Nocardia endophytica sp. nov., an endophytic actinomycete isolated from the oil-seed plant *Jatropha curcas* L.

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A novel actinomycete, designated strain KLBMP 1256^T, was isolated from a surface-sterilized stem of the oil-seed plant *Jatropha curcas* L. collected from Sichuan Province, south-west China, and was characterized to determine its taxonomic position. Phylogenetic analyses based on 16S rRNA gene sequences indicated that the isolate was closely related to members of the genus *Nocardia* in the family *Nocardiaceae*, being most closely related to *Nocardia callitridis* CAP 290^T (98.4 % similarity) and *Nocardia nova* JCM 6044^T (97.5 %). Levels of 16S rRNA gene sequence similarity between strain KLBMP 1256^T and the type strains of other recognized species of the genus *Nocardia* were less than 97 %. Chemotaxonomic data supported the affiliation of the new isolate to the genus *Nocardia*. However, the novel strain could be distinguished from its closest phylogenetic neighbour, *N. callitridis* CAP 290^T, by a range of phenotypic properties. The combination of low DNA–DNA relatedness values and phenotypic differences from *N. callitridis* CAP 290^T indicated that strain KLBMP 1256^T represents a novel species of the genus *Nocardia*, for which the name *Nocardia endophytica* sp. nov. is proposed. The type strain is KLBMP 1256^T (=KCTC 19777^T =CCTCC AA 2010004^T).

The genus *Nocardia* belongs to the family *Nocardiaceae*, and members of the genus are aerobic, Gram-positive, mycolic acid-containing actinobacteria that form an extensively branched mycelium, which fragments into bacillus- or coccoid-like elements (Goodfellow & Lechevalier, 1989). At the time of writing, the genus comprised more than 70 recognized species, including the recently described species *Nocardia acidivorans* (Kämpfer *et al.*, 2007), *N. altamirensis* (Jurado *et al.*, 2008), *N. jinanensis* (Sun *et al.*, 2009), *N. iowensis* (Lamm *et al.*, 2009), *N. mikamii* (Jannat-Khah *et al.*, 2010) and *N. niwae* (Moser *et al.*, 2011). There is only one record of the isolation of a novel species belonging to the genus *Nocardia* from the endophytic environment (Kaewkla & Franco, 2010). Here we describe the results of phenotypic and phylogenetic analyses of another endophytic actinobacterium and show that this strain represents a novel species of the genus *Nocardia*.

Strain KLBMP 1256^T was isolated from a healthy stem of the oil-seed plant *Jatropha curcas* L. collected from the city of Panzhihua, Sichuan Province, south-west China. Healthy stem samples of *J. curcas* were washed in running tap water to remove adhered epiphytes and surface-sterilized according to a five-step sterilization procedure (Qin *et al.*, 2008). The surface-sterilized samples were then aseptically crumbled into smaller fragments by using a commercial blender, spread onto sodium propionate agar (Qin *et al.*, 2009) and incubated at 28 °C for 2–8 weeks. The novel strain was picked from colonies present on isolation medium after incubation for 14 days and then pure cultures

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KLBMP 1256^T is HM153801.

One supplementary figure is available with the online version of this paper.

of this strain were maintained on yeast extract-malt extract agar [International *Streptomyces* Project medium 2 (ISP 2); (Shirling & Gottlieb, 1966)] slants at 4 °C and as 20 % (v/v) glycerol suspensions at −80 °C.

Cultural and morphological characteristics of strain KLBMP 1256^T were determined after 2–4 weeks at 28 °C on standard ISP 2, 3, 4 and 5 media (Shirling & Gottlieb, 1966) as well as by using potato-dextrose agar (PDA), Czapek's agar and nutrient agar (Waksman, 1967). The colours of soluble pigments of both substrate and aerial mycelia were determined by comparison with those described by Kelly (1964). The arrangement of hyphae and spores was observed by using light microscopy (SA3300-PL) and scanning electron microscopy (S-3400N; Hitachi) after 2 weeks of growth on ISP 2 medium. Physiological and biochemical characteristics of strain KLBMP 1256^T and the phylogenetically closely related *Nocardia callitridis* CAP 290^T were tested after incubation at 28 °C for 3 weeks. Growth was tested at 0, 4, 10, 15, 20, 28, 37, 40, 45 and 55 °C, pH 5.0–10.0 (at intervals of 0.5 pH units) and NaCl concentrations of 0–15 % (w/v) (at intervals of 0.5 %) by using ISP 2 as the basal medium. Physiological and biochemical features, including the range of substrates used as sole carbon and energy sources, were determined by using the procedures described by Gordon *et al.* (1974). A comparison of the phenotypic characteristics of strain KLBMP 1256^T and its closest phylogenetic neighbour is shown in Table 1.

Strain KLBMP 1256^T had morphological properties typical of members of the genus *Nocardia*. The organism was an aerobic, Gram-positive actinomycete which formed an extensively branched substrate mycelium that fragmented into irregular, non-motile, coccoid and rod-shaped elements. Aerial mycelium consisted of hyphae that fragmented into short to elongated rod-like elements and the surface was smooth (see Supplementary Fig. S1 available in IJSEM Online). Strain KLBMP 1256^T grew well on ISP 2, 3 and 5, PDA and nutrient agar, with moderate growth on ISP 4 and Czapek's media. White aerial mycelia were observed on all media tested. Yellowish to yellow–white substrate mycelia were formed on ISP 2, PDA and nutrient agar, while white substrate mycelia were formed on ISP 3, 4 and 5 and Czapek's media. No soluble pigments were observed on any of these media. Growth was observed at 4–28 °C, at pH 6.0–8.0 and in the presence of 0–7 % (w/v) NaCl. Detailed phenotypic characteristics are presented in Table 1 and in the species description below.

For cellular fatty acid analysis, strain KLBMP 1256^T was grown in trypticase soy broth (TSB) at 150 r.p.m. for 10 days at 28 °C. Fatty acids were extracted and prepared according to the standard protocol of the Microbial Identification System (MIDI, TSBA library version 4.0) (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Biomass for other chemotaxonomic studies was obtained after cultivation at 28 °C for 7–10 days in shaken ISP 2 cultures. Amino acid and sugar analyses of whole-cell hydrolysates were performed according to the procedures described by Hasegawa *et al.* (1983). Analysis of mycolic acids was performed by using the method described by Minnikin *et al.* (1980). Polar lipids were extracted, examined by two-dimensional TLC and identified by using published procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Menaquinones were extracted and purified as described by Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1997). The G+C content of the DNA was determined according to the method of Mesbah *et al.* (1989).

Table 1. Differential characteristics between strain KLBMP 1256^T and *Nocardia callitridis* CAP 290^T

Data were taken from the present study under identical conditions, except where indicated. +, Positive; −, negative; w, weakly positive.

Characteristic	KLBMP 1256^T	*N. callitridis* CAP 290^T
Growth on ISP 3 medium	Good	Poor
Growth on ISP 4 medium	Moderate	Poor
Growth on Czapek's medium	Moderate	Poor
Growth on ISP 5 medium		
Diffusible pigment	None	Brownish orange
Substrate mycelium colour	White	Brownish orange
Growth at 4 °C	+	−
Growth with 10 % (w/v) NaCl	−	+
pH range for growth	6.0–8.0	6.0–10.0
Utilization of:		
D-Arabinose	−	+
Cellobiose	+	−
D-Fructose	−	w
D-Galactose	−	+
Lactose	−	w
D-Mannose	+	w
Raffinose	+	w
D-Ribose	+	w
Dextrin	−	w
Inositol	−	+
Maltose	+	−
Sucrose	−	w
Xylitol	−	+
Decomposition of hypoxanthine	+	−
DNA G+C content (mol%)	68	68.7*

*Data from Kaewkla & Franco (2010).

Chemotaxonomic analyses revealed that strain KLBMP 1256^T displayed chemical characteristics that were consistent with those of the genus *Nocardia*. Whole-organism hydrolysates were rich in *meso*-diaminopimelic acid, arabinose and galactose (wall chemotype IV of Lechevalier & Lechevalier, 1970). The predominant menaquinone was MK-8($H_4\omega$-cycl). The phospholipid profile comprised diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides (phospholipid type II *sensu* Lechevalier *et al.*, 1977). Strain KLBMP 1256^T was also characterized by the presence of mycolic acids that

co-migrated (R_F value ~0.47) with those from *N. callitridis* CAP 290^T. The major fatty acids were $C_{16:0}$ (22.58 %), $C_{16:1}\omega7c$/$C_{16:1}\omega6c$ (17.53 %), $C_{18:1}\omega9c$ (8.65 %), $C_{18:0}$ (7.93 %), 10-methyl $C_{18:0}$ (7.34 %) and iso-$C_{16:1}$ H (5.58 %), with $C_{17:0}$ (3.20 %), $C_{18:1}\omega6c$ (3.09 %), $C_{17:1}\omega8c$ (2.66 %), $C_{17:1}\omega6c$ (2.57 %), $C_{18:1}\omega7c$ (2.37 %), iso-$C_{16:0}$ (2.16 %), $C_{16:1}\omega9c$ (2.11 %), $C_{14:0}$ (2.0 %), $C_{19:0}$ (1.92 %), $C_{19:1}\omega9c$/$C_{19:1}\omega11c$ (1.46 %) and $C_{15:1}\omega6c$ (1.12 %) present as minor components; $C_{12:0}$, $C_{13:0}$, $C_{14:1}\omega5c$, iso-$C_{15:0}$, $C_{15:1}\omega8c$, $C_{17:1}\omega5c$, 10-methyl $C_{17:0}$ and $C_{20:0}$ were detected at a level of <1 %. The DNA G+C content of strain KLBMP 1256^T was 68 mol%, which falls within the range of 64–72 mol% described for the genus *Nocardia* (Goodfellow & Lechevalier, 1989).

Extraction of genomic DNA and amplification of the 16S rRNA gene from strain KLBMP 1256^T were carried out as described by Li *et al.* (2007). The resultant sequences were first aligned via the BLAST search program in NCBI. The CLUSTAL X program (Thompson *et al.*, 1997) was then used to align the almost-complete 16S rRNA gene sequence of strain KLBMP 1256^T with corresponding sequences of members of the genus *Nocardia*. A phylogenetic tree was reconstructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods by using the software package MEGA version 4.0 (Tamura *et al.*, 2007). Genetic distances between sequences were calculated by using Kimura's two-parameter model (Kimura, 1980). The stability of the trees was assessed by performing bootstrap analyses (Felsenstein, 1985) of the neighbour-joining data based on 1000 resamplings. The level of DNA–DNA relatedness between strain KLBMP 1256^T and *N. callitridis* CAP 290^T was determined according to the fluorometric micro-well method (Ezaki *et al.*, 1989; He *et al.*, 2005).

The almost-complete 16S rRNA gene sequence of strain KLBMP 1256^T (1459 bp) was used for phylogenetic analysis. The neighbour-joining tree based on 16S rRNA gene sequences (Fig. 1) showed that strain KLBMP 1256^T was a member of the genus *Nocardia* and formed a separate line of descent in the phylogenetic cluster of the genus *Nocardia* with *N. callitridis* CAP 290^T, which was also supported by a very high bootstrap value. Sequence similarity calculations indicated that the closest relatives of strain KLBMP 1256^T were *N. callitridis* CAP 290^T (98.4 %) and *Nocardia nova* JCM 6044^T (97.5 %). Levels of 16S rRNA gene sequence similarity between strain KLBMP 1256^T and the type strains of other recognized species of the genus *Nocardia* were less than 97 %. The level of DNA–DNA relatedness between strain KLBMP 1256^T and *N. callitridis* CAP 290^T (27.4±3.1 %; mean±SD of 5 determinations) was well below the 70 % cut-off recommended for the circumscription of a bacterial genomic species (Wayne *et al.*, 1987), indicating that strain KLBMP 1256^T represents a novel species of the genus *Nocardia*. DNA–DNA hybridization

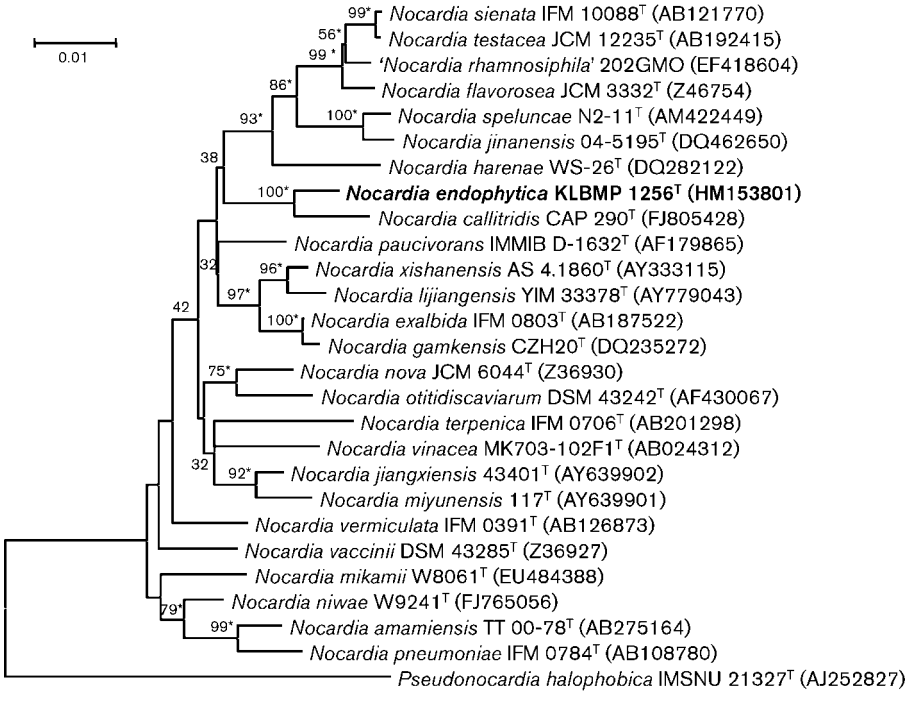

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain KLBMP 1256^T and related species of the genus *Nocardia*. Numbers at nodes are percentage bootstrap values (based on 1000 resamplings). Asterisks indicate the clades that were conserved when the neighbour-joining and maximum-parsimony methods were used to reconstruct phylogenetic trees. Bar, 0.01 substitutions per nucleotide position.

analysis was not carried out between strain KLBMP 1256^T and *N. nova* JCM 6044^T because they were positioned in different clusters in the phylogenetic tree and shared a relatively low level of 16S rRNA gene sequence similarity. It has been shown that some species of the genus *Nocardia* share high 16S rRNA gene sequence similarities, but have DNA–DNA relatedness values below the 70 % cut-off point for defining genomic species (Kageyama *et al.*, 2004; Yassin & Brenner, 2005; Kämpfer *et al.*, 2007; Sun *et al.*, 2009). For example, *N. nova* JCM 6044^T and *Nocardia pseudobrasiliensis* ATCC 51512^T shared 98 % 16S rRNA gene sequence similarity, but had a DNA–DNA relatedness level of 12 % (Ruimy *et al.*, 1996).

The present genotypic and phenotypic data distinguish strain KLBMP 1256^T from its closest phylogenetic neighbour, *N. callitridis* CAP 290^T. Thus, on the basis of data from the present polyphasic taxonomic study, strain KLBMP 1256^T is considered to represent a novel species of the genus *Nocardia*, for which we propose the name *Nocardia endophytica* sp. nov.

Description of *Nocardia endophytica* sp. nov.

Nocardia endophytica (en.do.phy′ti.ca. Gr. pref. *endo* within; Gr. n. *phyton* plant; L. fem. suff. *-ica* adjectival suffix used with the sense of belonging to; N.L. fem. adj. *endophytica* within plant, endophytic, pertaining to isolation of the type strain from plant tissues).

Gram-positive and aerobic. Forms white aerial mycelia and yellowish white to yellow substrate mycelia on the media tested. The aerial mycelia fragment into short to elongated rod-like elements. No soluble pigment is produced. Grows at 4–28 °C (optimum 28 °C), at pH 6.0–8.0 (optimum pH 7.0) and in the presence of 0–7 % (w/v) NaCl (optimum 3 %). Positive for catalase, milk coagulation and milk peptonization, but negative for nitrate reduction, gelatin liquefaction, cellulose and starch hydrolysis, and H_2S production. The cell wall contains *meso*-diaminopimelic acid. Whole-cell sugars are arabinose and galactose. The cell wall is of the glycolyl type and contains mycolic acids that co-migrate (R_F value ~0.47) with those from *N. callitridis* CAP 290^T. The phospholipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides. The major menaquinone is MK-8($H_4\omega$-cycl). The predominant cellular fatty acids (>5 % of the total) are $C_{16:0}$, $C_{16:1}\omega7c$/$C_{16:1}\omega6c$, $C_{18:1}\omega9c$, $C_{18:0}$, 10-methyl $C_{18:0}$ and iso-$C_{16:1}$ H. The G+C content of the genomic DNA of the type strain is 68 mol%.

The type strain, KLBMP 1256^T (=KCTC 19777^T =CCTCC AA 2010004^T), was isolated from a surface-sterilized stem of *Jatropha curcas* L. collected from the city of Panzhihua, Sichuan Province, south-west China.

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References

Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycan based on 2, 4-diaminobutyric acid. *J Appl Bacteriol* **48**, 459–470.

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.

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. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–789.

Goodfellow, M. & Lechevalier, M. P. (1989). Genus *Nocardia* Trevisan 1889, 9^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2348–2361. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H. N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.

Groth, I., Schumann, P., Rainey, F. A., Martin, K., Schuetze, B. & Augsten, K. (1997). *Demetria terragena* gen. nov., sp. nov., a new genus of actinomycetes isolated from compost soil. *Int J Syst Bacteriol* **47**, 1129–1133.

Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Microbiol* **29**, 319–322.

He, L., Li, W., Huang, Y., Wang, L., Liu, Z., Lanoot, B., Vancanneyt, M. & Swings, J. (2005). *Streptomyces jietaisiensis* sp. nov., isolated from soil in northern China. *Int J Syst Evol Microbiol* **55**, 1939–1944.

Jannat-Khah, D., Kroppenstedt, R. M., Klenk, H. P., Spröer, C., Schumann, P., Lasker, B. A., Steigerwalt, A. G., Hinrikson, H. P. & Brown, J. M. (2010). *Nocardia mikamii* sp. nov., isolated from human pulmonary infections in the USA. *Int J Syst Evol Microbiol* **60**, 2272–2276.

Jurado, V., Boiron, P., Kroppenstedt, R. M., Laurent, F., Couble, A., Laiz, L., Klenk, H. P., González, J. M., Saiz-Jimenez, C. & other authors (2008). *Nocardia altamirensis* sp. nov., isolated from Altamira cave, Cantabria, Spain. *Int J Syst Evol Microbiol* **58**, 2210–2214.

Kaewkla, O. & Franco, C. M. M. (2010). *Nocardia callitridis* sp. nov., an endophytic actinobacterium isolated from a surface-sterilized root of an Australian native pine tree. *Int J Syst Evol Microbiol* **60**, 1532–1536.

Kageyama, A., Yazawa, K., Nishimura, K. & Mikami, Y. (2004). *Nocardia inohanensis* sp. nov., *Nocardia yamanashiensis* sp. nov. and *Nocardia niigatensis* sp. nov., isolated from clinical specimens. *Int J Syst Evol Microbiol* **54**, 563–569.

Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.

Kämpfer, P., Huber, B., Buczolits, S., Thummes, K., Grün-Wollny, I. & Busse, H.-J. (2007). *Nocardia acidivorans* sp. nov., isolated from soil of the island of Stromboli. *Int J Syst Evol Microbiol* **57**, 1183–1187.

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 sequences. *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.

Lamm, A. S., Khare, A., Conville, P., Lau, P. C. K., Bergeron, H. & Rosazza, J. P. N. (2009). *Nocardia iowensis* sp. nov., an organism rich in biocatalytically important enzymes and nitric oxide synthase. *Int J Syst Evol Microbiol* **59**, 2408–2414.

Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.

Lechevalier, M. P., De Bievre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.

Li, W. J., Xu, P., Schumann, P., Zhang, Y. Q., Pukall, R., Xu, L. H., Stackebrandt, E. & Jiang, C. L. (2007). *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* **57**, 1424–1428.

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.

Minnikin, D. E., Collins, M. D. & Goodfellow, M. (1979). Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* **47**, 87–95.

Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980). Thin layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr A* **188**, 221–233.

Moser, B. D., Klenk, H.-P., Schumann, P., Pötter, G., Lasker, B. A., Steigerwalt, A. G., Hinrikson, H. P. & Brown, J. M. (2011). *Nocardia niwae* sp. nov., isolated from human pulmonary sources. *Int J Syst Evol Microbiol* **61**, 438–442.

Qin, S., Li, J., Chen, H.-H., Zhao, G.-Z., Zhu, W.-Y., Jiang, C.-L., Xu, L.-H. & Li, W.-J. (2009). Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. *Appl Environ Microbiol* **75**, 6176–6186.

Qin, S., Wang, H.-B., Chen, H.-H., Zhang, Y.-Q., Jiang, C.-L., Xu, L.-H. & Li, W.-J. (2008). *Glycomyces endophyticus* sp. nov., an endophytic actinomycete isolated from the root of *Carex baccans* Nees. *Int J Syst Evol Microbiol* **58**, 2525–2528.

Ruimy, R., Riegel, P., Carlotti, A., Boiron, P., Bernardin, G., Monteil, H., Wallace, R. J., Jr & Christen, R. (1996). *Nocardia pseudobrasiliensis* sp. nov., a new species of *Nocardia* which groups bacterial strains previously identified as *Nocardia brasiliensis* and associated with invasive diseases. *Int J Syst Bacteriol* **46**, 259–264.

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.

Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.

Sun, W., Zhang, Y.-Q., Huang, Y., Zhang, Y.-Q., Yang, Z.-Y. & Liu, Z.-H. (2009). *Nocardia jinanensis* sp. nov., an amicoumacin B-producing actinomycete. *Int J Syst Evol Microbiol* **59**, 417–420.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics 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.

Waksman, S. A. (1967). *The Actinomycetes. A Summary of Current Knowledge.* New York: Ronald Press.

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.

Yassin, A. F. & Brenner, S. (2005). *Nocardia elegans* sp. nov., a member of the *Nocardia vaccinii* clade isolated from sputum. *Int J Syst Evol Microbiol* **55**, 1505–1509.