**NOTE**

1 Department of Biological Sciences, KAIST, Daejeon, Republic of Korea
2 Institut für Mikrobiologie, Zentralklinik Emil v. Behring, Berlin, Germany
3 DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

**Nocardia pseudovaccinii sp. nov.**

K. K. Kim, A. Roth, S. Andrees, S. T. Lee and R. M. Kroppenstedt

Author for correspondence: Reiner M. Kroppenstedt. Tel: +49 531 2616 227. Fax: +49 531 2616 418. e-mail: kdt@dszm.de

Comparative 16S rDNA studies of Nocardia type and reference strains revealed that strain DSM 43406, identified as Nocardia vaccinii, was wrongly classified. The strain was aerobic, Gram-positive and produced scarce, white, branched aerial mycelium and a beige-red substrate mycelium. The reverse side of the colonies was yellow-orange. It showed chemotaxonomic markers that were consistent with its classification in the genus Nocardia. The mycolic acids had chain lengths from 50 to 58 carbon atoms. The 16S rDNA sequence showed the highest similarity to Nocardia nova (97.7%) and N. vaccinii (97.6%), but the strain could be clearly separated from these species and other members of the N. vaccinii cluster by significant differences in biochemical test results and unique fatty acid and mycolic acid patterns. These data led to the conclusion that the isolate represents a novel species within the genus Nocardia, for which the name Nocardia pseudovaccinii sp. nov. is proposed. The type strain is strain AR 368,38366-20T (= DSM 43406 = NRRL B-24154).

**Keywords:** Nocardia pseudovaccinii sp. nov., polyphasic taxonomy

Nocardiae are distributed ubiquitously in the environment and they are common in soil (Orchard et al., 1977; Wang et al., 2001). They are frequently isolated from rivers (Maldonado et al., 2000) and scumming activated sludge (Lemmer & Kroppenstedt, 1984). However, despite their essentially saprophytic nature, most attention has been focused on the taxonomy of clinically significant nocardiae. Most of the validly described Nocardia species have been isolated from humans and animals, where they cause a variety of suppurative diseases, notably actinomycete mycetoma (Schaal, 1972; Schaal & Lee, 1992) and pulmonary infections (Gürtler et al., 2001; Hamid et al., 2001) and nocardiosis in fish (Kudo et al., 1988; Isik et al., 1999) and oysters (Friedman & Smith, 1997). Species of the genus Nocardia form a homogeneous group within the order Actinomycetales. Nocardiae are well separated from related taxa such as Rhodococcus and Gordonia by their unique chemotaxonomic markers and genus-specific 16S rDNA signature sequences (Stackebrandt et al., 1997). The Nocardia strain AR 368,38366-20 was originally isolated by J. B. Routien, who identified the isolate as a streptomyecete. Later, Ruth Gordon of the Institute of Microbiology, Rutgers University, identified the strain as a strain of Nocardia vaccinii because of its morphology. In 1979, the strain was deposited at the DSMZ as N. vaccinii DSM 43406 (DSMZ, 2001). A recent study of the 16S–23S rDNA internal transcribed regions of Nocardia reference strains and unclassified Nocardia collection strains revealed that the strain N. vaccinii DSM 43406 was misclassified (unpublished results). Comparative 16S rDNA gene sequencing showed that this strain was a member of a novel Nocardia species. Our reported genotypic and phenotypic data of strain DSM 43406 show that this strain merits recognition as a novel species in the genus Nocardia, for which the name Nocardia pseudovaccinii sp. nov. is proposed. Strain DSM 43406 was cultivated on Trypticase/soy broth agar (TSB; DSMZ medium 535) and on glucose/yeast extract/malt extract agar (GYM; DSMZ medium 65) at 28 °C. For analysis of fatty acids, about 40 mg cells was scraped from TSB agar plates whereas, for the other chemical analyses, the cells were grown in liquid TSB and harvested by centrifugation. The strain was characterized biochemically by examining carbon-source utilization by means of the reduction of the redox dye 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.
Table 1. Physiological properties of strain DSM 43406T and type strains of Nocardia species

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<th>5, N. brevicatena</th>
<th>6, N. carneae</th>
<th>7, N. corynebacterioides</th>
<th>8, N. cummindsii</th>
<th>9, N. farcinica</th>
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<th>12, N. nova</th>
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<th>16, N. salmonidea</th>
<th>17, N. seriola</th>
<th>18, N. soli</th>
<th>19, N. transvalensis</th>
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triazolium bromide (MTT) and by quantitative enzyme tests. The test was performed in standard microtitre plates as described previously (Klatte, 1994). Whole-cell amino acid and sugar analyses were performed according to Stanek & Roberts (1974). The murein acyl type was determined by a modification of the colorimetric method of Uchida & Aida (1977). In contrast to the original procedure, our whole-cell hydrolysate was neutralized by passing it through an ion-exchange column (Analytichem Bond Elut SCX, Varian). Isoprenoid quinones and polar lipids were extracted and purified using the small-scale integrated procedure of Minnikin et al. (1984). Dried preparations were dissolved in 200 μL 2-propanol and 1–10 μL amounts were separated by HPLC without further purification and analysed as described by Kroppenstedt (1982, 1985). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin et al., 1977). Fatty acid methyl esters and mycolic acid trimethylsilyl esters were prepared and analysed as described previously (Klatte et al., 1994) using the standard Microbial Identification System (MIDI Inc.) for automated GC analyses (Sasser, 1990).

The complete 16S rDNAs of DSM 43406T and 22 type strains of validly described Nocardia species (strain sources and GenBank accession numbers shown in Fig. 1) were amplified by PCR and sequenced directly using a Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A, Applied Biosystems). A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987); distances were estimated by the method of Kimura (1980) using TREECON for Windows version 1.3b. The tree position of strain DSM 43406T was confirmed by parsimony analysis. Bootstrapping was not performed because of the high degree of similarity of nocardioid 16S rDNA sequences.
tests obtained from the microtitration plates revealed substrate mycelium. The results of the physiological stable, scant, white aerial mycelium and red-orange acid (tuberculostearic acid).

Values are percentages of total fatty acids. Examples of abbreviations: 16:0, hexadecanoic acid (palmitic acid); 18:1ω9c, cis-9-octadecenoic acid (oleic acid); 10me-18:0, 10-methyl octadecanoic acid (tuberculostearic acid).

<table>
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<th>Fatty acid</th>
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<th>N. africana DSM 4491T</th>
<th>N. veterana DSM 44445T</th>
<th>N. vaccinii DSM 43285T</th>
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</table>

Fig. 1. Phylogenetic dendrogram obtained by distance-matrix analysis showing the position of Nocardia pseudovaccinii sp. nov. DSM 43406T among 22 strains of the genus Nocardia. Tree topology was inferred by the neighbour-joining method. The tree was rooted by the sequence of Rhodococcus equi. Bar, 0.1 substitutions per nucleotide position.

Strain DSM 43406T, isolated from soil, produced stable, scant, white aerial mycelium and red-orange substrate mycelium. The results of the physiological tests obtained from the microtitration plates revealed that strain DSM 43406T was able to utilize 17 of the 32 carbon sources by means of the MTT reduction test and hydrolysed all three chromogenic substrates (Table 1). This utilization pattern did not match any of the known Nocardia species. The chemotaxonomic properties of the strain were also consistent with its classification in the genus Nocardia (Klatte et al., 1994; Stackebrandt et al., 1988). Whole-cell hydrolysates of strain DSM 43406T contained meso-diaminopimelic acid as the only diamino acid of the peptidoglycan and arabinose plus galactose as major cell-wall sugars (cell wall chemotype IV according to Lechevalier & Lechevalier, 1970). As expected for Nocardia and related taxa, the sugars of the peptidoglycan were glycolated. MK-8(H2) was the only menaquinone of this strain. The polar lipids were composed of diphosphatidyglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides. This pattern matched quite well with those reported by Minnikin et al. (1977) for Nocardia. The fatty acid pattern was composed of straight-chain saturated and unsaturated fatty acids plus tuberculostearic acid (Table 2). A homologous series of mono- and polyunsaturated mycolic acids were synthesized by this strain, ranging from 50 to 58 carbon atoms, with C50, C54 and C56 being the three principal mycolic acids. This chain length was in the range of the mycolic acids expected for Nocardia species, C50–C56, but the strain differed in its quantitative composition of mycolic acids (Klatte, 1994; Baba et al., 1997; Gürtler et al., 2001).

In order to ascertain the phylogenetic position of strain DSM 43406T, the complete 16S rDNA sequences of strain DSM 43406T and 22 type strains, consisting of 1492 nucleotides, were determined and subjected to a comparative analysis. Sequence database searches
comparing the sequence with those from representative
tatives of the main actinomycete sublines of descent
revealed that the strain was phylogenetically a member of
the genus *Nocardiaceae* (Stackebrandt et al., 1997). Strain DSM
43406\(^t\) shares 16S rDNA similarity of 97.7 and 97.6\%,
respectively, with its nearest relatives, *Nocardiaceae*
and *N. vaccinii*. Higher similarities have been recorded
between several validly described *Nocardiaceae* species,
e.g., *Nocardia paeonii* and *Nocardia brevicatenata* (Yassin et al., 2000), *Nocardia carnea* and *Nocardia
flavorosea* (Chun et al., 1998), *Nocardia ignorata* and
*Nocardia salmonicida* (Yassin et al., 2001) and *N.
Vaccinii* and *Nocardia veterana* (Gürtler et al., 2001);
the DNA–DNA relatedness values shown between
these pairs of type strains were found to be well below
the 70% cut-off point recommended for circumscrip-
tion of bacterial genomic species (Wayne et al., 1987;
Stackebrandt & Goebel, 1994). Treeing analysis clearly
demonstrated that strain DSM 43406\(^t\) was separate from
all described nocardiae. The phylogenetic tree
(Fig. 1), constructed by the neighbour-joining method,
shows the nearest relatives of strain DSM 43406\(^t\) to be
*N. vaccinii* DSM 43285\(^t\), *N. nova* DSM 44481\(^T\),
*Nocardia africana* DSM 44491\(^T\) and *N. veterana* DSM
44445\(^T\).

The results of our polyphasic taxonomic investigation
clearly showed that strain DSM 43406\(^t\), formerly
listed in the DSMZ catalogues as *N. vaccinii*,
was misclassified and represents a novel species of
the genus *Nocardiaceae*. Biochemically, strain DSM 43406\(^t\)
differs from all type strains of the genus *Nocardia*
(Table 1). Significant quantitative differences from
other *Nocardiaceae* species could be shown in the fatty
acid (Table 2) and mycolic acid (Baba et al., 1997)
distributions. Therefore, we propose to classify DSM 43406\(^t\)
as a novel species in the genus *Nocardiaceae*, *Nocardiaceae*
and *Nocardia pseudovaccinii* sp. nov.

**Description of *Nocardiaceae pseudovaccinii* sp. nov.**

*Nocardiaceae pseudovaccinii* (pseu.do.vac.cin.i.i. Gr. adj.
pseudoes false; N.L. gen. n. vaccinii of Vaccinium, the
generic name of blueberry; N.L. n. pseudovaccinii false
vaccini, referring to the earlier misclassification of the
type strain as a strain of *Nocardia vaccinii*).

Aerobic, Gram-positive, non-motile actinomycete that
forms a scarce, white, branched aerial mycelium and a
beige-red substrate mycelium. The reverse side of the
colonies is yellow-orange on GYM agar. Utilizes the
following substrates by means of the reduction of
MMT dye: L-alanine, D-arabitol, L-aspartate, 4-hydroxybenzoate, caprate, glucarate, D-glucosaminic acid,
N-acetyl glucosamine, 2-oxoglutarate, 2-glutarate,
L-inositol, L-leucine, pimelate, D-ribose, suc-
cinate, tryamine, 2-hydroxyvalerate and l-valine. No
reduction of MTT is found with acetamide, benzotoate,
4-aminobutyrate, 3-hydroxybenzoate, citrate, D-galac-
tose, gluconate, phenylacetate, L-proline, putrescine,
quinate, L-rhamnose, L-serine, sucrose or D-turanose.
All three chromogenic substrates tested are hydro-
lysed: p-nitrophenyl phosphorylcholine, p-nitrophenyl
β-D-xyloside and 2-deoxymyridine 5-‘p-nitrophenyl-
phosphate. Whole-cell hydrolysates contain mezo-
diaminopimelic acid and arabinose and galactose (cell-
wall chemotype IV sensu Lechevalier & Lechevalier,
1970). The sugars of the peptidoglycan are glycolated.
The predominant menaquinone is MK-8(H\(_4\)).
The polar lipids are diphasitidyglycerol, phosphadi-
tylethanolamine, phosphatidylinositol and phos-
phatidylinositol mannosides. The fatty acid pattern
is composed of C\(_{16:0}\) (12.2\%), C\(_{12:0}\) (0.7\%), C\(_{16:1}\)
(17.2\%), C\(_{16:0}\) (28.0\%), C\(_{17:1}\) (3.6\%), C\(_{17:0}\) (3.0\%),
C\(_{10\text{me}-17:0}\) (2.5\%), C\(_{18:1}\) (13.0\%), C\(_{19:0}\) (3.5\%)
and C\(_{10\text{me}-18:0}\) (20.3\%) and C\(_{19:1}\) (2.2\%). The principal
mycolic acids have chain lengths of 52, 54 and 56
carbon atoms. The isolation source is not recorded.
The type strain is strain AR 368,38366-20\(^T\) (= DSM
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