Streptomyces avicenniae sp. nov., a novel actinomycete isolated from the rhizosphere of the mangrove plant *Avicennia mariana*

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A novel isolate, designated strain MCCC 1A01535^T, belonging to the genus *Streptomyces* was isolated from the rhizosphere of the mangrove plant *Avicennia mariana* from Fujian Province, south China. Characterization of the isolate was based on a polyphasic approach. Pairwise comparison of 16S rRNA gene sequences revealed that strain MCCC 1A01535^T shared 97.7 and 97.5 % sequence similarities to *Streptomyces specialis* GW41-1564^T and *Streptomyces hainanensis* YIM47672^T, respectively, its most closely related neighbours, whereas the DNA–DNA relatedness value between strains MCCC 1A01535^T and GW41-1564^T was 50.2 %. The major fatty acids of strain MCCC 1A01535^T were iso-C_{16:0}, C_{16:0} and anteiso-C_{15:0}. These differed from the fatty acid compositions of related strains. Strain MCCC 1A01535^T exhibited an unusual menaquinone system that comprised MK-10(H₆) as the predominant component and moderate amounts of MK-9(H₆), MK-9(H₈) and MK-10(H₈); minor amounts of MK-9(H₄), MK-10(H₄), MK-9(H₁₀) and MK-10(H₁₀) were also present. Based on its chemotaxonomic, phenotypic and genotypic characteristics, strain MCCC 1A01535^T is considered to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces avicenniae* sp. nov. is proposed. The type strain is MCCC 1A01535^T (=DSM 41943^T=CGMCC 4.5510^T).

Members of the genus *Streptomyces*, proposed by Waksman & Henrici (1943), are a rich source of novel bioactive, commercially significant compounds. Although *Streptomyces* strains were frequently isolated from terrestrial environments, they have also been recovered from aquatic and symbiotic environments. Marine actinomycetes have been recognized as a resource for biotechnology in recent years (Bull *et al.*, 2000). Analysis of the diversity of actinomycetes isolated from Challenger-Deep sediment from the Mariana Trench has proven the presence of *Streptomyces* in the deep-sea environment (Pathom-Aree *et al.*, 2006).

Mangrove forests are highly productive ecosystems that comprise diverse flora and fauna of marine, freshwater and terrestrial species (Jennerjahn & Ittekkot, 2002). The exploitation of the profusion of mangrove micro-organism resources has been expanded in many aspects (Long *et al.*,

2005), including the screening of novel actinomycetes that could be a dormant source of new natural products. A systematic investigation of the actinomycete diversity in the National mangrove reserve in Fujian Province, China was performed, during which strain MCCC 1A01535^T was isolated. Here, we report the results of a polyphasic taxonomic investigation on strain MCCC 1A01535^T and propose that it represents the type strain of a novel species of the genus *Streptomyces* that exhibits an unusual menaquinone system.

Strain MCCC 1A01535^T was isolated after 2 weeks incubation at 28 °C on ISP 2 agar (Shirling & Gottlieb, 1966). Morphological observations of spores and mycelia on Gause's synthetic agar at 28 °C for 12 days were made by using light microscopy (COBER-015; Olympus) and scanning electron microscopy (XL30 ESEM-TMP; Philips-FEI). Cultural characteristics were determined after 3 weeks incubation at 28 °C by using methods in the ISP (Shirling & Gottlieb, 1966). Colours were assessed according to colour chips in the ISCC-NBS Colour Charts Standard no. 2106 (Kelly, 1964). Biomass of strain MCCC 1A01535^T for chemical and molecular analyses was grown in ISP 2 liquid

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MCCC 1A01535^T is EU399234.

Tables showing major quinone and major fatty acid compositions are available as supplementary material with the online version of this paper.

Table 1. Cultural characteristics of strain MCCC 1A01535^T on various media at 28 °C after 7 days

–, Not detected.

Medium	Growth	Soluble pigment	Colony colour	
			Aerial mycelium	Substrate mycelium
Yeast extract agar (ISP 2)	Moderate	Deep-brown	Deep orange-red	Deep orange-red
Oatmeal agar (ISP 3)	Good	–	Yellow-brown	Yellow-brown
Inorganic salts-starch agar (ISP 4)	Good	–	Olive	Grey
Glycerol-asparagine agar (ISP 5)	Moderate	–	Olive	Grey
Czapek's agar	Poor	–	Light yellow	White
Starch agar	Good	–	Light yellow	Light yellow
Glucose-asparagine agar	Good	–	Yellow-brown	Yellow-brown

broth for 3–5 days at 28 °C in a rotary shaker (180 r.p.m.) and harvested by centrifugation.

The isolate grew well on ISP 3 and 4 agar media with moderate growth occurring on other media. The colours of the aerial and substrate mycelium were media-dependent (Table 1). A deep-brown soluble pigment was produced on ISP 2 media. At maturity, the aerial mycelium developed well on Gause's synthetic agar. The spore chains were compact spiral and contained about eight helixes per chain. The spores were elliptical and non-motile with smooth surfaces (Fig. 1).

The temperature range for growth was determined on modified ISP 2 agar at 4, 10, 15, 20, 28, 37 and 45 °C for 1 to 2 weeks. Carbohydrate utilization was carried out according to the method of Kämpfer *et al.* (1991). Gelatin liquefaction, starch hydrolysis, milk coagulation and peptonization, melanin pigment production, nitrate reduction and H₂S production were tested using the methods of Goodfellow (1971) and Gordon *et al.* (1974). Resistance to various antibiotics was determined with antimicrobial susceptibility discs (Oxoid). Phenotypic characteristics that differentiate strain MCCC 1A01535^T from related *Streptomyces* species are given in Table 2.

Fig. 1. Scanning electron micrograph of spore chains of strain MCCC 1A01535^T grown on Gause's synthetic agar for 10 days at 28 °C. Bar, 5 μm.

Analyses of the diagnostic isomer of the diaminopimelic acid (A₂pm) and whole-cell sugars were done using freeze-dried cells that were hydrolysed with 6 and 0.5 M HCl at 120 °C for 30 min (Lechevalier & Lechevalier, 1980), and analysed by TLC (Hasegawa *et al.*, 1983). Phospholipid analysis was carried out as described by Komagata & Suzuki (1987). The cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI, Inc.) and Sherlock MIS Software.

Amino acids in the peptidoglycan layer of strain MCCC 1A01535^T were LL-A₂pm, alanine, glycine and glutamic acid. Whole-cell hydrolysates contained ribose, xylose, arabinose, galactose and glucose. Diphosphatidylglycerol

Table 2. Phenotypic characteristics that differentiate strain MCCC 1A01535^T from related *Streptomyces* species

Strains: 1, MCCC 1A01535^T (*S. avicenniae* sp. nov.); 2, *S. specialis* GW41-1564^T; 3, *S. hainanensis* YIM 47672^T. Data for *S. specialis* and *S. hainanensis* were from Kämpfer *et al.* (2008) and Jiang *et al.* (2007). All strains do not utilize D-mannose. +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3
Production of diffusible pigment			
On ISP 3 and ISP 4	–	+	+
On ISP 5	–	+	–
H ₂ S production	–	–	+
Nitrate reduction	–	–	+
Gelatin liquefaction	+	+	–
Milk coagulation	+	+	–
Milk peptonization	+	+	–
Utilization of:			
D-Xylose	+	–	ND
D-Galactose	+	–	–
Inositol	–	+	–
L-Arabinose	+	–	–
D-Ribose	–	+	–
L-Rhamnose	+	–	–
Sucrose	+	+	–

was the predominant polar lipid. Phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and an unknown phospholipid were present in minor amounts. The major cellular fatty acids were iso-C_{16:0} (32.0 %), C_{16:0} (15.1 %) and anteiso-C_{15:0} (10.7 %).

Menaquinones were extracted by using the method of Collins *et al.* (1977) and analysed by HPLC (Kroppenstedt, 1985). The quinone system of strain MCCC 1A01535^T consisted of the predominant menaquinone MK-10(H₆) (29 %) and moderate amounts of MK-9(H₆) (16.8 %), MK-9(H₈) (16.3 %) and MK-10(H₈) (14.8 %). Minor amounts (<10 %) of MK-9(H₄) (6.7 %), MK-10(H₄) (9.4 %), MK-9(H₁₀) (3.0 %) and MK-10(H₁₀) (4.0 %) were also present.

Genomic DNA was extracted as described by Weisburg *et al.* (1991). DNA was digested and dephosphorylated prior to the determination of the base composition by using HPLC (Tamaoka & Komagata, 1984). The G+C content of the DNA was calculated according to Mesbah *et al.* (1989). The G+C content of the DNA strain MCCC 1A01535^T was determined to be 73.3 mol%.

Amplification of the 16S rRNA gene sequence was performed as described by Cui *et al.* (2001). An almost-complete 16S rRNA gene sequence (1491 nt) of strain MCCC 1A01535^T was obtained and compared against the GenBank and EzTaxon databases using BLAST (Altschul *et al.*, 1997; Chun *et al.*, 2007). The 16S rRNA gene sequences of representative related actinomycetes were aligned using CLUSTAL_X (Thompson *et al.*, 1997) and a phylogenetic tree was constructed by using maximum-parsimony and neighbour-joining with MEGA 3.1 software (Kumar *et al.*, 2004; Saitou & Nei, 1987). The topology of the tree was evaluated by using a bootstrap analysis (Felsenstein, 1985) with 1000 replications. Strain MCCC 1A01535^T formed a distinct branch with *Streptomyces specialis* GW41-1564^T in the phylogenetic tree (Fig. 2). The highest 16S rRNA sequence similarities between the isolate and type strains of recognized species in the databases were 97.7 % to *S. specialis* GW41-1564^T (Kämpfer *et al.*, 2008) and 97.5 % to *Streptomyces hainanensis* YIM 47672^T (Jiang *et al.*, 2007).

DNA–DNA relatedness studies were carried out between strain MCCC 1A01535^T and the closely related strain GW41-1564^T using DIG-High prime DNA labelling and detection starter kit II (Roche), as described in the instruction manual. Chromosomal DNA of the strains was digested by *Hae*III. Fragments of 400 bp to 5 kb were collected and labelled as a probe. The total chromosomal DNA was spotted onto Hybond-N+ nylon membrane (Amersham) for hybridization. DNA–DNA relatedness values were 50.2 %, below the recommended criterion of 80 % for species delineation of the genus *Streptomyces* (Labeda, 1992).

According to Kim *et al.* (2003), the major menaquinones of the genus *Streptomyces* are MK-9(H₆) and MK-9(H₈). It was interesting that strains GW41-1564^T and YIM 47672^T both contained unusual quinone systems, but strain MCCC 1A01535^T exhibited a distinct quinone system that was different from both of them (see Supplementary Table S1, available in IJSEM Online). The detailed profile of the cellular fatty acids given in Supplementary Table S2 (in IJSEM Online) and the sugar pattern gave additional support for the separation of strain MCCC 1A01535^T from the related strains GW41-1564^T and YIM 47672^T.

Strain MCCC 1A01535^T showed a notable difference in the production of diffusible pigment from its closest relatives. The three strains could be discriminated based on the predominant menaquinones. Based on morphology, chemotaxonomy and 16S rRNA phylogeny, it can be concluded that strain MCCC 1A01535^T represents a novel species of the genus *Streptomyces*, for which we propose the name *Streptomyces avicenniae* sp. nov.

Description of *Streptomyces avicenniae* sp. nov.

Streptomyces avicenniae (a.vi.cen'ni.ae. N.L. fem. n. *Avicennia* scientific name of genus of tree; N.L. fem. gen. n. *avicenniae* of *Avicennia*, isolated from the rhizosphere of the plant *Avicennia mariana*).

Aerobic, Gram-positive actinomycete with branching aerial mycelium and substrate mycelium. Non-motile and ellipsoid spores with smooth surfaces are borne in compact spiral spore chains with up to eight helixes per chain.

Fig. 2. Phylogenetic dendrogram obtained by using neighbour-joining analysis based on 16S rRNA gene sequences (1458 bp, by omitting unaligned regions), showing the positions of strain MCCC 1A01535^T and related strains. The sequence of the type strain of *Micrococcus psychrophilum* was used as an outgroup. Only bootstrap values above 50 %, expressed as percentages of 1000 replications, are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

Colony colour is medium-dependent and diffusible pigments are produced on ISP 2 media. Aerial mycelia are light-yellow on Czapek's agar and Starch agar, yellow-brown on glucose-asparagine agar and ISP 3, olive on ISP 4 and ISP 5, and deep orange-red on ISP 2. Substrate mycelia are white, grey to light-yellow and deep orange-red on different media. Growth occurs at temperatures ranging from 15 to 37 °C, with optimum growth at 28 °C. Raffinose, D-xylose, D-galactose, L-arabinose, L-rhamnose, mannitol and sucrose are utilized, but D-mannose, inositol and D-ribose are not. Tests for gelatin liquefaction, milk coagulation and peptonization are positive and tests for nitrate reduction, H₂S production and melanin production are negative. Cell wall contains LL-diaminopimelic acid. Whole-cell hydrolysates contain ribose, xylose, arabinose, galactose and glucose. Resistant to ciprofloxacin (5 µg), clindamycin (2 µg), furazolidone (15 µg), metronidazole (5 µg), lincomycin (2 µg) and norfloxacin (10 µg). Sensitive to ampicillin (10 µg), chloramphenicol (30 µg), rocephin (30 µg), erythromycin (15 µg), kanamycin (30 µg), cefazolin (30 µg), neomycin (10 µg), penicillin G (10 µg), rifampicin (5 µg) and vancomycin (30 µg). Major menaquinone is MK-10(H₆) with moderate amounts of MK-9(H₆), MK-9(H₈), MK-10(H₈), MK-9(H₄), MK-10(H₄), MK-9(H₁₀) and MK-10(H₁₀). Diagnostic phospholipids are diphosphatidylglycerol with minor amounts of phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol and an unknown phospholipid. Major fatty acid components are iso-C_{16:0}, C_{16:0} and anteiso-C_{15:0}. The G+C content of the DNA of the type strain is 73.3 mol%.

The type strain, MCCC 1A01535^T (=DSM 41943^T=CGMCC 4.5510^T), was isolated from the rhizosphere of the mangrove plant *Avicennia mariana* from Fujian Province, south China.

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