Sphingobacterium anhuiense sp. nov., isolated from forest soil

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A Gram-negative bacterium, designated strain CW 186^T, was isolated from forest soil in Anhui province, China. Cells of strain CW 186^T were strictly aerobic, non-motile and rod-shaped. The strain grew optimally at 25–30 °C and pH 6.0–8.0. The major cellular fatty acids of strain CW 186^T were iso-$C_{15:0}$ (32.2 %), iso-$C_{17:0}$ 3-OH (9.8 %) and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; 33.7 %). The predominant isoprenoid quinone was MK-7. The G+C content of the genomic DNA was 36.3 mol%. Phylogenetic analysis using 16S rRNA gene sequences showed that strain CW 186^T formed a monophyletic cluster with *Sphingobacterium daejeonense* LMG 23402^T, *Sphingobacterium composti* LMG 23401^T, *Sphingobacterium composti* DSM 18850^T, *Sphingobacterium mizutaii* ATCC 33299^T and *Sphingobacterium spiritivorum* ATCC 33861^T. Sequence similarities were less than 94 % (the maximal similarity was about 93.9 % to *S. composti* LMG 23401^T) to *Sphingobacterium* species with validly published names. A polyphasic taxonomic study including chemotaxonomic and phylogenetic analyses demonstrated that strain CW 186^T should be classified as representing a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium anhuiense* sp. nov. is proposed. The type strain is CW 186^T (=KCTC 22209^T=CCTCC AB 207197^T).

The genus *Sphingobacterium* was first proposed by Yabuuchi *et al.* in 1983 (Yabuuchi *et al.*, 1983) and characterized as including Gram-negative, aerobic, non-motile, rod-shaped bacteria with colonies that are unusually yellow-pigmented. Chemotaxonomically, members of the genus contained MK-7 as the predominant isoprenoid quinone, and iso-branched fatty acids iso-$C_{15:0}$ and iso-$C_{17:0}$ 3-OH and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$) as major fatty acids. At the time of writing, the names of 11 species (*Sphingobacterium antarcticum*, *S. daejeonense*, *S. faecium*, *S. heparinum*, *S. mizutaii*, *S. multivorum*, *S. spiritivorum*, *S. thalpophilu*m, *S. piscium* and two *S. composti*) have been validly published (Yoo *et al.*, 2007; Kim *et al.*, 2006; Ten *et al.*, 2006; Shivaji *et al.*, 1992; Takeuchi & Yokota, 1992; Yabuuchi *et al.*, 1983). In the course of an investigation of the bacterial community in forest soil from Anhui Province, China, a bacterial strain, designated CW 186^T, was isolated and submitted to a polyphasic taxonomy study. We hereby propose that strain CW 186^T represents a novel species of the genus *Sphingobacterium*.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CW 186^T is EU364817.

To investigate the morphological, biochemical and physiological characteristics, strain CW 186^T was routinely cultivated using TYB medium (0.3 % yeast extract, 0.2 % beef extract, 0.6 % tryptone, 0.3 % NaCl, 0.001 % $FeCl_3$, w/v) at 30 °C. Unless otherwise indicated, the phenotypic characteristics were studied using standard procedures (Smibert & Krieg, 1994; Zhou *et al.*, 2007) and all experiments were conducted in triplicate. The morphological characteristics of strain CW 186^T were determined using transmission electron microscopy (model H-7650; Hitachi) after incubation for 24 h at 30 °C on TYB agar. The electron microscopy preparations were performed as described by Nedashkovskaya *et al.* (2005). For the various physiological tests, API 20NE and API 50 CHB test strips (bioMérieux) were used according to the manufacturer's instructions.

PCR amplification of the 16S rRNA gene was performed as described by Li *et al.* (2007). The 16S rRNA gene sequence was aligned manually with reference sequences retrieved from the GenBank database following BLAST searches. The phylogenetic tree was constructed using the software package MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignment of data using CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the

Kimura two-parameter model; Kimura, 1980, 1983) and clustering were based on the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Bootstrap analysis based on 1000 resamplings was used to evaluate the topology of the neighbour-joining tree (Felsenstein, 1985).

Genomic DNA was extracted and purified following the procedure of Marmur (1961). The DNA G+C content was determined by using reversed-phase HPLC (Supelcosil LC-18 S; Supelco) according to Mesbah *et al.* (1989), using *E. coli* DH5α to determine the standard deviation. Respiratory quinones were extracted from lyophilized cells and the samples were purified and analysed by HPLC using the procedures reported by Hu *et al.* (2001). The cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification system (MIDI, Inc.).

Strain CW 186^T showed good growth on TYB, MacConkey (Difco) and Luria–Bertani (Oxoid) agar at 25–30 °C. Cells of CW 186^T were non-motile rods (0.4–0.8 μm in width and 1.8–2.5 μm in length). The physiological and biochemical properties that differentiate strain CW 186^T from other recognized species of the genus *Sphingobacterium* are shown in Table 1.

An almost-complete 16S rRNA gene sequence (1382 nt) of strain CW 186^T was determined. The neighbour-joining phylogenetic tree constructed using 16S rRNA gene sequences showed that strain CW 186^T belongs to the genus *Sphingobacterium* (Fig. 1). Relatively low 16S rRNA gene sequence similarity values (the maximal similarity was about 93.9 % to *S. composti* LMG 23401^T) were obtained with other recognized species of the genus *Sphingobacterium*.

The DNA G+C content of strain CW 186^T was 36.3 mol%. The predominant respiratory quinone was MK-7 (content >99 %). Major fatty acids included iso-$C_{15:0}$ (32.2 %), iso-$C_{17:0}$ 3-OH (9.8 %) and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; 33.7 %). Detailed fatty acid profiles are given in Table 2.

Although the results from the chemotaxonomy (major fatty acids and quinone) and phylogenetic analyses unequivocally supported the proposal that the new isolate is a member of the genus *Sphingobacterium*, strain CW 186^T could be differentiated from other related *Sphingobacterium* species by means of phenotypic properties such as growth temperature range, starch hydrolysis and acid production from carbohydrates (API 50 CHB test strip; Table 1) and also by differences in minor fatty acid components (Table 2). On the basis of the phylogenetic and chemotaxonomic

Table 1. Phenotypic characteristics that differentiate strain CW 186^T from its closest phylogenetic neighbours

Strains: 1, CW 186^T (*Sphingobacterium anhuiense* sp. nov.); 2, *S. composti* LMG 23401^T (data from Ten *et al.*, 2006); 3, *S. composti* DSM 18850^T (Yoo *et al.*, 2007); 4, *S. daejeonense* LMG 23402^T (Kim *et al.*, 2006); 5, *S. spiritivorum* ATCC 33861^T (Takeuchi & Yokota, 1992); 6, *S. multivorum* NBRC 14947^T (Takeuchi & Yokota, 1992); 7, *S. mizutaii* ATCC 33299^T (Takeuchi & Yokota, 1992); 8, *S. thalpophilum* ATCC 43320^T (Takeuchi & Yokota, 1992); 9, *S. faecium* NBRC 15299^T (Takeuchi & Yokota, 1992); 10, *S. antarcticum* MTCC 675^T (Shivaji *et al.*, 1992). All strains are positive for aerobic growth at 30 °C, catalase and oxidase activities and the assimilation of D-glucose, D-mannose, maltose and sucrose. All strains are negative for Gram-staining, sporulation and indole production. +, Positive; (+), weakly positive; −, negative; V, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10
Growth at:										
5 °C	+	−	−	−	−	−	−	−	+	+
42 °C	−	+	+	+	−	−	−	+	−	−
Acid production from:										
Glucose	+	+	−	+	+	+	+	+	+	+
L-Rhamnose	−	−	−	−	−	V	−	+	−	−
L-Arabinose	+	−	+	−	−	+	+	+	+	−
Sucrose	+	+	+	+	+	+	+	+	+	−
D-Mannitol	−	−	−	−	+	−	−	−	−	−
Hydrolysis of:										
DNA	(+)	+	−	−	+	+	+	−	+	ND
Aesculin	+	−	+	−	+	+	+	+	+	+
Gelatin	−	−	−	−	−	−	−	−	−	+
Starch	+	−	−	−	+	+	+	+	+	−
Urease	−	−	−	−	+	+	+	+	+	+
Assimilation of:										
L-Rhamnose	−	−	−	−	+	+	−	+	+	+
L-Arabinose	+	−	+	−	−	+	V	+	+	+
D-Mannitol	−	+	−	−	+	−	−	−	−	+
Melibiose	+	−	+	+	+	+	+	+	+	−
DNA G+C content (mol%)	36.3	36.0	42.3	38.7	39.0	39.9−40.5	39.3−40.0	44.0−44.2	37.3	39.3

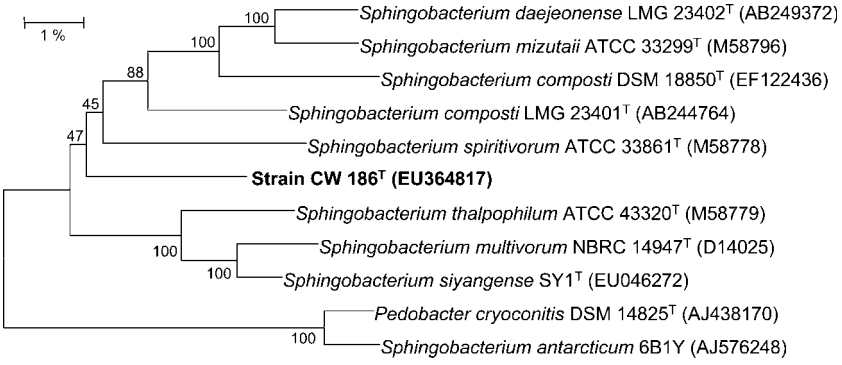

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain CW 186^T in the genus *Sphingobacterium*. Bootstrap percentages of 1000 replicates are given at branch points. GenBank accession numbers are given in parentheses. Bar, 1 % nucleotide substitutions per 100 nt.

evidence together with the phenotypic characteristics and low 16S rRNA gene sequence similarities presented in this study, strain CW 186^T should be classified as representing a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium anhuiense* sp. nov. is proposed.

Description of *Sphingobacterium anhuiense* sp. nov.

Sphingobacterium anhuiense (an.hu.i.en′se. N.L. neut. adj. *anhuiense* pertaining to Anhui, the province where the type strain was isolated).

Cells are Gram-negative, non-motile, non-spore-forming and rod-shaped (0.4–0.8 μm wide and 1.8–2.5 μm long). Colonies are circular, convex and bright yellow-coloured after 24 h cultivation at 30 °C on TYB agar. Growth occurs at 4–35 °C (optimum, 25–30 °C) and pH 6.0–8.0 (optimum, pH 6.5–7.5); growth occurs with 0–3 % NaCl, but not with 4 % NaCl in modified TYB broth. Starch and DNA are hydrolysed, but not Tween 80, casein or urea. Catalase, oxidase, methyl α-D-glucosidase and β-galactosidase activities are present; arginine dihydrolase, ornithine

Table 2. Cellular fatty acid contents of strain CW 186^T and other recognized *Sphingobacterium* species

Strains: 1, CW 186^T (*Sphingobacterium anhuiense* sp. nov.); 2, *S. composti* LMG 23401^T (data from Ten *et al.*, 2006); 3, *S. composti* DSM 18850^T (Yoo *et al.*, 2007); 4, *S. daejeonense* LMG 23402^T (Kim *et al.*, 2006); 5, *S. spiritivorum* ATCC 33861^T (Takeuchi & Yokota, 1992); 6, *S. multivorum* NBRC 14947^T (Takeuchi & Yokota, 1992); 7, *S. mizutaii* ATCC 33299^T (Takeuchi & Yokota, 1992); 8, *S. thalpophilum* ATCC 43320^T (Takeuchi & Yokota, 1992); 9, *S. faecium* NBRC 15299^T (Takeuchi & Yokota, 1992); 10, *S. antarcticum* MTCC 675^T (Shivaji *et al.*, 1992). Values are percentages of total fatty acids. +, Fatty acid was detected but its content was not reported; ND, not detected; tr, trace (<1 %). Fatty acids representing less than 1.0 % in all species were omitted.

Fatty acid	1	2	3	4	5	6	7	8	9	10
Straight-chain saturated										
$C_{14:0}$	1.3	ND	ND	ND	1.0	2.7	tr	3.2	tr	+
$C_{16:0}$	3.6	2.1	2.2	2.0	3.5	7.8	tr	6.0	4.5	+
$C_{18:0}$	1.7	1.2	ND	ND	ND	ND	ND	ND	ND	ND
$C_{16:0}$ 2-OH	ND	ND	ND	ND	ND	tr	ND	3.2	ND	ND
$C_{16:0}$ 3-OH	2.0	ND	1.2	ND	2.7	5.3	tr	6.3	2.1	ND
Branched saturated										
iso-$C_{15:0}$	32.2	45.6	29.5	26.1	30.1	22.2	30.0	17.7	24.6	29.0
iso-$C_{15:0}$ 3-OH	2.7	2.1	2.3	1.3	2.2	3.2	3.0	4.3	3.7	tr
iso-$C_{17:0}$ 3-OH	9.8	17.2	19.7	17.3	12.5	7.1	22.1	10.0	10.0	tr
anteiso-$C_{15:0}$	1.2	tr	ND	4.1	tr	ND	tr	ND	tr	ND
$C_{16:0}$ 10-methyl	ND	9.7	ND	ND	ND	ND	ND	ND	1.4	ND
Summed feature 3*	33.7	14.9	37.5	29.8	42.7	49.0	35.1	47.8	48.1	56.0
Monounsaturated										
$C_{16:1}\omega5c$	tr	tr	ND	ND	tr	ND	tr	ND	1.5	ND
$C_{17:1}$	ND	ND	ND	ND	ND	ND	ND	ND	ND	+
$C_{18:1}\omega9c$	1.1	tr	ND	ND	ND	ND	ND	ND	ND	ND
iso-$C_{15:1}$ G	1.1	1.8	ND	1.2	ND	ND	ND	ND	ND	ND
iso-$C_{17:1}\omega9c$	ND	2.9	2.9	3.5	1.7	tr	3.7	ND	ND	ND

*Summed features represent groups of two or three fatty acids that cannot be separated by GC with the MIDI system. Summed feature 3 contains one or more of iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$.

decarboxylase, lysine decarboxylase, tryptophan decarboxylase and urease activities are absent. H_2S is not produced. Voges–Proskauer test is positive. Citrate is not utilized and indole is not produced (API 20NE tests). *N*-Acetylglucosamine, aesculin, D-arabinose, L-arabinose, arbutin, cellobiose, fructose, D-fucose, L-fucose, D-galactose, glucose, inulin, D-lactose, laetrile, maltose, D-mannose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, raffinose, salicoside, starch and sucrose are utilized; D-adonite, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, D-gentiobiose, glycerol, glycogen, gluconate, inositol, 2-ketogluconate, 5-ketogluconate, D-lyxose, mannitol, melezitose, methyl β-D-xyloside, L-rhamnose, D-ribose, sorbitol, L-sorbitose, D-tagatose, turanose, xylitol, D-xylose and L-xylose are not utilized (API 50 CHB tests). Predominant isoprenoid quinone is MK-7. Major cellular fatty acids are iso-$C_{15:0}$ (32.2 %), iso-$C_{17:0}$ 3-OH (9.8 %) and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; 33.7 %). The DNA G+C content of the type strain is 36.3 mol% (HPLC).

The type strain, CW 186^T (=KCTC 22209^T=CCTCC AB 207197^T), was isolated from forest soil in Anhui province, China.

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References

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Hu, H.-Y., Lim, B.-R., Naohiro, G. & Koich, F.-J. (2001). Analytical precision and repeatability of respiratory quinones for quantitative study of microbial community structure in environmental samples. *J Microbiol Methods* **47**, 17–24.

Kim, K.-H., Ten, L. N., Liu, Q.-M., Im, W.-T. & Lee, S.-T. (2006). *Sphingobacterium daejeonense* sp. nov., isolated from a compost sample. *Int J Syst Evol Microbiol* **56**, 2031–2036.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence. *J Mol Evol* **16**, 111–120.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution.* Cambridge: Cambridge University Press.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Li, W. J., Xu, P., Schumann, P., Zhang, Y. Q., Pukall, R., Xu, L. H., Stackebrandt, E. & Jiang, C. L. (2007). *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China) and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* **57**, 1424–1428.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Nedashkovskaya, O. I., Kim, S. B., Suzuki, M., Shevchenko, L. S., Lee, M. S., Lee, K. H., Park, M. S., Frolova, G. M., Oh, H. W. & other authors (2005). *Pontibacter actiniarum* gen. nov., sp. nov., a novel member of the phylum '*Bacteroidetes*', and proposal of *Reichenbachiella* gen. nov. as a replacement for the illegitimate prokaryotic generic name *Reichenbachia* Nedashkovskaya *et al.* 2003. *Int J Syst Evol Microbiol* **55**, 2583–2588.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 16.

Shivaji, S., Ray, M. K., Rao, N. S., Saisree, L., Jagannadham, M. V., Kumar, G. S., Reddy, G. S. N. & Bhargava, P. M. (1992). *Sphingobacterium antarcticus* sp. nov., a psychrotrophic bacterium from the soils of Schirmacher Oasis, Antarctica. *Int J Syst Bacteriol* **42**, 102–106.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Takeuchi, M. & Yokota, A. (1992). Proposals of *Sphingobacterium faecium* sp. nov., *Sphingobacterium piscium* sp. nov., *Sphingobacterium heparinum* comb. nov., *Sphingobacterium thalpophilum* comb. nov., and two genospecies of the genus *Sphingobacterium* and synonymy of *Flavobacterium yabuuchiae* and *Sphingobacterium spiritivorum*. *J Gen Appl Microbiol* **38**, 465–482.

Ten, L. N., Liu, Q. M., Im, W. T., Aslam, Z. & Lee, S. T. (2006). *Sphingobacterium composti* sp. nov., a novel DNase-producing bacterium isolated from compost. *J Microbiol Biotechnol* **16**, 1728–1733.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Yabuuchi, E., Kaneko, T., Yano, I., Moss, C. W. & Miyoshi, N. (1983). *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium multivorum* comb. nov., *Sphingobacterium mizutae* sp. nov., and *Flavobacterium indologenes* sp. nov.: glucose-nonfermenting gram-negative rods in CDC groups IIK-2 and IIb. *Int J Syst Bacteriol* **33**, 580–598.

Yoo, S.-H., Weon, H.-Y., Jang, H.-B., Kim, B.-Y., Kwon, S.-W., Go, S.-J. & Stackebrandt, E. (2007). *Sphingobacterium composti* sp. nov., isolated from cotton-waste composts. *Int J Syst Evol Microbiol* **57**, 1590–1593.

Zhou, Y., Dong, J., Wang, X., Huang, X., Zhang, K.-Y., Zhang, Y.-Q., Guo, Y.-F., Lai, R. & Li, W.-J. (2007). *Chryseobacterium flavum* sp. nov., isolated from polluted soil. *Int J Syst Evol Microbiol* **57**, 1765–1769.