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Notes

Mycobacterium sherrisii isolation from a patient with pulmonary disease

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Abstract

The characterization of 2 clinical isolates of *Mycobacterium sherrisii* provides further phenotypic and genotypic information beyond that reported in the article that originally described this species. One of our strains was responsible for pulmonary disease in a middle-aged non-HIV patient; thus, confirming the potential pathogenicity of this species previously reported only in an HIV-positive patient.

Keywords: Genetic sequencing; *Mycobacterium sherrisii*; Pulmonary disease; Pathogenicity

The not yet officially recognized species, *Mycobacterium sherrisii*, belongs to a heterogeneous group of mycobacteria that share with *Mycobacterium simiae* a unique genetic marker. In such organisms, helix 18, one of the loops resulting from the secondary structure of 16S rRNA, is in fact 12 nucleotides shorter than in any other slow grower, which apparently reproduces the genetic trait characteristic of rapid growers (Stahl and Urbance, 1990). Although helix 18 is in hypervariable region B, which contains some single-base substitutions that differentiate most mycobacterial species, it is identical in all members of the group of *M. simiae*-related organisms (Tortoli, 2003). In the last 13 years, investigations of phenotypic and genotypic features of various *M. simiae*-like strains have led to the recognition of 14 new species.

Recently, the article describing the new species, *M. sherrisii* (Selvarangan et al., 2004), noted that the 16S rDNA sequence of the novel mycobacterium was identical, at least in the hypervariable regions A and B, to that of the 2 strains reported in our article characterizing several *M. simiae*-like mycobacteria (Tortoli et al., 1997). This remark urged us to retrieve the 2 strains from the laboratory collection for further characterization.

One of the strains had been isolated in 1996 from a 58-year-old man with a history of rheumatoid arthritis who was hospitalized because of articular pain, low-grade fever, and weight loss. A chest computed tomography scan revealed 2 small infiltrates in the upper lobe of the left lung and a pleural effusion. A standard tuberculin test was positive. Histopathologic examination of a pleural biopsy specimen showed noncaseating granulomas with central necrosis and Langerhans giant cells; no acid-fast bacilli were seen. After 3 weeks in liquid medium (Bactec 12B; Becton Dickinson, Sparks, MD), the pleural biopsy specimen yielded a slowly growing mycobacterium. The strain could not be fully identified using biochemical tests, whereas high-performance liquid chromatography revealed a pattern resembling that of *Mycobacterium lentiflavum*; the genetic sequencing, limited to the hypervariable regions A and B of the 16S rDNA, did not reveal identity with any of the species known at that time, but suggested that it was genetically related to the species *M. simiae* (Tortoli et al., 1997).

Antitubercular therapy with isoniazid, rifampin, ethambutol, and pyrazinamide was undertaken, but after 4 months, no significant reduction of lung infiltrates or pleural effusion was evident. Information about subsequent developments is not available.

The other strain had been isolated in the previous year. In this case, the source was a single smear-negative sputum sample from a 53-year old man with cough.

On both strains retrieved from the laboratory collection, the 1st third of the 16S rDNA (Cloud et al., 2002), the

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16S–23S rDNA internal transcribed spacer (ITS) (Roth et al., 1998), and a trait of about 400 bp within the gene encoding for the 65-kDa heat shock protein (*hsp65*) (McNabb et al., 2004) were sequenced. The 2 strains were 100% identical in all such regions. The sequence of the whole 5′ end (500 bp) of the 16S rDNA was in agreement with earlier sequences of their hypervariable regions A and B (Tortoli et al., 1997) and revealed 100% identity to the *M. sherrisii* sequence present in GenBank. The ITS sequence we deposited in GenBank (accession number DQ185132), where no other sequence of this region was present for *M. sherrisii*, turned out to be most closely related to that of *M. simiae* from which it differed by 13 bp. The *hsp65* sequence (accession number DQ523524) revealed 1 nucleotide difference (cytosine in place of adenine at position 631) from that of *M. sherrisii* and 3 nucleotide difference from *M. simiae*.

The similarity of *M. sherrisii* and *M. simiae* in the ITS led us to assay the test strains with the commercial inverse hybridization system INNO LiPA Mycobacteria (Innogenetics, Belgium), which relies on the ITS variability. As suspected, both hybridized with the line probe specific for *M. simiae*. Furthermore, both of our strains presented a 2nd band corresponding to the probe specific for *Mycobacterium gordonae*. This band was indeed very weak but, nevertheless, recognizable. Because the strains were tested at different times, the possibility of an accidental finding seems unlikely, particularly because the INNO LiPA strips are known to provide very clean patterns (Tortoli et al., 2003).

Results of biochemical and cultural tests of our strains were identical to the ones reported for the type strain (Selvarangan et al., 2004) as follows: nitrate reduction (−), catalase at 68 °C (+), Tween 80 hydrolysis (−), urease (+), and slow growth rate at temperatures ranging from 25 to 37 °C. Furthermore, they scored negative for 3-day arylsulfatase, growth on MacConkey agar without crystal-violet, and growth on Lowenstein–Jensen with 5% NaCl, whereas they scored positive for tellurite reduction. Niacin accumulation and semiquantitative catalase, variable in the original description, were negative and positive, respectively, in both of our strains. Although the colonies were initially white in both test strains, a pale yellow pigmentation developed after several hours of light exposure and a subsequent 2- to 3-day incubation at 37 °C.

Susceptibility testing performed by a macrodilution quantitative method in liquid medium (Siddiqi et al., 1993) revealed that the strains were fully resistant to ciprofloxacin, ofloxacin, isoniazid, and rifampin. They were borderline resistant to amikacin, ethambutol, and streptomycin, and moderately susceptible to clarithromycin and rifabutin. Such data, although achieved with different techniques, practically overlap the ones reported in the sp. nov. description and confirm the already reported multidrug resistance of most species genetically related to *M. simiae* (Tortoli et al., 1997).

No clinical information is provided in the article describing *M. sherrisii* sp. nov., but very recently, a pulmonary infection due to *M. sherrisii* has been reported in an AIDS patient (Gamperli et al., 2005). Both of our strains had been grown from the lungs of middle-aged males. Although the sputum isolate did not fulfill the ATS criteria for clinical significance (ATS, 1997), the pleural isolate unquestionably exhibited pathogenic activity. It had been obtained from a biopsy specimen which, on histology, presented typical granulomatous lesions. The case reported here is the 2nd in which *M. sherrisii* is responsible for pulmonary disease in humans and the 1st in a non–HIV-infected patient.

In conclusion, as for other nontuberculous species, only genetic sequencing seems suitable to correctly identify *M. sherrisii* and to differentiate it from *M. simiae*. The sequences of ITS and of a new sequevar of *hsp65*, made available by us in GenBank, prove that these targets represent effective alternative to the 16S rDNA. Attention should be paid by users of INNO LiPA system to the possible presence of a weak band level with that from the *M. gordonae* probe, on several of the strips presenting the hybridization pattern typical of *M. simiae*. This might in fact be a clue that the isolate belongs to the species *M. sherrisii*.

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