***Mycobacterium elephantis* sp. nov., a rapidly growing non-chromogenic *Mycobacterium* isolated from an elephant**

Hasan Shojaei,¹ John G. Magee,² Roger Freeman,² Malcolm Yates,³ Neil U. Horadagoda⁴ and Michael Goodfellow¹

Author for correspondence: Michael Goodfellow. Tel: +44 191 222 7706. Fax: +44 191 222 5228.
e-mail: m.goodfellow@ncl.ac.uk

¹ Department of Agricultural and Environmental Science, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

² Regional Centre for Mycobacteriology, Public Health Laboratory, General Hospital, Newcastle upon Tyne NE4 6BE, UK

³ Public Health Laboratory, Dulwich Hospital, Dulwich, London SE22 8QF

⁴ Department of Veterinary Paraclinical Studies, Faculty of Veterinary Medicine and Clinical Science, University of Peradeniya, Sri Lanka

A strain isolated from a lung abscess in an elephant that died from chronic respiratory disease was found to have properties consistent with its classification in the genus *Mycobacterium*. An almost complete sequence of the 16S rDNA of the strain was determined following the cloning and sequencing of the amplified gene. The sequence was aligned with those available on mycobacteria and phylogenetic trees inferred by using three tree-making algorithms. The organism, which formed a distinct phyletic line within the evolutionary radiation occupied by rapidly growing mycobacteria, was readily distinguished from members of validly described species of rapidly growing mycobacteria on the basis of its mycolic acid pattern and by a number of other phenotypic features, notably its ability to grow at higher temperatures. The type strain is *Mycobacterium elephantis* DSM 44368^T.

Keywords: *Mycobacterium elephantis* sp. nov., polyphasic taxonomy, lung abscess of adult elephant

INTRODUCTION

A rapidly growing, weakly acid–alcohol-fast organism isolated from a lung abscess of an elephant had properties consistent with its classification in the genus *Mycobacterium* (Lévy-Frébault & Portaels, 1992; Goodfellow & Magee, 1997). In the present investigation, the organism was examined for a selection of genotypic and phenotypic properties to clarify its taxonomic position. It is apparent from the results that the organism represents a new species, for which the name *Mycobacterium elephantis* is proposed.

METHODS

Organism and growth conditions. The organism (strain 484^T) was isolated on Löwenstein–Jensen medium after 7 d incubation at 37 °C. It was then cultivated on the same medium at 25, 36, 42 and 45 °C and on Columbia blood, MacConkey, Middlebrook 7H10 and 5% (w/v) sodium chloride agars at 25 and 36 °C for between 3 and 10 d. The strain was maintained on Middlebrook 7H10 agar and grown at 36 °C for the remaining phenotypic tests.

Phenotypic characterization. Standard methods were used throughout. The Gram and Ziehl–Neelsen stains were carried out on cells grown for 5 d on Middlebrook 7H10 agar. Catalase activity, niacin activity and nitrate reduction were determined after 10 d, aryl sulphatase production after 3 and 14 d, tellurite reduction after 5 d, Tween hydrolysis after 10 d and urease production after 3 d. Antibiotic-susceptibility studies were carried out using the resistance ratio method.

Extraction and analysis of mycolic acids. Lyophilized biomass from 10-d-old Middlebrook 7H10 agar plates was degraded by alkaline methanolysis (Saddler *et al.*, 1987) and two-dimensional TLC of the methanolysates was carried out according to the method of Minnikin *et al.* (1980).

16S rDNA sequencing. The test strain was shaken in 100 ml Sauton's broth (Mordarska *et al.*, 1972) at 150 r.p.m. for 14 d at 36 °C. The biomass was harvested by centrifugation at 10000 r.p.m. for 10 min and washed twice in sterile TE buffer (Tris/HCl, pH 8, 10 mM; EDTA 1 mM); approximately 100 mg wet weight biomass was used for DNA extraction. The extraction and purification of DNA and the amplification, cloning and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995; Shojaei *et al.*, 1997). The 16S rDNA sequence of strain 484^T was aligned manually with all available sequences of rapidly growing mycobacteria by using the AL16s program (Chun, 1995). The additional sequence data were obtained from the GenBank and EMBL databases.

The EMBL accession number for the 16S rDNA sequence of strain 484^T is AJ010747.

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rDNA sequences of rapidly growing mycobacteria (1446 nucleotides) showing the phylogenetic position of strain 484^T. The sequence of the type strain of *Mycobacterium hassiacum* was not included in this analysis as the phylogenetic position of this rapidly growing organism is with the slowly growing mycobacteria (Schroder *et al.*, 1997). The numbers at the nodes indicate the level of bootstrap support, based on a neighbour-joining analysis of 1000 resampled datasets; only values greater than 40% are given. Bar, 0.005 substitutions per nucleotide position.

Evolutionary trees were inferred by using three algorithms, namely the Fitch–Margoliash (1967), maximum-parsimony

(Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices for the Fitch–Margoliash and neighbour-joining methods were generated as described by Jukes & Cantor (1969). The PHYLIP package (Felsenstein, 1993) was used for all of the analyses. The resultant unrooted tree topologies were evaluated by performing bootstrap analyses (Felsenstein, 1985) of the neighbour-joining database on 1000 resamplings with the SEQBOOT and CONSENSE programs in the PHYLIP package. The root positions of the unrooted tree based on the neighbour-joining method was estimated by using three outgroup organisms, namely *Gordona terrae* DSM 43249^T (sequence accession no. X79286), *Nocardia asteroides* ATCC 19247^T (Z36934) and *Rhodococcus equi* DSM 20307^T (X80614), as described by Swofford & Olsen (1990).

RESULTS AND DISCUSSION

An almost complete 16S rDNA sequence (1517 nucleotides) was obtained for strain 484^T. Comparison of this nucleotide sequence with available sequences for representatives of the genus *Mycobacterium* showed that the organism fell within the evolutionary radiation occupied by rapidly growing mycobacteria (Fig. 1). The pairwise nucleotide similarity value found between strain 484^T and the rapidly growing mycobacteria ranged from 95.6 to 97.7%, giving a mean of $96.7 \pm 0.5\%$. The corresponding mean nucleotide similarity value found between strain 484^T and the representative slowly growing mycobacteria was $96.2 \pm 0.4\%$.

The nucleotide sequence of strain 484^T shows substantial differences from the corresponding sequences of its nearest neighbours, namely *Mycobacterium confluentis* (97.8%), *Mycobacterium madagascariense* (97.6%) and *Mycobacterium phlei* (97.7%); these values correspond to 29, 33 and 30 nucleotide differences, respectively. Comparable scales of difference exist between the nucleotide sequences of validly described species of rapidly growing mycobacteria (Shojaei *et al.*, 1997). The positions of the test and marker strains in the phylogenetic tree were not markedly affected by either the tree-making algorithms or the outgroup strains (Fig. 1). It is also clear from Fig. 1 that the relationship between strain 484^T and its nearest neighbour is not supported by high bootstrap values. However, the sequence of this strain, like those of other rapidly growing mycobacteria, has the charac-

Table 1. Characteristics that differentiate strain 484^T from related mycobacteria

Strain/species	Growth at 45 °C	Nitrate reduction	Aryl sulphatase (14 d)	Growth on 5% (w/v) NaCl agar	Pigmentation
Strain 484 ^T	+	+	—	—	n
<i>M. confluentis</i>	—	—	+	—	n
<i>M. madagascariense</i>	—	—	—	—	s
<i>M. phlei</i>	+	+	+	+	s

n, Non-chromogenic; s, scotochromogenic.

teristic short helix at positions 451–482 (*Escherichia coli* numbering system; Brosius *et al.*, 1978).

The phenotypic properties of strain 484^T are also consistent with its classification in the genus *Mycobacterium* (Lévy-Frébault & Portaels, 1992). The organism is aerobic, non-motile, Gram-positive and weakly acid–alcohol-fast. It is catalase- and urease-positive, reduces nitrate and is positive for Tween hydrolysis. Older cultures may show weak chromogenicity. The detection of α - and keto-mycolates and wax esters in methanolysates of the strain distinguishes it from the rapidly growing mycobacteria *Mycobacterium abscessus*, *Mycobacterium chelonae*, *M. confluentis* and *Mycobacterium fortuitum*, but not from *M. madagascariense* or *M. phlei*. However, these species are scotochromogenic and can be distinguished from strain 484^T by the phenotypic characteristics shown in Table 1.

It is evident from the chemical, 16S rDNA sequencing and microbiological data that strain 484^T shows a combination of properties that distinguish it from all other validly described species of *Mycobacterium*. It is proposed, therefore, that this organism be classified in the genus *Mycobacterium* as *Mycobacterium elephantis*.

Description of *Mycobacterium elephantis* sp. nov.

Mycobacterium elephantis (e.le.phan'tis. L. n. *elephas* elephant; L. gen. n. *elephantis* of the elephant).

Aerobic, Gram-positive, weakly acid–alcohol-fast, asporogenous, non-motile organism which is non-chromogenic. Cells are cocco-bacillary and 1.2–1.4 μ m long. The organism is positive for catalase (45 mm foam), nitrate reductase, Tween hydrolysis and urease. Negative for tellurite reduction (5 d) and aryl sulphatase activity (14 d). Good growth occurs at 25, 37, 42 and 45 °C. Growth is inhibited by isoniazid (1.4 mg l⁻¹), rifampicin (16 mg l⁻¹), pyrazinamide (66 mg l⁻¹), thiacetazone (10 mg l⁻¹) and thiophen-2-carboxylic acid hydrazide, but not by ciprofloxacin (2.5 mg l⁻¹) or ethambutol (3.2 mg l⁻¹). Isolated from a lung abscess of an adult elephant that had died from chronic respiratory disease. The type strain of *M. elephantis* is strain 484^T (= DSM 44368^T).

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