Dyella marensis sp. nov., isolated from cliff soil

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A novel, Gram-negative bacterium, designated CS5-B2^T, was isolated from soil that had been collected from a cliff on Mara Island, Republic of Korea. Phylogenetic analyses based on 16S rRNA gene sequences showed that the strain fell within the radiation of the genus *Dyella*. The closest relatives were the type strains of *Dyella koreensis*, *Dyella ginsengisoli* and *Dyella japonica* and 16S rRNA gene sequence similarities between strain CS5-B2^T and these strains were 99.0, 97.9 and 97.8 %, respectively. The sequence similarities between the novel isolate and other related taxa compared in the phylogenetic analysis were less than 96.7 %. The cells of strain CS5-B2^T were aerobic, oxidase-negative, catalase-positive, motile rods. The temperature range for growth was 20–37 °C, with optimal growth at 30–37 °C. Growth occurred at pH 5.1–9.1, with optimal growth at pH 6.1–9.1. NaCl tolerance for growth was from 1 to 2 % (w/v). Ubiquinone-8 was the predominant respiratory lipoquinone. The major fatty acids were iso-C_{15:0} and iso-C_{17:1ω9c}. The G + C content of the DNA was 65.7–66.6 mol%. The level of DNA–DNA relatedness with *D. koreensis* KCTC 12359^T was 20.2 and 29.6 % in duplicate measurements. On the basis of phenotypic features, phylogenetic analysis and DNA–DNA relatedness, a novel species of the genus *Dyella* is proposed, with the name *Dyella marensis* sp. nov. The type strain is CS5-B2^T (=JCM 14959^T =KCTC 22144^T).

The genus *Dyella* was first described by Xie & Yokota (2005). At the time of writing, the genus *Dyella* contained three recognized species, *Dyella japonica* (Xie & Yokota, 2005), *Dyella koreensis* (An *et al.*, 2005) and *Dyella yeosuensis* (Kim *et al.*, 2006). A fourth species, *Dyella ginsengisoli* (Jung *et al.*, 2009) has been described while this paper was in press. This genus is closely related to the genera *Fulvimonas*, *Frateriella* and *Rhodanobacter* in the family *Xanthomonadaceae* on the basis of 16S rRNA gene sequence data.

Strain CS5-B2^T was isolated from a soil sample that had been collected from a cliff on Mara Island, Republic of Korea. A 1 g sample was placed into a sterile plastic tube containing 9 ml sterile distilled water. After mixing for 30 min using a tube rotator, followed by serial dilution, 100 µl aliquots were transferred to plates of isolation medium, starch–casein agar (1⁻¹: 10 g soluble starch, 0.3 g casein, 2 g KNO₃, 2 g NaCl, 2 g KH₂PO₄, 0.02 g CaCO₃, 0.05 g MgSO₄ · 7H₂O, 0.01 g FeSO₄ · 7H₂O, 18 g agar). The plates were incubated at 30 °C for 2 weeks. A single colony was selected and streaked on YMG agar (1⁻¹: 4 g yeast extract, 10 g malt extract, 4 g glucose, 18 g agar; pH 7.2) to ensure purity. The pure culture was maintained at –20 and

–80 °C as a 20 % (v/v) glycerol solution. For phenotypic and genetic comparison, *D. japonica* DSM 16031^T and *D. koreensis* KCTC 12359^T were used as reference strains.

Cell morphology and motility were observed by using phase-contrast microscopy with cells grown on trypticase soy agar (TSA; Difco) for 3 days. To check for the presence of flagella, cells were stained with 2 % phosphotungstic acid, placed on a gold-coated grid and observed with a transmission electron microscope (JEM-1200EX II; JEOL). Growth was tested on YMG agar, TSA, nutrient agar (Difco) and R2A agar (Difco). The colonies of strain CS5-B2^T were deep to dark yellow in colour. Soluble pigments were not produced on most of the media tested, with the exception of a yellowish brown pigment on YMG agar. Growth was determined at 4, 10, 20, 30, 37 and 42 °C and initial pH 4.1–12.1 (with intervals of 1.0 pH unit). NaCl tolerance for growth was tested on YMG agar supplemented with 1–4 % (w/v) NaCl. Gram stain, oxidase and catalase activities, degradation abilities and utilization of carbohydrates were determined by using previously described methods (Lee & Lee, 2008). Other physiological and biochemical properties were tested using the API 20NE and API ZYM kits (bioMérieux) following the manufacturer's instructions. Cells of strain CS5-B2^T were aerobic, Gram-negative, motile rods (0.4–0.5 × 1.1–3.6 µm) (see Supplementary Fig. S1, available in IJSEM Online). Cultural, physiological and biochemical characteristics are given in Table 1 and the species description.

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain CS5-B2^T is AM939778.

A supplementary figure showing the cell morphology of strain CS5-B2^T and a supplementary table detailing the fatty acid profile of strain CS5-B2^T and related species are available with the online version of this paper.

Table 1. Characteristics that differentiate strain CS5-B2^T from some type strains of species of the genus *Dyella*

Strains/species: 1, CS5-B2^T; 2, *D. japonica* DSM 16301^T (data from Xie & Yokota, 2005 and this study); 3, *D. koreensis* KCTC 12359^T (An *et al.*, 2005 and this study); 4, *D. yejuensis* R2A16-10^T (Kim *et al.*, 2006). All strains are positive for aesculin degradation and utilization of D-glucose, D-mannose, N-acetyl-D-glucosamine and maltose, but negative for indole production, glucose fermentation and utilization of D-arabinose, D-mannitol, gluconate, caprate, adipate, citrate and phenylacetate (API 20NE). All strains are positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, α -glucosidase and β -glucosidase activities but negative for β -glucuronidase activity (API ZYM). All strains produce acid from D-glucose, but none produce acid from inulin, melezitose, methyl α -D-glucoside, methyl α -D-mannoside, raffinose, L-rhamnose, salicin, L-sorbose, sucrose, dulcitol, meso-erythritol, glycerol, myo-inositol, D-mannitol, D-sorbitol or D-xylitol. +, Positive; -, negative; w, weak; ND, no data available.

Characteristic	1	2	3	4
Growth at 10 °C	—	+	+	+
Motility	+	+	—	+
Enzyme activity				
Oxidase	—	—	+	+
Catalase	+	+	w	+
β -Galactosidase	+	—	+	+
Reduction of nitrate	—	+	+	—
Utilization of:				
Malic acid	+	—	+	—
Acid production from:				
D-Arabinose	+	—	+	—
Cellobiose	+	w	+	—
Lactose	+	—	—	—
Maltose	+	+	+	—
Trehalose	+	+	+	—
D-Xylose	+	—	—	—
Degradation of:				
Casein	—	—	+	+
Chitin	—	—	+	ND
CM-Cellulose	—	—	+	—
Starch	+	—	—	—
Tyrosine	—	—	—	+
Enzyme activity (API ZYM)				
N-Acetyl- β -glucosamidase	—	w	+	+
α -Chymotrypsin	+	—	—	—
Cystine arylamidase	w	—	w	+
Esterase (C4)	—	+	—	+
α -Fucosidase	—	+	+	—
Lipase (C14)	—	+	—	—
α -Mannosidase	—	+	—	—
Trypsin	—	+	—	—
DNA G + C content (mol%)	65.7–66.6	63.4–64	63.8	63.0

Genomic DNA was extracted and purified as described by Hopwood *et al.* (1985). The 16S rRNA gene was amplified and purified according to the method of Lee & Lee (2008).

Multiple alignments of sequences were performed using CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987) from evolutionary distances calculated with the coefficient of Jukes & Cantor (1969). The confidence levels of the tree topology were evaluated by bootstrap analysis (Felsenstein, 1985) using 1000 replications. The neighbour-joining tree was compared with trees constructed with the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods.

The partial 16S rRNA gene sequence (1377 nt) of strain CS5-B2^T determined in this study was compared with the corresponding sequences of members of the genus *Dyella* and related taxa. A neighbour-joining tree (Fig. 1) based on 16S rRNA gene sequences showed that strain CS5-B2^T lies within the radiation of the genus *Dyella*. The closest relatives were *D. koreensis* BB4^T (99.0 % sequence similarity), *D. ginsengisoli* Gsoil 3046^T (97.9 %) and *D. japonica*

Fig. 1. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain CS5-B2^T within the radiation encompassing species of the genus *Dyella* and related taxa. *Xanthomonas campestris* LMG 568^T was used as an outgroup. Bootstrap percentages (from 1000 replications) greater than 50 % are shown at branching points. *, Branches also found in trees generated with maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Bar, 1 inferred nucleotide substitution per 100 nucleotides.

XD53^T (97.8 %). The 16S rRNA gene sequence similarities between the novel isolate and the type strains of related taxa used in the phylogenetic analysis were less than 96.7 %.

Cell biomass for chemotaxonomic analyses was obtained from cultures grown in YMG broth for 3 days at 30 °C. Respiratory lipoquinones were extracted according to Collins (1985) and identified by HPLC (Kroppenstedt, 1985). For analysis of cellular fatty acids, strain CS5-B2^T and the type strains of *D. koreensis* and *D. japonica* were grown on TSA for 3 days at 30 °C. Cellular fatty acid methyl esters were prepared and analysed according to the standard protocol of the Microbial Identification System (version 6; MIDI). Ubiquinone-8 is the predominant respiratory lipoquinone. The cellular fatty acid profile of strain CS5-B2^T consisted of saturated, branched and hydroxy fatty acids (see Supplementary Table S1 in IJSEM Online). The major fatty acids were iso-C_{15:0} (23.2–27.0 %) and iso-C_{17:1ω9c} (23.4–26.1 %). The cellular fatty acid profiles of *D. koreensis* KCTC 12359^T and *D. japonica* DSM 16031^T as determined in this study contained smaller amounts of iso-C_{17:0} (10.7–12.4 and 7.5–12.4 %, respectively) than previously reported (15.6–26.0 and 20.0 %, respectively) (An *et al.*, 2005; Xie & Yokota, 2005).

The DNA G+C contents of strain CS5-B2^T, *D. koreensis* KCTC 12359^T and *D. japonica* DSM 16031^T were analysed by HPLC (Mesbah *et al.*, 1989) and the values were determined by using the following formula: G+C mol%=[(1+dT/dC)⁻¹ × 100 + (1+dA/dG)⁻¹ × 100]/2. Each experiment was performed in duplicate with DNA prepared from independent cultures. The DNA G+C content was 65.7–66.7 mol% for strain CS5-B2^T, 61.1–62.2 mol% for *D. koreensis* and 62.6–63.2 mol% for *D. japonica*. The values for *D. koreensis* and *D. japonica* were slightly lower in this study than previously reported figures (63.8 and 63.4–64 mol%, respectively) (An *et al.*, 2005; Xie & Yokota, 2005).

Along with low 16S rRNA gene sequence similarity, strain CS5-B2^T differed from *D. japonica* DSM 16031^T in many characteristics, such as nitrate reduction, utilization of malic acid, acid production from D-arabinose, lactose and D-xylose, starch hydrolysis and several enzyme activities (Table 1). Therefore, DNA–DNA relatedness was determined only between strain CS5-B2^T and *D. koreensis* KCTC 12359^T, as described previously (Lee & Lee, 2008). The DNA–DNA hybridization values in duplicate measurements were 20.2 and 29.6 %, lower than the threshold value of 70 % recommended for the definition of bacterial species (Wayne *et al.*, 1987). The physiological and biochemical characteristics that distinguish the novel isolate from *D. koreensis* KCTC 12359^T are also given in Table 1.

On the basis of the phenotypic, phylogenetic and DNA–DNA hybridization analysis presented, it is clear that strain CS5-B2^T represents a novel species of the genus *Dyella*, for which the name *Dyella marensis* sp. nov. is proposed.

Description of *Dyella marensis* sp. nov.

Dyella marensis (ma.ren'sis. N.L. fem. adj. *marensis* of Mara Island, Jeju, Republic of Korea, on which the type strain was isolated).

Cells are aerobic, Gram-negative, oxidase-negative, catalase-positive, motile rods (0.4–0.5 × 1.1–3.6 µm). Colonies are irregular, undulate, umbonate, dark yellow in colour and reach 2.5–4.0 mm in diameter after 3 days of incubation. Temperature range for growth is 20–37 °C, with good growth at 30–37 °C. Growth occurs at pH 5.1–9.1, with good growth at pH 6.1–9.1. NaCl tolerance for growth is up to 2 % (w/v). Gelatin liquefaction is observed. DNA and elastin are hydrolysed but hypoxanthine and xanthine are not. Arginine dihydrolase and urease are not detected. Acid is produced from dextrin, D-galactose and D-mannose but not from L-arabinose and D-fructose. Data for other physiological and biochemical properties are given in Table 1. Ubiquinone-8 is the predominant respiratory lipoquinone. Major fatty acids are iso-C_{15:0} and iso-C_{17:1ω9c}.

The type strain, CS5-B2^T (=JCM 14959^T =KCTC 22144^T), was isolated from a soil sample that had been collected from a cliff on Mara Island, Republic of Korea. The DNA G+C content of the type strain is 65.7–66.6 mol%.

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