Nocardia tenerifensis sp. nov.

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A Gram-positive, non-spore-forming bacterium (GW39-1573^T) was isolated from soil of the Spanish island of Tenerife. 16S rRNA gene sequence similarity studies showed that strain GW39-1573^T belonged to the genus *Nocardia* and was most closely related to *Nocardia brasiliensis* (98.0%), *Nocardia beijingensis* (97.3%), *Nocardia transvalensis* (97.5%), *Nocardia asteroides* (97.2%) and *Nocardia farcinica* (97.0%). Strain GW39-1573^T could be distinguished from all other validly described *Nocardia* species by sequence similarity values of less than 97%. Chemotaxonomic data [major menaquinone: MK-8(H₄, ω-cycl); major polar lipids: diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside and an unknown glycolipid and an unknown phospholipid; major fatty acids: C_{16:0}, C_{18:1}ω9c and 10 methyl C_{16:0}] and the presence of mycolic acids supported the affiliation of strain GW39-1573^T to the genus *Nocardia*. The results of DNA–DNA hybridizations and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain GW39-1573^T from those related species that showed 16S rRNA gene sequence similarity values of greater than 97%. Strain GW39-1573^T merits species status, and the name *Nocardia tenerifensis* is proposed with the type strain GW39-1573^T (=DSM 44704^T = CIP 107929^T).

The genus *Nocardia* encompasses 29 validly described species of mycolic-acid-containing actinomycetes, including the recently described species *Nocardia cerradoensis*, *Nocardia caishijiensis* and *Nocardia puris* (Albuquerque de Barros *et al.*, 2003; Zhang *et al.*, 2003; Yassin *et al.*, 2003). A comprehensive treatise of the history of the genus has been given by Goodfellow *et al.* (1999).

During the characterization of organisms isolated from different soils, strain GW39-1573^T was recovered from 1 g of a soil sample (heated for 100 °C) originating from the Spanish island of Tenerife, after a 2 h extraction in 10 ml of a 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; rifampicin, 5 mg] with incubation 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 that fragmented easily into irregular, rod-shaped cells. Orange–white aerial hyphae were formed.

The strain stained Gram-positive using the method of Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at ×1000, with cells grown for 3 days at 25 °C on medium 65. The 16S rRNA gene of the strain was analysed as described by Kämpfer *et al.* (2003). Phylogenetic analysis was performed using the ARB software package (Strunk *et al.*, 2000) and the MEGA (Molecular Evolutionary Genetics Analysis) software package version 2.1 (Kumar *et al.*, 2001) after multiple alignment of sequences by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to Kimura's two-parameter model) were calculated and clustering of the sequences was determined with the neighbour-joining method and maximum parsimony; bootstrap values were determined based on 1000 replications (results are available in IJSEM

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The EMBL accession number for the 16S rRNA gene sequence of *Nocardia tenerifensis* GW39-1573^T is AJ566157.

Details of the major fatty acids of *N. tenerifensis* GW39-1573^T and phylogenetic trees showing the relationship of *N. tenerifensis* with members of the genus *Nocardia* are available in IJSEM Online.

Online). The 16S rRNA gene sequence of strain GW39-1573^T was a continuous stretch of 1481 nt. Sequence similarity calculations after a neighbour joining analysis indicated that the closest relatives of strain GW39-1573^T were *Nocardia brasiliensis* (EMBL accession no.) X80608 (98.0%), *Nocardia beijingensis* AF154129 (97.3%), *Nocardia transvalensis* X80609 (97.5%), *Nocardia asteroides* X84850 (97.2%), *Nocardia farcinica* X80610 (97.0%) and *N. puris* AJ508748 (96.9%). Lower sequence similarities (<97.0%) were found with all other validly described species of the genus *Nocardia*.

Results of chemotaxonomic analyses are given in the species description. The following analytical procedures were performed as described: menaquinones (Tindall, 1990); polar lipids (Ventosa *et al.*, 1993); mycolic acids were

extracted and analysed by one-dimensional TLC and developed in petroleum benzene/acetone (95:5, v/v) (Minnikin *et al.*, 1980); fatty acids (Kämpfer & Kroppenstedt, 1996). The quinone system with the predominant compound (99%) MK-8(H₄, ω -cycl) supports affiliation of strain GW39-1573^T to the genus *Nocardia*, where all species have MK-8(H₄, ω -cycl) as the major quinone (Goodfellow *et al.*, 1999). The polar lipid profile of strain GW39-1573^T was similar to those reported for other nocardiae but the presence of significant amounts of phosphatidylglycerol has been reported only for *N. asteroides* ATCC 19247^T and *N. brasiliensis* ATCC 19296^T (Minnikin *et al.*, 1977). The mycolic acid of strain GW39-1573^T showed a chromatographic behaviour which was similar to that of *N. brasiliensis* DSM 43009, which was analysed in parallel. The fatty acid profile of strain GW39-1573^T (available in IJSEM Online)

Table 1. Physiological characteristics of the type strains of *Nocardia* species grouped into the same cluster on the basis of 16S rRNA gene sequence similarity studies (see Figs I and II in IJSEM Online)

Taxa: 1, GW39-1573^T; 2, *N. transvalensis* DSM 43405^T; 3, *N. brasiliensis* DSM 43758^T; 4, *N. farcinica* DSM 43665^T; 5, *N. beijingensis* DSM 44636^T. pNP, para-nitrophenyl; pNA, para-nitroanilide. +, Positive; -, negative; (+), weakly positive. All strains were positive for hydrolysis of bis-(pNP) phosphate, pNP phenylphosphonate, (pNPphosphoryl)-choline, 2-deoxythymidine 5'-pNP phosphate and L-alanine pNA. All strains were negative for hydrolysis of pNP β -D-glucuronide. All strains were also positive for assimilation of D-glucose, acetate*, propionate and oxoglutarate. All strains were negative for assimilation of L-arabinose, p-arbutin, D-cellobiose, gluconate, D-mannose, D-maltose, α -D-melibiose, sucrose, salicin, D-xylose*, maltitol, 4-aminobutyrate, ornithine*, L-phenylalanine and L-tryptophan.

Test	1	2	3	4	5
Hydrolysis of:					
Aesculin*, pNP α -D-glucopyranoside, pNP β -D-glucopyranoside, pNP β -D-xylopyranoside, L-glutamate γ -(3-carboxy-pNA), L-proline pNA	+	+	+	+	-
<i>o</i> -Nitrophenyl β -D-galactopyranoside	-	+	-	-	-
Assimilation of:					
DL- β -Hydroxybutyrate	+	(+)	+	+	+
Pyruvate*	+	(+)	+	+	(+)
Fumarate, L-malate	-	+	+	+	+
Citrate*, glutarate	+	+	+	-	+
Suberate	(+)	+	(+)	-	(+)
N-Acetyl-D-galactosamine, N-acetyl-D-glucosamine, D-galactose, D-ribose, i-inositol, L-alanine, L-proline	+	+	+	-	-
L-Leucine	+	(+)	+	-	-
D-Mannitol*, <i>cis</i> -aconitate	+	+	(+)	-	(+)
Azelate	+	+	-	-	+
L-Histidine	+	-	(+)	+	-
D-Trehalose	+	-	+	+	-
D-Fructose, DL-lactate	-	(+)	-	+	-
β -Alanine	(+)	(+)	-	-	-
L-Serine, 4-hydroxybenzoate	+	-	(+)	-	-
L-Rhamnose*, 3-hydroxybenzoate, phenyl acetate	-	(+)	-	+	-
Adonitol, D-sorbitol*, adipate	-	+	-	-	-
<i>trans</i> -Aconitate, itaconate, mesaconate	-	(+)	-	-	-
Putrescine	-	-	+	-	-
L-Aspartate	+	-	-	-	-

*Test (based on a different method) was also listed by Albuquerque de Barros *et al.* (2003) and gave congruent results for *N. transvalensis*, *N. beijingensis*, *N. farcinica* and *N. brasiliensis*.

was very similar to those of the other closely related species and was congruent with the fatty acid profiles reported for other *Nocardia* species.

Detailed results of the physiological characterization of strain GW39-1573^T are given in the species description, with methods as described previously (Kämpfer *et al.*, 1991). Degradation of polymeric substances was performed using standard procedures (Williams *et al.*, 1983) and results were read after 7 days incubation at 28 °C. Results of DNA–DNA hybridization experiments between strain GW39-1573^T and the type strains were: *N. brasiliensis* DSM 43758^T, 29.3/26.2%; *N. beijingensis* DSM 44636^T, 25.4/16.8%; *N. transvalensis* DSM 43405^T, 16.2/23.6%; *N. farcinica* DSM 43665^T, 15.8/15.2% (first value, performed with labelled DNA from strain GW39-1573^T; second value, performed with labelled DNA from the type strain; all values are mean values of two analyses). The method of Ziemke *et al.* (1998) was used, except that for nick translation, and 2 µg of DNA was labelled during a 3 h incubation at 15 °C.

Description of *Nocardia tenerifensis* sp. nov.

Nocardia tenerifensis (te.ne.ri'fen.sis. N.L. fem. adj. *tenerifensis* named after Tenerife, from where the organism was isolated).

Forms an orange-coloured vegetative mycelium that fragments very easily into irregular, rod-shaped elements. Aerial mycelium is yellowish-white. Gram-positive, oxidase-positive, showing an oxidative metabolism. Good growth occurs on nutrient agar and medium 65 at 25–30 °C. Major fatty acids are C_{16:0}, C_{18:1ω9c} and 10 methyl C_{16:0}. Carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters) are indicated in Table 1. Urea and allantoin are hydrolysed. Tyrosine, casein, starch (weak) and hypoxanthine are degraded, but adenine, xylan and xanthine are not.

The type strain is GW39-1573^T (=DSM 44704^T=CIP 107929^T), isolated from soil, Tenerife, Spain.

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