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.

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 flavescens (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.
The phenotypic properties of strain 73 are also consistent with its classification in the genus *Mycobacterium* (15). The organism is aerobic, nonmotile, gram positive, and weakly acid-alcohol fast and is arylsulfatase positive after 14 days. It is also photochromogenic but is negative for iron uptake and Tween opacity. The detection of α-mycolates and wax esters, including α-carboxymycolates, in methanolysates of the strain distinguishes it from other photochromogenic rapidly growing mycobacteria, namely, *M. parafortuitum* and *M. vaccae*, but not from representatives of 17 other rapidly growing species, including *M. flavescens* and *M. haussiacum* (8, 25). However, of these species are scotochromogenic; the remaining two, *M. mortokaenis* and *M. pulverbis*, are nonpigmented.

It is evident from the chemical, 16S rDNA sequencing, and microbiological data that strain 73 has properties which distinguish it from all other validly described species of *Mycobacterium*. It is, therefore, proposed that this organism be classified in the genus *Mycobacterium* as *Mycobacterium novocastrense*.

**Description of Mycobacterium novocastrense sp. nov.** *Mycobacterium novocastrense* (ivo.vo. ca. sten'se L.adj. novus, -a, -um; L. n. noun castrum, castle, M.L.n. adj. novocastrense, pertaining to Newcastle, a city in the northeast of England).

Aerobic, gram-positive, weakly acid-alcohol-fast, asporogenous, nonmotile organism which is moderately photochromogenic. Rod-shaped cells are produced, 3 to 4 μm long, with some longer rods and filamentous forms in older cultures. Visible yellow-pigmented growth from dilute inocula occurs when the organism is incubated for 3 to 7 days in the light; coloration is deeper yellow in older cultures. Colonies on Löwenstein-Jensen medium and Middlebrook 7H10 agar are smooth, butyrous, and have entire edges. Good growth occurs on Columbia blood agar (3 days); weak growth is formed on MacConkey and 5% (wt/vol) sodium chloride agars (14 days). The organism is positive for arylsulfatase (14 days), catalase (45-mm foam), and nitrate reductase but is negative for arylsulfatase (3 days), iron uptake, and Tween opacity. Niacin is not produced. Growth is inhibited by capreomycin sulfate (35.5 mg/liter), ciprofloxacin (2.5 mg/liter), cycloserine (16 mg/liter), ethambutol (3.2 mg/liter), ethionamide (18 mg/liter), and streptomycin sulfate (10 mg/liter) but not by hydroxyamphetamine (500 mg/liter), pyrazinamide (66 mg/liter), rifampin (32 mg/liter), thiacetazone (10 mg/liter), or thiopen-2-carboxylic acid hydrazide (5 mg/liter). The organism synthesizes α- and keto-mycolates and wax esters. Its phylogenetic position, based on an evaluation of an almost complete 16S rDNA 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 \textit{M. novocastrense} is DSM 44203 (strain 73).

It is possible that \textit{M. novocastrense} might be confused with \textit{M. marinum}, another photochromogenic organism which causes self-limiting granulomatous skin lesions. However, the two organisms can be distinguished readily as only \textit{M. novocastrense} reduces nitrate and grows at 42°C. In addition, \textit{M. marinum} is a slowly growing organism which has a mycolic acid profile different from that of \textit{M. novocastrense}.

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REFERENCES


\begin{table}
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\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Taxon} & \textbf{16S} & \textbf{165} & \textbf{1006} & \textbf{1023} \\
\hline
\textit{M. tuberculosis} & \textit{CCACATGCAGAAGGCGGC} & \textit{GAA} & \textit{GGGAC} & \textit{G} \\
\textit{M. chromogenicum} & \textit{GTCG} & \textit{ATACGTGC} & \textit{G} & \textit{G} \\
\textit{M. confuentis} & \textit{GTCG} & \textit{GTC} & \textit{G} & \textit{G} \\
\textit{M. flavesens} & \textit{GTCG} & \textit{GTCG} & \textit{G} & \textit{G} \\
\textit{M. fortuitum} & \textit{GTCG} & \textit{GTCG} & \textit{G} & \textit{G} \\
\textit{M. madagascarense} & \textit{GTCG} & \textit{GTCG} & \textit{G} & \textit{G} \\
\textit{M. phlei} & \textit{GTCG} & \textit{GTCG} & \textit{G} & \textit{G} \\
\textit{M. smegmatis} & \textit{GTCG} & \textit{GTCG} & \textit{G} & \textit{G} \\
\textit{M. thermoresistible} & \textit{GTCG} & \textit{GTCG} & \textit{G} & \textit{G} \\
\textit{Strain 75} & \textit{GTCG} & \textit{GTCG} & \textit{G} & \textit{G} \\
\hline
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\end{table}

\footnote{See footnotes to Table 1 for explanation of terms.}