Differentiation Between *Mycobacterium kansasii* and *Mycobacterium marinum* by Gas-Liquid Chromatographic Analysis of Cellular Fatty Acids

CHARLES O. THOEN, ALFRED G. KARLSON, AND RALPH D. ELLEFSON

*Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901*

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Comparison of the cellular fatty acids of 10 strains of *Mycobacterium marinum* and 35 strains of *Mycobacterium kansasii* revealed similarities within each species but differences between these two photochromogenic mycobacteria. A branched-chain fatty acid characteristic of *M. kansasii* was found in trace amounts in 2 of the 10 strains of *M. marinum.*

In a study of 35 strains of *Mycobacterium kansasii,* we found that the chromatographic profiles of cellular fatty acids were similar, as determined by gas-liquid chromatography (4). Also, each of the strains contained a branched-chain fatty acid with a methyl group at C4; this was not observed in fatty acid profiles of *Mycobacterium avium* and some other non-photochromogenic mycobacteria (5).

We report herein our studies on the analysis of cellular fatty acids of *M. marinum* which, like *M. kansasii,* is also photochromogenic. The 10 strains of *M. marinum* were found to have similar fatty acid profiles. All strains differed from those of *M. kansasii* in having no branched-chain fatty acid or only trace amounts of it. We also found that the fatty acid components of 3 strains of *M. kansasii* grown at 30°C was the same as that reported for the 35 strains grown at 37°C (4).

The 10 strains of *M. marinum* examined had been isolated from different patients with skin lesions. (Five strains each were supplied by Werner B. Schaefer, National Jewish Hospital, Denver, and E. H. Runyon, Veterans Administration Hospital, Salt Lake City.) The organisms were identified by tests for photochromogenicity (1), by nitrate reduction (6), and by seralloglutination (2); all were photochromogenic, failed to reduce nitrates, and were agglutinated by homologous antiserum.

Each of 10 strains of *M. marinum* were subcultured on Lowenstein-Jensen medium for 14 days. One loopful (2-mm loop) of growth was transferred to 300 ml of Proskaue and Beck medium in each of two 1,000-ml Erlenmeyer flasks and incubated at 30°C for 28 days in an atmosphere of air containing 5% carbon dioxide. Three strains of *M. kansasii* were incubated simultaneously under identical conditions. Flasks of uninoculated medium were included as controls. Smears and subcultures were made to detect contamination.

The bacterial cells were harvested by centrifugation for 20 min at 3,000 rev/min. The saponification of cells and extraction of fatty acids were accomplished as previously described (4). The fatty acids were esterified with diazomethane by the method of Schlenk and Gellerman (3).

The fatty acid methyl esters were analyzed by using a gas-liquid chromatograph equipped with a hydrogen flame ionization detector (F & M model 500-1609). The fatty acids were fractionated on 6-foot (1.8-m) columns packed with 15% diethylene glycol succinate on siliconized Chromosorb G-AN (60-80 mesh) or 5% methyl silicone SE-30 on Chromosorb W (60-80 mesh). The columns were operated isothermally at 200 and 250°C, respectively, with the detector at 275°C.

The cellular fatty acids were identified by comparing their chromatographic mobilities with those of known standards and by testing for unsaturation and for convertibility to known saturated acids with catalytic hydrogenation. The fatty acid profiles of *M. marinum* as determined by gas-liquid chromatography in-
Table 1. Comparison of the percentage of fatty acids of *M. kansasii* and *M. marinum*

| Carbon no. | Retention time (min) of methyl esters of fatty acids | *M. kansasii* (35 strains) | *M. marinum* (10 strains) |
|------------|-----------------------------------------------|---------------------------|--------------------------|
|            | DEGS | SE-30 | Mean per cent | SD | Mean per cent | SD |
| 8:0        | 1.7  | 0.8   | T             | T  |               | T  |
| 10:0       | 2.9  | 2.2   | T             | T  |               | T  |
| 11:0       | 3.7  | 3.0   | T             | T  |               | T  |
| 12:0       | 5.0  | 3.8   | 1.0           | 0.2|               | T  |
| 14:0       | 8.4  | 6.7   | 4.1           | 1.3| 2.8           | 0.5|
| BCFA<sup>c</sup> | 7.2  | 7.9   | 3.8           | 0.7| T             | T  |
| 15:0       | 10.7 | 8.8   | 1.3           | 0.5| 1.1           | 0.2|
| 16:0       | 14.5 | 11.0  | 35.0          | 3.5| 33.4          | 3.1|
| 16:1       | 17.3 | 13.4  | 15.3          | 3.7| 13.0          | T  |
| 17:0       | 19.2 | 14.8  | 4.7           | 1.4| 3.1           | 1.5|
| 17:B       | 19.6 | 12.8  | T             | T  |               | T  |
| 18:0       | 23.8 | 18.5  | 10.4          | 2.3| 12.4          | 2.2|
| 18:1       | 28.8 | 19.4  | 17.3          | 3.8| 14.3          | 2.4|
| 19:0       | 32.4 | 25.2  | T             | T  | 1.0           | 0.3|
| B<sup>+</sup> | 21.6 | 8.4   | 8.4           | 1.7| 13.6          | 3.2|
| 20:0       | 42.4 | 32.7  | 2.0           | 0.6| 4.2           | 1.4|
| 20:1       | 49.6 | 31.0  | T             | T  |               | T  |
| 21:0       | 56.0 | 42.4  | T             | T  | 2.1           | 0.6|
| 22:0       | 72.0 | 54.4  | 2.3           | 0.7| 1.0           | 0.4|
| 22:1       | 84.4 | 51.4  | 1.6           | 0.4| T             | T  |
| 23:0       | 96.4 | 70.2  | T             | T  | 1.1           | 0.3|
| 24:0       | 126.0| 90.0  | 2.8           | 0.6| 3.2           | 0.7|
| 24:1       | 144.4| 88.4  | 1.6           | 0.3| T             | T  |

<sup>a</sup>On two gas-liquid chromatography columns: DEGS, diethylene glycol succinate coated on Chromosorb G-AW; SE-30, methyl silicone rubber gum coated on Chromosorb W.

<sup>b</sup>Includes data previously described (4). Abbreviations: T, trace; SD, standard deviation.

<sup>c</sup>Tentatively identified by mass spectrographic analysis as a saturated branched-chain fatty acid with a methyl group at C<sub>2</sub>.

<sup>d</sup>Tentatively identified as tuberculostearic acid.

Included saturated fatty acids ranging from C<sub>4</sub> to C<sub>24</sub>, plus some unsaturated analogues and a branched-chain fatty acid tentatively identified as tuberculostearic acid (Table 1). Strong similarities were observed among the fatty acid profiles of the 10 strains of *M. marinum*. Those fatty acid profiles did not include more than traces of a saturated, branched-chain fatty acid that was a component of *M. kansasii*; thus far in our experience, that saturated branched-chain acid has been a peculiar characteristic of *M. kansasii*. As it was found also in a nonphotochromogenic mutant of *M. kansasii* but not in *M. marinum* in more than trace amounts, the formation of the peculiar saturated, branched-chain acid must have been unrelated to the chromogenicity of *M. kansasii*.

Fatty acid components of the three strains of *M. kansasii* grown at 30°C were similar to chromatographic profiles of the 35 strains grown at 37°C, indicating that such changes in incubation temperature did not significantly alter fatty acid metabolism.

On the basis of the consistent differences that we observed, an analysis of the cellular fatty acids seems to be a reliable chemical means of distinguishing *M. marinum* from *M. kansasii*.

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