeks after inoculation.

Serology. Antiserum was prepared [28] against whole cells of each of 13 isolates cultivated on amended glutamate citrate agar [27]. Cross reactions among isolates tested against the various antisera were determined by means of the gel diffusion method [26] using boiled cells as antigens.

Results

The origin of the strains and isolates of *Agrobacterium* used is shown in Table 1; 48 isolates from galls collected in the summer rainfall area and three from galls from the winter rainfall area, representing 14 vineyards in total, were identified as biotype-3 strains on the basis of their cell morphology, growth on selective media, and physiological properties. In one instance a pathogen (strain A5) and a serologically unrelated non-agrocin-producing nonpathogen were isolated from the same gall.

Examination of the 13 isolates selected for antiserum production showed that they included five serologically different strains. Antisera against these five strains were used to compare gel diffusion patterns of other isolates and to place them in serogroups (Tables 2 and 3). Each serogroup consisted of isolates whose gel patterns were indistinguishable, but which differed with regard to the spectrum of isolates sensitive to agrocins of different members of the group. Results obtained with pathogens that were not tested serologically are only shown when their sensitivity patterns to agrocins produced by the biotype-3 strains differed (Table 3).

In vitro sensitivity of the various strains and isolates to agrocins produced by pathogenic and nonpathogenic biotype-3 strains on Stonier's [27] modified medium showed the following patterns: (a) No agrocin activity of a nonpathogenic agrocin-producing biotype-3 strain was discernable against other nonpathogenic agrocin producers; agrocin activity against agrocin-producing pathogenic strains occurred with 11 of 60 strain combinations (Table 2). Agrocin activity was shown by one or more of these nonpathogenic strains against all non-agrocin-producing biotype-3 pathogens tested (Table 3). No biotype-1 (Table 4) or any of 20 biotype-2 strains tested was sensitive to agrocins of these strains. (b) Agrocins of pathogenic agrocin-producing biotype-

Table 1. Origin of isolates and strains of *Agrobacterium* studied

Strain/isolate designation	Biotype	Host	Origin
A, B, C, D, G, H, I, J, K[a]	3	Grapevine	PPRI[b], South Africa
F1, F2, V1, L1, Y1	3	Grapevine	PPRI, South Africa
At1, At2	3	Grapevine	G.H. Boelema, South Africa
W7	3	Grapevine	H.J. du Plessis, South Africa
1771	3	Grapevine	NCPPB[c], England
1477, 78, H3/1, K2/2	1	Grapevine	PPRI, South Africa
CHR2, M37g, $M37_{10}$, M51g, M523, M525	1	Chrysanthemum	PPRI, South Africa
2086A, 2080	1	Willow	PPRI, South Africa
1895, 2153	1	*Prunus* sp.	PPRI, South Africa
57	1	*Prunus* sp.	H.J. du Plessis, South Africa
E4, E6, LR1, LR2, LR6	1	*Rosa* sp.	PPRI, South Africa
B6	1	Apple	USA
T37	1	*Juglans* sp.	K. Kersters, USA
925	1	*Dahlia* sp.	NCPPB, England
TT9	1	unknown	J. De Ley, Belgium
198	1	unknown	A. Kerr, Australia
84	1	soil	A. Kerr, Australia
20 isolates	2[d]		PPRI, South Africa

[a] Numbers of 46 isolates used, start with one of the letters indicated.
[b] Plant Protection Research Institute.
[c] National Collection of Plant Pathogenic Bacteria.
[d] In addition to the biotype-1 and 3 strains and isolates, 20 biotype-2 isolates of various origin were used.

Table 2. In vitro sensitivity[a] of agrocin-producing biotype-3 strains to agrocins produced by other biotype-3 strains

Strains[b] tested for sensitivity		Biotype-3 strains producing agrocins[c]														
		Nonpathogenic strains						Pathogenic strains								
		A4	F2	L1	I2	B5	H6	A1	A3	F1	At1	H5	H8	H9	H10	H11
A4–NP		−	−	−	−	−	−	+	+	+	+	+	+	+	+	−
F2–NP		−	−	−	−	−	−	+	+	+	+	−	+	+	+	+
L1–NP	[d]	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
I2–NP		−	−	−	−	−	−	+	−	+	+	+	+	+	+	+
B5–NP		−	−	−	−	−	−	+	+	+	+	+	+	−	+	+
H6–NP		−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
A1–P		−	−	−	+	+	−	−	−	−	−	−	−	−	−	−
A3–P	[e]	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−
F1–P		−	−	−	+	+	−	−	−	−	−	−	−	−	−	−
At1–P		−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
H5–P		+	−	−	+	−	−	−	−	−	−	−	−	−	−	−
H8–P		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
H9–P	[d]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
H10–P		−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
H11–P		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] + denotes inhibition of test strain; − denotes no discernable inhibition (four replicates of each treatment).
[b] Pathogens and nonpathogens are indicated, respectively, by P and NP following the strain number.
[c] Agrocins produced on modified medium of Stonier [27].
[d] Solid vertical lines indicate strains of the same serogroup.
[e] Broken vertical line indicates strains that do not form a serogroup, but differ serologically from the other serogroups.

Table 3. In vitro sensitivity[a] of non-agrocin-producing biotype-3 pathogens to agrocins produced by other biotype-3 strains

Strains tested for sensitivity	Biotype-3 strains producing agrocins[b]														
	Nonpathogenic strains						Pathogenic strains								
	A4	F2	L1	I2	B5	H6	A1	A3	F1	At1	H5	H8	H9	H10	H11
A5 [c]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C3 [c]	+	−	+	+	+	−	+	+	+	+	−	+	−	−	−
D1 [c]	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
D6 [c]	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
G1 [c]	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−
G10 [c]	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H14 [c]	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+
J2 [c]	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+
J3 [c]	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
K1 [c]	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
K4 [c]	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
1771	+	−	+	+	−	−	−	+	+	−	−	+	−	−	−
Y1	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
W7	−	−	−	+	−	−	+	−	+	+	+	+	+	+	+
V1	−	−	−	+	−	−	+	+	+	+	+	+	+	+	+
D4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D6	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
G11	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+
H3	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
H7	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
H15	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+
J1	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
J6	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
J11	−	−	+	+	+	−	+	+	+	+	+	+	+	+	+
K3	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9 isolates	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] + denotes inhibition of test strain; − denotes no discernable inhibition (four replicates of each treatment.)
[b] As determined on modified medium of Stonier [27].
[c] Vertical line indicates strains of the same serogroup; other strains and isolates were not tested serologically.

3 strains showed no activity against other agrocin-producing biotype-3 pathogens (Table 2), but they were active against agrocin-producing nonpathogens (Table 2) and non-agrocin-producing biotype-3 pathogens (Table 3). In addition, both pathogenic and nonpathogenic biotype-1 strains were sensitive to these agrocins (Table 4). No biotype-2 strain tested was sensitive. When tested against biotype-1 strains the nonpathogenic biotype-2 strain 84 showed activity against eight of the 18 pathogens but not against nonpathogenic strains (Table 4).

The effect that each of five nonpathogenic agrocin-producing biotype-3 strains and the biotype-2 strain 84 had on gall formation on grapevines by biotype-3 pathogens is shown in Table 5. Only six of the 15 pathogens formed galls on each of the four replicates when applied alone. A strong tendency toward decreased tumor formation, both with regard to number of plants with galls and gall size, was noticeable when agrocin producers and pathogens were applied simultaneously; strains F2 and H6 were outstanding in this regard. The biotype-2 strain 84 also seemed to decrease gall production by some of the pathogens. Among the most virulent pathogens were the three agrocin-producing strains H8, At1, and At2 (Table 5).

Results of five of the biotype-3 strains that produced agrocins on the modified medium of Stonier [27] differed when modified AB medium [20] was used (Table 6). The agrocin-producing pathogens H8 and At1 were active against all nonpathogenic agrocin producers on modified Stonier's medium, but had no discernable effect on these strains on AB medium. Strain H8 failed to produce an agrocin active against other agrocin-producing pathogens on modified Stonier's medium, but did so against three of these strains, A3, F1, and At1, on AB medium. Biotype-2 strain 84, which also showed no agro-

Table 4. In vitro sensitivity[a] of biotype-1 strains to agrocins produced by biotype-3 strains and the biotype-2 strain 84

Strains[b] tested for sensitivity	Strains[c] producing agrocin on modified medium of Stonier [27] Biotype 3 Pathogenic									Biotype 2 Nonpathogenic 84
	A1	A3	F1	At1	H5	H8	H9	H10	H11	
CHR-P	−	−	−	+	+	+	+	+	+	+
M37g-P	−	−	−	−	−	−	−	−	−	−
M37-P	−	−	−	+	+	+	+	+	+	−
M51g-P	−	−	−	+	+	+	+	+	+	−
M525-P	−	−	−	+	+	+	+	+	+	−
M523-P	−	−	−	+	+	+	+	+	+	−
TT9-P	−	−	−	+	+	+	+	+	+	+
T37-P	−	−	−	+	+	+	+	+	+	+
198-P	−	−	−	−	−	−	−	−	−	+
925-P	−	−	−	−	−	−	−	−	−	−
78-P	−	−	−	−	−	−	−	−	−	−
1895-P	−	−	−	+	+	+	+	+	+	−
1479-P	−	−	−	+	+	+	+	+	+	+
2086-P	−	−	−	+	+	+	+	+	+	+
2080-P	−	−	−	−	−	−	−	−	−	−
B6-P	−	−	−	+	+	+	+	+	+	−
57-P	−	−	−	+	+	+	+	+	+	+
2153-P	−	−	−	+	+	+	+	+	+	+
E4-NP	−	−	−	+	+	+	+	+	+	−
E6-NP	−	−	−	+	+	+	+	+	+	−
H3-NP[d]	−	−	−	−	−	−	−	−	−	−
K2-NP[d]	−	−	−	+	+	+	+	+	+	−
LR1-NP	−	−	−	+	+	+	+	+	+	−
LR2-NP	−	−	−	+	+	+	+	+	+	−
LR6-NP	−	−	−	+	+	+	+	+	+	−

[a] + denotes inhibition of test strain; − denotes no discernable inhibition (four replicates of each treatment).
[b] Pathogens and nonpathogens are indicated, respectively, by P and NP following the strain number.
[c] No nonpathogenic biotype-3 strain produced agrocins active against biotype-1 strains.
[d] The nonpathogenic biotype-1 isolates H3 and K2 were isolated from galls formed by biotype-3 pathogens.

cinogenic effect on Stonier's medium, produced agrocins active against four agrocin-producing biotype-3 pathogens on AB medium.

Discussion

The relative ease with which *Agrobacterium* biotype-3 strains were isolated from gall tissue on grapevines is evidence of the suitability of the isolation technique. It also showed that gall tissue could serve as a valuable source of nonpathogenic strains, a fact probably overlooked in the past because non-tumor-inducing isolates from galls were discarded as contaminants. Further studies are warranted to determine whether agrocin and non-agrocin producers could occur in the same gall; in this study, in the only instance where a pathogen and a nonpathogenic strain were isolated from the same gall, both were non-agrocin producers.

The finding that most of the isolates tested fitted into one of only five serogroups was not unexpected in spite of the fact that they were isolated from vineyards that in some instances were hundreds of kilometers apart and subjected to diverse soil and climatic conditions. The grapevine industry in the northern summer rainfall areas has only become established over the last decade, with rooted propagation material obtained mostly from nurseries in the winter rainfall area of the western Cape Province, where grapevines have been cultivated for three centuries. A common origin of the biotype-3 isolates studied is therefore indicated.

Interesting patterns with regard to in vitro agrocin production by pathogenic and nonpathogenic biotype-3 isolates and sensitivity of strains to these

Table 5. Number of grapevines[a] with galls (and gall index[b]) after simultaneous inoculation with pathogenic and agrocin-producing nonpathogenic strains

Biotype-3 pathogens		Biotype 2	Agrocin-producing nonpathogenic strains Biotype 3					
	Uninoculated	r84	A4	I2	L1	F2	H6	Total[c]
Uninoculated	0	0	0	0	0	0	0	
A5	2(8)	1(1)	0	0	0	0	0	1(1)
C3	2(5)	2(2)	2(2)	1(1)	0	0	0	5(5)
D1	4(7)	2(3)	1(1)	0	0	1(1)	0	4(5)
D6	2(4)	1(2)	0	0	0	0	0	1(2)
F1[d]	3(6)	3(3)	0	0	0	0	0	3(3)
G1	4(8)	1(1)	0	3(3)	0	0	1(1)	5(5)
G10	1(1)	1(1)	1(1)	1(1)	0	0	0	3(3)
H8[d]	4(11)	2(4)	4(12)	1(1)	2(3)	0	2(2)	11(22)
J2	1(2)	2(3)	1(1)	0	1(1)	0	0	4(5)
J3	1(1)	3(4)	0	0	0	0	0	3(4)
K4	2(3)	2(4)	1(1)	2(2)	0	0	0	5(7)
At1[d]	4(10)	3(6)	2(7)	3(3)	1(1)	2(2)	0	11(19)
At2[d]	4(9)	2(3)	2(3)	2(2)	2(3)	0	0	8(11)
1771	4(7)	0	1(1)	0	0	0	0	1(1)
Y1	3(4)	2(2)	2(2)	0	1(1)	1(1)	1(1)	7(7)
Total	41(86)	27(39)	17(31)	13(13)	7(9)	4(4)	4(4)	

[a] Each pair of strains was used to inoculate four plants.
[b] Gall index = number of plants of each treatment with galls × average gall size. Gall size scored from 1 to 4: 1 = 1–5 mm diameter; 2 = 5–10 mm diameter; 3 = 10–15 mm diameter; and 4 > 15 mm diameter.
[c] Total refers to galls of plants inoculated with strain pairs.
[d] Agrocin-producing pathogens.

agrocins have emerged when a modified medium of Stonier [27] was used—notably, the restriction of in vitro agrocin activity of the nonpathogenic biotype-3 strains to biotype-3 pathogens (Tables 2 and 3). In this regard they resembled the agrocin-producing biotype-2 strain 84 whose activity is limited to pathogenic strains [14], as was also demonstrated in this study (Table 4). Although appreciable differences in sensitivities of individual biotype-3 strains to the nonpathogenic agrocin producers were evident when modified Stonier's medium was replaced by modified AB medium [20], the activity pattern with regard to sensitive "strain groups" as displayed on Stonier's medium remained unchanged (Table 6).

The pattern shown by agrocin-producing biotype-3 pathogens that were active in vitro against non-agrocin-producing biotype-3 pathogens and nonpathogens (agrocin producers) alike, but inactive against agrocin-producing biotype-3 pathogens (Tables 2 and 3), suggested a different mechanism. This is supported by agrocin activity of these strains against biotype-1 pathogens and nonpathogens, and by the marked change in the activity pattern of strain H8 when AB instead of Stonier's medium was used (Table 6); on the AB medium the reaction of this strain resembled those of the nonpathogenic agrocin producers by showing activity against pathogens only.

The change in the spectrum of strains showing in vitro sensitivity to the agrocin-producing pathogen H8, which paralleled a change in growth medium, may indicate the involvement of more than one agrocin; it may also reflect altered sensitivities of the test strains, or both. The mechanism involved needs clarification. It is of interest that medium composition also affected the reaction of biotype-3 strains to strain 84; the generally accepted insensitivity of these strains to agrocin 84 was confirmed on the modified Stonier's medium, but on AB medium four of 11 agrocin-producing biotype-3 pathogens were sensitive to strain 84. Other evidence indicating effects of nutrition on strain-84–pathogen interactions in vitro [22] and on agrocin production by the biotype-1 strain D286 [9] has been reported.

The marked decrease in galls on grapevines when nonpathogenic agrocin-producing strains were introduced into wounds concurrent with biotype-3 pathogens indicated the possibility of employing one or more of these strains for biological control of biotype-3 pathogens. In this regard, it is

Table 6. Agrocin production by biotype-3 strains and biotype-2 strain 84 on modified medium of Stonier [27] and modified AB [20] medium

Strains tested for sensitivity	Strains[a] producing agrocins[b] on Medium of Stonier						AB medium					
	L1	I2	H6	H8	At1	84	L1	I2	H6	H8	At1	84
A4[c]	−	−	−	+	+	−	−	−	−	−	−	−
F2	−	−	−	+	+	−	−	−	−	−	−	−
L1	−	−	−	+	+	−	−	−	−	−	−	−
I2	−	−	−	+	+	−	−	−	−	−	−	−
B5	−	−	−	+	+	−	−	−	−	−	−	−
H6	−	−	−	+	+	−	−	−	−	−	−	−
A1[d]	−	+	−	−	−	−	+	+	−	−	−	−
A3	−	+	−	−	−	−	−	+	−	+	−	−
F1	−	+	−	−	−	−	−	+	+	+	−	−
At1	−	−	−	−	−	−	+	+	+	+	−	+
At2	+	+	−	−	−	−	−	+	+	−	−	−
H5	−	+	+	+	+	+	+	−	+	−	−	+
H8	−	−	−	−	−	−	−	−	−	−	−	+
H9	−	−	−	−	−	−	+	−	−	−	−	−
H10	−	−	+	−	−	−	−	−	−	−	−	−
H11	−	−	−	−	−	−	−	−	+	−	−	+
A5[e]	+	+	+	+	+	−	−	+	+	−	−	−
C3	+	+	−	+	+	−	−	−	−	−	−	−
D1	+	+	+	+	+	−	−	−	+	−	−	−
D6	+	−	+	+	+	−	−	+	+	+	−	−
1771	+	+	−	+	+	−	−	−	−	−	−	−
Y1	+	+	+	+	+	−	+	+	+	−	−	−
V1	−	+	−	+	+	−	−	−	−	−	−	−

[a] L1, I2, H6, and 84 are nonpathogens; H8 and At1 are pathogens.
[b] + denotes inhibition of test strain; − denotes no discernable inhibition.
[c] Agrocin-producing nonpathogenic strains (A4 to H6).
[d] Agrocin-producing pathogens (A1 to H11).
[e] Non-agrocin-producing pathogens (A5 to V1).

of interest that reduced gall formation as a result of the application of a nonpathogenic agrocin producer (Table 5) did not necessarily correlate with in vitro sensitivity of the pathogen to the agrocin concerned (Tables 2 and 3).

Involvement of agrocin-producing capability in pathogen performance was also indicated by the in vivo results of the greenhouse experiment (Table 5). Among the 15 pathogens used, three of four agrocin-producing strains, H8, At1, and At2, formed galls on each of four replicate plants when used in the absence of the nonpathogenic agrocin producers; the third strain, F1, formed galls on three plants (Table 5). In contrast, only three of 11 non-agrocin-producing pathogens formed galls on four replicates. Three of the agrocin-producing pathogens, H8, At1, and At2, also appeared to be among the more resistant strains to the effects of the nonpathogenic agrocin producers, perhaps an indication of reverse inhibition of the nonpathogens that showed in vitro sensitivity to the agrocins produced by the pathogens involved (Table 2). Kerr [13] also attributed a case of ineffective biological control by strain 84 to agrocin formation by the pathogen.

The unsuitability of strain 84 as agent for biological control of biotype-3 strains on grapevines [25] has been confirmed by the greenhouse experiment. Although an overall tendency toward slightly reduced gall formation by plants inoculated with strain 84 was indicated, some pathogens tended to induce galls on more plants when strain 84 was present.

For biological control purposes the nonpathogenic agrocin-producing biotype-3 strains need to be further screened in field experiments. Attention should also be given to the agrocin-producing pathogens that could either be of value if cured of their pathogenicity or may have important implications in a biological control program owing to their apparent status as "super" pathogens.

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