JOURNAL OF BACTERIOLOGY, Apr. 1992, p. 2172–2177
0021-9193/92/072172-06$02.00/0

Vol. 174, No. 7

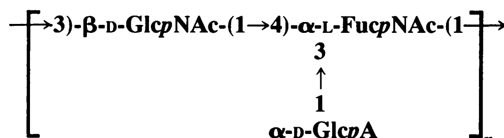
Capsule Structure of *Proteus mirabilis* (ATCC 49565)

LINDA M. BEYNON,[1] ANITA J. DUMANSKI,[2] ROBERT J. C. McLEAN,[2,3] LEANN L. MacLEAN,[1] JAMES C. RICHARDS,[1] AND MALCOLM B. PERRY[1]*

Institute for Biological Sciences, National Research Council, Ottawa, Ontario, Canada K1A OR6,[1] and Department of Microbiology and Immunology[2] and Department of Urology,[3] Queen's University, Kingston, Ontario, Canada K7L 3N6

Received 21 October 1991/Accepted 21 January 1992

Proteus mirabilis **2573 (ATCC 49565) produces an acidic capsular polysaccharide which was shown from glycose analysis, carboxyl reduction, methylation, periodate oxidation, and the application of one dimensional and two-dimensional high-resolution nuclear magnetic resonance techniques to be a high-molecular-weight polymer of branched trisaccharide units composed of 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine), 2-acetamido-2,6-dideoxy-L-galactose (*N*-acetyl-L-fucosamine), and D-glucuronic acid, having the structure:**



P. mirabilis **2573 also produces an O:6 serotype lipopolysaccharide in which the O-chain component has the same structure as the homologous capsular polysaccharide. This is the first report of a defined capsular polysaccharide in this bacterial genus.**

Proteus mirabilis is of great interest to the medical and scientific communities because it is implicated in urinary tract infections and the production of infectious urinary stones. These calculi generally contain struvite ($MgNH_4PO_4 \cdot 6H_2O$) or carbonate-apatite [$Ca_{10}(PO_4)_6CO_3$] crystals (22, 23). While urease activity is a major virulence determinant for this organism (14), this is not cause enough for stone formation. Capsules, frequently associated with pathogenic *Proteus* strains, had been hypothesized to be a potential virulence factor in that they may accelerate struvite crystal growth (7, 22) and aggregate urine-precipitated components to form a stone (23). Complete characterization of capsules may lead to a better understanding of their role in the production of these urinary calculi.

P. mirabilis 2573, which was used in this project, was deposited in the American Type Culture Collection as strain ATCC 49565. Serotyping by passive hemagglutination (25) (courtesy of J. L. Penner, Department of Microbiology, University of Toronto) identified it as O:6; the structural characterization of this antigen has not been reported. Although morphological evidence of the *P. mirabilis* capsule has been previously reported (13, 28), no biochemical characterization has been conducted. In this article, we report the complete chemical structure of the *P. mirabilis* capsule. This represents the first report of a capsule structure for the genus *Proteus*.

MATERIALS AND METHODS

Bacterial growth and PS isolation. *P. mirabilis* (ATCC 49565) was grown on tryptic soy agar supplemented with phenylalanine and D-xylose. Polysaccharide (PS) production under these conditions was verified by electron microscopy (19). After 20 h at 37°C, the cells were harvested in 1% phenol solution and were collected by low-speed centrifugation. The cells (139 g, wet weight) were extracted with 50% (wt/vol) aqueous phenol (10 min, 65°C), and after the dialyzed phenol-free separated (4°C) aqueous phase of the extract was lyophilized, it was digested sequentially with ribonuclease, deoxyribonuclease, and protease K (16). The aqueous solution of this dialyzed digest was subjected to ultracentrifugation (105,000 × *g*, 12 h, 4°C), and the precipitated gel was dissolved in water and lyophilized to give lipopolysaccharide (LPS) (yield, 580 mg).

The addition of ethanol (6 volumes) to the ultracentrifugate afforded crude PS (1.7 g) which was dissolved in water (75 ml) and treated with 1% (wt/vol) aqueous cetyltrimethylammonium chloride. The fine white precipitate was removed by centrifugation, a further portion of 1% cetyltrimethylammonium chloride (75 ml) was added, and the resulting precipitate was collected by centrifugation. This second precipitate was dissolved in 10% (wt/vol) sodium chloride solution, and the precipitate which was obtained with the stirred addition of ethanol (5 volumes) was collected, dialyzed, and lyophilized. Pure PS (142 mg) was obtained by Sephadex G-50 gel filtration of this latter material, by which it eluted at the void volume of the system.

Analytical methods. Quantitative colorimetric methods used were the modified Elson-Morgan reaction for 2-amino-2-deoxyhexoses (9), the phenol-sulfuric acid method for neutral glycoses (8), and the *p*-hydroxydiphenyl method for hexuronic acid (5). For analysis of constituent glycoses, samples (1 mg) were hydrolyzed with 6 M HCl (0.5 ml, 3 h, 100°C). Glycoses were determined by gas-liquid chromatography-mass spectrometry (GLC-MS) of their derived alditol acetates (15) and of their trimethylsilylated, derived 2-(−)-butyl glycosides (10).

Analytical GLC-MS was done by using a Hewlett-Packard 5885B system fitted with a flame ionization detector and an

* Corresponding author.

OV-17 fused silica column (Quadrex Corp.). The following programs were used: for alditol acetates, 180°C for 2 min and then 4°C/min to 240°C; for partially methylated alditol acetates, 180°C for 2 min and then 2°C/min to 240°C.

Methylation analyses (6). Samples (2 mg) were dissolved in dimethylsulfoxide (0.2 ml). Powdered NaOH (20 to 30 mg) and iodomethane (0.2 ml) were added, and the mixture was stirred for 15 min at 20°C. The methylated products were partitioned between dichloromethane and water (five times). Methylated and methylated and reduced samples were hydrolyzed with 4 M trifluoroacetic acid (0.5 ml) for 1 h at 125°C, and the products were analyzed by GLC-MS of their alditol derivatives.

For carboxyl group reductions, $NaBD_4$ (10 to 15 mg) was added to samples of methylated PS (2 mg) in dry tetrahydrofuran (1 ml), and the mixture was stirred for 15 h at 50°C. The mixture was diluted with water (1 ml), neutralized with 10% acetic acid, and deionized by passage through a column of Rexyn 101(H^+) and Duolite A4(OH^-) ion-exchange resins. The eluant and MeOH-H_2O (1:1) washings were concentrated to dryness, the residue was evaporated with MeOH (4 times with 2 ml), and the product was recovered by extraction with dichloromethane.

Periodate oxidation and Smith degradation (12). Capsular PS (25 mg) in water (10 ml) was treated with $NaIO_4$ (0.2 g), and, after 19 h at 20°C, ethylene glycol (0.5 ml) and then $NaBH_4$ (0.4 g) were added and the mixture was stirred for 12 h. The mixture was neutralized with 30% acetic acid, and the product was desalted on a Sephadex G-50 column (65 by 2 cm). The collected void volume product was treated with 2% acetic acid for 2 h at 100°C, and the mixture was dialyzed and lyophilized to yield the degraded PS (17 mg).

Carboxyl reduction of PS (29). Native PS (30 mg) in water (10 ml) was adjusted to pH 4.7 with 0.05 M HCl, and, after the addition of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (120 mg), the stirred mixture was maintained at pH 4.7 for 4 h by the addition of 0.05 M HCl. Sodium borodeuteride (150 mg) was added, and the mixture was stirred for 15 h at 20°C. The mixture was brought to pH 7 by 30% acetic acid, and, after extensive dialysis, the reduced PS (25 mg) was obtained by lyophilization.

Nuclear magnetic resonance (NMR) spectroscopy. Spectra were obtained with solutions in D_2O at 27 or 67°C by using a Bruker AMX-600 or AMX-500 spectrometer, each fitted with a 5-mm inverse broadband probe, using Bruker reverse electronics.

Proton spectra were obtained by using a spectral width of 2.2 kHz at a 16K data block and a 90° pulse. Chemical shifts are expressed relative to internal acetone (2.225 ppm).

Broadband proton-decoupled ^{13}C spectra were obtained at 125 MHz with a spectral width of 33 kHz by using a 32K data set and a 90° pulse employing WALTZ decoupling (27).

Two-dimensional correlated spectroscopy (COSY) and nuclear Overhauser effect spectroscopy (NOESY) experiments were carried out as previously described (4), and the data were processed to give either magnitude (2) or phase-sensitive (17, 18) spectra.

Heteronuclear two-dimensional ^1H-^{13}C chemical shift correlation experiments were obtained by ^1H detection via multiple-quantum coherence (3) in the phase-sensitive mode. In a typical experiment, a data matrix of 256 × 1,024 complex points was employed and 116 scans were acquired for each t_1 value. Sweep widths of 12,631 Hz and 2,513 kHz were used in F_1 and F_2, respectively. The data were zero filled to give 512 × 1,024 real points, and squared sine-bell window functions shifted 60° were applied in both dimensions. ^{13}C decoupling was achieved by using the GARP-1 composite pulse sequence during ^1H acquisition (26).

RESULTS AND DISCUSSION

P. mirabilis (ATCC 49565) cells grown on tryptic soy agar supplemented with phenylalanine and D-xylose (22) were collected and extracted with hot aqueous phenol, and the separated water phase after digestion with ribonuclease, deoxyribonuclease, and protease K (16) was subjected to ultracentrifugation. The precipitated gel was dissolved in water and lyophilized to give LPS (yield, ca. 4.2% on the basis of dry cell weight).

The addition of ethanol to the supernatant gave a precipitate of crude bacterial PS, from which purified PS was obtained via its insoluble cetyltrimethylammonium salt complex. This material was further purified by Sephadex G-50 gel filtration chromatography to give a final pure PS as a fraction eluting at the void volume of the system. This PS was homogeneous with respect to hexuronic acid and 2-amino-2-deoxyglycose components.

The PS had $[\alpha]_D$ −75° (c 1.4, water) and elemental analysis: C, 39.59; H, 5.40; N, 3.84; ash, 3.0%. Reduction of the carboxylic acid function in the PS by the borohydride-carbodiimide (29) method gave a neutral PS (88% yield) which had $[\alpha]_D$ −80° (c 1.1, water). Hydrolysis of the reduced ($NaBD_4$) native PS followed by GLC-MS analysis of the reduced and acetylated product showed it to be composed of 2-amino-2-deoxy-D-glucose (D-GlcNH_2), 2-amino-2-deoxy-L-fucose (L-FucNH_2), and D-glucose-6-d,d (D-Glc) (1:1:1). The configurations of the glycoses were determined by capillary GLC of their trimethylsilylated (R)-2-butyl glycoside derivatives (10), and their identities were confirmed from the unique GLC retention times (15) and mass spectra of their alditol acetate derivatives compared with those of authentic samples.

Methylation (6) of the native and carboxyl reduced PS followed by GLC-MS analysis of the derived alditol acetates of the methylated glycoses obtained by acid hydrolysis (Table 1) showed the PS to be composed of a repeating trisaccharide unit containing the structural residues D-Gal*p*A-(1→, →3,4)-L-Fuc*p*NAc-(1→, and →3)-D-Glc*p*NAc-(1→.

Periodate oxidation of the native PS followed by reduction ($NaBH_4$) and Smith-type hydrolysis (12) of the product resulted in the removal of the nonreducing D-Glc*p*A end-groups, leaving a high-molecular-weight polymer which was isolated as the void volume fraction from Sephadex G-50 gel filtration chromatography. This 2-deoxy-2-acetamidogly-

TABLE 1. Methylation analysis data for *P. mirabilis* PS and its polymeric derivatives

Methylated[a] glycose (as alditol acetate derivative)	T_{GM}[b]	Molar ratio[c]			
		PS	Reduced methylated PS	PS backbone (AG)	Reduced PS
2,3,4,6-Glc	0.7				1.3
2,3,4-Glc	1.0		1.3		
3-FucNHMe	1.5			1.3	
FucNHMe	1.7	1.3	2.0		1.2
4,6-GlcNHMe	2.1	1.0	1.0	1.0	1.0

[a] 2,3,4,6-Glc represents 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.
[b] Retention time relative to 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol.
[c] Molar ratios quoted relative to 4,6-GlcNHMe = 1.0.

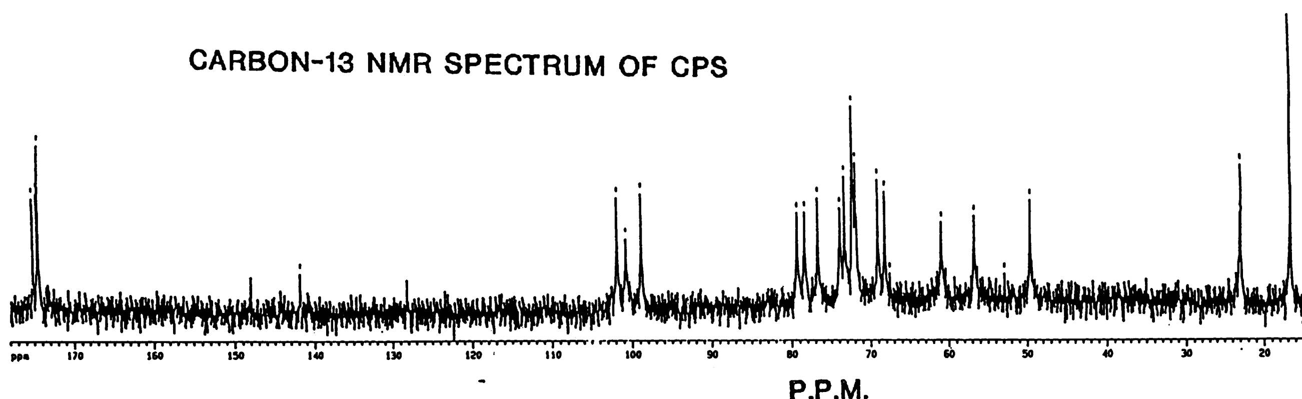


FIG. 1. ^{13}C NMR spectrum of the capsular PS (CPS) from *P. mirabilis* (ATCC 49565).

can (AG), composed of D-GlcNAc and L-FucNAc (1:1), on methylation analysis (Table 1) afforded 2-deoxy-4,6-di-*O*-methyl-2-(*N*-methylamino)-D-glucose and 2-deoxy-3-*O*-methyl-2-(*N*-methylamino)-L-fucose (1:1). The isolation of the 3-*O*-methyl L-fucosamine derivative indicates that it is substituted by the D-Glc*p*NAc at its O-4 position in the AG polymer and shows that in the native PS, the D-Glc*p*A residues were exclusively linked as single residues to the O-3 positions of the L-Fuc*p*NAc branch points.

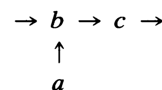
Confirmation of the trisaccharide nature of the repeating unit, glycosidic linkage positions, and a determination of anomeric configurations in the PS was made by one- and two-dimensional ^{1}H and ^{13}C NMR spectroscopy. The one-dimensional ^{13}C NMR spectrum (125 mHz) (Fig. 1 and Table 2) of the PS showed three anomeric carbon signals (99.0, 100.9, and 101.8 ppm), three carbonyl signals (174 to 175 ppm), two signals (49.6 and 56.9 ppm) indicative of C-2 carbon resonances of 2-acetamido-2-deoxyglycoses, two overlapping signals (23.08 ppm) from methyl carbons of *N*-acetyl functions, and one methyl carbon signal (16.7 ppm) indicative of a 6-deoxyhexose residue, results consistent with the recorded chemical analyses.

The ^{1}H NMR spectrum (500 MHz) of the PS showed three anomeric proton signals at 5.50 (d, 1H, $J_{1,2}$ = 2.0 Hz), 4.95 (d, 1H, $J_{1,2}$ = 3.2 Hz), and 4.68 ppm (d, 1H, $J_{1,2}$ = 7.9 Hz). Consideration of the H-1 coupling constants indicated that two of the glycopyranosyl residues have the α configuration and one residue has the β configuration. The spectrum of the PS backbone polymer (AG) showed two anomeric proton signals, one at 4.98 (d, 1H, $J_{1,2}$ = 3.2 Hz) and the other at 4.54 ppm (d, 1H, $J_{1,2}$ = 7.7 Hz), showing that one residue has the α configuration and the other has the β configuration. This evidence leads to the hexuronic acid end group in the PS being characterized as an α-D-Glc*p*A residue.

COSY NMR experiments on the native PS and the Smith product AG allowed the assignment of anomeric and ring proton signals (Table 3). The assignment of the ^{13}C resonances was then made by direct correlation with the ^{1}H resonances by heteronuclear ^{13}C-^{1}H chemical shift correlation (Table 2).

The anomeric proton signals in the COSY of the native PS were labelled *a* to *c* in order of decreasing chemical shifts. Residues *a* and *c* could be identified as pyranosyl units having the *gluco* configuration from the observed large values of the ring proton coupling constants (Table 3) (1). Direct correlation of the resonance H-2*c* (3.97 ppm) to the corresponding ^{13}C resonance (56.9 ppm) permitted *c* to be identified as the Glc*p*NAc residue as indicated by chemical analysis. Thus, *a* was identified as the GlcpA residue, and the absence of signals due to this residue in the polymer AG confirms this assignment. Similarly, *b* could be identified as the Fuc*p*NAc residue since the high field signal at 1.20 ppm for methyl protons exhibited a cross-peak, relating it to H-5*b* (4.39 ppm).

The sequence and linkage positions of the glycoses within the repeating unit of the native PS were established by a NOESY experiment (Fig. 2). Transglycosidic nuclear Overhauser effects establish connectivities between anomeric and aglyconic protons on adjacent glycosyl residues. The occurrence of a strong interresidue nuclear Overhauser effect between H-1*a*/H-3*b*, H-1*b*/H-3*c*, and H-1*c*/H-4*b* establishes the sequence of sugars in the repeating unit as



Furthermore, nuclear Overhauser effects relating the anomeric proton to other protons within the same ring system identified the configurations at the anomeric centers. The

TABLE 2. ^{13}C NMR data for the *P. mirabilis* PS and backbone polymer (AG)[a]

PS	Residue *a*, α-D-Glc*p*A-(1→						Residue *b*, →4)-α-L-Fuc*p*NAc-(1→						Residue *c*, →3)-β-D-Glc*p*NAc-(1→					
	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6
PS[b]	100.9	72.1	73.6	72.5	73.6	174.8	99.0	49.6	74.7	79.0	68.4	16.7	101.8	56.9	79.5	69.5	76.9	61.5
AG[c]							98.7	50.9	68.2	81.3	67.9	16.2	102.5	56.5	79.2	69.1	76.6	61.2

[a] Chemical shifts quoted in parts per million relative to internal acetone (δ 31.07).
[b] Measured at 67°C.
[c] Measured at 27°C.

NOESY SPECTRUM OF P. MIRABILIS CPS

H-1c H-4b
H-3c
H-1b
H-1a H-3b
P.P.M.

FIG. 2. Contour plot of the ^{1}H NMR spectral region 5.22 to 3.32 ppm of the NOESY spectrum of the capsular PS (CPS) from *P. mirabilis* (ATCC 49565). Cross-peaks arising from nuclear Overhauser effects involving anomeric protons are indicated.

occurrence of intraresidue nuclear Overhauser effects only between H-1 and H-2 of *a* and *b* showed both residues to have an α configuration, whereas intraresidue nuclear Overhauser effects between H-1 and H-3 and H-1 and H-5 of residue *c* were diagnostic of a β configuration.

From the combined chemical and NMR evidence it is concluded that the PS produced by *P. mirabilis* (ATCC 49565) is a high-molecular-weight polymer of a repeating trisaccharide unit having the structure

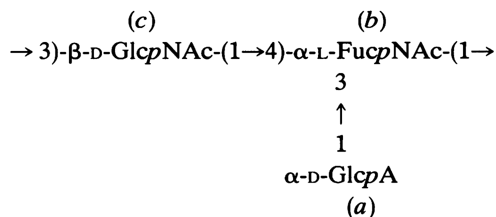


TABLE 3. ^{1}H NMR data for *P. mirabilis* PS and backbone polymer (AG)[a]

PS	Residue *a*, α-D-Glc*p*A-(1→					Residue *b*, →4)-α-L-Fuc*p*NAc-(1→						Residue *c*, →3)-β-D-Glc*p*NAc-(1→						
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5	H-6 ($J_{5,6}$)	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5	H-6 ($J_{5,6}$)	H-6′ ($J_{5,6'}$) ($J_{6,6'}$)
PS[b]	5.50 (2.0)	3.52 (9.3)	3.74 (9.3)	3.58 (8.9)	4.25	4.95 (3.2)	4.50 (10.2)	4.04 (—)[c]	4.06 (—)	4.39	1.20 (7.5)	4.68 (7.9)	3.97 (9.5)	3.69 (8.9)	3.54 (9.5)	3.41	3.99 (3.9)	3.80 (4.3) (11.8)
AG[d]						4.98 (3.2)	4.12 (10.1)	3.88 (—)	3.97 (—)	4.43	1.17 (6.4)	4.54 (7.7)	3.96 (9.6)	3.64 (9.0)	3.55 (9.6)	3.47	3.89 (—)	3.74 (6.4) (12.8)

[a] Chemical shifts are quoted in parts per million relative to internal acetone (δ 2.225), and *J* values (subscripts) are given in hertz.
[b] Measured at 67°C.
[c] —, Unresolved.
[d] Measured at 27°C.

It is of note that the PS has the same structure as the O chain of the S-type LPS produced by the same organism; however, since the analysis of the PS did not show it to contain aldoheptose or 3-deoxy-D-*manno*-octulosonic acid, components characteristic of *Proteus* LPS core oligosaccharide, the PS is not considered a product related to LPS synthesis or degradation. This unusual feature has previously been found in the polymers produced by *Escherichia coli* O111 (11).

It is interesting to speculate that the acidic nature of this PS may play a role in urinary stone formation (7, 22) in a fashion similar to the established roles of acidic proteins and glycoproteins during mineralization and control of crystal formation in biological tissues (20, 21). We are currently exploring this possibility and are identifying acidic glycans produced in other bacterial strains associated with urinary stone formation.

ACKNOWLEDGMENTS

This project was partially supported by an operating grant from the Natural Sciences and Engineering Council of Canada to R.J.C.M. A.J.D. is supported in part by a scholarship from the Faculty of Medicine, Queen's University. R.J.C.M. is supported by an Ontario Ministry of Health Career Scientist Fellowship.

We express our appreciation to J. L. Penner, University of Toronto, for serotyping the *P. mirabilis* strain. F. Cooper provided GLC-MS analyses, and large-scale cell production was carried out by D. W. Griffith.

REFERENCES

1. **Altona, C., and C. A. G. Haasnoot.** 1980. Prediction of *anti* and *gauche* vicinal proton-proton coupling constants in carbohydrates: a similar additivity rule for pyranose rings. Org. Magn. Reson. **13:**417–429.
2. **Bax, A., R. Freeman, and G. Morris.** 1981. Correlation of proton chemical shifts by two-dimensional Fourier transform NMR. J. Magn. Reson. **42:**164–168.
3. **Bax, A., R. H. Griffey, and B. L. Hawkins.** 1983. Correlation of proton and nitrogen-15 chemical shifts in multiple quantum NMR. J. Magn. Reson. **55:**301–315.
4. **Beynon, L. M., J. C. Richards, and M. B. Perry.** 1991. Structure of the capsular polysaccharide from *Actinobacillus pleuropneumoniae* serotype 7. Carbohydr. Res. **209:**211–223.
5. **Blumenkrantz, N., and G. Asboe-Hansen.** 1973. New method for quantitative determination of uronic acids. Anal. Biochem. **54:**484–489.
6. **Ciucanu, I., and F. Kerek.** 1984. A simple and rapid method for the permethylation of carbohydrates. Carbohydr. Res. **131:**209–217.
7. **Clapham, L., R. J. C. McLean, J. C. Nickel, J. Downey, and J. W. Costerton.** 1990. The influence of bacteria on struvite crystal habit and its importance in urinary stone formation. J. Cryst. Growth **104:**475–484.
8. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for the determination of sugars and related substances. Anal. Chem. **28:**350–356.
9. **Gatt, R., and E. R. Berman.** 1965. A rapid procedure for the estimation of aminosugars on a micro scale. Anal. Biochem. **15:**167–171.
10. **Gerwig, G. J., J. P. Kamerling, and J. F. G. Vliegenthart.** 1979. Determination of the absolute configuration of monosaccharides in complex carbohydrates by capillary G.L.C. Carbohydr. Res. **77:**1–7.
11. **Goldman, R. C., D. White, F. Ørskov, I. Ørskov, P. D. Rick, M. S. Lewis, A. K. Bhattacharjee, and L. Leive.** 1982. A surface polysaccharide of *Escherichia coli* O111 contains O-antigen and inhibits agglutination of cells by O-antiserum. J. Bacteriol. **151:**1210–1221.
12. **Goldstein, I. J., G. W. Hay, B. A. Lewis, and F. Smith.** 1970. Periodate oxidation of carbohydrates. Methods Carbohydr.

Chem. **5**:361–370.
13. **Graham, L. L., R. Harris, W. Villiger, and T. J. Beveridge.** 1991. Freeze-substitution of gram-negative eubacteria: general cell morphology and envelope profiles. J. Bacteriol. **173**:1623–1633.
14. **Griffith, D. P., D. W. Musher, and C. Itin.** 1976. Urease—the primary cause of infection-induced urinary stones. Invest. Urol. **13**:346–350.
15. **Gunner, S. W., J. K. N. Jones, and M. B. Perry.** 1961. The gas-liquid chromatography of carbohydrate derivatives. Part I. The separation of glycose and glycitol acetates. Can. J. Chem. **39**:1892–1895.
16. **Johnson, K. G., and M. B. Perry.** 1976. Improved techniques for the preparation of bacterial lipopolysaccharides. Can. J. Microbiol. **22**:29–34.
17. **Kumar, A., R. R. Ernst, and K. Wüthrich.** 1980. A two-dimensional nuclear Overhauser enhancement (2D NOE) experiment for elucidation of complete proton-proton cross-relaxation networks in biological macromolecules. Biochem. Biophys. Res. Commun. **95**:1–6.
18. **Kumar, A., G. Wagner, R. R. Ernst, and K. Wüthrich.** 1981. Buildup rates of the nuclear Overhauser effect measured by two-dimensional proton magnetic resonance spectroscopy: implications for studies of protein conformation. J. Am. Chem. Soc. **103**:3654–3658.
19. **Mackie, E. B., K. N. Brown, J. Lam, and J. W. Costerton.** 1979. Morphological stabilization of capsules of group B streptococci, types Ia, Ib, II, and III, with specific antibody. J. Bacteriol. **138**:609–617.
20. **Mann, S.** 1983. Mineralization in biological systems. Struct. Bonding **54**:125–174.
21. **Mann, S.** 1988. Molecular recognition in biomineralization. Nature (London) **332**:119–124.
22. **McLean, R. J. C., J. R. Lawrence, D. R. Korber, and D. E. Caldwell.** 1991. *Proteus mirabilis* biofilm protection against struvite crystal dissolution and its implications in struvite urolithiasis. J. Urol. **146**:1138–1142.
23. **McLean, R. J. C., J. C. Nickel, K.-J. Cheng, and J. W. Costerton.** 1988. The ecology and pathogenicity of urease-producing bacteria in the urinary tract. Crit. Rev. Microbiol. **16**:37–79.
24. **McLean, R. J. C., J. C. Nickel, V. C. Noakes, and J. W. Costerton.** 1985. An in vitro ultrastructural study of infectious kidney stone genesis. Infect. Immun. **49**:805–811.
25. **Penner, J. L., and J. N. Hennessy.** 1980. Separate O-grouping schemes for serotyping clinical isolates of *Proteus vulgaris* and *Proteus mirabilis*. J. Clin. Microbiol. **12**:304–309.
26. **Shaka, A. J., and J. Keeler.** 1987. Broadband spin decoupling in isotropic liquids. Prog. NMR Spectrosc. **19**:47–129.
27. **Shaka, A. J., J. Keeler, F. Frenkiel, and R. Freeman.** 1983. An improved sequence for broadband decoupling WALTZ-16. J. Magn. Reson. **52**:335–338.
28. **Stahl, S. J., K. R. Stewart, and F. D. Williams.** 1983. Extracellular slime associated with *Proteus mirabilis* during swarming. J. Bacteriol. **154**:930–937.
29. **Taylor, R. L., J. E. Shively, and H. E. Conrad.** 1976. Stoichiometric reduction of uronic acid carboxyl groups in polysaccharides. Methods Carbohydr. Chem. **5**:361–370.