**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† 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† belonged to the genus *Sphingobacterium* and showed the highest sequence similarity of 94.6% (*S. composti* T5-12T) with the type strains within the genus. The major fatty acids were summed feature 3 (iso-C15:0 2-OH and/or C16:1 ø7c, 34.4%), iso-C16:0 (22.4%), C16:0 3-OH (15.2%), and iso-C17:0 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† should represent a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium bambusae* sp. nov. is proposed. The type strain is IBFC2009† (=CCTCC AB 209162 =KCTC 22814†).

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

The genus *Sphingobacterium* was created by Yabuuchi et al. (1983), which includes *Sphingobacterium multivorans*, *S. spiritivorum*, and *S. mizutae*. To date, nine other species have been described: *S. antarcticum* (Shivaji et al., 1992), *S. fucicium* 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. styangense* (Liu et al., 2008), *S. kitalihiroshimense* (Matsuyama et al., 2008), and *S. anhuiense* (Wei et al., 2008). There were two different *S. composti* with validly published names. *S. composti* T5-12T was described by Ten et al. (2006) one year before the *S. composti* 4M24T of Yoo et al. (2007) The name *S. composti* 4M24T was then considered illegitimate and thus changed. Two other previously described species, *S. heparinum* and *S. pismicum*, 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† 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† 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† 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 IBFC2009† 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† 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 ×1,000, an atomic force microscope Nano-Scope IIIa (SPA 400, Seiko Inc & Multimode SPM, Digital Instruments, Co. Ltd) at ×20,000 and an electron microscope JEOL-1230 (JEOL Ltd., Japan) at ×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 K2HPO4, 0.5 Na2HPO4, 2.0 (NH4)2SO4, 0.2 MgSO4, and 0.1 CaCl2. 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 IBFC2009T, 5 ml of exponential phase cultures were centrifuged for 2 min at 10,000 rpm. The cell pellet was resuspended in 200 µl 100 mM (pH 7.4) Tris-EDTA (TE) buffer containing 400 µg/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 µl reaction mixture for PCR of 16S rRNA gene contained 0.5 µM 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 Chemstation 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 denaturation of genomic DNA (Marmur and Doty, 1962; Dong and Cai, 2001).

Results
Phenotypic and biochemical characteristics
Microscopically, the cells of IBFC2009T 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