

The new mycobacteria: an update

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Abstract

The continuous evolution of mycobacterial taxonomy may represent a source of confusion for laboratories and clinicians. Apart from the obvious pathogenic strains of the *Mycobacterium tuberculosis* complex, *Mycobacterium leprae* and *Mycobacterium ulcerans*, the role of other mycobacteria may be associated with varying conditions ranging from contamination to specific disease processes. Of the more than 120 mycobacterial species recognized currently, very few have not been reported as pathogenic in humans or animals. Although the attempt to keep pace with the steadily increasing number of mycobacterial species seems hopeless, a careful review of the recent literature relevant to the newly described species may be advantageous. The aim of this present update is to provide epidemiological and clinical information along with major phenotypic and genotypic characteristics of the species described in the last 3 years.

Introduction

The increasing availability of modern genetic techniques and instrumentation provides microbiologists with extremely powerful means for accurate identification of microorganisms (Nolte & Caliendo, 2003). It is not uncommon that, using such methods, strains not differentiated by conventional methods prove to be distinct and thus to belong to unique as yet undescribed taxa.

Within the genus *Mycobacterium*, these new methodologies have led to an extraordinary flourishing of new species, with the number being more than doubled in the last 15 years. The isolation of the large majority of strains from clinical specimens should not appear to conflict with the common hypothesis that the environment, including water, aerosols, soil and dust, is the natural reservoir of nontuberculous mycobacteria (NTM). Humans are, in fact, by far the most investigated samples for the presence of mycobacteria. It seems certain, however, that human isolates represent only the tip of the 'NTM-iceberg'.

Among the most important features distinguishing NTM from *Mycobacterium tuberculosis*, are the low virulence and the lack of human-to-human transmission within the NTM. Such a reassuring scenario should not, however, lead one to underestimate the pathogenicity of the NTM, especially in the immunocompromised host. The recent reduction of

NTM disease in HIV-infected patients, due to the introduction of highly active antiretroviral treatments, has been counterbalanced by the steadily increasing plethora of subjects with underlying iatrogenic immuno-suppression who develop NTM disease.

Although a short time has elapsed since the last review of new mycobacteria published in 2003 (Tortoli, 2003), almost 30 novel species have been described during the past 3 years and thus an update is needed. The outline of information adopted in the previous review will be followed with species listed in alphabetic order in this current review.

Pigmented slow growers***Mycobacterium nebraskense***

Mycobacterium nebraskense was described in 2004 on the basis of five strains isolated from the sputum of patients with symptomatic pulmonary infection (Mohamed *et al.*, 2004).

Phenotypic features

The species grows slowly at temperatures in the range 25–35 °C; the colonies are scotochromogenic and rough.

The commonly performed biochemical tests are negative with the only exception of Tween 80 hydrolysis which is variable. Growth is inhibited on MacConkey agar without crystal-violet and on Lowenstein–Jensen containing 5% NaCl (Table 1).

The only method used for investigating cell wall lipids has been HPLC, which reveals a novel pattern, described as grossly resembling that of *Mycobacterium avium* complex (MAC) strains (Mohamed *et al.*, 2004).

No information is available concerning antimicrobial susceptibility.

Genotypic features

The genetic sequences of the 16S rRNA gene, characterized by a long helix 18, and that of the 16S–23S internal transcribed spacer (ITS) do not match any officially recognized species. This species most closely resembles *Mycobacterium kansasii*, by 16S rRNA gene sequence (Fig. 1), and MAC, by ITS.

Clinical and epidemiological features

Although no clinical data are available concerning the patients from which the strains were isolated, the authors indicate an association with pulmonary disease (Mohamed *et al.*, 2004).

Type strains: ATTC BAA-837^T, DSM 44803^T.

Accession numbers: 16S rRNA gene, AY368456; ITS, AY368458.

Mycobacterium parascrofulaceum

Mycobacterium parascrofulaceum was described in 2004 (Turenne *et al.*, 2004a) following a thorough characteriza-

tion of 12 clinical strains, previously identified as *Mycobacterium scrofulaceum*. Subsequently, five reference strains of *M. scrofulaceum* and one of *Mycobacterium simiae* were identified as *M. parascrofulaceum*.

Phenotypic features

The species is scotochromogenic and grows slowly at 25–37 °C. Among conventional biochemical tests, only urease is positive, while Tween 80 hydrolysis, nitrate reduction and 3-day arylsulfatase are negative. No growth is achieved on Lowenstein–Jensen with 5% NaCl or on MacConkey agar without crystal-violet (Table 1).

The HPLC pattern is undistinguishable from that of some MAC strains (Turenne *et al.*, 2004a).

In vitro susceptibility testing has shown activity with amikacin, clarithromycin and rifampicin (Table 2).

Genotypic features

The genetic sequence of the 16S rRNA gene presents a short helix 18, thus adding this new species to the group of organisms related to *M. simiae* (Fig. 2). Furthermore, it shares characteristics with MCRO33 (Turenne *et al.*, 2004a) already present in public domain databases (Springer *et al.*, 1996).

In contrast with the 16S rRNA gene, the ITS and the gene encoding for the 65 kDa heat shock protein (*hsp65*) are not identical in all the test strains. Both such regions are characterized by five sequevars, with most of them similar to each other (1–2 bp mismatches) and with only few (one for ITS and two for *hsp65*) much more extensively divergent (13–23 bp). None of the sequevars have corresponding sequences in GenBank and all most closely resemble *M. scrofulaceum*.

Table 1. Phenotypic features of slowly growing species (no information is available for "*Mycobacterium tilburgii*")

<i>Mycobacterium</i> species	Pigmentation	Growth range (°C)	3-day arylsulfatase	Catalase > 45 mm	Nitrate reduction	Tween 80 hydrolysis	Urease
<i>M. arupense</i>	N	22–37	–	NA	–	+	–
<i>M. caprae</i>	N	37	NA	NA	NA	NA	NA
<i>M. chimaera</i>	N	25–37	–	–	–	–	–
<i>M. colombiense</i>	N	25–37	V	NA	–	–	+
<i>M. florentinum</i>	N	25–37	–	V	V	–	+
<i>M. montefiorensis</i>	N	25–30	–	–	–	–	–
<i>M. nebraskense</i>	S	25–35	–	–	–	V	–
<i>M. parascrofulaceum</i>	S	25–37	–	V	–	–	+
<i>M. parmense</i>	S	25–37	–	–	–	+	+
<i>M. pinnipedii</i>	N	37	NA	NA	–	NA	NA
<i>M. pseudoshottsii</i>	P	22–30	–	–	–	–	+
<i>M. saskatchewanense</i>	S	25–37	–	V	–	+	–
" <i>M. sherrisii</i> "	N	25–37	–	V	–	–	+

N, nonchromogenic; S, scotochromogenic; P, photochromogenic; NA, datum not available; V, variable; –, negative result; +, positive result.

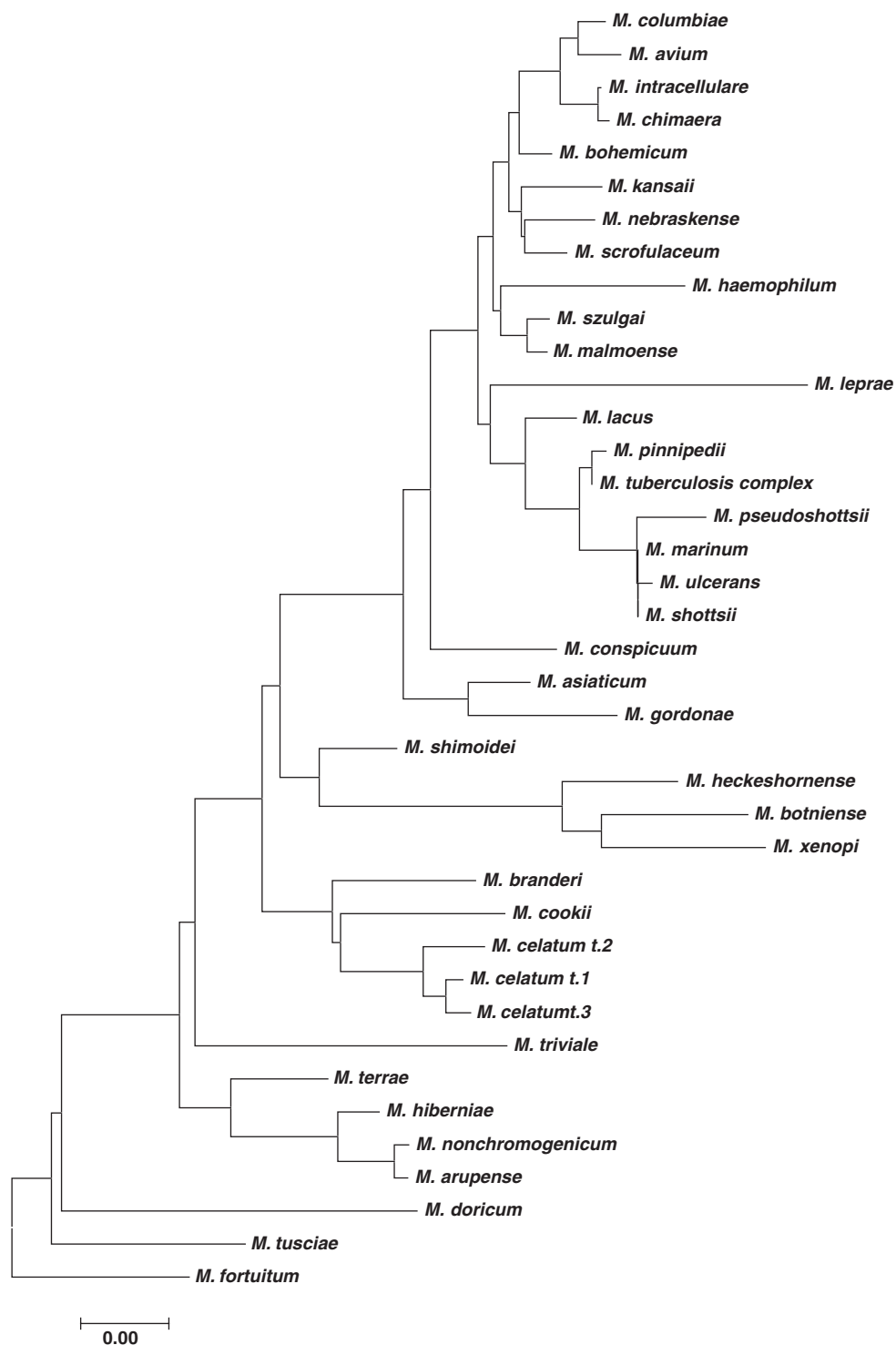


Fig. 1. The 16S rRNA gene-based phylogenetic tree of slow growers characterized by a long helix 18 (*Mycobacterium fortuitum* outgroup).

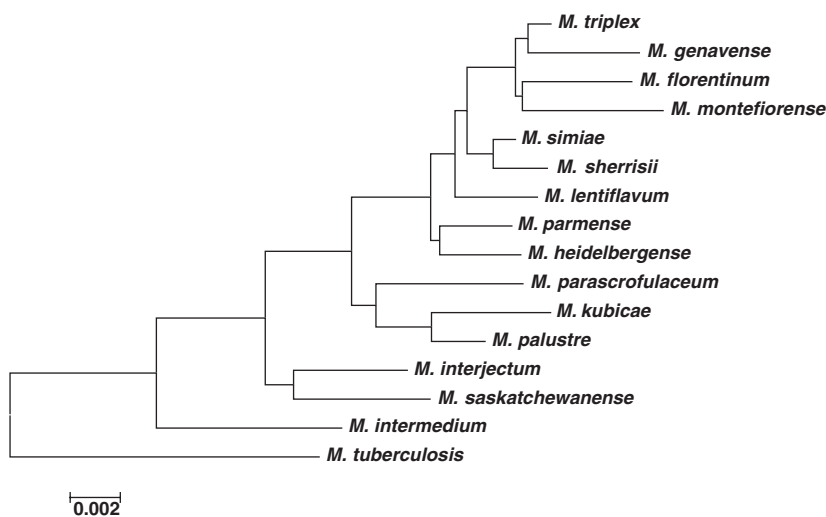
PCR restriction analysis (PRA) of *hsp65* produces a different pattern for each sequevar (Table 3). With the exception of one sequevar, all are distinct from previously reported species.

With the commercial line probe assays INNO LiPA (Innogenetics) and GenoType (Hein) *M. parascrofulaceum* is misidentified as *M. scrofulaceum* (Tortoli *et al.*, 2005a).

Table 2. Antimicrobial susceptibility of slowly growing species (no information is available for *Mycobacterium colombiense*, *M. montefiorensis*, *M. nebraskense* and "*M. tilburgii*")

<i>Mycobacterium</i> species	Amikacin	Ciprofloxacin	Clarithromycin	Ethambutol	Rifampicin	Streptomycin
<i>M. arupense</i>	V	R	S	S	R	R
<i>M. caprae</i>	S	S	–	S	S	S
<i>M. chimaera</i>	V	V	V	R	V	V
<i>M. florentinum</i>	V	R	S	V	V	–
<i>M. parascrofulaceum</i>	V	R	S	R	S	V
<i>M. parmense</i>	–	S	S	S	S	S
<i>M. pinnipedii</i>	–	–	–	S	S	S
<i>M. pseudoshottsii</i>	–	–	–	S	S	–
<i>M. saskatchewanense</i>	S	S	S	I	S	S
" <i>M. sherrisii</i> "	–	R	S	R	R	–

V, variable; S, susceptible; R, resistant; –, datum not available.

**Fig. 2.** The 16S rRNA gene-based phylogenetic tree of slow growers characterized by a short helix 18 (*Mycobacterium tuberculosis* outgroup). '*Mycobacterium tilburgii*' is not included because of the limited portion of 16S available in GenBank.

Clinical and epidemiological features

Repeated isolations were obtained from several sputum samples and one bronchial aspirate of a 44-year-old woman with history of pulmonary tuberculosis (Turenne *et al.*, 2004a). The patient presented lung cavitations, which, despite an antituberculosis treatment, worsened. Following a change in regimen to clarithromycin, ethambutol and rifampicin, cultures became negative. However, there was no reduction in the size of the patient's lung cavities.

Another case involved a pulmonary infection in a 63-year-old man who had a fatal outcome (Tortoli *et al.*, 2005a). In a third case of respiratory disease, the pathogenic role of *M. parascrofulaceum*, although isolated in multiple cultures, could not be confirmed (Tortoli *et al.*, 2005a).

Mycobacterium parascrofulaceum infections in two AIDS patients have been reported, with the strains, in one case, from sputum and, in the second case, from blood (Tortoli *et al.*, 2005a).

Type strains: ATTC BAA-614^T, DSM 44648^T.

Accession numbers: 16S rRNA gene, ITS and *hsp65*, AY337273-AY337283.

Mycobacterium parmense

Mycobacterium parmense was described in 2004 following its isolation from a lymph node in a case of childhood cervical lymphadenopathy (Fanti *et al.*, 2004).

Phenotypic features

Mycobacterium parmense is scotochromogenic and grows slowly between 25 and 37 °C. The species possesses urease and Tween 80 hydrolysis, whereas production of nitrate reductase, arylsulfatase at 3 days and semiquantitative catalase are negative. Growth is inhibited by 5% NaCl and on MacConkey agar without crystal-violet (Table 1).

Thin layer chromatography (TLC) of mycolic acids reveals alpha-, keto- and wax esters-mycolates in the cell

Table 3. PRA patterns of new mycobacterial species (no information is available for *Mycobacterium aubagnense*, *M. arupense*, *M. bollettii*, "*M. fluoranthenorans*", "*M. hackensackense*", "*M. massiliense*", *M. montefiorensis*, *M. parmense*, *M. phocaicum*, *M. pinnipedi*, *M. psychrotolerans*, *M. pyrenivorans*, *M. nebraskense*, "*M. sherrisii*" and "*M. tilburgii*". DNA fragments shorter than 50 bp have been omitted)

<i>Mycobacterium</i> species	<i>Bst</i> Ell	<i>Hae</i> III
<i>M. boenickei</i>	235, 210	140, 125 140, 120, 50
<i>M. brisbanense</i>	235, 115, 100	140, 125, 100
<i>M. canariensis</i>	325, 130	140, 90, 80
<i>M. chimaera</i>	240, 120, 100	140, 130, 60
<i>M. colombiense</i>	240, 210	130, 105, 60
<i>M. cosmeticum</i>	310, 130	140, 96, 80
<i>M. florentinum</i>	441	148, 130
<i>M. houstonense</i>	235, 210	140, 125
	235, 115, 85	140, 125, 100
" <i>M. lacticola</i> "	310, 116	145, 94, 87 172, 145
" <i>M. manitobense</i> "	235, 205	140, 85, 60
<i>M. neworleansense</i>	235, 115, 85	140, 125
<i>M. parascrofulaceum</i>	231, 116, 79	139, 94
	231, 210	127, 94
	231, 131, 79	127, 94, 81, 78 145, 139, 75
<i>M. pseudoshottsii</i>	235, 210	145, 105, 80
<i>M. saskatchewanense</i>	231, 210	145, 127
	231, 131, 79	127, 94

wall, a pattern present in a number of commonly isolated mycobacterial species.

Major compounds revealed by gas-liquid chromatography (GLC) include exadecanoic and octadecenoic acids.

The HPLC pattern, characterized by an early major cluster of peaks followed by two minor ones, grossly resembles MAC. However it differs, with shorter retention times of the first peaks (Fanti *et al.*, 2004).

Susceptibility testing reveals *in vitro* activity of ciprofloxacin, clarithromycin, ethambutol, rifabutin, rifampicin and streptomycin (Table 2).

Genotypic features

Phylogenetically, all of the most closely related species share with *M. parmense* the 12-nucleotide deletion in hypervariable region B. In contrast, the sequence of hypervariable region A is unique (Fig. 2).

Clinical and epidemiological features

Mycobacterium parmense grew from material obtained from cervical lymph nodes of a 3-year-old girl with bilateral lymphadenitis (Fanti *et al.*, 2004). After an unsuccessful antitubercular treatment, only the complete excision of lymph nodes led to complete recovery.

Type strains: CIP93171^T, DSM 44553^T.

Accession numbers: 16S rRNA gene+ITS, AF466821.

Mycobacterium pseudoshottsii

Several strains isolated from fish during a mycobacterial epizootic were initially considered members of the species *Mycobacterium shottsii*. A thorough genotypic investigation revealed substantial differences and led to the recognition of the new species in 2005 (Rhodes *et al.*, 2005). The possible overlapping of *M. pseudoshottsii* with mycobacteria isolated previously from animals [*Mycobacterium ulcerans*-like (Trott *et al.*, 2004) and '*Mycobacterium seriola*' Kusuda *et al.*, 1987] was not excluded by the authors.

Phenotypic features

Mycobacterium pseudoshottsii is photochromogenic and grows in about 2 months at 23–30 °C; no growth is achieved at 37 °C. Among major biochemical tests, niacin accumulation and urease are positive, while nitrate reduction, 3-day arylsulfatase and Tween 80 hydrolysis are negative (Table 1).

The mycolic acid pattern is characterized by a single late cluster of peaks and is undistinguishable from that of *M. shottsii* (Rhodes *et al.*, 2003, 2005).

Genotypic features

The sequence of the 16S rRNA gene is characterized by very close relatedness to *Mycobacterium marinum*, *M. shottsii* and *Mycobacterium ulcerans* (Fig. 1). Similarity with the same species was detected when the *hsp65* sequence was analyzed.

PRA is not able to distinguish *M. pseudoshottsii* from *M. ulcerans* (Table 3).

The species is characterized by the presence of two insertion elements (IS2404 and IS2606), which are associated with *M. ulcerans*.

Epidemiological features

Mycobacterium pseudoshottsii is pathogenic, causing granulomatous lesions of the spleen, in striped bass (Rhodes *et al.*, 2005). No information about the possibility of disease transmission to humans is available so far.

Type strains: ATCC BAA-883^T; NCTC 13318^T.

Accession numbers: 16S rRNA gene, AY570988; *hsp65*, AY571788.

Mycobacterium saskatchewanense

Sixteen strains were investigated for the description of the new species in 2004 (Turenne *et al.*, 2004b); its 16S rRNA gene sequence was already present in GenBank assigned to the uncharacterized mycobacterium MCRO8 (Springer *et al.*, 1996).

Phenotypic features

Mycobacterium saskatchewanense is a yellow-pigmented scotochromogenic species which grows slowly at 25–37 °C. Biochemical tests reveal positive Tween 80 hydrolysis and production of catalase. Production of nitrate reductase, arylsulfatase and urease are negative (Table 1).

HPLC analysis of cell wall mycolic acids exhibits a pattern presenting a single late cluster of peaks resembling *M. interjectum* type 2 (Tortoli *et al.*, 1996) and *Mycobacterium palustre* (Torkko *et al.*, 2002).

In vitro it is susceptible to amikacin, ciprofloxacin, clarithromycin, linezolid, rifabutin, rifampicin, streptomycin and trimethoprim-sulfamethoxazole (Table 2).

Genotypic features

All but one of the strains investigated gave a positive result with the commercial DNA probe AccuProbe (Gen-Probe) specific for MAC but not with the probes specific for *Mycobacterium avium* and *Mycobacterium intracellulare*. An identical anomalous reaction had been reported for another newly described species, *M. palustre* (Torkko *et al.*, 2002).

The 16S rRNA gene presents the short helix 18, typical of the species related to *M. simiae*. Among them, *Mycobacterium interjectum* (Fig. 2) is the more closely related. In this genetic region, one of the strains (the AccuProbe-negative one) differed from the others by a single nucleotide. Again the strain above differed from the others in the ITS, a region in which the genetic sequence of *M. saskatchewanense* clearly differs from any other species. Four different sequevars characterize *M. saskatchewanense* in the *hsp65*; *M. interjectum* is again the most closely related species.

The PRA pattern shared by all the strains investigated is unique among previously recognized species (Table 3).

Clinical and epidemiological features

Multiple respiratory cultures from an elderly woman with bronchiectasis and lung cavitations yielded, in a 2-year period, *M. saskatchewanense*. She was finally cured with a combination of ciprofloxacin, azithromycin and amikacin. Disease involvement was not proven for other *M. saskatchewanense* strains isolated, at times repeatedly, from the lungs of different patients (Turenne *et al.*, 2004b).

Type strains: ATCC BAA-544^T, DSM 44616^T.

Accession numbers: 16S rRNA gene+ITS, AY208856; *hsp65*, AY208857.

Nonpigmented slow growers

Mycobacterium arupense

Sixty-five clinical isolates presenting full identity, in the 5'-end of the 16S rRNA gene, with the unnamed

mycobacterium MCRO6 (Springer *et al.*, 1996) present in GenBank, gave rise to the new species in 2006 (Cloud *et al.*, 2006).

Phenotypic features

The phenotypic features unambiguously place *M. arupense* within the *M. terrae* complex; it is in fact nonpigmented, characterized by an intermediate growth rate, and grows at 22–27 °C. The species hydrolyzes Tween 80, and is negative for nitrate reductase and urease. No growth is achieved on MacConkey agar or on Lowenstein–Jensen containing 5% NaCl (Table 1).

The HPLC profile is undistinguishable from that of *Mycobacterium nonchromogenicum*.

The species is susceptible *in vitro* to rifabutin, ethambutol and clarithromycin (Table 2).

Genotypic features

The 16S rRNA gene is identical to MCRO6 and closely related to *M. nonchromogenicum* (Fig. 1). The sequence of the *hsp65* gene reveals the presence of two sequevars differing by 2 bp and clearly differentiates *M. arupense* from other members of *M. terrae* complex. Similar results are obtained by the sequence of ITS.

Clinical and epidemiological features

Most of the strains isolated from sputum were not thought to be clinically significant. In contrast, the suspicion for clinical significance was high for those grown from sterile body sites (lymph nodes, biopsies, pleural fluid) (Cloud *et al.*, 2006).

Type strains: ATCC BAA-1242^T, DSM 44942^T.

Accession number: 16S rRNA gene, DQ157760.

Mycobacterium caprae

The taxonomic position of *M. caprae* has been in transition. The species was first described as a subspecies of *M. tuberculosis* (Aranaz *et al.*, 1999) and then was considered a subspecies of *Mycobacterium bovis* (Niemann *et al.*, 2002). *Mycobacterium caprae* was finally elevated to species rank in 2003 (Aranaz *et al.*, 2003). The new species belongs to the *M. tuberculosis* complex.

Phenotypic features

Except for the susceptibility to pyrazinamide, the major biochemical and cultural features do not allow a clear distinction of *M. caprae* from *M. bovis* (Table 1).

Genotypic features

At the genetic level the differentiation from *M. bovis* is supported by the lack of *M. bovis*-specific mutation in *pncA* and in *gyrB* (Aranaz *et al.*, 2003), and by the presence of unique spoligotypes (at least 11) and RFLP patterns (at least 43). Such fingerprints are widely used for identification purposes (Erler *et al.*, 2004).

In the RD ('region of difference', which is missing, among the members of the *M. tuberculosis* complex, only in *M. bovis* BCG; *M. caprae* is the only species presenting Rv0577, IS1561', Rv1510 and Rv3877/8 genes and missing Rv1970 and Rv3120 (Huard *et al.*, 2003).

Clinical and epidemiological features

Mycobacterium caprae is a clearly pathogenic species as proven by the high number of isolates grown from symptomatic animals and, less frequently, from humans.

In addition to goats, it has been isolated, in various countries of central and southern Europe, from deer, wild boar, cattle, camel and even from a Siberian tiger (Aranaz *et al.*, 1999; Proding *et al.*, 2002; Kubica *et al.*, 2003; Lantos, 2003; Erler *et al.*, 2004; Gortazar *et al.*, 2005).

The most frequent human disease has pulmonary localization and concerned subjects with occupational contact with cattle. One case of fatal pericarditis in a 76-year-old male with malaise, weight loss and dyspnea (Blaas *et al.*, 2003) and one case in a 69-year-old woman with lupus vulgaris and with signs of previous tuberculosis are noteworthy (Meyer *et al.*, 2005). No epidemiological information is available for either case.

Type strain: CIP 105776^T.

Accession number: 16S rRNA gene, AJ131120.

Mycobacterium chimaera

The new species emerged in 2004 from the study of a heterogeneous group of mycobacteria included in the MAC but not belonging either to *M. avium* or *M. intracellulare* (Tortoli *et al.*, 2004). The description is based on the characterization of 12 independent strains, all but one isolated from respiratory specimens of elderly patients.

Phenotypic features

Mycobacterium chimaera is nonpigmented and grows slowly at 25–37 °C. All major biochemical tests are negative and no growth is achieved either on MacConkey agar without crystal-violet or Lowenstein–Jensen with 5% NaCl (Table 1).

By TLC and GLC, the lipid pattern is not distinguishable from that of *M. intracellulare*. In contrast, the HPLC profile differs from other isolates of MAC in that it presents a major

early cluster of peaks and only a late minor one; the pattern lacks the intermediate cluster consistently present in other members of the complex (Tortoli *et al.*, 2004).

The *in vitro* antimicrobial susceptibility is quite variable and strains susceptible and resistant to major antimycobacterial drugs have been reported (Table 2).

Genotypic features

Commercial DNA probes identify *M. chimaera* differently: AccuProbe and GenoType assign the strains to the species *M. intracellulare*; the first version of INNO LiPA includes it among the MAC other than *M. avium* and *M. intracellulare*, while the second version of LiPA identifies it as *M. intracellulare* type 2.

By analysis of the 16S rRNA gene, all of the strains characterized in the sp. nov. description, and others investigated in a subsequent study (Lebrun *et al.*, 2005) share a sequence not previously present in GenBank but considered the sequevar 5 of *M. intracellulare* in RIDOM (Harmsen *et al.*, 2003).

The same ITS sequence is shared by all of the strains and overlaps the sequence assigned in a previous study to the variant A (MAC-A) of isolates of MAC other than *M. avium* and *M. intracellulare*. MAC-A differs by at least 21 bp from the latter species (Frothingham, 1993).

Although several sequevars are present in the *hsp65* gene, only one PRA pattern is produced, which is unable to differentiate *M. chimaera* from *M. intracellulare* (Lebrun *et al.*, 2005) (Table 3).

Clinical and epidemiological features

In the paper describing the sp. nov., seven out of 12 clinical cases supported the pathogenic role of *M. chimaera* (Tortoli *et al.*, 2004). The patients were HIV-negative, prevalently male, had different predisposing conditions and pulmonary diseases of variable severity. Two further clinically significant strains are reported in a subsequent study (Lebrun *et al.*, 2005).

Type strains: CIP 107892^T, DSM 44623^T.

Accession number: 16S rRNA gene+ITS, AJ548480.

Mycobacterium colombiense

Mycobacterium colombiense is a new member of the *M. avium* complex described in 2006 following the characterization of seven MAC strains (Murcia *et al.*, 2006).

Phenotypic features

The organism grows slowly at 20–37 °C, forming nonpigmented colonies which may become yellowish with aging. The only consistently positive biochemical reaction is the production of urease (Table 1).

The HPLC pattern shows the typical structure of MAC, with a major early cluster of peaks and two minor late consecutive peaks. The peaks of the first and the second cluster occur consistently in rising order, which is different from the usual bell-shaped order characteristic of other isolates of MAC (Murcia *et al.*, 2006).

Genotypic features

Mycobacterium colombiense is identified, by AccuProbe, as MAC different from *M. avium* and *M. intracellulare*, and by INNO LiPA, as MAIS different from *M. avium*, *M. intracellulare*, *M. chimaera* and *M. scrofulaceum*.

The 16S rRNA gene and ITS sequences are identical in all of the strains; the first reveals the most close relatedness with *M. avium* (Fig. 1), the other, which had not been reported before, was named MAC-X.

All of the isolates match the *hsp65* PRA pattern of *M. avium* type I (Table 3) but display up to five mutations when the respective sequences are compared.

DNA–DNA hybridization reveals homology greater than 75% among different strains of *M. colombiense* and less than 40% in comparison with the other species of MAC. Therefore, isolates of *M. colombiense* belong to a single species which differs from any previously reported one.

Clinical and epidemiological features

The seven strains investigated for the sp. nov. description had been isolated in Colombia from four HIV-positive patients and all but one had been grown from blood. All the patients died from underlying immuno-compromised conditions (Murcia *et al.*, 2006).

Type strains: CIP 108962^T, CECT 3035^T.

Accession numbers: 16S rRNA gene+ITS, AM062764; *hsp65*, AM062765.

Mycobacterium florentinum

Mycobacterium florentinum emerged in 2005 from a cluster of eight independent strains (Tortoli *et al.*, 2005b), one of which had been previously considered a variant of *Mycobacterium triplex*.

Phenotypic features

The species is nonchromogenic and grows slowly at 25–37 °C. Among major biochemical tests, only urease production is consistently positive. Nitrate reduction and semiquantitative catalase are variable, while arylsulfatase production at 3 days and Tween 80 hydrolysis are negative. No growth is obtained on MacConkey agar or on Lowenstein–Jensen with 5% NaCl (Table 1).

The mycolic acid pattern revealed by TLC is common within the genus *Mycobacterium*, and is shared by *M. tuberculosis*. The GLC pattern is similar to that of *M. triplex* and *M. lentiflavum*, from which it is nevertheless distinguishable. Although the HPLC pattern shows the three-clustered pattern shared by the majority of species related to *M. simiae*, the emergence of such clusters of peaks, in particular the first one, is quite early (Tortoli *et al.*, 2005b), making HPLC a more discriminative analysis in comparison to GLC.

The strains are characterized by multidrugresistance with susceptibility only to clarithromycin and clofazimine (Table 2).

Genotypic features

The short helix 18 in the 16S rRNA gene places *M. florentinum* among the species related to *M. simiae* with the closest resemblance to *M. triplex* (Fig. 2).

The PRA pattern is identical to that of *M. lentiflavum* (Table 3), from which it can be distinguished using a third restriction enzyme (*Hha*I).

Clinical and epidemiological features

Six out of eight independent strains had been isolated, two or more times, from the sputum of elderly patients, the other strains were grown from the stools of an AIDS patient and from the cervical lymph node of a young girl (Tortoli *et al.*, 2005b). Most of the isolates met the ATS criteria for clinical significance (ATS, 1990). One of the strains, isolated in Finland from a severe pulmonary infection previously had been considered a sequevar of *M. triplex* (Suomalainen *et al.*, 2001).

Type strains: CIP 107385^T, DSM 44852^T.

Accession number: 16S rRNA gene+ITS, AJ616230.

Mycobacterium montefiorensis

The new species described in 2003 (Levi *et al.*, 2003) is based on strains isolated from captive moray eels presenting ulcerative masses. The growth of α -hemolytic rough colonies was obtained on blood agar after more than 3 months of incubation at 22 °C.

Phenotypic features

Mycobacterium montefiorensis is nonchromogenic and grows slowly at temperatures lower than 30 °C.

Most conventional biochemical and cultural tests, including 3-day arylsulfatase, semiquantitative catalase, urease, nitrate reduction, Tween 80 hydrolysis and 5% NaCl tolerance, are negative (Table 1).

The lipid pattern emerging from GLC analysis is similar to that of *M. triplex*. Due to the shorter retention times of single peaks, the HPLC profile is described as differing from the three-clustered pattern characterizing most of *M. simiae*-like organisms (Levi *et al.*, 2003).

Genotypic features

The 12-nucleotide gap in hypervariable region B which characterizes the mycobacteria related to *M. simiae* is present in the 16S rRNA gene. The sequences of ITS and *hsp65* are unique.

Epidemiological features

Mycobacterium montefiorensis was responsible for a fatal zoonotic outbreak in a captive population of moray eels. The eels presented extensive granulomatous lesions concentrated around the head and trunk within the derma and subcutaneous facial plane (Herbst *et al.*, 2001). The authors report the involvement of similar organisms in other marine infections but do not speculate on their pathogenicity for humans.

Scratch inoculation of mycobacterium produces lesions in recipient eels.

Type strains: ATCC BAA-256^T, DSM 44602^T.

Accession numbers: 16S rRNA gene, AF330038; *hsp65*, AY027785.

Mycobacterium pinnipedii

Mycobacterium pinnipedii is a new member of the *M. tuberculosis* complex described in 2003 (Cousins *et al.*, 2003) on the basis of 30 strains isolated from captive and wild seals in Australia, Argentina, Uruguay, Great Britain, and New Zealand.

Phenotypic features

Biochemical and cultural characteristics do not clearly differentiate *M. pinnipedii* from *M. bovis*. Niacin accumulation and nitrate reduction are negative. Growth is enhanced by the presence of pyruvate and inhibited by thyophen-carboxylic acid hydrazide. The colonies are dysgonic, rough and nonchromogenic (Table 1).

The HPLC pattern is consistent with *M. tuberculosis* and *M. bovis* (Cousins *et al.*, 2003).

Genotypic features

The 16S rRNA gene sequence differs from all the other species of *M. tuberculosis* complex by a single nucleotide (Fig. 1).

Spoligotyping is suitable to identify *M. pinnipedii* because of the unique pattern shared by all the strains thus far investigated. Although the *gyrB* RFLP can distinguish the new species from most members of the *M. tuberculosis* complex, it cannot separate *M. pinnipedii* from '*M. canettii*' and *M. africanum* type I (Chimara *et al.*, 2004). Two gene deletions in the RD2 region clearly differentiate *M. pinnipedii* from *M. bovis* (Bigi *et al.*, 2005). Thus, the new species appears to be more closely related to *M. tuberculosis* than to *M. bovis* (Cousins *et al.*, 2003).

Clinical and epidemiological features

At least 30 cases of *M. pinnipedii* tuberculosis, involving hosts of six species have been reported. In mammals, granulomatous lesions may be present in lymph nodes, lung, pleura, spleen and peritoneum; disseminated disease may also develop (Cousins *et al.*, 2003). The species is pathogenic for guinea pigs. The only case of human tuberculosis due to *M. pinnipedii* concerns a seal trainer with lung, and associated lymph node, infection (Thompson *et al.*, 1993).

Type strains: ATCC BAA-688^T, NCTC 13288^T.

Accession number: 16S rRNA gene, AF502574.

'*Mycobacterium sherrisii*'

The not-yet-valid species, '*M. sherrisii*' was described in 2004 (Selvarangan *et al.*, 2004) following the characterization of five isolates phenotypically resembling *M. simiae*.

Phenotypic features

The species grows slowly at 25–37 °C and is nonpigmented, although some strains develop a pale yellow pigmentation after prolonged light exposure. Urease is positive, while Tween 80 hydrolysis and nitrate reduction are negative; semiquantitative catalase is variable (Table 1).

The HPLC pattern of mycolic acids is characterized by three clusters of peaks and is not distinguishable from *M. simiae* (Selvarangan *et al.*, 2004). The GLC is poorly discriminative and is very similar, apart from minor differences, to that of other species sharing the same HPLC profile.

Like other species related to *M. simiae*, '*M. sherrisii*' is highly drug resistant, with susceptibility to only clarithromycin, rifabutin and sulfamethoxazole (Table 2).

Genotypic features

The 16S rRNA gene exhibits the typical deletion of 12 nucleotides marking the *M. simiae*-like group. The *hsp65* gene sequence, of which two different sequencings differing for one nucleotide have been reported (E. Tortoli *et al.*, 2006), is unique.

The very low percent DNA–DNA hybridization with other *M. simiae*-related species unquestionably supports its status as a new species.

With the commercial INNO LiPA, '*M. sherrisii*' is misidentified as *M. simiae* (E. Tortoli *et al.*, 2006).

Clinical and epidemiological features

The publication describing the new species does not provide any clinical information about the patients who yielded the '*M. sherrisii*' strains. However, two very recent case reports refer to pulmonary infections in a HIV-positive (Gamperli *et al.*, 2005) and in a nonimmunocompromised patient (E. Tortoli *et al.*, 2006). A further clinical isolate not fulfilling the criteria for clinical significance is also reported in the latter publication.

Type strain: ATTC BAA-832^T.

Accession numbers: 16S rRNA gene, AY353699; *hsp65*, AY365190, DQ523524.

'*Mycobacterium tilburgii*'

'*Mycobacterium tilburgii*' has been reported in two patients, but it is not yet a valid species (Richter *et al.*, 2000; Kolditz *et al.*, 2005).

Phenotypic features

The species is tentatively included here among nonchromogenic slowly growing mycobacteria, however, to date, no strain has been grown in culture.

Genotypic features

The 16S rRNA gene clearly includes '*M. tilburgii*' within the *M. simiae*-like group, with '*M. sherrisii*' being the most closely related species.

Clinical and epidemiological features

The DNA of '*M. tilburgii*' has been amplified from two AIDS patients with CD4+ count lower than 50 μL^{-1} ; one patient presented gastro-intestinal plaque-like lesions (Richter *et al.*, 2000), while the other had pulmonary nodules (Kolditz *et al.*, 2005). The patients improved following treatment with drugs usually used against *M. avium*.

Type strain: not available.

Accession number: 16S rRNA gene, Z50172.

Pigmented rapid growers

Mycobacterium cosmeticum

Several strains isolated from human lesions and from the sink drain of a nail salon were assigned to the new species *M. cosmeticum* in 2004 (Cooksey *et al.*, 2004).

Phenotypic features

Mycobacterium cosmeticum grows rapidly at 28–37 °C, forming smooth, opaque, pale yellow colonies. Growth is obtained on MacConkey agar but not on Lowenstein–Jensen with 5% NaCl. The isolate possesses nitrate reductase but is negative for arylsulfatase at 3 days (Table 4).

Table 4. Phenotypic features of rapidly growing species

<i>Mycobacterium</i> species	Pigmentation	Growth range (°C)	MacConkey	5% NaCl	3-day arylsulfatase	Catalase > 45 mm	Nitrate reduction	Tween 80 hydrolysis	Urease
<i>M. aubagnense</i>	N	25–37	NA	–	+	NA	–	NA	–
<i>M. boenickei</i>	N	28–35	+	+	+	+	+	NA	+
<i>M. bollettii</i>	N	25–37	NA	–	+	NA	–	NA	–
<i>M. brisbanense</i>	N	28–35	+	+	+	–	+	NA	+
<i>M. canariasense</i>	N	30–37	+	–	+	NA	–	+	NA
' <i>M. conceptionense</i> '	N	24–37	NA	+	+	NA	+	NA	–
<i>M. cosmeticum</i>	S	28–35	+	–	–	NA	+	NA	NA
' <i>M. fluoranthenvivans</i> '	N	20–37	NA	NA	NA	NA	–	–	–
' <i>M. hackensackense</i> '	N	24–37	–	–	–	+	–	+	+
<i>M. houstonense</i>	N	28–42	+	+	+	+	+	NA	+
' <i>M. lacticola</i> '	S	25–37	–	+	–	–	–	NA	+
' <i>M. manitobense</i> '	S	25–37	–	–	–	–	–	+	+
' <i>M. massiliense</i> '	N	24–37	+	+	+	–	–	NA	–
<i>M. neworleansense</i>	N	28–37	+	+	+	+	+	NA	+
<i>M. phocaicum</i>	N	25–37	NA	–	+	–	+	NA	–
<i>M. psychrotolerans</i>	S	4–37	NA	+	–	–	+	NA	+
<i>M. pyrenivivans</i>	S	24–37	NA	NA	NA	+	+	–	NA

N, nonchromogenic; S, scotochromogenic; NA, datum not available; –, negative result; +, positive result.

Table 5. Antimicrobial susceptibility of slowly growing species (no information is available for "*Mycobacterium lacticola*", *M. canariense*, "*M. fluoranthivorans*" and *M. pyrenivorans*)

<i>Mycobacterium</i> species	Amikacin	Cefoxitin	Ciprofloxacin	Clarithromycin	Doxycycline	Imipenem	Sulfamethoxazole	Tobramycin
<i>M. aubagnense</i>	S	S	S	S	S	S	S	R
<i>M. boenickei</i>	S	R	S	–	R	S	S	–
<i>M. bollettii</i>	I	R	R	R	R	R	R	R
<i>M. brisbanense</i>	S	R	S	–	R	S	S	–
" <i>M. conceptionense</i> "	–	–	S	S	S	S	–	–
<i>M. cosmeticum</i>	S	S	S	S	S	S	S	S
" <i>M. hackensackense</i> "	I	I	R	S	R	S	R	R
<i>M. houstonense</i>	S	R	S	–	R	S	S	–
" <i>M. manitobense</i> "	S	S	S	S	S	R	S	S
" <i>M. massiliense</i> "	S	R	R	S	S	R	R	R
<i>M. neworleansense</i>	S	R	S	–	S	S	R	–
<i>M. phocaicum</i>	S	S	S	S	S	S	R	R
<i>M. psychrotolerans</i>	–	–	S	–	–	–	–	–

I, intermediate; S, susceptible; R, resistant; –, datum not available.

The HPLC pattern is characterized by two late clusters of peaks (Cooksey *et al.*, 2004), a pattern shared by many rapid growers. Therefore, differentiation from other rapidly growing mycobacteria, especially *M. smegmatis*, is problematic.

The species is susceptible to the antimicrobials recommended by the CLSI for rapidly growing mycobacteria (NCCLS, 2002) (Table 5).

Genotypic features

The genetic sequences of 16S rRNA gene, *hsp65* and *rpoB* are unique, most closely resembling *Mycobacterium diernhoferi* (Fig. 3). The *hsp65* PRA differs from that of any other previously reported mycobacterial species (Table 3).

Clinical and epidemiological features

The only strain isolated so far from humans was grown from granulomatous subdermal lesions of a woman undergoing mesotherapy with an unknown substance. Considering that the isolation of rapidly growing mycobacteria has been reported repeatedly from similar preparations, it seems very likely the substance used for the treatment was the source of the infection (Winthrop *et al.*, 2002). The isolation of other strains of *M. cosmeticum* from footbaths and a sink drain in nail salon further strengthen the link between cutaneous mycobacterial infections and cosmetic treatments (Winthrop *et al.*, 2002).

Type strains: ATTC BAA-878^T, CIP 108170^T.

Accession numbers: 16S rRNA gene: AY449728; *hsp65*: AY449729.

'*Mycobacterium lacticola*'

Although the 16S rRNA gene sequence of '*M. lacticola*' was already present in GenBank, the case of catheter-related

sepsis published in 2004 (Kiska *et al.*, 2004) is the first report concerning this not yet validated species.

Phenotypic features

The species grows in less than 4 days at 25–30 °C. The yellow-orange colonies do not grow on MacConkey agar but are able to tolerate 5% NaCl. Urease is positive, while nitrate reduction and arylsulfatase are negative (Table 4). Such a phenotypic pattern is difficult to differentiate from *Mycobacterium neoaurum*.

No information is available about the lipid composition of the cell wall and the antimicrobial susceptibility.

Genotypic features

The only isolation of '*M. lacticola*' reported in the literature (Kiska *et al.*, 2004) is characterized by 16S rRNA gene sequence identical to that already present in GenBank. A 3 bp difference in the *hsp65* gene characterizes this species closely related to *M. neoaurum* (Fig. 3). The *hsp65* PRA is not identical to that of any other mycobacterial species (Table 3).

Clinical and epidemiological features

The strain was grown from peripheral blood and from the blood collected from a catheter of a 4-year-old girl, who had been previously transplanted with autologous stem cells (Kiska *et al.*, 2004). The catheter was removed and the patient rapidly recovered from the febrile episode.

Type strain: ATTC 9626^T.

Accession numbers: 16S rRNA gene, AF480582; *hsp65*, AY341032.

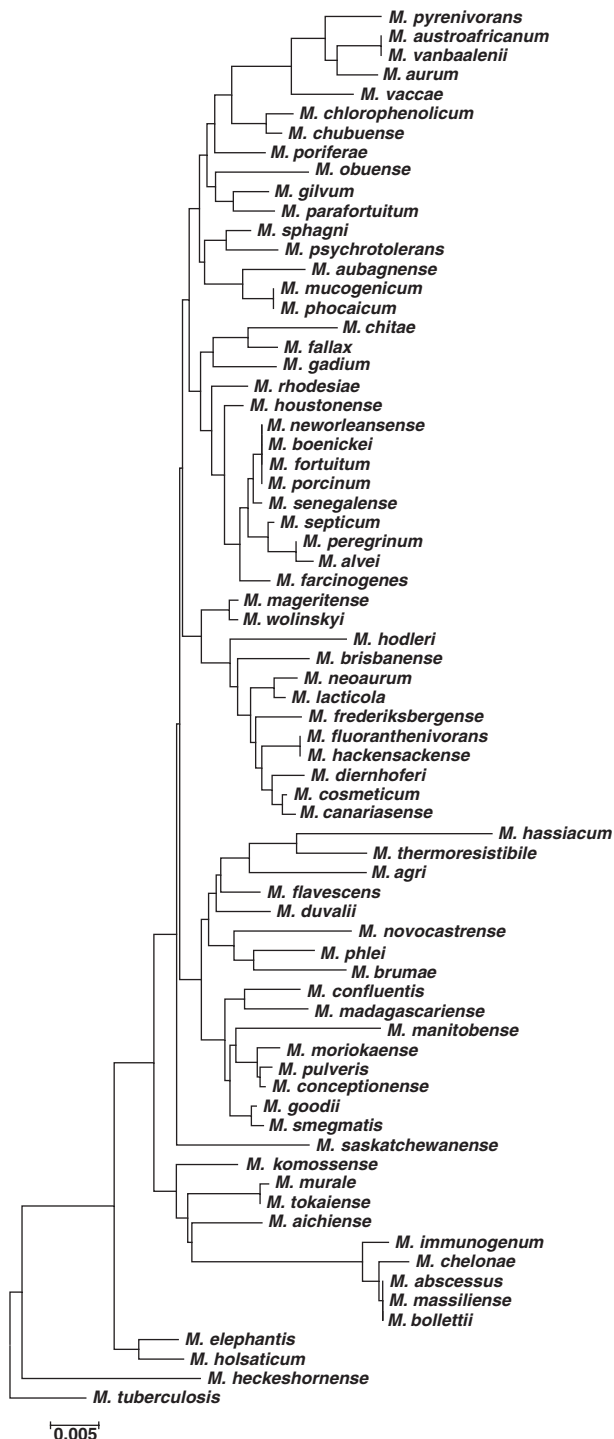


Fig. 3. The 16S rRNA gene-based phylogenetic tree of rapid growers (*Mycobacterium tuberculosis* outgroup).

'*Mycobacterium manitobense*'

This nonvalidated species is based on a single strain isolated from a posttraumatic wound in an immunocompetent man (Turenne *et al.*, 2003).

Phenotypic features

'*Mycobacterium manitobense*' grows rapidly at 25–37 °C and is characterized by orange scotochromogenic colonies. Major biochemical tests are negative and no growth is obtained on MacConkey without crystal-violet or in the presence of 5% NaCl (Table 4).

The HPLC pattern exhibits a major cluster of peaks followed, after an interval of about 2 min, by a minor cluster (Turenne *et al.*, 2003). This pattern is shared by several mycobacterial species, including *M. terrae*.

The strain is susceptible to the standard agents tested against rapidly growing mycobacteria (amikacin, ciprofloxacin, ceftazidime, doxycycline, tobramycin, clarithromycin, sulfonamides), except imipenem (NCCLS, 2002) (Table 5).

Genotypic features

The genetic sequence of the 16S rRNA gene is characterized by the signature of thermotolerant rapid growers, most closely related to *Mycobacterium madagascariense*. The ITS differs from all other mycobacterium present in GenBank. The *hsp65* PRA pattern is not distinguishable from that of *Mycobacterium obuense* and *Mycobacterium chubuense* (Table 3).

Clinical and epidemiological features

The only isolate of '*M. manitobense*' was grown from an ulcer swab at 31 °C on solid media. The growth failed, in contrast on the liquid medium inoculated simultaneously. The patient, a previously healthy 39-year-old male, was suffering from a traumatic wound of the ankle, which failed on heal despite the treatment with a wide spectrum of antimicrobials. Following the isolation of the mycobacterium, a treatment regimen including clarithromycin was undertaken but a marked improvement was obtained only when doxycycline was added (Turenne *et al.*, 2003).

Type strains: ATTC BAA545^T, DSM 44615^T.

Accession number: 16S rRNA gene+ITS, AY082001.

Nonpigmented rapid growers

Mycobacterium aubagnense

The species, based on two strains, emerged in 2006, using genetic sequencing of the *rpoB* gene, from the investigation of a cluster of rapidly growing mycobacteria (Adékambi *et al.*, 2006a).

Phenotypic features

Mycobacterium aubagnense is nonpigmented, grows rapidly at 24–37 °C, but not on Lowenstein–Jensen containing

5% NaCl. The species is positive for arylsulfatase at 3 days; nitrate reductase is not present (Table 4).

The GLC pattern is consistent with the genus *Mycobacterium*.

The antimicrobial pattern is characterized by susceptibility to most agents recommended for rapid growers (NCCLS, 2002) (Table 5).

Genotypic features

The genetic sequence of the 16S rRNA gene is very close to that of *Mycobacterium mucogenicum*, while other genetic regions (*hsp65*, *sodA* and *recA*) reveal substantial diversity with the same species.

Clinical and epidemiological features

One strain was isolated from a bronchial aspirate of a patient with chronic pneumonia, and another strain was isolated from the articular fluid of a patient with sepsis (Adékambi *et al.*, 2006a).

Type strains: CIP 108543^T, CCUG 50186^T.

Accession number: 16S rRNA gene, AY859683.

Mycobacterium boenickei

The new species was described in 2004 following a thorough revision of the sorbitol-negative strains of the *Mycobacterium fortuitum* third biovariant complex (Schinsky *et al.*, 2004).

Phenotypic features

Mycobacterium boenickei grows at 28–35 °C in less than one week and is not pigmented. Growth is obtained on Lowenstein–Jensen with 5% NaCl and on MacConkey. Among the major biochemical tests, 3-day arylsulfatase, semiquantitative catalase, nitrate reduction and urease are positive.

The GLC pattern is undistinguishable from that of other species previously grouped within the *M. fortuitum* third biovariant complex.

The HPLC profile overlaps with *M. fortuitum*, *Mycobacterium peregrinum* and *Mycobacterium septicum*.

Among the drugs currently recommended by the CLSI for rapidly growing mycobacteria (NCCLS, 2002), the species is susceptible to amikacin, ciprofloxacin, imipenem and sulfamethoxazole (Table 5).

Genotypic features

The 16S rRNA gene sequence is most closely related to *Mycobacterium porcinum* (Fig. 3).

DNA–DNA hybridization confirms the distinction from the other species previously grouped within the *M. fortuitum* third biovariant complex.

The five strains investigated present, apart from minor variations in two of them, the same unique PRA pattern (Table 3).

Clinical and epidemiological features

Out of six strains reported so far, four had been isolated from wounds, one from subcutis and one from sputum, with all but the latter proving to be clinically significant (Schinsky *et al.*, 2004).

Type strains: ATTC 49935^T, DSM 44677^T.

Accession number: 16S rRNA gene, AY012573.

Mycobacterium bollettii

The new species, based on four strains, was described in 2006 following the investigation of several rapidly growing mycobacteria by means of genetic sequencing of the *rpoB* gene (Adékambi *et al.*, 2006a).

Phenotypic features

Mycobacterium bollettii produces nonpigmented colonies in 3–5 days at 24–37 °C. No growth is obtained in presence of 5% NaCl. Three-day arylsulfatase is positive; nitrate reduction and urease are negative (Table 4).

Only GLC was used to investigate the lipid composition of the cell wall, but it does not provide useful information for the identification.

The most relevant phenotypic feature is the extremely high resistance to all antimycobacterial drugs, including clarithromycin (Table 5).

Genotypic features

The 16S rRNA gene sequence of *M. bollettii* is 100% identical to that of *Mycobacterium abscessus* (Fig. 3). However genetic sequencing of the *hsp65*, *sodA* and *recA* genes reveals substantial diversity from *M. abscessus*.

Clinical and epidemiological features

The four strains investigated thus far were isolated from sputum (three cases) and from a gastric aspirate from patients with chronic pneumonia (Adékambi *et al.*, 2006a).

Type strains: CIP 108541^T, CCUG 50184^T.

Accession number: 16S rRNA gene, AY859681.

Mycobacterium brisbanense

The new species, based on a single strain, emerged in 2004 from a thorough revision of the strains of the sorbitol

positive *M. fortuitum* third biovariant complex (Schinsky *et al.*, 2004).

Phenotypic features

Mucoid nonpigmented colonies grow rapidly at 28–37 °C, not only on conventional media but also on Lowenstein–Jensen with 5% NaCl and on MacConkey agar. Nitrate reduction, 3-day arylsulfatase and urease are present, while semiquantitative catalase is below the 45 mm limit (Table 4).

The GLC pattern is undistinguishable from that of other species previously grouped in the *M. fortuitum* third biovariant complex.

The HPLC profile overlaps those of *M. fortuitum*, *M. peregrinum* and *M. septicum*.

Amikacin, ciprofloxacin, imipenem and sulfamethoxazole are active *in vitro* (Table 5).

Genotypic features

Analysis of the sequence of the 16S rRNA gene reveals a close relatedness with *M. diernhoferi* (Fig. 3).

DNA–DNA hybridization distinguishes *M. brisbanense* from the other strains of the *M. fortuitum* third biovariant complex.

Clinical and epidemiological features

The only strain isolated thus far was responsible for an antral sinus infection (Schinsky *et al.*, 2004).

Type strains: ATCC 49938^T, DSM 44680^T.

Accession number: 16S rRNA gene, AY012577.

Mycobacterium canariense

The characterization of *M. canariense*, in 2004, was based on a cluster of strains isolated from blood cultures from patients with probable nosocomial infection (Jimenez *et al.*, 2004).

Phenotypic features

The colonies, which are nonpigmented and smooth, grow rapidly at 30–37 °C. Growth is obtained on MacConkey agar but not on 5% NaCl Lowenstein–Jensen. Isolates have arylsulfatase activity at 3 days and show hydrolysis in Tween 80 but nitrate reductase is not present (Table 4).

TLC reveals a pattern identical to *Mycobacterium chelonae* and *M. abscessus*, characterized by the presence of α - and α' -mycolates.

Genotypic features

Like almost all rapid growers (except for *M. chelonae* and *M. abscessus*) two copies of the 16S rRNA gene are present in isolates of *M. canariense*. Genetically, the species closely resembles *M. diernhoferi* and *M. neoaurum* (Fig. 3).

All of the strains share a unique *hsp65* PRA pattern.

DNA–DNA hybridization reveals less than 15% homology with other rapid growers. This datum unquestionably supports the assignment of the strains to a previously unknown species.

Clinical and epidemiological features

Mycobacterium canariense was isolated from the blood of 17 patients, with central venous catheters; 12 of them had fever most likely due to the mycobacterium. One patient, in whom the catheter was not removed, relapsed and two other patients died (Jimenez *et al.*, 2004).

Type strains: CIP107998^T; CCUG 47953^T.

Accession numbers: 16S rRNA gene: AY255478; *hsp65*: AY255477.

'*Mycobacterium conceptionense*'

'*Mycobacterium conceptionense*' emerged in 2006 from the strains of the sorbitol-negative variant of the *M. fortuitum* third biovariant complex. Currently, '*M. conceptionense*' is not considered a valid species. The only strain detected so far was isolated from a wound of a patient with an open fracture (Adékambi *et al.*, 2006b).

Phenotypic features

The colonies are nonpigmented and develop within 3 days at 24–37 °C. Growth is obtained on Lowenstein–Jensen with 5% NaCl. Nitrate reduction and 3-day arylsulfatase are positive, while urease is negative (Table 4).

Gas-chromatographic analysis of fatty acid methyl esters reveal a profile most closely resembling *M. porcinum* (Adékambi *et al.*, 2006b).

Clarithromycin, ciprofloxacin, doxycycline and imipenem are active *in vitro* (Table 5).

Genotypic features

The 16S rRNA gene sequence is reported close to *M. porcinum* (not confirmed in our phylogenetic tree, Fig. 3). *rpoB* is considered the most significant genetic region for the recognition of the species.

Clinical and epidemiological features

'*Mycobacterium conceptionense*' was isolated from secretions, bone and skin tissue, of a wound subsequent to open tibia fracture in a 31-year-old woman who was rescued following an extended period in the river water after an accident. The wound healed over 3 months using unspecified antibiotic treatment (Adékambi *et al.*, 2006b).

Type strains: ATCC 108544^T, CCUG50187^T.

Accession number: 16S rRNA gene, AY859685.

'*Mycobacterium hackensackense*'

'*Mycobacterium hackensackense*' is not a valid species; the only reported strain was isolated from blood of a young girl with leukemia (Hong *et al.*, 2003).

Phenotypic features

The strain produces smooth nonpigmented colonies in 2–4 days at 25–37 °C. No growth is obtained on MacConkey agar and Lowenstein–Jensen with 5% NaCl. Positive biochemical tests include nitrate reduction, semiquantitative catalase, Tween 80 hydrolysis and urease (Table 4).

The HPLC pattern is indistinguishable from *M. smegmatis* (Hong *et al.*, 2003).

Isolates are susceptible to clarithromycin and imipenem only (Table 5).

Genotypic features

The 16S rRNA gene sequence is typical of rapid growers and closely resembles *M. diernhoferi* (Fig. 3).

Clinical and epidemiological features

'*Mycobacterium hackensackense*' was isolated repeatedly from the central venous catheter and peripheral blood cultures of a 6-year-old girl with lymphocytic leukemia. The initial treatment with amikacin and clarithromycin produced rapid defervescence. Subsequently amikacin was replaced with meropenem. The girl was recovered at a 10-month follow-up (Hong *et al.*, 2003).

Type strain: ATCC BAA-823^T.

Accession number: 16S rRNA gene, AY266138.

Mycobacterium houstonense

The new species, based on two strains, emerged in 2004 from a thorough revision of the sorbitol-positive strains of the *M. fortuitum* third biovariant complex (Schinsky *et al.*, 2004).

Phenotypic features

Mycobacterium houstonense is nonpigmented, and grows rapidly at 28–42 °C. Growth can be achieved on Lowenstein–Jensen with 5% NaCl and on MacConkey agar. Positive biochemical tests include 3-day arylsulfatase, semiquantitative catalase, nitrate reduction and urease (Table 4).

The GLC pattern is undistinguishable from that of other species previously grouped with the *M. fortuitum* third biovariant complex.

The HPLC profile overlaps *M. fortuitum*, *M. peregrinum* and *M. septicum*.

Amikacin, ciprofloxacin, imipenem and sulfamethoxazole are active *in vitro* (Table 5).

Genotypic features

Mycobacterium fortuitum is the closest species on the basis of 16S rRNA gene sequence (Fig. 3).

The low percentage of DNA–DNA hybridization clearly separates this species from the others emerging from the *M. fortuitum* third biovariant complex.

The two strains investigated present different PRA patterns (Table 3).

Clinical and epidemiological features

One strain was isolated from a face wound while another was grown from a lung biopsy (Schinsky *et al.*, 2004).

Type strains: ATTC 49403^T, DSM44676^T.

Accession number: 16S rRNA gene, AY012579.

'*Mycobacterium massiliense*'

'*Mycobacterium massiliense*' is not yet a validated species. It is based on a single strain isolated from a patient with disseminated disease (Adékambi *et al.*, 2004). Pulmonary samples were cultured both in liquid medium and using the amoebal coculture method.

Phenotypic features

Growth, which is present also on MacConkey agar and Lowenstein–Jensen containing 5% NaCl, is rapid and occurs at 24–37 °C; the colonies are not pigmented. Among biochemical tests, 3-day arylsulfatase is positive, while nitrate reductase and urease are negative.

Among drugs to be used against rapidly growing mycobacteria, susceptibility testing reveals activity with amikacin, clarithromycin and doxycycline.

Genotypic features

The sequence of the 16S rRNA gene overlaps that of *M. abscessus* (Fig. 3), while ITS and *hsp65* differ from *M. abscessus* by two and five nucleotides respectively. The low similarity with *M. abscessus*, with the *rpoB* gene, is stressed by the authors to support the sp. nov. hypothesis.

Clinical and epidemiological features

A 50-year-old woman undergoing corticosteroid treatment presented hemoptysis, subcutaneous nodules and micro-nodular pulmonary lesions. '*Mycobacterium massiliense*' was isolated repeatedly from sputum and bronchial aspirates. The patient recovered following a 1-month treatment with

clarithromycin, and a 6-month treatment of minocycline (Adékambi *et al.*, 2004).

Type strains: CIP108297^T, CCUG 48898^T.

Accession numbers: 16S rRNA gene, AY593980; ITS AY593978; *hsp65*: AY596465.

Mycobacterium neworleansense

Mycobacterium neworleansense originated, in 2004, from the sorbitol-negative strains of the *M. fortuitum* third biovariant complex (Schinsky *et al.*, 2004). Only one isolate has been reported.

Phenotypic features

Rough nonpigmented colonies grow rapidly at 28–37 °C on various media including MacConkey agar and Lowenstein–Jensen with 5% NaCl. Nitrate reduction, 3-day arylsulfatase, semiquantitative catalase and urease are positive (Table 4).

The GLC pattern is not distinguishable from *M. boenickei*, *M. brisbanense* and *M. houstonense*, while the HPLC analysis is unsuitable to differentiate *M. neworleansense* from *M. fortuitum*, *M. peregrinum* and *M. septicum*.

Antimicrobial susceptibility testing reveals susceptibility to amikacin, ciprofloxacin, imipenem and sulfamethoxazole (Table 5).

Genotypic features

By 16S rRNA gene sequence, the species most closely resembles *M. porcinum*.

DNA–DNA hybridization confirms the distinction from any other previously described species.

Clinical and epidemiological features

The only strain reported so far was from a scalp wound infection (Schinsky *et al.*, 2004).

Type strains: ATTC 49404^T, DSM 44679^T.

Accession number: 16S rRNA gene, AY012575.

Mycobacterium phocaicum

Mycobacterium phocaicum was described in 2006 on the basis of three strains recognized within a group of rapid growers in which the genetic sequence of the *rpoB* gene was investigated (Adékambi *et al.*, 2006a).

Phenotypic features

The species is not pigmented and grows rapidly at 24–37 °C but does not tolerate the presence of 5% NaCl in the medium. Nitrate reductase and arylsulfatase at 3 days are present (Table 4).

The GLC is suitable only to confirm the assignment of the strains to the genus *Mycobacterium*.

It is susceptible *in vitro* to almost all CLSI recommended antimycobacterial drugs to be tested with the rapid growers (NCCLS, 2002) (Table 5).

Genotypic features

Although the 16S rRNA gene does not reveal any diversity from *M. mucogenicum* (Fig. 3), the number of mismatches with such species at the level of genes *hsp65*, *sodA* and *recA* is high.

Clinical and epidemiological features

The only strains of *M. phocaicum* isolated were from three patients with chronic pneumonia (Adékambi *et al.*, 2006a).

Type strains: CIP 108542^T, CCUG 50185^T.

Accession number: 16S rRNA gene, AY859682.

Solely environmental strains

'*Mycobacterium fluoranthenivorans*'

The only strain on which the description of this nonvalidated species is based was reported in 2004 following its isolation from soil polluted with polycyclic aromatic hydrocarbons (Hormisch *et al.*, 2004).

Phenotypic features

'*Mycobacterium fluoranthenivorans*' is nonchromogenic and grows rapidly at 20–37 °C (Table 4). The species is able to use fluoranthene as a sole source of carbon and to degrade aflatoxin B1; features that suggest the possible use of the species in detoxification processes.

TLC reveals the presence of α - and epoxy-mycolates. GLC is suitable to distinguish the new species from closely related rapid growers. The HPLC pattern is characterized by two late-emerging clusters of peaks (Hormisch *et al.*, 2004), a feature shared by many slow growers.

Genotypic features

The genetic sequence of the 16S rRNA gene is identical to that of another nonvalidated species, '*M. hackensackense*'. Other than '*M. hackensackense*', the most closely related species emerging from the analysis of such genetic region is *M. diernhoferi* (Fig. 3).

Type strains: DSM 44556^T; NRRL CIP 108203^T.

Accession number: 16S rRNA gene, AJ617741.

Mycobacterium psychrotolerans

The new species, described in 2004, grows at very low temperatures and was isolated from pond water near a uranium mine (Trujillo *et al.*, 2004).

Phenotypic features

Mycobacterium psychrotolerans is a rapid grower over a wide temperature range (4–37 °C); its colonies are smooth and scotochromogenic (bright orange). Growth is inhibited on MacConkey agar but supported on Lowenstein–Jensen with 5% NaCl. Nitrate reduction and urease, the only biochemical tests investigated, are positive (Table 4).

The TLC reveals α -, keto-, ω - and wax esters-mycolates. The HPLC pattern is characterized by two widely separated clusters of peaks, with the first one clearly prominent.

Only ciprofloxacin, among a large number of drugs, very few of which are however specific for rapid growers, is active *in vitro* (Table 5).

Genotypic features

The genetic sequence of 16S rRNA gene places *M. psychrotolerans* closest to *Mycobacterium sphagni*.

Type strains: DSM 44697^T; LMG 21953^T.

Accession number: 16S rRNA gene, AJ534886.

Mycobacterium pyrenivorans

Mycobacterium pyrenivorans, which is able to use pyrene as sole source of carbon, was isolated in 2004 from soil highly contaminated with polycyclic aromatic hydrocarbons (Derz *et al.*, 2004).

Phenotypic features

The strain is scotochromogenic and grows within 7 days at 35 °C on conventional media including trypticase soy agar. It is able to mineralize phenanthrene, fluoranthene and pyrene. The semi-quantitative catalase test is positive, while Tween 80 hydrolysis is negative (Table 4).

TLC analysis is characterized by the presence of α -, epoxy, ω -dicarboxy- and wax ester-mycolates. GLC and HPLC reveal lipid profiles close to *Mycobacterium aurum* and *Mycobacterium austroafricanum*.

Genotypic features

The 16S rRNA gene sequence confirms similarity with *M. aurum* and *M. austroafricanum* (Fig. 3).

Type strains: DSM 44605^T; NRRL B24244^T.

Accession numbers: 16S rRNA gene, AJ431371.

Conclusions

Differences within species previously considered homogeneous are emerging continuously due to modern improvements of genetic techniques. When a new organism that potentially may be responsible for human infection is recognized, the findings become not only merely of taxonomic interest but also may have significant clinical implications. The taxonomists are primarily concerned with the suitability of criteria that allow the division of a taxon into different species and with the reliability of phylogenetic deductions. Conversely, the clinician places more importance on the potential pathogenicity and the antimicrobial susceptibility of the newly described species. From the clinician's point of view, the recognition of new microorganisms often is perceived as a nuisance, without medical relevance.

Instead of siding with either argument from the scientist or clinician, it is preferable to let the numbers speak for themselves.

In the last 3 years, 31 new mycobacteria have been reported, 26 of which were isolated from humans.

Two new species, which preferably infect animals, were recognized within the *M. tuberculosis* complex, but their ability to produce severe pathologies in humans has also been proven.

One-third of the more than 150 isolates of NTM of human origin reported, have shown unquestionable clinical significance. Furthermore, antimicrobial susceptibility, among the 18 taxa for which such data are available, revealed that five species are characterized by a multidrug resistant pattern, while five species are fully susceptible. Such data take into account the esoteric differences in drug batteries to be used against rapidly and slowly growing NTM.

Additionally, these findings further emphasize that in several pathological conditions, especially wound infections and sepsis, the possible association with an emerging mycobacterial species should not be disregarded.

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