

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

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23 **Running title:** *Streptomyces sparsus* sp. nov.

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25

26 **Category:** New Taxa - Actinobacteria Actinobacteria

27

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

30

31 Salt and alkaline-tolerant actinomycete strain, YIM 90018^T, was isolated from a saline
32 and alkaline soil sample collected from Qinghai, China, and was then subjected to
33 polyphasic taxonomy. Aerial hyphae of strain YIM 90018^T were not produced on most
34 media tested except YIM 82# agar and the vegetative hyphae were well developed and
35 did not fragmented. Straight or flexuous (*Rectiflexibiles*) spore chains are produced. The
36 strain grew well in the presence of 25 % of MgCl₂·6H₂O and at pH 10. All of these
37 characters consistently assigned strain YIM 90018^T to the genus *Streptomyces*. Based on
38 phylogenetic analysis of 16S rRNA gene, DNA-DNA hybridization, phenotypic
39 characters and comparison with known species of the genus, strain YIM 90018^T can be
40 differentiated from all the validly described *Streptomyces* species. A novel species,
41 *Streptomyces sparsus* sp. nov. is proposed. The type strain of the new species is YIM
42 90018^T (CCTCC AA204019= DSM 41858^T).

43

44 The genus *Streptomyces* was proposed by Waksman & Henrici (Waksman & Herici
45 1943) and species of this genus have been of great interest owing to their production of
46 various natural products with considerable commercial value. In the course of screening
47 of actinomycetes for metabolites with bioactivity, strain YIM 90018^T was isolated from
48 a saline and alkaline soil sample collected from Qinghai Province, China. It was
49 determined to belong to the genus *Streptomyces* and the taxonomic results are reported in
50 this paper.

51

52 Strain YIM 90018^T was isolated from a saline and alkaline soil sample collected from
53 Qinghai, China by using starch-casein medium with 20 % MgCl₂. This medium contained
54 (g/l): Starch 10 g, casein 0.3 g, KNO₃ 2 g, MgSO₄·7H₂O 0.05 g, NaCl 2 g, K₂HPO₄ 2 g,
55 CaCO₃ 0.02 g, MgCl₂·6H₂O 200 g and agar 20 g (pH 7.2). The strain was maintained in
56 20 % glycerol and kept at -20 °C.

57

58 The strain was cultivated on YIM #82 agar [starch, 5 g; asparagine, 1 g; K₂HPO₄, 1 g;
59 vitamin mixture from HV agar (Hayakawa & Nonomura 1987), 3.7 mg; trace salts from
60 ISP 5 (Shirling & Gottlieb 1966), 1 ml; agar, 20 g; pH 7.2 or 10.0-11.0] for microscopic
61 observations of the sporophores, spore chains and spore surface using light and scanning

62 electron microscope (JEOL Ltd., JSM-5600LV, Tokyo, Japan). The cultural
63 characteristics were studied on ISP media (Shirling & Gottlieb 1966), Czapek's agar,
64 nutrient agar (Waksman 1961), YIM #81 agar (asparagine, 1 g; glycerol, 10 g; yeast
65 extract, 0.5 g; KNO₃, 0.5 g; K₂HPO₄, 1 g; agar, 20 g; pH 7.2 or 10-11) and YIM #82 agar
66 after incubation for 14 days at 28 °C. The colour of both substrate and aerial mycelia
67 together with the production of soluble pigments were determined by comparison with
68 chips from the ISCC-NBS color charts (Kelly 1964). All tests were done at 28 °C and pH
69 7.2 unless otherwise specified. The production of melanin was tested on ISP 7 medium.
70 Carbon source utilization was examined on ISP 9 as a basal medium supplemented with 1
71 % final concentration of the tested carbon sources. Utilization of different nitrogen
72 sources, catalase production, and degradation of starch and gelatin were detected in
73 modified Bennett's agar medium (MBA) after 7, 14 and 21 days as described by
74 Williams *et al.* (1983). Hydrogen sulphide production was detected by the method of
75 Shirling and Gottlieb (1966). The effect of temperature and pH on the growth and the
76 tolerance to salts was determined using MBA as a basal medium.

77

78 For chemotaxonomic studies, strain YIM 90018^T was grown in potato extract-glucose
79 broth (fresh potato, 200g; boiling for 30 min, filtrated, utilized the broth of 1000 ml), on a
80 shaking incubator at 200 rpm and 28 °C for 7 days. The mycelia were harvested by
81 centrifugation and washed three times with distilled water and then freeze-dried. The
82 determination of diamino acid in the cell wall and analysis of the whole-cell sugars were
83 performed as described by Lechevalier *et al.* (1970, 1980) and Stanek and Roberts
84 (1974), respectively. Polar lipids were extracted and detected by the method of Komagata
85 and Suzuki (1987). Menaquinones were extracted, purified and identified by HPLC as
86 described by Collins (1985). The composition of cellular fatty acid was analysed as
87 described by Sasser (1990). The DNA G+C base content was determined by HPLC
88 (Tamaoka & Komagata 1984) with an Agilent 1100 LC system (IRIS Technologies,
89 U.S.A). DNA-DNA hybridization of strain YIM 90018^T with related species was carried
90 out by the method described by Christensen *et al.* (2000).

91

92 Genomic DNA was extracted for 16S rDNA analysis by the method described by Orsini
93 and Romano-Spica (2001). PCR-mediated amplification of the 16S rDNA, purification of
94 PCR products and sequence analysis of purified products were done as described
95 previously (Cui *et al.* 2001). The resultant sequence was manually aligned against
96 bacterial sequences available from public databases. A more detailed comparison was
97 performed with members of the genus *Streptomyces* and evolutionary distance matrices
98 were calculated by the method of Jukes and Cantor (1969). Phylogenetic trees were
99 inferred by using the neighbour-joining (Saitou & Nei 1987) and maximum-likelihood
100 methods (Felsenstein 1981). The trees were rooted using *Streptomyces megasporus* DSM
101 41476^T (accession number Z68100) as outgroup. Bootstrap analysis was used to evaluate
102 the tree topology of the neighbour-joining data by performing 1000 resamplings
103 (Felsenstein 1985).

104

105 Morphological observation of 15-days-old culture of strain YIM 90018^T revealed that
106 aerial hyphae were not produced on most tested media except YIM #82. Vegetative
107 hyphae were abundant and not fragmented. Straight to flexuous (*Rectiflexibiles*) spore
108 chains were only present on YIM #82. Spores were short rod shaped and variable in size
109 (0.5-0.7×1.0-1.3 μm). The spore surface was smooth (Fig. 1).

110

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

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118 The 16S rDNA sequence (1466 nucleotides, accession number AJ849545) of strain YIM
119 90018^T was compared with the corresponding sequences of the representative reference
120 strains of the genus *Streptomyces*. The neighbour-joining tree based on 16S rDNA
121 sequences in 1000 resamplings was constructed to show relationships between the strain
122 YIM 90018^T and 19 other related *Streptomyces* species (Fig. 2). Phylogenetic analysis

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

128

129 Spore chains of *Streptomyces rimosus* subsp. *rimosus* are abundant and spiral the spore
130 mass is white or yellow. *Streptomyces sclerotialus* and *Streptomyces niger* were merged
131 into *S. phaeochromogenes* (Locci 1989, Skerman *et al.* 1980, Yan 1992). Their spore
132 chain is abundant and spiral, form sclerotia, aerial mycelium is grey, vegetative hyphae
133 are yellow-brown, green or black, diffusible pigments are yellow-brown or green,
134 melanin pigment is produced, gelatin liquefaction is positive, milk coagulation and
135 peptonization are negative, and no antimicrobial activities against bacteria and fungi are
136 produced. Spore chains of *Streptomyces erumpens* are abundant and spiral, and spore
137 mass is gray. Aerial mycelium of *Streptomyces kasugaensis* (Yan 1992, Tresner &
138 Bachus 1956) is abundant and white, spore chains are spiral, dark yellowish soluble
139 pigment is produced, gelatin liquefaction is positive, and milk coagulation is negative
140 (Table 2). Content of fatty acids of YIM 90018^T were remarkable different from closed
141 species of the genus *Streptomyces*. YIM 90018^T contained 38.1 % of 18:1 w9c.
142 *Streptomyces sclerotialus* DSM 43032^T only contained 0.7 %, and *S. kasugaensis* and *S.*
143 *niger* do not. YIM 90018^T contained 16:1 w9c and 20:1 w9c. But the three species do
144 not contain the two fatty acids. The three species contain 25.7 % to 34.0 % of 15:0
145 anteiso. But YIM 90018^T only contains 6.6 %. The three species contain 4.2 % to 12.0 %
146 of 17:0 ISO. But YIM 90018^T does not have (Table 3). The results of DNA-DNA
147 hybridization of strain YIM 90018^T with closed 6 species indicate that the chromosomal
148 DNA homology of them is below 60 % (Fig. 3). The diagnostic properties of strain YIM
149 90018^T that distinguish it from the related species were the absence of aerial mycelium,
150 flexuous spore chains (*Rectiflexibiles*) when produced, spores were short rod-shaped,
151 soluble pigments are not produced, growth in the presence of MgCl₂·6H₂O concentrations
152 of 25 %, and the presence of over 38 % 18:1 w9c fatty acid in the FAME profile.

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

155

156 **Description of *Streptomyces sparsus* sp. nov.**

157

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

160

161 No aerial hyphae are formed on most media tested, but extremely poor and pale grey
162 aerial mycelium formed on YIM #82 agar. Yellowish vegetative hyphae grows well and
163 does not fragment. Soluble pigments are not formed. Straight to flexuous (*Rectiflexibles*)
164 spore chains. Spores are short rod shaped and the surface is smooth. Milk coagulation and
165 peptonization, growth on cellulose and H₂S production are positive reactions. Gelatin
166 liquefaction, starch hydrolysis, nitrate reduction and melanin formation are negative.
167 Grow occurs at 0-15 % of NaCl, 0-5 % of KCl, 0-25 % of MgCl₂·6H₂O, 0-1 % of CaCl₂
168 and pH 6.0-10.0. Utilize glucose, galactose, rhamnose, arabinose, xylose, raffinose, starch,
169 ribose, inositol, mannitol, glycine, histidine, methionine and asparagine. Acid is produced
170 from glucose. Sobitol was not utilised. Antimicrobial activity against *Bacillus subtilis*
171 (ATCC 11060^T), *Staphylococcus aureus* (AS 1.72^T), *Micrococcus luteus* (ATCC
172 11001^T), *Sarcina lutea* (AS 1.241^T) and *Xanthomonas oryzae*(AS 1.843^T). The cell wall
173 peptidoglycan contains LL-diaminopimelic acid and glycine. The whole-cell hydrolysates
174 contain galactose and xylose. Predominant menaquinones were MK-9 (H₄) (48 %), MK-9
175 (H₆) (39 %) and MK-9 (H₈) (13 %). The diagnostic phospholipid was
176 phosphatidylethanolamine. The major fatty acid pattern consists of anteiso-C_{15:0} (6.6 %),
177 iso-C_{16:0} (16.04 %), C_{16:0} (14.4 %), and ω⁹c-C_{18:1} (38.1 %). G+C content of genomic
178 DNA is 71.2 mol %. The typical strain is YIM 90018^T (= CCTCC AA204019^T = DSM
179 41858^T), which was isolated from a saline and alkaline soil sample collected from
180 Qinghai Province, China.

181

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

Medium	Aerial mycelium		Substrate mycelium	
	Growth	Color	Growth	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***

	<i>Streptomyces rimosus</i> subsp. <i>rimosus</i>	<i>Streptomyces sclerotialus</i>	<i>Streptomyces niger</i>	<i>Streptomyces erumpens</i>	<i>Streptomyces kasugaensis</i>	<i>Streptomyces olivaceiscleroticus</i>	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 (<i>Rectiflexibiles</i>)
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

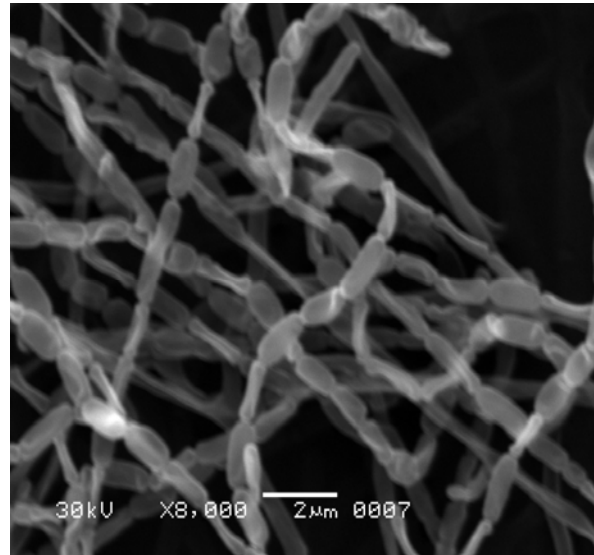
325 **Table 3. Comparison of fatty acids of YIM 90018^T with related species of the genus**
 326 ***Streptomyces***

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

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 328 Note: 1. *Streptomyces sparsus* sp.nov. (YIM 90018^T); 2. *Streptomyces kasugaensis* (DSM 40819^T);
 329 3. *Streptomyces sclerotialus* (DSM 43032^T); 4, *Streptomyces niger* (DSM 43049^T).
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341 **Fig. 1.** Scanning electron micrograph showing spores and spore chains of strain YIM
342 90018^T after growth on YIM 82# agar at 28 °C for 15 days.

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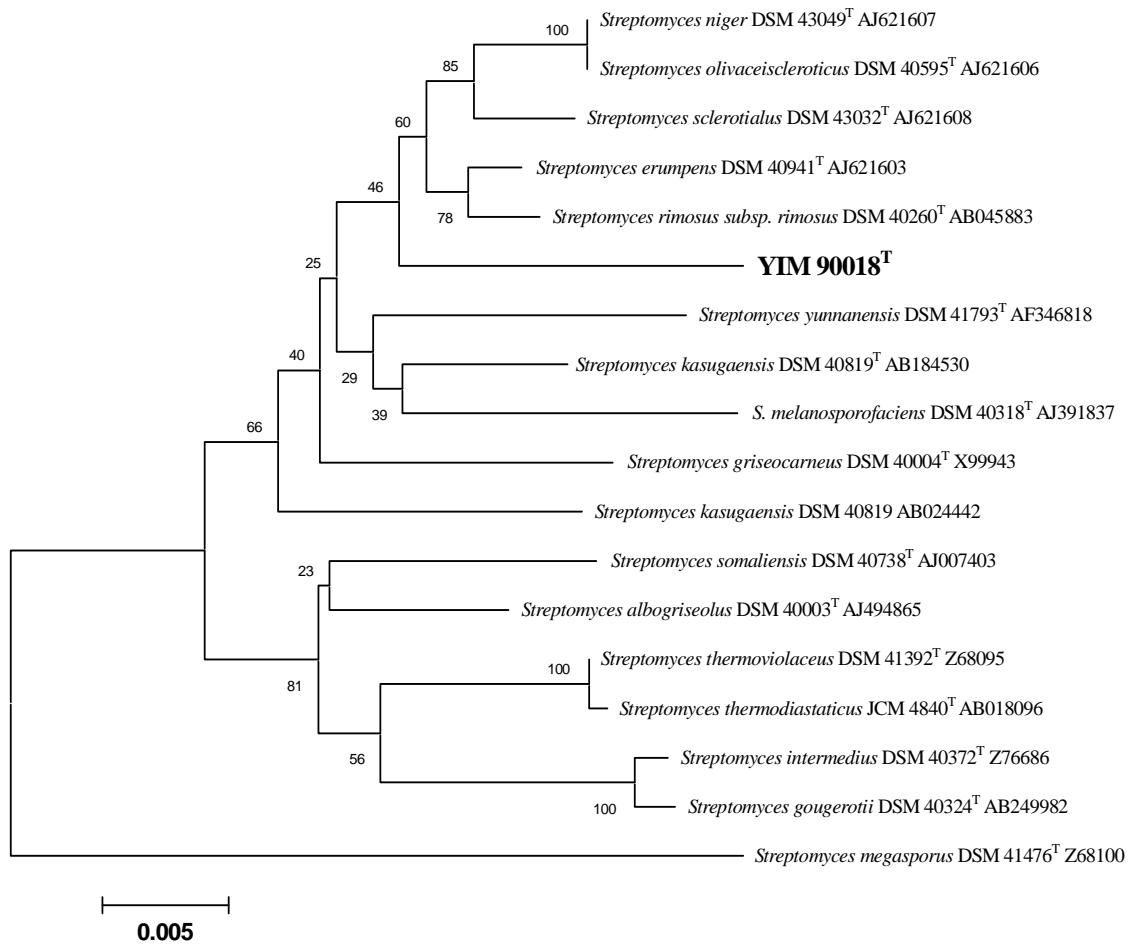
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363 **Fig. 2.** Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA
 364 sequences, showing the position of strain YIM 90018^T among phylogenetic neighbours.
 365 Numbers on branch nodes are bootstrap values (1000 resamplings). Bar indicated 0.5%
 366 sequence divergence.
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377 **Fig. 3.** Homology values of DNA-DNA hybridization of YIM 90018 with related species
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