

## *Paenibacillus ginsengiterrae* sp. nov., a ginsenoside-hydrolyzing bacteria isolated from soil of ginseng field

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Received: 12 September 2014 / Revised: 5 December 2014 / Accepted: 6 December 2014 / Published online: 17 December 2014  
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**Abstract** A novel bacterial strain DCY89<sup>T</sup> was isolated from soil sample of ginseng field and was characterized using a polyphasic approach. Cells were Gram-reaction-positive, rod-shaped, spore-forming and motile with flagella. The strain was aerobic, esculin and starch positive, catalase- and oxidase-negative, optimum growth temperature, and pH were 25–30 °C and 6.0–7.5, respectively. On the basis of 16S rRNA gene sequence analysis, strain DCY89<sup>T</sup> was shown to belong to the genus *Paenibacillus* and the closest phylogenetic relatives were *Paenibacillus cellulosityticus* KACC 14175<sup>T</sup> (98.2%), *Paenibacillus kobensis* KACC 15273<sup>T</sup> (98.1%), *Paenibacillus xylaniclasticus* KCTC 13719<sup>T</sup> (96.9%), and *Paenibacillus curdlanolyticus* KCTC 3759<sup>T</sup> (96.64%). The DNA G+C content was 52.5 mol%, and the predominant respiratory quinone was

MK-7. The major fatty acids were iso-C<sub>15:0</sub>, iso-C<sub>16:0</sub>, and anteiso-C<sub>15:0</sub>. The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylglycerol. The results of the genotypic analysis in combination with chemotaxonomic and physiological data demonstrated that DCY89<sup>T</sup> represented a novel species within the genus *Paenibacillus*, for which we propose the name *Paenibacillus ginsengiterrae*. The type strain is DCY89<sup>T</sup> (JCM 19887<sup>T</sup> = KCTC 33430<sup>T</sup>).

**Keywords** *Paenibacillus ginsengiterrae* · Ginseng soil · Taxonomy · Biotransformation

### Introduction

The members of the genus *Paenibacillus* are either Gram-reaction-positive or Gram-reaction-negative (Ash et al. 1993; Valverde et al. 2008), facultatively anaerobic or strictly aerobic, produced ellipsoidal spores, non-pigmented, rod-shaped, and motile (Lim et al. 2006; Zhou et al. 2012) with a G+C content 39–59 mol % (Yao et al. 2014). The genus *Paenibacillus*, which belongs to the family *Paenibacillaceae*, is a novel group of bacilli first described by Ash et al. (1993). This genus currently comprises 149 species and 4 subspecies (<http://www.bacterio.cict.fr/p/paenibacillus.html>). Members of genus *Paenibacillus* are widespread microorganisms commonly isolated from various sources, including food (Berge et al. 2002), fresh water (Baik et al. 2011), air (Rivas et al. 2005), human blood (Roux and Raoult 2004), heat-treated milk (Scheldeman et al. 2004), necrotic wounds (Glaeser et al. 2013), warm springs (Saha et al. 2005), and rhizospheric soil (Cheong et al. 2005). The cell wall peptidoglycan diamino acid of the *Paenibacillus* members is *meso*-DAP,

Communicated by Erko Stackebrandt.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DCY89<sup>T</sup> is KF915799.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00203-014-1073-0) contains supplementary material, which is available to authorized users.

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and menaquinone-7 (MK-7) is the predominant menaquinone. Diphosphatidylglycerol (DPG) is the major polar lipid in all the *Paenibacillus* members for which polar lipids data are available (Yao et al. 2014). The predominant cellular fatty acid is anteiso-C<sub>15:0</sub> (Ludwig et al. 2009). In this study, we describe a novel  $\beta$ -glucosidase producing bacterium, DCY89<sup>T</sup>, isolated from soil of a ginseng field in Kyung Hee University, based on a polyphasic taxonomic approach.

## Methods and materials

### Bacterial isolation

Soil samples were collected from a ginseng field, Kyung Hee University, Republic of Korea (37 ° 14' 43"N 127 ° 04' 56"E). Under 10 cm depth, around 100 g of the rhizosphere soil were carefully collected without any stones and particles, zipped lock covers and transferred to the laboratory. For bacterial isolation, five times diluted R2A agar (Difco) was used. The serial dilution was made up to 10<sup>-5</sup> by dissolving of one gram of soil sample in 10 ml of 0.85% sterile saline, and 100  $\mu$ l of each dilution was spread on five times diluted R2A plates. The plates were incubated at 30 °C for 3–5 days. The purification of single colony was carried out by transferring them to new R2A agar plates. Isolate was preserved at -70 °C in R2A broth (Difco) supplemented with 25 % (v/v) glycerol. For the comparative study, *Paenibacillus cellulosilyticus* KACC 14175<sup>T</sup> and *Paenibacillus kobensis* KACC 15273<sup>T</sup> were obtained from the Korean Agricultural Culture Collection (KACC), while *Paenibacillus xylaniclasticus* KCTC 13719<sup>T</sup> and *Paenibacillus curdlanolyticus* KCTC 3759<sup>T</sup> were obtained from Korean Collection for Type Cultures (KCTC) as reference type strains. These strains were cultured under the same conditions as strain DCY89<sup>T</sup>.

### Cell growth, physiology, morphology, and biochemical characteristics

Colonies of strain DCY89<sup>T</sup> were observed after culturing on R2A agar plate at 30 °C. Cell morphology and flagella were examined under microscope ( $\times$ 1,000 magnifications, MBL-2100) and transmission electron microscopy (LOE912AB) using cells that were grown on TSA broth for 24 h at 30 °C. The motility was checked on TSA broth supplemented with on 0.2 % agar (Weon et al. 2008). Gram reaction was tested by using a bioMerieux Gram stain kit. Nitrate reduction was tested in nitrate broth containing 0.2 % KNO<sub>3</sub> (Skerman 1967). Different media were tested for growth such as trypticase soy agar (TSA; Difco), R2A, MacConkey (Bacto), nutrient agar (NA; Difco), and LB

agar (Difco) at 30 °C for 7 days. For checking growth at different temperature, a range of 5, 10, 15, 20, 25, 30, 37, and 40 °C were tested for 7 days. Growth at different NaCl was tested by using of 0–10 % (w/v) NaCl in TSB at 0.5 % unit interval. Growth of strain DCY89<sup>T</sup> also checked at different pH values in TSB medium. Different initial pH values (4–10 at intervals of 1 pH units) were adjusted, respectively, by use of Tris HCl 0.1 M and 1N NaOH. According to the manufacturer's instruction, 1 % (w/v) *N,N,N,N*-tetramethyl-1,4-phenylenediamine reagents was used to test the oxidase activity. Catalase activity was determined by the production of bubble from 3 % (v/v) H<sub>2</sub>O<sub>2</sub> solution. Triple sugar iron agar was used to test for H<sub>2</sub>S production. Hydrolysis of DNA, gelatin, esculin, Tween 80, starch, skim milk, and tyrosinase were analyzed as described by Cowan and Steel (1974). Enzymes production and carbon source utilization were conducted by using API ZYM, API 20NE, and API 32GN strips according to the manufacturer's instructions (bioMérieux). 1 % tryptophan broth was used for indole production by using Kovács's reagent. To check anaerobic growth, strain DCY89<sup>T</sup> was cultured on TSA plates and incubated in a GasPak EZ (BD) for 14 days at 30 °C. The antibiotics susceptibility was checked according to Bauer et al. (1966) using Oxoid antibiotic disks, on Müller-Hinton agar plates incubated at 30 °C for 24–48 h under aerobic condition. The inhibition zone was measured following manufacturer's manual. The antibiotics were used including carbenicillin (CAR<sub>100</sub>, 100  $\mu$ g), vancomycin (VA<sub>30</sub>, 30  $\mu$ g), ceftazidime (CAZ<sub>30</sub>, 30  $\mu$ g), novobiocin (NV<sub>30</sub>, 30  $\mu$ g), neomycin (N<sub>30</sub>, 30  $\mu$ g), tetracycline (TE<sub>30</sub>, 30  $\mu$ g), cephalosin (KZ<sub>30</sub>, 30  $\mu$ g), erythromycin (E<sub>15</sub>, 15  $\mu$ g), oleandomycin (OL<sub>15</sub>, 15  $\mu$ g), penicillin G (P<sub>10</sub>, 10  $\mu$ g), rifampicin (RD<sub>5</sub>, 5  $\mu$ g), and lincomycin (MY<sub>15</sub>, 15  $\mu$ g).

### 16S rRNA sequence and phylogenetic analysis

The genomic DNA of strain DCY89<sup>T</sup> was isolated by using the DNA isolation kit (Gene All Biotechnology, Republic of Korea). The bacterial universal primer set 27F, 518F, 800R, and 1492R were used to amplify the 16S rRNA gene sequence (Lane 1991). The purified PCR product was sequenced by Genotech (Daejeon, Republic of Korea) according to Kim et al. (2005). Seq-Man software version 4.1 (DNASTAR, Inc.) was used to compile the nearly complete sequenced (1495 bp) of strain DCY89<sup>T</sup>. Then, the sequence was uploaded to EZTaxon-e server (<http://eztaxon-e.ezbiocloud.net/>) to find the pair wise similarity of the nearly full 16S rRNA gene sequence by performing the identity analysis. The 16S rRNA gene sequences of closely related strain were collected from gene bank and then aligned by CLUSTAL X program (Thompson et al.

1997), and distances were calculated according to Kimura two-parameter model (Kimura 1983). The phylogenetic tree was constructed with neighbor-joining (Saitou and Nei 1987) and MPM (Fitch 1971) by using the MEGA5 program (Tamura et al. 2011). In order to take the confidential levels for the branches (Felsenstein 1985), bootstrap analysis with 1,000 replications was also conducted.

#### G+C content and DNA–DNA hybridization

In order to analyze the G+C mol % of DNA, the genomic DNA of strain DCY89<sup>T</sup> 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. (1989). Subsequently, the obtained nucleoside mixture was separated using a reverse-phase HPLC column YMC-Triart C18 (4.6 × 250 mm × 5 μm).

DNA–DNA hybridization was performed with photobiotin-labeled probes as described by Ezaki et al. (1989). Optimal hybridization temperature was at 39.1 °C. Levels of DNA–DNA relatedness were determined by triplicate between strain DCY89<sup>T</sup> and references type strains of the closest phylogenetic neighbors *P. cellulosityticus* KACC 14175<sup>T</sup>; *P. kobensis* KACC 15273<sup>T</sup>; *P. xylaniclasticus* KCTC 13719<sup>T</sup>; and *P. curdlanolyticus* KCTC 3759<sup>T</sup> (mean ± SD, n = 3).

#### Chemotaxonomic characteristics

##### *Polar lipids and respiratory quinone*

Cells were grown in trypticase soy broth at 30 °C, shaken at 160 rpm for 1 day, centrifuged, and dried in freeze drier. Isoprenoid quinone was extracted from 100 mg freeze-dried cells with chloroform/methanol (2:1, v/v), and after concentrated at 40 °C using vacuum rotary evaporator, the residue was subsequently extracted with 10 ml hexane using Sep-Pak<sup>®</sup> Vac 6 cc silica cartridge for further purification. Then, the samples were analyzed by HPLC (Model, NS-6000A Futecs) according to Collins and Jones (1981).

Polar lipid of strain DCY89<sup>T</sup> and *P. cellulosityticus* KACC 14175<sup>T</sup> were extracted from dry cells (100 mg) (Minnikin et al. 1977). Polar lipids were dissolved in chloroform/methanol (2:1, v/v). The samples were spotted on the corner of two-dimensional thin layer chromatography (2D-TLC) using TLC Kiesel gel 60F<sub>254</sub> (Merck) plates (10 × 10 cm) and developed in the first direction by using chloroform/methanol/water (65:25:4, by v/v/v) while in the second direction developed by chloroform/acetic acid/methanol/water (80:15:12:4, by 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.

##### *Peptidoglycan and cell wall sugar analysis*

Peptidoglycan and cell wall sugar analysis of strain DCY89<sup>T</sup> and reference strain *P. cellulosityticus* KACC 14175<sup>T</sup> was analyzed as mentioned by Schleifer and Kandler (1972). The hydrolyzed peptidoglycans were determined by spotting the sample on the TLC plate by using of TLC cellulose Merck KGaA (20 × 20 cm). The solvent methanol/pyridine/6N HCl/water (100:12.5:6:32.5, by vol) was made 1 day before running. The hydrolyzed sugar was detected by spotting 5 μl sample and 5 μl of the standard mixture of sugars as a reference on the TLC plate (by using of TLC cellulose Merck KGaA (20 × 20 cm), (Staneck and Roberts 1974).

##### *Cellular fatty acid analysis*

The strain DCY89<sup>T</sup> and the four reference type strain cells (*P. cellulosityticus* KACC 14175<sup>T</sup>; *P. kobensis* KACC 15273<sup>T</sup>; *P. xylaniclasticus* KCTC 13719<sup>T</sup>; and *P. curdlanolyticus* KCTC 3759<sup>T</sup>) were grown on TSA agar at 30 °C for 1 day. Fatty acid was extracted, methylated, and saponified by the method described by Sherlock Microbial Identification System (MIDI) and analyzed by capillary GLC (Hewlett Packard 6890) using the TSBA library (version 6.1) (Sasser 1990). Then, fatty acid analysis was performed in duplicate.

##### *Polyamine analysis*

Polyamine of strain DCY89<sup>T</sup> and *P. cellulosityticus* KACC 14175<sup>T</sup> were extracted and analyzed as reported by Taibi et al. (2000). The polyamine standards spermine, spermidine, and putrescine 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 i.d.) 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.

##### *Biotransformation of ginsenoside Rb1 by crude enzyme of strain DCY89<sup>T</sup>*

Strain DCY89 was grown in TSB medium at 30 °C for overnight, and cells were collected by centrifuging at 5,000×g for 30 min at 4 °C. Then, cells were resuspended in 20 mM sodium phosphate buffer (pH 7.0) and lysed by sonication with short pulses, and debris was removed by

centrifugation (12,000×g, at 4 °C for 30 min). Supernatant was used as crude enzyme. This is the modification of Quan et al. (2012) protocol. The biotransformation procedure was carried out in 15-ml screw-capped tubes. First, 2 ml of crude enzyme was mixed with an equal volume of ginsenoside Rb1 at 1.0 mg/ml in 20 mM sodium phosphate buffer. The mixture was then incubated at 37 °C with shaking (160 rpm). During the reaction period, a 200-μl aliquot was removed every 1 h. Aliquots were extracted using water-saturated *n*-butanol and analyzed by TLC, HPLC, and LC/MS. TLC analysis was carried out using silica gel plates (60F<sub>254</sub>; Merck, Darmstadt, Germany) and developed with a solvent system of CHCl<sub>3</sub>:CH<sub>3</sub>OH:H<sub>2</sub>O (65:35:10 v/v/v). Spots on TLC plates were detected by spraying with 10 % (v/v) H<sub>2</sub>SO<sub>4</sub> followed by heating at 110 °C for 10 min. HPLC analysis was carried out with a C18 (250 × 4.6 mm, particle size 5 μm) column. LC/MS for the ginsenoside was analyzed with an Agilent QQQ/MS in positive polarity mode with an ion trap analyzer.

## Results and discussion

Cell growth, morphology, physiology, biochemical tests, and antibiotic susceptibility

Morphological observation of strain DCY89<sup>T</sup> colonies on TSA agar was spherical with beige color and raised colonies with approximate diameters 4–9 mm after incubation at 30 °C for 48 h. Strain DCY89<sup>T</sup> was found to grow on R2A, TSA, LB, and NA, but not to grow on MacConkey agar. The strain was observed to grow best in TSA at temperature 20–37 °C (optimum temp 30 °C); at pH 6–8 (optimum, pH 7) and up to 2 % NaCl (optimum, 0.5 %). Phenotypic analysis showed that strain DCY89<sup>T</sup> cells are Gram-positive, aerobic, rod-shaped, motile with monotrichous flagella (Fig. S2), and spore-forming (Fig. S3). Tests for catalase and oxidase activity were negative. Strain DCY89<sup>T</sup> did not hydrolyze skim milk, tyrosine, DNA, and Tween 80, but hydrolyzed starch, esculin, and gelatin after 2 days of incubation at 30 °C. Nitrate was reduced to nitrite. Indole was not produced. Other biochemical and physiological characteristics of strain DCY89<sup>T</sup> and four references strain are shown in Table 1. Strain DCY89<sup>T</sup> was susceptible to all antibiotics that were used in this study, especially cephalazolin (KZ<sub>30</sub>, 30 μg), ceftazidime (CAZ<sub>30</sub>, 30 μg), and rifampicin (RD<sub>5</sub>, 5 μg).

## 16S rRNA sequence and phylogenetic analysis

The nearly complete sequence (1,495 bp) of the 16S rRNA gene was obtained. The comparison of the 16S rRNA

**Table 1** The biochemical and physiological characteristics of strain DCY89<sup>T</sup> and the reference strains of genus *Paenibacillus*

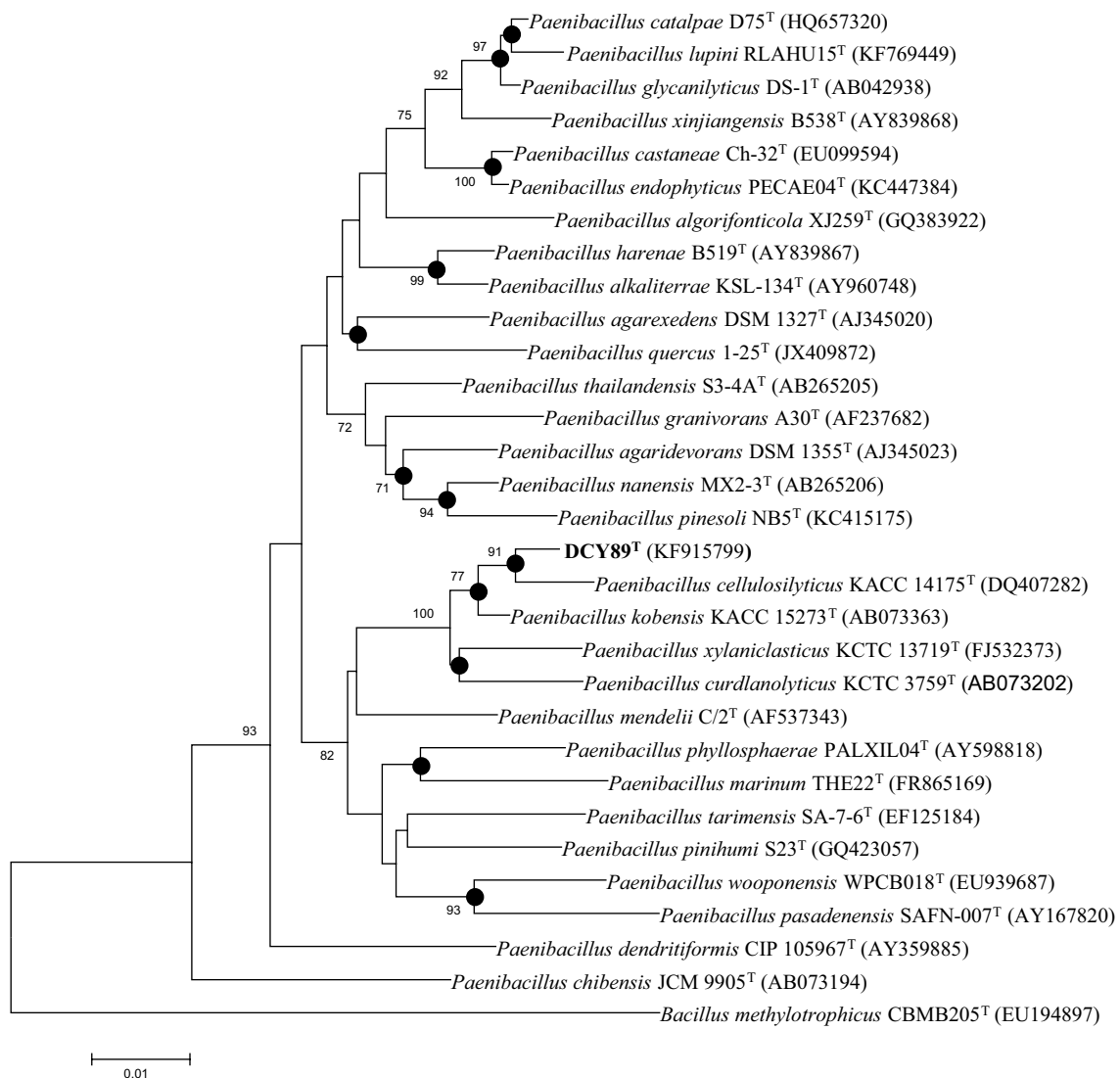
Characteristics	1	2	3	4	5
<i>Hydrolysis of:</i>					
Arginine	–	+	+	+	+
Urea	–	+	–	+	+
Gelatin	+	–	+	–	+
<i>Enzyme activity:</i>					
α-Glucosidase	–	w	–	–	+
β-Glucosidase	+	+	–	+	–
β-Galactosidase	+	+	–	+	+
<i>Assimilation of:</i>					
L-Rhamnose	+	–	+	–	–
N-Acetyl-glucose	+	–	+	–	+
D-Ribose	+	–	+	–	w
Inositol	+	–	+	–	+
D-Saccharose	+	+	+	–	+
Itaconic acid	w	w	w	–	–
Suberic acid	w	w	–	–	–
Sodium malonate	–	–	w	–	w
Sodium acetate	w	–	w	–	w
Lactic acid	w	–	w	–	–
L-Alanine	w	–	w	–	w
Potassium 5-ketogluconate	w	w	w	–	w
L-Serine	w	–	w	–	–
D-Mannitol	+	–	+	–	–
L-Fucose	w	w	w	–	w
D-Sorbitol	–	–	w	w	w
Valeric acid	–	–	w	–	w
Trisodium citrate	w	–	+	–	w
L-Histidine	+	–	+	–	+
Potassium 2-ketogluconate	–	–	+	–	–
3-Hydroxybutyric acid	–	–	w	w	–
4-Hydroxybenzoic acid	+	w	w	–	–
L-Proline	–	–	w	–	w

Strain: 1, *P. ginsengiterrae* DCY89<sup>T</sup>; 2, *P. cellulosityticus* KACC 14175<sup>T</sup>; 3, *P. kobensis* KACC 15273<sup>T</sup>; 4, *P. xyliclasticus* KCTC 13719<sup>T</sup>; and 5, *P. curdlanolyticus* KCTC 3759<sup>T</sup>

All strains were positive for the following reactions: esterase (C4) (API ZYM); hydrolysis of esculin, assimilation of D-glucose, L-arabinose (API 20NE); assimilation of D-maltose, glycogen, salicin, D-melibiose (API 32GN); and negative for the following reactions: α-mannosidase, α-fucosidase, N-acetyl-D-glucosaminidase (API ZYM); assimilation of 3-hydroxybenzoic acid, propionic acid, capric acid (API 32GN)

+, positive; w, weakly positive; –, negative

sequence of strain DCY89<sup>T</sup> (GenBank/EMBL/DDBJ accession number KF915799) with other *Paenibacillus* strains revealed that strain DCY89<sup>T</sup> is a novel strain of the genus *Paenibacillus*, sharing highest sequence similarity with *P. cellulosityticus* KACC 14175<sup>T</sup> (98.2 %), *P. kobensis*



**Fig. 1** The neighbor-joining tree based on 16S rRNA gene sequence analysis showing phylogenetic relationships of strain DCY89<sup>T</sup> and members of the genus *Paenibacillus*. Bootstrap values more than 70 % based on 1,000 replications are shown at branching points.

Filled circles indicate that the corresponding nodes were also recovered in the tree constructed with the maximum parsimony (MP) algorithm. Scale bar 0.01 substitutions per nucleotide position

KACC 15273<sup>T</sup> (98.1 %), *P. xylaniclasticus* KCTC 13719<sup>T</sup> (96.9 %), and *P. curdlanolyticus* KCTC 3759<sup>T</sup> (96.6 %). Strain DCY89<sup>T</sup> formed a reliable and monophyletic cluster with *P. cellulosityticus* PALXIL08<sup>T</sup> in NJ tree, and filled circles indicate that the corresponding nodes were also recovered in the tree constructed with the maximum parsimony (MP) algorithm (Fig. 1). This cluster was also recovered in the trees generated by the ML (Fig. S4).

#### G+C content and DNA–DNA hybridization

The DNA G+C content of strain DCY89<sup>T</sup> was 52.5 mol %, which is similar to related type strains (Shida et al. 1997; Rivas et al. 2006). The values for DNA–DNA relatedness

between strain DCY89<sup>T</sup> and the closest type strains *P. cellulosityticus* KACC 14175<sup>T</sup>; *P. kobensis* KACC 15273<sup>T</sup>; *P. xylaniclasticus* KCTC 13719<sup>T</sup>; and *P. curdlanolyticus* KCTC 3759<sup>T</sup> were  $22.7 \pm 3.4$ ,  $17.8 \pm 1.2$ ,  $31.3 \pm 3.2$ , and  $37.4 \pm 1.0$ , respectively. These values were well below the 70 % threshold proposed for species delineation (Wayne et al. 1987) suggesting that strain DCY89<sup>T</sup> represents a distinct genomic species of the genus *Paenibacillus*.

Fatty acid, quinone, polar lipid, polyamine, peptidoglycan, and cell wall sugar analysis

The major fatty acids of strain DCY89<sup>T</sup> was anteiso-C<sub>15:0</sub> (45.7 %), however, iso-C<sub>16:0</sub> (16.4 %), iso-C<sub>15:0</sub> (9.4 %),

**Table 2** Fatty acid profiles of strain DCY89<sup>T</sup> and related species of the genus *Paenibacillus*

Fatty acid	1	2	3	4	5
Saturated fatty acid					
C <sub>12:0</sub>	1.31	0.71	ND	ND	0.66
C <sub>14:0</sub>	1.52	1.01	0.66	0.63	1.07
C <sub>16:0</sub>	7.95	8.88	1.47	11.09	8.38
C <sub>17:0</sub>	ND	0.38	ND	1.48	0.24
C <sub>18:0</sub>	ND	0.37	4.21	1.46	0.45
C <sub>20:0</sub>	ND	ND	2.71	ND	0.14
Branched-chain fatty acid (iso)					
iso-C <sub>13:0</sub>	ND	ND	0.7	ND	0.13
iso-C <sub>14:0</sub>	2.89	2.71	1.25	0.72	3.6
iso-C <sub>15:0</sub>	9.41	6.21	14.01	1.53	7.24
iso-C <sub>16:0</sub>	16.41	25.37	0.48	23.64	17.44
iso-C <sub>17:0</sub>	3.43	4.81	5.36	1.67	3.95
iso-C <sub>18:0</sub>	ND	0.28	ND	0.37	0.15
iso-C <sub>19:0</sub>	ND	ND	3.29	ND	ND
Branched-chain fatty acid (anteiso)					
anteiso-C <sub>11:0</sub>	ND	ND	ND	1.36	ND
anteiso-C <sub>13:0</sub>	ND	1.13	ND	ND	0.16
anteiso-C <sub>15:0</sub>	45.68	37.65	51.46	35.76	49.17
anteiso-C <sub>17:0</sub>	7.09	9.79	9.12	19.51	5.02
anteiso-C <sub>19:0</sub>	ND	ND	2.53	ND	ND
Summed feature 4 <sup>a</sup>	1.4	ND	ND	ND	0.19
Summed feature 5 <sup>b</sup>	0.66	ND	ND	ND	0.52

Strain: 1, *P. ginsengiterrae* DCY89<sup>T</sup>; 2, *P. cellulossilyticus* KACC 14175<sup>T</sup>; 3, *P. kobensis* KACC 15273<sup>T</sup>; 4, *P. xylaniclasticus* KCTC 13719<sup>T</sup>; and 5, *P. curdlanolyticus* KCTC3759<sup>T</sup>

ND not detected

<sup>a</sup> Summed feature 4 contained anteiso-C<sub>17:1</sub> B and/or iso-C<sub>17:1</sub>

<sup>b</sup> Summed feature 5 contained C<sub>18:2</sub> ω6,9c and/or anteiso-C<sub>18:0</sub>

anteiso-C<sub>17:0</sub>, (7.0 %), and saturated-C<sub>16:0</sub>, (8.0 %) were also found little high amount in strain DCY89<sup>T</sup>. The isolated strain DCY89<sup>T</sup> showed a similar major fatty acid composition to the related type strains of the genus *Paenibacillus*, but there were significant quantitative differences when cultivated under the same conditions (Table 2). Thus, the results indicate that strain DCY89<sup>T</sup> is a new species in the genus *Paenibacillus*. The major menaquinone of strain DCY89<sup>T</sup> was MK-7 that was similar to other members of genus *Paenibacillus*. The major polar lipids of strain DCY89<sup>T</sup> were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) (Fig. S1), which have been reported in several members of the genus *Paenibacillus*. The major polyamine of strain DCY89<sup>T</sup> was spermidine. The peptidoglycan contained the major amino acid *meso*-DAP (diaminopimelic acid), also contained glutamic acid and alanine, which is similar with *P. cellulossilyticus* KACC 14175<sup>T</sup>. Whole cell sugars of strain DCY89<sup>T</sup>

were identified as ribose, rhamnose, glucose, and galactose, while *P. cellulossilyticus* KACC 14175<sup>T</sup> was found to contain ribose and galactose.

#### Biotransformation of ginsenoside Rb1

Ginsenoside Rb1 was converted to Rd by hydrolysis of a glucose unit at the C-20 position of the ginsenoside aglycone. As shown in Fig. S5, the concentrations of ginsenoside Rb1 and the decomposition product Rd exhibited regular changes with increasing reaction time. Within 3 h, Rb1 was fully hydrolyzed and converted into ginsenoside Rd. The conversion of ginsenosides Rb1 by DCY89<sup>T</sup> was confirmed by quantitative HPLC analysis (Fig. S6). The peaks with retention times of 20.68 and 22.59 min correspond to ginsenosides Rb1 and Rd, respectively (Fig. S6A). Fig. S6B shows the control of ginsenoside Rb1. As shown in Fig. S6C, the peak for ginsenoside Rb1 was fully disappeared (100 %) within 3 h, and a new peak appeared. The new peak had retention time consistent with that of ginsenosides Rd. The metabolite was determined as ginsenoside Rd based on the protonated molecular ion peak (Fig. S7).

All the result of the phylogenetic and phenotypic suggested that strain DCY89<sup>T</sup> belongs to the genus *Paenibacillus*. While the phylogenetic and chemotaxonomic distinctiveness of DCY89<sup>T</sup> supported that strain represents a novel species that is distinct from previously known *Paenibacillus* species. Strain DCY89<sup>T</sup> also can be differentiated from other related *Paenibacillus* species based on phenotypic characteristics. Therefore, on the basis of the data presented, we consider that strain DCY89<sup>T</sup> represents a novel species of the genus *Paenibacillus*, for which name *Paenibacillus ginsengiterrae* sp. nov. is proposed.

#### Description of *Paenibacillus ginsengiterrae* sp. nov.

*Paenibacillus ginsengiterrae* (gin.sen. gi. térrae. N. L. n. ginsengum ginseng; L. n. terra soil; N.L. gen. n. ginsengiterrae of soil of a ginseng field, the source of the type strain).

Cells are Gram-reaction-positive, catalase-negative, oxidase-negative, aerobic, rod-shaped, motile with monotrichous flagella and spore-forming. Colonies are circular, beige color on TSB agar and 0.4–0.9 mm in diameter after incubation for 2 days. Cells grow on TSA, R2A, NA, but not on MacConkey agar. Growth occurs at 20–37 °C, at pH 6–8 and at 0–2 % (w/v) NaCl. Nitrate is reduced to nitrite. Tyrosine, skim milk, Tween 80, and DNA are not hydrolyzed. Starch, gelatin, and esculine are hydrolyzed. Acid production from glucose, sucrose, and lactose is positive. H<sub>2</sub>S gas is not produced. In API ZYM tests, enzyme activity shows positive for esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase,

$\alpha$ -galactosidase,  $\beta$ -galactosidase, and  $\beta$ -glucosidase, but negative for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin,  $\alpha$ -chymotrypsin,  $\beta$ -glucuronidase,  $\alpha$ -glucosidase, *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase,  $\alpha$ -fucosidase. Positively assimilated compounds are L-rhamnose, *N*-acetyl-glucose, D-ribose, Inositol, D-saccharose, D-maltose, Itaconic acid, suberic acid, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, L-serine, D-mannitol, D-glucose, salicin, D-melibiose, L-fucose, L-arabinose, trisodium citrate, L-histidine, and 4-hydroxybenzoic acid. However, the following are negative for assimilation: sodium malonate, 3-hydroxybenzoic acid, D-sorbitol, propionic acid, capric acid, valeric acid, potassium 2-ketogluconate, 3-hydroxybutyric acid, and L-proline (API 20NE, ID 32GN and traditional methods). Cells are sensitive to neomycin (N<sub>30</sub>), tetracycline (TE<sub>30</sub>), cephalosporin (KZ<sub>30</sub>), carbenicillin (CAR<sub>100</sub>), vancomycin (VA<sub>30</sub>), ceftazidime (CAZ<sub>30</sub>), novobiocin (NV<sub>30</sub>), erythromycin (E<sub>15</sub>), oleandomycin (OL<sub>15</sub>), penicillin G (P<sub>10</sub>), rifampicin (RD<sub>5</sub>), and lincomycin (MY<sub>15</sub>) with more sensitive to cephalosporin (KZ<sub>30</sub>), ceftazidime (CAZ<sub>30</sub>), and rifampicin (RD<sub>5</sub>). The predominant quinone is MK-7. The major cellular fatty acid of strain DCY89<sup>T</sup> was anteiso-C<sub>15:0</sub>. Strain DCY89<sup>T</sup> also contain little high amount of iso-C<sub>16:0</sub>, iso-C<sub>15:0</sub>, anteiso-C<sub>17:0</sub>, and saturated-C<sub>16:0</sub>. The peptidoglycan contained the amino acids *meso*-DAP, glutamic acid, and alanine. The major polar lipids of strain DCY89<sup>T</sup> were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG). Cell wall sugars of strain DCY89<sup>T</sup> were ribose, rhamnose, glucose, and galactose. The DNA G+C content of the type strain is 52.47 mol %.

The type strain DCY89<sup>T</sup> (KCTC 33430<sup>T</sup> = JCM 19887<sup>T</sup>) was isolated from soil of a ginseng field in Kyung Hee University, Republic of Korea.

**Acknowledgments** This research was supported by Korea Institute of Planning & Evaluation for Technology in Food, Agriculture, Forestry & Fisheries (KIPET NO: 309019-03-3-SB010) and Next-Generation BioGreen 21 Program (SSAC, Grant#: PJ009529032014), Republic of Korea.

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