Amycolatopsis salitolerans sp. nov., a filamentous actinomycete isolated from a hypersaline habitat

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A novel actinomycete strain, designated TRM F103^T, was isolated from a hypersaline habitat of the Tarim basin in Xinjiang province, north-west China. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate belonged to the genus *Amycolatopsis* and was most closely related to *Amycolatopsis halophila* YIM 93223^T (99.3 % 16S rRNA gene sequence similarity). However, DNA–DNA relatedness between these two strains, based on triplicate experiments, was only 31.6 %. The isolate contained *meso*-diaminopimelic acid and ribose, glucose and galactose as the major whole-cell sugars. The predominant menaquinone was MK-8(H₄). The major fatty acids were iso-C_{16:0} and C_{16:0}. The polar lipids were diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine and glucosamine-containing phospholipids. The G + C content of the genomic DNA was 66.4 mol%. The phenotypic data clearly distinguished the isolate from its closest relatives. The combined phylogenetic, chemotaxonomic and phenotypic data indicate that the isolate represents a novel species of the genus *Amycolatopsis*. The proposed name is *Amycolatopsis salitolerans* sp. nov., with TRM F103^T (=JCM 15899^T=CCTCC AB 208326^T) as the type strain.

The genus *Amycolatopsis* (Lechevalier *et al.*, 1986), first proposed as a novel genus of nocardioform actinomycetes in 1986, was classified in the family *Pseudonocardiaceae*, suborder *Pseudonocardineae* (Stackebrandt *et al.*, 1997) on the basis of 16S rRNA gene sequence analysis. Not long ago, we described a halophilic species in the genus *Amycolatopsis*, namely *Amycolatopsis halophila* (Tang *et al.*, 2010), which was isolated from a salt lake in Xinjiang province, north-west China. Recently, during a study of the cultivable aerobic bacterial community in a hypersaline habitat, a novel halotolerant, filamentous, *Amycolatopsis*-like actinomycete was isolated from a similar hypersaline environment in the Tarim basin, Xinjiang province; the habitat and

precise location were described previously (Guan *et al.*, 2010).

Strain TRM F103^T was isolated under aerobic conditions on GTY agar (containing l⁻: 5 g glucose, 0.5 g tryptone, 2 g yeast extract, 1 g CaCO₃, 0.5 g K₂PO₄ and 16.0 g agar; pH 7.0–7.5) supplemented with 10 % (w/v) NaCl. The strain was purified and maintained on GTY agar slants supplemented with 5 % NaCl and stored as a suspension in 20 % (v/v) glycerol at –20 °C or as lyophilized cells. Biomass for chemical and molecular studies was obtained by cultivation in shake flasks (about 170 r.p.m.) using GTY broth supplemented with 5 % NaCl (pH 7.0) at 37 °C for 1 week. *A. halophila* YIM 93223^T was also analysed in this study to compare phenotypic characteristics and to determine DNA–DNA relatedness.

Amplification and sequencing of the 16S rRNA gene was performed as described by Cui *et al.* (2001) using a

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TRM F103^T is HQ436534.

A supplementary figure is available with the online version of this paper.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain TRM F103^T with members of the genus *Amycolatopsis*. Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes. Bar, 0.01 substitutions per nucleotide position.

PCR-product purification kit (Sangon). An almost-complete (1418 nt) 16S rRNA gene sequence of strain TRM F103^T was obtained. Multiple alignments and sequence similarity calculations with sequences of the closest neighbours were carried out using the EzTaxon server 2.0 (Chun *et al.*, 2007). Phylogenetic analysis was performed on a manual alignment with sequences of type strains of all recognized species in the genus *Amycolatopsis* using MEGA version 4.0 (Tamura *et al.*, 2007). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Evolutionary distance matrices were generated as described by Kimura (1980). Topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replications.

It was evident from 16S rRNA gene sequence analysis that strain TRM F103^T belonged to the genus *Amycolatopsis*. Strain TRM F103^T formed a monophyletic clade that was associated with *A. halophila* YIM 93223^T, which was supported by bootstrap values of 100% in the neighbour-joining analysis (Fig. 1) and 99% in the maximum-parsimony analysis (data not shown). Strain TRM F103^T exhibited highest sequence similarity with *A. halophila* YIM 93223^T (99.3% 16S rRNA gene sequence similarity). Other members of the genus displayed <96.0% 16S rRNA gene sequence similarity with the isolate. DNA–DNA hybridization was performed using the method described by He *et al.* (2005). Strain TRM F103^T and *A. halophila* YIM 93223^T showed 31.6% DNA–DNA relatedness (based on triplicate experiments). Considering 70% DNA–DNA relatedness as

the cut-off point for species delineation (Wayne *et al.*, 1987), strain TRM F103^T represents a novel species of the genus *Amycolatopsis*.

Mycelial morphological characteristics were examined by light microscopy (BH-2; Olympus) and scanning electron microscopy (JSM 5600LV; JEOL) of 13-day-old cultures grown on GTY agar supplemented with 5% NaCl. The Gram-reaction was performed as described by Gerhardt *et al.* (1994). Cultural characteristics were determined after incubation for 2–3 weeks by methods used by the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966). All media were supplemented with 5% (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). Unless specified otherwise, physiological properties were examined using ISP 4 as the basal medium. Growth was tested at 4, 10, 20, 30, 37, 42, 45, and 50 °C and pH 4.0–12.0 (at intervals of one pH unit), with the pH of media adjusted after autoclaving with 1 M HCl and 1 M NaOH. Growth with 0–20% (w/v) NaCl (at intervals of 1%) was tested on ISP 4. Standard physiological tests were carried out according to the methods described by Smibert & Krieg (1994). Enzyme activities were studied using the API ZYM substrate panels (bioMérieux). Susceptibility to antibiotics was tested by the method of Williams (1967).

Strain TRM F103^T grew well on inorganic salts-starch agar, Czapek's agar, oatmeal agar and potato agar and grew weakly on glycerol-asparagine agar and yeast extract-malt extract agar, but did not grow on nutrient agar. The aerial mycelium was white and the substrate mycelium was white–yellow. No soluble pigments were produced. Other phenotypic characteristics are given in Table 1 and the species description.

The isomer type of diaminopimelic acid in the cell-wall peptidoglycan was determined by the method of Hasegawa *et al.* (1983). Whole-cell sugars were analysed as described by Stanek & 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 the procedures of Minnikin *et al.* (1984). Cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI). Mycolic acids were checked by the acid methanolysis method of Minnikin *et al.* (1980). The G+C content of the DNA was determined using HPLC (Mesbah *et al.*, 1989). The G+C content of genomic DNA was 66.4 mol%, which is comparable with that of *A. halophila* YIM 93223^T (66.1 mol%) (Tang *et al.*, 2010). According to the chemotaxonomic analysis, strain TRM F103^T was clearly distinguishable from its closest phylogenetic neighbours. For example, the polar

Table 1. Differential characteristics of strain TRM F103^T and its closest phylogenetic neighbours

Strains: 1, *Amycolatopsis salitolerans* sp. nov. TRM F103^T; 2, *A. halophila* YIM 93223^T (Tang *et al.*, 2010); 3, *A. marina* Ms392A^T (Bian *et al.*, 2009); 4, *A. palatopharyngis* 1BDZ^T (Huang *et al.*, 2004). +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4
NaCl range (% w/v)	0–13	1–15	0.5–12	0–10
pH range	5.0–8.0	6.0–8.0	6.0–9.0	6.0–10.0
Temperature range (°C)	25–45	25–45	10–45	10–40
Catalase	+	–	+	–
Nitrate reduction	+	–	–	+
Decomposition of:				
L-Lysine	–	–	–	+
Starch	+	–	w	–
Urea	–	–	–	w
Utilization of:				
L-Arabinose	–	–	–	w
L-Rhamnose	+	+	+	–
Maltose	–	–	+	–
Raffinose	–	+	–	w
Major fatty acids (%):				
iso-C _{16:0}	44.6	28.9	40.4	50.3
C _{16:0}	12.2	18.6	–	21.7
C _{16:1} ω7c and/or iso-C _{15:0} 2-OH	–	12.3	–	–
iso-C _{16:0} 2-OH	–	–	11.4	–
anteiso-C _{17:0}	–	–	–	12.4
Predominant menaquinone	MK-8(H ₄)	MK-8(H ₄)	MK-9(H ₄)	MK-9(H ₄)
Major whole-cell sugars*	Rib, Glc, Gal	Glc, Gal	Gal, GlcN	Gal, Man, Glc
DNA G+C content (mol%)	66.4	66.1	70.1	65.8

*Gal, Galactose; Glc, glucose; GlcN, glucosamine; Man, mannose; Rib, ribose.

lipids of strain TRM F103^T included glucosamine-containing phospholipids and phosphatidylmethylethanolamine (Supplementary Fig. S1, available in IJSEM Online), which are not found in *A. halophila* YIM 93223^T. Also, the major fatty acids of strain TRM F103^T were iso-C_{16:0} (44.6 %) and C_{16:0} (12.2 %), but those of *A. halophila* YIM 93223^T were iso-C_{16:0} (28.9 %), C_{16:0} (18.6 %) and C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (12.3 %). In addition, the predominant menaquinone of strain TRM F103^T was MK-8(H₄) (88.1 %), whereas that of *Amycolatopsis marina* and *Amycolatopsis palatopharyngis*, which are also closely related to the isolate, was MK-9(H₄). Strain TRM F103^T also contained MK-8(H₆) (6.2 %), MK-9(H₄) (4.7 %) and MK-8(H₂) (1 %) as minor menaquinones. Details of other chemotaxonomic characteristics are given in Table 1 and the species description.

On the basis of the phenotypic, genotypic and DNA–DNA hybridization data presented here, strain TRM F103^T is considered to represent a novel species of the genus *Amycolatopsis*, for which the name *Amycolatopsis salitolerans* sp. nov. is proposed.

Description of *Amycolatopsis salitolerans* sp. nov.

Amycolatopsis salitolerans (sa.li.to'le.rans. L. n. *sal*, *salis* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *salitolerans* salt tolerating).

Cells are aerobic, Gram-positive, oxidase-negative, catalase-positive and non-motile. Substrate mycelium is branched and white–yellow. White aerial mycelium is produced on oatmeal agar and GTY agar. Diffusible pigments are not produced on the media tested. Grows at 25–45 °C (optimum 37 °C), at pH 5.0–8.0 (optimum pH 7.0) and with 0–13 % (w/v) NaCl (optimum 5 % NaCl). Tweens 20, 40 and 60, aesculin and starch are degraded, but casein, cellulose, chitin, Tween 80 and urea are not. Nitrate reduction is positive, but gelatin liquefaction, milk peptonization and coagulation, and H₂S and melanin production are negative. D-Galactose, L-rhamnose, maltose, sorbitol and glycerol are utilized as sole carbon sources, but trehalose, inositol, xylitol, erythritol, sodium acetate, raffinose, D-glucose, L-arabinose, cellobiose, D-ribose, D-fructose, D-mannose, D-xylose, lactose, dulcitol, mannitol, trisodium citrate and sodium propionate are not. L-Threonine, L-serine, L-proline, alanine, hypoxanthine, L-tyrosine, adenine and L-asparagine are utilized as nitrogen sources, whereas L-lysine, L-phenylalanine, L-arginine and xanthine are not. With API ZYM, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, α-chymotrypsin, arylamidase, cystine and N-acetyl-β-glucosaminidase are positive, but α- and β-galactosidase, acid phosphatase, β-glucuronidase, trypsin, α-mannosidase, α- and β-glucosidase, and α-fucosidase are negative. Susceptible to (μg per disc) novobiocin (5), tetracycline (30), tobramycin (10), clindamycin (15) and erythromycin (15), but resistant to netilmicin (10), penicillin (10), norfloxacin (10), ampicillin (10) and amikacin (30).

The cell wall contains *meso*-diaminopimelic acid. Mycolic acids are absent. Ribose, glucose and galactose are the major whole-cell sugars. The predominant menaquinone is MK-8(H₄) and the minor menaquinones are MK-8(H₆), MK-9(H₄) and MK-8(H₂). The phospholipid pattern mainly consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine and glucosamine-containing phospholipids. The major fatty acids (>10 %) are iso-C_{16:0} and C_{16:0}.

The type strain is TRM F103^T (=JCM 15899^T=CCTCC AB 208326^T), isolated from a hypersaline habitat of the Tarim basin, north-west China. The DNA G+C content of the type strain is 66.4 mol%.

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