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Mycobacterium alsiense, a Novel, Slowly Growing Species Isolated from Two Patients with Pulmonary Disease▽

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A previously undescribed, slowly growing *Mycobacterium* species was isolated from pulmonary specimens of two patients, one from Denmark and one from Italy. The isolates showed unique 16S rRNA internal transcribed spacers and *hsp65* sequences: the 16S rRNA was most closely related to *Mycobacterium szulgai* and *Mycobacterium malmoense*.

CASE REPORTS

Patient from Denmark. A 72-year-old patient with a history of pulmonary carcinoma and tumor excision presented, 3 years after surgery, with reduced pulmonary capacity, fever (39.6°C), dyspnea, and productive cough. Right and left lower lobe infiltrates were found by chest radiography and computerized tomography. The detection of *Pseudomonas aeruginosa* in the sputum initially led to treatment with ceftazidime and ciprofloxacin. Due to progression of the pulmonary infiltrates, four additional sputum specimens (collected within 7 weeks) were analyzed for the presence of mycobacteria. Initial acid-fast smears of these specimens were negative. Mycobacterial growth was detected in all four specimens with liquid (BacT/Alert; bioMérieux, Marcy l'Etoile, France) and solid (Löwenstein-Jensen and Stonebrink medium) media. The partial 16S rRNA gene sequence (8) of two isolate were identical but did not match that of any known mycobacterial species (GenBank Database; http://www.ncbi.nlm.nih.gov/BLAST/).

Antimicrobial treatment was expanded to include ethambutol (1,200 mg/day), pyrazinamide (2000 mg/day), isoniazid (300 mg/day), and rifampin (600 mg/day). Subsequent drug susceptibility testing with the radiometric BACTEC 460 system (Becton Dickinson Diagnostic Systems, Sparks, MD) (9) showed no growth in the presence of rifampin (2 mg/liter), rifabutin (1 mg/liter), ethambutol (3.75 mg/liter), clarithromycin (1 mg/liter), and streptomycin (3 mg/liter) but growth in the presence of isoniazid (0.1 mg/liter). However, therapy was unchanged for 60 days but was subsequently continued with ethambutol and rifampin only. Five months later, a chest radiograph showed a clear regression of the infiltrates and the antibiotic treatment was stopped after 6 months. The patient remained well, and all follow-up specimens (five sputa within 12 months) remained negative.

Patient from Italy. A 70-year old male with a history of two episodes of hemoptysis in the last 7 months was hospitalized because of productive cough. The chest radiograph revealed only signs of emphysema, and the hematologic parameters were normal. The only sputum sample investigated for mycobacteria was smear negative but grew a scotochromogenic mycobacterium on solid and in liquid cultures. The strain was identified as "*Mycobacterium szulgai*-like" on the basis of genetic sequence analysis. The patient was treated with clarithromycin and rifampin. Two months later, a subsequent chest radiograph revealed a bronchopneumonic focus with signs of healing.

Strain characteristics. The two strains presented identical sequences in the first one-third of the 16S rRNA gene, in the internal transcribed spacer (ITS) interposed between the 16S and 23S rRNA genes, and in the 422-bp segment of the gene coding for the 65-kDa heat shock protein (*hsp65*).

Sequence comparison of the 40- to 640-bp fragment of the 5′ end of the 16S rRNA gene revealed no match in the RIDOM database (6). However, the strains showed only 3- and 6-bp differences from *M. szulgai* and *Mycobacterium malmoense*, respectively. Moreover, in a comparison of the complete 16S rRNA gene sequence, there was no identical entry in the GenBank database. The highest similarity was obtained with *M. malmoense*, with a total of 16 bp of differences.

Furthermore, the ITS sequence (AJ938170), compared against GenBank and RIDOM databases, showed <91% and <86% identity, respectively. Additionally, by the *hsp65* sequence (DQ381733), the strains differed by 13 bp from the most closely related sequence of *Mycobacterium avium*.

We propose to name the new species *Mycobacterium alsiense*, pertaining to the Isle of Als (Denmark), the location of the hospital to which the first patient was admitted.

The phenotypic characteristics of *M. alsiense* were poorly distinctive. The strain grew at 25°C to 37°C, but not at 45°C, with a weak yellow pigment under both light and dark conditions on Löwenstein-Jensen and Stonebrink medium. It remained unpigmented on solid Middlebrook 7H10 medium. Both genotypic characteristics including the presence of a long helix 18 at positions 451 to 482 of 16S rRNA (according to the *Escherichia coli* numbering system) (1) and phenotypic char-

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TABLE 1. Analysis of growth, biochemical characteristics, and antimicrobial susceptibility of *M. alsiense* sp. nov. compared to those of *M. malmoense* and *M. szulgai*

Characteristic	Result for[a]:		
	M. alsiense	*M. malmoense*	*M. szulgai*
Growth at:			
25°C	+	+	+
37°C	+	+/-	+
45°C	−	−	−
Colony morphology	Smooth	Smooth	Rough/smooth
Pigmentation	s	n	s
Catalase at 68°C	+	v	+
Growth in the presence of:			
p-Nitrobenzoic acid	+	+	+
TCH[b]	+	+	+
Thioacetazone	+	+	+
Isoniazid	+	+	v
Oleate	+	−	−
NaCl	−	−	−
MacConkey agar	−	−	−
Niacin	−	−	−
Nitrate reductase	−	−	+
Semiquantitative catalase (mm)	<45	<45	>45
Tween 80 hydrolysis	−	+	v
Arylsulfatase activity	−	−	−
Urease activity	−	v	+
β-Glucosidase	−	−	−
Tellurite reduction	−	+	v

[a] v, variable; n, nonchromogenic; s, scotochromogenic.
[b] Thiophene-2-carboxylic acid hydrazide.

acteristics group the species among the slowly growing mycobacteria. However, phenotypic characteristics alone do not allow a clear distinction of *M. alsiense* from *M. malmoense* and *M. szulgai* (Table 1).

Mycolic acid methyl esters were analyzed by thin-layer chromatography (3, 7) and revealed the presence of α - and keto-mycolic acids, with methoxy-mycolic acids being present in minor quantities. This is a common pattern among mycobacterial species, including *Mycobacterium tuberculosis*.

Fatty acid methyl esters were identified by gas/liquid chromatography-mass spectrometry (12). The major lipid components were $C_{16:0}$ (26.2%), $C_{18:1\omega9}$ (18.2%), tuberculostearic acid (10Me-$C_{18:0}$; 14.9%), and $C_{18:0}$ (8.8%).

High-performance liquid chromatography, performed according to the CDC guidelines (2), revealed an identical profile characterized by a single, late cluster of peaks grossly resembling *Mycobacterium palustre* and *Mycobacterium lacus* (Fig. 1) (http://www.MycobacToscana.it/page4.htm).

Species identification of the nontuberculous mycobacteria based solely on biochemical and cultural characteristics is no longer considered reliable because of the increasing number of currently recognized species (approximately 130). Apart from the most frequently encountered species, many isolates of mycobacteria remain unclassified. The introduction of molecular methods for identification of mycobacteria in the past years has led to an increasing knowledge about the taxonomy of this genus (4). Although numerous new species have been described recently, several mycobacterial isolates in the laboratory remain unidentified (11). Reports of isolates like the current ones may lead to the correct establishment of new species, once a suitable number of isolates have been detected (11). Furthermore, a definite identification of a given isolate may lead to a better estimation of the pathogenicity and epidemiology of this organism.

In conclusion, slowly growing mycobacteria are often found to be pathogenic in both immunocompetent and immunocompromised patients (4, 5, 10). Although only two strains have been isolated, the potential pathogenicity of the proposed slowly growing mycobacterial species *M. alsiense* is supported by the presence of compatible clinical disease, laboratory findings, and the response to mycobacterial treatment.

Nucleotide sequence accession number. The complete 16S rRNA gene sequence has been submitted to the EMBL nucleotide sequence database under accession no. AJ938169.

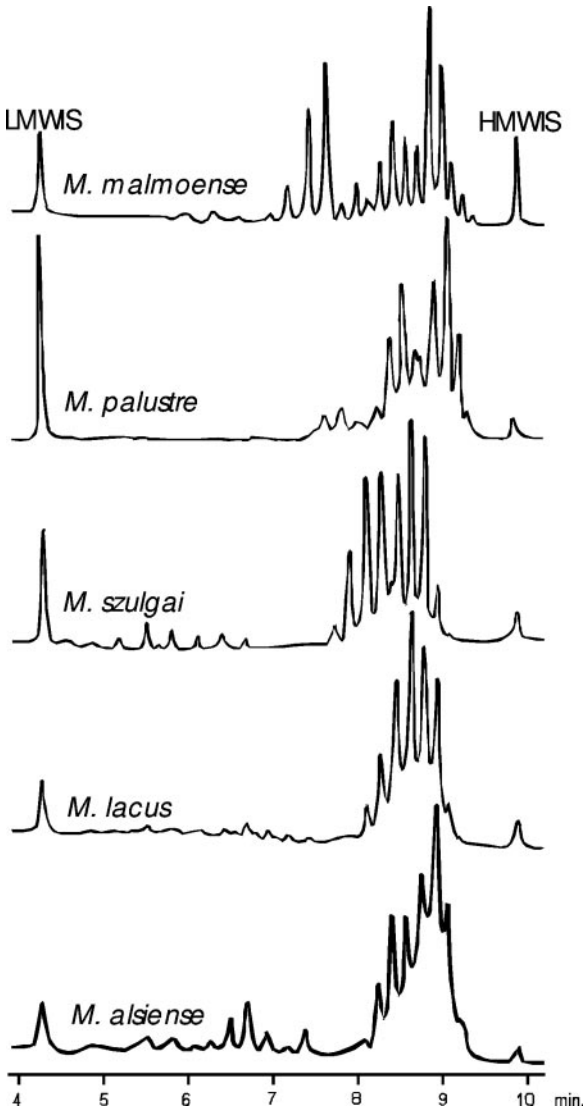

FIG. 1. High-performance liquid chromatography chromatograms from *M. malmoense*, *M. palustre*, *M. szulgai*, *M. lacus*, and *M. alsiense*. LMWIS, low-molecular-weight internal standard; HMWIS, high-molecular-weight internal standard.

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