

BACTERIAL CHARACTERISATION

Characterisation of a new isolate of *Mycobacterium shimoidei* from Finland

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This report describes the first isolation of *Mycobacterium shimoidei* in Finland from a sputum specimen obtained from an elderly female patient. *M. shimoidei*, a potential lung pathogen, is difficult to identify by routine methods and only a few cases have been reported. The present study demonstrated that *M. shimoidei* has a characteristic pattern for fatty acids and alcohols in gas liquid chromatography. This chromatogram and the pattern of mycolic acids on thin-layer chromatography allow it to be distinguished routinely. The unique sequence of the 16S rRNA gene and the 16S–23S rDNA spacer region allows identification by molecular methods.

Introduction

Mycobacterium shimoidei, first isolated from a respiratory infection in a Japanese patient in 1975 [1], has been detected in various parts of the world. It is regarded as a potential cause of disease in man, although few cases of infection have been documented [2]. *M. shimoidei* was revived as a valid species by Tsukamura [3] with a description of its growth and biochemical characteristics. It shares some characteristics with other slow-growing non-chromogenic species, mainly *M. malmoense* and the *M. terrae* complex, but is distinct on the basis of numerical taxonomy and DNA relatedness [4–7].

In this study, both the whole 16S rRNA gene and the spacer region of 16S–23S rRNA (ITS) of three *M. shimoidei* strains initially identified by Tsukamura and a suspected new clinical isolate were amplified and sequenced. The determined sequences were compared with those of species resembling *M. shimoidei* by growth and biochemical characteristics. The strains were also analysed for composition of fatty acids and alcohols and for mycolic acids. This report describes the first isolate of *M. shimoidei* in the Nordic Countries.

Materials and methods

M. shimoidei strains ATCC 27962, 903, 1360 (gifts from F. Portaels, Antwerp, Belgium) and the clinical isolate were grown on Middlebrook 7H11 agar at 37°C for 14 days before harvesting for DNA and chromatographic analyses. Nucleic acids were extracted as described previously [8]. The 16S rRNA gene and 16S–23S rDNA spacer region were amplified by PCR, and the sequences were determined as described previously by the solid-phase method [9]. Sequencing reactions were performed with the Auto Read kit (Pharmacia, Uppsala, Sweden) and analysed with an automated A.L.F. DNA sequencer (Pharmacia). The sequences were assembled by means of the Staden Package program on a Sun Workstation. The sequence obtained has been deposited in the EMBL databank under accession number AJ005005.

The strains were analysed for fatty acid and alcohol composition by gas liquid chromatography as described previously in detail [10]. The identity of eluted constituents was verified by mass spectrometry (Ion Trap 700, Finnigon-MAT, San Jose, CA, USA) [11]. Mycolic acids were analysed by two-dimensional thin-layer chromatography as described previously [10].

Results and discussion

All of the four strains examined had an identical unique 16S rRNA sequence. This sequence was similar to the one deposited earlier in the EMBL databank

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(accession no. X82459). However, discrepancies were detected at five positions in the sequence. At positions 39, 881 and 1220 there was an N in the database sequence which the present study determined as C, C and G, respectively. At positions 45 and 1407 a G was missing in both cases in the database sequence. *M. shimoidei* has been placed phylogenetically near *M. branderi*, a recently described species [9]. The sequence of the spacer region 16S–23S rRNA of *M. shimoidei* was unique and very distinct from all other mycobacterial sequences. The sequence obtained was identical to that reported earlier [12].

In lipid analyses, the strains examined contained a high amount of tuberculostearic acid (10Me18:0) (mean 19.0%; range 15.8–22.0%) and other fatty acids typical

of mycobacteria. The major mycolic acid cleavage product was tetracosanoic acid (24:0) (mean 14.7%; range 11.0–18.9%). A characteristic feature in the fatty-acid composition was the presence of two secondary fatty alcohols, 2-eicosanol (2-OH-20:0) (mean 9.2%; range 6.0–13.0%) and 2-docosanol (2-OH-22:0) (mean 2.8%; range 1.1–3.6%). The latter compound has been detected previously only in *M. xenopi* [13]. Another marker was a compound that eluted together with tetracosanoic acid (24:0) in an unpolar column. Due to its instability, this compound remains unidentified at present. The profile of fatty-acid composition is shown in Fig. 1. By GLC fatty-acid analysis alone, *M. shimoidei* is easily separated from all known slow-growing species. It resembles *M. avium* complex and *M. xenopi*, but the presence of 2-OH-

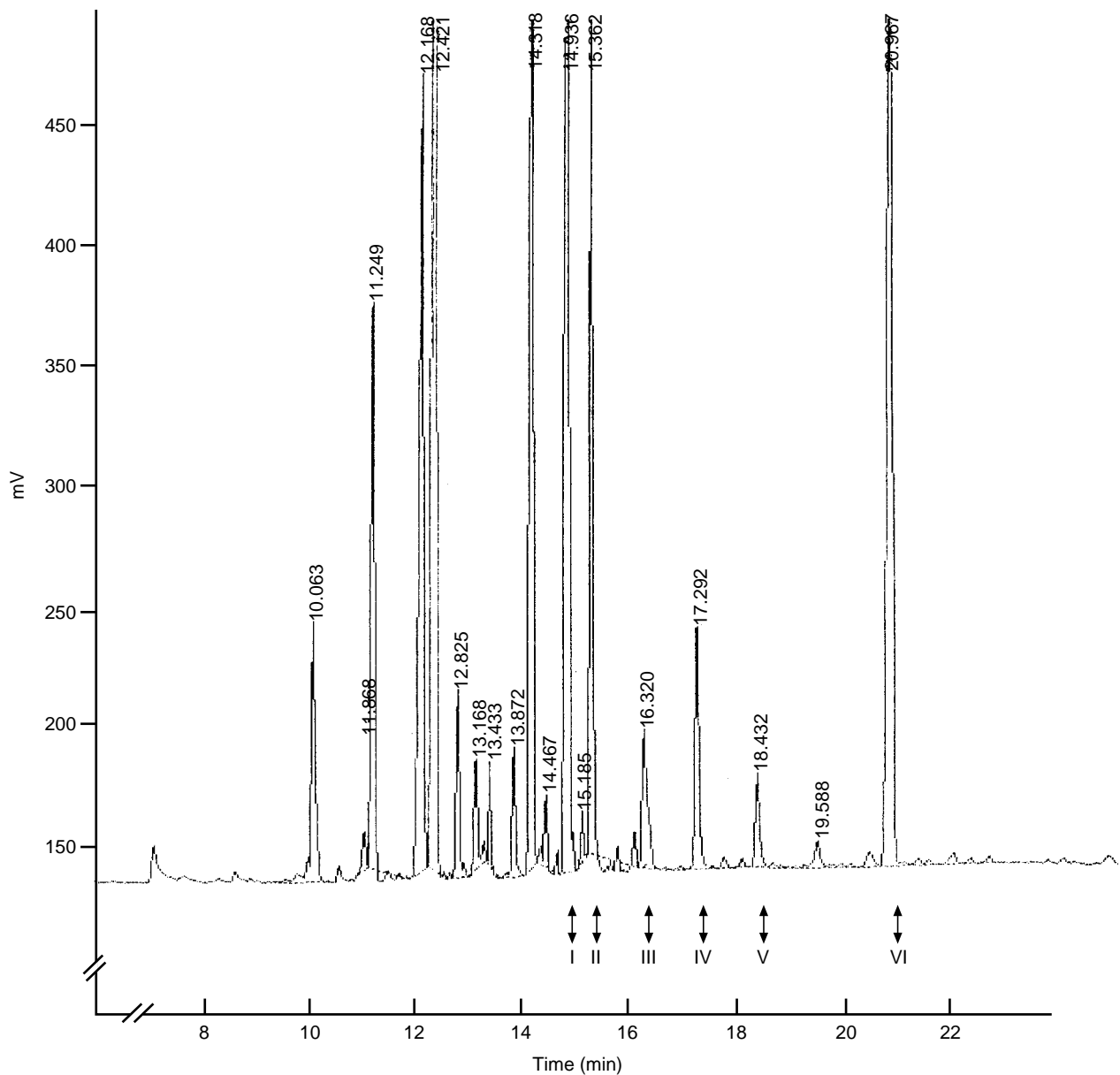


Fig. 1. The lipid profile of *M. shimoidei*. I, tuberculostearic acid (10 Me18:0); II, 2-eicosanol (2-OH-20:0-alcohol); III, eicosanoic acid (20:0); IV, 2-docosanol (2-OH-22:0-alcohol); V and VI, an unidentified unstable compound eluting together with tetracosanoic acid (24:0).

22:0-alcohol in *M. shimoidei* offers a definitive separation from the *M. avium* complex, and lack of hexacosanoic acid (26:0) from *M. xenopi*.

All strains analysed had an identical mycolic acid content, shared only by rapidly growing non-pathogenic mycobacteria, and unlike that of other potentially pathogenic slow-growing species (Fig. 2). In addition to α -, α' - and keto-mycolates, present in *M. malmoense* and *M. simiae*, it contained carboxymycolates detectable in several species, including the *M. avium* complex [14].

By conventional analysis, *M. shimoidei* shows similarities with other slowly growing species, including *M. malmoense*, *M. haemophilum* and the *M. terrae* complex. The key test results that distinguish it from other non-chromogenic mycobacteria include growth at 45°C and positive Tween 80 hydrolysis (10 days), no growth at 25°C and a weak positive result in the nitrate reductase test [10]. The typical fatty-acid and alcohol profile and the mycolic acid pattern are also very useful in identification. On the basis of 16S rRNA gene sequence homology, *M. shimoidei* is located close to *M. malmoense* and *M. haemophilum* in the phylogenetic tree. Its unique gene sequence in the spacer region of 16S–23S rRNA [12] also differentiates this potentially pathogenic mycobacterium from all other known mycobacterial species, including the non-pathogenic members of the *M. terrae* complex [15].

These results were used to demonstrate *M. shimoidei* infection in a 78-year-old Finnish woman with no chronic obstructive lung disease. She had suffered from a prolonged productive cough. Her chest radiograph was normal, but one colony of a non-chromogenic

slowly growing species grew from one of three sputum samples cultivated for mycobacteria and was presumptively identified as *M. shimoidei* by biochemical tests. The possibility of contamination was excluded as *M. shimoidei* had not been cultivated in the laboratory within the preceding 2 years. This species was verified by lipid analysis and sequencing of the 16S rRNA gene and its ITS region as described above. All later sputum samples have remained negative for *M. shimoidei*. However, *M. gordonae* subtype SZENT, as identified by Accuprobe (GenProbe) and glycolipid composition, has been isolated from two sputum samples taken 7 months later. Another set of three sputum samples taken 15 months later remained culture negative. High resolution computed tomography scans showed that the patient's lungs were devoid of inflammatory changes. The patient remains under observation. *M. shimoidei* and *M. gordonae* isolations were regarded as clinically insignificant.

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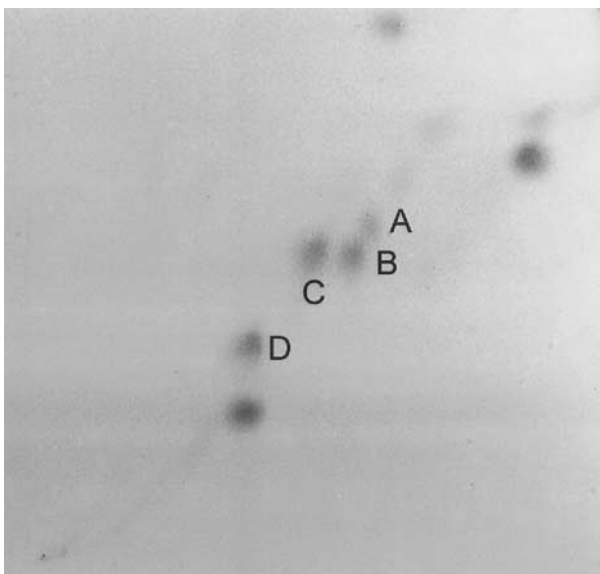


Fig. 2. The mycolic acid pattern of *M. shimoidei* contained α -mycolates (A), α' -mycolates (B), ketomycolates (C) and carboxymycolates (D).

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