Nocardiopsis salina sp. nov., a novel halophilic actinomycete isolated from saline soil in China

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A moderately halophilic actinomycete strain, designated YIM 90010^T, was isolated from a soil sample collected from a hypersaline habitat in Xinjiang Province, China, and then investigated using a polyphasic taxonomic approach. The strain produced abundant aerial mycelia and fragmented substrate mycelia on most media tested; the optimum NaCl concentration for growth was 10 % (w/v) and the optimum growth temperature and pH were 28 °C and 7·2, respectively. Chemotaxonomically and phylogenetically, the strain was related to members of the genus *Nocardiopsis*. The isolate contained chemotaxonomic markers that were diagnostic for the genus *Nocardiopsis*, i.e. *meso*-diaminopimelic acid, no diagnostic sugars, and MK-10(H_6), MK-10(H_8) and MK-12 as the predominant menaquinones. The major fatty acids were iso- and anteiso-branched acids combined with tuberculostearic acid (Me $C_{18:0}$), straight-chain saturated fatty acids and unsaturated fatty acids. The G+C content was 73·1 mol%. Phylogenetic analysis confirmed that strain YIM 90010^T was a member of the genus *Nocardiopsis* and most closely related to *Nocardiopsis kunsanensis* (97·6 % similarity) and *Nocardiopsis xinjiangensis* (98·1 % similarity). It can be differentiated from these species by using phenotypic characteristics, phylogenetic analysis and DNA–DNA hybridization results. On the basis of the polyphasic evidence, a novel species, *Nocardiopsis salina* sp. nov., is proposed. The type strain of the species is YIM 90010^T (=KCTC 19003^T=CCTCC AA 204009^T).

The genus *Nocardiopsis* was first described by Meyer (1976) and currently comprises 18 species with validly published names (Meyer, 1976; Grund & Kroppenstedt, 1990; Yassin *et al.*, 1993, 1997; Al-Tai & Ruan, 1994; Evtushenko *et al.*, 2000; Chun *et al.*, 2000; Peltola *et al.*, 2001; Al-Zarban *et al.*, 2002; Kämpfer *et al.*, 2002; Schippers *et al.*, 2002; M. G. Li *et al.*, 2003; Hozzein *et al.*, 2004; Sabry *et al.*, 2004). During our taxonomic study on extremophilic actinomycetes, we used phenotypic and genotypic approaches to facilitate the characterization of one moderately halophilic actinomycete, strain YIM 90010^T.

Strain YIM 90010^T was isolated from a saline soil sample by using modified International *Streptomyces* Project (ISP) 5 medium supplemented with 20 % (w/v) NaCl. The soil sample was collected from the same source as described previously (Cui *et al.*, 2001; M. G. Li *et al.*, 2003; W. J. Li *et al.*, 2003a, b, c). The strain was maintained on ISP 2 and ISP 5 slants containing 10 % (w/v) NaCl at 4 °C and as 20 % (w/v) glycerol suspensions at −20 °C. Biomass for chemical and molecular studies was obtained by cultivation in shake flasks (about 150 r.p.m.) using modified ISP 5 medium [10 % (w/v) NaCl, pH 7·0] broth at 28 °C for 1 week.

The morphological characteristics of strain YIM 90010^T were observed by using light microscopy (model BH 2; Olympus) and scanning electron microscopy with a JEOL model JSM5600LV after 14 days growth on ISP 5 medium containing 10 % (w/v) NaCl. Cultural characteristics were determined after 4 weeks at 28 °C by using the methods adopted in the ISP (Shirling & Gottlieb, 1966). All media were supplemented with 10 % (w/v) NaCl, and the colours of both substrate and aerial mycelia and the production of soluble pigments were determined by comparison with chips from the ISCC–NBS colour charts (Kelly, 1964). The detailed results are shown in Table 1.

Strain YIM 90010^T was found to be Gram-positive. Its vegetative hyphae were long, well-developed and fragmented.

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 90010^T is AY373031.

Table 1. Cultural characteristics of strain YIM 90010^T

All media were supplemented with 10 % (w/v) NaCl (pH 7·2); ISP (Shirling & Gottlieb, 1966). Colours were taken from ISCC–NBS colour charts (Kelly, 1964).

Medium	Growth	Aerial mycelium	Substrate mycelium
Yeast extract/malt extract (ISP 2)	Good	White	Moderate orange–yellow
Oatmeal agar (ISP 3)	Poor	White	Pale yellow
Inorganic salts/starch agar (ISP 4)	–	–	–
Glycerol/asparagine agar (ISP 5)	Good	White	Pale yellow
Czapek agar	Abundant	White	Yellow–white
Potato agar	Good	White	Soft yellow–brown
Nutrient agar	Poor	White	Light orange–yellow

Long spore chains were borne on the aerial hyphae. Spores (dimensions 0·4–0·6 × 0·8–1·2 μm) were rod-shaped, smooth and non-motile (Fig. 1).

The media and procedures used for determining physiological and biochemical features and carbon-source utilization were as described by Shirling & Gottlieb (1966). The results are given in Table 2 or in the species description.

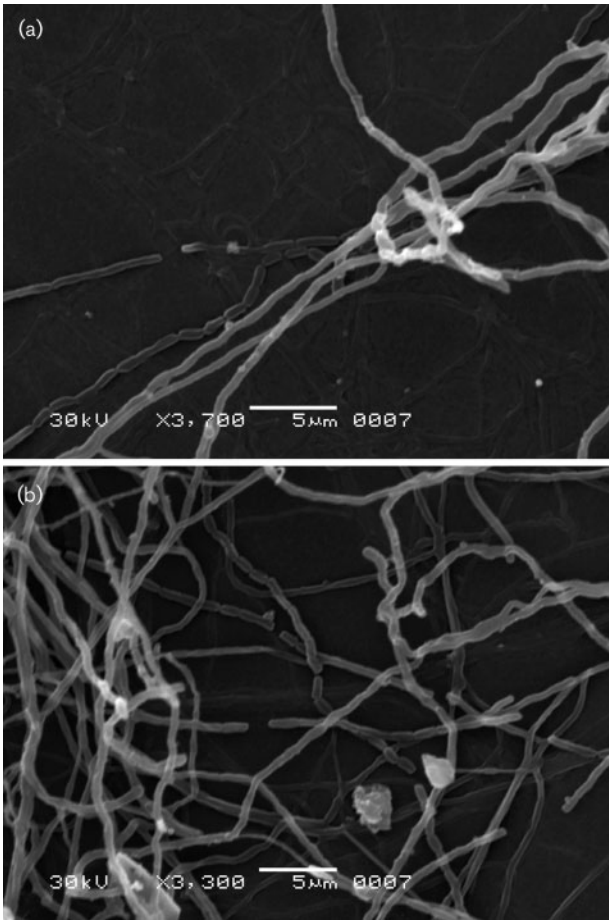

Fig. 1. Scanning electron micrographs of YIM 90010^T cultivated on modified ISP 5 medium (10 %, w/v, NaCl) for 14 days at 28 °C.

Analyses of the amino acids and sugars of the cell walls were performed as described by Stanek & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1984). Menaquinones were isolated using the methods of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). The cellular fatty acid composition was determined as described by Sasser (1990), using the Microbial Identification System (MIDI). The cell walls of strain YIM 90010^T contained *meso*-diaminopimelic acid and no diagnostic sugars. The phospholipids contained phosphatidylglycerol and phosphatidylinositol. The predominant menaquinones were MK-10(H_6), MK-10(H_8) and MK-12. The major cellular fatty acids were i-$C_{16:0}$ (37·80 %), 10Me $C_{18:0}$ (15·73 %) and $C_{18:1}\omega 9c$ (9·04 %).

Extraction of genomic DNA and amplification of the 16S rRNA gene were done as described by Cui *et al.* (2001). Phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein, 1993) and MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) (Kimura, 1980, 1983) and clustering were determined 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 genomic DNA of strain YIM 90010^T for the determination of G+C content was prepared according to the method of Marmur (1961). The G+C content was determined using the thermal denaturation method of Marmur & Doty (1962) and produced a value of 73·1 mol%.

The almost-complete 16S rRNA gene sequence (1449 bp) of strain YIM 90010^T was determined. Phylogenetic analyses based on a dataset consisting of 1430 unambiguous nucleotides between positions 53 and 1482 (*Escherichia coli* positions; Brosius *et al.*, 1978) showed that the novel

Table 2. Characteristics useful for differentiating between strain YIM 90010^T and closely related *Nocardiopsis* species

Abbreviations: PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; DPG, diphosphatidylglycerol. All three strains shared the following characteristics: negative results for arabinose, *meso*-inositol, mannitol, rhamnose, trehalose, xylitol, xylose and maltose; positive results for fructose, sodium citrate and L-alanine; growth on media with NaCl concentrations between 3 and 20 % (optimum 10 %); H_2S not produced; *meso*-diaminopimelic acid as the cell-wall peptidoglycan. +, Positive; −, negative; w, weak growth or reaction.

Characteristic	*N. salina* YIM 90010^T	*N. xinjiangensis* YIM 90004^T*	*N. kunsanensis* KCTC 9831^T*
Nitrate reduction	+	−	−
Gelatin liquefaction	w	w	−
Utilization as sole carbon source			
Glucose	−	−	+
Cellobiose	−	+	+
Ribose	+	−	−
Galactose	−	−	+
Melibiose	−	−	+
Raffinose	+	−	−
Sodium acetate	+	−	+
Sucrose	+	−	+
Utilization as sole nitrogen source			
Proline	+	+	−
Serine	+	+	−
Growth temperature range (°C) (optimum)	20–40 (28)	20–40 (28)	20–50 (37)
Growth pH range (optimum)	6·0–9·0 (7·2)	6·0–10·0 (7·2)	7·0–11·0 (9·0)
Diagnostic sugar(s)	None	Xyl, Ara, Gal	None
Phospholipids	PI, PG	PI, PG	PC, PG, DPG
Major menaquinone(s)	MK-10(H_6), MK-10(H_8), MK-12	MK-10(H_2), MK-10(H_4)	MK-10(H_8)
Major fatty acids	i-$C_{16:0}$, $C_{18:1}\omega9c$, Me $C_{18:0}$	i-$C_{14:0}$, ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{16:0}$, $C_{18:0}$, Me $C_{18:0}$	i-$C_{15:0}$, ai-$C_{16:0}$, Me $C_{18:0}$
G+C content (mol%)	73·1	74·3	71

*Some data were taken from Chun *et al.* (2000) and M. G. Li *et al.* (2003).

isolate falls into a distinct clade with two other recognized *Nocardiopsis* species, *Nocardiopsis kunsanensis* (KCTC 9831^T) and *Nocardiopsis xinjiangensis* (YIM 90004^T). A phylogenetic tree based on the 16S rRNA gene sequences of strain YIM 90010^T, the two aforementioned *Nocardiopsis* species and other related species is shown in Fig. 2. The 16S rRNA gene sequence of strain YIM 90010^T exhibited 97·6 % similarity with that of *N. kunsanensis* KCTC 9831^T and 98·1 % with that of *N. xinjiangensis* YIM 90004^T.

There are three recognized halophilic species of the genus *Nocardiopsis*: *N. kunsanensis*, *N. xinjiangensis* and *Nocardiopsis halophila*. However, the level of 16S rRNA gene sequence similarity between strain YIM 90010^T and *N. halophila* DSM 44494^T was low (below 96 %). In addition to this low level of similarity, the two strains lie in different clades within the phylogenetic tree based on the 16S rRNA gene sequences of all recognized *Nocardiopsis* species (Fig. 2).

Accordingly, comparative taxonomic studies were performed with strain YIM 90010^T, *N. kunsanensis* KCTC 9831^T and *N. xinjiangensis* YIM 90004^T to determine whether strain YIM 90010^T could be considered as a novel species of the genus *Nocardiopsis* or should be assigned to one of the two species.

Strain YIM 90010^T differed greatly from the two species in terms of some of its physiological and biochemical characteristics and some chemotaxonomic data (Table 2). DNA–DNA relatedness tests were performed with strain YIM 90010^T, *N. kunsanensis* KCTC 9831^T and *N. xinjiangensis* YIM 90004^T, using the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992). DNA–DNA reassociation similarity values between strain YIM 90010^T and *N. kunsanensis* KCTC 9831^T, and strain YIM 90010^T and *N. xinjiangensis* YIM 90004^T were 38·6 and 45·5 %, respectively, while the value for *N. kunsanensis* KCTC 9831^T and *N. xinjiangensis* YIM 90004^T was 22·4 % (repeated twice). DNA–DNA relatedness provided decisive evidence that the novel isolate, YIM 90010^T, and the other two related type strains, *N. kunsanensis* KCTC 9831^T and *N. xinjiangensis* YIM 90004^T, are members of different genomic species (Wayne *et al.*, 1987).

Therefore, on the basis of the above-mentioned phenotypic

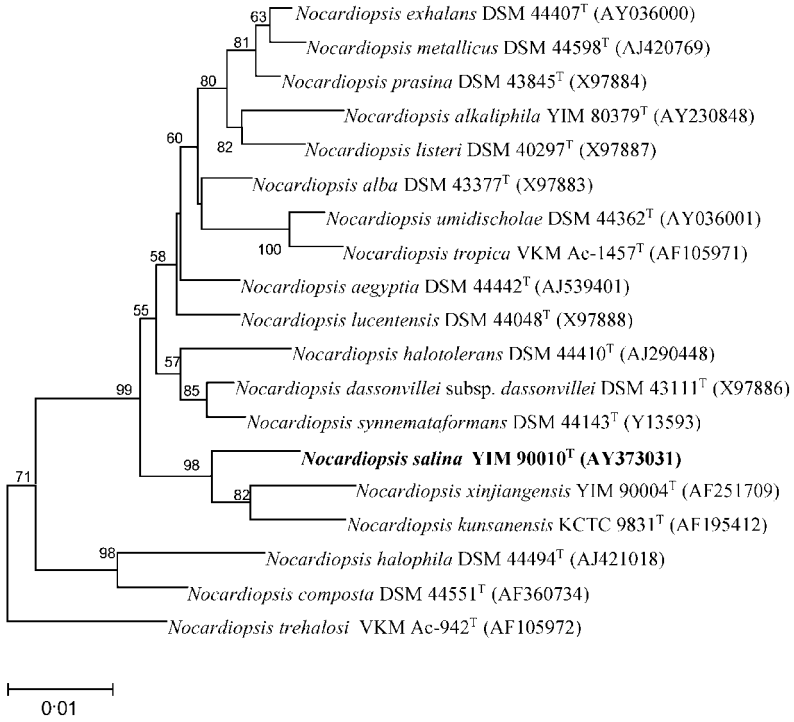

Fig. 2. Phylogenetic dendrogram, based on 16S rRNA gene sequence analysis, constructed using the neighbour-joining method, showing the phylogenetic position of strain YIM 90010^T within the genus *Nocardiopsis*. The sequence of *Actinomadura madurae* DSM 43067^T (GenBank accession no. X97889) was used as the outgroup (not shown). Bar, inferred nucleotide substitution per 100 nucleotides.

and genotypic results, we consider that strain YIM 90010^T represents a novel species of the genus *Nocardiopsis*, for which we propose the name *Nocardiopsis salina* sp. nov.

Description of *Nocardiopsis salina* sp. nov.

Nocardiopsis salina (sa.li′na. N.L. fem. adj. *salina* salty, saline).

Cells are aerobic, Gram-positive, non-acid-fast and non-motile. The colour of the aerial mycelium is white on most media tested and the substrate mycelium is pale yellow to light orange–yellow or yellow–white. No diffusible pigments are produced. The vegetative hyphae are long, well-developed and fragmented. Long or short spore chains are borne on the aerial hyphae. Spores (dimensions 0·4–0·6 × 0·8–1·2 μm) are rod-shaped, smooth and non-motile. Cell walls contain *meso*-diaminopimelic acid and have no diagnostic sugars. Polar lipids are phosphatidylglycerol and phosphatidylinositol. Major menaquinones are MK-10(H_6), MK-10(H_8) and MK-12. Major cellular fatty acids are i-$C_{16:0}$ (37·80 %), $C_{18:1\omega9c}$ (9·04 %) and 10Me $C_{18:0}$ (15·73 %). Ribose, sucrose, fructose, raffinose, sodium citrate and sodium acetate are utilized as carbon sources, while arabinose, glucose, cellobiose, galactose, inositol, mannitol, melibiose, rhamnose, trehalose, xylitol, xylose and maltose are not. Almost all nitrogen sources tested, such as asparagine, phenylalanine, serine, histidine, methionine, valine, threonine, arginine, adenine, hypoxanthine, glycine, proline and hydroxyproline, can be utilized. Negative in tests for milk coagulation, milk peptonization, starch hydrolysis, H_2S production, urease activity and melanin production. Doubtful result for gelatin liquefaction; positive for nitrate reduction. Grows optimally at 28 °C and at pH 7·2 with 10 % (w/v) NaCl; the temperature, pH and NaCl tolerance range are 20–40 °C, 6·0–9·0 and 3–20 % (w/v), respectively. The DNA G+C content is 73·1 mol%. Isolated from a saline soil sample in the west of China.

The type strain is YIM 90010^T (=KCTC 19003^T=CCTCC AA 204009^T).

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