JOURNAL OF CLINICAL MICROBIOLOGY, May 1979, p. 648-649
0095-1137/79/05-0648/02$02.00/0

Vol. 9, No. 5

Further Studies of the Cellular Fatty Acid Composition of Legionnaires Disease Bacteria

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Received for publication 5 March 1979

The cellular fatty acid composition of 36 strains of the Legionnaires disease bacterium was determined by gas chromatography after growth on different media. The fatty acid profile of each strain was essentially identical on each medium and was characterized by large amounts (>68%) of branched-chain acids.

The cellular fatty acid composition of the first six strains of the Legionnaires disease bacterium (LDB) to be recognized was reported earlier from this laboratory (3). The fatty acid profiles of these strains were essentially identical and apparently were different from other gram-negative bacteria in that they had large amounts of branched-chain acids. Since our initial study, 36 additional strains have been isolated both from clinical materials and from environmental sources at diverse geographical locations. We examined these isolates for cellular fatty acids after growing them on enriched Mueller-Hinton (M-H) agar and on other recently developed growth media.

The identity of each strain was established by conventional cultural and staining characteristics (5), by fluorescent antibody tests (1), and by gas-liquid chromatography of cellular fatty acids (3). Cells for fatty acid analysis were obtained after 24 to 72 h of growth on enriched M-H agar (2), on charcoal-yeast extract (C-YE) agar (2), and on semisynthetic medium (4). The cells were saponified and the fatty acids were methylated by the procedure described previously (3). Methyl esters were identified and quantitated by gas-liquid chromatography as described in the earlier report (3).

Each of the 36 strains grown on enriched M-H agar had a fatty acid composition similar to that reported for the first six strains grown on this medium (3). In each strain the most abundant acid was a saturated, branched-chain 16-carbon acid (i-16:0) with the methyl branch at the iso (penultimate) carbon atom. The next most abundant were a monounsaturated, 16-carbon straight-chain acid (16:1); a 15-carbon branched-chain acid (a-15:0) with the methyl branch at the anteiso (antepenultimate) carbon atom; a saturated, 14-carbon branched-chain acid (i-14:0); and a saturated, 17-carbon branched-chain acid (a-17:0). These five major acids constituted approximately 85 to 90% of the total acids. Other acids present in relatively small amounts (0 to 5%) were a monounsaturated, 16-carbon iso branched-chain acid (i-16:1) and normal straight-chain saturated acids (16:0, 17:0, 18:0, 19:0, 20:0). In addition, some strains contained small amounts (0 to 4%) of a 17-carbon cyclopropane acid (17Δ).

The effect of media composition on cellular fatty acids was evaluated with each new medium developed for LDB (2). Fatty acid compositions

TABLE 1. *Cellular fatty acid composition LDB strains Berkeley and Flint 2 grown on M-H and C-YE media*

Fatty acid[a]	Berkeley		Flint 2		Avg. of 36 strains	
	M-H	C-YE	M-H	C-YE	M-H	C-YE
i-14:0	7[b]	7	7	6	10	8
14:0	T	T	T	T	T	T
a-15:0	17	21	14	18	15	14
15:0	T	T	T	3	T	T
i-16:1	7	7	3	4	6	3
i-16:0	36	26	49	35	39	32
16:1	7	14	10	12	15	13
16:0	7	5	7	8	2	10
a-17:0	17	16	10	12	9	11
17Δ	T	T	T	T	T	3
17:0	T	T	T	T	T	2
18:0	2	2	T	2	2	2
19:0	T	T	T	T	T	T
20:0	T	2	T	T	2	2
Total of branched-chain acids	84	77	83	77	79	68

[a] The number to the left of the colon refers to the number of carbon atoms; the number to the right refers to the number of double bonds; i- indicates a methyl branch at the iso carbon atom; a- indicates a methyl branch at the anteiso carbon; and Δ indicates a cyclopropane ring.

[b] Number refers to percent of total fatty acids; T = less than 2%.

of two LDB strains grown on M-H and C-YE agar are presented in Table 1. Comparison of the data shows no qualitative difference in fatty acids on the two media. However, the total percentage of branched-chain acids was higher on the M-H medium than on C-YE (Berkeley, 7%; Flint 2, 6%). The same relationship was observed for other strains; the average value of total branched-chain acids for 36 strains on M-H medium was 79%, compared to 68% on C-YE. The fatty acid composition of cells grown on Feeley-Gorman (2) and semisynthetic (4) media was essentially identical to that of cells grown on M-H, with some slight increase (5 to 7%) in the relative amount of a-15:0 acid on the semisynthetic medium. Even though the relative amounts of total branched-chain acids for all strains was lower on C-YE medium than on M-H medium, the presence of relatively large amounts of i-16:0, a-15:0, a-17:0, and i-14:0 acids on all media were consistent, characteristic features of the LDB fatty acid profile. Thus, the determination of these acids provides a valuable test for rapid identification and classification of suspect isolates of LDB.

Excellent separation of the fatty acid methyl esters of LDB is obtained on nonpolar gas-liquid chromatographic stationary-phase materials, and strong preliminary identification is possible by comparing retention times to highly purified standards, which are available from several commercial sources. However, branched-chain isomers are not resolved on nonpolar phases, and their identity must be established by additional gas-liquid chromatographic analysis on polar stationary phases or by mass spectrometry (3).

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