# *Sphingobacterium composti* sp. nov., isolated from cotton-waste composts

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A Gram-negative, strictly aerobic, short rod-shaped, non-motile bacterial strain designated 4M24$^T$ was isolated from cotton-waste compost. Analysis of the 16S rRNA gene sequence of strain 4M24$^T$ revealed that it is a member of the genus *Sphingobacterium*, sharing 88.5–94.5 % sequence similarity with type strains of the genus *Sphingobacterium* and being most closely related to *Sphingobacterium daejeonense* TR6-04$^T$ (94.5 % sequence similarity) and *Sphingobacterium mizutaii* ATCC 33299$^T$ (92.2 % similarity). The major fatty acids of strain 4M24$^T$ grown on trypticase soy agar medium were summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; 37.5 %), iso-$C_{15:0}$ (29.5 %) and iso-$C_{17:0}$ 3-OH (19.7 %). The G+C content of the genomic DNA was 42.3 mol%. On the basis of phenotypic and genotypic characteristics, strain 4M24$^T$ represents a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium composti* sp. nov. is proposed. The type strain is 4M24$^T$ (=KACC 11313$^T$=DSM 18850$^T$).

Members of the genus *Sphingobacterium* Yabuuchi *et al.* 1983 have been isolated from Antarctic soil, clinical specimens (blood, urine and the uteruses of human patients) and compost composed of cow dung and rice straw (Holmes *et al.*, 1982; Yabuuchi *et al.*, 1983; Shivaji *et al.*, 1992; Kim *et al.*, 2006). To date, the following species have been described: *Sphingobacterium antarcticum*, *Sphingobacterium daejeonense*, *Sphingobacterium faecium*, *Sphingobacterium mizutaii*, *Sphingobacterium multivorum*, *Sphingobacterium spiritivorum* and *Sphingobacterium thalpophilum* (Yabuuchi *et al.*, 1983; Shivaji *et al.*, 1992; Takeuchi & Yokota, 1992; Kim *et al.*, 2006). Cotton-waste composts are used as media for the cultivation of oyster mushrooms (*Pleurotus ostreatus*) in Korea. During the composting process, the compost temperature is gradually increased to 65 °C. Strain 4M24$^T$ was isolated by plating on trypticase soy agar (TSA, pH 7.0; Difco) at 30 °C; cultures were maintained on TSA medium.

The morphological, physiological and biochemical characteristics of strain 4M24$^T$ were investigated using routine cultivation on TSA medium at 30 °C. Gram staining, catalase and oxidase activities, and hydrolysis of casein, chitin, CM-cellulose, DNA, gelatin, starch, Tweens 20, 40 and 80, and tyrosine were investigated as described by Smibert & Krieg (1994). Growth was assessed at 5, 10, 20, 25, 30, 37, 40, 45 and 50 °C, at pH 4, 5, 6, 7, 8, 9 and 10, and at 0, 1, 3, 5 and 7 % NaCl. The strain was additionally characterized using the whole test spectrum of the API 20NE, API ID 32 GN and API ZYM systems (bioMérieux) according to the manufacturer's instructions. Sensitivity to antibiotics was determined with the routine disc-diffusion plate method. The following antibiotics were tested: ampicillin (10 μg), benzylpenicillin (10 μg), carbenicillin (100 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (15 μg), neomycin (30 μg), oleandomycin, polymyxin (300 U), streptomycin (10 μg) and tetracycline (30 μg).

Cells were harvested after 48 h growth on TSA medium, and the identification of fatty acids was performed according to the standard protocol of the Microbial Identification System (MIDI; Microbial ID). The DNA G+C content was determined according to the method of Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18-S; Supelco).

The 16S rRNA gene of strain 4M24$^T$ was amplified using PCR with primers fD1 and rP2 (Weisburg *et al.*, 1991); the entire PCR fragment was directly sequenced (Hiraishi, 1992). For phylogenetic analyses, the 16S rRNA gene

The GenBank accession number for the 16S rRNA gene sequence of strain 4M24$^T$ is EF122436.

sequences of the type strains of *Sphingobacterium* species (and *Pedobacter heparinus* DSM 2366$^T$, serving as an outgroup) were used. The 16S rRNA gene sequences were aligned using the MEGALIGN program (DNASTAR). A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) in MEGA, version 3 (Kumar *et al.*, 2004). The stability of relationships was assessed by performing bootstrap analyses of the neighbour-joining data, based on 1000 resamplings. The phylogenetic analyses were also assessed using maximum-parsimony analysis.

The cells of strain 4M24$^T$ were found to be Gram-negative, non-motile short rods. The phenotypic and chemotaxonomic characteristics that differentiate strain 4M24$^T$ from previously described *Sphingobacterium* species are listed in Table 1.

The major fatty acids were summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$, 37.5 %), iso-$C_{15:0}$ (29.5 %) and iso-$C_{17:0}$ 3-OH (19.7 %), which is a scenario common to all strains except *S. antarcticum* MTCC 675$^T$. However, some differences in the proportions of the fatty acids could be observed between strain 4M24$^T$ and its phylogenetically closest relative, *S. daejeonense* TR6-04$^T$. Fatty acids anteiso-$C_{15:0}$, iso-$C_{16:0}$ and $C_{17:0}$ 2-OH were absent from strain 4M24$^T$, but each represented $>2$ % of the fatty acid content of *S. daejeonense* TR6-04$^T$. The cellular fatty acid profiles of strain 4M24$^T$ and *Sphingobacterium* species are presented in Table 2. The DNA G+C content for the genus *Sphingobacterium* ranges from 37.3 to 44.2 mol%. The DNA G+C content of strain 4M24$^T$ (42.3 mol%) was within this range.

An almost-complete 16S rRNA gene sequence of strain 4M24$^T$ was obtained (1470 bp). Preliminary sequence comparisons with 16S rRNA gene sequences deposited in the GenBank database indicated that our isolate belonged to the genus *Sphingobacterium* of the *Bacteroidetes*. Strain 4M24$^T$ showed sequence similarities of 88.5–94.4 % with respect to type strains of the genus *Sphingobacterium*. The closest relative was *S. daejeonense* TR6-04$^T$ (94.4 %). According

**Table 1.** Differential characteristics of strain 4M24$^T$ and related members of the genus *Sphingobacterium*

Strains: 1, 4M24$^T$; 2, *S. daejeonense* TR6-04$^T$ (Kim *et al.*, 2006); 3, *S. spiritivorum* NBRC 14948$^T$; 4, *S. multivorum* NBRC 14947$^T$; 5, *S. mizutaii* ATCC 33299$^T$; 6, *S. thalpophilum* NBRC 14963$^T$; 7, *S. faecium* NBRC 15299$^T$ [data in columns 3–7 are from Takeuchi & Yokota (1992) and Steyn *et al.* (1998)]; 8, *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, D-maltose and sucrose. All strains are negative for Gram staining, sporulation, indole production, motility, assimilation of acetate and acid production from inositol. With the exception of *S. antarcticum* MTCC 675$^T$, all strains are positive for the assimilation of *N*-acetyl-D-glucosamine and salicin, and for acid production from D-melibiose and amygdalin. All strains are negative for the assimilation of D-fucose, gluconate, propionate, valerate, caprate, phenylacetate, 3-hydroxybenzoate, malate, itaconate, adipate, suberate and L-alanine (data are not reported for *S. antarcticum*). +, Positive; −, negative; V, variable; ND, no data available.

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

**Table 2.** Cellular fatty acid composition of strains 4M24$^T$ and *Sphingobacterium* species

Strains: 1, 4M24$^T$; 2, *S. daejeonense* TR6-04$^T$; 3, *S. mizutaii* DSM 11724$^T$ (data in columns 1–3 are from this study); 4, *S. spiritivorum* NBRC 14948$^T$; 5, *S. multivorum* NBRC 14947$^T$; 6, *S. thalpophilum* NBRC 14963$^T$; 7, *S. faecium* NBRC 15299$^T$ (data in columns 4–7 are from Steyn *et al.*, 1998); 8. *S. antarcticum* MTCC 675$^T$ (Shivaji *et al.*, 1992). +, Fatty acid detected but its content was not reported; –, not detected or <1 %.

| Fatty acid | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| $C_{14:0}$ | – | – | – | 1.0 | 2.7 | 3.2 | – | + |
| anteiso-$C_{15:0}$ | – | 4.1 | 2.3 | – | – | – | – | – |
| iso-$C_{15:0}$ | 29.5 | 26.1 | 27.4 | 30.1 | 22.2 | 17.7 | 24.6 | 29.0 |
| iso-$C_{15:0}$ 3-OH | 2.3 | 1.3 | 1.4 | 2.2 | 3.2 | 4.3 | 3.7 | – |
| iso-$C_{15:1}$ G | – | 1.2 | 1.2 | – | – | – | – | – |
| $C_{16:0}$ | 2.2 | 2.0 | 2.3 | 3.5 | 7.8 | 6.0 | 4.5 | + |
| $C_{16:0}$ 2-OH | – | – | – | – | – | 3.2 | – | – |
| $C_{16:0}$ 3-OH | 1.2 | – | 1.0 | 2.7 | 5.3 | 6.3 | 2.1 | – |
| $C_{16:0}$ 10-methyl | – | – | – | – | – | – | 1.4 | – |
| iso-$C_{16:0}$ | – | 2.3 | 1.6 | – | – | – | – | – |
| iso-$C_{16:0}$ 3-OH | – | 1.6 | – | – | – | – | – | – |
| iso-$C_{16:1}$ H | – | 1.0 | – | – | – | – | – | – |
| $C_{16:1}\omega5c$ | – | – | – | – | – | – | 1.5 | – |
| $C_{17:0}$ 2-OH | – | 2.4 | 1.4 | – | – | – | – | – |
| iso-$C_{17:0}$ 3-OH | 19.7 | 17.3 | 17.4 | 12.5 | 7.1 | 10.0 | 10.0 | - |
| $C_{17:1}$ | – | – | – | – | – | – | – | + |
| $C_{18:1}\omega7c$ | – | 1.0 | – | – | – | – | – | – |
| iso-$C_{17:1}\omega9c$ | 2.9 | 3.5 | 3.6 | 1.7 | – | – | – | – |
| Summed feature 3* | 37.5 | 29.8 | 33.6 | 42.7 | 49.0 | 47.8 | 48.1 | 56.0 |
| Unknown (ECL 13.566)† | – | – | – | – | – | 1.3 | 1.4 | – |

*Summed feature 3 contains $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH.
†ECL, Equivalent chain-length.

to the phylogenetic tree (Fig. 1), strain 4M24$^T$ forms a compact cluster with *S. daejeonense* and *S. mizutaii*.

On the basis of our phenotypic and phylogenetic studies, it is clear that strain 4M24$^T$ represents a member of the genus *Sphingobacterium*. Therefore, we conclude that strain 4M24$^T$ represents a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium composti* sp. nov. is proposed.

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

*Sphingobacterium composti* (com.pos′ti. N.L. gen. n. *composti* of compost).

Cells are Gram-negative, non-motile rods, 0.5–0.6 μm long by 1.0–2.0 μm wide. Colonies grown on TSA are yellow, circular and convex with entire margins. The temperature, pH and NaCl ranges for growth are 10–45 °C, pH 6–9 and 0–5 % NaCl, respectively. Nitrate is not reduced. Indole is not produced. Glucose is not fermented. Catalase, oxidase, arginine dihydrolase, β-galactosidase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase activities are present. Negative for esterase (C4), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase activities. Hydrolyses Tweens 20 and 80. Does not hydrolyse aesculin, casein, chitin, CM-cellulose, DNA, gelatin, starch, Tween 40, tyrosine or urea. Assimilates D-glucose, L-arabinose, D-mannose, *N*-acetylglucosamine, D-maltose, D-sucrose, salicin and D-melibiose. Does not assimilate D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-

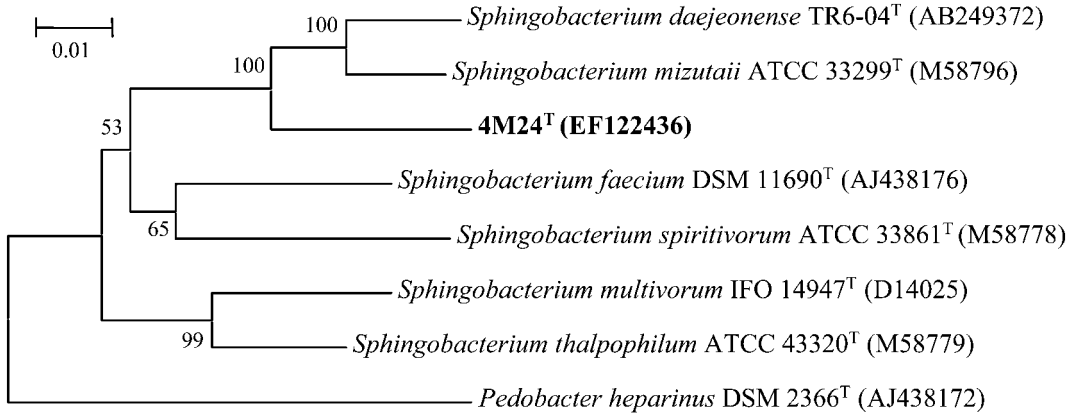


**Fig. 1.** Phylogenetic tree generated using the distance matrix and neighbour-joining method based on the 16S rRNA gene sequence of strain 4M24$^T$. Numbers at nodes represent bootstrap percentages based on 1000 samplings. Bar, 0.01 changes per nucleotide position.

alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or L-proline. Acids are produced from D-arabinose, L-arabinose, D-galactose, D-glucose, D-fructose, D-mannose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-sucrose, D-raffinose and L-fucose. Acids are produced weakly from D-xylose, D-melibiose, D-trehalose and gentiobiose. Acids are not produced from glycerol, erythritol, D-ribose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, *N*-acetylglucosamine, inulin, D-melezitose, starch, glycogen, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. Resistant to ampicillin (10 μg), benzylpenicillin (10 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (15 μg), neomycin (30 μg), oleandomycin, polymyxin (300 U) and streptomycin (10 μg). Sensitive to carbenicillin (100 μg) and tetracycline (30 μg). The major fatty acids are summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), iso-$C_{15:0}$ and iso-$C_{17:0}$ 3-OH. The G+C content of the genomic DNA is 42.3 mol%.

The type strain, 4M24$^T$ (=KACC 11313$^T$=DSM 18850$^T$), was isolated from cotton-waste composts in South Korea.

## Acknowledgements

This study was carried out with the support of the programme of international joint research by the Rural Development Administration (South Korea) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany).

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