Antonie van Leeuwenhoek (2014) 106:935–945
DOI 10.1007/s10482-014-0263-8

ORIGINAL PAPER

Paenibacillus yonginensis sp. nov., a potential plant growth promoting bacterium isolated from humus soil of Yongin forest

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Received: 9 June 2014 / Accepted: 14 August 2014 / Published online: 29 August 2014

Abstract Strain DCY84^T, a Gram-stain positive, rod-shaped, aerobic, spore-forming bacterium, motile by means of peritrichous flagella, was isolated from humus soil from Yongin forest in Gyeonggi province, South Korea. Strain DCY84^T shared the highest sequence similarity with *Paenibacillus barengoltzii* KACC 15270^T (96.86 %), followed by *Paenibacillus timonensis* KACC 11491^T (96.49 %) and *Paenibacillus phoenicis* NBRC 106274^T (95.77 %). Strain DCY84^T was found to able to grow best in TSA at temperature 30 °C, at pH 8 and at 0.5 % NaCl. MK-7 menaquinone was identified as the isoprenoid quinone. The major polar lipids were identified as phosphatidylethanolamine, an unidentified aminophospholipid, two unidentified aminolipids and an unidentified polar lipid. The peptidoglycan was found to contain the amino acids meso-diaminopimelic acid, alanine and D-glutamic acid. The major fatty acids of strain DCY84^T were identified as branched chain anteiso-$C_{15:0}$, saturated $C_{16:0}$ and branched chain anteiso-$C_{17:0}$. The cell wall sugars of strain DCY84^T were found to comprise of ribose, galactose and xylose. The major polyamine was identified as spermidine. The DNA G+C content was determined to be 62.6 mol%. After 6 days of incubation, strain DCY84^T produced 52.96 ± 1.85 and 72.83 ± 2.86 μg/ml L-indole-3-acetic acid, using media without L-tryptophan and supplemented with L-tryptophan, respectively. Strain DCY84^T was also found to be able to solubilize phosphate and produce siderophores. On the basis of the phenotypic characteristics, genotypic analysis and chemotaxonomic characteristics, strain DCY84^T is considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus yonginensis* sp. nov. is proposed. The type strain is DCY84^T (=KCTC 33428^T = JCM 19885^T).

Keywords *Paenibacillus yonginensis* · Gram-staining-positive · Plant growth promoting

Electronic supplementary material The online version of this article (doi:10.1007/s10482-014-0263-8) contains supplementary material, which is available to authorized users.

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Introduction

Direct use of microorganisms to promote plant growth and to control plant pests continues to be an area of rapidly expanding research (Weller 2007). Those bacteria which can promote plant growth, called plant growth-promoting bacteria (PGPB), include either those which form specific symbiotic relationships with plants or those which are free-living or endophytic bacteria which can colonize plant tissues. The members of the genus *Paenibacillus* are well known as PGPB, together with *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter* and *Burkholderia* (Hayat et al. 2010). Many new species from the genus *Paenibacillus* have been reported, along with more research ongoing. The genus *Paenibacillus* was first proposed by Ash et al. (1991) and later its description was emended by Shida et al. (1997). Members of this genus are ubiquitous in nature and species have been isolated from various environments such as petroleum-hydrocarbon-contaminated sediment (Montes et al. 2004), warm springs (Saha et al. 2005), alkaline soils (Yoon et al. 2005), rice fields (Sánchez et al. 2005), a spacecraft assembly facility (Osman et al. 2006), ginseng field soil (Park et al. 2007), poultry litter compost (Vaz-Moreira et al. 2007), phyllosphere (Valverde et al. 2008), gut (Park et al. 2009), rhizosphere (Beneduzi et al. 2010), a subsurface molybdenum mine (Benardini et al. 2011), tidal flat (Wang et al. 2012), nodules (Carro et al. 2013) and gamma-irradiated Antarctic soil (Dsouza et al. 2014). At the time of writing, there are nearly 150 species of the genus *Paenibacillus* with validly published names (http://www.bacterio.net/paenibacillus.html). Members of the genus *Paenibacillus* are Gram-positive or Gram-variable, rod-shaped bacteria, and strictly aerobic or facultatively anaerobic (Ash et al. 1991). The major fatty acid is anteiso-$C_{15:0}$, the predominant isoprenoid quinone is unsaturated menaquinone with seven units (MK-7) and meso-diaminopimelic acid is commonly found in the peptidoglycan (Priest 2009). In this study, we characterized a new isolate, strain DCY84^T, belonging to the genus *Paenibacillus* from humus soil from Yongin forest in Gyeonggi province, South Korea. The phenotypic and genotypic characterizations of the novel strain are described in this report and we propose that strain DCY84^T represents a novel species, for which the name *Paenibacillus yonginensis* sp. nov. is proposed.

Materials and methods

Isolation, morphological and physiological characterization

Samples from several random spots at Yongin forest were carefully collected in clean zip lock covers, without any stones or particles, and transferred to the laboratory. One gram of sample was dissolved in 10 ml of 0.85 % (w/v) saline solution, serially diluted up to 10^{-5} and spread on 5 times diluted Reasoneŕs 2A (R2A) agar (MB cell). The plates were incubated at 30 °C for 3 days. Single colonies were purified by transferring them to new R2A agar plates. A novel *Paenibacillus*, designated as DCY84^T, was isolated and characterized in this study. The isolate was routinely cultured on R2A agar at 30 °C and stored at −80 °C as a suspension in R2A broth with 30 % (v/v) glycerol. However, later the media was changed to Trypticase Soya Agar (TSA, MB cell) and Trypticase Soya Broth (TSB) after checking results for optimum growth of this strain. Strain DCY84^T has been deposited in the Korean Collection for Type Cultures (as KCTC 33428^T) and Japan Collection of Microorganisms (as JCM 19885^T). *Paenibacillus barengoltzii* KACC 15270^T and *Paenibacillus timonensis* KACC 11491^T were obtained from the Korean Agricultural Culture Collection and *Paenibacillus phoenicis* NBRC 106274^T was obtained from the National Institute of Technology and Evaluation (NITE) Biological Resource Center for use as reference type strains. These strains were cultured under the same optimum conditions as strain DCY84^T.

Based on the proposed minimal standards for the description of aerobic, endospore-forming bacteria (Logan et al. 2009), standard tests were performed for phenotypic characterization of strain DCY84^T. Strain DCY84^T colonies were observed after culturing on TSA agar plate at 30 °C after incubation for 48 h. Gram-reaction was tested by using a bioMérieux Gram stain kit. Cells were grown in TSB for 24 h at 30 °C and then tested for gliding motility by the hanging-drop technique (Skerman 1967). Cell morphology and flagella were detected by using transmission electron microscopy (Carl Zeiss LOE912AB). Suspended cells previously grown on TSA at 30 °C for 48 h were placed on carbon- and formvar-coated nickel grids for 30 s and grids were floated on one 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.

Different media were tested for growth such as R2A, TSA, Nutrient Agar (NA, MB cell), Luria–Bertani (LB, MB cell) agar, Potato Dextrose Agar (PDA, MB cell) and MacConkey Agar (Difco) at 30 °C for 7 days. For checking growth at different temperature, a range of 4, 8, 15, 20, 25, 30, 37, 40 and 45 °C were tested for 7 days. The salinity test was checked by using 0.5–9.5 % (w/v) NaCl in TSB using increments of 1 %. Growth of strain DCY84^T was also checked at different pH values, from 4 to 10 in TSB using increments of 1 pH units. The following buffers were used to adjust the pH values: citric acid/sodium citrate (pH 4.0–6.0), Na_2HPO_4/NaH_2PO_4 (pH 6.0–8.0), $Na_2CO_3/NaHCO_3$ (pH 8.0–10.0) and Na_2HPO_4/NaOH (pH 10.0) (Gomori 1955). Spore formation was checked by incubating the strains using Tryptone Yeast Extract (TYE) agar supplemented with 5 mg/l $MnSO_4$ for 7 days at 30 °C (Verma et al. 2013). The Becton–Dickinson (BD) GasPak™ EZ Gas Generating System was used to test anaerobic growth. Catalase activity was determined by the production of bubbles from 3 % (v/v) H_2O_2 solution mixed with freshly grown cells. Oxidase activity was checked by using of 1 % (w/v) *N*,*N*,*N*,*N*-tetramethyl-*p*-phenylenediamine reagent (Sigma) according to the manufacturer's instructions. Triple sugar iron agar was used to test for H_2S production. Nitrate reduction was tested in nitrate broth containing 0.2 % KNO_3 (Skerman 1967). Indole production was analyzed using Kovács's reagent in 1 % tryptone broth (Skerman 1967). Hydrolysis of gelatin (on a medium containing 12 % gelatin, 0.3 % beef extract and 0.5 % peptone), aesculin (on TSA containing 0.3 % aesculin and Fe^{3+}), Tween 20 and 80 (on TSA containing 1 % Tween 20/80 and 0.02 % $CaCl_2$), starch (on TSA containing 1 % starch) and casein (on TSA supplemented with 2 % skim milk) were analyzed as previously described by Cowan and Steel (1974). Antibiotic susceptibility was tested by using Oxoid antibiotic paper discs on Mueller–Hinton (Difco) agar at 30 °C for 48 h under aerobic conditions as described by Bauer et al. (1966). The antibiotics were used included carbenicillin (CAR_{100}, 100 μg), ceftazidime (CAZ_{30}, 30 μg), novobiocin (NV_{30}, 30 μg), neomycin (N_{30}, 30 μg), tetracycline (TE_{30}, 30 μg), cefazolin (KZ_{30}, 30 μg), erythromycin (E_{15}, 15 μg), oleandomycin (OL_{15}, 15 μg), lincomycin (L_{15}, 15 μg) and rifampicin (RD_5, 5 μg) (Sigma-Aldrich). Zones of inhibition were interpreted according to the manufacturer's instruction. Basic chemical tests, carbon source assimilation and enzyme activities were conducted using API 20E (bioMérieux), API 50CHB (bioMérieux), GP VITEK-2 compact system version 4.01 (bioMérieux) and API ZYM (bioMérieux), according to the manufacturer's instructions. API 20E, API 50CHB, GP VITEK-2 compact system version 4.01 (bioMérieux) were recorded after incubation for 48 h, under the optimal conditions for each strain while API ZYM was recorded after incubation for 10 h.

Molecular characterization

The genomic DNA of strain DCY84^T was isolated by using a DNA isolation kit (Gene All Biotechnology, Republic of Korea). The 16S rRNA gene was amplified using the universal bacterial primer sets including 27F/1492R (Lane 1991) and 518F/800R (Weisburg et al. 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 16S rRNA sequence of strain DCY84^T. The nearly complete (1,474 bp) 16S rRNA sequence of strain DCY84^T was compiled using Seqman software and edited using the BioEdit program (Hall 1999). Multiple alignments were performed with the CLUSTAL X program (Thompson et al. 1997) and distances were calculated according to Kimura two-parameter model (Kimura 1983). *Paenibacillus* members with 16S rRNA gene sequence similarities above 94 % were included in the phylogenetic tree. The phylogenetic tree was constructed with the neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971) and maximum-likelihood (Felsenstein 1981) methods by using the MEGA 6 program package (Tamura et al. 2013). In order to determine the confidence levels for the branches, bootstrap analysis with 1,000 replications was conducted (Felsenstein 1985). The strain DCY84^T sequence obtained was compared with those in public databases by using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al. 2012) and NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi; Johnson et al. 2008).

DNA G+C mol% content

For DNA G+C mol% content analysis, the genomic DNA of strain DCY84^T and the closest related strain, *P. barengoltzii* KACC 15270^T, were extracted and purified by using an Exgene TM Cell SV mini-kit (Gene All Biotechnology, Republic of Korea) according to the manufacturer's instructions. Nuclease and alkaline phosphatase enzymes were used to degrade DNA into single nucleotides. The obtained nucleotide mixture was separated using HPLC [Model NS-6000A, Futecs, reversed-phase column YMC-Triart C18 (4.6 × 250 mm × 5 µm), using a mixture of 0.02 M $(NH_4)H_2PO_4$ and acetonitrile (20:1, v/v) for the mobile phase, a flow rate of 1.2 ml/min and wavelength at 270 nm (Mesbah et al. 1989). The genomic DNA of *Escherichia coli* strain B (Sigma-Aldrich D4889) was used as a standard.

Chemotaxonomic characterization

For quinone analysis, cells were grown in TSB at 30 °C, shaken at 160 rpm for 1 day, centrifuged and freeze dried. Respiratory quinones were extracted from 100 mg freeze-dried cells with chloroform/methanol (2/1, v/v), separated by using hexane and eluted with hexane/diethyl ether (98/2, v/v), then eluent was evaporated by a rotatory evaporator and the residue dissolved in acetone, according to Collins (1985). Menaquinones were analysed by reverse-phase HPLC (Model, NS-6000A Futecs, wavelength 270 nm, solvent MeOH: Isopropanol = 7:3). Polar lipids of strain DCY84^T and *P. barengoltzii* KACC 15270^T were analyzed as described by Minnikin et al. (1984). Separately, each sample was spotted on the corner of two-dimensional thin layer chromatography (2D-TLC) using TLC Kiesel gel 60 F_{254} (Merck) plates (10 × 10 cm) and developed in the first direction by using of chloroform/methanol/water (65/25/4, by vol), then in the second direction developed by chloroform/acetic acid/methanol/water (80/15/12/4, by vol). TLC plates were sprayed with 5 % molybdatophosphoric acid to detect total lipids, 0.2 % ninhydrin reagent to detect aminolipids and 15 % α-naphthol reagent to detect glycolipids. After spraying, plates were heated at 120 °C for 10 min. TLC plates were also sprayed with Molybdenum blue reagent for detection of phospholipids. For fatty acid analysis, the cells were grown on TSA at 30 °C for 24 h. Fatty acids were extracted, methylated and saponified by the method described by the Sherlock Microbial Identification system (MIDI) and analyzed by capillary GLC (Hewlet Packard 6890) using the TSBA library version 6.1 (Sasser 1990). Fatty acid analysis was performed in duplicate.

The peptidoglycan of strain DCY84^T and *P. barengoltzii* KACC 15270^T was analyzed as described by Schleifer (1985). The hydrolysed peptidoglycans were analysed by spotting the samples on cellulose Merck KGaA (20 × 20 cm) TLC plates. The modified TLC solvent Methanol/Pyridine/HCl (12 N)/water (32/4/1/7, by vol) was prepared 1 day before running the analysis (Schumann 2011). The polyamines of strain DCY84^T and *P. barengoltzii* KACC 15270^T were extracted as described elsewhere (Taibi et al. 2000). Samples were analyzed by using HPLC using a reversed-phase Eclipse C18 column (30 × 50 mm^2 × 2.7 mm) with 60 % methanol and wavelength 234 nm (Scherer and Kneifel 1983). To analyze the whole cell sugars of strain DCY84^T and *P. barengoltzii* KACC 15270^T, freeze dried cells were collected and hydrolysed in 1 N H_2SO_4 for 2 h at 100 °C. After cooling, the pH of the samples pH was adjusted by adding saturated $Ba(OH)_2$ and methyl red solution as pH indicator. After centrifugation, the liquid phase was taken and dried in a freeze drier. The released sugars were detected by spotting 10 µl samples and 5 µl of a standard mixture of sugars as reference on TLC plates (TLC cellulose Merck KGaA, 20 × 20 cm) developed by using the solvent 1-butanol/pyridine/water (5/3/2, by vol) (Staneck and Roberts 1974).

In vitro assessment of plant growth promoting traits

The DCY84^T isolate was also evaluated for the presence of desirable plant growth promoting characteristics. To assess indole-3-acetic acid (IAA) production, the method described by Glickmann and Dessaux (1995) was used. Some modifications for in vitro IAA production were performed by using modified King B broth (Casein 10 g/l, Peptone no.3 10 g/l, dipotassium phosphate 1.5 g/l, magnesium sulfate 1.5 g/l) media with and without additional L-tryptophan (3 mg/ml), as described by Shokri and Emtiazi (2010). The production of IAA was measured after 6 days of incubation. Qualitative test of phosphate solubilizing ability of strain DCY84^T was checked by plate

screening methods and the media were prepared manually as described by Pikovskaya (1948). Strain DCY84^T was also analyzed for its siderophore production capacity in petri dishes containing Pseudomonas Agar F (Difco) medium (Glickmann and Dessaux 1995) supplemented with a chrome azurol S complex [CAS/iron(III)/hexadeciltrimethyl ammonium bromide], as described by Schwyn and Neilands (1987).

Results and discussion

The comparison of the 16S rRNA sequence of strain DCY84^T (GenBank/EMBL/DDBJ accession number KF915796) with other *Paenibacillus* strains revealed that strain DCY84^T is a novel strain of the genus *Paenibacillus*, sharing highest sequence similarity with *P. barengoltzii* KACC 15270^T (96.86 %), followed by *P. timonensis* KACC 11491^T (96.49 %) and *P. phoenicis* NBRC 106274^T (95.77 %). The similarities with other type strains of the genus *Paenibacillus* were 91.87–96.49 %. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain DCY84^T in the genus *Paenibacillus* was constructed (Fig. 1). The topologies of the phylogenetic trees built using maximum-parsimony method also supported the conclusion that strain DCY84^T forms a stable clade with the type strain *P. barengoltzii* KACC 15270^T (Fig. 1). The phylogenetic trees built using maximum-likelihood method are also available as Supplementary Fig. S1. On the basis of sequence analysis, it is evident that the novel strain belongs to the genus *Paenibacillus*.

The DNA G+C content of strain DCY84^T was determined to be 62.6 mol%, indicating it can be considered to belong to the high-G+C lineage (56–63 %) within the genus *Paenibacillus*, together with *Paenibacillus soli* DCY03^T (56.8 ± 0.2 mol%, Park et al. 2007), *Paenibacillus humicus* PC-147^T (58.3 ± 0.3 mol%, Vaz-Moreira et al. 2007) and *Paenibacillus pasadenensis* NBRC 101214^T (63.4 ± 0.6 mol%, Vaz-Moreira et al. 2007).

Morphological observation of strain DCY84^T colonies on TSA agar revealed white, round, smooth and raised colonies with approximate diameters 2–5 mm after incubation at 30 °C for 48 h. Strain DCY84^T was found to grow on LB, TSA, R2A and NA, but not to grow on PDA and MacConkey agar. The strain was observed to grow best in TSA at temperatures of 15–40 °C; at pH 5–9 and at 0.5–4.5 % NaCl (optimum, 0.5 %). Optimum growth occurs at 25–30 °C and pH 7–8. Phenotypic analysis showed that strain DCY84^T cells are Gram-positive, motile, aerobic and rod shaped with size range approximately 0.7–0.9 × 3.4–4.7 μm. Peritrichous flagella were observed by using TEM (Supplementary Fig. S2). Strain DCY84^T was also found to produce ellipsoidal endospores. Endospore formation is a general characteristic of members of the family *Bacillaceae* (Logan et al. 2009). Tests for oxidase activity and H_2S production were negative, whilst catalase activity and indole test was found to be positive. Strain DCY84^T is not able to hydrolyse casein and DNA but is able to hydrolyse starch, Tween 20, Tween 80, gelatin and aesculin. Nitrate was not found to be reduced to nitrite. The biochemical and physiological characteristics of strain DCY84^T and nearest *Paenibacillus* strains are shown on Table 1.

Strain DCY84^T is susceptible to all antibiotics tested in this study, notably to ceftazidime (CAZ_{30}, 30 μg) and erythromycin (E_{15}, 15 μg). In addition, strain DCY84^T was found to susceptible to carbenicillin ($CAR_{100,}$ 100 μg), novobiocin (NV_{30}, 30 μg), neomycin (N_{30}, 30 μg), tetracycline (TE_{30}, 30 μg), cefazolin (KZ_{30}, 30 μg), oleandomycin (OL_{15}, 15 μg), lincomycin (L_{15}, 15 μg) and rifampicin (RD_5, 5 μg). Furthermore, strain DCY84^T was also found to be resistant to bacitracin and optochin, but susceptible to novobiocin, O/129 (2,4-diamino-6,7-diisopropylpteridine) and polymyxin B (according GP VITEK-2).

Both strain DCY84^T and *P. barengoltzii* KACC 15270^T were shown to have MK-7 as the only isoprenoid quinone detected. As shown in Fig. 2, the major polar lipids were identified as phosphatidylethanolamine (PE), an unidentified aminophospholipid (APL), unidentified aminolipids (AL1, AL2) and an unidentified polar lipid (L1). This profile is similar to the polar lipids of *P. barengoltzii* KACC 15270^T which has two additional unidentified polar lipids among its major polar lipids. The cell wall peptidoglycan of strain DCY84^T was found to contain meso-diaminopimelic acids, alanine and D-glutamic acid, which is similar with *P. barengoltzii* KACC 15270^T. The major fatty acids of strain DCY84^T were identified as branched chain anteiso-$C_{15:0}$ (32.1 %), saturated $C_{16:0}$ (20.1 %) and branched chain anteiso-$C_{17:0}$ (18.3 %),as has also been seen in *P. barengoltzii*

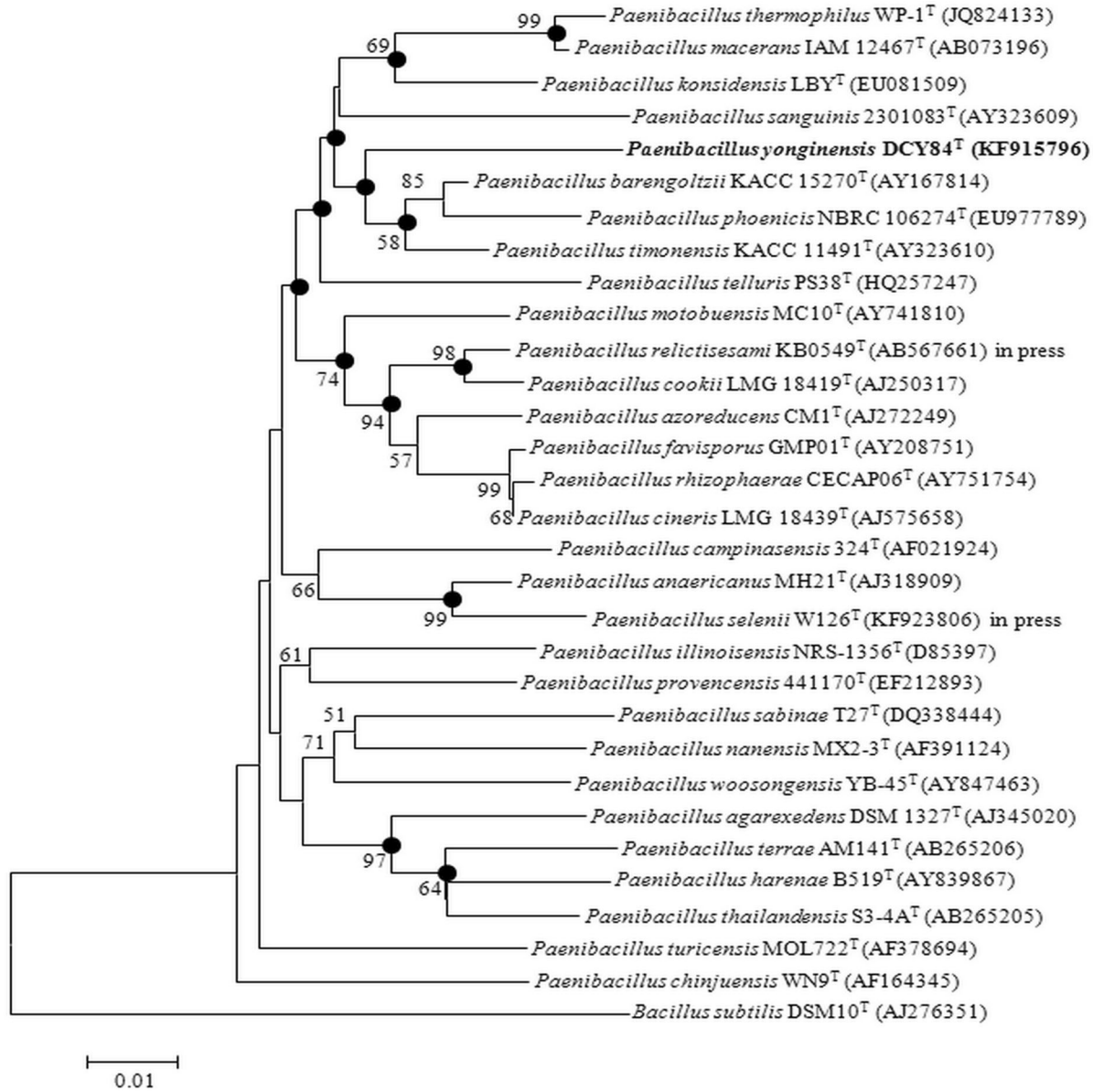

Fig. 1 16S rRNA gene sequence analysis showing phylogenetic relationships of strain DCY84^T and members of the genus *Paenibacillus* (neighbour-joining tree). *Filled circles* indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bootstrap values more than 50 % based on 1,000 replications are shown at branching points. *Bacillus subtilis* DSM10^T was used as an out group. *Scale bar*, 0.005 substitutions per nucleotide position

KACC 15270^T, *P. timonensis* KACC 11491^T and *P. phoenicis* NBRC 106274^T. The proportion of anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and $C_{16:0}$ was significantly different from that in the closely related species. Anteiso-$C_{17:0}$ from strain DCY84^T was the highest (18.3 %), while iso-$C_{16:0}$ from strain DCY84^T was the lowest (9.0 %), compared with other reference strains. The cellular fatty acid profile of strain DCY84^T and the related reference strains are shown on Table 2. The polyamine pattern of strain DCY84^T was found to be the same as that of *P. barengoltzii* KACC 15270^T. Both of these strains have spermidine as predominant polyamine. Cell wall sugars of the strain DCY84^T were identified as ribose, galactose and xylose, while

Table 1 The different biochemical, physiological and in vitro plant growth promoting characteristics of strain DCY84^T and the reference strains of the genus *Paenibacillus*

Characteristics	1	2	3	4
Bacitracin resistance	+	−	−	+
Hydrolysis of				
Cyclodextrin	+	−	+	−
Salicin	+	−	+	+
Inulin	−	+	+	−
Gelatin (bovine origin)	+	−	−	−
Glycogen	−	+	+	+
Potassium gluconate	−	+	+	+
Dulcitol	−	−	+	−
Xylitol	−	−	+	−
Enzyme activity				
Naphthol-AS-BI-phosphohydrolase	w	w	+	w
Methyl β-D-xylopyranoside	w	w	+	+
Methyl-β-D-glucopyranoside	+	−	+	+
Methyl α-D-mannopyranoside	−	w	w	+
α-fucosidase	w	−	−	−
β-galactopyranosidase	+	+	−	−
α-chymotrypsin	+	−	w	−
Lipase	−	+	+	w
Leucine arylamidase	+	+	+	−
Alanine arylamidase	+	−	−	−
Tyrosine arylamidase	+	+	+	−
Aminoexotripeptidase	−	+	−	−
L-Aspartate arylamidase	+	+	−	+
L-Pyrrolidonyl arylamidase	+	−	−	−
N-acetyl-D-glucosaminidase	+	−	+	w
Assimilation of				
D-Xylose	+	−	+	+
D-Ribose	+	−	+	−
D-Melezitose	−	−	w	−
D-Turanose	+	−	w	−
D-Lyxose	−	−	+	−
D-Arabitol	−	−	+	−
D-Mannitol	+	+	+	−
L-Fucose	−	−	w	w
L-Sorbose	−	−	+	−
D-Lactose	+	−	+	+
Saccharose/sucrose	+	+	+	−
N-acetyl-D-glucosamine	+	−	+	+
In vitro plant growth promoting traits				
IAA production w/o L-tryptophan (μg/ml)	52.96 ± 1.85	50.22 ± 0.45	12.27 ± 0.7	29.54 ± 1.05
IAA production with L-tryptophan (μg/ml)	72.83 ± 2.26	70.82 ± 1.91	32.87 ± 4.57	56.96 ± 3.55
Siderophore production	+	+	w	+

Table 1 continued

Characteristics	1	2	3	4
Phosphate solubilization	+	+	−	−

Strain 1, *Paenibacillus yonginensis* DCY84^T; 2, *Paenibacillus barengoltzii* KACC 15270^T; 3, *Paenibacillus phoenicis* NBRC 106274^T; 4, *Paenibacillus timonensis* KACC 11491^T

All strains are positive for arbutin, aesculin, gentiobiose, starch, optochin resistance, acetoin production (Voges Proskauer), indole production, citrate utilization, D-amygdalin, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-maltose, D-melibiose, D-raffinose, D-trehalose, L-arabinose, methyl α-D-glucopyranoside, *N*-acetylglucosamine, tryptophandeaminase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, esterase and esterase lipase. All strains are negative for glycerol, erythritol, inositol, pullulan, potassium 2-ketogluconate, potassium 5-ketogluconate, L-lactate alkalinization, novobiocin resistance, polymyxin B resistance, O/129 (2,4-diamino-6,7-diisopropylpteridine) resistance, growth in 6.5 % NaCl, H2S production, D-sorbitol, D-adonitol, D-arabinose, D-tagatose, D-fucose, L-xylose, L-rhamnose, L-arabitol, α-mannosidase, β-glucuronidase, urease, trypsin, arginine dihydrolase, arginine dihydrolase 2, alkaline phosphatase, valine arylamidase, cystine arylamidase, L-proline arylamidase, lysine decarboxylase, ornithine decarboxylase, acid phosphatase, phosphatase and phosphatidylinositol phospholipase C

+ Positive result, w weakly positive, − negative result

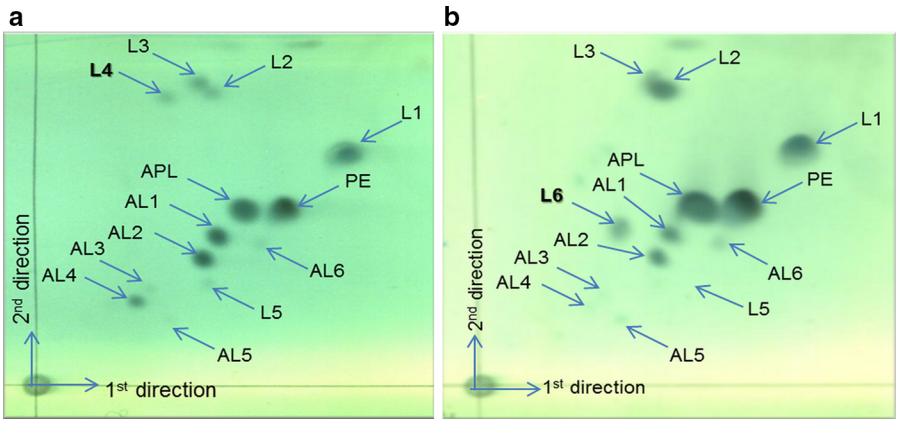

Fig. 2 Two-dimensional TLC of the total polar lipids of *Paenibacillus yonginensis* DCY84^T (**a**) and *Paenibacillus barengoltzii* KACC 15270^T (**b**), stained for total polar lipids with 5 % ethanolic molybdophosphoric acid. *APL* aminophospholipid, *PE* phosphatidylethanolamine, *AL1-6* unidentified aminolipids, *L1-5* unidentified polar lipids

P. barengoltzii KACC 15270^T was found to contain ribose, rhamnose and glucose.

Regarding the plant growth promoting characteristics, strain DCY84^T was found to produce 52.96 ± 1.85 μg/ml (without L-tryptophan) and 72.83 ± 2.86 μg/ml (with L-tryptophan) IAA, respectively. Clear halo regions observed around the colonies on opaque Pikovskaya medium indicated positive results for the phosphate solubilization test (Supplementary Fig. S3). Moreover, strain DCY84^T was able to produce siderophores as was confirmed by the development of yellow halo zones surrounding the colonies on blue-green coloured medium (Supplementary Fig. S3). A comparison of the in vitro plant growth promoting characteristics of strain DCY84^T and the reference strains of genus *Paenibacillus* is shown on Table 1.

On the basis of the phylogenetic, phenotypic and chemotaxonomic data, strain DCY84^T (=KCTC 33428^T = JCM 19885^T) is considered to represent a novel species of the genus *Paenibacillus*, for which name *Paenibacillus yonginensis* sp. nov. is proposed.

Description of *Paenibacillus yonginensis* sp. nov

Paenibacillus yonginensis (yon.gi.nen'sis. N.L. masc. adj. *yonginensis* of or belonging to Yongin, Korea, from where the type strain was isolated)

Table 2 Chemotaxonomic profiles of strain DCY84^T and related species of the genus *Paenibacillus*

	1	2	3	4
Fatty acid				
Saturated				
$C_{12:0}$	0.6	2.8	2.1	2.4
$C_{14:0}$	6.9	12.0	6.3	10.8
$C_{16:0}$	20.2	31.6	21.4	23.3
$C_{18:0}$	0.9	tr	tr	tr
Branched-chain				
iso-$C_{14:0}$	0.7	0.9	1.1	2.2
iso-$C_{15:0}$	4.9	2.6	5.1	3.5
iso-$C_{16:0}$	9.0	13.1	10.6	18.6
iso-$C_{17:0}$	3.6	2.7	3.7	3.5
anteiso-$C_{13:0}$	tr	tr	0.7	tr
anteiso-$C_{15:0}$	32.1	22.2	33.4	24.7
anteiso-$C_{17:0}$	18.3	9.8	13.0	8.9
$C_{18:1}$ ω7c	0.5	–	tr	–
Summed feature 5*	0.9	tr	tr	–
Cell wall sugar	Ribose galactose xylose	Ribose rhamnose glucose	ND	ND
Major Polyamine	Spermidine	Spermidine	ND	ND

Strain 1, *Paenibacillus yonginensis* DCY84^T; 2, *Paenibacillus barengoltzii* KACC 15270^T; 3, *Paenibacillus phoenicis* NBRC 106274^T; 4, *Paenibacillus timonensis* KACC 11491^T

For fatty acid analysis, all type strains were collected after 24 h of growth on TSA medium (Difco) at 30 °C. Summed feature 5 could not be separated by the Microbial Identification System (MIDI). Fatty acids amounting to <0.5 % in all strains were not listed

Summed feature 5* consisted of anteiso-$C_{18:0}$ and/or $C_{18:2}$ ω6,9c and/or $C_{18:1}$ ω9c

– not detected, *tr* trace amount (<0.5 %), *ND* no data

Gram-stain positive, rod-shaped, aerobic and motile by peritrichous flagella. Vegetative cells are approximately 0.7–0.9 μm in width and 3.4–4.7 μm in length. Colonies formed after 48 h of growth on TSA at 30 °C are white, round, smooth, raised, have a diameter of 2–5 mm, show no pigmentation and possess entire edges. Grows on LB, TSA, R2A and NA, but does not grow on PDA and MacConkey agar. Cells can grow in TSA over the temperature range of 15–40 °C, at pH range 5–9 and at 0.5–4.5 % NaCl. Optimal growth is observed in TSA at 30 °C, pH 8 and 0.5 % NaCl. Does not hydrolyse casein and DNA agar but is able to hydrolise starch, Tween 20, Tween 80, gelatin and aesculin. Tests for nitrate reduction, oxidase and H_2S production are negative, while catalase, indole and acetoin production are positive. Assimilation of the following sole carbon sources is positive: D-xylose, D-ribose, D-turanose, D-mannitol, D-amygdalin, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-maltose, D-melibiose, D-raffinose, D-trehalose, D-lactose, L-arabinose, saccharose and *N*-acetyl-D-glucosamine. Assimilation of the following sole carbon sources is negative: D-melezitose, D-lyxose, D-arabitol, D-fucose, D-sorbitol, D-adonitol, D-arabinose, D-tagatose, L-fucose, L-arabitol, L-xylose, L-sorbose and L-rhamnose. Methyl α-D-glucopyranoside, Methyl β-D-glucopyranoside, *N*-acetylglucosamine, tryptophandeaminase, α-glucosidase, α-galactosidase, α-chymotrypsin, β-glucosidase, β-galactosidase, β-galactopyranosidase, leucine arylamidase, alanine arylamidase, tyrosine arylamidase, L-aspartate arylamidase, L-pyrrolidonyl arylamidase, *N*-acetyl-D-glucosaminidase, esterase and esterase lipase are produced but methyl α-D-mannopyranoside, lipase, aminoexotripeptidase, α-mannosidase, β-glucuronidase, urease, trypsin, arginine dihydrolase, arginine dihydrolase 2, alkaline phosphatase, valine arylamidase, cystine arylamidase, L-proline arylamidase, lysine decarboxylase, ornithine decarboxylase, acid phosphatase, phosphatase and phosphatidylinositol phospholipase C are absent. Naphthol-AS-BI-phosphohydrolase, methyl β-D-xylopyranoside and α-fucosidase are weakly produced. Able to utilize arbutin, gentiobiose, salicin, cyclodextrin and citrate but not able to utilize inulin, glycogen, potassium gluconate, dulcitol, xylitol, glycerol, erythritol, inositol, pullulan, potassium 2-ketogluconate, potassium 5-ketogluconate and L-lactate. MK-7 menaquinone is the isoprenoid quinone. The major polar lipids are phosphatidylethanolamine, an unidentified aminophospholipid, two unidentified aminolipids and an unidentified polar lipid. The cell wall contains meso-diaminopimelic acid, alanine and D-glutamic acid. The major fatty acids are branched chain anteiso-$C_{15:0}$, saturated $C_{16:0}$ and branched chain anteiso-$C_{17:0}$. Spermidine is found as the predominant polyamine. Cell wall sugars contain ribose, galactose and xylose.

The DNA G+C content of the type strain, DCY84^T, is 62.6 mol%.

The type strain is DCY84^T (= KCTC 33428^T = JCM 19885^T) was isolated from humus soil of a Yongin forest of Gyeonggi province in South Korea. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DCY84^T is KF915796.

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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