Bacteriocin, Plasmid and Pectolytic Diversity in *Pseudomonas cepacia* of Clinical and Plant Origin

By CARLOS F. GONZALEZ* AND ANNE K. VIDAVER

*Department of Plant Pathology, University of Nebraska, Lincoln,
Nebraska 68583, U.S.A.*

(Received 25 July 1978)

Pseudomonas cepacia strains of plant and clinical origin were compared with the type strains of *P. cepacia*, *P. kingii* and *P. multivorans*. Conventional biochemical tests and antibiotic sensitivity patterns supported the previous proposals of synonymy between *P. cepacia*, *P. kingii* and *P. multivorans*. However, bacteriocin production patterns, onion maceration tests and hydrolysis of low pH pectate agar clearly differentiated strains of clinical and plant origin into two distinct groups; these tests may therefore be helpful in epidemiological studies. In contrast, plant and clinical strains were of equal lethality to mice. Agarose gel electrophoresis indicated the presence of one or more plasmids (molecular weights 9×10^6 to 120×10^6) in 15 out of 16 strains of both types examined.

INTRODUCTION

Pseudomonas cepacia has been described as the causal agent in decay of onions (Burkholder, 1950). Since then, *P. multivorans* isolated from soil and *P. kingii* from clinical sources also have been described as synonyms for *P. cepacia* (Sinsabaugh & Howard, 1975; Snell *et al.*, 1972). The synonymy of *P. cepacia* (Burkholder, 1950) and *P. multivorans* (Stanier *et al.*, 1966) was established by Ballard *et al.* (1970) on the basis of indistinguishable patterns of utilization of a wide range of carbon substrates and of similar levels of molecular homology as shown by DNA–DNA hybridization. Snell *et al.* (1972) have, in addition, proposed that *P. kingii* (Jonsson, 1970) is a synonym of *P. cepacia*. Sinsabaugh & Howard (1975) confirmed the synonymy of *P. multivorans* and *P. kingii* by subjecting a larger number of strains (15 of each) to a wider variety of morphological, nutritional, physiological and biochemical tests than employed in previous studies.

The importance of *P. cepacia*, *P. kingii*, and *P. multivorans* as aetiological agents of clinical infection is well documented (Bassell *et al.*, 1970; Dailey & Benner, 1968; Phillips *et al.*, 1971; Taplin *et al.*, 1971). *Pseudomonas kingii* strains have been reported as causing necrotizing pneumonitis (Dailey & Benner, 1968). *Pseudomonas multivorans*, isolated from sputum and blood cultures, has been implicated as significant in blood infections (Phillips *et al.*, 1971).

In view of these findings and the synonymy of *P. cepacia* with both *P. kingii* and *P. multivorans*, the question remained open as to whether *P. cepacia* of plant origin was equivalent to clinical isolates in characters not previously studied. We report here that *P. cepacia* of plant and clinical origin are distinguishable by differences in bacteriocin production, maceration of onion slices, hydrolysis of low pH pectate agar and the size of resident plasmids.

* Present address: Department of Plant Pathology, University of California, Davis, California 95616, U.S.A.

METHODS

Bacterial strains, medium and cultural conditions. The strains used in this study (Table 1) were maintained at 4 °C on nutrient yeast extract medium (NBY; Vidaver, 1967).

Biochemical tests. Acid production from nine sugars was tested by the method of Hugh & Leifson (1953), using Difco O-F basal medium and the ammonium salts medium of Smith *et al.* (1952). Results were read daily for 5 d. Arginine dihydrolase was tested by the method of Thornley (1960). Lysine and ornithine decarboxylase activities were detected using Moeller (Difco) decarboxylase base overlaid with sterile mineral oil. Oxidase activity was tested with cytochrome oxidase test paper (PathoTec).

Assay for pectolytic activity. Cells were streaked on NBY agar, incubated overnight at 30 °C, then suspended in sterile distilled deionized water and adjusted spectrophotometrically to 1×10^9 colony forming units (c.f.u.) ml⁻¹. Appropriate dilutions of the suspension were made to obtain isolated colonies. Hildebrand's (1971) medium A and medium B and Cuppels & Kelman's (1974) crystal violet pectate (CVP) medium, all of which contained sodium polypectate (Sigma), were used in testing for pectolytic activity. Hildebrand's medium A was adjusted to pH 4.9 by adding 3.0 ml 1 M-HCl to 500 ml basal medium, medium B was adjusted to pH 6.9 by adding 1.2 ml 1 M-NaOH to 500 ml basal medium, and CVP medium was adjusted to pH 7.2 by adding 1 M-NaOH as previously described (Cuppels & Kelman, 1974). Plates were incubated at 30 °C for *P. cepacia* and *Erwinia carotovora* and at 25 °C for *P. fluorescens*. Readings were taken at 24, 48 and 72 h. The type and extent of depression in the pectate gel were rated on a 0- to 5-point scale. Agar pitting was compared with the 5-point rating of *E. carotovora* on CVP medium.

Bacteriocin production and assay. Conditions for production were as previously described (Vidaver *et al.*, 1972) except that, for assay, producer plates were incubated at 25 or 30 °C, depending on the experiment. All indicator strains were grown at 30 °C. The conditions for bacteriocin production in liquid were as described by Haag & Vidaver (1974), except that incubation was at 20, 25 or 30 °C on a rotary shaker at 300 rev. min⁻¹, depending on the experiment. Assay plates were incubated at the temperature of production for 24 h.

Assay for ability to macerate plant tissue. Yellow onions (Utah yellow sweet spanish) were used throughout this study. The outer scale layers were carefully removed before slicing with an alcohol-flamed knife. The onion slices, approximately 3 to 5 mm thick, were cut transversely through the bulb; this usually gave five to eight leaf bases. The slices were then placed in a sterile Petri dish containing two sheets of sterile Whatman no. 1 filter paper pre-moistened with sterile distilled water.

For inoculation, bacterial isolates were grown overnight on NBY agar. A bacterial suspension was then made in sterile distilled water and washed twice by centrifugation. The A_{425} was adjusted to give between 10^4 and 10^9 c.f.u. ml⁻¹. (The bacterial concentrations were determined using calibration curves prepared by triplicate platings on NBY agar.) Onion slices were inoculated by transverse spreading of 0.1 ml bacterial suspension to give between 10^9 and 10^8 c.f.u. per slice. Plates were incubated at 25 and 30 °C; readings were taken at 24, 48 and 72 h.

Determination of LD₅₀ in mice. Bacteria were grown overnight in NBY broth, then centrifuged (12100g) and washed twice in sterile saline [0.85% (w/v) NaCl]. The final pellet was resuspended in sterile saline and adjusted spectrophotometrically to 10^9 c.f.u. ml⁻¹. Serial twofold dilutions of the suspensions were made in sterile saline. Female Swiss-Webster mice (20 to 25 g) were inoculated by intraperitoneal injection of 0.25 ml of a bacterial dilution (four mice per dilution). Mice inoculated with saline served as a control. Animals were observed for 5 d after inoculation, and the LD₅₀ was calculated by the method of Reed & Muench (1938). A minimum of 32 animals was used per experiment.

Minimal inhibitory concentration (m.i.c.). Bacteria were grown overnight on Mueller-Hinton agar (Difco), then suspended in sterile saline and diluted to less than 2000 c.f.u. ml⁻¹; 10 µl was spotted on to Mueller-Hinton agar plates containing doubling concentrations (5 to 1280 µg ml⁻¹) of different antibiotics, plates without antibiotic serving as controls. Plates were incubated at 30 °C and read after 24, 48 and 72 h. The m.i.c. given is the concentration of antibiotic which prevented colony formation. The antibiotics tested were penicillin, neomycin, nalidixic acid, streptomycin, kanamycin, gentamycin, tetracycline, chloramphenicol and polymyxin B (all from Sigma), carbenicillin (Geopen, from Ffizer) and minocycline (Minocin, from Lederle Laboratories, Pearl River, N.Y., U.S.A.).

Preparation of DNA for agarose gel electrophoresis. DNA for agarose analysis was isolated by a slight modification of the method of Currier & Nester (1976). Bacteria (30 ml) were grown in NBY to an A_{640} of 0.7, then washed twice in TE buffer (0.05 M-Tris, 0.02 M-EDTA, pH 8.0), resuspended in 11 ml TE buffer and treated with lysozyme (at 200 µg ml⁻¹) for 40 min at 37 °C. Pronase B (Sigma) in TE buffer was pre-digested at 37 °C for 90 min and then added at 500 µg ml⁻¹ to the bacteria. After 5 min at 37 °C, Sarkosyl (ICN-K & K Laboratories) was added to a final concentration of 1% (w/v) and incubation was continued at 37 °C for 30 min. Shearing, denaturation and renaturation were as described by Currier & Nester (1976). After neutralization, the lysate was adjusted to 1 M-LiCl. One volume of saturated phenol/1 M-LiCl (1:1,

Table 1. *Test strains*

	Strain no.*	Strain designation as received†	Source of isolation
<i>Pseudomonas cepacia</i>	1	B5912 ^a	Wound
<i>P. cepacia</i>	2	B6566 ^a	Blood
<i>P. cepacia</i>	3	B4648 ^a	Skin exudate
<i>P. cepacia</i>	4	B5910 ^a	Urine
<i>P. cepacia</i>	5	B4345 ^a	Sputum
<i>P. kingii</i>	6	ATCC 25609 ^b	Bronchial washing
<i>P. cepacia</i>	7	Hines no. 1 ^c	Nebulizer
<i>P. cepacia</i>	8	Hines no. 2 ^c	Nebulizer
<i>P. cepacia</i>	9	PC142 ^d	Onion
<i>P. cepacia</i>	10	64-22 ^e	Onion
<i>P. cepacia</i>	11	71-22 ^e	Onion
<i>P. cepacia</i>	12	72-108 ^e	Onion
<i>P. cepacia</i>	13	72-60 ^e	Onion
<i>P. cepacia</i>	14	74-34 ^e	Onion
<i>P. cepacia</i>	15	ATCC 10856 ^e	Onion
<i>P. cepacia</i>	16	ATCC 25416 ^b	Onion
<i>P. multivorans</i>	17	ATCC 17759 ^b	Soil
<i>P. allicola</i>	18	ATCC 19302 ^b	Onion
<i>P. aeruginosa</i>	19	ATCC 17793 ^f	Sputum
<i>P. fluorescens</i>	20	ATCC 13525 ^b	Pre-filter tank
<i>Micrococcus luteus</i>	21	ATCC 4698 ^b	Unknown
<i>Erwinia carotovora</i>	22	SR204 ^g	Potato

* Code number used for this study.

† Received from: a, R. Weaver, Center for Disease Control, Atlanta, Ga.; b, American Type Culture Collection (all type strains); c, S. M. Gelbarth, VA Hospital, Hines, Ill.; d, M. P. Starr, International Collection of Phytopathogenic Bacteria, University of California, Davis, Calif.; e, J. W. Lorbeer, Department of Plant Pathology, Cornell University, Ithaca, N.Y.; f, S. Berk, Department of Immunology and Microbiology, Wayne State University, Detroit, Mich.; g, A. Kelman, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

v/v) was then stirred with the lysate for 5 min. The aqueous phase was separated from the phenol phase by centrifuging, and DNA was precipitated by adding two volumes of 95% (v/v) ethanol.

Agarose gel electrophoresis. Ethanol-precipitated DNA was subjected to electrophoresis in 0.7% (w/v) agarose (ME grade; SeaKem, Marine Colloids, U.S.A.) dissolved in Tris/borate buffer (89 mM-Tris, 2.5 mM-EDTA and 89 mM-boric acid) containing 0.1% (w/v) sodium dodecyl sulphate (SDS). The dye solution contained 5% (w/v) SDS, 25% (v/v) glycerol and 0.025% (w/v) bromophenol blue. Electrophoresis was carried out using an ORTEC Electrophoresis System (model 4200; ORTEC, Oakridge, Tenn., U.S.A.). The dimensions of the gel were 8.0×10×0.3 cm. Sample wells were made by using a Teflon comb with 12 teeth. A 6% (w/v) acrylamide plug of about 1 cm was used to prevent the agarose from slipping out of the cell. Electrophoresis was carried out at 18 mA, 40 V for 3 h. Gels were stained in a solution of ethidium bromide (0.5 µg ml⁻¹) for 30 min. The DNA bands were visualized by the fluorescence from bound ethidium bromide. The gels were laid directly on the u.v. lamp filter (transilluminator C62; Ultraviolet Products, San Gabriel, Calif., U.S.A.) and photographed with a Polaroid MP-4 camera, equipped with a u.v. filter and orange (O2) filter, using Polaroid-type 665 film.

RESULTS

Biochemical characteristics

A distinguishing feature of *P. cepacia* is production of acid from a wide range of carbohydrates (Snell *et al.*, 1972). All isolates tested (Table 1), including the type strains of *P. kingii*, *P. multivorans* and *P. cepacia*, produced acid from glucose, mannose, xylose, lactose, sucrose, maltose and mannitol, and were variable for acid from fructose. No acid was produced from rhamnose.

Table 2. *Pectolytic activity of P. cepacia strains on Hildebrand's medium A and medium B*

Results were scored on a 5-point scale (0, no pitting; 5, depression to bottom of plate) after 24, 48 and 72 h.

Strain*	Medium A (pH 4.9)			Medium B (pH 6.9)		
	24 h	48 h	72 h	24 h	48 h	72 h
1	0.5	1	2	0	0	0
2	0	1	1	0	0.2	0.2
3	0	0	0	0	0	0
4	0.5	1	2	0	0	0
5	0	0	0	0	0	0
6	1	2.5	2.5	0	0.5	1
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	3	3	3	3	3	3
10	2	2.5	2.5	0	0	1
11	2	3	3	0	0	1
12	2	2	2	0	0	0
13	2	2	2	0	0	1
14	2	2	2	0	0	0
15	2	2	2.5	0	1	2
16	2	3	3	0	1	2
17	1	1.5	1.5	0	0	0
20	0	0	0	0	0	0
22	0	1	3	3	4	4

* Code numbers given for this study (see Table 1): strains 1 to 6 were from clinical sources; strains 7 and 8 were isolated from hospital equipment; strains 9 to 17 were of plant or soil origin.

Pectolytic activity

The pectolytic activity of the *P. cepacia* strains was assessed on plates containing polypectate. Strains plated on CVP medium, a selective medium for *Erwinia* spp., were all negative, whereas the control *E. carotovora* had strong pectolytic activity and caused typical deep pits in the medium.

The pectolytic activity of the *P. cepacia* strains varied with the source of isolation, the medium and the period of incubation (Table 2). Differentiation of strains by pectolytic activity was most apparent at 24 h, with Hildebrand's media A and B. All plant strains showed moderate activity on medium A (pH 4.9) at 24 h, as indicated by a wide shallow depression in the polypectate gel; only a trace of activity was shown by two clinical strains. None of the *P. cepacia* strains, except PC142, showed activity on medium B (pH 6.9). By 48 h, some plant and clinically derived strains showed a progressive utilization of the pectate at the lower pH (medium A), as indicated by wider and deeper pitting of the gel. Activity on medium B, at 48 h, was none to moderate; three plant strains showed weak to moderate activity, and two clinical strains showed only a trace of activity. At 72 h, activity on medium A had increased. Only a few isolates from either source showed activity on medium B. The pH dependence of the pectolytic enzymes was apparent. The 24 h reading appeared best for strain differentiation.

Bacteriocin production patterns

All *P. cepacia* strains and the type strains of *P. kingii*, *P. multivorans* and *P. allicola* were tested for bacteriocin production on solid medium. Each strain was used as an indicator against all the other strains. *Micrococcus luteus* was also tested as an indicator for all strains, since this strain is susceptible to all bacteriocin groups of the plant pathogens *P. syringae*, *P. phaseolicola* and *P. glycinia* (Vidaver *et al.*, 1972).

The Hines isolates (nos 7 and 8) enabled the clinical and phytopathogenic *P. cepacia* to

Table 3. *Bacteriocin production patterns of P. cepacia strains**

Indicator strain	Producer strain			
	1-6	7, 8†	9-17	18
1-6	—	—	—	—
7, 8	—	—	+‡	—
9-17	—	—	—	—
18	—	—§	—	—
21	+‡	+‡	+‡	+‡

* Production was assayed at 25 and 30 °C. Strains 1 to 6 (clinical strains) produced identical patterns; strains 9 to 17 (plant or soil strains) produced identical patterns (see Table 1 for strain numbers).

† Strains isolated from hospital equipment (see Table 1).

‡ All zones were clear and measured 15 to 20 mm from the margin of the colony.

§ Strain 7 produced a turbid zone on strain 18 at 25 °C but not at 30 °C.

be clearly differentiated; the former were negative and the latter were positive in bacteriocin production (Table 3). The *P. multivorans* type strain, which had previously been shown to be taxonomically equivalent to the plant isolates, was also grouped with the phytopathogenic *P. cepacia*. All strains produced bacteriocin-like activity against *Micrococcus luteus*. Results were the same whether plates were incubated at 30 or 25 °C, except for strain no. 7 (Table 3).

To test for inducibility of bacteriocins, mid-exponential phase liquid cultures of all isolates were treated with mitomycin C. The supernatants from the treated cultures were spotted on to lawns of all *P. cepacia* strains and *M. luteus*. No indication of bacteriocin activity was detected in any combination of indicator and producer. Therefore, under our conditions, bacteriocin production in liquid culture was not detected.

Onion maceration test

Onion slices were used to test the ability of the different strains to macerate plant tissue. The clinical isolates were inoculated at 10^8 , 10^6 and 10^4 bacteria per slice. The symptoms were rated on a 5-point scale for maceration, and discoloration of tissue was noted. No maceration or discoloration of tissue was observed with clinical isolates at 10^4 and 10^6 bacteria per slice, and only a trace of maceration and some discoloration (browning) occurred at the highest concentration tested (Table 4).

The plant strains were inoculated at 10^8 , 10^5 and 10^3 bacteria per slice. At the highest concentration, tissue was completely macerated. At the lower concentrations, these isolates gave reactions ranging from 2, with a brown-yellow discoloration, to 5 (complete maceration of tissue) accompanied by a sulphur yellow discoloration due to growth of the bacteria (Table 4). The *P. multivorans* strain caused little (1+) maceration and only slight discoloration of tissue at the 10^8 concentration; *Pseudomonas allicola* caused moderate (3+) maceration and a brown discoloration of tissue, when inoculated at 10^5 bacteria per slice.

Determination of LD_{50} in mice

Although pectolytic activity differentiated the phytopathogenic *P. cepacia* strains from those of clinical origin, the isolates tested differed only slightly in their lethality to mice (Table 5). The *P. aeruginosa* positive control and the *P. fluorescens* negative control gave LD_{50} values of 9×10^7 and 2×10^9 , respectively. This LD_{50} for *P. aeruginosa* was slightly lower than the 1.1×10^8 previously reported for the strain (Berk, 1977). No deaths were observed prior to 8 h. Diarrhoea was observed only in animals which eventually died.

Minimal inhibitory concentration studies

Since most clinical strains of *P. cepacia* are susceptible to chloramphenicol but not to antibiotics of the polymyxin group or to gentamycin (Gilardi, 1971), we examined the plant

Table 4. *Test for onion maceration by P. cepacia strains*

Results were scored on a 5-point scale (0, no maceration; 5+, tissue completely macerated) after 72 h.

Strain*	Inoculum (c.f.u. per slice)			
	10 ³	10 ⁴	10 ⁵	10 ⁸
1-8	ND	0	ND	≤ 0.5+
9	1+	ND	3+	5+
10	2+	ND	3+	5+
11	2+	ND	4+	5+
12	1+	ND	3+	5+
13	4+	ND	5+	5+
14	3+	ND	5+	5+
15	1+	ND	3+	5+
16	2+	ND	4+	5+
17	0	ND	0.5+	1+
18	1+	ND	3+	5+

ND, Not done.

* Code numbers given for this study (see Table 1); strains 1 to 6 were from clinical sources; strains 7 and 8 were isolated from hospital equipment; strains 9 to 18 were of plant or soil origin. Strains 1 to 8 gave indistinguishable results.

Table 5. *LD₅₀ of P. cepacia strains in mice*

The inoculum was injected intraperitoneally and the LD₅₀ was determined after 72 h.

Strain*	Source	LD ₅₀
1	Wound	5.1 × 10 ⁸
2	Blood	4.1 × 10 ⁸
6	Bronchial washing	2.6 × 10 ⁸
10	Plant	4.2 × 10 ⁸
14	Plant	5.4 × 10 ⁸
16	Plant	1.7 × 10 ⁸
17	Soil	1.9 × 10 ⁸

* Code number given for this study (see Table 1).

pathogens to see if they had different antibiotic susceptibility patterns (Table 6). The aminoglycoside antibiotics – kanamycin, gentamycin and neomycin – had limited activity against plant and clinical isolates. The majority of strains were resistant to polymyxin B, penicillin, streptomycin and carbenicillin at 640 to 1280 µg ml⁻¹. Resistance to tetracycline was generally high (160 to 320 µg ml⁻¹), except for the Hines isolates (nos 7 and 8) which were sensitive to less than 20 µg ml⁻¹. However, the tetracycline derivative minocycline (7-dimethylamino-6-deoxytetracycline) was inhibitory (< 10 µg ml⁻¹) to all strains. Sensitivity to nalidixic acid was moderate in most strains. As expected, susceptibility to chloramphenicol was high (usually 5 to 20 µg ml⁻¹).

Plasmid analysis of strains

Because of the high levels of antibiotic resistance and because many studies have shown that genetic determinants for such resistance may be carried on plasmids, experiments were initiated to detect their presence. Lysates from several strains were made and the salt/ethanol-precipitated DNA was analysed by agarose gel electrophoresis for the presence of plasmid DNA (Figs 1 and 2). Among *P. cepacia* from clinical sources, the plasmid number ranged from 0 to 4 (Fig. 1). The Hines isolates, which differ in bacteriocin and drug sensitivity, had four plasmids ranging in size from 9 × 10⁶ to 105 × 10⁶ daltons (Fig. 1j, k). Almost all the onion pathogens including the type strain (Fig. 2d to j) had a common plasmid of 120 × 10⁶ daltons. The *P. multivorans* type strain had a plasmid of 13 × 10⁶ daltons (Fig. 2h).

Table 6. *Minimal inhibitory concentrations of antibiotics* for various strains*

Strain†	Minimal inhibitory concentration ($\mu\text{g ml}^{-1}$)										
	Pc	Cb	Nm	Sm	Nal	Km	Gm	Tc	Mc	Cm	PolyB
1	320	2000	640	1280	40	80	640	320	< 10	5	> 1280
2	640	2000	640	1280	20	160	320	320	< 10	5	> 1280
3	80	125	320	> 1280	80	40	160	320	< 10	5	> 1280
4	320	250	320	> 1280	40	80	320	320	< 10	5	> 1280
5	640	2000	640	> 1280	20	160	640	160	< 10	5	> 1280
6	640	2000	320	1280	40	80	320	320	< 10	5	640
7	640	2000	160	640	40	40	80	20	< 10	80	1280
8	320	1000	80	640	40	40	160	20	< 10	20	> 1280
9	< 40	625	40	320	40	20	80	160	< 10	20	> 1280
10	640	500	320	> 1280	40	80	640	320	< 10	20	> 1280
11	640	2000	640	640	40	80	320	320	< 10	20	> 1280
12	640	2000	640	> 1280	40	80	320	320	< 10	20	> 1280
13	640	2000	320	1280	40	40	320	320	< 10	20	> 1280
14	640	2000	320	> 1280	40	40	160	320	< 10	20	> 1280
15	640	2000	320	1280	40	80	320	160	< 10	20	> 1280
16	640	2000	320	1280	40	40	320	160	< 10	20	> 1280
17	640	2000	640	> 1280	20	40	320	160	< 10	20	> 1280
18	< 40	< 40	40	20	20	< 5	< 5	< 20	< 10	40	> 1280

* Abbreviations: Pc, penicillin; Cb, carbenicillin; Nm, neomycin; Sm, streptomycin; Nal, nalidixic acid; Km, kanamycin; Gm, gentamycin; Tc, tetracycline; Mc, minocycline; Cm, chloramphenicol; PolyB, polymyxin B.

† Code number given for this study (see Table 1): strains 1 to 6 were from clinical sources; strains 7 and 8 were isolated from hospital equipment; strains 9 to 18 were of plant or soil origin.

DISCUSSION

Epidemiological surveillance of *P. cepacia* has not been possible previously as strains from clinical sources could not be distinguished from those from plants and soil (Sinsbaugh & Howard, 1975; Snell *et al.*, 1972). However, our results show that these strains can be separated into two distinct groups, based on bacteriocin production, maceration of onion slices, hydrolysis of low pH pectate agar and plasmid profile.

Bacteriocin production is more stable as an epidemiological marker than bacteriocin sensitivity (Farmer, 1972*a, b*) and has been used as an epidemiological tool for a number of Gram-negative bacteria (Abbott & Shannon, 1958; Farmer, 1972*a, b*; Gillies & Govan, 1966; Stouthamer & Tieze, 1966; Vidaver *et al.*, 1972). The production patterns obtained with the Hines strains as indicators clearly allowed differentiation between the plant isolates of *P. cepacia* and those of clinical origin. Interestingly, the *P. multivorans* strain also inhibited the Hines isolates and, therefore, belongs to the same group as the plant isolates.

The onion slice test for soft rotting of tissue also differentiated the two groups, the clinical isolates having little or no activity. Endopolygalacturonase from *P. cepacia* in diseased onions has a pH optimum between 4.4 and 4.6 (Ulrich, 1975). The pH dependence of the activity of the isolates tested here was also evident; all plant strains had moderate activity after 24 h at pH 4.9 whilst only one isolate had activity at pH 6.9. The clinical strains, with two exceptions, showed, at most, only a trace of activity at the lower pH and none at the higher.

Although strains of *P. cepacia* can be differentiated by their pectolytic activity, isolates of both types proved equally toxic to mice; the LD₅₀ for plant strains averaged to 3.7×10^8 , and that for clinical strains was 3.9×10^8 . Similarly, resistance to antibiotics was not significantly different between the two groups of isolates. The levels of resistance, in several instances, were in excess of normal levels of chromosomal resistance. In particular, the resistance to tetracycline (m.i.c. $320 \mu\text{g ml}^{-1}$) was much higher than previously recorded for strains of *P. aeruginosa*, *P. fluorescens* and *P. putida* (Shipley & Olsen, 1974) and this value

Fig. 1. Agarose gel electrophoresis of ethanol-precipitated plasmid DNA from cleared lysates of *P. cepacia* of clinical origin and of *E. coli* containing reference plasmids: (a) *E. coli* j53(RSF1030), 5.5×10^6 daltons (lower band), chromosomal DNA (upper band); (b) *E. coli* j53(RP4), 34×10^6 daltons; (c) *E. coli* j53(R1drd19), 62×10^6 daltons; (d) *P. cepacia* B5912, 105×10^6 and 30×10^6 daltons; (e) *P. cepacia* B6566, none; (f) *P. cepacia* ATCC 25609, 105×10^6 daltons; (g) *P. cepacia* B5910, 30×10^6 daltons; (h) *P. cepacia* B4648, 63.5×10^6 daltons; (i) *P. cepacia* B4345, 105×10^6 and 41×10^6 daltons; (j) *P. cepacia* Hines no. 1, 105×10^6 , 33×10^6 , 23×10^6 and 9×10^6 daltons; (k) *P. cepacia* Hines no. 2, 105×10^6 , 33×10^6 , 23×10^6 and 9×10^6 daltons. Chromosomal DNA migrated to the same position for all strains.

Fig. 2. Agarose gel electrophoresis of ethanol-precipitated plasmid DNA from cleared lysates of *P. cepacia* of plant and soil origin and of *E. coli* containing reference plasmids: (a) *E. coli* j53(RSF1030), 5.5×10^6 daltons (lower band), chromosomal DNA (upper band); (b) *E. coli* j53(RP4), 34×10^6 daltons; (c) *E. coli* j53(R1drd19), 62×10^6 daltons; (d) *P. cepacia* 74-34, 120×10^6 and 45×10^6 daltons; (e) *P. cepacia* 64-22, 120×10^6 and 45×10^6 daltons; (f) *P. cepacia* ATCC 25416, 120×10^6 daltons; (g) *P. cepacia* ATCC 10856, 120×10^6 daltons; (h) *P. multivorans* ATCC 17759, 13×10^6 daltons; (i) *P. cepacia* 71-22, 120×10^6 daltons; (j) *P. cepacia* 72-60, 120×10^6 daltons; (k) *P. allicola* ATCC 19302, 120×10^6 and 90×10^6 daltons. Chromosomal DNA migrated to the same position for all strains.

is in keeping with resistance being determined by R factors (Shipley & Olsen, 1974; Lacey, 1973). By analogy with the results and conclusions of Asheshov (1975) – who found that the tetracycline resistance determinant was plasmid borne in strains inducibly resistant to tetracycline, but sensitive to minocycline – we consider that the tetracycline resistance may be plasmid determined. Indeed, agarose gel analysis showed the presence of one or more plasmids of differing size in 15 out of 16 strains examined.

It is suggested that a combination of bacteriocin production and either the pectate gel test or the onion slice soft rot test would assist both clinicians and plant pathologists in determining the origin of *P. cepacia* isolates.

C.F.G. was supported by a predoctoral fellowship granted by the Ford Foundation. This work was supported, in part, by USDA-regional research funds for project NC-135. Published as Paper Number 5545, Journal Series, Nebraska Agricultural Experiment Station.

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