Sphingosinicella vermicomposti sp. nov., isolated from vermicompost, and emended description of the genus *Sphingosinicella*

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A Gram-stain-negative, rod-shaped bacterium, designated strain YC7378^T was isolated from vermicompost (VC) collected at Masan, Korea, and its taxonomic position was investigated by using a polyphasic approach. Strain YC7378^T grew optimally at 30 °C and at pH 6.5–8.5. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YC7378^T belongs to the genus *Sphingosinicella* in the family *Sphingomonadaceae*. The most closely related strains are *Sphingosinicella soli* KSL-125^T (95.7 %), *Sphingosinicella xenopeptidilytica* 3-2W4^T (95.6 %) and *Sphingosinicella microcystinivorans* Y2^T (95.5 %). Strain YC7378^T contained ubiquinone Q-10 as the major respiratory quinone system and sym-homospermidine as the major polyamine. The major fatty acids of strain YC7378^T were C_{18:1}ω7c, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, C_{14:0} 2-OH and C_{16:0}. The major polar lipids were sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The total DNA G + C content was 59.4 mol%. The phenotypic, phylogenetic and chemotaxonomic data showed that strain YC7378^T represents a novel species of the genus *Sphingosinicella*, for which the name *Sphingosinicella vermicomposti* sp. nov. is proposed. The type strain is YC7378^T (=KCTC 22446^T =DSM 21593^T).

The genus *Sphingosinicella*, grouped in the family *Sphingomonadaceae*, belongs to the *Alphaproteobacteria* and was proposed by Maruyama *et al.* (2006) with the description of a single species, *Sphingosinicella microcystinivorans* Y2^T. Subsequently, two other species of the genus *Sphingosinicella*, *Sphingosinicella xenopeptidilytica* 3-2W4^T and *Sphingosinicella soli* KSL-125^T were described (Geueke *et al.*, 2007; Yoon *et al.*, 2008). At the time of writing, the names of three species of the genus *Sphingosinicella* have been validly published.

Vermicompost (VC) is an organic manure (bio-fertilizer) produced as the vermicast by earthworms feeding on biological waste materials. Vermicomposting involves the bio-oxidation and stabilization of organic matter through the joint action of earthworms and micro-organisms (Aria

et al., 2002). During analysis of the bacterial community in VC, we succeeded in isolating many novel strains by using an improved isolation method (Aslam *et al.*, 2009). The majority of the novel bacteria isolated belonged to the phylum ‘Proteobacteria’. Among them only one strain, YC7378^T, was related to the genus *Sphingosinicella*. The other novel strains were related to the members of the genera *Xanthomonas* and *Aminobacter*, while some belonged to the *Betaproteobacteria*. Here we report the taxonomic characterization of the newly isolated bacterial strain YC7378^T, which represents a novel species in the genus *Sphingosinicella* for which we propose the name *Sphingosinicella vermicomposti* sp. nov.

The vermicompost sample, prepared with paper mill and dairy sludge, was collected from a commercial farm at Masan, Korea. One gram of VC (fresh weight) was dispersed in a 100 ml aliquot of sterile distilled water and was treated (sonication) or untreated prior to serial dilution (10⁻³–10⁻⁷) (Janssen *et al.*, 2002). A 200 µl aliquot of each dilution from each sample was spread on three replicate plates of 0.08 g nutrient broth l⁻¹ (Difco) supplemented with VC aqueous extract (20 % w/v, 0.2 µm filter-sterilized) and 1.5 % agar. The plates were incubated

Abbreviation: VC, vermicompost.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YC7378^T is FJ442859.

A transmission electron micrograph of strain YC7378^T, a two-dimensional TLC of polar lipids from strain YC7378^T, and a phylogenetic tree based on maximum-likelihood analysis of 16S rRNA gene sequences showing the relationship of strain YC7378^T with other related taxa are available with the online version of this paper.

at 25 °C for 2 weeks and isolates were purified by subculturing using 1/10 strength nutrient agar (NA) media without VC aqueous extract. The novel strain YC7378^T was isolated and identified on the basis of 16S rRNA gene sequence analysis, chemotaxonomic and phenotypic characteristics. It was routinely cultured on half-strength R2A agar media (1.5 g R2A broth l⁻¹ and 1.5 % agar) at 30 °C.

Cell morphology was observed under a Nikon light microscope at ×1000 magnification and the presence of flagella was investigated using a transmission electron microscope (Hitachi, model H-600) with cells grown for 24 h at 30 °C in half-strength R2A broth. The Gram reaction was determined by using a bioMérieux Gram staining kit according to the manufacturer's instructions. Catalase and oxidase tests were performed by using the procedures outlined by Cappuccino & Sherman (2002). The physiological properties of strain YC7378^T and related type strains were determined according to published methods: hydrolysis of casein, aesculin, gelatin, starch, tyrosine and urea (Brown, 2007), cellulose (Hendricks *et al.*, 1995), xylan (Broda *et al.*, 2000) and Tweens 40 and 80 (Atlas, 1993). Enzyme activities and acid production from different carbohydrates were determined by using API ZYM and API 20E kits, respectively, at 30 °C according to the instructions of the manufacturer (bioMérieux). Assimilation of various substrates was determined by using API 20NE and Biolog GN2 Micro Plate assays as recommended by the manufacturers. Growth at different temperatures (4, 10, 20, 25, 28, 30, 37, 40 and 45 °C) and various pH (5.0–12.0 at intervals of 0.5 pH units) was tested on half-strength R2A agar plates after 7 days of incubation. Growth under anaerobic conditions was determined on half-strength R2A agar plates for 7 days at 30 °C in an anaerobic GasPak jar containing an atmosphere of CO₂ (GasPak System; Becton Dickinson). Salt tolerance was tested in half-strength R2A broth supplemented with 0–6 % (at 0.5 % intervals) NaCl after 7 days of incubation at 30 °C. Duplicate antibiotic-sensitivity tests were performed using filter-paper discs containing 10 µg of tetracycline, gentamicin, penicillin, streptomycin or rifampicin, or 30 µg of chloramphenicol, kanamycin or vancomycin. Discs were placed on half-strength R2A agar plates spread with cells of strain YC7378^T or related type strains, and plates were incubated at 30 °C for 3 days.

Strain YC7378^T was Gram-stain-negative and non-motile. Cells were rod-shaped (0.4–0.5 × 1.2–1.4 µm) (Supplementary Fig. S1, available in IJSEM Online). The strain grew well on half-strength R2A, 1/10 strength NA and 0.1 % tryptic soy broth (TSB) supplemented with 1.5 % agar, but did not grow on full-strength NA, TSA or MacConkey agar. Growth did not occur under anaerobic conditions. Physiological and biochemical characteristics of strain YC7378^T are summarized in the species description, and comparison of selective characteristics with those of related type strains is given in Table 1.

Cellular fatty acids of strain YC7378^T and the three related type strains were analysed using colonies grown on half-

Table 1. Differential phenotypic characteristics between strain YC7378^T and type strains of related species

Strains: 1, *Sphingosinicella vermicomposti* sp. nov. YC7378^T; 2, *S. microcystinivorans* KCTC 12019^T; 3, *S. soli* KCTC 12482^T; 4, *S. xenopeptidilytica* DSM 17130^T. All strains were positive for oxidase. +, Positive; –, negative; w, weak positive. Data from this study unless indicated.

Characteristic	1	2	3	4
Nitrate reduction	–	+	–	+
Growth at 2.0 % NaCl	–	+	+	+
Catalase	–	+	+	+
Oxidase	w	+	+	+
Aesculin	+	–	–	–
Detection (by PCR) of:				
<i>mlrA</i>	–	+	–	–
<i>mlrB</i>	–	+	–	–
<i>puf</i>	–	+	–	–
Assimilation of:				
Tween 80	–	+	–	+
Tween 40	–	+	+	+
β-Hydroxybutyric acid	–	+	+	+
DL-Lactic acid	–	+	–	+
D-Alanine	+	–	–	+
L-Alaninamide	+	–	–	–
L-Ornithine	–	–	+	–
L-Proline	–	+	–	+
D-Serine	–	+	–	+
Trisodium citrate	–	–	–	+
Enzyme activity (API ZYM)				
Valine arylamidase	+	–	+	–
Cystine arylamidase	+	–	–	–
Trypsin	+	–	–	–
Acid phosphatase	+	–	–	+
N-acetyl-β-glucosaminidase	–	+	+	+
Susceptibility to antibiotics				
Ampicillin	+	–	–	+
Streptomycin	–	+	+	–
DNA G + C content (mol%)	59.4	63.6–63.7 ^{*a}	65.1 ^{*b}	65.0 ^{*c}

*Data from: a, Maruyama *et al.* (2006); b, Yoon *et al.* (2008); c, Geueke *et al.* (2007).

strength R2A for 72 h at 30 °C. The analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID). Isoprenoid quinones were extracted and analysed using reverse-phase HPLC according to the method described by Komagata & Suzuki (1987). Polyamines were analysed as described by Busse & Auling (1988) and Busse *et al.* (1997). Polar lipids were extracted according to the procedure described by Minnikin *et al.* (1984) and were identified using two-dimensional TLC followed by spraying with the appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). For the measurement of G + C content of the chromosomal DNA, genomic DNA of

strain YC7378^T was extracted and purified as described by Ausubel *et al.* (1995). It was then enzymically degraded into nucleosides and G+C content was determined as described by Mesbah *et al.* (1989) using a reverse-phase HPLC.

The major cellular fatty acids of strain YC7378^T comprised C_{18:1}ω7c (32.5 %), C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (28.2 %), C_{14:0} 2-OH (12.2 %) and C_{16:0} (11.1 %). This cellular fatty acid profile was generally similar to those of the other three species of the genus *Sphingosinicella*, but was distinguishable from them in terms of the content of some fatty acids, as shown in Table 2. The major respiratory quinone detected in strain YC7378^T was Q-10 and *sym*-homospermidine was the major polyamine. The polar lipids detected in strain YC7378^T were sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and unidentified phospholipids (Supplementary Fig. S2), which showed the relatedness of strain YC7378^T to the genus *Sphingosinicella* (Maruyama *et al.*, 2006). The DNA G+C content of strain YC7378^T was 59.4 mol%.

Extraction of genomic DNA was done using a commercial genomic DNA extraction kit (Core Biosystem). The 16S rRNA gene was PCR amplified from a small amount (1.5 ng) of purified genomic DNA by using primers 27F

and 1492R, and the purified PCR product was sequenced by GenoTech (Daejeon, Korea) (Lane, 1991). The 16S rRNA gene sequence was compiled using SeqMan software (DNASTAR) and the sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed by using the CLUSTAL_X program (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). Phylogenetic trees were reconstructed by using the neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1972) in the MEGA4 program (Tamura *et al.*, 2007) with bootstrap values based on 1000 replications (Felsenstein, 1985). A phylogenetic tree based on the maximum-likelihood algorithm was also constructed using the PHYLIP program, version 3.6 (Felsenstein, 2002). Pair-wise sequence similarity values between the novel strain YC7378^T and related taxa were computed by using a global alignment algorithm, which was implemented at the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). The occurrence of the *mlrA*, *mlrB*, *mlrC*, *mlrD* and *puf* genes was investigated by using primers and conditions as described by Geueke *et al.* (2007). The presence of the *bapA* gene was analysed by using primers and PCR conditions as described by Geueke *et al.* (2005) and the type strain *S. xenopeptidilytica* DSM 17130^T was used as a positive control.

The entire 1314 nucleotides of the 16S rRNA gene sequence of strain YC7378^T aligned with all sequences in the MEGA4 analysis. Phylogenetic analyses of 16S rRNA gene sequences showed that strain YC7378^T is closely related to species of the genus *Sphingosinicella* of the family *Sphingomonadaceae* (Fig. 1). In the phylogenetic tree, based on the neighbour-joining algorithm, strain YC7378^T joined the cluster comprising the three species of the genus *Sphingosinicella* with 98 % bootstrap support (Fig. 1). The topologies of phylogenetic trees based on the maximum-likelihood (Supplementary Fig. S3) and maximum-parsimony (data not shown) algorithms also supported the result of the neighbour-joining tree that strain YC7378^T is a novel member of the genus *Sphingosinicella*. Strain YC7378^T exhibited 16S rRNA gene sequence similarity values of 95.7, 95.6 and 95.5 % with the type strains of *S. soli*, *S. xenopeptidilytica* and *S. microcystinivorans*, respectively. Sequence similarity of less than 95.2 % was observed between strain YC7378^T and the other taxa used in the phylogenetic analysis (Fig. 1). The signature nucleotides of strain YC7378^T matched with those of members of the family *Sphingomonadaceae*, but varied from other species of the genus *Sphingosinicella* as described by Geueke *et al.* (2007). Strain YC7378^T was the first member of the genus *Sphingosinicella* that showed less than 96 % similarity with the other type strains of the genus. Geueke *et al.* (2007) identified signature nucleotides in the 16S rRNA gene sequences that had more than 98 % sequence similarity with each other. The low 16S rRNA gene sequence similarity and intragenomic microheterogeneity in the 16S rRNA gene sequence of strain YC7378^T were probably responsible for the variation of signature nucleotides, as described by Alperi

Table 2. Cellular fatty acid composition (%) of strain YC7378^T sp. nov. and type strains of related species

Strains: 1, *Sphingosinicella vermicomposti* sp. nov. YC7378^T; 2, *S. microcystinivorans* KCTC 12019^T; 3, *S. soli* KCTC 12482^T; 4, *S. xenopeptidilytica* DSM 17130^T. All data from this study. Strains were grown on half-strength R2A agar plates for 72 h at 30 °C. —, Not detected.

Fatty acid	1	2	3	4
Straight-chain				
C _{14:0}	1.5	—	1.8	—
C _{16:0}	11.1	8.7	9.8	8.0
C _{18:0}	5.5	—	4.1	—
Unsaturated				
C _{16:1} ω5c	2.0	3.1	—	1.5
C _{17:1} ω6c	1.8	0.9	—	2.3
C _{18:1} ω7c	32.5	39.1	31.2	34.7
C _{18:1} ω9c	—	—	2.5	—
Hydroxy				
C _{14:0} 2-OH	12.2	14.6	8.8	12.6
C _{15:0} 2-OH	1.1	—	—	—
C _{16:0} 2-OH	—	—	2.9	—
iso-C _{16:0} 3-OH	—	—	3.7	1.3
11-Methyl C _{18:1} ω7c	4.3	2.4	4.9	7.3
Summed feature 3*	28.2	34.5	30.4	32.5

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

Fig. 1. Phylogenetic tree reconstructed from the comparative analysis of 16S rRNA gene sequences showing the relationship of *Sphingosinicella vermicomposti* YC7378^T sp. nov. with other related taxa. The phylogenetic tree was reconstructed by using the neighbour-joining method and Jukes & Cantor evolutionary distance matrix with data obtained from aligned nucleotides. Bootstrap values (expressed as percentage of 1000 replications) greater than 50% are shown at the branch points. Bar, 1 substitution per 100 nucleotide positions.

et al. (2008). The *mlrA*, *mlrB*, *mlrC*, *mlrD*, *puf* and *bapA* genes were not detected in strain YC7378^T.

The 16S rRNA gene sequence similarity, phylogenetic analysis and fatty acid, polyamine and polar lipid profiles clearly indicate that strain YC7378^T belongs to the genus *Sphingosinicella*. Strain YC7378^T is distinguishable from the other species of the genus *Sphingosinicella* by several phenotypic characteristics (Table 1). In contrast to the related type strains, strain YC7378^T is positive for cystine arylamidase and aesculin hydrolysis, but negative for catalase, *N*-acetyl- β -glucosaminidase and assimilation of β -hydroxybutyric acid. Strain YC7378^T does not exhibit the signature nucleotides of the genus *Sphingosinicella*. The antibiotic sensitivity, phylogenetic tree and differential phenotypic properties clearly distinguish strain YC7378^T as a member of a novel species of the genus *Sphingosinicella*, for which the name *Sphingosinicella vermicomposti* YC7378^T sp. nov. is suggested.

Emended description of the genus *Sphingosinicella*

Characteristics are the same as given in the original description of the genus *Sphingosinicella* (Maruyama *et al.*, 2006) and the

amended description of the genus described by Geueke *et al.* (2007). However, the β -peptidyl aminopeptidase activity and the signature nucleotides in the 16S rRNA gene sequence described previously (Geueke *et al.*, 2007) are variable from species to species within the genus *Sphingosinicella*.

Description of *Sphingosinicella vermicomposti* sp. nov.

Sphingosinicella vermicomposti (ver.mi.com.pos'ti. L. masc. n. *vermis* worm; N.L. neut. n. *compostum* compost; N.L. gen. n. *vermicomposti* of vermicompost, referring to the isolation of the type strain from vermicompost).

Cells are Gram-stain-negative, non-spore-forming rods (0.4–0.5 $\times$ 1.2–1.4 μ m). Colonies are smooth, convex, slightly sticky, circular, yellow and 0.5–1.0 mm in diameter after 5 days of incubation at 30 $^{\circ}$ C on half-strength R2A agar. Grows at 10–37 $^{\circ}$ C (optimum 30 $^{\circ}$ C) and at pH 6.0 to 11.0 (optimum pH 6.5–8.5). Grows in the absence of NaCl but not in the presence of >1.5% (w/v) NaCl in half-strength R2A medium. No growth under anaerobic conditions. Resistant to 10 μ g streptomycin but susceptible to 10 μ g of tetracycline, penicillin, rifampicin or gentamicin and 30 μ g of vancomy-

cin, chloramphenicol or kanamycin. Weakly oxidase-positive. Can hydrolyse aesculin, but cannot hydrolyse gelatin, casein, starch, CM-cellulose, xylan, L-tyrosine, Tween 40 or Tween 80. Can use maltose, potassium gluconate, malic acid, L-alaninamide and D-alanine as a single carbon source available in API 20NE and Biolog GN2 MicroPlates. Urea is not hydrolysed. Indole and H₂S are not produced. Tryptophan deaminase is produced. Nitrate is not reduced to nitrite or nitrogen gas. Acid is not produced from different glucose sources available in the API 20E kit. In the API ZYM kit, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsinase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -glucosidase activities are present, but lipase (C14), α -chymotrypsin, β -glucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase activities are not present. The major quinone is Q-10 and sym-homospermidine is the major polyamine. The major fatty acids are C_{18:1 ω 7c}, C_{16:1 ω 7c} and/or iso-C_{15:0} 2-OH, C_{14:0} 2-OH and C_{16:0}. The major polar lipids are sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The DNA G + C content of the type strain is 59.4 mol%.

The type strain, YC7378^T (=KCTC 22446^T =DSM 21593^T), was isolated from vermicompost at Masan, Korea.

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