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# *Nonomuraea endophytica* sp. nov., an endophytic actinomycete isolated from *Artemisia annua* L.

Jie Li,[1,2] Guo-Zhen Zhao,[1] Hai-Yu Huang,[1] Wen-Yong Zhu,[1] Jae-Chan Lee,[3] Li-Hua Xu,[1] Chang-Jin Kim[3] and Wen-Jun Li[1,2]

Correspondence
Wen-Jun Li
wjli@ynu.edu.cn
Chang-Jin Kim
changjin@kribb.re.kr

[1]The Key Laboratory for Microbial Resources of the Ministry of Education, PR China, and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China

[2]CAS Key Laboratory of Marine Bio-resources Sustainable Utilization, RNAM Center for Marine Microbiology, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, PR China

[3]Korea Research Institutes of Biosciences and Biotechnology, 52 Eoeun-dong, Yuseong gu, Daejeon 305-333, Republic of Korea

A Gram-stain-positive, aerobic, non-motile actinomycete strain, designated YIM 65601$^T$, was studied by using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that strain YIM 65601$^T$ should be assigned to the genus *Nonomuraea*, with highest 16S rRNA gene sequence similarities to *Nonomuraea candida* HMC10$^T$ (98.8 %), *N. salmonea* DSM 43678$^T$ (98.7 %), *N. turkmeniaca* DSM 43926$^T$ (98.5 %), *N. roseola* DSM 43767$^T$ (98.4 %), *N. dietziae* IFO 14309$^T$ (98.2 %) and *N. kuesteri* GW 14-1925$^T$ (98.1 %). 16S rRNA gene sequence similarities to strains of other *Nonomuraea* species were below 98.0 %. Morphological and chemotaxonomic properties of the isolate were consistent with those of members of the genus *Nonomuraea*. The results of DNA–DNA hybridization and physiological and biochemical tests and fatty acid profiles allowed genotypic and phenotypic differentiation of strain YIM 65601$^T$ from closely related species. Thus, YIM 65601$^T$ represents a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea endophytica* sp. nov. is proposed, with YIM 65601$^T$ (=CCTCC AA 209037$^T$ =DSM 45385$^T$) as the type strain.

The genus *Nonomuraea* was first proposed by Zhang *et al.* (1998) as a member of the family *Streptosporangiaceae* and is characterized by organisms that form extensively branched substrate and aerial mycelia. At the time of writing, the genus comprised 22 species with validly published names and two subspecies. During preparation of this manuscript, *Nonomuraea rosea* was proposed by Kämpfer *et al.* (2010).

In the course of an investigation on endophytic actinomycetes present in surface-sterilized plant tissues of *Artemisia annua* L. collected from Yunnan province, south-west China, strain YIM 65601$^T$ was recovered on sodium propionate-asparagine-plant extract agar (pH 7.2) at 28 °C. This medium was prepared as follows: 100 g plant was boiled with 1 l water for 1 h and the suspension was collected and then evaporated under reduced pressure to yield 100 ml plant extract; 1 ml plant extract was added to each litre of sodium propionate-asparagine agar as described previously (Qin *et al.*, 2009). The purified strain was cultured routinely on yeast extract-malt extract agar (ISP 2) medium (Shirling & Gottlieb, 1966) at 28 °C and stored as a glycerol suspension (20 %, v/v) at −70 °C.

Gram staining was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Cultural characteristics were observed on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5) (Shirling & Gottlieb, 1966), Czapek's agar, potato-glucose agar and nutrient agar (Waksman, 1961) after growth at 28 °C for 7, 14, 21 and 28 days. 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 (Kelly, 1964). Morphological properties were examined using a light microscope (BH-2; Olympus) and a scanning electron microscope (Philips XL30; ESEM-TMP) after 7–21 days of incubation on ISP 2 medium at 28 °C. Physiological properties of the isolate, such as growth at 4, 10, 15, 20, 28, 37, 45, 55 and 65 °C, pH 4.0–10.0 and 1, 3, 5, 7, 10, 12, 15 and 20 % (w/v) NaCl, were examined using tryptic soy

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 65601$^T$ is GU367158.

A supplementary table and a supplementary figure are available with the online version of this paper.

broth (TSB) as the basal medium. Hydrolysis of starch, gelatin and Tweens 20, 40 and 80 was determined as described by Smibert & Krieg (1994). The ability to use a range of carbon and nitrogen sources for energy and growth and other physiological and biochemical features were tested according to Gordon *et al.* (1974).

Isolate YIM 65601$^T$ grew well on all tested media including ISP 2, ISP 3, ISP 4, ISP 5, potato-glucose, Czapek's and nutrient agars. White to pink aerial mycelium developed well on ISP 2, ISP 3, ISP 4, Czapek's agar and potato-glucose agar; yellowish pink aerial mycelium was found on ISP 5, but aerial mycelium was absent on nutrient agar medium. The substrate mycelium branched extensively and the colours on different media were red to deep red (ISP 2 and Czapek's agar), deep brown (ISP 3 and ISP 4), grey–reddish brown (ISP 5), deep reddish brown (potato-glucose agar) or brown–black (nutrient agar). Soluble pigments were produced on most test media, for example, yellowish pink (Czapek's agar), orange–brown (ISP 3 and nutrient agar) and reddish brown (ISP 2, ISP 5 and potato-glucose agar). The aerial hyphae developed masses of long, straight spore chains, each containing more than 20 non-motile spores, which showed a warty ornamentation (Supplementary Fig. S1, available in IJSEM Online). Spore chains were stalked, single or in clusters. Sporangia were not detected. Strain YIM 65601$^T$ grew well at 10–37 °C (optimum 20–28 °C) and pH 6.0–9.0 (optimum pH 7.0–8.0) and tolerated up to 5 % NaCl. Other physiological and biochemical characteristics are presented in the species description and in Table 1.

The cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI). For other chemotaxonomic analyses, freeze-dried cells were obtained from cultures grown in TSB for 7 days at 28 °C. Diaminopimelic acid isomers and the sugars of whole-cell hydrolysates were analysed according to procedures developed by Hasegawa *et al.* (1983) and Lechevalier & Lechevalier (1970). Phospholipids were identified according to published procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Menaquinones were extracted (Collins *et al.*, 1977) and separated by HPLC (Tamaoka *et al.*, 1983). The G+C content of the genomic DNA was determined by the HPLC method according to Mesbah *et al.* (1989).

**Table 1.** Differential phenotypic properties of strain YIM 65601$^T$ and related *Nonomuraea* type strains

Strains: 1, YIM 65601$^T$ (data from this study); 2, *N. kuesteri* GW 14-1925$^T$ (data from Kämpfer *et al.*, 2005, 2010); 3, *N. salmonea* DSM 43678$^T$ (Kämpfer *et al.*, 2010); 4, *N. candida* HMC10$^T$ (this study and Kämpfer *et al.*, 2010); 5, *N. turkmeniaca* DSM 43926$^T$ (Kämpfer *et al.*, 2010); 6, *N. dietziae* DSM 44320$^T$ (Stackebrandt *et al.*, 2001; Kämpfer *et al.*, 2010); 7, *N. roseola* DSM 43767$^T$ (Kämpfer *et al.*, 2010); 8, *N. longicatena* NRRL 15532$^T$ (Chiba *et al.*, 1999; Kämpfer *et al.*, 2010). +, Positive; −, negative; W, weak; ND, no data available.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Spore chains | Straight | Spirals | Hooked/ spirals | Hooked/ curled | Spirals | Straight/spirals | Straight/ spirals | Straight/irregular spiral |
| Spore ornamentation | Warty | ND | Warty | Smooth | Smooth | Cross-ridged, smooth or rough | Folded | Smooth |
| Number of spores | ⩾20 | ND | 4–30 | ND | 10–20 | ⩾30 | 6–20 | 10–30 |
| Growth on ISP 3 | | | | | | | | |
| Aerial mycelium | White | Trace | Pink | White | Trace | Yellow–white | Pink | White |
| Substrate mycelium | Deep brown | Yellow | Red | Yellow–white | Violet/red | Yellowish brown | Brown/red | Ochre |
| Soluble pigment | Orange–brown | None | None | None | Pink/violet | Yellow/grey–yellow | None | None |
| Nitrate reduction | − | ND | + | − | + | − | + | − |
| Degradation of: | | | | | | | | |
| Gelatin | − | − | + | + | + | + | + | − |
| Tween 40 | − | ND | ND | + | ND | − | ND | ND |
| Urea | − | − | + | + | + | + | + | + |
| Starch | − | − | − | − | + | − | − | + |
| Hypoxanthine | + | − | − | − | + | + | − | + |
| Tyrosine | + | − | + | − | − | + | + | − |
| $H_2S$ production | − | ND | ND | + | ND | + | ND | ND |
| Utilization of: | | | | | | | | |
| L-Arabinose | − | + | − | + | + | W | − | − |
| D-Mannose | − | + | − | + | + | W | − | + |
| Ribose | + | − | ND | − | ND | − | ND | + |
| Raffinose | + | ND | − | − | + | − | − | − |
| D-Xylose | − | + | − | + | + | − | − | − |
| Growth at 45 °C | − | ND | ND | + | ND | + | ND | − |
| NaCl tolerance (%) | 5 | ND | ND | 3 | ND | 10 | ND | 3 |
| pH range for growth | 6.0–9.0 | ND | ND | 4.0–8.0 | ND | 6.0–10.0 | ND | ND |

Chemotaxonomic analyses revealed that strain YIM 65601$^T$ exhibited characteristics that are typical of members of the genus *Nonomuraea*, such as the presence of *meso*-diaminopimelic acid and the presence of galactose, glucose, mannose, ribose and madurose as whole-cell sugars. The predominant menaquinones were MK-9($H_4$) (56.2 %) and MK-9($H_2$) (32.3 %); MK-9 (5.6 %), MK-9($H_6$) (3.3 %) and MK-10($H_2$) (2.6 %) were also present. The phospholipids were diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, phosphatidylinositol mannosides and phosphatidylinositol. The detailed fatty acid profile was 10-methyl $C_{17:0}$ (30.3 %), iso-$C_{16:0}$ (19.7 %), $C_{17:0}$ (10.5 %), $C_{15:0}$ (10.2 %), $C_{17:1}\omega6c$ (6.4 %), iso-$C_{15:0}$ (4.3 %), $C_{17:1}\omega8c$ (2.7 %), $C_{16:0}$ (2.2 %), $C_{14:0}$ (2.0 %), $C_{15:0}$ 2-OH (2.0 %), $C_{17:0}$ 2-OH (1.8 %), $C_{18:0}$ (1.8 %) and iso-$C_{16:1}$ G (1.2 %). Moreover, the fatty acid profiles of strain YIM 65601$^T$ and *Nonomuraea candida* HMC10$^T$ showed remarkable differences, e.g. quantitative differences in the proportions of 10-methyl $C_{17:0}$, iso-$C_{16:0}$, $C_{17:0}$, $C_{15:0}$, iso-$C_{15:0}$, $C_{17:1}\omega6c$, $C_{17:1}\omega8c$ and anteiso-$C_{17:0}$ (Supplementary Table S1). The genomic DNA G+C content of strain YIM 65601$^T$ was 67.4 mol%.

Extraction of genomic DNA and amplification of the 16S rRNA gene sequence from strain YIM 65601$^T$ were performed as described by Li *et al.* (2007). The resultant 16S rRNA gene sequence (1518 bp) was aligned with corresponding sequences of representatives of the genus *Nonomuraea* (retrieved from GenBank/EMBL/DDBJ) using CLUSTAL_X (Thompson *et al.*, 1997). Neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) trees were constructed using MEGA version 4.0 (Tamura *et al.*, 2007). The PHYLIP software package version 3.6 was used to construct a maximum-likelihood tree (Felsenstein, 1981). Topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. 16S rRNA gene sequence similarity between strain YIM 65601$^T$ and other *Nonomuraea* strains was calculated by using the global pairwise alignment tool provided by EzTaxon server 2.1 (Chun *et al.*, 2007).

16S rRNA gene sequence analysis revealed that strain YIM 65601$^T$ belongs to the genus *Nonomuraea* (Fig. 1); it shared highest 16S rRNA gene sequence similarities with *N. candida* HMC10$^T$ (98.8 %), *N. salmonea* DSM 43678$^T$ (98.7 %), *N. turkmeniaca* DSM 43926$^T$ (98.5 %), *N. roseola* DSM 43767$^T$ (98.4 %), *N. dietziae* IFO 14309$^T$ (98.2 %) and *N. kuesteri* GW 14-1925$^T$ (98.1 %). The 16S rRNA gene sequence similarity between the isolate and other members of the genus *Nonomuraea* was less than 98.0 %. Moreover, strain YIM 65601$^T$ formed a coherent subclade with *Nonomuraea longicatena* NRRL 15532$^T$ that was supported by the neighbour-joining, maximum-parsimony and maximum-likelihood tree-making algorithms; however, 16S rRNA gene sequence similarity between these two strains was 97.5 %.

Genomic relatedness between strain YIM 65601$^T$ and its closest relative, *N. candida* HMC10$^T$, was determined by using DNA–DNA hybridization according to the fluorometric microwell method (Ezaki *et al.*, 1989; He *et al.*, 2005). The experiment was repeated five times and the level of DNA–DNA relatedness between them was determined to be 41.4 ± 1.5 % (mean ± SD); this result is below the cut-off point recommended by Stackebrandt & Goebel (1994) for assigning strains to the same species and confirms the separation of strain YIM 65601$^T$ from its nearest phylogenetic neighbour. It has been shown that *Nonomuraea* species share high 16S rRNA gene sequence similarities (within the range 97.6–99.4 %), but have low DNA–DNA relatedness (Fischer *et al.*, 1983; Poscher *et al.*, 1985; Tamura *et al.*, 2000; Kämpfer *et al.*, 2005); Stackebrandt *et al.* (2001) reported

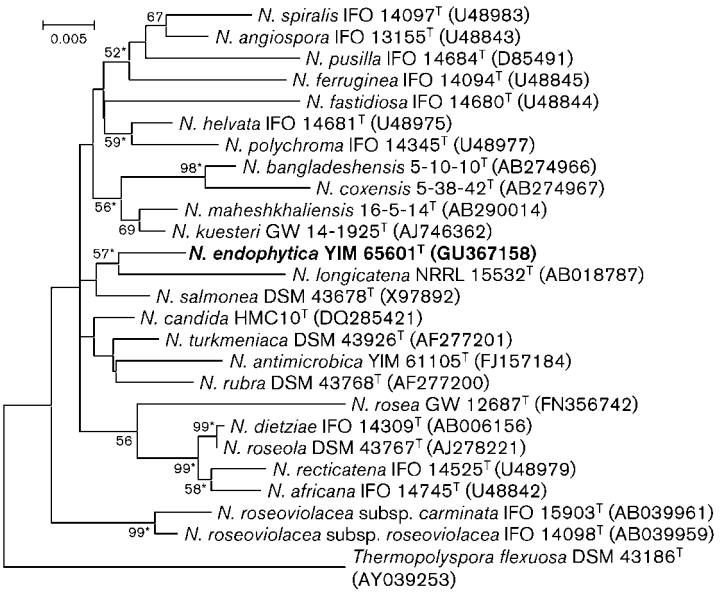


**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences showing relationships between YIM 65601$^T$ and all recognized species of the genus *Nonomuraea*. The sequence of *Thermopolyspora flexuosa* DSM 43186$^T$ was used as the outgroup. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are shown. Asterisks denote nodes that were also recovered using the maximum-parsimony and maximum-likelihood methods. Bar, 0.005 substitutions per site.

45–48 % as the highest DNA–DNA relatedness between the type strains of *Nonomuraea africana*, *N. dietziae* and *N. recticatena*, although these strains shared 98.9–99.8 % 16S rRNA gene sequence similarity. Based on these findings and results obtained in the present study, strain YIM 65601$^T$ was considered to represent a genetically distinct species.

Besides the genotypic evidence, strain YIM 65601$^T$ could also be distinguished from its close relatives by phenotypic characteristics (Table 1). The spore chain arrangement, spore ornamentation and cultural characteristics were clearly different between strain YIM 65601$^T$ and the most closely related phylogenetic neighbours, *N. candida* HMC10$^T$, *N. salmonea* DSM 43678$^T$, *N. kuesteri* GW 14-1925$^T$, *N. turkmeniaca* DSM 43926$^T$, *N. roseola* DSM 43767$^T$, *N. dietziae* DSM 44320$^T$ (=IFO 14309$^T$) and *N. longicatena* NRRL 15532$^T$. Furthermore, there were many physiological and biochemical features that differed between strain YIM 65601$^T$ and these strains, such as differences in nitrate reduction, degradation of gelatin, Tween 40, starch, urea, hypoxanthine and tyrosine, production of $H_2S$, utilization of sole carbon sources, tolerance of NaCl, temperature and pH ranges for growth and fatty acid compositions (Table 1 and Supplementary Table S1). Based on the genotypic and phenotypic evidence, strain YIM 65601$^T$ warrants classification as the type strain of a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea endophytica* sp. nov. is proposed.

### Description of *Nonomuraea endophytica* sp. nov.

*Nonomuraea endophytica* (en.do.phy′ti.ca. Gr. pref. *endo* within; Gr. *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 the isolation of the type strain from plant tissues).

Gram-stain-positive, aerobic, non-motile actinomycete that forms extensively branched substrate and aerial mycelia; soluble pigments are produced on most test media. The aerial mycelium develops masses of long, straight spore chains, each containing more than 20 non-motile spores, which show a warty ornamentation. Temperature range for growth is 10–37 °C, with optimal growth at 20–28 °C. pH range for growth is pH 6.0–9.0, with optimal growth at pH 7.0–8.0. Tolerates up to 5 % NaCl. Positive for catalase, milk coagulation, milk peptonization and $H_2S$ production. Negative for oxidase, gelatin liquefaction, hydrolysis of urea, cellulose and starch and nitrate reduction. Tween 20 is hydrolysed, but Tweens 40 and 80 are not. Utilizes cellobiose, D-fructose, D-galactose, glucose, *myo*-inositol, lactose, maltose, D-mannitol, raffinose, ribose, D-sorbitol and sucrose as sole carbon sources. L-Arabinose, dulcitol, glycerol, D-mannose, sodium acetate and D-xylose are not utilized. L-Alanine, L-arginine, L-asparagine, glycine, L-hydroxyproline, L-lysine, L-phenylalanine, L-serine and L-valine can be used as sole nitrogen sources. Hypoxanthine and tyrosine are decomposed, but not xanthine. The diagnostic amino acid of the peptidoglycan is *meso*-diaminopimelic acid. Cell hydrolysates contain galactose, glucose, mannose, ribose and madurose. Polar lipids include diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, phosphatidylinositol mannosides and phosphatidylinositol. The predominant menaquinones are MK-9($H_4$) and MK-9($H_2$). Major fatty acids (>10 % of the total) are 10-methyl $C_{17:0}$, iso-$C_{16:0}$, $C_{17:0}$ and $C_{15:0}$.

The type strain is YIM 65601$^T$ (=CCTCC AA 209037$^T$ =DSM 45385$^T$), isolated from a surface-sterilized sample of *Artemisia annua* L. collected from Yunnan province, south-west China. The DNA G+C content of the type strain is 67.4 mol%.

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## References

**Chiba, S., Suzuki, M. & Ando, K. (1999).** Taxonomic re-evaluation of '*Nocardiopsis*' sp. K-252$^T$ (=NRRL 15532$^T$): a proposal to transfer this strain to the genus *Nonomuraea* as *Nonomuraea longicatena* sp. nov. *Int J Syst Bacteriol* **49**, 1623–1630.

**Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y.-W. (2007).** EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

**Collins, M. D. & Jones, D. (1980).** Lipids in the classification and identification of coryneform bacteria containing peptidoglycans 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 microdilution 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.

**Fischer, A., Kroppenstedt, R. M. & Stackebrandt, E. (1983).** Molecular-genetic and chemotaxonomic studies on *Actinomadura* and *Nocardiopsis*. *J Gen Microbiol* **129**, 3433–3446.

**Fitch, W. M. (1971).** Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

**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.

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

**Kämpfer, P., Kroppenstedt, R. M. & Grün-Wollny, I. (2005).** *Nonomuraea kuesteri* sp. nov. *Int J Syst Evol Microbiol* **55**, 847–851.

**Kämpfer, P., Busse, H. J., Tindall, B. J., Nimtz, M. & Grün-Wollny, I. (2010).** *Nonomuraea rosea* sp. nov. *Int J Syst Evol Microbiol* **60**, 1118–1124.

**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.

**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.

**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.

**Poscher, J., Kroppenstedt, R. M., Fischer, A. & Stackebrandt, E. (1985).** DNA:DNA reassociation and chemotaxonomic studies on *Actinomadura*, *Microbispora*, *Microtetraspora*, *Micropolyspora* and *Nocardiopsis*. *Syst Appl Microbiol* **6**, 264–270.

**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.

**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. *USFCC Newsl* **20**, 16.

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

**Smibert, R. M. & Krieg, N. R. (1994).** Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

**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.

**Stackebrandt, E., Wink, J., Steiner, U. & Kroppenstedt, R. M. (2001).** *Nonomuraea dietzii* sp. nov. *Int J Syst Evol Microbiol* **51**, 1437–1441.

**Tamaoka, J., Katayama-Fujimura, Y. & Kuraishi, H. (1983).** Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J Appl Microbiol* **54**, 31–36.

**Tamura, T., Suzuki, S. & Hatano, K. (2000).** *Acrocarpospora* gen. nov., a new genus of the order *Actinomycetales*. *Int J Syst Evol Microbiol* **50**, 1163–1171.

**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. (1961).** *The Actinomycetes*, vol. 2. Baltimore: Williams & Wilkins.

**Zhang, Z., Wang, Y. & Ruan, J. (1998).** Reclassification of *Thermomonospora* and *Microtetraspora*. *Int J Syst Bacteriol* **48**, 411–422.