Amycolatopsis halophila sp. nov., a halophilic actinomycete isolated from a salt lake

Shu-Kun Tang,¹ Yun Wang,² Tong-Wei Guan,³ Jae-Chan Lee,⁴ Chang-Jin Kim⁴ and Wen-Jun Li¹

Correspondence

Wen-Jun Li
wjli@ynu.edu.cn or
liact@hotmail.com

¹The Key Laboratory for Microbial Resources of the Ministry of Education, and Laboratory for Conservation and Utilization of Bio-resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China

²Xinjiang Institute of Microbiology, Xinjiang Academy of Agricultural Science, Urumqi, Xinjiang 830091, PR China

³Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin of Xinjiang Production & Construction Corps, Tarim University, Alar, Xinjiang 843300, PR China

⁴Functional Metabolite Research Center KRIIBB, 52 Eoeun-dong, Yuseong gu, Daejeon 305-806, Republic of Korea

A novel halophilic actinomycete, designated strain YIM 93223^T, was isolated from a salt lake in Xinjiang Province, north-west China, and was subjected to a polyphasic taxonomic study. The isolate grew at 25–45 °C, at pH 6–8 and in the presence of 1–15 % (w/v) NaCl; no growth was observed in the absence of NaCl. Strain YIM 93223^T contained *meso*-diaminopimelic acid, glutamic acid and alanine as cell-wall amino acids, and glucose and galactose as major whole-cell-wall sugars. Major fatty acids were iso-C_{16:0}, C_{16:0} and C_{16:1}ω7c/iso-C_{15:0} 2-OH. MK-8(H₄) was the predominant menaquinone. The genomic DNA G+C content was 66.1 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YIM 93223^T belongs to the genus *Amycolatopsis*. It shared highest 16S rRNA gene sequence similarity with *Amycolatopsis palatopharyngis* 1BDZ^T (96.6 %) and *Amycolatopsis marina* Ms392A^T (96.4 %), but lower values (94.5–96.2 %) with the type strains of other recognized species of the genus *Amycolatopsis*. On the basis of the data from this polyphasic study, strain YIM 93223^T is considered to represent a novel species of the genus *Amycolatopsis*, for which the name *Amycolatopsis halophila* sp. nov. is proposed. The type strain is YIM 93223^T (=DSM 45216^T =KCTC 19403^T).

The genus *Amycolatopsis* was first established by Lechevalier *et al.* (1986) and was classified in the family *Pseudonocardiaceae*, suborder *Pseudonocardineae* (Stackebrandt *et al.*, 1997). At the time of writing, it comprises 41 recognized species, most of which have been described in the past few years: *Amycolatopsis australiensis* (Tan *et al.*, 2006), *A. benzoatilytica* (Majumdar *et al.*, 2006), *A. decaplanina* (Wink *et al.*, 2004), *A. echigonensis* (Ding *et al.*, 2007), *A. jejuensis* (Lee, 2006), *A. halotolerans* (Lee, 2006), *A. lurida* (Lechevalier *et al.*, 1986; Stackebrandt *et al.*, 2004), *A. marina* (Bian *et al.*, 2009), *A. minnesotensis* (Lee *et al.*, 2006),

A. nigrescens (Groth *et al.*, 2007), *A. niigatensis* (Ding *et al.*, 2007), *A. palatopharyngis* (Huang *et al.*, 2004), *A. plumensis* (Saintpierre-Bonaccio *et al.*, 2005), *A. regifaucium* (Tan *et al.*, 2007), *A. rifamycinica* (Bala *et al.*, 2004), *A. saalfeldensis* (Carlsohn *et al.*, 2007), *A. taiwanensis* (Tseng *et al.*, 2006), *A. tucumanensis* (Albarracín *et al.*, 2010) and *A. ultiminotia* (Lee, 2009). These species are non-halophilic actinomycetes, and have been isolated from geographically diverse soils, clinical material, vegetable matter, the wall of a hypogean cave, and ocean sediment.

During a study of halophilic actinomycetes from a soil sample collected from Qijiaoing Lake in Xinjiang Province, north-west China (43° 26' 52" N 91° 29' 18" E), an *Amycolatopsis*-like isolate, YIM 93223^T, was picked after 3 weeks of incubation at 37 °C on cellulose-casein multi-salt medium (Tang *et al.*, 2008). The strain was maintained on modified International *Streptomyces* Project (ISP) medium 4 slants containing 5 % (w/v) NaCl at 4 °C, and as a glycerol suspension (20 %, v/v) at –20 °C. Biomass for chemical and molecular studies was obtained

Abbreviation: *meso*-DAP, *meso*-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 93223^T is FJ606836.

A scanning electron micrograph of spore chains of strain YIM 93223^T, chromatograms of hydrolysates of whole-cell sugars and menaquinones of strain YIM 93223^T and reference taxa, and a figure showing the polar lipid composition of strain YIM 93223^T are available with the online version of this paper.

by cultivation in shaken flasks (about 150 r.p.m.) by using ISP medium 4 without agar [5% (w/v) NaCl, pH 7.5] at 37 °C for 2 weeks.

Cultural characteristics were determined after incubation for 3–4 weeks according to Shirling & Gottlieb (1966). All growth media were supplemented with 5% (w/v) NaCl. 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). Good growth was observed on inorganic salts-starch agar, oatmeal agar and potato agar, moderate growth was observed on Czapek's agar, and weak growth on glycerol/asparagine agar and yeast extract-malt extract; no growth was observed on nutrient agar. The aerial mycelium was white and the substrate mycelium was white–yellow. No soluble pigments were produced. Morphological characteristics of strain YIM 93223^T were observed by light microscopy (model BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 21 days of growth on ISP medium 4 containing agar and 5% (w/v) NaCl. The morphological characteristics of strain YIM 93223^T were typical for the genus *Amycolatopsis* (Lechevalier *et al.*, 1986; Stackebrandt *et al.*, 2004). The substrate mycelium was well developed and fragmented into squarish, rod-like elements. The aerial mycelium differentiated into long chains of spore-like structures at maturity (see Supplementary Fig. S1 in IJSEM Online).

Growth temperature was tested at 5–55 °C on ISP medium 4 containing 5% (w/v) NaCl, at intervals of 5 °C. For NaCl tolerance experiments, ISP medium 4 was used as the basal

medium, and salt concentrations ranging from 0 to 20% (w/v) at intervals of 1% were tested. The pH range for growth was investigated between 4.0 and 10.0 at intervals of 1 pH unit, by using the buffer system: 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 according to the method of Williams (1967). Strain YIM 93223^T was able to grow at 25–45 °C, at pH 6.0–8.0 and in the presence of 1–15% NaCl, but no growth occurred in the absence of NaCl, showing that strain YIM 93223^T was a halophilic actinomycete. Strain YIM 93223^T could be distinguished from recognized species of the genus *Amycolatopsis* based on a combination of phenotypic properties (Table 1). The detailed physiological and biochemical characteristics of strain YIM 93223^T are given in the species description below.

A. marina Ms392A^T and *A. palatopharyngis* 1BDZ^T were used as reference strains for studies of whole-cell sugars and menaquinones. A purified cell-wall preparation was obtained and was hydrolysed as described by Schleifer & Kandler (1972). Amino acids in cell-wall hydrolysates were analysed by precolumn derivatization with *o*-phthalaldehyde by HPLC (Tang *et al.*, 2009a). Whole-cell sugars were detected by precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone by HPLC (Tang *et al.*, 2009b). Mycolic acids were checked by the acid methanolysis method of Minnikin *et al.* (1980). Polar lipids were

Table 1. Differential phenotypic characteristics between strain YIM 93223^T and its nearest phylogenetic neighbours in the genus *Amycolatopsis*

Strains: 1, YIM 93223^T (data from the present study); 2, *A. marina* Ms392A^T (Bian *et al.*, 2009); 3, *A. palatopharyngis* 1BDZ^T (Huang *et al.*, 2004). +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3
NaCl range (% w/v)	1–15	0.5–12	0–10
pH range	6.0–8.0	6.0–9.0	6.0–10.0
Temperature range (°C)	25–45	10–45	10–40
Catalase	–	+	–
Nitrate reduction	–	–	+
Decomposition of:			
L-Lysine	–	–	+
Starch	–	w	–
Urea	–	–	w
Utilization as sole carbon source			
L-Arabinose	–	–	w
L-Rhamnose	+	+	–
Maltose	–	+	–
Raffinose	+	–	w
Major fatty acids (>10%)	iso-C _{16:0} (28.9%), C _{16:0} (18.6%), C _{16:1ω7c} / iso-C _{15:0} 2-OH (12.3%)	iso-C _{16:0} (40.4%), iso-C _{16:0} 2-OH (11.4%)	iso-C _{16:0} (50.3%), C _{16:0} (21.7%), anteiso-C _{17:0} (12.4%)
DNA G+C content (mol%)	66.1	70.1	65.8

extracted and examined by two-dimensional TLC and were identified by using the procedures described by Minnikin *et al.* (1984). Menaquinones were isolated according to Minnikin *et al.* (1984) and were separated by HPLC (Kroppenstedt, 1982) with *Amycolatopsis orientalis* subsp. *orientalis* CGMCC 4.1214^T and *Streptomyces griseus* subsp. *griseus* ATCC 23345^T as reference strains; identification was confirmed by atmospheric pressure photoionization (APPI) (+)-LC-MS (Tang *et al.*, 2008). For fatty acid analysis, cells of strain YIM 93223^T were cultured on trypticase soy agar (Difco) containing 5 % NaCl at 37 °C for 7 days. Analysis was performed as described by Sasser (1990) by using the Microbial Identification System (MIDI). Strain YIM 93223^T contained *meso*-diaminopimelic acid (*meso*-DAP), glutamic acid and alanine as cell-wall amino acids, and glucose and galactose as major whole-cell sugars (minor amounts of mannose and ribose were also detected, and trace amounts or no arabinose) (Table 2 and Supplementary Fig. S2). Phospholipids detected were diphosphatidylglycerol, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositolmannosides and an unknown phospholipid (Supplementary Fig. S3). The predominant menaquinone was MK-8(H₄) (87.3 %); MK-8(H₆) (5.2 %) and MK-9(H₄) (7.5 %) were detected as minor components (Supplementary Figs S4 and S5). Strain YIM 93223^T had a cellular fatty acid profile that contained major amounts of straight, saturated and branched components and minor

straight-chain, unsaturated and methyl components. Fatty acids present at >1 % were: C_{14:0} (2.6 %), iso-C_{15:0} (1.4 %), C_{15:0} (2.4 %), iso-C_{16:0} G (1.6 %), iso-C_{16:0} (28.9 %), C_{16:0} (18.6 %), C_{16:0} 10-methyl (2.1 %), iso-C_{17:0} (1.6 %), anteiso-C_{17:0} (3.1 %), C_{17:1}ω8c (4.7 %), C_{17:1}ω6c (1.8 %), C_{17:0} (4.9 %), C_{18:1}ω9c (5.5 %), C_{18:0} (4.1 %) and C_{16:1}ω7c/iso-C_{15:0} 2-OH (12.3 %).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Li *et al.* (2007). The 16S rRNA gene was amplified by PCR by using eubacterial primers 27F and 1492R (DeLong, 1992) and PCR products were purified by using a Sangon PCR purification kit. Multiple alignments with sequences of closely related species of the genus *Amycolatopsis* and calculations of levels of sequence similarity were carried out by using the EzTaxon server 2.0 (Chun *et al.*, 2007). Phylogenetic analyses were performed by using three tree-making algorithms, namely the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed with the neighbour-joining method from *K*_{nuc} values (Kimura, 1980) by 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) based on 1000 replicates. For determination of the G + C content of the genomic DNA of strain YIM 93223^T, DNA was prepared according to the method of Marmur (1961). The G + C content was determined by reversed-phase HPLC of nucleosides according to Mesbah *et al.* (1989).

The results of 16S rRNA gene sequence comparisons clearly demonstrated that strain YIM 93223^T was a member of the genus *Amycolatopsis*. In the phylogenetic tree based on the neighbour-joining algorithm, strain YIM 93223^T formed a monophyletic clade with *A. palatopharyngis* 1BDZ^T and *A. marina* Ms392A^T, supported by a bootstrap value of 51 % (Fig. 1). The topology of the phylogenetic tree built with the maximum-likelihood method was similar to that of the neighbour-joining tree but was different from that of the maximum-parsimony tree. In the phylogenetic tree based on the maximum-parsimony algorithm, strain YIM 93223^T formed a monophyletic clade with *A. taiwanensis* 0345M-7^T (data not shown). Levels of 16S rRNA gene sequence similarity between strain YIM 93223^T and its closest neighbours, *A. palatopharyngis* 1BDZ^T and *A. marina* Ms392A^T, were 96.6 and 96.4 %, respectively, while similarities with the type strains of other species of the genus *Amycolatopsis* were 94.5–96.2 %. The G + C content of the DNA of strain YIM 93223^T was 66.1 mol%.

It is evident from the phenotypic and phylogenetic data (Fig. 1 and Fig. S1) that strain YIM 93223^T represents a novel species of the genus *Amycolatopsis*. Although strain YIM 93223^T is similar to *A. palatopharyngis* 1BDZ^T and *A. marina* Ms392A^T in colony colour (white to yellow) and shares the same subclade in the phylogenetic tree, it differs in some morphological and physiological properties

Table 2. Menaquinone compositions and hydrolysates of whole-cell sugars of strain YIM 93223^T and closely related species of the genus *Amycolatopsis*

Strains: 1, YIM 93223^T; 2, *A. marina* Ms392A^T; 3, *A. palatopharyngis* 1BDZ^T. All data are from the present study. tr, Trace; –, not present.

Characteristic	1	2	3
Menaquinone composition (%)			
MK-8(H ₄)	88.4	8.2	8.7
MK-8(H ₆)	5.7	3.5	–
MK-9(H ₂)	–	–	2.5
MK-9(H ₄)	5.9	84.1	85.4
MK-9(H ₆)	–	1.9	1.4
MK-10(H ₄)	–	2.3	2.1
Whole-cell sugars (%)			
Mannose	7.3	9.9	17.7
Ribose	6.4	–	–
Unknown sugar A	–	4.6	1.4
Rhamnose	1.7	2.0	–
Glucosamine	1.4	24.9	5.8
Unknown sugar B	–	–	3.7
Unknown sugar C	11.0	–	–
Glucose	36.0	2.6	15.3
Unknown sugar D	–	6.9	10.8
Galactose	36.2	39.4	32.0
Arabinose	tr	9.7	13.3

Fig. 1. Neighbour-joining phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 93223^T among species of the genus *Amycolatopsis*. Asterisks indicate branches of the tree that were also found by using the maximum-likelihood algorithm. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets (only values >50% are shown). The sequence of *Prauserella rugosa* DSM 43194^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

(Table 1), i.e. strain YIM 93223^T can grow at 15% NaCl and no growth is observed in the absence of NaCl. Strain YIM 93223^T differed from *A. palatopharyngis* 1BDZ^T and *A. marina* Ms392A^T in a number of chemotaxonomic properties (Tables 1 and 2). Strain YIM 93223^T contained glucose and galactose as major whole-cell sugars, no or only trace arabinose in the cell wall, MK-8(H₄) as the predominant menaquinone, and iso-C_{16:0}, C_{16:0} and C_{16:1}ω7c/iso-C_{15:0} 2-OH as major fatty acids; by contrast, *A. palatopharyngis* 1BDZ^T and *A. marina* Ms392A^T contained galactose, arabinose, mannose and glucose or

glucosamine as major whole-cell sugars, MK-9(H₄) as the predominant menaquinone, and iso-C_{16:0} and iso-C_{16:0} 2-OH, or iso-C_{16:0}, C_{16:0}, and anteiso-C_{17:0} as major fatty acids.

On the basis of the phenotypic, chemotaxonomic and phylogenetic data presented, strain YIM 93223^T should be placed in the genus *Amycolatopsis* as the type strain of a novel species, for which we propose the name *Amycolatopsis halophila* sp. nov.

Emended description of the genus *Amycolatopsis* Lechevalier *et al.* 1986

The description is as given by Lechevalier *et al.* with the following additions. The genus encompasses alkaliphilic, mesophilic, thermophilic, pathogenic and halophilic species. Can tolerate up to 15% NaCl. Whole-cell-wall sugars contain no or only trace arabinose. Predominant menaquinone is MK-8(H₄) or MK-9(H₄).

Description of *Amycolatopsis halophila* sp. nov.

Amycolatopsis halophila (ha.lo.phi'la. Gr. n. *halos* salt; Gr. adj. *philos* loving; N.L. fem. adj. *halophila* salt-loving, referring to the ability of the type strain to grow at high NaCl concentrations).

Aerobic, Gram-positive, halophilic, filamentous actinomycete. Substrate mycelia are well developed and fragment into squarish, rod-like elements. Aerial mycelia differentiate into long chains of spore-like structure at maturity. Growth occurs at 25–45 °C, at pH 6.0–8.0 and in the presence of 1–15% NaCl. Optimal growth occurs at 37 °C, pH 7.0 and 5% NaCl; no growth occurs in the absence of NaCl. Tweens 20, 40 and 60 are degraded, but aesculin, casein, starch, cellulose, chitin, Tween 80 and urea are not. Positive for gelatin liquefaction, but negative for nitrate reduction, milk peptonization and coagulation, H₂S, and melanin production. Utilizes D-galactose, raffinose, L-rhamnose, trehalose, inositol, xylitol, erythritol, sodium acetate, glycerol and trisodium citrate as sole carbon source, but not D-glucose, L-arabinose, cellobiose, D-ribose, D-fructose, D-mannose, D-xylose, maltose, lactose, dulcitol, mannitol, sorbitol or sodium propionate. Utilizes L-threonine, L-arginine, glycine, L-serine, L-histidine, L-proline, L-tyrosine, alanine, L-methionine, hypoxanthine, L-asparagine and xanthine as sole nitrogen source, but not L-lysine, L-phenylalanine or adenine. In the API ZYM system, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase, but negative for acid phosphatase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and β-fucosidase. Susceptible (per disc) to ciprofloxacin (5 µg), amoxicillin (10 µg), novobiocin (5 µg), tetracycline (30 µg) and erythromycin (15 µg), but resistant to netilmicin (10 µg), norfloxacin

(10 µg), penicillin (10 µg), ampicillin (10 µg), tobramycin (10 µg), sulfamethoxazole (23.75 µg), amikacin (30 µg) and clindamycin. Contains *meso*-DAP, glutamic acid and alanine as cell-wall diamino acids, and glucose and galactose as major whole-cell sugars. The major fatty acids are iso-C_{16:0}, C_{16:0} and C_{16:1ω7cl}/iso-C_{15:0} 2-OH. MK-8(H₄) is the predominant menaquinone. Mycolic acids are not detected. Phospholipids present are diphosphatidylglycerol, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositolmannosides and an unknown phospholipid. The DNA G+C content of the type strain is 66.1 mol%.

The type strain, YIM 93223^T (=DSM 45216^T =KCTC 19403^T), was isolated from a salt lake in Xinjiang Province, north-west China.

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