Mycobacterium novocastrense sp. nov., a Rapidly Growing Photochromogenic Mycobacterium


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A strain isolated from a biopsy sample taken from a slowly spreading skin granulation on a child’s hand was found to have properties consistent with its classification in the genus Mycobacterium. An almost complete gene sequence of the 16S rRNA of the strain was determined following the cloning and sequencing of the amplified gene. The sequence was aligned with those available for mycobacteria, and phylogenetic trees were inferred with four tree-making algorithms. The organism, which formed a distinct phyletic line within the evolutionary radiation occupied by rapidly growing mycobacteria, was readily distinguished from members of validly described species of rapidly growing mycobacteria on the basis of its mycolic acid pattern and a number of other phenotypic features, notably its ability to form yellow pigmented colonies when incubated in the light. The name proposed for this new species is Mycobacterium novocastrense. The type strain is DSM 44203.

A relatively rapidly growing, weakly acid-alcohol-fast organism was isolated from biopsy tissue of a patient who had received six weeks of antituberculosis therapy. The strain had phenotypic properties consistent with its classification in the genus Mycobacterium, and these set it apart from other rapidly growing mycobacteria. In the present investigation, the organism was the subject of a polyphasic taxonomic study designed to clarify its taxonomic position. The results indicate that the organism represents a new species, for which the name Mycobacterium novocastrense is proposed.

MATERIALS AND METHODS

Organism and culture conditions. The organism (strain 73), which was isolated on Löwenstein-Jensen medium (9) after 9 weeks of growth at 36°C, was obtained from a biopsy sample taken from a slowly spreading skin granulation on the hand of a six-year-old child. It was then cultivated on Columbia blood agar (4), Löwenstein-Jensen medium (9), MacConkey agar (18), Middlebrook 7H10 agar (17), and 5% (wt/vol) sodium chloride agar (13) for between 3 and 10 days at 25°C and 36°C. The strain, which was maintained on Middlebrook 7H10 agar (17), was grown at 36°C in all of the remaining tests.

Phenotypic characterization. The growth, temperature, and sensitivity studies were carried out on Middlebrook 7H10 agar (17). The Gram (16) and Ziehl-Neelsen (20) stains were performed on cells grown for 5 days at 36°C on this medium. Catalase (14) and nicin (12) activity were examined after 10 days, and the production of arsulfatase (29) was examined after 3 and 14 days. Similarly, iron uptake (27) and Tween hydrolysis (28) were detected after 10 days at 36°C.

Extraction and analysis of mycolic acids. Lyophilized biomass from 10-day-old Middlebrook 7H10 agar plates was degraded by alkaline methanolysis (23) and two-dimensional thin-layer chromatography of the methanolysates carried out as described previously (19).

Sequencing of genes coding for 16S rRNA (16S rDNA). The biomass of the test strain needed for sequencing was obtained from a 5-day-old Löwenstein-Jensen (9) slant incubated at 36°C. The extraction and purification of DNA were carried out as described earlier (3), with specific modifications designed to optimize the isolation of DNA from the mycobacterial cells. Pretreatment of biomass with 0.5% (wt/vol) were applied to facilitate the susceptibility of cells to the standard digestion and extraction procedure of Pitcher et al. (22). The amplification, cloning, and sequencing of the 16S rDNA were carried out as described previously (3). The resultant 16S rDNA sequence was aligned manually with available sequences of rapidly growing mycobacteria by using the AL16S program (2). The additional sequence data were obtained from the GenBank and EMBL databases.

Evolutionary trees were inferred with four tree-making algorithms, namely, the Fitch-Margoliash (7), maximum-likelihood (5), maximum-parsimony (11), and neighbor-joining (24) methods. Evolutionary distance matrices for the neighbor-joining and Fitch-Margoliash methods were generated by the procedure of Jukes and Cantor (10). The PHYLIP package (6) was used for the neighbor-joining, Fitch-Margoliash, and maximum-parsimony analyses; the fast DNAml program (21) was employed for the maximum-likelihood method. The resultant unrooted tree topology was evaluated in bootstrap analyses (6) of the neighbor-joining method based on 1,000 resamplings with the SEQBOOT and CONSENSE programs in the PHYLIP package. The root positions of the unrooted tree based on the neighbor-joining method were estimated with four outgroup organisms (Gordonia terrae DSM 43249T [T = type strain], Rhodococcus erythropolis DSM 43066T, Rhodococcus equi DSM 20037T, and Rhodococcus fascians DSM 20669T), as described by Swafford and Olsen (26).

Nucleotide sequence accession number. The EMBL nucleotide sequence accession number for strain 73 is U96747.

RESULTS AND DISCUSSION

An almost complete 16S rDNA sequence (1,517 nucleotides) was obtained for strain 73. Comparison of this nucleotide sequence with available sequences for strains of the genus Mycobacterium showed that the organism fell within the evolutionary radiation encompassed by rapidly growing mycobacteria. The average nucleotide similarity value found between strain 73 and representative rapidly growing mycobacteria was 97 ± 0.5; the corresponding figure for slowly growing mycobacteria was 95 ± 0.5.

The nucleotide sequence of strain 73 shows substantial differences from the corresponding sequences of its nearest neighbors, namely, Mycobacterium flavescentis (98.4%), M. fortuitum (98.1%), M. phlei (97.4%), and M. senegalense (97.3%). A comparable scale of difference exists between the nucleotide sequences of validly described species of rapidly growing organisms. The positions of the test and marker strains in the phylogenetic tree were not markedly affected by either the tree-making algorithms or the outgroup strains used (Fig. 1). The sequence of strain 73, like those of other rapidly growing mycobacteria, contains the characteristic short helix at positions 451 to 482 (E. coli numbering system [1]). Nucleotide sequences which distinguish strain 73 from other rapidly growing mycobacteria are shown in Tables 1 and 2.
M. fortuitum.

M. chromogenicum

M. smegmatis

M. madagascariense

Strain 73

FIG. 1. Neighbor-joining tree (24) based on nearly complete 16S rDNA sequences of rapidly growing mycobacteria (1,398 nucleotides) showing the phylogenetic position of strain 73. The sequence for the type strain of *M. haisiacum* was not included in this analysis as the phylogenetic position of this rapidly growing organism is with the slowly growing mycobacteria (25). F, p, and m indicate branches that were also found by the Fitch-Margoliash (7), maximum-parsimony (11), and maximum-likelihood (5) tree-making methods, respectively; the asterisks indicate branches that were recovered with all four methods. The numbers at the nodes indicate the level of bootstrap support based on a neighbor-joining analysis of 1,000 resampled data sets; only values greater than 40% are given. The scale bar indicates 0.005 substitution per nucleotide position.

### TABLE 1. Selected stretches of the first nucleotide signature region at the 5′ end of the 16S rDNA

<table>
<thead>
<tr>
<th>Nucleotide signature&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. tuberculosis&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>M. chromogenicum</td>
</tr>
<tr>
<td>M. fortuitum</td>
</tr>
<tr>
<td>M. flavescens</td>
</tr>
<tr>
<td>M. madagascariense</td>
</tr>
<tr>
<td>M. phlei</td>
</tr>
<tr>
<td>M. smegmatis</td>
</tr>
<tr>
<td>M. thermoresistible</td>
</tr>
</tbody>
</table>

Strain 73

<sup>a</sup> Dashes indicate deletions. A dot indicates that the base pair was identical to the *M. tuberculosis* base pair. N, not determined.

<sup>b</sup> The numbers indicate the respective *E. coli* 16S rRNA positions (1).

<sup>c</sup> *M. tuberculosis* was used as the reference sequence.
places it as a distinct phylectic line within the evolutionary radiation of the rapidly growing mycobacteria. Isolated from a slowly spreading skin granulation of the hand of a child. The type strain of *M. novocastrense* is DSM 44203 (strain 73).

It is possible that *M. novocastrense* might be confused with *M. marinum*, another photochromogenic organism which causes self-limiting granulomatous skin lesions. However, the two organisms can be distinguished readily as only *M. thermoresistibile* is a slowly growing organism which has a mycolic acid profile different from that of *M. novocastrense*.

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**REFERENCES**


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bacteria. J. Chromatogr. 188:221-233.


### TABLE 2. Selected stretches of the second and third signature regions in the 16S rDNA corresponding to *E. coli* positions 439 to 494 and 1006 to 1023

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Nucleotide signature&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em></td>
<td>TCACCATCGACGAGTCGGGGTCTCTCAGGTTAGGGAGGCGTCTAGAGATAGGCGT</td>
</tr>
<tr>
<td><em>M. chromogenicum</em></td>
<td>GT.C-C...&lt;C-G.AA-G...AC...CGGG...GT.GN</td>
</tr>
<tr>
<td><em>M. confluens</em></td>
<td>G...&lt;C-GCAAG-G...A.C...TGGG...TCAG..GAC.GC...GT.GT</td>
</tr>
<tr>
<td><em>M. flavescens</em></td>
<td>G...&lt;C-GCAAG-G...CC..AT..AC.GG...GT.GT</td>
</tr>
<tr>
<td><em>M. fortuitum</em></td>
<td>AT.GG...&lt;C-GCAAG-G...CC..AT..AC.GG...GT.GT</td>
</tr>
<tr>
<td><em>M. madagascariense</em></td>
<td>G...&lt;C-GCAAG-G...T.C...TGGG...TCAG..GAC.GC...GT.GT</td>
</tr>
<tr>
<td><em>M. phlei</em></td>
<td>G...&lt;C-GCAAG-G...CC..AC..ACGGC...GT.GT</td>
</tr>
<tr>
<td><em>M. smegmatis</em></td>
<td>G...&lt;C-GCAAG-G...T.C...CCGGC...GT.GT</td>
</tr>
<tr>
<td><em>M. thermoresistibile</em></td>
<td>GTCG...&lt;C-GCAAG-G...CRC...CGGC...GT.GT</td>
</tr>
<tr>
<td>Strain 73</td>
<td><em>M. thermoresistibile</em></td>
</tr>
</tbody>
</table>

<sup>a</sup> See footnotes to Table 1 for explanation of terms.