Mycobacterium setense sp. nov., a Mycobacterium fortuitum-group organism isolated from a patient with soft tissue infection and osteitis

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A Gram-positive, rod-shaped acid-fast bacterium was isolated from a patient with a post-traumatic chronic skin abscess associated with osteitis. Morphological analysis, 16S rRNA, hsp65, sodA and rpoB gene sequence analysis, cell-wall fatty acid and mycolic acid composition analyses and biochemical tests showed that the isolate, designated ABO-M06T, belonged to the genus Mycobacterium. Its phenotype was unique and genetic and phylogenetic findings suggest that strain ABO-M06T represents a novel species within the Mycobacterium fortuitum group. The name Mycobacterium setense sp. nov. is proposed for this novel species, with the type strain ABO-M06T (=CIP 109395T=DSM 45070T).

The Mycobacterium fortuitum group traditionally included three taxa, Mycobacterium fortuitum, Mycobacterium peregrinum and the unnamed third biovariant complex. It is defined by growth in less than 7 days, absence of pigmentation, 3 day arylsulfatase production, nitrate reduction, iron uptake and growth in the presence of 5% NaCl. With the combination of molecular methods and phenotypic criteria, reports of novel species within the group have steadily increased during the past decade and the subject has become complicated. To date, the M. fortuitum 16S rRNA gene clade is composed of Mycobacterium fortuitum, Mycobacterium peregrinum, Mycobacterium senegalense, Mycobacterium porcinum, Mycobacterium houstonense, Mycobacterium neworleansense, Mycobacterium boenickei, Mycobacterium conceptionense, Mycobacterium septicum and Mycobacterium alvei (Schinsky et al., 2000, 2004; Brown-Elliott & Wallace, 2002; Adékambi et al., 2006). Debate still persists on whether Mycobacterium mageritense belongs to the M. fortuitum group (Brown-Elliott & Wallace, 2002; Adékambi & Drancourt, 2004).

M. fortuitum-group members cause a variety of human infections (Brown-Elliott & Wallace, 2002, 2005). Accurate bacterial identification, rising from a relevant classification, is crucial as antimicrobial resistance is species-dependent (Wallace et al., 1991).

Here we describe a new member of the M. fortuitum group. The isolate, designated ABO-M06T, was obtained from a patient with post-traumatic soft tissue infection and osteitis. A polyphasic study showed a unique genotype and phenotype, suggesting that this strain is representative of a novel species.

Strain ABO-M06T was isolated from a 52-year-old patient admitted to Bassin de Thau Hospital, Sète, France, for a chronic soft tissue infection of the left foot that occurred after he stepped on a nail. The local infection was later complicated with osteitis and tenosynovitis. The organism was initially isolated after a 21 day culture on Löwenstein–Jensen slants incubated at 37 and 30 °C. It was subcultured on Löwenstein–Jensen slants and blood agar plates, and formed colonies in less than 7 days at 20, 25 and 37 °C. Strain ABO-M06T was a Gram-positive, acid-fast bacterium initially identified as M. peregrinum with the INNO-LiPA method (Innogenetics) and the patient was treated...
with clarithromycin (1 g per day for 30 days). However, treatment failure led us to go further into bacterial identification. The initial isolate was maintained in glycerol suspension (10 % v/v) at −80 °C.

The isolate was examined for pigmentation and morphological characteristics as described previously (Schinsky et al., 2004). Arylsulfatase activity (3 day) and catalase activities, iron uptake and growth on Löwenstein–Jensen medium containing 5 % NaCl were tested as described by Vincent et al. (2003). We also inoculated API Coryne, API 20E and API 20NE strips (bioMérieux) as recommended by the manufacturer, and incubated them for 5 days at 30 and 37 °C in a highly humidified atmosphere (Adékambi et al., 2006). Standard fatty acid and mycolic acid analyses were performed by the Deutsche Sammlung von Mikroorganismen und Zellkulturen by means of gas chromatography and HPLC, respectively, as previously described (Butler et al., 1992), using standard Microbial Identification System software (MIDI). The broth microdilution method was used to test susceptibility to amikacin, clarithromycin, doxycycline, gatifloxacin, moxifloxacin, sulfamethoxazole and tobramycin, as recommended by the Clinical Laboratory Standards Institute (CLSI, 2006). Standard fatty acids included C 16 : 0 (37.8 %), C 18 : 1 (7.6 %) and C 14 : 0 (7.5 %). Cell wall analysis showed the presence of arylsulfatase activity at 14 days. Strains: 1, M. porcinum CIP 105392 T; 2, M. mycolatum CIP 105382 T; 3, M. fortuitum CIP 105384 T; 4, M. houstonense ATCC 49403 T; 5, M. conceptionense CIP 108544 T; 6, M. porcinum CIP 105392 T; 7, M. septicum ATCC 700731 T; 8, M. senegalense CIP 104941 T; 9, M. neworleansense ATCC 49404 T; 10, M. boenicei CIP 107829 T; 11, M. alvei CIP 103464 T; 12, M. wolinskyi ATCC 700010 T; 13, M. mageritense CIP 104973 T. Data from Adékambi et al. (2006), Brown et al. (1999), Schinsky et al. (2000, 2004) and Wallace et al. (2002). –, Negative; +, positive; ND, no data available.

Table 1. Differential phenotypic characteristics of Mycobacterium setense sp. nov. and related rapidly growing species

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<tr>
<td>Growth on Löwenstein–Jensen slant at 42 °C</td>
<td>−</td>
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<td>Arylsulfatase activity at 3 days</td>
<td>+</td>
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<td>−*</td>
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<td>Growth on carbon source: d-Mannitol</td>
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<td>Citrate utilization</td>
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<td>Gelatinase activity</td>
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*Positive for arylsulfatase activity at 14 days.
The fatty acid and mycolic acid elution profile did not correspond to a specific *Mycobacterium* species stored in the MIDI databases and was clearly distinct from *M. fortuitum* (Butler & Kilburn, 1990; Butler & Guthertz, 2001). Although it showed some similarities with patterns from members of the *M. fortuitum* group, the mycolic acid elution profile is characteristic and strain ABO-M06\textsuperscript{T} can be differentiated from these species by quantitative differences in the mycolic acid composition.

The isolate was susceptible to imipenem, moxifloxacin, gatifloxacin, cefoxitin and amikacin, intermediate to tobramycin and clarithromycin, and resistant to doxycycline, erythromycin and sulfamethoxazole. These results were in keeping with the clinical data, as clarithromycin was ineffective while levofloxacin–amikacin (1 g each per day) therapy for 30 days eradicated the infection.

The gene sequences of strain ABO-M06\textsuperscript{T} differed markedly from those of *Mycobacterium* species with validly published names. These differences were 0.68 % in the 16S rRNA gene sequence (corresponding to 9 differences among 1336 nucleotides) with *M. houstonense* ATCC 49403\textsuperscript{T} and *M. senegalense* CIP 104534\textsuperscript{T}, 4.64 % in *rpoB* gene sequence (32 differences among 690 nucleotides) with *M. conceptionense* CIP 108544\textsuperscript{T}, 1.56 % in *hsp65* gene sequence (6 differences among 387 nucleotides) with *M. houstonense* ATCC 49403\textsuperscript{T} and 3.8 % in *sodA* gene sequence (15 differences among 395 nucleotides) with *M. septicum* ATCC 700731\textsuperscript{T}. As previously described in the genus *Mycobacterium*, the *sodA* and *rpoB* genes were found to be more discriminatory than the 16S rRNA and *hsp65* genes for differentiation of strain ABO-M06\textsuperscript{T} from closely related species. Moreover, partial *rpoB* gene sequence analysis supported the affiliation of strain ABO-M06\textsuperscript{T} to a novel species according to Adékambi et al. (2003), who have shown that intraspecies and interspecies variabilities in partial *rpoB* gene sequences are <1.7 % and >3 %, respectively, for rapidly growing mycobacteria. This was confirmed by partial *sodA* gene sequence analysis. Although data on intraspecies variability are lacking, interspecies variability on this gene fragment was found to be ≥0.75 % for rapidly growing mycobacteria.

The evolutionary trees inferred from the three treeing algorithms gave congruent results (data not shown) and the neighbour-joining trees are shown in Figs 1 and 2 and Supplementary Figs S2 and S3. Phylogenetic analysis of the near-complete 16S rRNA gene sequence indicated that strain ABO-M06\textsuperscript{T} belonged to the *M. fortuitum* group (Fig. 1). A similar result was observed after phylogenetic analysis based on *rpoB* (Fig. 2), *hsp65* (Supplementary Fig. S2) and *sodA* (Supplementary Fig. S3) gene sequences. Independent lineage was observed in all the trees reconstructed for the strain ABO-M06\textsuperscript{T}. The species most closely related to strain ABO-M06\textsuperscript{T} differed with the phylogenetic marker, but the bootstrap values were too low (below 50 %) to induce much confidence on any phylogenetic relatedness within the *M. fortuitum* group. The percentages of gene sequence difference, together with the marked evolutionary distances and lack of congruence observed in four-gene sequence analysis, showed that isolate ABO-M06\textsuperscript{T} was clearly distinct from the nearest species. Together with the unique phenotype, these results warranted its classification as a novel *Mycobacterium* species belonging to the *M. fortuitum* group, for which we propose the name *Mycobacterium setense* sp. nov.

**Description of Mycobacterium setense** sp. nov.

*Mycobacterium setense* [se.ten’sé N.L. neut. adj. setense pertaining to Sète (France), the city from which the infected patient originated].

Cells are acid-fast, Gram-positive and pleomorphic rods. Colonies are smooth, convex, round, entire-edged, non-pigmented (beige) and small (approx. 1 mm in diameter). They do not produce aerial hyphae. The cells grow on 5 % sheep blood agar and Löwenstein–Jensen agar within 2 to 4 days at 25 °C, 30 °C (optimum) and 37 °C when

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**Fig. 1.** Phylogenetic tree based on the 16S rRNA gene sequence showing the relationship of *Mycobacterium setense* ABO-M06\textsuperscript{T} with the 14 most closely related *Mycobacterium* species. This tree was constructed by using the neighbour-joining method and was based on a comparison of a stretch of 1336 nt. The tree was rooted using *M. tuberculosis* H37Rv as the outgroup. Bootstrap values are indicated by the value at each node as a percentage of 1000 replications. The different branches were supported by the results of three different algorithms. Bar, 0.5 % difference in nucleotide sequence.

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subculturing. They also grow in the presence of 5% NaCl. No growth occurs at 42 °C. Positive for 3 day arylsulfatase production, iron uptake and thermostable catalase activity. It utilizes D-mannitol and D-glucose, but not D-inositol, L-rhamnose or L-arabinose, or citrate as the sole carbon source. It is positive for pyrazinamidase, alkaline phosphatase, nitrate reductase, urease and gelatinase activities. M. setense ABO-M06T sp. nov. belongs to the M. fortuitum group and can be differentiated phenotypically from other species of this group as follows. It differs from M. conceptionense CIP 108544T by D-glucose and D-mannitol utilization, lack of inositol degradation and gelatinase activity. It differs from M. porcinum CIP 105392T by D-glucose utilization and lack of inositol degradation, from M. houstonense ATCC 49403T by the lack of inositol degradation and failure to grow at 42 °C, from M. fortuitum CIP 105354T by failure to grow at 42 °C and by D-mannitol utilization, and from M. septicum ATCC 700731T by lack of inositol degradation. It exhibits unique cellular fatty acid and mycolic acid patterns. Strain ABO-M06T shows 99.32% 16S rRNA gene similarity with M. houstonense ATCC 49403T and 95.36% rpoB gene similarity with M. conceptionense CIP 108544T, the phylogenetically closest type strains.

The type strain, ABO-M06T (=CIP 109395T=DSM 45070T), was recovered from an excised skin and soft tissue specimen in a context of osteitis.

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References


Brown-Elliott, B. A. & Wallace, R. J., Jr (2005). Infections caused by Mycobacterium boneickei ATCC 49935T was not included because its rpoB gene sequence is absent from the GenBank database. Bar, 2% difference in nucleotide sequence.


