Nocardiopsis valliformis sp. nov., an alkaliphilic actinomycete isolated from alkali lake soil in China

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A strain (HBUM 20028^T) isolated from alkali lake soil in China was studied by a polyphasic taxonomic approach. The strain produced abundant aerial and substrate mycelia. Long spore chains were borne on the aerial mycelium, and the substrate mycelium was often arranged in a shape like a fence or palisade. The special characteristic of strain HBUM 20028^T was its abundant growth under alkaline conditions, at pH 8.0–14.0. The cell wall of strain HBUM 20028^T contained *meso*-diaminopimelic acid but no diagnostic sugar. Major phospholipids included diphosphatidylglycerol and phosphatidylcholine. The major menaquinones were MK-10(H₂), MK-10(H₄) and MK-10(H₆). The major cellular fatty acids were iso-C_{16:0} (31.66%), anteiso-C_{17:0} (14.85%) and C_{18:1ω9c} (14.73%). All of these characters consistently indicated that strain HBUM 20028^T belongs to the genus *Nocardiopsis*. DNA–DNA hybridization between the strain and type strains of related species gave relatedness values far below 70%. Based on 16S rRNA gene sequence analysis, DNA relatedness and phenotypic characteristics, a novel species with the name *Nocardiopsis valliformis* sp. nov. is proposed. The type strain is HBUM 20028^T (=DSM 45023^T =CGMCC 4.2135^T).

The genus *Nocardiopsis* was first described by Meyer (1976) based on morphological characteristics and the chemical composition of cells. The genus *Nocardiopsis* currently contains 24 species and two subspecies: *Nocardiopsis dassonvillei* subsp. *dassonvillei* (Meyer, 1976), *N. alba*, *N. listeri* (Grund & Kroppenstedt, 1990), *N. halophila* (Al-Tai & Ruan, 1994), *N. lucentensis* (Yassin *et al.*, 1993), *N. prasina*, *N. synnemataformans* (Yassin *et al.*, 1997), *N. kunsanensis* (Chun *et al.*, 2000), *N. tropica*, *N. trehalosi*, *N. dassonvillei* subsp. *albirubida* (Evtushenko *et al.*, 2000), *N. exhalans*, *N. umidischolae* (Peltola *et al.*, 2001), *N. halotolerans* (Al-Zarban *et al.*, 2002), *N. composta* (Kämpfer *et al.*, 2002), *N. metallicus* (Schippers *et al.*, 2002), *N. xinjiangensis* (Li *et al.*, 2003), *N. alkaliphila* (Hozzein *et al.*, 2004), *N. salina* (Li *et al.*, 2004), *N. aegyptica* (Sabry *et al.*, 2004), *N. baichengensis*, *N. chromatogenes*, *N. gilva*, *N. rhodophaea* and *N. rosea* (Li *et al.*, 2006). Most species are alkaliphilic or halophilic. Because of the discovery of novel taxa and many new valuable secondary metabolites, more and more scientists are now searching extreme environments and studying their unusual actinomycetes.

In this paper, we isolated some strains of alkaline actinomycetes from Xinjiang, a region of western China.

Strain HBUM 20028^T was identified by a polyphasic approach. Comparative studies of morphology, physiology, biochemistry and chemical composition of cells, phylogenetic analysis based on 16S rRNA gene sequences and DNA relatedness tests were carried out between strain HBUM 20028^T and type strains of the genus *Nocardiopsis*. The results indicated that the novel strain belongs to the genus *Nocardiopsis* and is different from known members of the genus. Therefore, we consider that strain HBUM 20028^T represents a novel species of the genus *Nocardiopsis*.

Strain HBUM 20028^T used in this study was isolated from a soil sample collected from an alkali lake near the county of Buerjin (Xinjiang, China) by using agar plates containing DSMZ medium no. 852 (GYE medium, adjusted to pH 10.0 with sterile saturated sodium carbonate solution after sterilization). Strain HBUM 20028^T was cultured for 3, 5, 7 and 14 days at 28 °C and pH 10.0 on GYE agar and then observed by using a model BH-2 Olympus light microscope and a model KYKY-AMRAY-100B scanning electron microscope.

Cultural characteristics were studied by using 7-day-old cultures grown at 28 °C and pH 10.0 on GYE agar media. Vegetative and aerial mycelium of strain HBUM 20028^T grew well on GYE medium and the aerial mycelium was usually abundant and white to yellowish, dividing into rod-shaped, smooth-surfaced and non-motile spores (0.3–0.5 × 1.2–2.5 µm), but the substrate mycelium was yellow

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HBUM 20028^T is AY336503.

to light-brown and was often arranged in a shape like a palisade (Fig. 1).

The physiological characteristics of the strain were tested according to Holt *et al.* (1994). The optimal growth temperature was 28 °C. The optimal pH for growth was pH 9.5–13, and the strain had a broad pH range for growth, from pH 8.0 to 14.0, but did not grow at pH 7.0, demonstrating that strain HBUM 20028^T is strongly alkaliphilic. Other physiological and biochemical properties are presented in Table 1.

Biomass used for chemotaxonomic and molecular systematic studies was cultivated in GYP liquid medium on a shaking incubator for 4 days at 28 °C; cells were then harvested by centrifugation and washed with distilled water. Amino acids of purified cell walls were analysed by using the method of Lechevalier & Lechevalier (1980). Diagnostic sugars of whole-cell hydrolysates were determined by using the methods of Hasegawa *et al.* (1983). Menaquinones were determined as described by Collins (1985). Phospholipids were prepared and identified by referring to the methods of Lechevalier & Lechevalier (1980). The cellular fatty acid composition was studied as described by Sasser (1990), using the Microbial Identification System (MIDI). Cell walls of strain HBUM 20028^T contained *meso*-diaminopimelic acid, but did not contain any diagnostic sugars. It can be considered to have a type III/C cell wall. Phospholipids consisted of diphosphatidylglycerol and phosphatidylcholine, which belong to the type PIII phospholipid pattern based on Lechevalier & Lechevalier (1980). The major menaquinones of the strain were MK-10(H₂), MK-10(H₄) and MK-10(H₆) and the major cellular fatty acids were iso-C_{16:0} (31.66 %), anteiso-C_{17:0} (14.85 %) and C_{18:1ω9c} (14.73 %).

Chromosomal DNA of strain HBUM 20028^T was prepared by referring to the methods of Marmur (1961) and Kutchma *et al.* (1998). The DNA G+C content was determined according to the methods of Mesbah *et al.*

(1989). The DNA was treated with BAL31 nuclease and alkaline phosphatase and mononucleotides were determined by HPLC. The 16S rRNA gene was amplified by PCR (TGRADIENT; Biometra) using *Taq* DNA polymerase (Sangon). Universal primers for actinomycetes were used, primers 27f (5'-GAGTTTGATCCTGGCTCAG-3'; *Escherichia coli* positions 8–27) and 1525r (5'-AGAAAGGAGGTGTACCAGCC-3'; *E. coli* positions 1525–1545) (Lane *et al.*, 1985), and the 1.5 kb amplified 16S rRNA gene fragment was purified and sequenced by Sangon Biological Engineering Co. Ltd (Shanghai). The same primers (27f and 1525r) were used for terminal sequencing. The internal sequencing primer used was P3 (5'-CTAACTACGTGCCAGCAGCC-3'). The 16S rRNA gene sequences of type strains of related species were obtained from GenBank. 16S rRNA gene sequences were aligned manually with sequences of members of the genus *Nocardiopsis* by using CLUSTAL_X 1.81 (Thompson *et al.*, 1997). The evolutionary tree was inferred by using the neighbour-joining method (Saitou & Nei, 1987). The topology of the resultant tree was evaluated by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining data based on 1000 replications. All phylogenetic analyses were inferred by using the PHYLIP software package (Felsenstein, 2001). The phylogenetic tree was rebuilt by TreeView (Page, 1996).

The nearly complete 16S rRNA gene sequence of strain HBUM 20028^T was determined and consisted of 1434 bp. Preliminary comparison of the sequence against those in GenBank indicated that members of the genus *Nocardiopsis* were the closest phylogenetic neighbours. Binary similarity values of this strain and type strains of other species of the genus *Nocardiopsis* ranged between 93.98 % (*N. rhodophaea* YIM 90096^T) and 99.93 % (*N. exhalans* ES10.1^T). The results show that strains HBUM 20028^T, *N. exhalans* ES10.1^T, *N. metallicus* KBS6^T and *N. prasina* DSM 43845^T were clustered into a branch. Fig. 2 shows the neighbour-joining tree. Sequence similarity calculations after a

Fig. 1. Scanning electron micrographs of strain HBUM 20028^T showing the long-branched hyphae (×3000) (a), aerial mycelium dividing into rod-shaped spores (b) and mycelium arranged in a palisade-like shape and divided (c). Original magnification, ×3000 (a, b) and ×5000 (c). Bars, 10 μm.

Table 1. Differential phenotypic characteristics of HBUM 20028^T and the type strains of closely related *Nocardiopsis* species

Strains: 1, *N. valliformis* sp. nov. HBUM 20028^T; 2, *N. prasina* DSM 43845^T; 3, *N. metallicus* DSM 44598^T; 4, *N. exhalans* DSM 44407^T. +, Positive; –, negative; v, not confirmed; ND, no data available. Data for reference strains were taken from Yassin *et al.* (1997), Peltola *et al.* (2001) and Schippers *et al.* (2002). All strains are positive for growth at 28 °C, nitrate reduction and utilization of L-arabinose, D-xylose and glycerol and negative for growth at 45 °C, H₂S production, formation of melanin on tyrosine agar (ISP 6) and peptone-yeast-iron agar (ISP 7) and utilization of *myo*-inositol.

Characteristic	1	2	3	4
Aerial mycelium	White to yellowish	White	White	White
Colony pigmentation (ISP 5)	White	White	Pale yellow	Light yellow
Optimal pH for growth	9.5–13	10.0	8.5	7.2
Utilization of:				
Sucrose	–	–	+	+
L-Rhamnose	v	–	+	+
Lactose	+	–	–	–
Cellobiose	v	–	+	+
Growth at:				
10 °C	–	–	+	+
42 °C	–	+	–	–
Growth in 10 % NaCl	–	+	+	+
Gelatin liquefaction	+	+	–	+
Major menaquinones	10(H ₂), 10(H ₄), 10(H ₆)	10(H ₄), 10(H ₆)	ND	10(H ₆), 10(H ₈)
Major phospholipids*	DPG, PC	PC, PME	ND	PC, PI, PG, PME, DPG
Major cellular fatty acids† (>10 %)	i-C _{16:0} (32 %), ai-C _{17:0} (15 %), C _{18:1ω9c} (15 %)	10-Me C _{18:0} (21 %), i-C _{16:0} (21 %), ai-C _{17:0} (18 %)	i-C _{16:0} (26 %), C _{18:1ω9c} (23 %), ai-C _{17:0} (15 %)	i-C _{16:0} (22 %), 10-Me C _{18:0} (12 %), ai-C _{17:0} (11 %)
DNA G + C content (mol%)	70.6	68.1	70.8	71.0

*DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmonomethanolamine.

†ai, Anteiso-branched; i, iso-branched; Me, methyl.

neighbour-joining analysis indicated that the closest relatives of strain HBUM 20028^T were *N. exhalans* ES10.1^T (99.93 % 16S rRNA gene sequence similarity), *N. metallicus* KBS6^T (99.58 %) and *N. prasina* DSM 43845^T (99.22 %). The G + C content of the DNA of strain HBUM 20028^T was 70.6 mol%.

DNA–DNA hybridization studies were performed by the initial reassociation-rate method (De Ley *et al.*, 1970) using a spectrophotometer (model CE9500; CECIL Company) equipped with a programmable melting temperature control unit. DNA of strain HBUM 20028^T was hybridized with that of *N. exhalans* DSM 44407^T, *N. metallicus* DSM 44598^T and *N. prasina* DSM 43845^T, the closest phylogenetic neighbours. The levels of DNA–DNA relatedness with the three strains were 31.4, 20.3 and 24.6 %, respectively.

Phenotypic and genotypic characteristics provided clear evidence that strain HBUM 20028^T belongs to the genus *Nocardiopsis*. The phylogenetic position of this strain is within a cluster that contains *N. exhalans*, *N. metallicus* and *N. prasina*. However, strain HBUM 20028^T can be differentiated from other species by its morphological, physiological and chemotaxonomic characteristics (Table 1). The values of DNA–DNA relatedness of strain HBUM

20028^T with related type strains were found to be <70 %. Based on the above results, it is concluded that strain HBUM 20028^T represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis valliformis* sp. nov. is proposed.

Description of *Nocardiopsis valliformis* sp. nov.

Nocardiopsis valliformis (val.li.for'mis. L. n. *vallum* palisade; L. adj. suffix *-formis* -like, in the shape of; N.L. fem. adj. *valliformis* shaped like a palisade, referring to the characteristic mycelium, which is often arranged in a shape like a palisade).

Aerial mycelium is abundant and white to yellowish, and fragments into rod-shaped, smooth-surfaced and non-motile spores (0.3–0.5 × 1.2–2.5 µm). Substrate mycelium is yellow to light-brown and is often arranged in a shape like a fence or palisade. Aerobic, Gram-positive. Optimal growth temperature is 28 °C; no growth at 10, 42 and 45 °C. Optimal pH for growth is pH 9.5–13; has a broad range of growth pH, from pH 8.0 to 14.0. No growth at pH 7.0. Growth occurs in the absence of NaCl and in 1, 3 and 5 % NaCl; no growth in 10 % NaCl. L-Arabinose, D-xylose, lactose and glycerol are utilized, but not sucrose or

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequence analysis, showing the phylogenetic position of strain HBUM 20028^T (*Nocardiopsis valliformis* sp. nov.) compared with the other species of the genus *Nocardiopsis*. The sequence of *Actinomadura madurae* DSM 43067^T served as an outgroup. Bootstrap values >50 % are shown at nodes of the tree. Bar, 0.01 substitutions per nucleotide position.

myo-inositol. Nitrate reduction and gelatin liquefaction are positive; H₂S production is negative. Melanin is not observed on either tyrosine agar or peptone-yeast-iron agar (ISP media 6 and 7). Whole-cell hydrolysates of the type strain contain *meso*-diaminopimelic acid but no diagnostic sugar. Major phospholipids include diphosphatidylglycerol and phosphatidylcholine, which belong to the type PIII phospholipids pattern based on Lechevalier & Lechevalier (1980). Major menaquinones are MK-10(H₂), MK-10(H₄) and MK-10(H₆). Major cellular fatty acids are iso-C_{16:0} (31.66 %), anteiso-C_{17:0} (14.85 %) and C_{18:1ω9c} (14.73 %). The G+C of the DNA of the type strain is 70.6 mol% (HPLC).

The type strain, HBUM 20028^T (=DSM 45023^T =CGMCC 4.2135^T), was isolated from a soil sample collected from an alkali lake in Xinjiang, China.

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