Streptomonospora alba sp. nov., a novel halophilic actinomycete, and emended description of the genus *Streptomonospora* Cui *et al.* 2001

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A halophilic actinomycete, strain YIM 90003^T, was isolated from a soil sample collected from Xinjiang Province, China, by using starch-casein agar with a salt concentration of 20 % (w/v), pH 7·0. The strain grew well on most media tested. No diffusible pigment was produced. Aerial mycelium and substrate mycelium were well developed on most media. The aerial mycelium formed short spore chains, bearing non-motile, straight to flexuous spores with wrinkled surfaces. The cell walls of strain YIM 90003^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid. Cell-wall hydrolysates contained galactose and arabinose. Menaquinone composition varied with the medium used for cell cultivation; on glucose-yeast extract medium supplemented with 10 % NaCl, the major menaquinone was MK-9(H_4), while, on vitamin-enriched ISP 2 medium, the major menaquinones were MK-10(H_2), MK-9(H_8) and MK-10(H_4). Phospholipids were phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, diphosphatidyl glycerol, methylphosphatidylethanolamine, phosphatidylserine, phosphatidylcholine and an unidentified phospholipid. 16S rRNA gene sequence analysis showed *Streptomonospora salina* as the closest phylogenetic neighbour. On the basis of these analyses, strain YIM 90003^T is a member of the genus *Streptomonospora*, though its properties do not match the generic description fully with respect to the menaquinone composition and peptidoglycan amino acid. Analyses of mechanically disrupted cell walls of the type species, *Streptomonospora salina* DSM 44593^T, and strain YIM 90003^T, purified by tryptic digestion and subsequent SDS treatment, revealed the exclusive presence of *meso*-diaminopimelic acid as the diagnostic diamino acid of peptidoglycan. Thus, the genus description of *Streptomonospora*, indicating the presence of several amino acids usually not found in the peptidoglycan moiety, is therefore emended. DNA–DNA hybridization and comparison of physiological and chemotaxonomic characteristics demonstrated strain YIM 90003^T to be different from *Streptomonospora salina*. The name *Streptomonospora alba* sp. nov. is proposed, with strain YIM 90003^T (=CCTCC AA001013^T=DSM 44588^T) as the type strain.

The halophilic actinomycete genus *Streptomonospora*, forming a distinct branch in the 16S rDNA phylogenetic tree adjacent to the genera *Nocardiopsis* and *Thermobifida*, suborder *Streptosporangineae* (Stackebrandt *et al.*, 1997), was proposed by Cui *et al.* (2001) with a single species, *Streptomonospora salina*. During a taxonomic study on

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Abbreviations: A_2pm, diaminopimelic acid; ISP, International *Streptomyces* Project.

The GenBank/EMBL accession number for the 16S rRNA gene sequence of *Streptomonospora alba* sp. nov. YIM 90003^T is AF462347.

A neighbour-joining tree based on 16S rDNA sequences showing the phylogenetic position of strain YIM 90003^T is available as supplementary material in IJSEM Online.

extremophilic actinomycetes, a second halophilic actinomycete, strain YIM 90003^T, was isolated from the same dry hypersaline soil sample (25 % NaCl, pH 7·5) from which *S. salina* YIM 90002^T was isolated. The sampling site was near a small salt lake in Xinjiang Province in western China.

Strain YIM 90003^T was enriched on starch-casein agar (salt concentration, 20 %, w/v; pH 7·0) and incubated at 28 °C for about 4 weeks. The strain was maintained on International *Streptomyces* Project (ISP) medium 2 and ISP medium 5 agar slants (Shirling & Gottlieb, 1966), supplemented with 15 % (w/v) salt, at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C. Other media used were ISP media 3 and 4, inorganic salt-starch agar, Czapek's agar, potato agar and nutrient agar, all supplemented with 15 % (w/v) salt. Morphological features were observed on modified ISP 2 and ISP 5 media for 4 weeks at 28 °C. Morphological observations of spores and mycelia were done by scanning electron microscopy with a JEOL model JSM35CF scanning electron microscope.

Strain YIM 90003^T grew well on most media, especially on ISP 5 medium, nutrient agar and Czapek's agar. No diffusible pigments were produced on any of the media tested. Investigations of 28-day-old cultures of strain YIM 90003^T, grown on ISP media 2, 4 and 5, revealed that the strain shared morphological characteristics described for the genus *Streptomonospora*. The aerial mycelium was white on all media used and the substrate mycelium was well developed but not fragmented (Fig. 1). It formed short chains of spores at maturity, which were straight to flexuous; spores were oval- to cylindrical-shaped (0·4–0·7 × 0·8–1·6 μm) with wrinkled surfaces and they were non-motile. The colour of the substrate mycelium, determined from the ISCC-NBS Color Charts (standard samples no. 2106; Kelly, 1964), was different on different media (see species description). Single, round to oval spores are borne on substrate mycelium (Fig. 2).

Physiological features and carbon source utilization were observed on media commonly used for the characterization of *Streptomyces* species (Shirling & Gottlieb, 1966; Williams *et al.*, 1989) supplemented with 10 or 15 % (w/v) NaCl. Cultural characteristics were determined after 4 weeks at 28 °C by ISP methods (Shirling & Gottlieb, 1966). Strain YIM 90003^T was catalase-positive and oxidase-negative. The range of carbon substrates of strain YIM 90003^T could not be determined definitely due to poor growth in basal medium supplemented with 10 or 15 %

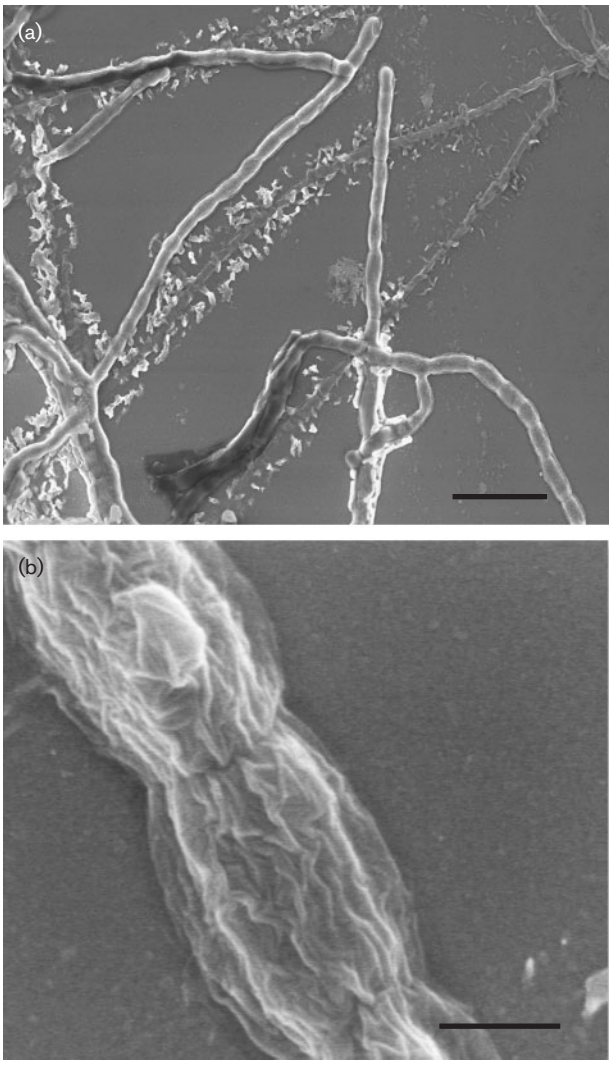

Fig. 1. Scanning electron micrographs of aerial mycelium of a spore chain (a) and spores (b) of *Streptomonospora alba* sp. nov. YIM 90003^T grown on ISP 5 medium (15 % NaCl, w/v; pH 7·0) for 28 days at 28 °C. Bars, 5 μm (a) and 500 nm (b).

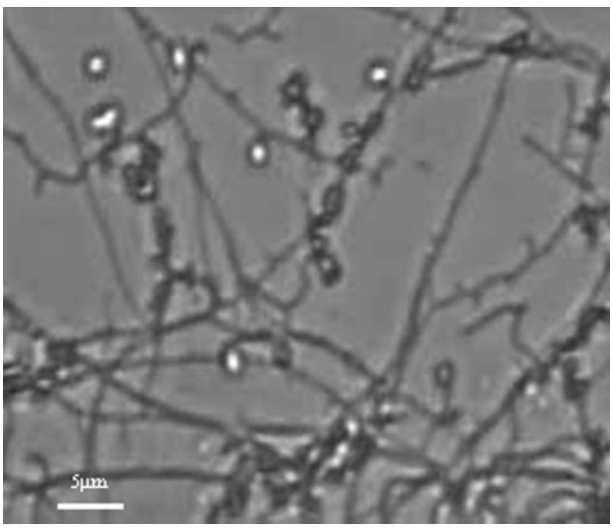

Fig. 2. Light micrograph of single spores borne on substrate mycelium of *Streptomonospora alba* sp. nov. YIM 90003^T grown on ISP 2 medium (10 % NaCl, w/v; pH 7·0) for 21 days at 28 °C. Bar, 5 μm.

(w/v) NaCl. The tests were repeated independently in the laboratories in Kunming and Braunschweig and were found to be identical except for the utilization of glucose. Other reactions are indicated in the species description.

Extraction of genomic DNA, as well as 16S rDNA amplification and sequencing, using TaKaRa Ex *Taq*, have been described previously (Cui *et al.*, 2001). Calculations 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 the K_{nuc} value of Kimura (1980, 1983) using sequences contained in the DSMZ sequence database. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The almost-complete 16S rRNA gene sequence (1487 nt) was obtained for strain YIM 90003^T. A neighbour-joining tree, generated from about 1440 bp resulting after removal of alignment gaps, is available as supplementary material in IJSEM Online. The sequence of strain YIM 90003^T was very similar to that of *S. salina* YIM 90002^T (99·3 %), and the two strains formed a distinct branch in the phylogenetic tree of members of the family *Nocardiopsaceae* (Rainey *et al.*, 1996), with which strain YIM 90003^T shares similarity values between 93·9 and 96·4 % (not all relatives are shown in the tree).

Chromosomal DNA was prepared following the method of Marmur (1961). The G+C content of DNA of strain YIM 90003^T, determined using the HPLC method of Mesbah *et al.* (1989), was 74·4 mol%. The DNA G+C content is similar to that of *S. salina* YIM 90002^T (72·9 mol%), which was not included in the original description of the species (Cui *et al.*, 2001).

Biomass for molecular systematic studies was obtained by growing at 28 °C for 3 weeks in 1000 ml shake flasks (about 500 r.p.m.) containing 200 ml ISP 2 medium (15 % NaCl, w/v) broth supplemented with vitamin mixtures of HV medium (Hayakawa & Nonomura, 1987). Menaquinones, peptidoglycan structure, phospholipids and cell-wall sugars were also determined from cells grown in medium GYM (medium no. 65; DSMZ, 2001), supplemented with 10 % NaCl. Cell walls were purified and amino acids analysed using TLC as described by Lechevalier & Lechevalier (1980) and Schleifer & Kandler (1972). Cell walls contained *meso*-diaminopimelic acid (*meso*-A_2pm), alanine, glutamic acid, muramic acid and acetylglucosamine. This result did not correspond to the description of the genus *Streptomonospora*, in which meso-A_2pm, DD-A_2pm, glycine and aspartic acid were reported as cell wall amino acids (Cui *et al.*, 2001). In order to investigate whether all amino acids reported by Cui *et al.* (2001) are really components of the peptidoglycan, elucidation of the peptidoglycan structure was performed with purified cell walls of strains YIM 90002^T and YIM 90003^T. It turned out that purification of peptidoglycan from residual contaminating proteins required prolonged mechanical disintegration (35 instead of 20 min) and intensive treatment with 2 % (w/v) SDS (15 instead of 3 min at 100 °C) in the case of strain YIM 90002^T. Total and partial hydrolysis of peptidoglycan of strain YIM 90002^T and subsequent two-dimensional TLC on cellulose plates (Merck) revealed the presence of *meso*-A_2pm, alanine, glutamic acid, muramic acid and acetylglucosamine and di- and tripeptides typical of peptidoglycan type A1γ (Schleifer & Kandler, 1972), as found for strain YIM 90003^T. Thus, it can be assumed that the additional amino acids reported for *S. salina* are constituents of a protein tightly attached to the peptidoglycan. GC/MS used for the quantitative determination of sugar content (Chen *et al.*, 2000) revealed the presence of mainly glucose and arabinose in strain YIM 90003^T, while glucose and galactose were present in *S. salina* YIM 90002^T (Cui *et al.*, 2001). In this study, galactose and arabinose were found by TLC on cellulose plates (Merck) in hydrolysates of purified cell walls of strain YIM 90003^T and only galactose was detected in those of the type strain of *S. salina*. The phospholipid composition of strain YIM 90003^T, determined according to Minnikin *et al.* (1979), was complex, consisting of phosphatidylglycerol, phosphatidylethanolamine (PE), phosphatidylinositol, diphosphatidylglycerol, methyl-phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine and an unidentified phospholipid. The phospholipid pattern of *S. salina* YIM 90002^T was found to consist of phosphatidylglycerol, PE and phosphatidylcholine as well as traces of two methylated PEs and phosphatidylinositol.

Following the procedures of Groth *et al.* (1999), the predominant menaquinones in strain YIM 90003^T grown on vitamin-enriched ISP 2 medium were MK-10(H_2), MK-10(H_4) and MK-9(H_8). The composition changed, however, when cells grown on glucose-yeast extract medium were analysed: the major menaquinone was MK-9(H_4), while MK-9, MK-10(H_4), MK-9(H_2), MK-9(H_6) occurred as minor compounds. In order to investigate whether the same phenomenon was detected in *S. salina*, strain YIM 90002^T grown on glucose-yeast extract agar was subjected to menaquinone analysis and found to contain MK-10(H_4), MK-10(H_6), MK-10(H_2), MK-10(H_8) and MK-10, while MK-9(H_2) and MK-9(H_4) were minor components. This composition differed from the published data (Cui *et al.*, 2001) obtained with cells grown on vitamin-enriched ISP 2 agar, which revealed the presence of MK-9(H_6), MK-10(H_2) and MK-10(H_4). The reason for this change has not been investigated, but medium- and age-dependent shifts in menaquinone composition have been reported before for *Actinobacteria* (Saddler *et al.*, 1986; Hiraishi & Komagata, 1989).

DNA–DNA hybridization was carried out between the phylogenetically highly related strains YIM 9003^T and *S. salina* YIM 90002^T, applying the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992) under optimal hybridization conditions (2× SSC, 20 % formamide, 69 °C). The binary value of 18 % (mean of duplicate measurements of 16 and 20 %) was significantly

lower than 70 %, considered to be the threshold value for the delineation of genospecies, clearly indicating the genomic differences between *S. salina* YIM 90002^T and strain YIM 90003^T. On the basis of morphological, phylogenetic and some common chemotaxonomic data, strain YIM 90003^T should be placed in the genus *Streptomonospora*. Differences between the two strains were found in the composition of cell-wall sugars and menaquinones and in the presence of phosphatidylserine in strain YIM 90003^T. *S. salina* is oxidase-positive, nitrate reduction-negative, starch hydrolysis-positive and produces melanin, while strain YIM 90003^T shows the opposite responses. Comparison of carbon utilization between *S. salina* and strain YIM 90003^T is hampered by the extremely poor growth of the latter strain in basal medium supplemented with 10 % (w/v) NaCl. These differences justify the description of a novel species for strain YIM 90003^T, for which the name *Streptomonospora alba* sp. nov. is proposed.

Emended description of *Streptomonospora* Cui *et al.* 2001

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

Gram-positive, aerobic organisms with branching hyphae. Non-fragmenting substrate mycelium present. The aerial mycelium, at maturity, forms short chains of non-motile spores; spores in short chains are oval- to rod-shaped with wrinkled surfaces. Substrate mycelium is extensively branched with non-fragmenting hyphae. Single, non-motile, oval to round spores are borne on sporophores or dichotomously branched sporophores of substrate hyphae. Peptidoglycan contains *meso*-diaminopimelic acid as diagnostic diamino acid. Cell walls contain galactose or galactose plus arabinose. The phospholipid pattern is complex, consisting of phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol; diphosphatidylglycerol, methylphosphatidylethanolamine and phosphatidylserine may occur. The menaquinone composition may depend on the growth medium and consists mainly of menaquinones with nine or ten isoprenoid chains and a varying degree of hydrogenation: i.e. a combination of one or more representative(s) of the series [MK-9(H_2), (H4), (H_6), (H_8)] plus [MK-10(H_2), (H_4), (H_6), (H_8)]. The DNA base composition ranges from 69 to 71 mol% G+C (HPLC). Phylogenetically, a neighbour of *Nocardiopsis*, *Thermobifida* and *Actinomadura*. The type species is *Streptomonospora salina*.

Description of *Streptomonospora alba* sp. nov.

Streptomonospora alba (al′ba. L. fem. adj. *alba* white).

Aerial mycelium and substrate mycelium are well developed but not fragmented on most media. The white aerial mycelium forms short chains of spores at maturity, which are straight to flexuous; spores are oval- to cylindrical-shaped (0·4–0·7 × 0·8–1·6 μm) with wrinkled surfaces and they are non-motile. Single, round to oval spores are borne on substrate mycelium. Grows well on most test media but no diffusible pigment is produced. Colour of the substrate mycelium is white (ISP 4, ISP 5, Czapek's agar), grey-white (ISP 3), moderate orange-yellow (ISP 2), deep orange-yellow (potato agar) or brilliant orange-yellow (nutrient agar). The diagnostic diamino acid of peptidoglycan is *meso*-diaminopimelic acid, while galactose and arabinose are cell-wall sugars. The predominant menaquinone is MK-9(H_4) (glucose-yeast extract-grown cells), while MK-10(H_2), MK-9(H_8) and MK-10(H_4) are found in vitamin-enriched ISP 2 medium. Major phospholipids are phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, diphosphatidylglycerol, methylphosphatidylethanolamine, phosphatidylserine, phosphatidylcholine and an unidentified phospholipid. Catalase-positive, oxidase-negative. Grows in medium supplemented with between 5 and 25 % NaCl (w/v), optimum growth in 10–15 % NaCl (w/v) at 28 °C and pH 7·0. Nitrate reduction is positive, starch hydrolysis and production of melanin are negative. The range of carbon utilization could not be determined because of negative reactions caused by extremely poor growth in basal media. The G+C content of DNA of the type strain is 74·4 mol% (HPLC).

The type strain, strain YIM 90003^T (=CCTCC AA001013^T=DSM 44588^T), was isolated from soil in a hypersaline habitat in Xinjiang Province, western China.

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