Isolation and Characterization of a Bacteriocin Produced by *Pseudomonas solanacearum*

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Strain B1, an avirulent non-slime-producing variant of *Pseudomonas solanacearum* K60, had greater bacteriocinogenic activity than did strain K60 both in liquid and on solid medium. The bacteriocin synthesized by strain B1 inhibited the growth of 43 of 51 *P. solanacearum* strains tested. It had no effect on strain B1, strain K60, or selected strains from 10 other bacterial species. Although the optimal growth temperature for strain B1 was 32 °C, the temperature most favourable for bacteriocin production was 30 °C. Bacteriocin titre could be increased by short exposure to ultraviolet light but not by chemical inducing agents.

The bacteriocin produced by strain B1 was purified from the culture supernatant by ammonium sulphate precipitation, anion exchange chromatography, membrane ultra-filtration and preparative electrophoresis. Purified bacteriocin, being non-sedimentable, thermolabile and sensitive to proteolytic enzymes, resembled the S-type bacteriocins produced by *P. aeruginosa*. As determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis, the purified bacteriocin had a molecular weight of approximately 65000. It was insensitive to chloroform, nucleases and phospholipase. Although the bacteriocin present in culture supernatant was stable for 3 months at 4 °C, the activity of purified bacteriocin declined rapidly under a variety of storage conditions. Maximum pH stability was between 6 and 8.

INTRODUCTION

Several bacterial plant pathogens synthesize bacteriocins, proteinaceous bactericidal agents that act specifically on strains of the same or closely related species (Echandi, 1976; Nakatani & Tsuyama, 1974; Vidaver *et al.*, 1972). However, only one of these bacteriocins has been isolated and characterized: syringacin 4A from *Pseudomonas syringae* (Haag & Vidaver, 1974).

Bacteriocin-like substances were first reported for the wilt pathogen, *Pseudomonas solanacearum*, by Okabe (Okabe, 1954). Ten of the 40 strains that Okabe had used to study lysogeny were sensitive to agents that diffused from the clones of five producer strains. For these strains, production of and sensitivity to the antibiotic-like compounds did not correlate with degree of virulence or host range (Okabe & Goto, 1961).

Pseudomonas solanacearum is pathogenic for many different species of plants in over 33 genera. As yet, no classification scheme based on laboratory-determined characteristics adequately defines the pathogenic profile of the various strains. Thus, a more comprehensive investigation into the nature of the bactericidal substances synthesized by this important plant pathogen might prove very useful. In addition, the bacteriocins have potential value as biological control agents (Vidaver, 1976).

The objectives of the present study were, firstly, to determine the optimal conditions for

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Table 1. *Pseudomonas solanacearum* indicator strains used

Strain no.	Host plant	Region of origin (Donor + donor's designation)	Isolation date
3	Banana	Armuelles, Panama (Sequeira S107)	1959
23	Potato	Israel (Volcani)	1955
26	Tomato	Georgia, U.S.A. (Kelman K74)	1954
27	Tobacco	Florida, U.S.A. (Kelman, K105)	1955
28	Potato	Cyprus (Dowson)	1955
37	Potato	Bogota, Colombia (Thurston)	1960
40	Banana	LaLima, Honduras (Buddenhagen)	1960
70	Plantain	Ibague, Colombia (Thurston 23)	1965
71	Plantain	Ibague, Colombia (Thurston 24)	1965
85	Tomato	Canada (Layne 12A)	1967
135	Banana	Buena Vista, Honduras (Berg 5513-4)	1964
143	Tomato	Nambour, Australia (Hayward 002a)	1965
267	Tomato	Taipei, Taiwan (Mew)	1974

production of bacteriocin-like compounds by *P. solanacearum* and, secondly, to isolate one such compound from the neotype strain of the species, $\kappa 60$. A preliminary report on this investigation has been presented (Cuppels *et al.*, 1975).

METHODS

Bacterial strains. *Pseudomonas solanacearum* $\kappa 60$ was isolated from tomato by A. Kelman (Husain & Kelman, 1958). The avirulent form of $\kappa 60$, strain B1, was obtained by a procedure described previously (Kelman & Hruschka, 1973). The *P. solanacearum* strains used as indicators of bacteriocinogenic activity are listed in Table 1.

Several bacterial species other than *P. solanacearum* were tested for sensitivity to *P. solanacearum* bacteriocin. The following strains were obtained from the stock culture collection maintained in the Department of Plant Pathology at the University of Wisconsin-Madison: *Erwinia carotovora* var. *atroseptica* SR8; *E. carotovora* var. *carotovora* SR38, SR34, SR204, SR117, SR40 and SR53; *E. chrysanthemi* SR29 and the corn pathotypes SR78, SR61, SR120 and SR90; *Pseudomonas marginalis* M13 and M12; *P. syringae* W3. Three cultures came from the Wisconsin State Laboratory of Hygiene: *Enterobacter cloacae* 15-561; *Escherichia coli* 86015; *Serratia marcescens* 11771. *Pseudomonas aeruginosa* PAO8 and R3725 were supplied by B. Holloway. Three cultures from the stock culture collection of the Department of Bacteriology at the University of Wisconsin-Madison were also used: *Salmonella enteritidis*; *Salmonella* sp. 1061; *Staphylococcus aureus* FDA209.

Culture media. Strains were grown routinely in Casamino acids/peptone/glucose broth (CPG broth) containing (g l⁻¹): Bacto-peptone (Difco), 10; glucose, 10; Bacto-Casamino acids (Difco), 1. Other media used were CPG agar [CPG broth supplemented with 1.5% (w/v) Bacto-agar (Difco)], CPG soft agar [CPG broth plus 0.6% (w/v) Bacto-agar] and TZC agar (Kelman, 1954).

Bacteriocin production and assay procedure. CPG broth (10 ml in a 125 ml flask) was inoculated with the producer strain to a final concentration of 5×10^8 bacteria ml⁻¹. After 12 to 16 h incubation (30 °C, aerobic conditions), mitomycin C (Nutritional Biochemicals) was added to the culture (final concentration 1 μ g ml⁻¹). The culture was incubated in the dark for an additional 4 h before being treated with chloroform (final concentration 5%, v/v). The chloroform/culture mixture was shaken vigorously for 60 s and then incubated for 4 h at 21 °C. A sample taken from the aqueous layer was diluted 1:100 in sterile distilled water. Lawns of the indicator organism were spotted with 0.02 ml each of the diluted and undiluted aqueous sample. The indicator lawn was prepared by adding 10^7 bacteria of the indicator strain to 3.5 ml CPG soft agar (molten and at 50 °C) and then pouring the soft agar over a CPG agar plate. The assay plates were incubated for 24 h at 32 °C. Bacteriocin concentration was measured in terms of arbitrary units (AU) ml⁻¹. The AU ml⁻¹ of a particular preparation was the reciprocal of the highest dilution that gave a clear zone of inhibition on an indicator lawn.

Determination of bacteriocin production by individual colonies. *Pseudomonas solanacearum*, diluted serially in sterile distilled water, was added to molten (50 °C) CPG soft agar, which then was poured over the surface of TZC agar. Soft agar was poured over the hardened inoculated soft agar to prevent spreading of colonies. As soon as individual colonies became visible (36 to 48 h at 32 °C), a third portion of soft agar, inoculated with the indicator strain, was poured over the plate. Bacteriocin production by the colonies was visible within 24 h.

Preparation and purification of bacteriocin from Pseudomonas solanacearum. Strain B1 was grown in 0.5 l CPG broth (2 l flask) on a rotary shaker at 29 °C for 14 h. This culture (5×10^8 bacteria ml⁻¹) served as inoculum for 12 l CPG broth containing 1.2 ml antifoam B emulsion (Sigma) (final concentration 2×10^8 bacteria ml⁻¹) which was aerated vigorously in a New Brunswick Microferm 14 l fermenter (400 rev. min⁻¹ at 30 °C). After 12 h incubation, when the density had reached 10^{10} bacteria ml⁻¹, the fermenter was connected to a reservoir of CPG broth. As CPG broth was pumped into the fermenter at the rate of 4 l h⁻¹, the culture was pumped out and cooled to 4 °C. After 10.5 h, 54 l of culture had been collected. The bacteria were sedimented in a Sharples continuous flow centrifuge and then frozen at -20 °C. The volume of the supernatant, which initially contained 200 AU bacteriocin ml⁻¹ (assayed on strain 40), was reduced immediately to 2 l by means of a Mojonniere evaporator. The following enzymes were then added: DNAase (DN-25; Sigma) dissolved in 0.01 M-Tris/0.2 M-NaCl, pH 8.0 (final concentration 10 µg ml⁻¹), RNAase T1 (RT1 54N476; Worthington Biochemical Corp.; final concentration 0.3 units ml⁻¹) and RNAase A (R-4875; Sigma; final concentration 1 µg ml⁻¹). The preparation was dialysed against buffer (0.01 M-Tris/0.02 M-NaCl, pH 8.0) for 3.5 h at 21 °C and then against distilled water (pH 7.1) for 12 h at 4 °C. All subsequent steps were performed at 4 °C. The bacteriocin preparation, which had increased in volume to 3.6 l during dialysis, was clarified twice by centrifuging at 12000 g for 15 min. The buffer concentration was adjusted to 0.05 M with 1 M-potassium phosphate buffer, pH 7.3. The preparation was eluted twice from 15 × 4.5 cm columns of DE52 (Whatman column chromedia, preswollen, anion-exchange cellulose) with 1.5 l of 0.05 M-potassium phosphate buffer, pH 7.3. None of the bacteriocin was retained on the column. The bacteriocin solution was clarified by centrifuging and then fractionated by ammonium sulphate precipitation into 0 to 25 % and 25 to 45 % precipitates. The precipitates were resuspended in 120 ml 0.05 M potassium phosphate buffer, pH 7.3. The solutions were dialysed against 0.05 M-potassium phosphate, pH 7.3, for 12 h and filter-sterilized. The 0 to 25 % fraction was not inhibitory for indicator strain 40 and was discarded. The 25 to 45 % fraction (2×10^4 AU ml⁻¹) was concentrated from 120 ml to 8 ml with a Diaflo ultrafiltration membrane XM50 (Amicon Corp.) and then filter-sterilized.

Polyacrylamide gel electrophoresis

(i) *Preparative polyacrylamide gel electrophoresis.* Gel preparation was essentially the same as that described for analytical gel electrophoresis by Maurer (1971). A Tris gel system in which the proteins were concentrated at pH 8.3 and separated at pH 9.5 (Jovin *et al.*, 1964) was used. Once the tracking dye [0.05 % (w/v) bromphenol blue] had entered the separation gel, 5 mg bacteriocin (10^5 AU) in 1 ml of stacking gel buffer containing 10 % (v/v) glycerol were applied to the surface of the stacking gel. The column was then subjected to electrophoresis for 20 h at 5 mA and 450 V. A Buchler fractionator set at 20 min per fraction was used to collect 70 fractions after the marker dye had eluted from the column. Each fraction was assayed for protein and inhibition of indicator strain 40.

(ii) *Analytical disc gel electrophoresis.* The gel system consisted of a 7.5 % (w/v) polyacrylamide (medium pore) separation gel (7 × 0.5 cm) prepared in 0.38 M-Tris/HCl, pH 8.8, and a 2.5 % (w/v) polyacrylamide (large pore) stacking gel (1 × 0.5 cm) prepared in a buffer containing 0.06 M-Tris and 0.032 M-H₂PO₄, pH 6.9. The reservoir buffer was 0.005 M-Tris and 0.038 M-glycine, pH 8.3. After electrophoresis (4 mA per gel, 4 h, 4 °C), the gels were either stained with Coomassie blue or sliced and assayed for bacteriocin activity. The procedure of Fairbanks *et al.* (1971) was employed for staining and destaining the gels. Gels to be assayed for bacteriocin activity were sliced into 1 mm slices and each slice was incubated at 4 °C for 12 h in 0.2 ml 0.09 M-Tris/HCl, pH 8.1. The material eluted from each slice was then assayed for activity using indicator strain 40.

(iii) *Sodium dodecyl sulphate-polyacrylamide gel electrophoresis.* Polyacrylamide gels with a sodium dodecyl sulphate (SDS) buffer were prepared as described by Laemmli (1970). Proteins used as standards for the molecular weight determination were: trypsin (23 800 mol. wt, Calbiochem), ribonuclease (13 700 mol. wt, ICN-Nutritional Biochemicals), lysozyme (17 500 mol. wt, ICN-Nutritional Biochemicals), cytochrome c (12 400 mol. wt, a Schwartz-Mann non-enzymic protein molecular weight marker) and crystalline bovine albumin (67 000 mol. wt, ICN-Pharmaceuticals).

Protein determination. The amount of protein in a bacteriocin extract was determined by means of the Folin phenol reagent (Lowry *et al.*, 1951) for crude extracts or by absorption at 280 nm (Mayr-Harting *et al.*, 1972) for purified preparations.

Agarose gel filtration. A jacketed column was packed with Bio-Rad Bio-Gel A-1.5 m, 100 to 200 mesh, agarose (8 %, w/v) to a height of 59.5 cm (1.5 cm diam.) and cooled to 8 °C. The void volume (V_0) was determined with 0.025 % (w/v) Blue dextran. The elution volumes (V_e) of four non-enzymic protein molecular weight markers (Schwartz-Mann no. 908109) were used to prepare a molecular weight standard curve: horse apo-ferritin (490 000 mol. wt), gamma-globulin (160 000 mol. wt), ovalbumin (45 000 mol. wt) and myoglobin (17 000 mol. wt).

Sensitivity of P. solanacearum bacteriocin to enzymes. Bacteriocin purified by preparative gel electrophoresis [2×10^4 AU (mg protein) $^{-1}$] was diluted in 0.005 M-Tris/0.39 M-glycine buffer containing 30 % (w/v) glycerol, pH 8.1, to 50 AU ml $^{-1}$. Samples (1 ml) were treated for 90 min at 30 °C with one of the following enzymes: DNAase (10 µg plus 10 mM-MgSO $_4$), RNAase (50 µg RNAase A plus 6 units RNAase T1), trypsin (50 µg), pronase (50 µg plus 0.01 M-CaCl $_2$), pepsin (50 µg plus 10 mM-MgSO $_4$) and phospholipase A (24 units). Each reaction mixture was serially diluted in 0.05 M-Tris/HCl, pH 8.0, and assayed on strain 40.

Sensitivity of P. solanacearum bacteriocin to chloroform. Bacteriocin (10 AU ml $^{-1}$) eluted from an 8 % (w/v) agarose column was treated with 1 % (v/v) chloroform for 60 min at 21 °C; the chloroform was then removed by evaporation. The sample was filter-sterilized and then its inhibitory activity for strain 40 was compared with that of untreated bacteriocin held at 21 °C for 60 min.

RESULTS

Cultural conditions for production of bacteriocin-like compounds by P. solanacearum k60

The abundant slime that *P. solanacearum* formed when grown on solid media (Husain & Kelman, 1958) prevented the detection of bacteriocin production by standard methods (Mayr-Harting *et al.*, 1972; Gillies & Govan, 1966). However, reproducible patterns of bacteriocin production were obtained when *P. solanacearum* was grown aerobically in a nutritionally rich liquid medium. In CPG broth the highest bacteriocin titre occurred during the late-exponential phase of growth: 50 AU ml $^{-1}$ as measured with indicator strain 40 or 10 AU ml $^{-1}$ as measured with indicator strain 143. Growth of strain k60 in the following complex media failed to increase the titre: CPG1 broth (same as CPG but with glycerol in place of glucose), trypticase soy broth (Difco), nutrient broth (Difco; 1:1 dilution of the manufacturer's recipe) and TDY broth [1 % (w/v) tryptone, 1 % (w/v) dextrose and 0.1 % (w/v) yeast extract].

In liquid medium, an avirulent non-slime-producing variant of *P. solanacearum* k60, designated B1, consistently released larger quantities of bacteriocin-like compounds than did k60. Isolated colonies of B1 were surrounded by significantly larger zones of inhibition than were isolated colonies of k60. Although the bacteriocin titre in CPG broth for both k60 and B1 was higher with indicator strain 40 (50 and 100 AU ml $^{-1}$, respectively) than with indicator strain 143 (10 and 50 AU ml $^{-1}$, respectively), the zones of inhibition around producer colonies on CPG agar were much larger with indicator strain 143 (9.2 ± 0.9 and 12.3 ± 0.8 mm, respectively) than with indicator strain 40 (3.0 ± 0.7 and 5.6 ± 0.6 mm, respectively).

Fifty-one *P. solanacearum* strains were tested as indicators of the ability of strains k60 and B1 to release bacteriophage or bacteriocin-like compounds. Although several other strains of *P. solanacearum* release bacteriophage in the presence of mitomycin C (Cuppels, 1976), strains k60 and B1 do not. If bacteriophage had been present in the test culture supernatants, the serial dilutions made of the supernatants would have resulted in the appearance of discrete plaques on the appropriate indicator strain. Bacteriocin from strain B1 inhibited 43 strains, whereas that from strain k60 inhibited only 39 strains (Cuppels, 1976). Strains k60 and B1 were sensitive to agents produced by the same 20 strains, out of 46 other *P. solanacearum* strains tested. Neither strain was inhibited by its own bacteriocin.

The bacteriocin titres of strain B1 grown at five different temperatures using indicator strains 40 and 143 were, respectively: 25 °C, 200 and 100 AU ml $^{-1}$; 30 °C, 500 and 100 AU ml $^{-1}$; 32 °C, 100 and 50 AU ml $^{-1}$; 34 °C, 10 and 0 AU ml $^{-1}$; 38 °C, 0 and 0 AU ml $^{-1}$. Although the optimal temperature for growth of strain B1 was 32 °C, the temperature most favourable for bacteriocin production was 30 °C. When producer strains k60 (tomato isolate), B1 and 40 (banana isolate) were grown at 30 °C instead of 32 °C, they inhibited additional *P. solanacearum* strains (Table 2). The incubation temperature for bacteriocin production in all subsequent assays was 30 °C.

Addition of mitomycin C (0.4, 1, 2 or 5 µg ml $^{-1}$), chloramphenicol (150 µg ml $^{-1}$) or trimethoprim (1, 2 or 5 µg ml $^{-1}$) to exponentially growing cultures of strains k60 or B1

Table 2. Sensitivity of 28 *Pseudomonas solanacearum* strains to bacteriocins produced by strains K60, B1 and 40, grown at 30 and 32 °C

The three producer strains were grown aerobically for 14 h in 10 ml CPG broth (125 ml flask) on a water-bath shaker set at 30 or 32 °C. Filter-sterilized supernatants of the cultures were spotted on to lawns of the indicator strains.

Producer strain	No. of bacteriocin-sensitive strains in indicator group					
	Tomato-Tobacco *(12)		Banana-Plantain (8)		Potato (8)	
	30 °C	32 °C	30 °C	32 °C	30 °C	32 °C
K60 (tomato)	6	4	7	6	6	6
B1	9	8	7	6	6	6
40 (banana)	9	6	6	3	5	4

* Numbers in parentheses indicate the number of strains in each indicator group tested for bacteriocin sensitivity.

Table 3. Preliminary steps in the purification of bacteriocin from the culture supernatant of *Pseudomonas solanacearum* B1

Step	Vol. (l)	10 ⁻³ × Activity* (AU ml ⁻¹)	10 ⁻⁶ × Total activity (AU)	10 ⁻² × Total protein (mg)	10 ⁻³ × Specific activity (AU mg ⁻¹)	Yield (%)
(1) Supernatant	50	0.2	10	—	—	100
(2) Concentrated supernatant	2	5.0	10	—	—	100
(3) Nucleases + dialysis	3.6	2.5	9	1300	0.07	90
(4) DE52 column, 0.05 M-potassium phosphate buffer, pH 7.3	3.6	2.5	9	340	0.27	90
(5) 25 to 45 % ammonium sulphate fraction	0.12	20	2.4	1.5	15	24
(6) Amicon XM50 concentration	0.008	300	2.4	0.22	100	24

* Bacteriocinogenic activity was measured in terms of arbitrary units, AU (the reciprocal of the highest dilution that gave an inhibition zone). The bacteriocin preparation was diluted in sterile distilled water and assayed on indicator strain 40.

did not increase the bacteriocin titre of either strain more than twofold. However, short exposure of strain B1 to ultraviolet light (366 nm; 2 min at a distance of 50 cm from a 4 W bulb) raised the titre 10-fold, from 100 to 1000 AU ml⁻¹, against indicator strain 40. The titre against indicator strain 143 was unaffected by ultraviolet irradiation.

Isolation of bacteriocin from *P. solanacearum* B1

Pseudomonas solanacearum B1 bacteriocin was obtained as described in Methods. The initial steps in the purification of bacteriocin from the culture supernatant are summarized in Table 3. The activity of the compound was unaffected during concentration with the Mojonner evaporator or treatment with nuclease. After centrifugation for 60 min at 34000 g, less than 1 % of the activity was sedimented.

The bacteriocin was further purified by preparative polyacrylamide gel electrophoresis. Approximately 10 % (5 × 10³ AU) of the bacteriocin activity added to the column was recovered. Although the specific activity of bacteriocin was reduced from 10⁵ to 2 × 10⁴ AU (mg protein)⁻¹, its purity increased substantially as demonstrated by analytical disc gel electrophoresis (Fig. 1). The location of the two protein bands on a stained gel (Fig. 1B) corresponded to the location of bacteriocin activity on an unstained gel (13 to 15 mm

Fig. 1. Analytical disc gel electrophoresis [7.5 % (w/v) acrylamide; running pH 9.5] of a bacteriocin preparation before (A; 140 μ g step 6 material; 1.4×10^4 AU) and after (B; 8 μ g; 1.2×10^2 AU) purification by preparative gel electrophoresis. The two gels (each 7 cm long) were not subjected to electrophoresis simultaneously. Bacteriocinogenic activity in gel B is indicated by the arrow. In gel A, bacteriocinogenic activity occurred 6 to 8 mm from the top. The gels were stained for protein with Coomassie blue.

below the top of the separation gel). The purified bacteriocin extract was subjected to analytical disc gel electrophoresis three more times with the same results. In contrast, bacteriocin from the concentrated ammonium sulphate fraction (Step 6, Table 3) produced many bands on a stained gel (Fig. 1 A) and bacteriocin activity could not be identified with any distinct protein band(s).

The molecular weight of bacteriocin purified by preparative gel electrophoresis was estimated by SDS-polyacrylamide gel electrophoresis. As with the analytical gel, two protein bands were visible near the top of the gel. The molecular weights of these two bands calculated from a standard plot of log molecular weight *v.* relative electrophoretic mobility were 64000 and 66000.

The molecular weight of the *P. solanacearum* B1 bacteriocin was also estimated by agarose gel filtration. Bacteriocin partially purified by the six steps listed in Table 3 was applied to an 8 % (w/v) agarose gel column (see Methods) and its molecular weight was calculated from its elution volume. The value obtained, 63000, approximated to those determined from SDS-polyacrylamide gels for bacteriocin purified by preparative gel electrophoresis.

Purified bacteriocin eluted from the agarose column, like the supernatant from a strain B1 broth culture, was active against strains 3, 23, 27, 28, 37, 40, 70, 71, 85, 135 and 143. Unlike the supernatant, it was inactive against strains 267 and 26. This difference, as in the case of colicins (McGeachie & McCormick, 1967), could be explained by the difference in bacteriocin concentration used for the assays (200 AU ml⁻¹ for the supernatant and 10 AU ml⁻¹ for purified bacteriocin).

Bacteriocin in strain B1 culture supernatant passed through an Amicon XM100 filter

(100 000 molecular weight cut-off), but not an XM50 filter (50 000 molecular weight cut-off). This estimate of molecular weight agreed well with those calculated using SDS-polyacrylamide gels and agarose gel filtration.

Characterization of the P. solanacearum bacteriocin

The bacteriocin titre of strain B1 culture supernatant was 200 AU ml⁻¹ provided the pH of the supernatant remained between 6 and 8. After 3 h incubation (21 °C) at pH 9 or 5, the titre dropped by 50%, while at pH 3 or 10, bacteriocin activity disappeared completely. Bacteriocin in filter-sterilized culture supernatant (200 AU ml⁻¹) retained full activity for 3 months at 4 °C. However, the activity of purified bacteriocin declined rapidly at 4 °C (50% loss in 2 d), whether in 0.05 M-Tris/HCl (pH 8), 0.05 M-Tris/0.2 M-NaCl (pH 8.1), 0.005 M-Tris/0.39 M-glycine (pH 8.3) or 0.01 M-potassium phosphate buffer (pH 7.3).

Lyophilization in 0.05 M-Tris/HCl (pH 8.0) completely removed the activity of bacteriocin purified by preparative gel electrophoresis. When it was stored at -20 °C in 30% (v/v) glycerol, there was a 50% reduction in activity within 2 weeks. Dimethylsulphoxide (5%, v/v) increased the rate of loss of activity. Activity in purified preparations and in culture supernatants was lost completely when heated at 65 °C for 10 min. Bacteriocin purified by preparative gel electrophoresis was insensitive to DNAases, RNAase A and T1, phospholipase A, pepsin and chloroform. It was completely inactivated by pronase. Trypsin reduced the titre from 50 to 5 AU ml⁻¹ in 90 min at 30 °C.

Four *P. solanacearum* strains (K60 from tomato, 128 from plantain, 71 from banana and 120 from potato), representing the three *P. solanacearum* races and producing very different patterns of inhibition against 53 *P. solanacearum* indicator strains (Cuppels, 1976), were unable to inhibit 23 strains selected from the following species: *Erwinia carotovora* var. *atroseptica*, *E. carotovora* var. *carotovora*, *E. chrysanthemi*, *Enterobacter cloacae*, *Escherichia coli*, *Serratia marcescens*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Pseudomonas marginalis*, *P. aeruginosa* and *P. syringae*. Thus, the *P. solanacearum* bacteriocins appeared to be species-specific.

DISCUSSION

Syringacin 4A of *P. syringae* (Haag & Vidaver, 1974), which resembles a bacteriophage tail, was the first bacteriocin produced by a phytopathogen to be thoroughly characterized. However, the *P. solanacearum* B1 bacteriocin was the first one to be isolated from a plant pathogen that belonged to the non-sedimentable, low molecular weight group (Bradley, 1967). It resembled pyocin S2 from *P. aeruginosa* (Ohkawa *et al.*, 1973), the one other pseudomonad bacteriocin of this group that has been described.

Avirulent *P. solanacearum* B1 produced a higher titre of bacteriocin and inhibited more indicator strains than did its parent strain, K60, whose bacteriocinogenic activity might be masked by extracellular polysaccharide. Avirulent B1 was sensitive to the same bacteriocins or bacteriocin-like compounds as its virulent parent strain, K60, suggesting that pathogenicity was not associated with bacteriocin sensitivity, as in biotype 1 strains of *Agrobacterium tumefaciens* (Roberts & Kerr, 1974).

Although easily inactivated during the purification process, the *P. solanacearum* B1 bacteriocin present in culture supernatant was stable and inhibited a large number of *P. solanacearum* strains. This bacteriocin and its avirulent producer, strain B1, could, therefore, be useful in the development of a bacteriocin-typing scheme for *P. solanacearum* or in the development of biological control measures for bacterial wilt.

Although the optimal growth temperature for *P. solanacearum* K60 and B1 was 32 °C, the optimal temperature for bacteriocin production was 30 °C (Table 2). Similar observations were made concerning bacteriocin production by *Pseudomonas aeruginosa* (Gillies & Govan, 1966) and *P. syringae*, *P. glycinea* and *P. phaseolicola* (Vidaver *et al.*, 1972).

The bacteriocin titre of a *P. solanacearum* B1 culture could not be increased substantially by treatment with chemical inducing agents. The titre of cell-bound colicins controlled by the self-transmissible Col factors of the I and V group likewise cannot be induced (Herschman & Helinski, 1967*b*), although Hardy & Meynell (1972) suggested this might be because they had not been totally released from the cells by chloroform treatment. For *P. solanacearum* bacteriocin, 95% of the total yield was present in the culture supernatant; only 5% was released from lysozyme-treated cells.

A sample of bacteriocin purified by preparative electrophoresis produced two major bands, representing molecular weights of 66000 and 64000, on SDS-polyacrylamide gels. Similar results had been obtained with colicin E2 by Herschman & Helinski (1967*a*) but their bacteriocin existed as two electrophoretically distinguishable forms of the same molecule and not as two structurally distinct molecules. Whether the bacteriocin activity of *P. solanacearum* B1 is associated with one or two distinct bacteriocins cannot be determined until the two bands seen on SDS gels are separated.

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