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Isolation of an Unusual Mycobacterium from an AIDS Patient

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A mycobacterium isolated from a clinical sample of an AIDS patient was identified as *Mycobacterium interjectum* by direct 16S rRNA sequence determination. High-performance liquid chromatography, however, revealed a mycolic acid pattern which was different from the one shared by the previously analyzed strains of this species.

Infections due to nontuberculous mycobacteria were occasionally described before the 1980s (20), but their frequency and importance have greatly increased with the spread of the AIDS epidemic. *Mycobacterium avium* is the most frequently isolated nontuberculous species worldwide (7), but many other acid-fast bacilli have been found to be responsible for important infections in AIDS patients (19). More recently, a number of previously unknown species have been described (1, 3, 11, 14, 15). Correct identifications of such organisms are important not only from a scientific point of view but mainly as they can aid in evaluating the clinical significance of an isolate and choosing the correct therapeutic regimen; many species are in fact characterized by different antimicrobial agent susceptibility patterns.

A major role in the recognition of new species has been played by direct nucleic acid sequencing of genomic regions which include hypervariable species-specific segments suitable for bacterial identification. The genomic region which has yielded the most important contributions to the present knowledge of mycobacterial taxonomy is probably the 16S rRNA gene (9).

Among phenotypic approaches, the analysis of cell wall mycolic acids by high-performance liquid chromatography (HPLC) has proved to be a reliable identification tool (2); its results are generally in agreement with those of genetic sequencing.

We describe here a clinical isolate characterized by an evident disagreement between phenotypic (HPLC) and genotypic (sequencing) features. A slowly growing mycobacterium (FI-3695) was isolated from the sputum of an immunocompromised (CD4 count = 47/ml) 36-year-old AIDS patient, a drug addict since the age of 17.

Conventional identification was performed with a broad panel of biochemical and cultural tests by standard procedures (12).

For HPLC of cell wall mycolic acids, the technique developed at the Centers for Disease Control and Prevention (4) was used with a reverse-phase C_{18} ultrasphere-XL cartridge column on an HPLC System Gold model (Beckman; Palo Alto, Calif.). Peaks were identified on the basis of their retention times relative to that of a high-molecular-weight internal standard (Ribi; ImmunoChem, Hamilton, Mont.).

Genetic analysis was performed, as reported previously (10), by direct sequence determination of a PCR-amplified 16S rRNA gene fragment. The regions used for alignment included nucleotides corresponding to positions 129 to 266 (hypervariable region A) and 430 to 468 (hypervariable region B) of *Escherichia coli* 16S rRNA.

The susceptibilities of this strain to antimicrobial agents were determined in liquid radiometric medium (Bactec 12B; Becton Dickinson, Sparks, Md.) by the previously described technique (13) for the *M. avium* complex; the growth kinetics of our isolate was very close to that of the *M. avium* complex, so all the requirements of this method were easily fulfilled.

This organism grown in radiometric broth (Bactec 12B) presented in subcultures on solid media smooth domed colonies whose morphology appeared to be microscopically indistinguishable from that of the opaque type of the *M. avium* complex. While the majority of them had a deep yellow pigmentation, a few were completely white. The two chromatic variants appeared to be invariable, and no pigmentation conversion was detected. Conventional procedures gave identical results for both colonial variants and revealed a biochemical and cultural pattern (Table 1) not quite compatible with that of any mycobacterial species. The maximum-likelihood identification, as determined by using a program for mycobacterial speciation (17), appeared to be *Mycobacterium malmoense*, *Mycobacterium interjectum*, or *Mycobacterium marinum* as a consequence of considering the isolate nonchromogenic, scotochromogenic, or photochromogenic, respectively.

The partial 16S rRNA gene sequence determined twice (on mixed chromatic variants and on isolated yellow colonies) revealed within hypervariable regions A and B the characteristic signature of the recently described new species *M. interjectum* (14) (Fig. 1).

The HPLC patterns (Fig. 2) of both variants of this isolate appear to be identical and differ from that of any other strain in our mycobacterium HPLC library, which includes 57 species. The profile with which that of our isolate presents the greatest similarity appears to be the one of the newly described species

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TABLE 1. Comparison of conventional biochemical, cultural, and inhibition test results for our isolate and some of the more closely related species[a]

Test or characteristic	FI-3695	*M. interjectum*	*M. malmoense*	*M. marinum*
Niacin	−	−	−	−
Nitrate reduction	−	−	−	−
Thermostable catalase	+	+	+	+
β-Glucosidase	−	−	−	−
Tween 80 hydrolysis (10 days)	+	V	+	+
Tellurite reduction	+	+	V	−
Arylsulfatase (3 days)	−	−	−	−
Urease	+	V	+	+
Catalase (over 45 mm of foam)	−	V	−	V
Photochromogenicity	−	−	−	+
Scotochromogenicity	±	+	−	−
Growth at:				
25°C	+	+	+	+
37°C	+	+	V	V
45°C	−	−	−	−
MacConkey agar	−	−	−	−
Growth rate	Slow	Slow	Slow	Slow
Colonial morphology	Smooth	Smooth	Smooth	Smooth
Tolerance to:				
p-Nitrobenzoate (500 μg/ml)	+	+	+	V
NaCl (5%)	−	−	−	−
Thiophene-2-carboxylic hydrazide (5 μg/ml)	+	+	+	+
Thiacetazone (10 μg/ml)	+	+	+	V
Hydroxylamine (500 μg/ml)	+	V	V	+
Isoniazid (1 μg/ml)	+	+	+	+
Oleate (250 μg/ml)	−	+	−	−

[a] +, positive result; −, negative result; V, variable; ±, positive and negative variants.

Mycobacterium intermedium (11). Although the two patterns share almost all the major peaks, the relative heights of some of them evidently differ; the pattern for *M. intermedium* (reference strain DSM 40049) is characterized by an unique cluster of peaks, whereas the low central peaks of our isolate give the impression of a double cluster. On the contrary, the HPLC pattern of FI-3695 is clearly different from the one previously reported for *M. interjectum* (16), which is shared by the reference strain (DSM 44064) on which the sp. nov. description was based (14) and by a subsequently reported clinical isolate (16).

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129                     172
TGA TCT GCC CTG CAC TTC / TAC CGG ATA GG-ACCA CGG GAT GCA TGTCT-TGT GGT   M. tuberculosis
.A. ... ... ... ... ...   ... ... ... ..-...T ..A .GC ... ..C..-... ...   M. interjectum
.A. ... ... ... ... ...   ... ... ... ..-...T ..A .GC ... ..C..-... ...   FI-3695
.A. ... ... ... ... ...   ... ... ... ..-.... .TT .GC ... ..G..-... ...   M. simiae
... ... ... ... ... ..T   ... ..A ... TTCC.TA TT. .TC ... ..G.CTG.. A.G   M. flavescens
... ... ... ... ... ..T   ... ..A ... ..-...G .AT .C. ... ..GTG-... ...   M. nonchromogenicum
... ... ... ... ... .CT   ... ... ... ..-.... T.. ... ... ...TC-... ...   M. terrae
... C.. ... ... ... ...   ... ... ... ..-.... TTC TGC ... ...GG-G.. ...   M. xenopi
.A. ... ... ... ... A..   ... ..A ... ..-.... .A. ..C A.. ....C-... ...   M. gordonae
C.. ... ... ... ... ...   ... ... ... ..-.... ... ... T.. ....C-... ...   M. marinum/M. ulcerans
CA. ... ... ... ... ...   ... ... ... ..-.... .TT .GC ... ..C..-... ...   M. scrofulaceum
.A. ... ... ... ... ...   ... ... ... ..-...C ..A .GC ... ..C..-..G ...   M. szulgai
.A. ... ... ... ... ...   ... ..A ... ..-...C ..A .GC ... ..C..-..G ...   M. malmoense
CA. ... ... ... ... AC.   ... ... ... ..-.... .TT .GC ... ..C..-... ...   M. gastri/M. kansasii
CA. ... A.. ... ... ...   ... ... ... ..-...T .AA ..C ... .....-.C. ...   M. avium
CA. ... ... ... ... ...   ... ... ... ..-...T TTA .GC ... .....-.TA ...   M. intracellulare
.A. ... ... ... ... ACT   ... ... ... ..-...T .TC .GC ... ..C..AG.A ...   M. intermedium
.A. ... ... ... ... ...   ... ... ... T.-.... ... A.C ... ...T.-... ...   M. genavense
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FIG. 1. Partial alignment within hypervariable region A of selected mycobacterial 16S rRNA sequences. The sequence from *Mycobacterium tuberculosis* was used as the reference sequence. Only nucleotides different from those in the *M. tuberculosis* sequence are shown; dashes indicate deletions; dots indicate identical nucleotides. The respective *E. coli* 16S rRNA positions are indicated above the sequences.

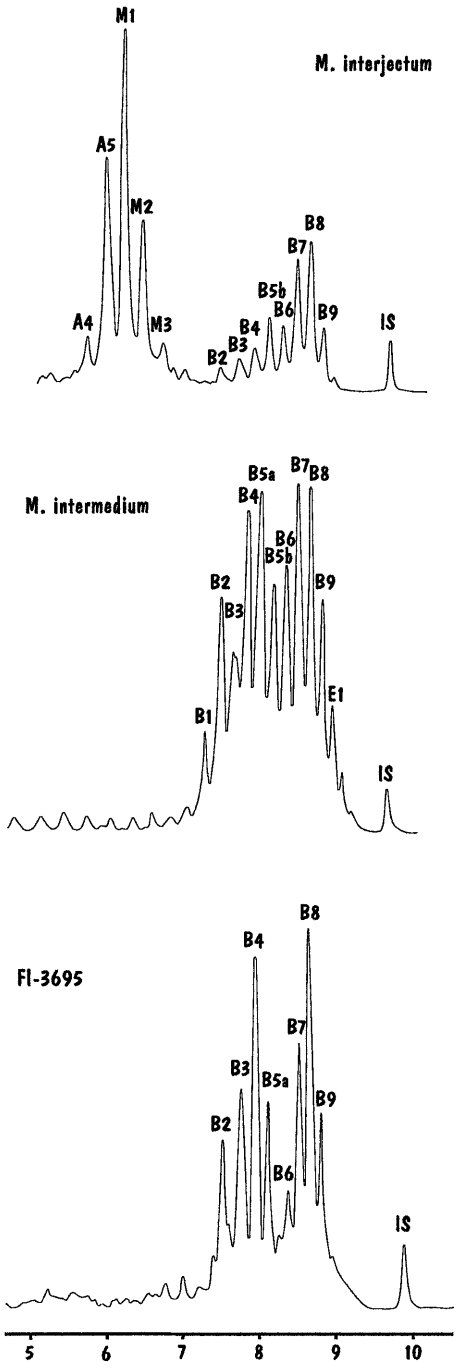

FIG. 2. Comparison of the HPLC mycolic acid profiles of *M. interjectum*, *M. intermedium*, and our isolate. IS, internal standard.

The antimicrobial agent susceptibility pattern of this strain (Table 2) is characterized by low MICs of all the drugs tested, except ethambutol.

A steadily expanding group of recently recognized mycobacteria is defined by a unique phylogenetic position. Phenotypically, this group is characterized by slow growth, yet it shows a short helix 18, the indicator signature of rapidly growing species. These species, including *Mycobacterium simiae*, *M. interjectum*, *Mycobacterium genavense*, and *M. intermedium*, have identical nucleic acid sequences in hypervariable region B but differ significantly in region A (10).

TABLE 2. Susceptibility pattern of our isolate

Drug	MIC (μg/ml)
Amikacin	≤1
Ciprofloxacin	≤0.5
Clarithromycin	≤1
Clofazimine	0.12
Ethambutol	8
Ofloxacin	≤0.5
Rifabutin	≤0.25
Rifampin	≤0.5
Sparfloxacin	≤0.5
Streptomycin	≤1

Sequences within the signature regions of 16S rRNA are characterized by species specificity; in fact, only the following two pairs of slowly growing mycobacteria present identical signatures (10): *Mycobacterium gastri* with *Mycobacterium kansasii* and *M. marinum* with *Mycobacterium ulcerans*; however, the latter taxa are differentiable by extending the sequence determination outside regions A and B (6).

HPLC mycolic acid patterns are characterized by an equally good species specificity; so far, only for *Mycobacterium gordonae* have two different profiles been reported (5); within this species, however, three genotypes, which differ at one or two nucleotides in hypervariable region A (8), are also present.

It is not possible at present to ascertain whether our strain indeed belongs to the species *M. interjectum*, thus establishing two different HPLC profiles within this taxon, or whether it is the first isolate of a new species which shares an identical signature region within the 16S rRNA with *M. interjectum*. An isolate of *M. interjectum* with an anomalous HPLC pattern was reported in a recent cooperative study of the International Working Group on Mycobacterial Taxonomy (18); the planned comparison of the two strains will hopefully provide further information.

No speculation about the clinical significance of our strain is possible; in fact, a single isolation from a nonsterile sample (sputum) does not allow a diagnosis of infection.

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