Monohydroxy and Dihydroxy Fatty Acid Composition of *Legionella* Species†

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A total of 28 strains, representing 10 species of *Legionella* and including strains belonging to most of the known serogroups of each species, were examined for location, content, and composition of cellular nonhydroxy, monohydroxy, and dihydroxy fatty acids. The nonhydroxy fatty acid profiles were in agreement with previously published values. All species contained 5 to 15 mol% (depending on species) monohydroxy fatty acid, the profile of each species being readily distinguishable from the profiles of the other species. Only two species, *Legionella pneumophila* and *Legionella micdadei*, contained detectable levels of dihydroxy fatty acids, which comprised 1 to 5 mol% of the total fatty acids. The dihydroxy fatty acid profiles of these two species were significantly different. In all species, the nonhydroxy fatty acids were readily extractable by lipid solvents and were labile when subjected to mild alkaline methanolysis (lipid associated, ester linked), whereas the hydroxylated components were not extractable, were stable when subjected to alkaline methanolysis, and were labile when subjected to acid hydrolysis (bound, amide linked). The monohydroxy fatty acids were shown to be acids of the 3-hydroxy family. The dihydroxy fatty acids were shown to be members of the 2,3-dihydroxy family. The profiles of the hydroxylated fatty acids provide a powerful method for differentiating the *Legionella* species, particularly those species whose nonhydroxy fatty acid profiles are quite similar.

One of the important criteria for species identification within the family *Legionellaceae* is cellular nonhydroxy fatty acid composition (1-5, 7-10, 16-20, 22-24). Profiles of these acids have most often been acquired from samples derived from alkaline saponification of whole-cell pellets. Little use has been made of the hydroxylated fatty acid component of these organisms.

In a previous study (12), five strains of *Legionella pneumophila*, the type species of the family, were shown to contain, in addition to the nonhydroxy acids reported by other workers, low but significant levels (ca. 5 to 10 mol% of the total cellular fatty acids) of 3-hydroxy acids, predominantly iso-branched C₁₄ and straight-chain C₂₀ acids. Additionally, these strains contained small amounts (1 to 3 mol%) of a novel class of 2,3-dihydroxy fatty acids, predominantly iso-branched C₁₄ acid. The hydroxylated fatty acids were not extractable with chloroform-methanol (i.e., wall associated, bound) and were alkali stable and acid labile (i.e., amide linked). In contrast, the nonhydroxy fatty acids, which comprised 90 to 95 mol% of the total cellular fatty acids, were extractable (i.e., lipid associated) and alkali labile (i.e., ester linked). The fatty acid composition in each of the classes seemed to be relatively insensitive to variations in growth medium or culture age.

In the current study, 28 strains, representing all 10 currently named *Legionella* species and including strains belonging to most of the known serogroups of these species, were examined for nonhydroxy, monohydroxy, and dihydroxy fatty acid content and composition. The fatty acid classes were acquired by three different methods.

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MATERIALS AND METHODS

Organisms. The strains of *Legionella* species studied were obtained from either the Biological Products Division, H. W. Wilkinson, or Leo Pine, Centers for Disease Control, Atlanta, Ga. These strains included *Legionella pneumophila* Philadelphia 1^T (T = type strain), Knoxville 1, Togus 1, Bloomington 2, Los Angeles 1, Dallas 1, Chicago 2, Chicago 8, Concord 3, and IN-23-G1-C2; *Legionella longbeachae* Long Beach 4^T, Tucker 1, and Atlanta 5; *Legionella micdadei* TATLOCK^T, PI-1, and HEBA; *Legionella bozemanii* WI-GA^T and MI-15; *Legionella dumoffii* NY-23^T and TEX-KL; *Legionella jordanis* BL-540^T and ABB-9; *Legionella gormanii* LS-13^T; *Legionella oakridgensis* OR-10^T, OR-23, and OR-24; "*Legionella feeleyi*" WO-44C^T; and *Legionella wadsworthii* 81-716A^T. During the course of this study, the strains were periodically checked serologically for confirmation of identity.

Growth media and conditions. Most of the experimental material was grown on charcoal-yeast extract agar prepared by the method of Feeley et al. (6), except that the NaOH used by these authors was replaced by a comparable amount (4 meq/liter) of Na₂CO₃. Occasional batches of medium were prepared to which individual organic buffers were substituted for the Na₂CO₃. These buffer components, which were supplied at levels of approximately 4 × 10⁻³ M, included tris(hydroxymethyl)aminomethane, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, and N-(2-acetamido)-2-aminoethanesulfonic acid (24). In these instances, the aqueous solution of the buffer was adjusted to pH 7.0 before addition of the dry medium components.

On occasion, batches of carbonate-containing charcoal-yeast extract agar were supplemented with dyes, either a mixture of bromthymol blue and bromcresol purple (26) or these two dyes along with crystal violet. The dye components were added at concentrations of 100 mg/liter.

In a typical run, a cell suspension approximating a McFarland no. 1 turbidometric standard was prepared in 1% yeast extract from a 48 to 72-h plate culture of the test strain. The

† Dedicated to the memory of Leo Roy Mayberry, 1904-1983.

cell suspension (0.1 ml) was applied to each of 10 plates and spread with a bent glass rod. A blood agar plate was similarly inoculated as a check for contamination. The plates were incubated at 35°C in enclosed containers (1-gallon [3.785-liter] paint cans) to maintain high humidity until heavy confluent growth occurred (usually 3 to 4 days). The cells were harvested with distilled water and were recovered by centrifugation at $2,600 \times g$ for 30 min at 4°C. Pellets were washed once by suspension in and centrifugation from 15 ml of distilled water. Each strain was subjected to at least four complete procedures (growth through analysis).

Acquisition of cellular fatty acid components. Three methods were used to obtain the cellular fatty acids separated into nonhydroxy, monohydroxy, and dihydroxy fatty acid classes. In the first method (total acid hydrolysis) cell pellets (1 to 1.5 g [wet weight]) were suspended in 5 ml of 2 N HCl and hydrolyzed overnight at 100°C. The hydrolysates were filtered through sintered glass to remove charred particulate matter. The filter cakes were washed sequentially with one 5-ml portion of methanol and two 5-ml portions of chloroform. To the combined filtrates and washings were added 5 ml of chloroform and 5 ml of water, with shaking. The aqueous layers were discarded, and the organic phases were evaporated to dryness in vacuo. The dried material was dissolved in 5 ml of chloroform and 3 ml of methanol (1 M in HCl) and esterified for 1 h at 55°C. Chloroform (10 ml) and water (10 ml) were added, with shaking. The organic phases were washed once with 10 ml of water and dried. The methyl esters were separated into nonhydroxy, monohydroxy, and dihydroxy classes by thin-layer chromatography.

In the second method, wet cell pellets were extracted with 15 ml of chloroform-methanol (2:1, vol/vol) for 12 h at room temperature. Samples were centrifuged at $2,600 \times g$ for 30 min at 4°C, and the lipid extract was decanted. The cell

pellets were suspended in and centrifuged from an additional 15 ml of the mixed solvent. The combined organic phases, which contained the "extractable lipids," were evaporated to dryness in vacuo and subjected to either acid methanolysis (1 M HCl in methanol for 2 h at 100°C) or alkaline methanolysis (0.2 M KOH in methanol for 2 h at room temperature) to obtain nonhydroxy fatty esters. The defatted cell pellets were hydrolyzed in 2 N HCl and extracted with chloroform. The chloroform phases were esterified, and the esters were separated into classes as described above for the total acid hydrolysates.

In the third method, wet cell pellets were subjected to direct alkaline methanolysis in 10 ml of 0.2 M KOH-methanol for 2 h at room temperature. The reaction mixtures were neutralized with 1 M HCl-methanol and centrifuged. The cell pellets were washed by suspension in and centrifugation from 15 ml of methanol. The combined methanolic phases, which contained methyl esters of ester-linked fatty acids along with some free fatty acid (owing to the small amounts of water in the reaction mixture), were evaporated to dryness and subjected to re-esterification to insure complete conversion to methyl esters. The partially deacylated cell pellets were subjected to acid hydrolysis, and extraction, esterification, and chromatographic separation of the released fatty acid esters were performed as described above.

Analytical methods. Thin-layer chromatography was carried out on 0.5-mm plates prepared from Silica Gel H type 60 (E. Merck, Brinkmann, Westbury, N.Y.) by using distilled water as the suspending liquid. The plates were predeveloped in chloroform-methanol (1:1, vol/vol) and activated at 110°C before they were used.

Nonhydroxy fatty esters were separated from hydroxylated compounds by development with chloroform. Monohy-

TABLE 1. Nonhydroxy fatty acid profiles of *Legionella* species^a

Fatty acid ^b	Mol% of nonhydroxy acid fraction in: ^c									
	<i>L. pneumo-</i> <i>phila</i>	<i>L. oakridg-</i> <i>ensis</i>	<i>L. long-</i> <i>beachae</i>	<i>L. gor-</i> <i>manii</i>	<i>L. boze-</i> <i>manii</i>	<i>L. dumoffii</i>	<i>L. jordanis</i>	<i>L. micda-</i> <i>dei</i>	<i>L. wads-</i> <i>worthii</i>	" <i>L. fee-</i> <i>leii</i> "
i14:0	5	1	5	4	3	2	1	tr	1	3
n14:0	tr	tr	tr	tr	tr	tr	tr	tr		tr
i15:0	tr	tr	tr	tr	tr	tr	1	1		1
a15:0	22	8	22	31	33	38	47	46	38	27
n15:1	1	1	1	2	1	1	1	2	1	2
n15:0	tr	1	1	2	2	1	tr	4	tr	2
i16:1	3	2		tr	tr	tr	1	2	tr	2
i16:0	38	32	28	20	16	16	15	11	9	17
n16:1	10	17	24	11	10	8	6	5	14	26
16 cyc (?)			3	tr	tr	tr			1	
n16:0	4	10	5	10	9	5	2	5	2	14
i17:1	tr						tr			
a17:1	tr	2					1	3		
i17:0	tr	tr	tr	tr	tr	1	2	tr	tr	tr
a17:0	12	11	10	14	14	18	20	20	26	8
17 cyc	1	4	5	5	8	7	6	tr	3	tr
n17:0	tr	2	tr	2	3	2	1	tr	2	tr
n18:1	tr	1								tr
n18:0	1	8	tr	tr	1	tr	tr	tr		tr
a19:0		1								tr
n19:0	tr	1	tr	tr	1	tr	tr	tr		tr
i20:0	tr	tr								tr
n20:0	1	1	tr	tr	1	tr	tr	1		tr
a21:0								1		tr
i21:0								tr		tr

^a Values are averages of four to eight analyses per strain. The fatty acids are listed in order of elution from nonpolar columns

^b Fatty acids are designated according to the following expression: xN:y, where x is the chain configuration (i, iso branched; a, anteiso branched; n, normal [straight chain]; cyc, cyclopropane), N is the number of carbons, and y is the number of double bonds.

^c Nonhydroxy fatty acids comprised 85 to 95% of the total cellular fatty acids.

droxy compounds were separated from dihydroxy compounds by using diethyl ether-hexane (60:40, vol/vol). Zones of interest were visualized by spraying the plates to transparency with water. Opaque bands denoting lipoidal compounds were eluted with 15 ml of chloroform-methanol (2:1, vol/vol).

Gas-liquid chromatography was carried out with a Hewlett-Packard model 5840A instrument equipped with flame ionization detectors. Samples were analyzed on a 50-m fused silica wall-coated open tubular column coated with polydimethoxysilane liquid phase OV-1 (Hewlett-Packard), which was operated in the splitless mode under temperature-programmed conditions.

Gas-liquid chromatography-mass spectrometry was performed by using a Finnigan model 4000 instrument equipped with a 25-m fused silica wall-coated open tubular column of DB-5. Chromatography was done in the splitless mode under temperature-programmed conditions. Mass spectrometry of peaks was done in the electron impact ionization mode at an ionization energy of 70 eV.

Derivative formation. The procedures used for formation of the trimethylsilyl, trifluoroacetyl, acetyl, and *n*-butylboronyl derivatives of the hydroxylated esters have been described previously (12). Methyl esters of dihydroxy fatty acids were incubated for 30 min at room temperature in 0.5 ml of a mixture containing chloroform, acetone, and concentrated HCl (10:1:0.1, vol/vol) to form the isopropylidene derivatives. For all derivatives, the reagents were removed under a stream of dry nitrogen, and the samples were reconstituted in chloroform for gas-liquid chromatography. Monohydroxylated and dihydroxylated esters were routinely analyzed as the trimethylsilyl and trifluoroacetyl derivatives. As part of the procedure for verification of identification, the monohydroxy esters were also analyzed as the acetyl derivatives, and the dihydroxy esters were analyzed

as the acetyl, isopropylidene, and *n*-butylboronyl derivatives.

Standards. Straight-chain saturated and unsaturated nonhydroxy fatty acids of both even and odd carbon numbers were purchased from Sigma Chemical Co., St. Louis, Mo. Branched-chain nonhydroxy fatty acids were obtained in BC-Mix L and BC-Mix 1 standard mixtures (Alltech Associates, Inc., Applied Science Div., State College, Pa.), as well as from previously characterized hydrolysates of *Bacteroides asaccharolyticus* and *Bacteroides fragilis* (11) and *L. pneumophila* (12). Straight- and branched-chain 3-hydroxy acids were prepared from hydrolysates of *Escherichia coli*, *Acholeplasma axanthum* (13), *B. asaccharolyticus* and *B. fragilis* (11), and *L. pneumophila* (12).

For quantitative evaluation of the three fatty acid classes, *n*-tetradecanoic acid, DL-12-hydroxyoctadecanoic acid, and 9,10-dihydroxyoctadecanoic acid were used as internal standards for the nonhydroxy, monohydroxy, and dihydroxy fatty acids, respectively. The molar response factors relative to methyl *n*-nonadecanoate were determined empirically for the straight-chain nonhydroxy acids and those branched and hydroxylated compounds (as all pertinent derivatives) for which sufficient material could be obtained. The molar response factors of other compounds were estimated by interpolation and extrapolation from the experimentally determined values.

RESULTS

Varying the growth medium by using various buffers had little effect on either cell yield or composition of the three cellular fatty acid classes. Growth on the medium buffered with tris(hydroxymethyl)aminomethane appeared to be somewhat less luxuriant (subjectively) than growth on the other media tested. On carbonate-containing charcoal-yeast extract agar supplemented with bromthymol blue and brom-

TABLE 2. Monohydroxy fatty acid composition of *Legionella* species^a

Monohydroxy fatty acid ^b	Relative abundance in: ^c									
	<i>L. pneumophila</i>	<i>L. oakridgensis</i>	<i>L. micdadei</i>	<i>L. jordanis</i>	<i>L. dumoffii</i>	<i>L. bozemanii</i>	<i>L. wadsworthii</i>	<i>L. gormanii</i>	<i>L. longbeachae</i>	" <i>L. feeleii</i> "
n12h				1	8	4				
n13h	1		5	3	3	7		4	1	2
i14h	100	100	100	45	12	9	12	12	13	100
n14h	10	10	15	17	100	100	100	100	80	42
a15h	5	4	60	100	63	33	44	40	26	33
n15h		1	1	3	24	57	31	50	22	1
i16h	1		1	3	19	46	62	73	100	1
n16h	4	2	1	5	27	57	100	48	33	3
i17h							6	1	2	
a17h			1	2	9	5	50	19	6	
n17h	1	1		4	9	22	44	26	12	1
i18h	4	2		2		4	6	4	1	
n18h	16	20	1	17	61	80	100	73	58	7
a19h	4	2	1	23	7	4	75	7	5	
n19h	11	12	4	8	19	40	12	50	21	4
i20h	12	3	1	2						3
n20h	82	54	39	41	49	79	31	82	87	77
i21h	1			2						
a21h	9	2	31	16						2
n21h	22	2	38	6	6	16		18	7	13
i22h	13	1	7	2	1	1		2	2	1
n22h	14	2	39	3						19
a23h	3		39							
n23h			2							

^a The major monohydroxy fatty acids are the 3-hydroxy saturated fatty acids.

^b See Table 1, footnote b. The suffix -h indicates a monohydroxy fatty acid.

^c The monohydroxy fatty acids comprise 5 to 15 mol% of the total cellular fatty acids. Values are expressed relative to the component(s) present at the highest level, which was given a value of 100.

cresol purple, the bacterial growth became colored, as described by other workers; growth was grayish white (little change) in *L. pneumophila*, a deep blue in *L. micdadei*, and various shades of green in the other species (24, 26). On medium containing all three dyes, some *L. pneumophila* cultures appeared to take up crystal violet and appeared violet, whereas the other species retained the colors attributable to the other two dyes. The colors remained with the cells after harvest and were extracted into the organic phase during extraction. No effect on yield or composition of the cellular fatty acid classes was noted as a result of growth on dye-containing media, but the extracted dye components complicated purification of the hydroxylated ester fractions. In those species which autofluoresced (*L. bozemanii*, *L. gormanii*, and *L. dumoffii* [1, 3, 4]), lipoidal components, presumably of the fluorescent pigment system, further complicated the purification of the hydroxylated esters.

The three methods of acquisition of the cellular hydroxy acids yielded essentially the same distribution of fatty acid classes (85 mol% or more nonhydroxy acids, 5 to 15 mol% monohydroxy acids, 0 to 3 mol% dihydroxy acids). The composition of the nonhydroxy fatty acid classes varied somewhat among the three methods. Direct acid hydrolysis yielded lower levels of cyclopropane fatty acid, whereas the other two methods showed much higher, essentially equal levels. The monohydroxy and dihydroxy profiles were essentially the same with all three methods.

Cellular fatty acid profiles. Table 1 shows the composite nonhydroxy fatty acid ester profile of each species. The values given are averages of at least four runs per strain and include results obtained by all three methods of extraction. These profiles are reported as moles percent to be consistent with previously reported values and are in good agreement with data published by other workers (1–5, 7–10, 16–20, 22–24).

Since the monohydroxy and dihydroxy fatty acid classes comprise 15 mol% or less (depending on species) of the total fatty acid composition in each species, the profiles of these classes are reported on a "relative abundance" basis, with the most abundant compound considered equal to 100. This method provides a relatively simple means for comparing profiles without reference to the absolute levels of the hydroxylated compounds.

Table 2 shows the composite monohydroxy fatty ester profile of each species. The profile shown for *L. pneumophila* is in good agreement with the profile published previously (12). The monohydroxy esters, as *O*-trimethylsilyl ethers, yielded mass spectra containing a major ion at an *M/z* value of 175, indicating 3-hydroxylation, and a strong (*M*-15)⁺ peak from which molecular weight could be calculated, to confirm the chain length assignment. The mass spectra did not yield sufficient information to unequivocally assign iso and anteiso branching. Such assignment was made on the basis of relative gas-liquid chromatographic retention on two columns and by comparison with available standards. The even-carbon branched acids appeared to be iso branched, whereas the odd-carbon branched acids were assigned the anteiso structure, not the iso structure, as reported previously (12). There was some evidence of trace amounts of 2-hydroxy fatty acids as well.

Table 3 shows the composite profiles of the 2,3-dihydroxy fatty acids of two species. Only these two species contained detectable quantities of the dihydroxy compounds, at levels approaching one-fifth to one-third of the total hydroxylated fatty acid component of the organisms. In *L. pneumophila*, the branched (presumably iso) 14-carbon component was predominant, as reported previously (12). In *L. micdadei*,

the branched 14-carbon, normal 14-carbon, and branched (presumably anteiso) 15-carbon compounds were well represented. Trace levels of the normal 12- and 13-carbon components were found in these species as well. The bis-trimethylsilyl ethers of the diol esters yielded mass spectral ions that were attributable to cleavage on either side of and between the derivatized hydroxyl groups, as described previously (12, 14, 15, 25).

DISCUSSION

Although the direct total acid hydrolysis procedure was the quickest method of obtaining the three fatty acid fractions, considerable (10 to 100%), unpredictable losses of cyclopropane fatty acids occurred in the nonhydroxy acid fraction. The acid sensitivity of the cyclopropane fatty acids has been well documented by Moss et al. (21).

The majority of the nonhydroxylated fatty acids (90 to 95 mol%) have been shown to be associated with the extractable lipids in *L. pneumophila* (12) and were shown to be similarly distributed in the other species by preliminary experiments for the present study. Thus, the second procedure (extraction of lipids, followed by methanolysis of the lipid extract and hydrolysis of the defatted cell pellet) appears to be a useful method of obtaining a rather quick separation of the nonhydroxylated fatty acids from the hydroxylated compounds. The lipid fraction can then be cleaved by alkaline methanolysis or mild acid methanolysis to yield most of the nonhydroxy fatty acids, without significant loss of acid-sensitive components. Approximately 5% of the nonhydroxy acids remain associated with the cell pellet along with nearly all of the hydroxylated compounds. This procedure, although considerably more time consuming than direct total acid hydrolysis, has the advantage of removing most of the substances which tend to interfere with subsequent purification of the hydroxylated compounds (e.g., pigments and dye components).

The nonhydroxy fatty acids are predominantly in ester linkage (98 to 99%) in *L. pneumophila*, whereas the hydroxylated fatty acids appear to be amide linked and associated with the cell pellet (12). Preliminary studies indicated a similar distribution in the other species. The third method (direct alkaline methanolysis of the cell pellet, followed by hydrolysis of the partially deacylated cell pellet) has the advantage of removing the nonhydroxylated fatty acids in a mild, nondestructive manner. The mild re-esterification step to insure complete conversion to esters is not sufficient to cause significant destruction of the acid-sensitive cyclopropane acids. This procedure, involving alkaline deacylation of the wet cell pellet, is similar to the procedure used by other workers (1–5, 8–10, 16–23) but differs in the following two respects: (i) the concentration of water in the deacylation reagent is much lower (approximately 13% [vol/vol], equivalent to 24 mol%) than in the commonly used method (approximately 60% [vol/vol], equivalent to 76 mol%) and (ii) the reaction is allowed to proceed at room temperature instead

TABLE 3. Dihydroxy fatty acid composition of *Legionella* species^a

Species	Relative abundance of: ^b				
	n12h ₂	n13h ₂	i14h ₂	n14h ₂	a15h ₂
<i>L. pneumophila</i>	2	3	100	8	2
<i>L. micdadei</i>	4	8	100	20	63

^a The dihydroxy fatty acids are 2,3-hydroxylated and are saturated.

^b The dihydroxy fatty acids comprise 2 to 5 mol% of the cellular fatty acids in those species in which they are found. For acid designations, see Table 1, footnote b; the suffix-h₂ indicates a dihydroxy fatty acid.

of at 100°C. The potential contaminating compounds are extracted in the methanolic reaction mixture.

Acid hydrolysis of the partially deacylated cell residue yields hydroxylated compounds only slightly contaminated with nonhydroxy fatty acids, which are easily removed by subsequent thin-layer chromatographic purification.

Cellular fatty acid profiles. The nonhydroxy fatty acid profiles shown in Table 1 are in good agreement with those published previously by other workers (1–5, 7–10, 16–20, 22–24). The 10 species can be separated into two groups on the basis of relative amounts of iso-C_{16:0} and anteiso-C_{15:0} acids. In one group, iso-C_{16:0} acid is the larger component, and in the other group anteiso-C_{15:0} acid is the larger component. In the first group, *L. pneumophila* and *L. oakridgensis* are quite similar, differing primarily in their levels of anteiso-C_{15:0}, straight-chain C_{18:0}, and cyclopropane C₁₇ acids, whereas *L. longbeachae* possesses a readily distinguishable profile with quite high levels of straight-chain C_{16:1} acid.

In the second group, *L. gormanii* and *L. bozemanii* are similar in that the iso-C_{16:0} acid component is the second most abundant component, followed by the anteiso-C_{17:0} component, and are essentially indistinguishable. In contrast, *L. dumoffii*, *L. jordanis*, and *L. micdadei* all contain the anteiso-C_{17:0} component as the second most abundant acid. These species can be distinguished, with some difficulty, on the basis of the relative amounts of the other major nonhydroxy fatty acids. In *L. wadsworthii*, the level of

straight-chain C_{16:1} acid is significantly higher than the level of iso-C_{16:0} acid, a condition also seen in "*L. feeleeii*," in which straight-chain C_{16:1} acid is the second most abundant (under some conditions, the most abundant) nonhydroxy acid.

The 10 species can be divided into three groups on the basis of monohydroxy acid composition (Table 2). The members of the first group contain high levels (at least 40% of the major component) of iso-C₁₄ 3-hydroxy acid and low levels (less than 20% of the major component) of straight-chain C₁₄ 3-hydroxy acid. In this group, *L. pneumophila* and *L. oakridgensis* show quite similar profiles, whereas *L. micdadei* and *L. jordanis* each show a distinctive monohydroxy acid profile.

The members of the second group contain high levels (at least 80% of the major component) of straight-chain C₁₄ 3-hydroxy acid and low levels (less than 20% of the major component) of iso-C₁₄ 3-hydroxy acid. In this group, *L. dumoffii*, *L. bozemanii*, *L. gormanii*, *L. wadsworthii*, and *L. longbeachae* each present a readily distinguishable monohydroxy fatty acid profile.

The members of the third group, represented by "*L. feeleeii*," contain both iso-C₁₄ and straight-chain C₁₄ 3-hydroxy acids at relatively high levels.

Dihydroxy fatty acids are found only in *L. pneumophila* and *L. micdadei*. The profiles are readily distinguishable (Table 3) and are quite reminiscent of the early portions of

TABLE 4. Similarity grouping of *Legionella* species by cellular fatty acid classes

Fatty acid class	Group	Species	Composition ^a
Nonhydroxy	Iso-C ₁₆ acid highest	<i>L. pneumophila</i> <i>L. longbeachae</i> <i>L. oakridgensis</i>	i16:0 > a15:0 > a17:0 > n16:1 > i14:0 i16:0 > a15:0 = n16:1 > a17:0 ≥ 17 cyc i16:0 > a17:0 = n16:1 > a15:0 ≥ n18:0
	Anteiso-C ₁₅ acid highest	<i>L. micdadei</i> <i>L. jordanis</i> <i>L. dumoffii</i> <i>L. wadsworthii</i> <i>L. bozemanii</i> <i>L. gormanii</i> " <i>L. feeleeii</i> "	a15:0 > a17:0 > i16:0 > n16:1 = n16:0 a15:0 > a17:0 > i16:0 > n16:1 = 17 cyc a15:0 > a17:0 > i16:0 ≥ 17 cyc = n16:1 a15:0 > a17:0 > n16:1 > i16:0 > 17 cyc a15:0 > i16:0 > a17:0 ≥ 17 cyc = n16:1 = n16:0 a15:0 > i16:0 > a17:0 > n16:1 ≥ n16:0 > 17 cyc a15:0 = n16:1 > i16:0 > n16:0 > a17:0
Monohydroxy	Significant iso-C ₁₄ acid	<i>L. pneumophila</i> <i>L. oakridgensis</i> <i>L. micdadei</i> <i>L. jordanis</i>	i14h > n20h > n21h > n18h > n22h i14h > n20h > n18h > n19h > n14h i14h > a15h > n20h = a23h = n22h > n21h > a21h a15h > i14h ≥ n20h > a19h > a21h = n18h
	Significant straight-chain C ₁₄ acid	<i>L. dumoffii</i> <i>L. bozemanii</i> <i>L. wadsworthii</i> <i>L. gormanii</i> <i>L. longbeachae</i>	n14h > a15h = n18h > n20h n14h > n18h = n20h > n16h = n15h n14h = n16h = n18h > a19h > i16h n14h > n20h ≥ n18h = i16h i16h > n20h ≥ n14h > n18h
	Both straight-chain C ₁₄ and iso-C ₁₄ acids significant	" <i>L. feeleeii</i> "	i14h > n20h > n14h > a15h > n22h ≥ n21h
Dihydroxy	2,3-Dihydroxy acids present	<i>L. pneumophila</i> <i>L. micdadei</i>	br14h ₂ , n14h ₂ , br15h ₂ (ratio, approx 12:1:trace) br14h ₂ , n14h ₂ , br15h ₂ (ratio, approx 5:1:3)
	2,3-Dihydroxy acids not detected	<i>L. bozemanii</i> , <i>L. jordanis</i> , <i>L. dumoffii</i> , <i>L. longbeachae</i> , <i>L. gormanii</i> , <i>L. wadsworthii</i> , <i>L. oakridgensis</i> , " <i>L. feeleeii</i> "	

^a See Table 1, footnote b, Table 2, footnote b, and Table 3, footnote b. br, Branched.

the respective monohydroxy acid profiles, suggesting a biosynthetic relationship.

Table 4 summarizes the arrangement of the 10 *Legionella* species into "similarity groups" on the basis of all three fatty acid classes. Table 4 also indicates the utility of rank ordering the major components of each class as an aid to species identification.

It is of interest that, in all of the *Legionella* species described here, the monohydroxy acid profiles include relatively long-chain-length compounds (up to 23 carbons). In fact, a 20-carbon compound, straight-chain C₂₀ 3-hydroxy acid, appears to be a major component common to these species.

Although determination of the hydroxylated fatty acid components of the members of the *Legionellaceae* most likely entails procedures which are too cumbersome and time consuming to be practical on a routine basis, such analyses provide additional information which may be quite useful in the overall taxonomy of the group. In addition, such analyses provide characters which might allow distinction between isolates which appear to be quite similar on the basis of the more readily determinable nonhydroxy fatty acid patterns. Finally, the monohydroxy and dihydroxy acid profiles described here indicate that the organisms currently comprising the family *Legionellaceae* possess a rather interesting array of synthetic capabilities with regard to chain length and branching specificity and, in two species, the ability to synthesize the unique 2,3-dihydroxy fatty acids.

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