JOURNAL OF BACTERIOLOGY, Dec. 1971, p. 1304-1309
Vol. 108, No. 3
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Polysaccharides of Type 6 *Klebsiella*

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Received for publication 15 September 1971

Water-extractable type 6 *Klebsiella* antigens were separated into a type 6-specific acidic polysaccharide and a neutral polysaccharide. The neutral polymer was devoid of type 6 activity although it was serologically active. The type 6-specific polymer contained fucose, glucose, and mannose, and pyruvic, galacturonic, and possibly glucuronic acids. The neutral polymer contained glucose, galactose, and mannose.

The type-specific *Klebsiella* capsular antigens studied to date have been found to be uronic acid and hexose-rich polysaccharides which often contain pyruvic acid (9, 11, 16, 29). The present work was carried out to provide information, previously unavailable, about the chemical nature of *Klebsiella* type 6 capsular materials.

MATERIALS AND METHODS

Bacterial cultures. A strain of *Klebsiella ozaenae*, capsular type 6, was obtained from W. H. Ewing, Communicable Disease Center, Emory, Ga.

Growth conditions. Organisms, grown aerobically for 36 hr at 26 C on a liquid medium containing glucose, salts, and yeast extract dialysate, were killed by ethanol and harvested as previously reported (9). Growth was poor at 37 C.

Preparation of capsular polysaccharides. Capsular polysaccharides were prepared as previously reported (9). Aqueous solutions of capsular polysaccharides were treated with 45% aqueous phenol by the method of Westphal et al. (28). After the combined aqueous phase fractions were dialyzed overnight against hot running tap water (about 60 C), followed by 24 hr of dialysis against deionized water at 4 C, polysaccharides were recovered by lyophilization. Lipopolysaccharide was removed insofar as possible by centrifugation of 5% aqueous polysaccharide solutions at 100,000 × *g* for 3 hr in a Beckman model L-2 ultracentrifuge.

Ribonucleic acid (RNA) and acidic and neutral polysaccharides were separated by cetyltrimethyl ammonium bromide (CTAB) fractionation by the method of Scott (23). RNA was first precipitated from 0.25 M NaCl containing 1.0% CTAB. The RNA precipitate was removed by centrifugation, and the supernatant solution was diluted with water until the acidic polymer was precipitated. After the CTAB-acidic polysaccharide precipitate was removed by centrifugation, 10 volumes of 95% ethanol were added to the supernatant solution to precipitate neutral polysaccharide materials. Neutral and CTAB-precipitated fractions were dissolved in water or 0.25 M NaCl and precipitated with 10 volumes of ethanol to remove CTAB. The alcohol-precipitated polysaccharides were redissolved and dialyzed against deionized water for 24 hr at 4 C with changes every 6 hr and then lyophilized. Two preparations from different cell batches were studied, i.e., preparations 1 and 2.

Elemental analyses of the CTAB-purified acid polymers, performed by Galbraith Laboratories, Knoxville, Tenn., were as follows. Preparation 1: C, 39.66; H, 5.34; N, 0.46; S, 0.97; ash, 7.91; water, 12.1%. Preparation 2: C, 38.37; H, 5.51; N, 0.83; S, 0.16; ash, 9.98; water, 11.2%. Water content was determined by weight loss upon drying in vacuo over P_2O_5 at 80 C.

Treatment of dialysis tubing. Dialysis tubing, 35 mm (Union Carbide Corp., Chicago, Ill.), was treated as previously reported (9).

Hydrolysis. Materials (10 mg/ml) were heated in sealed tubes for times indicated. HCl was removed from hydrolysates by distillation in a rotary evaporator at 40 C at reduced pressure with repeated additions of water and reevaporation.

Amino acid analysis. Amino acid analyses were carried out on a modified Beckman 120-C analyzer (24) as previously reported (9). Samples were prepared by hydrolysis for 18 hr in vacuo at 100 C in 6 N HCl. Acid was removed by repeated addition of water and evaporation to dryness at 40 C under reduced pressure.

Colorimetric methods. Pyruvic acid was released from polysaccharides by hydrolysis in 1 N HCl for 90 min (9) before quantification by the 2,4-dinitrophenylhydrazone method of Friedemann and Haugen (8).

Uronic acids were quantified colorimetrically in unhydrolyzed polysaccharides by the borate-carbazole method of Bitter and Muir (2) and the orcinol method of Brown (3).

Hydrolysis for monosaccharide determination. Time course reducing sugar analyses (19) indicated maximum release after hydrolysis of polysaccharides for 3 hr in 2 N H_2SO_4 at 100 C. Sulfate was removed by titration of the hydrolysate to *p*H 2 to 3 with Dowex-1-HCO_3^-. The resin was removed by filtration. The filtrate and washings were evaporated to dryness, redissolved in a known volume of water, and samples were used for various determinations.

Glucose and galactose assay. Glucose and galactose

concentrations were determined with glucostat and galactostat reagents, obtained from Worthington Biochemical Corp., Freehold, N.J.

Gas-liquid chromatography. Monosaccharides were separated, identified, and quantified by gas-liquid chromatography as alditol acetate derivatives after reduction and acetylation by the procedure of Sawardeker et al. (21). The acetylation reagents were removed by evaporation, the samples were dissolved in acetone, and portions were injected into a column of 3% ECNSS-M on gas chrom-Q 100/200 (Applied Science Laboratories, State College, Pa.) at 180 C, using a gas chromatograph (F & M Scientific Corp., model 400) equipped with a hydrogen flame ionization detector. Areas under peaks were determined by use of the Technicon model AAG integrator-calculator.

Thin-layer chromatography. Solvent systems for thin-layer cellulose chromatography were: (A) ethyl acetate-pyridine-acetic acid-water (5:5:1:3); (B) *n*-butanol-pyridine-water (6:4:3); and (C) *n*-butanol-acetic acid-water (5:1:3).

High-voltage electrophoresis. Samples were electrophoresed on Whatmann 3MM paper utilizing a Savant model 30A flatbed high-voltage electrophoresis apparatus. The method of Haug and Larsen (10), which employed 0.01 M borate buffer at *p*H 9.2 containing 0.005 M $CaCl_2$, was used for uronic acid separations at 55 v/cm for 2 hr. Acid-hydrolyzed samples were dissolved in the same buffer before being streaked onto the paper in order to hydrolyze uronic acid lactones. After ionophoresis, the papers were dried, and guide strips, cut from both sides and center, were sprayed to locate compounds.

Sprays. Reducing sugar spots were detected with alkaline silver nitrate followed by aqueous thiosulfate to reduce background. Hexoses, pentoses, deoxysugars, lactones, and uronic acids were also detected by spraying with Timell's reagent (26), *O*-aminobiphenyl, 3%, in 100 ml of glacial acetic acid containing 1.3 ml of 85% phosphoric acid.

RESULTS

Considerable purification of capsular polysaccharide preparations was achieved by 96 hr of dialysis of crude aqueous extracts of alcohol-precipitated, acetone-dried cultures. Pyruvic acid, uronic acid, and serological activity (*see below*) were all retained within the dialysis tubing. None could be detected in the dialyzable fraction. Further purification resulted from the separation by CTAB fractionation of the extensively dialyzed retentates into acidic and neutral polymers. These results are apparent from a comparison of the uronic acid and pyruvic acid content of the various fractions (Table 1).

Yields of the CTAB fractions varied between the two preparations examined. The first preparation yielded 2.6% RNA, 38.4% acidic, type-specific polysaccharide fraction, and 6.2% neutral fraction. The second preparation yielded 1.6% RNA, 12.6% acidic, type-specific polysaccharide, and 17.0% neutral fraction. No attempt was made to recover either the ultracentrifuge

TABLE 1. *Sugar constituents of Klebsiella type 6 acidic and neutral polymers*[a]

Polysaccharide fraction	Constituent (micromoles per milligram)[b]						
	Pyruvic acid	Uronic acid[c]	Glucose	Galactose	Mannose	Fucose	3-*O*-Methyl mannose
Crude extract							
Preparation 1	(<2)	(6.1, 5.9)	ND	ND	ND	ND	ND
Preparation 2	ND	ND	ND	ND	ND	ND	ND
Dialyzed extract							
Preparation 1	(8.9)	(16.4, 17.8)	ND	ND	ND	ND	ND
Preparation 2	ND	(16.6, 19.5)	ND	ND	ND	ND	ND
Acidic polymer							
Preparation 1	2.2 (19.4)	1.6 (31.0)	0.56 (10.1)	tr (0)	0.41 (7.4)	0.30 (4.9)	0.0 (0)
Preparation 2	1.2 (10.6)	1.5 (29.1)	0.74 (13.3)	tr (0)	0.54 (9.7)	0.38 (6.2)	0.0 (0)
Neutral polymer							
Preparation 1	0.0 (0)	—[d]	0.028 (0.5)	0.140 (2.5)	0.028 (0.5)	0.0 6(0) 1)	0.056 (1.1)
Preparation 2	0.0 (0)	—[d]	0.078 (1.4)	0.505 (9.1)	0.072 (1.3)	0.0 (0)	tr (0)

[a] Total uronic acids were determined as galacturonic acid by the carbazole procedure. Glucose and galactose were quantified by glucostat reagents and also by gas-liquid chromatography. Mannose and 3-*O*-methylmannose were quantified by gas-liquid chromatography, relative to glucose or galactose.

[b] Figures in parentheses indicate percentage of weight; tr, trace; ND, not done.

[c] Uronic acids were determined on unhydrolyzed samples of crude and dialyzed extracts by the carbazole and the orcinol methods, respectively.

[d] Could not be accurately determined due to formation of interfering colored compounds.

sedimentables or the CTAB-nonprecipitable ethanol-soluble materials, which may explain low over-all recoveries.

Immunodiffusion analysis with Difco type 6-specific antiserum confirmed that the antigenic species remained in the retentate after dialysis of the crude extracts. A single precipitin band was observed. No activity could be detected in the dialysate after concentration at 40 C under reduced pressure followed by lyophilization. The CTAB acidic fractions from both preparations 1 and 2, over a range of 5 μg to 5 mg/ml, gave single identical bands on immunodiffusion analysis when either Difco type 6-specific antisera or the nonspecific antiserum was used. The CTAB-nonprecipitable, neutral alcohol-precipitated fractions gave no precipitin bands with the Difco type 6-specific antisera, but, at concentrations near 5 mg/ml, a different or second precipitin band was observed nearer the antibody well with the neutral antigen fractions and the nonspecific antiserum. A similar observation was previously reported by Henriksen and Eriksen in their studies of *Klebsiella* type 3 capsular antigens (12).

Chemical composition. Glucose, mannose, and fucose and pyruvic and uronic acids were the major constituents identified in the acidic polymer, whereas glucose, galactose, and mannose were found in the neutral polysaccharide fractions (Table 2). Also found only in the neutral fractions was a material designated as 3-*O*-methylmannose which was detected only by gas-liquid chromatography and tentatively identified on the basis of retention time and co-chromatography with synthetic 3-*O*-methylmannose. Further identification of this material was not made.

Table 1 also shows the relative amounts of the various constituents of the acidic and neutral fractions. Only small traces of other constituents were found, e.g., trace amounts of arabinose and xylose were found by gas-liquid chromatography in the acidic fractions. Amino acids were found to constitute 1.1% and 0.6% total of the acidic, type-specific polymer preparations 1 and 2, respectively. Less than 0.2% amino sugars was found and identified on a modified Beckman 120-C amino acid analyzer (24) as glucosamine only. Significant glucosamine spots were detectable by thin-layer chromatography in hydrolysates of the neutral but not in the acidic polysaccharide fractions.

Identification of uronic acids. High-voltage electrophoresis of various acids from 2 N H_2SO_4 (3 hr, 100 C) hydrolysates of type 6 acidic polysaccharide resulted in the mobilities relative to glucuronic acid shown in Table 3. Areas of the electropherogram corresponding to standard known uronic acids were cut out, eluted with water, evaporated to small volumes, and spotted onto thin-layer cellulose plates. Thin-layer chromatography in solvent system A, sprayed with Timell's reagent, yielded the mobilities relative to glucose shown in Table 3, corresponding to both glucuronic and galacturonic acids. The value given, 0.45, is the center of the spot which covered both areas.

Further evidence for the identification of the uronic acid component was obtained as follows. Polysaccharides were esterified with propylene oxide, and the esters were reduced to alcohols, i.e., the parent sugars, with sodium borotritiide by the method of Hungerer et al. (13). The prep-

TABLE 2. *Thin-layer chromatography of Klebsiella type 6 acidic and neutral capsular polysaccharide components*

Compound source	$R_{glucose}$[a]			
	Solvent system A (1[b])	Solvent system B (2[b])	Solvent system B (1[b])	Solvent system C (1[b])
Standards				
Arabinose	1.10	1.10	1.07	1.04
Glucosamine	ND	ND	ND	0.75
Ribose	1.33	1.33	1.26	1.30
Mannose	1.06	1.06	1.08	1.11
Rhamnose	1.22	1.22	1.40	1.51
Galactose	0.93	0.93	0.91	0.96
Glucose	1.00	1.00	1.00	1.00
Fucose	1.18	1.18	1.24	1.38
Mannuronic acid	ND	ND	0.42	0.78
Glucuronic acid	0.55	ND	0.34	0.75
Galacturonic acid	0.45	ND	0.29	0.65
Acidic polysaccharides				
Preparation 1	0.47	[c]	[c]	0.65
	0.99	0.98	0.98	1.00
	1.06	1.09	1.10	1.08
	1.18	1.18	1.18	1.38
Preparation 2	0.46	[c]	[c]	0.65
	0.99	0.97	0.99	1.00
	1.06	1.11	1.10	1.08
	1.17	1.19	1.20	1.36
Neutral polysaccharides				
Preparation 1	0.92	0.92	0.88	0.96
	0.99	0.99	0.97	0.99
	1.06	1.06	1.12	1.10
Preparation 2	0.92	0.92	0.88	0.95
	0.98	0.99	0.96	0.98
	1.08	1.06	1.10	1.08

[a] $R_{glucose}$ values by thin-layer cellulose chromatography for components present in CTAB fractions hydrolyzed for 3 hr at 100 C with 2 N HCl.

[b] Detection reagents: 1, Timell reagent; 2, alkaline silver nitrate.

[c] Uronic acid spots were also present, but separations were poor.

TABLE 3. *Chromatographic and electrophoretic mobility of type 6 uronic acids*[a]

Compound	$R_{glucuronic\ acid}$ (electrophoresis)	$R_{glucose}$ (thin-layer cellulose)
Standards		
Glucuronic acid	1.00	0.51
Galacturonic acid	0.89	0.42
Mannuronic acid	0.78	ND
Ribose	ND	1.28
Glucose	ND	1.00
Mannose	ND	1.06
Galactose	ND	0.93
Fucose	ND	1.18
Type 6 hydrolysate		
Minor uronic acid spot	0.99	
Major uronic acid spot	0.89	
Electropherogram eluate		
Unknown uronic acid		0.43[b]

[a] Electrophoretic mobilities of uronic acids in type 6 crude dialyzed polysaccharide fractions (column labeled $R_{glucuronic\ acid}$) after hydrolysis in 2 N H_2SO_4 for 3 hr at 100 C and thin-layer chromatographic mobility in solvent A of the components eluted from the uronic acid area of the electropherogram (column labeled $R_{glucose}$). Timell's reagent was used to make spots visible.

[b] A lactone spot was also present.

arations were hydrolyzed in 2 N HCl for 3 hr at 100 C. After removal of acid and repeated evaporations from deionized water to remove tritium from the hydrolysate, a sample was chromatographed in solvent system A along with standards on either side. Guide strips were sprayed with Timell's reagent, and areas of the plate corresponding to standard glucose, galactose, and mannose were carefully scraped off, added to scintillation vials, and counted on a Beckman LS-150 liquid scintillation counter. The level of counts observed indicated low incorporation of tritium. The major tritium-labeled hexose was found to be galactose. However, counts were also present in the glucose and mannose areas of the chromatogram.

DISCUSSION

The *Klebsiella* type 6-specific capsular antigen is shown in the present work to be an acidic polysaccharide. No type-specific activity was found in the neutral (or CTAB-nonprecipitable) fraction, although this fraction was found to be serologically active when a nonspecific cellular antiserum was used.

The type 6-specific antigen preparations contained fucose, glucose, mannose, uronic acid, and pyruvic acid. The relative ratios of fucose, glucose, and mannose from the two different preparations were quite similar, i.e., 1:2:1. However, uronic acid and pyruvic acid contents varied considerably, i.e., 5:7 and 4:3, respectively, in preparations 1 and 2. The significance of these observations must await the isolation and characterization of oligosaccharides and the establishment of a possible repeating unit. However, our data appear to indicate the possibility that pyruvic acid may be bound to uronic acid or deoxysugar residues in addition to hexoses in type 6-specific polysaccharides, or that more than one pyruvic acid residue (side chain ?) is bound to given hexose units. Such linkages have not been previously reported.

Thin-layer chromatograms and high-voltage electropherograms of hydrolysates of extensively dialyzed polysaccharide extracts, sprayed with Timell's reagent which allows differentiation of uronic acids and pentoses from hexoses, etc., indicated the presence of large amounts of galacturonic acid and smaller amounts of glucuronic acid and lactone spots. A preliminary attempt to use the method of Hungerer et al. for uronic acid determination (13) on the purified acidic polymer indicated that galacturonic, glucuronic, and possibly even small amounts of mannuronic acids were present in the type 6-specific antigen fraction. The occurrence of a second or possible third uronic acid suggests the possibility of contamination. The presence of fucose and glucuronic acid might suggest the possible presence of contaminating colanic acid. However, colanic acid, the common mucus or M-antigen of *Enterobacteriaceae*, is reported to contain one mole of galactose per mole of fucose (14, 25). The absence of galactose in the type 6-specific antigen preparations appears to rule out colanic acid contamination.

The neutral polysaccharide fraction we obtained from type 6 preparations contained glucose, galactose, and mannose in apparent ratios of 1:5:1 (preparation 1) and 1:7:1 (preparation 2), whereas pyruvic acid was absent. However, the question of homogeneity may still be raised since Dudman and Wilkinson (5) and Wilkinson et al. (30) demonstrated than an intracellular galactan could be obtained from *Klebsiella* type 54 preparations. Similar results were also reported for *Klebsiella pneumoniae* type 1 by Barker et al. (1). For this reason, the molar ratios we observed may reflect the relative ratio of one or more neutral polymers. On the other hand, Eriksen (6, 7) also used cetylpyridinium fractionation to separate acidic, type-specific antigens from a common neutral antigen found in capsular antigens prepared from *Klebsiella* types 1, 2, 3, 4, and 5. (The old designations are A, B,

C, D, and E, respectively.) Eriksen identified galactose, glucose, and mannose as the major constituents of this common neutral polymer (6, 7). The neutral polymer which we describe in the present work from *Klebsiella* type 6 polysaccharides, therefore, appears to be the same as that described previously for types 1 to 5 by Eriksen. At first thought, our neutral polymer would appear to be distinct from a different neutral polymer, which contained glucose and rhamnose, which also was reported by Eriksen to occur in *Klebsiella aerogenes* B 1076/48 and "Enterobacter" strain 349, both of which produce the acidic, type 3 reactive rhinoscleromatis antigen (6). The possibility that this rhamnose-containing neutral antigen is related to the "Sm" antigen of Pickett and Cabelli (20) was raised by Eriksen (7).

The neutral polymers we studied from type 6 *Klebsiella* contained an additional component, not mentioned by Eriksen, which we tentatively identified as 3-*O*-methylmannose by gas-liquid chromatography. While our work was in progress, Nimmich reported finding 3-*O*-methylmannose in lipopolysaccharides of *Escherichia coli* and *Klebsiella* (17). Nimmich also indicated that 3-*O*-methylmannose and rhamnose exhibited similar mobilities in several paper chromatographic solvents. This raises the possibility that the 3-*O*-methylmannose we observed in *Klebsiella ozaenae* type 6 "neutral" polysaccharide may be a component of the common "neutral" *Klebsiella* polysaccharides observed by Eriksen, which may also be similar to or identical with the lipopolysaccharide studied by Nimmich, or that our "neutral" polymer fraction is contaminated with a 3-*O*-methylmannose-containing lipopolysaccharide or other material.

It is of interest to note that 3-*O*-methylmannose has also been found in *Coccidioides immitis* (22) and in *Streptomyces griseus* (4), while 3-*O*-methylgalactose has been isolated from *Actinomadura madurae* hydrolysates (15), and 6-*O*-methylglucose was found in mycobacteria (31).

ACKNOWLEDGMENTS

This work was supported by Public Health Service research grant AI-01659 from the National Institute of Allergy and Infectious Diseases and by Public Health Service training grant 5-T01-GM-00233 predoctoral support for B.J. Gormus from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Barker, S. A., J. A. Brimacombe, J. L. Eriksen, and M. Stacey. 1963. Capsular polysaccharide of *Klebsiella pneumoniae* type A (strain 1265). Nature (London) **197**: 899-900.
2. Bitter, T., and H. M. Muir. 1962. A modified uronic acid carbazole reaction. Anal. Biochem. **4**:330-334.
3. Brown, A. H. 1949. Determination of pentose in the presence of large quantities of glucose. Arch. Biochim. Biophys. **11**:269-278.
4. Candy, D. J., and J. Baddiley. 1966. 3-O-Methyl-D-mannose from *Streptomyces griseus*. Biochem. J. **98**:15-18.
5. Dudman, W. F., and J. F. Wilkinson. 1956. The composition of the extracellular polysaccharides of aerobacter-klebsiella strains. Biochem. J. **62**:289-295.
6. Eriksen, J. 1965. Immunochemical studies on some cross-reactions in the *Klebsiella* group 11. Structure of the capsular polysaccharide of *Klebsiella aerogenes* strain B 1076/48 and "Enterobacter" strain 349. Acta Pathol. Microbiol. Scand. **64**:511-521.
7. Eriksen, J. 1966. Immunochemical studies on some serological cross-reactions in the *Klebsiella* group 16. Neutral polysaccharide antigens. Acta Pathol. Microbiol. Scand. **66**:71-78.
8. Friedemann, T. E., and G. E. Haugen. 1943. Pyruvic acid. II. The determination of keto acids in blood and urine. J. Biol. Chem. **147**:415-442.
9. Gormus, B. J., R. W. Wheat, and J. F. Porter. 1971. Occurrence of pyruvic acid in capsular polysaccharides from various *Klebsiella*. J. Bacteriol. **107**:150-154.
10. Haug, A., and B. Larsen. 1961. Separation of uronic acids by paper electrophoresis. Acta Chem. Scand. **15**:1395-1396.
11. Heidelberger, M., W. F. Dudman, and W. Nimmich. 1970. Immunochemical relationships of certain capsular polysaccharides of klebsiella, pneumococci, and rhizobia. J. Immunol. **104**:1321-1328.
12. Henriksen, S. D., and J. Eriksen. 1962. Immunochemical studies on some cross-reactions in the *Klebsiella* group 7. Serological reactions of some strains of type 3(C) and some cross-reacting strains. Acta Pathol. Microbiol. Scand. **54**:391-397.
13. Hungerer, D., K. Jann, B. Jann, F. Ørskov, and T. Ørskov. 1967. Immunochemistry of K antigens of *E. coli*. 4. The K antigen of *E. coli* 09:K30:H12. Eur. J. Biochem. **2**:115-126.
14. Lawson, C. J., C. W. McCleary, H. T. Nakada, D. A. Rees, T. W. Sutherland, and J. F. Wilkinson. 1969. Structural analysis of colanic acid from *E. coli* by using methylation and base-catalyzed fragmentation. Biochem. J. **115**:947-958.
15. Lechavalier, M. P., and N. N. Gerber. 1970. The identity of madurose with 3-0-methyl-D-galactose. Carbohyd. Res. **13**:451-454.
16. Lüderitz, O., K. Jann, and R. W. Wheat. 1968. Somatic and capsular antigens of gram-negative bacteria. Biochem. J. **26A**:105-228.
17. Nimmich, W. 1970. Occurrence of 3-*O*-methylmannose in lipopolysaccharides of *Klebsiella* and *E. coli*. Biochim. Biophys. Acta **215**:189-191.
18. Ouchterlony, O. 1968. Handbook of Immunodiffusion and Immunoelectrophoresis. Ann Arbor Science Publishers, Inc., Ann Arbor, Mich.
19. Park, J. T., and M. J. Johnson. 1949. A submicrodetermination of glucose. J. Biol. Chem. **181**:149-151.
20. Pickett, M. J., and V. J. Cabelli. 1953. The precipitating antigens of Friedlander's bacillus. J. Gen. Microbiol. **9**: 249-256.
21. Sawardeker, J. S., J. H. Sloneker, and A. Jeans. 1965. Quantitative determination of monosaccharides as their alditol acetates by gas-liquid chromatography. Anal. Chem. **37**:1602-1604.
22. Scheer, E., T. Terai, S. Kulkarni, N. F. Conant, R. W. Wheat, and E. P. Lowe. 1970. Unusual reducing sugar from *Coccidioides immitis*. J. Bacteriol. **103**:525-526.
23. Scott, J. E. 1960. Aliphatic ammonium salts in the assay of acidic polysaccharide acids from tissues. Methods Biochem. Anal. **8**:145-197.
24. Steele, R. S., K. Brendel, E. Scheer, and R. W. Wheat. 1970. Ion-exchange separation and automated assay of

complex mixtures of amino acids and hexosamines. Anal. Biochem. **34**:206–225.
25. Sutherland, T. W. 1969. Structural studies on colanic acid, the common exopolysaccharide found in the enterobacteriaceae by partial acid hydrolysis. Biochem. J. **115**:935–945.
26. Timell, T. E., C. P. J. Glaudemans, and A. L. Currie. 1956. Spectrophotometric method for determination of sugars. Anal. Chem. **28**:1916–1920.
27. Westphal, O., and K. Jann. 1965. p. 86–88. *In* R. L. Whistler, J. N. BeMiller, and M. L. Wolfrom, (ed.), Methods in carbohydrate chemistry. Academic Press Inc., New York.
28. Westphal, O., O. Lüderitz, and F. Bister. 1952. Über die Extraktien von Bakterien mit Phenol-Wasser. Z. Naturforsch. Ser. B **7**:148–155.
29. Wheat, R. W., C. Dorsche, and G. Godoy. 1965. Occurrence of pyruvic acid in the capsular polysaccharide of *Klebsiella rhinoscleromatis*. J. Bacteriol. **89**:539.
30. Wilkinson, J. F., W. F. Dudman, and G. O. Aspinall. 1955. The extracellular polysaccharide of *Aerobacter aerogenes* A3(S1) (Klebsiella type 54). Biochem. J. **59**: 446–451.
31. Yuan, C. L., and C. E. Ballou. 1964. 6-0-Methyl-D-glucose from mycobacteria. J. Biol. Chem. **239**:PC3602–3603.