The Structure of the Colony Migration Factor from Pathogenic
Proteus mirabilis

A CAPSULAR POLYSACCHARIDE THAT FACILITATES SWARMING*

(Received for publication, March 8, 1999, and in revised form, May 18, 1999)

M. Mahhabur Rahman‡, Jean Guard-Petter‡, Kokila Asokan‡§, Colin Hughes§, and Russell W. Carlson¶

From the ‡Complex Carbohydrate Research Center, The University of Georgia, Athens, Georgia 30602, the §Southeast Poultry Research Laboratory, United States Department of Agriculture, Agricultural Research Service, Athens, Georgia 30605, and the ¶Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, United Kingdom

Swarming by Proteus mirabilis is characterized by cycles of rapid and coordinated population migration across surfaces following differentiation of vegetative cells into elongated hyperflagellated swarm cells. It has been shown that surface colony expansion by the swarm cell population is facilitated by a colony migration factor (Cmf), a capsular polysaccharide (CPS) that also contributes to the uropathogenicity of P. mirabilis (Gygli, D., Rahman, M. M., Lai, H.-C., Carlson, R., Guard-Petter, J., and Hughes, C. (1995) Mol. Microbiol. 17, 1167–1175). In this report, the Cmf-CPS was extracted with hot water, precipitated with ethanol, and further purified by gel permeation chromatography. Its structure was established by glycosyl composition and linkage analyses, and by one- and two-dimensional NMR spectroscopy. The Cmf-CPS is composed of the following tetrasaccharide repeating unit.

\[ \beta-D-GalpA \]

Proteus mirabilis is a pathogenic Gram-negative bacterium that frequently causes kidney infections, typically established by ascending colonization of the urinary tract (1–5). It exhibits a striking form of multicellular behavior, called swarming migration, in which motile vegetative rods growing on solid media differentiate into extremely elongated hyperflagellated swarm cells that undergo rapid and coordinated population migration away from the initial colony (4, 6, 7).

A transposon mutant of P. mirabilis, WT19, defective in mass migration of normally differentiated swarm cells, has been reported (8). Genetic analyses identified a lesion in a putative polysaccharide assembly locus, and electron microscopy and gel electrophoresis confirmed the specific loss of a capsular polysaccharide (CPS). This CPS, named Cmf (colony migration factor)-CPS, was suggested to facilitate population migration by enhancing medium surface fluidity and possibly influencing cell-cell interactions. The cmfA was also attenuated in experimental uropathogenicity, showing greatly reduced colonization of the urinary tract (9).

Little is known about the structures of Proteus polysaccharides, and serology indicates substantial heterogeneity, with P. mirabilis and the closely related Proteus vulgaris divided into 49 O-serogroups, and many smooth strains remain unclassified (10). Analysis of the Cmf-CPS from wild-type P. mirabilis WT19 (8) indicated that it is an acidic type II molecule rich in galacturonic acid and N-acetylgalactosamine and that it can have a phospholipid anchor. This composition showed that it must be structurally different from previously reported, functionally anomalous CPSs from P. mirabilis ATCC49565 (11) and P. vulgaris CP2-86 (12). The former was reported to consist of a branched trisaccharide repeating unit of N-acetylgalcosamine, N-acetylfucosamine, and glucuronic acid and the latter of a tetrasaccharide repeating unit of two glucosyl, one N-acetylgalactosaminosyl, and one glucuronosyl residue. To establish the nature of a functionally characterized Proteus polysaccharide and gain a view of the possible common structural features among the polysaccharides of this genus, we report the structure of the Cmf-CPS from P. mirabilis WT19.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Conditions—P. mirabilis WT19 strain U6450 (protocrine type P3/S1) (13) was isolated from a chronic urinary tract infection involving renal stone formation (13). The bacterial cells were grown overnight at 37 °C on the surface of Brilliant Green agar (BBL, Becton Dickinson, Cockeysville, MD), and colony morphology was examined the next day. After observing that this culture produced terraced, swarming colonies that extended across 50% of the agar surface, cells at the edge of the swarm colony were transferred to Brilliant Green agar again to confirm the absence of contaminants. Biochemical confirmation of this strain as Proteus was made using a commercial package of diagnostic reagents (Enterotube II, Becton-Dickinson) and was confirmed as P. mirabilis by the National Veterinary Services Laboratories (Ames, IA), although it atypically failed to ferment two sugars, maltose and xylose.

Isolation and Purification of Cmf-CPS—Bacteria were grown in BHI broth, harvested by centrifugation, and washed once in physiologically buffered saline as described by Lee and Cherniak (14). Bacterial cells (100 g, wet weight) were suspended in 900 ml of water and stirred vigorously in boiling water for 30 min. The suspension was cooled in an ice bath with stirring for 90 min. The cell residue was removed by centrifugation and washed in an ice bath with stirring for 90 min. The cell residue was removed by centrifugation.

This paper is available on line at http://www.jbc.org

22993
centrifugation (10,000 × g, 30 min, 4 °C), the supernatant adjusted to 1% acetic acid, and crude Cmf-CPS precipitated by the addition of ethanol (2.5 volumes, 24 h, −20 °C). The Cmf-CPS precipitate was collected by centrifugation (10,000 × g, 30 min, 4 °C), washed with ethanol, washed again with acetone, dried, dissolved in water, and lyophylized. This crude Cmf-CPS was suspended in a solution containing 6 ml of EDTA-phosphate (0.05 mM Na2HPO4/0.005 mM EDTA, pH 7.0), 3 mg of DNase (in 3 ml of 0.04 mM MgCl2), and 20 mg of RNase (in 3 ml of 0.04 mM MgCl2). This solution was incubated for 16 h at 37 °C followed by the addition of proteinase K (4 μg) and incubated again for 16 h at 37 °C. The resulting solution was dialyzed against distilled water for 48 h, evaporated to 5,000 × g for 30 min, and the supernatant was lyophilized. The final yield was 800 mg of crude Cmf-CPS. Crude Cmf-CPS was further purified by gel filtration column (90 × 1.6 cm) chromatography using Sephadex G-150 equilibrated with a buffer solution consisting of 0.2 mM NaCl, 1 mM EDTA, 50 mM Tris base, and 0.25% deoxycholic acid (DOC), pH 9.25. The content of each fraction was identified by polyacrylamide gel electrophoresis in the presence of DOC (DOC-PAGE) using 18% acrylamide (15). Gels were fixed in the presence or absence of Alcian blue (16) and silver-stained (17).

A fraction of the crude Cmf-CPS was also further purified by mild acid hydrolysis in aqueous 1% acetic acid at 100 °C for 2 h. After hydrolysis, the solution was cooled and centrifuged (10,000 × g). The supernatant was extracted with diethyl ether (3 × 10 ml), and the aqueous layer was fractioned on a Sephadex G-75 column (90 × 1.6 cm). The fractions were assayed for hexose with phenol-sulfuric acid. The resulting Cmf-CPS and oligosaccharide (OS) fractions were lyophilized.

**Nuclear Magnetic Resonance Spectroscopy**—Samples were prepared for NMR analysis by a 2-fold lyophilization from D2O and dissolved in 0.5 ml of D2O. Spectra were recorded at 60 °C. Chemical shifts are reported in ppm, using sodium 3-trimethylsilylpropanoate-d4 (δ4 0.00), and acetone (δ6 31.00) as internal references. All NMR spectra were recorded on Bruker AMX-500 or DRX-600 MHz spectrometers. Two-dimensional DQF-COSY (18), TOCSY (19, 20), and NOESY (21) data were acquired, with 32 and 64 scans per FID. The sweep widths in both dimensions of 6 ppm. The TOCSY experiments contained 512 (t1) complex points were collected with 16 scans per FID and a sweep width in both dimensions of 6 ppm. The TOCSYS experiments contained MLEV17 (23) mixing sequences ranging from 60 to 320 ms, and the NOESY mixing delay was 200 ms.

An gradient HSQC (24) data set was collected using the echo/anti-echo method for pure absorption data. A data set of 2048 (t2) × 512 (t1) complex points was acquired, with 32 and 64 scans per FID and a sweep width in both dimensions of 6 ppm. The TOCSYS experiments contained MLEV17 (23) mixing sequences ranging from 60 to 320 ms, and the NOESY mixing delay was 200 ms.

**Glucosyl Composition Analyses**—Glucosyl composition of Cmf-CPS was determined by GLC-MS analysis of the trimethylsilylated (S)-2-butyl and (S)-2-acetyl derivatives. The GLC-MS analyses were performed using capillary columns (length, 30 m; inner diameter, 0.32 mm) with helium as the carrier. A DB-5 column (J & W Scientific) was used for aminoglycosyl derivatives, and an SP2330 column (Supelco, Bellefonte, PA) was used for the neutral glycosyl derivatives.

**RESULTS**

**Isolation and Purification of Cmf-CPS**—Analysis of the crude Cmf-CPS by DOC-PAGE (Fig. 1) showed that the crude Cmf-CPS contained some contaminating LPS. The crude Cmf-CPS was separated from the contaminating LPS by a Sephadex G-150 column using buffer containing DOC. The DOC-PAGE analysis of the fractions collected in a fractionation and that silver-stained only after fixing the gel in the presence of Alcian blue (Fig. 1A), a property characteristic of acidic polysaccharides (32). The low molecular weight fraction silver-stained after being fixed in the presence or absence of Alcian blue, a feature that is characteristic of LPS (32). Thus, the crude Cmf-CPS fraction contained a high molecular weight Cmf-CPS and a low molecular weight LPS (a lipo-oligosaccharide, LOS). During the purification of Cmf-CPS described in the previous paragraph, the sample was subjected to alkaline conditions (pH 9.25) for an extended period of time. Thus, any O-acetyl substituents, if present, would have been removed. To purify Cmf-CPS with minimal removal of O-acetyl groups, a portion of crude Cmf-CPS (50 mg) was hydrolyzed with mild acid and purified by Sephadex G-75 column chromatography. Two fractions were obtained, the high molecular weight Cmf-CPS (50 mg) was hydrolyzed with mild acid and purified by Sephadex G-75 column chromatography. Two fractions were obtained, the high molecular weight Cmf-CPS and low molecular weight oligosaccharides (OS) derived from the LOS. The Cmf-CPS eluted just after the void volume, and the OS eluted at twice the void volume (not shown).

**FIG. 1.** DOC-PAGE analysis of the fractions from Sephadex G-150 column of the crude Cmf-CPS from *P. mirabilis* WT19. Well C contains crude Cmf-CPS prior to Sephadex G-150 column fractionation. Fractions (5 ml) were collected, of which 10-μl samples of every fraction starting with fraction 21 were applied to each well. The gel shown in Panel A was fixed in the presence of Alcian blue prior to silver-staining, whereas that shown in Panel B was fixed without Alcian blue. Fractions 31–45 were combined and comprise purified Cmf-CPS (Cmf-CPSDOC), and fractions 55–71 were combined and comprise pure low molecular weight lipopolysaccharide, i.e. the LOS.
shown). The yields of Cmf-CPS\textsubscript{G75} and OS\textsubscript{G75} were 35 and 5 mg, respectively.

**Composition Analysis**—The results of glycosyl and fatty acid composition analyses of Cmf-CPS\textsubscript{DOC}, LOS\textsubscript{DOC}, Cmf-CPS\textsubscript{G75}, and OS\textsubscript{G75} are shown in Table I. Both Cmf-CPS\textsubscript{DOC} and Cmf-CPS\textsubscript{G75} have very similar glycosyl compositions, i.e., mannuronic acid, galacturonic acid, N-acetyl glucosamine, and N-acetyl galactosamine in a molar ratio of 1:1:1:1. The LOS\textsubscript{DOC} contains glycosyl residues characteristic of LPS core oligosaccharides, i.e., glucose, galactose, mannos, L,D-heptose, and 3-OHC\textsubscript{14,0} (33). Fatty acid analysis showed that neither of the Cmf-CPS fractions contained detectable fatty acyl residues, whereas the LOS\textsubscript{DOC} contained myristic, palmitic, and \(\text{Cmf-CPS} \text{G75}\) fractions contained detectable fatty acyl residues, ppm. The 1\(H\) NMR spectrum of \(\text{Cmf-CPS} \text{G75}\) fraction (data not shown) analyses, most of the 1\(H\) and 13\(C\) NMR signals could be assigned (Tables III and IV). The four glycosyl residues were designated A–D according to their decreasing anomeric chemical shifts.

Residue A has an anomeric signal at 5.37 and a \(J_{\text{H-1,H-2}}\) coupling constant of 3 Hz, indicating that it is an \(\alpha\)-linked residue. The H-1 to H-5 proton signals (Table III) for residue A were assigned from the COSY and TOCSY (Fig. 3A) spectra. A large \(J_{\text{H-3,H-4}}\) coupling constant (>5 Hz) was observed for A, supporting the conclusion it has a glucosyl configuration. The carbon signals (Table IV) from C-1 to C-5 for residue A were determined from the HSQC spectrum. The C-2 chemical shift of residue A is 54.0, typical of a nitrogen bearing carbon. The downfield position of the C-3 carbon signal (δ 82.2) indicates that residue A is substituted at this position. Thus, A is the 3-linked N-acetylglucosaminyl residue. The carbon chemical shifts from C-1 to C-5 for residue A (Table IV) are also similar to those previously reported for a 3-linked \(\alpha\)-N-acetylglucosaminyl residue (34).

### Table I

**Glycosyl linkage analysis of Cmf-CPS\textsubscript{DOC} from P. mirabilis WT19**

| Glycosyl Residue | Linkage | Cmf-CPS\textsubscript{DOC} | C\textsuperscript{CR}Cmf-CPS\textsubscript{DOC} |
|------------------|---------|-----------------------------|----------------------------------|
| Man              | 3,6-linked | --                          | 22\(a\)                           |
| Glc              | 6-linked  | --                          | 25\(a\)                           |
| Gal              | 3-linked  | 57                          | 30                               |
| GalNAc           | 3,4-linked | 43                          | 23                               |

\(a\) These values are for the partially methylated alditol acetates from carboxyl-reduced (CR) Cmf-CPS\textsubscript{DOC}. The mass spectra of these partially methylated alditol acetates show that they both have two deuterium atoms at C-6 and indicate that they were derived from the 3-linked mannosyl residue and from terminally linked galacturonic acid present in the Cmf-CPS.

**Fig. 2. The proton NMR spectrum of P. mirabilis WT19 Cmf-CPS\textsubscript{DOC}**
The anomic signal for residue B is δ 5.17 (J_{1H,1H} not resolved), showing that it is α-linked. The proton chemical shifts (Table III) from H-1 to H-5 protons were assigned from COSY (spectrum not shown) and TOCSY (Fig. 3A) spectra. A relatively small J_{1H,1H} coupling constant (<5 Hz) for residue B indicates that it has a galacto configuration. The carbon chemical shifts (Table IV) from C-1 to C-5 for residue B were assigned from HSQC spectrum. The C-2 chemical shift of residue B is δ 50.5, typical of a nitrogen bearing carbon. The downfield shift of C-3 (δ 76.6) and C-4 (δ 79.1) indicates that residue B is substituted at C-3 and C-4. Glycosyl linkage analysis (Table II) showed that N-acetylgalactosamine is the only 3,4-linked aminoglycosyl residue found in the Cmf-CPS. Therefore, residue B is the 3,4-linked-α-N-acetylgalactosaminosyl residue.

Residue C has an anomic proton chemical shift at δ 5.00, (J_{1H,1H} not resolved) indicating that it is α-linked. The proton chemical shifts (Table III) from H-1 to H-5 for residue C were assigned from the COSY and TOCSY (Fig. 3A) spectra. A small J_{1H,1H} coupling constant (<5 Hz), indicates that the residue C has a mannose configuration. The carbon chemical shifts (Table IV) from C-1 to C-5 were assigned from HSQC spectrum. The downfield chemical shift of C-3 (δ 76.5), indicates that residue C is substituted at this position. Glycosyl linkage analysis (Table II) showed only one 3-linked mannuronosyl residue in the Cmf-CPS. Thus, residue C is the 3-linked mannuronosyl residue.

The anomic proton chemical shift for residue D is δ 4.79 (J_{1H,1H} 7 Hz) indicating that it is β-linked. The proton chemical shifts (Table III) from H-1 to H-5 for residue D were assigned from the COSY and TOCSY (Fig. 3A) spectra. The J_{1H,1H} coupling constant for residue D is similar to that for residue B (i.e., < 5 Hz), indicating that it has a galacto configuration. The carbon chemical shifts (Table IV) from C-1 to C-5 for residue D were determined from the HSQC spectrum. There is no downfield chemical shift for any carbon of residue D, indicating that it is not substituted. The only terminally linked hexosyl residue observed in the glycosyl linkage analysis of the Cmf-CPS (Table II) was galacturonic acid. Thus, residue D is the terminally linked β-galacturonic acid residue.

The sequence of glycosyl residues was determined from a NOESY experiment (Fig. 3B and Table V). In addition to intra-residue NOE contacts to H-2 and H-3, residue A has a NOE contact from H-1 to H-3 of residue C. Because residue C is 3-linked α-D-mannuronic acid, the sequence shown in Structure 1 was established.

Residue C has a strong NOE contact from H-1 to H-4 of residue B (in addition to intra-residue contacts to H-2 and H-4) indicating that residue C is linked to the 4-position of residue B. Thus, the trisaccharide element A-C-B was established, as shown in Structure 2.

Residue D has a strong NOE contact of H-1 to H-3 of residue B and a weak contact to H-4 of residue B. Because residue C is

| Glycosyl residue | Chemical shifts\(^a\) | Glycosyl residue | Chemical shifts |
|------------------|----------------------|------------------|----------------|
|                  | H-1 | H-2 | H-3 | H-4 | H-5 | H-6a | H-6b |
| A                | 5.37 [3] | 4.08 | 4.07 | 4.46 | 4.04 | 3.72\(^b\) | 3.94\(^b\) |
| B                | 5.17 [nr] | 4.59 | 4.29 | 4.34 | 4.43 | 3.72\(^b\) | 3.88\(^b\) |
| C                | 5.00 [nr] | 4.05 | 3.80 | 3.76 | 4.38 |
| D                | 4.79 [7] | 3.52 | 3.59 | 4.38 | 4.32 |

\(^a\) J_{1H,2} values are given in square brackets. nr, not resolved. \(^b\) These assignments may be interchanged.

**Table III**

1H NMR data at 60 °C for the Cmf-CPS\(_{DGC}\) isolated from *P. mirabilis* WT19

**Table IV**

13C NMR data at 60 °C for the Cmf-CPS\(_{DGC}\) isolated from *P. mirabilis* WT19

---

*Fig. 3.* The 1H-1H TOCSY (A) and 1H-1H NOESY (B) spectra of *P. mirabilis* WT19 Cmf-CPS\(_{DGC}\). The signals labeled in bold type on the NOESY spectrum indicate the strong inter-residue NOE contacts from which the glycosyl sequence was deduced. The mixing time for the TOCSY spectrum shown was 120 ms. Complete assignment required several TOCSY experiments requiring several mixing times ranging from 60 to 320 ms. The spectra for these other experiments are not shown.

---

**Structure 1**

→ 3)-α-D-GlepNAc-(1 → 3)-α-D-ManpA-(1 →

A C

**Structure 2**

→ 3)-α-D-GlepNAc-(1 → 3)-α-D-ManpA-(1 → 4)-α-D-GalpNAc-(1 →

A C B

---

**Structure 1**

Residue C has a strong NOE contact from H-1 to H-4 of residue B (in addition to intra-residue contacts to H-2 and H-4) indicating that residue C is linked to the 4-position of residue B. Thus, the trisaccharide element A-C-B was established, as shown in Structure 2.

**Structure 2**

Residue D has a strong NOE contact of H-1 to H-3 of residue B and a weak contact to H-4 of residue B. Because residue C is
TABLE V

| Anomeric proton | NOE contact to proton | Intensity | Assignment |
|-----------------|-----------------------|-----------|------------|
| $\beta$-GlcNAc-(1→3)$\alpha$-ManpA-(1→4)-GlcNAc-1 | A H-2 | 3.60 m | C-H-3 |
| $\beta$-GlcNAc-1 | B H-4 | 4.36 | A H-3 |
| $\beta$-GlcNAc-1 | C H-2 | 4.05 m | B H-3 |
| $\beta$-GlcNAc-1 | D H-3 | 4.56 m | C H-3 |
| $\beta$-GlcNAc-1 | D H-4 | 4.32 m | D H-5 |

* a The intensities are estimated from visual inspection of the NOESY spectrum shown in Fig. 3.

linked to the 4-position of residue B, residue D must be linked to the 3-position of residue B. Thus, the tetrasaccharide structure -A-C-B(D)- was established, as shown in Structure 3.

$$\rightarrow\beta\text{-GalpA}$$

Residue B has a strong NOE contact from H-1 to H-3 of residue A, indicating that residue B is linked to the 3-position of residue A. Thus, the P. mirabilis WT19 Cmf-CPS consists of a tetrasaccharide repeating unit, as shown in Structure 4.

$$\rightarrow\beta\text{-GalpA}$$

EXTRACELLULAR POLYSACCHARIDES

Extracellular polysaccharides are central to bacterial survival, particularly against the immune defenses of the mammalian host. In uropathogenic P. mirabilis, the Cmf capsular polysaccharide has also been shown to facilitate the rapid multicellular migration of elongated hyperflagellated swarm cells, which correlates with the ability to establish experimental ascending colonization of the urinary tract and may be coupled to the formation of biofilms (7). Proteus migration requires close cell-cell contact, with swarm cells aligning along their long axes in multicellular rafts. The Cmf-CPS may provide a matrix for surface migration of the swarm cell rafts (35), stabilizing cell-cell contact and facilitating intercellular communication (8, 35). In addition, the acidic CPS is thought to act as lubricant, creating a fluid environment through which Proteus can swarm by extracting water from the agar medium beneath the colony (4, 8). This latter hypothesis is supported by the observation that increased agar concentration or reduced polysaccharide biosynthesis, both of which result in a lowered agar/capsular polysaccharide osmotic activity ratio, reduce migration velocity but do not inhibit differentiation (4, 8). Surface active agents are produced by other bacteria that undergo population migration cell rafts, e.g. the unrelated Myxococcus produces an extracellular slime during fruiting body development (36).

Increasing the understanding of the apparently multiple roles of Proteus polysaccharides in colony expansion and virulence requires a knowledge of their structures. Including the Cmf-CPS structure of this report, three Proteus CPS structures have been described in the literature and are shown in Fig. 4. Although these three structures are quite different from one another, they have two general similarities. First, all three structures are acidic in that they all contain at least one uronosyl residue; the CPS from P. mirabilis ATCC49565 has a branching terminal linked $\alpha$-d-glucuronosyl residue, the P. vulgaris CPS contains a 4-linked $\alpha$-d-glucuronosyl residue, and the P. mirabilis WT19 CPS contains 3-linked $\alpha$-d-mannuronosyl and branching terminal $\beta$-d-galacturonosyl residues. Second, all three structures have amino sugars; P. mirabilis ATCC59565 CPS contains both N-acetylgalactosamine and N-acetylfucosamine, P. vulgaris CP2-96 CPS contains N-acetylgalactosamine, and P. mirabilis WT19 CPS contains both N-acetylgalactosamine and N-acetylgalactosamine. Understanding the molecular basis by which these acidic CPSs facilitate Proteus swarming will require further investigation.

Acknowledgment—The authors thank Dr. John Gluska for assistance in obtaining the two-dimensional NMR spectra.

REFERENCES

1. Kotelko, K. (1996) *Curr. Top. Microbiol. Immunol.* 129, 181–215.
2. Rubin, E., Tolkoff-Rubin, N. E., and Cotran, R. S. (1986) in *The Kidney* (Brenner, B. M., and Rector, F. C., eds) pp. 1085–1141, W. B. Saunders Co., Philadelphia.
3. Ehringer, A., Khalafour, S., and Wilson, C. (1989) *Rheumatol. Int.* 9, 223–228.
4. Rauprich, O., Matsuzaki, M., Weiger, C. J., Siegert, F., Eiclov, E. S., and Shapiro, J. A. (1996) *J. Bacteriol.* 178, 6525–6538.
5. Stickler, D. J., Glanderon, L., King, J. B., Nettleton, J., and Winters, C. (1993) *J. Urol.* 21, 407–411.
6. Allison, C., and Hughes, C. (1991) *Sci. Prog. Edinburgh* 75, 403–422.
7. Fraser, G. M., Furchner, R. H., and Hughes, C. (1999) in *Prokaryotic Development* (Shimkets, L., and Young, Y., eds) American Society for Microbiology, Washington, D. C., in press.
8. Gugy, D., Rahman, M. M., Lai, H.-C., Carlson, R., Guard-Petter, J., and Hughes, C. (1995) *Microb. Biol.* 17, 1167–1175.
9. Allison, A., Emoely, L., Coleman, N., and Hughes, C. (1994) *J. Infect. Dis.* 169, 1155–1158.
10. Larsson, P. (1984) in *Methods Microbiol.* 14, 187–214.
11. Beynon, L. M., Dumas, A., J. M. McLean, J. C., MacLean, L. L., Richards, J. C., and Perry, M. B. (1992) *J. Bacteriol.* 174, 2172–2177.
12. Rahman, M., Guard-Petter, J., Asookan, K., and Carlson, R. W. (1997) *Carbohydr. Res.* 301, 613–620.
13. Allison, C., and Hughes, C. (1991) in *Microb. Biol.* 17, 1955–1962.
14. Lee, L., and Cherwink, R. (1974) *Infect. Immun.* 9, 318–322.
15. Komuro, T., and Galanos, C. (1988) *J. Chromatogr.* 450, 381–387.
16. Corse, J., Perez-Galdona, R., Leon-Barrios, M., and Gutierrez-Navarro, A. M. (1991) *Ectrophoresis* 12, 439–441.
17. Tsai, C., and Friesch, C. E. (1982) *Anal. Biochem.* 119, 115–119.
18. Pianitini, U., Sorensen, O. W., and Ernst, R. R. (1982) *J. Am. Chem. Soc.* 104, 22997.
22998

P. mirabilis Capsular Polysaccharides

6800–6801
19. Braunschweiler, L., and Ernst, R. R. (1983) J. Magn. Reson. 53, 521–528
20. Bax, A., and Davis, D. G. (1985) J. Magn. Reson. 65, 355–360
21. Wider, G., Macura, S., Kumar, A., Ernst, R. R., and Wuthrich, K. (1984) J. Magn. Reson. 56, 207–213
22. Marion, D., Ikura, M., Tschudin, R., and Bax, A. (1989) J. Magn. Reson. 85, 383–399
23. Davis, D. G., and Bax, A. (1985) J. Am. Chem. Soc. 107, 2820–2821
24. Davis, A. L., Keeler, J., Laue, E. D., and Moskau, D. (1992) J. Magn. Reson. 96, 207–216
25. Shaka, A. J., Barker, P. B., and Freeman, R. (1985) J. Magn. Reson. 64, 547–552
26. York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T., and Albersheim, P. (1985) Methods Enzymol. 118, 3–40
27. Gerwig, G. J., Kamerling, J. P., and Vliegenthart, J. F. G. (1978) Carbohydr. Res. 62, 349–357
28. Gerwig, G. J., Kamerling, J. P., and Vliegenthart, J. F. G. (1979) Carbohydr. Res. 77, 1–7
29. Ciucanu, I., and Kerek, F. (1984) Carbohydr. Res. 131, 209–217
30. McConville, M. J., Homans, S. W., Thomas-Oates, J. E., Dill, A., and Bacic, A. (1990) J. Biol. Chem. 265, 7385–7390
31. Waeghe, T. J., Darvill, A. G., McNeil, M., and Albersheim, P. (1983) Carbohydr. Res. 123, 281–304
32. Kim, J. S., Rohl, B. I., Rahman, M. M., Ridley, B., and Carlson, R. W. (1996) Glycobiology 5, 433–437
33. Holst, O., and Brade, H. (1994) Mol. Biochem. Cell Biol. 1, 135–170
34. Bock, K., Pedersen, C., and Pedersen, H. (1984) Adv. Carbohydr. Chem. Biochem. 42, 193–225
35. Stahl, S. J., Stewart, K. R., and Williams, F. D. (1983) J. Bacteriol. 154, 930–937
36. Dworkin, M. (1996) Microbiol. Rev. 60, 79–102