Dyella yeojuensis sp. nov., isolated from greenhouse soil in Korea

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A novel strain, R2A16-10^T, was isolated from greenhouse soil in Yeosu, Korea. The taxonomy of strain R2A16-10^T was studied by using polyphasic methods. On the basis of 16S rRNA gene sequence analyses, strain R2A16-10^T was found to be phylogenetically related to type strains of *Dyella* species (96.7–96.9%), *Frateuria aurantia* DSM 6220^T (96.5%), *Fulvimonas soli* LMG 19981^T (96.3%) and *Rhodanobacter* species (94.9–95.7%). Strain R2A16-10^T, which produced yellow-coloured colonies, was Gram-negative, rod-shaped (0.3–0.4 × 1.5–3.5 µm) and motile. The predominant fatty acids were 17 : 1 iso ω9c (25.5%), 15 : 0 iso (18.7%) and 17 : 0 iso (14.6%), and the major hydroxy fatty acids were 11 : 0 iso 3-OH (5.0%), 13 : 0 iso 3-OH (3.4%) and 17 : 0 iso 3-OH (1.0%). The major isoprenoid quinone was Q-8. The G + C content of the DNA of the type strain was 63.0 mol%. On the basis of the data from this study, strain R2A16-10^T represents a novel species of the genus *Dyella*, for which the name *Dyella yeojuensis* sp. nov. is proposed. The type strain is R2A16-10^T (= KACC 11405^T = DSM 17673^T).

Greenhouse soil is characterized by high ionic strength and high concentrations of organic compounds in comparison with upland soil. We isolated bacterial isolates from greenhouse soil cultivated with lettuce (*Lactuca sativa* L.) in the Yeosu region of Korea. Of these, one yellow-coloured bacterial isolate, R2A16-10^T, was shown to be closely related to *Dyella* species and *Frateuria aurantia* on the basis of 16S rRNA gene sequences. The genus *Dyella* was described recently by Xie & Yokota (2005). At present, the genus *Dyella* includes two species, *Dyella japonica* and *Dyella koreensis* (An *et al.*, 2005). This genus is closely related to the genera *Fulvimonas*, *Frateuria* and *Rhodanobacter* on the basis of 16S rRNA gene sequence analysis. *D. japonica* can be differentiated from *Frateuria aurantia* on the basis of fatty acid profiles and some phenotypic features such as growth at pH 4.5, acid production from carbohydrates and enzyme reactions.

Strain R2A16-10^T was cultivated on R2A medium (Difco) at pH 7.0 and 28 °C, and maintained on R2A medium.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain R2A16-10^T is DQ181549.

Differentiating properties among strain R2A16-10^T, members of the genus *Dyella* and *Frateuria aurantia* are shown in Supplementary Table S1 available in IJSEM Online.

Frateuria aurantia DSM 6220^T, *D. japonica* DSM 16301^T and *D. koreensis* BB4^T were used as reference strains. Standard physiological and biochemical tests were performed at 28 °C. Gram staining was performed by using a Difco Gram-stain kit. Catalase activity was tested with a 3% (v/v) H₂O₂ solution. Oxidase activity, degradation of agar (1.5%, w/v), hydrolysis of aesculin, starch, casein, gelatin and DNA and indole production were tested according to the methods of Smibert & Krieg (1994). Hydrolysis of tyrosine (0.5%, w/v) was tested on R2A agar medium. Hydrolysis of carboxymethylcellulose and Whatman powder CF11 was tested by overlaying R2A agar with a thin layer of 0.1% each component in tap-water agar. The urease test was performed using the method described by MacFaddin (2000). Growth on AE broth [1.5% (w/v) glucose, 0.2% (w/v) yeast extract, 0.3% (w/v) peptone, 6.5% (v/v) acetic acid and 2% (v/v) ethanol] (Entani *et al.*, 1985) was checked at 28 °C on a rotary shaker for 30 days. The pH range for growth was determined in R2A broth adjusted with citrate-phosphate buffer or Tris/HCl buffer (Breznak & Costilow, 1994) to pH 4.0–10.0, using increments of 0.5 pH units. Growth at 1, 2, 3, 5 and 7% NaCl (w/v) was investigated in R2A broth. Growth at various temperatures (5–50 °C) was measured on R2A medium. Physiological and biochemical properties were further determined with API ZYM, API 20NE and API 50 CH kits (bioMérieux). Tests involving

commercial systems were generally performed according to the manufacturer's instructions. The API ZYM test strip was read after 4 h incubation at 30 °C, and API test strips were examined after 48 h at 28 °C. In the case of the API 50 CH test strips, *Frateruia aurantia* DSM 6220^T produced reaction results after 48 h at 28 °C, whereas the reactions of type strains of the genus *Dyella* were delayed, being observed after 7 days at 28 °C.

Cells grown on R2A agar plates for 48 h were used for the analysis of the cellular fatty acid composition. Fatty acid methyl esters were extracted and prepared by using the standard protocol of the Microbial Identification System (MIDI; Microbial ID). Isoprenoid quinones were analysed by HPLC as described previously (Groth *et al.*, 1996). The G + C content (mol%) was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989), using a reverse-phased column (Supelcosil LC-18-S; Supelco).

The 16S rRNA gene sequence was determined by PCR amplification (Kwon *et al.*, 2003) and direct sequencing (Hiraishi, 1992). A phylogenetic analysis was carried out using 16S rRNA gene sequences of 1498 nucleotide bases, from positions 37 to 1510 (*Escherichia coli* numbering system). The neighbour-joining and maximum-parsimony methods were carried out using MEGA, version 2.1 (Kumar *et al.*, 2001), and the maximum-likelihood method (DNAML) was carried out using PHYLIP, version 3.5 (Felsenstein, 1993). The resulting trees and topology were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings.

After 2 days growth on R2A, the colonies of strain R2A16-10^T were circular, yellow in colour and convex with clear margins. The strain grew well on R2A, tryptic soy agar

Table 1. Comparison of selected characteristics among strain R2A16-10^T and members of the genera *Dyella* and *Frateruia*

Strains: 1, strain R2A16-10^T; 2, *D. japonica* DSM 16301^T; 3, *D. koreensis* BB4^T; 4, *Frateruia aurantia* DSM 6220^T. Data are from An *et al.* (2005), Bernardet *et al.* (1996), Im *et al.* (2004), Nalin *et al.* (1999), Swings *et al.* (1984), Xie & Yokota (2005) and this study. All of the strains were negative for starch hydrolysis. Symbols: +, positive; −, negative; w, weak; ND, not determined.

Characteristic	1	2	3	4
DNA G + C content (mol%)	63.0	63.4–64.0	63.8	63.5
Motility	+	+	−	+
Oxidase/catalase	+/+	−/+	+/w	−/+
Hydrolysis of:				
Cellulose	−	−	+	−
Casein	+	−	+	−
Growth at pH 4.5	−	−	ND	+
Growth at 4% NaCl	+	−	w	−
Growth on AE medium	−	−	ND	+

(Difco) and nutrient agar (Difco) but grew weakly on MacConkey agar (Difco). Strain R2A16-10^T was a Gram-negative rod, 0.3–0.4 × 1.5–3.5 µm in size. In API 20NE tests, the micro-organism assimilated D-glucose, D-mannose, N-acetylglucosamine and D-maltose and showed positive reactions for aesculin and gelatin hydrolysis and β-galactosidase activity. In API 50 CH tests, there were positive reactions only for D-galactose, D-glucose, D-fructose, D-mannose and aesculin. In API ZYM tests, the micro-organism showed positive reactions for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase activities (see Supplementary Table S1 available in IJSEM Online). Differentiating properties among strain R2A16-10^T, members of the genus *Dyella* and *Frateruia aurantia* are shown in Table 1 and Supplementary Table S1.

Strain R2A16-10^T contained Q-8 as the major isoprenoid quinone. It had a fatty acid profile very similar to those of other *Dyella* species, having a predominance of branched fatty acids, with 17:1 iso ω9c (21.5%), 16:0 iso (21.3%),

Table 2. Cellular fatty acid composition of strain R2A16-10^T in comparison with those of related species

Strains: 1, strain R2A16-10^T; 2, *D. japonica* DSM 16301^T; 3, *D. koreensis* BB4^T; 4, *Frateruia aurantia* DSM 6220^T. Values are percentages of total fatty acids. −, Not detected or <1%. ECL, Equivalent chain length.

Fatty acid	1	2	3	4
11:0 iso	3.7	5.0	4.5	−
Unknown (ECL 11.799)	2.3	3.1	2.5	−
11:0 iso 3-OH	4.2	6.0	5.5	−
12:0 2-OH	−	−	−	2.2
12:0 3-OH	−	−	−	5.7
12:0 iso 3-OH	1.0	1.4	1.4	−
14:1ω5c	−	−	−	1.0
14:0 iso	1.1	−	1.1	−
14:0	−	−	−	3.1
13:0 iso 3-OH	2.4	4.0	2.3	1.2
15:0 iso	14.5	14.6	11.1	41.2
15:0 anteiso	6.9	5.1	3.6	2.4
16:0 iso	21.3	17.5	19.2	−
16:0	1.8	1.5	2.5	7.5
17:1 iso ω9c	21.5	20.3	19.0	1.8
17:0 iso	7.9	8.5	6.7	5.3
17:0 anteiso	1.6	1.8	2.9	−
17:0 cyclo	−	−	−	9.8
18:0 iso	1.2	1.3	2.6	−
18:0	−	−	−	1.2
17:0 iso 3-OH	−	1.5	−	−
Summed feature 3*	5.2	5.0	7.9	10.5

*Summed feature 3 comprises 15:0 iso 2-OH and/or 16:1ω7c.

Fig. 1. Phylogenetic position of strain R2A16-10^T with respect to some members of the Gammaproteobacteria on the basis of 16S rRNA gene sequences. Numbers at nodes indicate levels of bootstrap support (%), based on a neighbour-joining analysis of 1000 resampled datasets. Bootstrap values below 50 % are not shown. Bar, 1 nucleotide substitution per 100 nucleotides.

15:0 iso (14.5 %) and 17:0 iso (7.9 %) as the major constituents. The major hydroxy fatty acids were 11:0 iso 3-OH (4.2 %), 13:0 iso 3-OH (2.4 %) and 12:0 iso 3-OH (1.0 %). The fatty acid profiles of *Dyella* species and isolate R2A16-10^T could be clearly differentiated from that of *Frateuria aurantia* (Table 2). The DNA G+C content of strain R2A16-10^T was 63.0 mol%.

Strain R2A16-10^T displayed high levels of 16S rRNA gene sequence similarity with *Dyella* species (96.7–96.9 %) and *Frateuria aurantia* DSM 6220^T (96.5 %). It formed a sub-line, which included *Dyella* species and *Frateuria aurantia*, supported by a bootstrap value of 71 % (Fig. 1). Within the cluster, strains of *Frateuria aurantia* formed another compact cluster distinct from that for members of the genus *Dyella*. On the basis of the data from polyphasic studies including analyses of biochemical properties, fatty acid composition and the 16S rRNA gene sequence, strain R2A16-10^T represents a novel species within the genus *Dyella*, for which the name *Dyella yeojuensis* sp. nov. is proposed.

Description of *Dyella yeojuensis* sp. nov.

Dyella yeojuensis (yeo.ju.en'sis. N.L. fem. adj. *yeojuensis* pertaining to Yeosu, a city in Korea, from where the type strain originated).

Cells are Gram-negative, aerobic, motile, catalase-positive and oxidase-positive. Colonies on R2A medium are yellow. Colonies after 2 days growth on R2A medium are circular, yellow in colour and convex with clear margins. Optimal growth occurs at 28 °C. Growth occurs at temperatures in the range 5–37 °C, at pH values in the range 4.5–8.5 and at 0–5 % (w/v) NaCl. Does not grow on AE medium. Hydrolyses aesculin, casein, DNA, gelatin and tyrosine. Does not hydrolyse starch, urea, CM-cellulose or Whatman powder CF11. The major isoprenoid quinone is Q-8. The predominant fatty acids are 17:1 iso ω 9c, 16:0 iso, 15:0 iso and 17:0 iso. The major hydroxy fatty acids are 11:0 iso 3-OH, 13:0 iso 3-OH and 12:0 iso 3-OH. The DNA G+C content of the type strain is 63.0 mol%.

The type strain, R2A16-10^T (=KACC 11405^T=DSM 17673^T), was isolated from greenhouse soil in Yeosu, Korea.

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