Mycobacterium algericum sp. nov., a novel rapidly growing species related to the *Mycobacterium terrae* complex and associated with goat lung lesions

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A previously undescribed, rapid-growing, non-chromogenic *Mycobacterium* isolate from a goat lung lesion in Algeria is reported. Biochemical and molecular tools were used for its complete description and showed its affiliation to the *Mycobacterium terrae* complex. 16S rRNA, *rpoB* and *hsp65* gene sequences were unique. Phylogenetic analyses showed a close relationship with *M. terrae sensu stricto* and *Mycobacterium sensuense*. Culture and biochemical characteristics were generally similar to those of *M. terrae* and *M. sensuense*. However, in contrast to *M. terrae* and *M. sensuense*, the isolate was positive for urease production and had faster growth. The mycolic acid profile was distinct from those of *M. terrae* and *M. sensuense*, thus further supporting the new taxonomic position of the isolate. We propose the name *Mycobacterium algericum* sp. nov. for this novel species. The type strain is TBE 500028/10^T (=Bejaia^T=CIP 110121^T=DSM 45454^T).

Non-tuberculous mycobacteria are typically found in the environment, but can be the cause of occasional opportunistic infections in humans or animals (Tortoli, 2009).

Members of the *Mycobacterium terrae* complex (*Mycobacterium terrae sensu stricto*, *Mycobacterium non-chromogenicum*, *Mycobacterium triviale* and *Mycobacterium sensuense*) are ubiquitous and potentially opportunistic pathogens. Indeed, some reports showed potential human pathogenicity (Krisher *et al.*, 1988; Mayo *et al.*, 1998; Smith *et al.*, 2000). Disseminated *M. terrae* infections have also been reported among AIDS patients (Carbonara *et al.*, 2000). *M. sensuense* was recently described as a novel *Mycobacterium* species closely related to the *M. terrae*

complex (Mun *et al.*, 2008). It was isolated from a Korean patient with a symptomatic pulmonary infection.

In this report, we present a previously undescribed *Mycobacterium* species, for which we propose the name *Mycobacterium algericum* sp. nov. (type strain, TBE 500028/10^T). Previous publications have shown that phenotypic and biochemical analyses often do not provide an accurate identification of *Mycobacterium* species (Cloud *et al.*, 2006; Lee *et al.*, 2004; Mun *et al.*, 2008; Springer *et al.*, 1996). Therefore, using a combination of both biochemical and molecular techniques, we showed that isolate TBE 500028/10^T, collected from a pulmonary lesion on a goat in Algeria, was representative of a novel *Mycobacterium* species related to the *M. terrae* complex, in particular *M. terrae sensu stricto* and *M. sensuense*.

The isolate was collected in the Bejaia department (Wilaya) in Algeria in 2008, at the slaughterhouse of Souk El Tenine. Lung lesions observed on goat carcasses were sampled and sent to the Pasteur Institute in Alger. There, Ziehl–Neelsen

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *hsp65* and partial *rpoB* gene sequences of strain TBE 500028/10^T are GU564404, GU564405 and GU564406, respectively.

A supplementary table is available with the online version of this paper.

staining and microscopy were performed. After grinding and decontamination by Petroff's method (4 % NaOH) (Ghosh *et al.*, 1978; Petroff, 1915), samples were inoculated on Löwenstein–Jensen growth medium. Tubes were incubated at 37 °C (ambient atmosphere) and monitored weekly until colonies suggesting mycobacterial growth were observed. Tubes with potential mycobacterial colonies were sent to Switzerland for further biochemical and molecular characterization of the isolate.

Biochemical characterization of strain TBE 500028/10^T was performed by observing growth under various conditions, according to methods described by Murray *et al.* (2007); the determined characteristics were compared with those of the closely related *Mycobacterium* species *M. terrae* and *M. senusense*. To determine the optimal growth temperature, Middlebrook 7H10 agar plates were inoculated and incubated at temperatures ranging from 25 °C (with and without light) to 45 °C (ambient atmosphere). We also tested growth ability on MacConkey agar with and without 5 % NaCl at 37 °C (ambient atmosphere) and tellurite reduction, as well as nitrate reductase, urease, catalase and pyrazinamidase production. Colony morphology and pigmentation are described. Cell morphological analyses were conducted after growth in 7H9 liquid broth with 10 % OADC supplement (oleic acid, bovine albumin, glucose and catalase). Cells were harvested in the exponential-growth phase and stained with auramine–rhodamine. Microscopic imaging was performed on a Zeiss Axiovert 200M microscope using a 100-fold magnifying objective (Carl Zeiss). Cell dimensions were determined by using the measurement tool provided by the SlideBook version 4.1 software (Intelligent Imaging Innovations). Acid–alcohol fastness was assessed by Ziehl–Neelsen staining.

Susceptibility to the antibiotics rifampicin (1.0 and 10.0 mg l⁻¹), rifabutin (0.1 and 1.0 mg l⁻¹), ethambutol (5.0 and 50 mg l⁻¹), clarithromycin (4.0, 16.0, 32.0 and 64.0 mg l⁻¹), amikacin (1.0 and 10 mg l⁻¹), ofloxacin (2.0 and 10.0 mg l⁻¹) and moxifloxacin (0.5, 2.5 and 10 mg l⁻¹) was tested in mycobacterial growth indicator tubes (MGIT 960; Becton Dickinson) and monitored with EpiCenter software and the TB eXiST module (Springer *et al.*, 2009).

HPLC analyses were performed to investigate the profile of cell-wall mycolic acids (Butler & Guthertz, 2001; CDC, 1996). The profile obtained for our isolate was compared with those from other closely related *Mycobacterium* species.

DNA was extracted from cultures using the Bio-Rad InstaGene Matrix. In order to assess the phylogenetic relationships of the isolate, we performed sequencing of the nearly complete 16S rRNA gene (1521 bp, corresponding to *Mycobacterium tuberculosis* H37Rv^T rrs nt 18–1534) using previously described primers and protocols (Rogall *et al.*, 1990; Springer *et al.*, 1996) and two new primers (16s_742_fw, 5'-AGCGTGGGGAGCGAACAGG-3'; 16s_1408_rv, 5'-CCCGAAGCCGGTGGCCTAA-3'). Two regions of the *rpoB* gene were amplified and sequenced:

710 bp (*M. tuberculosis* H37Rv^T *rpoB* nt 2436–3145) according to Adékambi *et al.* (2003), and 553 bp (*M. tuberculosis* H37Rv^T *rpoB* nt 1092–1645) according to Kim *et al.* (1999), with a modified reverse primer: MR2, 5'-CAGCTCGTCGTCGTCCTC-3'. A 441 bp part of the *hsp65* gene (*M. tuberculosis* H37Rv^T *groEL2* nt 145–585) was sequenced according to Telenti *et al.* (1993). All amplifications included the reference strain *M. tuberculosis* H37Rv^T as positive control, and buffers without any added mycobacterial DNA as negative control.

Nucleotide sequences were obtained for both forward and reverse primers. Sequence contingency alignment was performed for each gene with the software SeqMan version 7.0 (DNASTAR Inc.). The obtained consensus sequences were compared with mycobacterial sequences by similarity search in GenBank, using the BLASTN algorithm. Phylogenetic trees comparing our isolate with other *Mycobacterium* species were obtained for each gene by using the MEGALIGN software (DNASTAR Inc.) with default settings for CLUSTAL V neighbour-joining multiple alignments. Note that the *rpoB* phylogeny was calculated based on the 553 bp sequence fragment. All obtained trees were established by bootstrap analyses with 1000 resamplings and 111 seeds.

The goat from which the isolation was made presented nodular lung lesions of caseous aspect. Direct microscopy of Ziehl–Neelsen-stained tissues showed low-density (six acid–alcohol-resistant bacilli in 300 observed fields) infections with slightly curved bacilli. The isolate was inoculated into Löwenstein–Jensen tubes in Algeria. Growth rate was slow on Löwenstein–Jensen medium: small, round, white–yellow colonies were observed after 35 days. Inversely, subculture on Middlebrook 7H10 agar plates was rapid; microcolonies could be observed after 1 week at temperatures ranging between 25 and 40 °C, with or without light. Optimal growth was seen at 37 and 40 °C, whereas no growth was observed at 45 °C. On Middlebrook 7H10 agar plates, colonies were small, polymorphic, white, non-chromogenic, smooth and with irregular edges. Ziehl–Neelsen and auramine–rhodamine staining showed acid-fast, 1.4 ± 0.2 µm long, rod-shaped bacilli. No spores, cords or filaments were observed.

No growth was observed on MacConkey agar with or without 5 % NaCl. Production of urease, a positive nitrate reductase reaction, a positive catalase reaction at 25 °C, and tellurite reductase activity were observed.

Drug-susceptibility testing in MGIT 960 showed susceptibility to all drugs tested (see Supplementary Table S1, available in IJSEM Online), with the exception of low-level resistance to aminoglycosides (1.0 mg amikacin l⁻¹), quinolones (0.5 mg moxifloxacin l⁻¹ and 2.0 mg ofloxacin l⁻¹) and rifampicin (1.0 mg l⁻¹). Pyrazinamidase production was detected. Strain TBE 500028/10^T is thus sensitive to pyrazinamide.

HPLC analysis of the cell wall's mycolic acids demonstrated a unique profile. Although related closely to the profiles

Fig. 1. Mycolic acid profiles obtained by HPLC. (a) TBE 500028/10^T; (b) *M. terrae* ATCC 15755^T. The arrow in (a) indicates an additional peak in the late cluster of the profile of strain TBE 500028/10^T.

observed for other *M. terrae* complex subspecies, the chromatogram obtained for our isolate distinctively presented an additional peak in the late cluster (Fig. 1).

The overall biochemical profile of the isolate was similar to those of *M. terrae* and *M. sensuense*, with the exception of an intermediate instead of a slow growth rate at 37 °C (Table 1). Furthermore, in contrast to the type strains of *M. terrae* and *M. sensuense*, strain TBE 500028/10^T had a positive urease activity and a different antibiotic-susceptibility pattern (Mun *et al.*, 2008; Murray *et al.*, 2007; Smith *et al.*, 2000). Indeed, unlike strain TBE 500028/10^T, *M. terrae* and *M. sensuense* are susceptible to rifampicin, amikacin and quinolones (*M. sensuense* only).

Based on the BLAST sequence match obtained for the 16S rRNA gene (GenBank accession no. GU564404), strain TBE 500028/10^T was shown to be most closely related to *M. sensuense* 05-832^T (1492/1522 nt, 98.0%), *M. terrae* ATCC 15755^T (1451/1470 nt, 98.7%) and *Mycobacterium arupense* AR30097^T (1458/1495 nt, 97.5%). The two combined partial sequences of the *rpoB* gene (GenBank accession no. GU564406) showed that strain TBE 500028/10^T was related most closely to *M. sensuense* 05-832^T (403/421 nt, 95.7%), *M. terrae* ATCC 15755^T (591/633 nt, 93.4%), *Mycobacterium confluentis* CIP 105510^T (632/703 nt, 89.9%) and *Mycobacterium chitae* CIP 105383^T

(632/703 nt, 89.9%). The *hsp65* gene sequence (GenBank accession no. GU564405) showed that our strain was related most closely to *M. sensuense* strain DSM 44999^T (421/431 nt, 97.7%) and *M. terrae* strain CIP 104321^T (414/426 nt, 97.2%).

Table 1. Cultural and biochemical characteristics for *M. algericum* sp. nov. in comparison with the closely related *M. terrae* and *M. sensuense*

Taxa: 1, *M. algericum* TBE 500028/10^T; 2, *M. terrae* ATCC 15755^T; 3, *M. sensuense* DSM 44999^T. Data for *M. terrae* and *M. sensuense* were retrieved from the literature (Mun *et al.*, 2008; Murray *et al.*, 2007). +, Positive; –, negative; +/–, variable; I, intermediate in roughness; S, smooth; W, white; Y, yellow. All taxa were non-photochromogenic, negative for growth at 45 °C and on MacConkey agar both with and without 5% NaCl, and positive for production of nitrate reductase, catalase, tellurite reductase and pyrazinamidase.

Characteristic	1	2	3
Growth within 7 days at 37 °C	+	+/–	–
Optimal growth temperature (°C)	37–40	35	37
Colony morphology on 7H10	IW	IWY	SWY
Urease production	+	–	–

The close relationship to *M. terrae* and *M. senusense* was confirmed by phylogenetic analyses (Fig. 2). Trees based on *rpoB* and *hsp65* gene sequences each showed similar groupings for strain TBE 500028/10^T, but slightly different

from that of the 16S rRNA gene tree. In the *rpoB* and *hsp65* trees, TBE 500028/10^T forms a branch dependent of *M. terrae* together with *M. senusense*, whereas in the 16S rRNA gene tree, strain 500028/10^T forms a branch directly

Fig. 2. Phylogenetic trees designed with the neighbour-joining method and bootstrapped 1000 times. Bootstrapping values >90% are indicated at nodes. Trees were based on: (a) 16S rRNA gene; (b) 553 bp partial sequence of the *rpoB* gene; (c) *hsp65* gene.

dependent from *M. terrae*, with *M. sensuense* being situated in another cluster. These topologies were also supported by high bootstrap values.

The species which our isolate resembles most closely, *M. terrae* and *M. sensuense*, are both slow-growing, non-chromogenic and potentially pathogenic to humans.

Description of *Mycobacterium algericum* sp. nov.

Mycobacterium algericum (al.ge'ri.cum. N.L. neut. adj. *algericum* pertaining to Algeria, the country where the strain was first isolated).

Bacillus that stains acid-alcohol-fast. Cells are rod-shaped and 1.4 ± 0.2 µm long. Cording, spores and filaments are not observed. On Middlebrook 7H10 agar plates, growth is fast, with optimal growth between 37 and 40 °C. Microcolonies can be observed after 1 week. However, 5 weeks are necessary to observe colonies on Löwenstein-Jensen medium. Colonies on Middlebrook 7H10 agar plates are small, polymorphic, white, non-chromogenic, smooth and with irregular edges. Colonies grown on Löwenstein-Jensen medium are small, round and white-yellow. No growth is observed on MacConkey agar plates with or without 5 % NaCl. Urease, nitrate reductase, catalase and tellurite reductase production is positive. Pyrazinamidase production is positive. Susceptible to clarithromycin, ethambutol and rifabutin, but low-level resistant to amikacin, moxifloxacin, ofloxacin and rifampicin. HPLC analysis of mycolic acids showed a unique profile. Genetically, 16S rRNA, *rpoB* and *hsp65* gene sequences are unique. Phylogenetic analyses showed that the species is related to *M. terrae sensu stricto* and *M. sensuense*.

The type strain is TBE 500028/10^T (=Bejaia^T=CIP 110121^T=DSM 45454^T), isolated from a lung lesion of an Algerian goat.

Acknowledgements

The University of Zurich supported this study technically and financially. M.B. was funded by the Swiss National Science Foundation (grant no. IZ 70-Z0-123988). For the HPLC profile, we thank Professor Enrico Tortoli, Mycobacteria Reference Center, Florence, Italy.

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