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 diphostatidylglycerol and phosphatidycholine. The major menaquinones were MK-10(H_2), MK-10(H_4) and MK-10(H_6). The major cellular fatty acids were iso-C\(_{16:0}\) (31.66 %), anteiso-C\(_{17:0}\) (14.85 %) and C\(_{18:1}\) \(\omega_9\)c (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 (Evushenko et al., 2000), N. exhalans, N. umidoscholae (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. alkaliophilia (Hozzein et al., 2004), N. salina (Li et al., 2004), N. aegyptica (Sabry et al., 2004), N. baichengensis, N. chromatogenes, N. gilva, N. rhodophaeae 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.

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 20028ᵀ 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 20028ᵀ 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 diphasphatidylglycerol 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-C₁6:0 (31.66 %), anteiso-C₁₇:0 (14.85 %) and C₁₈:1ω9c (14.73 %).

Chromosomal DNA of strain HBUM 20028ᵀ 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’-GAGTTTGATCTGCTGTCAG-3’; Escherichia coli positions 8–27) and 1525r (5’-AGAAAGGGTGTACCCAGGC-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’-CTAAGCTAGTCCAGCAGCC-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 20028ᵀ 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 90096ᵀ) and 99.93 % (N. exhalans ES10.1ᵀ). The results show that strains HBUM 20028ᵀ, N. exhalans ES10.1ᵀ, N. metallicus KB56ᵀ and N. prasina DSM 43845ᵀ 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 20028ᵀ 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.
neighbour-joining analysis indicated that the closest relatives of strain HBUM 20028T were \textit{N. exhalans} ES10.1T (99.93 % 16S rRNA gene sequence similarity), \textit{N. metallicus} KBS6T (99.58 %) and \textit{N. prasina} DSM 43845T (99.22 %). The values of DNA–DNA relatedness with \textit{N. prasina} DSM 44407T, the closest phylogenetic neighbours. The levels of DNA–DNA relatedness with \textit{N. exhalans} +, Positive; –, negative; \textit{N. metallicus} KBS6T, the closest phylogenetic neighbours. The levels of DNA–DNA relatedness with \textit{N. prasina} DSM 44407T, the closest phylogenetic neighbours. The levels of DNA–DNA relatedness with \textit{N. exhalans} DSM 43845T, \textit{N. metallicus} DSM 44598T and \textit{N. prasina} DSM 43845T, 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 20028T belongs to the genus \textit{Nocardiopsis}. The phylogenetic position of this strain is within a cluster that contains \textit{N. exhalans}, \textit{N. metallicus} and \textit{N. prasina}. However, strain HBUM 20028T can be differentiated from other species by its morphological, physiological and chemotaxonomic characteristics (Table 1). The values of DNA–DNA relatedness of strain HBUM 20028T with related type strains were found to be <70 %. Based on the above results, it is concluded that strain HBUM 20028T represents a novel species of the genus \textit{Nocardiopsis}, for which the name \textit{Nocardiopsis valliformis} sp. nov. is proposed.

**Description of \textit{Nocardiopsis valliformis} sp. nov.**

\textit{Nocardiopsis valliformis} (val.li.for\-'mis. L. n. vallum pali- sade; L. adj. suffix -\textit{formis} -like, in the shape of; N.L. fem. adj. \textit{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 x 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 55 °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 myo-inositol.

### Table 1. Differential phenotypic characteristics of HBUM 20028T and the type strains of closely related \textit{Nocardiopsis} species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial mycelium</td>
<td>White to yellowish</td>
<td>White</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Colony pigmentation (ISP 5)</td>
<td>White</td>
<td>White</td>
<td>Pale yellow</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Optimal pH for growth</td>
<td>9.5–13</td>
<td>10.0</td>
<td>8.5</td>
<td>7.2</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>v</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cellobioso</td>
<td>v</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 °C</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>42 °C</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth in 10 % NaCl</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Major menaquinones</td>
<td>10(H2), 10(H4), 10(H6) (10(H4), 10(H6) ND 10(H6), 10(H8)</td>
<td></td>
<td></td>
<td>10(H4), 10(H8)</td>
</tr>
<tr>
<td>Major phospholipids*</td>
<td>DPG, PC</td>
<td>PC, PME</td>
<td>ND</td>
<td>PC, PI, PG, PME, DPG</td>
</tr>
<tr>
<td>Major cellular fatty acids†</td>
<td>i-C16:0 (32 %), ai-C17:0 (15 %), C16:1ω9c (15 %)</td>
<td>10-Me C18:0 (21 %), ai-C17:0 (18 %)</td>
<td>i-C16:0 (26 %), C18:1ω9c (11 %)</td>
<td></td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>70.6</td>
<td>68.1</td>
<td>70.8</td>
<td>71.0</td>
</tr>
</tbody>
</table>

*DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmonomethanol-amine.
†ai, Anteiso-branched; i, iso-branched; Me, methyl.
The type strain, HBUM 20028T (ATCC 28904T), was isolated from a soil sample collected from an alkaline lake in Xinjiang, China.

Acknowledgements

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