Streptomyces nanshensis sp. nov., isolated from the Nansha Islands in the South China Sea

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A novel actinomycete strain, designated SCSIO 01066^T, was isolated from a marine sediment sample collected from the Nansha Islands in the South China Sea and was subjected to a polyphasic taxonomic study. A phylogenetic tree based on 16S rRNA gene sequences showed that strain SCSIO 01066^T had the highest similarity (96.5%) to members of the genus *Streptomyces* and was loosely associated with *Streptomyces armeniacus* JCM 3070^T, *Streptomyces cacaoi* subsp. *cacaoi* NBRC 12748^T and *Streptomyces sodiiphilus* YIM 80305^T. Predominant menaquinones, major fatty acids and morphological properties were also consistent with typical characteristics of the genus *Streptomyces*; however, the presence of phosphatidylglycerol in the phospholipid pattern differs greatly from those of members of the genus *Streptomyces*. Additionally, strain SCSIO 01066^T showed some physiological differences from its most closely related neighbours. Based on the polyphasic data, a novel species, *Streptomyces nanshensis* sp. nov., is proposed, with the type strain SCSIO 01066^T (=KCTC 19400^T=CCTCC AA 208005^T).

The deep sea, covered by water >2000 m deep and comprising >50% of the total sea surface, is a unique and extreme environment characterized by high pressure, low temperature, lack of light and little nutrition. In deep-sea sediments, marine micro-organisms occupy the main ecological niche, which carries out an important role in the recycling of carbon and nitrogen sources on the sea floor. Marine microbes have been paid more attention in recent years, especially Gram-positive bacteria, which comprise about 13% of the total bacteria in marine sediment environments (Stevens *et al.*, 2007). Streptomycetes exist not only in all kinds of terrestrial environments, but also ubiquitously in marine environments (Pathom-aree *et al.*, 2006), as one of the main groups of marine actinomycetes (Maldonado *et al.*, 2005). During our study on marine actinomycete resources in deep-sea sediments, strain

SCSIO 01066^T was recovered from an abyssal sediment sample. Based on the polyphasic data, this strain should be recognized as a member of a novel species of the genus *Streptomyces*, for which the name *Streptomyces nanshensis* sp. nov. is proposed.

Strain SCSIO 01066^T was isolated from a deep-sea sediment sample, collected in June 2007 from the sea area of the Nansha Islands in the South China Sea (6° 40' N 113° 33' E) at a depth of 2015 m. The top 10 cm of sediment surface was obtained aseptically for sampling and then processed for cultivation experiments by using a standard dilution-plating method on ship within 2 h of sampling. This organism was isolated on Gauze no. 1 medium, modified with 50% seawater instead of distilled water, after incubation at 28 °C for 3 weeks. The purified strain was maintained on ISP medium 2 and as 20% (w/v) glycerol suspensions at –20 °C. Biomass for chemotaxonomic and molecular systematic studies was obtained by cultivation using ISP 2 broth medium (28 °C, 1 week, 150 r.p.m.).

Strain SCSIO 01066^T grew well on ISP media 2, 3, 4 and 5 (Shirling & Gottlieb, 1966), Czapek's solution agar

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Abbreviations: PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides.

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(Waksman, 1961), nutrient agar (Difco) and potato agar (Waksman, 1961) at 28 °C after 7, 14, 21 and 28 days, but white aerial mycelia were observed more easily on ISP media 2, 4 and 5 and Czapek's solution agar. The micromorphology was examined by light microscopy (model BH2; Olympus) and scanning electron microscopy [model JEM-1010 (Fig. 1a); model JSM5600LV (Fig. 1b) (both from JEOL)], using cells incubated for 14 and 28 days on ISP 2 medium modified with 50 % seawater instead of distilled water. The organism forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long, spiral spore chains; the spore surface is smooth (Fig. 1a, b). No diffusible pigments were observed by comparing the cultures with the most suitable colour chips from the ISCC-NBS colour charts (Kelly, 1964).

Media and procedures used for determination of physiological features and carbon-source utilization were those described by Smibert & Krieg (1981) and Williams *et al.* (1989). The temperature, pH range and tolerance to NaCl for growth were determined as described by Xu *et al.* (2005), using ISP 2 as the basal medium. Antibiotic susceptibility was examined as described by Groth *et al.* (2004) using antibiotic discs on ISP 2 medium.

Cells of strain SCSIO 01066^T were aerobic and Gram-positive. Good growth occurred at pH 7.0 and 28 °C with 0–3 % (w/v) NaCl. This organism was susceptible to

amikacin (30 µg per disc), amoxicillin (10 µg per disc), gentamicin (10 µg per disc), lincomycin (2 µg per disc), neomycin (10 µg per disc), netilmicin (30 µg per disc), norfloxacin (10 µg per disc), novobiocin (30 µg per disc), penicillin G (10 µg per disc), rifampicin (5 µg per disc), streptomycin (10 µg per disc), tetracycline (30 µg per disc), tobramycin (10 µg per disc) and vancomycin (30 µg per disc). It was resistant to ampicillin (10 µg per disc), ciprofloxacin (5 µg per disc), erythromycin (15 µg per disc) and sulfamethoxazole (23.75 µg per disc). Detailed physiological characteristics of this strain are given in the species description.

The isomers of diaminopimelic acid and whole-cell sugars were analysed according to the procedures developed by Hasegawa *et al.* (1983). Menaquinones were isolated by using the methods of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). Phospholipids were extracted and examined by using published procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Fatty acid analysis was performed by using standard methods (Sasser, 1990) and the results were compared with the database of fatty acids in the Microbial Identification System (MIDI, Inc.).

The cell wall of strain SCSIO 01066^T contained L-diaminopimelic acid with whole-cell sugars of galactose and glucose. Phospholipids comprised phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylinositol mannosides (PIM) and an unknown phospholipid. The menaquinones were MK-9(H₈) (77 %), MK-9(H₆) (13.9 %), MK-10(H₈) (3.9 %), MK-8(H₈) (2.1 %), MK-9(H₁₀) (1.9 %) and MK-10(H₆) (0.7 %). The fatty acids were ai-C_{15:0} (32.42 %), i-C_{16:0} (25.66 %), i-C_{15:0} (12.35 %), ai-C_{17:0} (10.72 %), i-C_{14:0} (8.75 %), i-C_{17:0} (2.47 %), i-H-C_{16:1} (2.23 %), C_{16:0} (1.89 %), C_{15:0} (0.79 %), ai-C_{13:0} (0.57 %), i-C_{13:0} (0.48 %), C_{16:1 cis9} (0.53 %), ai-C_{17:1} C (0.44 %), ai-C_{14:0} (0.35 %) and C_{14:0} (0.35 %).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described previously (Li *et al.*, 2007). Multiple alignments with sequences of the most closely related taxa and calculations of levels of sequence similarity were carried out by using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analyses were performed by using three tree-making algorithms: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree and distance matrix were reconstructed by using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980, 1983) using MEGA version 4.0 (Tamura *et al.*, 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The G+C content of genomic DNA was determined by using the HPLC method (Mesbah *et al.*, 1989).

Based on the almost-complete 16S rRNA gene sequence (1405 bp) of strain SCSIO 01066^T, BLAST search results

Fig. 1. Scanning electron micrographs of (a) substrate mycelia and (b) spore chains of *S. nanshensis* SCSIO 01066^T after incubation on ISP medium 2 for 4 weeks at 28 °C. Bars, 10 µm (a); 5 µm (b).

showed that this new isolate had the highest similarity (96.5 %) to members of the genus *Streptomyces*. Preliminary phylogenetic analysis, which included available, almost full-length sequences of type strains of species in the genus *Streptomyces*, showed that strain SCSIO 01066^T was associated with these members of the genus *Streptomyces*. Morphological and chemotaxonomic characteristics, including cell-wall type, predominant menaqui-

nones and major fatty acids, were also consistent with those of members of the genus *Streptomyces*. All of the above data confirmed that the new isolate should be assigned to the genus *Streptomyces*.

However, a phylogenetic tree (Fig. 2) based on the closest neighbours of strain SCSIO 01066^T showed that the new isolate was associated most closely with *Streptomyces*

Fig. 2. Phylogenetic tree of strain SCSIO 01066^T and its closest relative neighbours within the genus *Streptomyces*, reconstructed by using the neighbour-joining method. Numbers at nodes are bootstrap values based on 1000 resamplings (only values >50 % are indicated). Bar, 1 % sequence divergence.

armeniacus JCM 3070^T, *Streptomyces cacaoui* subsp. *cacaoui* DSM 40057^T and *Streptomyces sodiiphilus* YIM 80305^T, and these relationships were supported by all tree-making methods used in this study (data not shown). However, the presence of PG in the phospholipid pattern for the new isolate differs greatly from that of other members of the genus *Streptomyces*. Additionally, strain SCSIO 01066^T can be readily distinguished from its most closely related neighbours by the phenotypic characteristics shown in Table 1. Thus, based on the phylogenetic position and phenotypic data, a novel species is proposed for strain SCSIO 01066^T, to be named *Streptomyces nanshensis* sp. nov.

Description of *Streptomyces nanshensis* sp. nov.

Streptomyces nanshensis (nan.shen'sis. N.L. adj. *nanshensis* pertaining to the sea area of the Nansha Islands in the southern part of the South China Sea, from where the type strain was isolated).

Aerobic, Gram-positive actinomycete that forms extensively branched substrate mycelia and aerial hyphae that differentiate into long, spiral spore chains. Spore surface is

Table 1. Comparison of characteristics of strain SCSIO 01066^T and its most closely related neighbours in the genus *Streptomyces*

Strains: 1, SCSIO 01066^T; 2, *S. armeniacus* JCM 3070^T; 3, *S. cacaoui* subsp. *cacaoui* NBRC 12748^T; 4, *S. sodiiphilus* YIM 80305^T. Abbreviations: W, white; GW, grey-white; RA, Retinaculiaperti; S, smooth; SP, Spirales; ST, straight; WS, wrinkled surface; +, utilized or reaction positive; –, not utilized or reaction negative; ND, no data available. Data are from this study and Waksman (1961), Wellington & Williams (1981), Lanoot *et al.* (2002) and Li *et al.* (2005).

Characteristic	1	2	3	4
Colony colour on ISP 2	W	W	W	GW
Spore surface	S	S	S	WS
Spore-chain morphology	SP	SP	SP	ST to RA
Production of diffusible pigment	–	ND	–	+
Hydrolysis of:				
Gelatin	–	+	+	+
Starch	–	+	+	–
Melanoid pigment	+	–	–	+
H ₂ S production	–	ND	–	–
Utilization of:				
Glucose	+	+	+	–
Arabinose	+	+	+	–
Mannitol	+	–	+	–
Fructose	+	+	+	–
Rhamnose	+	+	–	+
Sucrose	+	+	+	–
Raffinose	+	+	+	–
Inositol	+	ND	–	–
Xylose	+	+	+	–

smooth. Soluble pigments are not produced. Negative for hydrolysis of Tweens 40 and 80, gelatin, starch, cellulose, milk coagulation and peptonization, H₂S production, oxidase and nitrate reduction, but positive for catalase, hydrolysis of Tweens 20 and 60, melanin production and utilization of urea. D-Arabinose, cellobiose, citrate, fructose, D-galactose, D-glucose, inositol, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-ribose, sucrose, trehalose and D-xylose are used as sole carbon sources for growth, but not acetate, dulcitol, D-lactose, D-sorbitol or xylitol. The pH, NaCl concentration and temperature ranges for growth are pH 6.0–8.0, 0–7% and 10–37 °C, respectively. The cell wall contains L-diaminopimelic acid with whole-cell sugars of galactose and glucose. Phospholipids comprise PG, DPG, PE, PI, PIM and an unknown phospholipid. The predominant menaquinones are MK-9(H₈) and MK-9(H₆). The major fatty acids are ai-C_{15:0}, i-C_{16:0}, i-C_{15:0}, ai-C_{17:0} and i-C_{14:0}. The G+C content of genomic DNA is 72.6 mol%.

The type strain, SCSIO 01066^T(=KCTC 19400^T=CCTCC AA 208005^T), was isolated from a sediment sample taken at a depth of 2015 m, collected from the Nansha Islands in the South China Sea.

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