JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1981, p. 89-92
0095-1137/81/010089-04$02.00/0

Vol. 13, No. 1

Carbohydrate Fingerprints of Streptococcal Cells

DAVID G. PRITCHARD,[1]* JOHN E. COLIGAN,[2] SHERYL E. SPEED,[1] AND BARRY M. GRAY[3]

Department of Microbiology[1] and Department of Pediatrics,[3] University of Alabama in Birmingham, Birmingham, Alabama 35294, and Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205[2]

The carbohydrates of whole cells of group A, B, C, D, F, and G streptococci were analyzed with a highly sensitive gas chromatographic procedure. Characteristic chromatographic fingerprints were obtained for each group of streptococci grown in broth cultures or as single colonies on a blood agar plate. Rhamnose, glucose, and *N*-acetylglucosamine were major components of all the groups. Groups A, C, and F contained very little galactose, and groups A and B showed almost a complete absence of *N*-acetylgalactosamine. Chromatograms obtained for group B streptococci were distinguished by the presence of 1,4-anhydroglucitol.

Hemolytic streptococci are classified by the serological detection of the group-specific polysaccharide or "C-substance" (6, 9). The group-specific polysaccharides of streptococcal groups A (7), C (8), B, and G (3) have been shown to account for a major portion of the cell walls of the bacteria and to significantly contribute to the carbohydrate composition of the whole cells. The type-specific polysaccharides of group D streptococci were found to be the structural and chemical counterparts of the group-specific polysaccharides of other groups (2). In all cases, rhamnose was the major monosaccharide component, and it has been suggested that the polysaccharides have a common polyrhamnose core (8). This report describes the characteristic carbohydrate fingerprints of group A, B, C, F, and G streptococci obtained by gas chromatography of whole cells analyzed as their trifluoroacetate derivatives after methanolysis. The procedure gives remarkably clean chromatograms with no interference by proteins, lipids, and other cellular components.

MATERIALS AND METHODS

Bacteriological methods. Group B variant (090R), group B type III (D136C), and group G (D166B) streptococci were obtained from R. C. Lancefield of the Rockefeller University. Group B type Ia (090) and group B type Ib (H36B) streptococci were obtained from Hazel W. Wilkinson of the Center for Disease Control, Atlanta, Ga. Four strains of group A, eight strains of group B (types Ia, Ib, II, and III), three strains of group C, five strains of group D, three strains of group F, and four strains of group G were isolated from patients at the University of Alabama in Birmingham Medical Center Complex in ongoing studies of group B streptococcal colonization and infection (10). Grouping and typing of streptococci were carried out by methods described previously (4).

Streptococci for carbohydrate analysis were grown at 37°C for 12 h in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) containing 1% glucose. The bacterial cells were harvested by centrifugation, and the pellets were washed twice with buffered saline (0.15 M NaCl, 0.05 M sodium phosphate, pH 7.5), once with water, and then lyophilized. Cell suspensions (1 mg/ml) were prepared in degassed water, and 2- to 5-μl portions were added to glass capillary tubes (90 by 0.8 mm, Fisher Scientific Co., Atlanta, Ga). The tubes were centrifuged briefly, placed in a vacuum desiccator, and rapidly taken to dryness with continuous pumping.

Streptococci grown on sheep blood agar were prepared by transferring a portion of an isolated colony to a capillary tube by using an inoculating loop. The sample was rinsed off the loop into a conical plastic micropipette tip attached to a glass capillary tube by a 2-mm length of polyethylene tubing. After a brief centrifugation, the sample was rapidly dried under vacuum.

Derivatization. Sugars and alditols were obtained from Sigma Chemical Co., St. Louis, Mo. High-purity methanol, hexane, and *N,N*-dimethylformamide were obtained from Burdick and Jackson Laboratories, Muskegon, Mich. *N*-Methyl-bis(trifluoroacetamide) (MBTFA) was obtained from Pierce Chemical Co., Rockford, Ill.

Methanolic HCl was prepared by passing dry HCl gas through methanol until the concentration was 1.4 N (determined by titration with a standard base). A 10% (wt/vol) solution of MBTFA in *N,N*-dimethylformamide was prepared weekly. The derivatization mixture was prepared immediately before use by adding 10 μl of pyridine to 100 μl of the 10% MBTFA solution.

The monosaccharide analyses were carried out by using an improved modification of a highly sensitive gas chromatographic procedure described previously (11). Briefly, approximately 10 μl of 1.4 N methanolic HCl was added to each capillary tube by using a glass micropipette. The capillaries were then sealed and placed in a heating block for 24 h at 80°C. The capillaries were broken open, and the samples were rapidly taken to dryness under vacuum. Derivatization was

accomplished by adding 5 μl of the trifluoroacetylation reagent to each capillary and allowing it to react at room temperature for at least 2 h.

Gas chromatography. A Hewlett-Packard Model 5830A gas chromatograph equipped with a splitless capillary inlet system and a ^{63}Ni linear electron capture detector was used for the analyses. A 30-m glass WCOT column coated with OV-105 was obtained from Universal Scientific, Atlanta, Ga. The straightened ends of the column and the glass splitless injector insert were treated with 0.1% Carbowax 20 M in methylene chloride. Helium at an inlet pressure of 20 cm Hg (26.66 kPa) was used as the carrier gas (38 cm/s linear flow) and for the septum (3 ml/min) and inlet (60 ml/min) purges. Argon-methane (95:5) was used for the detector makeup gas at a flow rate of 60 ml/min.

The injector temperature was 195°C, and the detector was operated at 280°C. The chromatograph was held at 100°C for 1 min and was then temperature

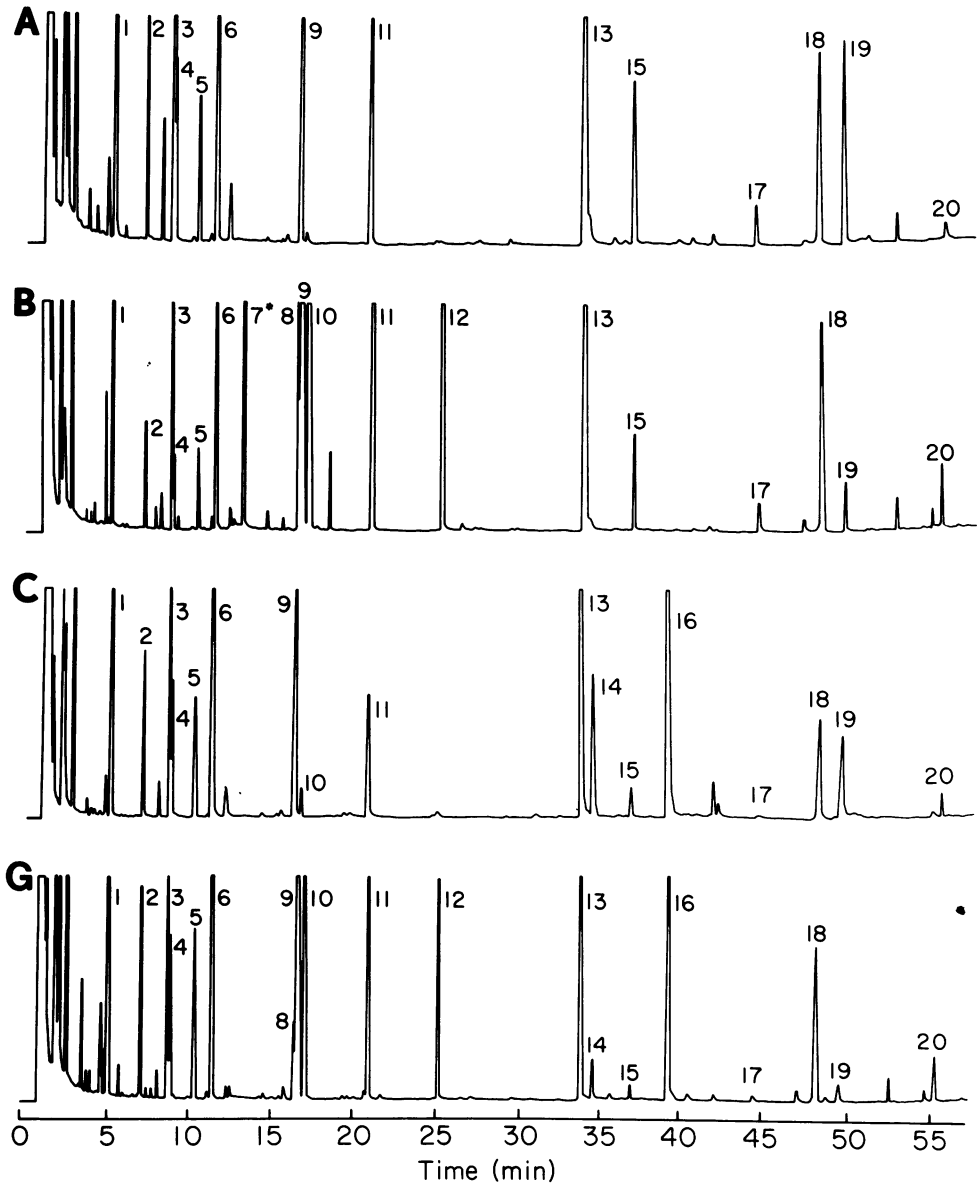

FIG. 1. *Gas-liquid chromatograms of 2-μg samples of lyophilized whole cells of group A, B, C, and G streptococci run as the trifluoroacetate derivatives of the methylglycosides. Peaks are numbered as follows: rhamnose (1, 3), ribose (2, 4, 5, 6), 1,4-anhydroglucitol (7), galactose (8, 10, 12), glucose (9, 11), N-acetylglucosamine (13, 15, 17, 19), N-acetylgalactosamine (14, 16), and N-acetylmuramic acid (18, 20).*

programmed to 155°C at 1°C/min and 225°C at 5°C/min. A 0.2-μl sample followed by 1 μl of hexane was injected, and the inlet purge gas flow was delayed for 18 s.

Peaks were identified on the basis of their retention times as compared with standards. Relative response factors for the various sugars were determined relative to glucitol by using standard sugar mixtures and were updated frequently.

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms obtained for 2-μg samples of group A, B, C, and G streptococci. The chromatograms were highly characteristic for a particular streptococcal group. Group A and C streptococci showed a virtual absence of galactose (peaks 8, 10, and 12). Groups A and B had little or no *N*-acetylgalactosamine (peaks 14 and 16), whereas this sugar is a major component of groups C and G. Only group B streptococci had 1,4-anhydroglucitol (peak 7), and its presence is diagnostic for the group (Pritchard et al., submitted for publication).

Table 1 summarizes compositional data obtained for streptococcal groups A, B, C, D, F, and G. Rhamnose was a major component of all the streptococcal groups. All organisms also contained ribose, glucose, *N*-acetylglucosamine, and *N*-acetylmuramic acid. Only minor differences were noted in the carbohydrate composition of types Ia, Ib, II, and III and the nontypable 090R strain of group B streptococci. Table 1 shows the average values for the group.

Ribose was present in all streptococci and presumably originated from ribonucleic acid since it was not present in purified cell walls. Deoxyribose is destroyed by the reaction conditions. *N*-Acetylmuramic acid is derived from cell wall peptidoglycan. Chromatograms obtained for group B streptococci were unique in containing 1,4-anhydroglucitol. This compound was derived from glucitol phosphate during methanolysis and is characteristic of group B streptococci.

TABLE 1. *Carbohydrate composition of whole streptococci*

Compound	Carbohydrate in following streptococcal group (%)					
	A (4)[a]	B (8)	C (3)	D (5)	F (3)	G (4)
Rhamnose	39.6	18.5	28.4	18.4	33.1	28.1
Ribose	12.4	4.8	17.4	9.9	8.9	12.1
Glucitol	0	4.0	0	0	0	0
Glucose	16.0	28.9	13.0	31.8	37.9	27.3
Galactose	0.2	25.1	0.8	17.1	3.9	17.3
N-Acetylglucosamine	28.7	16.1	16.0	12.1	6.4	6.1
N-Acetylgalactosamine	0	0	20.9	8.0	7.3	6.4
N-Acetylmuramic acid	3.1	2.6	3.5	2.7	2.5	2.7

[a] Number of strains analyzed is shown within parentheses; results are averages.

Unlike other groups, the C-substance of group D is a cytoplasmic rather than a cell wall constituent. The five group D strains examined here were quite uniform; however, different types are known to vary in composition (2) and would likely yield differing chromatograms.

The monosaccharide compositions obtained in this study for whole bacterial cells show general agreement with values obtained for cell walls and various group-specific carbohydrates of previous reports (2, 3, 7, 8). Precise comparisons are difficult, however, because only values for selected monosaccharides were reported by previous investigators, and their analytical methods often did not distinguish among the different hexoses.

The use of gas chromatography in bacterial chemotaxonomy has been reviewed (5). The analytical procedure employed in this study represents a major improvement over existing methods. Aluyi and Drucker (1) reported a combined gas chromatographic-mass spectrometric procedure for obtaining fingerprints on 5 mg of lyophilized cells of *Streptococcus mutans*. The procedure described in this report is several thousand times more sensitive, requiring less than a microgram of cells for analysis. In addition, the very high resolution obtained by using a capillary column and the great sensitivity and specificity for monosaccharide trifluoroacetates obtained by using an electron capture detector are major advantages. The ability to analyze bacteria from single colonies grown on solid medium eliminates the usual need for broth cultures and extraction of the bacterial cell pellets. The method is not intended for the routine grouping of hemolytic streptococci but should be especially useful in the area of bacterial chemotaxonomy.

The high sensitivity of the procedure makes it feasible to extend it to the examination of body fluids, including cerebrospinal fluids and synovial fluid. This method should be particularly useful in studies where immunological detection of antigens may be difficult because of their partial degradation or low concentration. Although rhamnose and *N*-acetylmuramic acid are present in most bacterial species, they are not found in mammalian cells, and their detection would indicate the presence of bacterial polysaccharides. A particular chromatographic pattern may indicate the presence of bacterial

products; the presence of glucitol would strongly suggest group B. Group A and group B streptococci are major human pathogens, and groups C, D, F, and G are also known to cause disease. The ability to obtain highly characteristic carbohydrate fingerprints of whole bacterial cells opens yet another dimension to the study of these organisms.

ACKNOWLEDGMENTS

D.G.P. is a research fellow of the Arthritis Foundation.

This investigation was supported in part by the Stolle Development Corporation and by a Public Health Service grant from the National Institute of Health Child and Human Development (HD10699, project no. 10).

LITERATURE CITED

1. **Aluyi, H. A. S., and D. B. Drucker.** 1979. Fingerprinting of carbohydrates of *Streptococcus mutans* by combined gas-liquid chromatography-mass spectrometry. J. Chromatogr. **178**:209-218.
2. **Bleiweis, A. S., and R. M. Krause.** 1965. The cell walls of group D streptococci. I. The immunochemistry of the type I carbohydrate. J. Exp. Med. **122**:237-249.
3. **Curtis, S. N., and R. M. Krause.** 1964. Antigenic relationships between groups B and G streptococci. J. Exp. Med. **120**:629-637.
4. **Dillon, H. C., M. A. Pass, and B. Buchanan.** 1978. Modified method for serological identification of group B streptococci. J. Clin. Microbiol. **7**:599-600.
5. **Drucker, D. B.** 1976. Gas-liquid chromatographic chemotaxonomy, p. 52-125. *In* J. R. Norris (ed.), Methods in microbiology, vol. IX. Academic Press, Inc., New York.
6. **Facklam, R. R.** 1976. A review of the microbiological techniques for the isolation and identification of streptococci. Crit. Rev. Clin. Lab. Sci. **6**:287-317.
7. **Krause, R. M., and M. McCarty.** 1961. Studies on the chemical structure of the streptococcal cell wall. I. The identification of a mucopeptide in the cell walls of groups A and A-variant streptococci. J. Exp. Med. **114**: 127-140.
8. **Krause, R. M., and M. McCarty.** 1962. Studies on the chemical structure of the streptococcal cell wall. II. The compositions of group C cell walls and chemical basis for serologic specificity of the carbohydrate moiety. J. Exp. Med. **115**:49-62.
9. **Lancefield, R. C.** 1933. A serological differentiation of human and other groups of hemolytic streptococci. J. Exp. Med. **57**:571-595.
10. **Pass, M. A., B. M. Gray, S. Khare, and H. D. Dillon.** 1979. Prospective studies of group B streptococcal infections in infants. J. Pediatr. **95**:437-443.
11. **Pritchard, D. G., and W. Niedermeier.** 1978. Sensitive gas chromatographic determination of the monosaccharide composition of glycoproteins using electron capture detection. J. Chromatogr. **152**:487-494.