Streptomyces xiamenensis sp. nov., isolated from mangrove sediment

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Actinomycete strain MCCC 1A01550^T, isolated from the national mangrove reserve in Fujian Province, China, was determined to belong to the genus *Streptomyces* based on a polyphasic approach. The cell wall of the novel strain contained LL-diaminopimelic acid and galactose. The predominant menaquinones were MK-9(H₄) and MK-9(H₆) and the major fatty acids were iso-C_{16:0}, cis9-C_{16:1} and C_{16:0}. The diagnostic phospholipid was phosphatidylethanolamine. The DNA G+C content of the novel strain was 71.6 mol%. Phylogenetic analysis of 16S rRNA gene sequences confirmed the separation of the novel strain from recognized members of the genus *Streptomyces* available in public databases. DNA–DNA relatedness values between strain MCCC 1A01550^T and the type strains of three related species ranged from 21.29 % to 43.38 %. Based on its phenotypic and genotypic characteristics, strain MCCC 1A01550^T (=DSM 41903^T=CGMCC 4.3534^T) represents the type strain of a novel species, for which the name *Streptomyces xiamenensis* sp. nov. is proposed.

Mangrove forests, important protectors of the coastline, are located in the tidal zones in tropical and subtropical areas. These highly productive ecosystems are home to a diverse flora and fauna of marine, freshwater and terrestrial species (Jennerjahn & Ittekkot, 2002). In contrast to the well-documented species diversity of larger animals and plants in these ecosystems, the microbial community diversity of mangrove forests has been poorly studied.

The regular changes in environmental factors seen in mangrove forests, such as salinity and tidal gradients, are believed to be effective selectors for metabolic pathway adaptations that could generate unusual metabolites. This has led to increasing exploitation of the mangrove micro-organism resources (Long *et al.*, 2005). The micro-organisms in mangrove forest sediments are not only involved in the decomposition and mineralization of litter-fall (Holguin *et al.*, 2001), but are also versatile producers of various enzymes and antibiotics.

In the course of our screening program for new antibiotic producers, groups of actinomycete strains, which contained both type I and type II polyketide biosynthesis pathway genes, were isolated from soil samples collected from the national mangrove reserve in the Fujian Province

of China. In this paper, the results of a polyphasic taxonomic study of a novel *Streptomyces* strain, MCCC 1A01550^T, are presented.

Strain MCCC 1A01550^T was isolated after 2 weeks incubation at 28 °C on ISP 3 agar (Shirling & Gottlieb, 1966). The novel strain was maintained on nutrient agar at 4 °C and stored in glycerol suspensions at –20 °C. For chemical and molecular analyses, biomass was prepared by culturing in trypticase soytone broth (TSB) for 3–5 days at 28 °C in a rotary shaker (180 r.p.m.) and was then harvested by centrifugation.

Morphological observations of spores and mycelia grown on Gause's synthetic agar at 28 °C for 12 days were made by light microscopy (COBER-015; Olympus) and scanning electron microscopy (XL30; ESEM-TMP Philips-FEI). Cultural characteristics were determined after 3 weeks at 28 °C by methods used in the ISP (Shirling & Gottlieb, 1966). Colours were assessed according to colour chips in the ISCC-NBS Color Charts Standard no. 2106 (Kelly, 1964). The temperature range for growth was determined on modified ISP 2 agar at 4, 10, 15, 20, 28, 37 and 45 °C for 1–2 weeks. Tolerance to NaCl was determined by adding 0, 5, 10, 15 or 20 % (w/v) NaCl to the ISP 2 agar medium, followed by incubation at 28 °C in a rotary shaker (180 r.p.m.) for 1 week.

The novel strain developed well on several media. The temperature range for growth was 15–37 °C and NaCl tolerance was 5 %, with an optimal temperature at 28 °C. At maturity, the aerial mycelium formed long, straight to *rectiflexibiles* spiral spore chains. The spores were non-motile and ellipsoid in shape (Fig. 1). Colonies were grey in

Abbreviations: ISP, International *Streptomyces* Project; PE, phosphatidylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA sequence of strain MCCC 1A01550^T is EF012099.

Details of the cellular fatty acid content of strain MCCC 1A01550^T are presented in a supplementary table that is available with the online version of this paper.

Fig. 1. Scanning electron micrographs of the spore chains of strain MCCC 1A01550^T grown on Gause's synthetic agar for 12 days at 28 °C, showing spore chains undergoing division into spores (a) and aerial mycelium and spore chains (b). Bars, 2 µm (a); 10 µm (b).

colour on ISP 2, ISP 3, Czapek's agar and starch agar, and white on ISP 4, ISP 5 and glucose-asparagine agar. Diffusible pigments were produced on most media (Table 1).

The media and procedures used to assess physiological and biochemical features were as described by Shirling &

Gottlieb (1966). Carbohydrate utilization was carried out according to the method of Kämpfer *et al.* (1991). Resistance to various antibiotics was determined on ISP 2 agar supplemented with different antibiotics (Shirling & Gottlieb, 1966). Gelatin liquefaction, starch hydrolysis and reaction with milk were tested according to the methods of Goodfellow (1971) and Gordon *et al.* (1974).

All physiological and biochemical tests were performed at 28 °C. The novel strain utilized mannose, arabinose, ribose, rhamnose and mannitol, but not raffinose, xylose or galactose. Tests for gelatin liquefaction, nitrate reduction and milk peptonization were positive and tests for H₂S and melanin production were negative. Good growth occurred in the presence of penicillin G (5 µg ml⁻¹), rifampicin (5 µg ml⁻¹), streptomycin (10 µg ml⁻¹) and ampicillin (10 µg ml⁻¹).

Tests to determine the diagnostic isomer of diaminopimelic acid and the whole-cell sugars were conducted using freeze-dried cells that were hydrolysed with 6 M and 0.5 M HCl at 120 °C for 30 min (Lechevalier & Lechevalier, 1980) and then analysed by TLC (Hasegawa *et al.*, 1983). Phospholipid analysis was performed as described by Komagata & Suzuki (1987). Menaquinones were extracted according to the method of Collins *et al.* (1977) and analysed by HPLC (Kroppenstedt, 1985). Cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI, Inc.) and Sherlock MIS software.

The amino acids in the peptidoglycan layer of the cell wall of strain MCCC 1A01550^T were LL-diaminopimelic acid, alanine, glycine and glutamic acid. The whole-cell sugar was galactose. The predominant menaquinones were MK-9(H₄) (71.2 %), MK-9(H₆) (16.3 %), MK-9(H₂) (4 %) and MK-9(H₈) (1.6 %). The diagnostic phospholipid was phosphatidylethanolamine (PE); other phospholipids detected were phosphatidylglycerol and phosphatidylinositol and phosphatidylinositol mannosides. The major cellular fatty acids were iso-C_{16:0} (43.82 %), *cis*-9-C_{16:1} (12.13 %) and C_{16:0} (11.30 %) (for details see Supplementary Table S1 in IJSEM Online).

Table 1. Culture characteristics of strain MCCC 1A01550^T on various media at 28 °C after 7 days

Medium	Growth	Sporulation	Diffusible pigment	Colony colour	
				Aerial mycelium	Substrate mycelium
Yeast extract agar (ISP 2)	Good	Good	Deep olive	Grey	Light yellow
Oatmeal agar (ISP 3)	Moderate	Poor	Grey-green	Grey-white	Moderate yellow
Inorganic salts-starch agar (ISP 4)	Good	Poor	—	White	Light yellow
Glycerol-asparagine agar (ISP 5)	Moderate	Poor	Light pink	White	White
Nutrient agar	Good	Good	Deep brown	Grey	Deep brown
Czapek's agar	Good	Good	Deep green	Grey	Deep brown
Starch agar	Good	Good	—	Grey-white	Dark olive
Glucose-asparagine agar	Moderate	Poor	—	White-yellow	White

Amplification of the 16S rRNA gene sequence was performed as described by Cui *et al.* (2001). Sequence analysis was then conducted using the BLAST network service provided by the NCBI (Altschul *et al.*, 1997). The 16S rRNA gene sequences of representative actinomycetes were aligned using CLUSTAL_X (Thompson *et al.*, 1997) and similarity values were used to construct a phylogenetic tree by the maximum-parsimony and neighbour-joining methods with MEGA3.1 software (Saitou & Nei, 1987). The topology of the tree was evaluated by performing a bootstrap analysis (Felsenstein, 1985) using 1000 replications.

Genomic DNA was extracted as described by Weisburg *et al.* (1991). DNA was digested and dephosphorylated before determination of base composition by HPLC analysis (Tamaoka & Komagata, 1984). Molar G+C content was calculated according to Mesbah *et al.* (1989). DNA–DNA relatedness studies were performed between strain MCCC 1A01550^T and *Streptomyces carpaticus* NRRL B-16359^T (Gause *et al.*, 1983), *Streptomyces cheonanensis* VC-A46^T (Kim *et al.*, 2006) and *Streptomyces sodiiphilus* YIM 80305^T (Li *et al.*, 2005). Chromosomal DNA of these strains was digested by *Hae*III and fragments of 400 bp to 5 kb were collected and labelled as probe. The total chromosomal DNA samples were spotted onto Hybond-N⁺ nylon membrane (Amersham) for hybridization. DNA–DNA hybridization studies were carried out using a DIG-High Prime DNA Labelling and Detection Starter kit II (Roche), as described in the instruction manual.

The G+C content of the DNA was determined to be 71.6 mol%. The almost complete 16S rRNA gene sequence (1493 nt) of strain MCCC 1A01550^T was obtained and was compared with the GenBank/EMBL/DBJ databases using

BLAST. The phylogenetic tree (Fig. 2) shows that strain MCCC 1A01550^T formed a distinct branch with *S. carpaticus* NRRL B-16359^T, *S. cheonanensis* VC-A46^T and *S. sodiiphilus* YIM 80305^T. The novel strain showed 97.5 % gene sequence similarity to *S. carpaticus* NRRL B-16359^T, 96.6 % similarity to *S. cheonanensis* VC-A46^T and 96.1 % similarity to *S. sodiiphilus* YIM 80305^T. DNA–DNA relatedness values below 80 % have been recommended for the recognition of novel genomic species of *Streptomyces* (Labeda, 1992). As determined by DNA hybridization, the DNA–DNA relatedness values between strain MCCC 1A01550^T and *S. carpaticus* NRRL B-16359^T (48.38 %), *S. cheonanensis* VC-A46^T (21.29 %) and *S. sodiiphilus* YIM 80305^T (25 %) were all below 70 %, and thus support the conclusion that strain MCCC 1A01550^T represents a separate genomic species.

The phylogenetic distinctiveness and DNA–DNA relatedness data were sufficient to categorize strain MCCC 1A01550^T as distinct from previously recognized species of the genus *Streptomyces*. This conclusion also was supported by the number of phenotypic differences between the novel strain and phylogenetically related species of the genus *Streptomyces* (Table 2). It is concluded that strain MCCC 1A01550^T represents a novel member of the genus *Streptomyces*, for which the name *Streptomyces xiamenensis* sp. nov. is proposed.

Description of *Streptomyces xiamenensis* sp. nov.

Streptomyces xiamenensis (xia.men.en'sis. N.L. masc. adj. *xiamenensis* of Xiamen, a coastal city where the mangrove sediment was collected from which the type strain was isolated).

Fig. 2. Phylogenetic dendrogram obtained by neighbour-joining analysis based on 1493 bp of 16S rRNA gene sequences, showing the positions of strain MCCC 1A01550^T and related strains. Only bootstrap values above 50 %, expressed as percentages of 1000 replications, are shown at the branch points. Asterisks indicate branches that were also recovered using the minimum-evolution tree-making algorithm. Bar, 0.005 substitutions per nucleotide position.

Table 2. Phenotypic properties that distinguish strain MCCC 1A01550^T from related species of the genus *Streptomyces*

Strains: 1, MCCC 1A01550^T; 2, *S. carpaticus* NRRL B-16359^T; 3, *S. cheonanensis* VC-A46^T; 4, *S. sodiophilus* YIM 80305^T. +, Positive; –, negative; ND, not detected.

Characteristic	1	2	3	4
Colony colour on ISP 5	White	Greyish-brown	Olive	Grey-white
Spore surface*	S	S	S	WS
Spore-chain morphology†	ST-RF	SP	RF	ST-RA
Production of diffusible pigment	Light pink	Dark brown	Olive	–
H ₂ S production	–	–	–	–
Nitrate reduction	+	–	–	+
Melanin pigment	–	–	+	–
Utilization of:				
Arabinose	+	+	+	–
Galactose	–	–	–	ND
Inositol	–	–	+	–
Mannitol	+	+	+	–
Mannose	+	–	+	ND
Raffinose	–	+	+	–
Rhamnose	+	+	+	+
Ribose	+	–	–	ND
Sucrose	+	–	+	–
Xylose	–	+	+	–

*S, smooth; WS, wrinkled surface.

†RF, *rectiflexibiles*; SP, spirals; ST, straight; RA, *retinaculiaperti*.

Aerobic, Gram-positive, non-acid-fast and non-motile organism with branching aerial mycelium and substrate mycelium. At maturity, aerial mycelium forms long, straight to *rectiflexibiles* spore chains. Spores are non-motile and ellipsoid with a smooth surface. Colony colour is medium-dependent and diffusible pigments are produced on most media. Aerial mycelia are grey on ISP 2, ISP 3 and Czapek's agars, but are white on ISP 4, ISP 5 and glucose-asparagine agars. Substrate mycelia are light yellow to brown. Grows well at 28, 30 and 37 °C, but does not grow at 45 or 10 °C. Mannose, arabinose, ribose, rhamnose, mannitol and sucrose are utilized as sole carbon and energy sources, but raffinose, xylose and galactose are not. Tests for gelatin liquefaction, nitrate reduction and milk peptonization are positive. Tests for H₂S and melanin production are negative. Tolerates NaCl concentrations of up to 5% (w/v). Resistant to penicillin G, rifampicin, streptomycin and ampicillin. Cell wall contains LL-diaminopimelic acid with trace amounts of meso-diaminopimelic acid. The predominant menaquinones are MK-9(H₄) and MK-9(H₆) and the diagnostic phospholipid is PE. Major fatty acid components are iso-C_{16:0}, *cis*-9-C_{16:1} and C_{16:0}.

The type strain, MCCC 1A01550^T (=DSM 41903^T=CGMCC 4.3534^T), was isolated from a mangrove sediment sample collected from Fujian Province, south China. The DNA G+C content of the type strain is 71.6 mol%.

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