Nocardia acidivorans sp. nov., isolated from soil of the island of Stromboli

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A Gram-positive, non-spore-forming bacterium (strain GW4-1778^T) was isolated from soil of the Italian island of Stromboli. 16S rRNA gene sequence similarity studies showed that strain GW4-1778^T is a member of the genus *Nocardia*, most closely related to *Nocardia pseudobrasiliensis* (GenBank accession no. DQ659914; 98.6%), *Nocardia nova* (Z36930; 98.6%), *Nocardia niigatensis* (AB092563; 98.4%), *Nocardia jiangxiensis* (AY639902; 98.0%), *Nocardia uniformis* (Z46752; 98.0%) and *Nocardia miyunensis* (AY639901; 97.8%). Strain GW4-1778^T could be distinguished from any other established *Nocardia* species by sequence similarity values of less than 97.5%. Strain GW4-1778^T exhibited a quinone system with the predominant compound MK-8 (H₄, ω -cycl) (99.5%) and traces of MK-8 (H₄), characteristic for the genus *Nocardia*. The polar lipid profile of strain GW4-1778^T consisted of the predominant compound diphosphatidylglycerol, moderate amounts of phosphatidylethanolamine, phosphatidylinositol, two phosphatidylinositol mannosides, a unknown polar lipid and trace amounts of two unknown lipids and the major fatty acids were C_{15:0}, C_{16:0}, C_{17:1 ω 8c} and 10-methyl C_{17:0}. The results of DNA–DNA hybridizations and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain GW4-1778^T from related species with 16S rRNA gene similarities of >97.5%. Therefore, strain GW4-1778^T merits species status, for which the name *Nocardia acidivorans* sp. nov. is proposed, with the type strain GW4-1778^T (=CCUG 53410^T = CIP 109315^T = DSM 45049^T).

The genus *Nocardia* encompasses more than 40 established species of mycolic acid-containing actinomycetes, including recently described species (Albuquerque de Barros *et al.*, 2003; Cui *et al.*, 2005; Iida *et al.*, 2006; Kageyama *et al.*, 2004a, b, c, 2005; Kämpfer *et al.*, 2004; Lee, 2006; Wang *et al.*, 2004; Xu *et al.*, 2005; Yamamura *et al.*, 2005; Yassin & Brenner, 2005; Yassin *et al.*, 2003; Zhang *et al.*, 2003, 2004). A comprehensive summary of the taxonomy of the genus has been given by Goodfellow *et al.* (1999).

During the characterization of organisms isolated from different soils, strain GW4-1778^T was recovered from 1 g of a soil sample (heated for 10 s at 100 °C) that originated from

the Italian island of Stromboli, after extraction for 2 h in 10 ml 0.1% (v/v) Tween 80 solution containing 5 mg ampicillin and dilution on mannitol-rifampicin agar [containing 1⁻¹: mannitol, 10 g; yeast extract, 7 g; Casamino acids, 2 g; peptone (Bacto), 1 g; NaCl, 1 g; CaCO₃, 0.2 g; nystatin, 100 mg; and rifampicin, 5 mg] for 6 weeks at 27 °C. The strain was maintained on Medium 65 (DSMZ, <http://www.dsmz.de/media/med065.htm>) at 25 °C and showed on this medium an orange-coloured substrate mycelium. Yellowish-white aerial hyphae were formed.

Cells of strain GW4-1778^T were Gram-positive using the staining procedure described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at $\times 1000$, with cells grown for 3 days at 25 °C on Medium 65. The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). Phylogenetic analysis was performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data using CLUSTAL_X (Thompson *et al.*, 1997).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GW4-1778^T is AM402972.

An extended neighbour-joining tree based on 16S rRNA gene sequences showing the positions of strain GW4-1778^T and other *Nocardia* species is available as supplementary material with the online version of this paper.

Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method and maximum-parsimony (data not shown) were performed by using bootstrap values based on 1000 replications. The sequenced length of the 16S rRNA gene of strain GW4-1778^T was 1493 bp. Sequence similarity calculations (based on 1299 nt) after a neighbour-joining analysis (Fig. 1 and Supplementary Fig. S1 available in IJSEM Online) indicated that the closest relatives of strain GW4-1778^T were *Nocardia pseudobrasiliensis* (GenBank accession no. DQ659914; 98.6 %), *Nocardia nova* (Z36930; 98.6 %), *Nocardia niigatensis* (AB092563; 98.4 %), *Nocardia jiangxiensis* (AY639902; 98.0 %), *Nocardia uniformis* (Z46752; 98.0 %) and *Nocardia miyunensis* (AY639901; 97.8 %). Lower sequence similarities (<97.5 %) were found with all other established species of the genus *Nocardia*.

Analysis of menaquinones was done as described by Tindall (1990) and Altenburger *et al.* (1997) and that of polar lipids as described by Ventosa *et al.* (1993). Strain GW4-1778^T exhibited a quinone system with the predominant compound MK-8 (H₄, ω -cycl) (99.5 %) and traces of MK-8 (H₄). This trait is characteristic of members of the genus *Nocardia* (Goodfellow *et al.*, 1999). The polar lipid profile of strain GW4-1778^T consisted of the predominant compound diphosphatidylglycerol, moderate amounts of phosphatidylethanolamine, phosphatidylinositol, two phosphatidyl-inositol mannosides, an unknown polar lipid and trace amounts of two unknown lipids. Traces of phosphatidylglycerol may be present, as assumed by the presence of a

weak spot showing the chromatographic behaviour for this lipid, but could not be stained with molybdenum blue, which is used to indicate the presence of phosphate groups (data not shown). However, the lack or presence only of trace amounts of phosphatidylglycerol has been reported for several nocardiae (Minnikin *et al.* 1977). The presence of only one aminolipid, phosphatidylethanolamine, demonstrates that strain GW4-1778^T exhibits phospholipid type II (Lechevalier *et al.*, 1977) and thus this polar lipid profile is in accordance with the characteristics of the genus *Nocardia*.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain GW4-1778^T given in Table 1 was similar to those of the other closely related species, but also showed pronounced quantitative differences.

Detailed results of the physiological characterization are given in Table 2 and the species description, using methods as described previously (Kämpfer *et al.*, 1991). DNA–DNA hybridization experiments were performed using labelled DNA from strain GW4-1778^T and type strains of other *Nocardia* species using the method described by Ziemke *et al.* (1998) except that, for nick translation, 2 µg of DNA was labelled during a 3 h incubation at 15 °C. The DNA–DNA hybridization values obtained were as follows: *N. pseudobrasiliensis* DSM 44290^T (46.1 %), *N. nova* DSM 44481^T (62.6 %), *N. jiangxiensis* DSM 17684^T (50.9 %), *N. miyunensis* DSM 17685^T (41.7 %), *N. niigatensis* DSM 44670^T (11.0 %) and *N. uniformis* DSM 43136^T (21.0 %).

Fig. 1. Phylogenetic analysis of selected *Nocardia* species most closely related to strain GW4-1778^T, based on 16S rRNA gene sequences available from GenBank/EMBL/DBJ (accession numbers are given in parentheses), constructed after multiple alignment of data by using CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were performed by using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001). Bootstrap percentages based on 1000 replications are given at branch points. Bar, 0.005 nucleotide substitutions per nucleotide position. An extended neighbour-joining tree is available as Supplementary Fig. S1 in IJSEM Online.

Table 1. Major fatty acid compositions (%) of strain GW4-1778^T and type strains of species of the genus *Nocardia* grouped in the same cluster on the basis of 16S rRNA gene sequence similarity (see Fig. 1)

Strains: 1, GW4-1778^T (*Nocardia acidivorans* sp. nov.); 2, *N. jiangxiensis* DSM 17684^T; 3, *N. miyunensis* DSM 17685^T; 4, *N. uniformis* DSM 43136^T; 5, *N. pseudobrasiliensis* DSM 44290^T; 6, *N. nova* DSM 44481^T; 7, *N. niigatensis* DSM 44670^T. All strains were grown on trypticase soy broth agar at 28 °C for 7 days prior to fatty acid analysis. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively. —, Not detected.

Fatty acid	1	2	3	4	5	6	7
Saturated fatty acids:							
C _{13:0}	0.7	—	—	—	—	—	—
C _{14:0}	1.9	0.7	—	1.0	0.4	1.0	1.6
C _{15:0}	20.3	2.1	6.0	5.7	1.4	1.1	5.0
C _{16:0}	11.7	35.7	38.2	33.4	40.2	37.7	32.1
C _{17:0}	6.6	2.7	2.9	2.3	1.9	1.9	4.2
C _{18:0}	—	2.1	0.8	0.7	8.6	7.2	3.0
C _{20:0}	—	0.5	—	—	—	—	—
Unsaturated fatty acids:							
C _{15:1ω5c}	4.4	—	1.1	—	—	—	1.5
C _{16:1ω9c}	—	0.7	—	—	—	—	—
C _{17:1ω9c}	—	—	—	—	—	—	—
C _{17:1ω8c}	13.8	1.8	4.9	2.4	0.8	—	4.0
C _{17:1ω5c}	0.6	1.0	1.0	—	—	—	—
C _{18:1ω9c}	5.7	13.1	11.5	19.0	6.3	15.6	12.1
C _{20:4ω6,9,12,15c}	—	—	—	—	—	—	0.7
C _{20:2ω6,9c}	—	—	—	1.1	—	—	—
C _{20:1ω9c}	—	2.2	—	—	—	—	—
Summed feature 2*	—	—	—	—	—	—	—
Summed feature 3*	12.1	6.7	12.3	16.4	14.4	20.0	16.1
Summed feature 5*	—	—	—	—	—	—	—
Summed feature 6*	2.8	1.5	0.1	2.1	—	—	1.7
10-Methyl C _{16:0}	—	—	—	—	—	—	0.8
10-Methyl C _{17:0}	11.3	1.0	3.4	1.0	0.9	—	2.5
10-Methyl C _{18:0} (TSBA) [†]	6.8	13.3	17.0	13.9	24.8	14.8	14.5
10-Methyl C _{19:0}	1.0	—	—	1.0	—	—	—
Unknown 14.959 [‡]	—	14.1	—	—	—	0.7	—

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C_{16:1 ω 7c} and/or C_{15:0} iso 2-OH. Summed feature 6 contains C_{19:1 ω 11c} and/or C_{19:1 ω 9c}.

[†]TSBA, Tuberculostearic acid.

[‡]Unknown fatty acid that has no name listed in the peak library file of the MIDI system and therefore cannot be identified; it is referred to by its equivalent chain length.

Description of *Nocardia acidivorans* sp. nov.

Nocardia acidivorans (a.cid.i.vo'rans. N.L. n. *acidum* an acid; L. v. *vorare* to devour; N.L. part. adj. *acidivorans* acid-devouring).

Forms a light orange-coloured vegetative mycelium. Aerial mycelium is yellowish-white. Gram-positive and oxidase-positive, showing an oxidative metabolism. Good growth occurs on nutrient agar and Medium 65 at 25–30 °C. The

menaquinone system contains predominantly MK-8 (H₄, ω -cycl) and traces of MK-8 (H₄). The polar lipid profile consists of the predominant compound diphosphatidylglycerol, moderate amounts of phosphatidylethanolamine, phosphatidylinositol, two phosphatidylinositol mannosides, an unknown polar lipid and trace amounts of two unknown lipids. Major fatty acids are C_{15:0}, C_{16:0}, C_{17:1 ω 8c} and 10-methyl C_{17:0}. Carbon source utilization and hydrolysis of chromogenic substrates (including differential characteristics) are given in Table 2.

Table 2. Physiological properties that distinguish strain GW4-1778^T from the type strains of the most closely related *Nocardia* species

Strains: 1, GW4-1778^T (*Nocardia acidivorans* sp. nov.); 2, *N. jiangxiensis* DSM 17684^T; 3, *N. miyunensis* DSM 17685^T; 4, *N. uniformis* DSM 43136^T; 5, *N. pseudobrasiliensis* DSM 44290^T; 6, *N. nova* DSM 44481^T; 7, *N. niigatensis* DSM 44670^T. +, Positive; –, negative; (+), weakly positive; pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl. All strains are positive for hydrolysis of aesculin, bis-pNP phosphate, pNP phenylphosphonate and L-alanine pNA and utilization of *N*-acetyl-D-glucosamine, D-glucose, D-ribose, inositol, glutarate and DL-3-hydroxybutyrate. All strains are negative for hydrolysis of pNP β -D-glucuronide, pNP phosphorylcholine, L-glutamate- γ -3-carboxy pNA, L-proline pNA and utilization of gluconate, maltose, D-xylose, adonitol, *trans*-aconitate, adipate, itaconate, DL-lactate, mesaconate, oxoglutarate, substrate, L-aspartate, L-ornithine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate.

Characteristic	1	2	3	4	5	6	7
Hydrolysis of:							
pNP β -D-galactopyranoside	–	–	–	(+)	–	–	–
pNP α -D-glucopyranoside	–	+	–	+	+	+	+
pNP β -D-glucopyranoside	+	+	–	+	+	+	+
pNP β -D-xylopyranoside	+	+	–	+	+	+	+
2-Deoxythymidine-5'-pNP phosphate	–	–	–	+	–	–	(+)
Utilization of:							
<i>N</i> -Acetyl-D-galactosamine	(+)	(+)	–	(+)	(+)	–	–
L-Arabinose	(+)	+	+	–	–	–	–
<i>p</i> -Arbutin	–	+	+	+	–	–	–
D-Cellobiose	–	+	+	–	–	–	–
D-Fructose	–	+	+	+	(+)	–	–
D-Galactose	(+)	(+)	(+)	–	(+)	(+)	(+)
D-Mannose	(+)	+	–	–	–	–	–
α -D-Melibiose	–	+	+	–	–	–	–
L-Rhamnose	–	+	+	–	–	–	–
Sucrose	–	+	+	–	–	–	–
Salicin	–	–	–	+	–	–	–
D-Trehalose	–	–	–	–	(+)	–	–
Maltitol	–	+	–	–	–	–	–
D-Mannitol	+	+	–	(+)	–	–	–
D-Sorbitol	–	–	–	–	(+)	–	–
Putrescine	+	–	–	–	–	–	–
Acetate	+	–	–	–	–	–	–
Propionate	+	–	–	–	–	–	–
<i>cis</i> -Aconitate	+	–	–	–	–	–	–
4-Aminobutyrate	–	–	+	–	–	–	–
Azelate	+	–	–	–	–	(+)	(+)
Citrate	+	–	–	–	(+)	–	–
Fumarate	+	(+)	+	+	+	+	(+)
L-Malate	–	–	+	+	+	(+)	(+)
Pyruvate	(+)	–	–	–	–	–	–
L-Alanine	(+)	(+)	+	(+)	(+)	–	–
β -Alanine	–	–	+	–	–	–	–
L-Histidine	(+)	–	–	–	–	–	–
L-Leucine	–	–	(+)	–	(+)	–	–
L-Proline	–	–	+	(+)	(+)	–	–
L-Serine	(+)	–	–	–	–	–	–

The type strain, GW4-1778^T (=CCUG 53410^T=CIP 109315^T=DSM 45049^T), was isolated from soil of Stromboli, Italy.

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