Streptomyces xinghaiensis sp. nov., isolated from marine sediment

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A novel actinomycete, strain S187^T, was isolated from a marine sediment sample collected from Xinghai Bay, Dalian, China. Growth occurred on ISP medium 2 containing 0–9% NaCl and at pH 6.0–9.0 and 10–45 °C. The cell wall of strain S187^T contained the isomer LL-diaminopimelic acid as the diagnostic diamino acid. The predominant menaquinones were MK-9(H₈) (40.8%), MK-9(H₆) (38.2%) and MK-9(H₂) (8.8%). The major fatty acids were iso-C_{16:0} (29.6%), anteiso-C_{15:0} (14.0%) and anteiso-C_{17:0} (11.6%). Cells contained phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, phosphatidylinositol mannosides and one unknown phospholipid. The G + C content of the genomic DNA was 72.01 mol%. The 16S rRNA gene sequence of the isolate had similarities of 98.1 and 97.5% with those of *Streptomyces flavofuscus* NRRL B-8036^T (=DSM 41426^T) and *Streptomyces albiacialis* DSM 41799^T, respectively, showing that the novel strain should be assigned to the genus *Streptomyces*. DNA–DNA hybridizations with the two above-mentioned *Streptomyces* species showed 31.4 and 46.9% relatedness, respectively. Moreover, the three strains differed in some physiological and biochemical properties. Thus, on the basis of phenotypic and genotypic analyses, it is proposed that strain S187^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces xinghaiensis* sp. nov. is proposed; the type strain is S187^T (=NRRL B-24674^T=CCTCC AA 208049^T=KCTC 19546^T).

Many marine actinobacteria have been screened and identified in recent years (Maldonado *et al.*, 2005; Ward & Bora, 2006). It has been demonstrated that a diverse assemblage of actinobacteria is present in the marine environment, including marine sediment (Jensen *et al.*,

2005; Bredholdt *et al.*, 2007), especially deep-sea sediment (Pathom-Aree *et al.*, 2006), as well as marine sponges (Montalvo *et al.*, 2005; Zhang *et al.*, 2006). Dalian is a coastal city located in the southernmost part of the Liaodong Peninsula, China, which has a very long coastline of 1906 km. However, the possible abundance of marine actinobacteria remains largely unexplored, except for some reports on sponge-derived marine actinobacteria (Zhang *et al.*, 2006). During our screening of marine actinobacteria from sediment samples taken from the sea in the Dalian area, strain S187^T was recovered from a sediment sample collected from Xinghai Bay. In this study, strain S187^T was characterized using a polyphasic taxonomic approach; it is

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S187^T is EF577247.

A scanning electron micrograph of strain S187^T, a phylogenetic tree based on 16S rRNA gene sequences of S187^T and related strains constructed using the maximum-parsimony method and antimicrobial activities of S187^T and closely related strains are available as supplementary material with the online version of this paper.

proposed that this strain represents a novel species of the genus *Streptomyces*.

Strain S187^T was isolated from a marine sediment sample from Xinghai Bay, Dalian, China, after 2 weeks incubation at 28 °C on Bennett's agar (Margalith & Beretta, 1960). Morphological, physiological and biochemical characterizations of strain S187^T were carried out following the standard protocol of the International *Streptomyces* Project (ISP; Shirling & Gottlieb, 1966, 1968a, b, 1969). Colour determination was assessed by comparison with the colour chips from ISCC–NBS Color Charts standard sample no. 2106 (Kelly, 1964). Formation of spores and mycelia was determined by light microscopy (BH-2; Olympus) and scanning electron microscopy (JSM-5600LV; JEOL) of strain S187^T grown on ISP medium 2 (ISP 2) at 28 °C for 4 weeks. Phenotypic properties of strain S187^T and closely related strains were examined using standard procedures (Shirling & Gottlieb, 1966; Williams *et al.*, 1983). The effects of various temperatures (4–65 °C), pH (4.0–10.0 at intervals of 1.0 pH unit; determined at 28 °C) and NaCl concentrations [0, 1, 2, 3, 4, 5, 10, 15, 20 and 25 % (w/v);

determined at 28 °C] on growth were determined on cells grown on ISP 2 for 3–4 weeks. Antimicrobial activities of strain S187^T and closely related strains were tested as follows. Cells were grown in tryptone soy broth (Becton Dickinson) at 28 °C for 6 days, culture broth was then centrifuged at 4 °C, 12 000 r.p.m. for 10 min, and 200 µl supernatant was applied to agar blocks on the assay plates spread with test strains. The test strains, purchased from the China General Microbiological Culture Collection Center (CGMCC), were *Escherichia coli* CGMCC 1.797, *Staphylococcus aureus* CGMCC 1.89, *Bacillus subtilis* CGMCC 1.1849, *Pseudomonas aeruginosa* CGMCC 1.2031, *Candida albicans* CGMCC 2.538 and *Aspergillus niger* CGMCC 3.2915.

Amino acids and peptides in cell-wall hydrolysates were analysed by the methods described by Hasegawa *et al.* (1983). Whole-cell sugars were analysed by the methods of Lechevalier & Lechevalier (1980). Menaquinones and phospholipids were extracted from freeze-dried cells and analysed according to Collins (1985) and Lechevalier *et al.* (1977). Fatty acid composition was determined according to Sasser (1990).

Table 1. Culture characteristics of strain S187^T and its most closely related neighbours

Strains: 1, S187^T; 2, *S. flavofuscus* NRRL B-8036^T; 3, *S. albiaxialis* DSM 41799^T. Diffusible pigments were not observed on any of the media listed. Colours were taken from ISCC–NBS Color Charts standard sample no. 2106 (Kelly, 1964). Plates were incubated for 4 weeks at 28 °C. –, No growth.

Culture medium	1	2	3
ISP 2			
Growth	Good	Good	Good
Aerial mycelium	White	White	Strong olive green
Substrate mycelium	Dark orange yellow	Dark orange yellow	Moderate reddish brown
ISP 3			
Growth	Good	Good	Good
Aerial mycelium	White	White	White
Substrate mycelium	Yellow white	Strong olive green	Deep orange yellow
Potato-dextrose agar			
Growth	Poor	Good	Good
Aerial mycelium	–	White	Vivid bluish green
Substrate mycelium	Pale orange yellow	Yellow white	Moderate reddish brown
Nutrient agar			
Growth	Poor	Good	Poor
Aerial mycelium	White	White	–
Substrate mycelium	Light orange yellow	Moderate orange yellow	Light orange yellow
Czapek's agar			
Growth	Poor	Moderate	Good
Aerial mycelium	–	White	Strong olive green
Substrate mycelium	Light yellow brown	Strong olive green	Light yellow brown
ISP 5			
Growth	Good	Good	Good
Aerial mycelium	Vivid bluish green	White	Vivid yellowish pink
Substrate mycelium	Yellow white	Pale orange yellow	Moderate reddish brown
ISP 4			
Growth	Good	Good	Good
Aerial mycelium	White	White	Deep olive green
Substrate mycelium	Pale orange yellow	Yellow white	Pale orange yellow

Genomic DNA was extracted as described by Lee *et al.* (2003) and the 16S rRNA gene was amplified according to Zhang *et al.* (2006). The DNA G+C content of strain S187^T was determined by using the HPLC method (Mesbah *et al.*, 1989). Closely related reference strains were chosen from BLAST (Altschul *et al.*, 1997) search results. Phylogenetic analysis was performed using the software package MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignment using CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987). The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Morphological observation of a 28-day-old culture of strain S187^T grown on ISP 2 (yeast extract-malt extract agar) revealed that strain S187^T had the typical characteristics of members of the genus *Streptomyces*. Aerial mycelium and substrate mycelium were well-developed without fragmentation. Long spore chains were straight or flexuous and spores were smooth and non-motile (see Supplementary Fig. S1, available in IJSEM Online). Strain S187^T grew well between pH 6.0 and 9.0, with optimum growth at pH 7.0–8.0. It grew at 10–45 °C, with optimum growth at 28 °C. Strain S187^T grew in the presence of 0–9% (w/v) NaCl. Mycelia of strain S187^T were well-

developed on some media tested, including ISP 2, ISP 3 (oatmeal agar), ISP 5 (glycerol-asparagine agar) and ISP 4 (inorganic salts-starch agar), but growth was poor on nutrient agar, Czapek's agar and potato-dextrose agar (Table 1). No diffusible pigments were observed on any media tested.

The cell wall of strain S187^T contained LL-diaminopimelic acid and glycine (cell-wall chemotype I; Lechevalier & Lechevalier, 1970). Whole-cell hydrolysates contained mainly ribose, glucose and galactose, with a small amount of mannose. Phospholipids of strain S187^T were phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, phosphatidylinositol mannosides and one unknown phospholipid. The predominant menaquinones were MK-9(H₆) (40.8%), MK-9(H₈) (38.2%) and MK-9(H₂) (8.8%). The major fatty acids found were iso-C_{16:0} (29.2%), anteiso-C_{15:0} (14.0%), anteiso-C_{17:0} (11.6%), iso-C_{14:0} (8.8%), iso-C_{15:0} (5.5%), iso-C_{16:1} H (4.6%), anteiso-C_{17:1} C (4.0%), C_{16:0} (4.0%) and iso-C_{17:0} (3.5%). All morphological characteristics and chemotaxonomic data showed that strain S187^T should be assigned to the genus *Streptomyces*.

The almost-complete 16S rRNA gene sequence (1490 nt) of strain S187^T was aligned manually with corresponding 16S rRNA gene sequences of representative type strains of members of the genus *Streptomyces* retrieved from

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence analysis and constructed using the neighbour-joining method showing the position of strain S187^T among its phylogenetic neighbours. Numbers at the branch nodes are bootstrap values, expressed as percentages of 1000 replicates (only values > 50% are shown). Bar, 0.01 substitutions per nucleotide position.

GenBank/EMBL/DDBJ using a BLAST search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>; Altschul *et al.*, 1997). Phylogenetic analyses based on 16S rRNA gene sequences showed that the novel isolate fell into one distinct subclade with *Streptomyces flavofuscus* NRRL B-8036^T (=DSM 41426^T) and *Streptomyces albiacialis* DSM 41799^T, with which it shared 16S rRNA gene sequence similarities of 98.1 and 97.5 %, respectively. Another closely related strain was *Streptomyces ferralitis* SFOP68^T, which showed 95.6 % 16S rRNA gene sequence similarity (1425 out of 1490 nt) to strain S187^T. Phylogenetic trees based on 16S rRNA gene sequences of strain S187^T and the most closely related type strains of the genus *Streptomyces* constructed using the neighbour-joining (Fig. 1) and maximum-parsimony (see Supplementary Fig. S2, available in IJSEM Online) methods are shown. DNA–DNA relatedness tests were performed between strain S187^T and its closest relatives, *S. flavofuscus* NRRL B-8036^T and *S. albiacialis* DSM 41799^T, using the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992). The DNA–DNA relatedness values of strain S187^T with *S. flavofuscus* NRRL B-8036^T and *S. albiacialis* DSM 41799^T were 31.4 and 46.9 %, respectively, which were significantly lower than 70 %, the threshold value for the delineation of genomic species (Wayne *et al.*, 1987). The antimicrobial activities of the three strains were investigated; strain S187^T displayed excellent activities against the four bacterial strains tested, whereas *S. flavofuscus* NRRL B-8036^T showed no activity against *P. aeruginosa* (see Supplementary Table S1, available in IJSEM Online). A phenotypic comparison between strain S187^T and closely related species of the genus *Streptomyces* was carried out and differential characteristics of the strains were identified (Table 2). On the basis of morphological, chemotaxonomic and phylogenetic evidence and its physiological and biochemical distinctiveness, it is confirmed that strain S187^T represents a novel species in the genus *Streptomyces*, for which the name *Streptomyces xinghaiensis* sp. nov. is proposed.

Description of *Streptomyces xinghaiensis* sp. nov.

Streptomyces xinghaiensis (xing.hai.en'sis. N.L. masc. adj. *xinghaiensis* pertaining to Xinghai Bay, Dalian, China, the site from which the type strain was isolated).

Gram-positive, aerobic and non-motile. Grows well on ISP media 2, 3, 4 and 5, but poorly on nutrient agar, potato-dextrose agar and Czapek's agar media (Table 1). Colonies are whitish to yellow with ruffled edges. Aerial mycelium and substrate mycelium are well-developed without fragmentation. Long spore chains are straight or flexuous; spores are smooth and non-motile. Casein, pectin and cellulose are degraded, but starch is not. Positive for catalase production and gelatin liquefaction. No soluble pigments are produced on any media tested. Temperature range for growth is 10–45 °C (optimum 28 °C). Growth occurs at pH 6.0–9.0 and in the presence of 0–9 % NaCl

Table 2. Physiological and biochemical features that differentiate strain S187^T from closely related species of the genus *Streptomyces*

Strains: 1, S187^T; 2, *S. flavofuscus* NRRL B-8036^T; 3, *S. albiacialis* DSM 41799^T. +, Positive; –, negative.

Characteristic	1	2	3
Degradation of:			
Casein	+	+	–
Cellulose	+	–	+
Growth in NaCl (% w/v):			
Range	0–9	0–10	0–6
Optimum	0–5	0–5	0–3
Growth at 5 °C	–	–	+
Growth pH	7–9	6–9	7–9
Carbon source utilization:			
L-Arabinose	–	–	+
Ribose	+	+	–
myo-Inositol	–	–	+
L-Rhamnose	+	+	–
Cellobiose	–	+	+
Sorbitol	–	–	+
Nitrogen source utilization:			
Lysine	–	+	–
Phenylalanine	+	–	+
Serine	+	+	–
Xanthine	+	+	–

(w/v). Good growth is found on almost all nitrogen sources tested. Utilizes D-glucose, sucrose, mannitol, fructose, mannose, galactose, ribose and rhamnose as carbon sources, but not trehalose, L-arabinose, myo-inositol, raffinose, cellobiose or sorbitol. More detailed phenotypic properties are shown in Table 2. Cell wall contains LL-diaminopimelic acid as the diagnostic diamino acid. Whole-cell hydrolysates contain mainly ribose, glucose and galactose, with a small amount of mannose. Chemotaxonomic properties are typical of the genus *Streptomyces*.

The type strain, S187^T (=NRRL B-24674^T=CCTCC AA 208049^T=KCTC 19546^T), was isolated from a marine sediment sample collected from Xinghai Bay, Dalian, Liaoning Province, China. The DNA G+C content of the type strain is 72.01 mol%.

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