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Streptomyces sparsus sp. nov., a novel member of the genus *Streptomyces* from saline and alkaline soil in China

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The 16S rRNA gene sequences of strain YIM 90018^T has been deposited in EMBL under the accession number AJ849545.

Salt and alkaline-tolerant actinomycete strain, YIM 90018^T, was isolated from a saline and alkaline soil sample collected from Qinghai, China, and was then subjected to polyphasic taxonomy. Aerial hyphae of strain YIM 90018^T were not produced on most media tested except YIM 82# agar and the vegetative hyphae were well developed and did not fragmented. Straight or flexuous (*Rectiflexibiles*) spore chains are produced. The strain grew well in the presence of 25 % of $MgCl_2 \cdot 6H_2O$ and at pH 10. All of these characters consistently assigned strain YIM 90018^T to the genus *Streptomyces.* Based on phylogenetic analysis of 16S rRNA gene, DNA-DNA hybridization, phenotypic characters and comparison with known species of the genus, strain YIM 90018^T can be differentiated from all the validly described *Streptomyces* species. A novel species, *Streptomyces sparsus* sp. nov. is proposed. The type strain of the new species is YIM 90018^T (CCTCC AA204019= DSM 41858^T).

The genus *Streptomyces* was proposed by Waksman & Henrici (Waksman & Herici 1943) and species of this genus have been of great interest owing to their production of various natural products with considerable commercial value. In the course of screening of actinomycetes for metabolities with bioactivity, strain YIM 90018^T was isolated from a saline and alkaline soil sample collected from Qinghai Province, China. It was determined to belong to the genus *Streptomyces* and the taxonomic results are reported in this paper.

Strain YIM 90018^T was isolated from a saline and alkaline soil sample collected from Qinghai, China by using starch-casein medium with 20 % $MgCl_2$. This medium contained (g/l): Starch 10 g, casein 0.3 g, KNO_3 2 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, NaCl 2 g, K_2HPO_4 2 g, $CaCO_3$ 0.02 g, $MgCl_2 \cdot 6H_2O$ 200 g and agar 20 g (pH 7.2). The strain was maintained in 20 % glycerol and kept at -20 ºC.

The strain was cultivated on YIM #82 agar **[s**tarch, 5 g; asparagine, 1 g; K_2HPO_4, 1 g; vitamin mixture from HV agar (Hayakawa & Nonomura 1987), 3.7 mg; trace salts from ISP 5 (Shirling & Gottlieb 1966), 1 ml; agar, 20 g; pH 7.2 or 10.0-11.0] for microscopic observations of the sporophores, spore chains and spore surface using light and scanning

electron microscope (JEOL Ltd., JSM-5600LV, Tokyo, Japan). The cultural characteristics were studied on ISP media (Shirling & Gottlieb 1966), Czapek's agar, nutrient agar (Waksman 1961), YIM #81 agar (asparagine, 1 g; glycerol, 10 g; yeast extract, 0.5 g; KNO_3 ,0.5 g; K_2HPO_4 ,1 g; agar, 20 g; pH 7.2 or 10-11) and YIM #82 agar after incubation for 14 days at 28 ºC. The colour of both substrate and aerial mycelia together with the production of soluble pigments were determined by comparison with chips from the ISCC-NBS color charts (Kelly 1964). All tests were done at 28 ºC and pH 7.2 unless otherwise specified. The production of melanin was tested on ISP 7 medium. Carbon source utilization was examined on ISP 9 as a basal medium supplemented with 1 % final concentration of the tested carbon sources. Utilization of different nitrogen sources, catalase production, and degradation of starch and gelatin were detected in modified Bennett's agar medium (MBA) after 7, 14 and 21 days as described by Williams *et al.* (1983). Hydrogen sulphide production was detected by the method of Shirling and Gottlieb (1966). The effect of temperature and pH on the growth and the tolerance to salts was determined using MBA as a basal medium.

For chemotaxonomic studies, strain YIM 90018^T was grown in potato extract-glucose broth (fresh potato, 200g; boiling for 30 min, filtrated, utilized the broth of 1000 ml), on a shaking incubator at 200 rpm and 28 ºC for 7 days. The mycelia were harvested by centrifugation and washed three times with distilled water and then freeze-dried. The determination of diamino acid in the cell wall and analysis of the whole-cell sugars were performed as described by Lechevalier *et al.* (1970, 1980) and Staneck and Roberts (1974), respectively. Polar lipids were extracted and detected by the method of Komagata and Suzuki (1987). Menaquinones were extracted, purified and identified by HPLC as described by Collins (1985). The composition of cellular fatty acid was analysesd as described by Sasser (1990). The DNA G+C base content was determined by HPLC (Tamaoka & Komagata 1984) with an Agilent 1100 LC system (IRIS Technologies, U.S.A). DNA-DNA hybridization of strain YIM 90018^T with related species was carried out by the method described by Christensen *et al.* (2000).

Genomic DNA was extracted for 16S rDNA analysis by the method described by Orsini and Romano-Spica (2001). PCR-mediated amplification of the 16S rDNA, purification of PCR products and sequence anaylsis of purified products were done as described previously (Cui *et al.* 2001). The resultant sequence was manually aligned against bacterial sequences available from public databases. A more detailed comparison was performed with members of the genus *Streptomyces* and evolutionary distance matrices were calculated by the method of Jukes and Cantor (1969). Phylogenetic trees were inferred by using the neighbour-joining (Saitou & Nei 1987) and maximum-likelihood methods (Felsenstein 1981). The trees were rooted using *Streptomyces megasporus* DSM 41476^T (accession number Z68100) as outgroup. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein 1985).

Morphological observation of 15-days-old culture of strain YIM 90018^T revealed that aerial hyphae were not produced on most tested media except YIM #82. Vegetative hyphae were abundant and not fragmented. Straight to flexuous (*Rectiflexibiles*) spore chains were only present on YIM #82. Spores were short rod shaped and variable in size (0.5-0.7×1.0-1.3 m). The spore surface was smooth (Fig. 1).

Cultural characteristics of strain YIM 90018^T are shown in Table 1. No aerial mycelium was produced on most tested media, and poor and pale gray on YIM #82 agar. Substrate mycelium grew well, and light or brilliant yellow. Soluble pigments were not produced on any used media. Physiological and biochemical characteristics, utilization of carbon and nitrogen sources, chemotaxonomic characteristics and anti-microbial activitites of YIM 90018^T are described in description of *Streptomyces sparsus* sp. nov.

The 16S rDNA sequence (1466 nucleotides, accession number AJ849545) of strain YIM 90018^T was compared with the corresponding sequences of the representative reference strains of the genus *Streptomyces*. The neighbour-joining tree based on 16S rDNA sequences in 1000 resamplings was constructed to show relationships between the strain YIM 90018^T and 19 other related *Streptomyces* species (Fig. 2). Phylogenetic analysis

revealed that YIM 90018^T is phylogenetically related to the genus *Streptomyces*, and formed a separate line in the tree. Highest sequence similarities were found with *Streptomyces rimosus* subsp. *rimosus* (98.55 %), *Streptomyces erumpens* (98.33 %), *Streptomyces sclerotialus* (98.04 %), *Streptomyces olivaceiscleroticus* (97.99 %), *Streptomyces niger* (97.99 %) and *Streptomyces kasugaensis* (97.6 %).

Spore chains of *Streptomyces rimosus* subsp. *rimosus* are abundant and spiralthe spore mass is white or yellow. *Streptomyces sclerotialus* and *Streptomyces niger* were merged into *S. phaeochromogenes* (Locci 1989, Skerman *et al.* 1980, Yan 1992). Their spore chain is abundant and spiral, form sclerotia, aerial mycelium is grey, vegetative hyphae are yellow-brown, green or black, diffusible pigments are yellow-brown or green, melanin pigment is producted, gelatin liquefaction is positive, milk coagulation and peptonization are negative, and no antimicrobial activities against bacteria and fungi are produced. Spore chains of *Streptomyces erumpens* are abundant and spiral, and spore mass is gray. Aerial mycelium of *Streptomyces kasugaensis* (Yan 1992, Tresner & Bachus 1956) is abundant and white, spore chains are spiral, dark yellowish soluble pigment is produced, gelatin liquefaction is positive, and milk coagulation is negative (Table 2). Content of fatty acids of YIM 90018^T were remarkable different from closed species of the genus *Streptomyces.* YIM 90018^T contained 38.1 % of 18:1 w9c. *Streptomyces sclerotialus* DSM 43032^T only contained 0.7 %, and *S. kasugaensis* and *S. niger* do not. YIM 90018^T contained 16:1 w9c and 20:1 w9c. But the three species do not contain the two fatty acids. The three species contain 25.7 % to 34.0 % of 15:0 anteiso. But YIM 90018^T only contains 6.6 %. The three species contain 4.2 % to 12.0 % of 17:0 ISO. But YIM 90018^T does not have (Table 3). The results of DNA-DNA hybridization of strain YIM 90018^T with closed 6 species indicate that the chromosomal DNA homology of them is below 60 % (Fig. 3). The diagnostic properties of strain YIM 90018^T that distinguish it from the related species were the absence of aerial mycelium, flexuous spore chains (*Rectiflexibiles*) when produced, spores were short rod-shaped, soluble pigments are not produced, growth in the presence of $MgCl_2 \cdot 6H_2O$ concentrations of 25 %, and the presence of over 38 % 18:1 w9c fatty acid in the FAME profile.

Therefore, a new species of the genus *Streptomyces* with the name *Streptomyces sparsus* sp.nov. is proposed.

Description of Streptomyces sparsus sp. nov.

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

No aerial hyphae are formed on most media tested, but extremely poor and pale grey aerial mycelium formed on YIM #82 agar. Yellowish vegetative hyphae grows well and does not fragment. Soluble pigments are not formed. Straight to flexuous (*Rectiflexibiles*) spore chains. Spores are short rod shaped and the surface is smooth. Milk coagulation and peptonization, growth on cellulose and H_2S production are positive reactions. Gelatin liquefaction, starch hydrolysis, nitrate reduction and melanin formation are negative. Grow occurs at 0-15 % of NaCl, 0-5 % of KCl, 0-25 % of $MgCl_2 \cdot 6H_2O$, 0-1 % of $CaCl_2$ and pH 6.0-10.0. Utilize glucose, glactose, rhamnose, arabinose, xylose, raffinose, starch, ribose, inositol, mannitol, glycine, histidine, methionine and asparagine. Acid is produced from glucose. Sobitol was not utilised. Antimicrobial activity against *Bacillus subtilis* (ACCC 11060^T), *Staphylococcus aureus* (AS 1.72^T), *Micrococcus luteus* (ACCC 11001^T), *Sarcina lutea* (AS 1.241^T) and *Xanthomonas oryzae*(AS 1.843^T). The cell wall peptidoglycan contains LL-diaminopimelic acid and glycine. The whole-cell hydrolysates contain galactose and xylose. Predominant menaquinones were MK-9 (H_4) (48 %), MK-9 (H_6) (39 %) and MK-9 (H_8) (13 %). The diagnostic phospholipid was phosphatidylethanolamine. The major fatty acid pattern consists of anteiso-$C_{15:0}$ (6.6 %), iso-$C_{16:0}$ (16.04 %), $C_{16:0}$ (14.4 %), and w9c-$C_{18:1}$ (38.1 %). G+C content of genomic DNA is 71.2 mol %. The typical strain is YIM 90018^T (= CCTCC $AA204019^T$ = DSM 41858^T), which was isolated from a saline and alkaline soil sample collected from Qinghai Province, China.

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306 **Table 1. Cultural characteristics of strain YIM 90018^{T}**

Medium	Aerial mycelium Growth	Color	Substrate Growth	mycelium Color
Czapek's agar	-	none	+	none
Glycerol-asparagine agar (ISP* 5)	-	none	+	Brilliant yellow
Glucose-asparagine agar	-	none	-	none
Inorganic salt-starch agar (ISP 4)	-	none	++	Light yellow
Yeast extract-malt extract agar (ISP 2)	-	none	++	Brilliant yellow
Potato extract agar	-	none	+++	Brilliant yellow
Nutrient agar	-	none	++	Brilliant yellow
YIM 81# agar	-	none	++	Light yellow
YIM 82# agar	+	Pale grey	+	Pale yellow

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308 Note: Colors taken from ISCC-NBS COLOR CHARTS Standard Samples No 2106（Kelly 1964）.

309 *ISP, International Streptomyces Project (Shirling & Gottlieb 1966).

310 +: weak; ++: modrate; +++: good; -: none

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322 **Table 2. Comparison of some morphological and cultural characteristics of YIM 90018^{T} with related species of the genus**
323 ***Streptomyces***

	Streptomyces rimosus* subsp. *rimosus	***Streptomyces sclerotialus***	***Streptomyces niger***	***Streptomyces erumpens***	***Streptomyces kasugaensis***	***Streptomyces olivaceiscleroticus***	**YIM 90018**
Aerial hyphae	Abundant, white, yellow	Abundant, bsclerotia, white, yellowish red, pale yellow green	Abundant, grey	Abundant, grey	Abundant, white	Abundant, pale white, grey black	Sparse, pale grey
Spore chain	Spiral	Spiral	Spiral	Spiral	loops and spiral	Spiral	Straight to flexuous (*Rectiflexibiles*)
Spore shape	Oval	Oval	Oval	/	/	Oval	Short rod
Substrate hyphae	Brown, red brown	Orange yellow, green, yellowish brown	Black	Brown	Brown, red brown	Black, brown	Yellow
Diffusible pigments	Yellow, yellowish brown	Yellowish brown, green	Brown	Yellow	Dark yellow, yellowish brown	Olive yellow, pale red	Non

324 Note: / = No test

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

Name of fatty acid	1	2	3	4
13:0 ISO				0.6
13:0 ANTEISO			0.3	0.6
14:0 ISO	1.2	5.2	2.8	4.2
14:0	0.9		0.4	0.8
15:0 ISO	2.0	17.6	7.4	13.0
15:0 ANTEISO	**6.6**	**25.7**	**34.0**	**31.5**
15:0		1.0	1.7	1.9
16:0 ISO	16. 0	8.9	14.8	13.3
16:0 ANTEISO	1.4			
16:0	14.4	19.1	5.9	10.0
16:1 ISOH	4.0		0.8	
16:1 w9c	1.1			
17:0 ISO		**12.0**	**4.2**	**6.8**
17:0 ANTEISO	4.8	8.1	17.7	13.5
17:0 CYCLO				0.5
17:0			0.9	1.1

Note: 1. *Streptomyces sparsus* sp.nov. (YIM 90018^T); 2. *Streptomyces kasugaensis* (DSM 40819^T); 3. *Streptomyces sclerotialus* (DSM 43032^T); 4, *Streptomyces niger* (DSM 43049^T).

Fig. 1. Scanning electron micrograph showing spores and spore chains of strain YIM 90018^{T} after growth on YIM 82# agar at 28 ºC for 15 days.

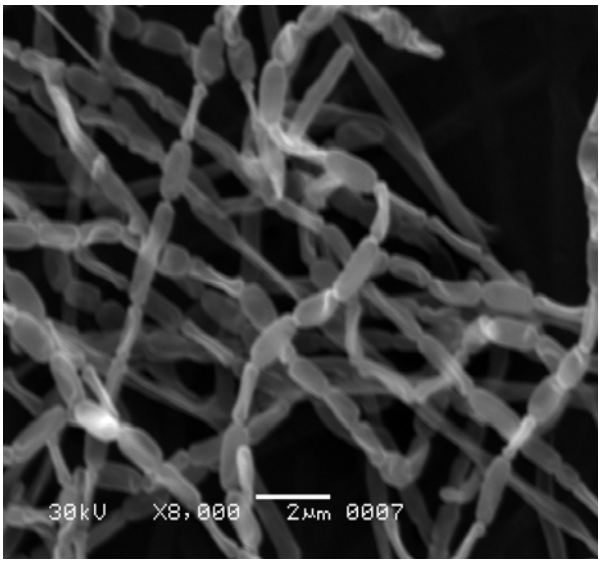

Fig. 2. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strainYIM 90018^{T} among phylogenetic neighbours. Numbers on branch nodes are bootstrap values (1000 resamplings). Bar indicated 0.5% sequence divergence.

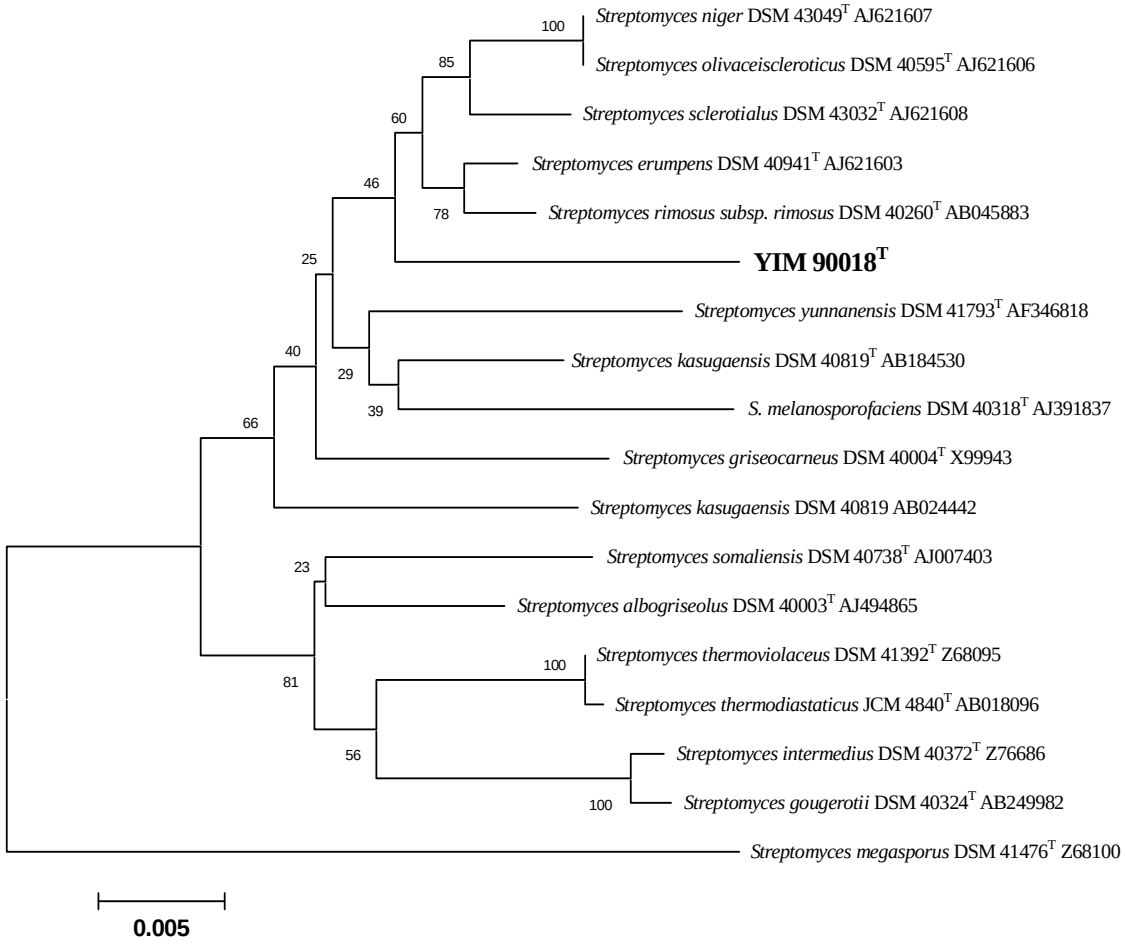

Fig. 3. Homology values of DNA-DNA hybridization of YIM 90018 with related species