**Mycobacterium grossiae** sp. nov., a rapidly growing, scotochromogenic species isolated from human clinical respiratory and blood culture specimens

Alberto Enrique Paniz-Mondolfi,1,* Alexander L. Greninger,2 Lynn Ladutko,3 Barbara A. Brown-Elliott,4 Ravikiran Vasireddy,4 Wesley Jakubiec,3 Swathi Vasireddy,4 Richard J. Wallace Jr,4 Keith E. Simmon,5 Bruce E. Dunn,6 Gary Jackoway,7 Surabhi B. Vora,8 Kevin K. Quinn,9 Xuan Qin2 and Sheldon Campbell1,3

**Abstract**

A previously undescribed, rapidly growing, scotochromogenic species of the genus *Mycobacterium* (represented by strains PB739T and GK) was isolated from two clinical sources – the sputum of a 76-year-old patient with severe chronic obstructive pulmonary disease, history of tuberculosis exposure and *Mycobacterium avium* complex isolated years prior; and the blood of a 15-year-old male with B-cell acute lymphoblastic leukaemia status post bone marrow transplant. The isolates grew as dark orange colonies at 25–37°C after 5 days, sharing features in common with other closely related species. Analysis of the complete 165 rRNA gene sequence (1492 bp) of strain PB739T demonstrated that the isolate shared 98.8 % relatedness with *Mycobacterium wolinskyi*. Partial 429 bp *hsp65* and 744 bp *rpaB* region V sequence analyses revealed that the sequences of the novel isolate shared 94.8 and 92.1 % similarity with those of *Mycobacterium neoaurum* and *Mycobacterium aurum*, respectively. Biochemical profiling, antimicrobial susceptibility testing, HPLC/gas-liquid chromatography analyses and multilocus sequence typing support the taxonomic status of these isolates (PB739T and GK) as representatives of a novel species. Both isolates were susceptible to the Clinical and Laboratory Standards Institute recommended antimicrobials for susceptibility testing of rapidly growing mycobacteria including amikacin, ciprofloxacin, moxifloxacin, doxycycline/minocycline, imipenem, linezolid, clarithromycin and trimethoprim/sulfamethoxazole. Both isolates PB739T and GK showed intermediate susceptibility to cefoxitin. We propose the name *Mycobacterium grossiae* sp. nov. for this novel species and have deposited the type strain in the DSMZ and CIP culture collections. The type strain is PB739T (=DSM 104744T=CIP 111318T).

Since its original description in 1896 by Lehman and Neumann [1, 2], the genus *Mycobacterium* has flourished as a rich group of micro-organisms with over 150 mycobacterial species and an increasing number of subspecies recognized to date [3]. Moreover, the increasing availability of molecular identification methods has provided microbiologists with ever-increasing opportunities to identify novel species [4, 5], driving an important expansion in mycobacterial taxonomy [3, 4]. Traditionally, mycobacteria are divided into two groups based on their growth rate on solid medium, with slowly growing mycobacteria (SGM) producing visible colonies after 7 days and rapidly growing mycobacteria (RGM) growing in less than 7 days [6]. Of the more than 150 species of non-tuberculous mycobacteria (NTM) currently having validly published names, approximately 75 are SGM and 75 are RGM [7, 8]. Slowly growing NTM include major pathogens such as *Mycobacterium avium* and

**Author affiliations:** 1Department of Laboratory Medicine, Yale School of Medicine/Yale-New Haven Hospital, New Haven, CT, USA; 2Department of Laboratory Medicine, University of Washington, Seattle, WA, USA; 3Department of Pathology and Laboratory Medicine, VA Connecticut Healthcare, West Haven, CT, USA; 4Department of Microbiology, The University of Texas Health Science Center, Mycobacteria/Nocardia Laboratory, Tyler, TX, USA; 5Department of Biomedical Informatics, University of Utah, Salt Lake City, UT, USA; 6Pathology and Laboratory Medicine, Clement J. Zablocki VA Medical Center, Milwaukee, WI, USA; 7MIDI, Inc., Newark, DE, USA; 8Pediatric Infectious Diseases, Kaiser Permanente Fontana Medical Center, Fontana, CA, USA.

*Correspondence:* Alberto Enrique Paniz-Mondolfi, alberto.paniz-mondolfi@yale.edu or albertopaniz@yahoo.com

**Keywords:** Mycobacterium grossiae; rapidly growing; scotochromogenic; respiratory; Mycobacterium; bacterial WGS; phylogenomics.

**Abbreviations:** ALL, acute lymphoblastic leukaemia; CLSI, Clinical and Laboratory Standards Institute; COPD, chronic obstructive pulmonary disease; MIC, minimal inhibitory concentration; NTM, non-tuberculous mycobacteria; RGM, rapidly growing mycobacteria; TMP/SMX, trimethoprim/sulfamethoxazole.

The GenBank/EMBL/DDJB accession numbers for the 16S rRNA, *hsp65* and *rpaB* gene sequences of strain PB739T are KM186137, KM186138 and KM186139, respectively. The genome sequence of strain GK was deposited under NCBI WGS accession MCHX00000000. Four supplementary figures are available with the online Supplementary Material.
Mycobacterium intracellulare, while RGM also include important human pathogens including Mycobacterium abscessus [9].

While environmental mycobacteria may colonize airways without causing clinical symptoms, a growing number of cases of RGM-related disease in both immunocompetent and immunosuppressed patients have been reported [10]. We herein report on a novel species of RGM isolated from a veteran patient with general symptoms of pulmonary infection (strain PB739T) and an adolescent with relapsed acute lymphoblastic leukemia (ALL) with fever, cough and hypoxia (strain GK).

Strain PB739T was originally isolated from a 76-year-old male from Connecticut (USA) who presented with a 1-year history of haemoptysis, a 4.5 kg weight loss, and recurrent signs and symptoms of broncho-pulmonary infection. Thirty years previously, he received treatment for tuberculosis (TB) exposure but did not complete isoniazid prophylaxis. At presentation, three serial sputum specimens resulted in a single culture positive for Mycobacterium avium complex for which he was not treated. He was an active smoker and had severe chronic obstructive pulmonary disease (COPD). Given the severity of his lung disease, aggressive interventions were deferred based on risk/benefit to the patient. Chest computed tomography showed peripheral basilar inflammatory disease, including a linear scar-like lesion in the right upper lobe, bronchiectatic changes, as well as several micronodules not present in previous studies suggestive of an infectious process. A subsequent sputum sample 7 months after presentation grew the first isolate of the novel species of the genus Mycobacterium we describe here. The patient was discharged and lost to follow up.

The second strain (GK) was isolated from a 15-year-old male with multiple relapsed pre-B-cell ALL who was admitted and enrolled in a chimeric antigen receptor T-cell therapy trial after his third disease relapse following an allogeneic bone marrow transplant from a fully matched sibling donor. One day prior to T-cell harvest, the patient developed fevers without signs of overt sepsis or localizing symptoms. Blood samples were sent for culture. The patient was treated with cefepime for neutropenic fever and continued on prophylactic voriconazole and acyclovir. The patient was treated with cefepime for neutropenic fever and continued on prophylactic voriconazole and acyclovir. The patient was treated with cefepime for neutropenic fever and continued on prophylactic voriconazole and acyclovir. The patient was treated with cefepime for neutropenic fever and continued on prophylactic voriconazole and acyclovir. The patient was treated with cefepime for neutropenic fever and continued on prophylactic voriconazole and acyclovir. The patient was treated with cefepime for neutropenic fever and continued on prophylactic voriconazole and acyclovir. The patient was treated with cefepime for neutropenic fever and continued on prophylactic voriconazole and acyclovir.

Isolate PB739T was recovered from a N-acetylcysteine/NaOH treated sputum sample after direct inoculation into BACTEC 960 MGIT broth (Becton Dickinson). It was then subcultured on Middlebrook 7H10 agar (BBL) and Lowenstein–Jensen media (BBL) at 37 °C under a 5% CO2 atmosphere.

Phenotypic characterization and biochemical traits were determined as described by Kent and Kubica [11, 12]. For PB739T, we observed colony morphology, pigmentaion, photo-induction and the ability of the isolate to grow at different temperatures (24, 30, 37, 42 °C) on Middlebrook 7H10 agar and Lowenstein–Jensen slants. Biochemical tests included nitrate reductase, Tween 80 hydrolysis, arylsulfatase, iron uptake and urease, arginine dihydrolase, ornithine decarboxylase and citrate synthase activities. Inhibition tests included 5% sodium chloride and ability to grow on McConkey agar without crystal violet. Additional biochemical testing was performed by inoculation of an API 20E (bioMérieux) strip as recommended by the manufacturer, with the exception that incubation time was increased to 7 days at 30 °C in a highly humid atmosphere as described previously [13].

Acid-fast microscopy of cultures of both strains revealed rod-shaped, acid-fast bacilli. Growth occurred after 5 days at an optimal temperature of 37 °C, with good growth also at 25 and 30 °C, as well as light growth at 42 °C. On both 7H11 Middlebrook and Lowenstein–Jensen solid medium, mature colonies grew as domed, smooth, dark orange/yellow colonies (Fig. S1, available with the online Supplementary Material). Photoreactivity testing confirmed their scotochromogenic nature. No growth was observed with 5% sodium chloride. The strains were positive for nitrate reductase, Tween 80 hydrolysis and urease activity but negative for three-day arylsulfatase, arginine dihydrolase, ornithine decarboxylase and citrate synthase activities. They utilized glucose, mannitol, sorbitol and rhamnose as carbon sources. These results were compared with those of three other closely related RGM species (Table 1).

Antimicrobial testing was performed on both isolates by broth microdilution using the recommended Clinical and Laboratory Standards Institute (CLSI) guidelines for RGM [14] and commercially available microdilution plates (RAPMYCO; Thermo-Fisher; previously TREK Diagnostics) using cation-adjusted Mueller–Hinton broth and incubation. The cultures were incubated at 30 °C until macroscopic growth was adequate for interpretation. Minimal inhibitory concentrations (MICs) were read using a mirrored light box. Antimicrobials tested included those recommended by the CLSI for RGM: amikacin, cefoxitin, ciprofloxacin, moxifloxacin, clarithromycin, doxycycline, imipenem, TMP/SMX and linezolid. Following CLSI recommendations, MICs for broth microdilution were established as the lowest concentration of drug to inhibit visible growth, except for TMP/SMX for which the MIC was determined at 80% inhibition of growth when compared with the growth control well. Strains PB739T and GK were susceptible to all agents but were intermediately susceptible to cefoxitin (Table 2).
Table 1. Phenotypic characteristics of Mycobacterium grossiae sp. nov. and comparison with selected closely related rapidly growing species of the genus Mycobacterium

Strains: 1, Mycobacterium grossiae sp. nov. PB739T; 2, Mycobacterium aurum ATCC 23365T; 3, Mycobacterium neoaurum ATCC 25795T (=DSM 44074T); 4, Mycobacterium wolinskyi ATCC 700010T (=DSM 44493T). ND, no data available.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RGM</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 ºC</td>
<td>+</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>30 ºC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>37 ºC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>42 ºC</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Arylsulfatase (3 days)</td>
<td>−</td>
<td>−</td>
<td>+/−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5% NaCl tolerance</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80 hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Growth on McConkey agar*</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Iron uptake</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>−</td>
<td>−/†</td>
<td>−</td>
<td>+/−</td>
<td>−</td>
</tr>
<tr>
<td>Carbon source</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>+</td>
<td>+†</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>−</td>
<td>−/†</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*McConkey agar without crystal violet.
†Some strains have shown variable reactions.

Samples from strain PB739T were later subjected to cell harvesting, saponification, extraction and clarification to isolate mycolic acids. HPLC analysis was performed on an Agilent 1100 HPLC system coupled to the Microbial identification system (MIDI) software. The HPLC pattern obtained with PB739T did not match any species-specific standard but was closest to the slowly growing NTM group Mycobacterium nonchromogenicum/Mycobacterium terrae cluster (with Sim Index of 0.500, below the cutoff for a reliable identification). The mycolic acid pattern exhibited two clusters of peaks after the elution, with the most prominent peaks located in the first cluster, thus suggesting a predominance of shorter-chain mycolic acids (at around 75%) emerging prior to 5 min (Fig. S2). The double cluster group includes a wide range of species which can be further resolved upon the prominence of the peaks and their time of emergence [15], sometimes with a few peak differences that appear not to affect their overall appearance. The prominent early eight-peak pattern produced by PB739T places it within the group of widely separated, early emerging, double-cluster patterns, such as those of Mycobacterium neoaureum, Mycobacterium phlei, Mycobacterium mucogenicum and Mycobacterium diernhoferi, but with no overlap with any previously reported mycobacterial pattern profile.

In addition, whole-cell fatty acid analyses was performed by gas-liquid chromatography with the Microbial Identification System (MIS; MIDI). The MIS included an HP model 6890A gas chromatograph equipped with a 25 m × 0.2 mm fused silica capillary column with a flame ionization detector. Peaks were identified automatically and quantified by the system. Organism identification was done by comparison with the MIDI mycobacterial library database (version 3.8). The manufacturer’s protocol was followed for all stages of cultivation, extraction, saponification, methylation and chromatography procedures. Fatty acid analyses of PB739T showed a profile with some similarity to members of the slowly growing NTM group, Mycobacterium avium-intracellularare-scrofulaceum (MAIS complex) (Sim Index: 0.397). Fatty acids typical of mycobacteria were present, comprising unbranched saturated/unsaturated fatty acid esters with chain lengths of 14, 15, 16, 17, 18 and 19 carbon atoms, with the majority of fatty acids being C18:0 (24.20%), C16:0 (24.01%) and tuberculostearic acid C18:1ω9c (24.20%).

Direct routine identification through matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was attempted but failed to render a reliable identification. Subsequently, isolate PB739T was then grown on 7H11 Middlebrook medium and subjected to an extraction protocol following the inactivated Mycobacterium/Nocardia bead preparation method as suggested by the manufacturer (Bucker Daltonics). The spectral fingerprint of the isolate provided a 1.301 score ID as the Mycobacterium farcinogenes/senegalense group, followed by Mycobacterium cosmeticum and Mycobacterium insubricum as the second (1.222) and third (1.218) best matches. The fact that the score was slightly below the cutoff recommended by the manufacturer for confident identification to

Table 2. Antimicrobial susceptibility pattern of Mycobacterium grossiae sp. nov. strains (µg ml⁻¹)

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Isolate PB739T</th>
<th>Isolate GK</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMP/SMX</td>
<td>≤0.25/4.75</td>
<td>≤0.25/4.75</td>
</tr>
<tr>
<td>Linezolid</td>
<td>≤1</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤2</td>
<td>≤2</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>≤0.25</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>3216</td>
<td>32</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≤1</td>
<td>≤1</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>≤0.12</td>
<td>≤0.12</td>
</tr>
<tr>
<td>Minocycline</td>
<td>≤1</td>
<td>≤1</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial (3 days)</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Extended (14 days)</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Downloaded from www.microbiologyresearch.org by
On: Fri, 15 Mar 2019 09:56:16
IP: 54.70.40.11
the genus level and that the acquired spectral profile matched to other species of the genus *Mycobacterium* precluded an accurate identification, probably due to the absence of this species in current MALDI-TOF microbial databases.

For genetic analyses, a loopful of mycobacteria biomass from isolated colonies of PB739\(^T\) was suspended in 100 \(\mu\)l of sample preparation reagent (Prepman Ultra; Life Technologies). The samples were held for 30 s and then heat-killed for 10 min at 100 °C, then centrifuged at maximum speed in a microcentrifuge for 2 min. The DNA was extracted, and sequencing of the complete 16S rRNA gene and partial RNA polymerase beta subunit (*rpoB*) and 65 kDa heat-shock protein (*hsp65*) genes was carried out as described below. Phylogenetic analysis was performed via alignment by performing BLAST searches and comparing them with the most closely related mycobacterial species in GenBank. Phylogenetic trees were reconstructed using the neighbour-joining method with Kimura’s two-parameter distance correction model and 1000 bootstrap replications in the MEGA5 software package [16], with all alignments including publicly available type strain reference sequences of pigmented mycobacteria for the 16S rRNA, *hsp65* and *rpoB* genes.

Complete amplification and sequencing of the 16S rRNA gene was performed using primers Pa, pC, pD, pE and pH, as previously described by Edwards et al. [17]. The BLAST search showed that, for the 16S rRNA gene, the sequence with highest similarity was that of *Mycobacterium wolinskyi* with 18 bp mismatches out of 1476 bp (98.8 % identity). The neighbour-joining tree based on the alignment of the 16S rRNA gene sequence and those of other species of the genus *Mycobacterium* revealed a very low robustness (Fig. 1). Isolates PB739\(^T\) and GK were 100 % matched to each other by 16S rRNA gene sequencing.

PCR amplification and restriction endonuclease analysis of the *hsp65* gene using primers TB11 and TB12 was performed following the procedure of Telenti et al. [18] as modified by Steingrube et al. [19]. Alignment of the 429 bp fragment of the *hsp65* gene sequence showed highest similarity with that of *M. neoaurum* (94.8 % identity). PCR restriction analysis (PRA) was performed on the *hsp65* gene sequences of both isolates using NEBcutter V2.0, a program available via a webserver (http://nc2.neb.com/NEBcutter2). The program is used to generate theoretical digests using the restriction enzymes *Bst*EII and *Hae*III [19]. The *Bst*EII digestion yielded a major band pattern of 223 bp/127 bp/79 bp and the *Hae*III digestion yielded a major band pattern of 145 bp/137 bp. Bands below 50 bp were not clearly visible and quantifiable on a 3 % Metaphor gel (Lona) used in experimental PRA from our PRA experience. The *Bst*EII and *Hae*III band patterns of these isolates did not match any known mycobacterial pattern present in the in-house PRA database (data not shown). The two isolates, PB739\(^T\) and GK, were a 100 % match to each other (Fig. S3).

Amplification and sequencing of region V of the *rpoB* gene of the amplified DNA fragments was carried out utilizing primers Myco F and Myco R as described by Adékambi et al. [20]. Sequences with highest similarity were those of *Mycobacterium aurum* with 51 base mismatches out of 644 bp (92.1 % identity) followed by *Mycobacterium vanbaalenii* (57 bp mismatches). Phylogenetic sequence analysis allocated the novel species within the group of RGM (Fig. S4). Trees based on the 723 bp gene (Fig. S4) showed no definitive relationship with any other previously described NTM. By *rpoB* region V, isolates PB739\(^T\) and GK were 100 % matched to each other.

By 16S rRNA, *hsp65* and *rpoB* region V gene sequencing, the two isolates PB739\(^T\) and GK matched 100 % to each other. Trees based on the 441 bp *hsp65* gene (Fig. S3) and 723 bp *rpoB* gene (Fig. S4) and 16S rRNA (Fig. 1) sequences showed no definitive relationship with other previously described mycobacteria, clearly depicting distinct lineages from other closely related species and a fair distance from other recognized RGM (Fig. 2).

For isolate GK, sequencing libraries were prepared using 100 ng genomic DNA with a Kapa HyperPlus kit followed by dual-index Truseq PCR with 1 ng genomic DNA using a Nextera XT with 12 cycles of dual-index PCR and then sequenced using portions of a 2 x 300 bp Truseq library, a 1 x 180 bp Truseq library and a 1 x 180 bp Nextera library on an Illumina MiSeq [21, 22]. Raw reads were quality- and adapter-trimmed using cutadapt, repaired using pairfq, de novo assembled using SPAdes v3.8 and annotated using prokka v1.11 [23, 24].

A total of 1 546 611 filtered reads split between the Nextera and Truseq libraries were de novo assembled into an assembly of 184 contigs comprising 6 147 628 bp with an average coverage of 37 x and an N50 of 72 082 bp. The genome contained 5889 coding sequences (CDSs) with most likely three copies of RNA loci based on coverage. DNA G+C content was 70.0 mol%. Of note, loci in the genome had 100, 99.9 and 99.5 % identity by nucleotide to the 16S rRNA (KM186137), *rpoB* (KM186139) and *hsp65* (KM186138) genes sequenced for strain PB739\(^T\) as described above. The complete *rpoB* CDS demonstrated 90 % nucleotide identity to M. wolinskyi ATCC 700010\(^T\) (AY262743) and *Mycobacterium vanbaalenii* PYR-1\(^T\) (CP000511) sequences; however, it showed only 76.6 % average nucleotide identity (ANiB) to these species. Tetra Correlation Search with JSpeciesWS showed highest correlation with *Mycobacterium chlorophenolicum* NBRC 15527\(^T\) and *Mycobacterium rufum* JS14\(^T\), but neither was >0.986 [25]. ANiB results to these species were 76.5 and 77.2 %, respectively; these alignment results were similar to those of *Mycobacterium obuense* UC1 and *Mycobacterium elephantis* Lipa [21, 22, 26].

The genome of strain GK contained a class A beta-lactamase that aligned between 62 and 67 % by amino acid to beta-lactamases present in multiple other species of the genus *Mycobacterium*. Biochemical genes included a *narB*
nitrate reductase that aligned 84% by amino acid sequence to *M. rufum* JS14T and a 4.3 kb *ureA–G* gene cluster that aligned 83% by nucleotide to *M. phlei* CCUG 21000T. Interestingly, given the lack of biochemical positivity, two putative arylsulfatase (*atsA*) genes and one sulfatase gene were annotated by prokka. The two *Mycobacterium grossiae* sp. nov. *AtsA* proteins aligned with 36% amino acid identity to each other and 85% amino acid identity to the closest mycobacterial arylsulfatase protein. It is unclear as to the mechanism of the discrepancy between the presence of the arylsulfatase genes and the lack of biochemical reaction.

Although RGM are generally considered saprophytic organisms which inhabit environments such as water, dust and soil in a variety of ecosystems, they are increasingly linked to human and animal disease [10]. NTM may colonize airways without causing disease [10, 27]; however, they have become emerging pathogens responsible for respiratory tract infections not only in patients with underlying respiratory disease [28], but also in immunocompetent hosts [29]. The patient from whom the first isolate was recovered presented with a clinical history of bronchiectasis due to smoking and severe COPD, possibly predisposing the patient to NTM infection, since RGM infection occurs mainly at sites of pre-existing mycobacterial disease or bronchiectasias due to other causes [30].

Even though the patient presented with a clinical history of pulmonary infection and displayed radiographic evidence of pulmonary disease, he did not fulfill the American Thoracic Society criteria for the diagnosis of NTM pulmonary disease [7]. On the basis of available information, the clinical significance of this isolate could not be verified.

Our second patient presented with relapsed ALL and failed bone marrow transplant and was highly immunosuppressed at the time of presentation with fever and respiratory symptoms. He also had a central line (catheter) and ultimately remained bacteraemic for several days until the organism was identified on blood culture. A wide variety of NTM are seen in patients with underlying bronchiectasis, including pigmented RGM. The pigmented RGM are generally nonpathogenic, but the one exception is central line (catheter)-related infections with mycobacteriaemia. Although infrequent, bloodstream infections by rapidly growing NTM have been described in immunocompromised patients [9, 31] with increasing reports on catheter-related bloodstream infections [32], particularly in those bone marrow transplant recipients with central lines [33]. Hence, it might not be a surprise that this new taxon would also be associated with a central catheter infection.

Diagnosis in such cases may be challenging, and accurate identification may be hindered by inadequate incubation times of conventional blood cultures to detect mycobacteria, as well as other factors such as long incubation time required for biochemical profiling, limited availability of commercial nucleic acid probes for RGM and potential misidentification with other closely related organisms.
In all, these genotypic findings, along with the distinct phenotypic characteristics, mycolic acid pattern, cell wall fatty acid composition and susceptibility profile, allow us to confirm the taxonomic status of isolates PB739^T and GK as representatives of a novel species within the genus *Mycobacterium*. The fact that PB739^T exhibited a >3 % rpoB region V sequence divergence strongly argues in favour of this being a representative of a novel mycobacterial species, as has been established by Adékambi *et al.* [20] using the partial 723 bp rpoB sequence to differentiate RGM isolates. In addition, this study also highlights the potential role of this novel species as an emerging pathogen in immunocompromised patients.

**DESCRIPTION OF MYCOBACTERIUM GROSSIAE SP. NOV.**

*Mycobacterium grossiae* (grossiae. N.L. gen. n. grossiae named after Wendy M. Gross in honour of, and in recognition for, significant contributions in mycobacterial research and clinical care).

Cells are acid-alcohol-fast and Gram-stain-positive bacilli. Colonies are domed, smooth, dark orange/yellow and grow on 5 % sheep blood agar, Middlebrook 7H10 agar and egg-based Lowenstein–Jensen slants after 5 days at temperatures between 24 and 42 °C. The optimal temperature is 37 °C while at 42 °C it exhibits light growth. Growth is observed on MacConkey agar medium without crystal violet, and no growth is observed with 5 % NaCl. It is positive for nitrate reductase, Tween 80 hydrolysis and urease activity, and negative for 3 day arylsulfatase. The type strain (PB739^T) and strain GK are susceptible in vitro to TMP/SMX, linezolid, clarithromycin, ciprofloxacin, imipenem, moxifloxacin, amikacin, doxycycline and minocycline and show intermediate susceptibility to cefoxitin. Has a low MIC to tigecycline (0.03 µg ml\(^{-1}\), see Table 2) but no susceptible breakpoints are yet available. HPLC analysis shows a distinct early emerging, double-cluster pattern. Genetically, it shares 94.8 % partial hsp65 gene sequence similarity with *M. neoaurum*, 92.1 % 16S rRNA gene sequence similarity with *M. aurum* and 98.8 % with *M. wolinskyi*, and 92.1 % similarity with *M. aurum* by rpoB region V gene sequencing. This species was isolated from two different clinical sources suggesting its role as an emerging human pathogen.

The type strain is PB739^T (=DSM 104744^T=CIP 111318^T), which was isolated from a human pulmonary specimen in
the USA. Strain GK is an additional strain of the species isolated from a human blood culture specimen.

Funding information

The authors received no specific grant from any funding agency.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References


