**Sphingomonas parvus** sp. nov. isolated from a ginseng-cultivated soil

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Strain GP20-2T was isolated from a soil cultivated with ginseng in Korea. The 16S rRNA gene sequence of this strain showed the highest sequence similarity with *Sphingomonas daechungensis* CH15-11T (96.7%) and *Sphingomonas sedimincola* Dae 20T (96.2%) among the type strains. The strain GP20-2T was a strictly aerobic, Gram-negative, non-motile, rod-shaped bacterium that formed very tiny colonies, less than 0.3 mm in diameter after 10 days on R2A agar. The strain grew at 10–35°C (optimum, 35°C), at a pH of 5.0–8.0 (optimum, pH 6.0), and in the absence of NaCl. The DNA G+C content of strain GP20-2T was 67.2 mol%. It contained ubiquinone Q-10 as the major isoprenoid quinone, and summed feature 8 (C18:0 606c and/or C16:0 7c, 49.8%) and C16:0 (17.0%) as the major fatty acids. On the basis of evidence from our polyphasic taxonomic study, we concluded that strain GP20-2T should be classified as a novel species of the genus *Sphingomonas* for which the name *Sphingomonas parvus* sp. nov. is proposed. The type strain is GP20-2T (=KACC 12865T =DSM 100456T).

**Keywords:** *Sphingomonas*, strain GP20-2, novel species, polyphasic taxonomy

**Introduction**

The genus *Sphingomonas* was so named by Yabuuchi et al. (1990) because of the presence of unique sphingoglycolipids in its cellular lipid. Strains of the genus *Sphingomonas* are characterized as Gram-negative, non-sporulating, strictly aerobic, motile or non-motile, and chemoorganotrophic rods. Colony color varies from orange to yellow to white to non-pigmented. They have Q-10 as the predominant quinone, and sym-homospermidine as the major polyamine. The G+C content of their DNA varies between 62% and 68% and the major fatty acids are C18:1, saturated C16:0, and/or C17:1. Phylogenetically, the genus *Sphingomonas* is a member of the α-4 subclass of Proteobacteria (Chen et al., 2012).

The members of the genus *Sphingomonas* are free living in natural and man-made environments such as air (Busse et al., 2003; Kim et al., 2014), soil (Zhang et al., 2010; Kim et al., 2014), desert sand (An et al., 2011), natural mineral water (Lee et al., 2001), wastewater (Fujii et al., 2001; Yoon et al., 2009), tidal flat sediment (Roh et al., 2009), seawater (Van-canneyt et al., 2001), and plants (Takeuchi et al., 1995; Xie and Yokota, 2006). Recent studies showed that the genus *Sphingomonas* is one of the dominant bacterial genera present on the phyllosphere and involved in plant protection against bacterial pathogens (Vorholt, 2012). At the time of this writing, the genus comprised 89 recognized species (www.bacterio.net).

During the course of an investigation of a bacterial community in soil cultivated with ginseng, one isolate was shown to represent a novel species of the genus *Sphingomonas* on the basis of phenotypic data and phylogenetic inference.

**Materials and Methods**

**Bacterial strains**

The soil sample was collected from a ginseng field in Yeongju region, Korea. It was diluted serially in 0.85% (w/v) saline solution, spread on R2A agar (Difco), and incubated for 7 days at 28°C. Bacterial colonies with unique morphologies were selected for the analysis of the 16S rRNA gene sequences and one of them, designated as strain GP20-2T, was selected for polyphasic characterization. Type strains of the genus *Sphingomonas* were obtained from the Korean Agricultural Culture Collection (KACC) for comparative taxonomic analysis.

**Phylogenetic analysis**

The 16S rRNA gene of the strain GP20-2T was amplified by the polymerase chain reaction (PCR) and sequenced as described by Ahn et al. (2014). The near-complete 16S rRNA gene sequence of strain GP20-2T (1,427 nt) and those of the selected type strains belonging to the genus *Sphingomonas* were aligned using the online tool SINA Alignment Service, version 1.2.11 (Pruesse et al., 2012) (www.arb-silva.de/align). The aligned sequences were exported to the Molecular Evolutionary Genetics Analysis (MEGA) software program, version 6.06 (Tamura et al., 2011) and maximum-likelihood, neighbor-joining, and maximum-parsimony trees were constructed. Nucleotide similarity values were calculated using the EzTaxon-e server (Kim et al., 2012) (www.ezbiocloud.net/eztaxon).
Nucleotide sequence accession number

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain GP20-2\textsuperscript{T} is KP025677.

Determination of DNA G+C content

The DNA G+C content was determined as described by Gonzalez and Saiz-Jimenez (2002) using the CFX96 real-time PCR system (Bio-Rad). Four bacterial strains were used to construct the calibration curve: Bacillus cereus KACC 11240\textsuperscript{T}, Bacillus amyloliquefaciens subsp. plantarum KACC 17177\textsuperscript{T}, Pseudomonas stutzeri KACC 10290\textsuperscript{T}, and Micrococcus luteus KACC 10488\textsuperscript{T}.

Morphological, physiological, and biochemical characterization

The cell morphology and motility of strain GP20-2\textsuperscript{T} was examined by using oil-immersion phase-contrast microscopy (Axioplan 2; Zeiss) with cells grown for 4 days on R2A agar at 30°C. In addition to R2A agar, growth of strain GP20-2\textsuperscript{T} was tested on trypticase soy agar (TSA) (Difco), nutrient agar (NA) (Difco), Luria-Bertani agar (LB) (Difco), and marine agar 2216 (MA) (Difco). Growth in the presence of 0%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0% NaCl (w/v), and at various temperatures (5–45°C at intervals of 5°C) was investigated by 2 weeks of incubation on R2A agar. The pH range for growth was observed for 10 days of incubation in R2A broth at a pH of 2.0–11.0 in increments of 1.0 units. In this experiment, no buffer was added due to the sensitivity of strain GP20-2\textsuperscript{T} to salt. Gram staining behavior was tested by the KOH test (Smibert and Krieg, 1994) and assessing L-alanine aminopeptidase activity (Bactident Aminopeptidase test kit; Merck). Catalase activity was detected by dispersing colonies in 3% (v/v) hydrogen peroxide and checking for bubble formation, and oxidative activity was determined by using the Oxidase Reagent (bioMérieux). Casein, carboxymethyl cellulose (CM-cellulose), starch, Tween 20/40/60/80, tyrosine, and lipase hydrolyses were examined on R2A plates containing milk powder [5% (w/v)], CM-cellulose [1% (w/v)], starch [1% (w/v)], Tween 20/40/60/80 [1% (w/v)], tyrosine [0.1% (w/v)], and tributyrin [1.0% (w/v)], respectively, for two weeks. Growth under anaerobic condition was tested by incubating R2A agar plates in AnaeroGen sachet pouches (Oxoid Ltd.) at 30°C for two weeks. Other biochemical characteristics were determined using

Fig. 1. Maximum-likelihood tree based on a comparative analysis of 16S rRNA gene sequences showing the relationship between strain GP20-2\textsuperscript{T} and selected type strains belonging to the genus Sphingomonas. Bootstrap values (>70%) based on 500 resamplings are shown at branching points. The dots indicate that the corresponding branches were also recovered in the neighbor-joining and maximum-parsimony trees. The scale bar indicates 0.02 estimated change per nucleotide. Escherichia coli KCTC 2441\textsuperscript{T} was used as an outgroup.