Prauserella salsuginis sp. nov., *Prauserella flava* sp. nov., *Prauserella aidingensis* sp. nov. and *Prauserella sediminis* sp. nov., isolated from a salt lake

Yan Li,^{1†} Shu-Kun Tang,^{1†} Yi-Guang Chen,² Jin-Yuan Wu,¹
Xiao-Yang Zhi,¹ Yu-Qin Zhang³ and Wen-Jun Li^{1,4}

Correspondence
Wen-Jun Li
wjli@ynu.edu.cn

¹The 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, Yunnan 650091, PR China

²College of Biology and Environmental Sciences, Jishou University, Jishou, Hunan 416000, PR China

³Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, PR China

⁴Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, PR China

Strains YIM 90625^T, YIM 90630^T, YIM 90636^T and YIM 90694^T were isolated from a salt lake in Xinjiang province, north-west China, and were subjected to a polyphasic analysis to determine their taxonomic positions. All isolates were moderately halophilic and were able to grow at NaCl concentrations up to 15 or 20% (w/v). The genomic DNA G+C contents of the strains ranged from 69.1 to 70.1 mol%. Based on the results of 16S rRNA gene sequence analysis, DNA–DNA relatedness, phenotypic characteristics and chemotaxonomic data, the isolates are proposed to represent four novel species of the genus *Prauserella* for which the names *Prauserella salsuginis* sp. nov. (type strain YIM 90625^T=CCTCC AA 208051^T=DSM 45264^T), *Prauserella flava* sp. nov. (type strain YIM 90630^T=CCTCC AA 208052^T=DSM 45265^T), *Prauserella aidingensis* sp. nov. (type strain YIM 90636^T=CCTCC AA 208053^T=DSM 45266^T) and *Prauserella sediminis* sp. nov. (type strain YIM 90694^T=CCTCC AA 208054^T=DSM 45267^T).

The genus *Prauserella* was first established by Kim & Goodfellow (1999) with a species that once belonged to the genera *Nocardia* (Di Marco & Spalla, 1957) and *Amycolatopsis* (Lechevalier *et al.*, 1986). Subsequently, it was emended by Li *et al.* (2003) with respect to morphology and the chemical composition of cell constituents. At the time of writing, the genus comprised three species with validly published names: *Prauserella rugosa*

(Kim & Goodfellow, 1999), *Prauserella halophila* and *Prauserella alba* (Li *et al.*, 2003). Members of this genus are aerobic, Gram-positive, non-acid-fast and halophilic or halotolerant. Substrate mycelia fragment into irregular rod-shaped elements and aerial mycelia form spores in chains. Hydrolysates of whole cells contain *meso*-diaminopimelic acid and major amounts of galactose and arabinose (some strains also ribose). MK-9(H₄) is the predominant menaquinone. The DNA G+C contents are 65.8–69.9% (Kim & Goodfellow, 1999; Li *et al.*, 2003).

Strains YIM 90625^T, YIM 90630^T, YIM 90636^T and YIM 90694^T were isolated from water from Aiding Lake, a salt lake in Xinjiang province, north-west China [89° 10' 32" E 42° 32' 10" N; containing (l⁻¹): 127.7648 g Na⁺, 529.1 mg K⁺, 597.8 mg Mg²⁺, 151.9 mg Ca²⁺, 177.5295 g Cl⁻, 29.6352 g SO₄²⁻ and 200.1 mg HCO₃⁻ as the major ions]. Cultured isolates were obtained after 3 weeks of incubation at 37 °C on cellulose-casein multi-salt medium, described

†These authors contributed equally to this work.

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIM 90625^T, YIM 90630^T, YIM 90636^T and YIM 90694^T are FJ444992, FJ444993, FJ444994 and FJ444995, respectively.

Scanning electron micrographs of cells of the four strains and tables showing their culture characteristics and menaquinone and fatty acid compositions are available as supplementary material with the online version of this paper.

by Tang *et al.*, (2008). The colony colour of strain YIM 90636^T was white, whereas the colonies of strains YIM 90625^T, YIM 90630^T and YIM 90694^T appeared as pale yellow to orange–yellow on the isolation plates. The isolates were maintained at 4 °C on slants of International *Streptomyces* Project (ISP) medium 2 agar (Shirling & Gottlieb, 1966) containing 10% (w/v) NaCl and as suspensions of mycelial fragments in glycerol (20%, v/v). Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (about 150 r.p.m.) using ISP 2 (containing 10%, w/v, NaCl; pH 7.0) at 37 °C for 2 weeks.

Morphology was observed by light microscopy (BH2; Olympus) and scanning electron microscopy (JSM-5600LV; JEOL) after growth for 28 days on ISP 2 agar containing 10% (w/v) NaCl. Cultural characteristics were examined using standard growth media (Shirling & Gottlieb, 1966). All media were supplemented with 10% (w/v) NaCl. The colours of substrate and aerial mycelia and any soluble pigments were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). For all four strains, substrate mycelia were well developed and fragmented. Spore chains were borne on aerial mycelia and cells were non-motile. Strain YIM 90630^T did not produce aerial mycelia or spores on any of the media tested (Supplementary Fig. S1, available in IJSEM Online). The four isolates could be distinguished from each other by using a battery of cultural characteristics (Supplementary Table S1, available in IJSEM Online).

Growth at various temperatures (4, 10, 15, 20, 28, 37, 40, 45, 50, 55, 60 and 65 °C) was tested on ISP 2 agar

containing 10% (w/v) NaCl. Tests for tolerance to NaCl concentrations and pH used ISP 2 as the basal medium. Growth with different NaCl concentrations was tested in the range 0–20% (w/v), in increments of 1%. Growth at various pH values was tested for the range pH 5.0–10.0, in increments of 0.5 pH units, using the following buffer system: pH 5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH₂PO₄/0.1 M NaOH; and pH 9.0–10.0, 0.1 M NaHCO₃/0.1 M Na₂CO₃. Media and procedures for the determination of physiological features and carbon source utilization patterns were those described by Williams *et al.* (1983). The detailed morphological and physiological properties of the four strains are given in the species descriptions. The four isolates could be distinguished from each other and from the other type strains of the genus *Prauserella* by means of morphological and physiological properties (Table 1).

Isomers of diaminopimelic acid and whole-cell sugars were analysed according to Hasegawa *et al.* (1983). Polar lipids were extracted and examined by two-dimensional TLC and identified using described procedures (Minnikin *et al.*, 1984). Menaquinones were isolated according to Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). Cellular fatty acids were analysed as described by Sasser (1990) using the Microbial Identification System (MIDI). All four strains, as well as two *Prauserella* reference strains, *P. halophila* YIM 90001^T and *P. alba* YIM 90005^T, contained *meso*-diaminopimelic acid as the diagnostic diamino acid, and galactose, arabinose and ribose as the major whole-cell sugars. The phospholipids for all four

Table 1. Phenotypic characteristics that differentiate the four isolates from each other and other type strains of the genus *Prauserella*

Strains: 1, YIM 90625^T (*P. salsuginis* sp. nov.); 2, YIM 90630^T (*P. flava* sp. nov.); 3, YIM 90636^T (*P. aidingensis* sp. nov.); 4, YIM 90694^T (*P. sediminis* sp. nov.); 5, *P. halophila* YIM 90001^T; 6, *P. alba* YIM 90005^T; 7, *P. rugosa* DSM 43194^T. Data in columns 1–4 were from this study, in columns 5 and 6 from Li *et al.* (2003) and column 7 from Kim & Goodfellow (1999) and Li *et al.* (2003). +, Positive; –, negative.

Characteristic	1	2	3	4	5	6	7
Aerial mycelium	+	–	+	+	+	+	–
Growth with NaCl (% w/v)							
Range	5–15	5–15	5–15	5–20	5–25	0–25	0–20
Optimum	8–10	8–10	8–10	10	10–15	10–15	5–10
Degradation of urea	–	–	–	–	+	–	–
Carbon utilization							
L-Arabinose	+	–	–	–	–	+	+
D-Galactose	+	–	–	–	–	+	+
<i>myo</i> -Inositol	+	–	–	–	+	+	–
Maltose	–	–	–	–	–	+	+
Trehalose	+	–	+	–	+	–	+
Nitrogen utilization							
L-Arginine	–	–	–	+	+	+	–
L-Glycine	–	–	–	–	+	+	+
L-Lysine	+	+	–	+	+	+	–
L-Serine	–	–	–	–	+	+	–
DNA G+C content (mol%)	69.1	69.9	70.1	69.1	65.8	66.7	69.2*

*Data from this study.

isolates comprised phosphatidylmethylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and some unknown phospholipids. The detailed menaquinone compositions and fatty acid profiles are given in Supplementary Tables S2 and S3 (in IJSEM Online), respectively. The predominant menaquinone was MK-9(H₄), but minor amounts of other menaquinones differentiated the strains from each other. The chemotaxonomic data for the four strains were consistent with their assignment to the genus *Prauserella* (Kim & Goodfellow, 1999; Li *et al.*, 2003).

For the determination of the DNA G+C content, the genomic DNA of the four strains were prepared according to the method of Marmur (1961) and the DNA G+C contents were determined by reversed-phase HPLC according to Mesbah *et al.* (1989). The G+C contents of the genomic DNA of strains YIM 90625^T, YIM 90630^T, YIM 90636^T and YIM 90694^T were 69.1, 69.9, 70.1 and 69.1 mol%, respectively.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene sequence were performed as described by Li *et al.* (2007). Multiple alignments with sequences from *Prauserella* species and other closely related species and calculation of levels of sequence similarity were carried out using the EzTaxon server version 2.0 (Chun *et al.*, 2007). Phylogenetic analyses were performed using three tree-making algorithms: neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) from *K*_{nuc} values (Kimura, 1980) using MEGA version 4.0 (Tamura *et al.*, 2007). The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The almost-complete 16S rRNA gene sequences of strains YIM 90625^T (1430 bp), YIM 90630^T (1457 bp), YIM 90636^T (1455 bp) and YIM 90694^T (1465 bp) were determined. These sequences were analysed by preliminary comparison with sequences from the GenBank database and the results indicated that the four isolates were most closely related to members of the genus *Prauserella*. Phylogenetic analysis showed that strains YIM 90625^T, YIM 90630^T and YIM 90694^T fell into one distinct subclade with *P. alba* YIM 90005^T and that strain YIM 90636^T fell into a separate subclade between *P. alba* YIM 90005^T and *P. halophila* YIM 90001^T (Fig. 1). The four strains showed 16S rRNA gene sequence similarities higher than 98 % with each other and with *P. alba* YIM 90005^T and *P. halophila* YIM 90001^T (Table 2).

DNA–DNA hybridization studies were performed using the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992) between the four isolates and *P. alba* YIM 90005^T and *P. halophila* YIM 90001^T. DNA–DNA relatedness values ranged from 20.8 to 60.3 % (mean values from three replicate hybridizations; Table 2), which were lower than the threshold value of 70 % recommended by Wayne *et al.*, (1987) for the delineation of genomic species. On the basis of these results, the isolates represent four novel species of the genus *Prauserella*.

Additionally, the four isolates could be distinguished from each other and from the two most closely related *Prauserella* species, *P. halophila* and *P. alba*, on the basis of physiological and biochemical characteristics and chemotaxonomic data (Table 1 and Supplementary Tables S1–S3). Based on the results of the phenotypic and genotypic analyses, we consider that strains YIM 90625^T, YIM 90630^T, YIM 90636^T and YIM 90694^T each represent a novel species of the genus *Prauserella*, for which the names proposed are *Prauserella salsuginis* sp. nov.,

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence analysis, constructed from evolutionary distances with the neighbour-joining method, showing the positions of strains YIM 90625^T, YIM 90630^T, YIM 90636^T and YIM 90694^T and related taxa. Bootstrap values (>50 %) based on 1000 replicates are shown at branch nodes. *Pseudonocardia compacta* IMSNU 20111^T was used as the outgroup. Bar, 0.5 % sequence divergence.

Table 2. DNA–DNA relatedness and 16S rRNA gene sequence similarity values (%) between the four novel strains and their two closest relatives

Values in parentheses are 16S rRNA gene sequence similarity values.

Strains	1	2	3	4	5	6
1. <i>P. halophila</i> YIM 90001 ^T	100 (100)					
2. <i>P. alba</i> YIM 90005 ^T	30.20 (98.5)	100 (100)				
3. <i>P. salsuginis</i> sp. nov. YIM 90625 ^T	20.80 (98.3)	47.15 (99.5)	100 (100)			
4. <i>P. flava</i> sp. nov. YIM 90630 ^T	41.25 (98.3)	43.60 (99.6)	56.90 (99.9)	100 (100)		
5. <i>P. aidingensis</i> sp. nov. YIM 90636 ^T	53.65 (98.9)	41.40 (99.2)	43.95 (99.0)	55.30 (99.3)	100 (100)	
6. <i>P. sediminis</i> sp. nov. YIM 90694 ^T	45.20 (98.3)	51.60 (99.5)	60.25 (99.9)	40.90 (99.9)	47.90 (99.2)	100 (100)

Prauserella flava sp. nov., *Prauserella aidingensis* sp. nov. and *Prauserella sediminis* sp. nov., respectively.

Description of *Prauserella salsuginis* sp. nov.

Prauserella salsuginis (sal.su'gi.nis. L. gen. n. *salsuginis* of salt water, from which the type strain was isolated).

Aerobic, Gram-positive, non-acid-fast and non-motile. Aerial mycelium is white and substrate mycelium is fragmented. Colony colours are light grey–white (ISP 5), brilliant yellow (ISP 2), deep yellow (Czapek's agar) and pale orange–yellow (potato agar and nutrient agar). Optimum growth occurs on ISP 2 agar supplemented with 8–10 % NaCl at 28–37 °C and pH 7.0. Temperature, pH and NaCl tolerance ranges are 15–45 °C, pH 6.0–9.0 and 5–15 % (w/v) NaCl, respectively. L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, lactose, D-mannitol, D-mannose, L-rhamnose, D-ribose, trehalose, D-xylitol and D-xylose are utilized as sole carbon sources, but maltose and raffinose are not. L-Alanine, L-histidine, L-hydroxyproline, L-lysine, L-phenylalanine, L-proline, threonine, DL-tryptophan, L-tyrosine, L-xanthine and L-valine are utilized as sole nitrogen sources, but adenine, L-arginine, glycine, DL-methionine and L-serine are not. Milk coagulation, gelatin liquefaction and catalase are positive, but H₂S and melanin production, starch hydrolysis, urease, nitrate reduction and oxidase are negative. The DNA G+C content of the type strain is 69.1 mol%.

The type strain, YIM 90625^T (=CCTCC AA 208051^T=DSM 45264^T), was isolated from Aiding Lake, a salt lake in Xinjiang province, north-west China.

Description of *Prauserella flava* sp. nov.

Prauserella flava (fla'va. L. fem. adj. *flava* yellow, referring to the colour of the substrate mycelium).

Aerobic, Gram-positive, non-acid-fast, non-motile organism. Aerial mycelium and spores are not produced on any of the media tested and substrate mycelium is fragmented. Colony colours are light yellow (ISP 5), brown (ISP 3), brilliant yellow (ISP 2) and grey–yellow–white (potato agar). Optimum growth occurs on ISP 2 agar supplement-

ted with 8–10 % NaCl at 28–37 °C and pH 7.0. Temperature, pH and NaCl tolerance ranges are 15–45 °C, pH 6.0–9.0 and 5–15 % (w/v) NaCl, respectively. D-Fructose, D-glucose, lactose, D-ribose and D-xylose are utilized as sole carbon sources, but L-arabinose, cellobiose, D-galactose, *myo*-inositol, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, trehalose and D-xylitol are not. L-Alanine, L-histidine, L-hydroxyproline, L-lysine, L-phenylalanine, L-proline, threonine, DL-tryptophan, L-tyrosine, L-xanthine and L-valine are utilized as sole nitrogen sources, but adenine, L-arginine, L-glycine, DL-methionine and L-serine are not. Milk coagulation, gelatin liquefaction and catalase are positive, but H₂S production, starch hydrolysis, urease, nitrate reduction and oxidase are negative. The DNA G+C content of the type strain is 69.9 mol%.

The type strain, YIM 90630^T (=CCTCC AA 208052^T=DSM 45265^T), was isolated from Aiding Lake, a salt lake in Xinjiang province, north-west China.

Description of *Prauserella aidingensis* sp. nov.

Prauserella aidingensis (ai.ding.en'sis. N.L. fem. adj. *aidingensis* from Aiding Lake, where the type strain was isolated).

Aerobic, Gram-positive, non-acid-fast, non-motile organism. Aerial mycelium is white and substrate mycelium is fragmented. Colony colours are light grey–white (ISP 5), slight grey–white (ISP 3), brilliant yellow (ISP 2) and brilliant yellow (potato agar). Optimum growth occurs on ISP 2 medium supplemented with 8–10 % NaCl at 28–37 °C and pH 7.0. Temperature, pH and NaCl tolerance ranges are 15–45 °C, pH 6.0–9.0 and 5–15 % (w/v) NaCl, respectively. D-Fructose, D-glucose, lactose, L-rhamnose, D-ribose, trehalose and D-xylose are utilized as sole carbon sources, but L-arabinose, cellobiose, D-galactose, *myo*-inositol, maltose, D-mannitol, D-mannose, raffinose and D-xylitol are not. L-Alanine, L-histidine, L-hydroxyproline, L-phenylalanine, L-proline, threonine, DL-tryptophan, L-tyrosine, L-xanthine and L-valine are utilized as sole nitrogen sources, but adenine, L-arginine, L-glycine, L-lysine, DL-methionine and L-serine are not. Milk coagulation, gelatin liquefaction and catalase are positive, but

H₂S production, starch hydrolysis, urease, nitrate reduction and oxidase are negative. The DNA G + C content of the type strain is 70.1 mol%.

The type strain, YIM 90636^T (=CCTCC AA 208053^T = DSM 45266^T), was isolated from Aiding Lake, a salt lake in Xinjiang province, north-west China.

Description of *Prauserella sediminis* sp. nov.

Prauserella sediminis (se.di'mi.nis. L. gen. n. *sediminis* of sediment).

Aerobic, Gram-positive, non-acid-fast, non-motile organism. Aerial mycelium is white on most media tested and substrate mycelium is fragmented. Colony colours are deep grey–white (ISP 5), pale yellow (ISP 3), orange–yellow (ISP 2) and grey reddish orange (potato agar). Optimum growth occurs on ISP 2 medium supplemented with 10 % NaCl at 28–37 °C and pH 7.0. Temperature, pH and NaCl tolerance ranges are 15–45 °C, pH 6.0–9.0 and 5–20 % (w/v) NaCl, respectively. D-Glucose, lactose and L-rhamnose are utilized as sole carbon sources, but L-arabinose, cellobiose, D-fructose, D-galactose, *myo*-inositol, maltose, D-mannitol, D-mannose, raffinose, D-ribose, trehalose, D-xylitol and D-xylose are not. L-Alanine, L-arginine, L-histidine, L-lysine, L-hydroxyproline, L-phenylalanine, L-proline, threonine, DL-tryptophan, L-tyrosine, L-xanthine and L-valine are utilized as sole nitrogen sources, but adenine, L-glycine, DL-methionine and L-serine are not. Milk coagulation, gelatin liquefaction and catalase are positive, but H₂S production, starch hydrolysis, urease, nitrate reduction and oxidase are negative. The DNA G + C content of the type strain is 69.1 mol%.

The type strain, YIM 90694^T (=CCTCC AA 208054^T = DSM 45267^T), was isolated from Aiding Lake, a salt lake in Xinjiang province, north-west China.

Acknowledgements

This research was supported by the National Basic Research Program of China (no. 2010CB833800), the National Natural Science Foundation of China (nos. 30600001, 30860002, 30870005), Key Project of International Cooperation (2007DFB31620) and Yunnan Provincial Science and Technology Department (nos. 2009AC017, 2009DA002).

References

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.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Di Marco, A. & Spalla, C. (1957). La produzione di cobalamina da fermentazione con una nuova specie di *Nocardia*: *Nocardia rugosa*. *Giorn Microbiol* **4**, 24–30 (in Italian).

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.

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

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

HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Jahnke, K. D. (1992). BASIC computer program for evaluation of spectroscopic DNA renaturation data from GILFORD SYSTEM 2600 spectrophotometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.

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.

Kim, S. B. & Goodfellow, M. (1999). Reclassification of *Amycolatopsis rugosa* Lechevalier *et al.* 1986 as *Prauserella rugosa* gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 507–512.

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.

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.

Lechevalier, M. P., Prauser, H., Labeda, D. P. & Ruan, J.-S. (1986). Two new genera of nocardioform actinomycetes: *Amycolata* gen. nov. and *Amycolatopsis* gen. nov. *Int J Syst Bacteriol* **36**, 29–37.

Li, W.-J., Xu, P., Tang, S.-K., Xu, L.-H., Kroppenstedt, R.-M., Stackebrandt, E. & Jiang, C.-L. (2003). *Prauserella halophila* sp. nov. and *Prauserella alba* sp. nov., moderately halophilic actinomycetes from saline soil. *Int J Syst Evol Microbiol* **53**, 1545–1549.

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.

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

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., 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.

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.

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.

Tang, S.-K., Tian, X.-P., Zhi, X.-Y., Cai, M., Wu, J.-Y., Yang, L.-L., Xu, L.-H. & Li, W.-J. (2008). *Haloactinospora alba* gen. nov., sp. nov., a

halophilic filamentous actinomycete of the family *Nocardiopsaceae*. *Int J Syst Evol Microbiol* **58**, 2075–2080.

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.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.