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Streptimonospora salina gen. nov., sp. nov., a new member of the family *Nocardiopsaceae*

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Actinomycete strain YIM 90002^T (= CCTCC 99003^T = CCRC 16284^T) was isolated from a soil sample collected from a salt lake in the west of China. The aerial mycelium of this organism is well developed but not fragmented and, at maturity, forms short chains of spores. Spores in short chains are oval- to rod-shaped and have wrinkled surfaces. Substrate mycelium is branched with non-fragmenting hyphae and forms single oval to round spores borne on sporophores or dichotomously branching sporophores. Single spores have wrinkled surfaces. Single spores and spores in short chains are non-motile. Strain YIM 90002^T contains *meso*-diaminopimelic acid, DD-diaminopimelic acid, glycine, lysine and aspartic acid in its cell wall and has glucose, galactose, ribose, xylose, arabinose and mannose as whole-cell sugars (no diagnostic sugars). The phospholipids are phosphatidylglycerol, phosphatidylinositol and phosphatidylethanolamine. The major menaquinones are MK-9(H_6), MK-10(H_2) and MK-10(H_4). Phylogenetic data indicate that this strain belongs to the family *Nocardiopsaceae*. The morphological and physiological characteristics and chemotaxonomic and phylogenetic data for this strain differ from those of previously described actinomycetes. Therefore, a new genus, *Streptimonospora*, is proposed for this organism; the type species of the genus is *Streptimonospora salina* gen. nov., sp. nov., and the type strain of *S. salina* is strain YIM 90002^T.

Keywords: *Nocardiopsaceae*, *Streptimonospora salina* sp. nov., 16S rDNA

INTRODUCTION

The family *Nocardiopsaceae* (Rainey *et al*., 1996) contains two genera, namely *Nocardiopsis* (Meyer, 1976) and *Thermobifida* (Zhang *et al*., 1998). Although 16S rRNA sequence-based phylogenetic analysis shows that they form a distinct clade in the suborder *Streptosporangineae* (Stackebrandt *et al*., 1997), *Nocardiopsis* and *Thermobifida* are morphologically and chemotaxonomically different. Currently, the genus *Nocardiopsis* contains the following validly described species and subspecies: *Nocardiopsis dassonvillei*, *Nocardiopsis alborubida*, *Nocardiopsis antarctica*, *Nocardiopsis listeri*, *Nocardiopsis lucentensis*, *Nocardiopsis halophila*, *Nocardiopsis alba* subsp. *alba*, *Nocardiopsis alba* subsp. *prasina* and *Nocardiopsis synnemataformans* (Meyer, 1976; Grund & Kroppenstedt, 1990; Abyzov *et al*., 1983; Yassin *et al*., 1993, 1997; Al-Tai & Ruan, 1994; Miyashita *et al*., 1984). The subspecies *N. alba* subsp. *prasina* has been elevated to species rank as *Nocardiopsis prasina* (type strain DSM 43845^T) on the basis of levels of DNA–DNA hybridization (24·25%) between *N. alba* subsp. *alba* and *N. alba* subsp. *prasina* (Yassin *et al*., 1997). Three species, *N. antarctica*, *N. alborubida* and *N. dassonvillei*, were designated as synonyms of *N. dassonvillei* on the basis of both 16S rDNA sequence similarity and DNA–DNA hybridization data (Yassin *et al*., 1997). The genus *Thermobifida* includes *Thermobifida fusca* and *Thermobifida alba* and both of them were transferred from the genus *Thermomonospora* (Zhang *et al*.,

Abbreviations: DAP, diaminopimelic acid, GL, phospholipids of unknown structure containing glucosamine; ISP, International *Streptomyces* Project; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmethylethanolamine.

The GenBank accession number for the 16S rDNA sequence of *Streptimonospora salina* strain YIM 90002^T (= CCTCC 99003^T = CCRC 16284^T) is AF178988.

1998). The species *Thermomonospora mesouvifomis* was proposed to be a synonym of *Thermomonospora alba* (Zhang *et al*., 1998; McCarthy & Cross, 1984).

During our taxonomic studies on extremophilic actinomycetes, we isolated a strain, YIM 90002^T, from soil samples collected from a salt lake in Xinjiang, the western province of China. After a primary analysis of the 16S rDNA sequence of the strain, together with analysis of the 16S rDNA sequences of many representative species from most genera of actinomycetes in databases, we found high levels of sequence similarity between *Nocardiopsis* species and *Thermobifida* species. Strain YIM 90002^T, however, formed a distinct branch in the phylogenetic tree. This strain was morphologically different from any other species of actinomycete. Therefore, strain YIM 90002^T differs phylogenetically and phenotypically from the closest genera, *Nocardiopsis* and *Thermobifida*. More detailed analyses were carried out subsequently.

In this work, we have analysed and classified strain YIM 90002^T by reconciling the genotypic and phenotypic features (Embley & Stackebrandt, 1994). We propose that the micro-organism should be included in a new genus, *Streptimonospora* gen. nov., as *Streptimonospora salina* sp. nov.

METHODS

Organism and culture conditions. Strain YIM 90002^T (as type species of the genus) was isolated from soil samples collected from a salt lake in Xinjiang, China, and deposited in the Chinese Centre for Type Cultures Collection as strain CCTCC 99003^T and the Culture Collection of the Food Industry Research & Development Institute, Taiwan, as strain CCRC 16284^T. International *Streptomyces* Project (ISP) medium 5 (salt concentration, 15%, w/v; pH 7·0) (Shirling & Gottlieb, 1966) was used for the isolation and pure-culture incubation of strain YIM 90002^T. Cell material for DNA extraction was grown on ISP medium 5 for YIM 90004^T at 28 °C for 28 d. The wet biomass used for whole-cell analysis of amino acids and sugars was obtained from cultures grown in ISP 5 broth (salt concentration, 15%, w/v) for 28 d at 28 °C. All strains investigated in this study are listed in Table 1.

Table 1 Strains investigated in this study

Nocardiopsis halophila was not included in this study.

Taxon	Strain	16S rDNA accession no.
Streptimonospora salina	YIM 90002^T	AF178988
Thermobifida alba	JCM 3077^T	AF002260
Thermobifida fusca	JCM 3262^T	AF002264
*Thermobifida mesouviformis**	JCM 3169^T	AF002265
Nocardiopsis prasina	DSM 43845^T	X97884
Nocardiopsis listeri	DSM 40297^T	X97887
Nocardiopsis alba	DSM 43377^T	X97883
Nocardiopsis lucentensis	DSM 44048^T	X97888
Nocardiopsis synnemataformans	DSM 44143^T	Y13593
Nocardiopsis dassonvillei	DSM 43111^T	X97886
Thermomonospora curvata	IFO 15933^T	D86945
Streptosporangium album	DSM 43023^T	X89934
Actinomadura madurae	DSM 43067^T	X97889
Microtetraspora glauca	DSM 43311^T	X93190
Saccharothrix australiensis	NRRL 11239^T	AF114803
Streptomyces megasporus	DSM 41476^T	Z68100

* Synonym of *Thermobifida alba* (Zhang *et al*., 1998).

Preparation of genomic DNA and amplification of the 16S rRNA gene. Genomic DNA was isolated from the test strain by using a procedure (Hopwood *et al*., 1985) that was modified slightly by us. 16S rDNA was amplified by PCR using TaKaRa Ex *Taq* (TaKaRa Biotechnology) and primers A 8–27f (5′-CCGTCGACGAGCTC**AGAGTTTGATCCTGGCTCAG**-3′) and B 1523–1504r (5′-CCCGGGTACCAAGCTT**AAGGAGGTGATCCAGCCGCA**-3′). The conditions used for thermal cycling were as follows: denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 56 °C for 1 min and extension at 72 °C for 3 min. At the end of the cycles, the reaction mixture was kept at 72 °C for 5 min and then cooled to 4 °C. The 1·5 kb amplified 16S rDNA fragment was separated by agarose gel electrophoresis and purified by using a Watson gel extraction kit. The purified fragment was sequenced directly by using the Big Dye terminator cycle sequencing ready reaction kit (Perkin-Elmer) and was analysed with an ABI PRISM 377 DNA sequencer. The sequencing primers were KMS098PB1r (5′-TAAGGAGGTGATCCAGCC-3′), KMS098PDr (5′-GGGTTGCGCTCGTTG-3′) and KMS098PCr (5′-TCTGCGCATTTCACCGCTAC-3′).

Sequence alignment and phylogenetic analysis. Reference strains were chosen from BLAST (Altschul *et al*., 1997) search results. Multiple alignments of sequences determined in this study together with reference sequences obtained from databases and calculations of levels of sequence similarity were carried out using CLUSTAL W 1·74 (Higgins *et al*., 1992). A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980, 1983). Maximum-likelihood and parsimony trees (not shown) were generated using the treeing algorithms contained in the PHYLIP package (Felsenstein, 1995). The topology of the neighbour-joining phylogenetic tree was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Nucleotide sequence accession numbers. The accession numbers of the reference strains, which are closely related to strain YIM 90002^T, are listed in Table 1.

Morphological and physiological characteristics. Morphological features were observed on glycerol/asparagine agar (ISP medium 5) (15% salt, w/v) and the incubation time was 28–30 d at 28 °C. Physiological features were observed on media commonly used for the characterization of *Streptomyces* species (Shirling & Gottlieb, 1966). Cultural characteristics were determined after 28–30 d at 28 °C by using ISP methods (Shirling & Gottlieb, 1966). Morphological observations of spores and mycelia were obtained by scanning electron microscopy, as described previously (O'Donnell *et al*., 1993), with a JEOL model JSM35CF scanning electron microscope. The media and procedures used to examine the physiological features and carbon-source utilization of strain

YIM 90002^T were those described by Shirling & Gottlieb (1966) and Locci (1989). Colour determinations were made by comparing pure cultures with colour chips from the ISCC–NBS colour charts (standard samples, no. 2106) (Kelly, 1964).

Analysis of chemotaxonomic characteristics. Cell wall was purified and analysed using the TLC protocol of Lechevalier & Lechevalier (1980). The procedures of Becker *et al.* (1964) and Lechevalier & Lechevalier (1980) were used for the analysis of whole-cell composition. GC/MS was used for the quantitative determination of sugar content (Saddler *et al.*, 1991). The phospholipid analysis was carried out by the method of Lechevalier *et al.* (1981). Menaquinones were determined using the procedures of Collins (1985).

RESULTS

Morphological observations

Morphological observation of a 28-d-old culture of strain YIM 90002^T grown on glycerol/asparagine agar (ISP medium 5) containing 15% (w/v) salt revealed that the vegetative hyphae with irregular branches were well developed but not fragmented (Fig. 1). The aerial mycelium, at maturity, formed short chains of spores; spores in short chains were oval- to rod-shaped (1·5–2 × 1 μm) with wrinkled surfaces (Fig. 1b). Substrate mycelium was extensively branched with non-fragmenting hyphae. Single spores, oval to round and 1·4–1·6 μm in diameter, were borne on sporophores of substrate mycelium (Fig. 1a) or dichotomously branched sporophores, and the surfaces of single spores were wrinkled. Therefore, the spores consist of two types, both of which are non-motile.

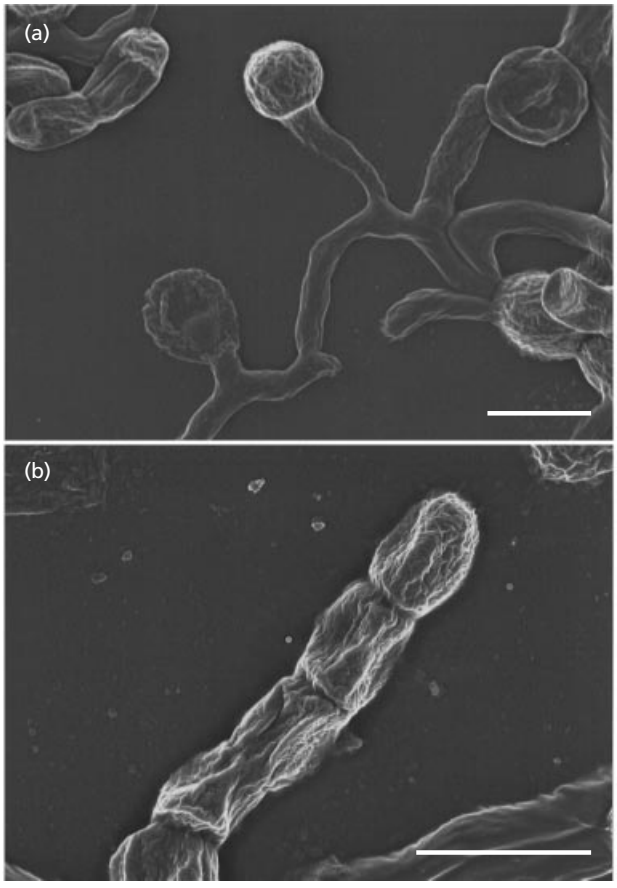

Fig. 1. Scanning electron micrographs of *Streptimonospora salina* (YIM 90002^T) grown on ISP medium 5 for 28 d at 28 °C, showing single spores (a) and a short chain of spores (b). Bars, 2 μm.

Cultural characteristics

As shown in Table 2, strain YIM 90002^T produced well-developed white to pale-yellow colonies on most media tested. It showed good growth on most media except oatmeal agar (ISP medium 3). No diffusible pigments were produced. It developed aerial hyphae on most media tested, especially Czapek's agar and glycerol/asparagine agar (ISP medium 5).

Physiological characteristics

Strain YIM 90002^T utilized glucose, sucrose, maltose, arabinose, raffinose, starch, glycerol, mannitol and histidine. It was positive for starch hydrolysis and melanin production, but negative for milk coagulation, milk peptonization, growth in cellulose, H_2S production and gelatin liquefaction.

Chemotaxonomic characteristics

The cell-wall amino acid composition and whole-cell sugar pattern of strain YIM 90002^T are shown in Table 3. The cell walls contained *meso*-diaminopimelic acid (DAP), glycine, aspartic acid and lysine and trace amounts of DD-DAP. Whole cells of strain YIM 90002^T contained large amounts of glucose and galactose and smaller amounts of ribose, arabinose, xylose and mannose. Rhamnose and madurose were not detected, indicating that the strain contains no characteristic wall sugars (according to the scheme of Lechevalier & Lechevalier, 1980). The phospholipids are phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidylethanolamine (PE). The major menaquinones are MK-9(H_6), MK-10(H_2) and MK-10(H_4).

Phylogenetic position

An almost complete 16S rDNA sequence was determined for strain YIM 90002^T (> 95% of the *Escherichia coli* sequence) from position 8 to position 1523 (*E. coli* numbering system; Brosius *et al.*, 1978). BLAST search results for strain YIM 90002^T came from non-redundant GenBank + EMBL + DDBJ + PDB; when reference sequences were chosen, unidentified and unpublished sequences were excluded (Table 1). The number of nucleotides compared was 1436 after elimination of sites for which the nucleotides were not

Table 2 Cultural characteristics of strain YIM 90002^T

Diffusible pigments were not produced on any of the media listed.

Medium*	Growth	Sporulation	Colour of colonies†	
			Aerial mycelium	Substrate mycelium
Yeast extract/malt extract (ISP medium 2)	Good	Moderate	Pale white	Mid-yellow
Oatmeal agar (ISP medium 3)	Poor	Poor	White	Deep yellow
Inorganic salt/starch agar (ISP medium 4)	Moderate	Moderate	White	Yellow/white
Glycerol/asparagine (ISP medium 5)	Good	Good	White	Mid-yellow
Czapek's agar	Good	Good	White	Mid-yellow
Potato agar	Good	Moderate	White	Deep yellow
Nutrient agar	Good	Moderate	White	Deep orange/yellow

* Containing 15% (w/v) salt; pH 7·0.

† Colours taken from ISCC–NBS colour charts (standard samples, no. 2106) (Kelly, 1964).

Table 3 Chemotaxonomic characteristics of strain YIM 90002^T and the genera of the *Nocardiopsaceae*

The genera *Nocardiopsis* and *Thermobifida* follow the classification of Zhang *et al*. (1998). None of the taxa exhibited any diagnostic sugars. Abbreviations: DAP, diaminopimelic acid; GL, phospholipids of unknown structure containing glucosamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmethylethanolamine.

Characteristic	Strain YIM 90002^T	*Nocardiopsis*	*Thermobifida*
Sugar content	15% Ribose, 6% xylose, 5% arabinose, 2% mannose, 34% galactose, 38% glucose*	Type C	Type C
Wall peptidoglycan	*meso*-DAP [glycine, lysine and aspartic acid (detected), DD-DAP (trace)]	Cell wall type III (*meso*-DAP)	Cell wall type III [*meso*-DAP, LL-DAP (trace)]
Phospholipids	PI, PE, PG	Type PIII (PC, PME, GL)	Type PII (PE, PME, GL)
Menaquinones	MK-9(H_6), MK-10(H_2), MK-10(H_4)	Type 4c [MK-10(H_2), MK-10(H_4), MK-10(H_6)]	Type 4d [MK-10(H_6), MK-10(H_8), MK-11(H_6)]

* Relative content. Rhamnose and madurose were not detected.

determined in all sequences. The BLAST search results and the phylogenetic tree (Fig. 2) generated from representative strains of the related genera showed that strain YIM 90002^T had high levels of sequence similarity to species of *Thermobifida* (Zhang *et al*., 1998) and members of the genus *Nocardiopsis*. The phylogenetic tree obtained by applying the neighbour-joining method to K_{nuc} values is shown in Fig. 2. 16S rDNA analysis revealed that strain YIM 90002^T is phylogenetically closely related to members of the genera *Nocardiopsis* and *Thermobifida* (the sequence similarity levels were 94·9–95·1% to *Thermobifida* and 93·7–94·7% to *Nocardiopsis*). All strains of the genera *Nocardiopsis* and *Thermobifida*, together with strain YIM 90002^T, formed a distinct clade that was strongly supported by a high bootstrap value (100%). Phylogenetically, strain YIM 90002^T forms a branch that is distinct from the groups containing the genera *Nocardiopsis* and *Thermobifida* and which lies between the two groups.

DISCUSSION

The results of 16S rDNA sequence comparisons clearly demonstrated that strain YIM 90002^T is a member of the family *Nocardiopsaceae*, and the 16S rDNA sequence of strain YIM 90002^T contains all the signature nucleotides defined for the family *Nocardiopsaceae*, as described by Stackebrandt *et al*. (1997). The high levels of 16S rDNA sequence similarity to species of the genera *Nocardiopsis* (93·7–94·7%) and *Thermobifida* (94·9–95·1%) place strain YIM 90002^T in the *Nocardiopsaceae* as a distinct member between *Nocardiopsis* and *Thermobifida* (Fig. 2).

Strain YIM 90002^T is morphologically different from members of the genera *Thermobifida* and *Nocardiopsis*. On aerial mycelium, strain YIM 90002^T forms short chains of spores that are somewhat like the spores formed on aerial mycelium by members of *Nocardiopsis*. Strain YIM 90002^T also forms single spores, borne on sporophores or dichotomously branched

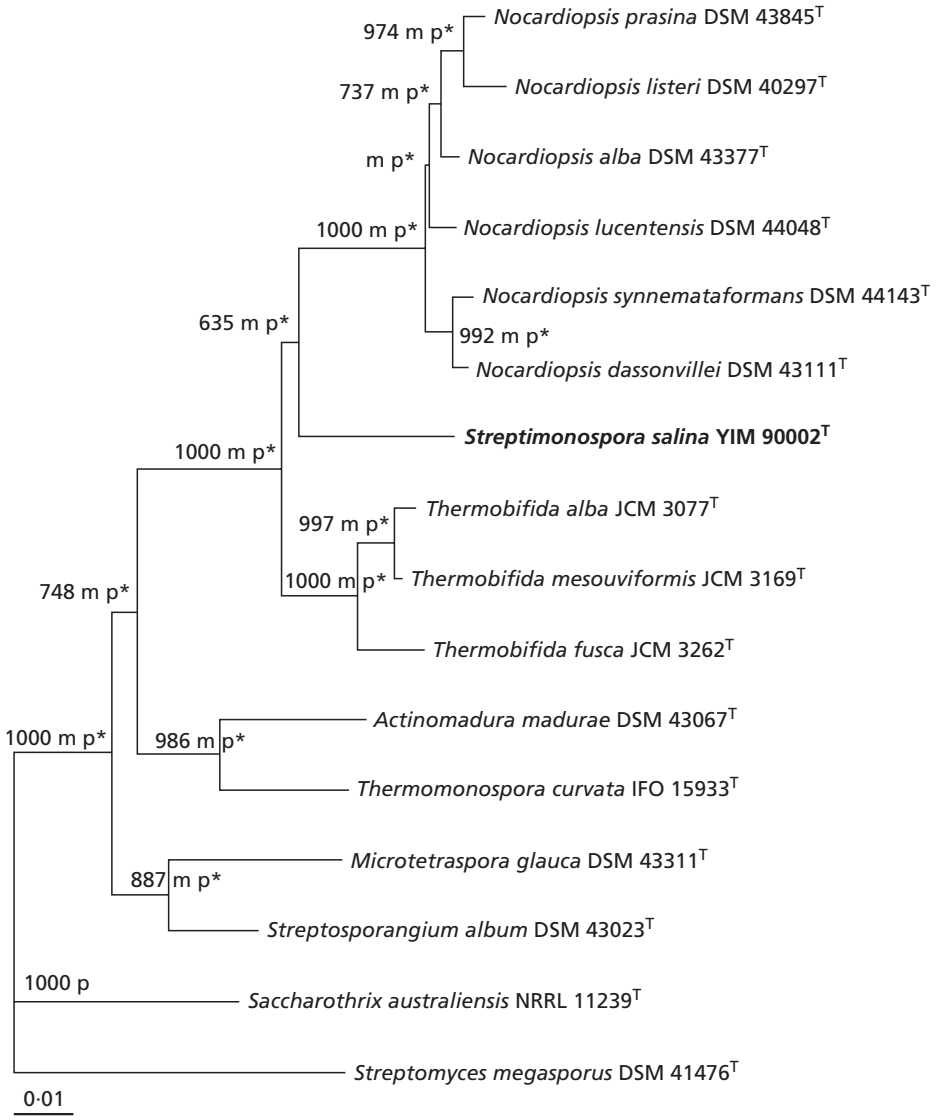

Fig. 2. Neighbour-joining tree showing the phylogenetic relationships among *Streptimonospora salina* and representative members of the families *Nocardiopsaceae*, *Streptosporangiaceae* and *Thermomonosporaceae* in the suborder *Streptosporangineae*. *Streptomyces megasporum* was used as the outgroup. The 'm' and 'p' labels indicate branches that were also found with the maximum-likelihood (Felsenstein, 1981) and parsimony (Kluge & Farris, 1969) algorithms, respectively: asterisks indicate branches that were recovered with all three methods. The analysis included 1436 unambiguous nucleotide positions. Bootstrap values (> 50 %) from 1000 analyses are shown at the nodes of the tree. The strain shown in bold is the type species of the new genus *Streptimonospora*. Bar, 1 nucleotide substitution per 100 nucleotides of 16S rDNA sequence.

sporophores of substrate hyphae, that are morphologically similar to the spores found on dichotomously branched sporophores (resulting in spore clusters on aerial hyphae) of members of the genus *Thermobifida*.

Strain YIM 90002^T is chemotaxonomically different from members of the genera *Nocardiopsis* and *Thermobifida*. The cell wall of strain YIM 90002^T contains *meso*-DAP, DD-DAP, glycine and aspartic acid; whole-cell hydrolysates contain no diagnostic sugars; the phospholipids are PG, PI and PE and the major menaquinones are MK-9(H_6), MK-10(H_2) and MK-10(H_4). For members of the genus *Thermobifida*, cell walls contain *meso*-DAP (cell wall type III), a trace amount of LL-DAP may be detected in whole-cell hydrolysates, the sugar pattern is type C (no diagnostic sugars), the predominant menaquinones are MK-10(H_6), MK-10(H_8) and MK-11(H_6) and the phospholipid pattern is type II [PE, PME (phosphatidylmethylethanolamine), GL (phospholipids of unknown structure, containing glucosamine)] (Zhang *et al.*, 1998). For members of the genus *Nocardiopsis*, the peptidoglycan contains *meso*-DAP, no diagnostic sugars are present (cell wall chemotype III/C) (Lechevalier & Lechevalier, 1970), the phospholipid pattern is type III (phosphatidylcholine, PME, GL)

and the menaquinone pattern is type 4c [MK-10(H_2), MK-10(H_4) and MK-10(H_6)] (Zhang *et al.*, 1998).

Therefore, we propose that strain YIM 90002^T should be classified as a member of a new genus, *Streptimonospora*. The type species is *Streptimonospora salina* gen. nov., sp. nov.

Description of *Streptimonospora* gen. nov.

Streptimonospora (Strep.ti.mo.no.spo′ra. Gr. adj. *streptos* pliant, bent; Gr. adj. *monos* single, solitary; Gr. fem. n. *spora* a seed, spore; M.L. fem. n. *Streptimonospora* indicating that this organism forms two types of spore, with wrinkled surfaces, on aerial mycelium and substrate mycelium).

Gram-positive, aerobic organisms with branching hyphae. Non-fragmenting substrate mycelia are present. The aerial mycelium, at maturity, forms short chains of spores; the spores in short chains are oval- to rod-shaped (1·5–2 × 1 μm) with wrinkled surfaces. Substrate mycelium is extensively branched with non-fragmenting hyphae. Single spores, which are oval to round (1·4–1·6 μm), are borne on sporophores or dichotomously branched sporophores of substrate hyphae; the surfaces of the spores are wrinkled. Both types of spore are non-motile. The cell wall contains *meso*-DAP, DD-DAP, glycine and aspartic acid. Whole-cell hydrolysates contain large amounts of glucose and galactose and smaller amounts of ribose, arabinose, xylose and mannose. The phospholipids are phosphatidylglycerol, phosphatidylinositol and phosphatidylethanolamine. The major menaquinones are MK-9(H_6), MK-10(H_2) and MK-10(H_4). The type species is *Streptimonospora salina*.

Description of *Streptimonospora salina* sp. nov.

Streptimonospora salina (sa.li′na. L. adj. *salina* salted, saline).

Aerial mycelium is well developed but not fragmented. Colonies are white on most media. Two types of spore with wrinkled surfaces are borne on aerial mycelium and substrate mycelium. No diffusible pigment is produced, but melanin is produced. Utilizes glucose, sucrose, maltose, arabinose, raffinose, starch, glycerol, mannitol and histidine. Positive for starch hydrolysis and melanin production, but negative for milk coagulation, milk peptonization, growth in cellulose, H_2S production and gelatin liquefaction. Optimum growth occurs in media supplemented with salt at a concentration of 15% (w/v) at 28 °C and pH 7·0.

Isolated from hypersaline habitats (a salt lake in China). The type strain is strain YIM 90002^T (= CCTCC 99003^T = CCRC 16284^T).

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