Journal of Microbiology (2017) Vol. 55, No. 10, pp. 767–774
DOI 10.1007/s12275-017-7033-z
eISSN 1976-3794
pISSN 1225-8873

Rhodoferax koreense sp. nov, an obligately aerobic bacterium within the family *Comamonadaceae*, and emended description of the genus *Rhodoferax*[§]

Mohamed El-Agamy Farh[1], Yeon-Ju Kim[2*], Priyanka Singh[2], Sun Young Jung[2], Jong-Pyo Kang[2], and Deok-Chun Yang[1,2*]

[1]Graduate School of Biotechnology and Ginseng Bank, [2]Department of Oriental Medicinal Biotechnology, College of Life Sciences, Kyung Hee University, Yongin 17104, Republic of Korea

(Received Jan 19, 2017 / Revised Jun 29, 2017 / Accepted Aug 28, 2017)

Gram-staining-negative, uniflagellated, rod-shaped, designated as DCY110^T, was isolated from sludge located in Gangwon province, Republic of Korea. The phylogenetic tree of 16S rRNA gene sequence showed that the strain DCY110^T belonged to the genus *Rhodoferax* with a close similarity to *Rhodoferax saidenbachensis* DSM 22694^T (97.7%), *Rhodoferax antarcticus* DSM 24876^T (97.5%), *Rhodoferax ferrireducens* DSM 15236^T (97.3%), and *Rhodoferax fermentans* JCM 7819^T (96.7%). The predominant isoprenoid quinine was ubiquinone (Q-8). DNA G + C content was 62.8 mol%. The major polar lipids were phosphatidylethanolamine and two unidentified phospholipids. The major fatty acids (> 10%) were $C_{12:0}$, $C_{16:0}$, summed feature 3 (which comprised $C_{16:1}$ *ω*7c and/or $C_{16:1}$ *ω*6c). The DNA-DNA relatedness values between the strain DCY110^T and the closely related relatives used in this study were lower than 70%. Based on the following polyphasic analysis, the strain DCY110^T is considered as a novel species of the genus *Rhodoferax*, for which the name *Rhodoferax koreense* sp. nov. is proposed. The type strain is DCY-110^T (= KCTC 52288^T = JCM 31441^T).

Keywords: taxonomy, *Proteobacteria*, *Rhodoferax koreense* sp. nov, sludge

Introduction

The genus *Rhodoferax* belongs to the family *Comamonadaceae* within the class *beta*-proteobacteria (Kuykendall, 2006). The type species, *R. fermentans* was considered previously as a *Rhodocyclus gelatinosus*-like bacterium, however the DNA-DNA relatedness analysis showed its low homology with the members of the genus *Rhodocyclus* (Hiraishi *et al.*, 1991). The genus *Rhodoferax* comprises Gram-negative members with vibroid or slightly curved shaped cell. They specially characterized by the existence of ubiquinone Q-8 or the rhodoquinone Q-8 and the major fatty acids $C_{16:1}$ *ω*7*c* and/or $C_{16:1}$ *ω*6*c*, $C_{16:0}$, and $C_{18:1}$ (Hiraishi *et al.*, 1991). Recently, four species of the genus *Rhodoferax* had been proposed. The genus *Rhodoferax* was initially proposed as purple, non-sulfur bacteria as the early descripted species are pigmented and able to grow phototrophically such as *R. fermentans* which isolated from ditchwater and activated sludge (Hiraishi *et al.*, 1991) and *R. antarcticus* from Antarctic region (Madigan *et al.*, 2000). But later, non-photosynthetic *Rhodoferax* species had been characterized such as *R. ferrireducens* from coastal sediment (Finneran *et al.*, 2003), and *R. saidenbachensis* from drinking water sediment (Kaden *et al.*, 2014). In this study, we proposed a non-photosynthetic strain designated as DCY-110^T as a novel species of the genus *Rhodoferax*, isolated from sludge located in Gangwon province, Republic of Korea.

Materials and Methods

Isolation and culture conditions

Sludge samples were collected from Hwacheon, Gangwon province, Republic of Korea, in April 2015, carefully kept in sterilized falcon tubes, directly brought to laboratory, and stored in 4°C until the time of the isolation. One gram of the sludge sample was mixed thoroughly with sterilized saline solution (0.85%, w/v) and then serially diluted to 10^{-7} by using sterilized saline water (0.85%, w/v). Subsequently, 100 μl of each dilution was plated on soil extract agar (SEA) prepared as described by (Hamaki *et al.*, 2005). The medium was prepared using the mud of the same source in which isolation sample was taken. Then, the inoculated plates were incubated for two weeks at 25°C. The strain designated as DCY110^T, was purified several times on the same medium and pure colonies were maintained in R2A (MB cell) broth supplied with glycerol (4:1, v/v).

Phylogenetic analysis

The genomic DNA of strain DCY110^T was extracted and purified using GeneAll Exgene TM Clinic SV kit (GeneAll) according to the manufacturer's instructions. The 16S rRNA gene sequence of strain DCY110^T was amplified by using universal bacteria primers 27F, 1492R, 518F and 800R (Lane, 1991; Anzai *et al.*, 2000) and then sequenced using the same

*For correspondence. E-mail: (D.C. Yang) dcyang@khu.ac.kr / (Y.J. Kim) yeonjukim@khu.ac.kr; Tel.: +82-31-201-2100; Fax: +82-31-202-2687
The NCBI GenBank accession number for the 16S rRNA gene sequence of strain DCY110^T is KU519435.
[§] Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

primers by Genotech company as described by Kim *et al.* (2005). Partial sequence (1,467 bp) of the 16S rRNA gene was assembled and corrected with SeqMan software version 4.1 (DNASTAR Inc.) and compared with 16S rRNA gene sequence available in the GenBank database using the BLASTn searches on NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and in the EzTaxon-e server (Kim *et al.*, 2012). The 16S rRNA gene sequence of strain DCY110^T along with the closely related species and genera were aligned together using CLASTAL_X (Thompson *et al.*, 1997). Phylogenetic trees were constructed by maximum likelihood, maximum-parsimony (Fitch, 1971), and neighbor joining methods (Saitou and Nei, 1987) using the MEGA 5 program (Tamura *et al.*, 2011). Bootstrap values were determined based on 1,000 replications (Felsenstein, 1985).

Phenotypic analysis

Colony morphology was observed after growth on R2A agar (MB cell) at 25°C for 24 h, using light microscope. Motility test was performed using hanging-drop technique (Bernardet *et al.*, 2002). Cells size, shape, and morphology were observed by transmission electron microscopy after grown on R2A agar (MB cell) for 24 h at 25°C. Gram reaction was determined by using a bioMérieux Gram staining kit according to the manufacturer's instructions. Growth was tested using several media such as nutrient agar (NA), trypticase soya agar (TSA), R2A, Luria-Bertani (LB), potato dextrose agar (PDA, all from MB cell), pseudomonas agar F (Difco), and MacConkey agar (Difco) at 25°C. Growth at various temperatures (4, 10, 15, 20, 25, 30, 37, and 40°C) was measured on the R2A agar. Salinity test was carried out on R2A broth supplemented with different concentrations of NaCl [0–10.0% (w/v), at 1.0% intervals] at 25°C. Growth at different pH values (3–10, at 0.5 pH unit intervals) was evaluated in R2A broth supplied with 100 mM phosphate-citrate buffer (pH 3–7) and 50 mM Tris-Cl (pH 8–10). Catalase activity was performed by bubble production in H_2O_2 solution (3.0%, v/v). Oxidase activity was determined by using 1% (w/v) *N*,*N*,*N*,*N*,-tetramethyl-1,4-phenylenediamine reagent (bioMérieux) according to the manufacturer's instructions. Hydrolysis of starch, skim milk, aesculin, Tween 20, Tween 80, and gelatin were tested by the standard described methods (Prescott and Harley, 2001). For anaerobic and phototrophic tests, the strain was grown on R2A agar medium at 25°C for 14 days under anaerobic condition by using a BD GasPakTM EZ pouch (Becton Dickinson) with an anaerobe sachet in the pouch, in the presence and absence of light. Other additional physiological and biochemical tests were carried out by using the API 20NE, API 32GN, and API ZYM according to the manufacturer's instruction (bioMérieux). The antibiotics susceptibility was estimated by using Oxoid antibiotic discs on Müller-Hinton agar plates (Bauer *et al.*, 1966). The inhibition zone was measured following manufacturer's manual (Oxoid). The antibiotic discs were used are carbenicillin (100 µg), vancomycin (30 µg), ceftazidime (30 µg), novobiocin (30 µg), neomycin (30 µg), tetracycline (30 µg), cephazolin (30 µg), erythromycin (15 µg), oleandomycin (15 µg), penicillin G (10 µg), rifampicin (5 µg), and lincomycin (15 µg).

Chemotaxonomic and genomic analyses

Cell biomass of DCY110^T and the closely related species *R. saidenbachensis* DSM 22694^T, were prepared for isoprenozid quinones and polar lipids analysis as follows. Strain were grown in R2A broth (MB cell) at 25°C for 48 h, and then cen-

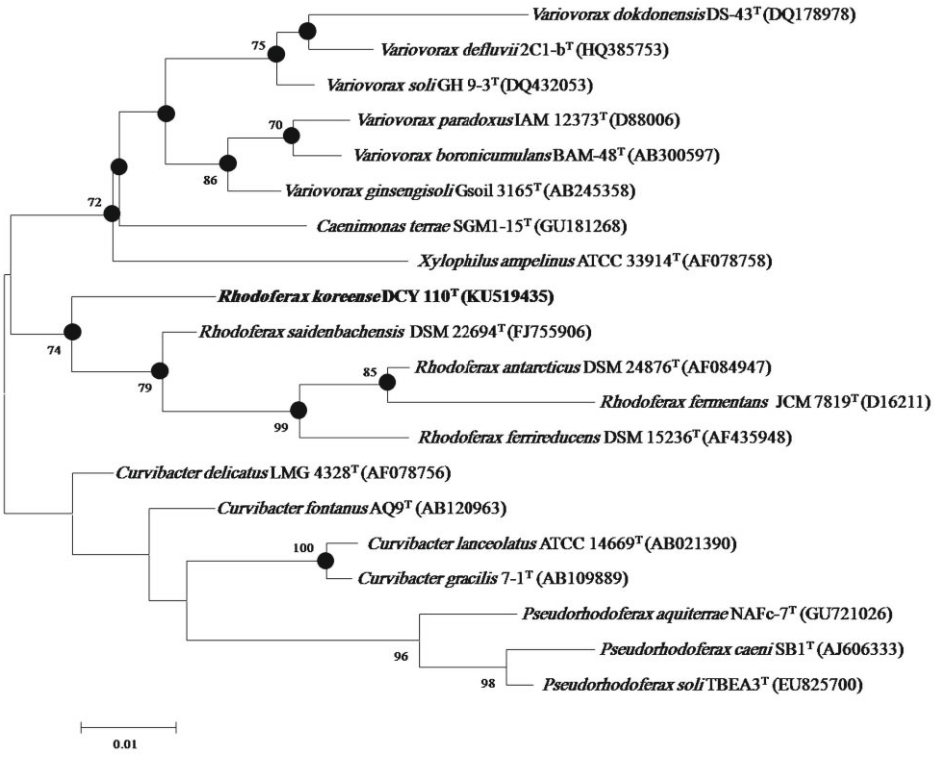

Fig. 1. Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences, showing the taxonomic position of strain DCY110^T in the genus *Rhodoferax*. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum parsimony algorithm. Bootstrap values > 70% based on 1,000 replications are shown at branching points. Bar, 0.01 substitutions per nucleotide position.

trifuged at 5,000 rpm for 15 min. The cell pellets were collected and then freeze dried. The isoprenoid quinones of DCY110^T and closely related species, *R. saidenbachensis* DSM 22694^T, were extracted and analyzed by using HPLC as described by Collins and Jones (1981). The polar lipids of the strains DCY110^T and *R. saidenbachensis* DSM 22694^T were extracted and analyzed as described by Minnikin *et al.* (1984). Analysis of total fatty acids was performed as follows. Strain DCY110^T and three references strains were grown on R2A agar for 72 h at 25°C. Then, cellular fatty acids were extracted, saponified, methylated, and purified according to the protocol of the Sherlock Microbial Identification System (Sasser, 1990). The fatty acid methyl esters were analyzed by GC (Hewlett Packard 6890) with Sherlock MIDI software (version 6.0) and a TSBA database (version 6.0). Analysis of the DNA G + C mol% content was performed as follows. The genomic DNA of strain DCY110^T was isolated by using GeneAll Exgene TM Clinic SV kit (GeneAll). 10 µg of DNA was denatured by boiling in water bath for 5 min and then cooled in ice. The DNA solution was then degraded into nucleosides by the addition of 10 µl of nuclease P1 solution [nuclease P1 20 U/ml of 40 mM acetate buffer (pH 5.4)] and incubated at 50°C for 1 h. After that, 10µl of alkaline phosphatase (40 U/ml) was added to the sample mixture, and incubated for 1 h at 37°C (Mesbah *et al.*, 1989). The nucleosides were separated using a reversed-phase HPLC column YMC-Triart C18 (4.6 × 250 mm × 5 µm). The genomic DNA of *Escherichia coli* strain B (D4889, Sigma-Aldrich) was treated by the same procedures and used as standard. DNA-DNA relatedness was carried out as described by Ezaki *et al.* (1989). The optimal hybridization temperature was 50°C. The hybridization was performed using five replications. DNA-DNA relatedness values were expressed as a mean of the nearest three different replicates (mean ± SD).

Results and Discussion

Phylogenetic analysis

Comparative 16S rRNA gene sequence analysis, showed that the strain DCY110^T was closely related to the members of genera *Rhodoferax*, *Curvibacter*, and *Variovorax*. The most closely related species of the genus *Rhodoferax* was *Rhodoferax saidenbachensis* DSM 22694^T (97.7%) and *Rhodoferax antarcticus* DSM 24876^T (97.5%) while the most closely related members of the genera *Curvibacter* and *Variovorax* were *Curvibacter delicates* (97.6%) and *Variovorax defluvii* (97.4%). However, the maximum-likelihood phylogenetic tree (Fig. 1) and neighbour-joining tree (Supplementary data Fig. S1) showed that the strain DCY110^T clustered with the members of the genus *Rhodoferax*.

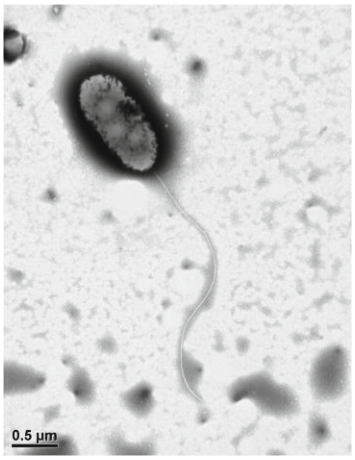

Fig. 2. Transmission electron microscope (TEM) showing the cell morphology and flagellation of the strain DCY110^T.

Phenotypic analysis

The colonies of the strain DCY110^T were observed to be circular with entire edges, colorless, with diameter ranged approximately from 0.5 to 2 mm, and tightly attached to the surface of R2A agar medium after growing 24 h at 25°C. Cells were rod-shaped, (2.0–3.0) µm long and (1.2–2.0) µm wide, uniflagellated (Fig. 2), motile, strictly aerobic, non-photosynthetic, Gram-reaction-negative, catalase positive, and oxidase negative. The strain DCY110^T was able to grow on all media listed above except LB and MacConkey agars. The growth of strain DCY110^T occurred at 4 to 30°C. No growth was observed at 37 or 40°C. This temperature range is characteristic to members of *Rhodoferax* genus among the family *Comamonadaceae* (Hiraishi *et al.*, 1991;

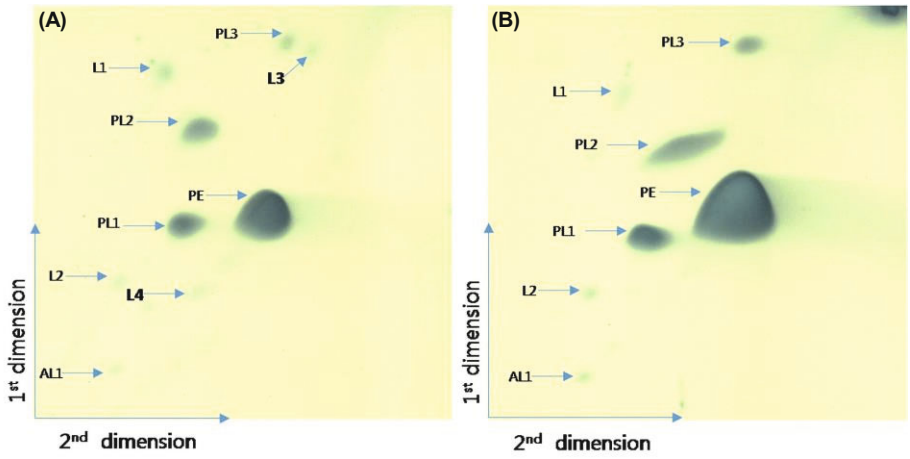

Fig. 3. Two-dimensional TLC of the total polar lipids of; (A), DCY110^T; (B), *R. saidenbachensis* DSM 22694^T, stained with 5% ethanolic molybdophosphoric acid. Abbreviations: PE, phosphatidylethanolamine; AL, unidentified aminolipids; PL, unidentified phospholipids; and L, unidentified lipids.

Table 1. Differential characteristics of strain DCY110^T and related taxa

Strains: 1, DCY110^T; 2, *R. saidenbachensis* DSM 22694^T; 3, *R. ferrireducens* DSM 15236^T; 4, *Rhodoferax antarcticus* ATCC 700587^T (Madigan *et al.*, 2000); 5, *R. fermentans* JCM 7819^T; 6, *Curvibacter delicates* IAM 14955^T (Ding and Yokota, 2004); 7, *Curvibacter fontanus* CCUG 49444^T (Ding and Yokota, 2010); 8, *Variovorax defluvii* 2C1-b^T (Jin *et al.*, 2012); 9, *Variovorax soli* NBRC 106424^T (Kim *et al.*, 2006). Data were obtained in this study unless indicated. All strains were positive for leucine arylamidase; assimilation of L-arabinose while being negative for production of indole and fermentation of glucose; lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase; assimilation of L-rhamnose, *myo*-inositol, salicin, D-melibiose, L-fucose, and valeric acid. Only the strains *Rhodoferax antarcticus* ATCC 700587^T, *Curvibacter delicates* IAM 14955^T, and *Curvibacter fontanus* CCUG 49444^T are not determined for these characteristics. +, positive; w, weakly positive; -, negative; ND, not determined; *, data were obtained from the reference study.

Characteristic	1	2	3	4*	5	6*	7*	8*	9*
Cell morphology	Rod	Short rod*	Rod*	Curved rod	Curved rod*	Curved rods	Spirilla to curved Rods	Rod	Short rod
Flagellation	Uniflagillate	Uniflagillate*	Uniflagillate*	Uniflagillate	Uniflagillate*	1-2	ND	ND	1-3
Colony color	Colorless	Colorless	Peach-brown	Peach-brown	Peach-brown	ND	Yellow–brown	Yellow	Light yellow
Growth at 4°C	+	+	+	+	+	-	+	-	-
Growth at 37°C	-	-	-	-	-	+	-	+	ND
DNA G + C (mol%)	62.8	60.3–61*	59.5*	61.5	59.8–60.3*	63.0	66.6	65.5	67.1
Oxygen requirement and photosynthesis	Aerobic; no photosynthesis	Aerobic; no photosynthesis*	Facultatively Anaerobic; no photosynthesis *	Facultatively Anaerobic; photosynthesis	Facultatively Anaerobic; photosynthesis*	Aerobic	Microaerobic	ND	Aerobic
Hydrolysis of :									
Tween 80	+	+	-	ND	-	ND	ND	ND	ND
1% *N,N,N,N,*-tetramethyl-1,4 phenylenediamine	-	-	-	ND	+	+	ND	+	+
H_2O_2	+	-	-	ND	w	+	+	+	+
Skim milk	-	-	-	ND	+	ND	ND	+	-
Starch	+	-	-	ND	+	ND	ND	-	-
L-Arginine	-	-	+	ND	-	-	-	-	-
Urea	+	-	+	ND	+	-	-	-	-
Aesculin	-	-	+	ND	-	-	-	+	-
Gelatin	-	-	-	ND	-	+	-	-	-
PNPG	-	-	-	ND	-	w	ND	-	-
Nitrate reduction	-	-	+	ND	-	ND	ND	+	+
Enzyme activity (API ZYM):									
Alkaline phosphatase	+	+	-	ND	-	ND	ND	-	+
Esterase (C4)	-	w	+	ND	-	ND	ND	+	+
Esterase lipase (C8)	+	+	+	ND	-	ND	ND	+	+
Valine arylamidase	-	w	-	ND	-	ND	ND	+	+
Acid phosphatase	+	+	+	ND	w	ND	ND	-	+
Naphthol-AS-BI-phosphohydrolase	+	w	w	ND	-	ND	ND	-	+
Assimilation of (API32 GN & API 20NE) :									
D-Glucose	+	+	w	+	+	w	+	-	+
D-Mannose	-	+	+	-	w	-	-	-	-
D-Mannitol	+	-	+	-	+	w	+	-	+
N-acetyl-glucosamine	-	-	-	ND	+	ND	ND	-	-
D-maltose	-	-	-	ND	-	W	+	-	-
Capric acid	-	-	+	-	-	ND	ND	-	-
Adipic acid	-	-	+	ND	-	ND	ND	+	+

Table 1. Continued

Characteristic	1	2	3	4*	5	6*	7*	8*	9*
Malic acid	-	-	-	+	+	ND	ND	+	+
Valeric acid	-	-	-	ND	-	ND	ND	+	+
Trisodium citrate	-	-	+	+	-	ND	ND	-	-
Phenylacetic acid	-	-	+	+	-	ND	ND	+	-
D-Ribose	+	-	+	-	w	ND	ND	-	-
Sucrose	-	-	-	+	-	ND	ND	-	-
Itaconic acid	-	-	-	ND	-	ND	ND	+	-
Suberic acid	-	-	-	ND	-	ND	ND	+	+
Sodium malonate	-	-	-	ND	-	ND	ND	+	-
Sodium acetate	-	-	-	+	-	ND	ND	+	-
Lactic acid	+	+	-	+	+	ND	ND	+	+
L-Alanine	+	-	+	ND	+	ND	ND	w	-
Potassium 5-ketogluconate	+	-	+	ND	+	-	+	-	-
Glycogen	-	-	+	ND	-	ND	ND	-	-
3-Hydroxybenzoic acid	+	+	-	-	-	ND	ND	+	+
L-Serine	-	-	-	ND	+	ND	ND	-	-
D-Sorbitol	-	-	-	ND	w	ND	ND	-	+
Propionic acid	-	-	W	-	-	ND	ND	+	+
L-Histidine	+	-	-	ND	-	ND	ND	+	+
Potassium 2-ketogluconate	+	-	+	ND	+	ND	+	+	+
3-Hydroxybutyric acid	+	+	+	-	+	ND	ND	+	+
4-Hydroxybenzoic acid	+	-	-	-	-	ND	ND	+	+
L-Proline	+	+	+	ND	+	ND	ND	-	-

Madigan *et al.*, 2000; Finneran *et al.*, 2003; Kaden *et al.*, 2014). The strain DCY110^T grew within the pH range 6 to 8 (optimal at pH 7). Regarding salinity tolerance, the strain DCY-110^T could grow only on 1% of NaCl, however the growth was lower than that in NaCl-free broth medium. The strain DCY110^T could hydrolyze starch, Tween 20 and 80 while could not hydrolyze aesculin, skim milk, and gelatin. The results of physiological and biochemical characteristics indicated by API 20NE, API 32GN, and API ZYM are summarized in the species description. The phenotypic characteristics that distinguished the strain DCY110^T from the other species of *Rhodoferax* as well as other taxa are summarized in Table 1. The strain DCY110^T was resistant to vancomycin (30 μg), lincomycin (15 μg), penicillin G (10 μg), erythromycin (15 μg), rifampicin (5 μg), novobiocin (30 μg), and oleandomycin (15 μg); intermediately sensitive to neomycin (30 μg); and completely sensitive to tetracycline (30 μg) and ceftazidime (30 μg), carbenicillin (100 μg), and cefazolin (30 μg).

Chemotaxonomic and genomic analyses

Ubiquinone was determined as the main isoprenoid quinones of the strain DCY110^T. This observation was similarly occurred in the reference strain, *R. saidenbachensis* DSM 22694^T (Kaden *et al.*, 2014). The major polar lipids of the strain DCY110^T were phosphatidylethanolamine (PE) and two unidentified phospholipids (PL1-2) which were typically similar to those of the closely related species, *R. saidenbachensis* DSM 22694^T. Both strains also have similar minor polar lipids except the unidentified polar lipids (L3-4) which exist only in DCY110^T, but absent in *R. saidenbachensis* DSM 22694^T (Fig. 3). The major cellular fatty acids (> 10%) of the strain DCY110^T were $C_{12:0}$ (12.1%), $C_{16:0}$ (22.1%), and summed feature 3 (26.1%). Same fatty acids were represented as majors in the reference strains except $C_{12:0}$ which observed to be major only in the strain DCY110^T. Some differences were recorded regarding the minor fatty acids of all strains; the profile of the minor fatty acids of the strain DCY 110^T were mostly similar to those of the genus *Rhodoferax* rather than other taxa, such as the presence of $C_{9:0}$, $C_{10:0}$, $C_{11:0}$, $C_{14:1}$ ω5c, *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:0}$, $C_{8:0}$ 3-OH, summed feature 2 and 8 and the absence of $C_{18:1}$ ω7c (Table 2). This is an indication to be a member of genus *Rhodoferax*. Nevertheless, the strain DCY 110^T is differed from the member of *Rhodoferax* by the presence of the minor fatty acids, $C_{18:1}$ ω9c (2.4%), $C_{10:0}$ 3-OH (8.5%), and $C_{17:0}$ cyclo (10.9%) and absence of $C_{10:0}$ 2-OH (Table 2). The DNA G + C of the strain DCY110^T was 62.8 mol%. Based on the phylogenetic tree of 16S rRNA gene sequence, DNA-DNA relatedness experiment was performed between the strain DCY110^T and the closely related species *R. saidenbachensis* DSM 22694^T, *R. ferrireducens* DSM 15236^T, and *R. fermentans* JCM 7819^T. The values obtained were 10.6 ± 0.2, 35.9 ± 8.5, and 14.2 ± 3.1%. The presented DNA-DNA relatedness values, which are lower than 70%, between the strain DCY110^T and the other reference strains, confirm that the strain DCY110^T is distinct species of the genus *Rhodoferax* (Wayne *et al.*, 1987). It was not able to obtain the DNA-DNA relatedness value between the strain DCY110^T and *R. antarcticus* because of the cultivation difficulty of the strain *R. antarcticus* DSM 24876^T, however the anaerobic and phototrophic characteristics of *R. antarcticus* DSM 24876^T is enough to be discriminated from our strictly aerobic, non-photosynthetic strain, DCY110^T.

Table 2. **Fatty acid composition of strain DCY110^T and related taxa**
Strains: 1, DCY110^T; 2, *R. saidenbachensis* DSM 22694^T; 3, *R. ferrireducens* DSM 15236^T; 4, *R. fermentans* JCM 7819^T; 5, *Curvibacter delicates* IAM 14955^T (Ding and Yokota, 2004); 6, *Curvibacter fontanus* CCUG 49444^T (Ding and Yokota, 2010); 7, *Variovorax defluvii* 2C1-b^T (Jin *et al.*, 2012); 8, *Variovorax soli* NBRC 106424^T (Kim *et al.*, 2006). Data were obtained in this study unless indicated. tr, fatty acids less than 0.5%; -, not determined. *, data were obtained from the reference study.

Fatty acids	1	2	3	4	5*	6*	7*	8*
Saturated								
$C_{9:0}$	0.6	0.45	0.8	0.7	-	-	-	-
$C_{10:0}$	1.4	tr	0.8	0.6	-	-	-	-
$C_{11:0}$	1.0	1.2	1.6	1.6	-	-	-	-
$C_{12:0}$	12.0	2.3	2.1	-	1.0	-	3.3	3.2
$C_{14:0}$	0.9	0.6	1.1	1.0	-	4.1	2.0	-
$C_{15:0}$	-	-	-	-	-	11.4	1.1	-
$C_{16:0}$	22.1	26.4	33.2	31.4	32.0	21.7	36.5	33.2
$C_{17:0}$	-	-	-	0.9	2.0	3.4	0.8	1.0
$C_{18:0}$	2.3	2.4	2.9	3.0	-	-	1.1	-
$C_{19:0}$	-	-	-	-	8.0	-	-	-
Unsaturated								
$C_{14:1}$ *ω5c*	1.1	1.3	1.4	1.4	-	-	-	-
$C_{15:1}$ *ω6c*	-	-	-	0.3	-	3.1	0.6	-
$C_{17:1}$ *ω6c*	-	-	-	-	-	3.3	-	-
$C_{18:1}$ *ω7c*	-	-	-	-	23.0	9.2	10.8	9.0
$C_{18:1}$ *ω9c*	2.4	-	-	-	-	-	-	-
Branched-chain								
iso-$C_{16:0}$	-	tr	tr	-	-	-	-	-
anteiso-$C_{15:0}$	0.7	0.5	0.7	1.0	-	-	-	-
anteiso-$C_{17:0}$	0.5	0.4	0.4	0.9	-	-	-	-
Hydroxy								
$C_{8:0}$ 3-OH	1.4	1.3	1.9	1.9	Present	-	-	-
$C_{10:0}$ 3-OH	8.5	-	-	-	-	5.3	2.1	5.1
$C_{10:0}$ 2-OH	-	tr	0.5	0.5	-	-	-	-
Branched hydroxyl								
iso-$C_{11:0}$ 3-OH	0.6	0.9	-	-	-	-	-	-
Cyclo								
$C_{17:0}$ cyclo	11.0	-	-	-	-	5.7	11.8	15.9
Summed features**								
2	0.7	1.0	1.4	1.5	-	-		-
3	26.1	54.4	49.1	51.5	35	29.4	27.8	29.3
7	-	-	-	0.7	-	-	-	-
8	5.8	5.4	1.4	0.5	-	-	-	-

**Summed feature 2 (unknown10.928), summed feature 3 (which comprises $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*), summed 7 (which comprises un 18.846 and/or 19:1 *ω6c*) and summed feature 8 (which comprises $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*).

Taxonomic conclusion

Based on the phylogenetic tree, the strain DCY110^T was proposed to belong to the genus *Rhodoferax*. The physiological, biochemical, and chemotaxonomic results distinguished the strain DCY110^T from the closely related strains. Therefore, the strain DCY110^T can be rendered as a novel species of the genus *Rhodoferax*, for which *Rhodoferax koorense* sp. nov has been proposed as a name for it.

Emended description of the genus *Rhodoferax*

The description of the genus *Rhodoferax* was first assigned by Hiraishi *et al.* (1991). Then, it was further emended by Kaden *et al.* (2014). Additionally, some species may be oxidase negative. The major polar lipids are phosphatidylethanolamine, and two unidentified phospholipids. The DNA G + C content is 59.5–62.8 mol%.

Description of *Rhodoferax koorense* sp. nov.

Rhodoferax koorense (ko.re.en'se. N.L. neut. adj. *koreense* of Korea, from where the novel organism was isolated).

Cells are Gram-reaction-negative, strictly aerobic, catalase positive, oxidase negative, motile, uniflagellated, and rod-shaped (2–3) μm long and (1.2–2) μm wide. Colonies are circular with entire edges, colorless, and tightly attached to the surface of agar medium with a diameter approximately 0.5 to 2 mm after incubation on R2A agar for 24 h at 25°C. Growth occurs at 4 to 30°C. The pH range for growth is 6–8 (optimal at pH 7). Growth occurs in 1% NaCl. Strain DCY110^T can hydrolyze Tween 20, 80, and starch, but aesculin, skim milk, and gelatin cannot. Cells are able to grow on NA, R2A, TSA, PDA, and Pseudomonas agar F, but not

on LB and MacConkey agars. According to the API ZYM test, positive results occur for alkaline phosphatase, esterase lipase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, while negative results occur for esterase, lipase, 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. According to the API20NE test, positive results occur for hydrolysis of urea and assimilation of D-glucose, L-arabinose, D-mannitol, and potassium gluconate while negative results occur for nitrate reduction, production of indole, fermentation of glucose, hydrolysis of L-arginine, aesculin, gelatin, and PNPG; assimilation of D-mannose, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenyl acetic acid. According to the API ID32 GN test, positive assimilations are obtained from D-ribose, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, D-mannitol, D-glucose, L-arabinose, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, and L-proline; and negative assimilations are obtained from L-rhamnose, *N*-acetyl-glucosamine, *myo*-inositol, sucrose, D-maltose, itaconic acid, suberic acid, sodium malonate, sodium acetate, glycogen, L-serine, salicin, D-melibiose, L-fucose, D-sorbitol, propionic acid, capric acid, valeric acid, and trisodium citrate. The predominant isoprenoid quinone of strain DCY110^T is ubiquinone (Q-8). The major polar lipids are phosphatidylethanolamine and two unidentified phospholipids. The major cellular fatty acids (> 10.0%) of strain DCY110^T are $C_{16:0}$, summed feature 3 (which comprises $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c), and the characteristic one, $C_{12:0}$. Other characteristic minor fatty acids are $C_{18:1}$ ω 9c, $C_{10:0}$ 3-OH, and $C_{17:0}$ cyclo.

The type strain is DCY110^T (= KCTC 52288^T = JCM 31441^T), was isolated from sludge sample taken from Hwacheon, Gangwon province, Republic of Korea. The DNA G + C content is 62.8 mol%.

Acknowledgements

This research was supported by a grant from the Next-Generation BioGreen 21 Program (SSAC, Grant Number: PJ0120342017), Rural Development Administration, Republic of Korea.

References

Anzai, Y., Kim, H., Park, J.Y., Wakabayashi, H., and Oyaizu, H. 2000. Phylogenetic affiliation of the *Pseudomonads* based on 16s rrna sequence. *Int. J. Syst. Evol. Microbiol.* **50**, 1563–1589.

Bauer, A., Kirby, W., Sherris, J.C., and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **45**, 493.

Bernardet, J.F., Nakagawa, Y., and Holmes, B. 2002. Proposed minimal standards for describing new taxa of the family flavobacteriaceae and emended description of the family. *Int. J. Syst. Evol. Microbiol.* **52**, 1049–1070.

Collins, M. and Jones, D. 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol. Rev.* **45**, 316–354.

Ding, L. and Yokota, A. 2004. Proposals of *Curvibacter gracilis* gen. nov., sp. nov. and *Herbaspirillum putei* sp. nov. for bacterial strains isolated from well water and reclassification of [*Pseudomonas*] *huttiensis*, [*Pseudomonas*] *lanceolata*, [*Aquaspirillum*] delicatum and [*Aquaspirillum*] autotrophicum as *Herbaspirillum huttiense* comb. nov., *Curvibacter lanceolatus* comb. nov., *Curvibacter delicatus* comb. nov. and *Herbaspirillum autotrophicum* comb. nov. *Int. J. Syst. Evol. Microbiol.* **54**, 2223–2230.

Ding, L. and Yokota, A. 2010. *Curvibacter fontana* sp. nov., a microaerobic bacteria isolated from well water. *J. Gen. Appl. Microbiol.* **56**, 267–271.

Ezaki, T., Hashimoto, Y., and Yabuuchi, E. 1989. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micrdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Evol. Microbiol.* **39**, 224–229.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783–791.

Finneran, K.T., Johnsen, C.V., and Lovley, D.R. 2003. *Rhodoferax ferrireducens* sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe (iii). *Int. J. Syst. Evol. Microbiol.* **53**, 669–673.

Fitch, W.M. 1971. Toward defining the course of evolution: Minimum change for a specific tree topology. *Syst. Biol.* **20**, 406–416.

Hamaki, T., Suzuki, M., Fudou, R., Jojima, Y., Kajiura, T., Tabuchi, A., Sen, K., and Shibai, H. 2005. Isolation of novel bacteria and actinomycetes using soil-extract agar medium. *J. Biosci. Bioeng.* **99**, 485–492.

Hiraishi, A., Hoshino, Y., and Satoh, T. 1991. *Rhodoferax fermentans* gen. nov., sp. nov., a phototrophic purple nonsulfur bacterium previously referred to as the "*Rhodocyclus gelatinosus*-like" group. *Arch. Microbiol.* **155**, 330–336.

Jin, L., Kim, K.K., Ahn, C.Y., and Oh, H.M. 2012. *Variovorax defluvii* sp. nov., isolated from sewage. *Int. J. Syst. Evol. Microbiol.* **62**, 1779–1783.

Kaden, R., Spröer, C., Beyer, D., and Krolla-Sidenstein, P. 2014. *Rhodoferax saidenbachensis* sp. nov., a psychrotolerant, very slowly growing bacterium within the family comamonadaceae, proposal of appropriate taxonomic position of *Albidiferax ferrireducens* strain t118t in the genus *Rhodoferax* and emended description of the genus *Rhodoferax*. *Int. J. Syst. Evol. Microbiol.* **64**, 1186–1193.

Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., and Yi, H. 2012. Introducing eztaxon-e: A prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* **62**, 716–721.

Kim, M.K., Im, W.T., Ohta, H., Lee, M., and Lee, S.T. 2005. *Sphingopyxis granuli* sp. nov., a β-glucosidase-producing bacterium in the family sphingomonadaceae in α-4 subclass of the proteobacteria. *J. Microbiol.* **43**, 152–157.

Kim, B.Y., Weon, H.Y., Yoo, S.H., Lee, S.Y., Kwon, S.W., Go, S.J., and Stackebrandt, E. 2006. *Variovorax soli* sp. nov., isolated from greenhouse soil. *Int. J. Syst. Evol. Microbiol.* **56**, 2899–2901.

Kuykendall, L. 2006. List of new names and new combinations previously effectively, but not validly, published. Validation list no. 107. *Int. J. Syst. Evol. Microbiol.* **56**, 1–6.

Lane, D.J. 1991. 16S/23S rRNA sequencing, pp. 115–176. *In* Stackebrandt, E. and Goodfellow, M. (eds.), Nucleic Acid Techniques in Bacterial Systematics. Chichester, Wiley.

Madigan, M.T., Jung, D.O., Woese, C.R., and Achenbach, L.A. 2000. *Rhodoferax antarcticus* sp. nov., a moderately psychrophilic purple nonsulfur bacterium isolated from an antarctic microbial mat. *Arch. Microbiol.* **173**, 269–277.

Mesbah, M., Premachandran, U., and Whitman, W.B. 1989. Precise

measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Evol. Microbiol.* **39**, 159–167.

Minnikin, D., O'donnell, A., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2**, 233–241.

Prescott, L. and Harley, J. 2001. Laboratory exercises in microbiology 5th edn. McGraw-Hill, New York, USA.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. MIDI Inc., Newark, DE, USA.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The clustal_x windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876–4882.

Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O., Krichevsky, M.I., Moore, L.H., Moore, W.E.C., Murray, R.G.E., Stackebrandt, E., *et al.* 1987. International committee on systematic bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* **37**, 463–464.