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Chemotaxonomic Differentiation of Legionellae by Detection and Characterization of Aminodideoxyhexoses and Other Unique Sugars Using Gas Chromatography-Mass Spectrometry

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Legionellae have been differentiated previously by analyzing their carbohydrate contents by gas chromatography with flame ionization detection. In the present study, total ion mode gas chromatography-mass spectrometry (GC-MS) was used to detect a number of unusual sugars, including one that is structurally related to *O*-methyldideoxyheptoses. Increased sensitivity and selectivity for carbohydrate detection was achieved by selected ion-monitoring GC-MS. Two of the uncommon sugars previously discovered in the legionellae (X1 and X2) were identified as quinovosamine and fucosamine, respectively. *Legionella pneumophila* contained rhamnose and quinovosamine but not the quinovosamine isomer fucosamine. *Tatlockia micdadei* and *Legionella maceachernii* contained large amounts of rhamnose, fucose, and fucosamine but not quinovosamine. These two species were the only legionellae studied that contained another unusual sugar that is referred to as X3, pending determination of its structure. *Fluoribacter dumoffii*, *Fluoribacter bozemanae*, and *Legionella anisa* were varied in their carbohydrate contents, both within and between species, but could be distinguished from *L. pneumophila* and the *T. micdadei* and *L. maceachernii* group. *Fluoribacter gormanii* was unique among the legionellae in that it lacked both quinovosamine and fucosamine. *Legionella jordanis* contained other unusual carbohydrates in addition to quinovosamine. GC-MS may have wide application in the differentiation of bacterial species.

The value of chromatographic profiling of organic components for bacterial identification is well established (18). By using gas chromatography (GC) with flame ionization detection (FID), fatty acids are the most commonly studied compounds used for microbial differentiation. The legionellae are easily recognized by this technique because of their unusual branched chain and hydroxy fatty acid compositions (16, 17). In addition, some species of legionellae are readily identified by visual inspection of their fatty acid chromatographic profiles; however, many *Legionella* species have similar compositions.

Members of the family *Legionellaceae* include 28 named species (20), with 45 serogroups and 3 subspecies (*Legionella pneumophila* subsp. *pneumophila*, *L. pneumophila* subsp. *fraseri*, and *L. pneumophila* subsp. *pascullei*) (4). *L. pneumophila* is the primary etiologic agent of Legionnaires disease, and *Tatlockia* (*Legionella*) *micdadei* is responsible for more than half of the legionellosis cases caused by members of this group of organisms other then *L. pneumophila* (8). Other species, predominantly of environmental origin, may occasionally cause opportunistic infections. Among these opportunistic pathogens, some species have been grouped in the proposed genus *Fluoribacter* (13). The systematics of this bacterial family is still being investigated. There are a number of simple biochemical tests and phenotypic characteristics which can distinguish species or groups of species among the legionellae. A battery of such tests has recently been evaluated for the clinical identification of these organisms, and a numerical algorithm has been developed (11).

Carbohydrate analysis by GC has been used to characterize legionellae (9). For example, the major pathogens (*L. pneumophila* and *T. micdadei*) are readily differentiated by their content of methylpentoses and aminodideoxyhexoses (21). Structural sugars have not been used extensively for the identification of other microbial groups. Sugars, in addition to their multiple hydroxyl groups, contain aldehyde or ketone moieties and, often, additional substituents such as amino groups. For GC (or mass spectrometric [MS]) analysis these groups must be in a form that allows volatility and minimizes interactions with the analytical system. Recently, these methods have been simplified and the manual steps have begun to be automated (10).

Unusual sugars, for which commercial standards are often not available and which may be present in trace amounts, are difficult to identify and quantitate by GC with only FID. Bench-top GC-MS systems, which are more sensitive, selective, relatively inexpensive, and simple to use, have only recently become available. Following a GC separation, MS can be used in the total ion mode to detect and identify sugars. In the selected-ion mode, chromatograms that are clear of background interference are produced, and these are easy to interpret and allow major components to be detected reliably (18).

After analysis of the carbohydrates of a limited number of strains, the superiority of GC-MS over GC with FID was established (14, 21). The purpose of the present study was to determine the utility of GC-MS (in the total ion mode) for the

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detection and characterization of unusual or low-abundance sugars from 26 strains representing eight species of the legionellae and to demonstrate the usefulness of selected ion monitoring for microbial identification.

MATERIALS AND METHODS

Cultures. The following strains (superscript 1's indicate environmental isolates; superscript 2's indicate clinical isolates) were studied: *L. pneumophila* Philadelphia-1[2] (serogroup 1), Los Angeles-1[1] (serogroup-4; *L. pneumophila* subsp. *fraseri*), Chicago-2[2] (serogroup 6), Dallas-1E[1] (serogroup-5, subspecies fraseri), Denver-1[1] (serogroup 1), and WAL[2] (serogroup-1); *T. micdadei* PPA-EK[2], PPA-PGH-12[1], PPA-JC[2], Tatlock[2], PPA-LR[2], and PPA-QUIG[2]; *Legionella maceachernii* PX-1-G2-E2[2]; *Fluoribacter* (*Legionella*) *dumoffii*, OIE[2], TEX-KL[2], and NY-23[1]; *Fluoribacter bozemanae* WIGA[2] (serogroup 1), MI-15[2] (serogroup 1), and Toronto-3[2] (serogroup 2); *Fluoribacter gormanii* LS-13[1]; *Legionella anisa* CH-47-C1[1], CH-47-C3[1], WA-316-C3[1], and E327-F[1]; and *Legionella jordanis* ABB9[1] and BL-540[1].

Legionellae were grown for 48 to 72 h on buffered charcoal yeast extract agar supplemented with α-ketoglutaric acid. Sufficient bacterial growth was harvested and suspended in 10 ml of supplemented buffered yeast extract broth to produce the turbidity of a McFarland no. 8 standard. Half of this suspension was inoculated into 350 ml of yeast extract broth, which was then incubated, with shaking, for 16 to 18 h at 37°C for exponential-phase growth. In some instances, duplicate flasks were incubated for 30 to 36 h to obtain stationary-phase organisms. The growth phase of the cultures was determined by observing their turbidity. Cells were harvested, washed, suspended in water, evaluated for contamination, placed in a boiling water bath for 1 h, and then lyophilized.

Carbohydrate analysis. Samples were analyzed by a modified alditol acetate procedure (10). In brief, 2 to 10 mg of each sample was hydrolyzed in 2 N sulfuric acid. The mixtures were neutralized with *N,N*-dioctylmethylamine (Fluka Chemical, Hauppauge, N.Y.). The aqueous phase was passed first through a C_{18} column (Analytichem, Harbor City, Calif.) to remove hydrophobic contaminants and then it was reduced with sodium borohydride. Acetic acid-methanol (1:200; vol/vol) was added several times, and the sample was dried to remove borate, which otherwise inhibits the acylation reaction. The samples were acylated with acetic anhydride (Alltech, Deerfield, Ill.) at 100°C overnight and then extracted with acid and alkaline solutions to remove polar contaminants. A mixture of deoxyribose, rhamnose, fucose, ribose, xylose, mannose, galactose, glucose, inositol, mannoheptulose, muramic acid, glucosamine, mannosamine, and galactosamine was used as an external standard. Mannoheptulose served as a standard for heptoses; both heptoses and heptuloses produce heptitols on reduction. A mixture of arabinose and methylglucamine was used as an internal standard.

GC-MS. GC-MS analyses were carried out with a mass-selective detector (5970; Hewlett-Packard Co., Palo Alto, Calif.) that was interfaced to a gas chromatograph (5890; Hewlett-Packard) equipped with an automated sample injector (7673A; Hewlett-Packard) and a SP-2330 fused-silica capillary column (Supelco, Bellefonte, Pa.) with splitless GC injection. The initial temperature of 100°C was held for 8.8 min, was increased at a rate of 20°C/min to 230°C, and was then increased at a rate of 5°C/min to a final temperature of 265°C. The injector temperature was 250°C and the mass-spectrometer-interface temperature was 280°C. The ions that were monitored and the retention time windows were as follows: 8.0 to 9.8 min, *m/z* 201.00 (deoxyribose, rhamnose, and fucose); 9.8 to 11.8 min, *m/z* 217.15 (ribose, arabinose, and xylose); 11.8 to 14.8 min, *m/z* 155.00 (X3), *m/z* 289.05 (hexoses), and *m/z* 209.95 (inositol); 14.8 to 17.0 min, *m/z* 258.90 (heptoses) and *m/z* 242.00 and 201.00 (aminodideoxyhexoses); 17.0 to 20.0 min, *m/z* 167.90 (muramic acid); 20.0 to 29.5 min, *m/z* 169.95 (methylglucamine) and *m/z* 318.05 (aminohexoses). Automated sample injection and macroprogramming on the workstation (Hewlett-Packard) automated the GC-MS analysis and the generation of integration reports.

RESULTS AND DISCUSSION

Ribose, mannose, glucose, muramic acid, and glucosamine were found in all legionellae and therefore were not useful diagnostically. Ribose was probably derived from RNA, muramic acid and some glucosamine presumably originated from peptidoglycan, and the rest of the glucosamine was likely released from the group antigen polysaccharide. As noted above, legionellae did not contain heptoses, which are usually found in gram-negative lipopolysaccharide. Deoxyribose (derived from DNA) was not detectable under the hydrolysis conditions that we used (9).

Two unusual sugars (X1 and X2) were identified in the legionellae by their mass spectra and chromatographic retention times (9, 21). The lipopolysaccharides (group antigens) of *L. pneumophila* serogroups 1 to 4 have since been found to contain the unusual aminodideoxyhexose, previously referred to as X1, as well as rhamnose, mannose, and glucosamine (19). Recently, the X1 present in the lipopoly-

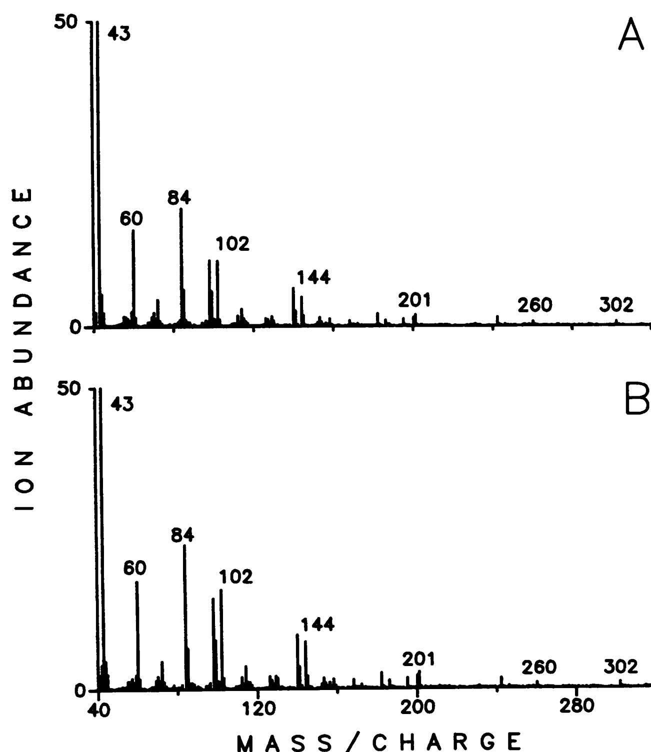

FIG. 1. Mass spectra of the alditol acetate of fucosamine separated from *T. micdadei* PPA-PGH12 (A) and an alkalophilic bacillus (strain C-125) teichuronic acid (B).

TABLE 1. Carbohydrate content of *L. pneumophila*

L. pneumophila	% (dry wt) of the following sugar components[a]:						
	Rha	Rib	Man	Glu	QuiN	Mur	GluN
Chicago-2	0.37	2.67	0.27	0.01	0.18	0.23	0.83
Dallas-1E	0.31	1.64	0.21	0.02	0.16	0.18	0.77
Philadelphia-1	0.37	1.28	0.32	0.03	0.15	0.29	0.65
Los Angeles-1	0.31	1.81	0.36	0.12	0.26	0.09	0.36
Denver-1	0.28	1.50	0.27	tr	0.19	0.09	0.30
WAL	0.29	1.79	0.21	0.01	0.25	0.05	0.32

[a] See Fig. 2 legend for sugar component abbreviation definitions.

saccharide of a strain of *L. pneumophila* was identified as quinovosamine (2-amino-2,6-dideoxyglucose) (A. Sonnesson, E. Jantzen, K. Bryn, L. Larsson, and J. Eng, Arch. Microbiol., in press). A related compound, fucosamine (2-amino-2-deoxyfucose or 2-amino-2,6-dideoxygalactose), was detected in a number of bacterial species and is present in the teichuronic acid of a particular alkalophilic *Bacillus* strain (1). This teichuronic acid was analyzed and found to contain a peak with the same retention time and mass spectrum as X2, thereby establishing its identity as fucosamine. Figure 1 compares the mass spectrum of fucosamine from the *Bacillus* teichuronic acid with that present in *T. micdadei* whole cells.

Table 1 summarizes carbohydrate content data for six strains of *L. pneumophila*, and Fig. 2 presents selected ion-monitoring chromatograms for three strains of *L. pneumophila*. Figure 2 illustrates that the carbohydrate profiles of different strains of *L. pneumophila* are essentially identical. We previously studied nine strains of *L. pneumophila*, including Philadelphia-1 (9, 21). In the present study, Philadelphia-1 was reanalyzed, along with five additional strains. In agreement with results of our previous studies (9, 21), all *L. pneumophila* strains tested contained rhamnose and quinovosamine, as well as several common sugars, while fucosamine and fucose were absent. In all, 13 strains of *L. pneumophila* representing both clinical and environmental isolates, six serogroups, and two of the three subspecies (*L. pneumophila* subsp. *pneumophila* and *L. pneumophila* subsp. *fraseri*) have essentially identical carbohydrate profiles.

Carbohydrate content data for six strains of *T. micdadei* and the single strain of *L. maceachernii* are provided in Table 2. Selected ion-monitoring chromatograms of a strain of *T. micdadei* and the strain of *L. maceachernii* are shown in Fig. 3. Both species contained large amounts of rhamnose and fucose. Fucosamine was present, but quinovosamine was not. Another unusual sugar (referred to as X3) with a retention time slightly less than that of mannose was detected in all six strains of *Tatlockia* as well as in *L. maceachernii*.

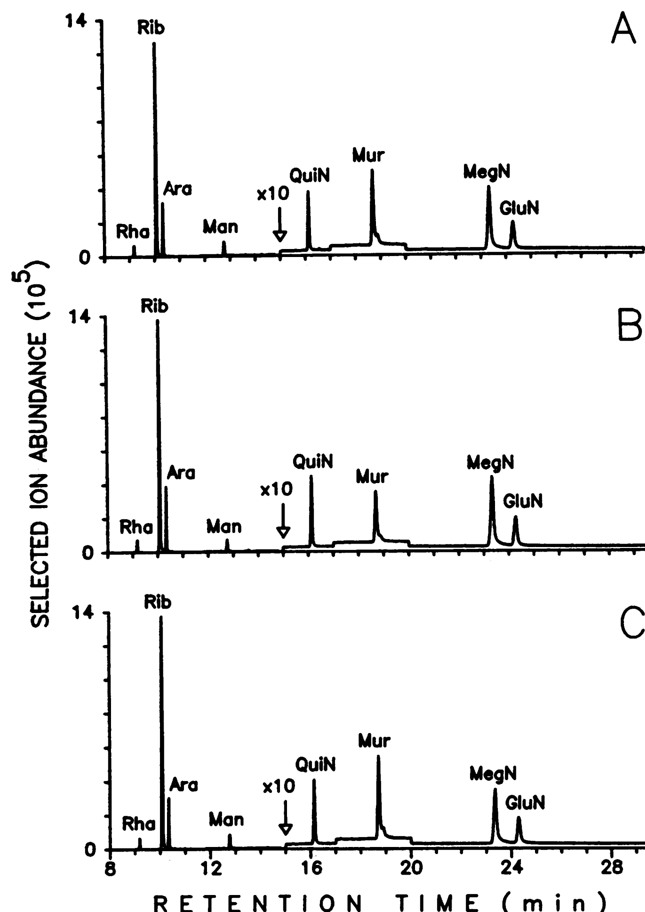

FIG. 2. Selected-ion chromatograms of alditol acetates of whole-cell hydrolysates of *L. pneumophila* Denver-1 (A), WAL (B), and Los Angeles-1 (C). Peak identifications are abbreviated as follows: Rha, rhamnose; Fuc, fucose; Rib, ribose; Ara, arabinose (internal standard); X3, *O*-methyldideoxyheptose; Man, mannose; Glu, glucose; QuiN, quinovosamine; FucN, fucosamine; Mur, muramic acid; MegN, methylglucamine (internal standard); GluN, glucosamine.

The ammonia chemical ionization mass spectrum of X3 indicated that the molecular weight of this compound is 362, based on the assumption that the ion at *m/z* 380 represented $M + NH_4^+$. The mass spectrum did not resemble that of common neutral or amino sugars. The compound, with a molecular weight of 362, was related in structure to the alditol acetate of an *O*-methyldideoxyheptose for the following reasons. In the electron impact mass spectrum, the largest mass observed was at *m/z* 275, which may have

TABLE 2. Carbohydrate content of *T. micdadei* and *L. maceachernii*

Species and strain	% (dry wt) of the following sugar components[a]:								
	Rha	Fuc	Rib	X3	Man	Glu	FucN	Mur	GluN
T. micdadei PPA-JC	2.01	1.80	1.54	0.29	0.28	0.08	0.07	0.27	0.42
T. micdadei PPA-PGH12	1.76	1.74	1.31	0.38	0.32	0.07	0.07	0.35	0.51
T. micdadei Tatlock	1.82	1.60	1.54	0.45	0.24	0.09	0.15	0.24	0.30
T. micdadei PPA-LR	3.35	3.11	2.70	0.45	0.41	0.08	0.26	0.10	0.29
T. micdadei PPA-QUIG	3.32	2.11	3.12	0.47	0.42	0.15	0.25	0.10	0.23
T. micdadei PPA-EK	2.82	2.32	3.13	0.46	0.33	0.37	0.20	0.11	0.18
L. maceachernii PX-1-G2-E2	2.17	1.89	2.21	0.72	0.55	0.10	0.16	0.29	0.49

[a] See Fig. 2 legend for sugar component abbreviation definitions.

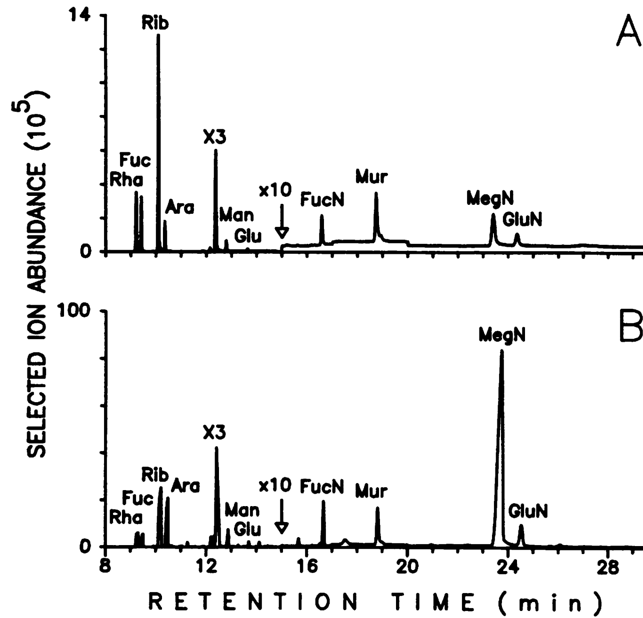

FIG. 3. Selected-ion chromatograms of alditol acetates of whole-cell hydrolysates of *T. micdadei* PPA-LR (A) and *L. maceachernii* (B). For peak identifications, see the legend to Fig. 2.

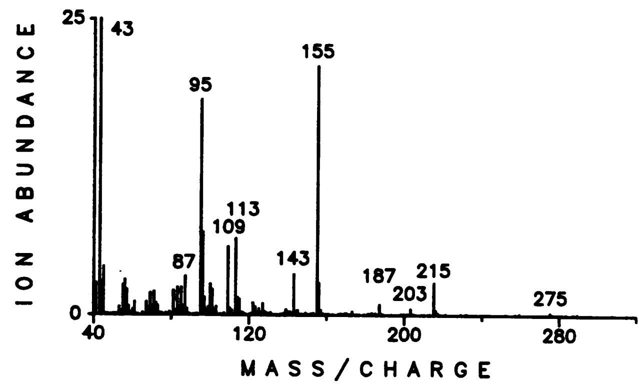

FIG. 4. Mass spectrum of the alditol acetate of X3 present in *T. micdadei* Tatlock.

resulted from primary cleavage between C-2 and C-3, and produced a complementary fragment ion of m/z 87. Losses of acetic acid (m/z 60) from a fragment ion of m/z 275 would produce the fragment ions at m/z 215, 155, and 95. The fragment at m/z 113 would result from the loss of ketene from the m/z 155 ion. The ion at m/z 203 represented a four-carbon-chain fragment, with two carbons being diacetylated, the third being a deoxy carbon, and the fourth being *O*-methylated. The m/z 143 ion would result from the loss of acetic acid from the m/z 203 ion. *O*-Methylated sugars are common among the mycobacteria, and the electron impact mass spectrum of the alditol acetate of a mycobacterial di-*O*-methylated deoxyhexose also contained prominent ions at m/z 203 and 143 (2). The electron impact mass spectrum of X3 is shown in Fig. 4; its structure is under investigation. X3 was not found in any other species of the legionellae studied.

In an earlier study (9) of seven strains of *T. micdadei*, aminodideoxyhexoses were not detected by GC with FID. Subsequently, GC-MS analysis of a single strain of *T. micdadei* (Tatlock) demonstrated the presence of fucosamine (21). In the present study, six strains of this species were analyzed by GC-MS along with the type strain of *L. maceachernii* because of its genotypic and phenotypic similarity to *T. micdadei* (3, 11). These strains contained fucosamine, but not quinovosamine. With the exception of the previously mentioned difference in fucosamine detection, the carbohydrate profiles obtained in the two studies were identical.

Of three strains of *F. (Legionella) dumoffii*, three strains of *F. bozemanae*, and four strains of *L. anisa*, seven strains contained both quinovosamine and fucosamine (Table 3 and Fig. 5). In the remaining three strains, only quinovosamine was detected. For the strains NY-23, WIGA, MI-15, and E327-F, the quinovosamine and fucosamine content was consistent between the present and the previous (9) studies. The only inconsistency noted between the two studies was that neither quinovosamine nor fucosamine was detected in *F. gormanii* LS-13 in this study, while it was previously reported to contain the lowest levels of quinovosamine and fucosamine of all the legionellae tested. We attribute this discrepancy to the better selectivity of GC-MS compared with that of GC with FID.

The carbohydrate profiles of two strains of *L. jordanis* are shown in Table 4, and a chromatogram of an *L. jordanis* strain is shown in Fig. 6. Both strains of *L. jordanis* had similar profiles. Quinovosamine was present; but rhamnose, fucose, fucosamine, and X3 were absent. A peak with a retention time slightly longer than that of fucose was ob-

TABLE 3. Carbohydrate content of *Fluoribacter* species and *L. anisa*

Species and strain	% (dry wt) of the following sugar components[a]:								
	Rha	Fuc	Rib	Man	Glu	QuiN	FucN	Mur	GluN
F. dumoffii OIE	0.02		1.46	0.21	0.02	0.30		0.27	0.66
F. dumoffii TEX-KL	0.12	0.12	1.38	0.47	0.03	0.18	0.39	0.23	0.46
F. dumoffii NY-23			2.30	0.24		1.09		0.21	0.82
F. bozemanae WIGA	0.14	0.20	1.40	0.39	0.01	0.10	0.21	0.10	0.22
F. bozemanae MI-15	0.21	0.27	1.41	0.32	0.02	0.21	0.44	0.16	0.32
F. bozemanae Toronto-3			4.42	0.34		0.54	0.60	0.14	0.35
L. anisa E327-F	0.03	0.03	2.61	0.70	0.05	1.01	1.10	0.28	0.75
L. anisa CH-47-C1			1.38	0.28	0.02	0.06	0.01	0.21	0.62
L. anisa WA-316-C3	0.13	0.01	1.26	0.36	0.04	0.15	0.31	0.25	0.52
L. anisa CH-47-C3	0.12	0.25	2.18	0.50	0.27	1.06	0.87	0.06	0.33
F. gormanii LS-13	0.02		1.13	0.36	0.02			0.33	0.86

[a] See Fig. 2 legend for sugar component abbreviation definitions. Peaks representing sugars that were present at levels of less than 0.04% were not considered reliable in chemotaxonomic differentiation.

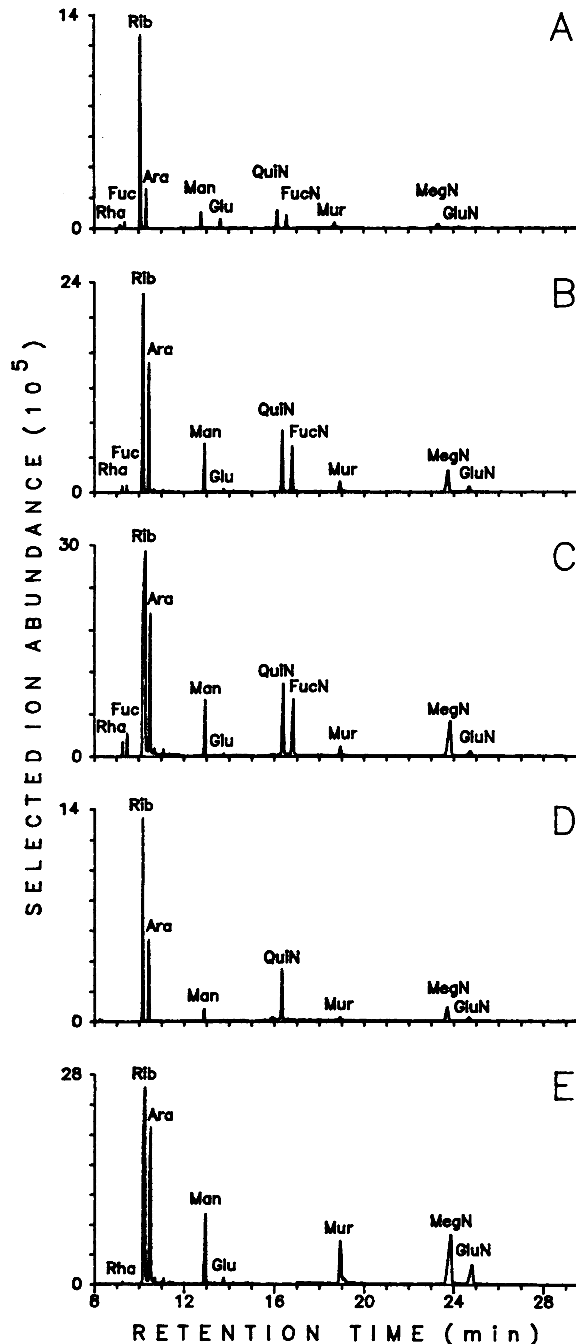

FIG. 5. Selected-ion chromatograms of alditol acetates of whole-cell hydrolysates of *L. anisa* CH-47-C3 (A) *F. dumoffii* TEX-KL (B), *L. bozemanae* WIGA (C), *F. dumoffii* NY-23 (D), and *F. gormanii* LS-13 (E). For peak identifications, see the legend to Fig. 2.

served in both strains of *L. jordanis*, but not in any of the other legionellae tested. Its mass spectrum matched that of an *O*-methylpentose (6). Another sugar peak with a retention time similar to that of X3 was noted in chromatograms of *L. jordanis*, but not in the other legionellae. The mass spectrum of this sugar did not resemble the mass spectrum of X3 found in *T. micdadei* but was essentially identical to the mass spectra of mannose, glucose, and galactose. This suggested that this sugar is one of the less common hexoses, and

TABLE 4. Carbohydrate content of *L. jordanis*

L. jordanis strain	% (dry wt) of the following sugar components[a]:							
	O-Met	Rib	Hex	Man	Glu	QuiN	Mur	GluN
ABB9	0.04	0.93	0.04	0.14	0.04	0.81	0.55	0.78
BL-540	0.05	2.26	0.07	0.09	0.01	0.41	0.28	0.41

[a] See Fig. 2 legend for sugar component abbreviation definitions. O-Met, *O*-Methylpentose.

illustrated the ability of MS to distinguish structurally different sugars that coelute.

T. micdadei and *L. maceachernii* have very similar carbohydrate profiles, which were readily distinguishable from those of the other six species of legionellae (19 strains) examined in this study. This was consistent with the observation that *T. micdadei* and *L. maceachernii* have very similar fatty acid profiles (16) and 16S-rRNA sequences (12; K. Fox, A. Brown, A. Fox, and G. Schnitzer, submitted for publication), which were distinct from those of the other legionellae.

F. dumoffii, *F. bozemanae*, and *L. anisa* have a small but significant degree of genetic relatedness detected by DNA-DNA hybridization (15), and each includes strains with fluorescing colonies. *F. bozemanae* and *F. dumoffii* also have essentially identical fatty acid profiles (16). *L. anisa* can only be distinguished from *F. bozemanae* and *F. dumoffii* by the ratio of $C_{a15:0}$ and $C_{i16:0}$ fatty acids; their fatty acid profiles are otherwise very similar (16). Of the 10 strains tested that represented these species, 7 strains contained quinovosamine and fucosamine. Of these seven strains, four, including at least one representative of each of three species (*L. anisa*, *F. dumoffii*, and *F. bozemanae*), also contained rhamnose and fucose and had indistinguishable carbohydrate profiles (Fig. 5). One of these seven strains contained rhamnose alone, but two other strains did not contain rhamnose or fucose. Three other strains (*F. dumoffii* OIE and NY-23 and *L. anisa* CH-47-C1) contained only quinovosamine without rhamnose or fucose. Thus, most strains (7 of 10) could be readily distinguished from *L. pneumophila* by the presence of fucosamine or, in the remaining three strains, by the absence of rhamnose. The carbohydrate contents of these strains are summarized in Table 3.

Three major features distinguished the *Fluoribacter* and *L. anisa* group from the *T. micdadei* and *L. maceachernii* group (Tables 2 and 3 and Fig. 3 and 5). Much less rhamnose and fucose was found in the former group; and unlike *T. micdadei* and *L. maceachernii*, with the exception of *F. gormanii*,

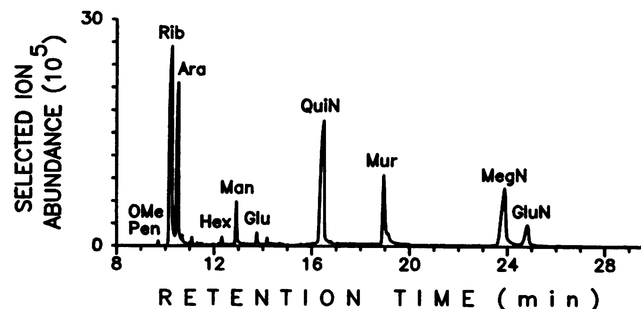

FIG. 6. Selected-ion chromatogram of alditol acetates of whole-cell hydrolysates of *L. jordanis* ABB9. For peak identifications, see the legend to Fig. 2.

TABLE 5. Effect of growth stage on carbohydrate content

Species and strain (growth phase)	% of the following sugar components[a]:						
	Rib	Man	Glu	QuiN	FucN	Mur	GluN
L. anisa E327-F							
Stationary	1.35	0.47		1.28	1.04	0.18	0.32
Exponential	2.03	0.34		0.87	0.93	0.13	0.59
F. gormanii LS-13							
Stationary	1.28	0.27	0.01			0.26	0.40
Exponential	2.56	0.29				0.15	0.30
F. dumoffii NY-23							
Stationary	1.36	0.14	0.03	1.53		0.21	0.35
Exponential	2.18	0.15		1.30		0.16	0.48

[a] See Fig. 2 legend for sugar component abbreviation definitions.

Fluoribacter and *L. anisa* strains contained quinovosamine but did not contain X3. *F. gormanii* was the only member of this family studied which lacked reliably detectable amounts of quinovosamine or fucosamine (Fig. 5E). Thus, all 11 *Fluoribacter* and *L. anisa* strains could be distinguished from the strains of *L. pneumophila* and of *Tatlockia* and *L. maceachernii* that were tested.

Since culture age might affect the carbohydrate content, several strains were analyzed during both the exponential growth and stationary phases. Table 5 gives the carbohydrate contents of representative strains of three species under these different growth conditions. The profiles of the strains in the two growth phases were similar. In addition, the profiles were similar to those obtained with the same strain harvested on a separate occasion (Table 3). As was expected, the only major difference noted was that the amount of ribose present during exponential growth was greater than that present during the stationary phase because of increased levels of RNA.

In summary, *L. pneumophila* and *T. micdadei*, the two major pathogens in the family *Legionellaceae*, were readily distinguished by their carbohydrate contents. On the other hand, the carbohydrate content of *L. maceachernii* was indistinguishable from that of *T. micdadei*. The group *Fluoribacter* and *L. anisa* was differentiated from the *T. micdadei* and *L. maceachernii* group and from *L. pneumophila*. In addition, certain strains of *F. bozemanae*, *F. dumoffii*, and *L. anisa* had indistinguishable carbohydrate profiles, which agreed with their significant genetic and phenotypic similarities. The carbohydrate profiles of *L. jordanis* and *F. gormanii* were distinct from those of any of the other six species studied.

By using the published DNA-DNA hybridization and the available 16S rRNA sequence data as a guide to relatedness, the following conclusions can be made: (i) *L. pneumophila* is a distinct group, (ii) the two species *T. micdadei* and *L. maceachernii* are related but are distinct from other species, and (iii) *Fluoribacter* species and *L. anisa* are a heterogeneous group of related organisms (3, 5, 15, 20). *L. jordanis* has no significant similarity to any other species tested so far (7). The similarities and differences observed in the carbohydrate profiles are in general agreement with genetic data. Carbohydrate profiling by GC-MS is useful for differentiation of the legionellae. GC-MS may also be a powerful tool for the study of other groups of microorganisms.

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