Haloactinospora alba gen. nov., sp. nov., a halophilic filamentous actinomycete of the family *Nocardiopsaceae*

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A novel halophilic, filamentous, actinomycete strain, designated YIM 90648^T, was isolated from a salt lake in Xinjiang Province, north-west China, and subjected to a polyphasic taxonomic study. Optimal growth occurred at 37 °C, pH 7.0–8.0 and 15 % (w/v) NaCl. The aerial mycelium of strain YIM 90648^T formed long chains of spores at maturity and the spores were cylindrical with smooth surfaces. Spore chains with pseudosporangia at the end were borne on the substrate mycelium and most spores had wrinkled surfaces. Strain YIM 90648^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid and galactose and ribose as the major whole-cell components. The phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and phosphatidylinositol mannoside. MK-10(H₈), MK-11(H₄), MK-11(H₆) and MK-11(H₈) were the predominant menaquinones. The major fatty acids were *i*-C_{16:0} and *ai*-C_{17:0}. The DNA G + C content was 68 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YIM 90648^T formed a distinct lineage within the family *Nocardiopsaceae* and showed 16S rRNA gene sequence similarity of 93.3–95.0 % with members of the family *Nocardiopsaceae*. On the basis of the polyphasic evidence, a novel genus and species, *Haloactinospora alba* gen. nov., sp. nov., is proposed to accommodate this isolate. The type strain of *Haloactinospora alba* is YIM 90648^T (=DSM 45015^T =CCTCC AA 206008^T).

The family *Nocardiopsaceae* was first described by Rainey *et al.* (1996) and currently comprises three genera, *Nocardiopsis* (Meyer, 1976), *Thermobifida* (Zhang *et al.*, 1998) and *Streptomonospora* (Cui *et al.*, 2001). With the exception of the genus *Thermobifida*, most strains of the family *Nocardiopsaceae* have been isolated from saline soils, and some of them are strictly halophilic filamentous actinomycetes. At present, the genus *Nocardiopsis* contains 24 species with validly published names, some of which have been isolated from hypersaline soils (Al-Tai & Ruan, 1994; Al-Zarban *et al.*, 2002; Li *et al.*, 2003a, 2004, 2006), and the genus *Streptomonospora* contains two species (Cui *et al.*, 2001; Li *et al.*, 2003b), both of which are halophilic filamentous actinomycetes.

During a study on isolation methods for halophilic filamentous actinomycetes from hypersaline soils, more

than 500 non-*Nocardiopsis* strains (determined by genus-specific primers as described by Salazar *et al.*, 2002; data not shown) were isolated from Xinjiang Province, north-west China. In this paper, the description of a moderately halophilic actinomycete, strain YIM 90648^T, which was isolated from a salt lake sample after 3 weeks incubation at 37 °C on cellulose-casein-multisalts (representing a combination of NaCl, KCl and MgCl₂) medium, is reported. The composition of the multisalts medium was [(1 distilled water)⁻¹]: 10 g microcrystalline cellulose, 0.3 g casein, 0.2 g KNO₃, 0.5 g K₂HPO₄, 0.02 g CaCO₃, 0.01 g FeSO₄, 100 g NaCl, 30 g MgCl₂ · 6H₂O, 20 g KCl and 15 g agar. The salts mixture was sterilized separately before being added to the medium. The medium was adjusted to pH 7.5 with 1 M NaOH. The isolate was maintained on ISP 4 agar slants containing 15 % (w/v) NaCl at 4 °C and as suspensions of mycelial fragments in 20 % (v/v) glycerol. Biomass for chemical and molecular studies was obtained by cultivation in shake flasks (about 150 r.p.m.) using ISP 4 supplemented with 15 % (w/v) NaCl (pH 7.5), incubated at 37 °C for 2 weeks.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 90648^T is DQ923130.

Morphological characteristics of strain YIM 90648^T were observed by light microscopy (model BH2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 4 weeks growth on ISP 2 containing 15% (w/v) NaCl. Cultural characteristics were determined after 3–4 weeks by methods used by the International *Streptomyces* Project (Shirling & Gottlieb, 1966). All media were supplemented with 15% (w/v) NaCl for growth and the colours of the substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). Media and procedures used for determination of physiological features and carbon source utilization were those described by Smibert & Krieg (1981) and Williams *et al.* (1989) using basal medium supplemented with 15% (w/v) NaCl. Growth was tested at 0, 4, 10, 15, 20, 28, 37, 45, 55 and 65 °C on ISP 4 containing 15% (w/v) NaCl. For endurance experiments involving concentrations of NaCl between 0–30% (at intervals of 1%), ISP 4 was used as the basal medium. The pH range for growth was investigated between pH 4.0 and 10.0 at intervals of 1 pH unit using the buffer system described by Xu *et al.* (2005).

Strain YIM 90648^T developed well on most media tested and detailed results are shown in the species description. The aerial and substrate mycelia were well developed, but not fragmented, on most media tested. The aerial mycelium formed long chains of spores at maturity that were straight and cylindrical in shape ($0.4\text{--}0.6 \times 1.0\text{--}1.2\text{ }\mu\text{m}$) with smooth surfaces; they were non-motile (Fig. 1a). Spore chains with pseudosporangia at the end were borne on the substrate mycelium and the spores had wrinkled surfaces (Fig. 1b, c). The morphological characteristics of strain YIM 90648^T were distinctly different from those of members of the other three genera of the family *Nocardiopsaceae*, shown in detail in Table 1. Growth occurred at 20–45 °C, pH 6.0–9.0 and in 9–21% (w/v) NaCl. Good growth occurred at 37 °C and pH 7.0–8.0 with 15% (w/v) NaCl. Detailed physiological properties of the strain are given in Table 1 and in the species description.

Diaminopimelic acid isomers and whole-cell sugars were analysed according to the procedures developed by Hasegawa *et al.* (1983). Polar lipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Minnikin *et al.*, 1984). The polar lipids were separated by two-dimensional TLC on silica gel 60 plates (Merck). The first direction was developed in chloroform/methanol/water (65:25:4, by vol.) and the second was developed in chloroform/methanol/acetic acid/water (80:12:15:4, by vol.). Total lipid material and specific functional groups were detected using Dittmer and Lester reagent (phosphate), ninhydrin (free amino groups), Dragendorff reagent (quaternary nitrogen) and anisaldehyde-sulfuric acid (glycolipids). For analysis of fatty acids, strain YIM 90648^T was cultured on trypticase soy agar (Difco) containing 15% NaCl at 37 °C for 1 week. The preparation and analysis of fatty acid methyl esters was performed as described by Sasser (1990)

Fig. 1. Scanning electron micrographs of spore chains and pseudosporangia of strain YIM 90648^T grown on ISP 2 [15% (w/v) NaCl] for 4 weeks at 37 °C. (a) Long spore chains on aerial mycelium; (b, c) short spore chain with pseudosporangium on substrate mycelium. Bars, 2 μm (a, b) and 1 μm (c).

using the Microbial Identification System (MIDI). Menaquinones were isolated and purified according to Minnikin *et al.* (1984). The purified menaquinones were dissolved in acetone and separated by atmospheric pressure photo-ionization-LC-MS. The chromatographic system consisted of an API QSTAR Pulsar I system and a column oven (ABI). A mixture of menaquinones (20 μl) was injected into the analytical column at 40 °C, equilibrated with 2-propanol/methanol (35:65, v/v) for 30 min and operated at a flow rate of $1000\text{ }\mu\text{l min}^{-1}$ (0–50 min, 2-propanol/methanol=35–70:65–30, v/v; 50–55 min, 2-propanol/methanol=70–35:30–65, v/v) for 60 min. Ionization conditions for atmospheric pressure photo-ionization were 1300 V and 450 °C.

The whole-cell hydrolysates of strain YIM 90648^T contained *meso*-diaminopimelic acid, galactose and ribose, with a minor amount of glucose. The polar lipids were

Table 1. Differential phenotypic and chemotaxonomic characteristics of strain YIM 90648^T and related genera of the family *Nocardiopsaceae*

Data for reference genera were taken from Kroppenstedt & Evtushenko (2006); Cui *et al.* (2001); Li *et al.* (2003b); Zhang *et al.* (1998). NA, No data available.

Characteristics	Strain YIM 90648 ^T	<i>Nocardiopsis</i>	<i>Streptomonospora</i>	<i>Thermobifida</i>
Spores formed on:				
Substrate mycelium	Sporangia	None	Single	Single, clusters some-times
Aerial mycelium	Long chains	Long chains	Short chains	Single, clusters
NaCl range (% w/v)	9–21	0–20	5–25	0–5
Temperature range (°C)	20–45	7–45	NA	35–60
Predominant menaquinones	10(H ₈), 11(H ₄), 11(H ₆), 11(H ₈)	10(H ₂), 10(H ₄), 10(H ₆), 9(H ₄), 9(H ₆)	9(H ₂), 9(H ₄), 9(H ₆), 9(H ₈), 10(H ₂), 10(H ₄), 10(H ₆), 10(H ₈)	10(H ₆), 10(H ₈), 11(H ₆)
Phospholipids*	DPG, PG, PC, PIM	PC, PME	PG, PE, PI	PE, PME, GL
Fatty acid type	3d	3d	NA	3e
DNA G + C content (mol%)	68	64–76	69–71	64–69

*DPG, Diphosphatidylglycerol; GL, glycolipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PME, phosphatidylmonomethylethanolamine.

diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol mannoside and some unidentified lipids (ninhydrin reagent-positive; Dittmer and Lester reagent-negative). Strain YIM 90648^T had a cellular fatty acid profile that contained predominantly branched fatty acids, with minor amounts of straight-chain and methyl fatty acids; it contained ai-C_{15:0} (6.7%), i-C_{16:0} (23.8%), ai-C_{16:0} (1.2%), i-C_{16:0} (2.2%), 10-methyl C_{16:0} (1.7%), i-C_{17:0} (3.2%), ai-C_{17:0} (36.0%), 10-methyl C_{17:0} (3.7%), i-C_{18:0} (4.6%), C_{18:0} (3.5%), tuberculo-stearic acid (10-methyl C_{18:0}) (4.9%) and ai-C_{19:0} (2.7%). The major fatty acids were i-C_{16:0} and ai-C_{17:0} and, according to Kroppenstedt (1985), this fatty acid pattern belongs to fatty acid type 3d. The predominant menaquinones were MK-10(H₈) (10.6%), MK-11(H₄) (11.3%), MK-11(H₆) (15.6%) and MK-11(H₈) (15.0%); the minor components MK-7(H₂) (1.1%), MK-10 (4.1%), MK-10(H₂) (3.1%), MK-10(H₄) (6.2%), MK-10(H₆) (8.1%), MK-11(8.2%), MK-11(H₂) (6.4%), MK-12 (1.7%), MK-12(H₂) (1.0%), MK-12(H₄) (2.1%) and MK-12(H₆) (2.6%) were also detected. The chemotaxonomic data are also distinctly different from those of members of the other three genera of the family *Nocardiopsaceae* (Table 1).

Genomic DNA for the determination of G + C content was prepared according to the method of Marmur (1961). The G + C content of the DNA was determined by reversed-phase HPLC of nucleosides according to Mesbah *et al.* (1989). The DNA G + C content of strain YIM 90648^T was 68 mol%.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li *et al.* (2007). Multiple alignments with sequences of related taxa in the family *Nocardiopsaceae* were performed by using CLUSTAL_X

(Thompson *et al.*, 1997). 16S rRNA gene sequence similarity values were calculated by using a web-based tool (<http://www.eztaxon.org>; Chun *et al.*, 2007). Phylogenetic analyses were performed using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree (Fig. 2) was constructed using the neighbour-joining method of Saitou & Nei (1987) from *K_{nuc}* values (Kimura, 1980) using MEGA version 2.1 (Kumar *et al.*, 2001). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The results of BLAST searches showed that strain YIM 90648^T had relatively high 16S rRNA gene sequence similarities (93.3–95.0%) to members of the genera *Thermobifida*, *Streptomonospora* and *Nocardiopsis* of the family *Nocardiopsaceae*. In the phylogenetic tree based on the neighbour-joining algorithm, all type strains of species of the three genera and strain YIM 90648^T clustered in a distinct clade that was strongly supported by a high bootstrap value (99%). This relationship was supported by all tree-making methods used in this study. Additionally, patterns of selected 16S rRNA gene signature nucleotides defined for the family *Nocardiopsaceae* (Stackebrandt *et al.*, 1997) were consistent with nucleotides determined for the 16S rRNA gene sequence of strain YIM 90648^T except that C–A was determined at positions 658:748. All the above data confirmed that the novel isolate should be assigned to the family *Nocardiopsaceae*.

However, within the clade described above, strain YIM 90648^T formed a branch that was distinct from that containing species of *Streptomonospora*, *Nocardiopsis* and *Thermobifida* and it could not be affiliated to any known

Fig. 2. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences showing the position of strain YIM 90648^T and its phylogenetic neighbours. Numbers on branch nodes are bootstrap values (1000 resamplings; only values over 50 % are given). The sequence of *Actinopolyspora halophila* ATCC 27976^T was used as the outgroup. Bar, 2 % sequence divergence.

genus of the family *Nocardiostrictaceae*. In addition, strain YIM 90648^T differed morphologically and chemotaxonomically from members of the other three genera of the family *Nocardiostrictaceae* (Table 1). Furthermore, distinctions could be made between strain YIM 90648^T and other members of the family *Nocardiostrictaceae* in some signature sites (Table 2). Thus, based on 16S rRNA gene sequence analysis, chemotaxonomic properties and the profile of metabolic properties, it is clear that strain YIM 90648^T represents a novel species in a new genus within the family *Nocardiostrictaceae*, for which the name *Haloactinospora alba* gen. nov., sp. nov. is proposed.

Description of *Haloactinospora* gen. nov.

Haloactinospora (Ha'lo.ac.ti.no.spo'ra. Gr. n. *hals*, *halos* salt; Gr. n. *actis*, *actinos* a ray; Gr. n. *spora* a seed; N.L. fem. n. *Haloactinospora* salt-loving and spored ray, referring to a halophilic and spore-forming actinomycete).

Gram-positive, aerobic, moderately halophilic, filamentous actinomycetes. The aerial mycelium forms long chains of spores and spores are non-motile. The substrate mycelium forms spore chains with pseudosporangia at the end. Whole-cell hydrolysates contain *meso*-diaminopimelic acid, galactose and ribose. The phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and phosphatidylinositol mannoside. The predominant menaquinones are MK-10(H₈), MK-11(H₄), MK-11(H₆) and

MK-11(H₈). The major fatty acids are *i*-C_{16:0} and *ai*-C_{17:0}. The type species is *Haloactinospora alba*.

Description of *Haloactinospora alba* sp. nov.

Haloactinospora alba (al'ba. L. fem. adj. *alba* white).

Displays the following properties in addition to those described for the genus. Growth is good on Czapek's agar, yeast extract-malt extract agar and potato agar, moderate on inorganic salts-starch agar, glycerol-asparagine agar and nutrient agar and poor on oatmeal agar. The colour of the aerial mycelium is white to yellow-white and the substrate mycelium is pale yellow to light yellow or deep yellow. No diffusible pigments are produced. The aerial and substrate mycelia are well developed, but not fragmented, on most media tested. Spores are straight and cylindrical with smooth surfaces. Tests for gelatin liquefaction, production of H₂S and melanin, hydrolysis of cellulose and starch and urease production are negative, whereas tests for milk peptonization and coagulation are positive. L-Arabinose, D-arabitol, cellobiose, D-fructose, D-galactose, D-glucose, inulin, raffinose, D-ribose, sucrose, D-tagatose and D-xylose are utilized as carbon sources, but D-adonitol, D-arabinose, dulcitol, erythritol, glycogen, inositol, D-lactose, maltose, D-mannitol, D-mannose, melezitose, L-rhamnose, L-sorbose, D-sorbitol, trehalose, xylitol and L-xylose are not. Temperature, pH and NaCl tolerance ranges are 20–45 °C,

Table 2. Selected 16S rRNA gene signature nucleotides detected among the various genera of the family *Nocardiopsaceae*

16S rRNA gene sequences of all type strains belonging to the family *Nocardiopsaceae* were included in this analysis. The signatures given below for each group were chosen for their presence in more than 95 % of the members of the respective genera.

Position(s)	Strain YIM 90648 ^T	<i>Nocard-iopsis</i>	<i>Streptomo-nospora</i>	<i>Thermo-bifida</i>
81:88	A-U	C-G	C-G	C-G
82:87	U-C	C-G	C-G	C-G
83	C	U	U	U
85	U	C	C	C
80:89	C-U	C-G	C-G	C-G
126:235	A-C	G-C	G-C	G-C
127:234	G-U	A-U	A-U	A-U
128:233	U-C	G-C	G-C	G-C
129:232	A-G	C-G	C-G	U-G
180	A	U	U	U
181	C	A	A	A
209	C	U	U	U
198:219	G-U	G-C	G-C	G-C
229	U	U	G	U
306	G	A	A	A
307	U	C	C	C
344	A	G	A	G
422	U	C	C	C
449	G	C	C	C
450:483	C-C	G-C	G-C	G-C
452	G	A	A	A
454	C	G	G	G
508	U	U	C	U
607	A	A	U	A
611:629	U-U	A-U	U-U	U-G
632	G	U	G	U
653	U	A	A	U
658:748	C-A	U-A	U-A	U-A
662:743	U-G	U-G	C-G	U-G
747	G	U	U	U
811	U	C	U	C
842	C	U	U	U
844	A	C	C	C
845	C	A	A	A
998:1043	C-G	C-G	G-C	C-G
1012:1017	A-U	A-U	G-C	A-U
1130	G	A	A	A
1131	C	G	G	G
1132:1142	A-G	C-G	C-G	C-G
1133:1141	C-G	A-U	A-U	A-U
1212	G	U	U	U
1252	U	U	U	A
1255:1282	C-C	G-C	G-C	G-C
1256	G	C	C	C
1264:1271	C-G	C-G	C-G	U-G
1254:1283	G-C	G-C	G-C	G-U
1285	A	U	U	U
1309:1328	G-U	G-C	G-C	G-C
1361	C	G	G	G

Table 2. cont.

Position(s)	Strain YIM 90648 ^T	<i>Nocard-iopsis</i>	<i>Streptomo-nospora</i>	<i>Thermo-bifida</i>
1362	A	C	C	C
1364	U	A	A	A
1414:1486	U-G	U-G	U-G	C-G
1438:1463	U-G	U-G	U-G	C-G
1447	C	A	A	A
1448:1455	G-G	C-G	C-G	C-G
1449:1454	G-G	C-G	C-G	C-G
1439:1462	G-U	G-U	G-U	G-C

pH 6–9 and 9–21 % (w/v) NaCl; good growth occurs at 37 °C, pH 7.0–8.0 and 15 % (w/v) NaCl.

The type strain is YIM 90648^T (=DSM 45015^T =CCTCC AA 206008^T), isolated from a salt lake in Xinjiang Province, north-west China. The G + C content of the genomic DNA of the type strain is 68 mol%.

Note added in proof

Since this article was accepted for publication, two additional taxa of the family *Nocardiopsaceae* have been described, *Streptomonospora halophila* (Cai *et al.*, 2008) and *Thermobifida halotolerans* (Yang *et al.*, 2008).

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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.
- Cai, M., Zhi, X.-Y., Tang, S.-K., Zhang, Y.-Q., Xu, L.-H. & Li, W.-J. (2008). *Streptomonospora halophila* sp. nov., a halophilic actinomycete isolated from a hypersaline soil. *Int J Syst Evol Microbiol* **58**, 1556–1560.
- 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.
- 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.

- 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 of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- 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.
- 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.
- Kumar, S., Tamura, K., Jakobsen, I.-B. & Nei, M. (2001).** MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.
- Li, M.-G., Li, W.-J., Xu, P., Cui, X.-L., Xu, L.-H. & Jiang, C.-L. (2003a).** *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., Xu, P., Zhang, L.-P., Tang, S.-K., Cui, X.-L., Mao, P.-H., Xu, L.-H., Schumann, P., Stackebrandt, E. & Jiang, C.-L. (2003b).** *Streptomonospora alba* sp. nov., a novel halophilic actinomycete, and emended description of the genus *Streptomonospora* Cui *et al.* 2001. *Int J Syst Evol Microbiol* **53**, 1421–1425.
- Li, W.-J., Park, D.-J., Tang, S.-K., 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 a 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.
- 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.
- Meyer, J. (1976).** *Nocardiopsis*, a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **26**, 487–493.
- 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.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996).** The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic tree. *Mol Biol Evol* **4**, 406–425.
- Salazar, O., González, I. & Genilloud, O. (2002).** New genus-specific primers for the PCR identification of novel isolates of the genera *Nocardiopsis* and *Saccharothrix*. *Int J Syst Evol Microbiol* **52**, 1411–1421.
- 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. (1981).** General characterization. In *Manual of Methods for General Microbiology*, pp. 409–443. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997).** Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- 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.
- Williams, S. T., Goodfellow, M. & Alderson, G. (1989).** Genus *Streptomyces* Waksman and Henrici 1943, 339^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2452–2492. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Xu, P., Li, W.-J., Tang, S.-K., Zhang, Y.-Q., Chen, G.-Z., Chen, H.-H., Xu, L.-H. & Jiang, C.-L. (2005).** *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family 'Oxalobacteraceae' isolated from China. *Int J Syst Evol Microbiol* **55**, 1149–1153.
- Yang, L.-L., Tang, S.-K., Zhang, Y.-Q., Zhi, X.-Y., Wang, D., Xu, L.-H. & Li, W.-J. (2005).** *Thermobifida halotolerans* sp. nov., isolated from a salt mine sample, and emended description of the genus *Thermobifida*. *Int J Syst Evol Microbiol* **58**, 1821–1825.
- Zhang, Z., Wang, Y. & Ruan, J. (1998).** Reclassification of *Thermomonospora* and *Microtetraspora*. *Int J Syst Bacteriol* **48**, 411–422.