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

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A halophilic actinomycete strain, designated YIM 91168^T, 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-C_{15:0}, iso-C_{16:0} and iso-C_{17:0}. 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 91168^T to the genus *Saccharopolyspora*. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM 91168^T had highest sequence similarity (95.4 %) with *Saccharopolyspora gregorii* NCIB 12823^T, 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 qijiaojiangensis* sp. nov. is proposed. The type strain is YIM 91168^T (=DSM 45088^T =KCTC 19235^T).

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 91168^T was isolated from a soil sample collected from Qijiaojiang 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⁻ (185 631.9), SO₄²⁻ (16 381.7) and HCO₃⁻ (318.1). 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 91168^T 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 91168^T were observed by light microscopy (model BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 21 days growth on ISP 4 agar

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 91168^T is EF687715.

medium containing 10 % (w/v) NaCl. Morphological features of YIM 91168^T 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 bead-like 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 sodium citrate; 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 91168^T 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 91168^T 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). Menaquinones were isolated according to Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). For fatty acid analysis, cells of strain YIM 91168^T 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 91168^T con-

tained *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 91168^T 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-C_{15:0} (27.0 %), iso-C_{17:0} (18.9 %), iso-C_{16:0} (13.9 %), anteiso-C_{17:0} (9.3 %), C_{16:0} (7.7 %), C_{18:0} (4.4 %), anteiso-C_{15:0} (3.6 %), C_{16:1ω7c}/iso-C_{15:0} 2-OH (2.7 %), C_{17:0} (2.6 %), C_{18:1ω9c} (2.3 %), 10-methyl C_{16:0} (1.4 %), C_{14:0} (1.3 %), C_{18:2ω6,9c}/anteiso-C_{18:0} (1.2 %), anteiso-C_{16:0} (1.1 %), C_{17:1ω8c} (1.1 %), iso-C_{14:0} (1.0 %) and N-alcohol C_{16:0} (0.7 %). This pattern represents fatty acid type 2d, according to Kroppenstedt (1985). The chemotaxonomic data for strain YIM 91168^T 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 rRNA 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 *K_{nuc}* 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 91168^T 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 rRNA gene sequence comparison clearly demonstrated that strain YIM 91168^T is a member of the genus *Saccharopolyspora*. In the phylogenetic tree based on the neighbour-joining algorithm, strain YIM 91168^T formed a monophyletic clade with *Saccharopolyspora gregorii* NCIB 12823^T, 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 rRNA gene sequence similarity between

Fig. 1. Scanning electron micrograph of fragmented substrate mycelium and bead-like spore chains of strain YIM 91168^T grown on ISP medium 4 (10 % NaCl, w/v) for 21 days at 28 °C. Bar, 5 µm.

Table 1. Differential phenotypic and chemotaxonomic characteristics of strain YIM 91168^T and its closest neighbours in the genus *Saccharopolyspora*

Strains: 1, YIM 91168^T; 2, *S. gregorii* NCIB (now NCIMB) 12823^T; 3, *S. rectivirgula* ATCC 33515^T; 4, *S. taberi* DSM 43856^T; 5, *S. erythraea* NRRL 2338^T; 6, *S. spinosporotrichia* AS4.198^T. Data for strain YIM 91168^T are from this study; data for the other strains are from Goodfellow *et al.* (1989), Korn-Wendisch *et al.* (1989), Labeda (1987) and Zhou *et al.* (1998). +, Positive; –, negative; ND, no data available. All strains degrade xanthine and utilize D-galactose, maltose and L-rhamnose.

Characteristic	1	2	3	4	5	6
Spore arrangement	Straight	Hooks or flexuous hyphae	Straight	NA	Open spirals	Spirals
Colour of:*						
Aerial mycelium	W–Y	W–Y	Y–O	NA	P–BRG–W	W–G
Substrate mycelium	W–Y	C–BF	W–LP	C–Y	OY–RBR	BR–R
Soluble pigment	None	None	None	O–R	Y–POBR	BR
Degradation of:						
Adenine	–	–	–	+	+	–
Casein	–	+	–	+	–	+
Chitin	–	ND	–	+	+	–
Hypoxanthine	+	+	+	+	+	ND
Starch	–	+	–	+	+	–
Tyrosine	–	+	+	+	+	–
Urea	–	–	+	+	+	ND
Nitrate reduction	–	–	+	+	+	–
Temperature for growth (°C)	20–40	10–35	37–63	20–45	20–42	28–37
NaCl tolerance (% w/v)	6–22	0–13	0–10	0–7	0–5	0–3
Utilization of:						
L-Arabinose	–	+	–	–	+	–
Lactose	+	–	+	+	–	–
Sucrose	–	+	+	+	+	+
D-Xylose	–	+	+	+	+	+
DNA G + C content (mol%)	70.1	74	70.4	70.8	71.1	70.4

*BF, Buff; BR, brown; C, colourless; G, grey; LP, light pink; O, orange; P, pink; R, red; W, white; Y, yellow.

strain YIM 91168^T and its closest neighbour, *S. gregorii* NCIB 12823^T, 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^T represents a novel species in the genus *Saccharopolyspora*, for which we propose the name *Saccharopolyspora qijiaojiangensis* sp. nov.

Description of *Saccharopolyspora qijiaojiangensis* sp. nov.

Saccharopolyspora qijiaojiangensis (qi.jiao'jing.en'sis. N.L. fem. adj. *qijiaojiangensis* pertaining to Qijiaojiang 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, H₂S 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 sulfamethoxazole/trimethoprim (23.75/1.25). The predominant menaquinone is MK-9(H₄). Major cellular fatty acids are iso-C_{15:0}, iso-

Fig. 2. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 91168^T and its phylogenetic neighbours. Numbers on branch nodes are bootstrap percentages (1000 resamplings; only values over 50 % are given). The sequence of *Thermocrisum agreste* DSM 44070^T 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 91168^T (=DSM 45088^T =KCTC 19235^T), isolated from a salt lake in Xinjiang Province, north-west China.

Acknowledgements

The authors are grateful to the anonymous reviewers for their helpful comments. This research was supported by the National Basic Research Program of China (no. 2004CB719601), the National Natural Science Foundation of China (nos 30600001, 30860002, 30870005), the Yunnan Provincial International Cooperative Program (no. 2005GH21), the Ministry of Science of Technology, PR China (2006DFA33550), and the Youth Technological Innovation Foundation of Xinjiang Academy of Agricultural Science (no. 2007Q07). W.-J.L. was supported by the Program for New Century Excellent Talents in University.

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