Note

Nocardiopsis xinjiangensis sp. nov., a halophilic actinomycete isolated from a saline soil sample in China

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An actinomycete strain (YIM 90004^T) was isolated from a saline soil sample from the Xinjiang Province, People's Republic of China. The strain displayed abundant aerial and substrate mycelia, and short spore chains were borne on the aerial mycelium. The spore chains were composed of non-motile, smooth-surfaced, rod-shaped spores. The cell wall of strain YIM 90004^T contained mainly *meso*-diaminopimelic acid, alanine and glutamic acid (cell wall type III). Glucose, ribose, galactose, xylose and arabinose were the whole-cell sugars of the strain. The predominant phospholipids were phosphatidylinositol and phosphatidylglycerol; MK-10(H₂) and MK-10(H₄) were the predominant menaquinones. The DNA G+C content of strain YIM 90004^T was 74.3 mol%. Chemotaxonomic properties and 16S rDNA analysis placed strain YIM 90004^T in the genus *Nocardiopsis*. On the basis of phenotypic and phylogenetic analyses and DNA–DNA hybridization results, strain YIM 90004^T (=CCRC 16285^T = CCTCC AA99004^T = DSM 44589^T) is considered to represent a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis xinjiangensis* is proposed.

At the time of writing, there were 15 validly described species of the genus *Nocardiopsis* (Al-Tai & Ruan, 1994; Kroppenstedt, 1992; Yassin *et al.*, 1993, 1997; Evtushenko *et al.*, 2000; Chun *et al.*, 2000; Peltola *et al.*, 2001; Al-Zarban *et al.*, 2002). Of these species, only *Nocardiopsis halophila* and *Nocardiopsis kunsanensis* are halophilic actinomycetes. Here, we report the characterization of a novel halophilic member of the genus *Nocardiopsis* that was isolated from a saline soil sample from the Xinjiang Province, People's Republic of China.

Strain YIM 90004^T was isolated after 4 weeks incubation at 28 °C on glycerol/asparagine agar (ISP5) containing 10 % (w/v) NaCl. Stock cultures were maintained on ISP2 and ISP5 slants containing 10 % NaCl and kept at 4 °C. The micromorphological characteristics of strain YIM 90004^T were observed by light microscopy (model BH2; Olympus) after 24–28 days growth on ISP2 and ISP5 agar containing 10 % NaCl. Spore characteristics were determined by examination of cells using a model EPMA-8705 scanning electron microscope (Shimadzu), following desiccation of cells and coating with gold.

Strain YIM 90004^T was Gram-positive. Its vegetative hyphae were long, slim (0.1–0.2 µm in width), well-developed and fragmented. Short spore chains were borne on the aerial hyphae. Spores (dimensions 0.3–0.5 × 0.5–1.0 µm) were rod-shaped, smooth-surfaced and non-motile (Fig. 1).

Procedures and media used for determination of the physiological properties of and carbon utilization by strain YIM 90004^T were based on described methods (Shirling & Gottlieb, 1966; Williams *et al.*, 1989). Strain YIM 90004^T could grow on almost all of the test media (Table 1). Phenotypic properties useful for distinguishing strain YIM 90004^T from related species are shown in Table 2. Strain YIM 90004^T did not produce any diffusible pigment during incubation. Its optimum pH, cultivation temperature and NaCl concentration were 7.2, 28 °C and 10 %, respectively. Colour of the strain's mycelium was determined by comparing the culture with the most suitable colour chips from the ISCC–NBS colour charts (standard sample, no. 2106) (Kelly, 1964).

DNA extraction was performed according to the methods of Hopwood *et al.* (1985) and Jiang & Xu (1995). The DNA G+C base content of strain YIM 90004^T, determined by the thermal denaturation method (Mandel & Marmur, 1968), was found to be 74.3 mol%. Amplification and analysis of the 16S rDNA sequence of strain YIM 90004^T were done as described previously (Cui *et al.*, 2001). The 16S rDNA

Published online ahead of print on 19 September 2002 as DOI 10.1099/ijs.0.02055-0.

The GenBank accession number for the 16S rDNA sequence of *Nocardiopsis xinjiangensis* YIM 90004^T is AF251709.

Fig. 1. Light micrograph (top panel) and scanning electron micrographs (middle and bottom panels) of strain YIM 90004^T grown on ISP5 plates for 28 days at 28 °C.

sequence of strain YIM 90004^T was aligned with previously published sequences obtained from GenBank. A more-detailed comparison was performed with members of the genus *Nocardiopsis* using the K_{nuc} value of Kimura (1980, 1983) and the neighbour-joining method of Saitou & Nei (1987). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. Similarities between strain YIM 90004^T and other actinomycetes indicated strain YIM 90004^T to be a member of the genus *Nocardiopsis* (94.8–98.1 %). Strain

YIM 90004^T was phylogenetically most closely related to *N. kunsanensis* (98.1 %). Fig. 2 shows a phylogenetic dendrogram displaying the relatedness of strain YIM 90004^T to type strains of the genus *Nocardiopsis*.

For chemotaxonomic analyses, strain YIM 90004^T was grown for 2 weeks in 100 ml ISP2 liquid medium. The mycelium was harvested by centrifugation at 3500 r.p.m. for 10 min and then washed several times with distilled water. Cell wall was purified and diaminopimelic acid analysed by the method of Lechevalier & Lechevalier (1980). Whole-cell sugar analysis was determined by GC and MS (GC-MS model HP-6890; Hewlett Packard) (Saddler *et al.*, 1991; Takahashi *et al.*, 1992). The analysis of phospholipids and menaquinones followed the methods of Lechevalier & Lechevalier (1980) and Collins (1985), respectively. *meso*-Diaminopimelic acid was the diagnostic amino acid of the peptidoglycan. Whole-cell sugars were quantitatively analysed and found to be glucose (32 %), ribose (23 %), xylose (22 %), arabinose (14 %) and galactose (8 %). Major menaquinones were MK-10(H₂) and MK-10(H₄), and phospholipid included phosphatidylglycerol and phosphatidylinositol. Some of these properties were consistent with the phylogenetic placement of strain YIM 90004^T into the genus *Nocardiopsis*.

Phylogenetically, strain YIM 90004^T was most closely related to *N. kunsanensis* (98.1 %), one of the two halophilic species of the genus *Nocardiopsis*. The second species, *N. halophila*, shared only 95.8 % 16S rDNA sequence similarity with strain YIM 90004^T, and can thus be clearly distinguished from strain YIM 90004^T by the primary structure of its 16S rDNA. At this level of 16S rDNA relatedness, strain YIM 90004^T and *N. kunsanensis* belong to different DNA–DNA reassociation groups and should not be combined in a single species (Stackebrandt & Goebel, 1994). These two taxa also differ phenotypically, morphologically and chemotaxonomically. Strain YIM 90004^T differs from the type strain of *N. kunsanensis* (KTCT 9831^T) in some of its chemotaxonomic properties (Table 2): the predominant menaquinones of strain YIM 90004^T are MK-10(H₂, H₄), while that of *N. kunsanensis* is MK-10(H₈); the phospholipids of strain YIM 90004^T included phosphatidylglycerol and phosphatidylinositol, while those of *N. kunsanensis* are composed of phosphatidylcholine, phosphatidylglycerol and diphosphatidylglycerol (phospholipid type III) (Chun *et al.*, 2000). Strain YIM 90004^T did not grow well in media above pH 8.0, had an optimum growth temperature of 28 °C and did not utilize glucose, xylose or alanine, whereas *N. kunsanensis* has optimum growth at pH 9.0 and 37 °C, and utilizes glucose, xylose and alanine. The DNA–DNA reassociation value determined for strains YIM 90004^T and *N. kunsanensis* KTCT 9831^T (only 20 %) reinforces the genomic differences between the two strains.

Based on the above results, we consider strain YIM 90004^T to represent a novel species of the genus *Nocardiopsis*, for which we propose the name *Nocardiopsis xinjiangensis*.

Table 1. Growth of strain YIM 90004^T on different media

Media*	Growth	Aerial mycelium†	Substrate mycelium†
Czapek agar	Poor	White	Pink white
Glycerol/asparagine (ISP5)	Strong	Very pink	Light orange yellow
Glucose/asparagine	Poor	Pale pink	Soft brilliant yellow
Inorganic salt starch (ISP4)	Moderate	Yellowish white	Poor yellow
Yeast extract/malt extract (ISP2)	Poor	White	Brilliant yellow
Oatmeal (ISP3)	Poor	White	Brilliant yellow
Potato extract	Moderate	Soft olive green	Moderate yellow
Nutrient agar	Moderate	White	Light yellow

*Contained 10 % (w/v) NaCl (pH 7.0). ISP, International *Streptomyces* Project (Shirling & Gottlieb, 1966).

†Colours taken from the ISCC–NBS colour charts (standard sample, no. 2106) (Kelly, 1964).

Table 2. Phenotypic properties useful for distinguishing strain YIM 90004^T from the halophilic members of the genus *Nocardiopsis*

Species: 1, *N. halophila*; 2, *N. kunsanensis*; 3, *Nocardiopsis* sp. YIM 90004^T. Data for *N. halophila* and *N. kunsanensis* are from this study and studies by Yassin *et al.* (1997) and Chun *et al.* (2000). +, Positive; w, weak positive; –, negative; v, variable result. *meso*-DAP, *meso*-diaminopimelic acid. PC, Phosphatidylcholine; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; PG, phosphatidylglycerol; DAP, diaminopimelic acid.

Characteristic	1	2	3
Carbon utilization			
Glucose	+	w	–
Maltose	–	+	–
Cellobiose	–	w	+
D-Galactose	–	+	+
Mannitol	–	v	–
Raffinose	–	+	–
Rhamnose	–	w	–
Sucrose	+	+	+
Xylose	v	+	–
Nitrogen utilization			
L-Alanine	+	–	+
Proline	–	+	+
Serine	–	–	+
Growth			
Optimum temperature	28	37	28
Optimum pH	7.2	9.0	7.2
Optimum NaCl concn (%)	15	10	10
Cell-wall peptidoglycan	<i>meso</i> -DAP	<i>meso</i> -DAP	<i>meso</i> -DAP
Diagnostic sugars	None	None	Xylose, arabinose, galactose
Phospholipids	PC, DPG	PI, PG, DAP	PI, PG
Menaquinone(s)	MK-10(H ₆ , H ₈)	MK-10(H ₈)	MK-10(H ₂ , H ₄)

Description of *Nocardiopsis xinjiangensis* sp. nov.

Nocardiopsis xinjiangensis (xin.ji.ang.en'sis. N.L. adj. *xinjiangensis* pertaining to Xinjiang, the Province of western China in which the sample was collected).

On most test medium, the fragmented substrate and aerial mycelia are well developed. Short spore chains are borne on the aerial mycelium; spores are rod-shaped with a smooth

surface. No diffusible pigment is produced. Whole-cell hydrolysates contain *meso*-diaminopimelic acid; whole-cell sugars include glucose, ribose, xylose, arabinose and galactose. Predominant menaquinones are MK-10(H₂, H₄), and the diagnostic phospholipids are phosphatidylglycerol and phosphatidylinositol. Utilizes cellobiose, galactose, alanine, proline and serine, but not glucose, xylose, maltose, mannitol, raffinose, rhamnose or sucrose. Gelatin liquefaction, urease activity and melanin production are positive. Milk

Fig. 2. Neighbour-joining tree, based on 16S rDNA sequences, showing the phylogenetic relationship of strain YIM 90004^T with recognized members of the genus *Nocardio-opsis*. *Streptomyces megasporus* was used as the outgroup. Only bootstrap values >50% (expressed as percentages of 1000 replications) are shown at the nodes of the tree. Bar, one nucleotide substitution per 100 nt.

coagulation, milk peptonization, starch hydrolysis, nitrate reduction and H₂S production are negative. Optimum growth temperature is 28 °C; optimum salt concentration for growth is 10%; optimum pH for growth is 7.2. DNA G+C content is 74.3 mol%. Isolated from a saline soil sample in the west of China. The type strain is YIM 90004^T (=CCRC 16285^T =CCTCC AA99004^T =DSM 44589^T).

Acknowledgements

The authors would like to thank Professor Sang-Jin Kim for kindly providing the type strain of *N. kunsanensis*. We also thank Professor Tseng Min for assistance in obtaining the scanning electron micrographs. This research was supported by the Key Laboratory for Microbial Resources of the Ministry of Education, People's Republic of China, the Yunnan Provincial Educational Administration and the Yunnan Provincial Natural Science Foundation.

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