

# *Paraburkholderia dokdonella* sp. nov., isolated from a plant from the genus *Campanula*<sup>§</sup>

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The novel Gram-stain-negative, rod-shaped, aerobic bacterial strain DCR-13<sup>T</sup> was isolated from a native plant belonging to the genus *Campanula* on Dokdo, an island in the Republic of Korea. Comparative analysis of the 16S rRNA gene sequence indicated that this strain is closely related to *Paraburkholderia peleae* PP52-1<sup>T</sup> (98.43% 16S rRNA gene sequence similarity), *Paraburkholderia oxyphila* NBRC 105797<sup>T</sup> (98.42%), *Paraburkholderia sacchari* IPT 101<sup>T</sup> (98.28%), *Paraburkholderia mimosarum* NBRC 106338<sup>T</sup> (97.80%), *Paraburkholderia denitrificans* KIS30-44<sup>T</sup> (97.46%), and *Paraburkholderia paradise* WA<sup>T</sup> (97.45%). This analysis of the 16S rRNA gene sequence also suggested that DCR-13<sup>T</sup> and the six closely related strains formed a clade within the genus *Paraburkholderia*, but that DCR-13<sup>T</sup> was clearly separated from the established species. DCR-13<sup>T</sup> had ubiquinone 8 as its predominant respiratory quinone, and its genomic DNA G + C content was 63.9 mol%. The isolated strain grew at a pH of 6.0–8.0 (with an optimal pH of 6.5), 0–4% w/v NaCl (with an optimal level of 0%), and a temperature of 18–42°C (with an optimal temperature of 30°C). The predominant fatty acids were C<sub>16:0</sub>; summed feature 8 (C<sub>18:1</sub> ω7c/C<sub>18:1</sub> ω6c), C<sub>17:0</sub> cyclo, C<sub>19:0</sub> cyclo ω8c, summed feature 3 (C<sub>16:1</sub> ω6c/C<sub>16:1</sub> ω7c) and summed feature 2 (C<sub>12:0</sub> aldehyde), and the major polar lipids were phosphatidylglycerol and phosphatidylethanolamine. On the basis of polyphasic evidence, it is proposed that strain DCR-13<sup>T</sup> (= KCTC 62811<sup>T</sup> = LMG 30889<sup>T</sup>) represents the type strain of a novel species, *Paraburkholderia dokdonella* sp. nov.

**Keywords:** *Paraburkholderia dokdonella* sp. nov., Dokdo, *Campanula*, novel species

## Introduction

The genus *Burkholderia* was first proposed by Yabuuchi *et al.* (1992) following the transfer of seven species of the genus *Pseudomonas* homology group II. Phylogenetically, the genus *Burkholderia* belongs to the family *Burkholderiaceae* within the *Betaproteobacteria*. Originally, it was considered a diverse group comprising more than 120 species; however, it was subsequently proven that *Burkholderia* was not monophyletic based on 16S rRNA gene sequences and conserved sequence indels (CSIs). As a result, the original genus was split into *Burkholderia* and the genus *Paraburkholderia*, which was first proposed by Sawana *et al.* (2014) with *Paraburkholderia graminis* as the type species. At the time of writing, *Paraburkholderia* includes 74 species with valid published names ([www.bacterio.net/paraburkholderia.html](http://www.bacterio.net/paraburkholderia.html)) isolated from various environments (e.g., soil, plants, and water), including the recently described *Paraburkholderia caseinilytica* (Gao *et al.*, 2018), *Paraburkholderia azotifigens*, which has been confirmed to have N<sub>2</sub>-fixing ability (Choi and Im, 2018), and *Paraburkholderia aromaticivorans*, which is an aromatic hydrocarbon-degrading strain (Lee and Jeon, 2018). *Paraburkholderia* species are Gram-negative, straight, slightly curved, or occasionally coccoid rods with one or more polar flagella. Their cell size varies (width of 0.4–1.2 μm, length of 1.2–3.0 μm), and they have a DNA G + C content in the range of 58.9–65.0 mol% (Dobritsa and Samadpour, 2016). In this study, we isolated and taxonomically characterized a putative novel strain belonging to the genus *Paraburkholderia*, referred to as strain DCR-13<sup>T</sup>. It was isolated from soil particles attached to the root surface of a native plant belonging to the genus *Campanula* on a Korean island.

## Materials and Methods

### Isolation and culturing conditions

The novel bacterial strain DCR-13<sup>T</sup> was isolated from *Campanula takesimana* plants sampled in April 2017 from Dokdo, an island in South Korea (37°14'N 131°51'E). The root portions of *C. takesimana* were excised using a sterilized knife and put into plastic bags without other soil debris. The residual soil particles attached to the root surface of the plant were then collected, suspended in phosphate-buffered saline (PBS, pH 7.5), and end-point diluted. Following this, a 100-μl aliquot of each dilution was spread onto Reasoner's 2A (R2A, Difco) agar plates, which were incubated at 30°C for 7 days. To obtain a pure colony, a single colony was repeatedly transferred onto fresh R2A agar plates and incubated again under the same conditions. Finally, a creamy

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colony was isolated and purified. The isolated DCR-13<sup>T</sup> was cultured at 30°C under aerobic conditions on R2A agar and preserved at -80°C in R2A broth supplemented with 15% (w/v) glycerol. The isolated colony was deposited in the Korean Collection for Type Cultures (KCTC 62811<sup>T</sup>) and the Belgian Co-Ordinated Collection of Microorganisms (BCCM; LMG 30889<sup>T</sup>). For comparison purposes, six reference type strains (*Paraburkholderia peleae* PP52-1<sup>T</sup>, *Paraburkholderia oxyphila* NBRC 105797<sup>T</sup>, *Paraburkholderia denitrificans* KIS30-44<sup>T</sup>, *Paraburkholderia sacchari* IPT101<sup>T</sup>, *Paraburkholderia paradisi* WA<sup>T</sup>, and *Paraburkholderia mimosarum* NBRC 106338<sup>T</sup>) were selected and obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and BCCM (LMG). Unless otherwise noted, the morphological, physiological, and biochemical characteristics of the novel isolate and reference strains were investigated with routine cultivation on R2A agar at 30°C for three days.

### Phylogenetic analysis

To determine the phylogenetic position of strain DCR-13<sup>T</sup>, genomic DNA (gDNA) extraction was conducted using a commercial genomic DNA extraction kit (GeneAll Biotechnology Co. Ltd.). The universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3'; *E. coli* position 8-27) and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'; *E. coli* position 1492-1510) (Weisburg *et al.*, 1991) were used for 16S rRNA gene amplification and the purified PCR product was sequenced by Macrogen Co. Ltd. using the ABI BigDye<sup>®</sup> Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturer protocols. Single-sequencing was conducted on each template using 27F, 518F, 900R, and 1492R primers to cover the entire region of the 16S rRNA gene. The fluorescent-labeled fragments were purified using the method recommended by Applied Biosystems because it removes the unincorporated terminators and dNTPs, and the samples then underwent electrophoresis in an ABI 3730 × 1 DNA Analyzer (Applied Biosystems).

An almost fully complete DCR-13<sup>T</sup> 16S rRNA gene sequence (about 1.5 kb) was obtained by assembling the sequences with SeqMan software (DNASTAR) and comparing with 16S rRNA gene sequence extracted from whole genome sequence of DCR-13<sup>T</sup> (see below). This sequence was then compared with the 16S rRNA gene sequences of related taxa obtained from the GenBank database and the EzBioCloud server (<https://www.ezbiocloud.net>; Yoon *et al.*, 2017). The 16S rRNA gene sequences of representative members of *Paraburkholderia* were aligned with the DCR-13<sup>T</sup> sequence using SILVA (<http://www.arb-silva.de/aligner>) considering the secondary structure of the rRNA gene (Pruesse *et al.*, 2007). Gaps between sequences were edited using the BioEdit program, and phylogenetic trees were generated using the neighbor-joining (Saitou and Nei, 1987), maximum-likelihood (Felsenstein, 1981), and minimum evolution (Nei *et al.*, 1998) algorithms implemented in the MEGA7 program (Kumar *et al.*, 2016). Kimura's two-parameter model (Kimura, 1980) was employed to calculate phylogenetic distances, and bootstrap analysis was conducted based on 1,000 resampled datasets.

### Morphological, physiological, and biochemical characterization

Cell morphology and size were observed using a JEOL JEM-1010 transmission electron microscope (80 kV) after negative staining with 1% w/v phosphotungstic acid. A Gram stain kit (Difco) was used according to the manufacturer's instructions. Growth at different NaCl concentrations (0% to 10% at intervals of 1%, w/v) was measured as previously described (Koh *et al.*, 2017) after four weeks at 30°C. Growth at different temperatures (5°C to 45°C at intervals of 5°C) and pH levels (pH 4.0 to 10.0 at intervals of 0.5 pH units) was tested in R2A broth. Four different buffers were used in the pH response analysis (final concentration, 10 mM): homopiperazine-1,4-bis (2-ethanesulfonic acid) (pH 4.0–5.0), 2-(N-morpholino) ethanesulfonic acid (pH 5.0–6.5), 1,3-bis[tris(hydroxymethyl)methylamino] propane (bispropane, pH 7.0–8.5), and 3-(cyclohexylamino)-1-propanesulfonic acid (pH 9.0–10.0), and if necessary, pH values were adjusted with 1 M NaOH and 1 M HCl. Carbon source utilization and extracellular enzyme activity for DCR-13<sup>T</sup> and the reference strains were tested with API 20NE, 32GN, and ZYM systems (bioMérieux) according to the manufacturer's instructions, with the tests carried out in duplicate and repeated twice to ensure reproducibility. An anaerobic growth test was conducted with the GasPak<sup>™</sup> EZ anaerobe pouch system (BD) over two weeks. Catalase activity was determined by bubble production in 3% (v/v) H<sub>2</sub>O<sub>2</sub> and oxidase activity was determined using 1% (w/v) tetramethyl-*p*-phenylenediamine (Merck). For the hydrolysis of DNA, cellulose, starch, skim milk, and Tween 60 were conducted with R2A medium as the basal medium as previously described (Koh *et al.*, 2015).

### Chemotaxonomic characterization

The cellular fatty acids of DCR-13<sup>T</sup>, *P. peleae* PP52-1<sup>T</sup>, *P. oxyphila* NBRC 105797<sup>T</sup>, *P. denitrificans* KIS30-44<sup>T</sup>, *P. sacchari* IPT101<sup>T</sup>, *P. paradisi* WA<sup>T</sup>, and *P. mimosarum* NBRC 106338<sup>T</sup> were analyzed using cultures grown on R2A agar for three days at 30°C. Mixtures of fatty acids and methyl ester were prepared according to the protocol published for the Sherlock Microbial Identification System (MIDI, version 6.1), and profiles were determined according to the MIDI/Hewlett Packard Microbial Identification System using gas chromatography (6890N and 7683 autosampler; Agilent Technologies) based on the manufacturer's instructions. Polar lipids were extracted from freeze-dried cells (100 mg) derived from the isolated strains and analyzed as previously described (Koh *et al.*, 2017). The quinones were extracted with a chloroform/methanol mixture (2:1 v/v; Hu *et al.*, 1999), evaporated in a vacuum, and re-extracted three times with n-hexane/water (1:1, v/v). They were then concentrated and moved to a Sep-Pak Plus silica column (Waters). Finally, the quinone profile were separated and identified using reversed-phase HPLC equipped with a photodiode array detector and internal and external quinone standards.

### Genomic analysis

The whole-genome sequence of DCR-13<sup>T</sup> was determined using the PacBio RS II sequencing platform and assembled

using the hierarchical genome-assembly process (HGAP4) *de novo* assembler, representing a coverage of ca. 220-fold. Genome annotation was conducted using the NCBI prokaryotic genome annotation pipeline (PGAP). Average nucleotide identity (ANI) was calculated using <http://enve-omics.ce.gatech.edu> (Rodriguez-R and Konstantinidis, 2014).

### DNA-DNA hybridization

DNA-DNA hybridization experiments were carried out with DCR-13<sup>T</sup>, *P. peleae* PP52-1<sup>T</sup>, *P. oxyphila* NBRC 105797<sup>T</sup>, *P. denitrificans* KIS30-44<sup>T</sup>, *P. sacchari* IPT101<sup>T</sup>, *P. paradisi* WA<sup>T</sup>, and *P. mimosarum* NBRC 106338<sup>T</sup> using a previously described method (Ezaki *et al.*, 1989). The genomic DNA of DCR-13<sup>T</sup> and the six reference strains was extracted using a genomic DNA extraction kit (GeneAll, Biotechnology Co. Ltd.) and a probe. Salmon sperm DNA was used as negative control. The probe DNA was biotinylated with photobiotin and hybridized with single-stranded, unlabeled genomic DNA fragments from the reference or test microorganisms. The mean of three independent measurements of DNA-DNA hybridization levels was calculated.

### Nucleotide and whole-genome sequence accession numbers

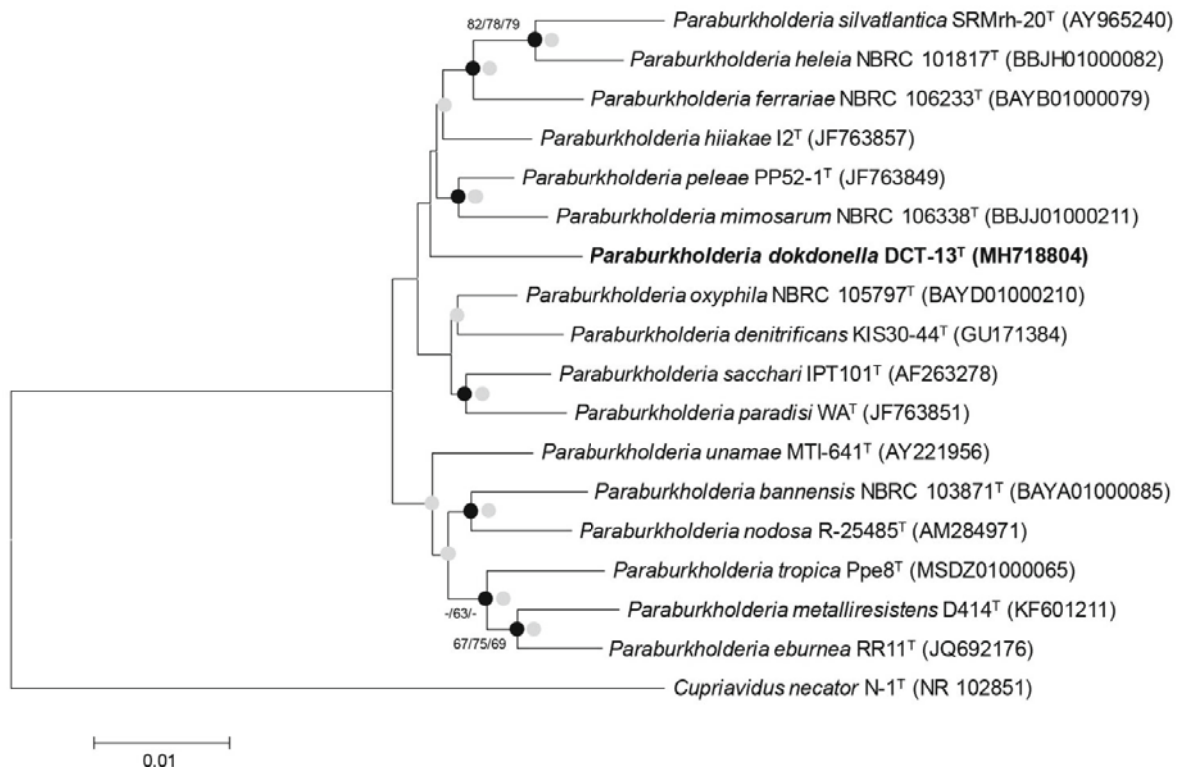
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene is MH718804 and those for the whole-genome sequences of DCR-13<sup>T</sup> are CP029640 and CP029641.

## Results and Discussion

### Phylogenetic and genomic analysis

A nearly complete 16S rRNA gene sequence for strain DCR-13<sup>T</sup> (1,533 bp) was subjected to comparative analysis. The highest sequence similarity as determined by the EzBio-Cloud database was to members of the genus *Paraburkholderia*: *P. peleae* PP52-1<sup>T</sup> (98.43%), *P. oxyphila* NBRC 105797<sup>T</sup> (98.42%), *P. sacchari* IPT 101<sup>T</sup> (98.28%), *P. mimosarum* NBRC 106338<sup>T</sup> (97.80%), *P. denitrificans* KIS30-44<sup>T</sup> (97.46%), and *P. paradise* WA<sup>T</sup> (97.45%). Phylogenetic analysis using the neighbor-joining method based on 16S rRNA gene sequences indicated that DCR-13<sup>T</sup> fell within the genus *Paraburkholderia*, a conclusion which is also supported by maximum-likelihood and minimum-evolution trees (Fig. 1).

Based on the genomic analysis, it was determined that DCR-13<sup>T</sup> consists of two circular chromosomes of 3.13 Mb (NZ\_CP029640) and 1.27 Mb (NZ\_CP029641), and the entire genome contains 3,910 coding sequences (CDSs), 12 rRNA genes, and 58 tRNA genes. The DCR-13<sup>T</sup> genome contains totally four identical copies of the 5S, 16S, and 23S rRNA genes. Three copies and one copy for rRNA genes were distributed in large and small size chromosome, respectively. Based on cluster orthologous group (COG) analysis, most of the CDSs were classified as functional unknown (S, 27.3% of all assigned COGs), followed by those identified as having roles in amino acid transport and metabolism (E, 8.5%),



**Fig. 1.** Neighbor-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationship between strain DCR-13<sup>T</sup> and other representative strains of the genus *Paraburkholderia*. Bootstrap values (based on 1,000 replications) greater than 60% are shown at the branch points. Black and gray filled circles indicate that the corresponding nodes were also recovered in the trees generated based on neighbor-joining method with the maximum-likelihood and minimum-evolution methods, respectively, with *Cupriavidus necator* N-1<sup>T</sup> included as an out-group. Bar: 0.01 substitutions per nucleotide position.

transcription (K, 7.7%) and energy production and conversion (C, 7.6%). The genomic G + C content of DCR-13<sup>T</sup> was 63.9 mol%, which is within the range of the values of other recognized *Paraburkholderia* species (Table 1). The

ANI values were calculated for DCR-13<sup>T</sup> and some of the related type strains within *Paraburkholderia* for which genome information is publicly available. The ANI values for DCR-13<sup>T</sup> and the type strains *P. oxyphila*, *P. sacchari*, *P.*

**Table 1. Phenotypic comparisons of strain DCR-13<sup>T</sup> and the type strains of closely related *Paraburkholderia* species**

Taxa: 1, strain DCR-13<sup>T</sup>; 2, *P. peleeae* PP52-1<sup>T</sup>; 3, *P. oxyphila* NBRC 105797<sup>T</sup>; 4, *P. denitrificans* KIS30-44<sup>T</sup>; 5, *P. sacchari* IPT101<sup>T</sup>; 6, *P. paradisi* WA<sup>T</sup>; 7, *P. mimosarum* NBRC 106338<sup>T</sup>.

All data were obtained in this study, unless otherwise indicated (\*). All strains are positive for the following characteristics: catalase, oxidase, nitrite reduction, assimilation of D-glucose, N-acetyl-glucosamine, D-sorbitol, L-histidine, 3-hydroxy-butyrate, inositol, malonate, L-alanine, enzyme activity of esterase (C4), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase. All strains are negative for the following characteristics: indole production, glucose acidification, arginine dihydrolase, assimilation of urease,  $\beta$ -glucosidase, protease, L-rhamnose, malonate, enzyme activity of trypsin,  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase,  $\alpha$ -glucosidase,  $\alpha$ -mannosidase,  $\alpha$ -fucosidase.

+, positive reaction; -, negative reaction; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phosphatidylinositol; DPG, diphosphatidylglycerol; APL, unidentified aminophospholipid; P, unidentified phosphotipid; AL, unidentified aminolipid \*ND, no data

Characteristics	1	2	3	4	5	6	7
Isolation source	Plant root	Volcanic soil <sup>a</sup>	Acidic soil <sup>b</sup>	Porest soil <sup>c</sup>	Agricultural soil <sup>d</sup>	Volcanic soil <sup>a</sup>	Root nodules <sup>e</sup>
Temperature range (°C)	18–42	15–40 <sup>a</sup>	15–40 <sup>a</sup>	15–30 <sup>c</sup>	25–27 <sup>d</sup>	15–45 <sup>a</sup>	28–37 <sup>e</sup>
pH range	6.0–8.0	5.7–8.5 <sup>a</sup>	3.5–8.5 <sup>a</sup>	5.0–9.0 <sup>b</sup>	6.0–8.0	5.7–8.5 <sup>a</sup>	6.0–8.0
NaCl range (% w/v)	0–3	0–3	0–2	0–3 <sup>b</sup>	0–3	0–3	0–4
Assimilation :							
$\beta$ -Galactosidase	-	-	-	-	-	-	+
L-Arabinose	+	-	-	-	+	-	+
D-Mannose	+	+	+	-	+	+	+
D-Mannitol	+	+	+	-	+	+	+
Gluconate	+	-	+	+	+	+	+
Caprate	+	-	+	-	+	+	+
Adipate	+	+	+	-	+	+	-
Malate	+	+	+	+	+	-	+
Citrate	+	-	+	+	+	-	-
Phenyl-acetate	+	+	+	-	+	+	+
Salicin	-	+	-	+	-	-	-
D-Melibiose	-	-	-	+	-	-	-
L-Fucose	+	+	-	+	+	+	-
Propionate	-	-	+	-	-	+	-
Valerate	-	-	+	+	+	+	+
2-Ketogluconate	+	-	+	-	+	+	+
4-Hydroxy-benzoate	+	+	+	-	+	+	-
L-Proline	+	-	+	-	+	+	+
D-Ribose	-	-	-	-	-	-	+
D-Sucrose	-	-	-	+	+	-	-
D-Maltose	-	+	-	+	-	-	-
Itaconate	-	-	+	-	-	-	-
Suberate	+	+	+	-	+	-	-
Acetate	-	-	+	+	+	+	-
DL-Lactate	-	+	+	+	+	+	-
5-Ketogluconate	+	+	-	+	-	-	-
Glycogen	-	-	-	-	+	-	-
3-Hydroxy-benzoate	-	-	+	+	-	+	+
L-Serine	-	-	+	+	+	+	+
Enzyme activity :							
Alkaline phosphatase	+	+	+	-	+	+	+
Esterase lipase (C8)	+	+	-	+	+	+	-
Lipase (C14)	-	-	-	+	-	-	-
Valine arylamidase	-	-	-	-	-	+	+
Cystine arylamidase	-	+	-	-	-	+	-
$\beta$ -Galactosidase	-	-	-	-	+	-	+
$\beta$ -Glucosidase	-	+	-	-	+	-	+
N-Acetyl- $\beta$ -glucosaminidase	-	+	-	-	-	-	-
Polar lipids composition	PE, PG, PL, DPG, P, APL1-2	ND*	ND	PE, PG, DPG, AL1-2 <sup>c</sup>	ND	ND	ND

Data from: <sup>a</sup> Weber and king (2017); <sup>b</sup> Otsuka et al. (2011); <sup>c</sup> Lee et al. (2012); <sup>d</sup> Brämer et al. (2001); <sup>e</sup> Chen et al. (2007)

*mimosarum* were 89.3, 84.9, 84.9, and 82.5%, respectively, which are clearly lower than the threshold (< 95%) generally accepted for species delineation (Richter and Rossello-Mora, 2009).

### Phenotypic and physiological characteristics

The cells of strain DCR-13<sup>T</sup> were Gram-stain-negative, strictly aerobic, catalase-positive, and oxidase-negative, and its colonies were creamy, circular, and raised, with entire margins. The cells had rod morphology (1.9 μm × 0.7 μm) with slight peanut shape and a single polar flagellum (Supplementary data Fig. S1). Cell growth occurred at 18–42°C (with an optimal temperature of 30°C), pH 6.0–8.0 (with an optimal temperature of 6.5), and 0–3% w/v NaCl (with an optimal level of 0%). Growth of DCR-13<sup>T</sup> was assessed at 30°C for three days on several forms of bacteriological agar media, including R2A, Luria-Bertani agar (LB; MP Biomedicals), nutrient agar (NA; BD), and tryptic soy agar (TSA; BD).

Other physiological and biological characteristics of strain DCR-13<sup>T</sup> are summarized in the species description, and a comparison of selected characteristics for DCR-13<sup>T</sup> and the selected type strains is provided in Table 1. DCR-13<sup>T</sup> also exhibited degradation activities on MacConky agar but not for DNA, cellulose, Tween 60, skim milk, or starch. As shown in Table 1, numerous other phenotypic characteristics distinguish strain DCR-13<sup>T</sup> from its closest phylogenetic neighbors *P. peleae* PP52-1<sup>T</sup>, *P. oxyphila* NBRC 105797<sup>T</sup>, *P. denitrificans* KIS30-44<sup>T</sup>, *P. sacchari* IPT101<sup>T</sup>, *P. paradisi* WA<sup>T</sup>, and *P. mimosarum* NBRC 106338<sup>T</sup>.

### Chemotaxonomic analysis

The most common fatty acid in strain DCR-13<sup>T</sup> was C<sub>16:0</sub> (25.12%), followed by summed feature 8 (C<sub>18:1</sub> ω7c/C<sub>18:1</sub> ω6c; 25.06%), C<sub>17:0</sub> cyclo (19.63%), C<sub>19:0</sub> cyclo ω8c (6.12%), summed feature 3 (C<sub>16:1</sub> ω6c/C<sub>16:1</sub> ω7c; 5.84%), and summed feature 2 (C<sub>12:0</sub> aldehyde; 5.67%). C<sub>16:0</sub> was also identified as the major fatty acid in the selected reference strains (17.37–31.54%). However, there were slight differences in the proportion of all fatty acids between this study and other experimental results for the reference type strains (Lee *et al.*, 2012; Weber and King, 2017), which may be have been caused by differences in cultivation, extraction, and/or analytical conditions. We also found that the proportion of other fatty acids slightly varied among the strains (Supplementary data Table S1).

The predominant respiratory quinone of strain DCR-13<sup>T</sup> was ubiquinone 8 (Q-8), in line with all other members of *Paraburkholderia*. The major polar lipids of DCR-13<sup>T</sup> were phosphatidylglycerol and phosphatidylethanolamine. Additionally, diphosphatidylglycerol, phosphatidylglycerol, unidentified phosphotidipid, and two unidentified aminophospholipids were detected using two-dimensional thin-layer chromatography (Supplementary data Fig. S2). These results support the conclusion that DCR-13<sup>T</sup> represents a species distinct from known *Paraburkholderia* species.

### DNA-DNA Hybridization

Because complete genome information is not available for

some of the reference species, and because it has been proposed that a novel species can be defined when DNA-DNA relatedness is below 70% (Wayne *et al.*, 1987), *in silico* DNA-DNA hybridization experiments were conducted between DCR-13<sup>T</sup> and the reference *Paraburkholderia* species. The levels of DNA-DNA relatedness between DCR-13<sup>T</sup> and *P. peleae* PP52-1<sup>T</sup>, *P. oxyphila* NBRC 105797<sup>T</sup>, *P. denitrificans* KIS30-44<sup>T</sup>, *P. sacchari* IPT101<sup>T</sup>, *P. paradisi* WA<sup>T</sup>, *P. mimosarum* NBRC 106338<sup>T</sup> were 29.1 ± 1.9, 28.5 ± 2.0, 24.8 ± 2.0, 21.6 ± 1.8, 21.9 ± 0.9, and 26.3 ± 4.1%, respectively. Thus, DCR-13<sup>T</sup> can be considered a new species within the genus *Paraburkholderia*.

### Taxonomic conclusion

Based on the phylogenetic analysis presented above, it can be concluded that strain DCR-13<sup>T</sup> belongs to the genus *Paraburkholderia*. However, the level of DNA-DNA relatedness was sufficiently low (< 30%) that the new strain could be clearly differentiated from the six closely related *Paraburkholderia* species analyzed in this study. Clear differences in other phenotypic, chemotaxonomic, and genotypic characteristics were also evident. Taken together, DCR-13<sup>T</sup> should be accepted as a novel species within *Paraburkholderia*, with a proposed name of *Paraburkholderia dokdonella* sp. nov.

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

*Paraburkholderia dokdonella* (Dok.do.nel'la. N.L. fem. dim. n. Dokdonella of Dokdo, a Korean island located in the East Sea, from which the organism was isolated).

Its cells are Gram-stain-negative, strictly aerobic, and rod shaped with a single polar flagellum, a length of 1.9 μm and a width of 0.7 μm. Optimum growth occurs at pH 6.0–8.0 (with an optimal level of pH 6.5), 0–4% w/v NaCl (with an optimal level of 0%), and at a temperature of 18–42°C (with an optimal level of 30°C). Its cells can grow on R2A, LB, NA, and TSA, and reduce nitrate to nitrite but not nitrogen. Its cells are positive for catalase but negative for oxidase, indole production, glucose acidification, arginine dihydrolase, urease. D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, gluconate, caprate, adipate, malate, citrate, phenylacetate, L-fucose, D-sorbitol, L-histidine, 2-ketoglucuronate, 3-hydroxybutyrate, 4-hydroxybenzoate, L-proline, inositol, malonate, suberate, L-alanine, 5-ketoglucuronate are utilized as assimilation sources, but β-glucosidase, protease, β-galactosidase, D-maltose, salicin, D-melibiose, propionate, valerate, L-rhamnose, D-ribose, D-sucrose, D-maltose, itaconate, acetate, DL-lactate, glycogen, 3-hydroxybenzoate, L-serine are not. Cells are positive for the enzymatic activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, but negative for 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. The predominant respiratory quinone is Q-8. The major cellular fatty acids are C<sub>16:0</sub>, summed feature 8 (C<sub>18:1</sub> ω7c/C<sub>18:1</sub> ω6c), C<sub>17:0</sub> cyclo, C<sub>19:0</sub> cyclo ω8c, summed feature 3 (C<sub>16:1</sub> ω6c/C<sub>16:1</sub> ω7c), and summed

feature 2 (C<sub>12:0</sub> aldehyde). The polar lipids consist primarily of phosphatidylglycerol, phosphatidylethanolamine, an unidentified phospholipid, and two unidentified aminophospholipids. The G + C content of the type strain is 63.9 mol%, as determined by genome sequencing.

The type strain DCR-13<sup>T</sup> (=KCTC 62811<sup>T</sup> =LMG 30889<sup>T</sup>) was isolated from a native plant that belongs to the genus *Campanula* on Dokdo, an island in the Republic of Korea.

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## Conflict of Interest

The authors declare that they have no conflicts of interest.

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