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Cross-Reactivity of Genetic Probe for Detection of *Mycobacterium tuberculosis* with Newly Described Species *Mycobacterium celatum*

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An acridinium ester-labeled DNA probe (AccuProbe; Gen-Probe Inc., San Diego, Calif.) for the identification of the *Mycobacterium tuberculosis* complex gave discrepant results with the newly described species *M. celatum*. Examination of 20 strains of *M. celatum* showed that 8 were positive with the probe; the remaining 12 were negative.

Prompt patient care for the treatment of *Mycobacterium tuberculosis* requires rapid identification of tubercle bacilli. For definitive diagnosis, the bacilli must be isolated and cultured. Conventional methods are laborious, time-consuming, and subject to error. Tuberculosis control efforts are further complicated by the emergence of drug resistance. The Centers for Disease Control and Prevention has advised using the latest technologies available for the rapid processing and identification of *M. tuberculosis* from clinical specimens (4, 8). Genetic probes that confirm the identity of members of the *M. tuberculosis* complex isolated in culture are in widespread use and allow recognition of this organism weeks before identification by conventional biochemical reactions (3). In actual laboratory use, the *M. tuberculosis* complex probe (AccuProbe; Gen-Probe Inc., San Diego, Calif.) has proven to be highly sensitive and specific (5). Discrepant reactions are rare but have been reported for *M. terrae* and for an unidentified species (6, 10).

Recently, we described a new species of mycobacteria, which we named *M. celatum* (1, 9). This species was initially recognized by biochemical reactions to be similar to *M. avium* but presented a mycolic acid pattern that was *M. xenopi* like (2, 7). *M. celatum* was further differentiated into types 1 and 2. Conventional biochemical identification methods were not able to separate the two types. Differentiation was made by restriction fragment length polymorphism (RFLP) analysis of the amplified sequence of the Hsp65 gene, multilocus enzyme electrophoresis, and 16S rRNA sequence analysis (1). Both types were deposited with the American Type Culture Collection as ATCC 51131^T and ATCC 51130, representative of types 1 and 2, respectively. The purpose of this correspondence is to report the cross-reactivity between *M. celatum* type 1 and the genetic probe for the *M. tuberculosis* complex and the subsequent ability of the probe to differentiate the *M. celatum* types. The frequency of isolation of *M. celatum* was determined.

Initial mycobacterial isolation procedures were used, and the isolates were processed by the clinical laboratory by testing for *M. tuberculosis* with the *M. tuberculosis* complex genetic probe (AccuProbe) according to the instructions of the manufacturer (Gen-Probe). Target RNA from a sonicated lysate of a mycobacterial cell suspension was hybridized to the acridinium ester-labeled DNA probe (AccuProbe). The amount of chemiluminescence emitted from hydrolysis of the DNA-RNA complex was estimated with a luminometer and quantified as relative light units (RLUs). Results were interpreted according to the criteria of the manufacturer. The expected values were ≥30,000 RLUs for a positive isolate and <30,000 RLUs for a negative isolate.

The sources of the 20 *M. celatum* strains, biochemical characteristics, varied geographic distribution, RFLPs, multilocus enzyme electrophoresis results, and partial 16S rRNA sequence have been described elsewhere (1). In brief, RFLP analysis grouped the strains into two types. Eight strains were type 1, with seven DNA bands, and 12 were type 2, with six bands, indicative of a single restriction site difference. Analysis of 14 enzymes by multilocus enzyme electrophoresis clustered the strains into two different groups. Each group contained the same strains as the two types demonstrated by RFLP analysis. Additionally, 16S rRNA sequencing indicated a 10-base difference between the *M. celatum* types. Because a cutoff point for the differentiation of species of mycobacteria has not been established for 16S rRNA sequence differences and because we were unable to recognize the two different types phenotypically, we defined the types together under a single designation, i.e., *M. celatum*.

To determine the clinical occurrence of *M. celatum* strains, a retrospective study for the last 5 years was conducted by the Veterans Affairs reference laboratory in West Haven, Conn. *M. celatum* was identified in this analysis by tabulating the number of isolates showing the following characteristics: inability to grow at 45°C, a positive 3-day arylsulfatase reaction, resistance to 2 μg of rifabutin per ml, and a negative reaction with the *M. avium* complex nucleic acid probe. These isolates had been identified as "nonphotochromogen, unable to identify."

Reference strains run in the genetic probe assay included *M. tuberculosis* ATCC 25177 as a positive control and *M. intracellulare* ATCC 13950 as a negative control. The RLUs for the positive control were 651,558, over 200 times the RLUs for the negative control. The eight strains of *M. celatum* type 1 had RLUs (average ± standard deviation) of 314,662 ± 68,882; values ranged from 203,175 to 394,467.

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FIG. 1. Alignment of partial 16S rRNA gene sequences for *M. tuberculosis*, *M. celatum* type 1 (ATCC 51131^T), and *M. celatum* type 2 (ATCC 51130). Boxed letters indicate the lack of a consensus among the three sequences.

The 12 strains of *M. celatum* type 2 had (average RLUs ± standard deviation) of 4,225 ± 1,783; values ranged from 2,288 to 8,152.

Since *M. celatum* type 1 reacted with the *M. tuberculosis* probe and *M. celatum* type 2 did not, we compared 16S rRNA sequences for the two types and for *M. tuberculosis* (Fig. 1). The homologous sequence alignment shown in Fig. 1 is for 450 bases of the partial 16S rRNA gene sequence. A comparison of the remaining 1,110 nucleotides between *M. celatum* types 1 and 2 and *M. tuberculosis* did not reveal any differences (1). Genetic probes are short oligonucleotide sequences; therefore, *M. tuberculosis* and *M. celatum* type 1 were expected to contain a limited, homologous sequence region. Furthermore, *M. celatum* type 2 was expected to demonstrate some oligonucleotide variability in this region. Bases found at positions 131, 132, 135, and 148 of the sequence meet this criterion. As expected, this region is within the target sequence region for the commercially available, proprietary probe (Gen-Probe) for the *M. tuberculosis* complex (2a, 10).

Since a false-positive result could result in an incorrect diagnosis and inappropriate treatment for tuberculosis, we attempted to define the occurrence of this species in the clinical laboratory. The rate of isolation of *M. celatum* was determined by examination of 13,530 laboratory isolates over a period of 5 years. The retrospective analysis revealed 24 isolates of *M. celatum* from 17 different patients (Table 1). The rate of isolation of both types of *M. celatum* was only 0.1%. Previous studies had shown the occurrence of *M. celatum* type 1 to be approximately 40% of the total number

TABLE 1. Five-year, retrospective examination of the rate of isolation of *M. celatum*

Yr	No. of mycobacterial isolates examined[a]	No. of isolates of *M. celatum*	No. of patients
1989	3,361	8	6
1990	3,264	5	5
1991	2,812	0	0
1992	3,110	10	5
1993	983	1	1

[a] Isolates were submitted for reference studies as clinically significant to the Veterans Affairs reference laboratory in West Haven, Conn.

of *M. celatum* isolates examined (1). Therefore, the rate of isolation of *M. celatum* type 1 is 0.05%.

In summary, *M. celatum* is a rarely encountered species that may be responsible for discrepant reactions with genetic probes for the *M. tuberculosis* complex. However, probes remain highly sensitive and specific for use in the clinical laboratory as a means for rapidly identifying isolates of the *M. tuberculosis* complex.

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