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Flavobacterium panaciterrae sp. nov., a β-glucosidase producing bacterium with ginsenoside-converting activity isolated from the soil of a ginseng field

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DCY69^T is JX233806.

A Gram-reaction-negative, oxidase- and catalase-positive, non-gliding motile strain, designated strain DCY69^T, was isolated from the soil of a ginseng field in the Republic of Korea. Colonies of strain DCY69^T were circular, 0.5−1.5 mm diameter, yellow, and convex on an R2A agar plate after 2 days. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain DCY69^T belonged to the genus *Flavobacterium* with 90.5−98.3% gene sequence similarity. The major predominant quinone was MK-6. The major cellular fatty acids were *iso*-$C_{15:0}$, *iso*-$C_{17:0}$ 3-OH, *iso*-$C_{15:0}$ 3-OH and summed feature 3 (containing $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). The major polar lipids were phosphatidylethanolamine, one unidentified aminolipid and unidentified polar lipids (L1, L2). The genomic DNA G+C content of strain DCY69^T was 35.0 mol%. The strain DCY69^T transformed ginsenoside Rb1 into Rd and F2. Based on the polyphasic taxonomic data, strain DCY69^T is considered to represent a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium panaciterrae* sp. nov. is proposed. The type strain is DCY69^T(= KCTC 32392^T = JCM 19161^T), isolated from the soil of a ginseng field in the Republic of Korea.

Key words: *Bacteroidetes*; *Flavobacteriaceae*; *Flavobacterium panaciterrae*; biotransformation

Introduction

The genus *Flavobacterium* was first described by Bergey et al. (1923). It is a member of the family *Flavobacteriaceae*, in the phylum *Bacteroidetes.* At the time of writing, more than 128 species of the genus *Favobacterium* have been recognized (Euzéby, 1997). The genus *Flavobacterium* comprises a very diverse group of bacteria that can be isolated from different sources such as soil (Yoon et al., 2006), freshwater lake sediment (Wang et al., 2006), fish tissues (Wiklund et al., 2000) and wastewater treatment plants (Ryu et al., 2007). Members of the genus *Flavobacterium* comprise Gram-reaction negative, aerobic, non-fermenting, oxidase-positive, predominantly gliding, yellow-to-orange-pigmented bacteria with menaquinone-6 (MK-6) as the primary respiratory quinone. The DNA G+C content is in the range of 30 to 41 mol% (Bernardet and Bowman, 2011). In the present study, we report the phenotypic, physiological, chemotaxonomic and phylogenetic characterization of the novel strain DCY69^T, which was isolated from the soil of a ginseng field. Based on these results, a new species of the genus *Flavobacterium*, with the name *Flavobacterium panaciterrae* sp. nov., is proposed.

Materials and Methods

Bacterial strain. Strain DCY69^T was isolated from the soil of a ginseng field in the Republic of Korea. The soil sample was inoculated on one-fifth-strength R2A agar (Difco, Franklin Lakes, NJ) using a standard dilution plating method and incubated at 30°C from 7 to 10 days. After primary isolation, the strains were cultured on R2A agar at 30°C and preserved in 30% (v/v) glycerol suspensions at −80°C.

Morphological, physiological and biochemical characteristics. Cell size, shape, morphology and flagella of the strain DCY69^T were observed by transmission electron

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microscopy using cells grown in R2A broth (Difco) for 24 h at 25°C. The suspended cells were placed on Carbon and Formvar-coated nickel grids for 30 s and the grids were floated on 1 drop of 0.1% (w/v) aqueous uranyl acetate, blotted dry and then viewed with a Carl Zeiss electron microscope (LOE912AB) at 100 kV under standard operating conditions. Gram staining of strain DCY69^T was performed using a Gram stain kit (bioMérieux, Marcy l'Etoile, France). Gliding motility was determined by the hanging-drop technique (Bernardet et al., 2002) with cells grown in R2A broth at 25°C for 24 h. Catalase activity was determined by bubble production in a 3% (v/v) H_2O_2 solution. Oxidase activity was tested using 1% (w/v) *N,N,N′,N′*-tetramethyl-1,4-phenylenediamine reagent (bioMérieux) according to the manufacturer's instructions. The hydrolysis of casein, esculin, DNA, gelatin, starch and Tween 80 were investigated on R2A agar after 3 days' incubation at 25°C according to Cowan and Steel (1974). The temperature range of growth was tested on R2A agar at 4, 10, 20, 25, 30, and 37°C. Growth at different pH values 3.0−11.0 was tested (at intervals of 1 pH units, 50 mM). The following buffer was used for different pH values, citric acid-Na_2HPO_4 (for pH 3−6), $NaHPO_4$-Na_2HPO_4 (for pH 7−8), glycine-NaOH (for pH 9−10), Na_2HPO_4-NaOH (for pH 11). Tolerance of salinity was evaluated on R2A broth supplemented with 0−5.0 % NaCl (0.5% intervals) at 25°C. Growth was tested on nutrient agar (NA), R2A agar, trypticase soy agar (TSA) and MacConkey agar. Growth under anaerobic conditions was assessed after 10 days of incubation on R2A at 25°C in the GasPak EZ anaerobic container system (BD). Biochemical and enzymatic characterizations, carbon and nitrogen substrates utilization and acid production were performed using API 20NE, API ZYM and API 32GN according to the manufacturer's instructions (bioMérieux). Antibiotic susceptibility was tested by using disc diffusion method (Bauer et al., 1966). The inhibition zone was interpreted according to the manufacturer's manual. The following antibiotics disks (Oxoid, Basingstoke, UK) were used: penicillin G (P_{10}, 10 units), erythromycin (E_{15}, 15 μg), cefazolin (KZ_{30}, 30 μg), oleandomycin (OL_5, 15 μg), ceftazidime (CAZ_{30}, 30 μg), vancomycin (VA_{30}, 30 μg), tetracycline (TE_{30}, 30 μg), novobiocin (NV_{30}, 30 μg), carbenicillin (CAR_{100}, 100 μg), rifampicin (RD_5, 5 μg) and neomycin (N_{30}, 30 μg).

Phylogenetic analysis, determination of DNA G+C content and DNA-DNA hybridization. Genomic DNA was extracted and purified using the Genomic DNA isolation kit (Core Bio System, Seoul, Republic of Korea). The 16S rRNA gene was amplified using the universal bacterial primer set 27F, 518F, 800R and 1492R (Lane, 1991; Anzai et al., 2000). The purified PCR products were sequenced by Genotech (Daejeon, Republic of Korea) (Kim et al., 2005). The sequence of 16S rRNA gene was compiled using SeqMan software (DNASTAR). The resulting 16S rRNA gene sequence (1,395 bp) was compared with available 16S rRNA gene sequences by using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) (Kim et al., 2012). The 16S rRNA gene sequences of closely related type strains were obtained from GenBank. Multiple alignments were performed with the CLUSTAL_X program (Thompson et al., 1997) and gaps were edited in the BioEdit program (Hall, 1999). The phylogenetic tree was constructed with neighbor-joining (Saitou and Nei, 1987) and maximum parsimony (Fitch, 1971) methods using MEGA 4 software (Tamura et al., 2007) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

The G+C content of the total DNA was measured by HPLC according to the method described by Mesbah et al. (1989). DNA-DNA hybridization was performed fluorometrically according to the method of Ezaki et al. (1989) using photobiotin-labelled DNA probes and microdilution wells. The DNA-DNA hybridization was performed with the strain DCY69^T and the references type strains of the closest phylogenetic neighbor. The relatedness values quoted are means of the three values (mean ± SD, $n = 3$).

Chemotaxonomic analysis. For analysis of fatty acid, strain DCY69^T and the three reference type strains were grown on TSA at 25°C for 24 h. The cellular fatty acids were extracted and analyzed according to the standard protocol of the Sherlock microbial identification system (MIDI). Extracts were analyzed by gas chromatograph HP 6890 using the Sherlock system MIDI 6.1 and the Sherlock Aerobic Bacterial Database (TSBA6.1) (Sasser, 1990).

Isoprenoid quinones were extracted from the dried cells with chloroform/methanol (2/1, v/v), evaporated under vacuum and re-extracted in *n*-hexane. The crude *n*-hexane quinone solution was purified using Sep-Pak®Vac 6 ml silica cartridges (Waters, Milford, MA) and subsequently analyzed by HPLC according to Collins (1985).

The polar lipids were extracted from dried cell as previously described by Minnikin et al. (1977). Extracts were identified by two-dimensional thin layer chromatography (TLC) and total polar lipids were detected by spraying the plates with 5% molybdophosphoric acid in ethanol and then heated at 120°C for 15 min.

Biotransformation of ginsenoside. The strain DCY69^T was cultured in R2A broth and then was added to 0.4 mg L^{-1} ginsenoside Rb1 in a shaking incubator (150 rpm) at 25°C. The reaction mixture was extracted with water-saturated *n*-butanol and analyzed by TLC and HPLC.

TLC analysis was carried out using Silica Gel 60 plates and a solvent system of $CHCl_3$-CH_3OH-H_2O (65 : 35 : 10, v/v/v, lower phase) as the mobile phase. The spots on the TLC plates were detected by spraying 10% (v/v) H_2SO_4 (in ethanol) followed by heating at 110°C for 10 min.

The HPLC-grade acetonitrile and water were purchased from SK Chemicals (Ulsan, Korea). The reactant(s) present in the *n*-butanol fraction was evaporated in vacuo and the residue was dissolved in CH_3OH and injected for HPLC analysis. Ginsenoside were detected by C18 column (250 × 4.6 mm, ID 5 μm) with acetonitrile (solvent A) and H_2O (solvent B) at A/B ratios of 20 : 80, 20 : 80, 29 : 71, 30 : 70, 48 : 52, 70 : 30, 90 : 10, 90 : 10, 20 : 80 and 20 : 80, with run times of 0, 10, 42, 52, 65, 82, 83, 93, 94 and 100 min, respectively. Finally, the flow rate was 1.6 ml min^{-1} and detection wavelength was 203 nm.

Results and Discussion

Morphological, physiological and biochemical characteristics

Cells of strain DCY69^T were Gram-reaction-negative, non-flagellated, aerobic oxidase- and catalase-positive, non-gliding, rods approximately 0.5 μm in diameter and 1−2 μm in length (Fig. S3). Colonies on R2A agar were

Table 1. Differential characteristics of *Flavobacterium panaciterrae* DCY69^T and related *Flavobacterium* species.

Characteristics	1	2	3	4
Reduction of nitrates	−	−	−	+
Enzyme activity (API ZYM):				
Esterase lipase	+	+	−	+
α-Chymotrypsin	+	−	+	+
α-Galactosidase	−	−	+	+
β-Galactosidase	−	+	+	+
β-Glucuronidase	−	−	w	w
α-Fucosidase	−	−	w	−
Assimilation of (API 20NE/API 32GN):				
D-Mannitol	−	−	+	+
L-Rhamnose	+	w	+	−
Sucrose	−	−	+	−
D-Melibiose	−	−	+	−
L-Fucose	−	−	+	+
L-Histidine	−	−	−	+
L-Proline	−	−	−	+

Strains: 1. *F. panaciterrae* DCY69^T; 2. *F. aquidurense* WB 1.1-56^T; 3. *F. frigidimaris* KUC-1^T; 4. *F. araucananum* LM-19-Fp^T. All data are from this study.

All strains were positive for the following: alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase; hydrolysis of D-glucose, esculin, gelatin, *p*-nitrophenyl-β-D-galactopyranoside, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose and glycogen. All strains were weakly positive for trypsin. All strains were negative for the following: lipase (C14), *α*-mannosidase, trypsin, indole production, L-arginine, urease, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, D-ribose, *myo*-inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, D-sorbitol, propionic acid, valeric acid, trisodium citrate, postassium 2-ketogluconate, 3-hydroxybutyric acid and 4-hydroxybenzoic acid. +, positive reaction; w, weakly positive; −, negative.

yellow, circular and slightly convex after 2 days. Growth occurred on NA, TSA and R2A agar. Growth occurred at 4−30°C (optimum, 25°C), at pH 5−9 (optimum, pH 7) and in the presence of 0−2.5% (w/v) NaCl (optimum, 0.5%). Nitrate was not reduced to nitrite. Skim milk, starch, DNA, Tween 80 and gelatin were hydrolyzed. The physiological and biochemical characteristics of strain DCY69^T are given in Table 1. Strain DCY69^T was resistant to penicillin G (P_{10}, 10 units), erythromycin (E_{15}, 15 μg), cefazolin (KZ_{30}, 30 μg), oleandomycin (OL_5, 15 μg), ceftazidime (CAZ_{30}, 30 μg), vancomycin (VA_{30}, 30 μg), carbenicillin (CAR_{100}, 100 μg) and neomycin (N_{30}, 30 μg); and sensitive to novobiocin (NV_{30}, 30 μg), tetracycline (TE_{30}, 30 μg) and rifampicin (RD_5, 5 μg).

Phylogenetic analysis, determination of DNA G+C content and DNA-DNA hybridization

The phylogenetic tree showed that strain DCY69^T was affiliated with the genus *Flavobacterium* (Fig. 1). On the basis of 16S rRNA gene sequence similarity, the closest recognized relatives of strain DCY69^T were the type strain of *Flavobacterium aquidurense* WB 1.1-56^T (98.3%), *Flavobacterium frigidimaris* KUC-1^T (98.2%) and *Flavobacterium araucananum* LM-19-Fp^T (97.9%).

The DNA G+C content of strain DCY69^T was 35.0 mol%. The value is consistent with other species of genus *Flavobacterium*, which ranges from 30 to 41 mol% (Bernardet and Bowman, 2011).

The DNA-DNA hybridization between strain DCY69^T and its nearest phylogenetic neighbors, *Flavobacterium aquidurense* WB 1.1-56^T, *Flavobacterium frigidimaris* KUC-1^T and *Flavobacterium araucananum* LM-19-Fp^T were 48.5 ± 1.3, 54.0 ± 2.1, 55.4 ± 0.5%, respectively. These values are well below the 70% threshold proposed for species delineation (Wayne et al., 1987). The above data clearly confirmed that strain DCY69^T belongs to a distinct genomic species of the genus *Flavobacterium*.

Chemotaxonomic analysis

The cellular fatty acid compositions of strain DCY69^T and the three references strains are summarized in Table 2. The major cellular fatty acids of strain DCY69^T were *iso*-$C_{15:0}$ (28.8%), *iso*-$C_{15:0}$ 3-OH (10.4%), *iso*-$C_{17:0}$ 3-OH (11.8%) and summed feature 3 containing ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; 16.9%), which were similar to other *Flavobacterium* species (Bernardet et al., 2002).

The major isoprenoid quinone was MK-6, which is in agreement with the menaquinone patterns reported for species of the genus *Flavobacterium* (Bernardet et al., 2002).

The major polar lipid of strain DCY69^T was phosphatidylethanolamine, one unidentified aminolipid and unidentified polar lipids (L1, L2), which was similar to that of reference strain (Fig. S1).

Biotransformation of ginsenoside

The HPLC profiles of the conversion of ginsenosides Rb1 of strain DCY69^T after incubation for 3 days are shown in Fig. S2. The peak for ginsenoside Rb1 disappeared and then new peaks appeared which had a retention time consistent with ginsenosides Rd and F2. However, the ginsenosides C-K, Rh2, and 20(S)-PPD were not detected. Hence, we conclude that strain DCY69^T transformed ginsenoside Rb1 into Rd and F2.

Description of *Flavobacterium panaciterrae* sp. nov.

Flavobacterium panaciterrae (pa.na.ci.ter′rae. N.L. n.

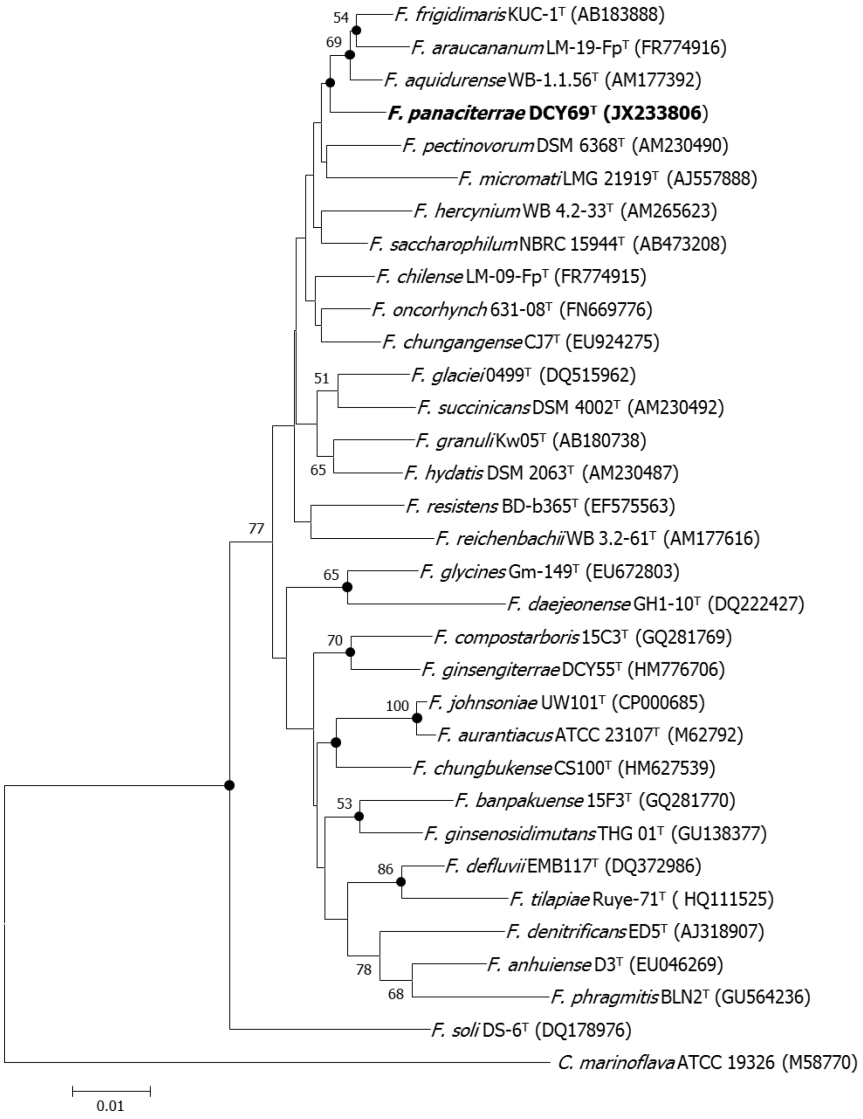

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain DCY69^T with other *Flavobacterium* species.

Bootstrap values >50% based on 1,000 replications are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. *Cytophaga marinoflava* ATCC19326^T was used as an out-group. Bar, 0.01 substitutions per nucleotide position.

Panax-acis scientific name for ginseng; L. n. *terra* soil; N.L. gen. n. *panaciterrae* of soil of a ginseng field)

Cells are Gram-reaction-negative, non-spore-forming, non-flagellated, aerobic, rod shaped (approximately 0.5 μm in width and 1−2 μm in length) and non-gliding. Colonies grown on R2A agar are yellow-pigmented, circular and slightly convex. Cells grow on NA, TSA and R2A, but do not on MacConkey or LB agars. Growth occurs at 4−30°C (optimum, 25°C), at pH 5−9 (optimum, pH 7) and in the presence of 0−2.5% (w/v) NaCl (optimum, 0.5%). Catalase and oxidase are positive. Nitrate is not reduced to nitrite. Skim milk, starch, DNA, Tween 80 and gelatine are hydrolyzed.

Cells are resistant to penicillin G (P_{10}, 10 units), erythromycin (E_{15}, 15 μg), cefazolin (KZ_{30}, 30 μg), oleandomycin (OL_5, 15 μg), ceftazidime (CAZ_{30}, 30 μg), vancomycin (VA_{30}, 30 μg), carbenicillin (CAR_{100}, 100 μg) and neomycin (N_{30}, 30 μg); and sensitive to novobiocin (NV_{30}, 30 μg), tetracycline (TE_{30}, 30 μg) and rifampicin (RD_5, 5 μg). In API ZYM, activities are positive for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase; and weakly positive for trypsin; but negative for lipase (C14) and α-mannosidase. In API 20 NE, they are positive for D-glucose, esculin, *N*-acetyl-glucosamine, hydrolysis of gelatin, *p*-nitrophenyl-β-D-galactopyranoside, L-arabinose, D-maltose, D-mannose, *N*-acetyl-glucosamine, but negative for indole production, and hydrolysis of L-arginine, urea, potassium gluconate, malic acid, trisodium citrate, capric acid, adipic acid and phenylacetic acid. In API 32 GN, they are positive for glycogen, D-maltose, D-glucose, L-arabinose, but negative for salicin, D-ribose, *myo*-inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, D-sorbitol, propionic acid, capric acid, valeric acid, trisodium citrate, postassium 2-ketogluconate, 3-hydroxybutyric acid and 4-hydroxybenzoic acid. The major cellular fatty acids (>9% of the total) are *iso*-$C_{15:0}$, *iso*-$C_{17:0}$ 3OH, *iso*-$C_{15:0}$ 3OH and summed feature 3

Table 2. Cellular fatty acid profile of strain DCY69^T and other related *Flavobacterium* type strains.

Fatty acids	1	2	3	4
Saturated				
$C_{14:0}$	tr	tr	tr	1.0
$C_{16:0}$	6.5	3.5	7.4	6.0
Branched-chain				
iso-$C_{15:0}$	28.8	26.4	17.0	26.9
iso-$C_{15:1}$ G	tr	2.3	tr	1.1
iso-$C_{16:0}$	tr	1.4	1.05	tr
iso-$C_{17:0}$	tr	1.3	1.1	tr
anteiso-$C_{15:0}$	2.9	3.0	7.4	3.2
$C_{15:1}$ ω6*c*	2.4	4.6	tr	1.8
$C_{17:1}$ ω8*c*	tr	1.8	tr	tr
$C_{17:1}$ ω6*c*	1.2	3.3	tr	1.3
Hydroxy				
$C_{15:0}$ 2OH	tr	tr	tr	1.3
$C_{15:0}$ 3OH	1.2	2.0	1.2	ND
$C_{16:0}$ 3OH	4.8	2.2	6.9	5.8
iso-$C_{15:0}$ 3OH	10.4	10.1	7.8	9.5
iso-$C_{16:0}$ 3OH	tr	2.0	1.3	tr
iso-$C_{17:0}$ 3OH	11.8	14.4	9.0	10.2
Summed feature				
2	1.0	tr	1.6	1.2
3	16.9	9.7	23.9	21.1
4	ND	ND	tr	1.4
9	4.5	6.8	4.9	4.3

Strains: 1. DCY69^T; 2. *F. aquidurense* WB 1.1-56^T; 3. *F. frigidimaris* KUC-1^T; 4. *F. araucananum* LM-19-Fp^T. All type strains were collected after 24-h growth on TSA medium (Difco) at 25°C. Summed feature 1 contained $C_{13:0}$ 3OH and/or *iso*-$C_{15:1}$ H; summed feature 2 contained $C_{14:0}$ 3OH and/or *iso*-$C_{16:1}$ I; summed feature 3 contained $C_{16:1}$ω7*c* and/or $C_{16:1}$ω6*c*; summed feature 4 contained $C_{17:1}$ *anteiso* B and/or *iso* I and summed feature 9 contained *iso*-$C_{17:1}$ω9*c* and/or 10-methyl $C_{16:0}$, and could not be separated by GCL with the Microbial Identification System (MIDI). All data are from this study; fatty acids amounting to less than 1.0% in any strain are not listed. ND: not detected, tr: traces (<1.0%).

($C_{16:1}$ω7*c* and/or $C_{16:1}$ω6*c*). The DNA G+C content is 35.0 mol%. The predominant respiratory quinone is MK-6. The major polar lipids of strain DCY69^T are phosphatidylethanolamine, one unidentified aminolipid and unidentified polar lipids (L1, L2).

The type strain DCY69^T (=KCTC 32392^T = JCM 19161^T), which transforms ginsenoside Rb1 into Rd and F2, was isolated from the soil of a ginseng field in the Republic of Korea.

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Supplementary Materials

Fig. S1. Two-dimensional TLC of polar lipid analysis.
Polar lipid extracts were obtained from strain DCY69^T (I) and the type strain *Flavobacterium araucananum* LM-19-FP^T (II) stained with 5% ethanolic molybdophosphoric acid for total polar lipids. PE, phosphatidylethanolamine; PS, phosphatidylserine; AL, unidentified aminolipid; L1-2, unidentified polar lipids.

Fig. S2. HPLC profiles of metabolites of ginsenoside Rb1 from strain DCY69^T.
A, Ginsenoside Rb1 control; B, Ginsenoside Rb1 metabolites.

Fig. S3. Transmission electron micrograph of strain DCY69^T.
Bar, 200 nm.

Supplementary figures are available in our J-STAGE site (http://www.jstage.jst.go.jp/browse/jgam).

References

Anzai, Y., Kim, H., Park, J. Y., Wakabayashi, H., and Oyaizu, H. (2000) Phylogenetic affiliation of the *Pseudomonads* based on 16S rRNA sequence. *Int. J. Syst. Evol. Microbiol.*, **50**, 1563−1589.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., and Turck, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, **45**, 493−496.

Bergey, D. H., Harrison, F. C., Breed, R. S., Hammer, B. W., and Huntoon, F. M. (1923) Genus II. *Flavobacterium* gen. nov. *In* Bergey's Manual of Determinative Bacteriology, Williams & Wilkins, Baltimore, pp. 97−117.

Bernardet, J. F., Nakagawa, Y., and Holmes, B. (2002) Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int. J. Syst. Evol. Microbiol.*, **52**, 1049−1070.

Bernardet, J. F. and Bowman, J. P. (2011) Genus I. *Flavobacterium* Bergey, Harrison, Hammer and Huntoon 1923, 97^{AL} emend. Bernardet, Segers, Vancanneyt, Berthe, Kersters and Vandamme 1996, 139. *In* Bergey's Manual of Systematic Bacteriology, 2nd ed., Vol. 4, ed. by Whitman, W., Williams & Wilkins, Baltimore, pp. 112−154.

Collins, M. D. (1985) Isoprenoid quinone analyses in bacterial classification and identification. *In* Chemical Methods in Bacterial Systematics (Society for Applied Bacteriology Technical Series No. 20), ed. by Goodfellow, M. and Minnikin, D. E., Academic Press, London, pp. 267−287.

Cowan, S. T. and Steel, K. J. (1974) Manual for the Identification of Medical Bacteria, Cambridge University Press, Cambridge.

Euzéby, J. P. (1997) List of Bacterial Names with Standing in Nomenclature: a folder available on the Internet. *Int. J. Syst. Bacteriol.,* **47**, 590−592, Last full update October, 2013. http://www.bacterio.net (List of Prokaryotic Names with Standing in Nomen-

clature).
Ezaki, T., Hashimoto, Y., and Yabuuchi, E. (1989) Fluorometric deoxyribonucleic acid — deoxyribonucleic acid hybridization in micro dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.*, **39**, 224−229.
Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783−791.
Fitch, W. M. (1971) Toward defining the course of evolution minimum change for specific tree topology. *Syst. Zool.*, **20**, 406−416.
Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.*, **41**, 95−98.
Kim, M. K., Im, W. T., Ohta, H., Lee, M., and Lee, S. T. (2005) *Sphingopyxis granuli* sp. nov., a ß-glucosidase-producing bacterium in the family *Sphingomonadaceae* in α-4 subclass of the *Proteobacteria*. *J. Microbiol.*, **43**, 152−157.
Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M. et al. (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.*, **62**, 716−721.
Lane, D. J. (1991) 16S/23S rRNA sequencing. *In* Nucleic Acid Techniques in Bacterial Systematics, ed. by Stackebrandt, E. and Goodfellow, M., Wiley, Chichester, pp. 115−176.
Mesbah, M., Premachandran, U., and Whitman, W. B. (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int. J. Syst. Bacteriol.*, **39**, 159−167.
Minnikin, D. E., Patel, P. V., Alshamaony, L., and Goodfellow, M. (1977) Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int. J. Syst. Bacteriol.*, **27**, 104−117.
Ryu, S. H., Park, M., Jeon, Y., Lee, J. R., Park, W. et al. (2007) *Flavobacterium filum* sp. nov., isolated from a wastewater treatment plant in Korea. *Int. J. Syst. Evol. Microbiol.*, **57**, 2026−2030.
Saitou, N. and Nei, M. (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406−425.
Sasser, M. (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101, MIDI, Inc., Newark, DE.
Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, **24**, 1596−1599.
Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, **25**, 4876−4882.
Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O. et al. (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.*, **37**, 463−464.
Wang, Z. W., Liu, Y. H., Dai, X., Wang, B. J., Jiang, C. Y. et al. (2006) *Flavobacterium saliperosum* sp. nov., isolated from freshwater lake sediment. *Int. J. Syst. Evol. Microbiol.*, **56**, 439−442.
Wiklund, T., Madsen, L., Bruun, M. S., and Dalsgaard, I. (2000) Detection of *Flavobacterium psychrophilum* from fish tissue and water samples by PCR amplification. *J. Appl. Microbiol.*, **88**, 299−307.
Yoon, J. H., Kang, S. J., and Oh, T. K. (2006) *Flavobacterium soli* sp. nov., isolated from soil. *Int. J. Syst. Evol. Microbiol.*, **56**, 997−1000.