

Actinopolyspora alba sp. nov. and *Actinopolyspora erythraea* sp. nov., isolated from a salt field, and reclassification of *Actinopolyspora iraqiensis* Ruan *et al.* 1994 as a heterotypic synonym of *Saccharomonospora halophila*

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Three actinomycetal strains, designated YIM 90479, YIM 90480^T and YIM 90600^T, were isolated from a salt field in Xinjiang province, North-west China and subjected to polyphasic taxonomic study. All three strains were moderately halophilic and were able to grow on agar at NaCl concentrations of up to 20–25% (w/v). No growth was observed in the absence of NaCl. Good growth occurred at 37 °C and 15% (w/v) NaCl. The cell walls of the three strains were of the type IV variety and their phospholipid patterns were type PIII. MK-9 (H₄) and MK-10 (H₄) or MK-9 (H₄) and MK-9 (H₂) were the predominant menaquinones. iso-C_{16:0} and anteiso-C_{17:0} or anteiso-C_{15:0} and anteiso-C_{17:0} were the major fatty acids. The G + C contents of the genomic DNA were 66.4–68.3 mol%. Based on differential phenotypic, chemotaxonomic and phylogenetic characteristics and the results of DNA–DNA hybridization tests, the three novel strains represent two novel species, for which the names *Actinopolyspora alba* sp. nov. and *Actinopolyspora erythraea* sp. nov. are proposed. The type strains of the species are YIM 90480^T (=DSM 45004^T =KCTC 19119^T) and YIM 90600^T (=CCTCC M 208247^T =KCTC 19372^T). In addition, we propose that *Actinopolyspora iraqiensis* (Ruan *et al.*, 1994) be transferred to the genus *Saccharomonospora* as a heterotypic synonym of *Saccharomonospora halophila* based on present research results.

Abbreviations: APPI, atmospheric pressure photo ionization; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositolmannoside; PL, phospholipid; PME, phosphatidylmethylethanolamine; PMP, 1-phenyl-3-methyl-5-pyrazolone.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIM 90479, YIM 90480^T, YIM 90600^T and *Actinopolyspora iraqiensis* AS 4.1193^T are DQ883811, GQ480940, GQ480939 and EF372522, respectively.

Three supplementary tables are available with the online version of this paper.

The genus *Actinopolyspora*, a group of extremely halophilic actinomycetes, was first proposed by Gochnauer *et al.* (1975) belonging to the suborder *Acintopolysporineae* (Zhi *et al.*, 2009). Since then, only three species have been described with validly published names: *Actinopolyspora halophila* (Gochnauer *et al.*, 1975), *Actinopolyspora mortivallis* (Yoshida *et al.*, 1991) and *Actinopolyspora iraqiensis* (Ruan *et al.*, 1994). Typically, members of the genus *Actinopolyspora* are Gram-positive, form long chains of spores on aerial mycelia, form substrate mycelia fragments, have type IV cell walls and a type PIII phospholipid pattern; the predominant menaquinones are MK-9 (H₄)

and MK-10 (H₄), the major fatty acids are iso-C_{15:0}, iso-C_{16:0}, iso-C_{17:0} and anteiso-C_{17:0} and the DNA G+C contents range from 64.2 to 68 mol% (Gochbauer *et al.*, 1975; Yoshida *et al.*, 1991). In the present study, we characterised three novel *Actinopolyspora*-like strains, designated YIM 90479, YIM 90480^T and YIM 90600^T, and propose the reclassification of *A. iraqiensis* (Ruan *et al.*, 1994) as a heterotypic synonym of *Saccharomonospora halophila*.

Three halophilic filamentous actinomycetal strains, designated YIM 90479, YIM 90480^T and YIM 90600^T, were isolated from a soil sample collected from Baicheng salt field in Xinjiang province, North-west China, after 3 weeks of incubation at 37 °C on cellulose-casein multi-salt (CCMS) medium as described by Tang *et al.* (2008). The three strains were maintained on a modified ISP medium 4 slant containing 15 % (w/v) NaCl at 4 °C or in 20 % (v/v) glycerol suspension at -80 °C. Biomass for chemical and molecular studies was obtained by cultivating in shaken flasks (~150 r.p.m.) using ISP 4 medium, or ISP 4 agar, containing 15 % (w/v) NaCl (pH 7.5) at 37 °C for 2 weeks. *A. iraqiensis* AS 4.1193^T was obtained from the CGMCC and *A. iraqiensis* DSM 44640^T, *S. halophila* DSM 44411^T, *A. halophila* DSM 43834^T and *A. mortivallis* DSM 44261^T were obtained from the DSMZ and were used as reference strains for comparing cultural, physiological and chemotaxonomic characteristics or for DNA-DNA hybridization tests.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Li *et al.* (2007). Multiple sequence alignments and calculations of evolutionary distances were carried out using CLUSTAL_X (Thompson *et al.*, 1997) software. Gaps at the 5' and 3' ends of the alignment were omitted for further analysis. Sequence similarity calculations were carried out using EzTaxon server 2.1 (Chun *et al.*, 2007). Phylogenetic analyses were performed using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was reconstructed using the neighbour-joining method using MEGA version 4.0 (Tamura *et al.*, 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method with 1000 replicates (Felsenstein 1985). For DNA hybridization tests, genomic DNA of the strains tested was prepared according to the method of Marmur (1961). Levels of DNA-DNA relatedness were determined according to the optical renaturation method using 81.5 °C as the hybridization temperature (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992). All DNA-DNA hybridizations were performed in triplicate.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that the three novel isolates clustered with *A. halophila* ATCC 27976^T and *A. mortivallis* DSM 44261^T but *A. iraqiensis* AS 4.1193^T clustered with *S. halophila* DSM 44411^T and *Saccharomonospora paurometabolica* YIM 90007^T (Fig. 1). 16S rRNA gene sequence similarities

between strain YIM 90600^T and strains YIM 90479, YIM 90480^T, *A. halophila* ATCC 27976^T, *A. mortivallis* DSM 44261^T, *A. iraqiensis* AS 4.1193^T, *S. halophila* DSM 44411^T and *S. paurometabolica* YIM 90007^T were 100, 97.6, 96.0, 94.4, 89.6, 90.0 and 89.3 %, respectively. Sequence similarities between strain YIM 90480^T and strains *A. halophila* ATCC 27976^T, *A. mortivallis* DSM 44261^T, *A. iraqiensis* AS 4.1193^T, *S. halophila* DSM 44411^T and *S. paurometabolica* YIM 90007^T were 95.9, 93.7, 89.5, 89.9, 89.6 and 89.3 %, respectively. Sequence similarities between strain *A. iraqiensis* AS 4.1193^T and strains *A. halophila* ATCC 27976^T, *A. mortivallis* DSM 44261^T, *S. halophila* DSM 44411^T and *S. paurometabolica* YIM 90007^T were 89.5, 89.7, 99.3 and 99.1 %, respectively. These results clearly revealed that strains YIM 90479, YIM 90480^T and YIM 90600^T belong to the genus *Actinopolyspora* and showed that *A. iraqiensis* AS 4.1193^T should be assigned to the genus *Saccharomonospora*. DNA-DNA relatedness values between strain YIM 90600^T and strains YIM 90479 and YIM 90480^T were 100 and 46.8 %, respectively. DNA relatedness values between *A. iraqiensis* AS 4.1193^T and *S. halophila* DSM 44411^T and *S. paurometabolica* YIM 90007^T were 95 and 56 %, respectively. Relatedness values greater than 70 %, the recommended threshold for the delineation of bacterial species, indicated that strains YIM 90479 and YIM 90600^T belong to the same species of the genus *Actinopolyspora* and that *A. iraqiensis* AS 4.1193^T and *S. halophila* DSM 44411^T belong to the same species of the genus *Saccharomonospora* (Wayne *et al.*, 1987).

Morphological characteristics of strains YIM 90480^T and YIM 90600^T were observed using light microscopy (model BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 21 days of growth on ISP medium 4 agar containing 15 % (w/v) NaCl. Aerial mycelia were well developed in the two strains and formed long spore chains. Fragments of substrate mycelia were also observed. All spores were oval or rod-shaped, 0.4–0.5 × 0.9–2.1 µm in size and non-motile with a smooth surface (Fig. 2). The morphological features of strains YIM 90480^T and YIM 90600^T were typical of members of the genus *Actinopolyspora* as described previously (Gochbauer *et al.*, 1975; Yoshida *et al.*, 1991), whereas *A. iraqiensis* AS 4.1193^T had single and paired spores with no long spore chains, which were more similar to those of members of the genus *Saccharomonospora* than those of the genus *Actinopolyspora*. Cultural characteristics of the strains tested were determined after incubation for 3–4 weeks by methods used in the International Streptomyces Project (ISP) (Shirling & Gottlieb, 1966). All media were supplemented with 15 % (w/v) NaCl for growth. Colours of aerial and substrate mycelia and any soluble pigments produced were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). Strains YIM 90480^T and YIM 90600^T developed well on most media tested and could be distinguished from *A. halophila* DSM 43834^T and *A. mortivallis* DSM 44261^T by some cultural characteristics (Table 1).

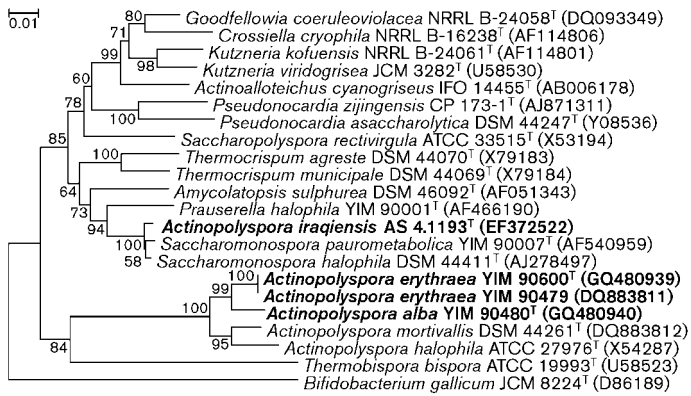


Fig. 1. Phylogenetic tree obtained by using distance matrix analysis of 16S rRNA gene sequences, showing the positions of strains YIM 90479, YIM 90480^T and YIM 90600^T and closely related species. Bootstrap values >50% (based on 1000 resamplings) are given at branch points. *Bifidobacterium gallicum* JCM 8224^T was used as outgroup. Bar, 1% sequence divergence.

Growth at 5–55 °C (intervals of 5 °C) was tested on ISP medium 4 containing 15% (w/v) NaCl. To determine tolerance of NaCl, ISP medium 4 was used as the basal medium and the salt concentration was adjusted from 0–30% (w/v) NaCl at intervals of 5%. Growth at pH 4–10 (intervals of 1 pH unit) was determined using the following buffer system: 0.1 M citric acid/0.1 M sodium citrate (pH 4–5); 0.1 M KH₂PO₄/0.1 M NaOH (pH 6–8); 0.1 M NaHCO₃/0.1 M Na₂CO₃ (pH 9–10). The media and procedures used for the determination of physiological features and carbon source utilization were those described by Williams *et al.* (1989). Enzyme activity was determined

by using the API ZYM system (bioMérieux) according to the manufacturer's instructions. The phenotypic characteristics of strains YIM 90480^T and YIM 90600^T were distinctly different from *A. halophila* DSM 43834^T and *A. mortivallis* DSM 44261^T (Table 2). The detailed physiological and biochemical characteristics of the novel isolates are given in the species description.

Isomers of diaminopimelic acids were analysed according to the procedures developed by Hasegawa *et al.* (1983). The whole-cell sugars were detected by precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP) by HPLC (Tang *et al.*, 2009). 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 atmospheric pressure photo ionization (APPI)-LC/MS (Tang *et al.*, 2008). For fatty acid analysis, the strains tested were cultured on tryptic soy agar (BD) containing 15% (w/v) NaCl at 37 °C for 7 days. Cellular fatty acid analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI). DNA G+C contents were determined by reversed-phase HPLC according to Mesbah *et al.* (1989). Both strains YIM 90480^T and YIM 90600^T contained *meso*-diaminopimelic acid as the cell-wall diamino acid. The detailed whole cell sugar, menaquinone, phospholipid and the fatty acid compositions of the strains tested are given in Supplementary Tables S1, S2 and S3, available in IJSEM online. Based on the test results, the chemotaxonomic properties of strains YIM 90480^T and YIM 90600^T were more similar to those of members of the genus *Actinopolyspora*. *A. iraqiensis* AS 4.1193^T contained galactose (40.6%) and glucose (33.5%) as the major whole-cell sugars; diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylmethylethanolamine (PME), an unknown phospholipid (PL), phosphatidylinositol (PI) and phosphatidylinositol-mannoside (PIM) (no phosphatidylcholine, PC) as the phospholipids; MK-9 (H₄) (66.7%) and MK-8 (H₄) (21.2%) as the predominant menaquinones and iso-C_{16:0} (63.9%) and C_{16:0} (12.1%) as the major fatty acids, making its chemotaxonomic properties more similar to

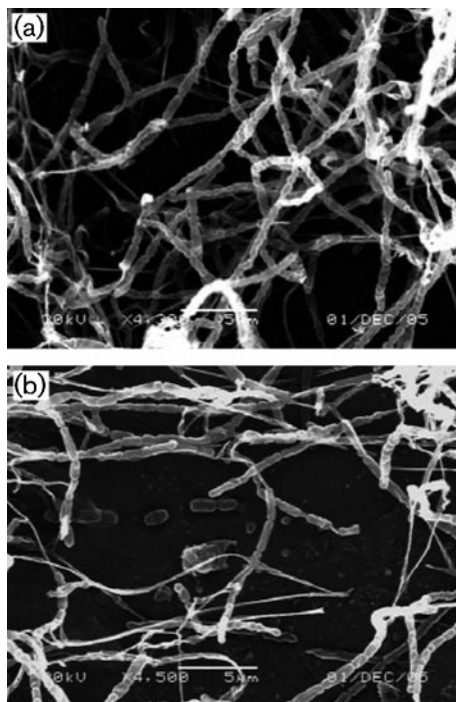


Fig. 2. Scanning electron micrograph showing long-chain spores of strains YIM 90480^T (a) and YIM 90600^T (b). Cells were grown on ISP 4 medium containing 15% (w/v) NaCl for 21 days at 37 °C. Bar, 5 μm.

Table 1. Cultural characteristics of strains YIM 90480^T, YIM 90600^T, *A. halophila* DSM 43834^T and *A. mortivallis* DSM 44261^T on various media

Strains: 1, YIM 90480^T; 2, YIM 90600^T; 3, *A. halophila* DSM 43834^T; 4, *A. mortivallis* DSM 44261^T. All data from this study. +, Good growth; (+), moderate growth; (-), poor growth; -, none/no growth; B, brown; DY, deep yellow; G-y, greyish-yellow; LY, light yellow; MY, moderately yellow; Y, yellow; Y-w, yellow-white. All media contained 15% (w/v) NaCl; pH 7. Colours were taken from ISCC-NBS colour charts (standard samples, no. 2106) (Kelly, 1964).

Medium*	1	2	3	4
Czapek agar				
Growth	+	+	+	+
Aerial mycelium	Y-w	Y-w	-	LY
Substrate mycelium	Y	Y	Y-w	B
Soluble pigment	Y	-	-	MY
Glycerol-asparagine agar (ISP 5)				
Growth	(-)	(+)	(+)	(+)
Aerial mycelium	-	Y-w	-	-
Substrate mycelium	Y-w	Y	Y-w	G-y
Soluble pigment	-	-	-	Y
Inorganic salts starch agar (ISP 4)				
Growth	+	+	+	+
Aerial mycelium	Y-w	Y-w	Y-w	Y-w
Substrate mycelium	Y-w	Y	Y-w	Y
Soluble pigment	-	-	-	-
Yeast extract-malt extract (ISP 2)				
Growth	(+)	(+)	(+)	(+)
Aerial mycelium	Y-w	Y-w	Y-w	Y-w
Substrate mycelium	DY	Y	Y	B
Soluble pigment	-	-	-	-
Oatmeal agar (ISP 3)				
Growth	(+)	+	-	(+)
Aerial mycelium	Y-w	Y-w	-	Y
Substrate mycelium	Y	Y-w	-	LY
Soluble pigment	-	-	-	-
Potato agar				
Growth	+	(+)	(+)	(+)
Aerial mycelium	Y-w	Y-w	-	Y-w
Substrate mycelium	DY	Y	Y	DY
Soluble pigment	-	-	-	-
Nutrient agar				
Growth	-	(-)	(+)	(+)
Aerial mycelium	-	-	-	Y-w
Substrate mycelium	-	Y	Y	B
Soluble pigment	-	-	-	MY

those of members of the genus *Saccharomonospora* than members of the genus *Actinopolyspora*. The DNA G+C contents of strains YIM 90480^T and YIM 90600^T were 68.3 and 66.4 mol%, respectively.

On the basis of their phenotypic and chemotaxonomic characteristics and on the results of phylogenetic analysis and DNA-DNA hybridization tests, strains YIM 90480^T and YIM 90600^T represent two novel species of the genus *Actinopolyspora*, for which we propose the names

Actinopolyspora alba sp. nov. and *Actinopolyspora erythraea* sp. nov., respectively.

Based on the deposition history records of the CGMCC and DSMZ culture collections, strain IQ-H1^T is the origin of *Actinopolyspora iraqiensis* and strains AS 4.1193^T and DSM 44640^T should also be equal to the original strain IQ-H1^T. However, according to the present results for *Actinopolyspora iraqiensis* strains AS 4.1193^T and DSM 44640^T, they did not contain PC as a whole-cell sugar, which showed a clear contradiction to the original description of strain IQ-H1^T by Ruan *et al.* (1994), which contained PC. However, for most taxonomic characteristics, including morphological and chemotaxonomic properties, *Actinopolyspora iraqiensis* strains AS 4.1193^T, DSM 44640^T and IQ-H1^T produced the same experimental results; all three strains formed single and paired spores as well as short spore chains, contained arabinose, galactose and ribose as whole-cell sugars and contained MK-9(H₄) as the predominant menaquinone. Thus, based on their morphological and chemotaxonomic properties, strains *Actinopolyspora iraqiensis* AS 4.1193^T, DSM 44640^T and IQ-H1^T should be reclassified as members of the genus *Saccharomonospora* as they all harbour characteristics typical of members of this genus rather than members of the genus *Actinopolyspora*. Additionally, although *Actinopolyspora iraqiensis* IQ-H1^T (=AS 4.1193^T =DSM 44640^T) differed from *S. halophila* DSM 44411^T in some phenotypic properties (Table 2), it was similar in some morphological, chemotaxonomic and physiological properties, in particular the results of DNA-DNA hybridizations. Therefore, we propose that *Actinopolyspora iraqiensis* Ruan *et al.*, 1994 be transferred to the genus *Saccharomonospora* as a heterotypic synonym of *Saccharomonospora halophila*.

Emended description of the genus *Actinopolyspora* Gochnauer *et al.* 1975

The genus description (Gochnauer *et al.*, 1975) is emended with respect to the chemical compositions of cell constituents. The major whole-cell sugars contain galactose and arabinose. The phospholipid pattern is type P III. MK-9 (H₄) and MK10 (H₄) or MK-9 (H₄) and MK9 (H₂) are the predominant menaquinones. iso-C_{16:0} and anteiso-C_{17:0} are the major fatty acids. The DNA G+C contents are 64–69 mol%.

Description of *Actinopolyspora alba* sp. nov.

Actinopolyspora alba (al'ba. L. fem. adj. *alba* white).

Cells are aerobic, Gram-reaction-positive, halophilic, filamentous actinomycetes that forms long chains of spores on aerial mycelium and produce fragments of substrate mycelium. Spores are non-motile, smooth-surfaced and oval or rod-shaped. Cultural characteristics are given in Table 1. Grows at 25–45 °C, at pH 6–8 and in 10–25% (w/v) NaCl. Good growth occurs at 37 °C, at pH 7–8 and in 15% (w/v) NaCl. Tests for milk peptonization and coagulation, hydrogen sulfide and melanin production, starch hydrolysis

Table 2. Differential characteristics of strains YIM 90480^T and YIM 90600^T and closely related species

Strains: 1, YIM 90480^T; 2, YIM 90600^T; 3, *A. halophila* DSM 43834^T; 4, *A. mortivallis* DSM 44261^T; 5, *A. iraqiensis* AS 4.1193^T; 6, *S. halophila* DSM 44411^T. +, Positive; –, negative; w, weakly positive. All data from this study.

Characteristic	1	2	3	4	5	6
NaCl range for growth (%)	10–25	10–25	15–30	10–30	10–25	10–30
Growth temperature range (°C)	25–45	25–45	10–45	10–50	25–55	25–50
Growth pH range	6–8	6–8	6–8	4–10	6–8	6–9
Decomposition of:						
Urea	–	–	–	+	–	–
Dextrin	–	+	–	–	–	+
Starch	–	+	–	–	–	–
Casein	–	+	+	+	+	+
Tween 80	–	+	+	+	+	+
Enzymic activity (API ZYM):						
Acid phosphatase	+	w	–	–	–	–
Lipase (C14)	–	–	+	–	w	w
α -Galactosidase	–	w	+	+	–	w
β -Galactosidase	+	w	w	+	–	w
α -Glucosidase	w	w	w	–	w	w
β -Glucosidase	w	w	w	–	+	w
α -Mannosidase	w	w	w	–	–	–
α -Fucosidase	w	–	w	–	–	–
Utilization of:						
D-Xylose	–	+	+	+	+	–
Maltose	+	+	–	–	+	+
D-Fructose	–	–	+	+	+	–
D-Galactose	–	+	–	+	–	–
Lactose	+	–	–	+	+	+
D-Mannitol	+	–	+	–	+	+
D-Mannose	+	+	–	+	–	+
Cellobiose	–	+	–	+	+	+
Xylitol	+	–	–	–	+	+
Trisodium citrate	+	+	+	+	–	+
L-Ornithine	+	+	–	+	+	+
L-Arginine	–	+	–	+	+	+
L-Lysine	+	+	+	–	+	–

and urease production are negative. Positive only for gelatin liquefaction. Tween 40 and aesculin are hydrolysed, but Tweens 20, 60 and 80, dextrin, starch and casein are not. Enzyme activities and carbon or nitrogen source utilization characteristics are given in Table 2. The type IV cell wall contains *meso*-diaminopimelic acid as the cell-wall peptidoglycan and galactose, arabinose and ribose as the major whole sugars. The phospholipid pattern is type P III. MK-9 (H₄) and MK10 (H₄) are the predominant menaquinones. *iso*-C_{16:0} and *anteiso*-C_{17:0} are the major fatty acids.

The type strain, YIM 90480^T (=DSM 45004^T =KCTC 19119^T), was isolated from a salt field in Xinjiang, North-west China. The DNA G + C content of the type strain is 68.3 mol%.

Description of *Actinopolyspora erythraea* sp. nov.

Actinopolyspora erythraea (e.ryth.rae'a. Gr. adj. *erythros* red, NL. fem. adj. *erythraea* referring to the production of the new erythromycin congeners erythronolides H and I).

Cells are aerobic, Gram-reaction-positive, halophilic, filamentous actinomycetes that form long chains of spores on aerial mycelium and produce fragments of substrate mycelium. Spores are non-motile, smooth-surfaced and oval or rod-shaped. Cultural characteristics are given in Table 1. Grows at 25–45 °C, at pH 6–8 and in 10–25 % (w/v) NaCl. Good growth occurs at 37 °C, at pH 7–8 and in 15 % (w/v) NaCl. Tests for milk peptonization and coagulation, hydrogen sulfide and melanin production and urease activity are negative. Positive for gelatin liquefaction. Aesculin, casein, dextrin, starch and Tweens 40 and 80 are hydrolysed but Tweens 20 and 60 are not. Produce the new erythromycin congeners erythronolides H and I. Enzyme activities and carbon and nitrogen source utilization characteristics are given in Table 2. The type IV cell wall contains *meso*-diaminopimelic acid as the cell-wall peptidoglycan and galactose and arabinose as the major whole-cell sugars. The phospholipid pattern is type PIII. MK-9 (H₄) and MK9 (H₂) are the predominant

menaquinones. anteiso-C_{15:0} and anteiso-C_{17:0} are the major fatty acids.

The type strain, YIM 90600^T (=CCTCC M 208247^T =KCTC 19372^T), was isolated from a salt field in Xinjiang, North-west China. The DNA G+C content of the type strain is 66.4 mol%.

Acknowledgements

This research was supported by the National Basic Research Program of China (2010CB833801), the 973 Pre-research Program of China (2008CB417214), the National Natural Science Foundation of China (30860002, 30870005), the Key Project of International Cooperation (2007DFB31620), the International Cooperation Research Program of Yunnan Province (2009AC017) and the Yunnan Provincial Natural Science Foundation (2007C167M).

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