Streptomyces spitsbergensis sp. nov.

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Isolate PCM 2404^T (T = type strain) from the Polish Collection of Microorganisms is proposed as the type strain of a new species, *Streptomyces spitsbergensis*. This new species is characterized by a nonfragmenting vegetative mycelium, rectiflexibles spore chains, a type I cell wall, type PII phospholipids, a lack of mycolic acids and major glycolipids, and a G+C content of 71 mol%. A comparison with related species of the genus confirmed the validity of the proposed taxon.

Actinomycete strain S-2^T (deposited in the Polish Collection of Microorganisms as strain PCM 2404^T) (T = type strain), which was isolated from soil in the course of a screening program, produces prodiginine-like metabolites (22), and has cultural, morphological, and chemical characteristics (3) of the genus *Streptomyces*, is proposed as a new species *Streptomyces spitsbergensis*. Data supporting the creation of the new taxon are described.

MATERIALS AND METHODS

Bacterial strains. Strain S-2^T was isolated from a soil sample from the Hornsund Gulf region that was plated onto organic

PCM 2371 (= JCM 4434) (JCM is the Japan Collection of Microorganisms).

Cultural and morphological characterization. Cultural characteristics of strain S-2^T were determined after incubation for 10 days on previously recommended substrata (12, 15) and organic medium 79. Morphology was ascertained by light microscopy and electron microscopy with a JEOL model 100 B electron microscope (20).

Physiological tests. The media and tests used to evaluate the physiological properties of strain S-2^T were the media and tests of the International Streptomyces Project (ISP), as well as standard media and tests recommended previously (1, 12, 15, 21).

TABLE 1. Cultural characteristics of strain S-2^T

Agar medium	Amt of growth	Color of:		Soluble pigment
		Vegetative mycelium	Aerial mycelium	
Yeast extract-malt extract (ISP medium 2)	Moderate	Red	Pink	None
Oatmeal (ISP medium 3)	Abundant	Red	Pink	None
Glycerol-asparagine (ISP medium 5)	Poor	Red	Light pink	None
Inorganic salts-starch (ISP medium 4)	Poor	Red	Light pink	None
Glucose-asparagine ^a	Moderate	Red	Pink	None
Peptone-yeast extract-iron (ISP medium 6)	Moderate	Brown or red	Violet	None
Tyrosine (ISP medium 7)	Poor	Red	Light pink	None
Sucrose-nitrate ^a	Poor	Brown	Violet	None
Glucose-nitrate ^a	Abundant	Brown or red	Dark pink	None
Glycerol-calcium malate ^a	Poor	Red	Light red	None
Glucose-peptone ^a	Abundant	Red	Pink	None
Nutrient ^a	Abundant	Red	Pink	None

^a See reference 12.

medium 79 (11) and was incubated for 1 week at 28°C.

The following type strains of *Streptomyces* taxa which on the basis of previously published data (5) seemed to be most similar to strain S-2^T were selected for genotaxonomic studies: *Streptomyces exfoliatus* PCM 2367 (= JCM 4366), *Streptomyces laurentii* PCM 2368 (= JCM 5063), *Streptomyces longispororuber* PCM 2396 (= JCM 4784), *Streptomyces murinus* PCM 2369 (= JCM 4333), *Streptomyces prunicolor* PCM 2370 (= JCM 4508), and *Streptomyces psammoticus*

Cell chemistry. Cell mass for chemotaxonomic analyses was obtained from 48-h submerged cultures grown in organic medium 79 at 28°C. Cell wall, whole-cell sugar, phospholipid, and glycolipid types were determined by thin-layer chromatography (4, 7, 14). The fatty acid spectrum of whole-cell methanolysates was determined by the method of Kroppenstedt (2). Methyl esters of fatty acids were analyzed by gas-liquid chromatography-mass spectrometry on a Hewlett-Packard model 5971A apparatus, using a type HP-1 glass capillary column (0.2 mm by 12 m) and a temperature program in which the temperature increased from 150 to 270°C at a rate of 8°C/min in electron ionization (70-eV) mode.

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FIG. 1. Electron micrograph of smooth-surfaced spores from a 10-day culture of strain S-2^T (= PCM 2404^T) grown on organic medium 79 at 28°C. Bar = 1 µm.

DNA isolation. The organisms which we studied were grown in submerged cultures in modified Sauton medium for 24 to 48 h at 28°C (8). The procedure used to obtain purified DNA has been described elsewhere (9).

DNA base composition. The average guanine-plus-cytosine (G+C) content was determined by thermal denaturation (6). Reference DNA was obtained from *Micrococcus luteus* (*Micrococcus lysodeikticus*) ATCC 4698.

Preparation of reference radioactive DNA. DNA from strain S-2^T was labeled in vitro by nick translation, using [1',2',5'-³H]dCTP (66 Ci/mmol) (13). The average specific activity obtained with this procedure was 5×10^6 cpm/µg of DNA.

DNA reassociation studies. The levels of DNA relatedness between test strains were examined by using a nitrocellulose membrane filter technique (9, 19). Hybridizations were done in quadruplicate at 60°C for 24 h in $3 \times$ SSC ($1 \times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 35% deionized formamide (i.e., under optimal reassociation conditions, which corresponded to approximately 25°C below the thermal melting point of the DNA).

RESULTS

Phenotypic characteristics. Strain S-2^T grew well on various organic media, but growth was poor on media containing

glycerol or sucrose as a carbon source and tyrosine or inorganic compounds as nitrogen sources (Table 1). The color of the mycelium ranged from red to dark red or violet or brown; the mycelium became pink to light pink when spores were formed. The spores on the aerial mycelium were oval and smooth and were arranged in long chains (Fig. 1) borne on rectiflexibles sporophores. The substrate mycelium was composed of long branching filaments that were 0.5 to 0.8 µm in diameter. No fragmentation of the cells was observed.

The results of physiological tests are as follows (most of the characteristics were determined as described by Williams et al. [21]): soluble pigments (including melanin) were formed; positive for lipolysis of olive oil, proteolysis of casein, hydrolysis of chitin, peptonization of milk, coagulation of milk, esculin hydrolysis, liquefaction of gelatin, starch hydrolysis, and H₂S production; negative for nitrate reduction, elastin hydrolysis, urease activity, and xanthine decomposition; growth occurred at 10 and 37°C but not at 4 and 45°C; the optimum growth temperature was 25 to 35°C; the optimum pH was 7.2 to 7.4; growth occurred in the presence of 0.001% (wt/vol) potassium tellurite and 0.01% (wt/vol) sodium azide but not in the presence of 0.1% (wt/vol) phenol or 4% (wt/vol) sodium chloride. In addition, strain S-2^T exhibited antibiosis to *Bacillus subtilis*, *Escherichia coli*, *M. luteus*, *Klebsiella* sp., *Proteus mirabilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Candida albicans*, *Candida tropicalis*, *Saccharomyces cerevisiae*, and human lymphocytes (immunosuppressive activity). Additional carbon assimilation tests performed on ISP basal medium (15) showed that glucose, fructose, mannose, L-rhamnose, D-xylose, maltose, trehalose, and raffinose were utilized by strain S-2^T (Table 2). L-Arabinose, lactose, glycerol, D-mannitol, and meso-inositol were not assimilated. Tests for utilization of amino acids (0.1%, wt/vol) showed that alanine, glycine, leucine, proline, serine, threonine, tyrosine, tryptophan, and valine served as nitrogen sources, but glutamic acid and cystine did not.

Chemical properties. Strain S-2^T had a type I cell wall composition; the cell walls contained L-diaminopimelic acid, glycine, glutamic acid, alanine, glucosamine, and muramic acid. No characteristic sugars were found. The phospholipid pattern was a type II pattern (4), containing phosphatidylethanolamine and traces of diphosphatidylglycerol and phosphatidylinositol. The glycolipid pattern was a type g pattern (7), meaning that no major components were found. The fatty acid pattern was a type 2c pattern (2), consisting mainly

TABLE 2. Carbon utilization by strain S-2^T and several of the most similar species of the genus *Streptomyces*^a

Organism	Utilization of the following sources of carbon:					
	L-Arabinose	Raffinose	L-Rhamnose	D-Xylose	D-Mannitol	Inositol
Strain S-2 ^T	— ^b	+	+	+	—	—
<i>Streptomyces exfoliatus</i> ^c	+	+	+	+	—	—
<i>Streptomyces laurentii</i> ^d	—	—	—	+	—	—
<i>Streptomyces longispororuber</i> ^e	+	—	—	D	+	+
<i>Streptomyces prunicolor</i> ^f	+	+	+	+	+	+
<i>Streptomyces psammoticus</i> ^f	W	—	—	—	—	—

^a As determined by ISP tests.

^b +, positive; —, negative; D, different (positive or negative); W, weak reaction.

^c Data from reference 16.

^d Data from reference 5.

^e Data from reference 18.

^f Data from reference 17.

TABLE 3. Fatty acid composition of strain S-2^T

Fatty acid ^a	Retention time (min)	Amt (%) ^b
iso-C _{14:0}	4.79	9
C _{14:0}	5.26	2
iso-C _{15:0}	6.15	7
anteiso-C _{15:0}	6.28	23
C _{15:0}	6.63	2
iso-C _{16:0}	7.51	22
C _{16:0}	7.98	20
iso-C _{17:0}	8.78	3
anteiso-C _{17:0}	8.89	11
C _{17:0}	9.22	1

^a The abbreviations for the fatty acids are in the following form: iso-C_{14:0} for 12-methyltridecanoic acid, etc.

^b Percentage of the total peak area from gas-liquid chromatography-mass spectrometry of methyl esters.

of palmitic, saturated, iso and anteiso fatty acids (C₁₄ to C₁₇) (Table 3). No mycolic acids were detected.

Genotaxonomic studies. The DNA base composition of strain S-2^T was 71 mol% G+C. The G+C content of the control strain, *M. luteus* ATCC 4698 (72 mol%), was in agreement with previously published values (10).

The levels of DNA-DNA relatedness between strain S-2^T and the type strains of the most similar *Streptomyces* spp. are shown in Table 4. All of the test strains had few DNA base sequences in common with strain S-2^T.

TABLE 4. Levels of DNA relatedness between strain S-2^T and the type strains of selected *Streptomyces* species

Organism	% Relative binding of labeled DNA from strain S-2 ^T
Strain S-2 ^T	100
<i>Streptomyces exfoliatus</i>	9
<i>Streptomyces laurentii</i>	7
<i>Streptomyces longispororuber</i>	13
<i>Streptomyces prunicolor</i>	12
<i>Streptomyces psammoticus</i>	9

DISCUSSION

The morphological and cultural characteristics, as well as the cell wall and whole-cell chemical characteristics, provide evidence that isolate S-2^T should be assigned to the genus *Streptomyces*.

Unfortunately, there is no simple and rapid procedure for objective classification and identification of streptomycete taxa. In general, physiological criteria are considered more useful for differentiating the microorganisms at the species level (1), whereas morphological or pigmentation characteristics do not provide adequate information.

A comparison of strain S-2^T with the previously described *Streptomyces* species (including the red series) that was based on previously published data (5, 21) and our own critical examination of phenotypic and chemical characteristics, as well as the results of DNA complementarity tests, showed that this strain is a representative of a new species, for which we propose the name *Streptomyces spitsbergensis*. This strain has been deposited in the Polish Collection of Microorganisms as strain PCM 2404^T.

Description of *Streptomyces spitsbergensis* sp. nov. *Streptomyces spitsbergensis* (spits.ber.gen'sis. L. deriv. *spitsbergensis*, of Spitsbergen, the source of the soil sample from which the type strain was isolated).

Spore chains are rectiflexibles. The spore surface is smooth. The color of branching substrate mycelium ranges from red to reddish brown, depending on the growth medium. Aerial mycelium is pink to violet.

The species produces some prodiginine-like metabolites with immunosuppressive activity. Soluble pigments (including melanin) are not produced. Hydrolyzes gelatin and starch. Nitrate is not reduced; other physiological properties are summarized above.

Grows well at 25 to 37°C; no growth occurs at 45°C.

Type I cell wall and type PII phospholipid pattern (saturated iso and anteiso fatty acids, lack of mycolic acids and major glycolipids).

The G+C content of the DNA is 71 mol% (as determined by the thermal denaturation method). Isolated from soil. The type strain is strain PCM 2404.

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