JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1993, p. 887–894
0095-1137/93/040887-08$02.00/0

Vol. 31, No. 4

Determination of Carbohydrate Profiles of *Bacillus anthracis* and *Bacillus cereus* Including Identification of O-Methyl Methylpentoses by Using Gas Chromatography-Mass Spectrometry

ALVIN FOX,* GAVIN E. BLACK, KAREN FOX, AND SOPHIA ROSTOVTSEVA

Department of Microbiology and Immunology, University of South Carolina, School of Medicine, Columbia, South Carolina 29208

Received 14 September 1992/Accepted 29 December 1992

Bacillus anthracis **and** ***Bacillus cereus*** **are closely related pathogenic organisms that are difficult to differentiate phenotypically or genotypically. It is well known that vegetative and spore forms of bacilli are quite distinct both morphologically and chemically, but spore-specific chemical markers allowing these species to be distinguished have not been previously described. By using gas chromatography-mass spectrometry, vegetative cells and spores of the two species were shown to exhibit distinct carbohydrate profiles. Profiles of vegetative *B. anthracis* typically contained high levels of galactose but did not contain galactosamine, whereas *B. cereus* contained galactosamine and generally low levels of galactose. Spore cultures exhibited unique carbohydrate profiles compared with those of vegetative cultures. *B. anthracis* spore profiles contained rhamnose alone, whereas *B. cereus* spore profiles contained rhamnose and fucose. Additionally, two spore-specific O-methylated methylpentoses were discovered. Both *B. anthracis* and *B. cereus* spores contained 3-*O*-methyl rhamnose, whereas *B. cereus* spores also contained 2-*O*-methyl rhamnose. Carbohydrate profiling is demonstrated to be a powerful tool for differentiating the two closely related species. Differentiation does not depend on whether organisms are in the vegetative or spore stage of growth.**

Many aspects of the clinical identification and taxonomic characterization of bacilli remain unresolved (6). The two major human pathogenic bacilli are *Bacillus anthracis*, the causative agent of anthrax, and *Bacillus cereus*, a food-poisoning organism. Clinical differentiation of these two organisms presents a challenge in that they display few distinguishing physiological characteristics (4, 9, 20). In recent years, there have been great advances in molecular biology- and analytical microbiology-based approaches for the differentiation of bacterial species. *B. anthracis* and *B. cereus* share a high degree of genetic similarity as demonstrated by DNA-DNA hybridization (18). Furthermore, the 16S and 23S rRNA sequences of these two species are almost identical (1, 2). Fatty acid profiles are an important means of differentiating many bacterial species. However, the fatty acid profiles of *B. anthracis* and *B. cereus* are extremely similar (17, 19). Therefore, there is a real need for another reference technique to provide additional parameters for discrimination.

Gas chromatography (GC)-mass spectrometry (MS) has previously been demonstrated to have great utility in the differentiation of legionellae, a fastidious group of gram-negative bacteria. Many of these organisms, like bacilli, are also closely related genetically. Discrimination by GC-MS is based on the identification of whole-bacterial-cell carbohydrate profiles, which are generally representative of a given bacterial species or genus (10, 13). In the present report, this approach was extended to a study of the gram-positive organisms *B. anthracis* and *B. cereus*. Differences in the profiles of cultures of vegetative and spore forms of these organisms were also compared.

* Corresponding author.

MATERIALS AND METHODS

Nonpathogenic strains of *B. anthracis* lacking one or both of the two virulence plasmids were kindly donated by Stephen Leppla, Fort Detrick, Md. ΔNH-1 (containing PX02), NNR-1 (containing PX01), and NNR-1-Δ1 (plasmid free) were derived from strain New Hampshire; Sterne is a veterinary vaccine strain (containing PX02) from which ΔSterne-1 (plasmid free) was derived; ΔTexas-1 is derived from Texas (which contains PX01) and is plasmid free; ΔAMES-1 (containing PX02) and ANR-1 (containing PX01) were both derived from the virulent Ames strain; and VNR-1-Δ1 (plasmid free) was derived from the virulent Vollum strain. Strains of *B. cereus* (1122, 14579, 12826, and 6464) were obtained from the American Type Culture Collection, and strains 6E1 and 6S1 were obtained from the *Bacillus* Genetic Stock Center (Ohio State University).

B. cereus is usually beta-hemolytic, unlike *B. anthracis* (4). All six strains of *B. cereus* used in the present study were hemolytic, and all nine strains of *B. anthracis* were nonhemolytic. One strain of *B. anthracis* (Texas) did exhibit hemolysis after extended growth.

After hemolysis had been determined on sheep blood agar plates, cultures were inoculated into nutrient broth and shaken at 37°C, since these organisms are strict aerobes. Flasks were tightly sealed to avoid aerosolization. Under these conditions, both *B. anthracis* and *B. cereus* were present during the first 6 to 24 h, predominantly as vegetative cells. After 7 to 10 days, cultures contained predominantly spores. The organisms were harvested by centrifugation, washed three times in distilled water, and sterilized by autoclaving. Sporulation was assessed by staining with malachite green or phase-contrast microscopy.

Carbohydrate profiles of bacilli were determined by using a method slightly modified from that previously described

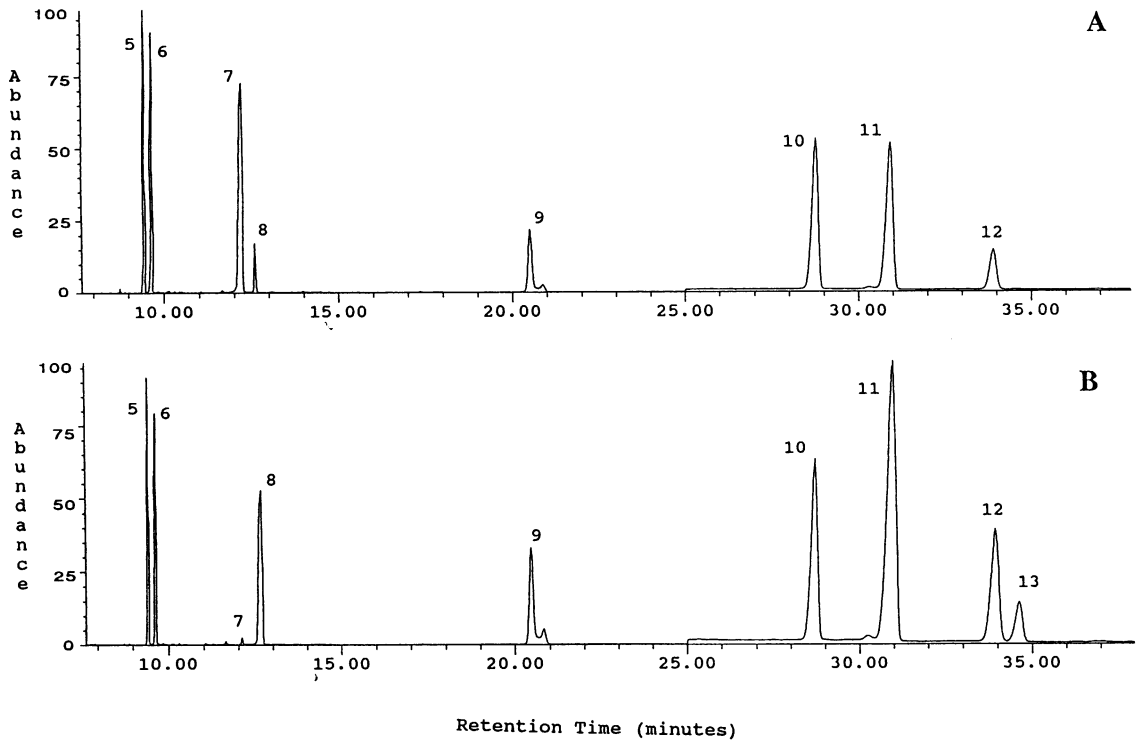

FIG. 1. Carbohydrate profiles of vegetative forms of *B. anthracis* VNR-1-Δ1 (A) and *B. cereus* 1112 (B). Peaks: 1, 2-*O*-methyl methylpentose; 2, 3-*O*-methyl methylpentose; 3, rhamnose; 4, fucose; 5, ribose; 6, arabinose (internal standard); 7, galactose; 8, glucose; 9, muramic acid; 10, methylglucamine (internal standard); 11, glucosamine; 12, mannosamine; 13, galactosamine.

(12). Sodium borodeuteride was substituted for sodium borohydride, allowing differentiation of alditols, aldoses, and ketoses. In brief, sugars were released by hydrolysis in sulfuric acid and converted to alditol acetates. Samples were purified by hydrophobic prederivatization cleanup and hydrophilic postderivatization cleanup. GC-MS analyses were carried out with a mass-selective detector (model 5970; Hewlett-Packard Co., Palo Alto, Calif.) interfaced to a GC (model 5890; Hewlett-Packard) equipped with an automated sample injector and an SP-2330 fused-silica capillary column (12). For GC conditions, the oven was held at 100°C for 1 min postinjection. This temperature was followed by a single ramp of 20°C/min to a final temperature of 245°C, which was held for 30 min. A constant helium flow of 1 ml/min served as the analyte carrier between GC and MS. Electron ionization was performed at 70 eV for both total spectrum scanning and selected ion monitoring. Ions monitored for standard sugars during selected ion runs have been previously described (12).

All sugar standards were purchased except 2-*O*- and 3-*O*-methyl rhamnoses and 2-*O*- and 3-*O*-methyl fucoses, which were not commercially available. O-methylated standards were prepared from rhamnose and fucose. One milligram of sugar was dissolved completely in 100 μl of anhydrous dimethyl sulfoxide (Fluka, Ronkonkoma, N.Y.). The addition of 10 mg of freeze-dried sodium hydroxide powder was followed by vigorous vortexing. Twenty-five microliters of puriss-grade methyl iodide (Fluka) was added to the mixture. The reactions were allowed to proceed at room temperature. Addition of 800 μl of acetic acid (2%) terminated the reaction and decomposed any unreacted methyl iodide. To remove any fully methylated derivatives, each reaction mixture was neutralized and extracted with 1 ml of 50% *N,N*-dioctylmethylamine in chloroform. Deuterated alditol acetates were then prepared from the aqueous phase as described above.

RESULTS

All strains of *B. anthracis* and *B. cereus* were found to contain ribose, glucose, muramic acid, glucosamine, and mannosamine, regardless of cellular form. Strains of vegetative *B. anthracis* typically displayed high levels of galactose but lacked galactosamine, whereas vegetative *B. cereus* contained galactosamine and generally had low levels of galactose (Fig. 1 and Table 1). In *B. anthracis* samples which contained spores (as demonstrated by malachite green staining and dark-field microscopy), rhamnose, galactosamine, and a sugar subsequently identified as 3-*O*-methyl rhamnose were found. *B. cereus* spores contained rhamnose and 3-*O*-methyl rhamnose (as did *B. anthracis*) as well as fucose and 2-*O*-methyl rhamnose. Figure 2 compares selected ion chromatograms of carbohydrates from sporulating cultures of *B. anthracis* and *B. cereus*. Table 2 shows the percent dry weight of each sugar present in spores of the two species.

One of the six strains of *B. cereus* analyzed, 6E1, showed an aberrant profile. The vegetative and spore forms of 6E1 contained galactose (characteristic of *B. anthracis*); the spore form, however, contained fucose and 2-*O*-methyl rhamnose (characteristic of *B. cereus*). The presence of galactose in unusual strains of *B. cereus* has been previously noted, and it has been suggested that these strains may represent a transition form between the two species (9).

At one stage of the alditol acetate procedure, sugars are

TABLE 1. Carbohydrate profiles of vegetative forms of *B. anthracis* and *B. cereus*

Species and strain	% dry wt of sugar[a]										
	2-*O*-Me	3-*O*-Me	Rha	Fuc	Rib	Gal	Glu	Mur	GlcN	ManN	GalN
B. anthracis											
ΔNH-1			0.006		0.578	3.064	1.584	1.262	3.380	0.517	
NNR-1			0.008		0.667	0.441	0.190	1.405	2.969	0.760	
NNR-1Δ1			0.024		1.531	2.294	0.649	1.696	2.821	0.530	
Sterne			0.113		0.832	3.094	1.029	1.009	2.877	0.656	
ΔSterne1		0.035	0.169		1.475	2.924	1.720	1.006	1.803	0.399	0.064
ΔTexas-1			0.006		0.821	3.125	0.816	0.687	2.456	0.585	
ΔAmes-1		0.052	0.256		2.243	3.216	0.643	1.676	2.932	0.583	0.003
ANR-1			0.032		1.604	2.356	0.760	0.678	2.080	0.400	
VNR-1Δ1			0.005		1.268	2.721	0.341	0.978	2.356	0.511	
B. cereus											
12826					1.099	0.030	2.271	1.063	3.975	1.194	1.412
14579					1.270	0.070	1.331	0.675	3.647	0.860	2.815
6464			0.009		2.191	0.026	1.313	0.859	4.027	0.772	1.144
1122			0.006		1.156	0.062	1.664	0.707	5.225	1.083	1.277
6E1	0.038	0.040	0.045		1.064	2.749	0.964	0.594	2.537	0.713	0.009
6S1			0.007		0.300	0.035	1.336	0.787	3.536	0.660	0.772

[a] 2-*O*-Me, 2-*O*-methyl methylpentose; 3-*O*-Me, 3-*O*-methyl methylpentose; Rha, rhamnose; Fuc, fucose; Rib, ribose; Gal, galactose; Glu, glucose; Mur, muramic acid; GlcN, glucosamine; ManN, mannosamine; GalN, galactosamine. The lowest levels reported are three digits beyond the decimal point. Thus, for consistency, all results are presented to three decimal places. Previous studies have indicated that relative standard deviation varies from 5 to 11%, depending on the sugar (26).

heated in methanol-acetic acid to remove borate. It is conceivable that this leads to artifactual O methylation. Substitution of deuterated (for nondeuterated) methanol in the analysis did not introduce deuterated *O*-methyl groups. Thus, *O*-methyl groups were naturally present on certain sugars in bacilli. The identities of the O-methylated sugars present in spores were confirmed by comparison of retention time and mass spectra of partially O-methylated derivatives of the two common methylpentoses, rhamnose and fucose, with the bacterial sugars. The retention times of the 2-*O*- and 3-*O*-methyl rhamnose standards (but not fucose) matched those of the two unidentified *O*-methyl methylpentoses found in spores of the bacilli.

The mass spectra of O-methylated sugars are dominated

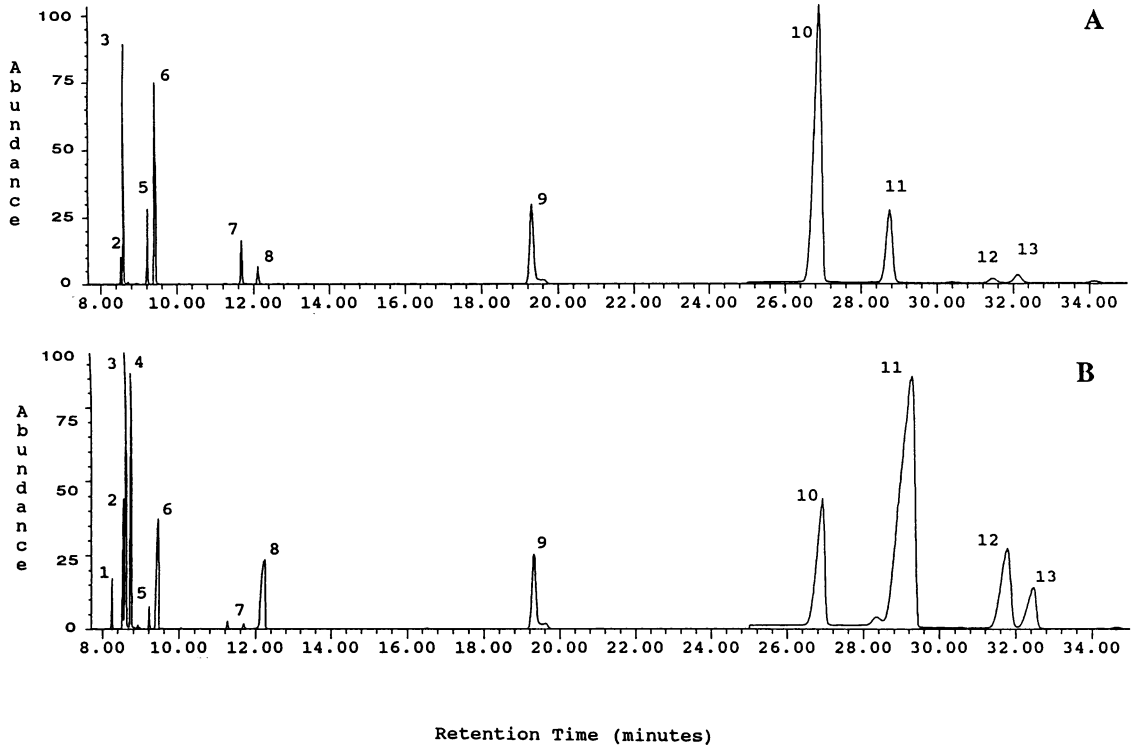

FIG. 2. Carbohydrate profiles of spore forms of *B. anthracis* Ames (A) and *B. cereus* 6S1 (B). See the legend to Fig. 1 for peak identification.

TABLE 2. Carbohydrate profiles of spore forms of *B. anthracis* and *B. cereus*

Species and strain	% dry weight of sugar[a]										
	2-*O*-Me	3-*O*-Me	Rha	Fuc	Rib	Gal	Glu	Mur	GlcN	ManN	GalN
B. anthracis											
ΔNH-1		0.089	0.193		0.812	0.268	0.854	0.957	1.195	0.035	0.143
NNR-1Δ1		0.196	0.979		0.703	2.027	0.616	1.737	2.908	0.334	0.288
ΔSterne-1		0.165	1.018		0.769	0.560	0.313	0.792	0.702	0.040	0.345
ΔAmes-1		0.073	0.593		0.294	0.197	0.130	0.534	0.385	0.012	0.101
ANR-1		0.198	0.866		0.688	3.089	0.591	2.411	3.240	0.522	0.559
VNR-1Δ1		0.107	0.083		0.460	0.932	0.108	0.556	0.834	0.091	0.096
B. cereus											
6464	0.131	0.078	0.378	0.123	0.219	0.028	0.701	0.362	2.071	0.306	0.510
14579	0.418	0.296	0.929	0.549	0.602	0.073	0.394	4.836	1.546	0.099	3.630
1122	0.071	0.049	0.355	0.051	0.234	0.087	0.472	0.229	1.323	0.164	0.471
6E1	0.176	0.338	0.724	0.009	0.216	2.414	0.182	1.634	2.593	0.375	0.019
6S1	0.331	0.322	0.727	0.710	0.094	0.027	1.545	0.761	6.163	1.115	1.604

[a] See Table 1, footnote *a*, for abbreviations.

by breakage of bonds between O-methylated carbon and adjacent O-acetylated carbons. For a deuterated 3-*O*-methyl methylpentose alditol acetate, this breakage would produce *m/z* 203 and 190 as primary fragments (5) (Fig. 3). For a deuterated 2-*O*-methyl methylpentose, the primary fragments are ions *m/z* 118 and 275 (3, 24) (Fig. 4). The 2-*O*- and 3-*O*-methyl sugars were readily observable in selected ion-monitoring chromatograms by monitoring for characteristic ions *m/z* 130 (a prominent secondary fragment of *m/z* 190 by loss of acetic acid) and 118. The mass spectra of 3-*O*-methylated derivatives of rhamnose and fucose can be readily differentiated (Fig. 3), in agreement with published reports (5, 24). Thus, comparison of the mass spectrum of a 3-*O*-methyl rhamnose standard with the 3-*O*-methyl methylpentose present in bacilli provided additional confirmation of the identity of the sugar. The mass spectra of 2-*O*-methyl rhamnose and 2-*O*-methyl fucose could not be differentiated in this fashion. Thus, identification of 2-*O*-methyl rhamnose in *B. cereus* was based on retention time alone.

By observation of mass spectra, all sugars present in bacilli were proven to be aldoses (as opposed to alditols). When sodium borodeuteride rather than sodium borohydride is used as the reducing agent, aldoses (on conversion to alditols) gain two deuteriums, one of which remains after acetylation. Thus, aldoses and alditols (after conversion to alditol acetates) can be distinguished by *m/z* differences of 1. For example, mannosamine, which is present in bacilli, contained *m/z* 85 and 145 as predominant ions, confirming its identity as an aldose (Fig. 5).

DISCUSSION

The degree of variability in composition of cellular polysaccharides among strains of *B. anthracis* and *B. cereus* has not previously been assessed. There have also been few investigations of the carbohydrate composition of the vegetative and spore forms of bacilli, even though there are other major chemical differences. For example, in the spore but not in the vegetative form, muramic acid of the peptidoglycan exists as a lactam (25). Additionally, dipicolinic acid (involved in heat resistance) is present only in the spore (23). The current study was thus undertaken to distinguish the whole-cell carbohydrate profiles of multiple strains of both the vegetative and the spore states of *B. anthracis* and *B. cereus* by employing GC-MS.

B. anthracis and *B. cereus* were shown in the present study to exhibit quite distinct carbohydrate profiles. Both contain ribose, glucose, muramic acid, glucosamine, and mannosamine. RNA components of the bacilli yield ribose, whereas muramic acid and some of the glucosamine are peptidoglycan components. The additional sugars are presumably released cell wall polysaccharide components. Vegetative *B. anthracis* is characterized by large amounts of galactose, and *B. cereus* is characterized by galactosamine. Spores of both organisms are distinct from vegetative cells (containing rhamnose and 3-*O*-methyl rhamnose). The spore form of *B. anthracis* additionally contains galactosamine. The spore form of *B. cereus* is quite distinct in containing fucose and 2-*O*-methyl rhamnose. Such O-methylated sugars are uncommon but have previously been described in mycobacteria (3) and annelid worms (24). O-methylated sugars have not previously been described in *B. anthracis* or *B. cereus*.

Although there have been few extensive investigations of the polysaccharide content of these bacilli, the presence of a polysaccharide containing galactose and glucosamine in *B. anthracis* has been known for many years (15). Indeed, *B. anthracis* can be differentiated from *B. cereus* by the presence of galactose-using lectins (7) or monoclonal antibodies (9). Only recently has the *B. anthracis* polysaccharide been analyzed by modern analytical methods. The polysaccharide of strain ΔSterne contains galactose, mannosamine, and glucosamine but not galactosamine (8). *B. cereus* AHU 1356 has been reported to contain two polysaccharides, one consisting of glucose, glucosamine, galactosamine, and mannosamine but lacking rhamnose, and a second containing rhamnose and glucosamine (22). The exosporium of the terminalis strain of *B. cereus* has also been noted to contain rhamnose, glucose, and glucosamine (21). Our observations entirely agree with and greatly extend these observations.

B. anthracis contains two virulence plasmids, PX01 and PX02. PX01 encodes the lethal toxin, while PX02 codes for genes involved in synthesis of the polyglutamic acid capsule necessary for survival in the host (16). One method of clinical identification of *B. anthracis* infections is to demonstrate the production of lethal toxin components (14). It is

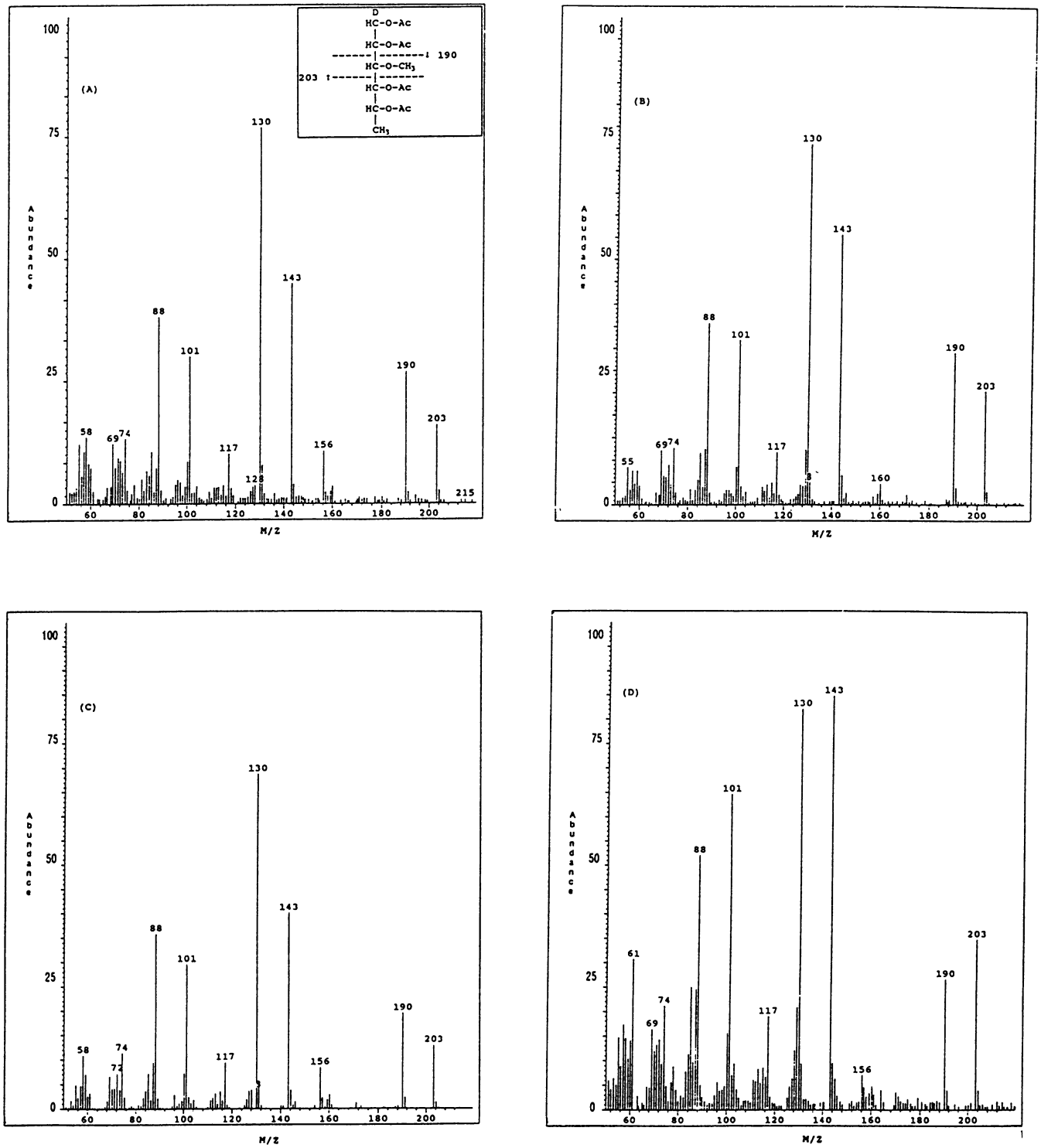

FIG. 3. Mass spectra of 3-*O*-methyl methylpentose from *B. anthracis* spore (A), 3-*O*-methyl methylpentose from *B. cereus* spore (B), 3-*O*-methyl rhamnose standard (C), and 3-*O*-methyl fucose standard (D) as deuterated alditol acetates.

desirable to have approaches to differentiate these species which do not depend on the presence of plasmids, which may be lost on culture. Carbohydrate profiles of *B. anthracis* were demonstrated not to be affected by the presence or absence of virulence plasmids.

Physiologically and chemically, these two species differ in few characteristics (9). For example, motility is often noted as a differentiating quality. Strains of *B. anthracis* are often nonmotile, while those of *B. cereus* are generally motile. However, technical limitations and the uncertainty of motil-

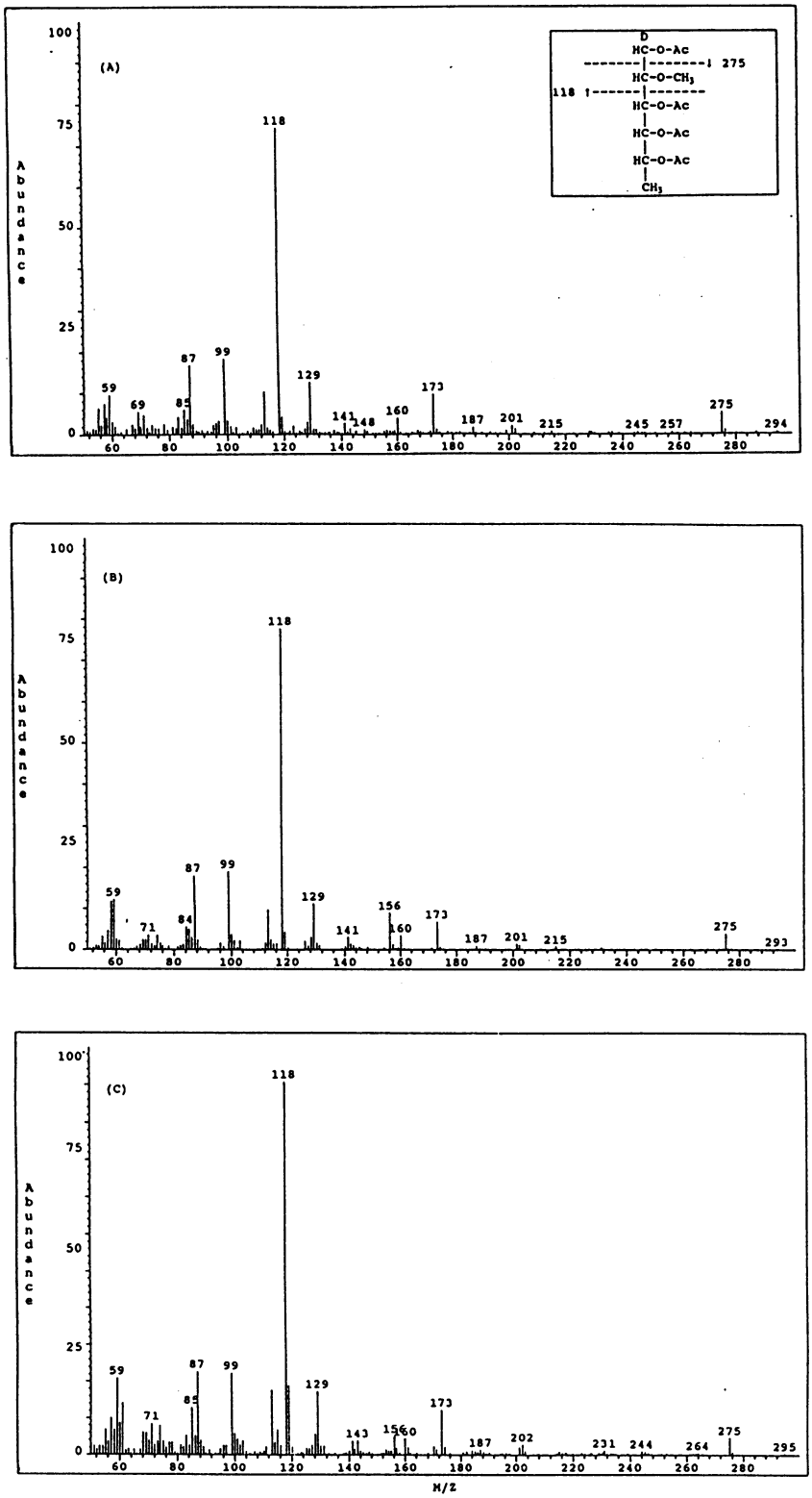

FIG. 4. Mass spectra of 2-*O*-methyl methylpentose from *B. cereus* spore (A), 2-*O*-methyl rhamnose standard (B), and 2-*O*-methyl fucose standard (C) as deuterated alditol acetates.

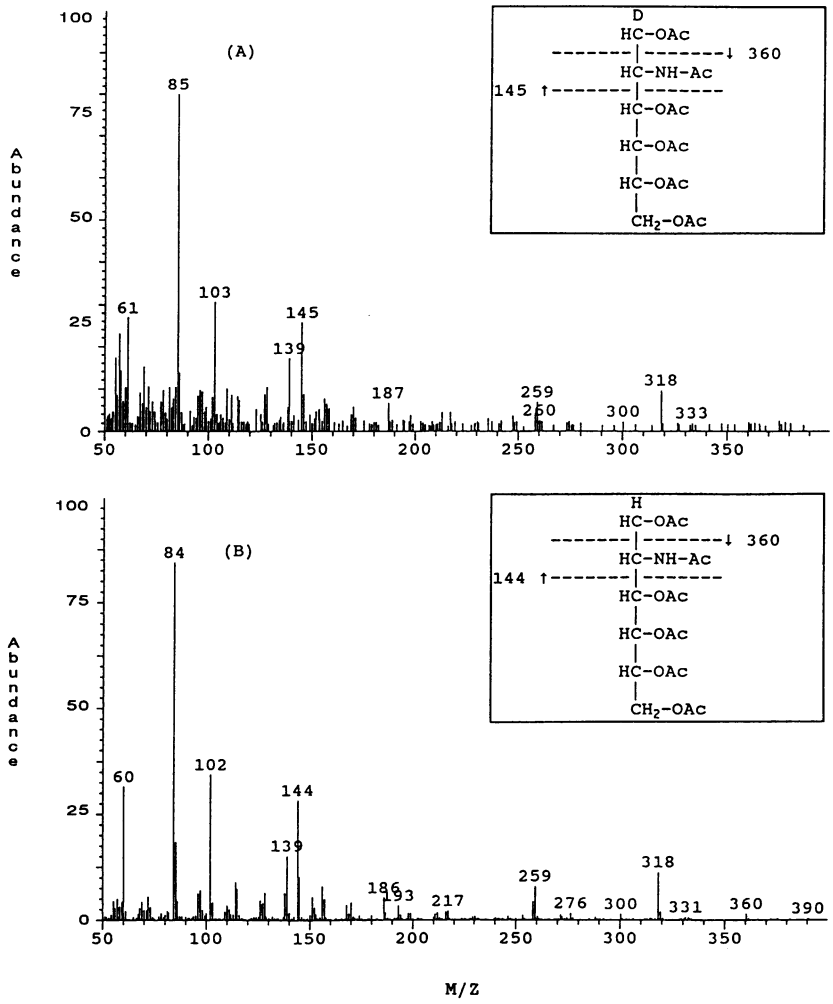

FIG. 5. Mass spectra of mannosamine from *B. anthracis* vegetative form as a deuterated alditol acetate (A) and mannosamine standard as alditol acetate (B).

ity as a differential criterion suggest that motility be eliminated as a diagnostic characteristic (4). It has been suggested that these organisms can be differentiated by using physiological tests, including fermentation patterns and growth characteristics (20). However, this scheme has not been widely accepted in clinical laboratories. Identical cellular fatty acids are also present in both species (17, 19). It has been suggested that after growth under carefully defined conditions, relative proportions of cellular fatty acids as determined by GC allow some discrimination of these organisms (19).

In many cases, bacterial species are readily differentiated by carbohydrate profiling. However, in some instances, species can have indistinguishable profiles, as demonstrated in a recent study of legionellae (13). Unusual sugars are often found in bacteria and are readily identified by GC-MS. Once identification has occurred, it is possible to use GC alone without MS for species differentiation (11, 13). Using GC in conjunction with MS allows chromatograms clear of background interference to be produced. Although GC-MS is preferred over GC, the cost may be prohibitive for many laboratories. Therefore, GC alone is a practical, economical alternative. Carbohydrate profiling provides an important means of differentiating *B. anthracis* and *B. cereus* and has great potential for differentiating many other bacterial species.

ACKNOWLEDGMENTS

This work was supported by Army Research Office grant DAAL03-92-0255 and by Center for Indoor Air Research contract 92-03A. Gavin E. Black was supported by a training award from the Army Research Office DoD EPSCoR program.

REFERENCES

1. **Ash, C., and M. D. Collins.** 1992. Comparative analysis of 23S ribosomal RNA gene sequences of *Bacillus anthracis* and emetic *Bacillus cereus* determined by PCR-direct sequencing. FEMS Microbiol. Lett. **94**:75–80.
2. **Ash, C., J. A. E. Farrow, M. Dorsch, E. Stackebrandt, and M. D. Collins.** 1991. Comparative analysis of *Bacillus anthracis*, *Bacillus cereus* and related species on the basis of reverse transcriptase sequencing of 16S rRNA. Int. J. Syst. Bacteriol. **41**:343–346.
3. **Brennan, P. J., H. Mayer, G. O. Aspinall, and J. E. Nam Shin.** 1981. Structures of the glycopeptidolipid antigens from serovars

in the *Mycobacterium avium/Mycobacterium intracellulare/Mycobacterium scrofulaceum* serocomplex. Eur. J. Biochem. **115:** 7–15.

4. **Burdon, K. L.** 1956. Useful criteria for the identification of *Bacillus anthracis* and related species. J. Bacteriol. **71:**25–42.
5. **Carpita, N., and E. Shea.** 1989. Linkage structure of carbohydrates by gas chromatography-mass spectrometry (GC-MS) of partially methylated alditol acetates, p. 157–216. *In* C. Biermann and G. McGinnis (ed.), Analysis of carbohydrates by GLC and MS. CRC Press, Inc., Boca Raton, Fla.
6. **Claus, D., and D. Fritze.** 1989. Taxonomy of *Bacillus*, p. 5–26. *In* C. Harwood (ed.), *Bacillus*. Plenum Press, New York.
7. **Cole, H. B., J. W. Ezzell, Jr., K. F. Keller, and R. J. Doyle.** 1984. Differentiation of *Bacillus anthracis* and other *Bacillus* species by lectins. J. Clin. Microbiol. **19:**48–53.
8. **Ekwunife, F. S., J. Singh, K. G. Taylor, and R. J. Doyle.** 1991. Isolation and purification of cell wall polysaccharide of *Bacillus anthracis* (Δ Sterne). FEMS Microbiol. Lett. **82:**257–262.
9. **Ezzell, J. W., T. G. Abshire, S. F. Little, B. C. Lidgerding, and C. Brown.** 1990. Identification of *Bacillus anthracis* by using monoclonal antibody to cell wall galactose-*N*-acetylglucosamine polysaccharide. J. Clin. Microbiol. **28:**223–231.
10. **Fox, A., J. Gilbart, and S. Morgan.** 1990. Analytical microbiology: a perspective, p. 1–17. *In* A. Fox, L. Larsson, S. Morgan, and G. Odham (ed.), Analytical microbiology methods: chromatography and mass spectrometry. Plenum Press, New York.
11. **Fox, A., P. Y. Lau, A. Brown, S. L. Morgan, Z.-T. Zhu, M. Lema, and M. D. Walla.** 1984. Capillary gas chromatographic analysis of carbohydrates of *Legionella pneumophila* and other members of the family *Legionellaceae*. J. Clin. Microbiol. **19:**326–332.
12. **Fox, A., S. L. Morgan, and J. Gilbart.** 1989. Preparation of alditol acetates and their analysis by gas chromatography and mass spectrometry, p. 87–117. *In* C. Biermann and G. McGinnis (ed.), Analysis of carbohydrates by GLC and MS. CRC Press, Inc., Boca Raton, Fla.
13. **Fox, A., J. Rogers, K. Fox, G. Schnitzer, S. Morgan, A. Brown, and R. Aono.** 1990. Chemotaxonomic differentiation of legionellae by detection and characterization of aminodideoxyhexoses and other unique sugars using gas chromatography-mass spectrometry. J. Clin. Microbiol. **28:**546–552.
14. **Harrison, L. H., J. W. Ezzell, Veterinary Laboratory Investigation Center, T. G. Abshire, S. Kidd, and A. F. Kaufmann.** 1989. Evaluation of serologic tests for diagnosis of anthrax after an outbreak of cutaneous anthrax in Paraguay. J. Infect. Dis. **160:**706–710.
15. **Ivanovics, G.** 1940. Untersuchungen über das Polysaccharid der Milzbrandbazillen. Z. Immunitaetsforsch. Exp. Ther. **97:**402–423.
16. **Ivins, B. E., J. W. Ezzell, Jr., J. Jemski, K. W. Hedlund, J. D. Ristroph, and S. H. Leppla.** 1986. Immunization studies with attenuated strains of *Bacillus anthracis*. Infect. Immun. **52:**454–458.
17. **Kaneda, T.** 1968. Fatty acids in the genus *Bacillus*. II. Similarity in the fatty acid compositions of *Bacillus thuringiensis*, *Bacillus anthracis*, and *Bacillus cereus*. J. Bacteriol. **95:**2210–2216.
18. **Kaneko, T., R. Nozaki, and K. Aizawa.** 1978. Deoxyribonucleic acid relatedness between *Bacillus anthracis*, *Bacillus cereus* and *Bacillus thuringiensis*. Microbiol. Immunol. **22:**639–641.
19. **Lawrence, D., S. Heitefuss, and H. S. H. Seifert.** 1991. Differentiation of *Bacillus anthracis* from *Bacillus cereus* by gas chromatographic whole-cell fatty acid analysis. J. Clin. Microbiol. **29:**1508–1512.
20. **Logan, N. A., J. A. Carman, J. Melling, and R. C. W. Berkeley.** 1985. Identification of *Bacillus anthracis* by API tests. J. Med. Microbiol. **20:**75–85.
21. **Matz, L. L., T. Cabrera Beaman, and P. Gerhardt.** 1970. Chemical composition of exosporium from spores of *Bacillus cereus*. J. Bacteriol. **101:**196–201.
22. **Murazumi, N., Y. Araki, and E. Ito.** 1986. Biosynthesis of the wall neutral polysaccharide in *Bacillus cereus* AHU 1356. Eur. J. Biochem. **161:**51–59.
23. **Murrel, W. G.** 1969. Chemical composition of spores and spore structures, p. 215–269. *In* G. W. Gould and A. Hurst (ed.), The bacterial spore, vol. 1. Academic Press, Inc., New York.
24. **Talmont, F., and B. Fournet.** 1991. Isolation and characterization of methylated sugars from the tube of the hydrothermal vent tubiculous annelid worm *Alvinella pompejana*. FEBS Lett. **281:**55–58.
25. **Warth, A. D., and J. L. Strominger.** 1972. Structure of the peptidoglycan from spores of *Bacillus subtilis*. Biochemistry **11:**1389–1396.
26. **Whiton, R. S., P. Lau, S. L. Morgan, J. Gilbart, and A. Fox.** 1985. Modifications in the alditol acetate method for analysis of muramic acid and other neutral and amino sugars by capillary gas chromatography-mass spectrometry with selected ion monitoring. J. Chromatogr. **347:**109–120.