Dyella marensis sp. nov., isolated from cliff soil

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A novel, Gram-negative bacterium, designated CS5-B2T, 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-B2T 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-B2T 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\textsubscript{15:0} and iso-C\textsubscript{17:1} \(\alpha\) \textbf{c}. The G+C content of the DNA was 65.7–66.6 mol%. The level of DNA–DNA relatedness with \textit{D. koreensis} KCTC 12359\textsuperscript{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 \textit{Dyella} is proposed, with the name \textit{Dyella marensis} sp. nov. The type strain is CS5-B2T (=JCM 14959\textsuperscript{T} =KCTC 22144\textsuperscript{T}).

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain CS5-B2\textsuperscript{T} is AM093978.

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

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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* Table 1.

**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. yeojuensis* 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, z-galactosidase, z-glucosidase and B-glucosidase activities but negative for B-glucuronidase activity (API ZYM). All strains produce acid from D-glucose, but none produce acid from inulin, melezitose, methyl D-glucoside, methyl 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.

![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.](image-url)

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XD53T (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-B2T 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-B2T consisted of saturated, branched and hydroxy fatty acids (see Supplementary Table S1 in IJSEM Online). The major fatty acids were iso-C15:0 (23.2–27.0%) and iso-C17:0 9c (23.4–26.1%). The cellular fatty acid profiles of *D. koreensis* KCTC 12359T and *D. japonica* DSM 16031T as determined in this study contained smaller amounts of iso-C17:0 (10.7–2.4% 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-B2T, *D. koreensis* KCTC 12359T and *D. japonica* DSM 16031T were analysed by HPLC (Mesbah et al., 1989) and the values were determined by using the following formula: G+C mol% = [(1 + dT/dC)×100 + (1 + dA/dG)×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-B2T, 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-B2T differed from *D. japonica* DSM 16031T in many characteristics, such as nitrate reduction, utilization of malic acid, acid production from d-arabinose, lactose and d-xyllose, starch hydrolysis and several enzyme activities (Table 1). Therefore, DNA–DNA relatedness was determined only between strain CS5-B2T and *D. koreensis* KCTC 12359T, 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 12359T 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-B2T represents a novel species of the genus *Dyella*, for which the name *Dyella marenensis* sp. nov. is proposed.

### Description of *Dyella marenensis* sp. nov.

*Dyella marenensis* (ma.ren’sis. N.L. fem. adj. *marenis* 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-C15:0 and iso-C17:0 9c.

The type strain, CS5-B2T (=JCM 14995T = KCTC 22144T), 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%.

### Acknowledgements

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### References


