Mycobacterium marseillense sp. nov., *Mycobacterium timonense* sp. nov. and *Mycobacterium bouchedurhonense* sp. nov., members of the *Mycobacterium avium* complex

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An *rpoB* sequence-based evaluation of 100 *Mycobacterium avium* complex (MAC) clinical isolates led to the identification of five respiratory tract isolates that were potential representatives of three novel MAC species. Distinctive phenotypic features of isolates 62863 and 5356591^T included a pseudomycelium morphology and both esterase and acid phosphatase activities. These two isolates exhibited sequence similarities of 99.8 % for the 16S rRNA gene, 86.3 and 86.1 % for 16S–23S rRNA gene internal transcribed spacer (ITS-1) sequence, 96.7 and 97.8 % for *rpoB* and 97.6 and 97.4 % for *hsp65*, respectively, with the type strain of *Mycobacterium chimaera*, the most closely related species. Isolates 3256799 and 5351974^T lacked α -mannosidase and β -glucosidase activities. They exhibited sequence similarities of 99.6 % for the 16S rRNA gene, 90.1 and 90.4 % for ITS-1, 97.8 % for *rpoB* and 98.0 and 98.1 % for *hsp65*, respectively, with the type strain of *M. chimaera*, the most closely related species. Isolate 4355387^T lacked urease and α -glucosidase activities, but it exhibited valine arylamidase, cystine arylamidase and acid phosphatase activities. It had sequence similarities of 99.3 % for the 16S rRNA gene, 51.8 % for ITS-1, 97.1 % for *rpoB* and 97.8 % for *hsp65* with the type strain of *Mycobacterium colombiense*, the most closely related species. A phylogenetic tree based on concatenated 16S rRNA gene, ITS-1, *rpoB* and *hsp65* sequences showed the uniqueness of these five isolates as representatives of three novel species, with bootstrap values ≥ 95 % in all nodes. On the basis of these phenotypic and genetic characteristics, these five isolates are proposed as representatives of three novel MAC species: *Mycobacterium marseillense* sp. nov., with strain 5356591^T (=CCUG 56325^T =CIP 109828^T =CSUR P30^T) as the type strain; *Mycobacterium timonense* sp. nov., with strain 5351974^T (=CCUG 56329^T =CIP 109830^T =CSUR P32^T) as the type strain; and *Mycobacterium bouchedurhonense* sp. nov., with strain 4355387^T (=CCUG 56331^T =CIP 109827^T =CSUR P34^T) as the type strain.

The *Mycobacterium avium* complex (MAC) initially comprised two species, namely *Mycobacterium avium* and *Mycobacterium intracellulare* (Pfyffer, 2007). However, recent studies have drawn attention to the wide diversity of isolates that can be detected in this complex (Lebrun *et al.*, 2005; Mijs *et al.*, 2002). Analysis of the 16S–23S rRNA gene internal transcribed spacer (ITS-1) sequence, a molecular approach used frequently for the identification of these mycobacteria, has revealed the existence of more than 30 different sequevars (Tortoli *et al.*, 2004). Only a

few of these sequevars have been assigned to *M. avium* (seven sequevars) and *M. intracellulare* (four sequevars). Recently, sequevar MAC-A organisms were assigned to a novel species, *Mycobacterium chimaera* (Tortoli *et al.*, 2004). Likewise, MAC-X organisms were gathered in a fourth species, *Mycobacterium colombiense* (Murcia *et al.*, 2006). We recently introduced partial *rpoB* sequencing for the molecular identification of MAC organisms at the species level and showed that a 99.3 % *rpoB* sequence similarity cut-off could be used to delineate MAC species (Ben Salah *et al.*, 2008). When applying *rpoB* sequencing to a collection of clinical MAC isolates cultured in our laboratory, five isolates were found whose partial *rpoB* sequence similarity with the four MAC species was below the 99.3 % cut-off. We further characterized these five isolates by using a polyphasic approach and herein present

Abbreviations: ITS, internal transcribed spacer; MAC, *Mycobacterium avium* complex.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene, *rpoB*, *hsp65* and ITS-1 sequences of the novel isolates are given in Tables 2 and 3.

phenotypic and genetic evidence for these isolates being representatives of three novel MAC species.

Mycobacterium isolates

Strains 62863 and 5356591^T were recovered from the bronchial aspirate and sputum, respectively, of two unrelated patients. Strains 3256799, 5351974^T and 4355387^T were recovered from sputum specimens of three other unrelated patients. All clinical specimens were inoculated into BACTEC 9000 MB broth after standard decontamination, according to the manufacturer's instructions (Beckton Dickinson Biosciences). The five clinical isolates, as well as seven MAC reference strains, *M. avium* subsp. *avium* ATCC 25291^T, *M. avium* subsp. *silvaticum* ATCC 49884^T, *M. avium* subsp. *paratuberculosis* ATCC 19698^T, '*M. avium* subsp. *hominissuis*' IWGMT 49 (IWGMT: International Working Group on Mycobacterial Taxonomy), a kind gift of D. van Soolingen (National Mycobacteria Reference Laboratory, Bilthoven, The Netherlands), *M. intracellulare* ATCC 15985, *M. chimaera* DSM 44623^T and *M. colombiense* CIP 108962^T, were subcultured on Middlebrook 7H10 agar.

Morphological features and cultivation

Initially, colony morphology, pigmentation and the ability of the isolates to grow at 25, 30, 37 and 45 °C were observed. Cultures were grown in the presence of 5 % NaCl on Middlebrook 7H10 agar, egg-based Löwenstein–Jensen slants (bioMérieux) and 5 % sheep blood agar (BioTechnologie Appliqué) at 37 °C under a 5 % CO₂ atmosphere. Subculture of the five clinical isolates on Middlebrook 7H10 agar yielded rough and non-pigmented colonies after 10 days of incubation at 30 °C. Neither clinical isolates nor MAC reference strains grew on MacConkey agar or Löwenstein–Jensen agar supplemented with 5 % NaCl after 10 days of incubation at 37 °C. Strains 3256799, 5351974^T and 4355387^T, as well as reference strains of *M. avium* subsp. *avium*, *M. avium* subsp. *silvaticum*, '*M. avium* subsp. *hominissuis*' and *M. intracellulare*, grew on Middlebrook 7H10 agar at 45 °C. All isolates were positive for Gram staining and they appeared as acid-fast bacilli following Ziehl–Neelsen staining.

Samples were prepared for electron microscopy as follows. A drop of mycobacterial suspension in PBS was deposited on a piece of Parafilm, which was placed on a 400-mesh Formvar carbon-coated copper grid and incubated for 15 min at 37 °C. The grid was then dried using filter paper (Macherey Nagel) and placed in 2 % phosphotungstic acid for 10 s. The grid was dried again and deposited on the filter paper with the Formvar facing upwards. This grid was observed using a Morgagni M 268D electron microscope (FEI) at 60 kV with × 28 000 magnification. Images were analysed using ANALYSIS PRO3.2 software. Electron microscopy observations indicated that all isolates looked like bacilli, with pseudomycelium formations apparent in strains 5356591^T and 62863. Cells of all five clinical isolates

were generally shorter than those of the MAC reference strains (Table 1).

Biochemical tests

Urease activity and the ability to take up iron were tested as described previously (Kent & Kubica, 1985; Vincent *et al.*, 2003). Additional biochemical tests were performed by inoculation of API Coryne, API 20E and API ZYM strips (bioMérieux) for 5 days at 30 °C under a highly humidified atmosphere (Table 1). With the exception of strain 5356591^T, all isolates were negative in the niacin test and were unable to hydrolyse Tween 80 and were positive for iron fixation and potassium tellurite reduction. Strains 5356591^T and 62863 differed from *M. chimaera* DSM 44623^T in their positive esterase and acid phosphatase activities. Strains 3256799 and 5351974^T differed from *M. chimaera* DSM 44623^T in their lack of α-mannosidase and β-glucosidase activities. Strain 4355387^T differed from *M. colombiense* CIP 108962^T by its lack of urease and α-glucosidase activities and the presence of cystine arylamidase, valine arylamidase and acid phosphatase activities, as well as use of citrate as a sole carbon source (Table 1).

Genotypic characterization

After inactivation (Djelouagji & Drancourt, 2006), genomic DNA was extracted from cells using a QIAamp DNA Mini kit (Qiagen). PCR amplification and sequencing were performed for the 16S rRNA gene (Weisburg *et al.*, 1991), the ITS-1 spacer (Frothingham & Wilson, 1993) and for parts of the *hsp65* (Turenne *et al.*, 2006) and *rpoB* genes (Ben Salah *et al.*, 2008). Products of the sequencing reactions were analysed with an ABI Prism 3100 DNA sequencer following the standard protocol of the supplier (Perkin Elmer Applied Biosystems). Accession numbers for the sequences of the 16S rRNA gene, *rpoB*, *hsp65* and ITS-1 are given in Tables 2 and 3. Similarities (%) between the sequences were determined by BIOEDIT (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) using the CLUSTAL W program supported by the PBIL website (<http://npsa-pbil.ibcp.fr/>; Tables 2 and 3). Sequences were trimmed to start and finish at the same nucleotide position for all of the isolates. Multiple sequence alignment was performed by use of the program CLUSTAL_X version 1.81 in the PHYLIP software package (Thompson *et al.*, 1997). Using *Mycobacterium tuberculosis* H37Rv^T as an outgroup, a phylogenetic tree was obtained from DNA sequences using the neighbour-joining method with Kimura's two-parameter distance correction model with 1000 bootstrap replications in the MEGA version 3.1 software package (Kumar *et al.*, 2004).

The 16S rRNA gene sequence, which is used widely for identification and phylogenetic analysis of novel bacterial species, is not informative for *Mycobacterium* species (Mignard & Flandrois, 2008). In the MAC, for example, the two species *M. intracellulare* and *M. chimaera* differ by

Table 1. Distinctive biochemical characteristics of MAC isolates and reference strains

Strains: 1, strain 5356591^T; 2, strain 62863; 3, strain 3256799; 4, strain 5351974^T; 5, strain 4355387^T; 6, *M. avium* subsp. *avium* ATCC 25291^T; 7, '*M. avium* subsp. *hominissuis*' IWGMT 49; 8, *M. avium* subsp. *paratuberculosis* ATCC 19698^T; 9, *M. avium* subsp. *silvaticum* ATCC 49884^T; 10, *M. intracellulare* ATCC 15985; 11, *M. chimaera* DSM 44623^T; 12, *M. colombiense* CIP 108962^T. +, Positive; –, negative; +/-, weakly positive.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Cell size (µm)												
Length	1.03	0.92	0.90	0.90	0.87	0.97	1.28	1.45	1.88	1.52	1.54	1.39
Width	0.50	0.54	0.44	0.45	0.49	0.55	0.47	0.48	0.39	0.43	0.50	0.44
Growth at 45 °C	+	+	+	+	+	+	+	–	+	+	–	–
Pigmentation	–	–	–	–	–	–	–	–	–	–	–	+
Tween 80 hydrolysis	+	–	–	–	–	–	–	–	–	–	–	–
Tellurite reduction	–	+/-	+/-	+/-	+	+	+	+	+	+/-	+/-	+/-
Aesculin hydrolysis	+/-	–	–	–	+/-	–	+	–	–	–	+	+
Acetoin production	+	+	+	+	+	+	+	+	+	–	+	+
Utilization as sole carbon source of:												
Citrate	–	–	–	–	+	–	–	–	–	–	–	–
D-Glucose	–	–	+/-	+/-	–	–	–	–	–	–	–	+/-
D-Ribose	+/-	+/-	+/-	+/-	+/-	–	–	–	–	–	+/-	+/-
Enzyme activity												
Esterase (C4)	+	+	–	+	–	–	–	–	–	+	–	–
Valine arylamidase	+	+	+	+	+	+	+	+	+	+	+	–
Cystine arylamidase	+	+	+	+	+	+	+	+	+	+	+	–
Pyrazinamidase	+	+	+	+	+	+	+	–	–	+	+	+
Pyrolidonyl arylamidase	+	–	+	+	+	–	+	–	–	–	+	+
Urease	–	–	–	–	–	–	–	–	–	–	–	+
Acid phosphatase	+	+	–	+	+	–	–	–	–	–	–	–
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	+	+	+	+	+	–	+	+
Alkaline phosphatase	+	+	+	+	+	–	+	–	–	+	+	+
α-Glucosidase	+	+/-	+/-	+	–	–	–	–	–	–	+	+
α-Mannosidase	+	+	–	–	–	–	–	–	–	–	+	–

*After 2 weeks of incubation.

only one mismatch at nucleotide position 403 (Tortoli *et al.*, 2004). In the present study, the proposed MAC species differed from the most closely related species by two positions in the 16S rRNA gene. We therefore completed genetic analyses by sequencing *hsp65*, *rpoB* and ITS-1.

Strain 4355387^T shared 99.3 % 16S rRNA gene sequence similarity, 51.8 % ITS-1 similarity, 97.1 % *rpoB* similarity and 97.8 % *hsp65* similarity with the most closely related type strain, *M. colombiense* CIP 108962^T (Tables 2 and 3). Strains 3256799 and 5351974^T exhibited an identical sequence, which is closely related to that of strains 62863 and 5356591^T and marginally related to the *M. intracellulare*/*M. chimaera* cluster. When this sequence was compared to that of *M. chimaera* DSM 44623^T, strains 3256799 and 5351974^T exhibited sequence similarities of 99.6 % for the 16S rRNA gene, 97.8 % for *rpoB*, 98.0 and 98.1 % for *hsp65*, and 90.1 and 90.4 % for ITS-1, respectively. Strains 62863 and 5356591^T exhibited sequence similarities of 99.8 % for the 16S rRNA gene, 86.3 and 86.1 % for ITS-1, 96.7 and 97.8 % for *rpoB*, and 97.6 and 97.4 % for *hsp65*, respectively, with *M. chimaera* DSM 44623^T. In total, the ≥2 mismatches in the 16S rRNA gene sequence, the >5

mismatches in *rpoB* and the ≥15 mismatches in the ITS and *hsp65* sequences between strains 5356591^T, 62863, 3256799 and 5351974^T and the closely related type strain *M. chimaera* DSM 44623^T, and between strain 4355387^T and *M. colombiense* CIP 108962^T were indicative of the existence of three novel species.

Furthermore, a phylogenetic tree derived from the 16S rRNA gene sequences demonstrated only 2/9 nodes (22 %) with bootstrap values ≥96 % (Fig. 1), giving poor confidence in this topology. The tree derived from the concatenated 16S rRNA gene, *rpoB*, *hsp65* and ITS-1 sequences (Devulder *et al.*, 2005; Stackebrandt *et al.*, 2002) yielded bootstrap values of ≥97 % in 8/11 nodes (72 %), indicating higher confidence in this representation (Fig. 2). This tree indicated that strain 4355387^T was distinct from but closely related to *M. colombiense* CIP 108962^T. Furthermore, strains 3256799 and 5351974^T formed a cluster that was distinct from but related to the cluster formed by strains 62863 and 5356591^T, and both clusters were marginally related to the *M. intracellulare*/*M. chimaera* cluster.

In this study, partial *rpoB* sequencing identified five MAC isolates that could not be classified at the species level.

Table 2. Sequence similarity (%) for *rpoB* (below the diagonal) and *hsp65* (above the diagonal) for MAC reference strains and the five atypical clinical isolates

Strains: 1, *M. avium* subsp. *avium* ATCC 25291^T; 2, *M. avium* subsp. *paratuberculosis* ATCC 19698^T; 3, *M. avium* subsp. *silvaticum* ATCC 49884^T; 4, '*M. avium* subsp. *hominissuis*' IWGMT 49; 5, *M. chimaera* DSM 44623^T; 6, *M. intracellulare* ATCC 15985; 7, *M. colombiense* CIP 108962^T; 8, 62863 (*rpoB* and *hsp65* sequence accession nos are EF584444 and EU239788, respectively); 9, 5356591^T (EF584434 and EU239787); 10, 4355387^T (EF584445 and EU239790); 11, 3256799 (EF584435 and EU239791); 12, 5351974^T (*rpoB* sequence identical to EF584444; *hsp65* sequence accession no. EU239792).

Strain	Sequence similarity (%) with:											
	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>M. avium</i> subsp. <i>avium</i> ATCC 25291 ^T		99.0	100	95.7	94.7	94.8	95.0	94.3	94.2	95.7	94.8	94.7
2. <i>M. avium</i> subsp. <i>paratuberculosis</i> ATCC 19698 ^T	99.7		99.2	95.3	94.5	94.6	94.6	94.1	94.0	95.3	95.0	94.5
3. <i>M. avium</i> subsp. <i>silvaticum</i> ATCC 49884 ^T	99.8	99.5		95.7	94.7	94.8	95.0	94.3	94.2	95.7	94.8	94.7
4. ' <i>M. avium</i> subsp. <i>hominissuis</i> ' IWGMT 49	99.7	99.7	99.5		91.0	91.1	91.5	90.9	90.8	92.0	91.1	91.0
5. <i>M. chimaera</i> DSM 44623 ^T	94.7	94.7	94.6	94.9		99.8	97.5	97.6	97.4	97.3	98.0	98.1
6. <i>M. intracellulare</i> ATCC 15985	94.3	94.3	94.2	94.5	99.2		97.6	97.6	97.5	97.0	98.1	98.2
7. <i>M. colombiense</i> CIP 108962 ^T	92.9	92.9	92.8	92.9	93.9	93.5		97.5	97.5	97.8	97.3	97.0
8. 62863	93.5	93.5	93.6	93.6	96.7	96.3	94.2		99.8	96.7	98.0	97.2
9. 5356591 ^T	94.6	94.6	94.7	94.7	97.8	97.4	93.3	98.8		96.6	97.6	97.1
10. 4355387 ^T	95.2	95.2	95.0	95.2	96.2	95.7	97.1	94.6	95.7		97.4	96.9
11. 3256799	94.6	94.6	94.5	94.7	97.8	97.4	93.9	97.1	98.1	96.3		99.0
12. 5351974 ^T	94.6	94.6	94.5	94.7	97.8	97.4	93.9	97.1	98.1	96.3	100	

Phylogenetic analyses indicated that four of these five isolates were more closely related to the *M. intracellulare*/*M. chimaera* group, although they belong to two different species that are distinct from both *M. intracellulare* and *M. chimaera*. The fifth isolate was more closely related to *M. colombiense*. Further polyphasic characterization of these five isolates indicated that these isolates were indeed

representative of three novel MAC species. These data indicate that partial *rpoB* sequencing was effective for the initial identification of MAC isolates. Moreover, the same PCR sequencing primer pair could be used to identify MAC isolates and rapidly growing mycobacteria, making *rpoB* a convenient tool for initial investigations of *Mycobacterium* isolates (Adékambi *et al.*, 2003). The data

Table 3. Sequence similarity (%) for 16S rRNA gene (below the diagonal) and ITS-1 (above the diagonal) sequences for MAC reference strains and the five atypical clinical isolates

Strains: 1, *M. avium* subsp. *avium* ATCC 25291^T; 2, *M. avium* subsp. *paratuberculosis* ATCC 19698^T; 3, *M. avium* subsp. *silvaticum* ATCC 49884^T; 4, '*M. avium* subsp. *hominissuis*' IWGMT 49; 5, *M. chimaera* DSM 44623^T; 6, *M. intracellulare* ATCC 15985; 7, *M. colombiense* CIP 108962^T; 8, 62863 (16S rRNA gene sequence identical to EU266632; ITS-1 sequence accession no. EU266630); 9, 5356591^T (16S rRNA gene and ITS-1 sequence accession nos EU266632 and EU266631, respectively); 10, 4355387^T (EF591053 and EF591051); 11, 3256799 (EF591054 and EF591050); 12, 5351974^T (16S rRNA gene sequence identical to EF591054; ITS-1 sequence accession no. EU266628).

Strain	Sequence similarity (%) with:											
	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>M. avium</i> subsp. <i>avium</i> ATCC 25291 ^T		100	100	99.7	45.4	44.2	62.5	35.5	35.5	43.3	38.8	38.3
2. <i>M. avium</i> subsp. <i>paratuberculosis</i> ATCC 19698 ^T	100		100	99.7	45.4	44.2	62.5	35.5	35.5	43.3	38.8	38.3
3. <i>M. avium</i> subsp. <i>silvaticum</i> ATCC 49884 ^T	100	100		99.7	45.4	44.2	62.5	35.5	35.5	43.3	38.8	38.3
4. ' <i>M. avium</i> subsp. <i>hominissuis</i> ' IWGMT 49	100	100	100		45.4	44.5	62.2	35.7	35.7	43.3	39.0	38.5
5. <i>M. chimaera</i> DSM 44623 ^T	96.3	96.3	96.3	96.3		95.6	51.1	86.3	86.1	95.4	90.1	90.4
6. <i>M. intracellulare</i> ATCC 15985	96.7	96.7	96.7	96.7	99.9		50.6	87.4	87.2	95.6	90.8	90.6
7. <i>M. colombiense</i> CIP 108962 ^T	96.7	96.7	96.7	96.7	99.4	99.4		50.4	50.4	51.8	50.2	49.7
8. 62863	96.4	96.4	96.4	96.4	99.8	99.5	99.5		99.7	86.3	86.5	86.5
9. 5356591 ^T	96.4	96.4	96.4	96.4	99.8	99.5	99.5	100		86.1	86.3	86.3
10. 4355387 ^T	96.6	96.6	96.6	96.6	99.9	99.9	99.3	99.5	99.5		90.4	90.6
11. 3256799	96.3	96.3	96.3	96.3	99.6	99.6	99.6	99.7	99.7	99.5		99.3
12. 5351974 ^T	96.3	96.3	96.3	96.3	99.6	99.6	99.6	99.7	99.7	99.5	100

Fig. 1. 16S rRNA gene sequence-based phylogenetic tree indicating the taxonomic relationships of the novel MAC species. Bootstrap support values $\geq 95\%$, determined from 1000 replicates, are indicated at nodes. The sequence of *Tsukamurella paurometabola* NCTC 10741 was used as the outgroup. Bar, 1% difference in nucleotide sequence.

presented here, in addition to the recent descriptions of *M. chimaera* (Tortoli *et al.*, 2004) and *M. colombiense* (Murcia *et al.*, 2006), suggest that the MAC consists of a heterogeneous group of mycobacteria that includes many novel species. More detailed phylogenetic and taxonomic descriptions of MAC species are warranted in order to provide a solid support from which to investigate MAC infections in animals and humans. Such descriptions could form the basis for analysis of mycobacterial sources and routes of transmission.

Description of *Mycobacterium marseillense* sp. nov.

Mycobacterium marseillense (mar.seil.len'se. N.L. neut. adj. *marseillense* pertaining to Marseille, where the first strains were isolated).

Cells are small ($0.92\text{--}1.03 \times 0.50\text{--}0.54\ \mu\text{m}$), acid-fast, Gram-positive bacilli. Colonies are non-pigmented and grow on 5% sheep blood agar, Middlebrook 7H10 agar and egg-based Löwenstein–Jensen slants after 10 days incubation at $30\ ^\circ\text{C}$. Exhibits a unique morphology with pseudo-mycelium formation. Hydrolyses Tween 80, but does not reduce potassium tellurite. It shares 96.4–99.8% 16S rRNA gene and 93.3–97.8% *rpoB*, 94.0–97.5% *hsp65* and 35.5–87.2% ITS-1 sequence similarities with MAC reference strains *M. avium* ATCC 25291^T, *M. intracellulare* ATCC 15985, *M. chimaera* DSM 44623^T and *M. colombiense* CIP 108962^T.

The type strain is 5356591^T (=CIP 109828^T =CCUG 56325^T =CSUR P30^T), sequevar MAC-X1, which was recovered from sputum. Strain 62863 (=CCUG 56324

Fig. 2. Phylogenetic tree based on concatenated 16S rRNA gene, ITS-1, *rpoB* and *hsp65* sequences, showing relationships between the clinical isolates 62863, 5351974^T, 3256799, 5356591^T and 4355387^T and strains of the four MAC reference species using the neighbour-joining method and Kimura's two-parameter distance correction model. Bootstrap support values $\geq 95\%$, determined from 1000 replicates, are indicated at nodes. *Mycobacterium tuberculosis* H37Rv^T was used as the outgroup. Bar, 0.05% difference in nucleotide sequence.

=CIP 109832 =CSUR P31), sequevar MAC-D, is a reference strain.

Description of *Mycobacterium timonense* sp. nov.

Mycobacterium timonense (ti.mo.nen'se. N.L. neut. adj. *timonense* pertaining to La Timone, the name of a hospital in Marseille, France, where the first strains were isolated).

Cells are acid-fast, Gram-positive bacilli with a mean size of $0.9 \times 0.45 \mu\text{m}$. Unpigmented colonies grow on 5% sheep blood agar, Middlebrook 7H10 agar and egg-based Löwenstein–Jensen slants after 10 days incubation at 30 °C. Positive for acid phosphatase activity. It shares 96.3–99.6% 16S rRNA gene, 96.3–99.6% *rpoB*, 91.0–98.2% *hsp65* and 38.3–90.6% ITS-1 sequence similarities with MAC reference strains.

The type strain is 5351974^T (=CIP 109830^T =CCUG 56329^T =CSUR P32^T), sequevar MAC-K, which was recovered from sputum. Strain 3256799 (=CCUG 56328 =CIP 109829 =CSUR P33), sequevar MAC-L, is a reference strain.

Description of *Mycobacterium bouchedurhonense* sp. nov.

Mycobacterium bouchedurhonense (bou.che.du.rho.nen'se. N.L. neut. adj. *bouchedurhonense* pertaining to Bouches du Rhône, the name of the département around Marseille, France, where the type strain was recovered).

Cells are small ($0.87 \times 0.49 \mu\text{m}$), acid-fast, Gram-positive bacilli. Colonies are non-pigmented and grow on 5% sheep blood agar, Middlebrook 7H10 agar and egg-based Löwenstein–Jensen slants after 10 days incubation at 30 °C. Differs from MAC species in its ability to use citrate as a carbon source. It shares 96.6–99.9% 16S rRNA gene, 95.0–97.1% *rpoB*, 43.3–51.8% *hsp65* and 92.0–97.8% ITS-1 sequence similarities with MAC reference strains.

The type strain is 4355387^T (=CIP 109827^T =CCUG 56331^T =CSUR P34^T), sequevar MAC-X2, which was recovered from sputum.

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