# *Sphingobacterium wenxiniae* sp. nov., a cypermethrin-degrading species from activated sludge

Jun Zhang,[1] Jin-Wei Zheng,[1] Byung Cheol Cho,[2] Chung Yeon Hwang,[2] Chengxiang Fang,[3] Jian He[1] and Shun-Peng Li[1]

Correspondence
Chengxiang Fang
cxfang@whu.edu.cn
Jian He
hejian@njau.edu.cn

[1]Key Laboratory for Microbiological Engineering of Agricultural Environment, Ministry of Agriculture, College of Life Sciences, Nanjing Agricultural University, Nanjing, Jiangsu 210095, PR China

[2]School of Earth and Environmental Sciences and Research Institute of Oceanography, Seoul National University, Shillim-dong, Kwanak-gu, Seoul 151-742, Republic of Korea

[3]College of Life Sciences, Wuhan University, Wuhan 430072, PR China

A Gram-negative, non-motile, non-spore-forming, non-flagellated rod capable of degrading cypermethrin, designated LQY-18$^T$, was isolated from activated sludge of a wastewater treatment plant in China. Strain LQY-18$^T$ grew at 8–40 °C (optimum 30 °C), at pH 5.0–10.0 (optimum pH 7.0) and with 0–5 % (w/v) NaCl (optimum 1 %). The predominant menaquinone was MK-7 (97 %) and the major fatty acids were summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), iso-$C_{15:0}$ and iso-$C_{17:0}$ 3-OH. The DNA G+C content was 40.3 mol%. Phylogenetic analysis revealed that the isolate belonged to the genus *Sphingobacterium* of the phylum *Bacteroidetes* and showed low 16S rRNA gene sequence similarity with recognized members of the genus *Sphingobacterium*. The closest neighbour was *Sphingobacterium mizutaii* ATCC 33299$^T$ (92.9 % 16S rRNA gene sequence similarity). On the basis of phenotypic, genetic and phylogenetic data, strain LQY-18$^T$ (=ACCC 05410$^T$=CCTCC AB 2010005$^T$=KCTC 23009$^T$) should be classified as a representative of a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium wenxiniae* sp. nov. is proposed.

The genus *Sphingobacterium* was proposed by Yabuuchi *et al.* (1983) to include Gram-negative rods that are positive for catalase and oxidase but negative for heparinase, gelatinase and indole production and contain iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, $C_{16:1}\omega7c$ and $C_{17:0}$ 3-OH as the main fatty acids (Takeuchi & Yokota, 1992; Steyn *et al.*, 1998). *Sphingobacterium* is the type genus of the family *Sphingobacteriaceae* (Steyn *et al.*, 1998) in the phylum *Bacteroidetes*. At the time of writing, the genus comprised 17 recognized species, including the recently described species *Sphingobacterium composti* (Ten *et al.*, 2006), *Sphingobacterium daejeonense* (Kim *et al.*, 2006), *Sphingobacterium anhuiense* (Wei *et al.*, 2008), *Sphingobacterium canadense* (Mehnaz *et al.*, 2007), *Sphingobacterium kitahiroshimense* (Matsuyama *et al.*, 2008), *Sphingobacterium siyangense* (Liu *et al.*, 2008), *Sphingobacterium bambusae* (Duan *et al.* 2009) and *Sphingobacterium shayense* (He *et al.*, 2010). The type species of the genus is *Sphingobacterium spiritivorum*.

Cypermethrin is a synthetic pyrethroid widely used to control insect pests (Katsuda, 1999). It shows acute toxicity to a number of non-target organisms such as bees, fish and aquatic invertebrates (Smith & Stratton, 1986; Saha & Kaviraj, 2008). A cypermethrin-degrading bacterial strain, designated LQY-18$^T$, was isolated from activated sludge of a wastewater treatment plant in a synthetic pyrethroid-manufacturing facility, using a procedure described elsewhere (Wang *et al.*, 2009). The primary enrichment medium was mineral salts medium [MSM, containing ($l^{-1}$): 4.8 g $K_2HPO_4$, 1.2 g $KH_2PO_4$, 1.0 g $NH_4NO_3$, 0.2 g $MgSO_4.7H_2O$, 0.4 g $Ca(NO_3)_2.4H_2O$, 0.5 g NaCl and 0.001 g $Fe_2(SO_4)_3$] with 100 mg cypermethrin as the sole carbon source. After five successive steps of subcultivation, the enrichment culture was spread on MSM agar and incubated at 30 °C for 4–5 days. Strain LQY-18$^T$ was obtained after several subcultivation steps on MSM agar.

Strain LQY-18$^T$ was grown aerobically on trypticase soy agar (TSA; Difco) or in trypticase soy broth (TSB; Difco) at 30 °C. *Sphingobacterium mizutaii* ATCC 33299$^T$, *S. composti* KCTC 12578$^T$, *S. shayense* CCTCC AB 209006$^T$, *S. spiritivorum* JCM 1277$^T$ and *S. daejeonense* KCTC 12579$^T$

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LQY-18$^T$ is GQ988781.

A supplementary figure is available with the online version of this paper.

were grown under the same conditions and used as reference strains in all physiological tests.

Cell morphology was determined by phase-contrast microscopy and transmission electron microscopy. Gliding motility was studied using the hanging-drop method (Bernardet *et al.*, 2002). Gram-staining, catalase and oxidase activities, and hydrolysis of starch, DNA, Tweens 20 and 80 and carboxymethyl-cellulose were investigated as described by Smibert & Krieg (1994). $H_2S$ production was tested as described by Bruns *et al.* (2001). Growth at pH 4.0–10.0 (at intervals of 0.5 pH units) was determined in TSB buffered with citrate/phosphate buffer or Tris/HCl buffer (Breznak & Costilow, 1994). Growth with 0–7 % NaCl (w/v) (at intervals of 0.5 %) was tested in TSB. Growth at 4, 5, 10, 20, 25, 28, 30, 37, 40, 41 and 45 °C was tested on TSA. Additional biochemical tests were performed using the API 20 NE, API 50 CH and API ID32 GN kits and enzyme activities were assessed using the API ZYM kit (bioMérieux), according to the manufacturer's instructions. Sensitivity to antibiotics was determined using the disc-diffusion method with 8 mm diameter discs (Sanofi Pasteur) containing the following (μg per disc): erythromycin (15), tetracycline (30), gentamicin (10), chloramphenicol (30), kanamycin (30), streptomycin (10), rifampicin (5), ampicillin (10), polymyxin B (30), penicillin G (10), cephradine (30), roxithromycin (15), lincomycin (2), carbenicillin (100), spectinomycin (100), amoxicillin (10), bacitracin (0.04) and vancomycin (30).

Cells of strain LQY-18$^T$ were strictly aerobic, Gram-negative, non-motile, non-sporulating, non-flagellated, short rods with rounded ends. Other phenotypic characteristics of strain LQY-18$^T$ are given in the species description and Table 1.

Extraction and purification of genomic DNA were carried out according to standard procedures (Sambrook & Russell, 2001). Amplification of the 16S rRNA gene was performed using a universal bacterial primer set (27F and 1492R; Lane, 1991). The PCR product was purified and sequenced. The 16S rRNA gene sequence of strain LQY-18$^T$ was aligned with relevant sequences obtained from the EzTaxon server (http://www.eztaxon.org) (Chun *et al.*, 2007) using MEGA4 and CLUSTAL W. Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and minimum-evolution (Rzhetsky & Nei, 1992) methods, with bootstrap values based on 1000 replications (Felsenstein, 1985, 1993). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1980, 1983).

An almost-complete 16S rRNA gene sequence (1453 nt) of strain LQY-18$^T$ was obtained. BLAST searches in the GenBank database and the EzTaxon server indicated that strain LQY-18$^T$ belonged to the genus *Sphingobacterium* of the phylum *Bacteroidetes* and showed 89.1–92.9 % 16S rRNA gene sequence similarity with members of the genus. Phylogenetic analysis (Fig. 1) confirmed that strain LQY-18$^T$ belonged to the genus *Sphingobacterium*, forming a distinct lineage in a cluster containing *S. shayense* HS39$^T$, and indicated that the isolate represents a novel member of the genus.

**Table 1.** Differential phenotypic characteristics of strain LQY-18$^T$ and closely related members of the genus *Sphingobacterium*

Strains: 1, *Sphingobacterium wenxiniae* sp. nov. LQY-18$^T$; 2, *S. shayense* CCTCC AB 209006$^T$; 3, *S. composti* KCTC 12578$^T$; 4, *S. mizutaii* ATCC 33299$^T$; 5, *S. daejeonense* KCTC 12579$^T$; 6, *S. spiritivorum* JCM 1277$^T$. All data were taken from this study. All strains are negative for Gram-staining, sporulation and indole production. +, Positive; W, weak; −, negative.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Temperature range (°C) | 8–40 | 10–40 | 10–42 | 10–40 | 10–42 | 5–42 |
| Hydrolysis of: | | | | | | |
| Starch | − | + | − | + | − | + |
| Aesculin | + | + | − | + | − | + |
| Urea | − | + | − | + | − | + |
| Assimilation of: | | | | | | |
| Sorbitol | − | − | + | + | − | + |
| L-Rhamnose | − | + | − | − | − | + |
| D-Mannitol | − | − | + | − | − | + |
| Glycogen | + | − | − | − | − | W |
| L-Proline | + | − | − | + | − | − |
| L-Fucose | + | − | − | − | − | − |
| Acid production from: | | | | | | |
| L-Rhamnose | − | + | − | − | − | − |
| D-Mannitol | − | − | − | − | − | + |
| L-Arabinose | − | + | − | + | − | − |
| Trehalose | + | + | W | − | − | − |

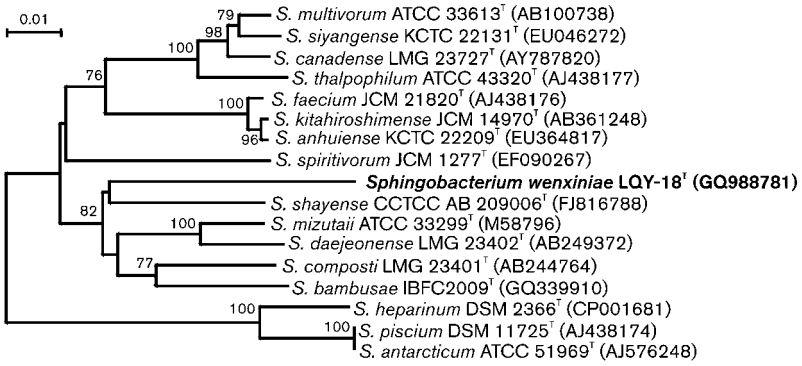


**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain LQY-18$^T$ and representative members of the genus *Sphingobacterium*. Bootstrap values (>70 %) based on 1000 replications are shown at branch nodes. Bar, 0.01 substitutions per nucleotide position.

Analysis of respiratory quinones was carried out by the identification service of the DSMZ and B. Tindall (Braunschweig, Germany). For fatty acid analysis, strain LQY-18$^T$ and *S. spiritivorum* JCM 1277$^T$ were cultured on LB agar at 30 °C for 48 h. The fatty acid methyl esters were obtained from cells by saponification, methylation and extraction as described elsewhere (Kämpfer & Kroppenstedt, 1996) and were separated by GC (Agilent 6890N). Peaks were automatically integrated and fatty acid names and percentages were determined using the MIDI Sherlock MIS system (TSBA6 library version 6.0B) (Sasser, 1990). Quinones were extracted using an integrated protocol as described elsewhere (Tindall 1990a, b; Altenburger *et al.*, 1996). Polar lipids were extracted by the method of Minnikin *et al.* (1984) and identified by two-dimensional TLC and spraying with specific reagents as described by Collins & Jones (1980). The DNA G+C content was determined by thermal denaturation (Marmur & Doty, 1962) using *Escherichia coli* K-12 as the standard.

Strain LQY-18$^T$ contained iso-$C_{15:0}$ (32.4 %), iso-$C_{17:0}$ 3-OH (15.2 %) and summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; 33.8 %) as the major fatty acids. The fatty acid profiles of strain LQY-18$^T$ and the reference strains were similar, although there were some differences in the proportions of some components. The detailed fatty acid compositions of strain LQY-18$^T$ and the reference strains are given in Table 2. The predominant isoprenoid quinone of strain LQY-18$^T$ was MK-7 (97 %). The major polar lipid was phosphatidylethanolamine; several unknown polar lipids were also detected (Supplementary Fig. S1, available in IJSEM Online). Strain LQY-18$^T$ and *S. spiritivorum* JCM 1277$^T$ contained sphingolipid, which is a distinct feature of members of the genus *Sphingobacterium*. The DNA G+C content of strain LQY-18$^T$ was 40.3 mol%, which fell within the range observed for the genus *Sphingobacterium* (36.0–44.2 mol%).

On the basis of phenotypic, genotypic and phylogenetic properties, strain LQY-18$^T$ should be classified as a representative of a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium wenxiniae* sp. nov. is proposed.

## Description of *Sphingobacterium wenxiniae* sp. nov.

*Sphingobacterium wenxiniae* (wen.xi.ni′a.e. N.L. fem. gen. n. *wenxiniae* of Wen-xin, to honour Wen-xin Chen, a respected soil microbiologist, for her great contributions to the investigation and development of *Rhizobia* resources in China).

Cells are Gram-negative, non-motile, non-spore-forming, non-flagellated, strictly aerobic rods, approximately 0.6–1.4 μm in length and 0.3–0.6 μm in diameter. After 3 days of incubation on TSA, colonies are 1.0–2.0 mm in diameter, yellowish, convex, circular and smooth with entire margins. Grows with 0–5 % (w/v) NaCl (optimum 1 %), at pH 5.0–10.0 (optimum pH 7.0–8.0) and at 8–40 °C (optimum 30 °C). $H_2S$ and indole are not produced. Nitrate is not reduced. Aesculin, Tweens 20 and 80, and carboxymethyl-cellulose are hydrolysed, but starch, urea, DNA and gelatin are not. Catalase, oxidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), cystine arylamidase, leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- and β-galactosidases, α- and β-glucosidases, *N*-acetyl-β-glucosaminidase and α-fucosidase are present, but arginine dihydrolase, urease, arginine decarboxylase, ornithine decarboxylase, lysine decarboxylase, lipase (C14), β-glucuronidase and α-mannosidase are absent. Assimilates D-glucose, D-mannose, *N*-acetylglucosamine, maltose, salicin, melibiose, L-fucose, glycogen, sucrose and L-proline, but not L-arabinose, gluconate, L-rhamnose, L-arabitol, L-histidine, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, D-mannitol, D-sorbitol, propionic acid, capric acid, valeric acid, trisodium, citrate, potassium 2-ketogluconate, 3-hydroxybutyric acid or 4-hydroxybenzoic acid. Acid is produced from D-arabinose, D-galactose, D-glucose, D-fructose, D-mannose, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, trehalose, sucrose, inulin, melezitose, raffinose, starch, glycogen, D-gentiobiose, turanose and L-fucose, but not from glycerol, mannitol, erythritol, L-arabinose, D-ribose, D- and

**Table 2.** Cellular fatty acid contents of strain LQY-18$^T$ and closely related members of the genus *Sphingobacterium*

Strains: 1, *Sphingobacterium wenxiniae* sp. nov. LQY-18$^T$; 2, *S. spiritivorum* JCM 1277$^T$; 3, *S. shayense* CCTCC AB 209006$^T$; 4, *S. composti* KCTC 12578$^T$; 5, *S. mizutaii* ATCC 33299$^T$; 6, *S. daejeonense* KCTC 12579$^T$. Data for columns 1 and 2 were taken from this study and for columns 3–6 from He *et al.* (2010). tr, Trace (<1 %); −, not detected.

| Fatty acid | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $C_{14:0}$ | tr | 2.1 | 1.3 | − | − | − |
| $C_{16:0}$ | 4.8 | 4.3 | 3.5 | 1.1 | 1.7 | 2.0 |
| $C_{18:0}$ | tr | tr | tr | 1.0 | 1.0 | 1.0 |
| $C_{16:0}$ 3-OH | 1.9 | 4.3 | 2.4 | tr | tr | tr |
| $C_{17:0}$ 2-OH | tr | tr | 1.1 | tr | tr | 3.4 |
| iso-$C_{15:0}$ | 32.4 | 25.5 | 28.6 | 38.0 | 38.3 | 32.3 |
| iso-$C_{15:0}$ 3-OH | 2.0 | 2.5 | 2.5 | 2.0 | 1.6 | 1.0 |
| iso-$C_{17:0}$ 3-OH | 15.2 | 9.5 | 13.5 | 17.4 | 18.0 | 15.7 |
| $C_{16:1}\omega5c$ | tr | 1.4 | 1.0 | tr | tr | − |
| anteiso-$C_{15:0}$ | 1.5 | tr | 3.2 | tr | 2.9 | 7.8 |
| Summed features* | | | | | | |
| 1 | tr | tr | tr | 1.9 | 1.3 | tr |
| 3 | 33.8 | 41.6 | 37.0 | 19.2 | 20.5 | 24.4 |
| 4 | tr | 1.6 | tr | tr | 1.1 | tr |
| 9 | 1.8 | 2.3 | tr | 9.5 | 5.1 | 2.8 |

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 1 consisted of $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ H. Summed feature 3 consisted of $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$. Summed feature 4 consisted of anteiso-$C_{17:1}$ B and/or iso-$C_{17:1}$ I. Summed feature 9 consisted of iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$.

L-xylose, D-adonitol, methyl α-D-xyloside, L-sorbose, L-rhamnose, dulcitol, inositol, sorbitol, xylitol, D-lyxose, D-tagatose, D-fucose, D- or L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. Sensitive to roxithromycin, erythromycin, chloramphenicol, rifampicin, spectinomycin and cephradine; resistant to tetracycline, gentamicin, kanamycin, streptomycin, ampicillin, polymyxin B, penicillin G, lincomycin, carbenicillin, amoxicillin, bacitracin and vancomycin. The major respiratory quinone is MK-7. The major polar lipid is phosphatidylethanolamine; an unknown aminolipid and several unknown polar lipids are also detected. Sphingolipid is present. The major cellular fatty acids are summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), iso-$C_{15:0}$ and iso-$C_{17:0}$ 3-OH.

The type strain, LQY-18$^T$ (=ACCC 05410$^T$=CCTCC AB 2010005$^T$=KCTC 23009$^T$), was isolated from activated sludge of a wastewater treatment plant in a synthetic pyrethroid-manufacturing facility of the Yangnong Chemical Group, Jiangsu Province, China. The DNA G+C content of the type strain is 40.3 mol%.

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