Streptomyces sparsus sp. nov., isolated from a saline and alkaline soil

Yi Jiang,[1] Yan-Ru Cao,[1,3] Jutta Wiese,[2] Shu-Kun Tang,[1] Li-Hua Xu,[1] Johannes F. Imhoff[2] and Cheng-Lin Jiang[1]

Correspondence
Yi Jiang
jiangyikm@hotmail.com
Johannes F. Imhoff
jimhoff@ifm-geomar.de

[1]Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, PR China

[2]Leibniz-Institut für Meereswissenschaften, IFM-GEOMAR, Düsternbrooker Weg 20, D-24105 Kiel, Germany

[3]College of Resources and Environment, Northwest A & F University, 712100 Yangling, Shaanxi, PR China

A salt- and alkali-tolerant actinomycete strain, YIM 90018^T, was isolated from a saline and alkaline soil sample collected from Qinghai, China. Aerial hyphae of strain YIM 90018^T were only produced on YIM 82 agar. Vegetative hyphae were well developed and did not fragment. Straight or flexuous (*rectiflexibiles*) spore chains were produced. The isolate grew well with 25 % (w/v) $MgCl_2 . 6H_2O$ and at pH 10. All of these characters indicated that strain YIM 90018^T belonged to the genus *Streptomyces*. On the basis of phylogenetic analysis of the 16S rRNA gene sequence, DNA–DNA hybridization and phenotypic characteristics, strain YIM 90018^T could be differentiated from all recognized species of the genus *Streptomyces*. A novel species, *Streptomyces sparsus* sp. nov., is proposed, with strain YIM 90018^T (=CCTCC AA204019^T=DSM 41858^T) as the type strain.

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) and species of this genus are of great interest owing to their production of various natural products with considerable commercial value. In the course of screening of actinomycetes for bioactive metabolites, strain YIM 90018^T was isolated from a saline and alkaline soil sample collected from Qinghai Province, China. Strain YIM 90018^T was isolated on starch-casein medium with 20 % (w/v) $MgCl_2$ (containing l^{-1}: 10 g starch, 0.3 g casein, 2 g KNO_3, 0.05 g $MgSO_4 . 7H_2O$, 2 g NaCl, 2 g K_2HPO_4, 0.02 g $CaCO_3$, 200 g $MgCl_2 . 6H_2O$, 20 g agar; pH 7.2). The isolate was stored in 20 % glycerol at −20 °C.

For observations of the sporophores, spore chains and spore surfaces, strain YIM 90018^T was cultivated on YIM 82 agar [containing l^{-1}: 5 g starch, 1 g asparagine, 1 g K_2HPO_4, 3.7 mg vitamin mixture from HV agar (Hayakawa & Nonomura, 1987), 1 ml trace salts from International *Streptomyces* Project (ISP) medium 5 (Shirling & Gottlieb, 1966), 20 g agar; pH 7.2 or pH 10.0–11.0] and examined by light and scanning electron microscopy (JSM-5600LV, JEOL). Cultural characteristics were studied on ISP media (Shirling & Gottlieb, 1966), Czapek's agar, nutrient agar (Waksman, 1961), YIM 81 agar (containing l^{-1}: 1 g asparagine, 10 g glycerol, 0.5 g yeast extract, 0.5 g KNO_3, 1 g K_2HPO_4, 20 g agar; pH 7.2 or pH 10–11) and YIM 82 agar after incubation for 14 days at 28 °C. The colour of both substrate and aerial mycelia, together with the production of soluble pigments, was determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). All tests were done at 28 °C and pH 7.2 unless otherwise specified. The production of melanin was tested on ISP 7. Carbon source utilization was examined using ISP 9 as the basal medium supplemented with 1 % final concentration of the tested substrate. Nitrogen source utilization, catalase production and starch and gelatin degradation were detected in modified Bennett agar after 7, 14 and 21 days, as described by Williams *et al.* (1983). Hydrogen sulphide production was detected by the method of Shirling & Gottlieb (1966). The effect of temperature, pH and salts on growth was determined using modified Bennett agar as the basal medium.

For chemotaxonomic studies, strain YIM 90018^T was grown in potato extract-glucose broth (200 g fresh potato boiled in 1 l water for 30 min and filtered) on a shaking incubator at 200 r.p.m. at 28 °C for 7 days. Mycelium was harvested by centrifugation, washed three times with distilled water and then freeze-dried. The determination of diamino acid in the cell wall and the analysis of the whole-cell sugars were performed as described by Lechevalier & Lechevalier (1970, 1980) and Staneck & Roberts (1974), respectively. Polar lipids were extracted

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 90018^T is AJ849545.

Table 1. Cultural characteristics of strain YIM 90018^T

Colours are according to ISCC-NBS colour charts, standard sample no. 2106 (Kelly, 1964). +++, Good; ++, moderate; +, weak; −, none. No growth took place on glucose-asparagine agar.

Medium	Aerial mycelium		Substrate mycelium	
	Growth	Colour	Growth	Colour
Czapek's agar	−	−	+	−
Glycerol-asparagine agar (ISP 5)	−	−	+	Brilliant yellow
Inorganic salt-starch agar (ISP 4)	−	−	++	Light yellow
Yeast extract-malt extract agar (ISP 2)	−	−	++	Brilliant yellow
Potato extract agar	−	−	+++	Brilliant yellow
Nutrient agar	−	−	++	Brilliant yellow
YIM 81 agar	−	−	++	Light yellow
YIM 82 agar	+	Pale grey	+	Pale yellow

and detected by the method of Komagata & Suzuki (1987). Menaquinones were extracted, purified and identified by HPLC as described by Collins (1985). The cellular fatty acid composition was analysed as described by Sasser (1990). The DNA G+C base content was determined by HPLC (Tamaoka & Komagata, 1984) using an Agilent 1100 LC system (IRIS Technologies). DNA–DNA hybridization between strain YIM 90018^T and its closest phylogenetic neighbours was carried out as described by Christensen *et al.* (2000).

For 16S rRNA gene sequence analysis, genomic DNA was extracted by the method described by Orsini & Romano-Spica (2001). PCR-mediated amplification of the 16S rRNA gene, purification of the PCR products and sequence analysis of the purified products were performed as described by Cui *et al.* (2001). The resultant sequence was manually aligned against bacterial sequences available from public databases. A more detailed comparison was performed with sequences from members of the genus *Streptomyces* and evolutionary distance matrices were calculated by the method of Jukes & Cantor (1969). Phylogenetic trees were inferred by using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Bootstrap analysis (Felsenstein, 1985) with 1000 resamplings was used to evaluate the topology of the neighbour-joining tree.

The cultural and morphological characteristics of strain YIM 90018^T are shown in Tables 1 and 2, respectively. Observation of 15-day-old cultures revealed that strain YIM 90018^T produced a poor, pale grey, aerial mycelium on YIM 82 agar but did not produce aerial hyphae on the other media tested. Vegetative hyphae were abundant, not fragmented and light or brilliant yellow. Straight to flexuous (*rectiflexibiles*) spore chains were only present on YIM 82 agar. Spores were short and rod-shaped and variable in size (0.5–0.7 × 1.0–1.3 μm). The spore surface was smooth (Fig. 1). Soluble pigments were not produced on any media.

The 16S rRNA gene sequence (1466 nt) of strain YIM 90018^T was compared with corresponding sequences of type strains of species of the genus *Streptomyces*. The

Table 2. Comparison of morphological characteristics of strain YIM 90018^T and some members of the genus *Streptomyces*

Strains: 1, YIM 90018^T (data from this study); 2, *S. rimosus* subsp. *rimosus* (Yan, 1992); 3, *S. sclerotialus* (Yan, 1992); 4, *S. niger* (Yan, 1992); 5, *S. erumpens* (Yan, 1992); 6, *S. kasugaensis* (Yan, 1992; Backus & Tresner, 1956); 7, *S. olivaceiscleroticus* (Yan, 1992). ND, No data available.

Characteristic	1	2	3	4	5	6	7
Aerial hyphae	Sparse, pale grey	Abundant, white, yellow	Abundant, sclerotia, white, yellowish red, pale yellow–green	Abundant, grey	Abundant, grey	Abundant, white	Abundant, pale white, grey–black
Spore chain	Straight to flexuous (*rectiflexibiles*)	Spiral	Spiral	Spiral	Spiral	Loops and spiral	Spiral
Spore shape	Short rod	Oval	Oval	Oval	ND	ND	Oval
Substrate hyphae	Yellow	Brown, red–brown	Orange–yellow, green, yellowish brown	Black	Brown	Brown, red–brown	Black, brown
Diffusible pigments	None	Yellow, yellowish brown	Yellowish brown, green	Brown	Yellow	Dark yellow, yellowish brown	Olive yellow, pale red

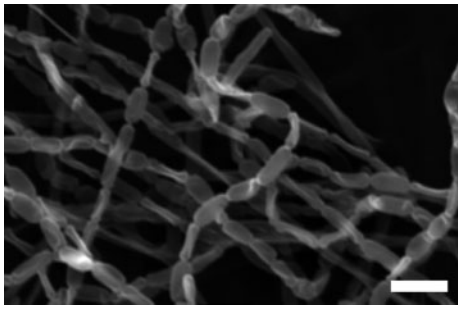

Fig. 1. Scanning electron micrograph showing spores and spore chains of strain YIM 90018^T after growth on YIM 82 agar at 28 °C for 15 days. Bar, 2 μm.

neighbour-joining tree based on 16S rRNA gene sequences is shown in Fig. 2. The phylogenetic analysis revealed that strain YIM 90018^T was phylogenetically related to the genus *Streptomyces* and formed a separate clade. The highest sequence similarities were found with *Streptomyces rimosus* subsp. *rimosus* JCM 4667^T (98.55 % 16S rRNA gene sequence similarity), *S. erumpens* DSM 40941^T (98.33 %), *S. sclerotialus* DSM 43032^T (98.04 %), *S. olivaceiscleroticus* DSM 40595^T (97.99 %), *S. niger* DSM 43049^T (97.99 %) and *S. kasugaensis* NBRC 13851^T (97.6 %).

The fatty acid content of strain YIM 90018^T was remarkably different from related members of the genus *Streptomyces* (Table 3). Strain YIM 90018^T contained 38.1 % $C_{18:1}\omega9c$, of which *S. sclerotialus* DSM 43032^T contained 0.7 % and *S. kasugaensis* DSM 40819^T and *S. niger* DSM 43049^T contained none. Strain YIM 90018^T also contained $C_{16:1}\omega9c$ and $C_{20:1}\omega9c$, which were not found in the reference strains. The reference strains contained 25.7–34.0 % anteiso-$C_{15:0}$, of which strain YIM 90018^T contained only 6.6 %, and 4.2–12.0 % iso-$C_{17:0}$, of which strain YIM 90018^T contained none. DNA–DNA relatedness between strain YIM 90018^T and its closest phylogenetic neighbours was <60 % (Fig. 3).

The physiological and biochemical characteristics, utilization of carbon and nitrogen sources, chemotaxonomic characteristics and antimicrobial activities of strain YIM 90018^T are given in the species description. In contrast to some of its closest phylogenetic neighbours, strain YIM 90018^T was negative for pigment production and gelatin liquefaction and positive for milk coagulation and peptonization and antimicrobial activity. *S. sclerotialus* and *S. niger*, which have been merged into *S. phaeochromogenes* (Locci, 1989; Skerman *et al.*, 1980; Yan, 1992), are positive for pigment production and gelatin liquefaction and negative for milk coagulation and peptonization and antimicrobial activity; *S. kasugaensis* is positive for pigment production and gelatin liquefaction and negative for milk coagulation.

The diagnostic properties of strain YIM 90018^T were the absence of aerial mycelium and soluble pigment, flexuous spore chains (*rectiflexibiles*), short rod-shaped spores, growth with 25 % $MgCl_2.6H_2O$ and $C_{18:1}\omega9c$ as the

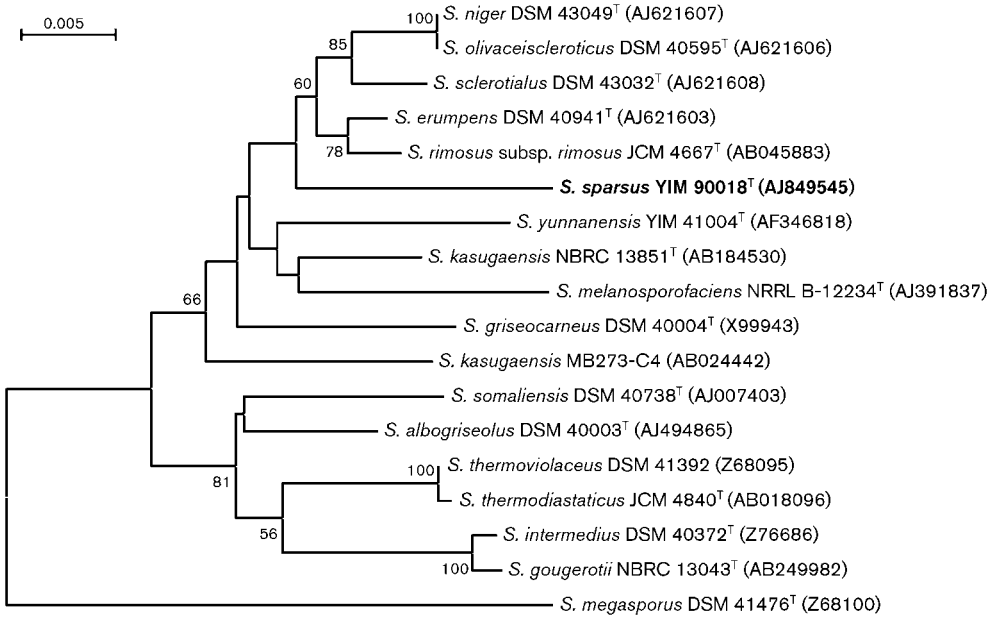

Fig. 2. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences showing the position of strain YIM 90018^T among its phylogenetic neighbours. Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. *Streptomyces megasporus* DSM 41476^T was used as an outgroup. Bar, 0.5 % sequence divergence.

Table 3. Comparison of fatty acids of strain YIM 90018^T with related members of the genus *Streptomyces*

Strains: 1, YIM 90018^T; 2, *S. kasugaensis* DSM 40819^T; 3, *S. sclerotialus* DSM 43032^T; 4, *S. niger* DSM 43049^T.
All data were taken from this study.

Fatty acid (%)	1	2	3	4
iso-$C_{13:0}$	–	–	–	0.6
anteiso-$C_{13:0}$	–	–	0.3	0.6
iso-$C_{14:0}$	1.2	5.2	2.8	4.2
$C_{14:0}$	0.9	–	0.4	0.8
iso-$C_{15:0}$	2.0	17.6	7.4	13.0
anteiso-$C_{15:0}$	6.6	25.7	34.0	31.5
$C_{15:0}$	–	1.0	1.7	1.9
iso-$C_{16:0}$	16.0	8.9	14.8	13.3
anteiso-$C_{16:0}$	1.4	–	–	–
$C_{16:0}$	14.4	19.1	5.9	10.0
iso-$C_{16:1}$ H	4.0	–	0.8	–
$C_{16:1}\omega 9c$	1.1	–	–	–
iso-$C_{17:0}$	–	12.0	4.2	6.8
anteiso-$C_{17:0}$	4.8	8.1	17.7	13.5
$C_{17:0}$ cyclo	–	–	–	0.5
$C_{17:0}$	–	–	0.9	1.1
$C_{18:1}\omega 9c$	38.1	–	0.7	–
$C_{20:1}\omega 9c$	1.5	–	–	–

major fatty acid. Therefore, a novel species of the genus *Streptomyces*, with the name *Streptomyces sparsus* sp. nov., is proposed to accommodate strain YIM 90018^T.

Description of *Streptomyces sparsus* sp. nov.

Streptomyces sparsus [spar′sus. L. masc. part. adj. *sparsus* (from L. v. *spargo*) scattered, sparse; referring to a streptomycete with sparse aerial mycelium].

No aerial hyphae are formed on most media tested, but extremely poor and pale grey aerial mycelium is formed on YIM 82 agar. Yellowish vegetative hyphae grow well and do not fragment. Soluble pigments are not formed. Straight to flexuous (*rectiflexibiles*) spore chains are produced. Spores are short and rod-shaped with smooth surfaces. Positive for milk coagulation and peptonization, growth on cellulose and production of H_2S. Negative for gelatin liquefaction, starch hydrolysis, nitrate reduction and melanin formation. Grows with 0–15 % NaCl, 0–5 % KCl, 0–25 % $MgCl_2.6H_2O$ and 0–1 % $CaCl_2$ and at pH 6.0–10.0. Utilizes glucose, galactose, rhamnose, arabinose, xylose, raffinose, starch, ribose, inositol, mannitol, glycine, histidine, methionine and asparagine, but not sorbitol. Acid is produced from glucose. Has antimicrobial activity against *Bacillus subtilis* (ACCC 11060), *Staphylococcus aureus* (AS 1.72), *Micrococcus luteus* (ACCC 11001), '*Sarcina lutea*' (AS 1.241) and *Xanthomonas oryzae* (AS 1.843). The cell-wall peptidoglycan contains LL-diaminopimelic acid and glycine. The whole-cell hydrolysate contains galactose and xylose. The predominant menaquinones are MK-9(H_4) (48 %), MK-9(H_6) (39 %) and MK-9(H_8) (13 %). The diagnostic phospholipid is phosphatidylethanolamine. The major fatty acids (>5 %) are $C_{18:1}\omega 9c$, iso-$C_{16:0}$, $C_{16:0}$ and anteiso-$C_{15:0}$.

The type strain, YIM 90018^T (=CCTCC AA204019^T=DSM 41858^T), was isolated from a saline and alkaline soil sample

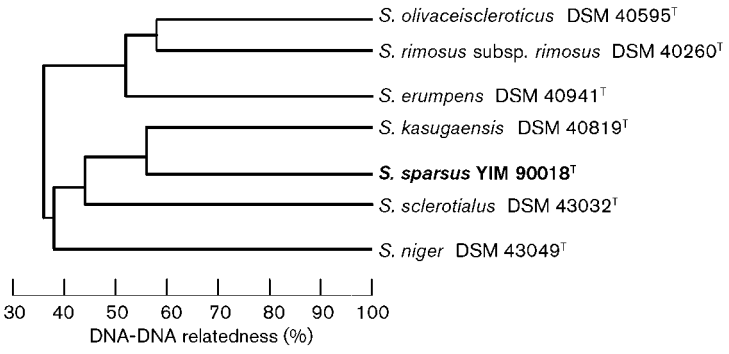

Fig. 3. DNA–DNA relatedness between strain YIM 90018^T and its closest phylogenetic neighbours.

collected from Qinghai Province, China. The DNA G+C content of the type strain is 71.2 mol%.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (grant numbers 30900002 and 30560001), the International Cooperative Key Project of Ministry of Science and Technology (grant number 2006DFA33550) and Talents in University and the 'Zentrum für Marine Wirkstoffe', which is funded by the Ministerium für Wirtschaft, Wissenschaft und Verkehr des Landes Schleswig-Holstein (Germany). We thank X.-F. Cai and Y. Chen for their technical assistance.

References

Backus, E. J. & Tresner, H. D. (1956). A broadened concept of the characteristics of *Streptomyces hygroscopicus*. *Appl Microbiol* **4**, 243–250.

Christensen, H., Angen, O., Mutters, R., Olsen, J. E. & Bisgaard, M. (2000). DNA–DNA hybridization determined in micro-wells using covalent attachment of DNA. *Int J Syst Evol Microbiol* **50**, 1095–1102.

Collins, M. D. (1985). Isoprenoid quinone analysis in bacterial classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

Cui, X. L., Mao, P. H., Zeng, M., Li, W. J., Zhang, L. P., Xu, L. H. & Jiang, C. L. (2001). *Streptimonospora salina* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae*. *Int J Syst Evol Microbiol* **51**, 357–363.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Hayakawa, M. & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J Ferment Technol* **65**, 501–509.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kelly, K. L. (1964). *Inter-Society Color Council – National Bureau of Standards Color Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.

Lechevalier, M. P. & Lechevalier, H. A. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy*, pp. 227–291. Edited by A. Dietz & D. W. Thayer. Arlington, VA: Society for Industrial Microbiology.

Locci, R. (1989). *Streptomyces* and related genera. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 24–77. Baltimore: Williams & Wilkins.

Orsini, M. & Romano-Spica, V. A. (2001). A microwave-based method for nucleic acid isolation from environmental samples. *Lett Appl Microbiol* **33**, 17–20.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 16.

Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.

Skerman, V. B., Mcgowan, D. V. & Sneath, P. H. A. (1980). Approved lists of bacterial names. *Int J Syst Bacteriol* **30**, 225–420.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Waksman, S. A. (1961). *The Actinomycetes, Classification, Identification and Description of Genera and Species*, vol. 2. Baltimore: Williams & Wilkins.

Waksman, S. A. & Henrici, A. T. (1943). The nomenclature and classification of the actinomycetes. *J Bacteriol* **46**, 337–341.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.

Yan, X. C. (1992). *Classification and Identification of Actinomycetes*. Beijing: Academic Press.