

Five novel species of the genus *Nocardiopsis* isolated from hypersaline soils and emended description of *Nocardiopsis salina* Li *et al.* 2004

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Five novel *Nocardiopsis* strains isolated from hypersaline soils in China were subjected to a polyphasic analysis to determine their taxonomic position. All of the novel isolates could grow on agar plates at NaCl concentrations of up to 18% (w/v), with optimum growth at 5–8%. The DNA G+C contents of the novel strains ranged from 67.9 to 73.2 mol%. The morphological and chemotaxonomic characteristics of the isolates matched those described for members of the genus *Nocardiopsis*. Based on their 16S rRNA gene sequence analysis, DNA–DNA hybridization values and phenotypic characteristics, including the composition of cell-wall amino acids and sugars, menaquinones, polar lipids and cellular fatty acids, the isolates are proposed as representing five novel species of the genus *Nocardiopsis*. The novel species are proposed as *Nocardiopsis gilva* sp. nov. [type strain YIM 90087^T (=KCTC 19006^T=CCTCC AA 2040012^T=DSM 44841^T), *Nocardiopsis rosea* sp. nov. [type strain YIM 90094^T (=KCTC 19007^T=CCTCC AA 2040013^T=DSM 44842^T), *Nocardiopsis rhodophaea* sp. nov. [type strain YIM 90096^T (=KCTC 19049^T=CCTCC AA 2040014^T=DSM 44843^T), *Nocardiopsis chromatogenes* sp. nov. [type strain YIM 90109^T (=KCTC 19008^T=CCTCC AA 2040015^T=DSM 44844^T) and *Nocardiopsis baichengensis* sp. nov. [type strain YIM 90130^T (=KCTC 19009^T=CCTCC AA 2040016^T=DSM 44845^T). On the basis of the chemotaxonomic data, the description of the recently described species *Nocardiopsis salina* Li *et al.* 2004 is emended.

The genus *Nocardiopsis* was first described by Meyer (1976) and, at present, the genus comprises 19 species with validly published names, including several recently described species (Hozzein *et al.*, 2004; Li *et al.*, 2004; Sabry *et al.*, 2004). Members of the genus *Nocardiopsis* have been reported to predominate in saline or alkaline soils (Tang *et al.*, 2003) and several recognized species have been isolated from such

sources (Al-Tai & Ruan, 1994; Chun *et al.*, 2000; Al-Zarban *et al.*, 2002; Li *et al.*, 2003; Hozzein *et al.*, 2004; Li *et al.*, 2004).

During a taxonomic study of extremophilic actinomycetes, more than 200 *Nocardiopsis*-like strains were isolated from hypersaline soils in Xinjiang Province, China, and analysed on the basis of their morphology and chemotype. In this paper, we report the phenotypic and genotypic characteristics of five novel isolates and it is proposed that they represent five novel species of the genus *Nocardiopsis*.

Strains YIM 90087^T, YIM 90094^T, YIM 90096^T, YIM 90109^T and YIM 90130^T were isolated from saline soil samples by using modified International *Streptomyces* Project (ISP) ISP5 medium supplemented with 15% NaCl (w/v). These

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIM 90087^T, YIM 90094^T, YIM 90096^T, YIM 90109^T and YIM 90130^T are AY619712–AY619716, respectively.

Scanning electron micrographs of the five novel strains and tables detailing their polar lipid profiles, menaquinone patterns, fatty acid contents and DNA–DNA relatedness are available as supplementary material in IJSEM Online.

strains were maintained on ISP5 or potato agar slants containing 5% NaCl (w/v) at 4 °C and as 20% (w/v) glycerol suspensions at -20 °C. Biomass for chemical and molecular systematic studies was obtained by cultivation for 1 week in shake flasks (about 150 r.p.m.) using modified ISP5 medium (5% NaCl, w/v; pH 7.0) broth at 30 °C (for strain YIM 90087^T) or 37 °C (for strains YIM 90094^T, YIM 90096^T, YIM 90109^T and YIM 90130^T). Additionally, the recently described species *Nocardiopsis salina* YIM 90010^T (Li *et al.*, 2004) was re-examined using standard DSMZ chemotaxonomic methods.

Morphological characteristics of the five novel strains were observed by light microscopy (BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 21 days growth on potato agar medium containing 5% NaCl (w/v). Cultural characteristics were determined after 2–3 weeks by methods used in the ISP (Shirling & Gottlieb, 1966). All media were supplemented with 5% NaCl (w/v) and the colours of substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964) (see Table 1).

For all five novel strains, vegetative hyphae were well developed and fragmented. Spore chains were borne on aerial hyphae and spores were non-motile. For strains YIM 90087^T, YIM 90094^T and YIM 90096^T, aerial hyphae did not develop very well on most of the media tested and their spore chains were short. For strains YIM 90109^T and YIM 90130^T, aerial hyphae developed well on most media tested and their spore chains were long (see Supplementary Fig. S1a–j in IJSEM Online).

Media and procedures used for physiological and biochemical features and carbon source utilization were as described by Shirling & Gottlieb (1966) and Locci (1989). The results are indicated in Table 2 or in the species descriptions.

Amino acid and sugar analysis of cell walls was conducted according to the procedures described by Hasegawa *et al.* (1983). Polar lipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Minnikin *et al.*, 1984). Menaquinones were isolated according to Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). Cellular fatty acid analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI). All five novel strains, together with strain YIM 90010^T, contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell wall; no diagnostic sugars were observed. The phospholipids comprised phosphatidylmethylethanolamine (PME), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG), together with some unknown phosphoglycolipids (PGL) and unknown phospholipids (PL). Strains YIM 90096^T, YIM 90109^T and YIM 90130^T also contained phosphatidylinositolmannosides (PIM). Many differences were found between the six strains in their menaquinone and fatty acid

characteristics. Detailed results of the analyses are given in Supplementary Tables S1–S3 in IJSEM Online.

Extraction of genomic DNA and amplification of the 16S rRNA genes were performed as described by Cui *et al.* (2001) and Xu *et al.* (2003). Phylogenetic analyses were performed using the PHYLIP (Felsenstein, 1993) and MEGA version 2.1 (Kumar *et al.*, 2001) software packages after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances, with distance options according to the Kimura two-parameter model (Kimura, 1980, 1983), and clustering were performed using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein, 1985).

The method of Marmur (1961) was used to prepare genomic DNA of the five novel isolates for the analysis of base composition. The DNA G + C contents were determined using the thermal denaturation method of Marmur & Doty (1962). The DNA G + C contents of the genomic DNAs from strains YIM 90087^T, YIM 90094^T, YIM 90096^T, YIM 90109^T and YIM 90130^T were 68.1, 67.9, 69.0, 71.8 and 73.2 mol%, respectively.

16S rRNA gene sequences of the five novel isolates ranged between 1438 bp and 1490 bp. Preliminary comparison of the sequences against the GenBank database indicated that the five novel isolates were closely related to members of the genus *Nocardiopsis* and were most closely related to *Nocardiopsis halophila* and *Nocardiopsis composta*. Phylogenetic analyses showed that the five novel isolates fall into one distinct clade with the type strains of *N. halophila* DSM 44494^T and *N. composta* DSM 44551^T (Fig. 1). Furthermore, three of the novel isolates, YIM 90087^T, YIM 90094^T and YIM 90096^T, formed a distinct subclade. The two other novel isolates formed a distinct subclade with *N. halophila* DSM 44494^T. The five novel isolates showed low 16S rRNA gene sequence similarities (below 96%) with other recognized *Nocardiopsis* species, except for *N. composta* (96.4–97.5% similarity) and *N. halophila* (96.1–99.9%). The 16S rRNA gene sequence similarity between the five novel strains was between 95.9 and 98.7% (detailed information on gene sequence similarity values between the five novel isolates and their two closest neighbours is presented in Supplementary Table S4 in IJSEM Online). However, high degrees of 16S rRNA gene sequence similarity (97% and higher) have been demonstrated to be of limited value for differentiating species and DNA–DNA hybridization studies need to be performed to determine species affiliation (Stackebrandt & Goebel, 1994).

Accordingly, DNA–DNA hybridization studies were performed using the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992) between the five novel isolates and *N. halophila* DSM 44494^T and *N. composta* DSM 44551^T. DNA–DNA relatedness values ranged from 0 to 55.9% (tests were repeated twice and the mean values are shown in Supplementary Table S4 in IJSEM Online). All of

Table 1. Culture characteristics of the five novel strains

Strains: 1, *N. gilva* YIM 90087^T; 2, *N. rosea* YIM 90094^T; 3, *N. rhodophaea* YIM 90096^T; 4, *N. chromatogenes* YIM 90109^T; 5, *N. baichengensis* YIM 90130^T. All media were supplemented with 5% NaCl (w/v, pH 7.2). All strains showed good growth on oatmeal agar (ISP3), potato agar and nutrient agar media. ISP, International *Streptomyces* Project (Shirling & Gottlieb, 1966). Only strain YIM 90109^T produced diffusible yellowish pink pigment on Czapek agar and ISP5 media, etc. Colours are taken from ISCC–NBS colour charts (Kelly, 1964).

Medium	1	2	3	4	5
Czapek agar					
Growth	Good	Poor	Good	Good	Good
Aerial mycelium	Pale yellow	Pink–white	Pale pink	White	White
Substrate mycelium	Pale yellow	Pale pink	Light reddish brown	Moderate reddish brown	Light yellow
Glycerol-asparagine agar (ISP5)					
Growth	Poor	Poor	Good	Good	Good
Aerial mycelium	Soft yellow	Grey–pink	Light reddish orange	White	Yellow–white
Substrate mycelium	Moderate yellow	Moderate reddish pink	Soft reddish brown	Soft brown	Moderate orange–yellow
Inorganic salts–starch agar (ISP4)					
Growth	Good	Good	Good	Poor	Poor
Aerial mycelium	Yellow–white	Pink–white	Pale yellow–pink	White	White
Substrate mycelium	Pale greenish yellow	Pale pink	Grey–reddish brown	Light reddish brown	White
Yeast extract–malt extract (ISP2)					
Growth	Good	Good	Good	Good	Poor
Aerial mycelium	Pale yellow	Pale pink	Pale pink	White	Yellow–white
Substrate mycelium	Brilliant yellow	Moderate pink	Deep reddish brown	Deep reddish brown	Deep orange–yellow
Oatmeal agar (ISP3)					
Aerial mycelium	Yellow–white	Pale pink	Light reddish brown	White	Yellow–white
Substrate mycelium	Pale yellow	Moderate red	Grey–reddish orange	Brown	Deep yellow
Potato agar					
Aerial mycelium	Yellow–white	Moderate reddish brown	Pale pink	White	Yellow–white
Substrate mycelium	Soft yellow	Deep red	Deep reddish brown	Reddish brown	Deep orange–yellow
Nutrient agar					
Aerial mycelium	Pale yellow	Pale pink	Pale pink	White	White
Substrate mycelium	Pale yellow	Moderate red	Soft reddish brown	Light reddish brown	Pale yellow

Table 2. Phenotypic characteristics that differentiate the five novel isolates and the two most closely related *Nocardiopsis* species

Strains: 1, *N. gilva* YIM 90087^T; 2, *N. rosea* YIM 90094^T; 3, *N. rhodophaea* YIM 90096^T; 4, *N. chromatogenes* YIM 90109^T; 5, *N. baichengensis* YIM 90130^T; 6, *N. halophila* DSM 44494^T (data from Al-Tai & Ruan, 1994); 7, *N. composita* DSM 44551^T (data from Kämpfer *et al.*, 2002). +, Positive; -, negative; (+), weakly positive; NT, not tested; TBSA, tuberculostearic acid, 10-methyl C_{18:0}.

Characteristic	1	2	3	4	5	6	7
Spore chains	Short	Short	Short	Long	Long	Long	Long
Colony pigmentation (ISP5)	Pale yellow	Pale pink	Pale pink	White	White	White	White
Temperature range for growth (°C)	10–40	20–60	20–60	20–60	20–50	15–36	15–50
Optimum temperature for growth (°C)	28–30	37–40	37–40	37–40	37–40	30	37
NaCl range for growth (%)	0–18	0–18	0–18	0–18	0–18	3–20	0–15
Optimum NaCl concentration (%)	5–8	5–8	5–8	5–8	5–8	5–15	10
Starch hydrolysis	–	–	–	+	–	NT	NT
H ₂ S production	–	–	–	–	–	NT	NT
Nitrate reduction	+	–	–	–	–	NT	NT
Gelatin liquefaction	–	–	–	–	+	NT	NT
Melanin production	–	–	–	+	+	NT	–
Carbon source utilization:							
D-Fructose	+	+	–	+	+	NT	+
D-Galactose	+	–	–	+	+	+	+
<i>myo</i> -Inositol	+	–	+	+	–	+	+
D-Maltose	–	+	–	+	+	+	–
D-Mannose	–	–	–	+	+	NT	+
L-Rhamnose	–	+	–	+	+	(+)	–
D-Ribose	–	+	+	+	+	NT	+
D-Sucrose	+	+	–	+	+	+	–
D-Xylose	+	–	–	+	–	+	–
Chemical characteristic:							
Polar lipids	PIII*; DPG, PC, PI, PG, PME, PE, PL, GL	PIII; DPG, PC, PI, PG, PME, PE, PL, GL	PIII; DPG, PC, PI, PG, PME, PE, PL, GL, PIM	PIII; DPG, PC, PI, PG, PME, PE, PL, GL, PIM	PIII; DPG, PC, PI, PG, PME, PE, PL, GL, PIM	PIII	PIII; PME, PC, DPG, PG, PL1, PL2
Major menaquinones	MK-11(H ₄), MK-11(H ₆), MK-11(H ₈)	MK-11, MK-11(H ₂), MK-11(H ₄)	MK-11(H ₆), MK-11(H ₈)	MK-10, MK-10(H ₂), MK-10(H ₄)	MK-9(H ₄), MK-10(H ₂), MK-10(H ₄), MK-10(H ₆)	MK-10(H ₆); MK-10(H ₈)	MK-10(H ₈), MK-11(H ₈), MK-10(H ₆), MK-12
Major cellular fatty acid (> 10%)	iso G C _{16:1} (21·69%), TBSA (36·51%) (30 °C)	iso-C _{16:0} (30·7%), anteiso-C _{17:0} (10·61%), TBSA (21·87%) (37 °C)	iso-C _{16:0} (30·77%), anteiso-C _{17:0} (11·21%), TBSA (12·17%) (37 °C)	iso-C _{16:0} (26·02%), anteiso-C _{17:0} (29·38%), TBSA (37 °C)	iso-C _{16:0} (24·17%), anteiso-C _{17:0} (13·64%), TBSA (33·19%) (37 °C)	NT	iso-C _{16:0} (16·0%), anteiso-C _{15:0} (18·9%), anteiso-C _{17:0} (12·8%) (28 °C)
DNA G+C content (mol%)	68·1	67·9	69·0	71·8	73·2	71·5	74·7

PIII refers to the phospholipid pattern as described by Lechevalier *et al.* (1977).

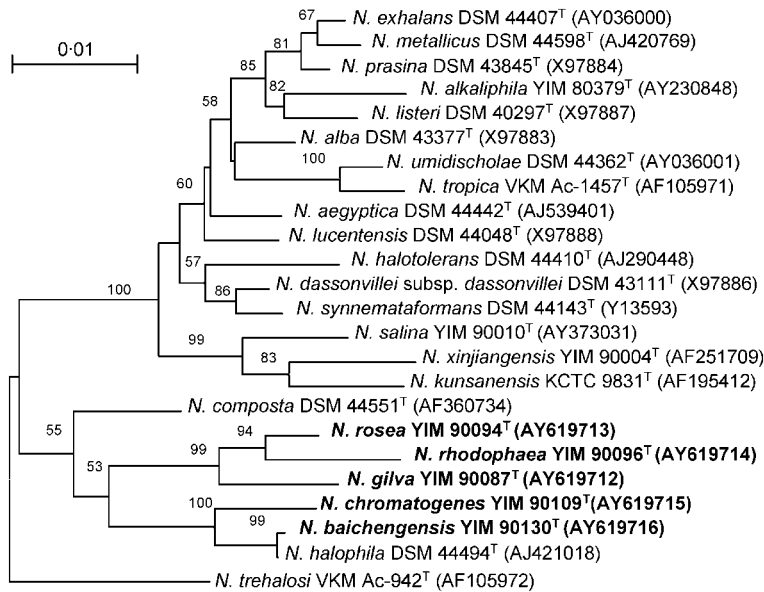


Fig. 1. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences showing the position of strains YIM 90087^T, YIM 90094^T, YIM 90096^T, YIM 90109^T and YIM 90130^T amongst their phylogenetic neighbours. Numbers at branch nodes are bootstrap values (1000 resamplings). The gene sequence of *Actinomadura madurae* DSM 43067^T (X97889) was used as the root (not shown). Bar, 1% sequence divergence.

the DNA–DNA relatedness values were lower than 70%, which is the recommended threshold value for the delineation of genomic species (Wayne *et al.*, 1987). On the basis of these results, the novel isolates represent five novel species of the genus *Nocardiopsis*.

Additionally, the five novel isolates could be distinguished from each other and from the two closely related *Nocardiopsis* species *N. halophila* and *N. composta* on the basis of physiological and biochemical characteristics and on chemotaxonomic data (Table 2).

Based on the results of phenotypic and genotypic analyses, we consider that the novel isolates represent five novel species of the genus *Nocardiopsis*, for which we propose the names *Nocardiopsis gilva* sp. nov., *Nocardiopsis rosea* sp. nov., *Nocardiopsis rhodophaea* sp. nov., *Nocardiopsis chromatogenes* sp. nov. and *Nocardiopsis baichengensis* sp. nov.

Emended description of *Nocardiopsis salina* Li *et al.* 2004

The description is the same as that given by Li *et al.* (2004) except for polar lipid and menaquinone compositions. In addition to PI and PG, the type strain also contains PC, DPG, phosphatidylethanolamine (PE), PME and four small phospholipid spots above DPG of unknown structure which are diagnostic for *Nocardiopsis* strains. The menaquinone pattern is mainly composed of MK-9(H₈) and MK-10(H₈) and smaller amounts of MK-9(H₄), MK-9(H₆), MK-10(H₄), MK-10(H₆) and MK-11(H₄).

Description of *Nocardiopsis gilva* sp. nov.

Nocardiopsis gilva (gil'va. L. fem. adj. *gilva* pale yellow).

Aerobic, Gram-positive, non-acid-fast, non-motile organism. Aerial mycelium is pale yellow to yellow–white and the substrate mycelium is pale yellow to pale greenish-yellow on media tested. No diffusible pigments are produced. Vegetative hyphae are well developed and fragmented. Spiral spore chains are short and are borne on the aerial hyphae. Spores are smooth surfaced and non-motile. Optimal growth at 28–30 °C and at pH 7.2 with 5–8% NaCl. Temperature, pH and NaCl tolerance ranges are 10–40 °C, pH 6.0–9.0 and 0–18% (w/v), respectively. L-Arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, D-lactose, D-mannitol, D-raffinose, sodium acetate, sodium citrate, D-sorbitol, starch, sucrose and D-xylose can be utilized as carbon sources, while D-maltose, D-mannose, L-rhamnose, D-ribose and D-xylitol can not be utilized. Alanine, arginine, asparagine, glycine, histidine, lysine, proline and threonine can be used as sole nitrogen sources, but adenine, cystine, glutamic acid, hydroxyproline, methionine, phenylalanine, tryptophan and valine can not be utilized. Milk coagulation, milk peptonization, gelatin liquefaction, starch hydrolysis, H₂S production, urease activity and melanin production are negative, but nitrate reduction is positive. Cell walls contain *meso*-diaminopimelic acid as the diagnostic diamino acid and have no diagnostic sugar. The polar lipid pattern is composed of PME, PC, PI, PG and DPG together with some unknown PGL and unknown PL. Major menaquinones are MK-11(H₄), MK-11(H₆) and MK-11(H₈). The cellular fatty acid profile contains iso-C_{16:1} (21.7%), C_{18:0} 10-methyl (36.5%), iso-C_{14:0} (0.7%), iso-C_{15:0} (1.1%), anteiso-C_{15:0} (2.1%), iso-C_{16:0} (6.6%), C_{16:0} (1.3%), C_{16:0} 10-methyl (3.7%), anteiso-C_{17:1} (8.5%), iso-C_{17:0} (1.8%), anteiso-C_{17:0} (3.6%), C_{17:0} 10-methyl (3.9%), iso-C_{18:0} (4.2%), C_{18:1}ω9c (1.5%), anteiso-C_{19:0} (0.6%) and C_{16:1}ω7c (2.0%). DNA G + C content is 68.1 mol%.

The type strain, YIM 90087^T (=KCTC 19006^T=CCTCC AA 2040012^T=DSM 44841^T), was isolated from a saline soil sample in the west of China.

Description of *Nocardiopsis rosea* sp. nov.

Nocardiopsis rosea (ro'se.a. L. fem. adj. *rosea* rose coloured).

Aerobic, Gram-positive, non-acid-fast, non-motile organism. Aerial mycelium is pink–white to pale pink and the substrate mycelium is pale pink to moderate red on media tested. No diffusible pigments are produced. Vegetative hyphae are well developed and fragmented. Spore chains are borne on the aerial hyphae. Spores are smooth surfaced and non-motile. Optimum growth at 37–40 °C and at pH 7.2 with 5–8% NaCl. Temperature, pH and NaCl tolerance ranges are 20–60 °C, pH 6.0–9.0 and 0–18% (w/v), respectively. L-Arabinose, D-fructose, D-glucose, D-lactose, D-maltose, L-rhamnose, D-ribose, sodium acetate, sucrose and starch can be utilized as carbon sources, but D-cellobiose, D-galactose, glycerol, *myo*-inositol, D-mannitol, D-mannose, D-raffinose, sodium citrate, D-sorbitol, D-xylitol and D-xylose can not be utilized. Alanine, arginine, asparagine, glycine, histidine and proline can be used as sole nitrogen sources, but adenine, cystine, glutamic acid, hydroxyproline, lysine, methionine, phenylalanine, threonine, tryptophan and valine can not be utilized. Milk coagulation, milk peptonization, gelatin liquefaction, starch hydrolysis, H₂S production, urease activity and melanin production are negative; nitrate reduction is positive. Cell walls contain *meso*-diaminopimelic acid as the diagnostic diamino acid and have no diagnostic sugar. The polar lipid pattern is composed of PME, PC, PI, PG and DPG together with some unknown PGL and unknown PL. Major menaquinones are MK-11, MK-11(H₂) and MK-11(H₄). The cellular fatty acid profile contains iso-C_{16:0} (30.7%), anteiso-C_{17:0} (10.6%), C_{18:0} 10-methyl (21.9%), iso-C_{10:0} (1.7%), C_{10:0} (0.2%), anteiso-C_{11:0} (0.2%), iso-C_{14:0} (1.4%), iso-C_{15:0} (0.6%), anteiso-C_{15:0} (2.2%), iso-C_{16:1} (1.3%), C_{16:0} (1.7%), C_{16:0} 10-methyl (1.6%), anteiso-A-C_{17:1} (0.9%), iso-C_{17:0} (2.7%), C_{17:0} (1.2%), C_{17:0} 10-methyl (3.9%), iso-C_{18:0} (6.7%), C_{18:1}ω9c (0.7%), C_{18:0} (8.7%), anteiso-C_{19:0} (0.5%), C_{19:0} (0.5%) and C_{16:1}ω7c (0.3%). DNA G+C content is 67.9 mol%.

The type strain, YIM 90094^T (=KCTC 19007^T=CCTCC AA 2040013^T=DSM 44842^T), was isolated from a saline soil sample in the west of China.

Description of *Nocardiopsis rhodophaea* sp. nov.

Nocardiopsis rhodophaea [rho.do.phae'a. Gr. n. *rhodos* the rose; Gr. adj. *phaeos* brown; N.L. fem. adj. *rhodophaea* rose-brown (after the colour of the substrate mycelium)].

Aerobic, Gram-positive, non-acid-fast, non-motile organism. Aerial mycelium is pale pink to light reddish brown and the substrate mycelium is light reddish brown to deep reddish brown on media tested. No diffusible pigments are

produced. Vegetative hyphae are well developed and fragmented. Short spore chains are borne on the aerial hyphae. Spores are smooth surfaced and non-motile. Optimum growth at 37–40 °C and at pH 7.2 with 5–8% NaCl. Temperature, pH and NaCl tolerance ranges are 20–60 °C, pH 6.0–9.0 and 0–18% (w/v), respectively. L-Arabinose, D-glucose, glycerol, *myo*-inositol, sodium acetate and D-ribose can be utilized as carbon sources, but D-cellobiose, D-fructose, D-galactose, D-lactose, D-maltose, D-mannose, D-mannitol, D-raffinose, L-rhamnose, sodium citrate, D-sorbitol, starch, sucrose, D-xylitol and D-xylose can not be utilized. Alanine, arginine, asparagine, glycine, histidine, proline and valine are used as sole nitrogen sources, while adenine, cystine, glutamic acid, hydroxyproline, lysine, methionine, phenylalanine, threonine and tryptophan are not be utilized. Milk coagulation, gelatin liquefaction, starch hydrolysis, H₂S production, urease activity, nitrate reduction and melanin production are negative, but milk peptonization is positive. Cell walls contain *meso*-diaminopimelic acid as the diagnostic diamino acid and have no diagnostic sugars. The polar lipid pattern is composed of PME, PC, PI, PG, DPG and PIM, together with some unknown PGL and unknown PL. Major menaquinones are MK-11(H₆) and MK-11(H₈). The cellular fatty acid profile contains iso-C_{16:0} (30.8%), anteiso-C_{17:0} (11.2%), C_{18:0} 10-methyl (12.2%), iso-C_{14:0} (1.6%), iso-C_{15:0} (2.4%), anteiso-C_{15:0} (5.8%), iso-C_{16:1} (1.3%), C_{16:0} (2.0%), C_{16:0} 10-methyl (1.3%), iso-C_{17:0} (4.1%), C_{17:1}ω8c (0.9%), C_{17:0} (1.1%), C_{17:0} 10-methyl (8.9%), iso-C_{18:0} (4.8%), C_{18:1}ω9c (3.4%), C_{18:0} (7.1%) and C_{16:1}ω7c (1.2%). DNA G+C content is 69.0 mol%.

The type strain, YIM 90096^T (=KCTC 19049^T=CCTCC AA 2040014^T=DSM 44843^T), was isolated from a saline soil sample in the west of China.

Description of *Nocardiopsis chromatogenes* sp. nov.

Nocardiopsis chromatogenes (chro'ma.to.gen.es. Gr. n. *chroma* -atos colour; Gr. v. *gennaio* to produce; N.L. part. adj. *chromatogenes* producing colour).

Aerobic, Gram-positive, non-acid-fast, non-motile organism. Aerial mycelium is white and the substrate mycelium is light reddish brown to deep reddish brown on most media tested. Yellowish pink pigments are produced on Czapek agar and inorganic salts–starch agar (ISP4) media. Vegetative hyphae are well developed and fragmented. Long spore chains are borne on the aerial hyphae. Spores are smooth surfaced and non-motile. Optimum growth at 37–40 °C and at pH 7.2 with 5–8% NaCl. Temperature, pH and NaCl tolerance ranges are 20–60 °C, pH 6.0–9.0 and 0–18% (w/v), respectively. L-Arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, D-lactose, D-maltose, D-mannitol, D-mannose, D-raffinose, L-rhamnose, D-ribose, sodium acetate, sodium citrate, sucrose and D-xylose can be utilized as carbon sources, but D-sorbitol, starch and D-xylitol can not be utilized. Alanine, arginine,

asparagine, glycine, histidine, lysine, phenylalanine, proline, threonine, tryptophan and valine can be used as sole nitrogen sources, but adenine, cystine, glutamic acid, hydroxyproline and methionine can not be utilized. Milk coagulation, milk peptonization, gelatin liquefaction, nitrate reduction, H₂S production and urease activity are negative, but starch hydrolysis and melanin production are positive. Cell walls contain *meso*-diaminopimelic acid as the diagnostic diamino acid and have no diagnostic sugars. The polar lipid pattern is composed of PME, PC, PI, PG, DPG and PIM, together with some unknown PGL and unknown PL. Major menaquinones are MK-10, MK-10(H₂) and MK-10(H₄). The cellular fatty acid profile contains iso-C_{16:0} (26.0%), anteiso-C_{17:0} (10.1%), C_{18:0} 10-methyl (29.4%), iso-C_{10:0} (1.3%), C_{10:0} (1.0%), anteiso-C_{11:0} (0.7%), iso-C_{14:0} (3.0%), iso-C_{15:0} (0.8%), anteiso-C_{15:0} (7.3%), i-C_{16:1} (2.2%), C_{16:0} (2.8%), C_{16:0} 10-methyl (1.7%), anteiso-A-C_{17:1} (1.5%), C_{17:0} 10-methyl (2.2%), iso-C_{18:0} (1.9%), C_{18:0} (5.6%) and C_{16:1}ω7c (1.0%). DNA G + C content is 71.8 mol%.

The type strain, YIM 90109^T (= KCTC 19008^T = CCTCC AA 2040015^T = DSM 44844^T), was isolated from a saline soil sample in the west of China.

Description of *Nocardiopsis baichengensis* sp. nov.

Nocardiopsis baichengensis (bai.cheng.en'sis. N.L. fem. adj. *baichengensis* pertaining to Baicheng, a county of Xinjiang Province in the west of China where the type strain was collected).

Aerobic, Gram-positive, non-acid-fast, non-motile organism. Aerial mycelium is white to yellow–white and the substrate mycelium is light yellow to deep orange–yellow on the media tested. No diffusible pigments are produced. Vegetative hyphae are well developed and fragmented. Long spore chains are borne on the aerial hyphae. Spores are smooth surfaced and non-motile. Optimum growth at 37–40 °C and at pH 7.2 with 5–8% NaCl. Temperature, pH and NaCl tolerance ranges are 20–50 °C, pH 6.0–9.0 and 0–18% (w/v), respectively. L-Arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, D-maltose, D-mannitol, D-mannose, L-rhamnose, D-ribose, sodium acetate, sodium citrate and sucrose can be utilized as carbon sources, but *myo*-inositol, D-lactose, D-raffinose, D-sorbitol, starch, D-xylitol and D-xylose can not be utilized. Alanine, arginine, asparagine, glycine, histidine, lysine, phenylalanine, proline, threonine, tryptophan and valine can be used as sole nitrogen sources, but adenine, cystine, glutamic acid, hydroxyproline and methionine can not be utilized. Milk coagulation, milk peptonization, starch hydrolysis, H₂S production, nitrate reduction and urease activity are negative, but gelatin liquefaction and melanin production are positive. Cell walls contain *meso*-diaminopimelic acid as the diagnostic diamino acid and have no diagnostic sugars. The polar lipid pattern is composed of PME, PC, PI, PG, DPG and PIM, together with some unknown PGL and

unknown PL. Major menaquinones are MK-10(H₂), MK-10(H₄) and MK-10(H₆). The cellular fatty acid profile contains iso-C_{16:0} (24.2%), anteiso-C_{17:0} (13.6%), C_{18:0} 10-methyl (33.2%), iso-C_{14:0} (1.0%), C_{14:0} (0.3%), iso-C_{15:0} (0.5%), anteiso-C_{15:0} (3.8%), i-C_{16:1} (1.7%), C_{16:0} (2.8%), C_{16:0} 10-methyl (1.7%), anteiso-A-C_{17:1} (1.9%), iso-C_{17:0} (1.1%), C_{17:0} (0.3%), C_{17:0} 10-methyl (1.7%), iso-C_{18:0} (3.5%), C_{18:1}ω9c (0.5%), C_{18:0} (6.1%), anteiso-C_{19:0} (0.5%) and C_{16:1}ω7c (1.5%). DNA G + C content is 73.2 mol%.

The type strain, YIM 90130^T (= KCTC 19009^T = CCTCC AA 2040016^T = DSM 44845^T), was isolated from a saline soil sample in the west of China.

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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.
- Chun, J., Bae, K.-S., Moon, E.-Y., Jung, S.-O., Lee, H.-K. & Kim, S.-J. (2000). *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete isolated from a saltern. *Int J Syst Evol Microbiol* **50**, 1909–1913.
- Cui, X. L., Mao, P. H., Zeng, M., Li, W. J., Zhang, L. P., Xu, L. H. & Jiang, C. L. (2001). *Streptimonospora salina* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae*. *Int J Syst Evol Microbiol* **51**, 357–363.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- Hozzein, W. N., Li, W.-J., Ali, M. I. A., Hammouda, O., Mousa, A. S., Xu, L.-H. & Jiang, C.-L. (2004). *Nocardiopsis alkaliphila* sp. nov., a novel alkaliphilic actinomycete isolated from desert soil in Egypt. *Int J Syst Evol Microbiol* **54**, 247–252.

- 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.
- Kämpfer, P., Busse, H.-J. & Rainey, F. A. (2002).** *Nocardiopsis compostus* sp. nov., from the atmosphere of a composting facility. *Int J Syst Evol Microbiol* **52**, 621–627.
- Kelly, K. L. (1964).** *Inter-Society Color Council–National Bureau of Standards Color Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office.
- Kimura, M. (1980).** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kimura, M. (1983).** *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Kroppenstedt, R. M. (1982).** Separation of bacterial menaquinones by HPLC using reverse phase (RP 18) and a silver loaded ion exchanger as stationary phases. *J Liquid Chromatogr* **5**, 2359–2387.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001).** MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.
- Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977).** Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.
- Li, M.-G., Li, W.-J., Xu, P., Cui, X.-L., Xu, L.-H. & Jiang, C.-L. (2003).** *Nocardiopsis xinjiangensis* sp. nov., a halophilic actinomycete isolated from saline soil sample in China. *Int J Syst Evol Microbiol* **53**, 317–321.
- 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.
- Locci, R. (1989).** Streptomycetes and related genera. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2451–2508. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Marmur, J. (1961).** A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962).** Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- 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 isoprenoid quinines and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Sabry, S. A., Ghanem, N. B., Abu-Ella, G. A., Schumann, P., Stackebrandt, E. & Kroppenstedt, R. M. (2004).** *Nocardiopsis aegyptia* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* **54**, 453–456.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC News* **20**, 16.
- Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- 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.
- Tang, S.-K., Li, W.-J., Wang, D., Zhang, Y.-G., Xu, L.-H. & Jiang, C.-L. (2003).** Studies of the biological characteristics of some halophilic and halotolerant actinomycetes isolated from saline and alkaline soils. *Actinomycetologica* **17**, 6–10.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 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.
- Xu, P., Li, W.-J., Xu, L.-H. & Jiang, C.-L. (2003).** A microwave based method for genomic DNA extraction from Actinomycetes. *Microbiology (Beijing)* **30**, 82–84 (in Chinese).