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Molecular Identification of a *Nocardiopsis dassonvillei* Blood Isolate

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***Nocardiopsis dassonvillei* is an environmental aerobic actinomycete seldom isolated in cutaneous and pulmonary infections. We herein report the first *N. dassonvillei* blood isolate in a patient hospitalized for cholangitis. Although morphological characteristics and biochemical tests allowed a presumptive identification of this isolate, cell wall fatty acid chromatographic analysis confirmed identification at the genus level, and 16S rRNA gene sequencing achieved definite identification. This study illustrates the usefulness of 16S rRNA gene sequencing as a routine method for the identification of actinomycetes.**

The genus *Nocardiopsis* includes aerobic, spore-forming actinomycetes that produce a branched, vegetative mycelium and aerial hyphae. *Nocardiopsis dassonvillei*, isolated from mildewed grain and originally classified under the name of *Streptothrix dassonvillei* (4), was subsequently transferred into the genera *Nocardia* (12) and *Actinomadura* (9). A new genus, *Nocardiopsis* (16), proposed on the basis of chemotaxonomic (5, 7, 11) and numerical taxonomic (2, 6) analysis, has been confirmed by genetic studies (5, 21). *N. dassonvillei* has recently been proposed as a new combination, including isolates previously designated *Nocardiopsis antarctica*, *Nocardiopsis alborubida*, and *N. dassonvillei* (26). *N. dassonvillei* was mostly isolated from environmental samples (16) but was seldom implicated in mycetoma (1, 22) and skin infections (20, 23), lung infections (3, 19), or conjunctivitis (12). Difficulties in accurate identification may have hampered the description of clinical conditions associated with *N. dassonvillei* infection. We herein report the first case of a *N. dassonvillei* blood isolate definitively identified on the basis of its cell wall fatty acid analysis and 16S rRNA gene sequence and confirm the usefulness of 16S rRNA gene sequencing (25) as a routine method for the accurate identification of *Nocardiopsis* species.

A 60-year-old Togolean man presented with cholangitis. His medical history included untreated rheumatoid spondylitis and arterial hypertension, which had been treated by timolol-amiloride-hydrochlorothiazide. The patient had spent an unremarkable 1-month journey in Togo 3 months before his admission to the hospital. He presented with a fever of 39°C, shivering, epigastrium pain, and icterus. Relevant laboratory data included a leukocyte count of 20×10^3/ml, with 86% of the leukocytes being polymorphonuclear, and an elevated level of hepatic enzymes. Three blood cultures grew *Enterobacter cloacae*. Acute pancreatitis developed 5 days after a retrograde cholangiography was performed under general anesthesia. An intravenous treatment with piperacillin (12 g per day) and ciprofloxacin (400 mg per day) resulted in the patient's recovery. A fever of 38.5°C and a leukocyte count of 11.7×10^3/ml, with 57% of the cells being polymorphonuclear, reappeared 5 days after the antibiotic treatment was stopped. Another set of blood cultures was collected, and the same antibiotic treatment was reintroduced for 6 days. All microbiologic investigations remained negative except for one blood culture which grew an isolate identified as *N. dassonvillei*.

The isolate grew over a period of 5 days in a BACTEC aerobic bottle (NR 6-A) that was incubated in a BACTEC NR-860 automated instrument (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.). It was then subcultured onto chocolate agar and Trypticase soy agar plates (BioMérieux, Marcy l'Etoile, France) and incubated at 37°C under a 10% CO_2 atmosphere for 48 h. Colonies presented with aerial hyphae and were white and farinaceous and dark brown-colored on the substrate side. Low magnification examination showed the presence of a well-developed aerial and substrate mycelium with zigzag hyphae. In a later stage of growth, hyphae fragmented into arthrospores. Colonies were formed of a gram-positive, not acid fast, branched bacterium susceptible to lysozyme. A test for catalase was positive. Biochemical characteristics determined by API Coryne and biotype 100 carbon source strips (BioMérieux) are presented in Table 1. The cell wall fatty acid composition, determined by gas chromatography (18) on a culture grown for 48 h on Trypticase soy agar (BioMérieux), included iso-$C_{16:0}$, 36.73%; $C_{16:0}$, 4.41%; anteiso-$C_{17:0}$, 13.12%; $C_{17:0}$ W8C, 4.48%; 10-methyl-$C_{17:0}$, 3.28%; iso-$C_{18:0}$, 3.55%; $C_{18:1}$ W9C, 15.26%; $C_{18:0}$, 6.98%; and 10-methyl-$C_{18:0}$, 12.21%. The mean and standard deviation of G+C content were determined five times by using high-pressure liquid chromatography (46200A system pump; Merck Clevenot, Nogent-sur-Marne, France) (24) and were 66.9 ± 0.27 mol%. DNA was later extracted from mycelium dissociated in 100 μl of Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, 0.1 M NaCl) at pH 8.0 and incubated for 1 h at 37°C. Digestion was supplemented by 40 μl of proteinase K solution (25 mg of proteinase K per ml) and 25 μl of 10% sodium dodecyl sulfate for 1 h at 55°C. A total of 200 μl of 4 M guanidine thiocyanate was added, left for 1 h at room temperature, and then heated at 100°C for 10 min with 50 μl of 0.5 M NaOH. Final extraction of nucleic acid was carried out by using a QIAmp kit (Qiagen, Hilden, Germany). PCR-mediated amplification of the 16S rRNA gene and sequence determination were performed as previously described (8, 25). The 1,523-bp sequence was aligned and compared with all eubacterial 16S rRNA gene sequences available in the GenBank database by using the multisequence Advanced Blast National Center for Biotechnology Information comparison software. The highest 16S rRNA gene sequence similarity value of 99.7% was obtained for the *N. dassonvillei* DSM 43111T 16S rRNA gene sequence (GenBank accession no. X97886). Our isolate exhibited mor-

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TABLE 1. Phenotypic characteristics of an *N. dassonvillei* blood isolate

Characteristic or test	Result(s) for isolate(s) in:	
	Present work	Type strains[a]
Aerial hyphae	+	
Color of:		
Aerial mycelium	White	White, yellow, grey
Substrate mycelium	Dark brown	Brown, orange, yellow
Fragmentation with arthrospores	+	+
Motile spores	−	−
Acid fastness	−	−
Growth in:		
Lysozyme	−	−
Anaerobiose	−	−
Catalase production	+	+
Urease activity	−	v[b]
Nitrate reductase activity	+	v
Hydrolysis of:		
Gelatin	+	+
Esculin	+	v
Carbon substrate assimilation tests		
D-Glucose	+	+
D-Galactose	+	v
D-Trehalose	+	+
Sucrose	+	+
Maltose	+	v
D-Cellobiose	+	+
D-Xylose	+	+
D-Mannitol	+	+
D-Gluconate	+	+
L-Rhamnose	+	v
Raffinose	−	−
Myoinositol	−	−
Hydroxybenzoate	−	−
G+C DNA content (mol%)	66.9 ± 0.27	

[a] Data compiled from references 10, 15, 17, and 26.
[b] v, variable results of the test.

phology, a G+C content, and a cell wall fatty acid profile characteristic of *Nocardiopsis* (5, 7, 14, 17), but only 16S rRNA gene sequence analysis provided species identification within 2 working days.

Although the actinomycete nature of an aerobic isolate was obvious in the presence of an aerial mycelium, weak or partial acid fastness, growth and morphological characteristics associated with further biochemical tests appeared inadequate for accurate genus determination (10, 15). Genus identification relies on the cell wall chemotype (9, 10, 17), menaquinone composition (5, 7), phospholipid pattern (10, 11), cell wall fatty acid profile (6, 7, 14), and DNA G+C content (17). The combination of chemotaxonomic data can differentiate *Nocardiopsis* from other actinomycetes, but it does not allow identification at the species level. Species of the genus *Nocardiopsis* may be distinguished by means of the color of their mature aerial and substrate mycelia, their degradation of different compounds, and their ability to use different carbon sources (17). However, *N. dassonvillei* is characterized by its phenotypic heterogeneity. The color of mycelium exhibits intraspecific variation and depends on the culture medium used to grow the isolate (17). Additional physiological tests are not routinely performed in clinical laboratories and were determined for a few reference strains, limiting their practical usefulness (17).

A cell wall fatty acid pattern, including a major percentage of branched-chain fatty acids with high percentages of $C_{17:0}$, anteiso-$C_{18:0}$, and $C_{18:0}$ 10-methyl fatty acids, was reported as characteristic of the *Nocardiopsis* genus (5, 7, 14). It allowed the identification of our isolate at the genus level. Cell wall fatty acid content, however, depends on culture conditions, extraction, and chromatography techniques (14) and requires large quantities of bacterial material. 16S rRNA gene sequencing allowed the definitive identification of this isolate, with a 99.7% sequence similarity with that reported for *N. dassonvillei* DSM 43111T. At present, a second 16S rRNA *N. dassonvillei* gene sequence is available in GenBank databases, presenting 99.1% similarity with strain DMS 43111T and 99.1% similarity with our query sequence, because of undetermined positions and gaps. This fact emphasizes the requirement for a high-quality sequence for comparison. Other closely related sequences (99.1% similarity) were those reported for *N. antarctica* and *N. alborubida*, two species recently considered to belong to the same species as *N. dassonvillei* on the basis of their high DNA-DNA hybridization (26). The usefulness of 16S rRNA gene sequencing has been greatly enhanced through the establishment of large public domain databases (13), which allow the comparison of a sequence with all other deposited eubacterial 16S rRNA gene sequences (13). Thus, molecular methods may provide quick and accurate identification independent of standard culture methods and the amount of isolate available. Indeed, the identification of our isolate at the species level relied on 16S rRNA gene sequence analysis.

No *N. dassonvillei* blood isolate has previously been reported. The isolate grew readily in pure culture, and no other *N. dassonvillei* strain was isolated in the same laboratory. *N. dassonvillei* is not part of the common skin flora and has never been reported as a blood culture contaminant. These facts suggest that this isolate was not a contaminant. The isolate was recovered from blood when the patient presented an acute onset of fever, with no other microorganism recovered from other appropriately timed specimens. The biliary and gastrointestinal tracts may be the entry route, since *N. dassonvillei* was recovered after retrograde cholangiography. Alternatively, an intravenous catheter inserted during the course of hospitalization may have been the entry route. Since *N. dassonvillei* was isolated after 10 days of hospitalization, it should be regarded as a potential nosocomial pathogen.

Accurate identification of *N. dassonvillei* (with other aerobic actinomycetes) is fastidious and is rarely available for most routine laboratory work. The 16S rRNA sequences offer a reliable and straightforward tool for their identification, and routine use of this method should increase our knowledge regarding the clinical spectrum of *N. dassonvillei* human infections.

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