Nocardiopsis litoralis sp. nov., a halophilic marine actinomycete isolated from a sea anemone

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A Gram-positive, moderately halophilic, alkalitolerant, filamentous, aerobic actinomycete, designated strain JSM 073097^T, was isolated from a sea anemone collected from a tidal flat in the South China Sea. Phylogenetic analyses based on 16S rRNA gene sequences indicated that the new isolate was a member of the genus *Nocardiopsis* and was most closely related to *Nocardiopsis kunsanensis* HA-9^T, *Nocardiopsis xinjiangensis* YIM 90004^T and *Nocardiopsis salina* YIM 90010^T (99.6, 98.5 and 98.1 % similarity, respectively). Phenotypic characteristics and chemotaxonomic data also indicated that strain JSM 073097^T was a member of the genus *Nocardiopsis*. The strain grew well on most of the media tested, producing white to yellow–white substrate mycelium and white aerial mycelium and straight to flexuous hyphae. The substrate mycelium was well developed and fragmented with age; the aerial mycelium produced long, straight to flexuous spore chains with non-motile, smooth-surfaced, rod-shaped spores. The strain grew in the presence of 1–15 % (w/v) total salts and at pH 6.0–10.5 and 20–35 °C; optimum growth occurred in the presence of 5–7 % (w/v) total salts and at pH 8.5 and 25 °C. Whole-cell hydrolysates of strain JSM 073097^T contained *meso*-diaminopimelic acid and no diagnostic sugars. The predominant menaquinones were MK-10(H₄), MK-10(H₆) and MK-10(H₈). The major cellular fatty acids were iso-C_{15:0}, iso-C_{16:0}, anteiso-C_{16:0} and 10-methyl C_{18:0}. Polar lipids comprised diphosphatidylglycerol, phosphatidylcholine and phosphatidylglycerol. The DNA G + C content of strain JSM 073097^T was 70.4 mol%. The combination of phylogenetic analysis, DNA–DNA relatedness data, phenotypic characteristics and chemotaxonomic data supported the suggestion that strain JSM 073097^T represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis litoralis* sp. nov. is proposed. The type strain is JSM 073097^T (=DSM 45168^T=KCTC 19473^T).

The genus *Nocardiopsis* was first described by Meyer (1976) and, at the time of writing, comprises 28 recognized species

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JSM 073097^T is EU583726.

A scanning electron micrograph of cells of strain JSM 073097^T and maximum-parsimony and maximum-likelihood phylogenetic trees showing the position of strain JSM 073097^T among members of the genus *Nocardiopsis* based on 16S rRNA gene sequences are available with the online version of this paper.

(Meyer, 1976; Grund & Kroppenstedt, 1990; Yassin *et al.*, 1993, 1997; Al-Tai & Ruan, 1994; Chun *et al.*, 2000; Evtushenko *et al.*, 2000; Peltola *et al.*, 2001; Al-Zarban *et al.*, 2002; Kämpfer *et al.*, 2002; Schippers *et al.*, 2002; Li *et al.*, 2003, 2004, 2006; Hozzein *et al.*, 2004; Sabry *et al.*, 2004; Chen *et al.*, 2008; Hozzein & Goodfellow, 2008; Yang *et al.*, 2008; Zhang *et al.*, 2008). During an investigation of the diversity of the microbial population of invertebrates inhabiting the South China Sea (Chen *et al.*, 2009a, b; Xiao *et al.*, 2009), a moderately halophilic, alkalitolerant,

filamentous, Gram-positive strain, designated JSM 073097^T, was isolated from a sea anemone collected from a tidal flat near Naozhou Island, southern China. Based on the results of the present taxonomic study, this strain is considered to represent a novel species of the genus *Nocardiopsis*.

Strain JSM 073097^T was isolated from homogenates of a sea anemone by plating 1 : 10 serial dilutions of the sample on marine agar 2216 (MA; Difco) supplemented with 10 % (w/v) NaCl and cultivating at 25 °C for 2 weeks. After primary isolation and purification, the isolate was preserved both on slants of MA supplemented with 3 % (w/v) NaCl (containing approximately 5 % NaCl and 6.4 % total salts, pH 8.5; hereafter referred to as MA3) at 4 °C and in marine broth 2216 (MB; Difco) supplemented with 20 % (v/v) glycerol at –80 °C. *Nocardiopsis kunsanensis* DSM 44524^T, *Nocardiopsis xinjiangensis* YIM 90004^T and *Nocardiopsis salina* YIM 90010^T were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) and the YIM (Yunnan Institute of Microbiology, Kunming, China). Morphological characteristics were observed by using light microscopy (BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 2 and 4 weeks growth on MA3. Cultural characteristics were determined after 2–4 weeks by using the methods applied in the International *Streptomyces* Project (Shirling & Gottlieb, 1966). The colours of both substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the colour charts of the Inter-Society Colour Council – National Bureau of Standards (Kelly, 1964). Growth was tested at various temperatures (5–45 °C, at increments of 5 °C) and pH (5.0–11.0, at pH increments of 0.5) in MB supplemented with 3 % (w/v) NaCl and on MA3. Turbidity was monitored at 610 nm after the MB tubes had been incubated for 5 days in a reciprocal water-bath shaker. Growth at different total salt contents (0, 0.1 and 0.5 %, w/v, and 1–20 %, w/v, at increments of 1 %) was determined on MA prepared according to the formula of Atlas (1993). The concentration of total salts contained in MA was changed (proportions maintained) as an integer in the salt-response experiment. Growth under anaerobic conditions and catalase and oxidase activities were detected as described previously (Chen *et al.*, 2007). The media and procedures used to determine physiological and biochemical features, as well as utilization of carbon and nitrogen sources, were those described by Shirling & Gottlieb (1966) and Kroppenstedt & Evtushenko (2006). Unless indicated otherwise, all tests were carried out with 6 % (w/v) NaCl at pH 8.5 with incubation at 25 °C.

Strain JSM 073097^T showed macroscopic and microscopic characteristics typical of most species of the genus *Nocardiopsis* (Meyer, 1976, 1994; Kroppenstedt & Evtushenko, 2006). The novel strain was Gram-positive, obligately aerobic and catalase-positive. It formed circular colonies that had white mycelia on MA3. Good growth was

also observed on yeast extract-malt extract, oatmeal, peptone-yeast extract-iron, Czapek's, nutrient and potato extract agar media supplemented with 6 % (w/v) NaCl, but poor growth occurred on inorganic salts-starch and glycerol-asparagine agar media supplemented with 6 % (w/v) NaCl. Substrate mycelia were well developed and fragmented with age. Aerial mycelia produced long, straight to flexuous spore chains with non-motile, smooth-surfaced, rod-shaped spores (see Supplementary Fig. S1 in IJSEM Online). No diffusible pigments were produced on any of the media tested. Strain JSM 073097^T was able to grow at 20–35 °C (optimum 25 °C) and at pH 6.0–10.5 (optimum pH 8.5) in the presence of 1–15 % (w/v) total salts (optimum 5–7 %). The results of other phenotypic tests are summarized in the species description below and in Table 1.

DNA was isolated according to the method of Hopwood *et al.* (1985) and the G+C content was determined by using the HPLC method (Mesbah *et al.*, 1989). The 16S rRNA gene was amplified and sequenced as described by Cui *et al.* (2001). The resulting 16S rRNA gene sequence was compared with sequences obtained from public databases (GenBank/EMBL/DBJ) to find the most closely related species. Multiple alignments with sequences of the most closely related strains were carried out by using CLUSTAL_X (Thompson *et al.*, 1997). Distances were calculated by using distance options according to Kimura's two-parameter model (Kimura, 1980). A neighbour-joining (Saitou & Nei, 1987) tree was constructed by using the software package MEGA version 4.1 (Tamura *et al.*, 2007). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees were generated by using the treeing algorithms contained in the PHYLIP package (Felsenstein, 2002). Confidence values for the branches of phylogenetic trees were determined by using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. DNA–DNA hybridization experiments were carried out by using photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989).

The DNA G+C content of strain JSM 073097^T was 70.4 mol%. An almost-complete 16S rRNA gene sequence (1404 bp) of this organism was determined. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain JSM 073097^T belonged to the genus *Nocardiopsis*, and was most closely related to the type strain of *N. kunsanensis* (99.6 % similarity; Chun *et al.*, 2000), followed by those of *N. xinjiangensis* (98.5 %; Li *et al.*, 2003) and *N. salina* (98.1 %; Li *et al.*, 2004). The four strains formed a robust lineage supported by a significant bootstrap resampling value (99 %) in the neighbour-joining tree (Fig. 1). The topology was similar to those of the phylogenetic trees constructed by using the maximum-likelihood and maximum-parsimony methods (see Supplementary Fig. S2). Levels of DNA–DNA relatedness between strain JSM 073097^T and the type strains of *N. kunsanensis*, *N. xinjiangensis* and *N. salina* were 30.7 ± 2.8 , 20.6 ± 2.3 and 22.1 ± 2.9 % (mean $\pm$ SD of 3 determinations),

Table 1. Differential characteristics between strain JSM 073097^T and phylogenetically related species of the genus *Nocardiopsis*

Taxa: 1, strain JSM 073097^T (data from the present study); 2, *N. kunsanensis* HA-9^T (Chun *et al.*, 2000); 3, *N. xinjiangensis* YIM 90004^T (Li *et al.*, 2003); 4, *N. salina* YIM 90010^T (Li *et al.*, 2004, 2006). +, Positive; –, negative; w, weak reaction; ND, no data available.

Characteristic	1	2	3	4
Fragmentation of substrate mycelia	+	–	+	+
NaCl range (optimum) (%; w/v)*	1–15 (5–7)	3–20 (10)	3–20 (10)†	3–20 (10)
Temperature range (optimum) (°C)	20–35 (25)	20–50 (37)†	20–40 (28)†	20–40 (28)
pH range (optimum)	6.0–10.5 (8.5)	7.0–11.0 (9.0)†	6.0–10.0 (7.2)†	6.0–9.0 (7.2)
Urease activity	–	+	+	–
Melanin production	–	–	+	–
Nitrate reduction	–	–	–	+
Hydrolysis of:				
Casein	–	+	ND	ND
Gelatin	+	–	+	w
Starch	–	+	–	–
Utilization of:				
D-Cellobiose	–	–	+	–
D-Fructose	–	+†	+†	+
D-Galactose	–	–	+	–
D-Glucose	+	+	–	–
L-Proline	–	–	+	+
Raffinose	–	–	–	+
L-Serine	–	–	+	+
D-Xylose	+	–†	–	–
Diagnostic sugars	None	None	Xylose, arabinose, galactose	None
Major phospholipids‡	DPG, PC, PG	DPG, PC, PG	PG, PI	DPG, PC, PE, PG, PI, PME
Major fatty acids	iso-C _{15:0} , iso-C _{16:0} , anteiso-C _{16:0} , 10-methyl C _{18:0}	iso-C _{15:0} , anteiso-C _{16:0} , 10-methyl C _{18:0}	iso-C _{14:0} , anteiso-C _{15:0} , iso-C _{16:0} , anteiso-C _{16:0} , C _{18:0} , 10-methyl C _{18:0}	iso-C _{16:0} , C _{18:1} ω9c, 10-methyl C _{18:0}
Major menaquinone(s)	MK-10(H ₄ , H ₆ , H ₈)	MK-10(H ₈)	MK-10(H ₂ , H ₄),	MK-9(H ₈), MK-10(H ₈)
DNA G + C content (mol%)	70.4	71	74.3	73.1

*Data for strain JSM 073097^T were for total salts.

†Data obtained from Li *et al.* (2004).

‡DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmethylethanolamine.

respectively. These values are far below the threshold of 70 % recommended by Wayne *et al.* (1987) for assignment of strains to the same species. The other closest phylogenetic neighbours of strain JSM 073097^T (showing 16S rRNA gene sequence similarity >97 %) were the type strains of *Nocardiopsis aegyptia* (97.7 %), *Nocardiopsis lucentensis* (97.5 %), *Nocardiopsis alba* (97.4 %), *Nocardiopsis dassonvillei* subsp. *dassonvillei* (97.4 %) and *Nocardiopsis dassonvillei* subsp. *albirubida* (97.2 %). These levels of sequence similarity are lower than those found for some other individual pairs of recognized *Nocardiopsis* species, as discussed previously (Al-Zarban *et al.*, 2002; Schippers *et al.*, 2002; Hozzein *et al.*, 2004; Sabry *et al.*, 2004; Yang *et al.*, 2008). Furthermore, Stackebrandt & Ebers (2006) suggested an increase from 97 to 98.7–99 % in the 16S rRNA gene sequence similarity threshold used for the delineation of separate species, provided that these data are supported by clear phenotypic differences. On this basis, together with

DNA–DNA hybridization data, the present phylogenetic analyses strongly suggested that strain JSM 073097^T represents a previously unknown species of the genus *Nocardiopsis*.

Biomass for chemical studies was obtained from cultures grown in MB (pH 8.5) supplemented with 3 % (w/v) NaCl on a rotary shaker (about 200 r.p.m.) at 25 °C for 3 days. Cells were harvested by centrifugation and were washed twice with distilled water. Amino acids and sugars of whole-cell hydrolysates were analysed as described by Stanek & Roberts (1974). Polar lipids were extracted, examined by using two-dimensional TLC and identified by using standard procedures (Minnikin *et al.*, 1984). Menaquinones were isolated by using the methods of Minnikin *et al.* (1984) and were then separated by HPLC (Kroppenstedt, 1982, 1985). Fatty acids were determined as described by Sasser (1990) by using the Microbial

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence analysis and constructed by using the neighbour-joining method showing the position of strain JSM 073097^T among members of the genus *Nocardiopsis*. Numbers at nodes indicate bootstrap values (>50%) based on a neighbour-joining analysis of 1000 resampled datasets. Bar, 1 substitution per 100 nt.

Identification System (MIDI; Microbial ID). Chemotaxonomic data for strain JSM 073097^T were consistent with its assignment to the genus *Nocardiopsis* (Meyer, 1976, 1994; Kroppenstedt & Evtushenko, 2006). Whole-cell hydrolysates contained *meso*-diaminopimelic acid as the diagnostic diamino acid and no diagnostic sugars. Polar lipids of the novel strain comprised diphosphatidylglycerol, phosphatidylcholine and phosphatidylglycerol. The predominant menaquinones were MK-10(H₄) (32.3%), MK-10(H₆) (36.8%) and MK-10(H₈) (16.3%), with MK-9(H₆) (5.8%), MK-9(H₈) (2.7%) and MK-10(H₂) (5.2%) present as minor components. The fatty acid profile of strain JSM 073097^T was similar to those of related *Nocardiopsis* species (Table 1). The major fatty acids of the novel strain were iso-C_{15:0} (10.1% of the total), iso-C_{16:0} (28.2%), anteiso-C_{16:0} (11.8%) and 10-methyl C_{18:0} (11.0%), with iso-C_{14:0} (4.4%), anteiso-C_{15:0} (5.7%), C_{16:1ω7c} alcohol (2.4%), C_{16:0} (3.7%), iso-C_{17:0} (1.1%), anteiso-C_{17:0} (5.1%), C_{17:0} (1.2%), 10-methyl C_{17:0} (1.5%), C_{18:1ω9c} (6.8%) and C_{18:0} (4.5%) present as minor components; iso-C_{13:0}, C_{14:0}, C_{15:0}, C_{17:1ω8c} and iso-C_{18:0} were detected at a level of <1%.

The results of phylogenetic analyses and of morphological and chemotaxonomic investigations supported the view that strain JSM 073097^T should be assigned to the genus *Nocardiopsis* (Meyer, 1976, 1994; Kroppenstedt &

Evtushenko, 2006). However, the comparatively low salinities [1–15% (w/v) total salts (optimum 5–7%)] and temperatures [20–35 °C (optimum 25 °C)] for growth, as well as its ability to utilize D-xylose as sole carbon source, markedly differentiated the new isolate from the phylogenetically related *Nocardiopsis* species (Table 1). Strain JSM 073097^T could also be distinguished from its closest phylogenetic neighbour, *N. kunsanensis*, based on other physiological, biochemical, nutritional and chemotaxonomic differences (see Table 1). Data from the present polyphasic taxonomic study, together with DNA–DNA hybridization data, indicated that strain JSM 073097^T represents a novel species of the genus *Nocardiopsis*, for which we propose the name *Nocardiopsis litoralis* sp. nov.

Description of *Nocardiopsis litoralis* sp. nov.

Nocardiopsis litoralis (li.to.ra'lis. L. adj. *litoralis* of the shore).

Gram-positive, moderately halophilic, alkalitolerant, non-motile, filamentous, aerobic actinomycete that forms white aerial mycelium and white to yellow–white substrate mycelium and straight to flexuous hyphae. Good growth occurs on most of the media tested. Diffusible pigments are not produced. Substrate hyphae are well developed and fragment with age. Long, straight to flexuous spore chains are borne on aerial hyphae, which fragment into elongated non-motile spores (0.5–0.7 × 1.5–1.8 μm in size) with smooth surfaces. Grows optimally at 25 °C and pH 8.5 and in the presence of 5–7% (w/v) total salts. Growth is not observed in the absence of salts. Positive for catalase but negative for oxidase. Unable to reduce nitrate to nitrite. H₂S and melanin are not produced. Degrades adenine, gelatin, hypoxanthine, tyrosine and xanthine, but not aesculin, casein, cellulose, chitin, DNA, starch, Tweens (20, 40, 60, 80) or urea. The following compounds are utilized as sole source of carbon and energy or sole source of carbon, nitrogen and energy: D-glucose, sucrose, D-xylose and L-alanine. The following substances are not utilized: L-arabinose, cellobiose, dextrin, D-fructose, D-galactose, D-lactose, maltose, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, D-salicin, trehalose, adonitol, D-arabitol, glycerol, *myo*-inositol, D-mannitol, D-sorbitol, acetate, citrate, gluconate, L-arginine, L-asparagine, L-glutamic acid, glycine, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine and L-valine. Whole-cell hydrolysates contain *meso*-diaminopimelic acid and no diagnostic sugars. The predominant menaquinones are MK-10(H₄), MK-10(H₆) and MK-10(H₈). The major cellular fatty acids are iso-C_{15:0}, iso-C_{16:0}, anteiso-C_{16:0} and 10-methyl C_{18:0}. Polar lipids comprise diphosphatidylglycerol, phosphatidylcholine and phosphatidylglycerol.

The type strain, JSM 073097^T (=DSM 45168^T=KCTC 19473^T), was isolated from homogenates of a sea anemone collected from a tidal flat on Naozhou Island in the South China Sea, near Zhanjiang City, southern China. The DNA

G+C content of the type strain is 70.4 mol% (HPLC method).

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References

- Al-Tai, A. M. & Ruan, J. S. (1994). *Nocardiopsis halophila* sp. nov., a new halophilic actinomycete isolated from soil. *Int J Syst Bacteriol* **44**, 474–478.
- Al-Zarban, S. S., Abbas, I., Al-Musallam, A. A., Steiner, U., Stackebrandt, E. & Kroppenstedt, R. M. (2002). *Nocardiopsis halotolerans* sp. nov., isolated from salt marsh soil in Kuwait. *Int J Syst Evol Microbiol* **52**, 525–529.
- Atlas, R. M. (1993). *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Chen, Y.-G., Cui, X.-L., Pukall, R., Li, H.-M., Yang, Y.-L., Xu, L.-H., Wen, M.-L., Peng, Q. & Jiang, C.-L. (2007). *Salinicoccus kunmingensis* sp. nov., a moderately halophilic bacterium isolated from a salt mine in Yunnan, south-west China. *Int J Syst Evol Microbiol* **57**, 2327–2332.
- Chen, Y.-G., Cui, X.-L., Kroppenstedt, R. M., Stackebrandt, E., Wen, M.-L., Xu, L.-H. & Jiang, C.-L. (2008). *Nocardiopsis quinghaiensis* sp. nov., isolated from saline soil in China. *Int J Syst Evol Microbiol* **58**, 699–705.
- Chen, Y.-G., Zhang, Y.-Q., Shi, J.-X., Xiao, H.-D., Tang, S.-K., Liu, Z.-X., Huang, K., Cui, X.-L. & Li, W.-J. (2009a). *Jeotgalicoccus marinus* sp. nov., a marine bacterium isolated from a sea urchin. *Int J Syst Evol Microbiol* **59**, 1625–1629.
- Chen, Y.-G., Zhang, Y.-Q., Xiao, H.-D., Liu, Z.-X., Yi, L.-B., Shi, J.-X., Zhi, X.-Y., Cui, X.-L. & Li, W.-J. (2009b). *Pontibacillus halophilus* sp. nov., a moderately halophilic bacterium isolated from a sea urchin. *Int J Syst Evol Microbiol* **59**, 1635–1639.
- Chun, J., Bae, K. S., Moon, E. Y., Jung, S.-O., Lee, H. K. & Kim, S.-J. (2000). *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete isolated from a saltern. *Int J Syst Evol Microbiol* **50**, 1909–1913.
- Cui, X.-L., Mao, P.-H., Zeng, M., Li, W.-J., Zhang, L.-P., Xu, L.-H. & Jiang, C.-L. (2001). *Streptomonospora salina* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae*. *Int J Syst Evol Microbiol* **51**, 357–363.
- Evtushenko, L. I., Taran, V. V., Akimov, V. N., Kroppenstedt, R. M., Tiedje, J. M. & Stackebrandt, E. (2000). *Nocardiopsis tropica* sp. nov., *Nocardiopsis trehalosi* sp. nov., nom. rev. and *Nocardiopsis dassonvillei* subsp. *albirubida* subsp. nov., comb. nov. *Int J Syst Evol Microbiol* **50**, 73–81.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Grund, E. & Kroppenstedt, R. M. (1990). Chemotaxonomy and numerical taxonomy of the genus *Nocardiopsis* Meyer 1976. *Int J Syst Bacteriol* **40**, 5–11.
- Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P. & Ward, J. M. (1985). Preparation of chromosomal, plasmid and phage DNA. In *Genetic Manipulation of Streptomyces: a Laboratory Manual*, pp. 79–80. Norwich, UK: John Innes Foundation.
- Hozzein, W. N. & Goodfellow, M. (2008). *Nocardiopsis arabia* sp. nov., a halotolerant actinomycete isolated from a sand-dune soil. *Int J Syst Evol Microbiol* **58**, 2520–2524.
- Hozzein, W. N., Li, W. J., Ali, M. I. A., Hammouda, O., Mousa, A. S., Xu, L.-H. & Jiang, C.-L. (2004). *Nocardiopsis alkaliphila* sp. nov., a novel alkaliphilic actinomycete isolated from desert soil in Egypt. *Int J Syst Evol Microbiol* **54**, 247–252.
- Kämpfer, P., Busse, H.-J. & Rainey, F. A. (2002). *Nocardiopsis compostus* sp. nov., from the atmosphere of a composting facility. *Int J Syst Evol Microbiol* **52**, 621–627.
- Kelly, K. L. (1964). *Inter-Society Color Council – National Bureau of Standards Color Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence. *J Mol Evol* **16**, 111–120.
- Kluge, A. G. & Farris, J. 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.
- Kroppenstedt, R. M. & Evtushenko, L. I. (2006). The family *Nocardiopsaceae*. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 3, pp. 754–795. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.
- Li, M.-G., Li, W.-J., Xu, P., Cui, X.-L., Xu, L.-H. & Jiang, C.-L. (2003). *Nocardiopsis xinjiangensis* sp. nov., a halophilic actinomycete isolated from a saline soil sample in China. *Int J Syst Evol Microbiol* **53**, 317–321.
- Li, W.-J., Tang, S.-K., Park, D. J., Wang, D., Lee, J. C., Xu, L.-H., Kim, C.-J. & Jiang, C.-L. (2004). *Nocardiopsis salina* sp. nov., a novel halophilic actinomycete isolated from saline soil in China. *Int J Syst Evol Microbiol* **54**, 1805–1809.
- Li, W.-J., Kroppenstedt, R. M., Wang, D., Tang, S.-K., Lee, J.-C., Park, D.-J., Kim, C.-J., Xu, L.-H. & Jiang, C.-L. (2006). Five novel species of the genus *Nocardiopsis* isolated from hypersaline soils and emended description of *Nocardiopsis salina* Li et al. 2004. *Int J Syst Evol Microbiol* **56**, 1089–1096.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Meyer, J. (1976). *Nocardiopsis*, a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **26**, 487–493.

- Meyer, J. (1994). The genus *Nocardiopsis* Meyer 1976. In *Bergey's Manual of Determinative Bacteriology*, 9th edn, pp. 2562–2568. Edited by J. G. Holt, N. R. Krieg, P. H. A. Sneath, J. T. Staley & S. T. Williams. Baltimore: Williams & Wilkins.
- 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.
- Peltola, J. S. P., Andersson, M. A., Kämpfer, P., Auling, G., Kroppenstedt, R. M., Busse, H.-J., Salkinoja-Salonen, M. S. & Rainey, F. A. (2001). Isolation of toxigenic *Nocardiopsis* strains from indoor environments and description of two new *Nocardiopsis* species, *N. exhalans* sp. nov. and *N. umidischolae* sp. nov. *Appl Environ Microbiol* 67, 4293–4304.
- Sabry, S. A., Ghanem, N. B., Abu-Ella, G. A., Schumann, P., Stackebrandt, E. & Kroppenstedt, R. M. (2004). *Nocardiopsis aegyptia* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* 54, 453–456.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Schippers, A., Bosecker, K., Willscher, S., Spröer, C., Schumann, P. & Kroppenstedt, R. M. (2002). *Nocardiopsis metallicus* sp. nov., a metal-leaching actinomycete isolated from an alkaline slag dump. *Int J Syst Evol Microbiol* 52, 2291–2295.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 16, 313–340.
- Stackebrandt, E. & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* 33, 152–155.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* 28, 226–231.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. *Mol Biol Evol* 24, 1596–1599.
- 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.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 37, 463–464.
- Xiao, H., Chen, Y., Liu, Z., Huang, K., Li, W., Cui, X., Zhang, L. & Yi, L. (2009). Phylogenetic diversity of cultivable bacteria associated with a sea anemone from coast of the Naozhou Island in Zhanjiang, China. *Wei Sheng Wu Xue Bao* 49, 246–250 (in Chinese).
- Yang, R., Zhang, L.-P., Guo, L.-G., Shi, N., Lu, Z. & Zhang, X. (2008). *Nocardiopsis valliformis* sp. nov., an alkaliphilic actinomycete isolated from alkali lake soil in China. *Int J Syst Evol Microbiol* 58, 1542–1546.
- Yassin, A. F., Galinski, E. A., Wohlfarth, A., Jahnke, K.-D., Schaal, K. P. & Trüper, H. G. (1993). A new actinomycete species, *Nocardiopsis lucentensis* sp. nov. *Int J Syst Bacteriol* 43, 266–271.
- Yassin, A. F., Rainey, F. A., Burghardt, J., Gierth, D., Ungerechts, J., Lux, I., Seifert, P., Bal, C. & Schaal, K. P. (1997). Description of *Nocardiopsis synnemataformans* sp. nov., elevation of *Nocardiopsis alba* subsp. *prasina* to *Nocardiopsis prasina* comb. nov., and designation of *Nocardiopsis antarctica* and *Nocardiopsis alborubida* as later subjective synonyms of *Nocardiopsis dassonvillei*. *Int J Syst Bacteriol* 47, 983–988.
- Zhang, X., Zhang, L.-P., Yang, R., Shi, N., Lu, Z., Chen, W. X., Jiang, C.-L. & Xu, L.-H. (2008). *Nocardiopsis ganjiahuensis* sp. nov., isolated from a soil from Ganjiahu, China. *Int J Syst Evol Microbiol* 58, 195–199.