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NOTES

Cellular Fatty Acid Composition of *Legionella longbeachae* sp. nov.†

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The cellular fatty acid composition of *Legionella longbeachae* was determined by gas-liquid chromatography. As in other *Legionella* species, the fatty acids of this new species are characterized by relatively large amounts of branched-chain acids.

Determination of the cellular fatty acid composition of the etiological agents of Legionnaires disease by gas-liquid chromatography has proved to be a valuable test for their identification and classification. The initial isolates from the 1976 epidemic in Philadelphia, which were later classified as *Legionella pneumophila*, family *Legionellaceae* (1), were found to differ from other gram-negative bacteria by the presence of relatively large amounts of branched-chain fatty acids (10). Subsequent studies confirmed the uniqueness of the cellular fatty acid profile of additional isolates of *L. pneumophila* (7), as well as of other more recently designated species of *Legionella* (4, 5, 8). Recently, McKinney et al. studied another group of *Legionella*-like organisms for which the name *Legionella longbeachae* has been proposed (5a). In this report, we describe the cellular fatty acid composition of *L. longbeachae* and compare it to the fatty acid composition of other *Legionella* species.

Each of the seven known strains of *L. longbeachae* was isolated from respiratory tract specimens by direct plating on charcoal-yeast extract (CYE) agar or buffered CYE (BCYE) agar (3, 11). They were identified by cultural, biochemical, and staining characteristics, by fluorescent-antibody tests, and by deoxyribonucleic acid-relatedness measurements (1, 2, 11). Cells for fatty acid analysis were obtained after 24 to 72 h of growth at 37°C on CYE. The cells were saponified, and the fatty acids were methylated by a procedure described previously (6, 10). Methyl esters were identified and quantitated by gas-liquid chromatography and by mass spectrometry as described in earlier reports (6, 9, 10).

Shown in Fig. 1 is a chromatogram of the cellular fatty acids of *L. longbeachae* strain Long Beach 4 (LB4). The fatty acids of this strain are qualitatively similar to those of other *Legionella* species, with the characteristic features of the presence of branched-chain i-14:0, a-15:0, i-16:1, i-16:0, and a-17:0 acids. Also present are the saturated and monounsaturated straight-chain 16-carbon acids (16:0 and 16:1), but the relative amounts of these acids are generally higher than in other species of *Legionella* (4, 5, 8, 10). The ratio of the i-16:0 and 16:1 acids differed among strains of *L. longbeachae*; a variation in the ratio of i-16:0 to 16:0 was also observed in the same strain upon repeated subculture. For example, in two of five separate subcultures of strain LB4, the relative concentration of the i-16:0 acid was slightly greater than that of 16:1 acid. Similar variations in the relative amounts of i-16:0 and 16:1 acids were observed upon repeated subculture of each of the other six *L. longbeachae* strains. This variation in fatty acid composition has not been observed in other *Legionella* strains (4, 5, 7, 8, 10). Reasons for the variations in the relative amounts of i-16:0 and 16:1 acids in *L. longbeachae* are not obvious, since the cultures were grown under identical conditions on the same culture medium. Also, no consistent differences were observed in the cellular fatty acid composition of cells grown for 24, 48, or 72 h.

The fatty acid compositions of known species of *Legionella* are presented in Table 1. Without exception, the single most abundant acid in more

† This paper is dedicated to William B. Cherry on the occasion of his retirement as Scientist Director, Centers for Disease Control, Atlanta, Ga.

TABLE 1. *Cellular fatty acid compositions of L. longbeachae and other Legionella species*

Species (no. of isolates tested)	Fatty acid content (% of total acids)															
	i-14:0[a]	14:0	a-15:0	15:1	15:0	i-16:1	i-16:0	16:1	16:0	a-17:1	a-17:0	17 CYC	17:0	18:0	19:0	20:0
L. longbeachae (7)	3	T[b]	11	2	T	T	19	31	13	T	9	2	2	3	T	2
L. pneumophila (298)	8	T	14	T	T	2	32	13	10	T	11	3	T	2	T	2
L. bozemanii (4)	4	T	31	T	T	T	17	11	12	T	24	2	T	T	T	T
L. micdadei (6)	T	T	40	T	T	T	11	10	10	3	24	T	T	T	T	T
L. dumoffii (2)	2	T	26	T	T	T	14	16	9	T	22	5	3	T	T	T
L. gormanii (1)	5	T	24	2	T	T	20	15	10	T	12	3	3	2	T	T

[a] Number to the left of the colon refers to the number of carbon atoms; number to the right refers to number of double bonds; i- indicates a methyl branch at the *iso* carbon atom; a- indicates a methyl branch at the *anteiso* carbon atom. CYC, Cyclopropane.

[b] T = <2%.

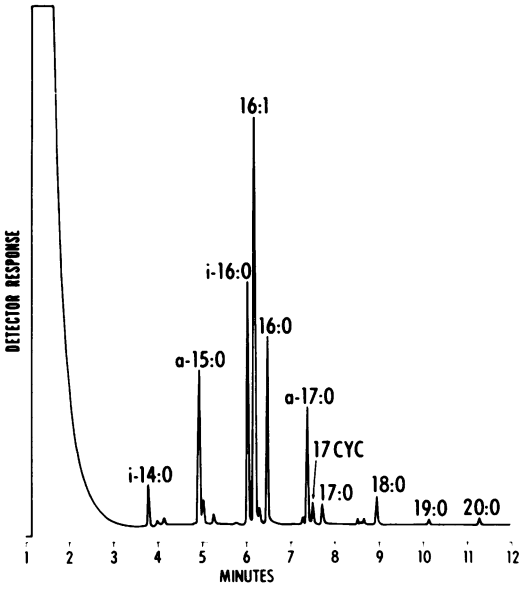

FIG. 1. *Gas chromatogram of esterified fatty acids (as methyl esters) of saponified whole cells of L. longbeachae LB-4 run on a 50-m by 0.2-mm fused silica OV-1 capillary column.*

than 290 strains of *L. pneumophila* has been i-16:0, whereas other species contain a-15:0 as the most abundant acid. However, with strains of *L. longbeachae*, either i-16:0 or 16:1 was the major acid, or these two acids were present in approximately equal concentrations as the major components. Thus, on the basis of fatty acid composition, *L. longbeachae* can be easily distinguished from all *Legionella* strains except *L. pneumophila*. Differentiation of *L. longbeachae* from *L. pneumophila* can be easily accomplished by direct fluorescent-antibody tests (5a).

Determination of the cellular fatty acid composition of suspect isolates is a rapid and reliable screening test for *Legionella*. The presence of relatively large amounts of the total acids as branched-chain i-14:0, a-15:0, i-16:0, and a-17:0 is characteristic of *Legionella*. The presence of these acids with major amounts of i-16:0 indicates *L. pneumophila* or possibly *L. longbeachae*; other *Legionella* species are recognized by the presence of these same acids, with a-15:0 as the major component. Identification of fatty acids by gas-liquid chromatography data alone is significantly increased by using capillary columns, since positional isomers of acids with the same carbon number can be resolved with these columns (6, 9).

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