

Proposal to elevate the genetic variant MAC-A, included in the *Mycobacterium avium* complex, to species rank as *Mycobacterium chimaera* sp. nov.

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The possibility that the strains included within the *Mycobacterium avium* complex (MAC), but not belonging either to *M. avium* or to *Mycobacterium intracellulare*, may be members of undescribed taxa, has already been questioned by several taxonomists. A very homogeneous cluster of 12 strains characterized by identical nucleotide sequences both in the 16S rDNA and in the 16S–23S internal transcribed spacer was investigated. Similar strains, previously reported in the literature, had been assigned either to the species *M. intracellulare* on the basis of the 16S rDNA similarity or to the group of MAC intermediates. However, several phenotypical and epidemiological characteristics seem to distinguish these strains from all other MAC organisms. The unique mycolic acid pattern obtained by HPLC is striking as it is characterized by two clusters of peaks, instead of the three presented by all other MAC organisms. All of the strains have been isolated from humans and all but one came from the respiratory tract of elderly people. The clinical significance of these strains, ascertained for seven patients, seems to suggest an unusually high virulence. The characteristics of all the strains reported in the literature, genotypically identical to the ones described here, seem to confirm our data, without reports of isolations from animals or the environment or, among humans, from AIDS patients. Therefore, an elevation of the MAC variant was proposed and characterized here, with the name *Mycobacterium chimaera* sp. nov.; this increases the number of species included in the *M. avium* complex. The type strain is FI-01069^T (= CIP 107892^T = DSM 44623^T).

Published online ahead of print on 16 February 2004 as DOI 10.1099/ijs.0.02777-0.

Abbreviations: HMMIS, high molecular mass internal standard; ITS, internal transcribed spacer; LMMIS, low molecular mass internal standard; MAC, *Mycobacterium avium* complex; MAIS, *M. avium*–*M. intracellulare*–*Mycobacterium scrofulaceum* group; MAIX, *M. avium*–*M. intracellulare* cluster X; PRA, PCR restriction enzyme pattern analysis.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA and ITS regions sequence of strain FI-01069^T is AJ548480.

Alignment of the ITS sequevars of MAC, PRA patterns and a table containing the fatty acid content are available as supplementary material in IJSEM Online.

The *Mycobacterium avium* complex (MAC) includes two species, *Mycobacterium avium* and *Mycobacterium intracellulare*, of which the first is split into four subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *silvaticum*, *M. avium* subsp. *paratuberculosis* and the recently described *M. avium* subsp. *hominissuis* (Mijs *et al.*, 2002a). Furthermore, a number of unnamed mycobacteria not belonging to any of these taxa (Frothingham & Wilson, 1994), sometimes referred to as *M. avium*–*M. intracellulare* cluster X (MAIX) (Viljanen *et al.*, 1993), are included within the MAC.

The need to create a group that includes closely related, nevertheless different, organisms emerged long before the boom of genetic-based taxonomy, because of the scant or near impossibility of differentiation by means of cultural and biochemical tests; in fact, at that time, the only universally accepted characteristic that discriminated *M. avium* from *M. intracellulare* was the virulence of the latter in chickens (Runyon, 1967; Anz *et al.*, 1970).

During the 1990s, genetic studies confirmed the relatedness of the organisms included in the MAC, although they revealed an intraspecies variability not present in other mycobacterial taxa (Frothingham & Wilson, 1993).

Apart from *M. avium* subsp. *paratuberculosis*, made easily recognizable by its mycobactin dependence and extremely slow growth, the differentiation of other members of the MAC remained unfeasible in diagnostic laboratories until the commercialization of DNA probes. The AccuProbe (Gen-Probe) allows easy and accurate differentiation between *M. avium* and *M. intracellulare* (Saito *et al.*, 1989) and their clear distinction from unnamed MAIX (Viljanen *et al.*, 1993). More recently, similar results became available using the INNO LiPA Mycobacteria (Innogenetics) reverse hybridization test (LiPA) (Tortoli *et al.*, 2001), which, in a novel formulation (Tortoli *et al.*, 2003), also allowed the differentiation of a subgroup [sequevar (sqv.)] of MAIX, so far tentatively assigned to the species *M. intracellulare*.

Our attention was first drawn to a number of strains identified by AccuProbe as *M. intracellulare* and by the first LiPA test as belonging to the *M. avium*–*M. intracellulare*–*Mycobacterium scrofulaceum* group (MAIS), but different from *M. avium*, *M. intracellulare* and *M. scrofulaceum*. An in-depth investigation of 12 such strains revealed both phenotypic and genotypic features that suggested their distinction from *M. intracellulare* and their belonging to a previously unrecognized species, within MAC, for which we propose the name *Mycobacterium chimaera* sp. nov.

Bacterial strains

The bacterial strains FI-01069^T, FI-01129, FI-99018, FI-02109, FI-02038, FI-02197, FI-02110, FI-02126, FI-01022, FI-02211, FI-02212 and FI-03048 were independent (Table 1). They had been isolated over a 5 year period

(1999–2003) from different patients in five Italian hospitals. Different media were used in various laboratories for culturing of the strains, including Middlebrook 7H9 agar, Lowenstein–Jensen medium and radiometric broth. All but one had been grown from respiratory specimens, and a single isolate had been obtained for six of them; the others (FI-01069^T, FI-99018, FI-02110, FI-02126, FI-02210 and FI-02211) had been excreted repeatedly by patients.

Biochemical and cultural tests

The most frequently investigated biochemical and cultural features (niacin accumulation, nitrate reduction, β -glucosidase, Tween 80 hydrolysis, 3-day arylsulfatase, urease, tellurite reduction, 68 °C and semiquantitative catalase, growth rate, pigmentation, growth at 25, 37 and 45 °C, MacConkey growth; and tolerance to NaCl, thio-phene carboxylic hydrazide, tiacetazone, *p*-nitrobenzoic acid, hydroxylamine, oleate and isoniazid) were tested using standard procedures described previously (Kent & Kubica, 1985).

TLC of mycolic acids

The mycolic acids present in the cell wall, extracted by means of methyl esterification, were separated by two-dimensional TLC on silica gel (Minnikin *et al.*, 1984). Identification of the spots was made by comparison with those from reference strains. Two strains, FI-01069^T and FI-02038, were chosen for this test.

Analysis of fatty acids

Fatty acid methyl esters were obtained from 40 mg (wet weight) of cells by saponification, methylation and extraction as described before (Miller, 1982). The methyl ester mixtures were separated by a gas chromatograph (model 5898A, Hewlett Packard) controlled by MIS software (Microbial ID). Peaks were integrated automatically and fatty acid names and percentages were determined using the Microbial Identification standard software package (Sasser, 1990). Strains FI-01069^T, FI-01129 and FI-02038 were chosen for fatty acid analyses.

HPLC of cell wall mycolic acids

The standard procedure of the Centers for Disease Control and Prevention (CDC, 1996) was used for the extraction and derivatization to bromophenacyl esters of mycolic acids. Separation was obtained with a 126 model System Gold (Beckman) HPLC instrumentation by using a gradient of methanol and methylene chloride with an Ultrasphere-XL column (Beckman). Chromatograms were compared visually with those of our laboratory library.

DNA probe hybridization

For each strain, DNA hybridization was attempted with AccuProbe *M. avium*, AccuProbe *M. intracellulare* and AccuProbe MAC (Kiehn & Edwards, 1987), with both

Table 1. Epidemiological, microbiological and clinical features of 12 strains of *M. chimaera* sp. nov.

+++ , Very probable; ++ , probable. *BAL, Bronchoalveolar lavage; BA, bronchial aspirate. †COPD, Chronic obstructive pulmonary disease.

Strain	Sex	Age	Specimen*	Microscopy	Isolation	Underlying disease†	Previous TB	Anti-mycobacterial treatment	Outcome	Isolation site	Clinical significance
FI-01069	F	56	Sputum; BAL	+	Multiple	Bronchiectasis	No	Clarithromycin; rifabutin	Relapse	A	+++
FI-01129	M	66	Sputum	-	Single	COPD	Yes	None	Unknown	B	Uncertain
FI-99018	F	82	Sputum; BA	-	Multiple	Non-Hodgkin's lymphoma	No	Azithromycin		C	+++
FI-02109	M	70	Sputum	-	Single	COPD	No	None	Unknown	B	Uncertain
FI-02038	M	62	Sputum	-	Multiple	COPD	No	None	Unknown	B	Uncertain
FI-02197	F	78	Urine	-	Single	Glomerulonephritis	Yes	None	Improvement	D	No
FI-02110	F	61	Sputum; BA	+	Multiple	Pulmonary cavitations	No	Clarithromycin; ethambutol; moxifloxacin; streptomycin	Recovery	B	+++
FI-02126	M	69	Sputum	-	Multiple	Pulmonary cavitations	No	Amikacin; ciprofloxacin; clarithromycin	Recovery	E	+++
FI-02210	M	78	BA	+	Multiple	Diabetes; respiratory failure	No	None	Death	E	++
FI-02211	M	64	Sputum	-	Multiple	COPD	Yes	Amikacin; clarithromycin	Unknown	E	+++
FI-02212	M	71	BA	-	Single	COPD	No	Ciprofloxacin; rifabutin	Recovery	E	+++
FI-03048	M	72	BA	-	Single	COPD	No	None	Unknown	B	No

formulations of LiPA [the first (Tortoli *et al.*, 2001) and the more recent one] and with a further reverse hybridization test GenoType Mycobacteria (HAIN). The tests were performed according to the recommendations of the manufacturers.

Genetic sequencing and phylogenetic analysis

The full-length 16S rRNA gene and the 16S–23S rDNA internal transcribed spacer (ITS) were amplified using primers and PCR protocols described previously (Kirschner *et al.*, 1993; Roth *et al.*, 1998). Sequencing of PCR products was carried out with an automated apparatus (ALFexpress DNA sequencer; Pharmacia Biotech) using the Thermo Sequenase fluorescent labelled primer cycle-sequencing kit with 7-deaza-dGTP and the Thermo Sequenase Cy5 dye terminator kit (Amersham Pharmacia Biotech). The sequences of the 16S rDNA and the ITS were compared with those present in the GenBank and Ridom (Harmsen *et al.*, 1999) databases; ambiguities in published databases were considered as identities. The complete ITS sequence was aligned with all known such sequences of MAC using the CLUSTAL W program [EMBL-European Bioinformatics Institute (<http://www.ebi.ac.uk/clustalw/>)]. The maximum-likelihood method was used for the construction of the phylogenetic tree (Felsenstein, 1993; PHYLIP). The tree was rooted with the ITS sequence of *Mycobacterium tuberculosis* as the outgroup. Tree branches were reproduced by performing 100 bootstrap replicates.

RFLP and insertion element investigations

The presence of IS1245 was investigated by means of a RFLP-based assay using *PvuII* restriction enzyme (van Soolingen *et al.*, 1998), in which the generated DNA fragments were separated electrophoretically on an agarose gel, blotted onto a nylon filter and tested by a peroxidase-labelled IS1245 probe. Other insertion elements characterizing various taxa included in MAC, IS900 (Green *et al.*, 1989) and IS901 (Kunze *et al.*, 1991), were investigated by PCR using previously reported primers (Sanderson, 1993; Kunze *et al.*, 1992; Ahrens *et al.*, 1995).

PCR restriction enzyme pattern analysis (PRA)

PRA was carried out as described previously (Telenti *et al.*, 1993). Briefly, primers Tb11 and Tb12 were used in a 50 µl

reaction volume, containing 1.5 mM MgCl₂; PCR conditions were the same as described by Telenti *et al.* (1993). Fifteen microlitres of the *hsp65* gene amplified product (441 bp) was digested with the restriction enzymes *BstEII* and *HaeIII* and separated electrophoretically on 4% NuSieve agarose gels. Digestion patterns were compared to the PRASITE database for identification purposes (<http://www.hospvd.ch:8005>).

Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MIC) were investigated on Middlebrook 7H11 agar plates using twofold dilutions of the following antibiotics: amikacin, ciprofloxacin, clarithromycin, ethambutol, rifampicin and streptomycin (Table 2).

Biochemical and cultural test results

All the strains were slow growers, negative for niacin, nitrate reduction, β-glucosidase, Tween 80 hydrolysis, 3-day arylsulfatase, urease, semiquantitative catalase, NaCl tolerance and MacConkey growth, and positive for 68 °C catalase, tolerated thiophene carboxylic hydrazide, tiacetazone and isoniazid and grew at 25 and 37 °C but not at 45 °C. Variability was detected for the remaining tests. The colonies, which were smooth, were unpigmented.

Antimicrobial susceptibility results

The susceptibility pattern was rather variable, with only ethambutol being ineffective on all the strains. The MICs were very inhomogeneous, quite low in four strains and high in the others (Table 2).

Lipid analysis results

The TLC revealed the presence of α-mycolates, ketomycolates and wax ester mycolates, which is the pattern shared by MAC and many other mycobacterial species (Luquín *et al.*, 1991).

On the basis of the pattern of fatty acids disclosed by GLC analysis, the MIDI system identified our strains as best resembling the species *M. intracellulare*. The similarity to the *M. intracellulare* pattern stored in the MIDI fatty acid database was, however, very close to 0.5, which is considered

Table 2. MICs (µg ml⁻¹) of strains of *M. chimaera* sp. nov.

Drug	FI-01069	FI-01129	FI-99018	FI-02109	FI-02038	FI-02197	FI-02110	FI-02126	FI-02210	FI-02211	FI-02212	FI-03048
Amikacin	≤2	4	≤2	≤2	8	>8	≤2	8	>8	>8	>8	>8
Ciprofloxacin	2	≤1	4	2	2	>4	≤1	>4	>4	>4	>4	>4
Clarithromycin	≤2	>32	16	≤2	32	>32	≤2	>32	32	>32	>32	>32
Ethambutol	8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8
Rifampicin	≤0.5	>8	4	≤0.5	4	>8	≤0.5	4	2	4	4	4
Streptomycin	≤2	>8	8	≤2	>8	8	≤2	4	4	4	4	≤2

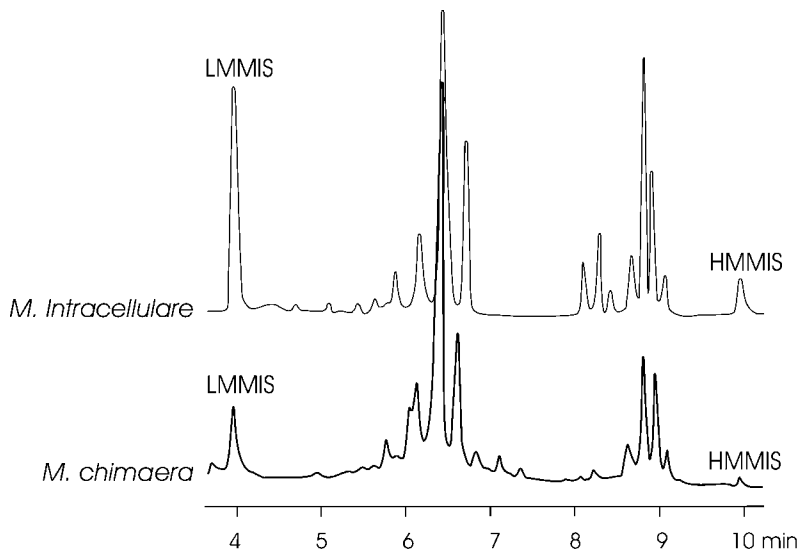


Fig. 1. Mycolic acid patterns of *M. chimaera* sp. nov. and *M. intracellulare* obtained by HPLC analysis. LMMIS, Low molecular mass internal standard; HMMIS, high molecular mass internal standard.

the lower limit for strains belonging to the same species. Details of the fatty acid contents are available as supplementary data, in IJSEM Online.

The mycolic acid HPLC pattern was characterized by two clusters of peaks: an early major one and a second one emerging 156 s later (Fig. 1). Only one of the isolates (FI-02110) presented a third minor cluster of peaks just before the second one.

DNA probe hybridization results

All the strains presented identical reactivity with the DNA probes tested. In the AccuProbe system, they hybridized with probes for MAC and *M. intracellulare* but not with the probe for *M. avium*. In the first LiPA, they were positive for the genus *Mycobacterium*-specific line and for the MAIS one, and negative for *M. avium* and *M. intracellulare*. In the new version of LiPA, the positive result with the newly added line-probe *M. intracellulare*-2 added to the previous ones. The strains only reacted with the *M. intracellulare* probe with the GenoType test.

Genetic sequences

Identical nucleotide sequences of the 16S rDNA and the 16S–23S rDNA ITS characterized our 12 strains. The 16S rDNA presented a single nucleotide mismatch (T→C) compared with *M. intracellulare* (GenBank accession no. AJ536036) at nucleotide position 403. In the Ridom database, there were only 442 bases of the 5' end of the 16S rDNA present; full identity emerged to *M. intracellulare* sqv. v (ATCC 35772). The sequence of the ITS was identical to that of sqv. MAC-A (accession no. L07847) (Frothingham & Wilson, 1993), which is characteristic of several MAC organisms other than *M. avium* and *M. intracellulare*. A supplementary figure showing the complete ITS alignment of known MAC sequevars is available in IJSEM Online.

Phylogenetic analysis (Fig. 2) shows three major branches in

which *M. avium*, *M. intracellulare* and most of MAC sequevars are clustered. However, nine sequevars, at present included in MAC, form two minor clusters and four isolated branches; among the latter lies sqv. MAC-A, which is, phylogenetically, the most distant both from *M. avium* and *M. intracellulare*.

PRA revealed the same pattern for all the isolates. Both restriction enzymes produced three bands of 240, 120 and 100 bp with *Bst*EII, and of 140, 130, 60 bp with *Hae*III. An identical pattern is characteristic of *M. intracellulare* PRA type I. PRA patterns are available as supplementary data in IJSEM Online.

Insertion sequences

None of the insertion elements (IS900, IS901 or IS1245) characterizing the various taxa included in the MAC was detectable in any of the strains.

Clinical significance of the isolates

Four of the strains isolated only once did not fulfil the criteria of the American Thoracic Society for clinical significance (Wallace *et al.*, 1990); one had been isolated from urine, while the others had been grown from patients with chronic obstructive pulmonary disease, for which the mycobacterial origin could not be demonstrated (Table 1). The other seven strains had been isolated from patients (four male and three female), 56 to 82 years old, whose cases were considered clinically significant. None of them was immunodeficient; two had pulmonary cavitations and haemoptysis, while the others presented pulmonary abscesses, chronic obstructive pulmonary disease and bronchiectasis (Table 1). One such patient (FI-02210), hospitalized in the intensive care unit because of acute respiratory failure, survived only a few days; the others were treated with multiple anti-mycobacterial drugs after which three of them recovered and have so far not relapsed. In all

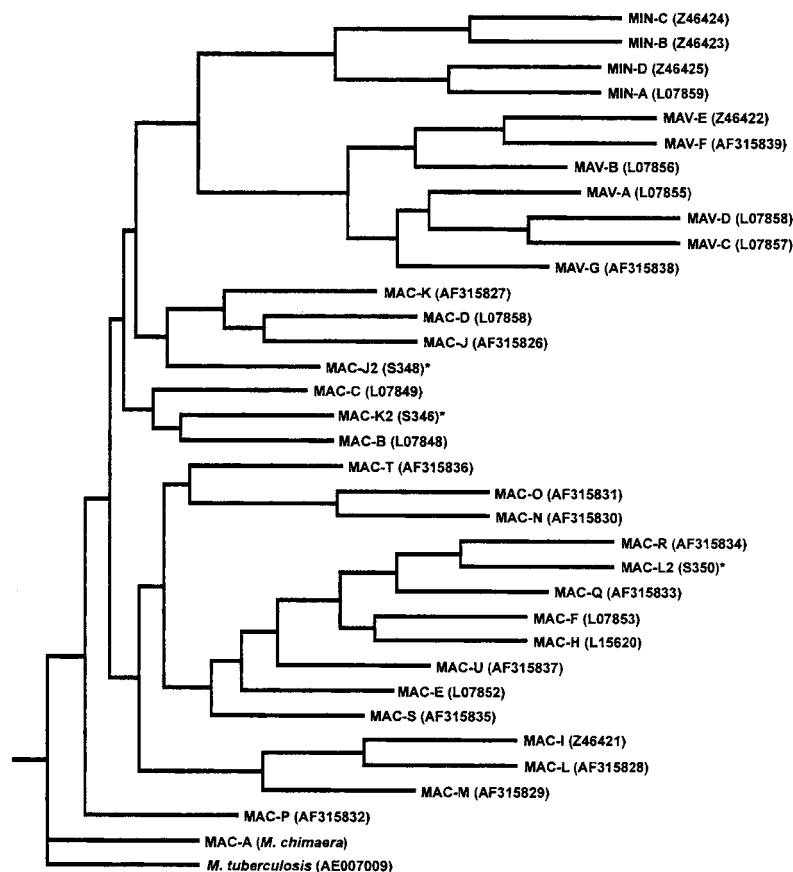


Fig. 2. Phylogenetic tree based on ITS sequences showing the relationships of *M. chimaera* and other sequevars of MAC. Each organism is indicated by the sequevar name followed by the GenBank accession number; for the asterisked sequevars, which are not present in GenBank database, the Ridom accession number is reported.

such cases no other plausible cause for the pulmonary disease was detected.

The aggregation, within the MAC, of strains that, although not identical, shared major traits arose from the impossibility of their differentiation by means of biochemical tests. While the commercialization of AccuProbe seemed to have resolved, at the level of routine diagnostic purposes, the dilemma of the distinction of *M. avium* from *M. intracellulare* and of both from MAIX, questions again arose with the subsequent introduction of LiPA, mainly because of the presence of strains with discrepant hybridization characteristics (Makinen *et al.*, 2002; Suffys *et al.*, 2001; Mijs *et al.*, 2002b; Miller *et al.*, 2000; Scarparo *et al.*, 2001; Tortoli *et al.*, 2001). In fact, strains do exist that are identified by AccuProbe as *M. intracellulare* while they are located by LiPA within the MAIX group, however, excluding their belonging to the species *M. avium*, *M. intracellulare* or *M. scrofulaceum*.

As the two commercial DNA probes are characterized by different targets, the 16S rRNA gene in the AccuProbe and the ITS in the LiPA, the above results only reflect the combination of the genetic sequences present in adjacent regions in such organisms. In fact, all the strains characterized here combine sqv. v of *M. intracellulare* in the 16S rDNA and sqv. MAC-A in the ITS; a genetic 'mosaic' reported for the first time by Frothingham *et al.* (1993). The

result achieved with GenoType, whose target is represented by the 23S rDNA, suggests that the sequence in this region is compatible with *M. intracellulare*.

The nucleotide sequences of 16S rDNA and ITS have confirmed the relatedness of the organisms included in the MAC, but, at the same time, revealed an unusual heterogeneity within it (Kirschner *et al.*, 1993; Roth *et al.*, 1998). Two and five sequevars of the 16S rDNA are present in *M. avium* and *M. intracellulare* (Ridom), respectively. With regard to the ITS, seven sequevars are known for *M. avium*, four for *M. intracellulare* and 23 for other members of the complex (Mijs *et al.*, 2002a) (in the latter case, a further cause of confusion is represented by two sets of three distinct sequevars, which received identical names, MAC-J, MAC-K and MAC-L, from independent researchers). The ITS sequevars of the species *M. avium* and *M. intracellulare* are characterized by close relatedness, while the heterogeneity is very high among strains unassigned to such taxa, an observation that led repeatedly to the hypothesis of the existence of other species within this group (Frothingham & Wilson, 1993; Wayne *et al.*, 1996; Wayne & Sramek, 1992).

The nucleotide sequences of the 16S rDNA of the strains investigated here present only one nucleotide mismatch compared with the most frequent sequevar (sqv. i) of *M. intracellulare*, but they are very distant from this species with regards to the ITS (sqv. MIN-A is the closest, with 20

mismatches, while 21 mismatches are present in each of the others).

Given the existence of mycobacterial species that differ by only one base in the 16S rDNA or that are even identical in this gene (e. g. *Mycobacterium kansasii* and *Mycobacterium gastri*; Kirschner *et al.*, 1993), nothing seems to contradict the hypothesis that the strains investigated here are members of a novel taxon. In contrast, there is evidence to suggest that the strains investigated do not belong to the species *M. intracellulare*, towards which very poor relatedness exists at the ITS level. Moreover, the variations in both officially recognized species of MAC are very limited; 1 to 3 bases in *M. avium* and 1 to 2 in *M. intracellulare*.

When the whole stretch of the rDNA operon, including both 16S and ITS, is considered, the maximum number of mismatches is four among various sequevars of *M. avium* and seven for those of *M. intracellulare*. In contrast, MAC-A differs from the first species by, at least, 20 nt and from the second by 21 or more. Furthermore, within this region, various genetic markers distinguish MAC-A from any other variant, such as the replacement of adenosine at position 149 with thymidine, that of guanosine at position 234 with adenosine and the deletion at position 230. Finally, in the phylogenetic tree (Fig. 2), MAC-A occupies a separate branch, far from other MAIX and further from *M. avium* and *M. intracellulare*.

The HPLC pattern of mycolic acid is very consistent among the strains included in the MAC, which are typically characterized by three clusters of peaks. The first cluster is the main one and includes four major peaks, while the others, which emerge later and close to each other, present three major peaks each. The relative heights of the peaks in the second and third cluster may vary with *M. avium* and *M. intracellulare* presenting, in most cases, lower peaks in the third and in the second cluster, respectively (Butler *et al.*, 1992). In contrast, the strains investigated here presented only the first and the third of such clusters (Fig. 1). The uniqueness of this phenotypic feature emerged also from the careful retrospective revision of over 300 HPLC profiles of MAC present in our laboratory file, which revealed only three such patterns. In only one of them (FI-99018), genetic sequencing revealed sqv. v and sqv. MAC-A in 16S rDNA and ITS, respectively; however, this was the only one which confirmed, once the HPLC was repeated, the typical two-cluster profile.

It seems conceivable that the GLC results, characterized by low similarity to typical *M. intracellulare*, are also somehow a confirmation of the peculiar lipid structure of the strains investigated here, but more strains have to be analysed before one can draw a final conclusion.

Several insertion elements characterize different taxa of MAC; IS900 is specific for *M. avium* subsp. *paratuberculosis* (Green *et al.*, 1989), IS901 is specific for the bird-type *M. avium* (Kunze *et al.*, 1991), while the number of elements

of IS1245 varies (Ritacco *et al.*, 1998). None of the above transposons was present in our strains. In contrast, two strains presenting DNA probe reactivity identical to ours, investigated in the recent paper describing *M. avium* subsp. *hominissuis* (Mijs *et al.*, 2002a), presented multiple bands of IS1245 in RFLP analysis.

The PRA pattern characteristic of *M. intracellulare* PRA type I does not exclude the possibility that the strains investigated here belong to a different species, as previously reported for other strains (Leclerc *et al.*, 2000).

There are a number of papers that emphasize the feature of drug resistance of MAC (Inderlied *et al.*, 1993); among our strains, along with some quite resistant, four were highly susceptible, except for ethambutol, to almost all antibiotics usually tested against mycobacteria.

Of importance is that the proposed species has been isolated, so far, only from humans (De Smet *et al.*, 1995; Frothingham & Wilson, 1994; Mijs *et al.*, 2002a), usually elderly males with pulmonary disorders, but never from AIDS patients (De Smet *et al.*, 1995; Frothingham & Wilson, 1994). The high frequency of cases in which it is clinically significant seems to suggest a virulence greater than other MAC organisms.

Description of *Mycobacterium chimaera* sp. nov.

Mycobacterium chimaera sp. nov. (chi.maer' a. L. n. *chimaera* the chimaera, the mythological being made up of parts of three different animals, referring to the apparent mix of genetic features characterizing the strains).

Slowly-growing, unpigmented mycobacterium characterized by acid-fast, non-motile and non-spore forming coccobacilli. AccuProbe and GenoType assign *M. chimaera* to the species *M. intracellulare*; LiPA has recently developed a specific probe, probably to comply with AccuProbe, which binds it to *M. intracellulare*. A unique taxonomic position emerges from genetic sequencing of both 16S rDNA and ITS, with the first being considered, at present, a sequevar of *M. intracellulare* and, the second, a sequevar of several MAC, other than *M. avium* and *M. intracellulare*. The typical HPLC profile easily differentiates *M. chimaera* from any other MAC. Additional signals may be provided by the anti-mycobacterial susceptibility pattern, characterized by full resistance to ethambutol, by the almost exclusive isolation from respiratory samples and by the frequent involvement in pulmonary disease of elderly people.

The type strain of *M. chimaera* is FI-01069^T (= CIP 107892^T = DSM 44623^T). Strains FI-02038 and FI-01129 were deposited in the DSMZ as DSM 44621 and DSM 44622, respectively.

Acknowledgements

The work of L. R., N. L. and C. G. was supported in part by National Research Program on AIDS grant no. 50C.11 and 50D.11 from the Italian 'Istituto Superiore di Sanità'. M. I. M. was supported by the National University of Colombia, Bogotá.

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