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Cellular Fatty Acid Compositions and Isoprenoid Quinone Contents of 23 *Legionella* Species

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The cellular fatty acid compositions and ubiquinone contents of 182 *Legionella* strains representing 23 species were determined by capillary gas-liquid chromatography and reverse-phase high-performance liquid chromatography, respectively. Except for the type strain of *Legionella erythra* (ATCC 35303^T), all *Legionella* species contained large (40 to 90%) amounts of branched-chain fatty acids and only trace to small (<0.5 to 5%) amounts of ester-linked hydroxy acids. The 23 species were placed in three major fatty acid groups on the basis of differences in the relative amounts of 14-methylpentadecanoic ($C_{i16:0}$), hexadecanoic ($C_{16:1}$), and 12-methyltetradecanoic ($C_{a15:0}$) acids. All *Legionella* species contained ubiquinones with 9 to 14 isoprene units in the side chains and were divided into five different ubiquinone groups. The species were further differentiated into 16 groups on the basis of qualitative and quantitative differences in their fatty acid compositions and ubiquinone contents. Both of these chemical characteristics can be used to distinguish *Legionella* species from other gram-negative bacteria and rapidly and accurately identify suspected isolates before serologic and other tests are done.

Since the causative agent of Legionnaires disease, *Legionella pneumophila*, was isolated and identified over 11 years ago (2, 4, 12), 24 additional species (2, 3, 20, 24) and 41 serogroups (20, 23, 24) of the genus *Legionella* have been reported. Genetic, phenotypic, and serologic data indicate that at least nine additional presently unnamed *Legionella* species have been isolated (2). Many of the *Legionella* species were derived from the environment and have not been implicated in human disease; however, 15 species have been shown to cause human disease by direct isolation from patients or serologic testing (2, 19, 20, 24). *Legionella* species are nonreactive in most biochemical tests (2, 3, 23); their phenotypic characteristics are very similar, and most cannot be differentiated on this basis (2, 3, 23). Legionellae do not grow on common laboratory media but require specialized media containing L-cysteine and iron salts (2, 3, 5, 6, 18, 23). Occasionally, species in other genera can mimic the growth, phenotypic, or serologic characteristics of members of the family *Legionellaceae* (22, 23). Serologic identification can be done using direct immunofluorescence assay or the slide agglutination test (SAT); although cross-reactions occur in these, most can be removed by absorption (1, 21, 22). Reagents used for these tests are not commercially available for all *Legionella* species and serogroups, and production of the species-specific antisera and absorbing antigens is time-consuming and expensive (2, 3, 21, 22). A suspected isolate with phenotypic characteristics like those of legionellae and which does not stain or agglutinate in serologic tests could represent a new serogroup or species or a different genus. Although DNA hybridization reactions can determine new serogroups of previously described *Legionella* species (2, 3), results from genetic testing must be used in conjunction with other tests to define new species. This is necessary due to the low levels of genetic relatedness of most of the *Legionella* species (2, 3).

Two useful tests that can tentatively identify *Legionella* species are chemical analyses of cellular fatty acid composition and isoprenoid quinone content (7, 15, 16). These bacteria differ from other gram-negative organisms by their large amounts of branched-chain fatty acids and only trace or minor amounts of hydroxy acids (15). Their isoprenoid quinone content is also unusual because *Legionella* species contain ubiquinones with side chains of 10 or more isoprenoid units (7, 15). In this report we summarize the cellular fatty acid compositions and ubiquinone contents of 23 of the named *Legionella* species, and we describe the usefulness of these chemical data in differentiating among them.

MATERIALS AND METHODS

Cultures. A total of 183 *Legionella* cultures including the type strains of 23 species and additional isolates of 19 of these species were examined (Tables 1, 2, and 3). The cultures were obtained from the stock culture collection at the Centers for Disease Control, Atlanta, Ga. They were isolated from human and environmental samples and identified by Centers for Disease Control personnel or submitted to the Centers for Disease Control by other laboratories for identification. The identity of the species was confirmed by DNA hybridization reactions (2, 3) or serologic testing (21, 22). Cultures were grown on CYE (6), BCYE (18), or BCYEα agar (5) plates or slants. Incubation was at 35 to 37°C in cans or plastic bags to maintain adequate moisture (23). Cultures that grew poorly were grown in a CO_2 incubator or a candle extinction jar. Cultures were incubated for 48 to 72 h or until confluent to ensure that cells were in the stationary phase of the growth cycle and would give reproducible and consistent fatty acid results (15).

Cellular fatty acid analysis. Growth from one plate or slant (70 to 150 mg, wet weight) was removed with approximately 1 ml of sterile distilled water and placed in a screw-cap tube fitted with a Teflon-lined cap. Cells were saponified with either 5% NaOH (8, 16) or 15% NaOH (9; method B) in 50% aqueous methanol, methylated, and extracted. Since 1986, the procedure for preparing cellular fatty acid methyl esters

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TABLE 1. Cellular fatty acid compositions of nine *Legionella* species in the 16C group

Species[a]	No. of strains	Fatty acid composition[b] (%)															
		$C_{14:0}$	$C_{15:0}$	$C_{a15:0}$	$C_{i16:1A}$	$C_{i16:1B}$	$C_{16:0}$	$C_{16:1A}$	$C_{16:1\omega7c}$	C_{Un16}	$C_{16:0}$	$C_{a17:1}$	$C_{i17:0}$	$C_{a17:0}$	$C_{17:0cyc}$	$C_{18:0}$	$C_{18:0}$	$C_{a19:0}$	$C_{21:0}$
L. feeleii	6	3 (1)[c]	tr	18 (2)	2 (1)	–	16 (4)	2 (1)	22 (3)	–	16 (5)	–	tr	7 (2)	tr	tr	1	–	1
L. oakridgensis	9	tr	–	2	–	2 (1)	26 (3)	tr	14 (3)	–	10 (2)	1 (1)	–	5 (1)	12 (4)	2	13 (2)	1	–
L. pneumophila	70	5 (2)	–	13 (3)	–	3 (1)	32 (7)	–	16 (4)	–	7 (3)	tr	tr	8 (2)	2 (2)	–	3 (1)	–	1
L. spiritensis[d] ATCC 35249[T]	1	2 (1)	tr	20 (2)	–	5 (2)	28 (1)	–	15 (2)	–	5 (2)	3 (1)	tr	16 (2)	1	tr	1	tr	–
L. sainthelensi	7	5 (1)	tr	20 (3)	–	–	22 (2)	–	25 (3)	–	7 (2)	–	–	10 (2)	1 (1)	tr	1	–	–
L. longbeachae	7	5 (2)	–	15 (4)	–	–	25 (4)	–	24 (4)	1 (1)	9 (5)	–	tr	8 (2)	4 (3)	tr	1 (1)	–	–
L. santicrucis[d] ATCC 35301[T]	1	6 (2)	–	10 (1)	–	–	30 (2)	–	22 (2)	tr	10	–	tr	4 (1)	8 (4)	–	1	–	–
L. erythra[d] ATCC 35303[T]	1	tr	1	8 (2)	–	–	4 (1)	–	38 (3)	–	26 (4)	–	1	10 (2)	–	–	4 (1)	–	tr
L. erythra	3	2	1	12 (1)	–	–	24 (2)	–	27 (2)	–	14 (3)	–	1	10 (1)	–	–	2	–	tr
L. rubrilucens	5	2 (1)	tr	12 (2)	–	–	23 (6)	–	27 (4)	–	15 (5)	–	tr	9 (2)	–	tr	2	–	–

[a] ATCC, American Type Culture Collection, Rockville, Md.; T, type strain.

[b] The number before colon is the number of carbon atoms; the number after colon is the number of double bonds; i, methyl branch at the iso-carbon atom; a, methyl branch at the anteiso-carbon atom; A, isomers of i16:1 and 16:1 present in *L. feeleii*; B, isomer of i16:1 present in *Legionella* species except *L. feeleii*; ω7c, double-bond position from hydrocarbon end of *cis* isomer; Un16, unidentified 16-carbon fatty acid; cyc, cyclopropane fatty acid; 21:0, a21:0 or a21:0 and 21:0 if both are present.

[c] The first number is the arithmetic mean of the relative percentage of the fatty acid (rounded to the nearest whole number); (*n*) is the standard deviation rounded to the nearest whole number; absence of (*n*) indicates that the standard deviation was less than 0.6; tr, <0.5%; –, not detected in any (or <80%) of the strains tested. With the exception of *L. erythra* ATCC 35303[T], data from the type and other strains were combined.

[d] Cultured and assayed at least three times.

(FAME) has been done with approximately one-half of the cell suspension from a BCYEα agar plate (Carr-Scarborough Microbiologicals, Inc., Stone Mountain, Ga.) and smaller amounts of reagents. The cells were saponified by heating with 1 ml of 15% NaOH in 50% aqueous methanol at 100°C for 30 min; the cooled sample was mixed with 1.5 ml of 25% HCl in methanol (pH 2 to 3) and heated at 85°C for 15 min. The methylated sample was cooled and extracted with 1.3 ml of a 1:1 mixture of diethylether-hexane. After the organic and aqueous layers separated, the aqueous phase (lower layer) was discarded. The organic phase was mixed with 1.5 ml of 0.3 M phosphate buffer (8); after 5 min, most of the organic phase (top layer) that contained the FAME was transferred to a sample vial and analyzed by gas-liquid chromatography with an automatic injector.

Gas-liquid chromatography. FAME samples were analyzed on either a 50-m by 0.2-mm fused silica capillary column with cross-linked methyl silicone (OV-101) as the stationary phase and a Hewlett-Packard (Hewlett-Packard Co., Palo Alto, Calif.) 5880 level 4 gas chromatograph and data system (15) or a 25-m by 0.2-mm methyl phenyl silicone (SE-54) fused silica capillary column and the Hewlett-Packard 5898A microbial identification system (13). In addition, many of the FAME samples were analyzed on both systems, and the resultant cellular fatty acid data were found to be almost identical. The identities of the FAME were confirmed by hydrogenation of unsaturated acids (8), trifluoracetylation of hydroxy acids (8), and mass spectrometry (14).

Isoprenoid quinone analysis. Cultures were grown on three to eight plates (15 by 100 mm) of CYE or BCYE for 48 to 72 h. Cells (0.3 to 1.0 g, wet weight) were harvested with sterile distilled water and placed in a 20- by 150-mm screw-cap tube fitted with a Teflon-lined cap. The quinones were extracted, analyzed by reverse-phase high-performance liquid chromatography, and identified by mass spectrometry as described previously (7, 15–17).

RESULTS AND DISCUSSION

The overall fatty acid compositions of the 23 *Legionella* species were similar, with most species containing high concentrations (>50%) of branched-chain fatty acids. The major or predominant acids included 14-methylpentadecanoic ($C_{i16:0}$), 12-methyltetradecanoic ($C_{a15:0}$), and 14-methylhexadecanoic ($C_{a17:0}$) acids; trace (<0.5%) to 8% amounts of several other branched-chain fatty acids, including 12-methyltridecanoic ($C_{i14:0}$), 13-methyltetradecanoic ($C_{i15:0}$), 14-methylpentadecanoic ($C_{i16:1}$), 15-methylhexadecanoic ($C_{i17:0}$), 16-methylheptadecanoic ($C_{i18:0}$), and 16-methyloctadecanoic ($C_{a19:0}$) acids, were also present. Straight-chain and other acids detected included tetradecanoic ($C_{14:1}$), tetradecanoic ($C_{14:0}$), pentadecanoic ($C_{15:1}$), pentadecanoic ($C_{15:0}$), isomers of hexadecanoic ($C_{16:1}$), hexadecanoic ($C_{16:0}$), heptadecanoic ($C_{17:0}$), nonadecanoic ($C_{19:0}$), and eicosanoic ($C_{20:0}$) acids and a 17-carbon cyclopropane acid tentatively identified as *cis*-9,10-methylene hexadecanoic ($C_{17:0cyc}$) acid. Small amounts (trace to 5%) of branched- or straight-chain, ester-linked hydroxy acids were detected in some species when cells were saponified with 15% NaOH in 50% aqueous methanol (9). Generally, they were not observed in all strains of a given species. Only in the case of *L. longbeachae*, *L. santicrucis*, *L. bozemanii*, and *L. dumoffii* were ester-linked hydroxy acids useful for classification and differentiation.

Examination of the qualitative and quantitive fatty acid data showed that the 23 species could be placed into three

TABLE 2. Cellular fatty acid compositions of seven *Legionella* species in the A15 group[a]

Species	No. of strains	Fatty acid composition (%)														
		$C_{i14:0}$	$C_{i15:0}$	$C_{a15:0}$	$C_{i16:1B}$	$C_{i16:0}$	$C_{16:1\omega7c}$	C_{Un16C}	$C_{16:0}$	$C_{a17:1}$	$C_{i17:0}$	$C_{a17:0}$	$C_{17:0cyc}$	$C_{18:0}$	$C_{a19:0}$	$C_{21:0}$
L. maceachernii	4	tr	–	28 (3)	3 (1)	11 (2)	14 (3)	–	6 (2)	5 (1)	–	22 (2)	–	tr	–	2 (1)
L. micdadei	11	tr	1	32 (4)	2	12 (2)	8 (3)	–	6 (2)	5 (1)	tr	21 (3)	1 (1)	tr	–	2 (1)
L. jordanis	3	2	2	46 (2)	1	18 (2)	4 (1)	–	1 (1)	1	2	19 (3)	2 (1)	tr	–	–
L. hackeliae	2	2 (1)	4 (2)	33 (1)	–	15 (2)	13 (3)	–	6 (2)	–	2 (1)	14 (3)	3 (2)	1	–	–
L. wadsworthii	3	1 (1)	tr	37 (7)	–	10 (2)	8 (3)	1 (1)	6 (2)	–	1 (1)	19 (5)	6 (4)	1 (1)	2 (1)	–
L. dumoffii	10	2 (1)	tr	30 (4)	–	14 (3)	8 (3)	1 (1)	7 (3)	–	–	17 (5)	8 (4)	1 (1)	–	–
L. bozemanii	14	3 (1)	tr	29 (3)	–	14 (3)	9 (3)	1 (1)	11 (4)	–	1	11 (2)	9 (3)	2 (1)	–	–

[a] See footnotes *b* and *c* to Table 1.

major groups on the basis of the relative amounts of $C_{i16:0}$, $C_{16:1}$, and $C_{a15:0}$ acids. The nine species containing $C_{i16:0}$ or $C_{16:1}$ or both $C_{i16:0}$ and $C_{16:1}$ as major acids were designated as the 16C group (Table 1); seven species containing $C_{a15:0}$ as the major acid at concentrations approximately twice that of $C_{i16:0}$ were designated as the A15 group (Table 2); and the remaining seven species, which contained both $C_{a15:0}$ and $C_{i16:0}$ as major acids in about equal amounts, were designated as group A15/16C (Table 3). Some acids, including $C_{14:1}$, $C_{14:0}$, $C_{15:1}$, $C_{15:0}$, $C_{17:0}$, $C_{19:0}$, $C_{20:0}$, and hydroxy acids, were omitted from the tables because they were not useful for differentiating the *Legionella* species or they were present in <80% of the strains.

Comparing relative amounts of $C_{i16:0}$, $C_{16:1\omega7c}$, and other acids enabled further differentiation of species in the 16C group (Table 1). *L. feeleii* was distinguished from all other species by the presence of two unsaturated 16-carbon acids that were designated $C_{i16:1A}$ and $C_{16:1A}$ (Tables 1, 2, and 3). The chain lengths of $C_{i16:1A}$ and $C_{16:1A}$ were established by hydrogenation and rechromatography, but the positions of unsaturation were not determined. Although these two acids were present in small amounts (1 to 2%), they were consistently found in each of the six *L. feeleii* strains and absent in all other named species. *L. oakridgensis* differed from all other *Legionella* species by the presence of higher amounts of $C_{18:0}$ and smaller amounts of $C_{a15:0}$ (Tables 1, 2, and 3) and from other species in the 16C group by the presence of small amounts (1 to 2%) of $C_{i18:0}$ and $C_{a19:0}$ and by larger amounts of $C_{17:0cyc}$ (Table 1).

The presence of $C_{i16:0}$ at higher concentrations than that of any other acid distinguished *L. pneumophila* and *L. spiritensis* from all other *Legionella* species. In addition, these two species contained a branched-chain unsaturated 16-carbon acid, designated $C_{i16:1B}$, that was absent in all other species in the 16C group except *L. oakridgensis* (Table 1, Fig. 1A). Although *L. spiritensis* contained approximately twice the amount of anteiso branched-chain acids as *L. pneumophila* (39 versus 21%; Table 1), differentiation between these two species requires testing of additional strains of *L. spiritensis* to confirm these apparent quantitative differences.

L. sainthelensi was distinguished from the remaining four species in the 16C group by the presence of approximately equal amounts of $C_{a15:0}$, $C_{i16:0}$, and $C_{16:1}$ acids; *L. longbeachae* and *L. santicrucis* contained $C_{17:0cyc}$, which was absent in *L. erythra* and *L. rubrilucens* (Table 1). The fatty acid compositions of *L. longbeachae* and *L. santicrucis* differed in that the total concentrations of the iso and anteiso acids in *L. longbeachae* were approximately equal (30 and 24%, respectively), but in *L. santicrucis* the concentrations of the iso acids (36%) were more than twice that of the anteiso acids (14%). In addition, six of seven *L. longbeachae* strains contained an unsaturated branched-chain 17-carbon hydroxy acid (OH $C_{17:1}$, Fig. 1B) in trace to 3% amounts that was not detected in the type strain of *L. santicrucis*. This acid coeluted with an isomer of octadecanoic acid ($C_{18:1}$) on the 25-m SE-54 column but was resolved from $C_{18:1}$ on the 50-m OV-101 column. The OH $C_{17:1}$ was tentatively identified by observing retention time shifts after hydrogenation and after treatment with trifluoroacetic anhydride to form the diester derivative (8). The identity was confirmed by combined gas-liquid chromatography and mass spectrometry (14).

The fatty acid composition of the type strain of *L. erythra* (ATCC 35303^T) differed significantly from those of three other DNA-confirmed strains of this species by the presence of $C_{16:1\omega7c}$ and $C_{16:0}$ as major acids rather than $C_{i16:0}$ and $C_{16:1\omega7c}$ (Table 1). Only small amounts (4%) of $C_{i16:0}$ acid were detected in the type strain regardless of the length of incubation (1 to 6 days) or the growth medium. The latter three *L. erythra* strains were essentially identical in fatty acid composition to strains of *L. rubrilucens*.

L. maceachernii, *L. micdadei*, and *L. jordanis* (Table 2) differed from the other four species in the A15 group by the presence of small amounts (2 to 5%) of branched-chain

TABLE 3. Cellular fatty acid compositions of seven *Legionella* species in the A15/16C group[a]

Species	No. of strains	Fatty acid composition (%)													
		$C_{i14:0}$	$C_{i15:0}$	$C_{a15:0}$	$C_{i16:1B}$	$C_{i16:0}$	$C_{16:1\omega7c}$	C_{Un16}	$C_{16:0}$	$C_{a17:1}$	$C_{i17:0}$	$C_{a17:0}$	$C_{17:0cyc}$	$C_{18:0}$	$C_{21:0}$
L. israelensis	3	2 (1)	1	27 (3)	1	22 (1)	7 (1)	–	7 (1)	1	1	17 (1)	5 (1)	1	2 (1)
L. jamestowniensis	3	2	1 (1)	30 (4)	3 (1)	21 (3)	4 (3)	tr	3 (1)	3 (1)	1	21 (1)	5 (2)	1	1
L. anisa	11	6 (1)	1	24 (3)	–	23 (4)	9 (3)	1 (1)	8 (3)	–	1 (1)	7 (1)	9 (3)	1	–
L. cherrii	5	5 (1)	tr	27 (4)	–	29 (3)	9 (1)	tr	6 (4)	–	1	12 (2)	3 (2)	1 (1)	–
L. gormanii	2	4 (1)	–	22 (4)	–	17 (3)	11 (2)	1 (1)	11 (3)	–	1	9 (2)	10 (3)	2	–
L. parisiensis ATCC 34299^T	1	3	tr	23 (2)	–	17 (1)	7 (2)	2 (1)	9 (1)	–	1	9 (1)	16 (3)	1	–
L. steigerwaltii ATCC 35302^T	1	2	tr	23 (1)	–	15 (2)	8 (2)	1 (1)	18 (4)	–	–	8	14 (2)	3	–

[a] See footnotes to Table 1.

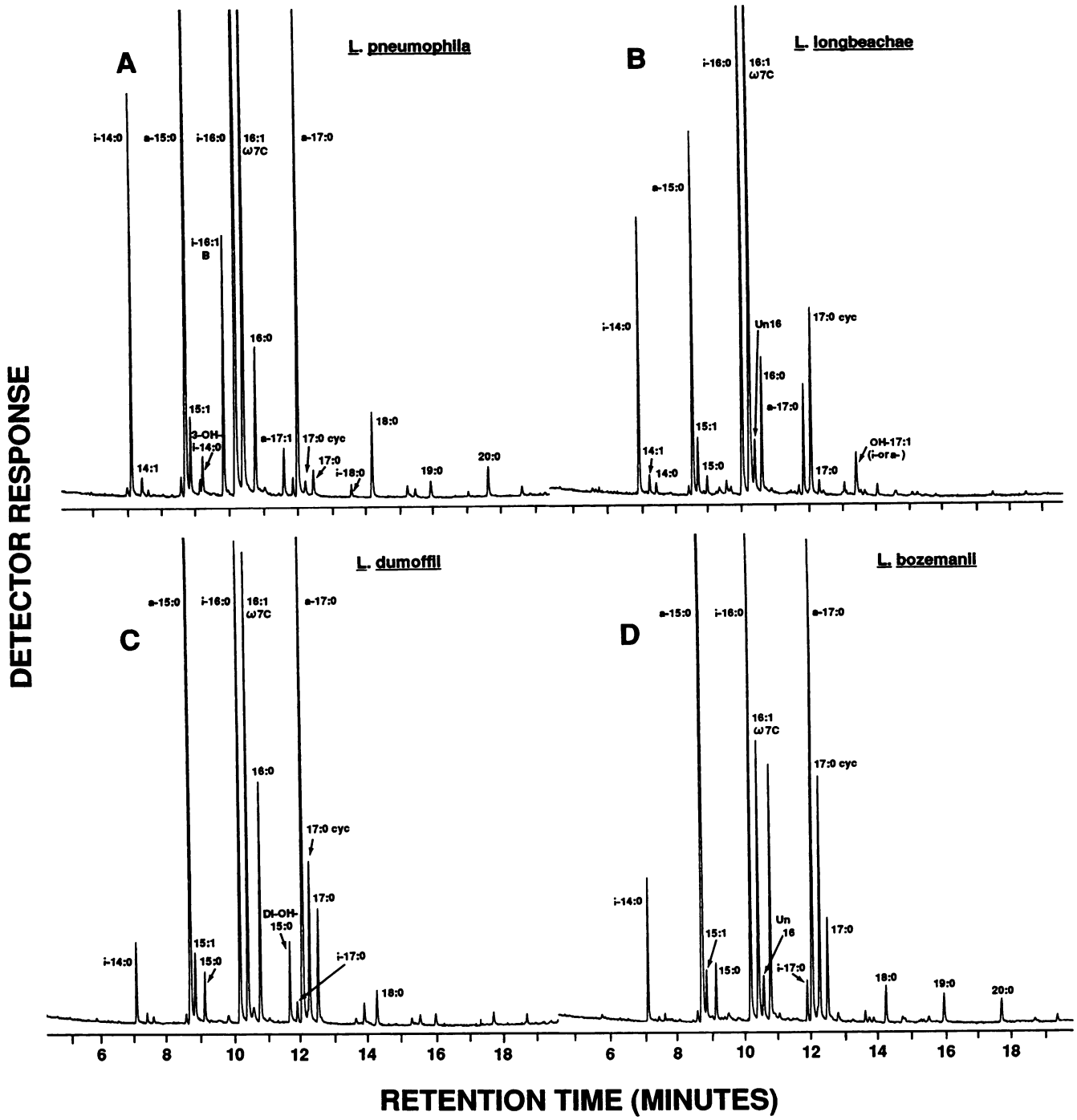

FIG. 1. Gas chromatograms of methylated cellular fatty acids of *L. pneumophila* (A), *L. longbeachae* (B), *L. dumoffii* (C), and *L. bozemanii* (D) analyzed on a 50-m by 0.2-mm OV-101 fused-silica capillary column. See the text and footnote *b* of Table 1 for an explanation of fatty acid abbreviations.

monounsaturated 16-carbon ($C_{i16:1B}$) and 17-carbon ($C_{a17:1}$) acids. *L. maceachernii* and *L. micdadei* had essentially the same fatty acid compositions, but both differed from *L. jordanis* by the presence of $C_{21:0}$, larger amounts of $C_{a17:1}$ (5 versus 1%), and smaller amounts of $C_{a15:0}$ (28 and 32 versus 46%). *L. hackeliae* contained small amounts (4%) of $C_{i15:0}$, which was absent or present in only trace amounts in *L. wadsworthii*, *L. dumoffii*, and *L. bozemanii*. *L. wadsworthii* was the only species in the A15 group that contained $C_{a19:0}$. This species also differed from *L. dumoffii* and *L. bozemanii* in the relative amounts of $C_{a17:0}$ and $C_{i16:0}$ acids; in *L. wadsworthii* the concentration of $C_{a17:0}$ was almost twice that of $C_{i16:0}$, whereas the other two species contained approximately equal amounts (Table 2). The fatty acid compositions of *L. dumoffii* and *L. bozemanii* were essentially identical except for trace to 5% amounts of a dihydroxy 15-carbon acid (di-OH $C_{15:0}$) present in 8 of 10 strains of *L. dumoffii*. This acid coeluted with $C_{i17:0}$ on the 25-m SE 54

column but was resolved from $C_{i17:0}$ on the 50-m OV-101 column (Fig. 1C). Di-OH $C_{15:0}$ was identified by acetylation of the two free hydroxyl groups and by observing the chromatographic retention time shifts of the resulting triester derivative. Combined gas-liquid chromatography and mass spectrometry was used to confirm the identification (14). The di-OH $C_{15:0}$ acid was not detected in *L. bozemanii* (Fig. 1D) or in any of the other *Legionella* species tested.

L. israelensis and *L. jamestowniensis* (Table 3) had almost identical fatty acid compositions and could not be differentiated further; they were differentiated from the other five species in the A15/16C group by the presence of $C_{i16:1B}$, $C_{a17:1}$, $C_{21:0}$, and approximately equal amounts of $C_{i16:0}$ and $C_{a17:0}$ acids. The fatty acid compositions of *L. anisa*, *L. cherrii*, *L. gormanii*, *L. parisiensis*, and *L. steigerwaltii* were very similar. Although quantitative differences were found in the concentrations of several of the acids ($C_{i16:0}$, $C_{16:0}$, and $C_{17:0cyc}$), these species were not differentiated further because only two strains of *L. gormanii* and the type strains of *L. parisiensis* and *L. steigerwaltii* were available for testing.

The acid listed in Tables 1, 2, and 3 as C_{Un16} eluted between $C_{16:1\omega7c}$ and $C_{16:0}$ (Fig. 1B and D) and was found in trace to 3% amounts in several of the *Legionella* species. In all species except *L. longbeachae*, this acid was tentatively identified as a 16-carbon cyclopropane acid ($C_{16:0cyc}$) because its retention time was not affected by either hydrogenation or trifluoracetylation (8). However, the C_{Un16} acid in *L. longbeachae* was converted to $C_{16:0}$ by hydrogenation, was unaffected by acetylation, and thus was identified as an unsaturated 16-carbon fatty acid ($C_{16:1}$). Since both acids were present in small concentrations, their identities have not yet been confirmed by mass spectrometry.

The isoprenoid contents of 23 *Legionella* species are summarized in Table 4. All species contained ubiquinones with 9 to 14 isoprene units in the side chains, and no menaquinones were detected (15, 16). Five different ubiquinone groups were observed. The first was designated group A and contained Q12 as the major ubiquinone, with small amounts of Q11 and Q13. Ubiquinone group B contained Q9, Q10, Q11, and Q12, and all but two species contained trace to 1% amounts of Q13. In general, the concentrations of Q9, Q10, Q11, and Q12 were approximately the same for the 10 species in ubiquinone group B. The concentration of Q9, however, was somewhat lower in *L. parisiensis*, *L. santicrucis*, and *L. steigerwaltii*, whereas *L. cherrii* and *L. steigerwaltii* contained lower concentrations of Q10 than were present in the others. The C group included only two species and was characterized by large amounts of Q10, smaller amounts of Q9 and Q11, and only trace amounts of Q12. Group D included seven species that contained Q13 as the major component. Most of the species also contained Q12 as a major component and Q11 and Q14 as minor components. Four of the species in group D (*L. israelensis*, *L. jamestowniensis*, *L. jordanis*, and *L. micdadei*) contained approximately equal concentrations of Q12 and Q13; in the other three (*L. hackeliae*, *L. maceachernii*, *L. spiritensis*), the relative concentration of Q12 was always less than one-half that of Q13. The E group contained Q13 as the major component with smaller amounts of Q14 and only trace amounts of Q12.

Differentiation of the *Legionella* species on the basis of both FAME composition and ubiquinone content is summarized in the decision trees shown in Fig. 2, 3, and 4. The placement and differentiation of some species, especially those in which only one to three strains are available, are preliminary and may change when additional strains are isolated and tested. *L. feeleii* was distinguished from all other named *Legionella* species by both its fatty acid and quinone patterns (Fig. 2). This species does not share close genetic (2) or serologic (21) relationships with any of the others of the 23 named *Legionella* species but was found to have a high level of DNA relatedness (72%) to unnamed species 31 (2). Recently, we observed that the fatty acid composition of species 31 is the same as that of *L. feeleii* (unpublished observations), and some evidence shows serologic cross-reactivity between them.

L. oakridgensis (Fig. 2) can be differentiated from *L. wadsworthii* (Table 4) and from all other *Legionella* species by its FAME composition. *L. oakridgensis* has low values of DNA relatedness to the other species (2) but is antigenically related to *L. sainthelensi* (21). However, *L. oakridgensis* is different from *L. sainthelensi* and other *Legionella* species because it is nonmotile (2).

L. pneumophila and *L. spiritensis* can be distinguished by differences in their ubiquinone patterns (Fig. 2). These two species also differ in the hippurate reaction (2) and are not closely related genetically (2, 3) or serologically (21) to each other or to any of the other named *Legionella* species, including those which have some chemical characteristics in common (Table 4).

The three species *L. sainthelensi*, *L. longbeachae*, and *L. santicrucis* differ phenotypically from the other seven species with a ubiquinone group B pattern (Table 4) because they do not autofluoresce blue-white (2). Except for weak or variable production of β-lactamase in *L. longbeachae* (2), *L. sainthelensi*, *L. santicrucis*, and *L. longbeachae* are alike

TABLE 4. Ubiquinone contents of 23 *Legionella* species

Species	Ubiquinone content[a]						Ubiquinone group
	Q9	Q10	Q11	Q12	Q13	Q14	
L. erythra	–	–	1–2	4	1–2	–	A
L. pneumophila	–	–	1–2	4	1–2	–	A
L. rubrilucens	–	–	1–2	4	1–2	–	A
L. anisa	2–3	4	2–3	3	tr–1	–	B
L. bozemanii	2–3	4	3–4	4	tr–1	–	B
L. cherrii	2	3	3–4	4	1	–	B
L. dumoffii	2–3	3–4	3–4	4	1	–	B
L. gormanii	3	4	4	4	–	–	B
L. longbeachae	3–4	4	3–4	3–4	tr	–	B
L. parisiensis	1–2	3–4	3	4	1	–	B
L. sainthelensi	3	4	3–4	3–4	tr	–	B
L. santicrucis	1–2	4	3–4	3–4	tr	1	B
L. steigerwaltii	1	2	2–3	4	–	–	B
L. oakridgensis	2	4	1–2	tr	–	–	C
L. wadsworthii	2	4	2–3	tr–1	–	–	C
L. hackeliae	–	–	–	2	4	tr–1	D
L. israelensis	–	tr	1–2	4	3–4	–	D
L. jamestowniensis	–	tr	1–2	4	4	tr	D
L. jordanis	–	tr	tr	3–4	4	tr	D
L. maceachernii	–	–	–	tr–1	4	1	D
L. micdadei	–	–	tr–1	3	4	tr	D
L. spiritensis	–	–	–	tr–1	4	1	D
L. feeleii	–	–	–	tr	4	2–3	E

[a] Numbers refer to visual estimates of the relative amounts of ubiquinones with the major component designated by 4; 2, half the amount of 4; 1, half that of 2; –, not detected. Ranges indicate differences between strains within a species.

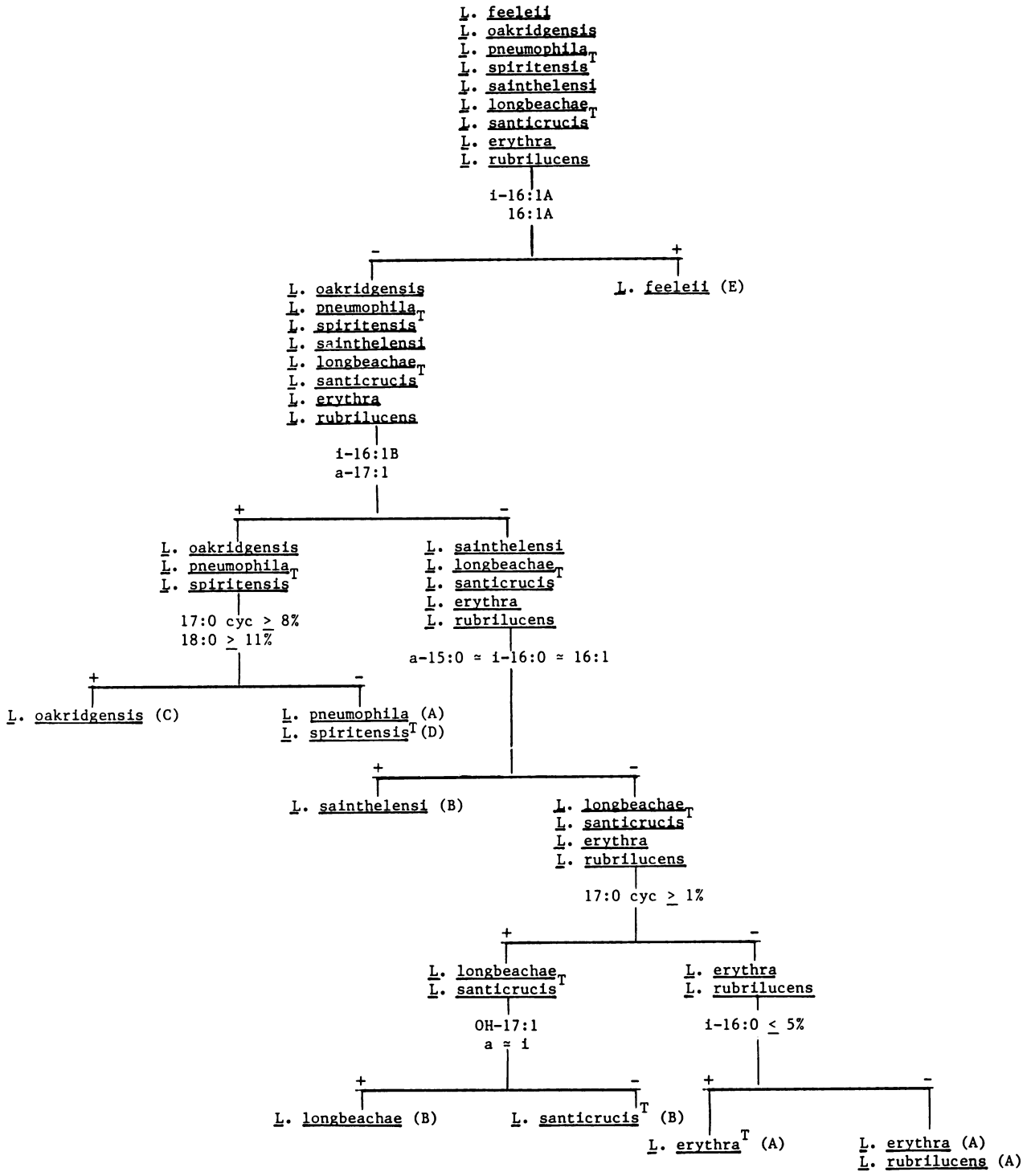

FIG. 2. Decision tree constructed for nine *Legionella* species in the 16C cellular fatty acid group. The superscript T indicates that only the type strain was tested or included. See text and footnote *b* of Table 1 for an explanation of acid designations. The letter within parentheses indicates the ubiquinone group of the species.

biochemically. *L. sainthelensi* and *L. santicrucis* share close genetic relatedness (64%) (2, 3), but the genetic relationships of *L. sainthelensi* and *L. santicrucis* to *L. longbeachae* are less close (37 and 38%, respectively); however, these three species are antigenically related in both the direct immunofluorescence assay (3) and the SAT (21). Although all three species have the same ubiquinone patterns, they can be distinguished by cellular fatty acid composition (Table 1, Fig. 2).

L. erythra and *L. rubrilucens* have the same ubiquinone pattern as *L. pneumophila*, but they are not closely related to *L. pneumophila* by genetic (2, 3) or serologic (21) tests.

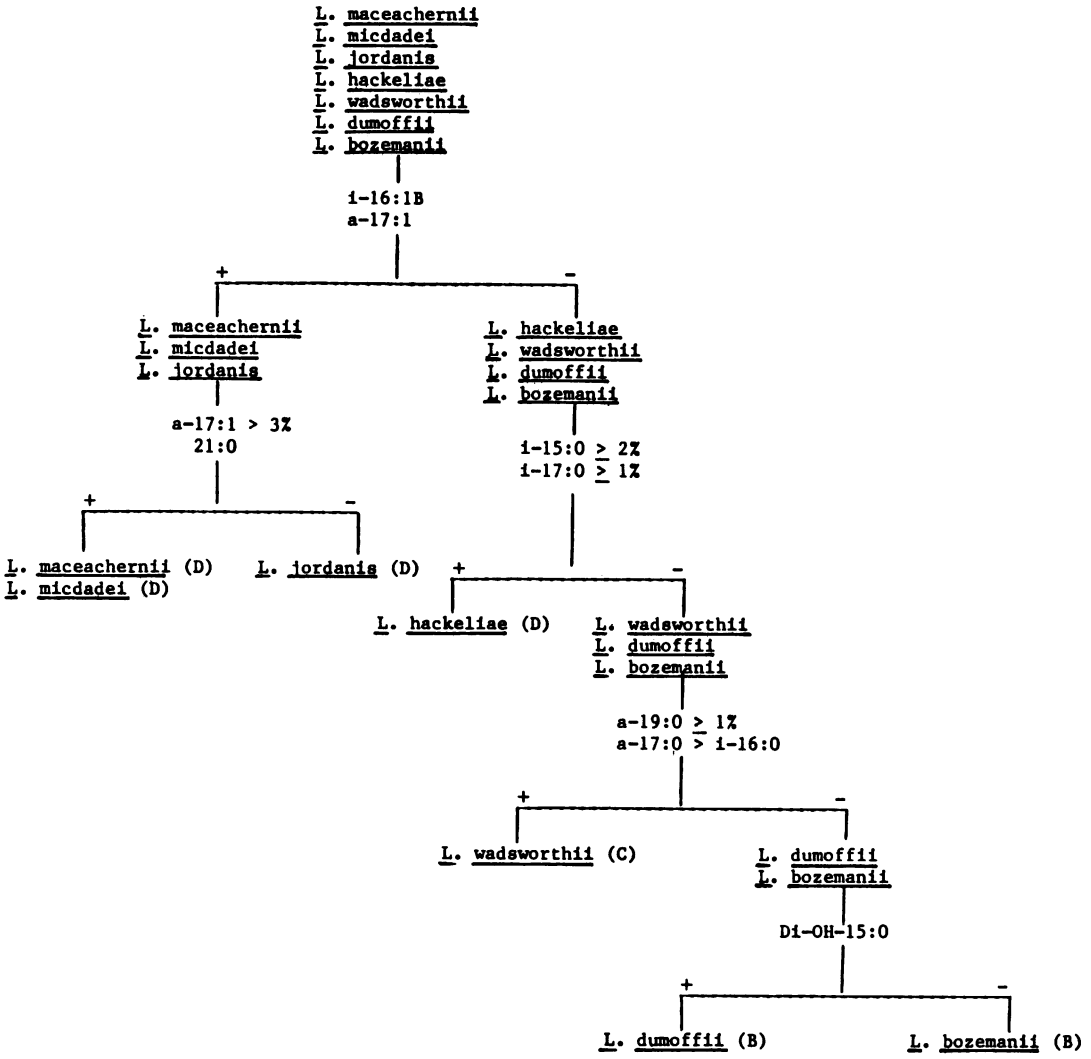

FIG. 3. Decision tree constructed for seven *Legionella* species in the A15 cellular fatty acid group. See the text and footnote *b* of Table 1 for an explanation of acid designations. The letter within parentheses indicates the ubiquinone group of the species.

Both *L. erythra* and *L. rubrilucens* autofluoresce red (2, 3) but can be separated because *L. erythra* is oxidase positive (2, 3). *L. erythra* and *L. rubrilucens* are close genetic relatives (approximately 60%) (2, 3), but antisera prepared against these two species do not cross-react in the direct immunofluorescence assay (3) or the SAT (21). With the exception of the low concentration of branched-chain fatty acids found only in the type strain of *L. erythra*, the chemical compositions of *L. erythra* and *L. rubrilucens* are the same. Additional strains of these two species need to be tested to determine whether the fatty acid composition found for *L. erythra* ATCC 35303^T is indeed an exception for this species.

The species in the A15 group with a ubiquinone group D pattern, *L. maceachernii*, *L. micdadei*, *L. jordanis*, and *L. hackeliae* (Fig. 3), have low genetic relatedness to each other and to most other *Legionella* species (2). *L. maceachernii* and *L. micdadei* share fatty acid profiles and exhibit some genetic relatedness (23%) (2, 3) and serologic relatedness by the direct immunofluorescence assay (3) but not by SAT (21). Cross-reactions do occur between these two species and *L. anisa* in SAT (21). *L. anisa* can be differentiated chemically from both *L. maceachernii* and *L. micdadei* because of differences in both fatty acid and ubiquinone

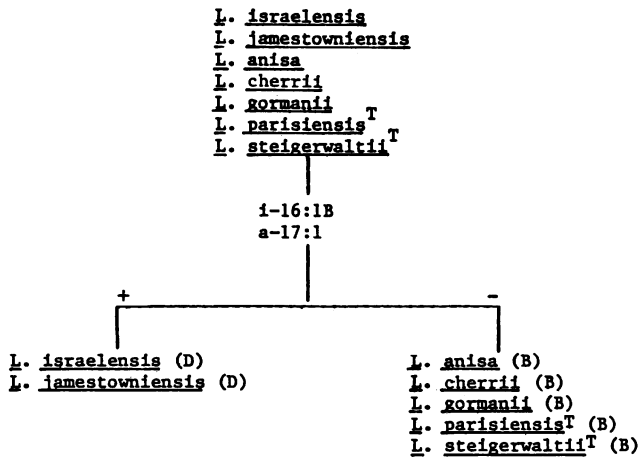

FIG. 4. Decision tree constructed for seven *Legionella* species in the A15/16C cellular fatty acid group. The superscript T indicates that only the type strain was tested. See the text and footnote *b* of Table 1 for an explanation of acid designations. The letter within parentheses indicates the ubiquinone group of the species.

patterns (Tables 3 and 4). *L. jordanis* has very low genetic relatedness to the other *Legionella* species (2) but is serologically related to *L. bozemanii*, *L. longbeachae*, and *L. anisa* (21). *L. jordanis* can be differentiated from these three species by cellular fatty acid composition and ubiquinone content. *L. hackeliae* is not closely related genetically or serologically to any of the other named *Legionella* species (2, 3, 21).

L. wadsworthii differs from all other species by its FAME composition and is the only species in the A15 group with a ubiquinone group C profile (Fig. 3). With the exception of a cross-reaction between *L. wadsworthii* SAT antigens and *L. israelensis* antiserum (1), *L. wadsworthii* is not serologically related to 21 other *Legionella* species (21). The closest genetic relatives of *L. wadsworthii* are *L. parisiensis* and *L. steigerwaltii* (2, 3); however, *L. parisiensis* and *L. steigerwaltii* are in fatty acid group A15/16C and have a ubiquinone group B profile.

L. bozemanii and *L. dumoffii*, the only two species in the A15 group with a ubiquinone group B profile, could usually be differentiated because most strains of *L. dumoffii* tested contained di-OH $C_{15:0}$ (Fig. 1C and 3). *L. bozemanii* and *L. dumoffii* differ genetically (2), serologically (21), and phenotypically in producing oxidase and β-lactamase. They both exhibit blue-white autofluorescence (2) and have close genetic relationships to the other blue-white-autofluorescing species (*L. anisa*, *L. cherrii*, *L. gormanii*, *L. parisiensis*, *L. steigerwaltii*). In addition, *L. bozemanii* has close serologic relationships to most of these five species (21). *L. anisa*, *L. cherrii*, *L. gormanii*, *L. parisiensis*, and *L. steigerwaltii* also have a ubiquinone group B pattern, but all are in the A15/16C fatty acid group and cannot be differentiated by cellular fatty acid composition (Table 3, Fig. 4). These close chemical relationships are not unexpected because the DNA relatedness values among the blue-white-autofluorescing species are higher than those in most of the other legionellae (2). With the exception of the oxidase reaction, *L. anisa*, *L. cherrii*, *L. gormanii*, *L. parisiensis*, and *L. steigerwaltii* are very similar biochemically (2).

L. israelensis and *L. jamestowniensis* are closely related chemically (Table 3, Fig. 4) but not by genetic (1, 2) or serologic (1, 21) tests. These two species can be differentiated phenotypically because *L. israelensis* does not produce a brown pigment on tyrosine-containing medium (1, 2).

With the exception of *L. dumoffii* and *L. longbeachae*, the other *Legionella* species contained only trace amounts of hydroxy fatty acids that were liberated by alkaline saponification (i.e., ester linked). This feature and the large amount of branched-chain nonhydroxy acids distinguish legionellae from other gram-negative bacteria (2, 15). Mayberry (10, 11) reported the presence of both monohydroxy and dihydroxy fatty acids in legionellae after acid hydrolysis (i.e., wall associated, amide linked) and used the hydroxy fatty acid profiles for differentiating some species (11, 20, 24). We have not used the acid-labile hydroxy fatty acids of *Legionella* in our identification scheme, since their accurate determination requires extensive and time-consuming analytical procedures that include multiple hydrolyses, extractions, and thin-layer chromatography (10, 11).

In summary, the results of this study show that all *Legionella* species contain large amounts of branched-chain acids (with only trace to small amounts of ester-linked hydroxy acids) and ubiquinones in which the isoprenoid side chain contains nine or more isoprene units. These two chemical features serve to distinguish legionellae from other gram-negative bacteria, and either or both of these can be used as a rapid, accurate means of identifying suspected isolates of *Legionella* before serologic and other tests are done. Some species, such as *L. feeleii* and *L. oakridgensis*, can be tentatively identified by analysis of cellular fatty acid data alone, whereas many of the other species can be identified using FAME analysis in combination with ubiquinone data. Confirmation of the identity can then be done by serologic and genetic testing with much saving of time and reagents. Identifying a suspect *Legionella* isolate to the genus level is easily accomplished by visual comparison of chromatographic profiles. Identifying to the species level, however, without a computer-based identification system is tedious, time-consuming, and subject to human error and bias. For these reasons we are currently using the Hewlett-Packard microbial identification system to develop a computer-based scheme for identifying legionellae by cellular fatty acid composition. When completed, the *Legionella* library will be available for use in the microbial identification system.

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