INFECTION AND IMMUNITY, May 1990, p. 1391–1395
0019-9567/90/051391-05$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 58, No. 5

# Antigenic Relationships of the Lipopolysaccharides of *Escherichia hermannii* Strains with Those of *Escherichia coli* O157:H7, *Brucella melitensis*, and *Brucella abortus*†

MALCOLM B. PERRY* AND DAVID R. BUNDLE

*Division of Biological Sciences, National Research Council, Ottawa, Ontario, Canada K1A OR6*

Received 18 October 1989/Accepted 17 January 1990

**Clinical isolates of *Escherichia hermannii* which showed serological cross-reaction with polyclonal antisera to the O-polysaccharide portion of the lipopolysaccharide of *E. coli* O157 strains and with antisera to the O antigens of *Brucella abortus* and *B. melitensis* were found by chemical and nuclear magnetic resonance analyses to have lipopolysaccharide O chains composed of linear polymers containing 1,2- and 1,3-linked 4-acetamido-4,6-dideoxy-α-D-mannopyranosyl (α-D-Rha*p*4NAc) residues. Two O-antigen structures were identified; each had an unbranched pentasaccharide repeating unit, and one was composed of three 1,2- and two 1,3-linked α-D-Rha*p*4NAc residues and the other had two 1,2- and three 1,3-linked α-D-Rha*p*4NAc residues. The above-described cross-serological reactivities, which have led to false-positive identifications, are related to the common occurrence of epitopes involving the presence of *N*-acyl derivatives of 4-amino-4,6-dideoxy-D-mannopyranosyl residues in the O-polysaccharide portions of the respective lipopolysaccharides of the organisms. Strains of *E. hermannii* which did not show serological cross-reactions with *E. coli* O157 and *Brucella* antisera were found to have unique lipopolysaccharide O chains devoid of D-Rha*p*4NAc residues, demonstrating the existence of serotypes of *E. hermannii* that are distinct on the basis of their lipopolysaccharide components.**

*Escherichia hermannii* has been described as an atypical biogroup of *Escherichia coli* characterized as gram-negative, oxidase-negative, fermentative, motile rods which produced a characteristic yellow pigment and, among other properties, gave positive KCN and cellobiose tests (2). Wounds accounted for 50% of human isolates of *E. hermannii*, followed by sputum or lung isolates (25%) and stool isolates (20%); however, its true incidence and clinical significance have not been investigated.

Our interest in *E. hermannii* was stimulated by the fact that some strains of the organism could, by use of polyclonal rabbit antisera, be falsely identified as *E. coli* O157:H7, an important pathogen which is associated with hemorrhagic colitis (1, 13). Earlier studies in our laboratory involving the structural analysis of the antigenic O chains of lipopolysaccharides (LPS) had shown that the serological cross-reaction among *E. coli* O157:H7, *Brucella abortus*, and *B. melitensis* cells and their respective polyclonal antisera (9) could be related to the occurrence in their LPS O chains of a common epitope involving 1,2-substituted *N*-acyl derivatives of 4-amino-4,6-dideoxy-α-D-mannopyranosyl residues (3, 4, 8, 14, 17). Similar serological cross-reactions involving the LPS of *Yersinia enterocolitica* serotype O:9 (6, 7), *Pseudomonas maltophilia* 555 (10), *Vibrio cholerae* (12), and *Salmonella* sp. group N (O:30) (5, 16) could also be related to the occurrence of epitopes involving *N*-acetyl derivatives of 4-amino-4,6-dideoxy-α-D-mannopyranosyl residues in their respective LPS O-chain antigens.

This report contains the results of structural analyses of LPS O antigens of five strains of *E. hermannii* and provides a rational basis for interpretation of *E. hermannii* serology, especially as it relates to cross-reactions with polyclonal rabbit antisera to *E. coli* O157 antigen.

## MATERIALS AND METHODS

**LPS and constituent O chains.** Five strains of *E. hermannii* (LCDC 86-1269, NRCC 4262; LCDC 86-958, NRCC 4297; LCDC 86-344, NRCC 4298; LCDC 86-333, NRCC 4299; and LCDC 88-1766, NRCC 4300) were grown from frozen stocks by being grown in brain heart infusion broth (50 ml) in 250-ml baffled Erlenmyer flasks incubated overnight at 37°C and 250 rpm and then transferred to brain heart infusion broth (1 liter) in 4-liter baffled Erlenmyer flasks and incubated at 37°C for 7 h at 200 rpm. The growth (1 liter) was used to inoculate a fermentor (28-liter Microferm; New Brunswick Scientific Co., Inc.) containing 28 liters of brain heart infusion broth and antifoam (5 ml; Mazu DF60P; Mazer Chemicals Inc., Gurnee, Ill.). The organisms were grown at 37°C and 200 rpm with aeration at 25 liters/min for 18 h. The growths were killed by the addition of phenol to a final concentration of 0.75%, and after the mixture was kept at 6°C for 2 h, the cells (ca. 300 g [wet weight]) were collected by using a Sharples continuous centrifuge. The collected cells were extracted directly with stirred 48% aqueous phenol (10 min at 67°C) (11), and following low-speed centrifugation at 4°C, the separated phenol and aqueous phases were collected and dialyzed against running tap water until free from phenol. The retained portions were lyophilized. The lyophilized products were dissolved in water (100 ml) and treated with RNase and deoxy-RNase (4 h, 37°C), followed by protease K (2 h, 37°C), and following removal of insoluble material by low-speed centrifugation, the LPS were obtained as precipitated gels on ultracentrifugation (105,000 × *g*, 4°C, 18 h). The gels were taken up in distilled water (40 ml) and lyophilized.

Samples of LPS (550 mg) dissolved in 2% (vol/vol) aqueous acetic acid (100 ml) were heated on a boiling water bath

* Corresponding author.

† Publication 31280 from the National Research Council of Canada.

for 2 h, and the precipitated lipids A were collected from the cooled solutions (4°C) by low-speed centrifugation. The lyophilized centrifugation products were dissolved in 0.05 M pyridinium acetate buffer (pH 4.7, 4 ml) and fractionated on a column of Sephadex G-50 (2 by 80 cm; Pharmacia Fine Chemicals) with the same buffer. The column eluates were monitored by using a Waters 402 refractometer, and fractions (10 ml) of the eluates were collected.

**Analytical methods.** Hydrolysis of glycans with anhydrous hydrofluoric acid or 1 M sulfuric acid, glycose analyses, and methylation analyses were performed as previously described (4). Paper chromatography was done by using pyridine-ethyl acetate-water (2:5:5 [vol/vol]; top layer) as the mobile phase, and mobilities are quoted relative to that of D-galactose. Gas-liquid chromatography–mass spectroscopy was done with a Hewlett-Packard 5985B system by using either (i) a capillary column (25 m) coated with OV17 and a program temperature rise from 180 to 240°C at 2°C/min or (ii) a capillary column of DB1701 (30 m; J & W Scientific Inc.) and a program temperature rise from 180 to 240°C at 2°C/min; an ionization potential of 70 eV was used. Retention times are quoted relative to that of D-glucitol hexaacetate or 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

**NMR.** One- and two-dimensional $^{13}C$ and $^{1}H$ nuclear magnetic resonance (NMR) spectroscopy was done at 125 MHz ($^{13}C$) and 500 MHz ($^{1}H$) by using a Bruker AM500 spectrometer with polysaccharide samples lyophilized once from a deuterium oxide solution and under conditions previously described (4, 5).

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analyses were done with 14% gels, and staining was done by the periodate-silver nitrate technique (18).

**Immunodiffusion.** Gel immunodiffusion experiments were performed with 1% agarose gels containing 1% NaCl with solutions (ca. 1%) of LPS, alkali O-deacylated LPS, and derived LPS O polysaccharides. A monoclonal antibody (BM10) (3) specific for the *Brucella* M antigen was used.

## RESULTS AND DISCUSSION

Fermentor-grown cells of five clinical isolates of *E. hermannii* which had been selected because of their diverse origins, agglutination by polyclonal rabbit *E. coli* O157 antisera, and identification by biochemical tests (2) were extracted by hot aqueous phenol, and the LPS present in the separated phenol and water layers of the cooled extract were obtained by ultracentrifugation of the concentrated dialyzed phenol-free phases. The major yields (ca. 90%) of LPS were found in the phenol phases (5 to 6% yields based on dry weight of cells), a characteristic of S-type LPS composed of O chains with a relatively high content of acylated aminodeoxyglycose residues.

Hydrolysis of the five phenol-phase LPS with hot, dilute acetic acid yielded insoluble lipid A (ca. 15% yield), while Sephadex G-50 column gel filtration of the aqueous soluble products produced similar yields of O polysaccharides ($K_{av}$, 0.02; ca. 55%), small amounts of core oligosaccharides ($K_{av}$, 0.47; ca. 3%), and fractions containing 3-deoxy-D-*manno*-octulosonic acid and phosphate ($K_{av}$, 0.99; ca. 12%) eluting in the monosaccharide region.

The five isolated O polysaccharides had similar specific optical rotations ($[\alpha]_D$; +80 to 82°; c 0.5, water) and elemental analysis results. Following hydrolysis with anhydrous hydrofluoric acid (8), the O polysaccharides all afforded 4-acetamido-4,6-dideoxy-D-mannose (ca. 90% yield), which had an $[\alpha]_D$ of +15° (c 1.2, water); they gave single spots on paper chromatography (retention time relative to D-galactose, 2.40) and had $^{13}C$-NMR spectra identical to those of authentic samples of 4-acetamido-4,6-dideoxy-D-mannose. On reduction ($NaBD_4$) and acetylation, the aminodideoxyglycose afforded 1,2,3,5-tetra-*O*-acetyl-4-acetamido-4,6-dideoxy-D-mannitol-1-*d*, which gave a single peak on gas-liquid chromatography–mass spectroscopy (program A) (10.4 min; retention time relative to that of D-glucitol hexaacetate; 1.31) with a retention time and a mass spectrum identical to those of an authentic reference sample. Hydrolysis of the methylated O polysaccharides with anhydrous hydrofluoric acid, followed by gas-liquid chromatography–mass spectroscopy (program B) of the reduced ($NaBD_4$) and acetylated products revealed well-separated 2-*O*-methyl (13.88 min; retention time relative to that of 1,5-di-*O*-acetyl-

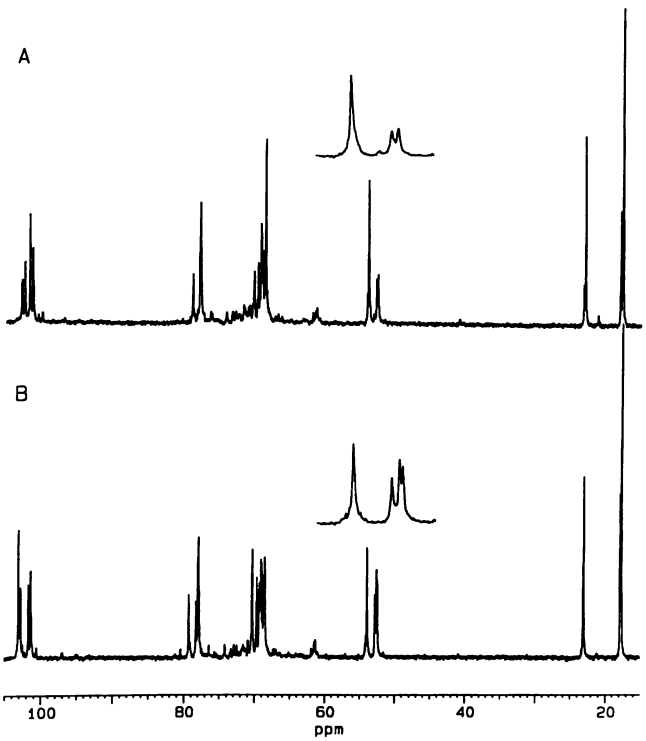


FIG. 1. $^{13}C$-NMR (125 MHz) spectra of *E. hermannii* NRCC 4262 LPS O polysaccharide (A) and *E. hermannii* NRCC 4300 LPS O polysaccharide (B).

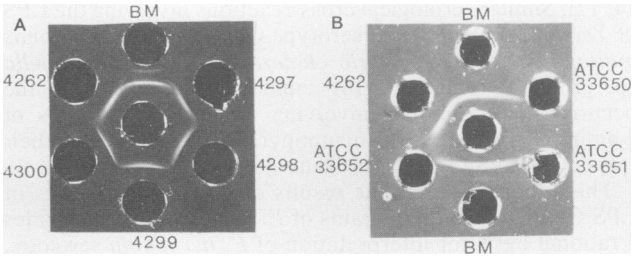


FIG. 2. Immunodiffusion of alkali O-deacylated LPS from *E. hermannii* NRCC 4262, NRCC 4297, NRCC 4298, NRCC 4299, NRCC 4300, ATCC 33650, ATCC 33651, and ATCC 33652 against *B. melitensis* M-antigen-specific monoclonal antibody BM10 (center well). BM, *B. melitensis* 16M LPS.

2,3,4,6-tetra-*O*-methyl-D-glucitol, 2.58) and 3-*O*-methyl (13.55 min; retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, 2.52) fully acetylated alditol derivatives of 4,6-dideoxy-4-(*N*-methylacetamido)-D-mannose, suggesting that the O chains were linear, unbranched polymers of 4-acetamido-4,6-dideoxy-α-D-mannopyranosyl (α-D-Rha*p*4NAc) residues in repeating units containing 1,2 and 1,3 linkages in ratios of 3:2 for *E. hermannii* NRCC 4262 and 2:3 for *E. hermannii* NRCC 4297, NRCC 4298, NRCC 4289, and NRCC 4300 O polysaccharides. The high positive specific optical rotations of the polymers suggest that the glycose units probably had the α-D configuration, a conclusion consistent with subsequent NMR analytical data, which also supported the relative ratios of 1,2 and 1,3 linkages determined by methylation analysis.

Analysis of the $^{1}H$ and $^{13}C$ NMR spectra revealed that *E. hermannii* NRCC 4297, NRCC 4298, NRCC 4299, and NRCC 4300 produced LPS O polysaccharides that were identical to one another but distinct from that of the O polysaccharide of *E. hermannii* NRCC 4262. This difference is clear, since the latter polysaccharide gave a $^{13}C$-NMR spectrum that was related to but distinct from those of the other four polysaccharides (Fig. 1A and B). The general features of the NMR spectra of both polysaccharide types showed the presence of 6-deoxy and acetamidodeoxy functions, which corroborated the component glycose analysis that revealed that 4-acetamido-4,6-dideoxy-D-mannose was the only monosaccharide present in the O polysaccharides.

NMR data for polysaccharides of this type have been well documented (4), and $^{13}C$ chemical spectra, in particular, exhibit several spectral windows at 170 ppm (carbonyl function), 100 ppm (anomeric carbon atoms), 53 ppm (C-4 carbon atoms), 23 ppm (acetamido methyl carbon atoms), and 17 ppm (6-deoxyhexose carbon atoms) that aid structure determinations. The $^{13}C$-NMR spectrum of the unique 4262 polysaccharide showed three carbonyl signals, four anomeric carbon resonances (integral ratio, 1:1:2:1), suggesting a repeating unit with a minimum of five monosaccharide residues, three C-4 resonances (integral ratio, 3:1:1), two acetamido methyl signals, and three 6-deoxyhexose methyl signals. The most significant structural information was derived from the C-4 chemical shifts, which previous work (4) has shown to be sensitive to the pattern of glycosyl substitution. When α-D-Rha*p*4NAc was glycosylated at O-2, the chemical shift of C-4 was found at 53.8 ppm as opposed to 52.7 ppm if glycosylation occurred at O-3 (4). It may therefore be concluded that two O-3- and three O-2-substituted residues are present in the pentameric repeating unit. This conclusion was corroborated by $^{1}H$ chemical shift data on the same polysaccharide, which showed four anomeric proton signals (integral ratio, 1:1:1:2) and, on the basis of two-dimensional homonuclear shift correlation and relayed coherence transfer experiments, three H-2 resonances at 4.17, 4.13, and 4.12 ppm and two H-2 signals at 3.85 and 3.79 ppm. These two ranges of H-2 chemical shifts are characteristic of residues linked α-1,2 and α-1,3 to the next glycose residue (4). The approximate 0.35-ppm shielding of the H-2 resonances had been linked to the conformation that results when a α-D-Rha*p*4NAc residue is 1,3 linked to the following residue (4), and this H-2 chemical shift datum indicates that the polysaccharide repeating unit is composed of five monosaccharide residues joined by three 1,2 and two 1,3 glycosidic linkages.

The similar chemical shift data for each of the five monosaccharide residues may be used to infer a linear arrangement of monosaccharides in the repeating unit, and usually the linkage sequence can be obtained by performing nuclear Overhauser (nOe) measurements. Two-dimensional phase-sensitive nOe experiments (NOESY) established correlations between each of the five anomeric protons and the H-2 proton of the same pyranose ring through space (4, 5). In addition, a cross-peak was also observed across the glycosidic linkage correlating anomeric protons attached to the carbon atoms bearing the glycosyl substituent (4, 5). It was not possible to trace the interresidue network of nuclear Overhauser connectivities for the two types of O polysaccharide reported here, because the H-3 resonances of each constituent monosaccharide residue possessed similar chemical shifts and therefore could not be unambiguously assigned to a given spin system. The two-dimensional nuclear Overhauser cross-peaks did suggest that only two of the three α-1,2 linkages of the strain NRCC 4262 polysaccharide were present in a contiguous sequence. This could be ascertained because α-D-Rha*p*4NAc residues linked 1,3 to the next residue have H-2 resonances shifted upfield from those 1,2 linked to the next residue. By correlating the chemical shifts of nuclear Overhauser cross-peaks that represent interresidue connectivities, it was possible to identify partial residue sequences that allowed possible structures to be excluded. Since there are only two possible sequences of monosaccharide residues (I and II), the nOe data identify structure II as the repeating unit of the strain NRCC 4262 O polysaccharide.

I

→3)-α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→

II

→3)-α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→

Similar arguments based on the observed NMR data were used to determine the fine structure and linkage sequences of the strain NRCC 4297, NRCC 4298, NRCC 4299, and NRCC 4300 O polysaccharides. Thus, the $^{13}C$ NMR chemical shift data also indicated a pentameric repeating unit, but the C-4 resonances of these polysaccharides showed three, with chemical shifts characteristic of monosaccharide residues bearing substituents at O-3 and two substituted at O-2. In support of the $^{13}C$ data, the $^{1}H$ chemical shift data for the H-2 atoms indicated a similar substitution pattern. The two-dimensional nuclear Overhauser experiment for the strain NRCC 4300 polysaccharide also established that only two of the three α-1,3 monosaccharide residues occurred as a contiguous sequence, again permitting discrimination of

the possible structures (III and IV) in favor of IV as the correct sequence for the NRCC 4297, NRCC 4298, NRCC 4299, and NRCC 4300 O-polysaccharide repeating units.

the *E. hermannii* strains discussed in this report. Thus, the LPS O chain of *E. hermannii* ATCC 33651 was an unusual homopolymer of D-rhamnopyranosyl residues, while the

III

→2)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→

IV

→2)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→2)-α-D-Rha*p*4NAc-(1→3)-α-D-Rha*p*4NAc-(1→

The results of methylation analysis were consistent with the ratios of 1,2 and 1,3 glycosidic linkages determined by NMR data, since the ratio of the 3-*O*-methyl to 2-*O*-methyl ether derivatives of α-D-Rha*p*4NAc from the strain NRCC 4297 polysaccharide was 3:2 and that for the NRCC 4297, NRCC 4298, NRCC 4299, and NRCC 4300 polysaccharides was 2:3.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the five *E. hermannii* phenol-phase LPS fractions was consistent with the postulated complete smooth LPS structures, and on the basis of the narrow-band region of type S LPS, it can be concluded that all of the LPS had a narrow range of chain lengths corresponding to a mean estimated molecular weight of ca. 20,000.

In imunodiffusion analysis (Fig. 2), the alkali *O*-deacylated *E. hermannii* LPS and their corresponding free O polysaccharides gave precipitin lines with monoclonal antibody BM10 to the *Brucella* M antigen (3). This antibody has been characterized (3) as having a binding requirement for at least the trisaccharide sequence →2)-α-D-Rha*p*4NR-(1→3)-α-D-Rha*p*4NR-(1→2)-α-D-Rha*p*4NR→(1→, where R is HCO or $CH_3CO$.

On the basis of the above-described results, it can be concluded that the strains of *E. hermannii* that react with polyclonal *E. coli* O157:H7 antiserum have very similar LPS O-antigenic polysaccharide components and that the basis for their serological cross-reactivities resides in epitope features involving the common α-D-Rha*p*4NAc residues present in the O antigens.

The LPS O chains examined were not differentiated from one another by monoclonal antibody BM10. It is of note that monoclonal antibodies specific for *E. coli* O157 antigen (15) did not give positive serological reactions with any strains of *E. hermannii*.

In the present study, we found that the LPS of five representative clinical isolates of *E. hermannii* which showed positive serological reaction with polyclonal rabbit antisera to *E. coli* O157:H7 had structurally related antigenic O-polysaccharide chains which were identified as unbranched homopolymers of 1,2- and 1,3-linked α-D-Rha*p* 4NAc residues very similar in basic structure to the corresponding 4-formamido derivatives found in the *Brucella* M antigen (4). It is believed that the structural relationship between the antigenic features found in the LPS O chains accounts for the observed serological cross-reactions with polyclonal antisera.

In subsequent investigations (Perry et al., unpublished data), it was found that strains of *E. hermannii* that did not show serological cross-reaction with polyclonal antisera to *E. coli* O157:H7 or *B. melitensis*, had LPS O chains with structures unrelated to the homopolymer LPS O chains of LPS O chains of *E. hermannii* ATCC 33650 and ATCC 33652 were composed of repeating tetrasaccharide units containing D-rhamnose, D-glucose, and D-galactose (2:1:1) (Perry, unpublished data). As might be expected, these latter strains of *E. hermannii* did not show serological cross-reactivity with monoclonal antibody BM10 (Fig. 2) (3), and this is evidence for the existence of defined serotypes of *E. hermannii* based on LPS antigenic O chains.

The core oligosaccharides of the five strains of *E. hermannii* had an $[\alpha]_D$ of +85° (c 0.8, water) and were composed of D-glucose, 2-acetamido-2-deoxy-D-glucose, and L-*glycero*-D-*manno*-heptose in a molar ratio of 1:1:1. Analysis of the lipid A isolates showed that they were composed of 2-amino-2-deoxy-D-glucose (ca. 8%), lauric acid (8%), myristic acid (19%), palmitic acid (31%), and β-hydroxymyristic acid (18%).

In conclusion, the strong serological cross-reactions among the strains of *E. hermannii* and *B. abortus* and *B. melitensis* described in this report can be attributed to the presence of common epitopes involving 1,2- and 1,3-linked *N*-acyl derivatives of 4-amino-4,6-dideoxy-α-D-mannopyranosyl units in linear homopolymers, whereas the fairly weak serological cross-reaction of *E. coli* O157 strains when polyclonal antisera were used can be related to the occurrence of an α-D-Rha*p*4NAc residue found in the repeating tetrasaccharide unit of its LPS O chain.

## ACKNOWLEDGMENTS

We thank H. Lior for strains of *E. hermannii*, D. W. Griffith for production of cells, F. Cooper for gas-liquid chromatography–mass spectroscopy analyses, and Leann MacLean for experimental assistance.

## LITERATURE CITED

1. **Borczyk, A. A., H. Lior, and B. Ciebin.** 1987. False positive identifications of *Escherichia coli* O157:H7 in foods. Int. J. Food Microbiol. **4**:247–249.
2. **Brenner, D. J., B. R. Davis, A. G. Steigerwalt, C. F. Riddle, A. C. McWhorter, S. D. Allen, J. J. Farmer, Y. Saitoh, and G. R. Fanning.** 1982. Atypical biogroups of *Escherichia coli* found in clinical specimens and description of *Escherichia hermannii* sp. nov. J. Clin. Microbiol. **15**:703–713.
3. **Bundle, D. R., J. W. Cherwonogrodzky, M. A. J. Gidney, P. J. Meikle, M. B. Perry, and T. Peters.** 1989. Definition of *Brucella* A and M epitopes by monoclonal typing reagents and synthetic oligosaccharides. Infect. Immun. **57**:2829–2836.
4. **Bundle, D. R., J. W. Cherwonogrodzky, and M. B. Perry.** 1987. Structural elucidation of the *Brucella melitensis* M antigen by high resolution nuclear magnetic resonance at 500 MHz. Biochemistry **26**:8717–8726.
5. **Bundle, D. R., M. Gerken, and M. B. Perry.** 1986. Two-dimensional nuclear magnetic resonance at 500 MHz: the structural elucidation of *Salmonella* serogroup N polysaccharide

antigen. Can. J. Chem. **64**:255–264.
6. **Bundle, D. R., M. A. J. Gidney, M. B. Perry, J. R. Duncan, and J. W. Cherwonogrodzky.** 1984. Serological confirmation of *Brucella abortus* and *Yersinia enterocolitica* serotype O:9 O-antigens by monoclonal antibodies. Infect. Immun. **46**:389–393.
7. **Caroff, M., D. R. Bundle, and M. B. Perry.** 1984. Structure of the O-chain of the phenol-phase soluble lipopolysaccharide of *Yersinia enterocolitica* serotype O:9. Europ. J. Biochem. **139**: 195–200.
8. **Caroff, M., D. R. Bundle, M. B. Perry, J. W. Cherwonogrodzky, and J. R. Duncan.** 1984. Antigenic S-type lipopolysaccharide of *Brucella abortus* 1119-3. Infect. Immun. **46**:384–388.
9. **Corbel, M. J., F. A. Stuart, and R. A. Brewer.** 1984. Observations on serological cross reactions between smooth *Brucella* species and organisms of other genera. Dev. Biol. Stand. **56**:341–348.
10. **DiFabio, J. L., M. B. Perry, and D. R. Bundle.** 1987. Analysis of the lipopolysaccharide of *Pseudomonas maltophilia* 555. Biochem. Cell Biol. **65**:968–977.
11. **Johnson, K. G., and M. B. Perry.** 1976. Improved techniques for the preparation of bacterial lipopolysaccharides. Can. J. Microbiol. **22**:29–34.
12. **Kenne, L., B. Lindberg, P. Unger, B. Gustafsson, and T. Holme.** 1982. Structural studies of the *Vibrio cholerae* antigen. Carbohydr. Res. **100**:342–349.
13. **Lior, H., and A. A. Borczyk.** 1987. False positive identifications of *Escherichia coli* O157:H7. Lancet **i**:333.
14. **Meikle, P. J., M. B. Perry, J. W. Cherwonodrodzky, and D. R. Bundle.** 1989. Fine structures of A and M antigens from *Brucella* biovars. Infect. Immun. **57**:2820–2828.
15. **Perry, M. B., D. R. Bundle, M. A. J. Gidney, and H. Lior.** 1988. Identification of *Escherichia coli* O157 strains by using a monoclonal antibody. J. Clin. Microbiol. **26**:2391–2394.
16. **Perry, M. B., D. R. Bundle, L. MacLean, J. A. Perry, and D. W. Griffith.** 1986. The structure of the antigenic lipopolysaccharide O-chain produced by *Salmonella urbana* and *Salmonella godesberg*. Carbohydr. Res. **156**:107–122.
17. **Perry, M. B., L. MacLean, and D. W. Griffith.** 1985. Structure of the O-chain polysaccharide of the phenol phase soluble lipopolysaccharide of *Escherichia coli* O157:H7. Can. J. Biochem. Cell Biol. **64**:21–28.
18. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in acrylamide gels. Anal. Biochem. **119**:115–119.