NOTE

***Nocardia pseudovaccinii* sp. nov.**

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Comparative 16S rDNA studies of *Nocardia* type and reference strains revealed that strain DSM 43406^T, 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 368,38366-20^T (= DSM 43406^T = NRRL B-24154^T).

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 *et al.*, 1998), 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.

Strain DSM 43406^T 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-diphenylte-

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

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

Table 1. Physiological properties of strain DSM 43406^T and type strains of *Nocardia* species

Species: 1, *N. pseudovaccinii* sp. nov. DSM 43406^T; 2, *N. abscessus*; 3, *N. asteroides*; 4, *N. brasiliensis*; 5, *N. brevicatena*; 6, *N. carnea*; 7, *N. corynebacterioides*; 8, *N. cunmideiensis*; 9, *N. farcinica*; 10, *N. flavorosea*; 11, *N. fluminea*; 12, *N. nova*; 13, *N. otitidiscaviarum*; 14, *N. paucivorans*; 15, *N. pseudobrasiliensis*; 16, *N. salmonicida*; 17, *N. seriolae*; 18, *N. soli*; 19, *N. transvalensis*; 20, *N. uniformis*; 21, *N. vaccinii*; 22, *N. veterana*. No physiological data were available for *N. africana*, *N. beijingensis*, *N. coeliaca*, *N. crassostreae*, *N. cyriacigeorgica*, *N. globerula*, *N. ignorata* or *N. vinacea*. Assimilation of auxonographic substrates was detected photometrically by means of reduction of the redox dye MTT: +, $A_{540}(\text{test}) - A_{540}(\text{control}) > 0.129$; -, $A_{540}(\text{test}) - A_{540}(\text{control}) < 0.129$; v, variable. pNP, *p*-Nitrophenyl.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Utilization of:																						
D-Galactose	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	-	-	-
L-Rhamnose	-	+	v	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	-	+
D-Ribose	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	v	+	+	-
D-Sucrose	-	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-
D-Turanose	-	+	-	-	+	+	+	+	-	+	-	+	-	-	+	+	-	-	-	-	-	-
D-Arabinose	+	+	-	-	+	+	+	+	-	-	+	+	-	+	-	+	-	-	-	-	-	-
D-Inositol	+	-	-	+	+	+	-	+	-	+	-	+	+	+	+	+	+	v	-	-	-	+
N-Acetyl D-glucosamine	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+	+	-
Glucuronic acid	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
Glucosamine	-	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	-	-	-
D-Glucosaminic acid	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	v	+	-	+
Caprate	+	+	v	-	-	-	-	-	+	+	-	+	-	+	-	-	-	-	+	-	-	-
Citrate	-	+	-	v	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	+	-	-
4-Aminobutyrate	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-
2-Hydroxyvalerate	+	-	v	-	+	+	-	+	-	+	+	-	-	-	+	+	-	+	-	+	+	-
2-Oxoglutarate	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+
Pimelate	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-
Succinate	+	+	v	v	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-	-
Benzoate	-	+	-	-	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	-	-	-
3-Hydroxybenzoate	-	-	-	-	+	+	-	+	+	-	-	-	+	+	+	-	+	-	-	-	-	-
4-Hydroxybenzoate	+	+	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Phenylacetate	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
Quinate	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-
L-Alanine	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	v	-	-	-	+
L-Aspartate	+	+	-	-	-	+	+	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-
L-Leucine	+	+	-	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	-	-	-
L-Proline	-	+	-	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	-	+	+
L-Serine	-	+	-	+	+	+	+	+	-	+	+	+	-	+	+	-	-	+	-	-	-	-
L-Valine	+	-	-	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	-	+	-	-
Putrescine	-	-	-	+	+	+	+	+	-	+	+	-	-	+	+	+	+	-	-	-	-	+
Tyramine	+	+	-	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-	-	-	-	-
Acetamide	-	+	v	-	+	+	-	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-
Hydrolysis of:																						
pNP β-D-Xyloside	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+	v	+	-	-
pNP Phosphorylcholine	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	-	-	+	v	-	-	+
2-Deoxythymidine 5'-pNP phosphate	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	-	-	+	+	-	-	-

trazolium bromide (MTT) and by quantitative enzyme tests. The test was performed in standard micro-titration 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 43406^T 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 43406^T was confirmed by parsimony analysis. Bootstrapping was not performed because of the high degree of similarity of nocardial 16S rDNA sequences.

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

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

Fatty acid	DSM 43406 ^T	<i>N. africana</i> DSM 44491 ^T	<i>N. veterana</i> DSM 44445 ^T	<i>N. vaccinii</i> DSM 43285 ^T	<i>N. nova</i> DSM 44481 ^T
14:0	1.2	2.0	2.0	0.5	0.9
15:1	0.7	—	—	—	—
15:0	4.1	—	1.2	0.4	—
16:1 ω 7c	—	—	0.4	0.5	—
16:1 ω 9c	17.2	6.9	13.8	8.0	9.7
16:0	28.0	26.0	37.4	44.1	36.9
17:1 ω 10c	3.6	—	0.7	1.5	—
17:0	3.0	1.2	1.1	0.4	1.2
10me-17:0	2.5	—	—	—	—
18:1 ω 9c	13.0	19.1	25.8	16.7	24.7
18:0	3.5	22.6	1.0	0.9	10.0
10me-18:0	20.3	18.70	15.3	25.2	16.6
19:1	2.2	—	0.8	0.5	—
19:0	—	—	—	0.3	—
20:1	—	1.4	—	—	—

Fig. 1. Phylogenetic dendrogram obtained by distance-matrix analysis showing the position of *Nocardia pseudovaccinii* sp. nov. DSM 43406^T 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 estimated substitutions per nucleotide position.

Strain DSM 43406^T, 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 43406^T 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^T 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(H₄ ω -cyc₁) was the only menaquinone of this strain. The polar lipids were composed of diphosphatidylglycerol, 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 C₅₂, 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–C₆₂, 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 43406^T, the complete 16S rDNA sequences of strain DSM 43406^T 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 representatives 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^T shares 16S rDNA similarity of 97.7 and 97.6%, respectively, with its nearest relatives, *Nocardia nova* and *N. vaccinii*. Higher similarities have been recorded between several validly described *Nocardia* species, e.g. *Nocardia paucivorans* and *Nocardia brevicatena* (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 circumscription 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 *Nocardia*. Biochemically, strain DSM 43406^T 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 mycolic acid (Baba *et al.*, 1997) distributions. Therefore, we propose to classify DSM 43406^T 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. *pseudes* 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-hydroxybenzoate, caprate, glucarate, D-glucosaminic acid, *N*-acetyl glucosamine, 2-oxoglutarate, 2-glutarate, *i*-inositol, L-leucine, pimelate, D-ribose, succinate, tyramine, 2-hydroxyvalerate and L-valine. No reduction of MTT is found with acetamide, benzoate, 4-aminobutyrate, 3-hydroxybenzoate, citrate, D-galactose, gluconate, phenylacetate, L-proline, putrescine,

quininate, L-rhamnose, L-serine, sucrose or D-turanose. All three chromogenic substrates tested are hydrolysed: *p*-nitrophenyl phosphorylcholine, *p*-nitrophenyl β -D-xyloside and 2-deoxythymidine 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(H₄ ω -cyc1). The polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides. The fatty acid pattern is composed of C_{14:0} (1.2%), C_{15:0} (0.7%), C_{16:1} (17.2%), C_{16:0} (28.0%), C_{17:1} (3.6%), C_{17:0} (3.0%), C_{10me-17:0} (2.5%), C_{18:1} (13.0%), C_{18:0} (3.5%), C_{10me-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 43406^T = NRRL B-24154^T).

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