ELSEVIER

www.elsevier.com/locate/carres

Carbohydrate Research 333 (2001) 295–302

CARBOHYDRATE RESEARCH

Extracellular polysaccharides of modified strains of *Erwinia* spp.

Byung Yun Yang, John M. Brand,[1] James S.S. Gray, Rex Montgomery*

Department of Biochemistry, College of Medicine, University of Iowa, Iowa City, IA 52242, USA

Received 4 January 2001; accepted 10 May 2001

Abstract

The structure of the extracellular polysaccharide (EPS) produced by *Erwinia chrysanthemi* strain A2148 has been determined using low pressure size-exclusion and anion-exchange chromatographies, high pH anion-exchange chromatography, glycosyl-linkage analysis, and 1D ^{1}H NMR spectroscopy. The polysaccharide is structurally similar, if not identical, to the EPS produced by *E. chrysanthemi* strain A350. A streptomycin-resistant strain of *E. chrysanthemi* Ech6 (Ech6S$^+$) has been generated and has an elevated production of EPS, as does a streptomycin-resistant strain (Ech9Sm6) of *E. chrysanthemi* Ech9. These modified *E. chrysanthemi* spp. have been ribotyped and found to be closely related to their parent strains. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Erwinia* spp.; Streptomycin; Extracellular polysaccharide; Structure; Ribotyping

1. Introduction

Erwinia chrysanthemi spp. are Gram-negative bacterial phytopathogens that cause soft rot in a number of plants.[1–6] A considerable amount of work has been performed on the taxonomy, serology and host range of *E. chrysanthemi* spp.[2–5,7,8] Specific biochemical tests were used by Dickey[2] to subdivide strains of *E. chrysanthemi* into five biovars; this was later amended to six biovars.[3,9] These six biovars could be assigned to six pathovars (I, pv. *dieffenbachia*; II, pv. *parthenii*; III, pv. *chrysanthemi*; IV, pv. *zeae*; V, *dianthicola*; VI, pv. *paradisiaca*) depending on their hosts.[2,3,8,9] *E. chrysanthemi* strains isolated from corn, saintpaulia, philodendron, and *allium* spp. (onion) are included in pv. *zeae*; those isolated from potatoes, depending on their origin, are included either in pv. *zeae* or pv. *parthenii*.[3] Boccara et al.,[7] using an extended set of biochemical tests, classified *E. chrysanthemi* strains into nine biovars. Restriction fragment-length polymorphism (RFLP) analysis of the pectate lyase genes (*pel* genes) of *E. chrysanthemi* gives rise to six clusters that correlate well with the biovar and pathovar classifications[2,3,8,9] of the strains but not well to their geographical distribution.[10] Ribotyping of *E. chrysanthemi* from different hosts gave rise to seven clusters that correlated well with their biovar and pathovar classification.[11]

Many of the *E. chrysanthemi* spp. produce extracellular polysaccharide (EPS), the role of

Abbreviations: EPS, extracellular polysaccharide; EPS A350 etc, EPS produced from Ech A350, etc; GLC, gas–liquid chromatography; GLC–MS, gas liquid chromatography–mass spectrometry; HPAEC-PAD, high pH anion-exchange chromatography with pulsed amperometric detection; RFLP, restriction fragment length polymorphism.

* Corresponding author. Fax: +1-319-3359570.

E-mail address: rex-montgomery@uiowa.edu (R. Montgomery).

[1] Present address: Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa.

0008-6215/01/$ - see front matter © 2001 Elsevier Science Ltd. All rights reserved.
PII: S0008-6215(01)00155-0

which in host-specificity and virulence is not well known. There is some evidence, however, that EPS production is necessary for infection of plants when the pathogen is present at low levels.[12] Also, EPS^- mutants are less virulent than EPS^+ strains.[12]

The structures of a number of *E. chrysanthemi* EPSs determined in this laboratory[13–18] can be classified into four families.[15] Interestingly, the ribotypes studied to date of the limited number of strains producing these polysaccharides tended to track the EPS chemotypes.[15]

The EPSs can be produced on agar plates or in liquid culture, where the yields are sometimes poor. One means of increasing the yield of EPS is to isolate hyperproducing strains. *E. chrysanthemi* strain 3937, and its *lacZ* (A350) and *pecT* (A2148) mutants are pathogenic to saintpaulia. Strain A2148 differs from 3937 and A350 in expressing elevated levels of the pectate lyase genes.[19] Strain A2148 also produces elevated levels of EPS on minimal media.[19] The interruption of the regulatory gene, *pecT*, is associated with both of these phenotypes.

E. chrysanthemi Ech9Sm6, another hyperproducer of EPS, is a streptomycin-resistant strain of the potato phytopathogen, Ech9. The structure of the EPS from Ech9Sm6 has been shown to be similar to that of the parent (unpublished work)

In this paper, the isolation of a streptomycin mutant from *E. chrysanthemi* Ech6 (named $Ech6S^+$) is shown to be a hyperproducer of EPS and by ribotyping to be closely related to its parent strain, Ech6. Similarly, the EPS from A2148 is structurally similar, if not identical, to that produced by A350.[15] Again, A2148 and A350 are closely related by ribotyping. Finally, a number of strains (Table 3), whose EPSs have not yet been studied in detail, have been ribotyped and the results are presented here.

2. Experimental

Production of streptomycin-resistant strain of E. chrysanthemi *Ech6.—E. chrysanthemi* Ech6 was streaked on an agar plate with a streptomycin gradient, similar to that described by Carlton and Brown.[20] Difco nutrient agar (Difco Laboratories, Detroit, MI) was made up, sterilized, and supplemented aseptically with filter-sterilized streptomycin sulfate to a final concentration of 500 μg/mL. This nutrient agar (15 mL) was poured into a 100-mm square Petri dish and angled so that one edge of the agar was flush with the intersection between the bottom and side of one edge of the plate. The plate was allowed to set, laid on a flat surface, and overlaid with 15 mL of plain Difco nutrient agar. The streptomycin gradient (0–500 μg/mL) was established overnight by diffusion. The plate was streaked with *E. chrysanthemi* Ech6 from low to high streptomycin concentration and incubated at rt (22–25 °C). After 10 days, a single colony was observed growing in the low to zero streptomycin region of the plate. This colony was restreaked over the entire plate, and grew everywhere. The strain, designated $E. chrysanthemi$ $Ech6S^+$, was purified by repeated streaking of single colonies on Difco nutrient agar supplemented with 500 μg/mL streptomycin.

Preparation of extracellular polysaccharides.

On agar plates. An extracellular polysaccharide was produced on a modified Scott's medium supplemented with 1.5% glucose and 1.5% Difco agar. The warm liquid agar medium (1 L) was dispensed into 40 plates and the EPS isolated as described previously.[13,14,18] The crude EPS was precipitated twice from 5% (w/v) NaCl with 3 vols of EtOH, dialyzed against three changes of distilled water and lyophilized. The yield of the EPS from $Ech6S^+$ was about 1.7 g/L of medium. The preparation of the EPS from strain A2148 was as described previously for strain A350.[15]

Liquid medium. The medium for preparation of EPS in Fernbach flasks was: glucose, 20 g/L; Difco proteose peptone, 2.0 g/L; yeast extract, 0.5 g/L; $MgSO_4 \cdot 7\ H_2O$, 0.5 g/L; KH_2PO_4, 0.5 g/L; $CaCO_3$, 2.5 g/L. Calcium carbonate was added aseptically to the autoclaved medium. This medium (100 mL in a 250-mL flask) was inoculated from a fresh

plate of the *E. chrysanthemi* strain of interest and then shaken for 3 days at 30 °C and 200 rpm. This culture (25 mL) was used to inoculate each liter of medium in a Fernbach flask. The flasks were shaken at 30 °C and 150 rpm for the first day, and for 2 days at 200 rpm. The resulting viscous culture was centrifuged in a Beckman J-21 (14,000 rpm (19,200*g*, av.), Beckman JA14 rotor, 4 °C) for 2 h or until the supernatant was clear. The supernatant was decanted, made 3% (w/v) with respect to NaCl, and the EPS was precipitated with EtOH as just described. The crude EPS was dissolved in 5% (w/v) NaCl, centrifuged, and recovered by EtOH precipitation. Yield from Ech6S$^+$ was 3.0 g/L.

Analytical and general methods.—The methods used for chromatographic purification of polysaccharide, glycosyl-linkage analysis by methylation, GLC and GLC–MS analyses, uronic acid reduction by Super-Deuteride after methylation, determination of the absolute configuration of the monosaccharides, monosaccharide analysis by HPAEC-PAD and by GLC, and degradation of polysaccharides by lithium in ethylenediamine, and 600 MHz 1D ^{1}H NMR spectroscopy have been described previously.[13–18]

Ribotyping.—Ribotyping was performed on a DuPont Qualicon Riboprinter® using the procedures described by the manufacturer. Single colonies of bacteria grown overnight on Brain–Heart Infusion agar were transferred to a sample carrier and heated for 22 min at 80 °C to kill the bacteria. The sample carrier was inserted into the Riboprinter®; all subsequent manipulations are automatic and consist of DNA extraction, digestion with EcoR1, electrophoresis and blotting onto a membrane, and probing with a labeled cDNA probe derived from *E. coli* 16S and 23S rRNA. After blotting, the image on the membrane is captured with a digital camera. The resulting patterns are analyzed and stored using software supplied by Qualicon. A software program, BIONUMERICS (v 2, Applied Maths, Kortrijk, Belgium), was used for subsequent analysis and plotting of the dendrogram directly from the archived data.

3. Results and discussion

Crude A2148 EPS recovered by fractional precipitation with ethanol was further purified by low-pressure gel-permeation (ToyoPearl HW65F, TosoHaas, Montgomeryville, PA) and anion-exchange chromatographies (ToyoPearl DEAE-650M). No neutral oligo- or polysaccharides were present in the A2148 EPS preparations. Analysis of the monosaccharide composition across the polysaccharide peaks from both chromatographies by HPAEC-PAD revealed that the EPS is homogeneous, having a composition of L-Rha, D-Gal and D-GalA in the ratio, 4:1:1. The absolute configurations of the sugar residues were determined as the TMS derivatives of the *R*-(−)-butan-2-ol glycosides by GLC analysis.[21] The presence of a GalA residue in the EPS from strain A2148 was confirmed by reduction of the methylated polysaccharide with Super-Deuteride (lithium triethylborodeuteride), and the identification of 1,4,5,6-tetra-*O*-acetyl-1,6,6′-trideuterio-2,3-di-*O*-methylgalactitol by GLC and GLC–MS analyses after hydrolysis, reduction with $NaBD_4$ and acetylation.

Glycosyl-linkage analysis of the A2148 EPS after reduction of the per-*O*-methylated EPS with Super-Deuteride (to convert the GalA to Gal), showed the presence of one residue of terminal Rha, two residues of 2-linked Rha and one residue each of 2,3-linked Rha, 3-linked Gal and 4-linked GalA (Table 1). It is evident from the glycosyl-linkage analysis that all of the residues in the EPS are in the pyranose form and that the EPS has a branched hexasaccharide repeating unit.

Treatment of the A2148 EPS by lithium in ethylenediamine,[22,23] where the uronic acid residue is cleaved and degraded to non-sugar compounds, generated two fragments, which were fractionated by gel-permeation chromatography (Bio-Gel P-2 200-400 mesh, 1.0 × 45 cm column) to give a polysaccharide and low molecular weight components containing Rha-ol and a small amount of Rha (determined by GLC–MS analysis of the acetylated derivatives). HPAEC-PAD analysis of acid hydrolyzates of the modified polysaccharide revealed the presence of Rha and Gal

in a 3:1 molar ratio. Glycosyl-linkage analysis by methylation of the modified polysaccharide disclosed the presence of two residues of 2-linked Rha, and one residue each of 3-linked Rha and 3-linked Gal (Table 1). These data suggest that the modified polysaccharide, which is the backbone of the native EPS, consists of a linear tetrasaccharide repeat that is substituted by a side chain (Rha*p*1→ 4GalA*p*1–) at O-2 of a branch point Rha residue in the native EPS.

The presence of a hexasaccharide repeat in the native EPS is confirmed by the presence of six anomeric protons in the 1D ^{1}H NMR spectrum (Fig. 1 and Table 2). The chemical shifts and coupling constants (Table 2) indicate that one signal, δ 4.645 ppm ($J_{1,2}$ 7.2 Hz, 1 H), arises from a Gal or GalA residue with

Table 1
Comparison of methylation analyses of two extracellular polysaccharides, A2148 and A350, before and after Li–ethylenediamine treatment

Methylated sugar [a]	Relative mol. ratio			
	A2148		A350 [c]	
	EPS native	EPS backbone	EPS native	EPS backbone
2,3,4-*O*-Me_3Rha	1.0		1.0	
3,4-*O*-Me_2Rha	1.8	2.0	1.8	1.9
2,4-*O*-Me_2Rha		0.8		0.8
4-*O*-MeRha	1.1		1.2	
2,4,6-*O*-Me_3Gal	1.0	1.0	1.0	1.0
2,3,-*O*-Me_2Gal(A) [b]	1.0		1.0	

[a] 2,3,4-*O*-Me_3Rha = 1,5-di-*O*-acetyl-1-deuterio-2,3,4-tri-*O*-methyl rhamnitol, etc.
[b] Observed as 1,4,5,6-tetra-*O*-acetyl-1,6,6′-trideuterio-2,3-di-*O*-methylgalactitol derived from the reduction of the methyl ester of 2,3-Me_2GalA with Super-Deuteride.
[c] Ref. 14.

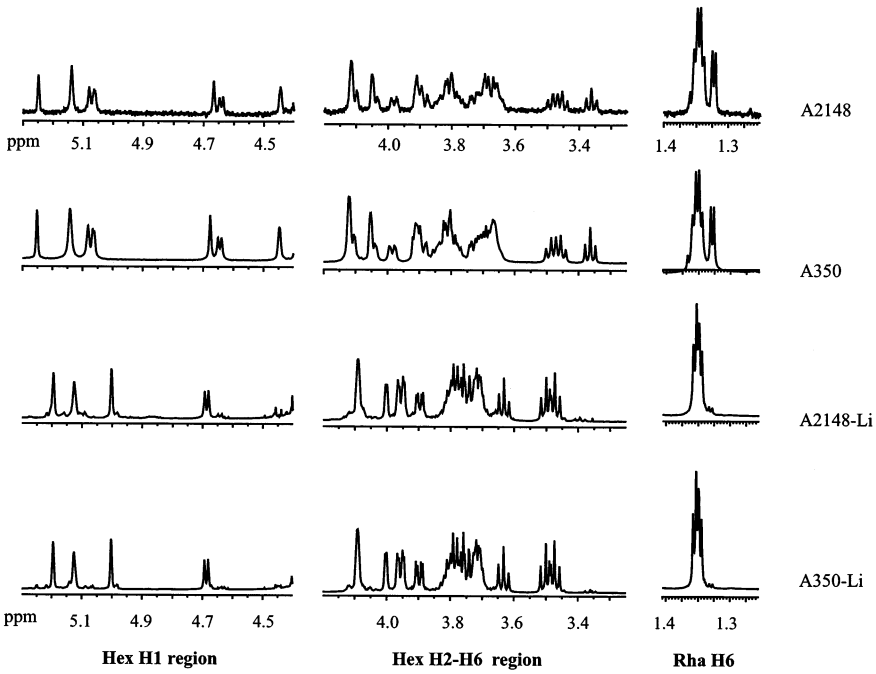


Fig. 1. 1D ^{1}H NMR spectra of native A350 EPS and native A2148 EPS, and the corresponding backbone recovered after Li-treatment. The spectra were acquired at 338 K and 600.14 MHz in D_2O containing 1% NaCl.

Table 2
NMR data of anomeric protons for *E. chrysanthemi* A2148 EPS [a]

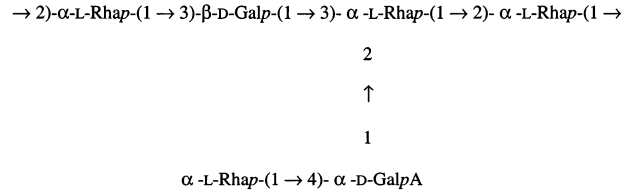
Residue	δ_H (ppm) [b]/$J_{1,2}$ (Hz)			
	A2148		A350 [d]	
	Native	Backbone	Native	Backbone
α-L-Rha*p*-(1→	5.253 (1.2)		5.252 (1.2)	
→2)-α-L-Rha*p*-(1→	5.142 (<1.0)	5.195 (<1.0)	5.141 (<1.0)	5.196 (<1.0)
→2)-α-L-Rha*p*-(1→	5.142 (<1.0)	5.126 (<1.0)	5.141 (<1.0)	5.126 (<1.0)
→3)-α-L-Rha*p* [c]-(1→		5.003 (<1.0)		5.004 (<1.0)
→2,3)-α-L-Rha*p*-(1→	5.084 (<1.0)		5.082 (<1.0)	
→4)-α-D-Gal*p*A-(1→	5.067 (3.6)		5.064 (3.6)	
→3)-β-D-Gal*p*-(1→	4.645 (7.2)	4.687 (7.5)	4.645 (7.2)	4.687 (7.5)

[a] The EPS was exchanged into D_2O containing 1% NaCl. The spectra were acquired at 338 K and 600.14 MHz.
[b] Chemical shifts relative to acetone (δ_H 2.225 ppm).
[c] Residue modified after lithium-treatment on EPS.
[d] Ref. 14.

a β configuration. The three resonances with unresolved coupling constants at δ 5.253, 5.142, 5.084 ppm, are characteristic of Rha residues with the α configuration and the resonance at δ 5.067 ppm ($J_{1,2}$ 3.6 Hz, 1 H) is characteristic of the α-galacto configuration. The anomeric proton resonating at δ 5.067 ppm is assigned to an α-GalA residue since the signals at δ 4.447 and 4.676 ppm are non-anomeric and are typical of H-5 and H-4, respectively, of 4-linked α-GalA.[15,24–27] Thus, the Gal residue has the β configuration. Twelve protons (four doublets, $J_{5,6}$ 6.0 Hz) corresponding to the four 6-deoxy groups of Rha, are observed at δ 1.246, 1.285, 1.295, and 1.305 ppm.

The four anomeric resonances at δ 5.195 ppm ($J_{1,2}$ unresolved, 1 H), δ 5.126 ($J_{1,2}$ unresolved, 1 H), δ 5.003 ($J_{1,2}$ unresolved, 1 H) and δ 4.687 ppm ($J_{1,2}$ 7.5 Hz, 1 H) in the 1D ^{1}H NMR spectrum of the A2148 EPS backbone confirms a tetrasaccharide repeat consisting of three Rha residues and one Gal residue (Fig. 1) as had been found in A350.[15] Moreover, the retention of the signal at δ 4.687 ppm and the loss of the signal at δ 5.064 ppm in the EPS backbone supports the assignment of these two resonances to the anomeric protons of β-D-Gal and α-D-GalA, respectively. The three doublets at δ 1.285, 1.292 and 1.301 ppm ($J_{5,6}$ 6.0 Hz) are assigned to the 6-deoxy groups of three Rha residues. Consequently, the doublet at δ 1.246 must arise from the side chain Rha, as found in *E. chrysanthemi* A350.[15] Examination of the 1D spectra reveals similarity of the ring-proton resonances, particularly those that are well separated from their neighbors (Fig. 1). No other substituents were detected in the A2148 EPS.

A comparison of the glycosyl residues compositions and ^{1}H NMR data for the EPSs from *E. chrysanthemi* strain A2148 and from strain A350 (Tables 1 and 2 and Fig. 1) suggests that the two polysaccharides have identical glycosyl sequences:



The structure of the EPS from strain A2148 and A350 is different from those produced by *E. chrysanthemi* strains SR260,[13,14] Cu643,[16] Ech6,[17] Ech1 and Ech9,[18] all of which contain glucuronic acid residues rather than galacturonic acid. The composition of the A2148 (and A350) EPS is similar to that from strain CU643 [16] except for the presence of GalA in the former and GlcA in the latter. A further major structural difference between the EPSs from A2148 and A350, and CU643 is that the former is a branched polysaccharide whereas the latter is a linear polysaccharide.

Ribotyping.—Many of the *Erwinia* strains studied in this laboratory have been subjected to biochemical tests in the clinical laboratory and were classified most often as *Pantoea agglomerans* (previously classified as *Enterobacter agglomerans*) or *Enterobacter cloacae*. They are all oxidase-negative Gram-negative rods, which are strongly pectolytic except for strain Ech6. The inability to easily distinguish between the strains biochemically, particularly those that produce similar EPSs (namely strains SR260, Ech1, Ech9, Ech378, SR31), was the initial impetus for ribotyping the strains. It was shown that these strains could be distinguished by ribotyping despite the similarity of the structures of their EPSs.[15]

Ribotyping has now been performed on a larger sampling of *Erwinia* spp. (Fig. 2) and the following conclusions can be drawn:

- *E. chrysanthemi* A350, A2148 and A2571 have nearly identical ribotypes as expected from their lineage (Table 3). Moreover, they share a common branch in the dendrogram with strain CU643 with about 70% correlation. The composition of their EPSs is similar (other than the difference in the uronic acid) although structurally, they are different, one being a branched polysaccharide and the other a linear polysaccharide.
- Ech1, Ech 9, and its streptomycin-resistant strain, Ech9Sm6, are closely related (Fig. 2). The structures of their EPSs are similarly related[18] and have similar physical properties. Similarly, the ribotypes of Ech6 and its streptomycin-resistant strain, Ech6S^{+}, are nearly identical (Fig. 2) as are their polysaccharides[17] (unpublished work).
- The ribotypes of ISU RA3 (isolated from onion, *Allium* spp.) and RA3/W are similar but the two strains differ phenotypically. ISU RA3 produces a black pigment on nutrient agar and modified YS-Glucose agar and produces no EPS on the latter medium. Strain RA3/W, a spontaneous

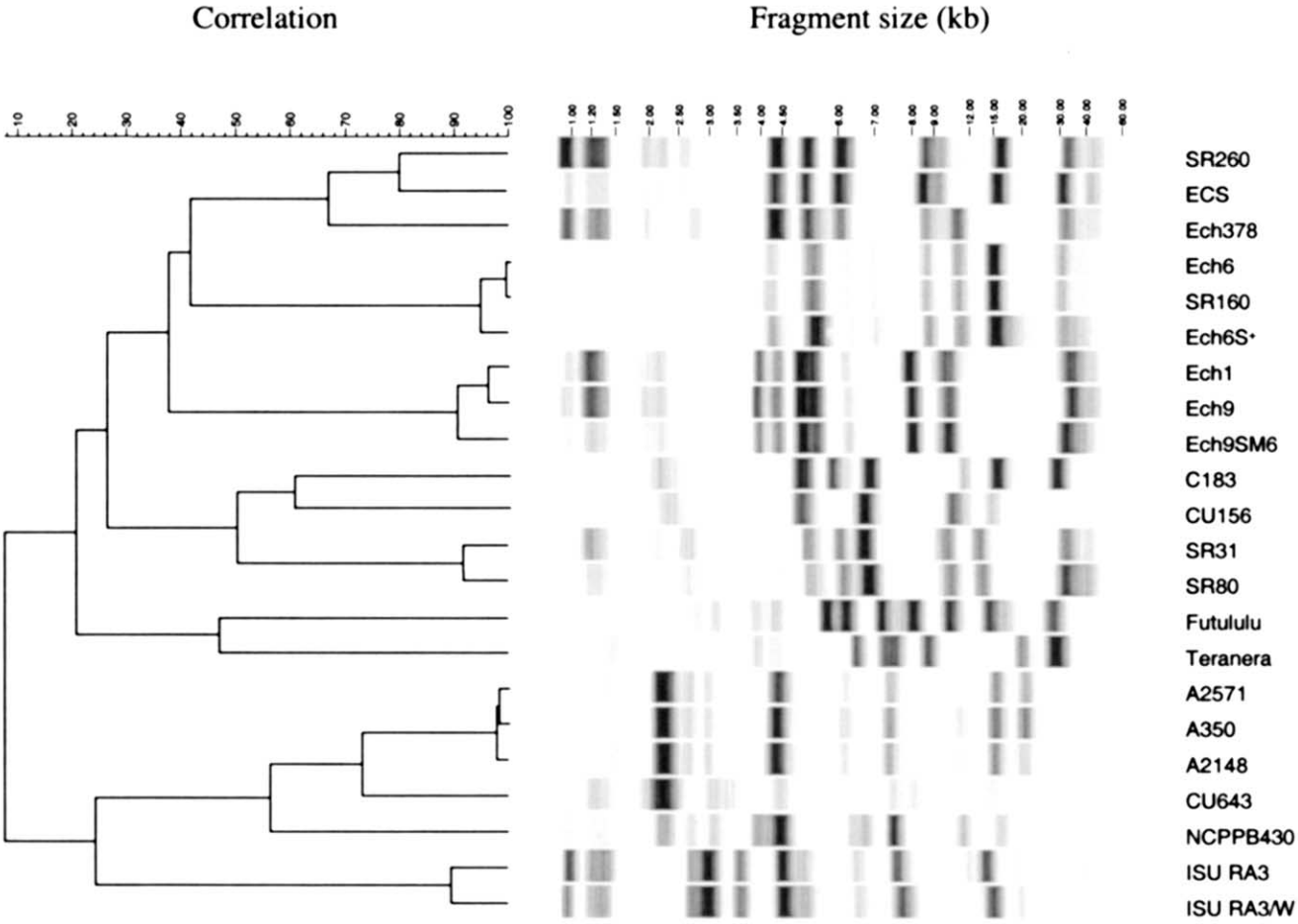


Fig. 2. Nearest neighbor analysis of the ribotyping data from the *E. chrysanthemi* strains used in this study.

Table 3
E. chrysanthemi strains and their origin

Strain	Host	Source	Notes
A350	saintpaulia	Condemine	$LacZ^-$ derivative of 3937
A2148	saintpaulia	Condemine	PecT mutant of A350, hyper EPS producer
A2571	saintpaulia	Condemine	EPS^- mutant of A350
SR120A	maize	Braun	isolated by Hayward, Hawaii
SR80	maize	Braun	isolated by Kelman, Wisconsin
RA3	onion	Braun	isolated by Tzeng, China. Black pigmented strain, no EPS production
RA3/W	onion	Gray	spontaneous non-pigmented EPS^+ strain derived from RA3
Ech378	dieffenbachia	Braun	isolated by Garibaldi, Italy
SR229	carnation	Braun	
CU643	philodendron	Braun	
SR260	maize	Mildenhall	Mildenhall, South Africa
ECS	maize	Gray	spontaneous Lac^+ mutant derived from SR260[28]
NCPPB 2347	maize	Vidaver	U. Mazzuchi, Italy
SR75	maize	Vidaver	Nebraska
Ech1	potato	Marasas	Vegetable and Ornamental Plant Research Institute, South Africa, Erwinia wilt, Douglas. Biotype IV
Ech6 [a]	potato	Marasas	Vegetable and Ornamental Plant Research Institute, South Africa, Erwinia wilt, Highveld. Biotype V
Ech9	potato	Marasas	Vegetable and Ornamental Plant Research Institute, South Africa, Black Leg, Douglas
SR31	maize	Kelman	ICPB-EC16, EC16. Burholder, Starr, New York
EC183		Chatterjee	
CU156		Chatterjee	
NCPPB430	dianthicola	Chatterjee	
Futululu [a]	Eucalyptus	Van Zyl	South Africa, Futululu Region
Teranera [a]	Eucalyptus	Van Zyl	South Africa, Teranera Region

[a] *E. chrysanthemi* not confirmed.

mutant isolated from ISU RA3, is white on both media and produces EPS on YS-Glucose agar.

- Although the structures of the EPSs from strains SR260, Ech1, Ech9 and Ech9Sm6 are similar, their ribotypes are different. They do, however, share a common branch in the dendrogram.
- When the correlation is less than 60%, the general structures of EPS are not closely related. One exception known to date is SR80 (unpublished work), its EPS has structure and properties similar to Ech 9, but does not correlate well by ribotyping.

All of the strains whose EPSs have been structurally characterized in this laboratory belong to the same pathovar (pv. *zeae*) with the possible exception of Ech6, Futululu and Teranera. It is thus evident that members of the same pathovar (and the same Biovar) can produce EPSs with different structures. Moreover, *E. chrysanthemi* strains isolated from different hosts produce EPSs with similar structures, e.g., SR260 and SR31 (corn), Ech1 and Ech9 (potato). Strong indications of chemotaxonomic similarity can be derived from the ribotyping data, giving assistance to the decision of which *Erwinia* spp. might produce different EPS or are worthy of more detailed study. Although the present procedure, using the restriction enzyme EcoR1, does not give such exclusive information on the structures of the EPSs as desired, it has been demonstrated in the comparison of the EPSs from A350 and A2148 that knowing the detailed structure of one and the nearly identical ribotype of the bacteria simplifies the structural characterization of the other.

Acknowledgements

The authors thank the Biotechnology Byproducts Consortium (USDA Grant No.

98-34188-5902) and the Carbohydrate Structure Facility for the use of its equipment. We also wish to thank John Snyder for recording the ^{1}H NMR spectra and for many discussions; Richard J. Hollis for the ribotyping and Carol Yang for the clinical microbiological characterizations.

References

1. Chatterjee, A. K.; Starr, M. P. *Annu. Rev. Microbiol.* **1980**, *34*, 645–676.
2. Dickey, R. S. *Phytopathology* **1979**, *69*, 324–329.
3. Dickey, R. S. *Phytopathology* **1981**, *71*, 23–29.
4. Dickey, R. S.; Zumoff, C. H.; Uyemoto, J. K. *Phytopathology* **1984**, *74*, 1388–1394.
5. Dickey, R. S.; Claflin, L. E.; Zumoff, C. H. *Phytopathology* **1987**, *77*, 426–430.
6. Perombelon, M. C. M. In *The Prokaryotes. A Handbook on the Biology of Bacteria*: *Ecophysiology*, *Isolation*, *Identification*, *Applications*; Balows, A.; Truper, H. G.; Dworkin, M.; Harder, W.; Schleifer, K.-H., Eds.; Springer–Verlag: New York, 1992; pp. 2899–2921.
7. Boccara, M.; Vedel, R.; Lalo, D.; Lebrun, M. H.; Lafay, J. F. *Mol. Plant Microb. Int.* **1991**, *4*, 293–299.
8. Slade, M. B.; Tiffin, A. I. *Methods Microbiol.* **1984**, *15*, 227–293.
9. Dickey, R. S.; Victoria, J. I. *Int. J. Syst. Bacteriol.* **1980**, *30*, 129–134.
10. Nassar, A.; Darrasse, A.; Lemattre, M.; Kotoujansky, A.; Dervin, C.; Vedel, R.; Bertheau, Y. *Appl. Environ. Microbiol.* **1996**, *62*, 2228–2235.
11. Nassar, A.; Bertheau, Y.; Dervin, C.; Narcy, J.-P.; Lemattre, M. *Appl. Environ. Microbiol.* **1994**, *60*, 3781–3789.
12. Condemine, G.; Castillo, A.; Passeri, F.; Enard, C. *Mol. Plant. Microb. Int.* **1999**, *12*, 45–52.
13. Gray, J. S. S.; Brand, J. M.; Koerner, T. A. W.; Montgomery, R. *Carbohydr. Res.* **1993**, *245*, 271–287.
14. Gray, J. S. S.; Koerner, T. A. W.; Montgomery, R. *Carbohydr. Res.* **1995**, *266*, 153–159.
15. Gray, J. S. S.; Yang, B. Y.; Montgomery, R. *Carbohydr. Res.* **2000**, *324*, 255–267.
16. Yang, B. Y.; Gray, J. S.; Montgomery, R. *Carbohydr. Res.* **1999**, *316*, 138–154.
17. Yang, B. Y.; Gray, J. S. S.; Montgomery, R. *Int. J. Biol. Macromol.* **1994**, *16*, 306–312.
18. Yang, B. Y.; Gray, J. S. S.; Montgomery, R. *Int. J. Biol. Macromol.* **1996**, *19*, 223–226.
19. Surgey, N.; Robert-Baudouy, J.; Condemine, G. *J. Bacteriol.* **1996**, *178*, 1593–1599.
20. Carlton, B. C.; Brown, B. J. In *Manual of Methods for General Bacteriology*; Gerhardt, P.; Murray, R. G. E.; Costilow, R. N.; Nester, E. W.; Krieg, N. R.; Philips, G. B., Eds.; ASM: Washington, DC, 1981; pp. 222–242.
21. Gerwig, G. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1978**, *62*, 349–357.
22. Lau, J. M.; McNeil, M.; Darvill, A. G.; Albersheim, P. *Carbohydr. Res.* **1987**, *168*, 219–243.
23. Lau, J. M.; McNeil, M.; Darvill, A. G.; Albersheim, P. *Carbohydr. Res.* **1987**, *168*, 245–274.
24. Zhan, D. F.; Janssen, P.; Mort, A. J. *Carbohydr. Res.* **1998**, *308*, 373–380.
25. Tjan, S. B.; Voragen, A. G. J.; Pilnik, W. *Carbohydr. Res.* **1974**, *34*, 15–23.
26. Schols, H. A.; Voragen, A. G. J.; Colquhoun, I. J. *Carbohydr. Res.* **1994**, *256*, 97–111.
27. Colquhoun, I. J.; de Ruiter, G. A.; Schols, H. A.; Voragen, A. G. J. *Carbohydr. Res.* **1990**, *206*, 131–144.
28. Gray, J. S. S.; Lindner, W. A.; Brand, J. M.; Mildenhall, J. P. *J. Bacteriol.* **1986**, *168*, 886–891.