Sphingobacterium bambusae sp. nov., Isolated from Soil of Bamboo Plantation

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A Gram-negative, non-motile, non-spore-forming bacterial strain designated IBFC2009^T was isolated from soil of a bamboo plantation. The strain could grow at 11°C~39°C, pH 6.0~9.0, and in the presence of 0~5% NaCl. Based on 16S rRNA gene sequence analysis, Strain IBFC2009^T belonged to the genus *Sphingobacterium* and showed the highest sequence similarity of 94.6% (*S. composti* T5-12^T) with the type strains within the genus. The major fatty acids were summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c, 34.4%), iso-C_{15:0} (22.4%), C_{16:0} 3-OH (15.2%), and iso-C_{17:0} 3-OH (12.8%). The G+C content of the genomic DNA was 41.0 mol%. According to the phenotypic and genotypic characteristics, Strain IBFC2009^T should represent a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium bambusae* sp. nov. is proposed. The type strain is IBFC2009^T (=CCTCC AB 209162^T =KCTC 22814^T).

Keywords: Sphingobacterium bambusae sp. nov., novel bacterium, taxonomy

The genus Sphingobacterium was created by Yabuuchi et al. (1983), which includes Sphingobacterium multivorum, S. spiritivorum, and S. mizutae. To date, nine other species have been described: S. antarcticum (Shivaji et al., 1992), S. faecium and S. thalpophilum (Takeuchi and Yokota, 1992), S. daejeonense (Kim et al., 2006), S. composti (Ten et al., 2006), S. composti (Yoo et al., 2007), S. siyangense (Liu et al., 2008), S. kitahiroshimense (Matsuyama et al., 2008), and S. anhuiense (Wei et al., 2008). There were two different S. composti with validly published names. S. composti T5-12^T was described by Ten et al. (2006) one year before the S. composti $4M24^{T}$ of Yoo *et al.* (2007) The name *S. composti* $4M24^{T}$ was then considered illegitimate and thus changed. Two other previously described species, S. heparinum and S. piscium, were reclassified in the genus Pedobacter (Steyn et al., 1998). Sphingobacterium species have been isolated from soil (Shivaji et al., 1992; Matsuyama et al., 2008), clinical specimens (Holmes et al., 1982; Yabuuchi et al., 1983), and compost (Ten et al., 2006; Yoo et al., 2007).

In this study, we isolated Strain IBFC2009^T from the soil of a bamboo plantation. Its phenotypic and chemotaxonomic characteristics were examined and a phylogenetic analysis was carried out. The results suggest that Strain IBFC2009^T represents a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium bambusae* sp. nov. is proposed.

Materials and Methods

Isolation and culture of the bacterial strain Strain IBFC2009^T was originally isolated from the soil of a bamboo plantation, which was collected from Hangzhou, China. The soil sample (5 g) was thoroughly shaken (250 rpm, 30°C) in 200 ml sterile water for 10 min. The suspension, following serial dilution, was spread onto Nutrient Agar (NA, Sinopharm Chemical Reagent Co., Ltd, China) plates (g/L): 10.0 peptone, 3.0 beef extract, 5.0 NaCl, and 20 agar. The plates were incubated at 31°C for 2 d. Single colonies on the plates were purified by transferring them onto fresh plates and subsequent reincubation. Strain IBFC 2009^{T} was one of the isolates that appeared on the NA plates in aerobic conditions. The strain was cultured routinely on nutrient broth medium (NB) at 31°C and maintained as a glycerol suspension (20%, w/v) at -70°C.

Phenotypic and biochemical characteristics

IBFC2009^T was grown for 2 d on NA plates at 31°C, and cell morphology was observed under Olympus light microscope (Olympus Corporation, Japan) at a magnification of \times 1,000, an atomic force microscope Nano-Scope IIIa (SPA 400, Seiko Inc & Multimode SPM, Digital Instruments, Co. Ltd) at \times 20,000 and an electron microscope JEOL-1230 (JEOL Ltd., Japan) at \times 60,000. Growth was assessed at 4, 10, 11, 16, 30, 31, 32, 33, 34, 37, 39, 40, 42, and 45°C, at pH 4, 5, 6, 7, 8, 9, and 10 in the presence of 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 9, and 11% NaCl. Bacterial concentration was measured at 550 nm using a spectrophotometer (UV-755B; Shanghai Precision & Scientific Instrument Co., Ltd., China).

For single-carbon-source assimilation study, a defined liquid medium containing basal salts was used (g/L): $0.5 \text{ K}_2\text{HPO}_4$, $0.5 \text{ NaH}_2\text{PO}_4$, $2.0 (\text{NH}_4)_2\text{SO}_4$, 0.2 MgSO_4 , and 0.1 CaCl_2 . Filter-sterilized carbohydrate, alcohol, amino acid, or organic acids were added to the medium, and final concentration was adjusted to 0.5%. After one to three serial culture transferring sessions to the fresh medium, the strain, which still grew in the medium, was identified as positive character.

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For the studies of acid production from carbohydrates and oxidative fermentation from glucose, a defined liquid medium containing basal salts was used (g/L): 2.0 peptone, 5.0 NaCl, 0.2 K₂HPO₄, 10.0 glucose, 6.0 agar, and 0.03 bromothymol blue, at pH 7.0~7.2. After stab culture for about 2 d, the strain that caused the medium to turn yellow was identified as positive character (Dong and Cai, 2001).

Oxidase activity was tested by determining the oxidation of 1% (w/v) tetramethyl-p-phenylenediamine, and catalase activity was evaluated by determining the production of oxygen bubbles in a 5% (v/v) aqueous hydrogen peroxide solution (Dong and Cai, 2001; Cappuccino and Sherman, 2002). Nitrate reduction, indole, H₂S production, methyl red, and Voges-Proskauer reaction, were investigated as described by Dong and Cai (2001). Gram staining, endospore-forming features, hydrolysis of CM-cellulose, DNA, gelatin, starch, Tween 80, urease, and aesculin were tested as described by Gerhardt *et al.* (1994).

16S rRNA gene sequencing and phylogenetic analysis For the extraction of genomic DNA from IBFC2009^T, 5 ml of exponential phase cultures were centrifuged for 2 min at 10,000 rpm. The cell pellet was resuspended in 200 μ l 100 mM (pH 7.4) Tris-EDTA (TE) buffer containing 400 μ g/ml lysozyme and incubated at 55°C for 5 min. The genomic DNA was then extracted using UNIQ-10 Column Bacteria Genomic DNA Extraction kit (Shanghai Sangon, China) according to the manufacturer's instruction.

The 50 μ l reaction mixture for PCR of 16S rRNA gene contained 0.5 μ M each of the forward and reverse primers, 1× PCR buffer, 50 ng of DNA, and other ingredients in TaKaRa 16S rDNA Bacterial Identification PCR kit (Dalian TaKaRa, China). The cycling conditions were as follows: 5 min at 95°C for pre-denaturation, followed by 0.5 min at 94°C for denaturation, 1 min at 52°C for annealing, 1 min at 72°C for extension for 30 cycles, and, finally, preservation at 72°C for 10 min. Purification and sequencing were carried out by Dalian TaKaRa Co., Ltd. using the dideoxy chain termination method with 3730 XL DNA sequencer (Applied Biosystems, USA).

For phylogenetic analyses, the 16S rRNA gene sequences of the type strains of *Sphingobacterium* species (and some *Pedobacter* species, serving as an out-group) were used. The 16S rRNA gene sequences were aligned using the CLUSTAL X program (Thompson *et al.*, 1997). A phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987) and the maximum-parsimony method (Fitch, 1972) in the MEGA3 program (Kumar *et al.*, 2004), with bootstrap values based on 1,000 replications (Felsenstein, 1985).

Analysis of cellular fatty acids and determination of G+C content

Cellular fatty acids were analyzed in organisms grown on NA at 31°C for 48 h. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI, 1999). The cellular fatty acids were analyzed by gas chromatography (Hewlett-Packard 6890) with the Microbial Identification software package (Sasser, 1990), workstation (HP ChemstaJ. Microbiol.



Fig. 1. Transmission electron micrograph of cell of Strain IBFC 2009^{T} after growth on NA for 2 d at 31°C. Bar, 0.5 µm. Magnification, ×60,000.

tion ver. A 5.01), and chromatographic column Ultra-2 (Agilent).

The genomic DNA was extracted and purified; the DNA G+C content was determined by a spectrophotometer with a thermal controller (DU800; Beckman Coulter, Inc., USA) according to absorbance during the thermal denaturalization of genomic DNA (Marmur and Doty, 1962; Dong and Cai, 2001).

Results

Phenotypic and biochemical characteristics

Microscopically, the cells of $IBFC2009^{T}$ appeared to be short rods, 1.2~2.5 µm in length and 0.5~0.8 µm in diameter (Fig. 1 and 2). The cells were Gram-negative and did not produce spores during incubation. After 2 d incubation at 31°C on NA, colonies were 1.0 to 2.0 mm in diameter, circular in shape, smooth, convex, glossy, and yellow in color. They were able to grow at a range of 11°C to 39°C. The



Fig. 2. Transmission atomic force micrograph of cell of Strain $IBFC2009^{T}$ after growth on NA for 2 d at 31°C. Bar, 1.0 µm. Magnification, ×20,000.

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Table 1. Differential phenotypic characteristics of Strain IBFC2009^T and other type strains of *Sphingobacterium* species Strains: 1, IBFC2009^T; 2, *S. composti* 4M24^T; 3, *S. composti* T5-12^T; 4, *S. kitahiroshimense* $10C^{T}$ (Matsuyama *et al.*, 2008); 5, *S. siyangense* SY1^T (Liu *et al.*, 2008); 6, *S. daejeonense* TR6-04^T (Kim *et al.*, 2006); 7, *S. spiritivorum* NBRC 14948^T; 8, *S. multivorum* NBRC 14947^T; 9, *S. mizutaii* ATCC 33299^T; 10, *S. thalpophilum* NBRC 14963^T; 11, *S. faecium* NBRC 15299^T [data in Columns 7~11 are from Takeuchi and Yokota (1992) and Steyn *et al.* (1998)]; 12, *S. antarcticum* MTCC 675^T (Shivaji *et al.*, 1992); 13, *S. anhuiense* CW 186^T (Wei *et al.*, 2008). +, Positive; -, negative; V, variable; ND, no data available.

Characteristic	1^{a}	2^{a}	3 ^a	4	5	6	7	8	9	10	11	12	13
Growth at:													
5°C	-	-	-	+	+	-	-	-	-	-	+	+	+
42°C	-	+	+	-	+	+	-	-	-	+	-	-	-
Hydrolysis of:													
DNA	-	-	+	-	+	-	+	+	+	-	+	ND	+
Starch	-	-	-	ND	+	-	+	+	+	+	+	-	+
Aesculin	+	+	-	+	-	-	+	+	+	+	+	+	+
Tween 80	+	+	+	+	-	ND	+	V	+	-	V	+	-
Gelatin	-	-	-	-	-	-	-	-	-	-	-	+	-
Urea	-	-	-	+	+	-	+	+	+	+	+	+	-
Assimilation of:													
D-Mannitol	-	-	-	-	+	-	+	-	-	-	-	+	-
D-Melibiose	+	+	-	+	+	+	+	+	+	+	+	-	ND
D-Raffinose	-	-	-	ND	+	+	+	+	+	+	+	+	+
D-Ribose	-	-	-	ND	+	-	-	-	-	-	-	+	-
D-Xylose	-	-	-	ND	+	+	+	+	+	+	-	+	-
Glycerol	-	-	-	-	+	-	V	-	-	+	+	+	-
Inositol	-	-	-	ND	-	-	-	-	-	-	-	+	-
Inulin	+	+	-	ND	+	-	V	V	-	+	+	+	+
L-Arabinose	-	+	-	-	+	-	+	+	-	+	+	+	+
L-Rhamnose	-	-	-	-	+	-	-	+	v	+	+	+	-
L-Sorbose	-	-	-	ND	+	-	-	-	-	-	-	ND	ND
Acid production from:													
D-Glucose	+	-	+	ND	-	+	+	+	+	+	+	+	+
D-Mannitol	-	-	-	ND	-	-	+	-	-	-	-	-	-
D-Sucrose	+	+	+	+	-	-	+	+	+	+	+	-	ND
D-Trehalose	+	+	+	+	+	-	-	-	-	-	-	ND	ND
L-Arabinose	+	+	-	+	-	-	-	+	+	+	+	-	+
L-Rhamnose	+	-	-	-	+	-	-	V	-	+	-	-	-
DNA G+C content (mol%)	41.0	45.0	38.9	36.9	38.5	38.7	39.0	39.9- 40.5	39.3- 40.0	44.0- 44.2	37.3	39.3	36.3

^a Data from this study utilize the same conditions as described in this paper.

pH growth range was between pH 6.0 and pH 9.0, and the NaCl growth range was between 0 and 5% (w/v). The optimum temperature, pH, and NaCl concentration were 31°C, pH 7.0, and 0.5~1.0% NaCl (w/v), respectively. The phenotypic and chemotaxonomic characteristics that differentiated Strain IBFC2009^T from previously described *Sphingobacterium* species are listed in Table 1.

16S rRNA gene sequence and phylogenetic analysis

Almost complete 16S rRNA gene sequence of Strain IBFC2009^T was obtained, and the GenBank accession no. for the 16S rRNA gene sequence of Strain IBFC2009^T was GQ339910 (1,453 bp). Preliminary sequence comparisons with 16S rRNA gene sequences deposited in the GenBank database indicated that Strain IBFC2009^T belonged to the genus *Sphingobacterium*. The strain showed the highest sequence similarities with the type strains of *S. composti* T5-12^T and 4M24^T (94.6% and 94.4%, respectively). Accor-

ding to the phylogenetic tree (Fig. 3), Strain IBFC2009^T formed a compact cluster with *S. composti* T5-12^T, *S. composti* 4M24^T, *S. daejeonense* TR6-04^T, and *S. mizutaii* ATCC 33299^T.

Cellular fatty acids and G+C content

The major fatty acids were summed feature 3 (iso- $C_{15:0}$ 2-OH and/or $C_{16:1} \omega 7c$, 34.4%), iso- $C_{15:0}$ (22.4%), $C_{16:0}$ 3-OH (15.2%), and iso- $C_{17:0}$ 3-OH (12.8%), including typical features for members of the genus *Sphingobacterium* (Steyn *et al.*, 1998; Takeuchi and Yokota, 1992). In contrast to other *Sphingobacterium* species, Strain IBFC2009^T contained a unique $C_{14:0}$ 2-OH (3.2%) content, had a larger of $C_{16:0}$ 3-OH (15.2%) and iso- $C_{15:0}$ 3-OH (5.8%) content, but had a smaller of $C_{16:0}$ (1.8%) content. The cellular fatty acid profiles of Strain IBFC2009^T and *Sphingobacterium* species are presented in Table 2. The DNA G+C content for the genus *Sphingobacterium* ranged from 36.0 mol% (Ten *et al.*, 2006)

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Fig. 3. Phylogenetic tree generated using the neighbor-joining method based on the 16S rRNA gene sequence of Strain $IBFC2009^{T}$. Numbers at nodes represent bootstrap percentages based on 1,000 samplings. Bar, 0.01 changes per nucleotide position. The maximum-parsimony tree showed essentially the same topology (data not shown).

Table 2. Cellular fatty acid of Strain IBFC2009^T and Sphingobacterium species

Strains: 1, IBFC2009^T; 2, *S. composti* 4M24^T; 3, *S. composti* T5-12^T; 4, *S. kitahiroshimense* 10C^T (Matsuyama *et al.*, 2008); 5, *S. siyangense* SY1^T (Liu *et al.*, 2008); 6, *S. daejeonense* TR6-04^T (Kim *et al.*, 2006); 7, *S. spiritivorum* NBRC 14948^T; 8, *S. multivorum* NBRC 14947^T; 9, *S. mizutaii* ATCC 33299^T; 10, *S. thalpophilum* NBRC 14963^T; 11, *S. faecium* NBRC 15299^T [data in Columns 7~11 are from Takeuchi and Yokota (1992) and Steyn *et al.* (1998)]; 12, *S. antarcticum* MTCC 675^T (Shivaji *et al.*, 1992); 13, *S. anhuiense* CW 186^T (Wei *et al.*, 2008). +, Fatty acid is detected but its content was not reported; -, <1% or not detected; Summed features are groups of two or three fatty acids that could not be separated by GLC using the MIDI system. Summed feature 3 contained C_{16:1} ω 7c/iso-C_{15:0} 2-OH.

Fatty acid	1^{a}	2 ^a	3 ^a	4	5	6	7	8	9	10	11	12	13
C _{14:0}	2.0	-	1.3	-	3.9	-	-	2.7	-	3.2	-	+	1.3
C _{14:0} 2-OH	3.2	-	-	-	-	-	-	-	-	-	-	-	-
iso-C _{15:0}	22.4	27.9	26.1	28.9	32.9	45.6	30.1	22.2	30.0	17.7	24.6	29.0	32.2
anteiso-C _{15:0}	-	-	-	-	2.7	2.6	-	-	-	-	-	-	1.2
iso-C _{15:0} 3-OH	5.8	3.3	2.4	-	3.0	1.5	2.2	3.2	3.0	4.3	3.7	-	2.7
iso-C _{15:1} G	-	-	-	-	-	-	-	-	-	-	-	-	1.1
C _{16:0}	1.8	-	4.9	-	10.9	3.4	3.5	7.8	-	6.0	4.5	+	3.6
C _{16:0} 2-OH	-	-	-	-	-	-	-	-	-	3.2	-	-	-
C _{16:0} 3-OH	15.2	2.3	1.2	-	6.4	-	2.7	5.3	-	6.3	2.1	-	2.0
C _{16:0} 10-methyl	-	-	-	-	-	-	-	-	-	-	1.4	-	-
C _{16:1} w5c	-	-	1.4	-	-	-	-	-	-	-	1.5	-	-
iso-C _{17:0} 3-OH	12.8	20.1	11.3	12.8	5.9	16.6	12.5	7.1	22.1	10.0	10.0	-	9.8
C _{17:1}	-	-	-	-	-	-	-	-	-	-	-	+	-
C _{18:0}	-	-	2.2	-	-	-	-	-	-	-	-	-	1.7
iso-C _{17:1} ω9c	-	1.6	7.4	-	1.1	2.9	1.7	-	3.7	-	-	-	-
iso-C _{15:1} F	-	-	3.1	-	-	-	-	-	-	-	-	-	-
C _{18:1} w5c	-	-	1.9	-	-	-	-	-	-	-	-	-	
Summed feature 3 ^a	34.4	37.6	25.8	40.3	24.1	23.8	42.7	49.0	35.1	47.8	48.1	56.0	33.7
Unknown (ECL 13.566)	-	-	-	-	-	-	-	-	1.3	1.3	1.4	-	-

^a Data from this study utilize the same conditions as described in this paper. Strains were grown on NA at 31°C for 48 h.

to 44.2 mol% (Takeuchi and Yokota, 1992). The DNA G+C content of Strain IBFC2009^T (41.0 mol%) was within this range.

Discussion

The genus *Sphingobacterium* was proposed to be negative for Gram staining and gelatinase and indole production, and positive for catalase and oxidase (Dong and Cai, 2001). Aside from the above-mentioned common characteristics, Strain IBFC2009^T had several other unique vital characteristics when compared with other *Sphingobacterium* species: aesculin was hydrolyzed, but DNA was not hydrolyzed; D-melibiose and inulin were assimilated, but L-arabinose was not assimilated; and acids were produced from D-glucose, L-arabinose, and L-rhamnose.

The genus Sphingobacterium had iso-C_{15:0} 2-OH, C_{16:1} ω 7c, iso-C_{15:0} and iso-C_{17:0} 3-OH as the main fatty acids (Takeuchi and Yokota, 1992; Steyn et al., 1998). Fatty acid results were, to some extent, dependent on the medium used, culturing time, and temperature (Yongmanitchai and Ward, 1991). The reported data of fatty acids on the genus Sphingobacterium were obtained using different methods or media (e.g., R2A, NB, TSA, TYB, and TSA, among others). In this study, the fatty acids of two closest relative species (two S. composti species) were studied on the same conditions with Strain IBFC2009^T (grown on NA at 31°C for 48 h). Strain IBFC2009^T contained a unique $C_{14:0}$ 2-OH (3.2%) content, had a greater C_{16:0} 3-OH (15.2\%) and iso-C_{15:0} 3-OH (5.8%) content, but had a lesser C_{16:0} (1.8%) content. The cellular fatty acid profiles suggested that it should be classified in the genus Sphingobacterium as a novel species.

The hybridization method applied could be disregarded if the highest 16S rDNA sequence similarity was less than 97.0% (Stackebrandt and Goebel, 1994; Keswani and Whitman, 2001). Strain IBFC2009^T showed the highest sequence similarities with the type strains of *S. composti* T5-12^T and $4M24^{T}$ (94.6% and 94.4%, respectively). In addition, according to the phylogenetic tree (Fig. 1), Strain IBFC2009^T formed a compact cluster with *S. composti* T5-12^T, *S. composti* 4M24^T, *S. daejeonense* TR6-04^T, and *S. mizutaii* ATCC 33299^T; therefore, the results demonstrated that Strain IBFC2009^T was not related to any other species of the genus *Sphingobacterium* at the species level.

On the basis of phenotypic and phylogenetic studies, it is demonstrable that Strain IBFC2009^T should represent a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium bambusae* sp. nov. is proposed.

Description of *Sphingobacterium bambusae* **sp. nov.** *Sphingobacterium bambusae* (bam.bu'sa.e. L. gen. n. Bambusa, of/from bamboo).

Cells are Gram-negative, non-motile, non-spore-forming rods, $0.5 \sim 0.8 \ \mu\text{m}$ wide, and $1.2 \sim 2.5 \ \mu\text{m}$ long for aerobic growth at 31°C. Colonies grown on NB are smooth, convex, round, glossy, yellow, and $1.0 \sim 2.0 \ \text{mm}$ in diameter after 2 d of incubation at 31°C on NA. The temperature, pH, and NaCl concentration ranges for growth are $11^{\circ}\text{C} \sim 39^{\circ}\text{C}$, pH $6.0 \sim 9.0$, and $0 \sim 5\%$ NaCl (w/v), respectively. Strain IBFC

2009^T tests are positive for catalase and oxidase, but negative for arginine decarboxylase, H₂S production, indole, lysine decarboxylase, methyl red, nitrate reduction, ornithine decarboxylase, phenylalanine ammonia lyase, tryptophan ammonia lyase, and the Voges-Proskauer reaction. Glucose is not fermented. Aesculin and Tween 80 are hydrolyzed, but CM-cellulose, DNA, gelatin, mannan, pectine, starch, urea, and xylan are not. The strain assimilates D-glucose, D-maltose, D-melibiose, D-sucrose, and inulin; however, it does not assimilate arginine monohydrochloride, ascorbic acid, benzaldehyde, citric acid, cysteine, D-fructose, D-fucose, D-galactose, D-galacturonic acid, D-mannitol, D-mannose, D-raffinose, D-ribose, D-sorbitol, D-xylose, glycerol, glycin, glycogen, inositol, kreatine, lactic acid, lactoflavin, lactose, L-arabinose, L-arginine, L-cystine, L-cystine mono hydrochloride, L-lactamine, L-lysine, L-phenyl alanine, L-proline, L-rhamnose, L-sorbose, L-threonine, malic acid, maltose, oxalic acid, propionic acid, pyro, sodium malonate, sodium oxalate, starch, tannin, tryptophan, and valeric acid. Acids are produced from D-fructose, D-galactose, D-glucose, Dlactose, D-maltose, D-mannose, D-raffinose, D-sucrose, Dtrehalose, D-xylose, ethanol, glycerol, inulin, L-arabinose, and L-rhamnose, but not from D-mannitol, D-ribose, Dsorbitol, glycogen, inositol, L-sorbose, and starch. The major fatty acids are summed feature 3 (iso-C15:0 2-OH and/or C_{16:1} ω7c), iso-C_{15:0}, C_{16:0} 3-OH, and iso-C_{17:0} 3-OH. The G+C content of the genomic DNA is 41.0 mol%.

The type strain, $IBFC2009^{T}$ (=CCTCC AB 209162^T =KCTC 22814^T), was isolated from the soil of a bamboo plantation, which was collected from Hangzhou, China.

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