**Sphingobacterium daejeonense** sp. nov., isolated from a compost sample

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A Gram-negative, strictly aerobic, rod-shaped, non-motile, non-spore-forming bacterial strain, designated TR6-04T, was isolated from compost and characterized taxonomically by using a polyphasic approach. The organism grew optimally at 30 °C and at pH 6.5-7.0. The isolate was positive for catalase and oxidase tests but negative for gelatinase, indole and H2S production. Comparative 16S rRNA gene sequence analysis showed that strain TR6-04T fell within the radiation of the cluster comprising *Sphingobacterium* species and clustered with *Sphingobacterium mizutaii* ATCC 33299T (96.7 % sequence similarity); the similarity to sequences of other species within the family *Sphingobacteriaceae* was less than 92.0 %. The G+C content of the genomic DNA was 38.7 mol%. The predominant respiratory quinone was MK-7. The major fatty acids were iso-C15:0, iso-C17:0 3-OH and summed feature 4 (iso-C15:0 2-OH and/or C16:1ω7c). These chemotaxonomic data supported the affiliation of strain TR6-04T to the genus *Sphingobacterium*. However, on the basis of its phenotypic properties and phylogenetic distinctiveness, strain TR6-04T (=KCTC 12579T =LMG 23402T =CCUG 52468T) should be classified as the type strain of a novel species, for which the name *Sphingobacterium daejeonense* sp. nov. is proposed.

The genus *Sphingobacterium* was created by Yabuuchi et al. (1983), who reclassified two former *Flavobacterium* species (Holmes et al., 1981, 1982) as *Sphingobacterium multivorum* and *Sphingobacterium spiritivorum* and proposed a novel species, *Sphingobacterium mizutae* [Holmes et al. (1988) corrected the spelling of the specific epithet to *mizutaii*]. At present, the genus *Sphingobacterium* also includes three other species, *Sphingobacterium antarcticum* (Shivaji et al., 1992) [the original spelling of the specific epithet, *antarcticus*, was corrected by Euzéby (1998)], *Sphingobacterium thalpophilum* (previously classified as *Flavobacterium thalpophilum*) and *Sphingobacterium faecium* (Takeuchi & Yokota, 1992). Two other previously described species, [*Sphingobacterium* *heparinum* and [*Sphingobacterium* *pis-cium* (Takeuchi & Yokota, 1992), were reclassified in the genus *Pedobacter* (Steyn et al., 1998). *Sphingobacterium* species have been isolated from Antarctic soil and from clinical specimens, including blood, urine and the uteruses of human patients with opportunistic infections (Holmes et al., 1982; Yabuuchi et al., 1983; Shivaji et al., 1992). Members of the genus are Gram-negative rods that are positive for catalase and oxidase, negative for heparinase, gelatinase and indole production and contain iso-C15:0, iso-C15:0 2-OH, C16:1ω7c and C17:0 3-OH as the main fatty acids (Takeuchi & Yokota, 1992; Steyn et al., 1998).

In this study, we have characterized an aerobic, non-spore-forming strain, TR6-04T, isolated from compost. Phenotypic, chemotaxonomic and phylogenetic analyses establish the affiliation of the isolate to the genus *Sphingobacterium*. The data obtained also suggest that the isolate represents a novel species of the genus.

Strain TR6-04T 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 3 weeks. Single colonies on the plates were purified by transferring them onto new plates. The purified colonies were tentatively identified by analysis of partial 16S rRNA gene sequences. Strain TR6-04T was one of the isolates that predominated on the R2A agar plates under aerobic conditions. Strain TR6-04T was routinely cultured on R2A agar at 30 °C and maintained as a glycerol suspension (20 %, w/v) at −70 °C.

A nearly complete 16S rRNA gene sequence of strain TR6-04T was determined as follows. DNA was extracted using the GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain TR6-04T is AB249372.
a commercial genomic DNA extraction kit (Core Bio-
system) and amplification of the 16S rRNA gene by PCR
and sequencing of purified PCR product were carried
out according to Kim et al. (2005). Partial 16S rRNA
gene sequences were assembled using SeqMan software
(DNASTAR). The 16S rRNA gene sequences of related
taxa were obtained from the GenBank database. Multiple
alignments were performed using the CLUSTAL_X pro-
gram (Thompson et al., 1997). Gaps were edited in BioEdit
(Hall, 1999). Evolutionary distances were calculated us-
ing the Kimura two-parameter model (Kimura, 1983).
Phylogenetic trees were constructed by using the neighbour-
joining method (Saitou & Nei, 1987) and the maximum-
parsimony method (Fitch, 1972) using the MEGA3 program
(Kumar et al., 2004) with bootstrap values based on 1000
replications (Felsenstein, 1985).

The 16S rRNA gene sequence of strain TR6-04T was a con-
tinuous stretch of 1472 bp. Comparative 16S rRNA gene
sequence analyses showed that strain TR6-04T is phylo-
genetically affiliated to the genus Sphingobacterium. The
phylogenetic tree based on the neighbour-joining algorithm
showed that strain TR6-04T fell within the radiation of the
cluster comprising Sphingobacterium species and joined
Sphingobacterium mizutaii ATCC 33299T (96-7 % similarity)
with a bootstrap resampling value of 100 % (Fig. 1).
Strain TR6-04T exhibited less than 92-0 % 16S rRNA gene
sequence similarity with respect to the type strains of other
recognized Sphingobacterium species. The phylogenetic de-
nition of a species generally includes strains with approxi-
ately 70 % or greater DNA–DNA relatedness (Wayne et al.,
1987). According to the available compilation of data, orga-
isms that have less than 97-0 % 16S rRNA gene sequence
similarity will not reassociate to more than 60 %, irrespec-
tive of the hybridization method applied (Stackebrandt &
Goebel, 1994; Keswani & Whitman, 2001). This phylo-
genetic result demonstrated that strain TR6-04T was not
related to any of the previously described Sphingobacterium
taxa at the species level.

A 16S rRNA gene sequence of the type strain of S.
antarcticum was not available. Instead, we noticed a 16S
rRNA gene sequence from S. antarcticum strain 6B1Y
(strain 6BY in the original paper of Shivaji et al., 1992),
which had shown 100 % DNA–DNA relatedness with
strain 4BYT, the type strain of S. antarcticum (Shivaji et al.,
1992; S. Shivaji, personal communication). Thus
this sequence can represent the species S. antarcticum. How-
ever, neighbour-joining trees showed that strain 6B1Y
clustered in the genus Pedobacter and showed close affilia-
tion with Pedobacter piscium DSM 11725T (Fig. 1). Strain
6B1Y and P. piscium DSM 11725T showed high 16S rRNA
genue sequence similarity (99-9 %) in a pairwise compari-
sion. The 16S rRNA gene of strain 6B1Y was sequenced only
after the original description of the genus Pedobacter was
published. Taxonomic rearrangement is required for this
species.

The Gram reaction was performed by the non-staining
method as described by Buck (1982). Cell morphology was
observed under a Nikon light microscope at ×1000, with
cells grown for 3 days at 30 °C on R2A agar. Catalase ac-
ceptivity was determined by bubble production in 3 % (v/v)
H₂O₂ and oxidase activity was determined using 1 % (w/v)
tetramethyl p-phenylenediamine. For single-carbon-source
assimilation studies, a defined liquid medium containing
basal salts was used (1 L⁻¹): K₂HPO₄ 1-8 g, KH₂PO₄ 1-08 g,
NaNO₃ 0-5 g, NH₄Cl 0-5 g, KCl 0-1 g, MgSO₄ 0-1 g, CaCl₂
0-05 g. To this medium, a vitamin solution (Widdel &
Bak, 1992), trace element solution SL-10 (Widdel et al., 1983)
and selenite/tungstate solution (Tschech & Pfennig, 1984)
were added and the pH of the medium was adjusted to 6-8.
This liquid medium was aliquotted into 96-well trays and
filter-sterilized carbon sources were added into each well
(individually at 0-1 % w/v). The plates were incubated at
30 °C for 7 days and growth in was examined visually. A
negative control well did not contain an added carbon
source, and a positive control well contained R2A broth.
Some physiological characteristics were determined with
API 20E galleries according to the instructions of the manu-
facturer (bioMérieux). Anaerobic growth was examined in
serum bottles with sodium thioglycolate (1 g L⁻¹) added
to R2A broth and the upper air layer replaced with nitro-
gen gas. For examination of the reduction of nitrate as
the final electron acceptor under anaerobic conditions,
10 mM KNO₃ was added to the medium for anaerobic
growth and the reduction of nitrate was monitored by
using an ion chromatograph (model 790 personal IC;
Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain TR6-04T among strains of the genera Sphingo-
bacterium and Pedobacter. GenBank accession numbers are shown in parentheses. Bootstrap values (expressed as percentages of
1000 replications) greater than 50 % are shown at branch points. Dots indicate that the corresponding nodes were also recovered
in the tree generated with the maximum-parsimony algorithm. Bar, 0-01 substitutions per nucleotide position.
Metrohm) equipped with a conductivity detector and an anion-exchange column (Metrosep Anion Supp 4; Metrohm). Degradation of DNA (using DNase agar from Scharlau, supplemented with 1 M HCl), casein, chitin and starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987) and xylan and cellulose (Ten et al., 2004) was also investigated; reactions were read after 5 days. Growth at different temperatures (4, 15, 25, 30, 37 and 42 °C) and various pH values (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on R2A medium supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypti- case soy agar (TSA; Difco) and MacConkey agar was also evaluated at 30 °C.

Strain TR6-04T is a Gram-negative, non-spore-forming, non-motile and rod-shaped bacterium. After 3 days incubation at 30 °C, colonies on R2A agar medium are round, slightly yellowish, convex and 1.0–2.0 mm in diameter. On R2A agar, the optimal growth temperature for TR6-04T was 30 °C. Strain TR6-04T was able to grow at 42 °C, but not at 10 or 45 °C. The pH growth range was between pH 5.0 and 9.0, with an optimum between pH 6.5 and 7.0. Growth occurred in the absence of NaCl and in the presence of 5.0 % (w/v) NaCl, but not 7 % (w/v) NaCl. Phenotypic and chemotaxonomic characteristics that differentiate strain TR6-04T from related Sphingobacterium species are listed in Table 1. In contrast to all other Sphingobacterium species, strain TR6-04T assimilates L-xylose, does not

### Table 1. Differential phenotypic characteristics of strain TR6-04T and related type strains of Sphingobacterium species

<table>
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<th>Characteristic</th>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>42 °C</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>Hydrolysis of:</td>
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<tr>
<td>DNA</td>
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<td>+</td>
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<td>+</td>
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<td>ND</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Aesculin</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>Gelatin</td>
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<td>−</td>
<td>−</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Assimilation of:</td>
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<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>L-Arabinit</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>+</td>
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<td>+</td>
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<td>D-Mannitol</td>
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<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<td>D-Melibiose</td>
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<td>Glycerol</td>
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<td>Inulin</td>
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<td>+</td>
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<td>+</td>
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<td>Pyruvate</td>
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<td>−</td>
<td>V</td>
<td>−</td>
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<td>+</td>
<td>+</td>
<td>−</td>
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<td>+</td>
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<td>Acid production from:</td>
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<tr>
<td>L-Rhamnose</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-Arabinit</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>38.7</td>
<td>39.0</td>
<td>39.9–40.5</td>
<td>39.3–40.0</td>
<td>44.0–44.2</td>
<td>37.3</td>
<td>39.3</td>
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</table>
hydrolyse ascinul and does not produce urease. Strain TR6-04T could be distinguished from *S. antarcticum* MTCC 675T on the basis of its inability to assimilate L-rhamnose, L-arabinose, D-ribose, pyruvate, formate, DL-3-hydroxybutyrate, lactate, malate, succinate, inositol, D-mannitol, D-sorbitol, glycerol and L-glutamate, to produce gelatinase and hydrogen sulphide, to grow at temperatures below 15°C and to grow on MacConkey agar.

For the measurement of G+C content of the chromosomal DNA, genomic DNA was extracted and purified as described by Moore (1995) and was then enzymically degraded into nucleosides. G+C content was determined as described by Mesbah et al. (1989) using a reverse-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum and re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane quinone solution was then purified using silica Sep-Pak Vac cartridges (Waters) and subsequently analysed by HPLC, as described previously (Hiraishi et al., 1996).

Cellular fatty acid profiles were determined for strains grown on TSA for 2 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI) and then analysed by a gas chromatograph (Hewlett Packard 6890) and identified by the Microbial Identification software package (Sasser, 1990). The cellular fatty acid profiles of strain TR6-04T and related *Sphingobacterium* species are presented in Table 2. Strain TR6-04T contained large amounts of iso-branched and iso-branched hydroxy fatty acids; the major components were iso-C15:0, iso-C17:0 3-OH and summed feature 4 (iso-C15:0 2-OH and/or C16:1ω7c), typical of members of the genus *Sphingobacterium* (Takeuchi & Yokota, 1992; Steyn et al., 1998). Some qualitative and quantitative differences in fatty acid Table 2. Fatty acid compositions of strain TR6-04T and related *Sphingobacterium* species

<table>
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<th>Fatty acid</th>
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<tr>
<td><em>Straight-chain saturated</em></td>
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<tr>
<td>C14:0</td>
<td>–</td>
<td>1·0</td>
<td>2·7</td>
<td>tr</td>
<td>3·2</td>
<td>tr</td>
<td>+</td>
</tr>
<tr>
<td>C16:0</td>
<td>3·4</td>
<td>3·5</td>
<td>7·8</td>
<td>tr</td>
<td>6·0</td>
<td>4·5</td>
<td>+</td>
</tr>
<tr>
<td>C16:0 2-OH</td>
<td>–</td>
<td>–</td>
<td>tr</td>
<td>–</td>
<td>3·2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C16:0 3-OH</td>
<td>–</td>
<td>2·7</td>
<td>5·3</td>
<td>tr</td>
<td>6·3</td>
<td>2·1</td>
<td>–</td>
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<tr>
<td><em>Branched saturated</em></td>
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<tr>
<td>iso-C15:0</td>
<td>45·6</td>
<td>30·1</td>
<td>22·2</td>
<td>30·0</td>
<td>17·7</td>
<td>24·6</td>
<td>29·0</td>
</tr>
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<td>iso-C17:0</td>
<td>tr</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>iso-C15:0 2-OH</td>
<td>–*</td>
<td>21·5</td>
<td>17·4</td>
<td>25·6</td>
<td>24·6</td>
<td>15·9</td>
<td>tr</td>
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<tr>
<td>iso-C15:0 3-OH</td>
<td>1·5</td>
<td>2·2</td>
<td>3·2</td>
<td>3·0</td>
<td>4·3</td>
<td>3·7</td>
<td>tr</td>
</tr>
<tr>
<td>iso-C17:0 3-OH</td>
<td>16·6</td>
<td>12·5</td>
<td>7·1</td>
<td>22·1</td>
<td>10·0</td>
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<tr>
<td>anteiso-C15:0</td>
<td>2·6</td>
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<tr>
<td>C16:1 10-methyl</td>
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<td>–</td>
<td>–</td>
<td>1·4</td>
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<tr>
<td><em>Summed feature 4†</em></td>
<td>23·8</td>
<td>–</td>
<td>–</td>
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<tr>
<td><em>Monounsaturated</em></td>
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<tr>
<td>C16:1ω5c</td>
<td>tr</td>
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<td>–</td>
<td>tr</td>
<td>–</td>
<td>1·5</td>
<td>–</td>
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<tr>
<td>C16:1ω7c</td>
<td>–*</td>
<td>21·1</td>
<td>31·6</td>
<td>9·5</td>
<td>23·2</td>
<td>32·2</td>
<td>56·0</td>
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<tr>
<td>iso-C17:1ω9c</td>
<td>2·9</td>
<td>1·7</td>
<td>tr</td>
<td>3·7</td>
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<td>ECL 13·566</td>
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<td>ECL 16·580</td>
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*Components included in summed feature 4.
†Summed features represent groups of two or three fatty acids that can not be separated by GLC with the MIDI system. Summed feature 4 contains iso-C15:0 2-OH and/or C16:1ω7c.
contents could be observed between strain TR6-04T and the phylogenetically closest relative, S. mizutaii ATCC 33299T. In comparison with this strain, strain TR6-04T contained larger amounts of iso-C15:0 and smaller amounts of iso-C15:0 2-OH and/or C16:1iso7c and it did not contain C14:0 or C16:0 3-OH. The G+C content of genomic DNA for members of the genus Sphingobacterium ranges from 37.3 mol% (reported for S. faecium) to 44.2 mol% (reported for S. thalpophilum) (Takeuchi & Yokota, 1992; Steyn et al., 1998). The G+C content of the DNA of strain TR6-04T was 38.7 mol% and falls within this range.

All of the characteristics determined for strain TR6-04T are in accordance with those of the genus Sphingobacterium. On the basis of phylogenetic distance from established Sphingobacterium species, also indicated by relatively low 16S rRNA gene sequence similarities (< 97 %) and the combination of unique phenotypic characteristics, it is demonstrable that TR6-04T is not affiliated with any species of this genus. Therefore, on the basis of the data presented, strain TR6-04T should be placed in the genus Sphingobacterium as the type strain of a novel species, for which the name Sphingobacterium daejeonense sp. nov. is proposed.

Description of Sphingobacterium daejeonense sp. nov.

Sphingobacterium daejeonense (dae.jeon.en’se. N.L. neut. adj. daejeonense pertaining to Daejeon, a city in Korea, where the type strain was isolated).

Cell are Gram-negative, strictly aerobic, non-spore-forming, non-motile and rod-shaped (0.5–1.0 µm wide and 1.2–1.8 µm long). After 3 days incubation at 30 °C on R2A, colonies are 1–2 mm in diameter, smooth, convex, round and slightly yellowish. Growth occurs between 15 and 42 °C; the optimum temperature for growth is 30 °C. The pH range for growth is pH 5.0–9.0, with an optimum between pH 6.5 and 7.0. Tolerates 5 % (w/v) NaCl, but not 7 %. Growth occurs on TSA, but not on MacConkey agar. Positive for catalase and oxidase. Negative for hydrolysis of chitin, starch, cellulose, DNA, xylan, casein and aesculin. The following substrates are utilized for growth: D-glucose, D-galactose, D-mannose, D-fructose, D-arabinose, D-lyxose, D-xylose, L-xylitol, N-acetyl-D-glucosamine, salicin, D-cellobiose, D-lactose, D-maltose, D-melibiose, sucrose, D-trehalose, D-raffinose, D-adonitol and amygdalin. The following substrates are not utilized for growth: D-fucose, ethanol, L-rhamnose, L-sorbitose, L-arabinose, 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, dulcitol, inositol, D-mannitol, D-sorbitol, xylitol, glycerol, mannitol, 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-tryptophan, L-tyrosine and L-valine. In API 20E tests, β-galactosidase and the Voges–Proskauer test are positive; tests for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, gelatinase, hydrogen sulphide and indole production are all negative. Acid is produced from D-glucose, D-melibiose and amygdalin, but not from L-arabinose, D-mannitol, inositol, D-sorbitol, L-rhamnose or sucrose. MK-7 is the predominant menaquinone, and major fatty acids are iso-C15:0, summed feature 4 (iso-C15:0 2-OH and/or C16:1iso7c) and iso-C17:0 3-OH. The G+C content of genomic DNA of the type strain is 38.7 mol% (as determined by HPLC).

The type strain, TR6-04T ( = KCTC 12579T = LMG 23402T = CCUG 52468T), was isolated from compost that was collected near Daejeon city in South Korea.

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References


