Chryseobacterium ginsengiterrae *sp. nov.*, *with Beta-Glucosidase Activity Isolated from Soil of a Ginseng Field*

Jong-Hun Noh, Van-An Hoang, Yeon-Ju Kim, Jong-Pyo Kang & Deok-Chun Yang

Current Microbiology

ISSN 0343-8651

Curr Microbiol
DOI 10.1007/s00284-017-1335-6

Springer

Curr Microbiol
DOI 10.1007/s00284-017-1335-6

Chryseobacterium ginsengiterrae sp. nov., with Beta-Glucosidase Activity Isolated from Soil of a Ginseng Field

Jong-Hun Noh[1] · Van-An Hoang[1] · Yeon-Ju Kim[1] · Jong-Pyo Kang[1] · Deok-Chun Yang[1,2]

Received: 23 January 2017 / Accepted: 10 August 2017

Abstract The isolated *Chryseobacterium ginsengiterrae* sp. nov DCY68^T was found to be Gram-negative, aerobic, non-motile, non-flagellate and rod-shaped. Their size was approximately 0.40–0.46 × 1.0–1.27 μm. The colonies were yellow-pigmented, convex, circular and 0.5–1.3 mm in diameter when grown on R2A agar for 2 days. DNA, esculin, skim milk, gelatine, starch, Tween 20, and Tween 80 were hydrolyzed, but not cellulose. The cells grew on R2A, TSA, and NA but not on MacConkey agars. Growth occured at 4–33 °C (optimum, 30 °C), at pH 5.0–8.0 (optimum, pH 6.5), and 0–2.5% NaCl. Nitrate was not reduced to nitrite. Oxidase and catalase activity were positive. Strain DCY68^T contained *β*–glucosidase activity in which ginsenoside Rb1 was enzymatically converted to ginsenoside F2. Analysis of the16S rRNA gene sequence revealed that strain *C. ginsengiterrae* sp. nov DCY68^T belonged to the family *Flavobacteriaceae* and was most closely related to *C. limigenitum* SUR2^T (97.4%). The genomic DNA G+C content was 42.0 mol%. The predominant quinones were MK-6 (74.5%) and MK-7 (25.5%). The major fatty acids were *iso*-$C_{15:0}$, summed feature 3 (containing $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and *iso*-$C_{17:0}$ 3-OH. On the basis of these phenotypic, genotypic and chemotaxonomic studies, strain DCY68^T represents a novel species of the genus *Chryseobacterium*, for which name *C. ginsengiterrae* sp. nov. is proposed. The type strain is DCY68^T (=KCTC 32089^T = JCM 18517^T).

Jong-Hun Noh and Van-An Hoang have contributed equally to this work.

The GenBank/EMBL/DDBJ Accession Number for the 16S rRNA gene sequence of strain DCY68^T is JX141783. The DPD Taxonumber of the type strain DCY68^T is TA00221.

Electronic supplementary material The online version of this article (doi:10.1007/s00284-017-1335-6) contains supplementary material, which is available to authorized users.

✉ Yeon-Ju Kim
yeonjukim@khu.ac.kr

✉ Deok-Chun Yang
dcyang@khu.ac.kr

[1] Department of Oriental Medicinal Biotechnology, College of Life Sciences, Kyung Hee University, Giheung-Gu, Yongin-Si, Gyeonggi-do, Republic of Korea

[2] Graduate School of Biotechnology and Ginseng Bank, College of Life Sciences, Kyung Hee University, Yongin 446-701, Republic of Korea

Introduction

Ginseng (*Panax ginseng* C. A. Meyer) is one of the most popular medicinal plants in Asian countries. Ginseng is mainly grown in northeastern Asia (e.g., China, Korea and eastern Siberia) where there is typically a cooler climate [44]. Ginseng plant grows best under conditions that simulate its natural habitat. It requires 70–90% natural of artificial shade. Ginseng thrives in a climate with 4–10 cm of annual precipitation and an average temperature of 10 °C. It requires several weeks of cold temperature for adequate dormancy. Ginseng generally prefers a loamy, deep (20–30 cm), well-drained soil with a high organic content and a pH near 5.5. Extremely sandy soils tend to produce long, slender roots of inferior quality. Ginseng requires 3–5 years to produce a marketable crop. There is no artificial or chemical fertilizer applied for ginseng growth [30]. Ginseng saponins (ginsenosides) are the main active constituents of ginseng, which possess a variety of properties and pharmacological activities [8]. Until now,

Published online: 19 August 2017

more than 128 ginsenosides have been isolated with six major ginsenosides including Rb1, Rb2, Rd, Rc, Re, and Rg1 constituting over 90% of the total ginsenosides [36].

Biotransformation of ginsenoside (deglycosylation) can be achieved by hydrolyzing and removing a sugar moiety from the major ginsenosides using bacterial and fungal strains [25, 38, 51]. For screening of ginsenosides-converting aerobic bacteria from ginseng soil samples, several novel isolates that possess ginsenosides converting activity. Among them, strain DCY68^T, isolated from a ginseng soil sample, could convert ginsenoside Rb1 to ginsenoside F2. This strain is the subject of a taxonomic investigation that is covered in this paper.

The genus *Chryseobacterium* (type species, *Chryseobacterium gleum*), established by Vandamme et al. [48], is a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes* [4]. Two former *Chryseobacterium* species, namely *Chryseobacterium meningosepticum* and *Chryseobacterium miricola*, were transferred to the genus *Elizabethkingia* [22]. The genus *Chryseobacterium* was described to contain Gram-staining negative, yellow-pigmented members, characterized by containing menaquinone-6 (MK-6) as the major predominant respiratory quinone and menaquinone-7 (MK-7) as the minor predominant respiratory quinone. *iso*-$C_{15:0}$, *iso*-$C_{17:0}$ 3-OH, *iso*-$C_{17:1}$ $\omega 9c$ as the major fatty acids and a DNA G+C content with 29–39 mol% [5]. Members of genus *Chryseobacterium* have been identified from a variety of environments such as sewage and wastewater [18], raw chicken [12], freshwater sediment [21], fermented beverages [42], plant roots [50], bioreactor sludge [39], cow's milk [15], diseased fish [17], arthropod faeces [19], clinical samples [49], and ginseng soil [16]. The genus presently contains more than 100 species (http://www.bacterio.net/chryseobacterium.html).

Materials and Methods

Bacterial Isolation

Strain DCY68^T was isolated from soil of a ginseng field in from Gochang County, Republic of Korea (38°04′00″N 126°57′00″E). 100–200 g of the rhizosphere soil was carefully collected without any stones and particles at 10 cm depth, in zipped lock covers and transferred to the laboratory. One gram of soil sample was dissolved in 10 ml of sterile normal saline. Serial dilution prepared up to 10^{-5} using 0.85% (w/v) sterile saline solution. Subsequently, 100 µl of each diluted sample was plated onto the five times diluted R2A agar (Difco). The plates were then incubated at 30 °C for 7 days. Single colonies were selected and transferred onto new plates for purification. Routine cultivation was performed on R2A agar at 30 °C and the isolates was stored at −80 °C in R2A broth (Difco) supplemented with 25% glycerol. The novel specie of genus *Chryseobacterium* for which the name DCY68^T was isolated and characterized in this study. A novel strain was deposited into the Korean Collection for Type Cultures and Japan Collection of Microorganisms with collection numbers are KCTC 32089^T and JCM 18517^T.

Bacterial Growth, Morphology, Physiological, and Biochemical Characteristics

Cell morphology was observed after growth on R2A agar (Difco) at 30 °C for 2 days. Gliding motility was tested using a hanging-drop technique [3]. Cell size, shape, morphology, flagellation, and motility of strain DCY68^T were observed by microscopy (×1000 magnification, Nikon Optiphot-2) and by Transmission Electron Microscopy after grown in R2A agar (Difco) for 1 day at 30 °C. 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. Hydrolysis of the following substrates was tested: gelatin (on a medium containing 0.3% beef extract, 0.5% peptone, and 12% gelatin), starch [on R2A agar containing 1% starch (Difco)], DNA [on DNase agar medium (Scharlau) flooded with 1 N HCl to reveal DNase activity], casein [on R2A agar supplemented with 2% skim milk (Difco)] [11], esculin [on R2A agar containing esculin (0.3%, Sigma) and ferric citrate (0.02%, Fluka)], Tweens 20 and 80 [on R2A agar containing (1% Tween 20 or 80) and 0.02% $CaCl_2$]. Indole production was analyzed using Kovács' reagent in 1% tryptone broth. Nitrate reduction was tested in nitrate broth containing 0.2% KNO_3 [43]. Urease activity was evaluated in Christensen's medium [9]. Gram staining was determined using a bioMérieux Gram stain kit according to the manufacturer's instructions. Growth was tested using several bacterial media such as nutrient (NA; Difco), trypticase soy (TSA; Difco), R2A (Difco), and MacConkey (Difco) agars at 30 °C. The temperature range of growth was tested by checking growth of strain DCY68^T in R2A broth and R2A agar at 4, 10, 20, 28, 30, 33, 34, 35, and 37 °C. Tolerance of salinity was evaluated on R2A broth supplemented with [0–5.0% (w/v) NaCl, at 0.5% intervals] at 30 °C. Growth at pH values 3.0–10.0 at 0.5 pH unit intervals was assessed in R2A broth supplemented with 10 mM phosphate-citrate buffer (pH 5.0), MES buffer (pH 5.5–6.5), HEPES buffer (pH 7.0–8.0), Tris buffer (pH 8.5–9.0) and $NaHCO_3$/Na_2CO_3 (pH 9.5–10.0). pH lower than 5.0 was adjusted by 1 N HCl. Growth under anaerobic conditions was assessed after 10 days of incubation on R2A at 30 °C in the GasPak EZ

anaerobic container system (BD). Carbon utilization, enzyme production, and other tests were carried out using the API 20NE, API 32GN, and API ZYM according to the manufacturer's instruction (bioMérieux). The strips were incubated at 30 °C and recorded after 24, 48 h.

Antibiotic Susceptibility Test

Antibiotic susceptibility was evaluated using Oxoid antibiotic disks, on Mueller–Hinton agar plates incubated at 30 °C for 48 h as described by Bauer et al. [2]. Inhibition zone was interpreted according to the manufacturer's manual. The antibiotic disks contained the following: erythromycin (E_{15}, 15 μg), cefazolin (KZ_{30}, 30 μg), penicillin G (P_{10}, 10 units), oleandomycin (OL_5, 15 μg), novobiocin (NV_{30}, 30 μg), carbenicillin (CAR_{100}, 100 μg), ceftazidime (CAZ_{30}, 30 μg), vancomycin (VA_{30} μg), tetracycline (TE_{30}, 30 μg), rifampicin (RD_5 μg), and neomycin (N_{30}, 30 μg).

Biotransformation of Ginsenosides

Ginsenosides Rg1, Re, Rf, Rg2(S), Rb1, Rc, Rb2, Rd, F2, Rg3(S), F2, Rh2(S), and compound K, used as standards, were purchased from Dalian Green Bio Ltd (China). The reaction mixture of ginsenoside Rb1 was obtained from ginseng resource bank, Kyung Hee University, Republic of Korea. The biotransformation ginsenosides of strain DCY68^T was carried out according to Hoang et al. [16].

16S rRNA Sequence, Phylogenetic, and G+C Content Analysis

Genomic DNA of strain DCY68^T was extracted and purification by using an Exgene^{TM} Cell SV mini-kit (GeneAll Biotechnology, Republic of Korea) according to the manufacturer's instructions. The 16S rRNA gene sequence of strain DCY68^T was amplified from the chromosomal DNA using universal bacteria primer sets 27F, 518F, 800R and 1492R [1, 29]. Subsequently, the purified PCR products were sequenced by Genotech (Daejeon, Republic of Korea) as described by Kim et al. [24]. An almost complete (1444 bp) 16S rRNA sequence was assembled with SeqMan software version 4.1 (DNASTAR Inc.) and with BioEdit program [14]. The 16S rRNA gene sequences of strain DCY86^T and related taxa were obtained from GenBank database. Multiple alignments of the sequences were performed with the CLUSTAL X program [47]. The Kimura 2-Parameter method was used to calculate distances matrices for aligned sequences according to Kimura [27]. A phylogenetic tree was constructed with the neighbor-joining method [40] and maximum-parsimony method [28] using the MEGA5 Program [46]. Bootstrap analysis with 1000 replication was also conducted to obtain confidential levels for the branches [13]. The 16S rRNA sequence similarities between strain DCY68^T and related *Chryseobacterium* species were measured EzTaxon-e server (http://www.ezbiocloud.net) [26].

In order to analyze the G+C mol% of DNA, the genomic DNA of strain DCY68^T was extracted and purified using the Genomic DNA isolation kit (Gene All, Republic of Korea), then degraded enzymatically into nucleosides as described by Mesbah et al. [34]. Subsequently, the obtained nucleoside mixture was separated by HPLC according to Hoang et al. [16].

Chemotaxonomic Characteristics

Polar Lipid Analysis, Isoprenoid Quinone, Fatty Acids, and Polyamine Analysis

The polar lipids of strain DCY68^T and two reference strains *C. defluvii* B2^T and *C. aquaticum* KCTC 12483^T were extracted and analyzed by two-dimensional thin layer chromatography followed by spraying with molybdatophosphoric acid, molybdenum blue, α-naphthol, and ninhydrin reagents as described previously [35].

For isoprenoid quinone analysis, cell biomass was grown on R2 broth media (MB-Cell, South Korea) at 30 °C for 48 h and freeze-dried. Respiratory quinones were extracted and purified according to Collins [10]; purification menaquinones were determined by reverse-phase HPLC [16] with the MK-6 and MK-7 from of *C. yeoncheonense* DCY67^T used as a reference.

For fatty acid analysis, strain DCY68^T, *C. limigenitum* SUR2^T, *C. aahli* T68^T, *C. soldanellicola* PSD1-4^T, and *C. aquaticum* KCTC 12483^T were grown on TSA agar (Difco) for 1 day at 30 °C. Whole cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI) and analyzed by capillary GLC (Hewlett Packard 6890) using the Sherlock system MIDI 6.1 and the Sherlock Aerobic Bacterial Database (TSBA6.1) [41].

Polyamines of strain DCY68^T and *C. defluvii* B2^T were extracted as described by Busse and Taibi [6, 7, 45]. The polyamine standards including spermine, spermidine, and putrescine were purchased from Sigma-Aldrich. The polyamine profile of *C. yeoncheonense* DCY67^T was used as reference. The internal standard was 1,8-diaminooctane (D22401, Sigma-Aldrich). The sample was analyzed by using Agilent 1260 infinity HPLC system and an Agilent Poroshell 120 EC–C18 column (30 × 50 mm, 2.7 μm). The methanol (60%) was applied as mobile phase at the flow rate of 0.6 ml min^{-1}. Oven column was 40 °C. The benzylated polyamines were detected at 260 nm.

Table 1 Differential characteristics of strain DCY68^T and related *Chryseobacterium* type species

Characteristics	1	2	3	4	5
DNA G+C content (mol%)	42	nd[a]	34.1[b]	38.5[c]	28.8[d]
Fermentation of glucose	+	+	−	−	−
Urease activity	+	−	−	+	+
Hydrolysis of	+	−	−	−	+
Arginine	+	−	−	−	+
Tween 80	+	+	+	+	−
Starch	+	+	−	+	−
Indole production	−	W	−	−	−
Rang of					
Temperature (°C)	4–33	4–36[a]	4–20[b]	5–37[c]	5–37[d]
pH	5.0–8.0	nd[a]	6–8[b]	6–8[c]	5–7[d]
Growth in NaCL (%, v/v)	0–2.5	1.0–3.0[a]	0–2[b]	1–3[c]	0–4[d]
API Zyme					
Esterase lipase	+	−	+	+	+
Cystine arylamidase	+	+	+	+	−
Trypsin	+	+	+	+	−
α-chymotrypsin	+	+	−	−	−
Naphthol-AS-BI-phosphohydrolase	−	+	+	+	+
β-glucosidase	+	+	+	−	+
N-acetyl-β-glucosaminidase	+	−	−	+	+
α-mannosidase	−	+	+	−	−
α-fucosidase	−	+	+	−	−
Assimilation of: (In API 20 NE and API 32GN)					
L-rhamnose	+	−	+	+	+
N-acetyl-glucose	+	−	−	−	−
D-saccharose	+	−	+	+	−
Suberic acid	+	−	−	−	−
Sodium malonate	−	−	−	+	−
Sodium acetate	−	−	−	+	+
Lactic acid	+	−	−	−	+
L-alanine	−	−	−	−	+
Glycogen	+	−	+	+	+
Salicin	−	−	+	−	−
D-melibiose	+	−	−	+	−
L-fucose	+	−	−	−	−
D-sorbitol	+	−	−	+	−
L-arabinose	−	−	+	−	+
Propionic acid	−	−	−	+	+
Valeric acid	+	−	−	−	−
Potassium 2-ketogluconate	+	−	−	−	−
L-proline	+	−	−	+	+
Arabinose	−	−	+	−	+
Maltose	+	−	−	+	+
Potassium gluconate	−	+	−	−	−
Adipic acid	+	−	−	−	−
Malate	−	−	−	−	+

Strains: 1, DCY68^T; 2, *Ch. limigenitum* SUR2^T; 3, *Ch. aahli* T68^T; 4, *Ch. aquaticum* KCTC 12483^T; 5, *Ch. soldanellicola* PSD1-4^T. All data are from this study except the DNA G+C content of the reference strains and *Ch. greenlandense* UMB34^T, which were from the following sources

+ positive, − negative, *w* weekly positive

[a] Data from Kämpfer et al. [20]

[b] Data from Loch et al. [32]

[c] Data from Kim et al. [23]

[d] Data from Park et al. [37]

Results and Discussion

Growth, Morphology, and Physiochemical Characteristics

Cells were Gram-staining negative, aerobic, non-motile, non-flagellate, and rod-shaped with approximately 0.40–0.46 × 1.0–1.27 μm in size (Fig. S1). Colonies were yellow-pigmented, convex, circular, and 0.5–1.3 mm in diameter of grown on R2A medium for 2 days. The physiological and biochemical characteristics of strain DCY68^T and related strains are given in Tables 1 and S1 and the species description. Strain DCY68^T could be clearly distinguished from their closest phylogenetic neighbor (Fig. 1).

Antibiotics Susceptibility Test

Strain DCY68^T was resistant to penicillin G (P_{10}), cefazolin (KZ_{30}), oleandomycin (OL_5); intermediate to neomycin (N_{30}), erythromycin (E_{15}), tetracycline (TE_{30}), ceftazidime (CAZ_{30}); and sensitive to vancomycin (VA_{30}), carbenicillin (CAR_{100}), novobiocin (NV_{30}), and rifampicin (RD_5).

Biotransformation of Ginsenosides

Based on results from the biotransformation of ginsenoside Rb1, ginsenoside Rb1 was converted into F2 after 1 day reaction (Figs. 2, S3). The result of HPLC was coincident with TLC profiles (results were similar to the TLC finding). The HPLC profiles of the ginsenoside Rb1 using culture broth after incubation were shown in Fig. S3. The transformed ginsenosides were determined by HPLC and TLC analysis in the course of reaction time. Strain DCY68^T produced ginsenosides hydrolyzing enzymes in the culture broth. The biotransformation of ginsenoside Rb1 was almost completely hydrolyzed into ginsenosides F2 after 3 days.

16S rRNA Sequences, Phylogenetic, and G+C Content Analysis

Phylogenetic analysis, based on 16S rRNA gene sequences, showed that strain DCY68^T belonged to the genus *Chryseobacterium*. The highest similarity value was observed with the type strain of *C. limigenitum* SUR2^T (97.4%), *C. aahli* T68^T (97.4%), *C. greenlandense* UMB34^T (97.3%), *C. soldanellicola* PSD1-4^T (97.0%), and *C. aquaticum* KCTC 12483^T (97.0%). In the Maximum Likelihood tree, strain DCY78^T formed a reliable and monophyletic cluster with *C. aquaticum* KCTC 12483^T (97.0%) and *C. greenlandense* UMB34^T. This cluster was also recovered in the trees generated by the neighbor-joining (Fig. S1) and maximum-parsimony algorithms (Fig. 1). The G+C content of genomic DNA of strain DCY68^T was 42.0 mol%

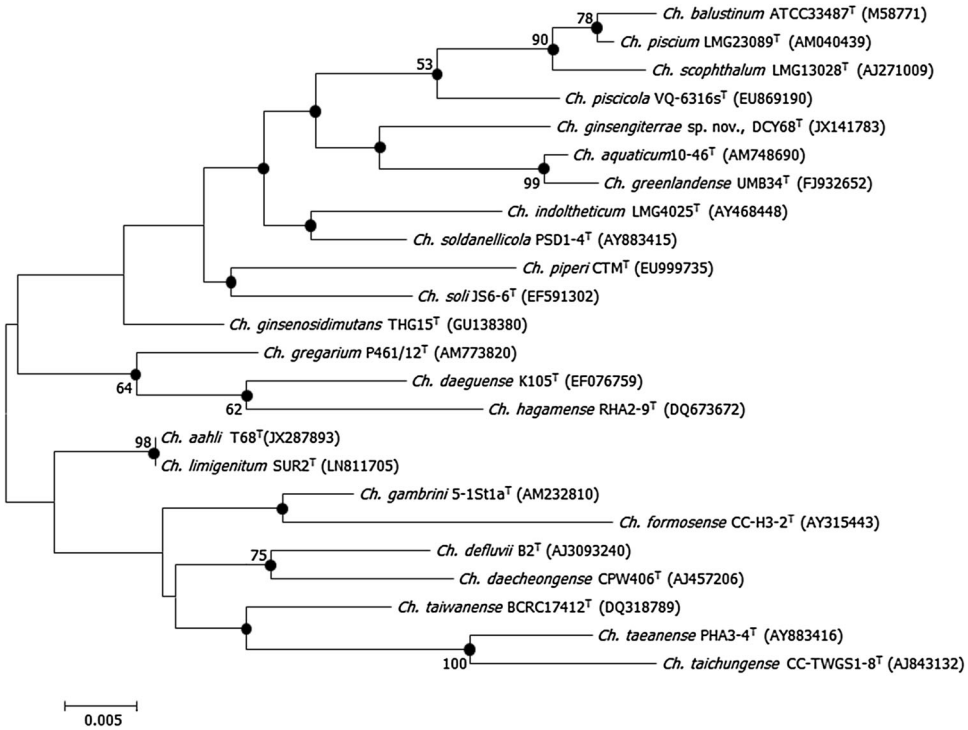

Fig. 1 Maximum–likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships of strain DCY68^T with other *Chryseobacterium* species. Boot strap values >50% were based on 1000 replications as shown at branching points. *Filled circles* indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. *Bar*, 0.005 substitutions per nucleotide position

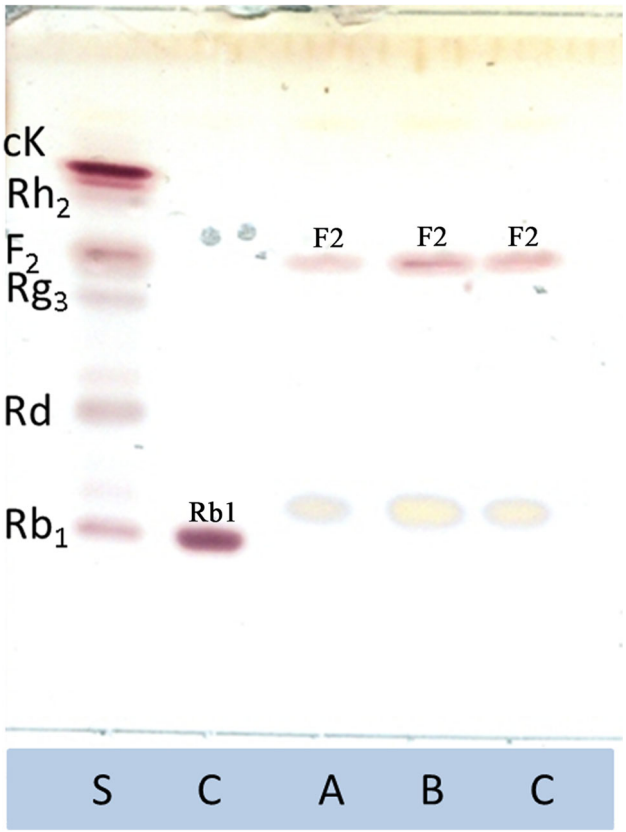

Fig. 2 TLC analysis of time-course transformation of ginsenoside Rb1 by strain DCY68^T. Developing solvent: $CHCl_3$/MeOH/H_2O (65/35/10, v/v/v, lower phase). S, saponin standards; C, control, standard of ginsenoside Rb1; A, reaction for 1 day; B, 2 days; C, 3 days. *cK* compound K

(HPLC), which is the range described for the members of genus *Chryseobacterium* (29–42.1 mol%) [5, 31].

Chemotaxonomic Characteristics

The major polar lipids of strain DCY68^T were phosphatidylethanolamine (PE), one unidentified aminolipid (AL4), and two unidentified polar lipids (L2, L3), while the minor lipids were several unidentified aminolipids (AL1, AL2, AL3) and unidentified polar lipids (L1 and L4). Phosphatidylethanolamine (PE), the unidentified polar lipids (L2, L4) were also present in *C. aquaticum* KCTC 12483^T (97.0%) and *C. defluvii* DSM B2^T displayed a similar polar lipid profile (as shown in Supplementary Fig. S2).

Strain DCY68^T contained MK-6 (74.5%) as the major isoprenoid quinone and MK-7 (25.5%) as the minor quinone. The major fatty acids were *iso*-$C_{15:0}$, summed feature 3 (containing $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and *iso*-$C_{17:0}$ 3-OH (Table 2). The major polyamine of strain DCY68^T, *sym*-homospermidine, was similar to those of *C. defluvii* B2^T and *C. yeoncheonense* DCY67^T.

Table 2 Cellular fatty acids profile of strain DCY68^T and other related *Chryseobacterium* type strains

Fatty acid	1	2	3	4*	5	6
Saturated						
C14:0	0.8	1.7	1.9	nd	1.3	0.5
C16:0	8.7	13.4	10.4	6.6	10.5	2.0
Branched-chain						
iso-C13:0	0.8	nd	1.7	nd	2.2	2.1
iso-C15:0	34.6	27.9	29.8	30.4	37.3	39.9
iso-C16:0	0.5	1.5	1.2	nd	tr	tr
iso-C17:0	1.3	nd	tr	2.4	2.5	1.3
anteiso-C15:0	3.6	3.6	3.5	2.5	2.6	3.9
Unsaturated						
C16:1ω5c	1.1	3.5	1.7	nd	0.4	nd
Hydroxy						
C17:0 2-OH	1.2	1.7	1.9	nd	1.1	0.6
C16:0 3-OH	5.1	1.1	2.0	3.3	3.9	1.5
iso-C15:0 3-OH	2.3	2.4	2.3	2.2	2.3	2.9
iso-C16:0 3-OH	0.7	nd	0.9	nd	tr	0.6
iso-C17:0 3-OH	16.4	10.9	17.2	19.0	17.0	19.6
Summed feature 3*	17.8	17.8	21.8	17.7	8.7	15.6
Summed feature 4*	0.2	nd	nd	nd	nd	nd
Summed feature 9*	4.3	10.7	1.7	8.5	7.9	8.9

Strains: 1, DCY68^T; 2, *Ch. limigenitum* SUR2^T; 3, *Ch. aahli* T68^T; 4, *Ch. greenlandense* UMB34^T; 5, *Ch. aquaticum* KCTC 12483^T; 6, *Ch. soldanellicola* PSD1-4^T

Summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 4 contained $C_{17:1}$ *anteiso* B and/or *iso* I and summed feature 9 contained *iso* $C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$ could not be separated by GCL with the Microbial Identification System (MIDI). All data are from this study except strain *Ch. greenlandense* UMB34^T, which were from the following sources. Fatty acids of less than 0.5% in all strains were not listed

nd not detected, *tr* traces (<0.5%)

* Data from Loveland-Curtze et al. [33]

Taxonomic Conclusion

Based on phylogenetic analysis, we suggested that strain DCY68^T was closely related to the members of the genus *Chryseobacterium*. The physiological, biochemical characteristics and chemotaxomic results strongly suggested that strain DCY68^T was distinguished from the related *Chryseobacterium* species. Therefore, it is proposed that strain DCY68^T to be classified as a novel species of the genus *Chryseobacterium*, which is named *Chryseobacterium ginsengiterrae* sp. nov.

Description of *Chryseobacterium ginsengiterrae* sp. nov

Chryseobacterium ginsengiterrae (gin.sen.gi.te.r'rae. N.L. n. *ginsengum* ginseng; L. n. *terra* soil; N.L. gen. n. *ginsengiterrae* of soil from a ginseng field).

Cells were Gram-staining negative, aerobic, non-motile, non-flagellate, and rod-shaped, with approximately 0.40–0.46 × 1.0–1.27 μm in size. Colonies were yellow-pigmented, convex, circular, and 0.5–1.3 mm in diameter of grown on R2A medium for 2 days. DNA, esculin, skim milk, gelatine, starch, Tween 20, and Tween 80 were hydrolyzed, but not cellulose, arginine, and urea. Cells were grown on R2A, TSA, and NA but not on MacConkey agar. Growth occurs at 4–33 °C (optimum, 30 °C), at pH 5.0–8.0 (optimum, pH 6.5), and 0–2.5% NaCl. Nitrate was not reduced to nitrite and indole was not produced. Oxidase and catalase activity were positive. Positive results were obtained for acidification of glucose, β-galactosidase activity, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, acid phosphatase, *N*-acetyl-β-glucosaminidase, β-glucosidase, naphthol-AS-BI-phosphohydrolase, trypsin and α-chymotrypsin; negative results were obtained for lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, α-mannosidase, and α-fucosidase. Assimilation of D-mannose, D-maltose, D-glucose, adipic acid, *N*-acetyl-glucosamine, L-rhamnose, glycogen, L-fucose, L-proline, D-melibiose, suberic acid, and lactic acid. No assimilation of L-arabinose D-mannitol, gluconic acid, capric acid, malic acid, potassium gluconate, trisodium citrate, phenylacetic acid, D-ribose, *myo*-inositol, sucrose, itaconic acid, sodium malonate, sodium acetate, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, D-sorbitol, L-arabinose, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, and 4-hydroxybenzoic acid was observed. The predominant quinones were MK-6 and MK-7. The major polar lipids were phosphatidylethanolamine (PE) and two unidentified polar lipids (L2, L3). The polyamine pattern was *sym*-homospermidine. The major fatty acids were *iso*-$C_{15:0}$, summed feature 3 (containing $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and *iso*-$C_{17:0}$ 3-OH. The G+C content of the genomic DNA was 42.0 mol%. The type strain DCY68^T (=KCTC 32089^T = JCM 18517^T) was isolated from soil of a ginseng field in Gochang County (38°04′00″N 126°57′00″E) Republic of Korea.

Acknowledgements This research was supported by a Grant from Korea Institute of Planning & Evaluation for Technology in Food, Agriculture, Forestry & Fisheries (KIPET No. 317007-3).

References

1. Anzai Y, Kudo Y, Oyaizu H (1997) The phylogeny of the genera *Chryseomonas, Flavimonas,* and *Pseudomonas* supports synonymy of these three genera. Int J Syst Bacteriol 47:249–251
2. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J ClinPathol 45:493–496
3. Bernardet J-F, Nakagawa Y, 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
4. Bernardet J-F, Hugo C, Bruun B (2006) The genera *Chryseobacterium* and *Elizabethkingia*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The *Prokaryotes*: a handbook on the biology of bacteria, vol 7, 3rd edn. Springer, New York, pp 638–676
5. Bernardet J-F, Hugo C, Bruun B (2010) Genus X. *Chryseobacterium* Vandamme et al. 1994a. In: Whitman W (ed.), Bergey's manual of systematic bacteriology, 2nd edn, vol. 4, Edited by The Williams & Wilkins Co., Baltimore. Springer, New York, pp. 180–196
6. Busse H-J, Bunka S, Hensel A, Lubitz W (1997) Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. Int J Syst Bacteriol 47:698–708
7. Busse HJ, Auling G (1988) Polyamine pattern as a chemotaxonomic marker within the Proteobacteria. Syst Appl Microbiol 11:1–8
8. Choi KT (2008) Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* C.A Meyer. Acta Pharmacol Sin 29:1109–1118
9. Christensen WB (1946) Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. J Bacteriol 52:461–466
10. Collins MD (1985) Isoprenoid quinone analyses in bacterial classification and identification. In:Goodfellow M, Minnikin DE (eds) Chemical methods in bacterial systematics (Society for Applied Bacteriology Technical Series no. 20), Academic Press, London, pp. 267–287
11. Cowan ST, Steel KJ (1974) Manual for the identification of medical bacteria. Cambridge University Press, Cambridge
12. de Beer H, Hugo CJ, Jooste PJ, Willems A, Vancanneyt M, Coenye T, Vandamme P (2005) *Chryseobacterium vrystaatense* sp. nov., isolated from raw chicken in a chicken processing plant. Int J Syst Evol Microbiol 55:2149–2153
13. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
14. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids SympSer 41:95–98
15. Hantsis-Zacharov E, Senderovich Y, Halpern M (2008) *Chryseobacterium bovis* sp. nov., isolated from raw cow's milk. Int J Syst Evol Microbiol 58:1024–1028
16. Hoang VA, Kim YJ, Nguyen NL, Yang DC (2013) *Chryseobacterium yeoncheonense* sp. nov., with ginsenoside converting activity isolated from soil of a ginseng field. Arch Microbiol 195:463–471
17. Ilardi P, Fernández J, Avendaño-Herrera R (2009) *Chryseobacterium piscicola* sp. nov., isolated from diseased salmonid fish. Int J Syst Evol Microbiol 59:3001–3005
18. Kämpfer P, Dreyer U, Neef A, Dott W, Busse H-J (2003) *Chryseobacterium defluvii* sp. nov., isolated from wastewater. Int J Syst Evol Microbiol 53:93–97
19. Kämpfer P, Arun AB, Young C-C, Chen W-M, Sridhar KR, Rekha PD (2010) *Chryseobacterium arthrosphaerae* sp. nov.,

isolated from the faeces of the pill millipede *Arthrosphaera magna* Attems. Int J Syst Evol Microbiol 60:1765–1769
20. Kämpfer P, Trček J, Skok B, Šorgo A, Glaeser SP (2015) *Chryseobacterium limigenitum* sp. nov., isolated from dehydrated sludge. Antonie Van Leeuwenhoek 107(6):1633–1638
21. Kim KK, Bae HS, Schumann P, Lee ST (2005) *Chryseobacterium daecheongense* sp. nov., isolated from freshwater lake sediment. Int J Syst Evol Microbiol 55:133–138
22. Kim KK, Kim M-K, Lim JH, Park HY, Lee ST (2005) Transfer of *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* to *Elizabethkingia* gen. nov. as *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov. Int J Syst Evol Microbiol 55:1287–1293
23. Kim KK, Lee KC, Oh HM, Lee JS (2008) *Chryseobacterium aquaticum* sp. nov., isolated from a water reservoir. Int J Syst Evol Microbiol 58:533–537
24. Kim MK, Im W-T, Ohta H, Lee M, 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
25. Kim MK, Lee JW, Lee KY, Yang DC (2005) Microbial conversion of major ginsenoside Rb1 to pharmaceutically active minor ginsenoside Rd. J Microbiol 43:456–462
26. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequences database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721
27. Kimura M (1983) The neutral theory of molecular evolution. Cambridge University Press, Cambridge
28. Kumar S, Tamura K, Jakobsen IB, Nei M (2001) *MEGA*2: molecular 9 evolutionary genetics analysis software. Bioinformatics 17:1244–1245
29. Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley: Chichester, pp. 115–176
30. Lee SS (2007) Korean ginseng (ginseng cultivation), Korean ginseng and T. Res Inst. 18–40
31. Li Z, Zhu H (2012) *Chryseobacterium vietnamense* sp. nov., isolated from forest soil. Int J Syst Evol Microbiol 62:827–831
32. Loch TP, Faisal M (2014) *Chryseobacterium aahli* sp. nov., isolated from lake trout (Salvelinus namaycush) and brown trout (Salmo trutta), and emended descriptions of *Chryseobacterium ginsenosidimutans* and *Chryseobacterium gregarium*. Int J Syst Evol Microbiol 64:1573–1579
33. Loveland-Curtze J, Miteva V, Brenchley J (2010) Novel ultramicrobacterial isolates from a deep Greenland ice core represent a proposed new species, Chryseobacterium greenlandense sp. nov. Extremophiles 14:61–69
34. Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. Int J Syst Bacteriol 39:159–167
35. Minnikin DE, ODonnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241
36. Park JH (2004) Sun ginseng—a new processed ginseng with fortified activity. Food Ind Nutr 9:23–27
37. Park MS, Jung SR, Lee KH, Lee MS, Do JO, Kim SB, Bae KS (2006) *Chryseobacterium soldanellicola* sp. nov. and *Chryseobacterium taeanense* sp. nov., isolated from roots of sand-dune plants. Int J Syst Evol Microbiol 56:433–438
38. Park CS, Yoo MH, Noh KH, Oh DK (2010) Biotransformation of ginsenosides by hydrolyzing the sugar moieties of ginsenosides using microbial glycosidases. Appl Microbiol Biotechnol 87:9–19
39. Quan Z-X, Kim KK, Kim M-K, Jin L, Lee S-T (2007) *Chryseobacterium caeni* sp. nov., isolated from bioreactor sludge. Int J Syst Evol Microbiol 57:141–145
40. Saitou N, Nei M (1987) The neighbour – joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
41. Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. Newark, De: MIDI Inc
42. Shimomura K, Kaji S, Hiraishi A (2005) *Chryseobacterium shigense* sp. nov., a yellow-pigmented, aerobic bacterium isolated from a lactic acid beverage. Int J Syst Evol Microbiol 55:1903–1906
43. Skerman VBD (1967) A Guide to the Identification of the Genera of Bacteria, 2nd edn. Williams & Wilkins, Baltimore
44. Sun H, Wang HT, Kwon WS, Kim YJ, In JG, Yang DC (2011) A simple and rapid technique for the authentication of the ginseng cultivar, Yunpoong, using an SNP marker in a large sample of ginseng leaves. Gene 487:75–79
45. Taibi G, Schiavo MR, Gueli MC, Rindina PC, Muratore R, Nicotra CM (2000) Rapid and simultaneous high-performance liquid chromatography assay of polyamines and monoacetylpolyamines in biological specimens. J Chromatogr B Biomed Sci Appl 745:431–437
46. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739
47. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acids Res 25:4876–4882
48. Vandamme P, Bernardet J-F, Segers P, Kersters K, Holmes B (1994) New perspectives in the classification of the *flavobacteria*: description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov., and *Empedobacter* nom. rev. Int J Syst Bacteriol 44:827–831
49. Yassin AF, Hupfer H, Siering C, Busse HJ (2010) *Chryseobacterium treverense* sp. nov., isolated from a human clinical source. Int J Syst Evol Microbiol 60:1993–1998
50. Young CC, Kämpfer P, Shen FT, Lai WA, Arun AB (2005) *Chryseobacterium formosense* sp. nov., isolated from the rhizosphere of *Lactuca sativa* L. (garden lettuce). Int J Syst Evol Microbiol 55:423–426
51. Zhao X, Wang J, Li J, Fu L, Gao J, Du X, Bi H, Zhou Y, Tai G (2009) Highly selective biotransformation of ginsenoside Rb1 to Rd by the phytopathogenic fungus *Cladosporium fulvum* (*syn. Fulvia fulva*). J Ind Microbiol Biotechnol 36:721–726