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Streptomyces fildesensis sp. nov., a novel streptomycete isolated from Antarctic soil

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Abstract A novel actinomycete strain, $GW25-5^{T}$, was isolated from a soil sample collected from the Fildes Peninsula, King George Island, West Antarctica. The strain was characterized by white to grey aerial mycelia, which were differentiated to straight to flexuous spore chains, with rod-shaped smooth spores. The cell wall of strain $GW25-5^{T}$ contained LL-diaminopimelic acid (A₂pm) and traces of *meso*-A₂pm. Whole-cell sugars were galactose and minor amounts of mannose and glucose. The predominant menaquinones were MK-9(H₆) (49%), MK-9(H₈) (24%) and MK-9(H₄) (12%). The phospholipids contained DPG, PE, PI, PIM and PL(s). The major

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Key Laboratory of Marine Bio-resources Sustainable Utilization, CAS; RNAM Center for Marine Microbiology, CAS; Guangdong Key Laboratory of Marine Materia Medica; South China Sea Institute of Oceanology, Chinese Academy Sciences, Guangzhou 510301, People's Republic of China cellular fatty acids were iso-C_{16:0} and anteiso-C_{15:0}. Genomic DNA G+C content of strain $GW25-5^{T}$ was 70.0 mol%. BLAST result showed that strain GW25-5 has the 16S rRNA gene sequence highest similarity of 97.5% with members of genus Streptomyces and phylogenetic analysis indicated that this strain belongs to the genus Streptomyces. DNA-DNA relatedness values of strain GW25-5^T with the closest species of *Streptomyces purpureus* LMG 19368^T and Streptomyces beijiangensis YIM 6^T were significantly lower than 70% of the threshold value for the delineation of genomic species. A polyphasic taxonomic investigation based on a judicious combination of genotypic and phenotypic characteristics revealed that the organism represents a novel species of the genus Streptomyces. Thus, we propose strain GW25- 5^{T} as the type strain of this novel species, *Streptomy*ces fildesensis (=CGMCC 4.5735^{T} = YIM 93602^{T} = $DSM 41987^{T} = NRRL B 24828^{T}$).

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The Key Laboratory for Microbial Resources of the Ministry of Education and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, People's Republic of China e-mail: wjli@ynu.edu.cn **Keywords** Streptomyces fildesensis sp. nov. · 16S rRNA · Antarctic

Introduction

The genus Streptomyces was first described by Waksman and Henrici (1943) and as of now, contained over five hundred validly published species (588) plus subspecies (38) (Euzéby 2011; http://www.bacterio. cict.fr/s/streptomycesa.html). Members of this genus are well known for their ability to produce antibiotics, enzymes, enzyme inhibitors and pharmacologically active agents (Bérdy 2005; Chun et al. 1997; Labeda et al. 1997). In the course of a screening program for new cytotoxic antibiotics, one streptomycete strain GW25-5^T was isolated from a soil sample collected from the west coast of the Fildes Peninsula near the Chinese Antarctic Great Wall Station, King George Island, West Antarctica. Based on phenotypic and genotypic evidence, it is proposed that this isolate represents a novel species of the genus Streptomyces, for which the name Streptomyces fildesensis sp. nov., is proposed.

Materials and methods

Strains and culture conditions

Strain GW25-5^T, was isolated on inorganic saltsstarch agar [International *Streptomyces* project (ISP) medium 4 (Shirling and Gottlieb 1966)] after incubation at 28°C for 2 weeks. The purified strain was maintained on ISP medium 2 (Shirling and Gottlieb 1966) slants at 4°C and as 20% (v/v) glycerol suspensions at -80° C. Strain GW25-5^T was deposited in the China General Microbiological Culture Collection Center (CGMCC) as strain CGMCC 4.5735^T and in the German Collection of Microorganisms and Cell Cultures (DSMZ) as strain DSM 41987^T.

Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (about 200 rpm) using ISP medium 2 at 28°C for 1 week.

Phenotypic characteristics

Morphological, cultural, physiological and biochemical characterizations of strain GW25-5^T were studied by following the guidelines of the International Streptomyces project (Shirling and Gottlieb 1966, 1968a, 1968b). The morphological characteristics of strain GW25-5^T, including spore-chain morphology, spore size and surface ornamentation, were assessed by light microscopy (Olympus microscope BH-2) and scanning electron microscopy (JSM5600LV; JEOL) of 14-day-old cultures on ISP medium 2. Aerial spore-mass colour, substrate mycelium pigmentation and coloration of the diffusible pigments of strain GW25-5^T were recorded on ISP media, Czapek's agar, potato-glucose agar and nutrient agar prepared as described by Dong and Cai (2001). Colour determination was compared with colour chips from the ISCC-NBS COLOUR CHARTS standard sample No.2106 (Kelly 1964). This organism was examined for physiological and biochemical characteristics according to previously described methods (Shirling and Gottlieb 1966; Williams et al. 1983). Growth at different temperatures (4, 10, 20, 28, 37, 42, 45, 50 and 55°C), various pH values (4.0-10.0 at intervals of 1.0 pH unit; using the buffer system described by Xu et al. 2005) and NaCl concentrations [0, 1, 2, 3, 4, 5, 6, 7, 10, 15 and 20 (w/v)] were determined by using ISP 2 agar plates, incubation at 28°C for 14–21 days. Media and procedures used for the determination of other physiological characteristics, carbon source utilization and acid production from carbohydrates were those described by Gordon et al. (1974). Resistance to antibiotics was examined as described by Al-Tai et al. (1999).

Chemotaxonomy

Biomass for chemotaxonomy studies was obtained by cultivation in shaken flasks (200 rpm) using ISP medium 2 at 28°C for 1 week. Amino acid and sugar analysis of cell walls were carried out according to the procedures described by Hasegawa et al. (1983). Phospholipids were extracted, isolated by two dimensional TLC and identified by using previously described methods (Minnikin et al. 1984). Menaquinones were isolated according to the method of Minnikin et al. (1984) and separated by HPLC (Kroppenstedt 1982). Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer's instructions. The fatty acid methyl esters were analysed by using the Microbial Identification software package (Sherlock Version 4.0; MIDI database: TSBA40). The G+C content of the genomic DNA was determined by using the HPLC method (Mesbah et al. 1989) with *E. coli* JM-109 as the reference strain.

Molecular analysis

Extraction of genomic DNA and amplification of the 16S rRNA gene sequences were performed as described by Li et al. (2007). The 16S rRNA gene sequence of strain GW25-5^T was compared against a database of cultured species via BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the Ez-Taxon Database (http://www.eztaxon.org, Chun et al. 2007) of type strains to retrieve most similar sequences of recognized bacteria. Multiple alignments with sequences of the most closely related Streptomycetes and calculations of levels of sequence similarity were carried out using CLUS-TAL_X (Thompson et al. 1997). Phylogenetic analyses were performed using the PHYLIP version 3.6 (Felsenstein 2002) and MEGA version 4.1 (Tamura et al. 2007). Bootstrap analysis was used to evaluate the tree topology of the neighbourjoining data by performing 1000 resamplings (Felsenstein 1985).

DNA–DNA hybridization studies between strain $GW25-5^{T}$ and the closest neighbours were performed using the thermal renaturation method (De Ley et al. 1970; Huss et al. 1983; Jahnke 1992).

The 16S rRNA gene sequence of strain $GW25-5^{T}$ has been deposited in GenBank under the accession number DQ408297.

Results and discussion

The cells of strain $GW25-5^{T}$ were Gram-positive, aerobic and non-motile. Morphological features were observed on different ISP media and other related media after incubation for 2 weeks at 28°C. Strain GW25-5^T grew well on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic saltsstarch agar (ISP 4), glycerol-asparagine agar (ISP 5) and potato agar (Table 1). It showed moderate growth on nutrient agar and Czapek's agar medium. A dark yellow-green diffusible pigment was produced on potato agar. Strain GW25-5^T had typical characteristics of the genus Streptomyces. Morphological observation of the 14-day-old culture of strain GW25-5^T revealed that the aerial mycelia formed long, straight to flexuous spore chains and the spores were non-motile (Fig. 1). The physiological features are indicated in Table 2 and in the species description.

The cell wall of strain GW25-5^T contained LL-diaminopimelic acid (A₂pm) and trace amounts of *meso*-A₂pm. Whole-cell hydrolysates contained galactose and small quantities of mannose and glucose. The menaquinones were MK-9(H₆) (49%), MK-9(H₈) (24%), MK-9(H₄) (12%), MK-8(H₆) (6%), MK-9(H₂) (4%) and MK-8(H₈) (4%). The phospholipid composition was diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIM) and other unidentified phospholipids (PLs). The major cellular fatty acids were iso-C_{16:0} (30.08%), anteiso-C_{17:0} (10.25%), anteiso-C_{17:1} (3.95%), iso-C_{16:1} (3.71%) and iso-C_{14:0} (6.98%). The G+C content of the

Table 1Culturecharacteristics of strain $GW25-5^T$ as observed ondifferent growth media afterincubation for 2 weeks at $28^{\circ}C$

Medium	Growth	Sporulation	Colony colour	
			Aerial mycelium	Substrate mycelium
Yeast-malt extract agar (ISP 2)	Good	Good	Pale white	Yellowish white
Oatmeal agar (ISP 3)	Good	Good	Light gray	Yellowish white
Inorganic salts-starch agar (ISP 4)	Good	Good	Yellowish white	Vivid yellow
Glycerol-asparagine agar (ISP 5)	Good	None	_	Yellowish white
Potato agar	Good	Good	Light gray	Olive gray
Nutrient agar	Moderate	None	_	Yellowish white
Czapek's agar	Moderate	Moderate	White	Yellowish white

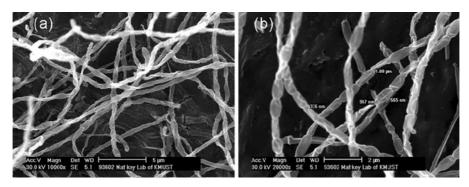


Fig. 1 Scanning electron micrograph of spore chains of strain GW25-5^T after incubation at 28°C for 14 days on yeast malt extract agar (ISP 2) medium. **a** *Bar* 5 μ m, **b** *Bar* 2 μ m

Table 2Phenotypiccharacteristics thatdifferentiate strainGW25-5 ^T from its closestphylogenetic neighbours	Characteristic	Strain GW25-5 ^T	<i>S. purpureus</i> LMG 19368 ^T	S.beijiangensis YIM 6 ^T
	Colony colour on ISP 2	Pale white	White	Pale white
	Spore shape	Rod	Ellipsoidal	Cylindrical
	Spore chain morphology	Straight to flexuous	Straight to flexuous	Straight to flexuous to retinaculiaperti
	Production of diffusible pigment	+	+	_
	Starch hydrolysis	_	_	+
	Degradation of Tween 80	_	+	+
	Melanoid pigment	+	_	+
	Growth at/with			
	NaCl (%, w/v)	0–3	0–7	0–5
	Temperature (°C)	10–37	10–37	4–37
	Utilization of:			
	Arabinose	+	+	_
	Fructose	_	+	_
	Galactose	+	_	-
	Glycerol	+	_	_
	Maltose	+	+	_
	Ribose	_	_	+
	Sorbitol	+	_	-
	Xylose	+	+	-
	Sodium oxalate	+	_	_
	Sodium citrate	+	_	+

genomic DNA from strain $GW25-5^{T}$ was 70.0 mol %, which is in accordance with the values for the genus *Streptomyces* (69 to 76 mol %). The chemotaxonomic characteristics of strain $GW25-5^{T}$, such as amino acid and sugar of whole-cell hydrolysates, menaquinones, major fatty acids and phospholipids were consistent with its assignment to the genus *Streptomyces*.

(1,44 nts) of strain GW25-5^T was aligned with the corresponding sequences of representative members of the genus *Streptomyces* using BLAST (Altschul et al. 1997), and the results revealed the highest similarity under 97.5% with *Streptomyces caeruleus* NRBC12804, *Streptomyces purpureus* LMG 19368^T, *Streptomyces beijiangensis* YIM 6^T and *Streptomyces*

The almost complete 16S rRNA gene sequence

candidus NRRL ISP-5141^T. Streptomyces caeruleus, however, was reclassified as Actinoalloteichus cyanogriseus (Tamura et al. 2008). Phylogenetic analyses based on 16S rRNA gene sequences showed that strain GW25-5^T fell into one distinct subclade with *S. purpureus* LMG 19368^T and *S. beijiangensis* YIM 6^T and was loosely associated with *S. candidus* NRRL ISP-5141^T. The phylogenetic tree reconstructed based on the 16S rRNA gene sequences of strain GW25-5^T and most closely related members of the genus *Streptomyces* is shown in Fig. 2. The DNA–DNA relatedness values of strain $GW25-5^{T}$ with *S. purpureus* LMG 19368^T and *S. beijiangensis* YIM 6^T were 53.8 ± 2.0 and $47.2 \pm 2.0\%$, respectively, and both values were significantly lower than 70% of the threshold value for the delineation of genomic species (Stackebrandt and Goebel 1994) suggesting that the strain $GW25-5^{T}$ should be considered as a different genomic species of the genus *Streptomyces.* in Addition, phenotypic and physiological characteristics showed great differences between strain $GW25-5^{T}$ and its closest

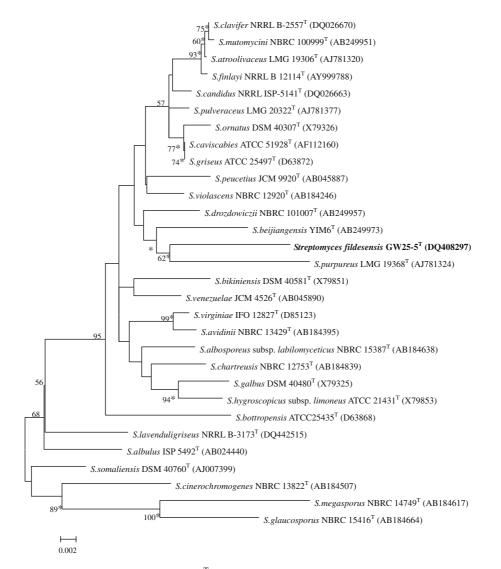


Fig. 2 Phylogenetic relationships of strain $GW25-5^{T}$ and other closely related *Streptomyces* species based on 16S rRNA gene sequences. The branching pattern was generated by the neighbour-joining method. *Asterisks* indicate branches that

were also recovered using the maximum-parsimony method. Bootstrap values (expressed as percentages of 1000 replications) of above 50% are shown at branch points. *Bar*, 0.002 substitutions per nucleotide position neighbours (Table 2). Thus, on the basis of polyphasic taxonomic evidence, it is suggested that strain $GW25-5^{T}$ represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces fildesensis* sp. nov. is proposed.

Description of Streptomyces fildesensis sp. nov

Streptomyces fildesensis [fil.de.sen'sis. N.L. masc.adj. *fildesensis* pertaining to the Fildes Peninsula (King George Island, West Antarctica), from where the type strain was isolated].

Aerial mycelium and substrate mycelium are well developed on most media. Aerial mycelium in maturity forms long and straight to flexuous spore chains. A vellow-green diffusible pigment is produced on potato agar. Optimal growth was observed at 28°C and at pH 7.0 and in the absence of salt. Temperature, pH and NaCl tolerance ranges are 10-37°C, pH 6.0-9.0 and 0-3% (w/v), respectively. Galactose, arabinose, maltose, xylose, glycerol, sorbitol, sodium oxalate and sodium citrate are utilized as sole carbon sources for growth, but not glucose, fructose, sucrose, lactose, mannose, trehalose, ribose, cellobiose or raffinose. Melanin production and degradation of Tween 20, 40 and 60 are positive. Starch hydrolysis and urea utilization are negative. The organism is sensitive to erythromycin (15 μ g/ disk), gentamicin (10 µg/disk), vancomycin (30 µg/ disk), lincomycin (2 µg/disk), chloramphenicol (30 µg/disk), netilmicin (30 µg/disk) and neomycin (10 µg/disk). Whole-cell hydrolysates contain galactose and trace amounts of mannose and glucose. The predominant menaquinones are MK-9(H₆), MK- $9(H_8)$ and MK- $9(H_4)$. The phospholipid composition is DPG, PE, PI, PIM and PL(s). The major cellular fatty acids are iso-C_{16:0}, anteiso-C_{15:0}, C_{15:0}, anteiso- $C_{17:0}$, anteiso- $C_{17:1}$, iso- $C_{16:1}$ and iso- $C_{14:0}$. The G+C content of the genomic DNA is 70.0 mol%.

The type strain, $GW25-5^{T} = CGMCC \ 4.5735^{T} =$ YIM 93602^T = DSM 41987^T = NRRL B 24828^T, was isolated from a soil sample collected from the west coast of Fildes Peninsula, near the Chinese Antarctic Great Wall Station, King George Island, West Antarctica.

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