Saccharopolyspora halotolerans sp. nov., a halophilic actinomycete isolated from a hypersaline lake

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A novel actinomycete strain, designated TRM 45123T, was isolated from a hypersaline habitat in Xinjiang Province (40° 20’ N 90° 49’ E), north-west China. The isolate was characterized using a polyphasic approach. 16S rRNA gene sequence analysis indicated that strain TRM 45123T belonged to the genus Saccharopolyspora and was closely related to Saccharopolyspora gloriosae (96.7 % similarity). The G+C content of the DNA was 69.07 mol%. The isolate contained meso-diaminopimelic acid as the diagnostic diamino acid, and arabinose and ribose as the major whole-cell sugars. The diagnostic phospholipids were phosphatidylethanolamine, phosphatidylcholine and phosphatidylglycerol. The predominant menaquinone was MK-9(H4). The major fatty acids were iso-C15 : 0, anteiso-C17 : 0, iso-C15 : 0 and anteiso-C15 : 0. On the basis of the evidence from this polyphasic study, a novel species, Saccharopolyspora halotolerans sp. nov., is proposed. The type strain of Saccharopolyspora halotolerans is TRM 45123T (=CCTCC AA 2013006T =DSM 45990T).

The genus Saccharopolyspora was first described by Lacey & Goodfellow (1975) and was assigned to the family Pseudonocardiaee (Warwick et al., 1994). At the time of writing, the genus Saccharopolyspora contained 22 species and one subspecies with validly published names: Saccharopolyspora hirsuta (Lacey & Goodfellow, 1975), S. erythreae (Labeleda, 1987), S. taberi (Labeleda, 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. hirsuta subsp. kobensis (Lacey, 1989), S. spinosa (Mertz & Yao, 1990), S. spinosporotrichia (Zhou et al., 1998), S. flava (Lu et al., 2001), S. thermophila (Lu et al., 2001), S. antitracibica (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. jiexiuensis (Zhang et al., 2009), S. rosea (Yassin, 2009), S. qijiaojingensis (Tang et al., 2009a, b), S. tripeterita (Li et al., 2009), S. gloriosa (Qin et al., 2010), S. phatthalungensis (Duangmal et al., 2010), S. lacinisai (Guan et al., 2011) and S. dendranthema (Zhang et al., 2013). The present study is a part of our long-term investigations into the diversity of halophilic actinomycetes from Lop Nor, in Xinjiang Province, China. In this study, a halophilic actinomycete, designated strain TRM 45123T, was isolated. Based on data from the present polyphasic taxonomic research, this strain is considered to represent a novel species of the genus Saccharopolyspora.

Strain TRM 45123T was isolated from a playa sample collected from the Lop Nor Lake, Xinjiang Province, north-west China (40° 20’ N 90° 49’ E). The strain was isolated from a glycerol-arginine agar plate that had been seeded with a soil suspension, followed by aerobic incubation at 37 °C for 3 weeks. The composition of the glycerol-arginine agar medium was (l): 2 g arginine, 12.5 g glycerol, 0.01 g FeSO₄·7H₂O, 2 g K₂HPO₄·3H₂O, 0.05 g MgSO₄·7H₂O, 0.001 g CuSO₄·5H₂O, 0.001 g ZnSO₄·7H₂O, 0.001 g MnSO₄·H₂O, 15 g agar. Complex salts, including 76 g NaCl, 8 g MgCl₂·6H₂O, 8 g KCl, and 8 g CaCl₂·2H₂O, were sterilized separately before being added to the medium. The pH of the medium was adjusted to pH 7.5 with 1 M NaOH. The strain was maintained on ISP 4 (Shirling & Gottlieb, 1966) agar slants containing 10 % (w/v) NaCl at 4 °C. The pure culture was maintained as a 20% (v/v) glycerol suspension at −20 °C, or as lyophilized cells for long-term preservation. Biomass for chemical and molecular studies was obtained by cultivation in tryptic soy broth on a shaker at 180 r.p.m., at 37 °C for 10 days. The tryptic soy broth was prepared by adding 10.67 g tryptic soy broth to a flask, and then sterilized in a water bath at 121 °C for 20 min. After cooling, 6 g glucose, 7.5 g NaCl, 2.5 g KCl, and 1 g MgSO₄·7H₂O were added and sterilized separately before being added to the medium. After the strain was inoculated into the medium, the pH was adjusted to pH 7.5 with 1 M NaOH, and the medium was adjusted to 121 °C for 30 min.
broth medium was (g\textsuperscript{-1}): Tryptic Soy Broth (OXOID) 30g, NaCl 100g.

Cultural characteristics were determined after 2 weeks by methods used in the International Streptomyces Project (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). Morphological characteristics of strain TRM 45123\textsuperscript{T} were observed by light microscopy (Axioskop 20; Zeiss) and scanning electron microscopy (Quanta; FEI) after 28 days of growth on ISP 4 containing 10 % (w/v) NaCl. Growth was tested at 4, 10, 15, 20, 28, 37, 40, 45, 55 and 65 °C on ISP 4 containing 10 % (w/v) NaCl. For NaCl tolerance experiments, ISP 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 12.0 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 KH\textsubscript{2}PO\textsubscript{4}/0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO\textsubscript{3}/0.1 M Na\textsubscript{2}CO\textsubscript{3}; pH 11.0–12.0, 0.05 M Na\textsubscript{2}HPO\textsubscript{4}/0.1 M NaOH. Media and procedures were used for determination of physiological features and carbon source utilization were those described by Williams et al. (1989). Enzyme activity and acid production from carbohydrates were determined by using the API ZYM and API 50 CH systems (bioMérieux) according to the manufacturer’s instructions. Antibiotic susceptibility was determined by the method of Williams (1967). Other physiological characteristics, including utilization of sole carbon and nitrogen sources for energy and growth, and decomposition of test substances, were assessed by using the media and methods of Gordon et al. (1974).

Isomers of diaminopimelic acid were analysed according to the procedures developed by Hasegawa et al. (1983). Amino acid and sugar analysis of whole-cell hydrolysates was performed according to the procedures described by Stanek & Roberts (1974). Polar lipids were examined by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Menaquinones were extracted using the method of Collins (1985) and analysed by HPLC (Groth et al., 1997). Cellular fatty acid composition was determined using the Microbial Identification System (Sherlock version 6.0; MIDI). Mycolic acids were checked by the acid methanolysis method of Minnikin et al. (1975). The G+C content of the genomic DNA for strain TRM 45123\textsuperscript{T} was determined by HPLC as described by Tamaoka & Komagata (1984).

Genomic DNA extraction and PCR amplification of the 16S rRNA gene from strain TRM 45123\textsuperscript{T} were performed using an established method (Chun & Goodfellow, 1995). Multiple alignments with sequences of the most closely related taxa and calculations of sequence similarity were carried out using EzTaxon-e (Kim et al., 2012). Phylogenetic analyses were performed using three tree-making algorithms: neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981). Phylogenetic trees were reconstructed using MEGA version 5 (Tamura et al., 2011) and the topology of the phylogenetic trees was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Strain TRM 45123\textsuperscript{T} grew well on ISP 2 agar, ISP 3 agar, ISP 4 agar, ISP 6 agar, nutrient agar and Czapek solution agar, and weakly on ISP 5 agar, ISP 7 agar and potato-glucose agar. The colour of aerial mycelia was white on ISP 4 agar. The colour of substrate mycelia was yellowish on ISP 3 agar, ISP 5 agar, ISP 6 agar, potato-glucose agar and nutrient agar, yellow on Czapek solution agar, and light brown on ISP 2 agar and ISP 7 agar. No soluble pigments were produced on any of the media tested. The aerial mycelium was unbranched, straight or helical, and spores were non-motile, rough-surfaced, spherical or oval, and budded from the aerial mycelium (see Fig. 1). Strain TRM 45123\textsuperscript{T} grew at 15–42 °C, at pH 4.0–9.0 and with 3–20 % NaCl, with optimum growth at pH 7.0 and 10 % NaCl (w/v) at 37 °C. The organism could be distinguished from members of the genus Saccharopolyspora with validly published names by using a battery of phenotypic tests (Table 1). It is clear from the comparisons that strain TRM 45123\textsuperscript{T} is phenotypically different from the closest related species of the genus Saccharopolyspora.

The cell wall of strain TRM 45123\textsuperscript{T} contained meso-diaminopimelic acid. Whole-cell hydrolysates contained mainly arabinose and ribose. The predominant menaquinone was MK-9(H\textsubscript{4}) (87.50 %), with minor amounts of MK-8(H\textsubscript{4}) (5.23 %), MK-9(H\textsubscript{8}) (4.11 %) and MK-8(H\textsubscript{8}) (0.78 %). The diagnostic phospholipids were phosphatidylcholine, diphasphatidylglycerol, phosphatidylinositol, and phosphatidylglycerol (Table 2). The 16S rRNA gene of strain TRM 45123\textsuperscript{T} was 99.99 % similar to the 16S rRNA gene of strain TRM 45123\textsuperscript{T} (Table 2). The phylogenetic analysis placed strain TRM 45123\textsuperscript{T} within the genus Saccharopolyspora, and the closest related species were "Saccharopolyspora" halotolerans (95.1 % similarity) and "Saccharopolyspora" mediterranea (95.0 % similarity). The phylogenetic tree is shown in Fig. 2.

**Fig. 1.** Scanning electron micrograph of aerial mycelium and spore of strain TRM 45123\textsuperscript{T} on ISP 4 (10 %, w/v, NaCl) after incubation for 4 weeks. Bar, 5 μm.
phosphatidylinositol mannosides, and phosphatidylglycerol (Fig. S1, available in the online Supplementary Material). Major cellular fatty acids (>10%) were iso-C16:0 (19.74%), anteiso-C17:0 (16.62%), iso-C15:0 (15.24%) and anteiso-C15:0 (10.75%). Fatty acids present in smaller amounts (<1%) were iso-C14:0 (7.28%), iso-C17:0 (5.40%), C17:1 ω8c (3.59%), iso-C16:1 ω (2.39%), C16:0 (1.90%), C17:0 (1.51%) and C18:1 ω9c (1.19%). The organism did not contain mycolic acids. The G+C content of the DNA was 69.07 mol%.

In the phylogenetic tree based on the neighbour-joining algorithm, strain TRM 45123T formed a distinct clade, and belonged to the genus Saccharopolyspora (Fig. 2). The highest level of 16S rRNA gene sequence similarity was shown with S. gloriosae YIM 60513T (GenBank accession no. EU005371; 96.67%). The sequence similarity between strain TRM 45123T and members of other species of the genus Saccharopolyspora was 93.96–96.67%. This relationship was supported by other two tree-making methods used in this study. The low level of 16S rRNA gene sequence similarity (<97%) between species of the genus Saccharopolyspora with validly published names and the novel isolate indicates that the isolate represents a novel genomic species of the genus Saccharopolyspora.

Strain TRM 45123T was different from members of other species of the genus Saccharopolyspora in some morphological and physiological properties: scanning electron microscopic observations of strain TRM 45123T showed the aerial mycelium was unbranched, straight or helical, and spores were non-motile, rough-surfaced, spherical or oval, and budded from the aerial mycelium. These features enabled it to be differentiated from S. gloriosae YIM 60513T, the nearest phylogenetic neighbour, and phylogenetically closely related species. TRM 45123T was also negative for hydrolysis of hypoxanthine and xanthine, and for H2S production, and positive for gelatin liquefaction and casein hydrolysis. Trehalose, raffinose and L-rhamnose were utilized as sole carbon sources, while lactose, D-galactose and sucrose were not. Moreover, strain TRM 45123T exhibited some chemotaxonomic differences from the species S. gloriosae and S. gregorii: strain TRM 45123T contained arabinose and ribose as whole-cell hydrolysates; MK-9(H4) as the predominant menaquinone; and phosphatidylinositol, phosphatidylglycerol, and phosphatidylinositol mannosides and phosphatidylglycerol as diagnostic phospholipids (Table 1). Additionally, strain TRM 45123T exhibited comparatively low 16S rRNA gene sequence similarity with the closest neighbour, S. gloriosae YIM 60513T (GenBank accession no. EU005371; 96.67%). In the phylogenetic tree based on the neighbour-joining algorithm, strain TRM 45123T formed a distinct clade, belonged to the genus Saccharopolyspora (Fig. 2).
On the basis of a combination of phylogenetic distinctness and differences in chemotaxonomic and morphological characteristics, strain TRM 45123\textsuperscript{T} represents a novel species in the genus \textit{Saccharopolyspora}, for which the name \textit{Saccharopolyspora halotolerans} sp. nov. is proposed.

**Description of \textit{Saccharopolyspora halotolerans} sp. nov.**

\textit{Saccharopolyspora halotolerans} (ha.lo.to.\textit{le.rans}. Gr. n. hals haloes salt; L part. tolerans tolerating; N.L. part. adj. halotolerans tolerating high salt concentrations).

Aerobic, Gram-stain-positive, non-acid-fast, filamentous actinobacterium. The aerial mycelium is unbranched, straight or helical; spores are non-motile, rough-surfaced, spherical or oval, and bud from the aerial mycelium. Grows well on ISP 2 agar, ISP 3 agar, ISP 4 agar, ISP 6 agar, nutrient agar and Czapek solution agar, and weakly on ISP 5 agar, ISP 7 agar and potato-glucose agar. No soluble pigments are produced on any media tested. Grows at 15–42°C (optimum 37°C), at pH 4.0–9.0 (optimum pH 7.0) and with 3–20% (w/v) NaCl (optimum 10% NaCl). Negative for decomposition of urea and cellulose, hypoxanthine hydrolysis, xanthine hydrolysis, H\textsubscript{2}S production, nitrate reduction and oxidase test. Positive for milk coagulation and peptonization, gelatin liquefaction, starch hydrolysis, melanin production, casein hydrolysis and catalase reaction. Tweens 20, 40, 60 and 80 are degraded. Trehalose, raffinose, L-rhamnose, L-arabinose, glucose, chitosan, inositol, mannitol, cellulose, D-fructose, sorbitol, starch and D-xylene are utilized as sole carbon sources, while lactose, D-galactose and sucrose are not. Acid is not produced from these carbon sources. Utilizes L-histidine and L-cysteine as sole nitrogen sources. The whole-cell hydrolysates contain \textit{meso}-diaminopimelic acid as the cell-wall diamino acid, and arabinose and ribose as the major whole-cell sugars. The diagnostic phospholipids are phosphatidylcholine, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and phosphatidylglycerol. The predominant menaquinone is MK-9(H\textsubscript{4}), with minor amounts of MK-8(H\textsubscript{4}), MK-9(H\textsubscript{6}) and MK-8(H\textsubscript{6}). Major cellular fatty acids are iso-C\textsubscript{16:0}, anteiso-C\textsubscript{17:0}, iso-C\textsubscript{15:0} and anteiso-C\textsubscript{15:0}. Lacks mycolic acids.

The type strain is TRM 45123\textsuperscript{T} (\textsuperscript{5}CCTCC AA 2013006\textsuperscript{T} = DSM 45990\textsuperscript{T}), isolated from a playa sample collected from the Lop Nor located at Xinjiang in north-west China. The G+C content of the DNA of type strain is 69.07 mol%.

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References


