Fatty and Mycolic Acids of *Mycobacterium malmoeense*

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The fatty acids and mycolic acids of 16 clinical isolates of *Mycobacterium malmoeense* were studied by gas chromatography and thin-layer chromatography. All strains contained 2-methyleicosanoic and 2,4,6-trimethyltetracosanoic acids and alpha-, alpha'-, and keto-mycolic acids. The reported findings suggest that lipid analysis is a very useful approach in the species identification of *M. malmoeense*.

Mycobacterium malmoeense, a lung pathogen first described by Schröder and Juhlin (9), is being isolated with increasing frequency from clinical specimens in Europe (3). This bacterium is classified as belonging to the slow-growing, nonchromogenic group of mycobacteria, sharing several characteristics with such species as *M. avium* and *M. intracellulare* (3, 12). The present investigation was done to study the cellular fatty acids and mycolic acids of *M. malmoeense*.

A total of 16 strains isolated from 16 patients were studied. Eight of the clinical isolates were recovered from patients in southern Sweden, and eight were from patients in the United Kingdom. The mycobacteria were cultivated for 4 to 5 weeks at 37°C in Middlebrook 7H9, Middlebrook 7H10, Dubos, or Löwenstein-Jensen medium and identified by several well-established methods (4-6, 11). The fatty acids and mycolic acids were extracted from 5 to 10 mg of bacteria by both acid methanolysis (7) and saponification followed by esterification with iodomethane (1, 2). The fatty acid methyl esters were separated by gas chromatography on a fused-silica capillary column (25 m by 0.25 mm) coated with SE-30, a nonpolar, stationary-phase agent. The column was temperature programmed from 140 to 280°C at a rate of 5°C/min. The methyl mycolates were separated by two-dimensional thin-layer chromatography with silica gel plates (10 by 10 cm) and dichloromethane (first direction, once) and petroleum ether (bp, 60 to 80°C)-acetone (95:5 [vol/vol]) (second direction, three times) as solvents (10). The components were identified by comparing the chromatograms with those of known methyl mycolates.

The thin-layer chromatography analyses revealed alpha-, alpha'-, and keto-mycolates in all strains (Fig. 1), whereas the gas chromatography analyses revealed the presence of saturated straight-chain fatty acids ranging from 14 to 26 carbon atoms (the C_{26:0} acid resulting from pyrolysis of the methyl mycolates in the heated gas chromatography injector), monounsaturated C₁₆ and C₁₈ acids, and tuberculostearic acid. In addition, 2-methyleicosanoic (2-CH₃ 20:0) and 2,4,6-trimethyltetracosanoic (2,4,6-CH₃ 24:0) acids were detected in all strains (Fig. 2). The identities of these compounds were confirmed by mass spectrometry with electron

ionization at an energy of 70 eV. The molecular ion of the former compound was found at *m/z* 340, and the base peak was found at *m/z* 88, indicating a methyl branch in position 2. The latter compound was identified analogously.

The mycolic acid composition of *M. malmoeense* found in the present study confirms previously reported results (8). The same mycolic acid pattern has been found in *M. simiae* but not in any other species of mycobacteria studied thus far (1, 2). Apart from *M. simiae* and *M. xenopi*, pyrolysis of the mycolates to yield C_{26:0} has been reported only for the *M. tuberculosis* complex (1). 2-Methyl branched-chain fatty acids are present in several slow-growing, chromogenic mycobacteria (e.g., *M. kansasii*, *M. marinum*, *M. szulgai*, and *M. goodii*) (1), but the combination of 2-methyl C_{20:0} and 2,4,6-trimethyl C_{24:0} in a *Mycobacterium* species seems to be unique for *M. malmoeense*. Analyses of fatty acids and

FIG. 1. Typical thin-layer chromatography pattern representing the methyl mycolates of *M. malmoeense*. Symbols: a, alpha-mycolate; a', alpha'-mycolate; c, keto-mycolate.

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FIG. 2. Gas chromatography profile representing the fatty acid methyl esters of a strain of *M. malmoense*. Peak designations refer to the number of carbon atoms and double bonds. TBSA, Tuberculostearate; 2-CH₃ 20:0, 2-methyleicosanoate; 2,4,6-CH₃ 24:0, 2,4,6-trimethyltetracosanoate.

mycolic acids are therefore very useful techniques in the identification of this important *Mycobacterium* species.

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