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# *Caulobacter ginsengisoli* sp. nov., a Novel Stalked Bacterium Isolated from Ginseng Cultivating Soil

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**A Gram negative, aerobic, nonspore-forming, straight or curved rod-shaped bacterium, designated Gsoil 317$^T$, was isolated from soil of a ginseng field in Pocheon Province (South Korea) and was characterized using a polyphasic approach. Cells were dimorphic, with stalk (or prostheca) and nonmotile or nonstalked and motile, by means of a single polar flagellum. Comparative analysis of 16S rRNA gene sequences revealed that strain Gsoil 317$^T$ was most closely related to *Caulobacter mirabilis* LMG 24261$^T$ (97.2%), *Caulobacter fusiformis* ATCC 15257$^T$ (97.1%), *Caulobacter segnis* LMG 17158$^T$ (97.0%), *Caulobacter vibrioides* DSM 9893$^T$ (96.8%), and *Caulobacter henricii* ATCC 15253$^T$ (96.7%). The sequence similarities to any other recognized species within *Alphaproteobacteria* were less than 96.0%. The detection of Q-10 as the major respiratory quinone and a fatty acid profile with summed feature 7 ($C_{18:1}$ ω7c and/or $C_{18:1}$ ω9t and/or $C_{18:1}$ ω12t; 56.6%) and $C_{16:0}$ (15.9%) as the major fatty acids supported the affiliation of strain Gsoil 317$^T$ to the genus *Caulobacter*. The G+C content of the genomic DNA was 65.5 mol%. DNA–DNA hybridization experiments showed that the DNA–DNA relatedness values between strain Gsoil 317$^T$ and its closest phylogenetic neighbors were below 11%. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 317$^T$ should be classified as representing a novel species in the genus *Caulobacter*, for which the name *Caulobacter ginsengisoli* sp. nov. is proposed. The type strain is Gsoil 317$^T$ (=KCTC 12788$^T$= DSM 18695$^T$).**

**Keywords:** *Caulobacter ginsengisoli*, polyphasic taxonomy, ginseng-field soil

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The genus *Caulobacter*, with *Caulobacter vibrioides* as the type species, was defined by Henrici and Johnson [7] in 1935, based on microscopic finding of organisms attached to slides that had been suspended in a freshwater lake, but they did not isolate *Caulobacter vibrioides* and therefore no type strain was given. Later, Poindexter [13] proposed strain CB 51 as the neotype strain of *Caulobacter vibrioides* and described nine species and two subspecies including *Caulobacter maris* and *Caulobacter halobacteroides*, the first two caulobacteria from seawater. At that time, the taxonomy of the members of the genus was based almost exclusively on the characteristics of morphology and mode of reproduction, mainly on the basis of their characteristic prosthecate. Stahl *et al.* [18] were the first to sequence the 16S rRNA genes of several *Caulobacter* representatives and concluded that members of *Caulobacter* formed two different lineages, one comprising freshwater and brackish-water forms, and the other comprising marine species. Abraham *et al.* [2] established that all freshwater caulobacteria belong to the genera *Caulobacter* and *Brevundimonas* and transferred several *Caulobacter* species or subspecies to the genus *Brevundimonas*. According to their results, the species *Caulobacter henricii*, *Caulobacter fusiformis*, *Caulobacter vibrioides*, and *Caulobacter segnis* form a paraphyletic group and belong *sensu stricto* to *Caulobacter*. At the time of writing, the genus *Caulobacter* includes the four above-mentioned species and the recently described *Caulobacter mirabilis* [3]. Members of this genus are characterized by their asymmetric cell division and chemotaxonomically by having Q-10 as the predominant ubiquinone, summed feature 7 ($C_{18:1}$ ω7c and/or $C_{18:1}$ ω9t and/or $C_{18:1}$ ω12t) and $C_{16:0}$ as the major fatty acids, and DNA G+C contents of 62–68 mol% [2, 3, 14, 20].

During a course of study on the culturable aerobic bacterial community in soil of a ginseng field in Pocheon Province (South Korea), a large number of bacteria were

isolated. Some of the strains were already characterized and classified as novel species of the genera *Bacillus* [19], *Chitinophaga* [1], *Cohnella* [22], *Lysobacter* [8], and *Sphingopyxis* [10, 11]. In this study, we have characterized one of these isolates, strain Gsoil 317$^T$. The phenotypic, chemotaxonomic, and phylogenetic analyses established the affiliation of the isolate to the genus *Caulobacter* in the *Alphaproteobacteria*. The data obtained also suggest that the isolate represents a novel species of this genus.

Strain Gsoil 317$^T$ was isolated from soil sample by using modified R2A agar as described previously [19]. The isolate was routinely cultured on R2A agar at 25°C and preserved in a glycerol solution (20%) at −70°C. The Gram reaction was performed by the nonstaining method, as described by Buck [4]. Flagellation and stalk generation were examined by transmission electron microscopy, using cells grown for 3 days at 25°C on R2A agar. The cells were negatively stained with 1% (w/v) phosphotungstic acid, and after air-drying, grids were examined with a transmission electron microscope (model CM-20, Philips). Enzyme activities, the assimilation of single carbon sources, and other physiological characteristics were determined with API ZYM, API 32GN, API 20NE, and API 20E galleries according to the manufacturer's instructions (bioMérieux). Catalase and oxidase activities, anaerobic growth, growth at different temperatures and pH values, and degradation of natural biopolymers were tested as reported previously [19, 22].

Strain Gsoil 317$^T$ was Gram-negative, aerobic, nonspore-forming, straight or curved rod-shaped. Cells were dimorphic, motile by means of a single polar flagellum (Fig. 1A) and nonmotile or nonstalked (Fig. 1B) and with stalk (or prostheca) (Fig. 1C, 1D); these are typical cell characteristics of *Caulobacter* species [2, 3, 13, 14]. After 3 days grown on R2A, colonies were 0.5–1.0 mm in diameter, convex, circular, transparent, and yellowish. On R2A agar, the optimum temperature for growth was 25°C. Strain Gsoil 317$^T$ grew well on nutrient agar medium and utilized a fairly narrow spectrum of carbon sources. The isolate was positive for oxidase but negative for catalase, and showed degradation of starch, cellulose, xylan, casein, and chitin. The physiological characteristics of strain Gsoil 317$^T$ are summarized in the species description in Table 1, and selective characteristics that differentiate strain Gsoil 317$^T$ from other members of the genus *Caulobacter* are also listed.

For phylogenetic analysis of strain Gsoil 317$^T$, genomic DNA was extracted using a commercial genomic DNA extraction kit (Solgent, Korea). PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* [9]. Other details of phylogenetic analysis were reported previously [19, 22].

A nearly complete 16S rRNA gene sequence of strain Gsoil 317$^T$ was obtained (1,421 bp). By using the EzTaxon server (http://www.eztaxon.org/; Chun *et al.* [5]), the novel isolate was shown to belong to the family *Caulobacteraceae*. The phylogenetic tree (Fig. 2) based on the neighbor-joining algorithm showed that strain Gsoil 317$^T$ fell within the radiation of the cluster comprising *Caulobacter* species and joined *Caulobacter fusiformis* ATCC 15257$^T$. Comparative analysis of 16S rRNA gene sequences revealed that strain Gsoil 317$^T$ was most closely related to *Caulobacter mirabilis* LMG 24261$^T$ (97.2%), *Caulobacter fusiformis* ATCC 15257$^T$ (97.1%), *Caulobacter segnis* LMG 17158$^T$ (97.0%), *Caulobacter vibrioides* DSM 9893$^T$ (96.8%), and *Caulobacter henricii* ATCC 15253$^T$ (96.7%). The sequence similarities to any other recognized species within *Alphaproteobacteria* were less than 96.0%.

A value of 97% 16S rRNA gene sequence similarity has been proposed to be the threshold for possible species delineation [17]. According to 16S rRNA gene sequence analysis and subsequent phylogenetic analysis, the DNA–DNA hybridization test between strain Gsoil 317$^T$ and *Caulobacter mirabilis* LMG 24261$^T$, *Caulobacter fusiformis* ATCC 15257$^T$, and *Caulobacter segnis* LMG 17158$^T$, were performed to define a novel species, using the method of Ezaki *et al.* [6]. The DNA–DNA relatedness values between

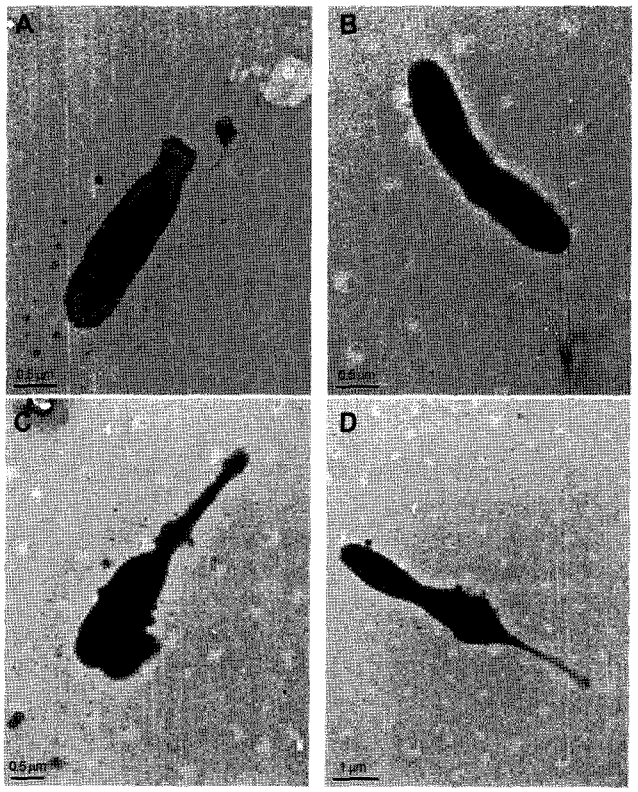


**Fig. 1.** Transmission electron micrographs of negatively stained cells of strain Gsoil 317$^T$.
**A.** Cell showing a single polar flagellum. **B.** Cell showing the loss of flagellum or nonstalked. **C** and **D.** Cells containing the typical stalk near the cell pole.

**Table 1.** Differential phenotypic characteristics of strain Gsoil 317$^T$ and recognized species of the genus *Caulobacter*.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Growth at 2% (w/v) NaCl[a] | – | + | + | + | – | – |
| Prostheca[a] | + | + | – | + | + | + |
| Arginine dihydrolase | – | – | – | + | – | – |
| Protease (gelatin hydrolysis) | – | + | + | – | – | + |
| Assimilation (API 20NE & ID 32GN): | | | | | | |
| D-Glucose | – | – | + | – | – | – |
| L-Arabinose | – | + | + | – | – | – |
| D-Mannitol | + | – | – | – | – | – |
| *N*-Acetyl-D-glucosamine | – | – | + | + | – | – |
| D-Mannitol | + | – | – | – | – | – |
| Salicin | – | – | + | – | – | – |
| L-Fucose | – | – | – | – | – | + |
| L-Arabinose | – | + | + | – | – | – |
| Propionate | – | – | – | + | – | – |
| Valerate | – | – | – | + | – | – |
| L-Histidine | – | – | – | + | – | – |
| 3-Hydroxybutyrate | + | – | + | + | – | – |
| 4-Hydroxybenzoate | – | – | + | | – | – |
| L-Proline | – | – | + | + | – | – |
| L-Rhamnose | + | – | + | + | – | – |
| D-Sucrose | – | – | + | – | – | – |
| Suberate | – | – | – | + | – | – |
| Acetate | – | – | – | + | – | – |
| Glycogen | – | – | + | – | – | – |
| 3-Hydroxybenzoate | – | – | + | – | – | – |
| API ZYM tests: | | | | | | |
| Esterase (C4) | – | – | w | – | – | – |
| Esterase lipase (C8) | + | – | – | – | + | – |
| Valine arylamidase | – | – | – | – | + | – |
| Trypsin | + | + | – | + | + | + |
| α-Chymotrypsin | w | – | – | – | – | – |
| Naphthol-AS-BI-phosphohydrolase | + | + | – | + | + | + |
| β-Galactosidase | + | + | + | – | + | – |
| α-Glucosidase | + | + | – | – | – | – |
| β-Glucosidase | + | + | + | – | + | – |
| *N*-Acetyl-β-glucosaminidase | - | + | – | – | + | + |
| DNA G+C content (mol%)[a] | 65.5 | NA | 67–68 | 68 | 64–65 | 62–65 |

Strains: 1, Gsoil 317$^T$; 2, *Caulobacter fusiformis*; 3, *Caulobacter segnis*; 4, *Caulobacter mirabilis*; 5, *Caulobacter vibrioides*; 6, *Caulobacter henricii*.
Symbols: +, positive; –, negative; NA, no data available.
All data are from this study, except where indicated otherwise. In API 20NE, API 32GN, API ZYM tests, all type strains produced positive results for alkaline phosphatase, leucine arylamidase, and acid phosphatase, and negative results for nitrate reduction, indole production, glucose acidification, urease, lipase (C14), cystine arylamidase, α-galactosidase, β-glucosidase, α-mannosidase, and α-fucosidase. None of the strains assimilated D-mannose, D-maltose, gluconate, caprate, adipate, malate, citrate, phenylacetate, D-melibiose, D-sorbitol, 2-ketogluconate, D-ribose, inositol, itaconate, malonate, lactate, L-alanine, 5-ketogluconate, and L-serine.
[a]Data taken from Abraham *et al.* [2, 3], Poindexter [14] and Stahl *et al.* [18], except data of Gsoil 317$^T$.
w$^l$:weak reaction.

strain Gsoil 317$^T$ and the 3 species of genus *Caulobacter* were 6–11% (*i.e.*, to *Caulobacter mirabilis* LMG 24261$^T$, 9%; *Caulobacter fusiformis* ATCC 15257$^T$, 11%; *Caulobacter segnis* LMG 17158$^T$, 6%), which is low enough to assign strain Gsoil 317$^T$ as a novel species of genus *Caulobacter* [21].

Isoprenoid quinones were extracted and purified according to the method reported previously [19, 22] and analyzed by HPLC, as described by Shin *et al.* [16]. Cellular fatty acids were extracted and analyzed according to the instructions of the Sherlock Microbial Identification System (MIDI) [15]. Strain Gsoil 317$^T$ and all related type strains were

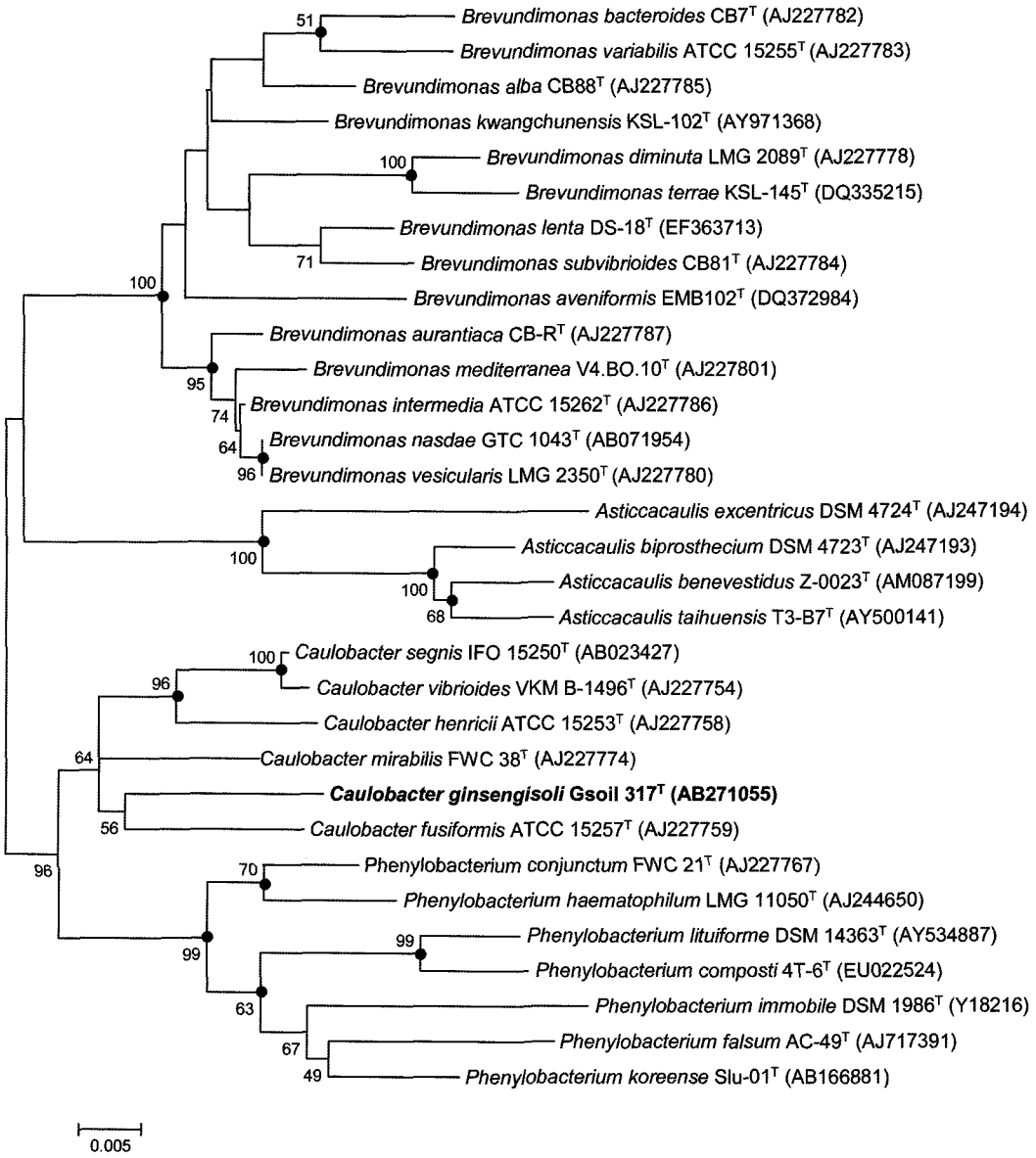


**Fig. 2.** Phylogenetic relationship of strain Gsoil 317$^T$ with *Caulobacter* species and other related species of *Alphaproteobacteria*.
The tree was constructed by the neighbor-joining method based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1,000 replications) greater than 50% are shown at branch points. Filled circles at nodes indicate generic branches that were also recovered by using maximum-parsimony algorithms. Bar, 0.005 substitutions per 1 nucleotide position.

cultivated on R2A for 7 days at 30°C. Ubiquinone Q-10 was the predominant respiratory quinone that supports affiliation of strain Gsoil 317$^T$ to the genus *Caulobacter*, where all recognized members have Q-10 as the major quinone [2, 3]. The fatty acids found in isolate Gsoil 317$^T$ are shown in Table 2 and are compared with values available for related *Caulobacter* species. Summed feature 7 and straight-chain saturated $C_{16:0}$, the major fatty acids in the genus *Caulobacter* [2, 3], were also the predominant fatty acid components of strain Gsoil 317$^T$, comprising 56.6% and 15.9 % of the total, respectively. $C_{12:1}$ 3-OH and summed feature 4, which are common fatty acids for all members of the genus *Caulobacter*, were also identified in our strain. Strain Gsoil 317$^T$ as well as other *Caulobacter* species (except *C. segnis*) additionally contained $C_{16:1}$ ω11c, $C_{17:1}$ ω6c, and $C_{17:1}$ ω8c. However, strain Gsoil 317$^T$ could be differentiated from other *Caulobacter* species by the presence of a relatively significant amount of $C_{17:0}$ and by the absence of $C_{12:0}$, $C_{14:0}$, $C_{16:1}$ 2OH, and $C_{12:0}$ 3OH. The G+C content of the chromosomal DNA was determined, as described by Mesbah *et al.* [12], using reversed-phase HPLC. The DNA G+C content of strain Gsoil 317$^T$ was 65.5 mol%, which lies within the range observed for members of the genus *Caulobacter* [3].

**Table 2.** Cellular fatty acids profile of *Caulobacter ginsengisoli* Gsoil 317$^T$ and related *Caulobacter* species.

| Fatty acids | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Straight-chain saturated | | | | | | |
| $C_{12:0}$ | – | – | 3.24 | 2.90 | – | – |
| $C_{14:0}$ | – | 3.99 | 2.24 | 0.68 | 4.32 | 5.62 |
| $C_{15:0}$ | 2.6 | 2.85 | - | 2.45 | 3.52 | 1.16 |
| $C_{16:0}$ | 15.9 | 25.62 | 22.43 | 28.42 | 23.48 | 29.83 |
| $C_{17:0}$ | 13.7 | – | – | 3.52 | – | – |
| $C_{18:0}$ | 2.1 | – | – | 1.19 | – | – |
| Mono-unsaturated | | | | | | |
| $C_{16:1}$ ω11c | 0.6 | – | – | – | – | – |
| $C_{17:1}$ ω6c | 3.5 | – | – | – | – | – |
| $C_{17:1}$ ω8c | 0.8 | – | – | – | – | – |
| Hydroxy fatty acids | | | | | | |
| $C_{16:1}$ 2OH | – | 4.80 | 2.82 | – | 6.69 | – |
| $C_{12:0}$ 3OH | – | 2.77 | 1.51 | - | 1.71 | 1.10 |
| $C_{12:1}$ 3OH | 3.6 | 2.79 | 2.00 | 2.52 | 2.74 | 1.09 |
| Summed feature[a] | | | | | | |
| Summed feature 4 | 0.6 | 19.75 | 21.07 | 1.04 | 22.47 | 15.52 |
| Summed feature 7 | 56.6 | 28.93 | 21.32 | 9.21 | 27.24 | 41.04 |
| Unknown[b] | | | | | | |
| ECL 11.798 | - | 3.64 | - | - | 3.45 | 2.39 |
| ECL 18.079 | - | 4.88 | 13.60 | 48.10 | 4.39 | 2.24 |
| ECL 18.149 | - | - | 9.77 | - | - | - |

Strains: 1, Gsoil 317$^T$; 2, *Caulobacter fusiformis*; 3, *Caulobacter segnis*; 4, *Caulobacter mirabilis*; 5, *Caulobacter vibrioides*; 6, *Caulobacter henricii*. Symbols: –, not detected; ECL, equivalent chain length.

[a]Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contained iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ ω7c and/or $C_{16:1}$ ω7t. Summed feature 7 contained $C_{18:1}$ ω7c and/or $C_{18:1}$ ω9t and/or $C_{18:1}$ ω12t.

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

All of the characteristics determined for strain Gsoil 317$^T$ are in accordance with those of the genus *Caulobacter*. However, the phylogenetic distinctiveness of strain Gsoil 317$^T$ confirmed that this isolate represents a species that is distinct from recognized *Caulobacter* species. There are some phenotypic differences between strain Gsoil 317$^T$ and phylogenetically related *Caulobacter* species (Table 1). Therefore, on the basis of the data presented, strain Gsoil 317$^T$ should be classified within the genus *Caulobacter* as the type strain of a novel species, for which the name *Caulobacter ginsengisoli* sp. nov. is proposed.

**Description of *Caulobacter ginsengisoli* sp. nov.**

*Caulobacter ginsengisoli* (gin.sen.gi.so'li. N.L. n. ginsengum, ginseng; L. n. solum, soil; N.L. gen. n. *ginsengisoli*, of soil of a ginseng field, the source of the organism).

Gram-negative, aerobic, nonspore-forming straight or curved rods (0.4–0.5 μm wide and 1.5–2.0 μm long). Cells are dimorphic, with stalk (or prostheca) and nonmotile or nonstalked and motile, by means of a single polar flagellum. After 3 days grown on R2A, colonies are 0.5–1.0 mm in diameter, convex, circular, transparent, and yellowish. Oxidase is positive but catalase is negative. Nitrate is not reduced to nitrite. Grows at 20–30°C and pH 5.5–8.0; optimum growth occurs at 25°C and pH 6.5–7.0. Grows without NaCl, but not at 1% (w/v) NaCl. Growth occurs on nutrient agar but not on TSA and MacConkey agar. Does not hydrolyze DNA, chitin, starch, cellulose, xylan, olive oil, and casein. D-Mannitol, L-rhamnose, and 3-hydroxybutyrate are utilized as sole carbon and energy sources, whereas D-glucose, D-maltose, D-mannose, D-melibiose, D-ribose, L-fucose, L-arabinose, D-sucrose, D-sorbitol, inositol, salicin, gluconate, propionate, caprate, phenylacetate, 3-hydroxybenzoate, 4-hydroxybenzoate, malonate, acetate, valerate, citrate, lactate, malate, 5-ketogluconate, 2-ketogluconate, itaconate, adipate, suberate, glycogen, *N*-acetyl-D-glucosamine, gelatin, urea, L-serine, L-tryptophan, L-alanine, L-arginine, L-histidine, and L-proline are not utilized. In API 20E tests, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, gelatin hydrolysis, the Voges–Proskauer test, and indole and hydrogen sulfide production are all negative. Acid is not produced from amygdalin, L-arabinose, D-glucose, inositol, D-mannitol, D-melibiose, L-rhamnose, D-sorbitol, and D-sucrose. Produces alkaline phosphatase, esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, and weakly α-chymotrypsin.

Does not produce *N*-acetyl-β-glucosaminidase, cystine arylamidase, esterase (C4), α-galactosidase, valine arylamidase, α-fucosidase, β-glucuronidase, lipase (C14), and α-mannosidase. Q-10 is the predominant ubiquinone. The major fatty acids are summed feature 7 ($C_{18:1}$ ω7c and/or $C_{18:1}$ ω9t and/or $C_{18:1}$ ω12t), $C_{16:0}$, and $C_{17:0}$. The G+C content of genomic DNA is 65.5 mol%.

The type strain Gsoil 317$^T$ (=KCTC 12788$^T$=DSM 18695$^T$) was isolated from a soil sample of a ginseng field of Pocheon Province (South Korea). The 16S rRNA gene sequence of strain Gsoil 317$^T$ has been deposited in NCBI GenBank under the accession number AB271055.

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