

Characterization of an Isolate Belonging to the Newly Described Species *Mycobacterium hassiacum*

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The isolation, from a urine sample, of a rapidly growing acid-fast mycobacterium assigned to the thermophilic species Mycobacterium hassiacum led to further insight into present knowledge of this newly described organism. Already known phenotypic traits of M. hassiacum were extended and its susceptibility to additional antimicrobials was investigated. The

high-performance liquid chromatography pattern of mycolic acids is, for the first time, presented. So far, no clinical relevance was proved for our isolate; likewise for the one which led to the species' original description. © 1998 Elsevier Science Inc.

INTRODUCTION

Several previously unrecognized mycobacterial species have been recently described; most of them have been isolated from HIV-infected patients (Böttger et al. 1993; Butler et al. 1993; Floyd et al. 1996; Koukila-Kähkölä et al. 1995; Meier et al. 1993; Springer et al. 1993, 1995, 1996). In many cases, the description of new mycobacteria was feasible only by resorting to sophisticated techniques that allow the differentiation of organisms otherwise undistinguishable on the basis of conventional tests alone. The recently described *Mycobacterium hassiacum* (Schröder et al. 1997) seems to escape this rule, as it can be easily characterized by phenotypic features, such as its extreme thermotolerance, representing an evident clue for its recognition.

The present characterization of an isolate of *M. hassiacum*, cultured from the urine of an immuno-

competent patient, reports the first isolation of this organism after the species' original description.

PATIENT AND METHODS

In 1994, a 45-year-old female suffering from hypertension and asthma was hospitalized with symptoms compatible with recurrent cystitis. Bacterial cultures from urine remained negative or presented scanty growth, clearly below the limit of 10^5 CFU discriminating contaminations; equally negative were cultures for mycobacteria. The patient was treated with a broad spectrum cephalosporin without success. When hospitalized again 5 months later, a homemade polymerase chain reaction (PCR) assay indicated presence of DNA from *Mycobacterium tuberculosis*, and treatment was started with isoniazid, ethambutol, and rifampin, followed by initial improvement. However, 6 weeks later, urine culture on Lowenstein-Jensen medium did not yield *M. tuberculosis*, but about 20 colonies of an unknown mycobacterial species, later identified as *M. hassiacum*. Therefore, the treatment was changed for ofloxacin according to the results of drug susceptibility testing. When symptoms went worse, ofloxacin was changed again for the former antituberculosis regimen, which was maintained for 4 months; at that moment the

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Received 6 August 1997; accepted 3 December 1997.

patient reported recurrent slight fever; another urine samples were collected but remained negative for mycobacteria, as well as a home-made PCR assay for *M. tuberculosis*.

The identification of the unique isolate from urine was first attempted by conventional biochemical and cultural tests, following standard procedures (Nolte et al. 1995). The strain was then submitted to high-performance liquid chromatography (HPLC) of cell wall mycolic acids according to the standard procedure (Butler et al. 1992) using a C₁₈ Ultrasphere XL cartridge column on a System Gold model HPLC instrumentation. Genetic analysis by sequencing of PCR-amplified 16S rDNA was finally performed and hypervariable regions of the gene were compared to published sequences (Kirschner et al. 1993; Schröder et al. 1997).

Antimicrobial susceptibility was tested following the agar disk elution method recommended for rap-

TABLE 1 Comparison of Our Results of Conventional Biochemical, Cultural, and Inhibition Tests Performed in Parallel on Our Isolate and the Reference Strain of *M. hassiacum*

Test	Our Isolate	DSM 44199
Niacin	-	-
Nitrate reduction	-	-
Thermostable catalase	+	+
β -glucosidase	-	-
Tween 80 hydrolysis (10 days)	+	+ ^a
Tellurite reduction	+	+
Arylsulfatase (3 days)	-	-
Urease	+	+
Catalase (over 45 mm of foam)	+	+
Photochromogenicity	-	-
Scotochromogenicity	+ ^b	+ ^b
Growth at 25°C	+ ^c	+ ^c
Growth at 37°C	+	+
Growth at 45°C	+	+
Growth at 65°C	+	+
MacConkey	-	-
Tolerance to:		
<i>p</i> -nitrobenzoate (500 μ g/ml)	+	+
NaCl (5%)	+ ^d	+ ^d
Thiophene-2-carboxylic hydrazide (5 μ g/ml)	+	+
Thiacetazone (10 μ g/ml)	+	+
Hydroxylamine (500 μ g/ml)	-	-
Isoniazid (1 μ g/ml)	+	+
Oleate (250 μ g/ml)	+	+
Growth rate	6 days	5 days
Colonial morphology	Smooth	Smooth

^a Reported as negative in the original species description.

^b The pigmentation increases after exposition to light.

^c Growth evident only after several weeks of incubation, reported as negative in the original species description.

^d Growth scanty and delayed.

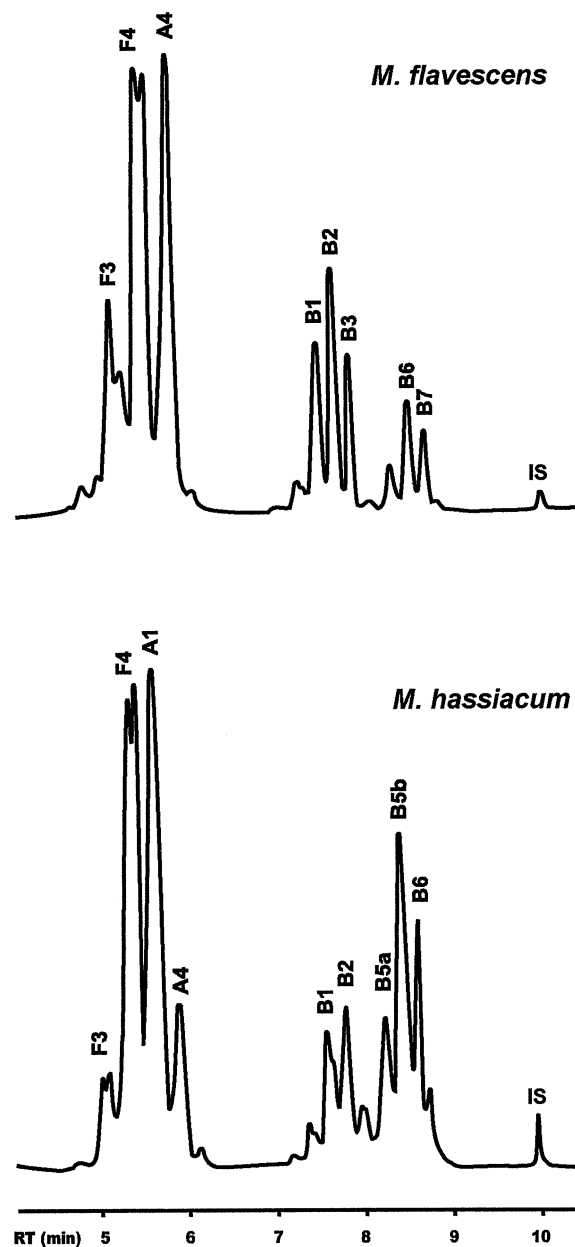


FIGURE 1 HPLC representative patterns of mycolic acid bromophenacyl ester of *M. flavescens* and *M. hassiacum*. RT, retention time; IS, internal standard.

idly growing mycobacteria (Brown et al. 1992) using an enlarged antimicrobial panel, which also included antituberculosis drugs.

RESULTS

The isolate, characterized by short, partially acid-fast rods, grew in culture in a week, yielding smooth, rubbery, pale yellow colonies whose pigmentation increased after exposition to light. In 1995, the results of conventional biochemical tests (Table 1) did not allow us to assign the mycobacterium to any species

known at that time; the growth at 45°C, the highest temperature usually applied in our panel of conventional tests, was not considered a discriminating feature and did not suggest the investigation of tolerance to higher temperatures. HPLC analysis yielded a mycolic acid pattern (Figure 1) partially resembling the one of *Mycobacterium flavescens*. Sequencing of the 16S rDNA, finally performed several months later, revealed a sequence identical to the newly described *M. hassiacum* (Genbank MHU49401). Subsequent extension of investigation of phenotypic traits, carried out in parallel on our isolate and on the reference strain (DSM 44199), revealed the identity of further conventional features including 65°C tolerance (Table 1) and overlapping of HPLC patterns. Parallel antimicrobial susceptibility testing of our isolate and of the reference strain revealed sensitivity to amikacin (6 µg/ml), ciprofloxacin (2 µg/ml), clarithromycin (1 µg/ml), doxycycline (6 µg/ml), ethambutol (5 µg/ml), imipenem (8 µg/ml), streptomycin (2 µg/ml), tobramycin (8 µg/ml), and trimethoprim (1.5 µg/ml)-sulfamethoxazole (28.5 µg/ml), and resistance to isoniazid (1 µg/ml) and rifampin (1 µg/ml); discordant results were found for cefoxitin (30 µg/ml), where our strain was found to be sensitive and the reference strain turned out to be resistant.

DISCUSSION

Several features support the evidence that our isolate belongs to the newly described species *M. hassiacum*: conventional biochemical tests (Table 1) yielded identical results to those reported for the reference strain. Tween hydrolysis was positive in our isolate, but was reported negative in the original species description; however, in our hands, this test was positive for the reference strain as well. Furthermore, concordant results were obtained for previ-

ously unreported biochemical features that were tested in parallel on our strain and the reference strain: β-glucosidase, tellurite reduction, tolerance to *p*-nitrobenzoate, thiophene-2-carboxylic acid hydrazide, thiacetazone, oleate hydroxylamine, and growth on MacConkey agar (Table 1). When subjected to HPLC, our isolate showed the same distinct pattern of mycolic acid as could be obtained for the reference strain. The resemblance to *M. flavescens* (Figure 1) turned out to be only partial, as single peaks, once identified on the basis of their relative retention times, appeared to be differently arranged.

The comparison of the entire 16S rDNA sequence of >1400 bp to the reference sequence published confirmed the identification of the strain as *M. hassiacum*. The antimicrobial susceptibility of *M. hassiacum*, practically overlapping for our strain and the reference strain, appeared to be characterized by resistance to the major antituberculosis drugs isoniazid and rifampin and by susceptibility to a large panel of broad-spectrum molecules; this pattern is not rare among rapidly growing mycobacteria. The positive *M. tuberculosis* PCR can be interpreted as a result of a laboratory contamination; urine samples spiked with cells of *M. hassiacum* in fact, failed the amplification. No information is yet available on the clinical significance of *M. hassiacum*. The isolation of a new, atypical mycobacterium from one single urine sample is difficult to interpret; the pathogenicity of the strain in the particular case presented here cannot be excluded in the presence of reported symptoms and of an apparent response to antituberculosis treatment. With regard to the original species description, originating also from a urine sample (Schröder et al. 1997), it would therefore be of interest to investigate clinical data from similar cases to assess the pathogenicity of the novel species.

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