**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 senuense*. Culture and biochemical characteristics were generally similar to those of *M. terrae* and *M. senuense*. However, in contrast to *M. terrae* and *M. senuense*, the isolate was positive for urease production and had faster growth. The mycolic acid profile was distinct from those of *M. terrae* and *M. senuense*, 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 500028/10T (=BejaiaT=CIP 110121T=DSM 45454T).

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 senuense*) 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. senuense* 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. senuense*.

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 500028/10T 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. senense. 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 H37RvT rrs nt 18–1534) using previously described primers and protocols (Rogall et al., 1993; Springer et al., 1996) and two new primers (16s_742_fw, 5′-AGCGTGGGGAGGCAAACAGG-3′; 16s_1408 rv, 5′-GCCGGAGCCGGTGCTAA-3′). Two regions of the rpoB gene were amplified and sequenced: 710 bp (M. tuberculosis H37RvT rpoB nt 2436–3145) according to Adekambi et al. (2003), and 553 bp (M. tuberculosis H37RvT rpoB nt 1092–1645) according to Kim et al. (1999), with a modified reverse primer: MR2, 5′-CAGCTGCTGTCGGTCCCTC-3′. A 441 bp part of the hsp65 gene (M. tuberculosis H37RvT groEL2 nt 145–585) was sequenced according to Telenti et al. (1993). All amplifications included the reference strain M. tuberculosis H37RvT 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 1−1), quinolones (0.5 mg moxifloxacin 1−1 and 2.0 mg ofloxacin 1−1) and rifampicin (1.0 mg l−1). Pyrazinamidase production was detected. Strain TBE 500028/10T is thus sensitive to pyrazinamide.

HPLC analysis of the cell wall’s mycolic acids demonstrated a unique profile. Although related closely to the profiles...
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. senuense, 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. senuense, strain TBE 500028/10T 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 500028/10T, M. terrae and M. senuense are susceptible to rifampicin, amikacin and quinolones (M. senuense only).

Based on the BLAST sequence match obtained for the 16S rRNA gene (GenBank accession no. GU564404), strain TBE 500028/10T was shown to be most closely related to M. senuense 05-832T (1492/1522 nt, 98.0 %), M. terrae ATCC 15755T (1451/1470 nt, 98.7 %) and Mycobacterium arupense AR30097T (1458/1495 nt, 97.5 %). The two combined partial sequences of the rpoB gene (GenBank accession no. GU564406) showed that strain TBE 500028/10T was related most closely to M. senuense 05-832T (403/421 nt, 95.7 %), M. terrae ATCC 15755T (591/633 nt, 93.4 %), Mycobacterium confluentis CIP 105510T (632/703 nt, 89.9 %) and Mycobacterium chitae CIP 105383T (632/703 nt, 89.9 %). The hsp65 gene sequence (GenBank accession no. GU564405) showed that our strain was related most closely to M. senuense strain DSM 44999T (421/431 nt, 97.7 %) and M. terrae strain CIP 104321T (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. senuense*

<table>
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<th>Characteristic</th>
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<th>2</th>
<th>3</th>
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<tr>
<td>Growth within 7 days at 37 °C</td>
<td>+</td>
<td>+/−</td>
<td>−</td>
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<tr>
<td>Optimal growth temperature (°C)</td>
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<td>35</td>
<td>37</td>
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<tr>
<td>Colony morphology on 7H10</td>
<td>IW</td>
<td>IWY</td>
<td>SWY</td>
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<tr>
<td>Urease production</td>
<td>+</td>
<td>−</td>
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The close relationship to *M. terrae* and *M. senuense* was confirmed by phylogenetic analyses (Fig. 2). Trees based on *rpoB* and *hsp65* gene sequences each showed similar groupings for strain TBE 500028/10, but slightly different from that of the 16S rRNA gene tree. In the *rpoB* and *hsp65* trees, TBE 500028/10 forms a branch dependent of *M. terrae* together with *M. senuense*, whereas in the 16S rRNA gene tree, strain 500028/10 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. senueuse 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. senueuse, 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. senueuse*.

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

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**References**


