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Sphingobacterium composti sp. nov., a Novel DNase-Producing Bacterium Isolated from Compost

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Abstract A Gram-negative, strictly aerobic, nonmotile, and nonspore-forming bacterial strain, designated T5-12^T, was isolated from compost and characterized using a polyphasic taxonomical approach. The isolate was positive for catalase and oxidase tests. It could degrade DNA, but was negative for degradation of macromolecules such as casein, collagen, starch, chitin, cellulose, and xylan. The DNA G+C content was 36.0 mol%. The predominant isoprenoid quinone was menaquinone 7 (MK-7). The major fatty acids were iso-$C_{15:0}$ (45.6%), iso-$C_{17:0}$ 3OH (17.2%), and summed feature 4 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2OH, 14.9%). Comparative 16S rRNA gene sequence analysis showed that strain T5-12^T fell within the radiation of the cluster comprising members of the genus *Sphingobacterium*. Strain T5-12^T exhibited lower than 94% of 16S rRNA gene sequence similarity with respect to the type strains of recognized *Sphingobacterium* species. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain T5-12^T (=KCTC 12578^T=LMG 23401^T=CCUG 52467^T) should be classified in the genus *Sphingobacterium* as the type strain of a novel species, for which the name *Sphingobacterium composti* sp. nov. is proposed.

Key words: *Sphingobacterium composti* sp. nov., polyphasic taxonomy, compost

The genus *Sphingobacterium* was created by Yabuuchi *et al*. [32], who transferred two former *Flavobacterium* species [7, 8] as *Sphingobacterium multivorum* and *Sphingobacterium spiritivorum* and proposed a new species *Sphingobacterium mizutae* (Holmes *et al*. [9] corrected the spelling of the specific epithet, *mizutae*, to *mizutaii*). Currently, it also comprises three other species, *Sphingobacterium antarcticum* [21], *Sphingobacterium thalpophilum* (previously classified as *Flavobacterium thalpophilum*), and *Sphingobacterium faecium* [24]. At the time of this writing, novel species of the genus *Sphingobacterium daejeonense* sp. nov. is listed under "papers in press" in IJSEM online [12] and will be validated soon. Two other previously described species [*Sphingobacterium*] *heparinum* and [*Sphingobacterium*] *piscium* [24] were reclassified in the genus *Pedobacter* [22]. *Sphingobacterium* species have been isolated from antarctic soil, and clinical specimens including blood, urine, and the uterus of human patients with opportunistic infections [8, 21, 32]. The members of the genus *Sphingobacterium* are Gram-negative rods that are positive for catalase and oxidase, negative for heparinase, gelatinase, and indole production, and contain iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, $C_{16:1}$ ω7c, and $C_{17:0}$ 3-OH as main fatty acids [22, 24].

In this study, we have characterized an aerobic, nonspore-forming strain, T5-12^T, isolated from compost. Phenotypic, chemotaxonomic, and phylogenetic analyses established the affiliation of the isolate to the genus *Sphingobacterium*. The data obtained also suggest that the isolate represents a novel species of the genus *Sphingobacterium*, and the name *Sphingobacterium composti* sp. nov. is proposed.

Materials and Methods

Isolation of Bacterial Strain and Culture Condition

Strain T5-12^T was originally isolated from compost composed of cow dung and rice straw, which was collected near Daejeon city in South Korea. This compost sample was thoroughly suspended in 50 mM phosphate buffer (pH 7.0) and the suspension, following serial dilution, was spread onto R2A agar (Difco) plates. The plates were incubated at 30°C for three weeks. Single colonies on the plates were purified by transferring them onto new plates. The purified colonies were tentatively identified by analysis of partial

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16S rRNA gene sequences. Strain T5-12^T was one of the isolates that appeared on the R2A agar plates in aerobic conditions. Strain T5-12^T was routinely cultured on R2A agar at 30°C and maintained as a glycerol suspension (20%, w/v) at −70°C.

Phenotypic and Biochemical Characteristics

Gram reaction was performed by the nonstaining method described by Buck [2]. Cell morphology was observed under a Nikon light microscope at ×1,000, with cells grown for 3 days at 30°C on R2A agar. Catalase and oxidase tests were performed as outlined by Cappuccino and Sherman [3]. For single-carbon-source assimilation studies, a defined liquid medium containing basal salts was used (g/l): 1.8 g K_2HPO_4, 1.08 g KH_2PO_4, 0.5 g $NaNO_3$, 0.5 g NH_4Cl, 0.1 g KCl, 0.1 g $MgSO_4$, and 0.05 g $CaCl_2$. To this medium, a vitamin solution [31], trace elements solution SL-10 [30], and selenite/tungstate solution [28] were added and the pH of the medium was adjusted to 6.8. This liquid medium was aliquoted (0.25 ml) into 96-well trays and filter-sterilized carbon sources were added into each well [individually at 0.1% (w/v)]. Growth in the 96-well plates, which were incubated at 30°C for 7 days, was examined visually. Negative-control wells did not contain an added carbon source. Positive controls included a well containing R2A broth. Tests for acid production from carbohydrates and some other phenotypic characteristics were determined with API 20E galleries according to the instructions of the manufacturer (bioMérieux). Tests for anaerobic growth were performed in serum bottles containing R2A broth supplemented with thioglycolate (1 g/l) under a nitrogen atmosphere. Tests for degradation of DNA, casein, chitin, starch [1]; lipid [14]; and xylan, cellulose, and collagen [10, 25, 26] were performed and evaluated after 7 days. Growth at different temperatures and pH was assessed after 5 days of incubation. Salt tolerance was tested on R2A medium supplemented with 1–15% (w/v) NaCl after 5 days of incubation. Growth on nutrient agar, trypticase soy agar (TSA), and MacConkey agar was also valuated at 30°C.

PCR Amplification, 16S rRNA Gene Sequencing, and Phylogenetic Analysis

DNA was extracted using a genomic DNA extraction kit (Core Biosystem, Korea); the 16S rRNA gene was amplified by PCR and sequencing of the purified PCR product was carried out according to Kim *et al.* [11]. The 16S rRNA gene full sequences were compiled using the SeqMan software (DNASTAR, Madison, WI, U.S.A.). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. The multiple alignments were performed by the Clustal_X program [27]. Gaps were edited in the BioEdit program [6]. The evolutionary distances were calculated using the Kimura two-parameter model [13]. The phylogenetic trees (based on 1,277 bps) were constructed by using the neighbor-joining method [18] and the maximum-parsimony method [5] using the MEGA3 Program [15] with bootstrap values based on 1,000 replications [4].

DNA Extraction and Determination of DNA G+C Content

For the measurement of G+C content of the chromosomal DNA, the genomic DNA of the strain was extracted and purified as described by Moore [17], enzymatically degraded into nucleosides, and then the G+C content of DNA was determined as described by Mesbah *et al.* [16] using reverse-phase HPLC.

Cellular Fatty Acids and Isoprenoid Quinones

Cellular fatty acids were analyzed in organisms grown on R2A agar for two days. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI, 1999). The fatty acids were then analyzed by gas chromatography (Hewlett Packard 6890) using the Microbial Identification software package [19]. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions, and reextracted in *n*-hexane-water (1:1, v/v). The crude quinone in *n*-hexane was purified using Sep-Pak Vac Cartridges Silica (Waters) and subsequently analyzed by HPLC, as previously described by Shin *et al.* [20].

Nucleotide Sequence Accession Numbers

The 16S rRNA gene sequence of strain T5-12^T determined in this study has been deposited in NCBI GenBank under

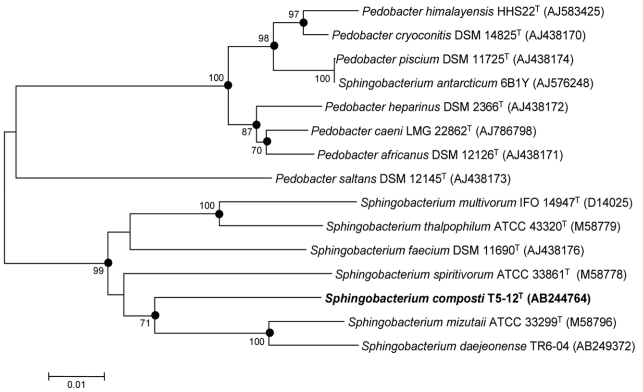

Fig. 1. Neighbor-joining tree (based on 16S rRNA gene sequences) showing the phylogenetic positions of strain T5-12^T among the type strains of the genera *Sphingobacterium* and *Pedobacter*.

Numbers on branch nodes are bootstrap values (1,000 resamplings; only values over 50% are given). Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 1% sequence divergence.

the accession number AB244764. The accession numbers of the reference strains, which are closely related to strain T5-12^T, are indicated in Fig. 1.

RESULTS AND DISCUSSION

Morphological and Phenotypic Characteristics

Strain T5-12^T was Gram-negative, strictly aerobic, nonmotile, nonspore-forming, and rod-shaped organism that was about 0.4–0.8 μm wide and 2.0–2.5 μm long. Colonies of T5-12^T were smooth, convex, round, glossy, slightly yellowish, and 1.0–1.5 mm in diameter after 6 days incubation at 30ºC on R2A agar. Strain T5-12^T was able to grow at 42°C, but not at 10 or 45°C. The pH growth range was between pH 5.5 and 8.5, with an optimum between pH 6.5 and 7.0. Growth occurred in the absence of NaCl and in the presence of 4.0% (w/v) NaCl, but not in the presence of 5% (w/v) NaCl. Other phenotypic and chemotaxonomic characteristics that differentiate strain T5-12^T from related *Sphingobacterium* species are listed in Table 1. In contrast to all other members of the genus *Sphingobacterium*, strain T5-12^T is negative for assimilation of D-fructose and D-raffinose, hydrolysis of aesculin, and urease production.

Cellular Fatty Acid and Menaquinone Compositions

The predominant menaquinone was MK-7. The cellular fatty acid profiles of strain T5-12^T and related *Sphingobacterium* species are presented in Table 2. Strain T5-12^T contained a large amount of iso-branched hydroxy and iso-branched fatty acids; the major components were iso-$C_{15:0}$, iso-$C_{17:0}$ 3OH, iso-$C_{15:0}$ 2OH, and/or $C_{16:1}$ ω7c, typical for members

Table 1. Comparison of the phenotypic characteristics of *Sphingobacterium composti* sp. nov. and related *Sphingobacterium* species.

Characteristics	1	2	3	4	5	6	7
Growth at							
5°C	–	–	–	–	–	+	+
42°C	+	–	–	–	+	–	–
Hydrolysis of							
DNA	+	+	+	+	–	+	ND
Gelatin	–	–	v	-	–	–	+
Starch	–	+	+	+	+	+	-
Aesculin	–	+	+	+	+	+	+
Urease production	–	+	+	+	+	+	+
Assimilation of							
L-Rhamnose	–	+	+	–	+	+	+
D-Ribose	–	–	-	–	–	–	+
L-Arabinose	–	–	+	v	+	+	+
D-Melibiose	–	+	+	+	+	+	–
D-Raffinose	–	+	+	+	+	+	+
D-Xylose	–	+	+	+	+	–	+
D-Fructose	–	+	+	+	+	+	+
D-Mannitol	+	+	–	–	–	–	+
Glycerol	–	v	–	–	+	+	+
Inositol	–	–	–	–	–	–	+
Inulin	–	v	v	–	+	+	+
Pyruvate	–	–	–	v	–	–	+
L-Glutamate	–	–	+	+	–	+	+
Succinate	–	–	v	–	–	–	+
Malate	–	–	v	–	–	–	+
Acid production from							
L-Rhamnose	–	–	v	–	+	–	–
L-Arabinose	–	–	+	+	+	+	–
D-Sucrose	+	+	+	+	+	+	–
D-Mannitol	–	+	–	–	–	–	–
G+C content (mol%)	36.0	39.0	39.9–40.5	39.3–40.0	44.0–44.2	37.3	39.3

Strains: 1, *Sphingobacterium composti* sp. nov. T5-12^T (present study); 2, *Sphingobacterium spiritivorum* IFO 14948^T [22, 24]; 3, *Sphingobacterium multivorum* IFO 14947^T [22, 24]; 4, *Sphingobacterium mizutaii* IFO 14946^T [22, 24]; 5, *Sphingobacterium thalpophilium* IFO 14963^T [22, 24]; 6, *Sphingobacterium faecium* IFO 15299^T [22, 24]; 7, *Sphingobacterium antarcticum* MTCC 675^T [21].
Symbols: +, positive; –, negative; v, variable; ND, not determined.

Table 2. Fatty acid composition of *Sphingobacterium composti* sp. nov. and phylogenetically related *Sphingobacterium* species[a].

Fatty acid	1	2	3	4	5	6	7
Straight-chain saturated							
$C_{14:0}$	–[b]	1.0	2.7	tr[c]	3.2	tr	+[d]
$C_{16:0}$	2.1	3.5	7.8	tr	6.0	4.5	+[d]
$C_{18:0}$	1.2	–	–	–	–	–	–
$C_{16:0}$ 2OH	–	–	tr	–	3.2	–	–
$C_{16:0}$ 3OH	–	2.7	5.3	tr	6.3	2.1	–
Branched saturated							
iso-$C_{15:0}$	45.6	30.1	22.2	30.0	17.7	24.6	29.0
iso-$C_{17:0}$	tr	–	–	–	–	–	–
iso-$C_{15:0}$ 2OH	Unknown[f]	21.5	17.4	25.6	24.6	15.9	tr
iso-$C_{15:0}$ 3OH	2.1	2.2	3.2	3.0	4.3	3.7	tr
iso-$C_{17:0}$ 3OH	17.2	12.5	7.1	22.1	10.0	10.0	tr
anteiso-$C_{15:0}$	tr	tr	–	tr	–	tr	–
$C_{16:0}$ 10methyl	9.7	–	–	–	–	1.4	–
Summed feature 4[e]	14.9	–	–	–	–	–	–
Monounsaturated							
$C_{16:1}$ ω5c	tr	tr	–	tr	–	1.5	–
$C_{16:1}$ ω7c	Unknown[f]	21.1	31.6	9.5	23.2	32.2	56.0
$C_{17:1}$	–	–	–	–	–	–	+[d]
$C_{18:1}$ ω9c	tr	–	–	–	–	–	–
$C_{18:1}$ ω5c	tr	–	–	–	–	–	–
iso-$C_{15:1}$ G	1.8	–	–	–	–	–	–
iso-$C_{17:1}$ ω9c	2.9	1.7	tr	3.7	–	–	–
Unknown[g]							
ECL[h] 13.566	–	tr	–	1.3	1.4	tr	–
ECL 16.580	tr	tr	tr	tr	–	tr	–

Strains: 1, *Sphingobacterium composti* sp. nov. T5-12^T (present study); 2, *Sphingobacterium spiritivorum* IFO 14948^T [22]; 3, *Sphingobacterium multivorum* IFO 14947^T [22]; 4, *Sphingobacterium mizutaii* IFO 14946^T [22]; 5, *Sphingobacterium thalpophilium* IFO 14963^T [22]; 6, *Sphingobacterium faecium* IFO 15299^T [22]; 7, *Sphingobacterium antarcticum* MTCC 675^T [21].

[a]Values are shown as a percentage of the total fatty acid content for each strain.

[b]Not detected.

[c]tr, trace (<1.0%).

[d]Fatty acid was detected but its content was not reported [22].

[e]Summed features represent groups of two fatty acids that could not be separated by GC with the MIDI system. Summed feature 4 contained iso-$C_{15:0}$ 2OH and/or $C_{16:1}$ ω7c.

[f]The fatty acid comprised into Summed feature 4.

[g]The unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified.

[h]ECL, equivalent chain length.

of the genus *Sphingobacterium* [22, 24]. Some qualitative and quantitative differences in fatty acid content could be observed between the strain T5-12^T and the phylogenetically closest relatives. In contrast to other *Sphingobacterium* species, strain T5-12^T contained a relatively large amount of $C_{16:0}$ 10-methyl (9.7%), larger amount of iso-$C_{15:0}$, smaller amount of iso-$C_{15:0}$ 2OH and/or $C_{16:1}$ ω7c, and it did not contain $C_{14:0}$ and $C_{16:0}$ 3OH.

DNA G+C Content

G+C content for the genus *Sphingobacterium* ranges from 37.3 mol% (reported for *S. faecium)* to 44.2 mol% (reported for *S. thalpophilium*) [22, 24]. The DNA G+C content of strain T5-12^T is not within this range, but it has a closely related value of 36.0 mol%.

Phylogenetic Analysis Based on 16S rRNA Gene Sequences

The 16S rRNA gene sequence of strain T5-12^T was a continuous stretch of 1,433 bp. Preliminary comparison of the sequence against the sequences in the GenBank database indicated that members of the genus *Sphingobacterium* were the closest phylogenetic neighbors. Strain T5-12^T exhibited 16S rRNA gene sequence similarity levels of 93.7% to *Sphingobacterium faecium* DSM 11690^T and *Sphingobacterium daejeonense* TR6-04 and lower than 92.5% with respect to the type strains of other recognized *Sphingobacterium* species. The phylogenetic definition of a species generally includes "strains with approximately 70% or greater DNA-DNA relatedness" [29]. According to the available compilation of data, organisms that have less

than 97.0% sequence similarity will not reassociate to more than 60%, irrespective of the hybridization method applied [10, 23]. The phylogenetic tree based on the neighbor-joining algorithm showed that strain T5-12^T fell within the radiation of the cluster comprising *Sphingobacterium* species and occupied a distinct phylogenetic position within the genus (Fig. 1). This phylogenetic result demonstrated that strain T5-12^T was not related to any of the previously described *Sphingobacterium* taxa as well as to *Sphingobacterium daejeonense* TR6-04 at the species level.

Taxonomic Conclusions

All of the characteristics determined for strain T5-12^T are in accordance with those of the genus *Sphingobacterium*. On the basis of phylogenetic distance from established *Sphingobacterium* species and *Sphingobacterium daejeonense* TR6-04, also indicated by relatively low 16S rRNA gene sequence similarities (<94%) and the combination of unique phenotypic characteristics, it is demonstrable that T5-12^T should be classified as a novel species of this genus, for which the name *Sphingobacterium composti* sp. nov. is proposed.

Description of *Sphingobacterium composti* sp. nov.

Sphingobacterium composti (N.L. n. compostum -i, compost; N.L. gen. n. composti, of compost). Colonies are 1.0–1.5 mm, smooth, convex, round, glossy, and slightly yellowish after 6 days of incubation at 30°C on R2A. Cells are rod-shaped (0.4–0.8 μm wide and 2.0–2.5 μm long), strictly aerobic, Gram-negative, and nonspore-forming. Motility was not observed. Grows between 15°C and 42°C; the optimum temperature for growth is 30°C. The bacterium grows within pH values of between 5.5 and 8.5; the optimum pH is 6.5–7.0. The strain tolerates 4% (w/v) NaCl, but not 5%. Growth occurs on TSA, but not on MacConkey agar. It is positive for catalase and oxidase, but negative for lipase. It is also positive for hydrolyses of DNA, but not for chitin, starch, cellulose, xylan, casein, collagen and aesculin. The following substrates are utilized for growth: D-glucose, D-galactose, D-mannose, D-lyxose, L-xylose, *N*-acetyl-D-glucosamine, salicin, D-cellobiose, D-lactose, D-maltose, D-sucrose, D-trehalose, D-mannitol, and D-sorbitol. The following substrates are not utilized for growth: D-arabinose, L-arabinose, D-fructose, D-fucose, D-xylose, D-melibiose, D-raffinose, L-rhamnose, L-sorbose, D-ribose, pyruvate, formate, acetate, propionate, DL-3-hydroxybutyrate, valerate, caprate, maleate, fumarate, phenylacetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, citrate, lactate, malate, malonate, succinate, glutarate, tartrate, itaconate, adipate, suberate, oxalate, gluconate, ethanol, D-adonitol, dulcitol, inositol, xylitol, glycerol, amygdalin, methanol, glycogen, inulin, dextran, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-histidine, glycine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophane, L-tyrosine, and L-valine. In API 20E tests, beta-galactosidase activity and the Voges-Proskauer test are positive; arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophane deaminase, urease, and gelatinase activities are not present. Hydrogen sulfide and indole were not produced. Acid is produced from D-glucose, D-melibiose, D-sucrose, and amygdalin, but not from L-arabinose, D-mannitol, inositol, D-sorbitol, and L-rhamnose. The major fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3OH, iso-$C_{15:0}$ 2OH, and/or $C_{16:1}$ ω7c. The DNA G+C content is 36.0 mol%.

The type strain, T5-12^T (=KCTC 12578^T=LMG 23401^T =CCUG 52467^T), was isolated from compost that was collected near Daejeon city in South Korea.

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REFERENCES

1. Atlas, R. M. 1993. In L. C. Parks (ed.). *Handbook of Microbiological Media*. CRC Press, Boca Raton, Florida.
2. Buck, J. D. 1982. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl. Environ. Microbiol.* **44:** 992–993.
3. Cappuccino, J. G. and N. Sherman. 2002. *Microbiology: A Laboratory Manual*, 6th Ed. Benjamin Cummings, San Francisco.
4. Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39:** 783–791.
5. Fitch, W. M. 1972. Toward defining the course of evolution: Minimum change for a specific tree topology. *Syst. Zool.* **20:** 406–416.
6. Hall, M. G. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* **41:** 95–98.
7. Holmes, B., R. J. Owen, and R. E. Weaver. 1981. *Flavobacterium multivorum*, a new species isolated from human clinical specimens and previously known as group IIk, biotype 2. *Int. J. Syst. Bacteriol.* **31:** 21–34.
8. Holmes, B., R. J. Owen, and D. G. Hollis. 1982. *Flavobacterium spiritivorum*, a new species isolated from human clinical specimens. *Int. J. Syst. Bacteriol.* **32:** 157–165.
9. Holmes, B., R. E. Weaver, A. G. Steigerwalt, and D. J. Brenner. 1988. A taxonomic study of *Flavobacterium spiritivorum* and *Sphingobacterium mizutae*: Proposal of

Flavobacterium yabuuchiae sp. nov. and *Flavobacterium mizutaii* comb. nov. *Int. J. Syst. Bacteriol.* **38:** 348–353.
10. Keswani, J. and W. B. Whitman. 2001. Relationship of 16S rRNA sequence similarity to DNA hybridization in prokaryotes. *Int. J. Syst. Evol. Microbiol.* **51:** 667–678.
11. Kim, M. K., W.-T. Im, H. Ohta, M. Lee, and S.-T. Lee. 2005. *Sphingopyxis granuli* sp. nov., a β-glucosidase producing bacterium in the family *Sphingomonadaceae* in α-4 subclass of the *Proteobacteria*. *J. Microbiol.* **43:** 152–157.
12. Kim, K.-H., L. N. Ten, Q.-M. Liu, W.-T. Im, and S.-T. Lee. 2006. *Sphingobacterium daejeonense* sp. nov., isolated from a compost sample. *Int. J. Syst. Bacteriol.* (in press).
13. Kimura, M. 1983. *The Neutral Theory of Molecular Evolution*. Cambridge University Press, Cambridge.
14. Kouker, G. and K.-E Jaeger. 1987. Specific and sensitive plate assay for bacterial lipases. *Appl. Environ. Microbiol.* **53:** 211–213.
15. Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment briefings. *Bioinformatics* **5:** 150–163.
16. Mesbah, M., U. Premachandran, and W. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39:** 159–167.
17. Moore, D. D. 1995. Preparation and analysis of DNA, pp. 2–11. *In* F. W. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (eds.), *Current Protocols in Molecular Biology.* Wiley, New York, U.S.A.
18. Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molec. Biol. Evol.* **4:** 406–425.
19. Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. Newark, DE: MIDI. U.S.A.
20. Shin, Y. K., J.-S. Lee, C. O. Chun, H.-J. Kim, and Y.-H. Park. 1996. Isoprenoid quinone profiles of the *Leclercia adecarboxylata* KTCT 1036^T. *J. Microbiol. Biotechnol.* **6:** 68–69.
21. Shivaji, S., M. K. Ray, S. N. Rao, L. Saisree, M. V. Jagannadham, G. S. Kumar, G. S. N. Reddy, and P. M. Bhargava. 1992. *Sphingobacterium antarcticus* sp. nov., a psychotrophic bacterium from soils of Schirmacher Oasis, Antarctica. *Int. J. Syst. Bacteriol.* **42:** 102–106.
22. Steyn, P. L., P. Segers, M. Vancanneyt, P. Sandra, K. Kersters, and J. J. Joubert. 1998. Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov., and *Pedobacter saltans* sp. nov. Proposal of the family *Sphingobacteriaceae* fam. nov. *Int. J. Syst. Bacteriol.* **48:** 165–177.
23. Stackebrandt, E. and B. M. Goebel. 1994. Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44:** 846–849.
24. Takeuchi, M. and A. Yokota. 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.
25. Ten, L. N., W.-T. Im, M.-K. Kim, M.-S. Kang, and S.-T. Lee. 2004. Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J. Microbiol. Meth.* **56:** 375–382.
26. Ten, L. N., W.-T. Im, M.-K. Kim, and S.-T. Lee. 2005. A plate assay for simultaneous screening of polysaccharide- and protein-degrading microorganisms. *Lett. Appl. Microbiol.* **40:** 92–98.
27. Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. Higgins. 1997. The Clustal_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **24:** 4876–4882.
28. Tschech, A. and N. Pfennig. 1984. Growth yield increase linked to caffeate reduction in *Acetobacterium woodii*. *Arch. Microbiol.* **137:** 163–167.
29. Wayne, L. G., D. J. Brenner, R. R. Colwell, *et al.* 1987. International Committee on Systematic Bacteriology. Report of the *ad hoc* committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* **37:** 463–464.
30. Widdel, F., G. W. Kohring, and F. Mayer. 1983. Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. 3. Characterization of filamentous gliding *Desulfonema limicola* gen. nov. sp. nov., and *Desulfonema magnum* sp. nov. *Arch. Microbiol.* **134:** 286–294.
31. Widdel, F. and F. Bak. 1992. Gram-negative mesophilic sulfate reducing bacteria, pp. 3352–3378. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (eds.), *The Prokaryotes,* 2nd Ed. Springer, New York.
32. Yabuuchi, E., T. Kaneko, I. Yano, C. W. Moss, and N. Miyoshi. 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.
33. Yeo, H. S., O. S. Lee, I. S. Lee, H. S. Kim, T. S. Yu, and Y. J. Jeong. 2004. *Gluconacetobacter persimmonis* sp. nov., isolated from Korean traditional persimmon vinegar. *J. Microbiol. Biotechnol.* **14:** 276–283.