Nocardiopsis quinghaiensis sp. nov., isolated from saline soil in China

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A previously unknown Gram-positive, obligately aerobic actinomycete, YIM 28A4^T, was isolated from a sample of saline soil collected from the Qaidam Basin in Qinghai Province, north-west China, and was investigated using a polyphasic taxonomic approach. The strain grew well on most of the media tested, producing white to pale-yellow substrate mycelium, white aerial mycelium and straight to flexuous hyphae. The substrate mycelium was well developed and fragmented with age; the aerial mycelium produced long, straight spore chains. The spore chains were composed of non-motile, smooth-surfaced, rod-shaped spores. No diffusible pigments were produced on any of the media tested. The strain grew in the presence of 0–10% (w/v) NaCl and at pH 6.0–8.0, with optimum growth occurring at 3% (w/v) NaCl and pH 7.0. It grew at 10–37 °C, the optimum growth temperature being 28 °C. Whole-cell hydrolysates of strain YIM 28A4^T contained *meso*-diaminopimelic acid and no diagnostic sugars. The predominant phospholipids were phosphatidylcholine, phosphatidylglycerol and diphosphatidylglycerol. The predominant menaquinones were MK-10, MK-10(H₂), MK-11 and MK-11(H₂). The major cellular fatty acids were iso-C_{16:0}, anteiso-C_{15:0} and anteiso-C_{17:0}. The DNA G + C content was 67.1 mol%. The morphological and chemotaxonomic characteristics of the isolate matched those described for *Nocardiopsis* species. A phylogenetic analysis based on 16S rRNA gene sequence comparisons confirmed that strain YIM 28A4^T was a member of the genus *Nocardiopsis* and most closely related to the type strains *Nocardiopsis aegyptia* DSM 44442^T and *Nocardiopsis halotolerans* DSM 44410^T, showing 98.1 and 97.8% 16S rRNA gene sequence similarity, respectively. Strain YIM 28A4^T can be differentiated from these type strains by using phenotypic, phylogenetic and DNA–DNA hybridization data. On the basis of the polyphasic evidence, strain YIM 28A4^T represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis quinghaiensis* sp. nov. is proposed. The type strain is YIM 28A4^T (=DSM 44739^T =CGMCC 4.3494^T).

The genus *Nocardiopsis* was first described by Meyer (1976) and currently comprises 24 species with validly published names (Meyer, 1976; Grund & Kroppenstedt, 1990; Yassin *et al.*, 1993, 1997; Al-Tai & Ruan, 1994; Chun *et al.*, 2000; Evtushenko *et al.*, 2000; Peltola *et al.*, 2001; Al-Zarban *et al.*, 2002; Kämpfer *et al.*, 2002; Schippers *et al.*, 2002; Li *et al.*, 2003, 2004; Hozzein *et al.*, 2004; Sabry *et al.*, 2004). During a recent study on the diversity of microbiological

communities in the soils of Qinghai Province (north-west China), a *Nocardiopsis*-like strain, designated YIM 28A4^T, was isolated from a sample of saline soil collected from the Chaidam Basin (Cui *et al.*, 2004; Schumann *et al.*, 2004; Li *et al.*, 2005a, b, c, d, 2006; Zhang *et al.*, 2005, 2007). On the basis of the results of this polyphasic taxonomic study, it is proposed that strain YIM 28A4^T represents a novel species of the genus *Nocardiopsis*.

Strain YIM 28A4^T was isolated from a sample of saline soil by plating 1:10 serial dilutions of the sample on marine agar 2216 (Difco) at 28 °C for 2 weeks. The strain was maintained both on a marine agar 2216 slant at 4 °C and at

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 28A4^T is EF597511.

–80 °C in marine broth (Difco) supplemented with 20 % (v/v) glycerol. Two type strains of the genus *Nocardiopsis*, *Nocardiopsis aegyptia* DSM 44442^T and *Nocardiopsis halotolerans* DSM 44410^T, were used in some parallel tests. Morphological characteristics were observed by using light microscopy (BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 2 and 4 week periods of growth on yeast extract-malt extract agar (ISP 2; Shirling & Gottlieb, 1966) supplemented with 3 % (w/v) NaCl. Anaerobic growth was determined using a GasPak Anaerobic System pot (BBL) according to the manufacturer's instructions. Cultural characteristics were determined after 2–4 weeks by using the methods applied in the International *Streptomyces* Project (Shirling & Gottlieb, 1966). All media were supplemented with 3 % (w/v) NaCl (pH 7.0). The colours of both substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the colour charts of the Inter-Society Color Council – National Bureau of Standards (Kelly, 1964).

Strain YIM 28A4^T showed the macroscopic and microscopic appearance typical of most species of the genus *Nocardiopsis* (Meyer, 1976, 1994). The strain was Gram-positive and obligately aerobic. The substrate mycelium was white to pale-yellow, well developed and fragmented with age. The white aerial mycelium was well developed on most of the media tested (Table 1) and the spore chains were long, containing rod-shaped, smooth-surfaced, non-motile spores. Hyphae from the aerial mycelium were straight to flexuous (Fig. 1). No diffusible pigments were produced on any of the media tested.

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene, purification of PCR products and electrophoresis of sequencing reactions were performed as described previously (Cui *et al.*, 2001). The resulting 16S rRNA gene sequence was compared with sequences obtained from databases, to find the most closely related species. A phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein, 1993) and MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997).

Fig. 1. Scanning electron micrograph of strain YIM 28A4^T grown on Czapek agar supplemented with 3 % (w/v) NaCl at 28 °C for 28 days. Bar, 2 µm.

Distances were calculated using distance options according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). A maximum-parsimony (Kluge & Farris, 1969) phylogenetic tree (not shown) was generated using the treeing algorithms contained in the PHYLIP package (Felsenstein, 1993). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by means of 1000 resamplings (Felsenstein, 1985).

The almost-complete 16S rRNA gene sequence (1384 bp) of strain YIM 28A4^T was determined. A preliminary comparison of the sequence against the GenBank database indicated that the isolate was closely related to the members of the genus *Nocardiopsis* and that it was phylogenetically most closely related to *N. aegyptia* DSM 44442^T and *N. halotolerans* DSM 44410^T, with sequence similarities of 98.1 and 97.8 %, respectively. The other closest phylogenetic neighbours of strain YIM 28A4^T (sequence similarity value >97 %) were *Nocardiopsis dassonvillei* subsp. *dassonvillei* DSM 43111^T (97.6 % sequence similarity), *Nocardiopsis umidischolae* DSM 44362^T (97.4 %), *Nocardiopsis lucentensis* DSM 44048^T (97.3 %), *Nocardiopsis alba* DSM 43377^T (97.2 %),

Table 1. Cultural characteristics of strain YIM 28A4^T

All media were adjusted to pH 7.0 and supplemented with 3 % (w/v) NaCl. ISP, International *Streptomyces* Project (Shirling & Gottlieb, 1966). The strain formed white aerial mycelium on all media tested.

Medium	Growth	Substrate mycelium
Yeast extract-malt extract agar (ISP 2)	Good	White
Oatmeal agar (ISP 3)	Good	White
Inorganic salts-starch agar (ISP 4)	Moderate	White
Glycerol-asparagine agar (ISP 5)	Poor	White
Peptone-yeast extract-iron agar (ISP 6)	Poor	Pale yellow
Czapek agar	Abundant	White
Potato extract agar	Good	Pale yellow

Nocardiopsis tropica DSM 44381^T (97.1 %) and *Nocardiopsis salina* YIM 90010^T (97.1 %). These sequence similarity values are lower than those found for some other individual pairs of *Nocardiopsis* species, as discussed previously (Al-Zarban *et al.*, 2002; Schippers *et al.*, 2002; Hozzein *et al.*, 2004; Sabry *et al.*, 2004). In the neighbour-joining phylogenetic tree for *Nocardiopsis* species (Fig. 2), strain YIM 28A4^T and *N. halotolerans* DSM 44410^T formed a sub-branch with low bootstrap support (66 %). To establish the precise taxonomic position of YIM 28A4^T, DNA–DNA hybridization studies were performed, using the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992), between the novel strain and the type strains *N. aegyptia* DSM 44442^T and *N. halotolerans* DSM 44410^T. The DNA–DNA relatedness between strain YIM 28A4^T and *N. aegyptia* DSM 44442^T was 20.0 %, while the value for *N. halotolerans* DSM 44410^T was 24.5 %. These values are far below the threshold value of about 70 % recommended by Wayne *et al.* (1987) for the assignment of strains to the same species. On the basis of phylogenetic analysis and the DNA–DNA hybridization data, a clear differentiation of strain YIM 28A4^T from *N. aegyptia* DSM 44442^T and *N. halotolerans* DSM 44410^T was possible.

DNA for the determination of the G+C content was isolated according to Hopwood *et al.* (1985). The G+C content, determined using the thermal denaturation

method of Mandel & Marmur (1968) with a Shimadzu UV-visible spectrophotometer (UV1601), was 67.1 mol%.

The media and procedures used to determine physiological and biochemical features and to identify the carbon sources utilized were those described by Kroppenstedt & Evtushenko (2006), Shirling & Gottlieb (1966) and Williams *et al.* (1989). Unless otherwise indicated, all tests were carried out with 3 % NaCl (w/v) at pH 7.0 with incubation at 28 °C. The results are listed in Table 2 and in the species description.

Biomass for chemical and systematic molecular studies was obtained from cultures grown in trypticase soy broth (BBL) at 28 °C for 3 days on a rotary shaker (about 200 r.p.m.). Cells were harvested by centrifugation and washed twice with distilled water. Analyses of the amino acids and sugars in whole-cell hydrolysates were performed according to the procedures described by Stanek & Roberts (1974). Polar lipids were extracted, examined using two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1984). Menaquinones were isolated using the methods of Minnikin *et al.* (1984) and then separated by HPLC (Kroppenstedt, 1982, 1985). Cellular fatty acids were analysed according to Kämpfer & Kroppenstedt (1996) with cells grown on trypticase soy agar (BBL) for 4 days at 28 °C.

Chemotaxonomic data for YIM 28A4^T were consistent with the assignment of the strain to the genus *Nocardiopsis* (Meyer, 1976; Kroppenstedt & Evtushenko, 2006). Whole-cell hydrolysates contained *meso*-diaminopimelic acid as the only diamino acid of the peptidoglycan. Diagnostic sugars were not found. The polar lipid pattern was composed of phosphatidylcholine, phosphatidylglycerol and diphosphatidylglycerol. The major menaquinones were MK-10 (29.7 %), MK-10(H₂) (23.3 %), MK-11(H₂) (17.7 %), MK-11 (15.2 %) and MK-9(H₄) (5.2 %). Traces of MK-9(H₆) and MK-12 were also found. The fatty acid profiles of strain YIM 28A4^T, *N. aegyptia* DSM 44442^T and *N. halotolerans* DSM 44410^T are given in Table 3. The major cellular fatty acids of strain YIM 28A4^T were iso-C_{16:0} (30 %), anteiso-C_{15:0} (15 %) and anteiso-C_{17:0} (10 %).

Phenotypically, the properties of strain YIM 28A4^T supported its placement in the genus *Nocardiopsis* (Meyer, 1976; Kroppenstedt & Evtushenko, 2006). However, the novel strain could be differentiated from its closest relatives by using a combination of morphological, physiological and chemotaxonomic data. A comparison of selected characteristics of strain YIM 28A4^T with those of its closest relatives, *N. aegyptia* DSM 44442^T and *N. halotolerans* DSM 44410^T, is presented in Table 2. Strain YIM 28A4^T differed from *N. aegyptia* DSM 44442^T and *N. halotolerans* DSM 44410^T in terms of colony colour, physiological test results and chemotaxonomy. Strain YIM 28A4^T produced white aerial mycelium and pale-yellow substrate mycelium, whereas DSM 44442^T and DSM 44410^T produce beige or dirty-white aerial mycelium and brown or beige-brown substrate mycelium, respectively. In

Fig. 2. Neighbour-joining phylogenetic dendrogram, based on 16S rRNA gene sequence analysis, showing the phylogenetic position of strain YIM 28A4^T among members of the genus *Nocardiopsis*. Filled circles indicate branches that were also found with the maximum-parsimony (Kluge & Farris, 1969) algorithm. Bootstrap values (>50 %) are indicated at the relevant branching points. Bar, 1 substitution per 100 nucleotides.

Table 2. Characteristics that distinguish strain YIM 28A4^T from its closest phylogenetic relatives

Data for *N. aegyptia* DSM 44442^T and *N. halotolerans* DSM 44410^T were taken from Sabry *et al.* (2004) and Al-Zarban *et al.* (2002), respectively, unless indicated. +, Positive; –, negative; ND, no data. All strains were positive for the hydrolysis of casein and negative for H₂S and melanin production and urease activity (data from our study).

Characteristic	Strain YIM 28A4 ^T	<i>N. aegyptia</i> DSM 44442 ^T	<i>N. halotolerans</i> DSM 44410 ^T
Pigmentation of mycelium			
Aerial mycelium	White	Beige to light yellow	Dirty white to yellow–grey
Substrate mycelium	White to pale yellow	Brown	Beige to brown
NaCl concentration for growth (% w/v)			
Range	0–10	0–5*	0–15
Optimum	3	ND	10
Temperature for growth (°C)			
Range	10–37	10–40*	10–35
Optimum	28	28	28
Hydrolysis of:†			
Starch	+	–	–
Gelatin	–	+	+
Tween 80	+	+	–
Nitrate reduction†	–	+	–
Utilization of:‡			
L-Arabinose	+	+	–
Galactose	–	+	+
D-Lactose	–	+	ND/–†
Melibiose	–	ND/–†	+
L-Rhamnose	–	+	ND/–†
D-Xylose	–	+	–
Fatty acid profile (%)			
Major fatty acids	iso-C _{16:0} (30); anteiso-C _{15:0} (15)	iso-C _{16:0} (19); anteiso-C _{17:0} (18)	iso-C _{16:0} (23); anteiso-C _{17:0} (18)
Unbranched saturated fatty acids	12	13	10
10-Methyl-branched fatty acids	9	16	4
Unsaturated fatty acids	7	11	17
Major menaquinones	MK-10, MK-10(H ₂), MK-11, MK-11(H ₂)	MK-10(H ₆), MK-10(H ₈), MK-10(H ₄), MK-10(H ₂)	MK-10, MK-10(H ₂), MK-10(H ₄), MK-11
Phospholipids§	PC, PG, DPG	PC, PI, PG, PME, DPG	PC, PI, PG, PME, DPG
DNA G + C content (mol%)	67.1	ND	68.0

**N. aegyptia* DSM 44442^T grew at 10–40 °C and in 5 % NaCl, but not at 45 °C or with 10 % NaCl (Sabry *et al.*, 2004).

†Our results.

‡Unless otherwise indicated, results of these tests for strains *N. aegyptia* DSM 44442^T and *N. halotolerans* DSM 44410^T were confirmed in parallel tests with strain YIM 28A4^T under identical conditions.

§DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmethylethanolamine.

contrast to those two strains, YIM 28A4^T was able to hydrolyse starch but not gelatin. It could utilize L-arabinose as a sole source of carbon but failed to grow on galactose, D-lactose, melibiose, L-rhamnose or D-xylose. All of these sugars (except melibiose) could be assimilated by DSM 44442^T, whereas DSM 44410^T could only grow on galactose and melibiose (Table 2). YIM 28A4^T showed a typical *Nocardiopsis* fatty acid profile containing a relatively large amount of 14-methyl hexadecanoic acid (anteiso-C_{17:0}) in combination with 10-methyl octadecanoic acid (tuberculo-stearic acid; 10-methyl C_{18:0}). YIM 28A4^T synthesized relatively small amounts of unsaturated fatty acids in

comparison with DSM 44442^T and DSM 44410^T (Tables 2 and 3). The salt tolerance of the novel strain also differed with respect to those two strains: YIM 28A4^T was able to grow at NaCl concentrations up to 10 % (w/v), DSM 44442^T can grow only at 5 % NaCl (w/v) and DSM 44410^T grows well at 15 % NaCl (w/v).

Thus, on the basis of the phylogenetic analysis, phenotypic distinctiveness (Table 2) and DNA–DNA relatedness data presented above, strain YIM 28A4^T represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis quinghaiensis* sp. nov. is proposed.

Table 3. Fatty acid compositions (%) of strain YIM 28A4^T and its closest phylogenetic relatives

Strains: 1, YIM 28A4^T; 2, *N. aegyptia* DSM 44442^T (data from Sabry *et al.*, 2004); 3, *N. halotolerans* DSM 44410^T (data from this study). –, Not present. Trace components in the fatty acid composition of strain YIM 28A4^T were iso-C_{12:0} (0.20 %), iso-C_{13:0} (0.25 %), anteiso-C_{13:0} (0.47 %), C_{14:0} (0.27 %) and C_{15:0} (0.75 %). A small amount of anteiso-C_{13:0} (0.57 %) was also found to be present in *N. halotolerans* DSM 44410^T. Trace components (<1 % of total) in the fatty acid composition of *N. aegyptia* DSM 44442^T were not listed (Sabry *et al.*, 2004).

Fatty acid	1	2	3
Unbranched saturated fatty acids			
C _{16:0}	3.66	4.21	3.21
C _{17:0}	2.83	1.72	3.08
C _{18:0}	4.45	7.32	3.46
Unsaturated fatty acids			
C _{16:1} <i>cis</i> -9	–	1.10	1.33
C _{17:1} <i>cis</i> -9	2.65	1.72	6.78
C _{18:1} <i>cis</i> -9	4.57	7.76	8.52
Terminally branched fatty acids			
iso-C _{14:0}	5.58	12.12	2.40
iso-C _{15:0}	2.59	1.70	3.38
anteiso-C _{15:0}	14.63	6.49	16.69
iso-C _{16:0}	30.02	19.14	23.30
iso-C _{17:0}	2.22	5.10	4.23
anteiso-C _{17:0}	9.99	17.85	18.12
anteiso-C _{17:1} <i>cis</i> -9	–	1.35	–
iso-C _{18:0}	1.40	3.16	1.34
10-Methyl-branched fatty acids			
10-Methyl C _{16:0}	1.75	1.50	–
10-Methyl C _{17:0}	2.78	2.58	1.61
10-Methyl C _{18:0}	4.51	12.19	1.32

Description of *Nocardiopsis quinghaiensis* sp. nov.

Nocardiopsis quinghaiensis (qing.hai.en'sis. N.L. fem. adj. *qinghaiensis* pertaining to Qinghai, a province of China in which the sample was collected).

Gram-positive, obligately aerobic, non-motile, catalase-positive nocardioform actinomycete that forms white aerial mycelium. The colour of the substrate mycelium is white to pale yellow. Diffusible pigments are not produced. Substrate hyphae are well developed and fragment with age. Long spore chains are borne on aerial hyphae. Spores are rod-shaped, smooth and non-motile. Optimal growth occurs on marine agar 2216 and Czapek agar with 3 % (w/v) NaCl at 28 °C and pH 7.0. The temperature, pH and NaCl concentration ranges for growth are 10–37 °C, pH 6.0–8.0 and 0–10 %. Positive in tests for hydrolysis of casein, chitin, starch, Tween 20 and Tween 80, but negative for hydrolysis of cellulose and gelatin. Nitrate is not reduced to nitrite. H₂S, melanin and urease are not produced. L-Arabinose, cellobiose, citrate, D-fructose, D-glucose, glycerol, *myo*-inositol, D-mannitol, D-mannose,

raffinose, D-ribose, sucrose, starch and trehalose are used as sole carbon and energy sources, whereas acetate, adonitol, D-galactose, D-lactose, maltose, melibiose, D-rhamnose, salicin, D-sorbitol, D-xylitol and D-xylose cannot be used for growth. L-Alanine, L-asparagine, L-glycine, L-histidine, hydroxy-L-proline, L-methionine, L-proline, L-threonine and L-tyrosine can be utilized as sole nitrogen sources, whereas adenine, L-arginine, L-cystine, glutamic acid, L-lysine, phenylalanine, L-tryptophan and L-valine cannot. Whole-cell hydrolysates contain *meso*-diaminopimelic acid and no diagnostic sugars. The polar lipids comprise phosphatidylcholine, phosphatidylglycerol and diphosphatidylglycerol. The major menaquinones are MK-10 (29.7 %), MK-10(H₂) (23.3 %), MK-11(H₂) (17.7 %), MK-11 (15.2 %) and MK-9(H₄) (5.2 %). The major cellular fatty acids are iso-C_{16:0} (30.0 %), anteiso-C_{15:0} (14.6 %), anteiso-C_{17:0} (10.0 %), iso-C_{14:0} (5.9 %) and C_{18:1} *ω*9c (4.6 %). The DNA G+C content is 67.1 mol%.

The type strain, YIM 28A4^T (=DSM 44739^T =CGMCC 4.3494^T), was isolated from a sample of saline soil collected from the Qaidam Basin, Qinghai Province, north-west China.

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