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Dyella jejuensis sp. nov., Isolated from Soil of Hallasan Mountain in Jeju Island[§]

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A novel bacterium, designated JP1^T, was isolated from soil of Hallasan Mountain in Jeju Island. The isolate was a Gram-negative, aerobic, motile and rod-shaped (0.2–0.4 × 1.2–2.0 μm) bacterium. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain JP1^T was closely related to *Dyella koreensis* with 97.6% similarity. Growth of strain JP1^T occurred at 10–37°C, pH 5–7 and 0–1% (w/v) NaCl. The genomic DNA G+C content of strain JP1^T was 62.1 mol%. The major fatty acids were iso-$C_{16:0}$, iso-$C_{17:1}$ ω9c, and iso-$C_{15:0}$. The predominant quinone was ubiquinone-8. The major polar lipids of strain JP1^T were phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, unidentified aminolipids and unidentified aminophospholipids. The DNA-DNA relatedness values between strain JP1^T and previously reported *Dyella* species were <10%. Based on phenotypic, genotypic, and phylogenetic distinctness, strain JP1^T represents a novel species in the genus *Dyella*, for which the name *Dyella jejuensis* sp. nov. is proposed. The type strain is JP1^T (=KACC 17701^T =JCM 19615^T).

Keywords: *Dyella*, *Dyella jejuensis* sp. nov., taxonomy

Introduction

The genus name *Dyella* was first described by Xie and Yokota (2005) and belongs to the family *Xanthomonadaceae* of the phylum *Proteobacteria*. The genus *Dyella* is comprised of 10 recognized species, including *D. japonica* (Xie and Yokate, 2005), *D. ginsengisoli* (Jung *et al*., 2009), *D. jiangningensis* (Zhao *et al*., 2013), *D. koreensis* (An *et al*., 2005), *D. kyungheensis* (Son *et al*., 2013), *D. marensis* (Lee and Lee, 2009), *D. soli* (Weon *et al*., 2009), *D. terrae* (Weon *et al*., 2009), *D. thiooxydans* (Anandham *et al*., 2011), and *D. yeojuensis* (Kim *et al*., 2006). These bacteria were isolated mainly from soil and the rhizophere. We isolated strain JP1^T from the soil on Hallasan Mountain, Jeju Island, Republic of Korea, and we describe, here, the morphological, biochemical, chemical, genotypic, and phylogenetic characteristics of *Dyella jejuensis* sp. nov.

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Materials and Methods

Bacterial strains

Strain JP1^T was isolated from soil on Hallasan Mountain, Juju Island using the standard dilution-plating method at 30°C on carboxymethycellulose (CMC; Difco, USA) agar, composed of: 10 g CMC, 1 g yeast extract, 2.5 g $(NH_4)_2SO_4$, 0.25 g $K_2HPO_4{\cdot}3H_2O$, 0.1 g NaCl, 0.125 g $MgSO_4{\cdot}7H_2O$, 0.0025 g $FeSO_4{\cdot}7H_2O$, 0.025 g $MnSO_4$, 10 g agar, and distilled water to 1,000 ml. Once an individual colony was obtained, it was subcultured several times to obtain a pure culture. For comparative analyses, the type strains of *D. koreensis* BB4^T (=KACC 11427^T), *D. marensis* CS5-B2^T (=KACC 14035^T), *D. soli* JS12-10^T (=KACC 12747^T) and *D. ginsengisoli* Gsoil 3046^T (=KACC 12866^T) were obtained from the Korean Agricultural Culture Collection (KACC).

Morphological, physiological, and biochemical characterization

Growth was tested on tryptic soy agar (Difco), nutrient agar (Difco), R2A agar (Difco) and CMC agar. Various temperatures, pHs, and NaCl concentrations were tested using R2A medium to determine the optimum culture conditions for strain JP1^T. Growth was tested at 4, 10, 15, 20, 25, 30, 37, 40, and 47°C. The pH range for growth was determined in R2A broth at pH 4.0–10.0 (in increments of 0.5 pH units) adjusted with 50 mM MES for pH 4–6, 50 mM TAPs for pH 7–8, and 50 mM Na_2HPO_4 for pH 9–10 (Hyun *et al*., 2013). NaCl tolerance and requirements for growth were tested on R2A broth supplemented with 0–5% (w/v, at intervals of 1%) NaCl. Strain JP1^T was routinely cultured in R2A medium under optimum growth conditions to investigate its morphological and physical characteristics. Exponential growth phase cells in R2A broth were stained using Gram staining reagent (Difco) according to the manufacturer's instructions. Anaerobic growth was determined after incubation for 7 days at 30°C on R2A agar in an oxygen-free N_2:CO_2:H_2 (90:5:5) anaerobic chamber. Cellulose-degradation activity was confirmed by staining CMC medium using 0.01% trypan blue. Motility was determined using semi-solid R2A agar (Tittsler and Sandholzer, 1936). Morphological

characteristics of strain JP1^T were observed by phase-contrast microscope (Eclipse 50*i*; Nikon, Japan) using cells cultured on R2A agar at 30°C for 48 h. Oxidase and catalase activities were determined using a 1% (w/v) tetramethyl-*p*-phenylenediamine oxidase reagent (bioMérieux, France) and a 3% (v/v) H_2O_2 solution for bubble production, respectively. Enzyme activities were determined by 19 hydrolytic enzymes using an API ZYM kit (bioMérieux), according to the manufacturer's instructions. Sole carbon sources were determined using the API 20NE and API 50CH kits (bioMérieux), according to the manufacturer's instructions.

Phylogenetic and genomic analyses

The 16S rRNA gene was amplified by colony PCR using PCR pre-mix (*i*NtRon Biotechnology, Korea) and two universal bacteria-specific primers: 27F (5′-AGAGTTTGATCCTGG CTCAG-3′) and 1492R (5′-GGYTACCTTGTTACGACTT-3′) (Lane, 1991). The amplified PCR product was purified using a QIAquick PCR purification kit (Qiagen, USA) and sequenced with an automated DNA analyzer system (PRISM 3730XL DNA analyzer; Applied Biosystems, USA). The 16S rRNA gene sequence fragments of strain JP1^T were assembled using SeqMan (DNASTAR), and the assembled sequences were aligned with sequences of the closely related species using ClustalW2 (http://www.ebi.ac.uk/Tools/phylogeny/clustalw2_ phylogeny/). Phylogenetic relationships among strain JP1^T and representative species in the genus *Dyella* were determined using the MEGA5.2 program (Tamura *et al*., 2011). Phylogenic trees were constructed based on the neighbor-joining (Saitou and Nei, 1987), maximum-likelihood (Kluge and Farris, 1969) and maximum-parsimony (Kluge and Farris, 1969) algorithms. Distance matrices were calculated as described by Jukes and Cantor (1980). Bootstrap analyses were conducted with 1000, 1000 and 300 replicates to evaluate the statistical reliability of the tree. The genomic DNAs of strain JP1^T and the reference species were extracted and purified using the method described by Sambrook *et al*. (1989). DNA G+C contents were determined using a fluorometric method with SYBR Gold I and a real-time PCR thermocycler (Gonzalez and Saiz-Jimenez, 2002). The genomic DNA of *Bacteroides thetaiotaomicron* VPI-5482^T, *D. soli* JS12-10^T and *D. terrae* JS14-6^T were used as calibration references (Gonzalez and Salz-Jimenez, 2002). DNA–DNA hybridization was performed reciprocally to elucidate the genetic relatedness of strain JP1^T to the four type strains in the genus *Dyella* using genome-probing microarrays (Chang *et al*., 2008).

Chemotaxonomy

The cellular fatty acid composition of strain JP1^T was identified using cells grown on R2A agar for 48 h at 30°C as reported previously (Zhao *et al*., 2013). Fatty acid methyl esters were extracted and analyzed using the standard protocols provided by the Sherlock Microbial Identification System (MIDI, 1999). A gas chromatograph (Agilent 7890, Agilent Technologies, USA) and the Microbial Identification software package (Sherlock ver. 6.2) were used for the analysis and identification of the fatty acids, respectively (Sasser, 1990). The isoprenoid quinone was extracted using chloroform: methanol (2:1, v/v) as described previously (Collins and Jones, 1981), and identified by reverse-phase HPLC (Collins and Jones, 1981) using a reversed-phase Hydrosphere C18 column (150 × 2 mm). Polar lipids of strain JP1^T were extracted and analyzed as described by Tindall *et al*. (2007). The polar lipid extract was separated by two-dimensional TLC on a silica gel 60 F_{254} plate (Merck, USA) with two solvents: chloroform/methanol/water (65:25:4, v/v) for the first direction and chloroform/methanol/acetic acid/water (80:12:15:4, v/v) for the second direction. Polar lipids

Table 1. Differential characteristics of strain JP1^T and phylogenetically related species of the genus *Dyella*

Strain: 1, JP1^T; 2, *D. koreensis* KACC 11427^T; 3, *D. marensis* KACC 14035^T; 4, *D. soli* KACC 12747^T; 5, *D. ginsengisoli* KACC 12866^T. All of the data were obtained in this study. All strains were positive for leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities, and α-glucosidase, and utilize D-xylose, D-glucose, *N*-acetylglucosamine, and D-maltose. All taxa were negative for lipase, trypsin, *β*-glucuronidase, indole production, and urease activities, and did not assimilate L-arabinose, D-mannitol, potassium gluconate, adipic acid, trisodium citrate, and phenylacetic acid. All strains could not utilize glycerol, L-arabinose, D-ribose, L-xylose, D-adonitol, methyl-*β*-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-*α*-D-mannopyranoside, D-melezitose, xylitol, D-tagatose, D-fucose, D-arabitol, L-arabitol, potassium gluconate, and potassium 5-ketogluconate. Symbol: +, positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4	5
Motility	+	–	+	+	+
Catalase	+	–	+	+	+
Oxidase	–	–	–	+	+
Assimilation (API 50CH) of:					
Erythritol	–	–	+	–	–
D-Arabinose, D-raffinose, D-lyxose	–	+	–	–	–
D-Fructose, D-mannose	+	+	+	–	–
D-Galactose, methyl-α-D-xylopyranoside, amygdalin, arbutin, salicin, D-lactose, D-melibiose, D-saccharose, D-turanose, L-fucose, potassium 2-ketogluconate	–	+	+	–	–
Asculin, D-cellobiose, D-trehalose, glycogen, gentiobiose	–	+	+	–	+
Inulin	+	–	–	–	–
Starch	–	+	+	+	+
Biochemical characteristics (API 20NE):					
Nitrate reduction	–	+	–	–	–
Arginine dihydrolase	–	–	–	+	–
Gelatin	–	+	+	–	+
Assimilation of (API 20NE):					
Capric acid	+	–	–	–	–
Malic acid	–	+	–	–	–
Enzyme activities (API ZYM):					
Alkaline phosphatase	–	+	+	–	+
Esterase (C4)	–	–	–	+	+
Esterase lipase (C8)	w	–	–	–	+
Cystine arylamidase	w	w	–	–	+
α-Chymotrypsin	–	–	+	–	w
α-Galactosidase	–	+	–	–	–
β-Galactosidase	–	+	+	–	–
N-Acetyl-*β*-glucosaminidase	+	+	–	–	+
α-Mannosidase	w	–	–	–	–
α-Fucosidase	–	+	+	–	–
DNA G+C content (mol%)	62.1	63.8	66.2	67.0	63.0

were detected by spraying four reagents (Tindall, 1990), as follows: 10% ethanolic molybdatophosphoric acid reagent for total lipids, ninhydrin reagent for amino-containing lipids and Zinzadze reagent for phospholipids. The phospholipids were also identified by one-dimensional TLC with four standard compounds (Sigma, USA): phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG).

Results and Discussion

Morphological, physiological, and biochemical characteristics

Strain JP1^T grew at 10–37°C (optimum, 30°C), at pH 5–7 (optimum, pH 6–7) and in the presence of 0–1% NaCl (optimum, 0%). Colonies grown on R2A agar were circular, convex, clear-margined and yellow-colored. Cells were Gram-negative, aerobic, rod-shaped, and motile. The hydrolysis of cellulose was not observed in the CMC medium stained with trypan blue, indicating that strain JP1^T does not have cellulose-degrading activity. A comparison of the physiological and biochemical characteristics between strain JP1^T and the type strains of four closely related *Dyella* species are summarized in Table 1.

Phylogenetic and genomic analysis

The 16S rRNA gene sequence of strain JP1^T (1405 bp; KF-709700) was compared with 16S rRNA sequences of other reported strains in the genus *Dyella* using the EzTaxon-e server (Kim *et al*., 2012). The 16S rRNA gene sequence analysis indicated that strain JP1^T belongs to the genus *Dyella*, with the highest sequence similarities to sequences from *D. koreensis* BB4^T (97.58%), *D. marensis* CS5-B2^T (97.51%), *D. soli* JS12-10^T (97.51%), *D. ginsengisoli* Gsoil 3046^T (97.08%), *D. japonica* XD53^T (96.94%), and *D. jiangningensis* SBZ3-12^T (96.94%). As shown in the phylogenetic tree based on the neighbor-joining method (Fig. 1), strain JP1^T lay within the radiation of the genus *Dyella*, and formed a monophyletic cluster with *D. koreensis* BB4^T and *D. marensis* CS5-B2^T. The genomic G+C content of strain JP1^T was 62.1 mol%. DNA relatedness values among strain JP1^T and its four closest relatives (16S rRNA gene sequence similarity >97%),

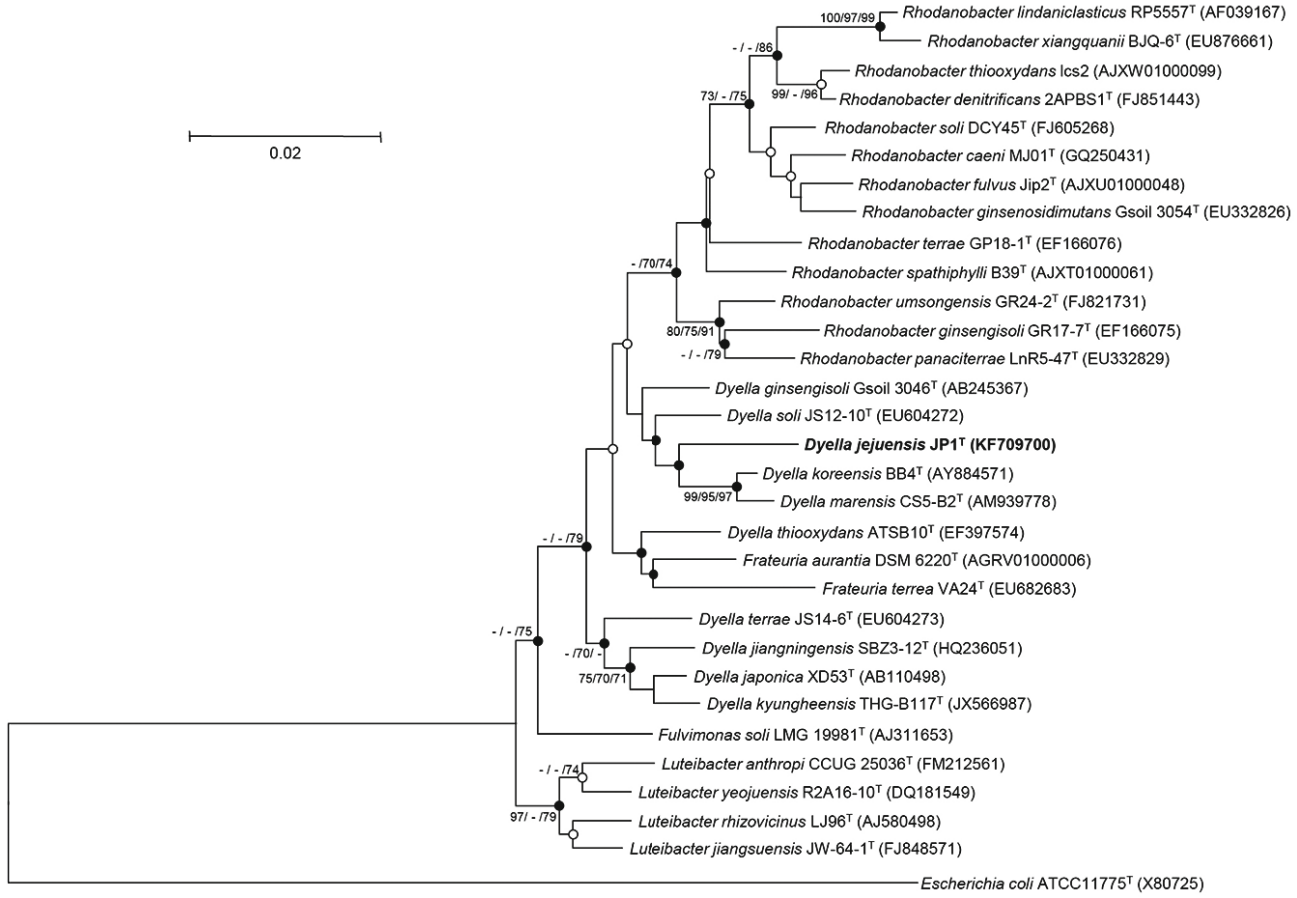

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationship between strain JP1^T, representative species of the genus *Dyella* and related taxa. The tree was generated based on the neighbor-joining method. Filled circles indicate generic branches that were also recovered in the maximum-likelihood/maximum-parsimony methods. Open circles indicate nodes also recovered either by the maximum-likelihood or maximum-parsimony methods. *Escherichia coli* ATCC 11775^T (GenBank no. X80725) served as an out-group. The numbers at nodes indicate bootstrap values, calculated by neighbor-joining/maximum-likelihood/maximum-parsimony probabilities in percent (%) and only values above 70% are shown. GenBank accession nos. are shown in parentheses. Bar, 0.02 substitutions per nucleotide.

namely *D. soli* JS12-10^T, *D. koreensis* BB4^T, *D. marensis* CS5-B2^T, and *D. ginsengisoli* Gsoil 3046^T were 10.0±4.0, 6.4±1.5, 5.1±2.0, and 5.3±4.4%, respectively. The low DNA-DNA relatedness values (<70%) between strain JP1^T and the reference species of *Dyella* confirmed that strain JP1^T represents a distinct species (Wayne *et al.*, 1987).

Chemotaxonomy

The predominant cellular fatty acids of strain JP1^T were iso-$C_{16:0}$ (20.2%), iso-$C_{17:1}$ ω9c (18.4%), and iso-$C_{15:0}$ (18.1%). A large proportion of these three fatty acids have also been reported for other *Dyella* species (Jung *et al.*, 2009; Zhao *et al.*, 2013). The cellular fatty acid contents of strain JP1^T and four *Dyella* species are shown in Table 2. The major respiratory quinone of strain JP1^T was ubiquinone-8, which has also been recognized in other species of the genus *Dyella* (Zhao *et al.*, 2013). The polar lipid profile of strain JP1^T comprised PE, PG, DPG, unidentified aminolipids (AL1-2), aminophospholipids (APL1-2), and unidentified lipids (UL1-5) (Supplementary data Fig. S1). This is similar to corresponding profiles of other species in the genus *Dyella* that contain PE, PG, DPG, ALs, APLs, and ULs (Anandham *et al.*, 2011; Son *et al.*, 2013). Taken together, strain JP1^T was similar to known members of the genus *Dyella*.

Taxonomic conclusion

In conclusion, strain JP1^T differed from *D. soli* JS12-10^T by the low 16S rRNA gene sequence similarity, different phenotypic characteristics, acid production from carbon sources, enzyme activity, and chemotaxonomic properties such as cellular fatty acid composition. Based on the phenotypic, genotypic, and phylogenetic characteristics, strain JP1^T is a novel species in the genus *Dyella*, for which the name *Dyella jejuensis* sp. nov. is proposed.

Table 2. Cellular fatty acid profiles of strain JP1^T and type strains of closely related species of the genus *Dyella*
1, JP1^T; 2, *D. koreensis* KACC 11427^T; 3, *D. marensis* KACC 14035^T; 4, *D. soli* KACC 12747^T; 5, *D. ginsengisoli* KACC 12866^T. Data were obtained in this study. All strains were grown on R2A agar for 48 h at 30°C. –, Not detected; tr, Trace (<1 % of the total).

Fatty acid	1	2	3	4	5
iso-$C_{11:0}$	3.3	5.2	4.4	5.4	4.7
iso-$C_{11:0}$ 3-OH	3.5	7.5	5.0	6.6	5.5
$C_{12:0}$ 3-OH	1.1	tr	tr	–	–
iso- $C_{12:0}$ 3-OH	1.1	1.7	tr	1.5	2.3
iso- $C_{13:0}$ 3-OH	2.1	3.0	3.2	3.6	2.5
$C_{14:0}$	tr	tr	tr	tr	tr
iso-$C_{14:0}$	1.6	tr	tr	1.4	2.2
anteiso-$C_{15:0}$	6.3	3.7	2.2	3.8	8.8
iso-$C_{15:0}$	18.1	9.4	14.3	21.4	6.6
$C_{16:0}$	2.5	3.4	2.8	1.6	2.6
iso-$C_{16:0}$	20.2	17.6	19.2	19.5	27.3
iso-$C_{16:1}$ H	1.6	tr	tr	1.1	2.0
$C_{17:0}$ cyclo	–	tr	2.5	–	–
anteiso-$C_{17:0}$	2.0	2.2	1.4	tr	1.8
iso-$C_{17:0}$	6.1	7.2	13.9	8.3	4.3
iso- $C_{17:0}$ 3-OH	–	1.1	1.1	tr	tr
iso-$C_{17:1}$ ω9c	18.4	15.5	16.7	19.1	19.0
$C_{18:0}$	–	tr	tr	–	1.1
iso-$C_{18:0}$	1.8	2.5	1.7	tr	2.0
Summed feature 3*	3.61	4.3	5.2	3.2	3.8

*Summed feature 3 contains $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c.

Description of *Dyella jejuensis* sp. nov.

Dyella jejuensis: jejuensis (je.ju.en'sis. N.L. fem. adj. jejuensis). Cells are Gram-negative, aerobic, motile, and rod-shaped (0.2–0.4 × 1.2–2.0 μm). Colonies are circular, convex, clear-margined, and yellow-colored on R2A agar medium. Growth occurs at 20–37°C, pH 5–7 and in the presence of 0–1% (w/v) NaCl on R2A broth and agar. Positive results for the test for catalase activity and negative results for tests for nitrate reduction, indole production, and oxidase and arginine dihydrolase activities. The following substrates are utilized: D-xylose, D-glucose, D-fructose, D-mannose, *N*-acetylglucosamine, D-maltose, inulin, and capric acid. The following substrates are not utilized for growth: glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, culcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, amygdalin, arbutin, asculin, salicin, D-cellobiose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Positive for the following enzyme activities: leucine arylamidase, valine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-glucosidase, and *N*-acetyl-glucosamine. Weak positive activities for esterase lipase (C8), cystine arylamidase, and α-mannosidase. Negative for the following enzyme activities: alkaline phosphatase, esterase (C4), lipase (C14), trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, and *α*-fucosidase. The genomic DNA G+C content of strain JP1^T is 62.1 mol%. The major fatty acids are iso-$C_{16:0}$, iso-$C_{17:1}$ ω9c, and iso-$C_{15:0}$ when grown on R2A agar at 30°C for 48 h. The ubiquinone-8 is the major component of the respiratory quinone system. The major polar lipids of strain JP1^T are comprised of phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, unidentified aminolipids and unidentified aminophospholipids. The type strain, JP1^T (=KACC 17701^T =JCM 19615^T), was isolated from soil collected from Hallasan Mountain in Jeju Island.

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References

An, D.S., Im, W.T., Yang, H.C., Yang, D.C., and Lee, S.T. 2005. *Dyella koreens*is sp. nov., a β-glucosidase-producing bacterium. *Int. J. Syst. Evol. Microbiol.* **55**, 1625–1628.

Anandham, R., Kwon, S.W., Indira Gandhi, P., Kim, S.J., Weon, H.Y., Kim, Y.S., Sa, T.M., Kim, Y.K., and Jee, H.J. 2011. *Dyella thiooxydans* sp. nov., a facultatively chemolithotrophic, thiosulfate-oxidizing bacterium isolated from rhizosphere soil of sunflower (*Helianthus annuus* L.). *Int. J. Syst. Evol. Microbiol.* **61**, 392–398.

Chang, H.W., Nam, Y.D., Jung, M.Y., Kim, K.H., Roh, S.W., Kim, M.S., Jeon, C.O., Yoon, J.H., and Bae, J.W. 2008. Statistical superiority of genome-probing microarrays as genomic DNA-DNA hybridization in revealing the bacterial phylogenetic relationship compared to conventional methods. *J. Microbiol. Methods* **75**, 523–530.

Collins, M.D. and Jones, D. 1981. A note on the separation of natural mixtures of bacterial ubiquinones using reverse-phase partition thinlayer chromatography and high performance liquid chromatography. *J. Appl. Bacteriol.* **51**, 129–134.

Gonzalez, J.M. and Saiz-Jimenez, C. 2002. A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ. Microbiol.* **4**, 770–773.

Hyun, D.W., Kim, M.S., Shin, N.R., Kim, J.Y., Kim, P.S., Whon, T.W., Yun, J.H., and Bae, J.W. 2013. *Shimia haliotis* sp. nov., a bacterium isolated from the gut of an abalone, *Haliotis discus hannai*. *Int. J. Syst. Evol. Microbiol.* **63**, 4248–4253.

Jukes, T.H. and Cantor, C.R. 1969. Evolution of Protein Molecules. *In* Munro, H.N. (eds). pp. 21–132. New York, Academic Press, USA.

Jung, H.M., Ten, L.N., Kim, K.H., An, D.S., Im, W.T., and Lee, S.T. 2009. *Dyella ginsengisoli* sp. nov., isolated from soil of a ginseng field in South Korea. *Int. J. Syst. Evol. Microbiol.* **59**, 460–465.

Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi, H., Won, S., and Chun, J. 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, B.Y., Wheon, H.Y., Lee, K.H., Seok, S.J., Kwon, S.W., Go, S.J., and Stackebrandt, E. 2006. *Dyella yeojuensis* sp. nov., isolated from greenhouse soil in Korea. *Int. J. Syst. Microbiol.* **56**, 2079–2082.

Kluge, A.G. and Farris, F.S. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* **18**, 1–32.

Lane, D.J. 1991. 16S/23S rRNA sequencing. Nucleic Acid Techniques in Bacterial Systematics. *In* Stackebrandt, E. and Goodfellow, M. (eds.). pp. 115–175. Chichester, Wiley, UK.

Lee, D.W. and Lee, S.D. 2009. *Dyella marensis* sp. nov., isolated from cliff soil. *Int. J. Syst. Evol. Microbiol.* **59**, 1397–1400.

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

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor, New York, Cold Spring Harbor Laboratory Press, USA.

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

Son, H.M., Yang, J.E., Yi, E.J., Park, Y.J., Won, K.H., Kim, J.H., Han, C.K., Kook, M.C., and Yi, T.H. 2013. *Dyella kyungheensis* sp. nov., isolated from soil of a cornus fruit field. *Int. J. Syst. Evol. Microbiol.* **63**, 3807–3811.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.

Tindall, B.J. 1990. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol. Lett.* **66**, 199–202.

Tindall, B.J., Sikorski, J., Smibert, R.M., and Kreig, N.R. 2007. Phenotypic characterization and the principles of comparative systematics. Methods for General and Molecular Microbiology 3rd edn. pp. 330–393. *In* Reddy, C.A., Beveridge, T.J., Breznak, J.A., Marzluf, G., Schmidt, T.M., and Snyder, L.R. (eds.) ASM Press, Washington, D.C., USA.

Tittsler, B.P. and Sandholzer, L.A. 1936. Use of semi-solid agar for the detection of bacterial motility. *J. Bacteriol.* **31**, 575.

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., and *et al.* 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* **37**, 463–464.

Weon, H.Y., Anandham, R., Kim, B.Y., Hong, S.B., Jeon, Y.A., and Kwon, S.W. 2009. *Dyella soli* sp. nov. and *Dyella terrae* sp. nov., isolated from soil. *Int. J. Syst. Evol. Microbiol.* **59**, 1685–1690.

Xie, C.H. and Yokota, A. 2005. *Dyella japonica* gen. nov., sp. nov., a γ-proteobacterium isolated from soil. *Int. J. Syst. Evol. Microbiol.* **55**, 753–756.

Zhao, F., Guo, X.Q., Wang, P., He, L.Y., Huang, Z., and Sheng, X.F. 2013. *Dyella jiangningensis* sp. nov., a γ-proteobacterium isolated from the surface of potassium-bearing rock. *Int. J. Syst. Evol. Microbiol.* **63**, 3154–3157.