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Cellular Fatty Acid Composition of *Francisella tularensis*

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Several unusual fatty acids characterized strains of *Francisella tularensis*. Long-chain (C_{20}-C_{26}) acids and the hydroxy acids 2-hydroxy-decanoate, 3-hydroxy-hexadecanoate, and 3-hydroxy-octadecanoate appeared to be of special diagnostic value.

Fatty acids of a high variety of structures have been encountered in bacterial lipids (1, 9, 12). Gram-negative bacteria often contain saturated and monoenoic straight-chain acids together with one or a few hydroxy fatty acids. Typical of many gram-positive bacteria are methyl branched acids and lack of hydroxy fatty acids. Cyclopropane-substituted alkyl chains have been found in acids of both gram stain groups. Such characteristic fatty acid features have proved useful in delineation of relationships among bacterial groups and species (8, 9, 12) and may provide valuable information for diagnosis of unusual clinical isolates (3, 5, 10).

Francisella tularensis is the etiological agent of tularemia and is extremely pathogenic for humans and animals. Probably due to the danger of handling this organism and its poor growth on usual media, its taxonomic position among gram-negative bacteria is unsatisfactorily elucidated. As part of a taxonomic study of small gram-negative rod-shaped bacteria, cellular fatty acid compositions of nine strains of *F. tularensis* are described in this report.

Strains 1, 6, 7, and 9 in Table 1 were received from K. A. Karlsson, Statens Veterinärmedicinska Anstalt, Stockholm, Sweden. Strain 2 (Jap Down) was a gift from G. Holt, Veterinärinstituttet, Oslo. Strain 3 (ATCC 6223) was obtained from American Type Culture Collection. Strain 4 (isolated from hare) was a gift of S. D. Henriksen, Kapt. W. Wilhelmsen og Frues Bakteriologiske Institutt, University of Oslo. Strain 5 (vaccination strain) was received from U.S. Army Medical Research Institute for Infectious Diseases, Fort Detrick, Md. U.S.A. Strain 8 (isolated from insect) is our own isolate.

The identity of the strains was established by conventional cultural and biochemical tests (6). For fatty acid analysis, bacteria were grown for 24 h on Thayer-Martin agar (with 7% horse blood) without antibiotics at 37°C. Harvesting, methyl ester formation, and extraction of cellular fatty acids were performed as described (7, 8). The chromatographic conditions for analysis of the methyl esters and their quantitation were mainly as previously described (7, 8). Two glass columns, a 25-m-long SCOT SE-30 (Chrompack Inc., The Netherlands) and a 2-m-long 10% CP-10 (Applied Science Laboratories, State College, Pa.), were used, and the detection was performed by a flame ionization detector. The percentage value of each fatty acid was calculated on the basis of the individual peak areas and printed out as percentage of total peak area by the electronic integrator. These values were then adjusted by the response factor of each fatty acid.

Peak identifications were obtained by comparison of retention times to those of authentic standards on both columns. Hydroxy fatty acid peaks were located by trifluoroacetylation and rechromatography (8). All structural elucidations were verified by gas chromatography-mass spectrometry (7; E. Jantzen and J. Lassen, submitted for publication).

A typical gas chromatogram of a *F. tularensis* strain (no. 2) is shown in Fig. 1. Most characteristic is the relatively large amounts of the long-chain saturated and monoenoic C_{20}-C_{26} acids. Such long fatty acids are unusual among bacteria and have, to our knowledge, only been found in appreciable amounts in mycobacteria (4, 16) and the gram-positive species *Lactobacillus heterohiochii*, an alcoholophilic bacterium (15).

For the C_{24} and C_{26} acids, no standards were available for retention time comparison. Their straight-chain nature and degree of saturation were tentatively established by comparison to standards of lower homologs. Thus, both the method of log retention-time plotting and mass spectrometry confirmed that these four compounds represented tetracosenoate (24:1), *n*-tetracosanoate (24:0), hexacosenoate (26:1), and *n*-hexacosanoate (26:0).

Three peaks changed their position by trifluo-

TABLE 1. *Cellular fatty acid composition (percent) of F. tularensis strains*

Strain[a]	Fatty acid																
	10:0[b]	2-OH-10:0	14:0	16:1	16:0	3-OH-16:0	18:2	18:1	18:0	3-OH-18:0	20:1	20:0	22:1	22:0	24:1	24:0	26:1	26:0
1	tr[c]	3.1	5.2	tr	10.4	3.8	tr	6.2	2.8	28.7	0.9	2.2	2.8	7.3	14.6	8.5	2.0	1.6
2	0.5	2.8	5.5	tr	10.2	3.2	tr	4.9	3.1	25.5	0.9	3.0	2.0	9.9	12.8	12.0	1.4	1.9
3	0.7	1.0	5.8	tr	12.1	1.9	tr	3.2	4.6	14.6	0.7	3.9	2.3	14.6	11.7	18.1	1.8	3.3
4	—	5.0	4.7	0.9	10.5	3.7	tr	5.5	2.8	27.3	0.9	2.7	2.7	9.3	13.0	9.1	1.2	1.0
5	tr	1.2	5.7	0.3	9.9	3.6	0.2	7.5	2.4	23.7	1.5	2.3	4.0	8.1	19.3	7.4	1.8	1.2
6	0.2	2.5	5.7	tr	9.9	2.9	0.1	5.7	4.0	24.0	0.9	3.2	2.3	11.0	11.7	12.4	0.9	1.4
7	0.2	1.8	5.4	0.2	11.0	3.1	tr	7.1	4.7	22.7	1.9	3.5	2.6	11.4	11.2	11.1	0.8	1.1
8	—	2.7	6.3	tr	10.2	3.4	0.5	5.7	3.5	26.6	1.0	3.0	2.7	9.9	13.7	9.0	1.1	0.5
9	0.5	2.2	6.2	0.2	10.0	2.9	tr	6.2	3.9	23.2	1.2	3.1	2.5	9.8	12.2	12.6	1.2	1.9

[a] See text for description.

[b] Key to the fatty acid designation: the figure before the colon indicates the number of carbon atoms in the chain; the figure after the colon indicates the number of double bonds; the symbols 2-OH and 3-OH indicate a hydroxy group and its position.

[c] tr, Trace, below 0.1%; —, below the detection limit.

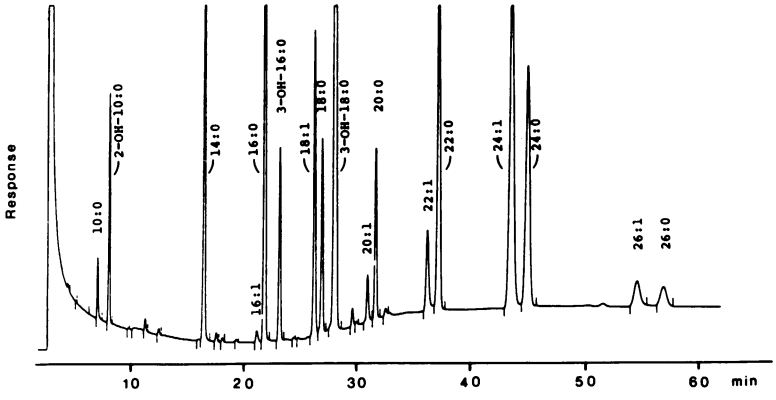

FIG. 1. *A typical gas chromatographic fatty acid methyl ester profile of a F. tularensis strain. The bacterial cells (strain no. 2 in Table 1) were digested by 2 N HCl in methanol, and the hydroxy fatty acids were trifluoroacetylated before injection on the 25-m SCOT SE 30 column.*

roacetylation, thus indicating the presence of an acid with a free hydroxy group in the chain. By gas chromatography-mass spectrometry, the first of these peaks (Fig. 1) gave a molecular ion of *m/e* 202 and a large M-59 fragment which is characteristic of the methyl esters of 2-hydroxy fatty acid (11). Both the mass spectra and the retention-time data fit precisely with those given by the 2-hydroxy-decanoate standard. The two other polar fatty acids showed the characteristic *m/e* 103 fragment of 3-hydroxy fatty acids (11). Comparison to reference compounds firmly established the identity of these peaks as 3-hydroxy-hexadecanoate and 3-hydroxy-octadecanoate, respectively. These three hydroxy fatty acids, especially 2-hydroxy-decanoate and 3-hydroxy-octadecanoate are rare among bacteria (1, 9, 12) and represent valuable taxonomic characteristics of *F. tularensis*.

Table 1 shows percentage composition of the cellular fatty acids of the nine examined strains. Evidently, in terms of fatty acids these organisms form a homogeneous group, and no distinction between geographical variants (e.g. palaearctica and nearctica) was revealed. The fatty acid pattern is distinctly different from all other gram-negative bacteria analyzed so far, including species of *Brucella* (2, 13), *Bordetella pertussis* (13), *Pasteurella multocida*, and species of *Haemophilus* (unpublished data). Species of *Yersinia*, i.e., *Y. pestis* and *Y. pseudotuberculosis*, have been reported to contain small amounts of 3-hydroxy-hexadecanoate and a compound tentatively identified as hydroxy-octadecanoate (14). Otherwise, the fatty acid composition of this genus is characterized by relatively large amounts of 16:1, 16:0, and a C_{17} cyclopropane fatty acid (14; Jantzen and Lassen,

submitted for publication). Thus, this bacterial group is also easily distinguishable from *F. tularensis* by fatty acid analysis.

Determination of cellular fatty acid composition seems to be a relatively rapid and selective identification method for strains of *Francisella*, and it will be interesting to see whether this method can distinguish *F. tularensis* from *F. novicida*, the other species of the genus.

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