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# RESEARCH NOTE

## Isolation of a novel sequevar of *Mycobacterium flavescens* from the synovial fluid of an AIDS patient

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### ABSTRACT

This report describes the characterisation of a mycobacterium involved in a case of septic arthritis in an AIDS patient that was treated successfully with specific anti-mycobacterial drugs. The biochemical and cultural features, and the mycolic acid pattern as assessed by high-performance liquid chromatography, were fully compatible with the isolate being *Mycobacterium flavescens*. However, the isolate's 16S rDNA sequence differed by five nucleotides from the two known sequevars of *M. flavescens*, thus indicating that this isolate belonged to a new 16S rDNA sequevar.

**Keywords** AIDS, identification, *Mycobacterium flavescens*, 16S rDNA

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*Mycobacterium flavescens* is a scotochromogenic mycobacterium, characterised by an intermediate

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growth rate, that was described for the first time in 1962 following isolation from a drug-treated tuberculous guinea-pig [1]. Although the isolation of *M. flavescens* from human specimens is not uncommon, only in extremely rare cases has this organism been considered to be responsible for disease. The authoritative review by Wayne and Sramek [2] reported only five human infections 'tentatively or confidently attributed to *M. flavescens*', and noted that none of these cases provided 'sufficient description of the organism to permit the identification to be evaluated'. Five further cases, again with few details of the identification of the organism, have been reported subsequently [3–7].

The present report describes a case of septic arthritis attributed to an organism with the distinctive phenotypic features of *M. flavescens* and a unique nucleotide sequence within the 16S rRNA gene that closely resembled the sequences characterising this species. Conventional investigations included all of the biochemical and cultural tests performed most frequently [8]. The cell-wall mycolic acids were investigated by high-performance liquid chromatography (HPLC) analysis as recommended by the Centers for Disease Control and Prevention for the UV detection of mycolic acid following its derivatisation to bromophenacyl esters [9]. Antimicrobial susceptibilities to ciprofloxacin, clarithromycin, ethambutol, rifabutin, rifampicin and streptomycin were investigated by determining the MICs in liquid radiometric medium following the procedure recommended for organisms belonging to the *Mycobacterium avium* complex [10]. The complete sequence of both strands of the 16S rDNA was determined as described previously [11].

The organism, coded FI-28796, was isolated in 1996 from a 34-year-old haemophilic male, who had been HIV-seropositive since the age of 22 years and was recognised as an AIDS case 11 years later following the development of oesophageal candidosis. The patient was hospitalised because of symptoms consistent with acute septic arthritis (fever, pain, swollen joints). The synovial fluid yielded by arthrocentesis was yellowish in colour and dense in appearance, and microscopy revealed giant-cell phlogosis. The $CD4^+$ lymphocyte count was 1 cell/mL and the HIV-1 load was 446 911 copies/mL. Following the isolation of an acid-fast bacillus from the fluid, empirical treatment with clarithromycin, ciprofloxacin and ethambutol was initiated. MICs for the isolate were as follows: ciprofloxacin ≤1 mg/mL, clarithromycin ≤2 mg/mL, ethambutol ≤2 mg/mL, rifabutin 1 mg/mL, rifampicin 2 mg/mL and streptomycin ≤2 mg/mL, thus confirming that the empirical treatment was appropriate. Treatment was discontinued after 4 months (clarithromycin was discontinued 1 month earlier), at which time the articular symptoms had resolved completely; there has been no relapse to date.

The mycobacterial isolate was identified initially as *M. flavescens* by HPLC and cultural features such as an intermediate growth rate at 25–37°C, the scotochromogenic pigmentation of the smooth colonies, tolerance to NaCl 5% w/v, and positive tests for nitrate, 68°C catalase, semiquantitative catalase (> 45 mm), Tween-80 hydrolysis and urease. The 16S nucleotide sequence (GenBank accession number AJ536296) of the isolate differed from any others reported so far. Three species, i.e., *Mycobacterium brumae*, *Mycobacterium goodii* and *M. flavescens*, shared

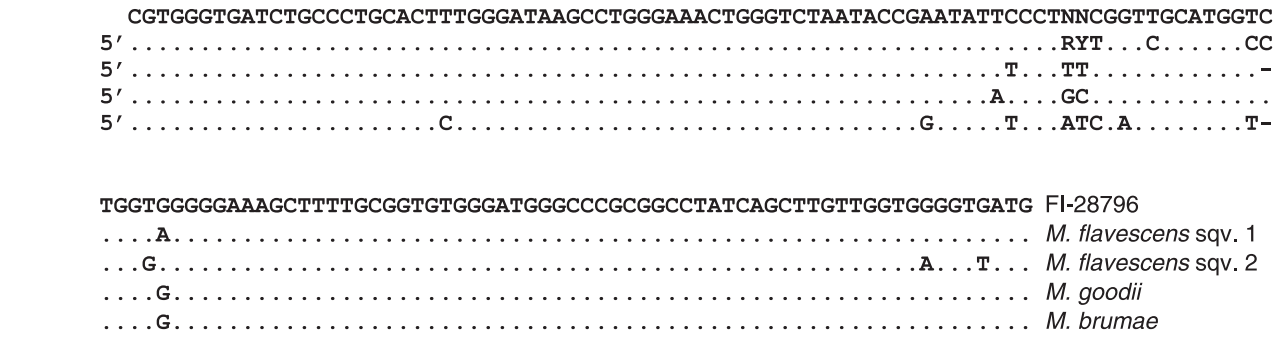


**Fig. 1.** Alignment of 16S rDNA, including the hypervariable region A, from FI-28796, *Mycobacterium flavescens*, *Mycobacterium brumae* and *Mycobacterium goodii*, commencing with position corresponding to base 118 on the *Escherichia coli* 16S rDNA. sqv., sequevar.

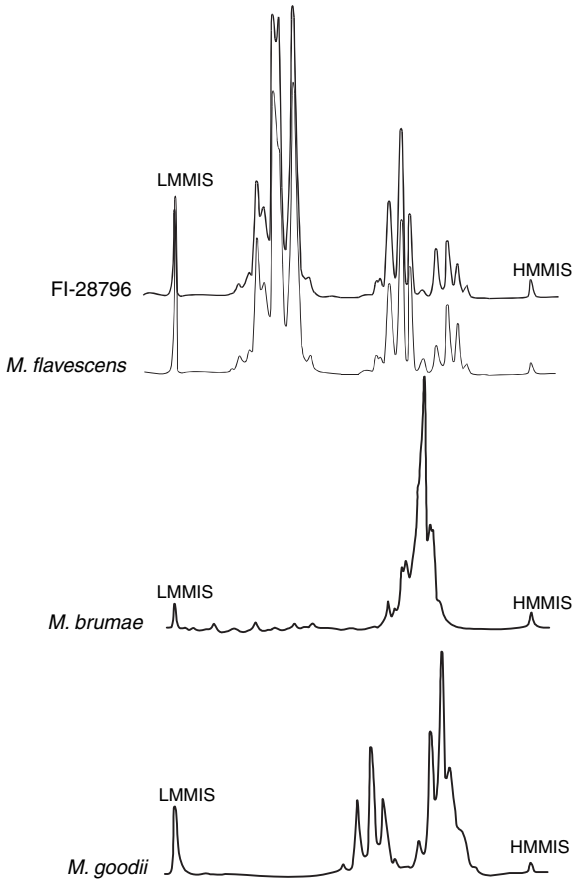


**Fig. 2.** Representative HPLC patterns of FI-28796, *Mycobacterium flavescens*, *Mycobacterium brumae* and *Mycobacterium goodii*. LMMIS, low molecular mass internal standard; HMMIS, high molecular mass internal standard.

the highest matching score (95% identity) [12]. If indeterminate nucleotides were considered as matching, each species had five mismatches in the hypervariable region A (Fig. 1). Of the three possibilities, only *M. flavescens* appeared to be phenotypically compatible with FI-28796, as the other species are non-chromogenic and grow rapidly with completely different HPLC patterns (Fig. 2). The mismatch of five nucleotides within a stretch of 150 bases compares with the discrepancy of six bases located in the hypervariable region A and in the adjacent trait (Fig. 1) that distinguishes sequevars 1 and 2 of *M. flavescens*. Therefore, combined with the excellent match of the phenotypic features, it seems that FI-28796 represents a third, previously undescribed, sequevar of the species *M. flavescens*.

In conclusion, this is the first report in which the in-depth characterisation of the isolate, according to recent recommendations [13], is consistent with the involvement in human infection of an organism belonging, or at least highly related, to the species *M. flavescens*. Although repeated joint bleeding and the patient's immunosuppression were the predisposing factors for the arthritis, the systemic (fever) and local (swelling, pain) signs of infection, coupled with response to specific anti-mycobacterial treatment, suggests that the isolated organism was the causative agent of the infection.

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