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 et al., 1999) or they produce stem galls on blueberry (Demaree & Smith, 1952). 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 streptomycete. 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strains and unclassified Nocardia collection strains revealed that the strain N. vaccinii DSM 43406 was misclassified (unpublished results).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.

Comparative 16S rDNA studies of Nocardia type and reference strains revealed that strain DSM 43406T, 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 (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.

Keywords: Nocardia pseudovaccinii sp. nov., polyphasic taxonomic

Abbreviation: MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.

The GenBank accession number for the 16S rDNA sequence of strain DSM 43406T is AF430046.

The Nocardia pseudovaccinii sp. nov. was described by K. K. Kim, A. Roth, S. Andrees, S. T. Lee and R. M. Kroppenstedt.

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

International Journal of Systematic and Evolutionary Microbiology (2002), 52, 1825–1829 DOI: 10.1099/ijs.0.02219-0

K. K. Kim,1,3 A. Roth,2 S. Andrees,2 S. T. Lee1 and R. M. Kroppenstedt3

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

Nocardia pseudovaccinii sp. nov.

Comparative 16S rDNA studies of Nocardia type and reference strains revealed that strain DSM 43406T, 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 43406T = NRRL B-24154T).

Keywords: Nocardia pseudovaccinii sp. nov., polyphasic taxonomic

Abbreviation: MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.

The GenBank accession number for the 16S rDNA sequence of strain DSM 43406T is AF430046.
Table 1. Physiological properties of strain DSM 43406T and type strains of Nocardia species

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| n-Glucose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| l-Rhamnose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| n-Ribose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| n-Sucrose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| n-Turanose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| n-Arabinose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| l-Inositol | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| N-Acetyl-D-glucosamine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Glucurionate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Glucuronate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| n-Glucosaminic acid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Caprate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Citrate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 4-Aminobutyrate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2-Hydroxybutyrate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2-Oxoglutarate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Putrescine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Phenylacetate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Quinate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-Alanine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-Aspartate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-Leucine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-Proline | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-Serine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-Valine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Putrescine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Tyramine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Acetamide | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hydrolysis of: | | | | | | | | | | | | | | | | | | | | | | | | | |
| pNP β-D-Xyloside | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| pNP Phosphorylcholine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2-Dexoyribosiduronic 5′-pNP phosphate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

Utilization of (test) — A450(control) > 0.129; (control) < 0.129; v, variable. pNP, p-Nitrophenyl.

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. Bootstrapting was not performed because of the high degree of similarity of nocardial 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).

Stain DSM 43406 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 43406 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(H₂0-cyc) was the only menaquinone of this strain. The polar lipids were composed of diphasphatidylglycerol, 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 \( C_{50} \) to \( C_{58} \) being the three principal mycolic acids. This chain length was in the range of the mycolic acids expected for Nocardia species, \( C_{50} \) to \( C_{58} \), but the strain differed in its quantitative composition of mycolic acids (Klatte, 1994; Baba et al., 1997; Görtler et al., 2001).

In order to ascertain the phylogenetic position of strain DSM 43406, the complete 16S rDNA sequences of strain DSM 43406 and 22 type strains, consisting of 1492 nucleotides, were determined and subjected to a comparative analysis. Sequence database searches

---

**Table 2. Comparison of fatty acid methyl esters of strain DSM 43406** and related strains

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>DSM 43406</th>
<th>N. africana DSM 44491</th>
<th>N. veterana DSM 44445</th>
<th>N. vaccini DSM 43285</th>
<th>N. nova DSM 44481</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1:2</td>
<td>2:0</td>
<td>2:0</td>
<td>0:5</td>
<td>0:9</td>
</tr>
<tr>
<td>15:1</td>
<td>0:7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:0</td>
<td>4:1</td>
<td></td>
<td>1:2</td>
<td>0:4</td>
<td></td>
</tr>
<tr>
<td>16:1o7c</td>
<td></td>
<td></td>
<td>0:4</td>
<td>0:5</td>
<td></td>
</tr>
<tr>
<td>16:1o9c</td>
<td>17:2</td>
<td>6:9</td>
<td>13:8</td>
<td>8:0</td>
<td>9:7</td>
</tr>
<tr>
<td>16:0</td>
<td>28:0</td>
<td>26:0</td>
<td>37:4</td>
<td>44:1</td>
<td>36:9</td>
</tr>
<tr>
<td>17:1o10c</td>
<td>3:6</td>
<td></td>
<td>0:7</td>
<td>1:5</td>
<td></td>
</tr>
<tr>
<td>17:0</td>
<td>3:0</td>
<td>1:2</td>
<td>1:1</td>
<td>0:4</td>
<td>1:2</td>
</tr>
<tr>
<td>10me-17:0</td>
<td>2:5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:1o9c</td>
<td>13:0</td>
<td>19:1</td>
<td>25:8</td>
<td>16:7</td>
<td>24:7</td>
</tr>
<tr>
<td>18:0</td>
<td>3:5</td>
<td>22:6</td>
<td>1:0</td>
<td>0:9</td>
<td>10:0</td>
</tr>
<tr>
<td>10me-18:0</td>
<td>20:3</td>
<td>18:70</td>
<td>15:3</td>
<td>25:2</td>
<td>16:6</td>
</tr>
<tr>
<td>19:1</td>
<td>2:2</td>
<td></td>
<td>0:8</td>
<td>0:5</td>
<td></td>
</tr>
<tr>
<td>19:0</td>
<td></td>
<td></td>
<td></td>
<td>0:3</td>
<td></td>
</tr>
<tr>
<td>20:1</td>
<td></td>
<td>1:4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Fig. 1. Phylogenetic dendrogram obtained by distance-matrix analysis showing the position of Nocardia pseudovaccinii sp. nov. DSM 43406 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.**

**http://ij.sgmjournals.org**
comparing the sequence with those from representative- 
tives of the main actinomycete sublines of descent 
revealed that the strain was phylogenetically a member of 
the genus Nocardia and that the sequence contains 
all of the signature nucleotides defined for the family 
Nocardiaceae (Stackebrandt et al., 1997). Strain DSM 
43406\(^{3}\) shares 16S rDNA similarity of 97.7 and 97.6\% 
respectively, with its nearest relatives, N. vaccinii 
and N. vaccinii. Higher similarities have been recorded 
between several validly described Nocardia species, 
e.g. Nocardia pacificors and Nocardia brevicatena 
(Yassin et al., 2000), Nocardia carneae 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). Treering analysis clearly 
demonstrated that strain DSM 43406\(^{3}\) 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 
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\(^{3}\), formerly 
listed in the DSMZ catalogues as N. vaccinii, 
was misclassified and represents a novel species of 
the genus Nocardia. Biochemically, strain DSM 43406\(^{4}\) 
differed from all type strains of the genus Nocardia 
(Table 1). Significant quantitative differences from 
other Nocardia species could be shown in the fatty acid 
(Table 2) and mycic acid (Baba et al., 1997) distribu-
tions. Therefore, we propose to classify DSM 43406\(^{6}\) 
as a novel species in the genus Nocardia, Nocardia 
pseudovaccinii sp. nov.

Description of Nocardia pseudovaccinii sp. nov.

Nocardia pseudovaccinii (pseu.do.vac.cin’i.i. Gr. adj. 
peudes false; N.L. gen. n. vaccinii of Vaccinium, the 
generic name of blueberry; N.L. n. pseudovaccinii false 
vaccinii, 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 
MTT dye: L-alanine, D-arabitol, L-aspartate, 4-hy-
droxybenzoate, caprate, glucarate, D-glucosaminic 
acid, N-acetylglucosamine, 2-oxoglutarate, 2-glut-
tarate, i-inositol, L-leucine, pimelate, D-ribose, suc-
cinate, tyramine, 2-hydroxyvalerate and L-valine. No 
reduction of MTT is found with acetamide, benzoate, 
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-xiloside and 2-deoxymyridine 5′-p-nitrophenyl-
phosphate. Whole-cell hydrolysates contain meso-
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\(_{4}\)-ooyl).
The polar lipids are diphosphatidylglycerol, phospha-
tidylenolamine, phosphatidylinositol and phosph-
athylinositol mannosides. The fatty acid pattern 
is composed of C\(_{16}:0\) (1.2\%), C\(_{16}:0\) (0.7\%), C\(_{17}:0\) 
(17.2\%), C\(_{18}:0\) (28.0\%), C\(_{17}:1\) (3.6\%), C\(_{17}:0\) (3.0\%), 
C\(_{10}:0\)-me-17:0 (2.5\%), C\(_{18}:1\) (13.0\%) C\(_{18}:0\) (3.5\%) 
C\(_{10}:0\)-me-18:0 (20.3\%) and C\(_{19}:1\) (2.2\%). The principal 
mycic acids have chain lengths of 52, 54 and 56 
carbon atoms. The isolation source is not recorded. 
The type strain is strain AR 368, 38366-20T (= DSM 
44306\(^{4}\) = NRRL B-24154\(^{7}\)).

References

molecular species as a criterion in nocardial classification. Int J Syst 

Chun, J., Seong, C.-N., Bae, K. S., Lee, K.-J., Kang, S.-O., Good-
Bacteriol 48, 901–905.

Demaree, J. B. & Smith, N. R. (1952). Nocardia vaccinii n. sp. causing 
galls on blueberry plants. Phytopathology 42, 249–252.

DSMZ (2001). Catalogue of Strains. Braunschweig: Deutsche Samm-
lung von Mikroorganismen und Zellkulturen.

Friedman, C. S., Beaman, B. L., Chun, J., Goodfellow, M., Gee, A. 

Gürtler, V., Smith, R., Mayall, B. C., Potter-Reinemann, G., Stacke-
isolated from human bronchial lavage. Int J Syst Ecol Microbiol 51, 
933–936.

Hamid, M. E., Maldonado, L., Sharaf Eldin, G. S., Mohamed, M. F., 
Saeed, N. S. & Goodfellow, M. (2001). Nocardia africana sp. nov., a 
new pathogen isolated from patients with pulmonary infections. J Clin 
Microbiol 39, 625–630.


of base substitutions through comparative studies of nucleotide 

Gattungen Corynebacterium Lehmann & Neumann 1896 und Rhodococcus 

molecular species as a criterion in nocardial classification. Int J Syst 

HPLC using reverse phase (RP-18) and a silver loaded ion exchanger. 
J Liq Chromatogr 5, 2359–2367.

Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of 
actinomycetes and related organisms. In Chemical Methods in Bacterial 
Systematics, no. 20 SAB Technical Series, pp. 173–199. Edited by M. 


International Journal of Systematic and Evolutionary Microbiology 52
Nocardia pseudovaccinii sp. nov.


