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Biocidal activity in plant pathogenic *Acidovorax*, *Burkholderia*, *Herbaspirillum*, *Ralstonia* and *Xanthomonas* spp.

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F.-P. HU AND J.M. YOUNG. 1998. Antibacterial and antifungal activity was investigated for strains of *Acidovorax* spp., *Burkholderia* spp., *Herbaspirillum rubrisubalbicans* and *Ralstonia solanacearum*; strains representing 118 species and pathovars of *Xanthomonas* were also tested for phytotoxic capacity. Antibacterial activity was present in all *Burkholderia* spp. except *B. andropogonis*, in biovars II and III of *R. solanacearum* but not in biovars I and IV, and in two strains of *Xanthomonas.* Little antibacterial activity was recorded for *Acidovorax* spp. Antifungal activity was expressed by most strains of *A. avenae* ssp. *avenae* and *A. avenae* ssp. *cattleyae.* Weak or variable antifungal reactions were given by strains of *A. avenae* ssp. *citrulli* and no activity was expressed by *A. konjaci.* Most strains of *B. caryophylli, B. cepacia, B. gladioli* pv. *agaricicola, B. gladioli* pv. *alliicola, B. gladioli* pv. *gladioli*, *B. glumae* and *B. plantari* produced extensive inhibition zones against *Rhodotorula mucilaginosa.* Strains of *H. rubrisubalbicans* and *R. solanacearum* gave negative, weak or variable reactions. Strains of *Xanthomonas* spp. exhibited no antifungal activity. In all cases antifungal activity was caused by a low molecular weight toxin. Three *Xanthomonas* strains exhibited phytotoxic activity. The ecological implications of these data are discussed.

INTRODUCTION

Many kinds of biocidal compounds have been reported for plant pathogenic bacteria and suggestions have been made for their function and application. Although most are non-specific biocides, some doubtless play a role in predisposing plant tissue to invasion. In the ecology of toxigenic bacteria, it has been suggested that biocides have protective effects against microbial antagonists and may enable bacteria to invade biological environments by inhibiting competitors. In practical terms, biocidal compounds may also have utility as medicinal antibiotics, and effective organisms have been claimed to have utility as biocontrol agents (Arie *et al.* 1987). As they are simple to assay, biocides may also be useful as determinative characters for identification (Hayward 1991; Rudolph 1991; Young 1991; Young and Triggs 1994). Thus, bacteriocins have sometimes been used to type bacteria in diagnostic protocols (Pitt and Gaston 1995).

Biocides produced by bacteria may be antibacterial, antifungal or phytotoxic, and commonly express more than one of these properties. Thus, phaseolotoxin, a potent inhibitor of plant metabolism, also has antibacterial properties (Rudolph 1991) while syringomycin has antibacterial, antifungal and phytotoxic properties (Iacobellis *et al.* 1992).

In this paper, part of an on-going survey of toxin production on Gram-negative plant pathogenic bacteria, a survey of antibacterial and antifungal activity in the plant pathogenic species of *Acidovorax avenae* and *A. konjaci, Burkholderia andropogonis, B. caryophylli, B. cepacia, B. gladioli, B. glumae,* and *B. plantarii, Herbaspirillum (Pseudomonas) rubrisubalbicans,* and *Ralstonia (Burkholderia) solanacearum* is reported. For species and pathovars of *Xanthomonas,* data are presented on antibacterial, antifungal and phytotoxic reactions.

METHODS AND MATERIALS

Strains

Strains were from the International Collection of Microorganisms from Plants (ICMP; formerly Plant Diseases Div-

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ision Culture Collection) Landcare Research, Auckland. Cultures of *Acidovorax, Burkholderia*, *Herbaspirillum* and *Ralstonia* were maintained by monthly subculture on slopes of yeast-extract phosphate salts agar, YPA containing (g l^{-1}) NH_4Cl 0·5, KCl 0·2, $MgSO_4.7H_20$ 0·2, K_2HPO_4 1·0, yeast extract (Difco, Detroit, MI, USA) 3·0, agar (Davis, Christchurch, New Zealand) 12, or held at room temperature as faintly turbid suspensions in sterile de-ionized water (SDW). Cultures of *Xanthomonas* were maintained on slopes of glucose-yeast-carbonate agar, GYCA containing glucose 5, yeast extract 5, $CaCO_3$ precipitated 60, agar 12, and subcultured at monthly intervals. All cultures were incubated at 25 °C and stored at 8 °C.

Antibacterial activity

Antibacterial activity of strains was tested using the inverted agar method of Kékessy and Pigeut (1970). Strains tested of *Acidovorax, Burkholderia* and *Herbaspirillum rubrisubalbicans* are indicated in Table 3. Strains of *Xanthomonas* tested are in Table 2. Strains of *Ralstonia solanacearum* were:

Biovar I: 5712^T (tomato), 6523 (tobacco), 7858 (banana), 7961 (potato)
Biovar II: 775 (potato), 777 (tomato), 6782 (banana), 7959 (capsicum)
Biovar III: 782 (potato), 7971 (capsicum), 8060 (tomato), 8091 (banana)
Biovar IV: 7960 (capsicum), 8109 (tomato), 8115 (potato), 8121 (eggplant).

Indicator strains were incubated on potato dextrose agar (PDA) medium for 7 d at 25 °C, after which plates were inoculated with indicator strains. Three Gram-positive bacterial species, *Clavibacter michiganensis* ICMP 5966, *Listeria innocua* 112989 and *Staphylococcus epidermidis* 12988, and five Gram-negative species, *Acetobacter pasteurianus* 3878, *Acidovorax avenae* 3183, *Burkholderia plantari* 9424, *Escherichia coli* 6107 and *Pseudomonas fluorescens* 3512, were used as indicator strains. Inhibitory reactions were recorded after incubation for 3 d. Positive reactions were recorded when no growth of indicator bacteria was observed within 3 mm of the indicator strain. Weak reactions were recorded if growth occurred closer to the indicator strain but inhibition occurred over the test strain. No inhibition was recorded if the indicator strain grew confluently over the test strain. Ambiguous reactions were repeated.

Antifungal activity

Plates containing PDA were spot-inoculated at four equidistant points with test strains and incubated for 7 d at 25 °C. Strains tested of *Acidovorax, Burkholderia* and *Herbaspirillum rubrisubalbicans* are indicated in Table 3. Strains of *Xanthomonas* tested are in Table 2. Strains of *Ralstonia solanacearum* tested for antifungal activity were:

Biovar I: 748 (potato), 756 (*Symphytum* sp.), 767 (*Musa* sp.), 769 (*Pelargonium* sp.), 770 (peanut), 5712^T (tomato), 6523 (tobacco), 7860 (*Musa* sp.), 7958 (potato), 8059 (tomato), 8090 (*Melampodium* sp.), 8281 (*Ageratum* sp.), 9915 (ginger)
Biovar II: 750 (tomato), 7868 (potato), 7954 (*Portulaca* sp.), 7959 (*Capsicum* sp.), 8166, 8220 (nightshade), 9914 (tumeric)
Biovar III: 771 (*Casuarina* sp.), 773 (ginger), 774 (*Dahlia* sp.), 779 (*Physalis* sp.), 780 (*Eclipta* sp.), 781 (teak), 783 (cabbage), 7862 (*Heliconia* sp.), 7955 (eggplant), 7969 (tobacco), 7971 (*Capsicum* sp.), 7981 (*Capsicum* sp.), 8079 (potato), 8091 (*Musa* sp.), 8110 (potato)
Biovar IV: 755 (ginger), 8118 (tomato), 8121 (eggplant), 8202 (potato).

Plates were then spray-inoculated with faintly turbid cellular suspensions of *Rhodotorula mucilaginosa* ICMP 12474 (CBS 5804) prepared from 48 h PDA spread plates. After 24–48 h the appearance of an inhibitory zone around bacteria colonies was recorded according to the annotations described in Table 3.

Extraction of antifungal toxins

Toxigenic bacteria were grown in a medium called IMM (Surico *et al.* 1988) or in potato dextrose broth incubated at 27 °C as still cultures for 4–5 d. The medium was clarified by centrifugation (2500 rcf, 5 min) and sterilized by membrane filtration (0·2 μm). The medium was inoculated into metal tubes with a capacity of 170 μl on surface-dried PDA plates which were then sprayed with *Rhodotorula mucilaginosa*. The presence of inhibition zones was recorded after incubation at 27 °C for 48 h.

Diffusible antifungal activity

Toxigenic bacteria were spot-inoculated onto dialysis membranes (sterilized by immersion in 10% commercial hypochlorite solution for 5 min and rinsed in SDW) laid onto PDA in plates, and then incubated for 4 d. The colony margins were then marked on the reverse side of the agar plate, the membranes with their bacterial culture were removed, and plates were assayed for the presence of toxin in the medium using *Rhodotorula mucilaginosa*. Strains of *Burkholderia andropogonis* and *Herbaspirillum rubrisubalbicans* tended to spread more rapidly over the membrane surface and hence, diffusion of toxins in these species was tested after 2 d.

Phytotoxic activity

Phytotoxic activity of strains of *Xanthomonas* was tested by inoculating the leaf laminae of tobacco cv. 'White Burley'

using a hypodermic syringe charged with turbid (5×10^8 cfu ml^{-1}) bacterial suspensions prepared from 48 h cultures in SDW. The appearance of necrosis in the lamina after 48 h was recorded as positive for the production of toxin.

RESULTS

Antibacterial activity

Acidovorax. Of all strains of *Acidovorax* spp., only *A. avenae* ssp. *cattleyae* 2826 and 8654 inhibited growth of *L. innocua* (Table 1).

Burkholderia. No strains of *B. andropogonis* were inhibitory to any bacterial indicator strains. All strains of *B. cepacia, B. gladioli* (all pathovars) and *B. glumae* were inhibitory to all indicator bacteria (except *B. plantarii* 9424, which is discussed separately). *Burkholderia plantarii* 9424 was inhibited by all strains of *B. gladioli* pv. *gladioli* and *B. plantarii,* but by none of *B. gladioli* pv. *alliicola*. *Burkholderia cepacia* 5796 and 5981 were not effective against *B. plantarii*. Only strain 3729 of *B. glumae* was inhibitory and only strain 12220 of *B. gladioli* pv. *agaricicola* was not inhibitory. All strains of *B. caryophylli* were inhibitory to *A. pasteurianus*, *C. michiganensis, L. innocua,* and *S. epidermidi*s, weakly inhibitory to *Ps. fluorescens* and not inhibitory to *A. avenae, B. plantarii* or *E. coli* (Table 1).

Herbaspirillum rubrisubalbicans. Strains of this species gave differing reactions. All strains inhibited the growth of *Acidovorax avenae* and *C. michiganensis.* No strains inhibited *B. plantarii*. Only strain 3108 did not inhibit *E. coli*. Only strain 8777 did inhibit *Acetobacter pasteurianus*. Positive, weak and negative reactions were given by the remaining strains to indicator bacteria. The variable reactions of this species are indicated in Table 1.

Ralstonia solanacearum. Strains of biovars I and IV, and the banana strain of biovar II (6782), did not produce inhibitory reactions against any indicator bacteria. Strains of biovars II and III were inhibitory to all strains except *Acetobacter pasteurianus* and *Burkholderia plantarii* (Table 1).

Xanthomonas. Xanthomonas campestris pv. *sesami* 621 produced an antibacterial reaction to all indicator strains except *A. pasteurianus and B. plantarii. Xanthomonas campestris* pv. *secalis* 5749 was inhibitory to *Acidovorax avenae.* No other strains of *Xanthomonas* produced inhibitory reactions to any indicator bacteria.

Antifungal activity

Acidovorax. Most strains of *Acidovorax avenae* ssp. *avenae* (22/27) and *Acidovorax avenae* ssp. *cattleyae* (3/3) gave positive reactions in the test for inhibition to *Rhodotorula mucilaginosa*. Most strains of *Acidovorax avenae* ssp. *citrulli* (6/8) produced weak or negative reactions. No strains of *Acidovorax konjaci* (3/3) produced antifungal reactions. All positive strains which were tested for diffusibility of toxin gave positive reactions in the test using dialysis membrane except *A. avenae* ssp. *avenae* 3186 and *A. avenae* ssp. *citrulli* 7713 (Table 3).

Burkholderia. All strains of *B. caryophylli* (5), *B. cepacia* (12), *B. gladioli* pv. *agaricicola* (5), *B. gladioli* pv. *alliicola* (16), *B. gladioli* pv. *gladioli* (3), *B. glumae* (5) and *B. plantarii* (6) gave positive reactions in the test for inhibition to *R. mucilaginosa*. Most strains of *B. andropogonis* (9/12) gave positive reactions. Of these, 2807 and 7855 gave weak or variable reactions. With the exception of *B. andropogonis* 2806, all strains tested for toxin diffusion gave positive reactions (Table 3).

Herbaspirillum rubrisubalbicans. Most strains of *Herbaspirillum rubrisubalbicans* (8/11) gave positive reactions in the test for inhibition to *Rhodotorula mucilaginosa*. All positive strains which were tested for diffusibility of toxin gave positive reactions (Table 3).

Ralstonia solanacearum. With the exception of two strains (5712 and 6523) in biovar I, no members of this species gave inhibitory reactions against *Rhodotorula mucilaginosa*.

Xanthomonas. No inhibitory zones were produced by any strains listed in Table 2.

Phytotoxic activity

Xanthomonas. Xanthomonas campestris pv. *graminis* 5733, *X. campestris* pv. *vesicatoria* 63 and *X. campestris* pv. *zantedechiae* 2372, gave rapid necrotic reactions within 48 h. No other strains were effective (Table 2).

Behaviour of toxins

The antifungal compound or compounds demonstrated in *Acidovorax* spp. and *Burkholderia* spp. are not produced in IMM medium or in potato dextrose broth in sufficient concentration to produce inhibitory reactions, but are a product of growth of the bacterium on PDA and able to pass through dialysis membranes (Table 3), indicating a molecular weight of $< 12\,000$.

Table 1 Antibacterial reactions by *Acidovorax* spp., *Burkholderia* spp., *Herbaspirillum rubrisubalbicans* and *Ralstonia solanacearum*

		Bacterial indicator strain							
	No. of strains	1*	2	3	4	5	6	7	8
Acidovorax avenae subsp. *avenae*	10	−	−	−	−	−	−	−	−
Acidovorax avenae subsp. *citrulli*	7	−	−	−	−	−	−	−	−
Acidovorax avenae subsp. *cattleyae*	3	−	+/−	−	−	−	−	−	−
Acidovorax konjaci	3	−	−	−	−	−	−	−	−
Burkholderia andropogonis	5	−	−	−	−	−	−	−	−
Burkholderia caryophylli	5	+	+	+	−	+	−	−	(+)
Burkholderia cepacia	3	+	+	+	+	+	+	+	+
Burkholderia gladioli pv. *agaricicola*	5	+	+	+	+	+	+/−	+	+
pv. *alliicola*	4	+	+	+	+	+	−	+	+
pv. *gladioli*	3	+	+	+	+	+	+	+	+
Burkholderia glumae	5	+	+	+	+	+	+/−	+	+
Burkholderia plantarii	5	+	+	+	+	+	+	+	+
Herbaspirillum rubrisubalbicans	6	+	+/−	+/−	+	+/−	−	+/−	+/−
Ralstonia solanacearum biovar I	4	−	−	−	−	−	−	−	−
biovar II†	4	+	+	+	+	−	−	+	+
biovar III	4	+	+	+	+	−	−	+	+
biovar IV	4	−	−	−	−	−	−	−	−

+, Positive inhibition of indicator strain.
(+), Weak positive inhibition of indicator strain.
−, No inhibition of indicator strain.
+/−, Positive, weak and negative reactions between strains in duplicated experiments.

* Bacteria indicator strains
Gram-positive
1. *Clavibacter michiganensis* 5966
2. *Listeria innocua* 12989
3. *Staphylococcus epidermidis* 12988

Gram-negative
4. *Acidovorax avenae* 3183
5. *Acetobacter pasteurianus* 3878
6. *Burkholderia plantarii* 9424
7. *Escherichia coli* 6107
8. *Pseudomonas fluorescens* 3512

†A banana strain of biovar II (6782) did not produce inhibitory compounds to any indicator bacteria.

DISCUSSION

Biocidal activity has been demonstrated in many of the species now classified in *Acidovorax, Burkholderia, Herbaspirillum* and *Ralstonia*. This activity may be correlated to earlier reports of biocidal production for some species. In the past, *A. niger* has been used in bioassays for biocidal activity (Young 1991; Young and Triggs 1994). Preliminary results indicated that the reactions of *A. niger* and *R. mucilaginosa* were quantitatively similar and that the latter organism was more easy to use in assays. Furthermore, our unpublished data including strains of *Burkholderia* spp., *Pseudomonas* spp. and *Xanthomonas* spp., showed that *A. niger* and *R. mucilaginosa* gave qualitatively similar reactions. Therefore, the experimental series were completed using only *R. mucilaginosa*.

For *Acidovorax*, only two strains of *A. avenae* ssp. *cattleyae* produced antibacterial reactions against *L. innocua*. In our tests using *R. mucilaginosa*, antifungal compounds were detected for most strains of *A. avenae* ssp. *avenae* and *A. avenae* ssp. *cattleyae*. This suggests that the biocide found in these strains has specific antifungal, rather than general biocidal, properties. It is presumably the same substance as

Table 2 Strains used in the study of *Xanthomonas* spp. and pathovars of *X. campestris*, with their ICMP numbers

X. albilineans 196	*daturae* 12546	*phleipratensis* 5744
X. bromi 12545	*desmodiilaxiflori* 6502	*phormiicola* 4294
X. codiaei 9513	*durantae* 5728	*phyllanthi* 5745
X. fragariae 5715	*erythrinae* 446	*physalidicola* 586
X. populi 5816	*esculenti* 5729	*pisi* 570
X. campestris 13	*eucalypti* 5382	*plantaginis* 1028
	euphorbiae 5730	*poae* 7726
X. campestris pvs:	*fici* 3036	*poinsetticola* 5779
aberrans 4805	*graminis* 5733	*populi* 8923
alangii 5717	*guizotiae* 5734	*pruni* 51
alfalfae 5718	hederae 453	*raphani* 1404
amorphophalli 3033	*holcicola* 3103	*rhynchsiae* 5748
arecae 5719	*hordei* 5752	*ricini* 5747
argemones 1617	*hyacinthi* 189	*secalis* 5749
armoraciae 7	*incanae* 574	*sesami* 621
arrhenathri 7727	*ionidii* 5736	*sesbaniae* 367
asclepiadis 10007	*juglandis* 35	*spermacoces* 5751
azadirachtae 3102	*khayae* 671	*tamarindi* 572
barbareae 438	*laureliae* 84	*tataxaci* 579
bauhiniae 5720	*lawsoniae* 319	*tardicrescens* 4295
betae 8917	*leeana* 5738	*theicola* 6774
biophyti 2780	*leersiae* 8788	*thespesiae* 7466
blepharidis 5722	*maculifoliigardeniae* 318	*thirumalacharii* 5852
boerhaaviae 9423	*malvacearum* 5739	*translucens* 5752
brunneivaginae 9991	*mangiferaeindicae* 5740	*tribuli* 5753
cajkani 444	*manihotis* 5741	*trichodesmae* 5754
cannabis 6570	*melonis* 8682	*undulosa* 5755
cannae 8306	*merremiae* 6747	*uppalii* 5756
cassavae 204	*musacearum* 2870	*vasculorum* 5757
cassiae 358	*nakataecorchori* 5742	*vesicatoria* 63
celebenis 1488	*nigromaculans* 80	*viegasii* 9261
centellae 6746	*oryzae* 3125	*vignaeradiatae* 5759
cerealis 1409	*oryzicola* 5743	*vignicola* 333
citri 24	*papavericola* 220	*viticola* 3867
clitoriae 6574	*patelii* 167	*vitistrifoliae* 5761
convolvuli 5380	*paulliniae* 8919	*vitiswoodrowii* 3965
coracanae 5724	*pedalii* 3030	*zantedeschiae* 2372
coriandri 5725	*pelargonii* 4321	*zingibericola* 8787
orylina 5726	*pennamericanum* 9627	*zinniae* 5762
cucurbitae 2299	*phaseoli* 5834	
cyamopsidis 616	*phlei* 7725	

the antifungal substance from *Acidovorax avenae*, effective against the rice blast fungus, reported elsewhere (Kunitake *et al.* 1988; Kunitake and Matsuyama 1989). Weak variable or negative reactions were recorded for *A. avenae* ssp. *citrulli* and *A. konjaci.*

Most species of *Burkholderia* exhibited a wide spectrum antibacterial activity (Table 1) although strains of *B. caryophylli* gave variable results and activity was lacking in *B. andropogonis.* Wakimoto *et al.* (1986) earlier reported anti-

Table 3 Strains of *Acidovorax* spp., *Burkholderia* spp., and *Herbaspirillum* sp. from ICMP used in the study, with their ICMP numbers. Inhibitory reactions to *Rhodotorula mucilaginosa* are recorded. Strains used in tests for antibacterial activity are marked with an asterix*

Bacterial name	Antifungal activity	Diffusible toxin
Acidovorax avenae subsp. *avenae*		
251	+	+
254*	+	nt
255	+	+
1656	+	nt
3106*	+	nt
3139	+	+
3168	+	+
3179*	+	+
3180	+	nt
3182	+	+
3183^{T}*	+	+
3184*	−	nt
3186*	+	−
3960*	+	+
5811*	+	nt
7083	+	nt
9906	+	(+)
9907	+	+
9908	+	+
9909	+	(+)
9910	+	nt
9913	+	(+)
10126	+	+
10130	−	−
11900*	+	nt
11992	−	−
11993	−	−
Acidovorax avenae subsp. *citrulli*		
6521*	(+)	nt
6522*	(+)	nt
7500^{T}*	(+)	nt
7713*	+	−
7714	−	−
7715*	+	nt
7716*	(+)	nt
8663*	nt	nt
12190	−	−
Acidovorax avenae subsp. *cattleyae*		
2826^{T}*	+	+
8654*	+	(+)
12228*	(+)	+
Acidovorax konjaci		
7733^{T}*	−	nt
7734*	−	nt
7851*	−	nt

Table 3 *(Continued)*

Bacterial name	Antifungal activity	Diffusible toxin
Burkholderia andropogonis		
2806*	+	−
2807T*	+/−	+
3377	+	+ (2 days)
3994	−	nt
3996*	−	nt
3998*	nt	nt
4001	+	+
5980	+	nt
6779	+	+ (2 days)
7854*	−	−
7855	(+)	+ (2 days)
8039	+	(+) (2 days)
8665	+	(+) (2 days)
Burkholderia caryophylli		
512*	nt	nt
2824*	(+)	(+)
2825	+	+
5848*	+	nt
8691*	+	nt
8692*	+	nt
Burkholderia cepacia		
2831	++	nt
3177	++	+
5796T*	++	++
5837	++	nt
5841	++	nt
5952	++	nt
5981*	++	++
5982	++	++
8655	++	nt
9162*	+	nt
9163	+	+
9164	+	+
Burkholderia gladioli pv. *agaricicola*		
7845*	+	nt
11096*	++	++
11097*	++	++
12220*	++	++
12222*	++	++
Burkholderia gladioli pv. *alliicola*		
2804T*	++	++
3410*	++	nt
3411	++	nt
3412	++	nt
5838	++	++
5839	++	nt
5840	++	nt
7845*	++	++

Table 3 *(Continued)*

Bacterial name	Antifungal activity	Diffusible toxin
9021	++	++
9022	++	nt
9023	++	nt
9209	++	++
9210*	++	++
9211	++	++
9212	++	nt
9213	++	++
3950T*	++	++
3951*	++	++
3952*	++	++
Burkholderia glumae		
3655T*	+	+
3727*	++	++
3728*	++	++
3729*	++	++
8657*	++	++
Burkholderia plantarii		
9424T*	++	++
9425	++	++
9426*	++	++
9427*	++	++
9428*	++	++
9429*	++	++
Herbaspirillum rubrisubalbicans		
792*	+	nt
793*	−	−
2850*	−	−
3108*	+	+
3109	+	+ (2 days)
3110	+	+ (2 days)
3112	(+)	(+)
5714	(+)	+ (2 days)
5777*	+	+
6268*	−	(+) weak
8664	+	+ (2 days)

T, Type strain.
−, No inhibitory zone produced.
(+), Inhibitory zone <0·5 mm wide around colony.
+, Inhibitory zone >0·5 mm; <10 mm wide around colony.
++, Inhibitory zone >10 mm wide around colony.
nt, Not tested for antifungal activity or diffusibility of toxin.

bacterial activity for *B. cepacia*, *B. gladioli* and *B. glumae*. We have no explanation for the observation that *B. plantarii* was inhibitory to itself.

Antifungal activity also occurs in most *Burkholderia* spp. The spectrum of activity is similar to that of antibacterial

activity, strains of most species except *B. andropogonis* giving uniformly positive reactions. This suggests that antibacterial and antifungal activity may be caused by the same compound or compounds. These have a low molecular weight and are either produced in large amounts or are potent biocides, as indicated by the size of the zones, which can almost cover a 9 cm Petri dish from a point inoculation after 4 d.

A number of antifungal compounds has been reported for *Burkholderia*. Cepacin A and B (Parker *et al.* 1984), cepalycin (Abe and Nakazawa 1994), cepacidine A and B (Lee *et al.* 1994; Lim *et al.* 1994), pyrrolnitrin (Homma *et al.* 1989) and tropolone (Korth *et al.* 1982) have been isolated from strains of *B. cepacia*. It remains to be shown whether these compounds are produced by all *Burkholderia* spp., and which are primarily responsible for expressed activity.

The antifungal compound or compounds demonstrated in *Acidovorax* spp. and *Burkholderia* spp. are not produced in IMM medium or in potato dextrose broth in sufficient concentration to produce inhibitory reactions, but have a molecular weight of $< 12\,000$. This is consistent with the compounds such as cepalycin and cepacidine. Inhibition zones by strains of *Acidovorax* spp. were smaller and zone margins appeared more diffuse than those of *Burkholderia,* suggesting different compounds.

The strains of *Xanthomonas* species and pathovars showed very little biocidal activity as expressed in antibacterial and antifungal tests. In their plant interactions, many pathogenic bacteria, inoculated in high concentrations, have the capacity to cause necrosis in plants to which they are not pathogenic. Tobacco has been commonly used to assay for the generalized hypersensitivity reaction (HR, Lelliott *et al.* 1966) but also usefully serves to test for generalized herbicidal actitivity. In this context, these reactions are to be found in *Acidovorax, Burkholderia* and *Herbaspirillum* (Hu *et al*. 1991). Strains of *Xanthomonas* species and pathovars expressed little herbicidal or HR activity as indicated in this assay. This is supported by the observation of Hildebrand and Riddle (1971) that a generalized necrosis could be induced in tobacco by xanthomonad strains only in a limited number of specified and unusual (continuous dark, elevated temperature) conditions. Several compounds have been reported for *Xanthomonas* as having toxigenic characteristics. Several of these are of carboxylic acids or other low molecular weight compounds reported as phytotoxins (Egawa *et al.* 1968; Noda *et al.* 1980; Robeson and Cook 1985; Ewbank and Maraite 1990). Proof that a particular compound extracted from plant tissue has a role as a phytotoxin initiating or supporting pathogenicity *in planta* requires more than its extraction from affected tissue and a demonstration of toxic capacity. The absence of expression of toxin production in our assays raises questions as to whether specific *in planta* conditions are needed for the production of toxic compounds or, if the compounds are produced, their concentration is sufficient to act as biocides.

Inhibitory reactions may be useful for identification. The uniform production of antifungal compounds by several species, subspecies and pathovars of *Acidovorax* and *Burkholderia* offers a useful confirmatory test for identifying isolates of these taxa from specific plants. The antibacterial reactions of biovars of *R. solanacearum* offer a useful guide to distinguish between biovars I and IV, and biovars II and III (Table 1). In this case, attention is drawn to the banana isolate of biovar II which may be incorrectly identified.

Strains *Herbaspirillum rubrisubalbicans* gave variable reactions in both antibacterial and antifungal assays.

In the plant pathogenic genera *Acidovorax, Burkholderia, Herbaspirillum* and *Ralstonia,* biocidal compounds are produced in several species, *Burkholderia* being notable in that strains of most species produced both antibacterial and antifungal reactions. Even within this genus, *B. andropogonis* was notable for the absence of antibacterial activity and for the variability of strain reactions in the tests for antifungal activity. Biovars II and III of *Ralstonia solanacearum* expressed antibacterial activity to a range of indicator bacteria, but no antifungal activity. The genus *Xanthomonas* is striking for the almost complete absence of expressed biocidal activity.

Explanations for wide spectrum biocidal activity commonly centre on the role such compounds play in supporting the producing organism against competitors (Rudolph 1995). The data here do not support such a role. Of all plant pathogenic bacteria, *Ralstonia solanacearum* appears to survive for the longest periods of time in soil independent of living host plants or of host plant residues (Buddenhagen and Kelman 1964; Sequeira 1994). The competitive saprophytic ability of this organism may be more limited than has been previously thought but there is little difference in the capacity for the different biovars (as races) to survive (Sequeira 1994). In the present study, strains of biovars II and III demonstrated antibacterial activity while strains of biovars I and IV species did not. None demonstrated antifungal activity. There appears to be no obvious association between biocide production and competitive saprophytic ability for *R. solanacearum*. Strains of *Xanthomonas* spp. have also been shown to survive in soil-associated environments in which competitive saprophytic ability is necessary (Goto 1972, 1992; Goto *et al.* 1976). The notable absence of activity against the selection of Gram-positive and Gram-negative bacteria, as well as against *Rhodotorula*, a representative fungus, indicates the absence of activity against a component of the natural microflora which is potentially competitive in soil. This suggests that biocide production may not be essential for successful competition of plant pathogenic bacteria against other micro-organisms.

Most plant pathogenic bacteria have evolved to form a close association with their host plants. Active bacterial metabolism and multiplication is in plant intercellular environments which are almost completely free of microbial competitors. In these circumstances, biocide production would be redun-

dant. It remains an open question whether or not biocidal compounds have a significant role in the ecological interactions of bacteria with their hosts or with associated microflora.

Phytotoxic compounds, where they are present, may be involved in specific plant associated interactions. For instance, strains of *P. syringae* pv. *syringae* and closely related or synonymous pathovars appear to be the only ones which produce the syringomycin complex (Young and Triggs 1994). These pathovars are also unusual in that they are able to invade mature plant tissue (Young 1991); it is a characteristic of most pathogenic bacteria that they are usually active only in juvenile plant tissue. If they have phytotoxic properties, then the wide-spectrum biocides with antifungal and haemolytic properties, such as cepalycin produced by *B. cepacia*, may have a role similar to syringomycin for this pathogen, and the antibiosis of related *Burkholderia* spp. may be similarly explained. Biocidal compounds have been demonstrated in high nutritional conditions in these and similar studies. Elsewhere, it has been proposed that such production is an artefact which may not occur in natural environments (Brian 1960). The possibility should be considered that these compounds are excreted waste residues and that their biocidal activity is incidental to bacterial function or their environmental interactions.

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