Saccharopolyspora qijiaojingensis sp. nov., a halophilic actinomycete isolated from a salt lake

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A halophilic actinomycete strain, designated YIM 91168T, was isolated from a salt lake in Xinjiang province, north-west China. The isolate grew at 20–40 °C, pH 5–8 and 6–22 % (w/v) NaCl; there was no growth in the absence of NaCl. The whole-cell hydrolysate contained meso-diaminopimelic acid, galactose and arabinose. The major fatty acids were iso-C₁₅ : 0, iso-C₁₆ : 0 and iso-C₁₇ : 0. MK-9(H₄) was the predominant menaquinone and the genomic DNA G+C content was 70.1 mol%. These chemotaxonomic data, together with its morphological properties, were consistent with the assignment of strain YIM 91168T to the genus Saccharopolyspora. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM 91168T had highest sequence similarity (95.4 %) with Saccharopolyspora gregorii NCIB 12823T, and showed lower 16S rRNA gene sequence similarity (93.0–95.1 %) with the other species of the genus Saccharopolyspora. On the basis of evidence from this polyphasic study, the novel species Saccharopolyspora qijiaojingensis sp. nov. is proposed. The type strain is YIM 91168T (=DSM 45088T =KCTC 19235T).

The genus Saccharopolyspora was first described by Lacey & Goodfellow (1975). At the time of writing, it comprises 16 species. The following species have been described in the last two years: Saccharopolyspora antimicrobica (Yuan et al., 2008), S. cebuensis (Pimentel-Elardo et al., 2008), S. shandongensis (Zhang et al., 2008), S. endophytica (Qin et al., 2008), S. halophila (Tang et al., 2009), S.jiangxiensis (Zhang et al., 2009) and S. rosea (Yassin, 2009). Members of the genus Saccharopolyspora are aerobic, Gram-positive, non-acid-fast actinomycetes. The substrate mycelium fragments into rod-shaped elements and the aerial mycelium forms bead-like chains of spores. Whole-cell hydrolysates contain meso-diaminopimelic acid, galactose and arabinose. MK-9(H₄) is the predominant menaquinone. The DNA G+C content is 66–77 mol% (Lacey & Goodfellow, 1975; Korn-Wendisch et al., 1989).

Strain YIM 91168T was isolated from a soil sample collected from Qijiaojing salt lake in Xinjiang Province, north-west China (GPS coordinates for the sampling site 43° 26′ 15″ N 91° 26′ 43″ E). The major ions (mg L⁻¹) were Na⁺ (107 788.4), K⁺ (1658.3), Mg²⁺ (10 357.6), Cl⁻ (26 014.7), SO₄²⁻ (138 318.7) and HCO₃⁻ (2166 009860). The strain was isolated after 3 weeks of incubation at 28 °C on cellulose-casein-multisalts (CCMS) medium, described by Tang et al. (2008). The strain was maintained on International Streptomyces Project (ISP) medium 4 agar slants containing 10 % (w/v) NaCl at 4 °C and as suspensions of mycelium fragments in glycerol (20 %, v/v). Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (about 150 r.p.m.) using ISP medium 4 [10 % (w/v) NaCl, pH 7.5] at 28 °C for 2 weeks.

Cultural characteristics were determined after 3–4 weeks by methods used in the ISP (Shirling & Gottlieb, 1966). All media were supplemented with 10 % (w/v) NaCl for growth. The colours of substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). Strain YIM 91168T grew well on ISP 4 agar and potato agar and showed no growth on Czapek’s agar, ISP 2 agar, nutrient agar and oatmeal agar. Aerial mycelium was less abundant on ISP 4 agar. The colour of aerial and substrate mycelia was white–yellow. No soluble pigments were produced. Morphological characteristics of strain YIM 91168T were observed by light microscopy (model BH 2; Olympus) and scanning electron microscopy (JSM600LV; JEOL) after 21 days growth on ISP 4 agar.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain YIM 91168T is EF687715.
medium containing 10 % (w/v) NaCl. Morphological features of YIM 91168T were consistent with those of members of the genus Saccharopolyspora described previously (Korn-Wendisch et al., 1989). The substrate mycelium was well developed, but fragmented into rod-shaped elements. The aerial mycelium formed beaded chains of spores (Fig. 1).

Growth was tested at 4, 10, 15, 20, 28, 37, 40, 45, 55 and 65 °C on ISP medium 4 containing 10 % (w/v) NaCl. For NaCl tolerance experiments, ISP medium 4 was used as the basal medium, with NaCl added at 0–30 % (w/v), at intervals of 1 %. The pH range for growth was investigated between pH 4.0 and 10.0 at intervals of 1 pH unit, using the following buffers: pH 4.0–5.0, 0.1 M citric acid/0.1 M NaCl; pH 6.0–8.0, 0.1 M KH₂PO₄/0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO₃/0.1 M Na₂CO₃. Media and procedures used for determination of physiological features and carbon source utilization were those described by Williams et al. (1989). Antibiotic susceptibility was determined by the method of Williams (1967). Strain YIM 91168T grew at 20–40 °C, pH 5.0–8.0 and 6–22 % NaCl; there was no growth in the absence of NaCl, showing that strain YIM 91168T is moderately halophilic. The organism can be distinguished from members of Saccharopolyspora with validly published names by using a battery of phenotypic tests (Table 1).

Isomers of diaminopimelic acid and whole-cell sugars were analysed according to the procedures developed by Hasegawa et al. (1983). Polar lipids were extracted and examined by two-dimensional TLC and identified using previously described procedures (Minnikin et al., 1984). Menaquinoles were isolated according to Minnikin et al. (1984) and separated by HPLC (Kroppenstedt, 1982). For fatty acid analysis, cells of strain YIM 91168T were cultured on tryptic soy agar (TSA; Difco) containing 10 % NaCl at 28 °C for 4 days. Cellular fatty acid analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI). Strain YIM 91168T contained meso-diaminopimelic acid as the cell-wall diamino acid, with galactose and arabinose as the major whole-cell sugars (wall chemotype IV; Lechevalier & Lechevalier, 1970). The phospholipids were diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylinositol mannosides and an unknown phospholipid, representing phospholipid pattern III (Lechevalier et al., 1977). The predominant menaquinone was MK-9(H₄) (72.0 %); minor amounts of MK-8(H₄) (17.5 %), MK-9(H₆) (9.7 %) and MK-9(H₈) (2.0 %) were detected. Strain YIM 91168T had a cellular fatty acid profile that contained major amounts of branched fatty acids and minor amounts of straight-chain, unsaturated and methyl fatty acids; it contained iso-C₁₅ : 0 (27.0 %), iso-C₁₇ : 0 (18.9 %), iso-C₁₆ : 0 (13.9 %), anteiso-C₁₇ : 0 (9.3 %), C₁₆ : 0 (7.7 %), C₁₈ : 0 (4.4 %), anteiso-C₁₅ : 0 (3.6 %), C₁₆ : 0 (0.7 %), iso-C₁₅ : 0 2-OH (2.7 %), C₁₇ : 0 (2.6 %), C₁₈ : 0 (2.3 %), 10-methyl C₁₆ : 0 (1.4 %), C₁₄ : 0 (1.3 %), C₁₈ : 0 (1.2 %), anteiso-C₁₆ : 0 (1.1 %), C₁₇ : 0 (1.1 %), iso-C₁₄ : 0 (1.0 %) and N-alcohol C₁₆ : 0 (0.7 %). This pattern represents fatty acid type 2d, according to Kroppenstedt (1985). The chemotaxonomic data for strain YIM 91168T are consistent with its assignment to the genus Saccharopolyspora (Lacey & Goodfellow, 1975; Korn-Wendisch et al., 1989).

Extraction of genomic DNA and PCR amplification of the 16S rDNA gene were done as described by Li et al. (2007). Multiple alignments with sequences of the most closely related Saccharopolyspora species and calculations of levels of sequence similarity were carried out using EzTaxon server 2.0 (Chun et al., 2007). Phylogenetic analyses were performed using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) from K₅₀ values (Kimura, 1980) using MEGA version 4.0 (Tamura et al., 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. Genomic DNA of strain YIM 91168T for the determination of G + C content was prepared according to the method of Marmur (1961). The G + C content of the DNA was determined by reversed-phase HPLC of nucleosides according to Mesbah et al. (1989). The G + C content of the DNA was 70.1 mol %.

Results of 16S rDNA gene sequence comparison clearly demonstrated that strain YIM 91168T is a member of the genus Saccharopolyspora. In the phylogenetic tree based on the neighbour-joining algorithm, strain YIM 91168T formed a monophyletic clade with Saccharopolyspora gregorii NCIB 12823T, and the two strains shared a branch with a bootstrap value of 70 % (Fig. 2). Topologies of phylogenetic trees built using the maximum-likelihood and maximum-parsimony algorithms were similar to that of the tree constructed by neighbour-joining analysis (not shown). The 16S rDNA gene sequence similarity between...
strain YIM 91168<sup>T</sup> and its closest neighbour, <i>S. gregorii</i> NCIB 12823<sup>T</sup>, was 95.4 %, while the similarity with the type strains of other species of the genus was 93.0–95.1 %. It is evident from the phenotypic, chemotaxonomic and phylogenetic data (Table 1 and Fig. 2) that strain YIM 91168<sup>T</sup> represents a novel species in the genus <i>Saccharopolyspora</i>, for which we propose the name <i>Saccharopolyspora qijiaojingensis</i> sp. nov.

**Description of Saccharopolyspora qijiaojingensis sp. nov.**

<i>Saccharopolyspora qijiaojingensis</i> (qi.jiao’jing.en sis. N.L. fem. adj. qijiaojingensis pertaining to Qijiaojing Lake, Xinjiang Province, north-west China, where the sample from which the type strain was isolated was collected).

Aerobic, Gram-positive-staining, moderately halophilic, filamentous actinomycete. Substrate mycelium is well-developed and fragments into rod-shaped elements. Aerial mycelium is less abundant and forms bead-like chains of spores; spores are non-motile and smooth-surfaced, 0.6–0.7 × 0.7–1.1 μm. Temperature, pH and NaCl ranges for growth are 20–40 °C, pH 5.0–8.0 and 6–22 % (w/v) NaCl; optimal growth occurs at 28 °C, pH 7.0 and 10–15 % (w/v) NaCl. Gelatin, hypoxanthine, Tween 20 and xanthine are degraded, but aesculin, casein, starch, cellulose, chitin, Tween 80, adenine, L-tyrosine and urea are not. Test for gelatin liquefaction is positive; tests for nitrate reduction, milk peptonization and coagulation, H<sub>2</sub>S and melanin production and starch hydrolysis are negative. Lactose, D-mannitol, D-mannose, L-rhamnose, D-galactose, maltose and trehalose are utilized as sole carbon sources, while L-arabinose, cellobiose, D-fructose, glycerol, glycogen, sucrose, starch and D-xylose are not. Growth is observed on DL-alanine, L-proline, hypoxanthine, L-histidine, L-asparagine, xanthine and L-hydroxyproline as nitrogen sources; growth is not observed on L-lysine, L-serine, adenine, L-tyrosine or L-phenylalanine. Sensitive to the following antibiotics (μg per disc): amoxicillin (10), ampicillin (10), tetracycline (30), rifampicin (5), erythromycin (15), ciprofloxacin (5), chloramphenicol (30) and vancomycin (30). Resistant to (μg per disc) tobramycin (10), gentamicin (10), streptomycin (10) and sulfaethoxazole/tri-methoprim (23.75/1.25). The predominant menaquinone is MK-9(H<sub>4</sub>). Major cellular fatty acids are iso-C<sub>15:0</sub>, iso-
Fig. 2. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 91168T and its phylogenetic neighbours. Numbers on branch nodes are bootstrap percentages (1000 resamplings; only values over 50% are given). The sequence of Thermocrispum agrestis DSM 44070T was used as outgroup. Bar, 1% sequence divergence.

C_{16:0} and iso-C_{17:0}. The G+C content of the DNA of the type strain is 70.1 mol%.

The type strain is YIM 91168T (=DSM 45088T =KCTC 19235T), isolated from a salt lake in Xinjiang Province, north-west China.

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