Sphingobacterium cibi sp. nov., isolated from the food-waste compost and emended descriptions of *Sphingobacterium spiritivorum* (Holmes *et al.* 1982) Yabuuchi *et al.* 1983 and *Sphingobacterium thermophilum* Yabe *et al.* 2013

Wei-An Lai,[1,2] Asif Hameed,[2] You-Cheng Liu,[2] Yi-Han Hsu,[2] Shih-Yao Lin[2] and Chiu-Chung Young[1,2]

Correspondence
Chiu-Chung Young
ccyoung@mail.nchu.edu.tw

[1]Agricultural Biotechnology Center, National Chung Hsing University, Taichung, Taiwan, ROC

[2]Department of Soil and Environmental Sciences, College of Agriculture and Natural Resources, National Chung Hsing University, Taichung, Taiwan, ROC

A novel, pale yellow-coloured, Gram-staining-negative, aerobic, rod-shaped and non-motile bacterium, designated as strain CC-YY411^T, was isolated from the food-waste compost and subjected to polyphasic taxonomy. Strain CC-YY411^T exhibited the highest pairwise 16S rRNA gene sequence similarity to *Sphingobacterium thermophilum* JCM 17858^T (94.6 %) while sharing 94.1 to 89.7 % similarities with other *Sphingobacterium* species. Novel strain established a discrete phylogenetic lineage within the clade that accommodated validly established members of the genus *Sphingobacterium*. The polar lipid profile of strain CC-YY411^T contained major amounts of phosphatidylethanolamine, one unidentified lipid and two unidentified aminolipids besides accommodating trace amounts of a sphingolipid, two phospholipids, an unidentified aminophospholipid and an unidentified glycolipid. The DNA G+C content of strain CC-YY411^T was 34.5 mol%. The major and minor respiratory quinones were MK-7 (89.1 %) and MK-6 (10.9 %), respectively. The predominant fatty acids were iso-$C_{15:0}$ (24.0 %), iso-$C_{17:0}$ 3-OH (11.9 %), iso-$C_{15:0}$ 3-OH (6.9 %), $C_{15:1}\omega5c$ (5.5 %) and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) (32.0 %). Based on the phylogenetic, phenotypic and chemotaxonomic distinctiveness, strain CC-YY411^T represents a novel species of *Sphingobacterium*, for which the name *Sphingobacterium cibi* sp. nov. is proposed. The type strain is CC-YY411^T (=BCRC 80430^T=JCM 18407^T). Amended species descriptions of *Sphingobacterium spiritivorum* (Holmes *et al.* 1982) Yabuuchi *et al.* 1983 and *Sphingobacterium thermophilum* Yabe *et al.* 2013 are also proposed based on new data obtained in this study.

Sphingobacterium, the type genus of family *Sphingobacteriaceae* in phylum *Bacteroidetes*, was first described by Yabuuchi *et al.* (1983) based mainly on its high concentration of sphingolipids. The *Sphingobacterium* strains are Gram-staining-negative rods that are positive for activities of catalase and oxidase but negative for gelatinase activity (Sun *et al.*, 2015). Their main fatty acids are iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, $C_{16:1}\omega7c$ and $C_{17:0}$ 3-OH. At the time of writing, there were one 'in press' and two published *Sphingobacterium* descriptions (*Sphingobacterium populi* Li *et al.*, 2016; *Sphingobacterium jejuense* Siddiqi *et al.*, 2016; *Sphingobacterium chuzhouense* Wang *et al.*, 2016), an invalid publication ('*Sphingobacterium yamdrokense*' Xiao *et al.*, 2015) and a total of 36 validly established *Sphingobacterium* species as listed in the LPSN (http://www.bacterio.net/sphingobacterium.html; Parte, 2014). *Sphingobacterium* species have been isolated from diverse habitats including clinical specimen (Holmes *et al.*, 1982; Yabuuchi *et al.*, 1983), fresh water (Albert *et al.*, 2013), compost (Kim *et al.*, 2006; Siddiqi *et al.*, 2016; Ten *et al.*, 2006), contaminated soil (Choi & Lee, 2012), wetland soil (Feng *et al.*, 2014), forest and farm soil (He *et al.*, 2010; Liu *et al.*, 2008, 2013; Peng *et al.*, 2014; Wang *et al.*, 2016; Wei *et al.*, 2008; Xiao *et al.*, 2013; Yabe *et al.*, 2013), rhizosphere soil (Sun *et al.*, 2015), sandy or desert soil (Jiang *et al.*, 2014; Zhao *et al.*,

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Four supplementary figures are available with the online Supplementary Material.

2014), raw milk (Schmidt *et al.*, 2012), activated sludge (Sun *et al.*, 2013; Zhang *et al.*, 2012), plants (Kämpfer *et al.*, 2016; Lee *et al.*, 2013; Li *et al.*, 2016; Liu *et al.*, 2012) and insects (Long *et al.*, 2016). Here, we describe the isolation and polyphasic taxonomic characterization of a novel strain designated CC-YY411^T, presumably affiliated to the genus *Sphingobacterium*.

Strain CC-YY411^T was isolated from a food-waste compost sample collected at Taichung, Taiwan, through the standard serial dilution-to-extinction plating technique using nutrient agar (NA; HiMedia) as culture media. The NA plates were incubated at 30 °C for 48 h, and the pale yellow-coloured colonies of strain CC-YY411^T were picked up and purified. Two type strains of *Sphingobacterium*, *Sphingobacterium thermophilum* JCM 17858^T and *Sphingobacterium* spiritivorum JCM 1277^T (type species) were used for direct comparative taxonomic analysis.

Bacterial genomic DNA was extracted by using UltraClean microbial DNA isolation kit (Mo Bio) according to the manufacturer's instructions. The 16S rRNA gene of strain CC-LY411^T was amplified by using the total genomic DNA as template by PCR (ABI 9700) and sequenced using oligonucleotide primers 1F (5′-GAG TTT GAT CAT GGC TCA G-3′) and 9R (5′-AAG GAG GTG ATC CAA CCG CA-3′), which are complementary to conserved regions of the 16S rRNA gene of *Escherichia coli* (Brosius *et al.*, 1978; Edwards *et al.*, 1989). The PCR product was purified by using the QIAquick gel extraction kit (Qiagen) and sequenced as described in Young *et al.* (2005).

The 16S rRNA gene sequence similarity values were calculated using EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007) and NCBI GenBank through BLAST search. Closely related sequences were retrieved from the public databases, and phylogenetic analyses were done using the software MEGA-6 (Tamura *et al.*, 2013) after multiple alignment of the sequences by CLUSTAL_X (1.83) program (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed using neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms. A bootstrap analysis (Felsenstein, 1985) was performed according to the algorithm of Kimura's two-parameter model (Kimura, 1980) based on 1000 resamplings (Felsenstein, 1993).

16S rRNA gene amplicon of strain CC-YY411^T contained 1495 bp (*E. coli* positions 22–1558) (Brosius *et al.*, 1978). Strain CC-YY411^T shared the highest sequence similarity (94.6 %) with *S. thermophilum* CKTN2^T, whereas with other *Sphingobacterium* species, it shared sequence similarities of 94.1 to 89.7 %. In particular, the pairwise similarity between strain CC-YY411^T and the type species of *Sphingobacterium* (*S. spiritivorum* NCTC 11386^T) was estimated to be 93.0 %. Given several species delimitation threshold of the 16S rRNA gene sequence similarity, such as 98.7 % (Kim *et al.*, 2014), 98.7 to 99.0 % (Stackebrandt & Ebers, 2006) and 98.2 to 99.0 % (Meier-Kolthoff *et al.*, 2013), the similarity values obtained for CC-YY41^T with regard to *Sphingobacterium* species were well below the reported threshold.

The exact taxonomic position of strain CC-YY411^T with respect to other *Sphingobacterium* species was assessed by multiple phylogenetic analyses. Strain CC-YY411^T was found to establish a discrete phyletic lineage within the clade that accommodated validly established *Sphingobacterium* species irrespective of low bootstrap support (Fig. 1). The low bootstrap confidence of the node could be considered as an indication of phylogenetic instability of strain CC-YY411^T with regard to other *Sphingobacterium* species, as no further evidence was obtained to state that it could be a novel representative of a new genus. Taken together, pairwise similarity values and phylogenetic analyses indicated that strain CC-YY411^T is most likely to be a novel species of *Sphingobacterium*.

Different culture media including actinomycete isolation agar (AIA; HiMedia), JCM medium no. 22 (NA no. 2), R2A agar (BD), tryptic soy agar (TSA; BBL), yeast mannitol agar (YMA) and NA (HiMedia) were used to test the growth of all three strains at 30 °C for 2 days. Strain CC-YY411^T exhibited good growth on NA no. 2 and NA and weak growth on R2A, but growth was not observed on AIA, YMA and TSA. On the contrary, *S. thermophilum* JCM 17858^T and *S. spiritivorum* JCM 1277^T grew well on TSA, R2A, NA no. 2 and NA and grew weakly on AIA and YMA.

Colonies were examined for morphological features such as colony appearance, size, shape, texture and pigmentation. Cell morphology including presence of flagella of CC-YY411^T was determined by placing the cells (1–2 days old) on a carbon-coated copper grid followed by staining with 0.2 % uranyl acetate for 5 to 10s, brief air drying and observation under transmission electron microscope (JEOL JEM-1400). Colonies of strain CC-YY411^T were 2 to 3.3 mm diameter, white, opaque and circular with entire margins on NA (HiMedia) after 48 h cultivation at 30 °C for 2 days. Microscopic examination showed that cells of strain CC-YY411^T were rod shaped without any flagella. Cells were 0.4 to 0.5 μm in width and 0.9 to 1.4 μm in length (Fig. S1, available in the online Supplementary Material).

Gram staining of strain CC-YY411^T was performed according to Murray *et al.* (1994). Cells of CC-YY411^T showed Gram-negative reaction. Swimming, swarming and twitching motilities were tested by point inoculation of the cells on the centre of NB no. 2 plates supplemented with 0.3, 0.5 and 1.0 % agar, respectively, followed by incubation at 30 °C for 72 h (modified from Rashid & Kornberg, 2000). No detectable spreading growth of these three strains occurred on media containing 0.5 and 1.0 % agar. However, *S. thermophilum* JCM 17858^T exclusively showed distinctive spreading growth on medium containing 0.3 % agar (Fig. S2). All strains found to lack flagella when evaluated through the microscopy of cells stained with the dyespecific for flagella (Clark, 1976). *Sphingobacterium* may exhibit locomotion based on a flagellum-independent mechanism known as gliding motility (Yabuuchi *et al.*, 1983), which

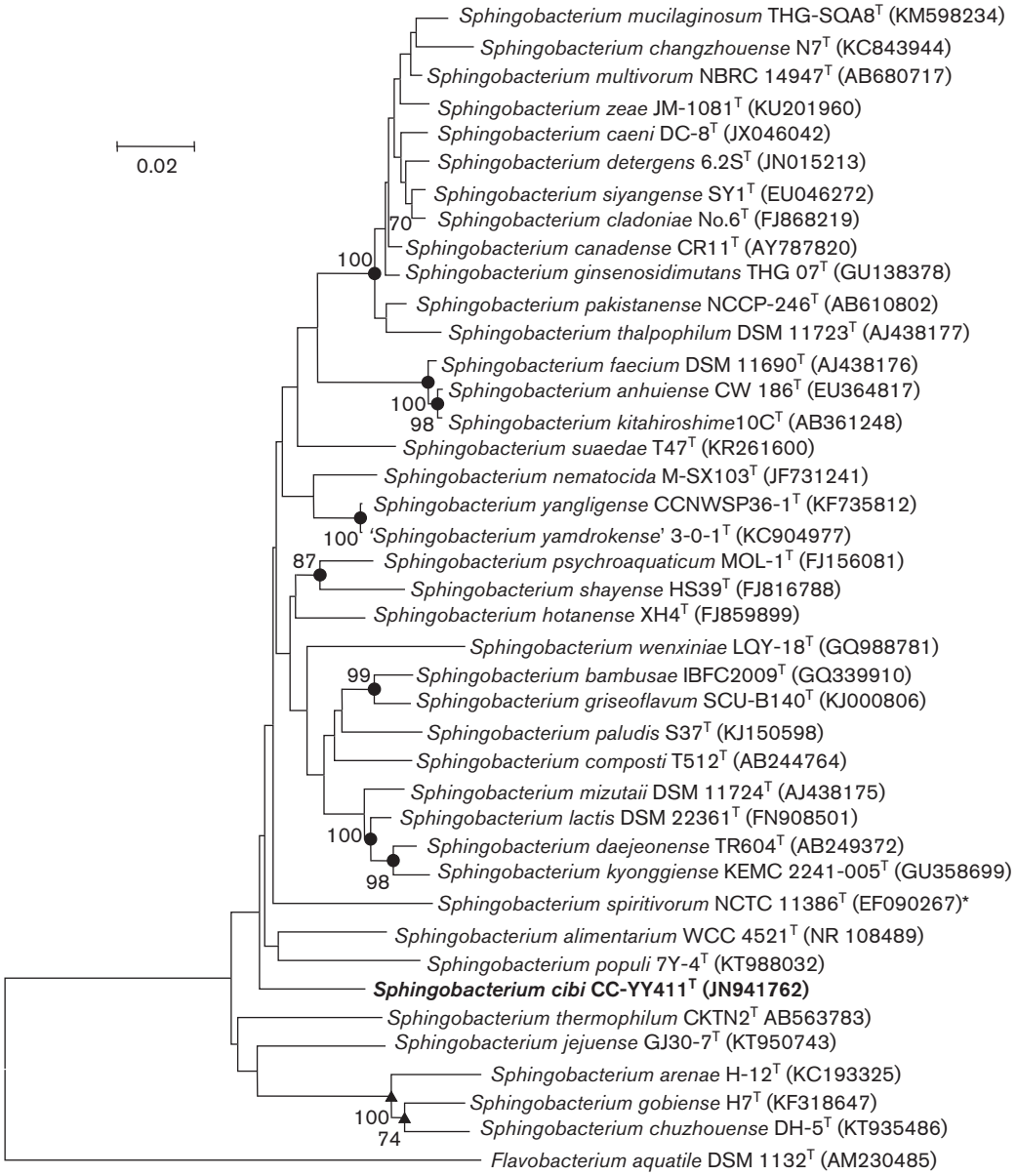

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing phylogenetic relationship of strain CC-YY411^T and other known *Sphingobacterium* species. Bootstrap values (>70 %) after 1000 replications are shown at the branching points. *Flavobacterium aquatile* DSM 1132^T was used as an outgroup. Filled triangle (▲), or circles (●) indicate that the corresponding nodes are also recovered in the trees generated using maximum-likelihood (ML), maximum-parsimony (MP) or ML and MP algorithms (with >70 % bootstrap support). Bar, 0.02 substitutions per nucleotide position.

could be a possible explanation for the spreading growth of *S. thermophilum* JCM 17858^T detected in this study.

The pH range for growth was determined in NB no. 2 at pH 3.0 to 10.0 (at 1.0 pH unit intervals) using 100 mM buffer solutions (citric acid–sodium citrate for pH 3–5, disodium hydrogen phosphate–sodium dihydrogen phosphate for pH 6–8 and sodium carbonate–sodium bicarbonate for pH 9–10). Salinity tolerance was tested using NB no. 2 supplemented with 0 to 20 % (w/v) NaCl (at 0.5 % intervals). Growth at various temperatures (10, 15, 20, 25, 30, 37, 42, 47 and 50 °C) was measured in NB. Anaerobic growth was tested using NB no. 2 supplemented with 0.1 % (w/v) KNO_3 by incubating the culture plates in an anaerobic chamber (Coy, USA) for 1 week. Carbon sources utilization (oxidation) was determined by using Biolog GN2 (bioMérieux). Acid productions from 49 carbohydrates were determined by using API 50 CH system (bioMérieux). Nitrate reduction, indole production, activities of β-galactosidase, urease, hydrolysis of

aesculin and gelatin and assimilation of 12 substrates were tested with API 20 NE strips (bioMérieux). The activities of various enzymes were detected by using API ZYM system (bioMérieux). Catalase activity was determined by assessing bubble production by cells in 3 % (v/v) H_2O_2, and oxidase activity was determined by using 1 % (w/v) *N*,*N*,*N*,*N*-tetramethyl-1,4-phenylenediamine reagent (bioMérieux).

Strain CC-YY411^T can be distinguished particularly from *S. thermophilum* JCM 17858^T based on its lack of motility, absence of growth on AIA, YMA, TSA and R2A, relatively moderate temperature range for growth, lack of α-glucosidase and β-glucosidase activities and lack of assimilation of *N*-acetylglucosamine and glucose (Table 1). Additional characteristics that differentiated strain CC-YY411^T from the reference strains are summarized in Table 1. The detailed phenotypic characteristics of strain CC-YY411^T are given in the species description.

Cells were grown on NB no. 2 media supplemented with 1.5 % agar at 30 °C for 2 to 3 days and harvested during mid-exponential growth phase for fatty acid, polar lipid and quinone analysis. Fatty acid methyl esters were prepared, separated and identified according to the standard protocol (Paisley, 1996) of the Microbial Identification System (MIDI) (Sasser, 1990) by using a gas chromatograph (Agilent 7890A) fitted with a flame-ionization detector. The detailed fatty acid profile of strain CC-YY411^T is compared with those of the type strains of related recognized *Sphingobacterium* species (Table 2). The major cellular fatty acids of strain CC-YY411^T were iso-$C_{15:0}$ (24.0 %), iso-$C_{17:0}$ 3-OH (11.9 %), iso-$C_{15:0}$ 3-OH (6.9 %), $C_{15:1}\omega5c$ (5.5 %) and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) (32.0 %). Minor amounts of anteiso-$C_{15:0}$ (4.3 %), $C_{16:0}$ (4.7 %), $C_{16:0}$ 3-OH (1.7 %), iso-$C_{16:0}$ 3-OH (1.7 %) and $C_{17:0}$ 2-OH (1.2 %) were also found. All three strains consistently accommodated iso-$C_{15:0}$, $C_{15:1}\omega5c$, iso-$C_{15:0}$ 3-OH and $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ in major amounts. Nevertheless, strains can be distinguished from each other based on the qualitative and quantitative variation in terms of several fatty acid components as shown in Table 2. Fatty acid profile of strain CC-YY411^T was found to be more similar to *S. spiritivorum* JCM 1277^T than *S. thermophilum* JCM 17858^T. Thus, fatty acid data validated the distinction between the novel strain and reference strains in general and *S. thermophilum* JCM 17858^T in particular.

The polar lipids of strain and two reference strains were extracted, dried using rotary vacuum evaporator and analysed by two-dimensional TLC (Embley & Wait, 1994). Ethanolic molybdatophosphoric acid (10 %), Zinzadze reagent, 0.2 % ninhydrin and α-naphthol reagents were used to visualize the spots corresponding to total lipids, phospholipids, aminolipids and glycolipids, respectively. Sphingolipids were analysed according to Kato *et al.* (1995) with slight modifications: aliquots of dry crude lipids were hydrolysed at 37 °C for 20 h with 100 µl of 0.5 M KOH in methanol, followed by neutralization with 50 µl of 1 M HCl. Hydrolysed lipids were extracted through chloroform/methanol (2 : 1, v/v) and loaded (20 µl) on to TLC Silica gel 60 F254 (Merck), dried and subjected to one-dimensional chromatography using chloroform/methanol/6.7 M ammonium hydroxide (33 : 15 : 1.25, v/v/v). TLC plates after chromatography were air dried and initially sprayed with 0.2 % ninhydrin and heated at 100 °C for 5 min. Alkali-stable sphingolipids were unambiguously localized after ninhydrin staining. Since phosphatidylethanolamine spot readily get bleached as a consequence of KOH treatment, it was used as a marker to indicate efficacy of lipid hydrolysis mediated by KOH. Polar lipids were quantified by densitometry with ImageJ software (http://imagej.nih.gov/ij/; Abràmoff *et al.*, 2004).

The total polar lipids of all three strains are shown in Fig. 2 and summarized in Table 2. Strain CC-YY411^T contained phosphatidylethanolamine, one unidentified lipid (L1) and two unidentified aminolipids (AL1–2, stained poorly with 0.2 % ninhydrin; Fig. S3) as major polar lipids besides accommodating trace amounts of a sphingolipid (SL1, Fig. S4), two unidentified phospholipids (PL1–2), an unidentified aminophospholipid and an unidentified glycolipid. All three strains consistently accommodated phosphatidylethanolamine, L1 and AL1–2 as major polar lipids. In addition, all three strains found to possess sphingolipids irrespective of qualitative and quantitative variations (Figs 2, S3 and Table 2). Strain CC-YY411^T strikingly lacked co-migrating phosphoglycolipids that were found in other two species and two unidentified phospholipids (PL3–4) found in *S. spiritivorum* JCM 1277^T. Sphingolipids are one of the important chemotaxonomic markers used to distinguish *Sphingobacterium* species (Yabuuchi *et al.*, 1983). The present polar lipid data indicated possible species-specific disparity existing particularly regarding the synthesis of sphingolipids, unidentified phospholipids and unidentified glycolipids in *Sphingobacterium* species. Nonetheless, consistent detection of sphingolipids and other major lipids in all three strains supported the classification of strain CC-YY411^T as a *Sphingobacterium* species.

The respiratory quinones of strain were extracted according to Minnikin *et al.* (1984) and analysed by Reversed phase HPLC according to Collins (1985). Menaquinone with seven isoprenoid units (MK-7) was detected as the predominant (89.1 %) respiratory quinone in strain CC-YY411^T. In addition, MK-6 was also detected as minor amounts (10.9 %). MK-7 was identified as a major respiratory quinone in *S. thermophilum* JCM 17858^T, although no information is available on possible minor quinones (Yabe *et al.*, 2013). *Sphingobacterium* species commonly produce MK-7 as a major respiratory quinone (Kämpfer *et al.*, 2016; Kim *et al.*, 2006; Liu *et al.*, 2012; Li *et al.*, 2015, 2016; Siddiqi *et al.*, 2016; Wang *et al.*, 2016; Yabe *et al.*, 2013). In addition, the presence of MK-6 as minor quinone has been reported in *Sphingobacterium psychroaquaticum* (Albert *et al.*, 2013; 3 %) and *Sphingobacterium zeae* (Kämpfer *et al.*, 2016; 1 %). Thus, the quinone profile of strain CC-YY411^T is in excellent agreement with *Sphingobacterium* species.

Table 1. Differential characteristics of the novel strain and related *Sphingobacterium* species

Taxa: 1, Strain CC-YY411^T; 2, *S. thermophilum* JCM 17858^T; 3, *S. spiritivorum* JCM 1277^T. All data are from this study. All strains can grow on NA (HiMedia) and NB no. 2 or NB no. 2 supplemented with 1.5 % agar and are non-motile and positive for catalase, cytochrome oxidase, *N*-acetyl-glucosaminidase, alkaline phosphatase, acid phosphatase, butyrate (C4) esterase, caprylate (C8) esterase, cystine arylamidase, leucine arylamidase, naphthol-AS-BI-phosphorylase and valine arylamidase. All strains are negative for nitrite reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase, myristate esterase and β-glucuronidase and assimilation of adipic acid, arabinose, capric acid, citric acid, mannitol, phenylacetic acid and potassium gluconate. +, Positive; −, negative; w, weakly positive. AIA, actinomycete isolation agar; YMA, yeast mannitol agar; TSA, tryptic soy agar.

Characteristic	1	2	3
Motility	−	w	−
Growth on:			
AIA, YMA	−	w	w
TSA	−	+	+
R2A	w	+	+
Growth at/with (in NB no. 2):			
Temperature (°C)	20–42	25–50	15–30
NaCl (%, w/v)	0–3	0–3	0–3.5
pH	6–8	7–8	6–9
Anaerobic growth	−	−	+
Enzymes:			
Chymotrypsin, trypsin	+	+	w
α-Galactosidase	w	w	+
β-Galactosidase (PNPG)	+	w	w
α-Glucosidase, β-glucosidase	−	+	+
α-Mannosidase	−	w	−
Urease, α-fucosidase	−	−	+
Assimilation from (API 20 NE):			
N-Acetylglucosamine, glucose	−	+	+
Maltose	−	w	+
Mannose	−	−	+
Oxidation of carbon sources (Biolog GN2):			
Acetic acid	−	w	−
L-Arabinose	−	+	+
D-Arabitol, citric acid, dextrin, D-galacturonic acid, D-gluconic acid, α-D-glucose-1-phosphate, D-glucose-6-phosphate, D,L-α-glycerol phosphate, glycyl-L-aspartic acid, α-hydroxybutyric acid, *N*-Acetyl-D-galactosamine, D-psicose, raffinose, D-saccharic acid, D-sorbitol	−	−	w
L-Asparagine, α-ketobutyric acid, L-serine	+	−	−
L-Aspartic acid	w	−	−
Cellobiose, L-fucose, D-glucosaminic acid, glucuronamide, glycerol, D-mannitol, D-mannose	−	−	+
α-Cyclodextrin	−	w	w
D-Fructose	w	w	w
D-Galactose, α-D-glucose, lactose	−	w	+
Gentibiose	+	w	+
L-Glutamic acid	+	+	w
Glycogen, hydroxy-L-proline, lactulose, threonine	−	w	−
Glycyl-L-glutamic acid	−	+	−
α-Ketovaleric acid, L-ornithine, propionic acid	w	−	−
D,L-Lactic acid	w	−	w
Maltose, *N*-acetyl-D-glucosamine, sucrose	+	−	+
Melibiose	−	w	w
Methyl β-D-glucoside	−	+	+
L-Proline	+	+	−
Succinic acid methyl ester	+	w	−
Trehalose	w	−	+
Turanose	w	w	−

Table 2. Differential chemotaxonomic and genotypic characteristics of the novel strain and related *Sphingobacterium* species

Taxa: 1, Strain CC-YY411^T; 2, *S. thermophilum* JCM 17858^T; 3, *S. spiritivorum* JCM 1277^T. All data are from this study unless indicated otherwise. Values of fatty acid (%) are shown as means ±SD (n=4). The values more than 5 % are shown in boldtype; –, not detectable or <1 %. Values of polar lipid (%) are shown by means of image analysis estimation. Relative quantities of polar lipids were estimated by densitometry with ImageJ software (http://imagej.nih.gov/ij/; Abràmoff *et al.*, 2004).

Characteristic	1	2	3
Fatty acid (%)			
$C_{14:0}$	–	–	2.4±0.3
Iso-$C_{15:0}$	**24.0±0.1**	**40.0±2.1**	**24.0±0.3**
Iso-$C_{15:0}$ 3-OH	**6.9±0.5**	2.8±0.1	**4.9±0.1**
Anteiso-$C_{15:0}$	4.3±0.1	–	1.4±0.2
$C_{15:1}\omega5c$	**5.5±0.3**	**8.2±0.1**	**5.2±0.1**
$C_{16:0}$	4.7±0.0	–	4.4±0.1
$C_{16:0}$ 3-OH	1.7±0.1	–	**5.0±0.1**
Iso-$C_{16:0}$ 3-OH	1.7±0.1	–	–
$C_{16:1}$ 2-OH	–	–	1.3±0.0
$C_{17:0}$ 2-OH	1.2±0.1	–	–
Iso-$C_{17:0}$ 3-OH	**11.9±0.7**	**17.0±1.1**	**8.6±0.7**
Iso-$C_{17:1}\omega9c$	1.3±0.0	**5.5±0.4**	2.3±0.8
Summed feature 3*	**32.0±1.7**	**21.1±1.6**	**32.3±0.7**
Sphingolipids			
SL1	+(minor)	+(minor)	+(9.0 %)
SL2	–	+(minor)	+(18.1 %)
Major polar lipids			
Phosphatidylethanolamine	+(28.6 %)	+(24.5 %)	+(19.3 %)
Unidentified phosphoglycolipid	–	+(20 %)	+(2.6 %)
Unidentified polar lipid (L1)	+(28.7 %)	+(18.8 %)	+(20.4 %)
Unidentified aminolipids (AL1, AL2)	+(23.0 %, 15.4 %)	+(24.5 %, 7.4 %)	+(16.3 %, 10.0 %)
G+C content (mol%)	34.5	40.3†	40.0‡

*Summed feature 3 consisted of $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$.
†Data from Yabe *et al.* (2013).
‡Data from Yabuuchi *et al.* (1983).

The genomic DNA G+C content of strain CC-YY411^T was determined by HPLC according to Mesbah *et al.* (1989). Strain CC-YY411^T was estimated to contain 34.5 mol% genomic DNA G+C content, which is slightly lesser as compared to that of other two reference strains investigated in this study (Table 2). Species of *Sphingobacterium* such as *Sphingobacterium cladoniae* No.6^T (Lee *et al.*, 2013; 36.8 %), *Sphingobacterium composti* (Ten *et al.*, 2006; 35.4 %), *Sphingobacterium daejeonense* (Kim *et al.*, 2006; 38.7 %), *S. faecium* (Takeuchi & Yokota, 1992; 37.3 %), *Sphingobacterium siyangense* (Liu *et al.*, 2008; 38.5 %) and *Sphingobacterium rhinocerotis* (Li *et al.*, 2015; 38.9 %) identified to possess <40 mol% G+C content. In general, a difference of <5 mol% in G+C content is adequate to assign two micro-organisms to the same species, and the difference by more than 10 mol% G+C should not be classified within the same genus (Goodfellow & O'Donnell, 1993). Thus, the genomic DNA G+C content data clearly favoured the classification of strain CC-YY411^T within the genus *Sphingobacterium*.

Taken together, strain CC-YY411^T can be readily distinguished at the species level from other *Sphingobacterium* representatives in general and from *S. thermophilum* JCM 17858^T in particular based on pairwise 16S rRNA gene sequence similarity values, phylogenetic instability, polar lipid profile, DNA G+C content, fatty acid profile, quinone composition and many other biochemical characteristics. Thus, strain CC-YY411^T considered to represent a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium cibi* sp. nov. is proposed. In addition, based on new data obtained in this study, we amend the species descriptions of *S. spiritivorum* (Holmes *et al.*, 1982) Yabuuchi *et al.* (1983) and *S. thermophilum* Yabe *et al.* (2013).

Emended description of *Sphingobacterium spiritivorum* (Holmes *et al.* 1982) Yabuuchi *et al.* 1983

The species description is according to Holmes *et al.* (1982) and Yabuuchi *et al.* (1983) with following amendments: besides having two distinct sphingolipids in large amounts, phosphatidylethanolamine, one unidentified lipid, two unidentified aminolipids and several unidentified co-migrating phosphoglycolipids are also present as the major polar lipid components. In addition, four unidentified phospholipids are present in moderate-to-trace amounts.

Emended description of *Sphingobacterium thermophilum* Yabe *et al.* 2013

The species description is according to Yabe *et al.* (2013) with following amendments: polar lipid profile contains major amounts of phosphatidylethanolamine, one unidentified lipid, two unidentified aminolipids and several unidentified co-migrating phosphoglycolipids. In addition, two distinct sphingolipids and an unidentified phospholipid are present in moderate amounts, and an unidentified aminophospholipid is present in trace amounts.

Description of *Sphingobacterium cibi* sp. nov.

Sphingobacterium cibi (ci'bi. L. gen. n. *cibi* of food).

The cells are rod shaped, 0.4 to 0.5 μm in diameter and 0.9 to 1.4 μm in length, non-motile and Gram-stain-negative. Good growth occurs in NB no. 2 or on NB no. 2 supplemented with 1.5 % agar and NA (HiMedia) and weak

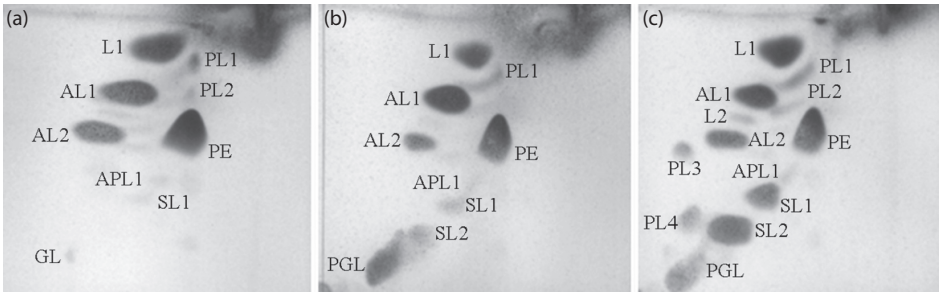

Fig. 2. Total polar lipids of *Sphingobacterium* species as determined by two-dimensional TLC. (a) Strain CC-YY411^T; (b) *S. thermophilum* JCM 17858^T; (c) *S. spiritivorum* JCM 1277^T. Total polar lipids were visualized by spraying the TLC plates with 10 % ethanolic molybdatophosphoric acid. SL1–2, sphingolipids; PE, phosphatidylethanolamine; PL1–PL4, unidentified phospholipids; AL1–2, unidentified aminolipid; APL, unidentified aminophospholipid; GL, unidentified glycolipid; PGL, unidentified phosphoglycolipid; L1–2, unidentified polar lipids, which were not detectable with any of the spray reagents specific for phosphate-, amino- or sugar moieties.

growth on R2A (BD) at 30 °C. Colonies grown on NA are opaque, circular, with entire margins and pale yellow coloured and usually 2 to 3.3 mm in diameter after 48 h of incubation at 30 °C. The conditions for growth are 20 to 42 °C, pH 6 to 8 and 0 % to 3 % NaCl. Cells are positive for cytochrome oxidase, catalase, aesculin hydrolysis (β-glucosidase), alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphorylase, butyrate (4C) esterase, caprylate (8C) esterase, trypsin, α-chymotrypsin, leucine arylamidase, cystine arylamidase, valine arylamidase and α-galactosidase, *N*-acetyl-glucosaminidase and β-galactosidase but are negative for indole production, nitrate reduction, urease, glucose fermentation, arginine dihydrolase, gelatinase, myristate esterase, α-fucosidase, α-glucosidase, β-glucosidase, α-mannosidase and β-glucuronidase activities and assimilation of adipic acid, arabinose, capric acid, citric acid, mannitol, phenylacetic acid and potassium gluconate (API ZYM and API 20 NE systems). Acid is produced from aesculin but is not produced from the other tested substrates (API 50 CH system). The following substrates in the Biolog GN2 MicroPlate are oxidized: L-asparagine, gentibiose, L-glutamic acid, α-ketobutyric acid, maltose, *N*-acetyl-D-glucosamine, L-proline, L-serine, succinic acid methyl ester and sucrose. L-Aspartic acid, D-fructose, α-ketovaleric acid, D,L-lactic acid, L-ornithine, propionic acid, trehalose and turanose are weakly positive. Other substrates in the Biolog GN2 MicroPlate are not oxidized. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:0}$ 3-OH and $C_{15:1}\omega5c$ and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). MK-7 and MK-6 are the major and minor respiratory quinones, respectively. Phosphatidylethanolamine, one identified lipid and two unidentified aminolipids are present in major amounts. In addition, a sphingolipid, two unidentified phospholipids, an unidentified aminophospholipid and an unidentified glycolipid are also present in trace amounts.

The type strain is CC-YY411^T (=BCRC 80430^T=JCM 18407^T), which was isolated from a food-waste compost sample collected at Taichung, Taiwan. The genomic DNA G+C content of the type strain is 34.5 mol%.

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