Isoptericola chiayiensis sp. nov., isolated from mangrove soil

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A novel actinomycete, designated strain 06182M-1^T, was isolated from a mangrove soil sample collected from Chiayi County in Taiwan. Phylogenetic analysis based on 16S rRNA gene sequences revealed levels of similarity of 97.0–98.8% to the type strains of recognized species of the genus *Isoptericola*. Chemotaxonomic data also supported the placement of strain 06182M-1^T within the genus *Isoptericola*. However, the low levels of DNA–DNA relatedness between the novel strain and the type strains of recognized species of the genus *Isoptericola*, in combination with differential phenotypic data, demonstrate that strain 06182M-1^T represents a novel species of the genus *Isoptericola*, for which the name *Isoptericola chiayiensis* sp. nov. is proposed. The type strain is 06182M-1^T (=BCRC 16888^T =KCTC 19740^T).

The genus *Isoptericola* was proposed by Stackebrandt *et al.* (2004) with the reclassification of *Cellulosimicrobium variabile* (Bakalidou *et al.*, 2002). At the time of writing, the genus *Isoptericola* comprises five recognized species (Stackebrandt *et al.*, 2004; Groth *et al.*, 2005; Zhang *et al.*, 2005; Wu *et al.*, 2010). Members of the genus are aerobic to microaerophilic organisms with a well-developed primary mycelium that fragments into short, irregular, non-motile rods and cocci. They are Gram-positive and non-acid-alcohol-fast. The peptidoglycan type is A4 α , containing L-Lys–L-Ala–L-Glu–D-Asp. Whole-cell sugars comprise glucose, rhamnose, galactose and mannose. The acyl type is acetyl. The predominant menaquinone is MK-9(H₄), and major fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, C_{16:0}, iso-C_{16:0} and anteiso-C_{17:0}. The phospholipids comprise diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylinositol (PI), two glycolipids and two unknown phospholipids. Mycolic acids are absent. The G+C content of the DNA is 70–72 mol%.

Strain 06182M-1^T was isolated from a mangrove soil sample collected in Chiayi County, Taiwan, by using HV agar (Hayakawa & Nonomura, 1987) with incubation at 30 °C for 28 days. The strain was maintained on trypticase soy agar (TSA; BBL) and as a suspension of cells in glycerol (20%, v/v) stored at –20 °C.

Morphological characteristics of strain 06182M-1^T were observed by scanning electron microscopy (S-4700; Hitachi), following incubation on TSA plates for 3 days at 30 °C, fixation with 4% osmium tetroxide solution,

dehydration in an ethanol series followed by acetone, and critical-point drying (Itoh *et al.*, 1989). All physiological tests were performed at 30 °C. Growth temperature, hydrolysis of adenine, aesculin, casein, hypoxanthine, L-tyrosine and xanthine, and production of amylase, nitrate reductase, urease and melanin were assessed according to the methods of Gordon *et al.* (1974). Sole carbon source utilization was tested by using the method of Shirling & Gottlieb (1966).

Biomass for chemotaxonomic studies was prepared following growth in shake flasks (125 r.p.m.) of trypticase soy broth (TSB; BBL) at 30 °C for 3 days. The isomer of diaminopimelic acid and sugars in whole-cell hydrolysates were determined according to Hasegawa *et al.* (1983). The presence of mycolic acids was examined by TLC following Minnikin *et al.* (1975), and phospholipids were extracted and identified following the method of Minnikin *et al.* (1984). Menaquinones were extracted and purified according to the method of Collins *et al.* (1977) and were then analysed by HPLC (model 600; Waters) with a Nova-Pak C18 column. For quantitative analysis of the cellular fatty acid content, strain 06182M-1^T was cultured in TSB at 30 °C on a shaking incubator for 3 days at 125 r.p.m. Extracts of the methylated fatty acids were prepared according to the protocol provided by the manufacturer.

For extraction of DNA for sequencing of the 16S rRNA gene, strain 06182M-1^T was grown in TSB at 30 °C for 3 days. Cells were removed from the broth by using a pipette tip and total DNA was extracted by using a Qiagen genomic DNA kit. The G+C content of the DNA was determined by the HPLC method of Tamaoka & Komagata (1984). DNA was prepared by using the same method as above. The 16S rRNA gene was PCR-amplified by using the methods of Nakajima *et al.* (1999) and was directly

Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 06182M-1^T is FJ469988.

Fig. 1. Morphological characteristics of strain 06182M-1^T grown on TSA for 3 days. Bar, 2 μ m.

sequenced on an ABI model 3730 automatic DNA sequencer by using a BigDye Terminator V3.1 kit (Applied Biosystems). Phylogenetic analysis was performed by using the software packages PHYLIP (Felsenstein, 1993) and MEGA version 4.0 (Tamura, *et al.*, 2007) after multiple alignments of the data by using CLUSTAL X (Thompson *et al.*, 1997). Calculation of evolutionary distances (distance

options according to the Kimura two-parameter model; Kimura, 1980, 1983) and clustering were conducted by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein, 1985). DNA–DNA hybridization was carried out according to the method of Ezaki *et al.* (1989).

Growth of strain 06182M-1^T was aerobic and cells stained Gram-positive. Strain 06182M-1^T produced substrate mycelia but no aerial mycelia. The substrate mycelia fragmented into short, irregular, non-motile rods and cocci (diameter 0.2 μ m) (Fig. 1). The colour of colonies on various media was light orange–yellow. No soluble pigment was produced in any of the media tested. The results of physiological and biochemical tests are given in the species description below and in Table 1.

Strain 06182M-1^T had peptidoglycan type A4 α , comprising L-Lys–L-Ala–L-Glu–D-Asp, and glucose, galactose and rhamnose in whole-cell hydrolysates. The predominant menaquinones were MK-9(H₄) and MK-9(H₂); mycolic acids were not detected. DPG, PG, PI, two glycolipids and two unknown phosphoglycolipids were detected. The major fatty acid methyl esters were iso-C_{16:0} (14.82%), C_{16:0} (14.63%), C_{17:0} (13.79%) and 10-methyl C_{17:0}

Table 1. Differential phenotypic characteristics between strain 06182M-1^T and the type strains of recognized species of the genus *Isoptericola*

Strains: 1, 06182M-1^T; 2, *I. variabilis* BCRC 16877^T; 3, *I. halotolerans* BCRC 16878^T; 4, *I. hypogaeus* BCRC 16879^T; 5, *I. dokdonensis* BCRC 16880^T; 6, *I. jiangsuensis* BCRC 16913^T. +/–, Variable reaction. Data from this study.

Characteristic	1	2	3	4	5	6
Growth temperature range (°C)	15–40	15–40	15–35	15–35	15–35	15–35
NaCl tolerance (%)	12	8	6	6	2	6
Hydrolysis of:						
Adenine	+	+	+	+	–	–
Casein	–	+	–	+	–	–
Aesculin	+	+	+	+	+	+/–
Hippurate	+	+	+	+	–	–
Hypoxanthine	–	+	–	+	–	–
Milk	+	+	+	–	+	–
Tyrosine	–	+	–	+/–	–	–
Xanthine	–	+	–	+	–	–
Nitrate reductase	–	+	–	+	+	+
Carbon source utilization						
Arabinose	–	+	+	+	–	–
Cellobiose	+	+	+	+	+	–
Fructose	+	+	+	+	+	–
Glucose	+	+	+	+	+	–
Inositol	–	–	–	+	–	–
Mannitol	+	–	+	+	–	–
Raffinose	–	+	+	–	–	–
Rhamnose	+	–	–	+	+	–
Sucrose	+	–	+	+	+	–
Xylose	+	–	+	+	+	–

Table 2. Fatty acid profiles of strain 06182M-1^T and the type strains of recognized *Isoptericola* species

Strains: 1, 06182M-1^T; 2, *I. variabilis* BCRC 16877^T; 3, *I. halotolerans* BCRC 16878^T; 4, *I. hypogaeus* BCRC 16879^T; 5, *I. dokdonensis* BCRC 16880^T; 6, *I. jiangsuensis* BCRC 16913^T. Values are percentages of the total fatty acids. Data from this study.

Fatty acid	1	2	3	4	5	6
C _{14:0}	1.75	2.18	1.28	2.97	3.09	2.92
C _{16:0}	9.72	3.39	17.63	3.70	8.97	8.11
C _{17:0}	1.48	0.16	0.97	0.17	1.16	1.94
iso-C _{13:0}					1.29	3.04
iso-C _{14:0}	3.01	1.93	0.50	1.17	2.54	3.55
iso-C _{15:0}	12.02	20.11	4.46	15.65	11.28	10.75
iso-C _{16:0}	5.02	10.16	1.93	3.45	1.93	2.39
iso-C _{17:0}	1.37	1.24	0.92	0.65	0.63	0.96
anteiso-C _{15:0}	55.04	50.70	53.62	61.77	61.98	56.49
anteiso-C _{17:0}	8.77	10.02	18.01	10.11	5.61	7.45

(23.77 %) (Table 2). The G + C content of the DNA of strain 06182M-1^T was 72.8 mol%.

The almost-complete 16S rRNA gene sequence (1514 nt) of strain 06182M-1^T was determined. Preliminary comparison of the 16S rRNA gene sequence of strain 06182M-1^T against the GenBank database revealed high levels of similarity with the type strains of recognized species of the genus *Isoptericola*: *Isoptericola dokdonensis* BCRC 16880^T (97.9 %), *Isoptericola halotolerans* BCRC 16878^T (98.8 %), *Isoptericola hypogaeus* BCRC 16879^T (97.0 %), *Isoptericola jiangsuensis* BCRC 16913^T (98.0 %) and *Isoptericola variabilis* BCRC 16877^T (97.9 %). The neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain 06182M-1^T and the type strains of recognized species of the genus *Isoptericola* and other related species is shown in Fig. 2. DNA–DNA hybridization experiments revealed that levels of DNA–DNA relatedness between strain 06182M-1^T and the type strains of recognized species of the genus *Isoptericola* were relatively low: *I. dokdonensis* BCRC 16880^T (16.7 %), *I. halotolerans* BCRC 16878^T (16.8 %), *I. hypogaeus* BCRC 16879^T (8.8 %), *I. jiangsuensis* BCRC 16913^T (15.4 %) and *I. variabilis* BCRC 16877^T

(5.3 %). These values are below the 70 % cut-off point recommended for the assignment of organisms to the same species (Wayne *et al.*, 1987). The phylogenetic data thus indicate that strain 06182M-1^T represents a novel species, which is confirmed by comparison of phenotypic characteristics with those of the type strains of recognized species of the genus *Isoptericola* (Table 1). Therefore, on the basis of the phenotypic and genotypic data presented, strain 06182M-1^T is considered to represent a novel species of the genus *Isoptericola*, for which the name *Isoptericola chiayiensis* sp. nov. is proposed.

Description of *Isoptericola chiayiensis* sp. nov.

Isoptericola chiayiensis (chia.yi.en'sis. N.L. masc. adj. *chiayiensis* of Chiayi County, from where the type strain was isolated).

Cells are Gram-positive and aerobic. Produces substrate mycelia but no aerial mycelia. Substrate mycelia fragment into short, irregular, non-motile rods and cocci (diameter 0.2 µm). The colour of colonies on various media is light orange–yellow; no soluble pigment is produced. Growth occurs between 15 and 40 °C (optimal growth at 30 °C). Tolerates up to 12 % (w/v) NaCl in the culture medium. Positive for adenine, aesculin, hippurate and starch hydrolysis and milk peptonization, but negative for casein, chitin, hypoxanthine, tyrosine and xanthine hydrolysis, gelatin liquefaction, and urease, melanin and nitrate reductase production. Utilizes cellobiose, fructose, glucose, mannitol, rhamnose, sucrose and xylose, but not arabinose, inositol or raffinose. The peptidoglycan type is A4α, with L-Lys–L-Ala–L-Glu–D-Asp, and glucose, galactose and rhamnose in whole-cell hydrolysates. The predominant menaquinones are MK-9(H₄) and MK-9(H₂); mycolic acids are not detected. DPG, PG, PI, two glycolipids and two unknown phosphoglycolipids are detected. The major fatty acid methyl esters are iso-C_{16:0}, C_{16:0}, C_{17:0} and 10-methyl C_{17:0}. The G + C content of the DNA of the type strain is 72.8 mol%.

The type strain, 06182M-1^T (=BCRC 16888^T =KCTC 19740^T), was isolated from mangrove soil collected from Chiayi County, Taiwan.

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain 06182M-1^T, the type strains of recognized species of the genus *Isoptericola* and some other related taxa. Numbers at nodes indicate percentages of 1000 bootstrap resamplings; only values >50 % are given. *Jonesia denitrificans* DSM 20603^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

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