View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Digital.CSIC

International Journal of Systematic and Evolutionary Microbiology (2008), 58, 2210–2214 DOI 10.1099/ijs.0.65482-0

Nocardia altamirensis sp. nov., isolated from Altamira cave, Cantabria, Spain

Valme Jurado,[1] Patrick Boiron,[2,3,4] Reiner M. Kroppenstedt,[5] Frédéric Laurent,[2,3,4] Andrée Couble,[2,3,4] Leonila Laiz,[1] Hans-Peter Klenk,[5] Juan M. González,[1] Cesareo Saiz-Jimenez,[1] Delphine Mouniée,[2,3,4] Emanuelle Bergeron[2,3,4] and Verónica Rodríguez-Nava[2,3,4]

Correspondence
Verónica Rodríguez-Nava
veronica.rodriguez@sante.univ-lyon1.fr

[1]Instituto de Recursos Naturales y Agrobiologia, CSIC, Apartado 1052, E-41080 Sevilla, Spain

[2]Research group on 'Bacterial Opportunistic Pathogens and Environment', Université de Lyon, F-69003 Lyon, France

[3]Université Lyon 1 and CNRS, UMR 5557, Ecologie Microbienne, F-69622 Villeurbanne, France

[4]Institut des Sciences Pharmaceutiques et Biologiques and Observatoire Français des Nocardioses, F-69373 Lyon, France

[5]Deutsche Sammlung von Mikroorganismen und Zellkulturen, D-38124 Braunschweig, Germany

A novel actinomycete strain, OFN S17^{T}, was isolated from a sample collected from Altamira Cave, Cantabria, Spain. This strain was identified by using a polyphasic taxonomic approach. The 16S rRNA, *hsp65* and *sod* gene sequences of the strain were determined and compared with those of representative *Nocardia* species. The results showed that strain OFN S17^{T} should be assigned to the genus *Nocardia*. Phylogenetic analysis indicated that strain OFN S17^{T} was most closely related to the type strain of *Nocardia tenerifensis* (98.6, 96.2 and 96 % similarity, respectively, for the 16S rRNA, *hsp65* and *sod* gene sequences). The DNA G+C content was 64.4 mol%. DNA–DNA hybridization analyses revealed 29 % relative reassociation between the DNA of strain OFN S17^{T} and *N. tenerifensis* DSM 44704^{T}. The phenotypic and genotypic data show that strain OFN S17^{T} merits recognition as a representative of a novel species of the genus *Nocardia*, for which the name *Nocardia altamirensis* sp. nov. is proposed. The type strain is OFN S17^{T} (=CIP 109606^{T} =DSM 44997^{T}).

Members of the genus *Nocardia* are aerobic, Gram-positive bacteria that are ubiquitous in the environment and can be found worldwide as saprophytic components in fresh and salt water, soil, dust, decaying vegetation and decaying faecal deposits from animals (Brown-Elliott *et al.*, 2006). The application of polyphasic taxonomic procedures has led to marked improvements in the classification of the genus *Nocardia* (Goodfellow, 1998). Numerous novel species have been described in recent years by using 16S rRNA gene sequencing as the reference method; at the time of writing, the genus encompasses 70 species with validly published names (http://www.bacterio.cict.fr/qr/nocardia.html). It seems likely that the soil is the primary reservoir of nocardiae, although members of the genus are better known as causal agents of suppurative and granulomatous infections of humans and animals (McNeil & Brown, 1994). There is also evidence that nocardial species diversity in both non-extreme and extreme habitats is grossly underestimated (Orchard & Goodfellow, 1980). Investigations of bacterial biodiversity from natural caves have revealed the presence of several novel actinomycetes (Groth & Saiz-Jimenez, 1999; Groth *et al.*, 1999, 2002; Jurado *et al.*, 2005a, b, 2006; Lee, 2006a, b, c). This is illustrated by the recent description of *Nocardia jejuensis* and *Nocardia speluncae* from natural caves in South Korea (Lee, 2006b; Seo *et al.*, 2007). In a preliminary report, Groth & Saiz-Jimenez (1999) investigated the presence of actinomycetes in Altamira and Tito Bustillo caves. Approximately 350 actinomycetes were identified by morphological, physiological and chemotaxonomic methods, among which the genus *Nocardia* was well represented.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *hsp65* and *sod* gene sequences of strain OFN S17^{T} are respectively EU006090, EU007653 and EU432372.

Neighbour-joining trees based on sequences of the 16S rRNA (with an extended set of reference sequences), *hsp65* and *sod* genes are available as supplementary material with the online version of this paper.

 65482 © 2008 IUMS Printed in Great Britain

In this work, a polyphasic approach was used to determine the taxonomic position of bacterial strain OFN S17^T. This strain was retrieved from Altamira cave (Cantabria, Spain) as a member of a complex microbial community forming a grey-coloured colonization on the walls of the cave.

Strain OFN S17^T was isolated on tryptose soy agar (TSA; Oxoid) at 28 °C. This strain was examined for a broad range of biochemical properties. The cultural characteristics of strain OFN S17^T was determined on Bennett's agar after 10 days at 30 °C. The well-developed substrate mycelium was yellowish orange in colour with irregular branches penetrating the agar, while the aerial hyphae were white. On Bennett's agar, the isolate was able to grow at 25–37 °C. The bacterial cells were Gram-positive and slightly acid-fast in a modified Ziehl–Neelsen method (1 % acid decoloration). Phenotypic characteristics were determined for strain OFN S17^T and the type strains of the closely related species *Nocardia tenerifensis* (DSM 44704^T) and *Nocardia brasiliensis* (ATCC 19296^T). Catalase activity, urea hydrolysis and the ability to hydrolyse adenine, casein, hypoxanthine, tyrosine, uric acid and testosterone were examined by following the protocol of Boiron *et al.* (1993). Tests for the utilization of various substrates as sole carbon sources (at 1 %, w/v) were carried out using previously described methods (Goodfellow & Lechevalier, 1989; Goodfellow, 1992, 1998) and utilization was observed over a period of 2 weeks. The more relevant results that allow discrimination between strain OFN S17^T and the type strains of *N. brasiliensis* and *N. tenerifensis* are the inability of strain OFN S17^T to utilize L-arabinose and its ability to decompose uric acid. The complete phenotypic results from strain OFN S17^T and the two reference type strains are summarized in Table 1.

Table 1. Phenotypic characteristics that differentiate strain OFN S17^T from the type strains of *N. tenerifensis* and *N. brasiliensis*

Strains: 1, *N. altamirensis* sp. nov. OFN S17^T; 2, *N. tenerifensis* DSM 44704^T; 3, *N. brasiliensis* ATCC 19296^T. All three strains were positive in tests for growth on D-fructose, D-galactose, D-glucose, glycerol, mannose and D-ribose (all at 1.0 %, w/v), urea hydrolysis, degradation of casein, activity of catalase and growth on Bennett's agar at 25 °C and negative for growth on raffinose (1.0 %, w/v). –, Negative; +, positive; W, weakly positive.

Test	1	2	3
Growth on carbon sources (%, w/v)			
L-Arabinose (1.0)	–	+	+
D-Mannitol (1.0)	+	+	W
L-Rhamnose (1.0)	–	W	–
Sucrose (1.0)	–	–	+
Sorbitol (1.0)	–	W	–
Growth on Bennett's agar at:			
37 °C	W	+	+
45 °C	–	–	+
Decomposition of (%, w/v):			
Adenine (0.4)	–	+	–
Hypoxanthine (0.4)	W	W	+
Testosterone (0.1)	+	+	W
Tyrosine (0.5)	W	–	+
Uric acid (0.5)	+	–	–

Extraction of genomic DNA, PCR amplification and sequencing of the 16S RNA, *hsp65* and *sod* genes were carried out as described by Rodríguez-Nava *et al.* (2004, 2006). Sequence analysis was performed using an Applied Biosystems model 373A DNA sequencer. Overlapping DNA segments from the forward and reverse strands were analysed to determine a consensus sequence for each gene. The nearly complete 16S rRNA gene sequence for strain OFN S17^T (1348 nt; corresponding to positions 46–1400 of the *Escherichia coli* numbering system) was obtained by using primers SQ1 and SQ6 (Rodríguez-Nava *et al.*, 2004). The *hsp65* and *sod* genes of strain OFN S17^T were partially amplified (around 441 nt for each gene) by using primers TB11 and TB12 for *hsp65* and Z205 and Z212 for *sod*, as described by Rodríguez-Nava *et al.* (2006, 2007). For phylogenetic analysis, nucleotide sequences were aligned using the program CLUSTAL_X (Thompson *et al.*, 1997) with the corresponding sequences of representative *Nocardia* species from the GenBank and BIBI (Devulder *et al.*, 2003) databases. Phylogenetic trees were reconstructed by using the software packages MEGA (Kumar *et al.*, 2004) and PHYLO_WIN (Galtier *et al.*, 1996). The overall topologies of phylogenetic trees obtained by the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods were similar (data not shown). The robustness of the trees was assessed by bootstrap resampling (1000 replicates).

The results of our phylogenetic analysis showed that strain OFN S17^T fell within the evolutionary radiation encompassed by the genus *Nocardia*. According to 16S rRNA gene sequence similarity calculations, strain OFN S17^T was most closely related to *N. brasiliensis* ATCC 19296^T (98.67 % sequence similarity; 18 differences out of 1348 nt) and *N. tenerifensis* GW39-1573^T (98.60 % sequence similarity; 19 differences out of 1348 nt). In the phylogenetic tree based on the 16S rRNA gene sequence (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online), strain OFN S17^T forms a clade with the type strains of *N. brasiliensis* and *N. tenerifensis* that is supported by a bootstrap value of 77 % in the neighbour-joining analysis. The 16S rRNA gene sequence similarity between our strain and the type strains of *N. brasiliensis* and *N. tenerifensis* was almost identical, so we decided to extend our study to the *hsp65* and *sod* genes.

For the *hsp65* gene, strain OFN S17^T was more closely related to *N. tenerifensis* DSM 44704^T, showing 96.2 % sequence similarity (15 differences among 401 nt, excluding the primers), than to *N. brasiliensis* ATCC 19296^T, which showed only 95.7 % sequence similarity (17 differences among 401 nt, excluding the primers). The phylogenetic tree reconstructed from *hsp65* gene sequences

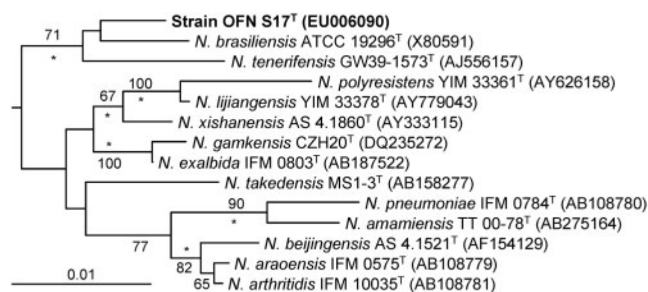

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences showing the relationships between *Nocardia altamirensis* sp. nov. OFN S17^{T} and species belonging to the genus *Nocardia*. The tree was constructed by using the neighbour-joining method and was based on a comparison of 1348 nucleotides. Bootstrap values are expressed as percentages of 1000 replications. Asterisks indicate branches of the tree that were also recovered using maximum-likelihood and maximum-parsimony treeing algorithms. Bar, 0.01 substitutions per nucleotide position. An extended version of this tree including a wider selection of reference strains is available as Supplementary Fig. S1.

did not contain the clade encompassing our isolate and *N. tenerifensis* and *N. brasiliensis* previously observed with the 16S rRNA gene tree (Supplementary Fig. S2). Instead, the clades formed with OFN S17^{T} showed non-significant bootstrap values (<50 %), meaning that these phylogenetic relationships are very weak.

For the *sod* gene, strain OFN S17^{T} was also more closely related to *N. tenerifensis* DSM 44704^{T}, showing 96 % sequence similarity (16 differences among 406 nt, excluding the primers), than to *N. brasiliensis* ATCC 19296^{T}, which showed 95.3 % sequence similarity (19 differences among 406 nt, excluding the primers). Similar to the 16S rRNA gene, in the phylogenetic tree reconstructed from the *sod* gene sequence, strain OFN S17^{T} forms a clade with the type strains of *N. tenerifensis* and *N. brasiliensis* and, in this case, the clade is supported by a bootstrap value of 92 % in the neighbour-joining analysis (Supplementary Fig. S3).

Phylogenetic analysis of *hsp65* and *sod* gene sequences showed that isolate OFN S17^{T} was more closely related to *N. tenerifensis* DSM 44704^{T} than to *N. brasiliensis* ATCC 19296^{T}. In light of these results, we decided that it was more pertinent to perform DNA–DNA hybridization between OFN S17^{T} and *N. tenerifensis* DSM 44704^{T}.

The degree of DNA–DNA relatedness between strain OFN S17^{T} and *N. tenerifensis* DSM 44704^{T} was determined by the fluorometric method as described by Gonzalez & Saiz-Jimenez (2005). This method measures the divergence between the thermal denaturation midpoint of homoduplex DNA and heteroduplex DNA (ΔT_m) using a real-time PCR thermocycler that obtains fluorescence determinations. The degree of DNA–DNA relatedness was also determined using the digoxigenin labelling/antibody detection system described by Ziemke *et al.* (1998). DNA was labelled and detected colorimetrically with *p*-nitrophenyl phosphate as substrate. Chromosomal DNA was extracted following the method described by Marmur (1961).

The difference in melting temperature between genomic DNA from strain OFN S17^{T} and *N. tenerifensis* DSM 44704^{T} was 7 °C. This is above the 5 °C cut-off point recommended for the delineation of species by Stackebrandt & Goebel (1994). Using DNA from strain OFN S17^{T} as a labelled probe, *N. tenerifensis* DSM 44704^{T} and strain OFN S17^{T} showed 29 % DNA relatedness. The DNA–DNA relatedness results confirmed that strain S17^{T} represents a novel genospecies clearly differentiated from *N. tenerifensis*.

The G+C content of genomic DNA of strain OFN S17^{T} was 64.4 mol%, as determined by the fluorometric method described by Gonzalez & Saiz-Jimenez (2002).

Strain OFN S17^{T} was analysed chemotaxonomically by using the following procedures. The isomer of diaminopimelic acid was analysed by TLC of whole-organism hydrolysates as described by Boiron *et al.* (1993). Whole-cell sugars were analysed following the methods of Staneck & Roberts (1974). Standard procedures (Kroppenstedt, 1982, 1985; Minnikin *et al.*, 1975, 1984) were used for analyses of fatty acids, mycolic acids and isoprenoid quinones by HPLC and analysed with the standard Microbial Identification System (MIDI) for automated GC analyses. The results support the assignment of strain OFN S17^{T} to the genus *Nocardia*. Analysis of the cell wall revealed mycolic acids with a chain length of 52–58 carbon atoms. Strain OFN S17^{T} presented mainly hexahydrogenated menaquinone with eight isoprene units where the two end units are cyclized [MK-8($H4_{\omega\text{-cycl}}$)]. This menaquinone is observed only in members of the genera *Nocardia* and *Skermania*. The isolate contained mainly straight-chain saturated and unsaturated fatty acids including palmitic acid ($C_{16:0}$; 33.48 %), oleic acid ($C_{18:1}$; 18.40 %), palmitoleic acid ($C_{16:1}$; 15.93 %) and tuberculostearic acid (10-methyl $C_{18:0}$; 13.09 %).

It is clear from the genotypic and phenotypic data obtained that strain OFN S17^{T} represents a novel species of the genus *Nocardia*, for which the name *Nocardia altamirensis* sp. nov. is proposed.

Description of *Nocardia altamirensis* sp. nov.

Nocardia altamirensis (al.ta.mi.ren′sis. N.L. fem. adj. *altamirensis* referring to Altamira cave, Cantabria, Spain, where the type strain was isolated).

Aerobic, Gram-positive, slightly acid–alcohol-fast, catalase-positive, non-motile actinomycete that forms a branched substrate mycelium which fragments into irregular rod-shaped to coccoid elements. The colour of the substrate mycelium is yellowish-orange. The aerial mycelium is white and has a patchy distribution. Colonies are 2–3 mm in diameter on Bennett's agar. Grows well at 30 °C but does not grow at 45 °C. D-Fructose, D-galactose, D-glucose,

glycerol, D-mannitol, D-mannose and ribose can be utilized as carbon sources, while raffinose, L-rhamnose, sucrose and sorbitol cannot be utilized. Urea is hydrolysed. Substrates that are decomposed are indicated in Table 1. Major fatty acids are $C_{16:0}$ (33.48 %), $C_{18:1}$ (18.40 %) and tuberculostearic acid (10-methyl $C_{18:0}$; 13.09 %). The mycolic acids are 52–58 carbon atoms in length. The DNA G+C content of the type strain is 64.4 mol%.

The type strain, strain OFN S17^T (=CIP 109606^T =DSM 44997^T), was isolated from Altamira cave (Cantabria, Spain).

Acknowledgements

This work received financial support from the Centre National de la Recherche Scientifique (CNRS). The authors acknowledge funding from the Spanish Ministry of Culture for the microbiological study of Altamira Cave and from the Spanish Ministry of Education and Science (project CGL2006-07424). Use of facilities provided by the Museo de Altamira is gratefully acknowledged.

References

Boiron, P., Provost, F. & Dupont, B. (1993). Technical protocols. In *Méthodes de laboratoire pour le diagnostic de la nocardiose*, pp. 107–126. Edited by Institut Pasteur. Paris: Institut Pasteur (in French).

Brown-Elliott, B. A., Brown, J. M., Conville, P. S. & Wallace, R. J. (2006). Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. *Clin Microbiol Rev* **19**, 259–282.

Devulder, G., Pierre, G., Baty, F. & Flandrois, J. P. (2003). BIBI, a bioinformatics bacterial identification tool. *J Clin Microbiol* **41**, 1785–1787.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Galtier, N., Gouy, M. & Gautier, C. (1996). SeaView and PHYLO_WIN, two graphic tools for sequence alignment and molecular phylogeny. *Comput Appl Biosci* **12**, 543–548.

Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* **4**, 770–773.

Gonzalez, J. M. & Saiz-Jimenez, C. (2005). A simple fluorimetric method for the estimation of DNA-DNA relatedness between closely related microorganisms by thermal denaturation temperatures. *Extremophiles* **9**, 75–79.

Goodfellow, M. (1992). The family *Nocardiaceae*. In *The Prokaryotes*, 2nd edn, pp. 1188–1213. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Goodfellow, M. (1998). *Nocardia* and related genera. In *Topley & Wilson's Microbiology and Microbial Infections*, 9th edn, vol. 2, pp. 462–489. Edited by A. Balows & B. I. Duerden. London: Arnold.

Goodfellow, M. & Lechevalier, M. P. (1989). Genus *Nocardia* Trevisan 1889, 9[AL]. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2350–2361. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Groth, I. & Saiz-Jimenez, C. (1999). Actinomycetes in hypogean environments. *Geomicrobiol J* **16**, 1–8.

Groth, I., Schumann, P., Schuetze, B., Augsten, K., Kramer, I. & Stackebrandt, E. (1999). *Beutenbergia cavernae* gen. nov., sp. nov., an L-lysine-containing actinomycete isolated from a cave. *Int J Syst Bacteriol* **49**, 1733–1740.

Groth, I., Schumann, P., Schütze, B., Augsten, K. & Stackebrandt, E. (2002). *Knoellia sinensis* gen. nov., sp. nov. and *Knoellia subterranea* sp. nov., two novel actinobacteria isolated from a cave. *Int J Syst Evol Microbiol* **52**, 77–84.

Jurado, V., Groth, I., Gonzalez, J. M., Laiz, L. & Saiz-Jimenez, C. (2005a). *Agromyces salentinus* sp. nov. and *Agromyces neolithicus* sp. nov. *Int J Syst Evol Microbiol* **55**, 153–157.

Jurado, V., Groth, I., Gonzalez, J. M., Laiz, L. & Saiz-Jimenez, C. (2005b). *Agromyces subbeticus* sp. nov., isolated from a cave in southern Spain. *Int J Syst Evol Microbiol* **55**, 1897–1901.

Jurado, V., Gonzalez, J. M., Laiz, L. & Saiz-Jimenez, C. (2006). *Aurantimonas altamirensis* sp. nov., a member of the order *Rhizobiales* isolated from Altamira Cave. *Int J Syst Evol Microbiol* **56**, 2583–2585.

Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.

Kroppenstedt, R. M. (1982). Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *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* (Society for Applied Bacteriology Technical Series vol. 20), pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. New York: Academic Press.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Lee, S. D. (2006a). *Amycolatopsis jejuensis* sp. nov. and *Amycolatopsis halotolerans* sp. nov., novel actinomycetes isolated from a natural cave. *Int J Syst Evol Microbiol* **56**, 549–553.

Lee, S. D. (2006b). *Nocardia jejuensis* sp. nov., a novel actinomycete isolated from a natural cave on Jeju Island, Republic of Korea. *Int J Syst Evol Microbiol* **56**, 559–562.

Lee, S. D. (2006c). *Actinocorallia cavernae* sp. nov., isolated from a natural cave in Jeju, Korea. *Int J Syst Evol Microbiol* **56**, 1085–1088.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

McNeil, M. M. & Brown, J. M. (1994). The medically important aerobic actinomycetes: epidemiology and microbiology. *Clin Microbiol Rev* **7**, 357–417.

Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium, Nocardia*, and related taxa by thin-layer chromatographic analyses of whole-cell methanolysates. *J Gen Microbiol* **88**, 200–204.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Orchard, V. A. & Goodfellow, M. (1980). Numerical classification of some named strains of *Nocardia asteroides* and related isolates from soil. *J Gen Microbiol* **118**, 295–312.

Rodríguez-Nava, V., Couble, A., Molinard, C., Sandoval, H., Boiron, P. & Laurent, F. (2004). *Nocardia mexicana* sp. nov., a new pathogen isolated from human mycetomas. *J Clin Microbiol* **42**, 4530–4535.

Rodríguez-Nava, V., Couble, A., Devulder, G., Flandrois, J.-P., Boiron, P. & Laurent, F. (2006). Use of PCR-restriction enzyme pattern analysis and sequencing database for *hsp*65 gene-based identification of *Nocardia* species. *J Clin Microbiol* **44**, 536–546.

Rodríguez-Nava, V., Khan, Z. U., Pötter, G., Kroppenstedt, R. M., Boiron, P. & Laurent, F. (2007). *Nocardia coubleae* sp. nov., isolated

from oil-contaminated Kuwaiti soil. *Int J Syst Evol Microbiol* **57**, 1482–1486.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Seo, J. P., Yun, Y. W. & Lee, S. D. (2007). *Nocardia speluncae* sp. nov., isolated from a cave. *Int J Syst Evol Microbiol* **57**, 2932–2935.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Ziemke, F., Höfle, M. G., Lalucat, J. & Rosselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.