

## *Dankookia rubra* gen. nov., sp. nov., an alphaproteobacterium isolated from sediment of a shallow stream<sup>§</sup>

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**WS-10<sup>T</sup>**—a Gram-negative, non-motile, and aerobic bacterial strain—was isolated from the sediment of a shallow stream in Korea. The optimum ranges of temperature and pH for growth were 20–40°C (optimum 28°C) and pH 6.0–8.0 (optimum pH 7.0), respectively. The DNA G+C content of strain WS-10<sup>T</sup> was 72.7 mol%. The major fatty acids (>5%) were summed feature 8 (C<sub>18:1</sub> ω7c), summed feature 3 (C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c), C<sub>16:0</sub>, and C<sub>18:1</sub> 2-OH. The major polar lipids consisted of phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, and unidentified aminolipids. Q-10 was the predominant respiratory quinone. The highest similarities in the 16S rRNA gene sequence were shown with *Paracraurococcus ruber* (95.3%), *Belnapia soli* (95.3%), *B. moabensis* (95.1%), and *B. rosea* (95.0%). A phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that strain WS-10<sup>T</sup> formed a distinct line within a clade containing the genera *Paracraurococcus*, *Craurococcus*, and *Belnapia* in the family *Acetobacteraceae*. On the basis of polyphasic evidence, strain WS-10<sup>T</sup> represents a novel species of a new genus in the family *Acetobacteraceae*, for which the name *Dankookia rubra* gen. nov., sp. nov. is proposed. The type strain of the type species is WS-10<sup>T</sup> (= KACC 18533<sup>T</sup> = JCM 30602<sup>T</sup>).

**Keywords:** *Dankookia rubra*, alphaproteobacterium

### Introduction

The family *Acetobacteraceae* was proposed as a member of the class *Alphaproteobacteria* (Gillis, 1980). Currently, a total of 33 genera have been identified in the family according to the database (<http://www.bacterio.net/acetobacteraceae.html>). Common characteristics of most species in the family *Acetobacteraceae* are as follows: Gram-negative, aerobic, and rod-shaped. In the present study, a novel Gram-negative, non-motile, and aerobic bacterial strain, WS-10<sup>T</sup>, was isolated

during an investigation of the microbial diversity of a shallow stream in South Korea. The isolate differs from the closest species of the genus *Belnapia* in that it has Q-10, while *B. rosea*, *B. moabensis*, and *B. soli* contain Q-9 as the major respiratory quinone (Reddy *et al.*, 2006; Jin *et al.*, 2012, 2013). Distinct phenotypic, chemotaxonomic, and phylogenetic differences from previously described taxa suggest that the isolate does not belong to any of the genera. Therefore, strain WS-10<sup>T</sup> was classified into a new genus, *Dankookia* gen. nov., with the name *Dankookia rubra* sp. nov.

### Materials and Methods

#### Isolation of bacterial strain and culture conditions

Samples were collected from the sediment of a shallow stream (36° 49'N 127° 10'E) in Cheonan, South Korea. Sediment samples were inoculated onto R2A agar (Difco) using standard dilution plating techniques and incubated at 28°C for three days. After the incubation, a red-pigmented colony was isolated and designated as WS-10<sup>T</sup>. Isolate WS-10<sup>T</sup> was routinely cultured on R2A agar plates at 28°C, suspended in distilled water containing 20% glycerol (v/v), and preserved at -80°C, as described previously (Kim *et al.*, 2014). Strain WS-10<sup>T</sup> has been deposited in the Korean Agricultural Culture Collection (KACC 18533<sup>T</sup>) and Japan Collection of Microorganisms (JCM 30602<sup>T</sup>).

#### Phenotypic and biochemical characteristics

The Gram staining was conducted using a Gram stain kit (BD Gram stain kit) according to the manufacturer's instructions. Cell morphology was investigated using a light microscope (Zeiss Axioskop 40) at × 1,000 magnification with cells grown on R2A agar at 28°C for three days. Motility was examined by the hanging-drop method (Skerman, 1967) using the light microscopy. Gliding motility was investigated by observing the spread of colony edges on R2A agar (Perry, 1973) and by microscopic observation of the hanging drops of an R2A broth culture (Bernardet *et al.*, 2002) under a CHT microscope (Olympus). For pigment characterization, cells were grown on R2A plates, scraped off the surface, suspended in 50 mM phosphate buffer or methanol and sonicated for 10 min at 4°C. The suspension was centrifuged at 10,000 rpm to remove unbroken cells. Bacteriochlorophyll a (bchl a) was extracted from whole cells of strain WS-10<sup>T</sup> with acetone/methanol (7:2, v/v) as described previously (Yurkov *et al.*, 1994). Absorption spectra were measured by a spectrophotometer (Mecasys Optizen 2120UV) from 400 to 900 nm (Alarico *et al.*, 2002). Oxidase activity was examined using

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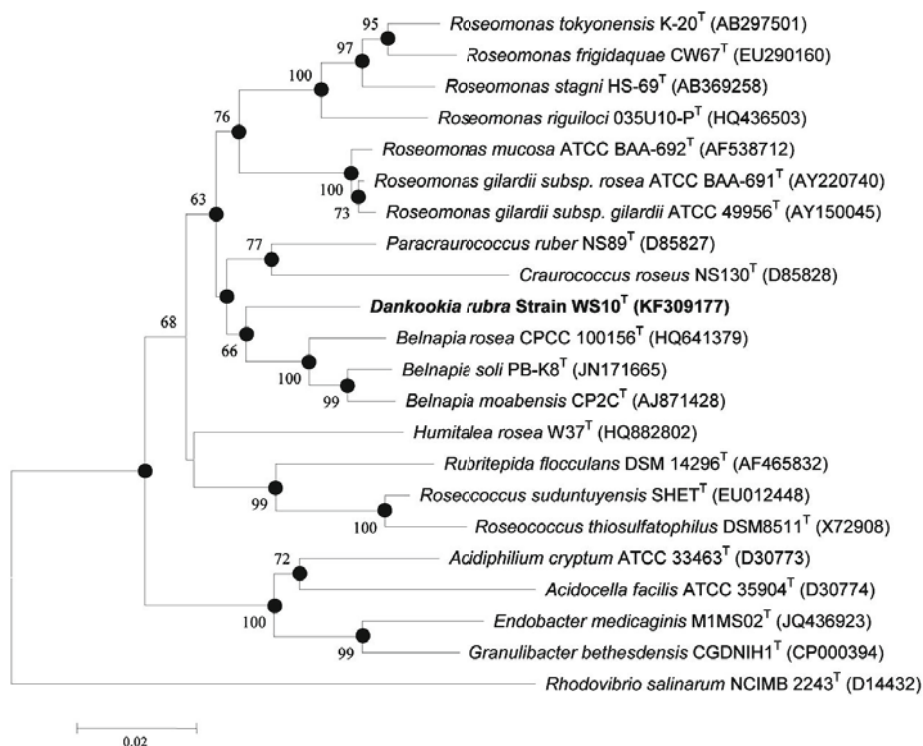
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1% N,N,N,N-tetramethyl-1,4-phenylenediamine and catalase activity was determined in 3% aqueous hydrogen peroxide solution. Growth ranges and optima of pH (pH 5, 6, 7, 9, and 10), NaCl tolerance (0 to 4.0 at intervals of 1.0%, w/v), and temperature (4, 10, 15, 20, 28, 37, 40, and 50°C) were measured using R2A agar (0.05% yeast extract, 0.05% meat peptone, 0.05% casamino acids, 0.05% glucose, 0.05% starch, 0.03% dipotassium hydrogen phosphate, 0.005% magnesium sulphate, 0.03% sodium pyruvate, and 1.5% agar) as well as R2A broth for three days. The following buffers were used to adjust the pH of R2A broth. Citrate buffer was used for pH 4–5. Tris (hydroxymethyl) aminomethane-maleate (Tris-maleate) buffer and glycine-NaOH buffer were used for pH 6–8 and pH 9–10, respectively. Growth was also tested on tryptone soy agar (TSA; 1.7% tryptone, 0.3% soy peptone, 0.5% sodium chloride, 0.25% dipotassium hydrogen phosphate, 0.25% dextrose, and 1.5% agar), potato dextrose agar (PDA; 0.4% potato extract, 2.0% dextrose, and 1.5% agar), mannitol salt agar (MSA; 1% proteose peptone, 0.1% beef extract, 1% D-mannitol, 7.5% sodium chloride, 1.5% agar, and 0.00025% phenol red), and 0.8% NaCl nutrient agar (NA; 0.8% sodium chloride, 0.3% peptone, 0.5% meat extract, and 1.5% agar). No substantial growth differences between the media at the same temperature, NaCl concentration, and pH were observed. Anaerobic growth was examined on R2A agar (0% NaCl) at 28°C for 7 days according to the manufacturer's instructions (BD GasPak EZ Anaerobe Container System Sachets). For the following biochemical tests, cells were grown on R2A agar at 28°C for three days. Hydrolysis of agarose, casein, starch, Tween 60, gelatin, and urea was determined by the method of Smibert and Krieg (1994). Enzyme activity, carbon utilization and assimilation tests were performed by the API 20NE, ZYM, 50CHB, and 32GN

(bioMérieux), according to the manufacturer's instructions.

### 16S rRNA gene sequencing and phylogenetic analysis

The genomic DNA of strain WS-10<sup>T</sup> was isolated using an InstaGene Matrix Kit (Bio-Rad). The 16S rRNA gene was amplified using the universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGYTACCTTGTACGACTT-3') (Lane, 1991). Sequencing of the 16S rRNA gene was carried out as described previously (Lee *et al.*, 2012). A fragment (1,412 bp) of the 16S rRNA gene was obtained. Sequences of related species were retrieved by using the BLAST (Altschul *et al.*, 1990) and EzTaxon-e (Kim *et al.*, 2012) identification applications with the sequenced 16S rRNA. The 16S rRNA sequence of WS-10<sup>T</sup> was aligned with those of the retrieved species using the ClustalW application (Thompson *et al.*, 1994). Hierarchical clustering analysis based on the sequence identity was performed using the heatmap application with the Euclidean distance matrix (Kolde, 2012). Phylogenetic trees were constructed using the MEGA program (version 5.10) with different algorithms based on the neighbor-joining, unweighted pair group method with arithmetic averages (UPGMA), and minimum-evolution methods (Tamura *et al.*, 2007). *Rhodovibrio salinarum* NCIMB 2243<sup>T</sup> (D14432) was used as an outgroup. The robustness of the trees was assessed by the bootstrap resampling method with 1,000 iterations. To ascertain the phylogenetic position of WS-10<sup>T</sup>, structure-based multiple sequence alignment analysis of the sequenced 16S rRNA fragment was also performed using the SSU-ALIGN algorithm (Nawrocki *et al.*, 2009). According to secondary structure prediction of 16S rRNA, the algorithm discards ambiguously aligned regions by masking the sequences in those sites. Another set of phylogenetic trees



**Fig. 1.** Phylogenetic tree reconstructed using neighbor-joining method based on 16S rRNA gene sequences, showing the position of strain WS-10<sup>T</sup> and closely related species of genera within the family Acetobacteraceae. Bootstrap values, generated from 1,000 resampling iterations, at or above 50% are indicated at branching points. Filled circles indicate branches found in phylogenetic consensus trees generated with the minimum-evolution and maximum-likelihood methods. *Rhodovibrio salinarum* NCIMB 2243<sup>T</sup> was used as an outgroup. Bar, 0.02 nucleotide substitutions per nucleotide position.

was constructed with the masked sequence using the same procedure described above.

### Chemotaxonomic and genomic analyses

Cells of strain WS-10<sup>T</sup> were grown on R2A agar for three days at 28°C and subsequently used to conduct the following analyses. Whole-cell fatty acid profiles were analyzed using gas chromatography/mass spectrometry with the prepared samples according to the instructions for the Microbial Identification System (Sasser, 1990). Respiratory quinones and polar lipids were extracted, resolved by two-dimensional TLC, and

identified as described previously (Minnikin et al., 1984; Collins, 1985). The DNA G+C content was determined by reversed-phase HPLC of nucleosides according to the protocol of Gonzalez and Saiz-Jimenez (2002).

## Results and Discussion

### Morphological and phenotypic characteristics

Cells of strain WS-10<sup>T</sup> were Gram-negative, coccus-shaped (2 µm in diameter), non-motile, non-gliding, and aerobic

**Table 1. Distinguishing features of strain WS-10<sup>T</sup> compared with closely related species**

Strains: 1, Strain WS-10<sup>T</sup> sp. nov.; 2, *Paracraurococcus ruber* NS89<sup>T</sup> (data from Saitoh et al., 1998; Alarico et al., 2002); 3, *Craurococcus roseus* NS130<sup>T</sup> (Saitoh et al., 1998; Alarico et al., 2002); 4, *Belnapia moabensis* CP2C<sup>T</sup> (Reddy et al., 2006; Jin et al., 2013); 5, *Belnapia rosea* CPCC 100156<sup>T</sup> (Jin et al., 2012, 2013); 6, *Belnapia soli* PB-K8<sup>T</sup> (Jin et al., 2013); 7, *Roseomonas gilardii* subsp. *gilardii* ATCC 49956<sup>T</sup> (Rihs et al., 1993; Han et al., 2003; Furuhashi et al., 2008); 8, *Roseomonas stagni* HS-69<sup>T</sup> (Furuhashi et al., 2008).

+, Positive; -, negative; v, variable; ND, not determined.

AL, unidentified aminolipid; APL, unidentified aminophospholipid; DPG, diphosphatidylglycerol; OH-PE, hydroxy PE; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unidentified phospholipid.

Characteristic	1*	2	3	4	5	6	7	8
Cell size (µm, wide × long)	2	0.8–1.5	0.8–2.0	1–2	1.8	0.7–1.0	ND	0.5–1.0
Motility	-	-	-	-	-	-	v	+
Gliding motility	-	ND	ND	ND	ND	ND	ND	ND
Growth media								
R2A	+	ND	ND	+	+	+	ND	+
TSA	-	ND	ND	+	+	+	+	ND
NA	+	ND	ND	+	+	+	ND	ND
MSA	-	ND	ND	-*	-*	-*	ND	ND
PDA	-	ND	ND	+	-*	-*	ND	ND
YM	ND	ND	ND	ND	+	ND	ND	ND
Blood agar	ND	ND	ND	ND	ND	ND	+	ND
Morphology								
Colony pigmentation	Red	Red	Pink	Red	Pink-rose	Red	Pink	Pale-pink
Growth range of temperature (°C)	20–40	20–42	20–37	15–30	20–37	4–37	4–35	25–35
Growth range of pH	6–8	ND	ND	6–8	5–8	5–8	ND	7–10
Growth range of NaCl (% w/v)	0–2	0–4	0–4	0	0–1	0–3	0–6	0–0.5
Catalase	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	-	-	+	+
Bchl <sub>a</sub> (nm)	-	+	+	-	ND	ND	-	ND
Hydrolysis of:								
Tween 60	+	+	+	-*	-*	-*	-	+
Urea	-	ND	ND	+	-	+	+	+
Enzyme activities:								
Esterase lipase (C8)	-	ND	ND	+	+	+	+	+
Leucine arylamidase	+	ND	ND	+	+	+	-	-
N-acetyl-β-glucosaminidase	+	ND	ND	-	-	-	-	-
Assimilation of:								
L-Alanine	+	+	+	-	-	-	ND	ND
L-Arabinose	+	+	+	-	-	-	v	-
D-Glucose	-	+	+	v	-	-	v	-
D-Mannose	-	+	+	v	-	-	ND	ND
L-Rhamnose	-	+	+	v	-	-	ND	ND
D-Ribose	-	+	+	v	-	+	ND	-
Polar lipids	PC, PG, PE, AL	ND	ND	PC, DPG, PG	PC, OH-PE, DPG, PG, AL, NPG	PME, PE, PG, APL2, PL2	PG, DPG, PE, PC	ND
Quinone	Q-10	Q-10	Q-10	Q-9	Q-9	Q-9	Q-10	Q-10
DNA G+C content (mol%)	72.7	71.0	70.5	75.0	70.3	72.1	67.6	72.0

\*Data from this study.

(Supplementary data Fig. S1). They formed red-pigmented colonies on R2A agar after incubation at 28°C for three days. Growth occurred in the pH range of 6.0–8.0 (optimum pH 7.0), and in the temperature range of 20–40°C (optimum 28°C). The isolate grew well in R2A and NA but not in TSA, MSA, or PDA media. Strain WS-10<sup>T</sup> did not grow under anaerobic condition on R2A media at 28°C. The isolate were both catalase- and oxidase-positive. The phenotypic characteristics of strain WS-10<sup>T</sup> demonstrated that the strain was quite different from its close relatives. Strain WS-10<sup>T</sup>, *P. ruber*, *C. roseus*, and *R. stagni* hydrolyzed Tween 60, while none of the species in the genus *Belnapia* did (Table 1). However, strain WS-10<sup>T</sup> differed from *P. ruber* and *C. roseus* in that it assimilated D-glucose, D-mannose, L-rhamnose, and D-ribose. In addition, only strain WS-10<sup>T</sup> (and not its close relatives) had the activity of N-acetyl-β-glucosaminidase. Additional differences are presented in Table 1.

### Phylogenetic analysis

Strain WS-10<sup>T</sup> was distinguished from the related genera according to the analyses of the 16S rRNA gene sequence and phylogenetic trees. The highest similarities in the 16S rRNA gene sequence were observed with *Paracraurococcus ruber* (95.3%), *Belnapia soli* (95.3%), *B. moabensis* (95.1%), *B. rosea* (95.0%), and *Roseomonas mucosa* (94.9%). Hierarchical clustering analysis based on the sequence identity showed that strain WS-10<sup>T</sup> was clustered with *P. ruber*, *B. soli*, *B. moabensis*, and *B. rosea* (Supplementary data Fig. S2). Since the sequence similarity was less than 96% with all of the clustered species and the cluster contained more than two genera, it could represent a novel species belonging to an uncharacterized genus. In accordance with the result,

strain WS-10<sup>T</sup> was placed between the genera *Belnapia* and *Paracraurococcus* according to phylogenetic trees constructed by the neighbor-joining, UPGMA, and minimum-evolution methods (Supplementary data Fig. S3). Another set of phylogenetic trees based on structural alignments followed by the neighbor-joining, UPGMA, and minimum-evolution algorithms showed slightly better phylogenetic trees in terms of bootstrap values (Supplementary data Fig. S4). They also indicated that strain WS-10<sup>T</sup> was positioned between the genera *Belnapia* and *Paracraurococcus*. Collectively, the phylogenetic signatures and clustering analysis suggested that strain WS-10<sup>T</sup> could be placed at the genus level in the family *Acetobacteraceae*.

### Chemotaxonomy

The major fatty acids (>5%) were summed feature 8 (C<sub>18:1</sub> ω7c), summed feature 3 (C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c), C<sub>16:0</sub>, and C<sub>18:1</sub> 2-OH. The polar lipids consisted of phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and unidentified aminolipids (AL) (Supplementary data Fig. S5). The major respiratory quinone is Q-10. The DNA G+C content of strain WS-10<sup>T</sup> was 72.7 mol%. In addition, methanolic extracts of pigments recorded *in vivo* did not show any peaks indicating the absence of bchl α and carotenoids.

The chemotaxonomic properties of strain WS-10<sup>T</sup> also indicated that the strain was distinguished from its close relatives. Strain WS-10<sup>T</sup> contained substantially lower levels of fatty acid C<sub>16:0</sub> as compared to *C. roseus*, *R. gilardii*, and the species in the genus *Belnapia* (Table 2). On the other hand, the strain had significantly higher levels of fatty acid C<sub>16:1</sub> ω7c, which set it apart from *P. ruber* and *R. stagni*. Notably,

**Table 2. Cellular fatty acid composition of strain WS-10<sup>T</sup> and its phylogenetically closest relatives**

Strains: 1, Strain WS-10<sup>T</sup> sp. nov.; 2, *Paracraurococcus ruber* NS89<sup>T</sup> (data from Reddy *et al.*, 2006); 3, *Craurococcus roseus* NS130<sup>T</sup> (Reddy *et al.*, 2006); 4, *Belnapia moabensis* CP2C<sup>T</sup> (Reddy *et al.*, 2006); 5, *Belnapia rosea* CPCC 100156<sup>T</sup> (Jin *et al.*, 2013); 6, *Belnapia soli* PB-K8<sup>T</sup> (Jin *et al.*, 2013); 7, *Roseomonas gilardii* subsp. *gilardii* ATCC 49956<sup>T</sup> (Reddy *et al.*, 2006); 8, *Roseomonas stagni* HS-69<sup>T</sup> (Furuhata *et al.*, 2008). -, Not detected or no data available; tr, Trace amount (< 1%).

Fatty acid	1	2	3	4	5	6	7	8
C <sub>11:0</sub>	3.1	-	-	-	-	-	-	-
aldehyde-C <sub>12:0</sub>	1.0	-	-	2.9	1.2	1.3	-	-
C <sub>14:0</sub>	1.1	tr	1.5	-	1.1	1.1	-	-
iso-C <sub>15:0</sub>	1.3	-	-	tr	-	-	-	-
anteiso-C <sub>15:0</sub>	2.3	-	-	-	-	-	-	-
C <sub>16:0</sub>	9.7	8.7	12.5	17.0	22.2	16.9	16.9	8.5
C <sub>16:1</sub> ω5c	tr	tr	1.1	1.3	tr	tr	tr	-
C <sub>16:1</sub> ω7c	18.5 <sup>†</sup>	5.8	12.7	7.7	18.5 <sup>†</sup>	17.1 <sup>†</sup>	1.3	9.6
C <sub>17:1</sub> ω7c	-	-	-	3.2	-	-	-	-
C <sub>18:0</sub>	tr	1.3	2.0	2.0	-	3.0	1.2	-
C <sub>18:1</sub> ω5c	tr	-	tr	1.7	-	-	tr	-
C <sub>18:1</sub> ω7c	52.6 <sup>‡</sup>	73.4	53.2	53.8	40.7 <sup>‡</sup>	35.7 <sup>‡</sup>	60.6	72.7
cyclo-C <sub>19:0</sub> ω8c	-	-	-	-	4.1	10.1	4.2	-
C <sub>14:0</sub> 3-OH	-	tr	1.1	2.9	-	-	tr	-
C <sub>16:0</sub> 2-OH	tr	-	-	-	-	-	-	-
C <sub>16:0</sub> 3-OH	tr	-	-	1.4	1.8	1.4	2.9	-
C <sub>18:1</sub> 2-OH	5.7	6.2	12.3	7.6	7.1	10.1	11.3	-
C <sub>18:0</sub> 3-OH	-	tr	1.8	tr	tr	tr	-	-

<sup>†</sup> Identified as summed feature 3, which contained C<sub>16:1</sub>ω7c and/or C<sub>16:1</sub>ω6c.

<sup>‡</sup> Identified as summed feature 8, which contained C<sub>18:1</sub>ω7c and/or C<sub>18:1</sub>ω6c.



the predominant quinone of strain WS-10<sup>T</sup> was Q-10, which was distinct from those of the genus *Belnapia*. Detailed chemotaxonomic characteristics of the isolate are presented in Table 2.

### Taxonomic conclusion

Based on the phenotypic, phylogenetic, biochemical, and chemotaxonomic properties, strain WS-10<sup>T</sup> (= KACC 18533<sup>T</sup> = JCM 30602<sup>T</sup>) exhibits different polyphasic characteristics from the closest genera, implying that the strain represents a species belonging to a novel genus of the family *Acetobacteraceae*. Therefore, the name *Dankookia rubra* gen. nov., sp. nov. is proposed.

### Description of *Dankookia* gen. nov.

*Dankookia* gen. nov. (Dan.kook'i.a. N.L. fem. n. Dankookia, referring to Dankook University in Republic of Korea, where taxonomic studies of this taxon were performed).

Cells are Gram-negative, non-motile, non-gliding, and aerobic. Oxidase- and catalase-positive. The major fatty acids are summed feature 8 (C<sub>18:1</sub> ω7c), summed feature 3 (C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c), C<sub>16:0</sub>, and C<sub>18:1</sub> 2-OH. Polar lipids include phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, and several unknown aminolipids. Q-10 is the predominant respiratory quinone which is distinct from its close relative *Belnapia*. The G+C contents of the genomic DNA are 72.7 mol%. Phylogenetically positioned in the family *Acetobacteraceae*, phylum *Proteobacteria*. The type species is *Dankookia rubra*.

### Description of *Dankookia rubra* sp. nov.

*D. rubra* sp. nov. (ru'bra. L. fem. adj. rubra, red, referring to the colour of the colonies)

The species exhibits the following characteristics in addition to those given in the genus description. Cells are coccus-shaped (2 μm in diameter). Colonies are approximately 1–2 mm and red-pigmented after three days of incubation at 28°C on R2A medium. It grows on R2A and NA but not on TSA, PDA, and MSA media. Growth occurs at 20–40°C (optimum 28°C), pH 6.0–8.0 (optimum pH 7.0), and in the presence of 0–2.0% NaCl (optimum 0%). Tween 60 (but not agarose, casein, starch, or gelatin) is hydrolyzed. Activity tests for the following enzymes were positive (API ZYM test strips): alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and N-acetyl-β-glucosaminidase. Activity tests for the following enzymes were negative: esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, and α-fucosidase. It utilizes glycerol (weakly), erythritol (weakly), D-arabinose, L-arabinose, D-ribose, D-xylose (weakly), L-xylose, D-lyxose (weakly), D-fucose (weakly), and L-fucose (weakly). However, it does not utilize D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose (bovine origin), D-melibiose,

D-saccharose, D-trehalose, inulin, D-melezitose, D-raffinose, amidon, glycogen, xylitol, gentiobiose, D-turanose, D-tagatose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate, or potassium 5-ketogluconate (API 50CHB test strips). The strain assimilates acid itaconique, acid subérique, sodium malonate, L-alanine, potassium 5-cetogluconate, L-arabinose, potassium 2-cetogluconate and acide 3-hydroxybutyrique, potassium gluconate, adipic acid, and malic acid (weakly). However, it does not assimilate L-rhamnose, N-acetyl-glucosamine, D-ribose, inositol, D-saccharose, D-maltose, sodium acetate, acide lactique, glycogen, acide 3-hydroxybenzoïque, L-serine, D-mannitol, D-glucose, salicin, D-melibiose, L-fucose, D-sorbitol, acide propionique, acide caprique, acide valerique, trisodium citrate, L-histidine, acide 4-hydroxybenzoïque, L-proline, D-mannose, trisodium citrate, or phenylacetic acid. (API 32 GN and API 20NE test strips).

The type strain, isolated from the sediment of a shallow stream, Republic of Korea, is WS-10<sup>T</sup> (= KACC 18533<sup>T</sup> = JCM 30602<sup>T</sup>).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of WS-10<sup>T</sup> is KF309177.

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