# *Mycobacterium eburneum* sp. nov., a non-chromogenic, slowly growing species isolated from sputum

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## Abstract

A non-pigmented slowly-growing *Mycobacterium* strain isolated from sputum was characterised using a polyphasic approach. The isolate, strain X82$^T$, has chemotaxonomic properties consistent with its classification in the genus *Mycobacterium*. The organism formed a distinct subclade in the mycobacterial 16S rRNA gene tree together with *Mycobacterium paraense* DSM 46749$^T$. The two strains were distinguished based on their fatty acid, mycolic acid and polar lipid profiles and by a combination of phenotypic features. These data support the conclusion that the isolate represents a novel mycobacterial species for which the name of *Mycobacterium eburneum* sp. nov. is proposed. The type strain is X82$^T$ (DSM 44358$^T$ = CECT 8775$^T$ ).

*Mycobacterium* (Lehmann & Neumann, 1896) is the type genus of the family *Mycobacteriaceae* (Magee & Ward, 2012) in the order *Corynebacteriales* (Goodfellow & Jones, 2012). The genus can be distinguished from other genera in the order *Corynebacteriales* using a combination of chemotaxonomic and morphological properties. At the time of writing the genus contains 175 species with validly published names and 13 subspecies (http://www.bacterio.net) which can be divided into two groups based on growth rate. Slowly-growing mycobacteria require seven or more days of incubation at optimal temperature to form visible colonies from highly diluted inocula, whereas colonies of rapidly growing strains are seen in less than seven days under compatible conditions (Wayne & Kubica, 1986). Species assigned to the two groups can be distinguished using a combination of genotypic and phenotypic criteria (Magee & Ward, 2012), including the characterisation of mycolic acids (Teramoto *et al.*, 2015). The genus encompasses obligate pathogens, saprophytes, and non-tuberculosis mycobacteria which are common in the environment and can cause opportunistic infections in humans (Tortoli, 2009).

The aim of the present study was to establish the taxonomic position of a slowly-growing mycobacterial strain that was isolated many years ago from sputum collected in Switzerland in 1998 and was deposited in a public culture collection without thorough taxonomic characterisation. During a systematic screening of mycobacterial cultures for distinct 16S rRNA gene sequences strain X82$^T$ was identified as a potential type strain of new species and subjected to polyphasic taxonomic analyses which showed that the strain represents a novel species for which we propose the name *Mycobacterium eburneum*.

*Mycobacterium eburneum* strain X82$^T$ and *Mycobacterium paraense* DSM 46749$^T$ (Costa *et al.*, 2015) were obtained from the Spanish Type Culture Collection (CECT) and Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, respectively. Both strains were maintained in 50% v/v glycerol at -80°C. DNA extraction and PCR amplification on strain X82$^T$ were achieved using the method of Amaro *et al.* (2008). An almost complete 16S rRNA gene sequence (1394 nucleotides [nt]) has been deposited in the GenBank NCBI database under the accession number KX879093.

A BLAST search of the 16S rRNA gene sequence of strain X82$^T$ in EzTaxon database (Kim *et al.*, 2012) was performed and the pairwise sequence similarities were calculated using the

method of Meier-Kolthoff *et al.* (2013a). Maximum likelihood (ML) and maximum parsimony (MP) trees were constructed based on a comparison of the sequences of strain X82$^T$ and its closest relatives using the DSMZ phylogenomics pipeline (Meier-Kolthoff *et al*., 2014) adapted to single genes with RAxML (Stamatakis, 2014) and TNT (Goloboff *et al.*, 2008), respectively. This pipeline is accessible through the GGDC web server (Meier-Kolthoff *et al.*, 2013b) available at http://ggdc.dsmz.de/. A multiple sequence alignment was performed with MUSCLE (Edgar, 2004). Rapid bootstrapping replicates in conjunction with the auto MREbootstopping criterion (Pattengale *et al.*, 2010) and tree-bisection-and-reconnection branch swapping were used for the ML and MP analyses, respectively. The $X^2$ test, as implemented in PAUP* (Swofford, 2002), was used to check the sequences for compositional bias. A neighbour joining (NJ) tree (Saitou & Nei, 1987) was constructed using MEGA software version 7.0 (Kumar *et al.*, 2015) and the evaluation of its robustness was performed in a bootstrap analyses based on 1000 replicates. The alignment of the sequences were achieved using ClustalW (Thompson *et al.*, 1997).

In the BLAST analyses of the 16S rRNA gene sequence of strain X82$^T$, in the EzTaxon database, the closest similarity, 97.9 %, was with *Mycobacterium conceptionense* DSM 45102$^T$. However using the method recommended by Meier-Kolthoff *et al.* (2013a), *M. conceptionense* DSM 45102$^T$ kept the same 16S rRNA gene similarity value to strain X82$^T$ and the highest 16S rRNA gene similaritiy was 98.0% between strain X82$^T$ and *M. paraense* DSM 46749$^T$ which was found in the 14$^{th}$ position in EzTaxon. These results are in line with the close phylogenetic position of strain X82$^T$ to *M. paraense* DSM 4674$^T$ highlighting the distinct phyletic position of *M. conceptionense* DSM 45102$^T$ (Adékambi *et al.*, 2006) from the present studied strain X82$^T$ (Fig. 1). The pairwise 16S rRNA gene similarities between strain X82$^T$ and its closest relatives, namely *M. paraense* DSM 46749$^T$ (Costa *et al.*, 2015), *M. kumamotonense* DSM 45093$^T$ (Masaki *et al.*, 2007), *M. longobardum* DSM 45394$^T$ (Tortoli *et al.*, 2013), *M. poriferae* DSM 44585$^T$ (Padgitt & Moshier, 1987), *M. rufum* DSM 45406$^T$ (Hennessee *et al.*, 2009) and *M. iranicum* DSM 44162$^T$ (Shojaei *et al.*, 2013) were 98.0%, 98.0%, 97.8%, 97.6%, 97.4%, 97.9% respectively. All of these strains were recovered in the same subclade. *M. paraense* DSM 46749$^T$ and *M. kumamotonense* DSM 45093$^T$ showed the highest 16S rRNA gene similarities, 98.0%, with strain X82$^T$ but the *M. paraense* strain was most closely related to strain X82$^T$ with 33 nt differences in the 16S rRNA gene sequences. The phyletic relatedness of strain X82$^T$ to *M. paraense* DSM 46749$^T$ was underpinned in the ML, MP (Fig. 1a) and NJ (Fig. 1b) analyses.

According to Meier-Kolthoff *et al*. (2013a), up to half of the currently conducted DDH experiments could safely be omitted without a significant risk for wrongly classifying species. For *Actinobacteria* they recommended a 16S rRNA sequence identity threshold of 99.0% for a maximal 1% probability of error in case of omitted DDH experiments. The 98.0% 16S rRNA sequence similarity determined for strain X82$^T$ and *M. paraense's* type strain DSM 46749$^T$ indicate a 0.05% [meaning 1 in 2000] chance for missing a positive DDH value (above 70%) in omitting wet lab DDH experiments. All other type strains in the close vicinity of X82$^T$ (see Fig. 1a) indicate even lower error probabilities of 0.01-0.025% [meaning 1 in 4000 to 10000]. High costs for DDH experiments and low risk for missed DDH values above the established threshold for species discrimination (Wayne *et al*., 1987) prompted us to relinquish wet lab DDH experiments.

Biomass preparation of the strains for the chemotaxonomic and phenotypic tests were harvested from flasks of Middelbrook 7H10 broth supplemented with oleic acid, albumin, dextrose and catalase (MB; Lorian, 1968) that was incubated at 37°C for 7 days. Bacterial cells for the chemotaxonomic analyses were washed three times in 0.9%, v/v sodium chloride solution and freeze dried. Phenotypic tests relevant to mycobacterial systematics, as recommended by Kent and Kubica (1985), were performed from fresh cultures prepared at mid logarithmic phase ($OD_{600}$ nm 0.30 - 0.60). Strain X82$^T$ and its closest phylogenetic neighbour were examined for chemotaxonomic properties known to be of value in mycobacterial systematics (Magee & Ward, 2012). Cellular fatty acids were extracted from fresh biomass of the strains and fatty acid methyl esters (FAMES) prepared following saponification, methylation and extraction using the protocol introduced by Miller (1982), as modified by Kuykendall *et al*. (1998). The FAMEs were analyzed using a gas chromatography instrument (Agilent 6890 N) and the resultant peaks automatically integrated fatty acids names and properties using the standard Microbial Identification (MIDI) system version 4.5 and the Myco 6 database (Sasser, 1990). Mycolic acid methyl esters prepared according to Minnikin and Goodfellow (1976) were analysed by matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (TOF-FMS) using Spiral Ion Trajectory (MALDI spiral-TOFMS), according to Teramoto *et al.* (2015). Standard chromatographic procedures were used to establish additional chemotaxonomic properties. Isomers of diaminopimelic acids were determined following the procedure of Schleifer and Kandler (1972). Whole cell sugars extract (Lechevalier & Lechevalier, 1970) were examined according to Staneck and Roberts (1974). Isoprenoid quinones were extracted according to Collins (1985) and identified by high pressure liquid

chromatography as designed by Kroppenstedt (1982). Polar lipids were extracted and identified following the protocol established by Minnikin *et al.* (1984), as modified by Kroppenstedt and Goodfellow (2006). Functional groups were revealed using spraying reagent namely α-naphthol-sulfuric acid (40.5 ml ethanol, 4 ml distilled water, 6.5 ml concentrated sulfuric acid and 10.5 ml α-naphthol (15g/l ethanol)) to detect sugar containing lipids, Dragendorff's solution (Merck) to identify choline-containing lipids (Tindall, 1990), ninhydrin reagent (0.2 % ninhydrin in acetone) to reveal amino-groups (Skipski *et al.*, 1964), molybdenum blue (Sigma 119KG123) to detect lipids with phospho-groups and molybdatophosphoric acid (Sigma P1518) to identify total lipid content.

Isolate X82$^T$ and *M. paraense* DSM 46747$^T$ were found to have chemotaxonomic properties in accordance with their classification in the genus *Mycobacterium* (Magee & Ward, 2012). They produced whole-organism hydrolysates rich in *meso*-2,6-diaminopimelic acid, arabinose, galactose, glucose and ribose (wall chemotype IV; Lechevalier & Lechevalier, 1970), straight chain saturated, unsaturated and 10 methyl-octadecanoic (tuberculostearic) fatty acids (Table S1), mycolic acids, dihydrogenated menaquinones with nine isoprene units as the predominant menaquinone (>50%) and polar lipid patterns that included diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), aminolipids (AL) and unidentified lipids ($L_{1-3}$) and glycolipids ($GL_{1-5}$) (Fig. S1; phospholipids pattern 2 according to Lechevalier *et al.* (1977)). However, the two strains can be distinguished using several chemotaxonomic features. The fatty acid profile of strain X82$^T$ showed major amounts of $C_{16:0}$ (38.2%) and $C_{10Me}$-$C_{18:0}$ (25.5%) with summed feature 3 (16.8%) containing 20:0 ALC 18.838 ECL /20 alcohol / 19:0 Cycloprop $\omega$10c / 19:0 Cycloprop $\omega$8c (Table S1) while *M. paraense* strain DSM 46747$^T$ exhibited $C_{18:0,}$ higher amounts of $C_{18:1}$ $\omega$9c (39.4%) and $C_{16:0}$ (32.1%), lower amount of $C_{10Me}$-$C_{18:0}$ (8.1 %) and lacked summed feature 3. In addition, the polar lipid profile of strain X82$^T$ contains phosphatidyl glycerol (PG) and an unknown phospholipid while *M. paraense* DSM 46747$^T$, has two aminolipids ($AL_{1-2}$) and lacked PG. Finally, strain X82$^T$ was characterised by the presence of dicarboxy-mycolic acids with 64 carbons while the *M. paraense* strain has α – *keto* –mycolic acids with 87 carbons (Fig. 2).

Strain X82$^T$ was examined for its ability to grow on GYM (glucose-yeast-malt extract) *Streptomyces* agar media (http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium 65), peptone-meat extract-glycerol (PMG; http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium 250), Lowenstein-Jensen

(LJ; Jensen, 1932), MB (Lorian, 1968) and on tryptic soy agar (TSA; MacFaddin, 1985) after 7 days at 25°C, 28°C, 37°C and 45°C.

Rough, non-pigmented colonies of isolate X82$^T$grew on almost all of the media (GYM, 7H10, LJ, MB, PMG,) at 37 °C after 14 days irrespective of whether plates were incubated in the light or dark. Optimal growth was observed on the MB and PMG agar plates after 7 days. Growth was not observed on plates incubated at either 25°C or 45°C.

Strains X82$^T$ and *Mycobacterium paraense* DSM 46749$^T$ were examined for a broad range of phenotypic properties. To this end, they were tested for their ability to use different carbon compounds, to grow at a range of pH values and in presence of sodium chloride and antibiotics using GENIII, PM 11 and PM 12 microplates and an Omnilog device (BIOLOG Inc. Hayward, CA, USA). All of the microplates were inoculated with a mycobacterial suspension in IFC solution (Inoculating Fluid provided by the manufacturer) and in MB 7H10 broth supplemented with Dye mix G, following the protocols of Khatri *et al.* (2013). The inoculation solution showed 71 % transmittance. The tests were carried out in triplicate using freshly prepared inocula harvested at mid-logarithmic phase ($OD_{600}$ nm 0.30 - 0.60) (Khatri *et al.,* 2013). The plates were incubated at 37 °C for 7 days and the resultant data were exported and analysed using the opm package version 1.3.36 (Vaas *et al.,* 2012; 2013). Strain X82$^T$ can be distinguished from *M. paraense* DSM 44749$^T$ using a combination of phenotypic properties through it is evident that these strains have many properties in common (Table 1).

The strains were also tested for their ability to produce catalase (Palomino *et al.,* 2007), heat stable catalase (Organización Panamericana de la Salud, 2008), arylsulfatase after 3 and 20 days (Tomioka *et al.,* 1990), niacin accumulation (Kent & Kubica, 1985), nitrate reductase (Bernardelli, 2007), growth in presence of potassium tellurite (Kilburn *et al.,* 1969; Kent & Kubica, 1985), degradation of Tween 80 (Ribón, 2012) and urease hydrolysis (Palomino *et al.,* 2007). Enzymatic features were determined using API ZYM kits following the manufacturer's Instructions (BioMérieux, France). Strain X82$^T$, unlike *M. paraense* strain, produced arylsulfatase after 3 and 20 days, acid phosphatase, β glucosidase, valine arylamidase, degraded Tween 80 and metabolised esterase (C4) while only the *M. paraense* DSM 46747$^T$ was positive for niacin accumulation, nitrate reduction and α-chymotrypsin. Additional phenotypic features for each of these strains are displayed in Table 1.

In conclusion, strain DSM 44358$^T$ can be distinguish from *M. paraense* DSM 44749$^T$, its nearest phylogenetic neighbour, using a combination of chemotaxonomic, genotypic and phenotypic data. Consequently, it is proposed that this strain be recognised as a new species in the genus *Mycobacterium*, *Mycobacetrium eburneum* sp. nov.

**Description of *Mycobacterium eburneum* sp. nov.**

*Mycobacterium eburneum (e. bur'ne.um.* N. L. masc. adj. referring to the cream coloured colonies produced by the organism).

Gram-stain-positive, acid-alcohol fast, non-motile, non-spore forming organism which produces cream coloured colonies on Middelbrook 7H10 and peptone- meat extract-glycerol agar media after 7 days at 37 °C in both the dark and light. Grows from 28 °C to 37 °C, optimally at 37 °C, from pH 7 to 8; optimal pH is 7.0. Produces arylsulfatase after 3 and 20 days, acid phosphatase, alkaline phosphatase, β glucosidase, catalase, catalase (68 °C), esterase lipase (C8), esterase (C4), lipase (C14), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, urease, valine arylamidase and grows in presence of potassium tellurite and degrades Tween 80. The major cellular fatty acids (>20%) are $C_{16:0,}$ and $C_{10Me}C_{18:0}$, contains dicarboxy mycolic acids with 64 carbons chain length. The predominant menaquinone (>50%) is MK-9 ($H_2$). The phospholipid pattern is composed of phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine.

The type strain of *M. eburneum* is X82$^T$ (= DSM 44358$^T$ = CECT 8775$^T$). The GenBank accession number of the 16S rRNA gene sequence is KX879093.

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## Figure legends

**Fig. 1.** Maximum likelihood (ML) (a) and neighbour joining (b) phylogenetic trees based on 16S rRNA gene sequences. The branches are scaled in terms of the expected number of substitutions per site. The numbers above the branches are bootstrap support values greater than 60%.

**Fig. 2.** Mycolic acid profiles of *M. eburneum* strain X82$^T$ (a) and *M. paraense* DSM 46749$^T$ (b) using high through put MALDI TOF according to Teramoto *et al*. (2015).

385 **Table 1**. Phenotypic features that distinguish strain X82$^T$ from *M. paraense* DSM 46749$^T$

386

| | ***M. eburneum* X82$^T$** | ***M. paraense* DSM 46749$^T$** |
|---|---|---|
| **Biochemical tests** | | |
| Arylsulfatase 3 and 20 days, esterase (C4), Tween 80 | + | - |
| Niacin accumulation, nitrate reduction, α-chymotrypsin | - | + |
| **Biolog GENIII microplates** | | |
| **Utilisation of sugars**[#] | | |
| D-cellobiose, D-fructose, D-fucose, D-galactose, D-glucose-6-phosphate, D-maltose, L-fucose, β-methyl-d-glucoside, *myo*-inositol, *N*-acetyl-D-glucosamine, stachyose, sucrose, turanose | + | - |
| Dextrin, D-raffinose, D-sorbitol, glucuronamide | - | + |
| **Utilisation of amino acids**[#] **:** | | |
| D-serine | - | + |
| L-alanine, L-glutamic acid | + | - |
| **Utilisation of organic acids :** | | |
| Acetoacetic acid, citric acid, D-malic acid, L-malic acid, mucic acid | - | + |
| D-gluconic acid, D-glucuronic acid, D-saccharic acid, α-*keto*-butyric acid | + | - |
| **Inhibition tests**[#] **:** | | |
| Sodium bromate, sodium chloride 1% ,4%, 8% , | - | + |
| Sodium formate | + | - |
| **Growth in presence of**[#] **:** | | |
| Fusidic acid, guanidine hydrochloride, lincomycin lithium chloride, nalidixic acid, niaproof tetrazolium blue, troleandomycin, vancomycin | - | + |
| **Biolog PM 11 microplates** | | |

| | | |
|---|---|---|
| **Growth in presence of** | | |
| Amikacin, cephalothin, chlortetracycline | + | - |
| Amoxicillin, cefazolin, ceftriaxone, demeclocycline, enoxacin, gentamicin, lomefloxacin, minocycline, neomycin | - | + |
| **Biolog PM 12 microplates** | | |
| **Growth in presence of**[#] | | |
| Carbenicillin, spiramycin, tetracycline, tobramycin, | + | - |
| 2,4-diamino-6,7-diisopropylpteridine, d, l-serine hydroxamate, 5-fluoroorotic acid, l-aspartic-β-hydroxamate, polymyxin b, spectinomycin, sulfamethazine, sulfamethoxazole, sulfathiazole | - | + |
| Polar lipids profile | PI, DPG, PG, PE, GPL, $GL_{1-5}$, $L_{1-3}$, PL, AL | PI, DPG, PE, GPL, $GL_{1-5}$, $L_{1-3}$, $AL_{1-2}$ |
| Mycolic acids (carbon chain length) | dicarboxy-mycolic acids (C64) | α–*keto*–mycolic acids (C87) |

+ Positive reaction; - negative reaction; [#] concentrations of antibiotics as specified for Biolog.

Isolate X82$^T$ and *M. paraense* DSM 46747$^T$ were positives for catalase, catalase (68 °C), potassium tellurite, urease, produced alkaline phosphatase, esterase lipase (C8), lipase (C14), leucine arylamidase and naphthol-AS-BI-phosphohydrolase (API ZYM tests), metabolised acetic acid, butyric acid, D- glucose, D-fructose-6-phosphate, dodecyltrimethyl ammonium bromide, D-trehalose, glycerol, propionate, Tween 40, α-*keto*-glutaric acid and β-hydroxy-butyric acid. Able to grow in presence of methyl pyruvate , tetrazolium violet and 1% sodium lactate. In contrast, neither strains metabolised aspartic acid, D -arabitol, D-aspartic acid, D-lactic acid methyl ester, D-mannitol, D-mannose, D-melibiose, gelatin, glycine-proline, inosine, L-arginine, L-galactonic acid-γ-lactone, L-histidine, L-lactic acid, L-pyroglutamic acid, L-serine, *N*-acetyl-d-galactosamine, *N*-acetyl-neuraminic acid, *N*-acetyl-β-D-mannosamine, pectin, *p*-hydroxy-phenylacetic acid, L-rhamnose, α-D-lactose, β-gentiobiose and 3-*O*-methyl-D-glucose. Both strains were resistant to aztreonam, capreomycin, carbenicillin, cephalothin, chloramphenicol, cloxacillin, colistin, demeclocycline, d-salicin (anti-inflamatory), nafcillin, ofloxacin, oxacillin, paromomycin, penicillin G and sisomicin but sensitive to amikacin, benzethonium chloride,

bleomycin, chlortetracycline, erythromycin, kanamycin, lincomycin, minocycline, novobiocin, penimepicycline, rifampicin, spiramycin, sulfadiazine and vancomycin.

Figure 1a

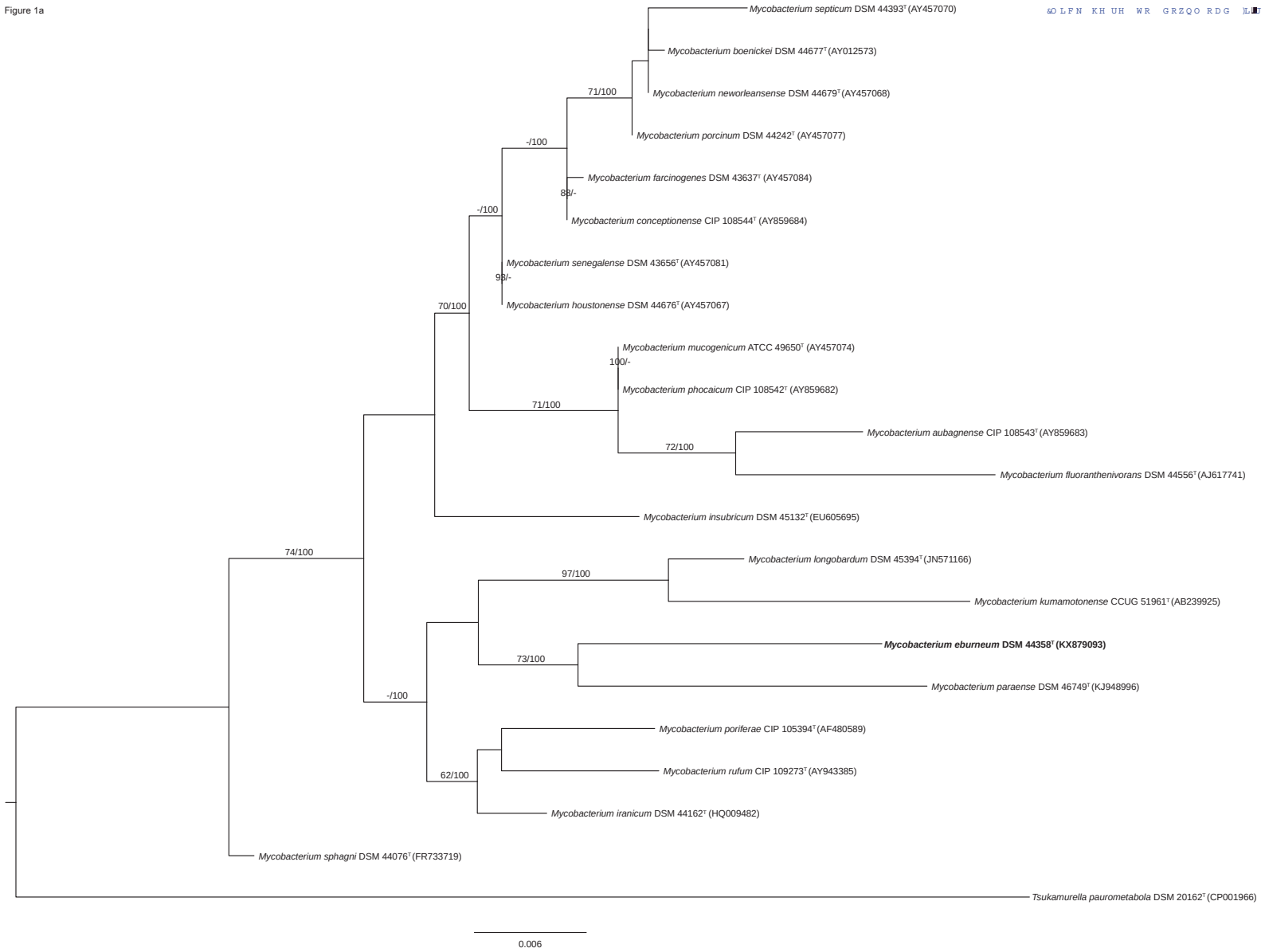
Mycobacterium septicum DSM 44393T (AY457070)
Mycobacterium boenickei DSM 44677T (AY012573)
Mycobacterium neworleansense DSM 44679T (AY457068)
Mycobacterium porcinum DSM 44242T (AY457077)
Mycobacterium farcinogenes DSM 43637T (AY457084)
Mycobacterium conceptionense CIP 108544T (AY859684)
Mycobacterium senegalense DSM 43656T (AY457081)
Mycobacterium houstonense DSM 44676T (AY457067)
Mycobacterium mucogenicum ATCC 49650T (AY457074)
Mycobacterium phocaicum CIP 108542T (AY859682)
Mycobacterium aubagnense CIP 108543T (AY859683)
Mycobacterium fluoranthenivorans DSM 44556T (AJ617741)
Mycobacterium insubricum DSM 45132T (EU605695)
Mycobacterium longobardum DSM 45394T (JN571166)
Mycobacterium kumamotonense CCUG 51961T (AB239925)
Mycobacterium eburneum DSM 44358T (KX879093)
Mycobacterium paraense DSM 46749T (KJ948996)
Mycobacterium poriferae CIP 105394T (AF480589)
Mycobacterium rufum CIP 109273T (AY943385)
Mycobacterium iranicum DSM 44162T (HQ009482)
Mycobacterium sphagni DSM 44076T (FR733719)
Tsukamurella paurometabola DSM 20162T (CP001966)
71/100
-/100
88/-
-/100
98/-
70/100
100/-
71/100
72/100
74/100
97/100
73/100
-/100
62/100
0.006

Figure 1b

Click here to download Figure Fig 1b NJ Tree.pdf

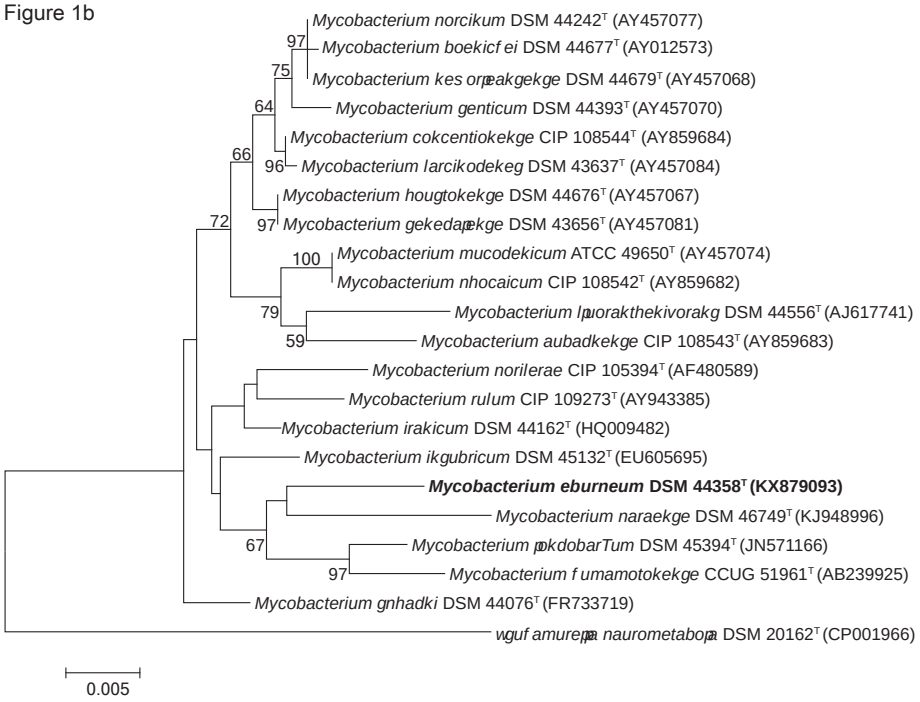

Figure 2

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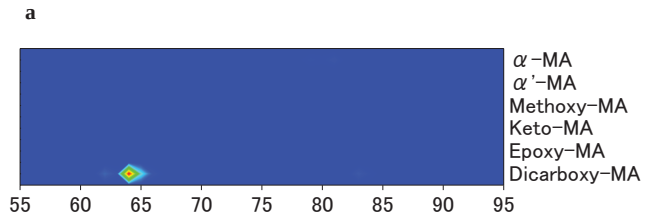
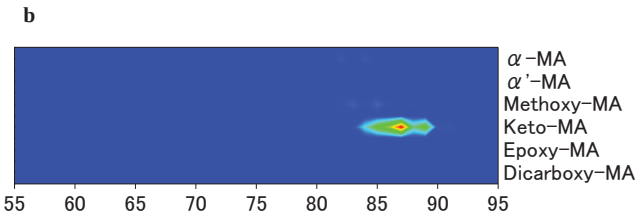
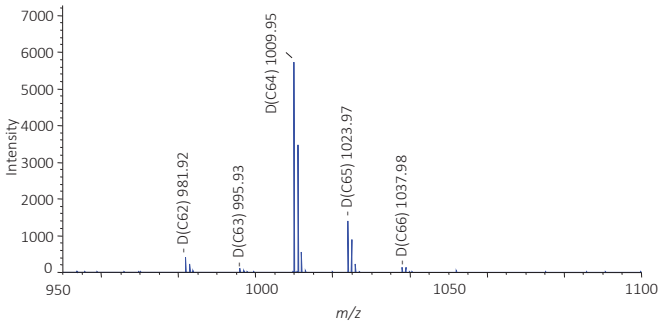
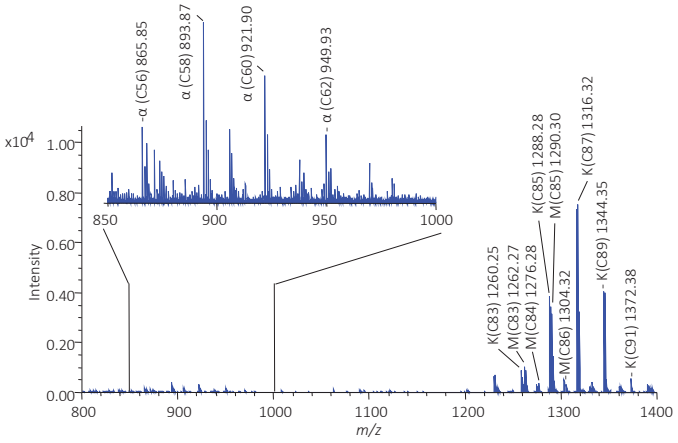



D: dicarboxy-mycolic acid




α: α-mycolic acid, M: methoxy-mycolic acid, K: keto-mycolic acid

Supplementary Material Files Fig S1

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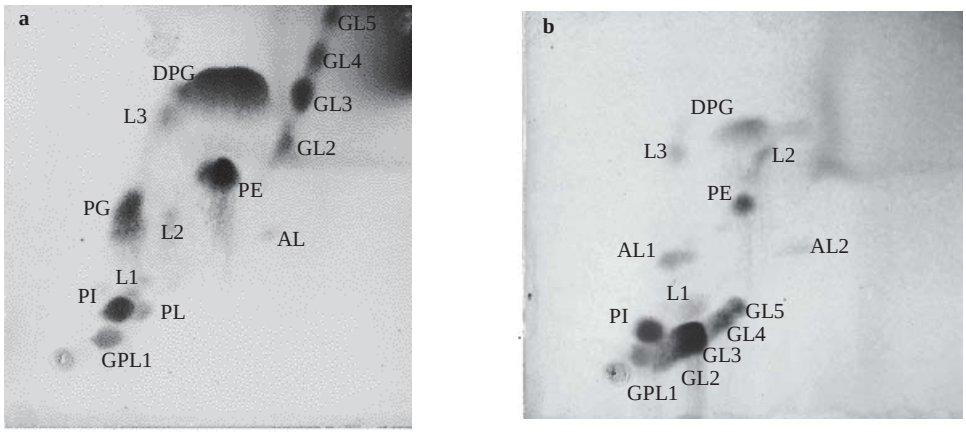


**Fig. S1**. Two-dimensional TLC plates of polar lipids extracted from *M. eburneum* X82$^{T}$ (a) and *M. paraense* DSM 46749$^{T}$ (b) using molybdatophosphoric acid (Sigma P1518) to identify total lipid content.

**Table S1.** Fatty acids patterns of *M. eburneum* X82$^T$ and its closest relatives *M. paraense* DSM 46749$^T$.

| | *M. eburneum* X82$^T$ | *M. paraense* DSM 46749$^T$ |
|---|---|---|
| $C_{16:0}$ | 38.2 | 32.1 |
| $C_{18:1}$ *w*9c | 19.4 | 39.4 |
| C18:2 w6,9c | - | - |
| $C_{18:0}$ | - | 9.1 |
| $C_{10Me}$-$C_{18:0}$ | 25.5 | 8.1 |
| C14:0 | - | - |
| C16:1 w9c | - | - |
| C16:1 w6c | - | - |
| Summed feature 2* | - | - |
| Summed feature 3** | 16.8 | - |

* 2 17:1 W7c / C17:1 w6c; ** 20:0 ALC 18.838 ECL /20 alcohol / 19:0 Cycloprop w10c / 19:0 Cycloprop w8c