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ORIGINAL PAPER

Burkholderia ginsengiterrae sp. nov. and *Burkholderia panaciterrae* sp. nov., antagonistic bacteria against root rot pathogen *Cylindrocarpon destructans*, isolated from ginseng soil

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Abstract Strain DCY85^T and DCY85-1^T, isolated from rhizosphere of ginseng, were rod-shaped, Gram-reaction-negative, strictly aerobic, catalase positive and oxidase negative. 16S rRNA gene sequence analysis revealed that strain DCY85^T as well as DCY85-1^T belonged to the genus *Burkholderia* and were closely related to *Burkholderia fungorum* KACC 12023^T (98.1 and 98.0 % similarity, respectively). The major polar lipids of strain DCY85^T and DCY85-1^T were phosphatidylethanolamine, one unidentified aminolipid and two unidentified phospholipids. The major fatty acids of both strains are $C_{16:0}$, $C_{18:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$). The predominant isoprenoid quinone of each strain DCY85^T and DCY85-1^T was ubiquinone (Q-8) and the G+C content of their genomic DNA was 66.0 and 59.4 mol%, respectively, which fulfill the characteristic range of the genus *Burkholderia*. The polyamine content of both DCY85^T and DCY85-1^T was putrescine. Although both DCY85^T and DCY85-1^T have highly similar 16S rRNA and identical *RecA* and *gyrB* sequences, they show differences in phenotypic and chemotaxonomic characteristics. DNA–DNA hybridization results proved the consideration of both strains as two different species. Based on the results from our polyphasic characterization, strain DCY85^T and DCY85-1^T are considered novel *Burkholderia* species for which the name *Burkholderia ginsengiterrae* sp. nov and *Burkholderia panaciterrae* sp. nov are, respectively, proposed. An emended description of those strains is also proposed. DCY85^T and DCY85-1^T showed antagonistic activity against the common root rot pathogen of ginseng, *Cylindrocarpon destructans*. The proposed type strains are DCY85^T (KCTC 42054^T = JCM 19888^T) and DCY85-1^T (KCTC 42055^T = JCM 19889^T).

Keywords Taxonomy · *Proteobacteria* · *Burkholderia ginsengiterrae* · *Burkholderia panaciterrae* · Antagonistic activity

Communicated by Erko Stackebrandt.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA, *gyrB* and *recA* gene sequence of strain DCY85^T and DCY85-1^T are KF915802, KF999960, KM501455, KM501454, KM495734 and KM495735, respectively.

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Introduction

The genus *Burkholderia* that belongs to *Burkholderiaceae* family (Sheu et al. 2013; Tian et al. 2013) was previously described as members of RNA homology group II of the genus *Pseudomonas*. (Yabuuchi et al. 1992). The genus *Burkholderia* comprises 89 species isolated from a wide range of niches (Coenye et al. 2004; Tian et al. 2013). Members of *Burkholderia* genus are gram-negative, aerobic, non-spore-forming, non-fermentative, straight rod-shaped and catalase-positive bacteria. Some strains are motile by using a single polar flagellum or a tuft of polar flagella (Gillis et al. 1995; Kim et al. 2006). *Burkholderia*

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strains can be found as plant or animal pathogens or as plant growth promoting, antagonists with diverse secondary metabolites production (Vandamme et al. 2007; Da et al. 2011; Mishra et al. 2012; Sua´rez-Moreno et al. 2012; Lu et al. 2012). Strains were also isolated from contaminated soil (Vanlaere et al. 2008) and from nodules of acid-adapted legumes (Howieson et al. 2008).

Panax ginseng is one of the most well-known medicinal plants in Asian countries (Leung and Wong 2010). Generally, high-quality ginseng can be obtained in slightly acidic soil (pH 5.5–6) (Lee 2007; Hankins 2009). Low quality and diseased roots were usually found in highly acidic soil. Root rot is the major disease that ginseng crop suffers from. It is frequently caused by the soil-born fungus, *Cylindrocarpon destructans,* which was found to be growing well in acidic soil (pH 5) (Rahman and Punja 2005). Detection and propagation of beneficial microorganisms that have ability to adapt on acidic condition may be useful for reducing the disease incidence and increasing the crop quality.

Materials and methods

Sampling and isolation

Soil samples were taken from Gochang province, Republic of Korea (35°23′56″N; 126°33′26″E) directly to lab and kept in 4 °C till isolation process. One gram of the soil sample was serially diluted, and 100 µl of each dilution was spread onto glucose yeast peptone agar (GYPA; glucose 4 %, yeast 0.5 %, peptone 1 %, agar 2 %) acidified using HCl. pH was adjusted to be 4.5 (±0.2), and cycloheximide (150 mg/l) was added to inhibit the fungal growth. Plates were kept for 2–3 days in 30 °C. The grown single colonies were purified three times in GYPA, and different single colonies were stocked in 30 % glycerol in GPY broth at −70 °C. Candidates of the genus *Burkholderia*, which were labeled DCY85^T and DCY85-1^T, were isolated and characterized in this study. *B. fungorum* KACC 12023^T was obtained from the Korean Agricultural Culture Collection (KACC), *Burkholderia caledonica* KCTC 12919^T from the Korean Collection for Type Cultures (KCTC); *Burkholderia bryophila* LMG 23644^T and *Burkholderia megapolitana* LMG 23650^T were obtained from the Belgian Coordinated Collections of Microorganisms (BCCM) as references type strains.

Bacterial growth, morphology, physiological and biochemical characteristics

Cells morphology was observed by transmission electron microscopy after grown in tryptic soy agar (TSA, MB cell) for 1 day at 30 °C; suspended cells were placed on Carbon and Formvar-coated nickel grids for 30 s; and 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 was determined using Gram staining kit (Fluka) according to the manufacturers' instructions. Growth of both strains DCY85^T and DCY85-1^{T}was tested using several media such as Reasoner's 2A (R2A, Difco), tryptic soy agar (TSA, MB cell), MacConkey agar (MacA, Difco), GYPA, nutrient agar (NA, Difco), potato dextrose agar (PDA, Difco), Luria–Bertani agar (LBA, Difco) and DNase agar (Difco) at 30 °C. The temperature for optimal growth was tested by checking growth of the strains DCY85^T and DCY85-1^T on TSA at 4, 10, 15, 20, 25, 28, 30, 37 and 40 °C. Tolerance of salinity was evaluated in tryptic soy broth (TSB, Difco) supplemented with 0–2.0 % (w/v) NaCl, at 0.5 % intervals at 30 °C. pH was ranged from 3 to 10 (1 pH value interval); the pH were adjusted using 100 mM phosphate-citrate buffer (pH 3–7) and 50 mM Tris Cl (pH 8–10). 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 manufacturers' instructions. Hydrolysis of the following substrates was tested too: starch on R2A agar containing 1 % starch (Difco), DNA on DNase agar medium (Scharlau) flooded with 1 N HCl, casein on R2A agar supplemented with 2 % skim milk (Difco) (Cowan and Steel 1974), Tween 20 and 80 on R2A agar containing (1 % Tween 20 or 80) and 0.02 % $CaCl_2$, gelatin on a medium containing (0.3 % beef extract, 0.5 % peptone and 20 % gelatin), esculin on TSA agar containing 0.3 % esculin (Sigma) and 0.02 % ferric citrate (Fluka). Growth under anaerobic conditions was assessed after 10 days of incubation of the DCY85^T and DCY85-1^T on GYPA at 30 °C in the GasPak EZ Anaerobic Container System (BD). Carbon utilization, enzyme production and other tests were carried out on the strains DCY85^T and DCY85-1^T in parallel with the reference strains using the API 32GN, API 20NE and API ZYM according to the manufacturers' instruction (bioMérieux), and results were recorded after 6 h for API ZYM and 48 h for API 32 GN and API 20NE.

Antibiotic susceptibility test

Antibiotic susceptibility was done using disk diffusion assay according to Kirby–Bauer method (Prescott and Harley 2001) with some modification; 200 ml of the bacterial broth grown in TSB were spread on TSA and the following antibiotics were used: rifampicin (5 µg), erythromycin (15 mg), vancomycin (30 µg), penicillin (10 µg), neomycin (30 µg), lencomycin (15 µg), tetracyclin (30 µg), novobiocin (30 µg), carbenicillin (100 µg), ceftazidime (30 µg), cephazoline (30 µg), and oleandomycin (15 µg).

16S rRNA, *recA*, *gyrB* sequences and phylogenetic analysis

Genomic DNA of strains DCY85^T and DCY85-1^T was extracted and purified using a DNA isolation kit (Promega) according to the manufacturers' instructions. The 16S rRNA gene sequence of strain DCY85^T and DCY85-1^T was amplified from the chromosomal DNA using universal bacteria primer sets 27F, 518F, 800R and 1492R (Lane 1991; Anzai et al. 1997). Subsequently, the purified PCR products were sequenced by Genotech (Daejeon, Republic of Korea). The partial sequence (1,453 bp) of the 16S rRNA was assembled with SeqMan software version 4.1 (DNASTAR Inc.) and with BioEdit program (Hall 1999). The 16S rRNA gene sequences of related taxa were obtained from EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/). Multiple alignments of the sequences were performed with the CLUSTAL X program (Thompson et al. 1997). A phylogenetic tree was constructed with the neighbor joining (NJ; Saitou and Nei 1987) by Kimura 2-parameter model (Kimura 1983), maximum likelihood (ML) by Tamura-Nei model and maximum parsimony (MP; Fitch 1971) using the MEGA4 program (Tamura et al. 2007). Bootstrap analysis with 1,000 replication was also conducted to obtain confidential levels for the branches (Felsenstein 1985). Additionally, *recA* and *gyrB* sequences were amplified from the strains, DCY85^T and DCY85-1^T as described by Spilker et al. (2009) by setting the annealing temperatures of *rec A* and *gyr B* to 60.8 and 62.6 °C, respectively. Sequences were checked on NCBI using blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and related sequences of other *Burkholderia* taxa were collected. Phylogenetic trees were performed using NJ method as described above for 16 rRNA.

G+C content mol% and DNA–DNA hybridization

In order to analyze the G+C mol% of DNA, the genomic DNA of strains DCY85^T and DCY85-1^T was extracted and purified as mentioned above then degraded enzymatically into nucleosides as described by Mesbah et al. (1989). Subsequently, the obtained nucleoside mixture was separated by HPLC (Model, NS-6000A, Futecs; reversed-phase column YMC-Triart C18 (4.6 × 250 mm × 5 µm); flow rate 1.0 ml/min; wavelength at 270 nm; using solvent mixture of 25 mM $NH_4H_2PO_4$/acetonitrile (20/1, v/v) as mobile phase).

DNA–DNA hybridization was performed with photo biotin-labeled probes as described by Ezaki et al. (1989). Levels of DNA–DNA relatedness were determined by triplicate between strain DCY85^T, DCY85-1^T and references type strains, reciprocally (mean ± SD, $n = 3$).

Chemotaxonomic characteristics

Polar lipid analysis

The polar lipids of strains DCY85^T and DCY85-1^T and the closest reference strain *B. fungorum* KACC 12023^T were extracted from fresh cell. Polar lipids were examined by two–dimensional thin layer chromatography (2D-TLC) using TLC silica gel 60F_{254} (Merck) plates (10 × 10 cm). Chromatograms were developed in the first dimension with chloroform/methanol/water (65/25/4, v/v/v) and in the second dimension with chloroform/methanol/acetic acid/water (80/12/15/4, v/v/v/v) as solvent systems. The total polar lipids, aminolipids, glycolipids and phospholipids were detected by staining the plates with 5 % ethanolic molybdophosphoric acid, ninhydrin, α-naphthol and molybdenum blue, respectively (Minnikin et al. 1984).

Isoprenoid quinone and fatty acid analysis

For isoprenoid quinone analysis, cell biomass of both novel strains, DCY85^T and DCY85^T grown on TSB media (Difco) at 30 °C for 48 h were prepared. Isoprenoid quinones were extracted from fresh cells with chloroform/methanol (2/1, v/v) and then concentrated at 40 °C using vacuum rotary evaporator. The residue was subsequently extracted with 20 ml hexane: water (1:1, v/v) then purified using Sep-Pak®Vac 6 cc silica cartridge; the samples were analyzed by HPLC system (Model, NS-6000A, Futecs; reversed-phase column YMC-Triart C18 (4.6 × 250 mm × 5 µm); flow rate 1.2 ml/min; wavelength at 270 nm; using Acetonitrile/isopropanol (65/35, v/v) as mobile phase) according to Collins (1985).

For fatty acid analysis, strains DCY85^T, DCY85-1^T and the four reference type strains were grown on TSA (Difco) for 36 h 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) (Sasser 1990).

Polyamine analysis

Polyamine was extracted and analyzed following the method as reported by Busse and Auling (1988) and Taibi et al. (2000). The polyamine standards spermine, spermidine, putrescine and *sym*-homospermidine were purchased from Sigma-Aldrich. The analysis of polyamine was performed using a HPLC system (Agilent technology 1260 infinity). The separation was carried out on a Poroshell 120 EC-C18 column (3.0 × 50 mm, 2.7 µm) using 60 % MeOH

as mobile phase with flow rate: 0.3 ml/min, and detection was performed by monitoring absorbance at 234 nm, with an injection volume of 5 µl.

Antagonistic activity

Antagonistic ability of DCY85^T and DCY85-1^T was checked as following; some ginseng pathogenic fungi, *Botrytis cinerea* KACC 41298, *Fusarium oxysporum* KACC 40052, *Fusarium solani* KACC 41092, *Colletotrichum gloeosporioides* KACC 40003 and *Cylindrocarpon destructans* KACC 44656 were tested for their susceptibility to strain DCY85^T and DCY85-1^T. *B. fungorum* KACC 12023^T was used for comparison. Each strain was streaked in a square shape centrally on PDA media and then incubated at 25 °C for 24 h. Five-millimeter-diameter fungal blocks were taken from edge of 7-day colonies and transferred in the middle of the PDA-containing bacteria plates and then incubated in 25 °C for up to 10 days (20 days for *C. destructans* KACC 44656). Fungal blocks were inoculated in PDA without bacterial inoculum as a control. The pathogens growth was checked two times (after 4 and 10 days; except *C. destructans* KACC 44656, 10 and 20 days) and compared with the control. Rate of inhibition was calculated according to (Zhao et al. 2012) by the following equation: (dc-dt)/(dc-5) × 100, where dc is the diameter of the control by millimeter and dt is the diameter of the treated strain by the bacteria by millimeter.

Results and discussion

Growth, morphology and physiochemical characteristics

Cells of both strains DCY85^T and DCY85-1^T were Gram-reaction-negative, strictly aerobic, catalase weakly positive, oxidase negative and rod shaped. Colonies were circular with entire margins, transparent yellow and intermediate in size (0.3–0.5 mm diameter) after 2 days on TSA but they looked different after growing on GYPA for 2 days; the former was opaque yellow (0.5–1 mm), while the latter was larger (2–2.5 mm diameter), with milky color and shiny surface. Both strains grew on TSA, GYPA, R2A Agar and LB agar, but they did not grow on MacConkey, NA and DNase agar. Growth of both strains occurred at 20–37 °C (optimum, 30 °C), but they showed differences in both pH and salinity tolerance tests; both strain could grow at pH 4–7 but DCY85^T optimally grew at pH 4.0, while DCY85-1^T at pH 5.0. In case of the salinity, both strains could grow in the presence of 0–1 % NaCl, DCY85^T optimally grew at 0.5 % salinity, while DCY85-1^T at 0 %. Tween 20 and Tween 80 were hydrolyzed by both strains, but skim milk, gelatine, esculin and starch were not hydrolyzed. The physiological and biochemical characteristics of strains DCY85^T and DCY85-1^T are given in Table 1 with its description.

Antibiotics susceptibility test

Strains DCY85^T and DCY85-1^T were found to be resistant to vancomycin (30 µg), lencomycin (15 µg), oleandomycin (15 µg), cephazoline (30 µg), tetracycline (30 µg), novobiocin (30 µg), neomycin (30 µg), penicillin P (10 µg), refampicin (5 µg) and erythromycin (15 µg), but susceptible to ceftazidime (30 µg). In case of carbenicillin (100 µg), they showed a difference in susceptibility; DCY85^T was intermediately susceptible, while DCY85-1^T was highly susceptible.

16S rRNA, *recA*, *gyrB* sequences and phylogenetic analysis

The 16S rRNA sequences of strains DCY85^T and DCY85-1^T were compared with EzTaxon-e server database. Strains DCY85^T and DCY85-1^T was found to be phylogenetically related to *B. fungorum* KACC 12023^T (98.1 and 98.0 %, respectively), *B. megapolitana* LMG 23650^T (97.5, 97.4 %), *B. caledonica* KCTC 12919^T (97.4, 97.3 %) and *B. bryophila* LMG 23644^T (97.1, 97.0 %) as shown in Fig. 1. Since 16 rRNA sequence failed to discriminate between *Burkholderia* at species level, *recA* and *gyrB* sequences were used for the discrimination (Vandamme et al. 2013). *RecA* and *gyrB* also could not distinguish between those two strains (Fig. S1 and S2).

G+C content and DNA–DNA hybridization

G+C content of strains DCY85^T and DCY85-1^T fulfilled the characteristic range of the genus *Burkholderia* as well as the reference strains (Coenye et al. 2001); however, they showed inconsistent amount (66.0 and 59.4 %, respectively). The DNA–DNA relatedness values between the strains DCY85^T, DCY85-1^T and reference strains are summarized in Table 3. Interestingly, the DNA–DNA relatedness values between DCY85^T and DCY85-1^T was lower than 70 % (58.0 ± 1.5 and 58.8 ± 1.4, respectively) and hence they are considered two different species according to Wayne et al. (1987).

Chemotaxonomic characteristics

The major polar lipids of DCY85^T, DCY85-1^T and *B. fungorum* KACC 12023^T were found to be consistent; phosphatidylethanolamine (PE), one unidentified aminolipids (AL1) and two unidentified phospholipids (PL1-2). On the

Table 1 Differential characteristics of strain DCY85^T, DCY85-1^T and related *Burkholderia* type strains

Characteristic	1	2	3	4	5	6
Hydrolysis of:						
H_2O_2	w	w	+	-	+	+
1 %*N,N,N,N*,-tetramethyl-1,4-phenylenediamine	–	–	+	-	-	+
2 % skim milk	–	–	ND	[+]	ND	[-]
Nitrate reduction	–	–	+	–	–	–
Enzyme activity (API ZYM)						
Alkaline phosphatase	+	w	+	+	+	+
Acid phosphatase	+	w	+	+	+	+
Naphthol-AS-BI-phosphohydrolase	+	w	+	+	+	w
Assimilation of (API32 GN and API 20NE):						
D-Ribose	–	–	+	+	+	+
Sodium acetate	+	+	+	w	+	+
Lactic acid	+	+	+	+	w	+
3-Hydroxybenzoic acid	+	+	–	–	–	–
L-serine	–	–	–	w	-	w
Capric acid	w	–	+	+	+	+
Valeric acid	w	–	+	–	–	+
Trisodium citrate	–	–	+	–	–	+
Adipic acid	-	-	+	-	-	-

Strains: 1, *B. ginsengiterrae* DCY85^T; 2, *B. panaciterrae* DCY85-1^T; 3, *B. fungorum* KACC 12023^T; 4, *B. megapolitana* LMG 23650^T; 5, *B. caledonica* KCTC 12919^T; 6, *B. bryophila* LMG 23644^T

+ Positive, *W* weakly positive, − negative, [] results were taken from Vandamme et al. (2007); *ND* not determined.

All strains were positive in the following: hydrolysis of Tween 80, L-rhamnose, *N*-acetyl-glucosamine, *myo*inositol, L-alanine, D-mannitol, D-glucose, D-sorbitol, L-arabinose, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, L-proline, β-galactosidase (PNPG), D-mannose, potassium gluconate, malic acid, phenylacetic acid, esterase (C4), esterase lipase (C8) and leucine arylamidase. Weakly positive for the following: L-fucose and propionic acid

All strains were negative in following: hydrolysis of DNase, D-saccharose, D-maltose, itaconic acid, suberic acid, sodium malonate, potassium 5-ketogluconate, glycogen, salicin, D-melibiose, 4-hydroxybenzoic acid, indole production, D-glucose (fermentation), L-arginine dihydrolase, urease, esculin hydrolysis, gelatinase, D-maltose, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase

other hand, they showed differences regarding the minor polar lipids. DCY85-1^T was characterized from DCY85^T by an additive minor unidentified polar lipid (L3) (Fig.S4.). Ubiquinone (Q-8) was the major Quinone in the strains DCY85^T and DCY85-1^T. The major fatty acids of both strains as well as the reference strains were qualitatively $C_{16:0}$, $C_{18:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), while minor fatty acids were $C_{14:0}$, $C_{17:0}$ *cyclo*, $C_{16:12}$ OH, $C_{16:0}$ 2-OH, $C_{16:0}$ 3-OH, $C_{19:0}$ *cyclo* $\omega8c$, $C_{18:1}$ 2-OH, summed feature 2 ($C_{12:0}$ aldehyde, unknown fatty acid with an equivalent chain-length value of 10.928 and/or $C_{14:0}$ 3-OH and/or $C_{16:1}$ *iso I*) (Coenye et al. 2001); the quantitative results are summarized in Table 2. DCY85^T distinguished from DCY85-1^T by a higher amount of $C_{16:0}$ and $C_{17:0}$ *cyclo* and lower amount of $C_{18:1}$ $\omega7c$. The major polyamine of both strains DCY85^T and DCY85-1^T as well as the closest species *B. fungorum* KACC 12023^T was putrescine Table 3.

Antagonistic activity

DCY85^T and DCY85-1^T showed an antagonistic activity against *C. destructans* that was not shown by *B. fungorum* KACC 12023^T. They did not show any activity against *F. oxysporum* and *F. solani* in contrast with *B. fungorum* KACC 12023^T. All tested strains could not inhibit the other remaining pathogens (Fig. S5.).

Description of *Burkholderia ginsengiterrae* sp. nov.

Burkholderia ginsengiterrae (gin.sen.gi.ter'ra.e. N.L. n. *ginsengum*, ginseng; L. n. *terra -ae*, soil; N.L. gen. n. *ginsengiterrae* of soil of a ginseng field).

Cells are Gram-reaction-negative, strictly aerobic, catalase weakly positive, oxidase negative and rod shaped. Colonies are circular with entire margins, transparent yellow and intermediate in size after 2 days on TSA, opaque

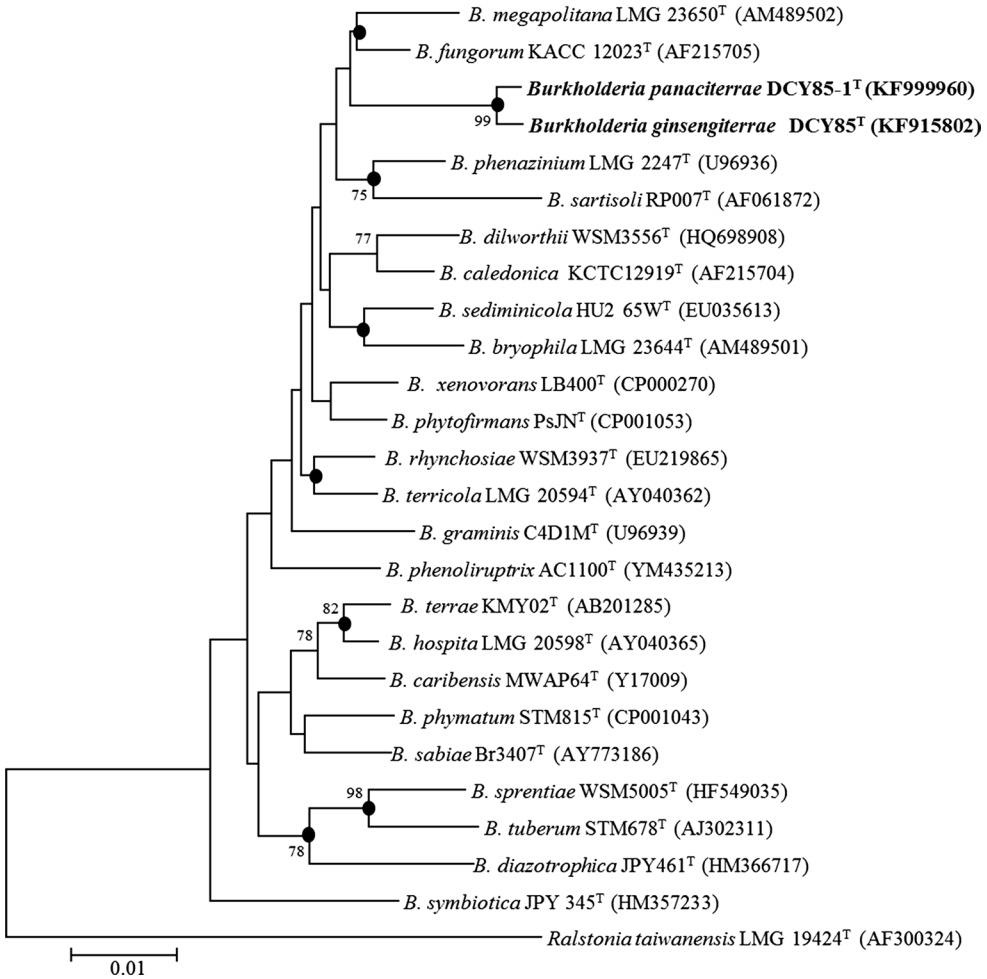

Fig. 1 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the taxonomic position of strain DCY85^T and DCY85-1^T in the genus *Burkholderia*. *Filled circles* indicate that the corresponding nodes were also recovered in the tree generated with the maximum parsimony algorithm. Bootstrap values >70 % based on 1,000 replications are shown at branching points. *Bar*, 0.01 substitutions per nucleotide position. *Ralstonia pickettii* ATCC 27511 ^{T} was used as an out-group

Table 2 Fatty acid composition of strain *B. ginsengiterrae* DCY85^T, *B. panaciterrae* DCY85-1^T and related *Burkholderia* type strains

Fatty acid	1	2	3	4	5	6
C14:0	1.0	1.2	4.4	0.6	4.0	0.8
C16:0	20.0	15.6	14.3	22.3	16.9	16.4
C17:0 *cyclo*	9.4	3.0	2.7	12.0	9.7	2.8
C16:12 OH	1.1	1.3	1.8	1.9	0.9	1.8
C16:0 2-OH	2.0	1.5	1.8	2.6	0.9	2.0
C16:0 3-OH	4.8	6.0	5.1	4.5	5.2	4.2
C18:1 *ω7c*	31.1	39.3	38.5	25.7	33.2	37.7
C19:0 *cyclo ω8c*	4.9	1.7	0.9	7.3	4.8	1.4
C18: 1 2-OH	1.0	1.4	1.1	0.9	tr	0.8
Summed feature 2	6.3	8.2	7.7	6.2	4.3	5.0
Summed feature 3	12.6	13.7	17.8	10.5	15.6	20.4

Strains: 1, *B. ginsengiterrae* DCY85^T; 2, *B. panaciterrae* DCY85-1^T; 3, *B. fungorum* KACCC 12023^T; 4, *B. megapolitana* LMG 23650^T; 5, *B. caledonica* KCTC 12919^T; 6, *B. bryophila* LMG 23644^T. All data from this study, tr, less than 0.5 %. Summed feature 2 contained $C_{12:0}$ aldehyde, unknown fatty acid with an equivalent chain-length value of 10.928 and/or $C_{14:0}$ 3-OH and/or $C_{16:1}$ *iso I*; summed feature 3 contained $C_{16:1}\omega 6c$ and/or $C_{16:1}\omega 7c$

yellow on GYPA. Growth occurs at 20-37 °C (optimum 30 °C), at pH 4–7 (optimum pH 4.0) and in the presence of 0–1 % NaCl (optimum, 0.5 %). Skim milk, starch, esculin, DNA and gelatin are not hydrolyzed, while Tween 80, 20 are hydrolyzed. In the API 20 NE strip, nitrate cannot be reduced to nitrite; D-glucose is not fermented; indole is

Table 3 DNA–DNA relatedness values of all *Burkholderia* strains examined

Strain	DNA relatedness	
	B. ginsengiterrae DCY85T	*B. panaciterrae* DCY85-1T
B. ginsengiterrae DCY85T	100	58.8 ± 1.4
B. panaciterrae DCY85-1T	58.0 ± 1.5	100
B. fungorum KACC 12023T	39.7 ± 1.2	39.8 ± 1.2
B. megapolitana LMG 23650T	23.0 ± 1.2	32.8 ± 3.0
B. caledonica KCTC 12919T	43.2 ± 1.6	36.6 ± 2.7
B. bryophila LMG 23644T	41.0 ± 1.7	23.8 ± 3.2

not produced; urease, arginine dihydrolase, β-glucosidase and gelatinase activities are absent; β-galactosidase activity is present; and D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, malic acid and phenylacetic acid are assimilated. Capric acid is weakly assimilated; but D-maltose, adipic acid and trisodium citrate are not assimilated. In the API 32GN strip, L-rhamnose, *N*-acetyl-glucosamine, myoinositol, sodium acetate, lactic acid, L-alanine, 3-hydroxybenzoic acid, D-mannitol, D-glucose, D-sorbitol, L-arabinose, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid and L-proline are assimilated. L-fucose, propionic acid, capric acid and valeric acid are weakly assimilated. D-ribose, D-saccharose (sucrose), D-maltose, itaconic acid, suberic acid, sodium malonate, potassium 5-ketogluconate, glycogen, L-serine, salicin, D-melibiose, trisodium citrate and 4-hydroxybenzoic acid are not assimilated. In the API ZYM strip alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present. Lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. It is resistant to rifampicin (5 μg), erythromycin (15 μg), vancomycin (30 μg), lencomycin (15 μg) and oleandomycin (15 μg), penicillin P (10 μg), neomycin (30 μg), cephazoline (30 μg), tetracycline (30 μg) and novobiocin (30 μg), but highly susceptible to ceftazidime (30 μg), and intermediately susceptible to carbenicillin (100 μg). The major polar lipids are phosphatidylethanolamine (PE), one unidentified aminolipid and two unidentified phospholipids. The major fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$). The predominant isoprenoid quinone is ubiquinone (Q-8). The G+C content of the genomic DNA is 66.0 mol%. Major polyamines of DCY85^T is putrescine. DCY85^T show antagonistic effect against root rot pathogen of ginseng, *Cylindrocarpon destructans* KACC 44656.

The type strain is DCY85^T (=KCTC 42054^T = JCM 19888^T), isolated from the rhizosphere of ginseng, Gochang province (35°23′56″N; 126°33′26″E) in the Republic of Korea.

Description of *Burkholderia panaciterrae* sp. nov.

Burkholderia panaciterrae (pa.na.ci.ter'rae. N.L. n. *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, strictly aerobic, catalase weakly positive, oxidase negative and rod shape. Colonies are circular with entire margins, transparent yellow and intermediate in size after 2 days on TSA and big, with milky color and shiny surface after 2 days on GYPA. Growth occurs at 20–37 °C (optimum 30 °C), at pH 4–7 (optimum pH 5.0) and in the presence of 0–1 % NaCl (optimum 0 %). Skim milk, starch, esculin, DNA and gelatin are not hydrolyzed, while Tween 80, 20 are hydrolyzed. In the API 20 NE strip, nitrate cannot reduced to nitrite; D-glucose is not fermented; indole is not produced; urease, arginine dihydrolase, β-glucosidase and gelatinase activities are absent; β-galactosidase activity is present; and D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, malic acid and phenylacetic acid are assimilated. D-maltose, capric acid, adipic acid and trisodium citrate are not assimilated. In the API 32GN strip, L-rhamnose, *N*-acetyl-glucosamine, myoinositol, sodium acetate, lactic acid, L-alanine, 3-hydroxybenzoic acid, D-mannitol, D-glucose, D-sorbitol, L-arabinose, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid and L-proline are assimilated. L-fucose and propionic acid are weakly assimilated. D-ribose, D-saccharose (sucrose), D-maltose, itaconic acid, suberic acid, sodium malonate, potassium 5-ketogluconate, glycogen, L-serine, salicin, D-melibiose, capric acid, valeric acid, trisodium citrate and 4-hydroxybenzoic acid are not assimilated. In the API ZYM strip esterase (C4), esterase lipase (C8), leucine arylamidase are present, while alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are weakly present. Lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase are absent. It is resistant to vancomycin (30 μg), lencomycin (15 μg), oleandomycin (15 μg), cephazoline (30 μg), tetracycline (30 μg), novobiocin (30 μg), neomycin (30 μg), penicillin P (10 μg), rifampicin (5 μg) and erythromycin (15 μg), but highly susceptible to ceftazidime (30 μg), and carbenicillin (100 μg). The major polar lipids are phosphatidylethanolamine (PE), one unidentified aminolipid and two unidentified phospholipids. The major fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$).

The predominant isoprenoid quinone is ubiquinone (Q-8). The G+C content of the genomic DNA is 59.4 mol%. Major polyamine of DCY85-1^T is putrescine. DCY85-1^T shows antagonistic effect against root rot pathogen of ginseng, *Cylindrocarpon destructans* KACC 44656.

The type strain is DCY85-1^T (KCTC 42055^T = JCM 19889^T), isolated from the rhizosphere of ginseng, Gochang province (35°23′56″N; 126°33′26″E) in the Republic of Korea.

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