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ORIGINAL PAPER

Saccharopolyspora lacisalsi sp. nov., a novel halophilic actinomycete isolated from a salt lake in Xinjiang, China

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Abstract A novel actinomycete strain, designated TRM 40133^T, was isolated from a hypersaline habitat of Tarim basin in Xinjiang Province, north-west China. Its taxonomic status was determined using a polyphasic approach. Phylogenetic analysis based on an almost-complete 16S rRNA gene sequence of the strain showed that it formed a well-seperated sub-branch within the radiation of the genus *Saccharopolyspora*. The highest levels of 16S rRNA gene sequence similarity was found between the strain TRM 40133^T and *Saccharopolyspora qijiaojingensis* YIM 91168^T (96.5%). The chemotaxonomic characteristics of the isolate are typical for the genus *Saccharopolyspora*. It contained *meso*-DAP as the diagnostic diamino acid. Whole cell hydrolysate contained arabinose, xylose, ribose and glucose. The diagnostic phospholipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol and two unknown phospholipids. The main menaquinone was MK-9(H_6) and MK-9(H_4). No mycolic acid was detected. The predominant cellular fatty acids were iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The G+C content of the genomic DNA was 68.2 mol%. In addition, the strain TRM 40133^T had a phenotypic profile that readily distinguished it from the recognized representatives of the genus *Saccharopolyspora*. The strain TRM 40133^T therefore represents a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora lacisalsi* sp. nov. is proposed. The type strain is TRM 40133^T (=KCTC 19987^T =CCTCC AA 2010012^T).

Keywords *Saccharopolyspora lacisalsi* sp. nov. · Halophilic · Salt lake

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Introduction

The genus *Saccharopolyspora* was first described by Lacey and Goodfellow (1975) and was assigned to the family Pseudonocardiaceae (Warwick et al. 1994). Currently, the genus comprises 20 species, namely *S. hirsuta* (Lacey and Goodfellow 1975), *S. erythraea* (Labeda 1987), *S. taberi* (Labeda 1987; Korn-Wendisch et al. 1989), *S. gregorii* (Goodfellow et al. 1989), *S. hordei* (Goodfellow et al. 1989), *S. rectivirgula* (Korn-Wendisch et al. 1989),

S. spinosa (Mertz and Yao 1990), *S. spinosporotrichia* (Zhou et al. 1998), *S. flava* (Lu et al. 2001), *S. thermophila* (Lu et al. 2001), *S. antimicrobica* (Yuan et al. 2008), *S. shandongensis* (Zhang et al. 2008), *S. cebuensis* (Pimentel-Elardo et al. 2008), *S. halophila* (Tang et al. 2009a, b), *S. jiangxiensis* (Zhang et al. 2009), *S. rosea* (Yassin 2009), *S. qijiaojingensis* (Tang et al. 2009a, b), S. *tripterygii* (Li et al. 2009), *S. gloriosae* (Qin et al. 2010) and *S. phatthalungensis* (Duangmal et al. 2010). Most species in the genus *Saccharopolyspora* were not halophilic except for *S. halophila* (Tang et al. 2009a, b) and *S. qijiaojingensis* (Tang et al. 2009a, b). The present study is a part of our long-term investigations into the diversity of halophilic actinomycetes from salt lakes of Xinjiang Province, China. In this paper, a halophilic actinomycete, designated strain TRM 40133^T, was isolated. Based on data from the present polyphasic taxonomic research, this strain is considered to represent a novel species of the genus *Saccharopolyspora*.

Materials and methods

Organism

Strain TRM 40133^T was isolated from a soil sample collected from the Lop Nur salt lake, Xinjiang Province, north-west China (GPS coordinates for the sampling site is 39°41′79″ N89°54′53″ E) using YC medium. The soil sample had a temperature of 23°C with a pH of 7.6. The lake environment was described previously (Guan et al. 2010). Sample was collected in sterile tube and brought to the laboratory in Tarim University, China, where it was stored at 4°C for several weeks before the experiments were started. This YC medium contained (g l^{-1}): yeast extract, 3.0 g; casein hydrolysate acid, 0.5 g; KNO_3, 0.5 g; $CaCO_3$, 0.1 g; NaCl, 200 g; KCl, 20 g; $MgCl_2$, 2 g; 1.2 ml trace element solution (Slobodkin et al. 1997); agar, 20.0 g; pH 7.5. The organism was grown and maintained on ISP 4 medium (Shirling and Gottlieb 1966) containing 15% (w/v) NaCl, and glycerol suspension 20% (v/v) at −20°C or lyophilized cell for long term storage at −4°C.

Phenotypic characteristics

Cultural characteristics of the strain TRM 40133^T were examined on ISP medium (Shirling and Gottlieb 1966). All media were supplemented with 15% (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). For the observation of cell morphology, the strain was grown on ISP 4 medium at 37°C for 3–4 weeks and examined using light microscopy (Olympus microscope BH-2) and scanning electron microscopy (JSM 5600LV; JEOL). The Gram staining and the KOH lysis test were carried out according to Doetsch (1981) and Gregersen (1978), respectively. The temperature range (4–55°C) and pH values (4.0–12.0) for growth were determined on ISP 4 medium supplemented with 15% NaCl as described by Xu et al. (2005). NaCl tolerance was studied on ISP medium 4 containing NaCl at final concentration of 0–30% (w/v) (at intervals of 1.0%). 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).

Chemotaxonomic characterization

Biomass for chemotaxonomic and molecular studies was obtained after incubation at 37°C for 1 week by growing in shake flasks of tryptic soy (Difco) containing 15% NaCl. The isomer type of diaminopimelic acid (DAP) in cell-wall peptidoglycan was determined by the method of Hasegawa et al. (1983). Mycolic acids were checked by the acid methanolysis method of Minnikin et al. (1980). Whole-cell sugars were analysed as described by Staneck and Roberts (1974). Menaquinones were extracted using the method of Collins et al. (1977) and analysed by HPLC (Groth et al. 1997). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin et al. 1979; Collins and Jones 1980). The fatty acids were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification system (Sasser 1990; Kämpfer and Kroppenstedt 1996). Genomic DNA of the strain TRM 40133^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).

Determination of 16S rRNA gene sequence and phylogenetic analysis

The phylogenetic position of the isolate TRM 40133^T was determined by 16S rRNA gene sequence. Amplification and sequencing of the 16S rRNA gene sequence were performed as described according to Cui et al. (2001) and PCR products were purified using the PCR purification kit (Sangon in Shanghai, China). The almost-complete 16S rRNA gene sequence of the strain TRM 40133^T (1,418 nucleotides) was obtained. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of *Saccharopolyspora lacisalsi* TRM 40133^T is JF411070. Multiple alignments with sequences of most closely related *Saccharopolyspora* 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 and Nei 1987) and maximum-parsimony (Fitch 1971) methods. Phylogenetic analysis was performed using the software package 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 1,000 replicates. Evolutionary distance matrices were generated as described by Kimura (1980).

Results and discussion

Phenotypic characteristics

The growth of the isolate TRM 40133^T was good on inorganic salts-starch agar, Czapek's agar and yeast extract-malt extract agar, moderate on oatmeal agar, weak on glycerol/asparagine agar and nutrient agar. The colour of aerial mycelia was white on most tested media except for yellowish grey on nutrient agar. Substrate mycelia were yellow or yellow brown. No soluble pigments were produced. The substrate mycelium was well developed, but fragmented into rod-shaped elements. The aerial mycelium formed bead-like chains of spores. Spores are non-motile, elliptical and smooth-surfaced. Strain TRM 40133^T growth occurred in the presence of 5–25% (w/v) NaCl (optimum 15%), at pH 6–8 (optimum pH 7.5) and at 25–42°C (optimum 37°C). Other detailed phenotypic properties that differentiate the strain TRM 40133^T from recognized *Saccharopolyspora* species are summarized in Table 1 and also mentioned in the species description below.

Phylogenetic analysis based on 16S rRNA gene sequence comparison

The almost-complete 16S rRNA gene sequence (1,418 bp) of the organism was determined. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the strain TRM 40133^T was a member of the genus *Saccharopolyspora* and was most closely related to the type strain

Table 1 Comparison of the phenotypic properties of the strain TRM 40133^T and its nearest phylogenetic neighbours

Characteristics	TRM 40133^T	*S. qijiaojingensis*	*S. gloriosae*	*S. gregorii*
Spore arrangement	Straight	Straight	Hooks and curved hyphae	Hooks, flexuous hyphae
Colour of				
Aerial mycelium	White, yellowish grey	Yellowish white	White	Yellowish white
Substrate mycelium	Yellow, yellowish buff	Yellowish white	White, yellowish white, pale orange-yellow	Colourless, buff
Degradation of				
Adenine	−	−	−	−
Casein	−	−	−	+
Hypoxanthine	−	+	+	+
Starch	−	−	+	+
Tyrosine	−	−	+	+
Gelatin	+	+	−	+
Xanthine	−	+	+	−
Nitrate reduction	+	−	−	−
Temperature range (°C)	25–42	20–40	10–32	10–35
NaCl range (%, w/v)	5–25	6–22	0–11	0–13
Utilization of				
L-arabinose	−	−	+	+
D-xylose	−	+	+	−
Raffinose	+	−	−	+
Sucrose	+	−	−	+
D-galactose	+	+	−	+
Lactose	−	+	−	−
L-rhamnose	−	+	+	−
Trehalose	+	+	+	−
DNA G+C content (mol%)	68.2	70.1	71.6	74

Data are from this study and from Goodfellow et al. (1989), Tang et al. (2009a, b) and Qin et al. (2010)

+ positive, − negative

S. qijiaojingensis YIM 91168^T (sequence similarity 96.5%). Lower than 96.1% sequence similarity was observed with other *Saccharopolyspora* species. It is generally accepted that organisms displaying 16S rRNA sequence similarity values of 97% or less belong to different species (Stackebrandt and Goebel 1994). The recognized species *S. qijiaojingensis* YIM 91168^T, *S. gloriosae* YIM 60513^T, *S. gregorii* NCIMB 12823^T and the strain TRM 40133^T form the closely phylogenetic neighbours in the phylogenetic tree (Fig. 1). The topology was similar to those of the phylogenetic trees constructed using maximum-parsimony methods (see Supplementary Fig. S2). Therefore, it would appear that, on the basis of the phylogenetic analysis, the strain TRM 40133^T represents a new species of the genus *Saccharopolyspora* according to the accepted criteria (Stackebrandt and Goebel 1994).

Chemotaxonomic characteristics and DNA base composition

The chemotaxonomic characteristics of the isolate TRM 40133^T are typical for the genus *Saccharopolyspora*. The isolate contains *meso*-diaminopimelic acid as the wall diamino acid and no mycolic acids. The phospholipid pattern consists of phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and two unknown phospholipids. Major cell-wall sugars were arabinose, xylose, ribose and glucose. The predominant menaquinones of the strain TRM 40133^T are MK-9(H_6) (79%) and MK-9(H_4) (21%). The major fatty acids (>5% of the total) of the strain TRM 40133^T were iso-$C_{16:0}$ (23.0%), anteiso-$C_{17:0}$ (24.9%), iso-$C_{15:0}$ (8.8%), anteiso-$C_{15:0}$ (5.7%), $C_{16:0}$ (5.7%), and iso-$C_{17:0}$ (9.7%). This fatty acid profile was similar to those of recognized *Saccharopolyspora* species, although there were differences in the proportions of some components (Goodfellow et al. 1989; Tang et al. 2009a, b; Qin et al. 2010). The DNA G+C content of the strain TRM 40133^T was 68.2 mol%.

Taxonomic conclusion

The results of the phylogenetic analysis and morphological and chemotaxonomic investigations supported the affiliation of the strain TRM 40133^T to the genus *Saccharopolyspora*.

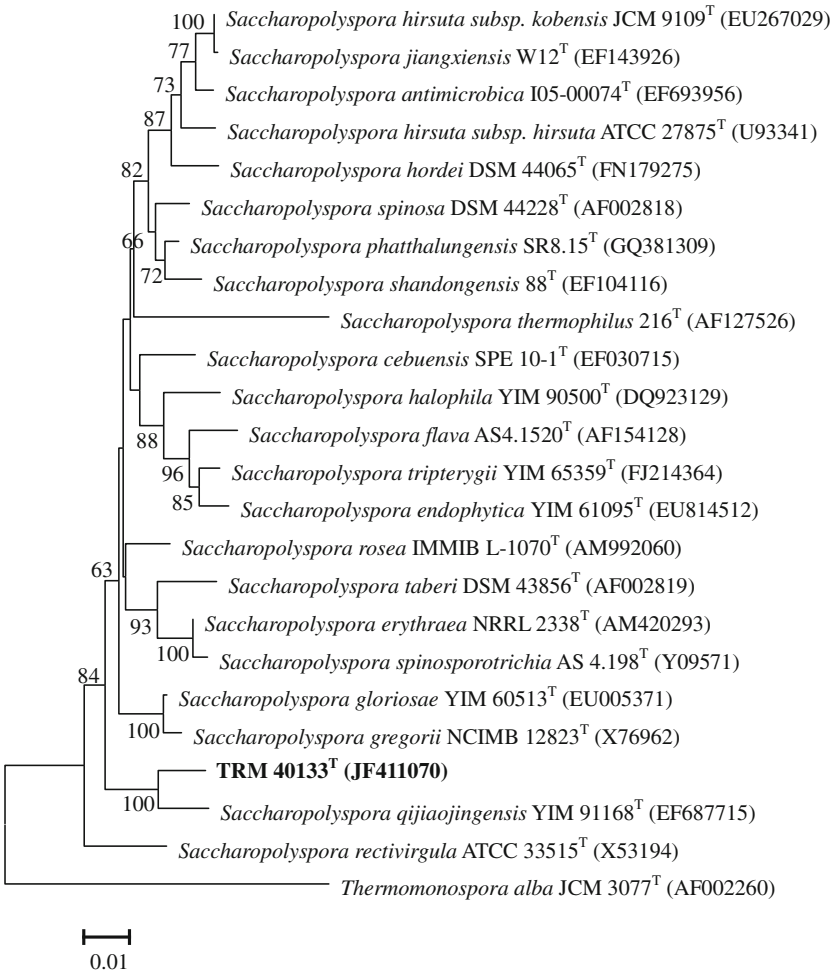

Fig. 1 Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationship of the novel isolate and related taxa. Bootstrap values are based on 1,000 replicates; only values >50% are shown. *Bar* 0.01 substitutions per nucleotide position

What is more, the novel strain could be clearly distinguished from the three recognized *Saccharopolyspora* species by differences in several phenotypic characteristics, including cell morphology, growth condition, utilization of substrates, activity of some enzymes, DNA base composition and so on (Table 1). And the strain TRM 40133^T exhibited comparatively low 16S rRNA gene sequence similarity with the closest neighbour *S. qijiaojingensis* (96.5%). In conclusion, the results of phylogenetic analysis based on 16S rRNA gene sequences, the phenotypic and chemotaxonomic data presented here supported the proposal of the strain TRM 40133^T representing a novel species of the genus *Saccharopolyspora*, for which the name *S. lacisalsi* sp. nov. is proposed.

Description of *S. lacisalsi* sp. nov

S. lacisalsi (L. n. lacus, lake; L. adj. salsus, salted; N.L. gen. n. lacisalsi, of a salt lake).

Aerobic, non-motile, Gram-positive-staining, halophilic (optimum 15%, range 5–25% NaCl), filamentous actinomycete. Oxidase-negative and catalase-positive. Gelatin, Tweens 20, 40, 60, 80 and cellulose are degraded, but casein, xanthine, adenine, hypoxanthine, L-tyrosine and urea are not. Nitrate reduction and methyl red test are positive. Tests for indole production, Voges-Proskauer, milk peptonization and coagulation, H_2S and melanin production and starch hydrolysis are negative. D-mannitol, D-galactose, D-mannose, D-glucose, L-maltose, trehalose, sorbitol, starch, L-raffinose, cellobiose and sucrose are utilized as sole carbon sources, while xylitol, lactose, erythritol, L-rhamnose, L-arabinose, D-ribose, D-fructose, D-xylose, dextrine and dulcitol are not. Susceptible (per disc) to novobiocin (5 μg), ofloxacin (5 μg), tetracycline (30 μg), amoxicillin (10 μg), vancomycin (30 μg), tobramycin (10 μg), ciprofloxacin (5 μg), neomycin (30 μg), chloramphenicol (30 μg), rifampin (5 μg), amikacin (30 μg), clindamycin and erythromycin (15 μg); but not to netilmicin (10 μg), streptomycin (10 μg), norfloxacin (10 μg), gentamicin (10 μg), ampicillin (10 μg) and amikacin (30 μg). The cell wall of the strain TRM 40133^T contains *meso*-DAP. Mycolic acids are absent. The predominant menaquinones are MK-9(H_4) and MK-9(H_6). Major fatty acids (>10%) are iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The G+C content of genomic DNA is 68.2 mol%.

The type strain, TRM 40133^T (=KCTC 19987^T =CCTCC AA 2010012^T), was isolated from a sediment of Lop Nur salt lake in Xinjiang, north-west China.

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