Dyella agri sp. nov., isolated from reclaimed grassland soil

Dhiraj Kumar Chaudhary and Jaisoo Kim*

Abstract

A novel strain, DKC-1T, was isolated from reclaimed grassland soil and was characterized taxonomically by a polyphasic approach. Strain DKC-1T was a Gram-staining-negative, light-yellow-coloured and rod shaped bacterium, motile with polar flagellum. It was able to grow at 20–37°C, at pH 4.5–9.0 and with 0–3 % (w/v) NaCl concentration. Based on the 16S rRNA gene sequence analysis, strain DKC-1T formed a clade within the members of the genus Dyella and showed highest sequence similarities to Dyella japonica XD53T (98.36 %), Rhodanobacter aciditrophus sjH1T (97.92 %), Rhodanobacter koreensis THG-DD7T (97.74 %), Dyella kyungheensis THG-B117T (97.65 %) and Rhodanobacter terrae GP18-1T (97.40 %). The only respiratory quinone was ubiquinone-8. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, diphasphatidylglycerol and phosphatidyl- \( \text{N} \)-methyl ethanolamine. The predominant fatty acids of strain DKC-1T were iso-C\(_{15:0}\), iso-C\(_{16:0}\), summed feature 9 (iso-C\(_{17:1}\) \( \omega \)9c and/or C\(_{16:0}\) 10-methyl), iso-C\(_{17:0}\), iso-C\(_{11:0}\) 3-OH and iso-C\(_{11:0}\). The genomic DNA G+C content of this novel strain was 63.1 mol%. The DNA–DNA relatedness between strain DKC-1T and its reference strains (D. japonica XD53T, R. aciditrophus sjH1T, R. koreensis THG-DD7T, D. kyungheensis THG-B117T and R. terrae GP18-1T) was 52.3, 44.7, 38.7, 49.0 and 32.7 %, respectively, which falls below the threshold value of 70 % for the strain to be considered as novel. The morphological, physiological, chemotaxonomic and phylogenetic analyses clearly distinguished this strain from its closest phylogenetic neighbours. Thus, strain DKC-1T represents a novel species of the genus Dyella, for which the name Dyella agri sp. nov. is proposed. The type strain is DKC-1T (=KEMB 9005-571T=KACC 19176T=JCM 31925T).

The genus Dyella of the family Xanthomonadaceae, belonging to the phylum Proteobacteria, was proposed by Xie and Yokota [1]. At the time of writing, the genus Dyella comprises 12 species with validly published names (http://www.bacterio.net/dyella.html), among which Dyella japonica is the type species of the genus [1].

The species of the genus Dyella are yellow-pigmented, aerobic, motile by polar flagella, rod-shaped and Gram-staining-negative bacteria. Cells are catalase-positive and oxidase-negative. Chemotaxonomically, species of the genus Dyella are characterized by the presence of ubiquione-8 as the predominant respiratory quinone; iso-C\(_{16:0}\), iso-C\(_{15:0}\), iso-C\(_{17:1}\) \( \omega \)9c, iso-C\(_{17:0}\), C\(_{11:0}\) iso 3-OH, C\(_{13:0}\) iso 3-OH and C\(_{17:0}\) iso 3-OH as the major fatty acids; and DNA G+C contents ranging from 62.0 to 64.0 mol% [1, 2]. The members of the genus Dyella have been isolated from a wide variety of habitats including soil, a fruit field, a ginseng field, a potassium-bearing rock, rhizosphere, cliff soil, and greenhouse soil [1–7]. Recently, Dyella lipolytica [8] and Dyella humi [9] were isolated from forest soil.

This study describes strain DKC-1T, a novel member of the genus Dyella, isolated from reclaimed grassland soil in Hwaseong, South Korea. Based on the results obtained from phenotypic, genotypic, chemotaxonomic and phylogenetic analyses, strain DKC-1T is proposed as a representative of a novel species of the genus Dyella.

Strain DKC-1T was isolated using a modified culture technique with 6-well polycarbonate transwell plates as described previously [10]. For pure colonies, the bacterial growth was repeatedly streaked on R2A medium (MB Cell). The pure cultures obtained on R2A agar plates were stored at 4°C for short-term maintenance and restreaked every 2 weeks. Before use, the strain was routinely cultivated by streaking on R2A agar and incubating at 28 °C for 48 h. For long-term maintenance, the cultures were preserved at −70°C in R2A broth supplemented with 20 % (v/v) glycerol.

Genomic DNA of strain DKC-1T was extracted according to the procedure described by Marmur [11]. The 16S rRNA gene was amplified by PCR using forward primer 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and reverse primer

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DKC-1T is KY313412. Two supplementary figures are available with the online Supplementary Material.
Cloud server (http://ezbiocloud.net/) [13] revealed that rRNA gene sequences from GenBank by using the EZBioCloud server [13]. The comparison of the evolutionary distances were calculated using the Kimura two-joining method [16], the maximum-parsimony algorithm [15], and the maximum-likelihood (Fig. 1), neighbour-joining and maximum-likelihood algorithms. Phylogenetic trees were subjected to multiple alignment with the sequences of closely related bacteria using CLUSTAL X 2.1 [14]. After multiple alignment, gaps at the 5′ and 3′ ends were deleted using the software package BioEdit [15]. Phylogenetic trees were reconstructed by three different methods: the neighbour-joining method [16], the maximum-parsimony algorithm [17] and the maximum-likelihood algorithm [18], using the MEGA6 program [19]. During the phylogenetic analysis, evolutionary distances were calculated using the Kimura two-parameter model [20], and bootstrap values were calculated based on 1000 replications [21]. The comparison of the resulting 16S rRNA gene sequence with the available 16S rRNA gene sequences from GenBank by using the EZBioCloud server (http://ezbiocloud.net/) [13] revealed that strain DKC-1 T represents a member of the family Xanthomonadaceae and showed highest sequence similarities with D. japonica XD53 T (98.36 %), Rhodanobacter aciditrophicus sjiH T (97.92 %), Rhodanobacter koreensis THG-DD7 T (97.74 %), Dyella kyungheensis THG-B117 T (97.65 %) and Rhodanobacter terrae GP18-1 T (97.40 %). The pairwise sequence similarities to all other members of the family Xanthomonadaceae were below 97.30 %. These values are at the level suggested to allocate a strain to a novel species [22, 23]. Furthermore, phylogenetic tree analyses using the maximum-likelihood (Fig. 1), neighbour-joining and maximum-parsimony methods showed that strain DKC-1 T formed a distinct clade with the members of the genus Dyella (D. japonica XD53 T, Dyella terrae JS14-6 T, Dyella jiangningensis SBZ3-12 T and D. kyungheensis R2A16-10 T). Based on the 16S rRNA gene sequence similarities and the phylogenetic tree analysis of D. japonica XD53 T (=KACC 11396 T), R. aciditrophicus sjiH T (=KCTC 42660 T), R. koreensis THG-DD7 T (=KACC 17560 T), D. kyungheensis THG-B117 T (=KACC 16981 T) and R. terrae GP18-1 T (=KACC 11761 T) were selected as reference strains for DNA–DNA hybridizations, physiological tests, biochemical studies and chemotaxonomic analysis.

Culture media was assessed by using R2A agar (MB Cell), nutrient agar (NA; Oxoid), tryptone soya agar (TSA; Oxoid), soylent MacConkey agar (MA; Oxoid), marine agar 2216 (Difco), Luria–Bertani agar (LBA; Oxoid) and brain heart infusion agar (BHI; BD Bacto). Growth at different temperatures was observed on R2A plates incubated at 10–40 °C (10, 15, 20, 25, 28, 30, 32, 37 and 40 °C). Growth at different NaCl concentrations was examined by using R2A broth as the basal medium supplemented with 0–5 % NaCl (w/v, at intervals of 0.5 %) [26]. The optimum pH for growth was assessed in R2A broth and the medium was adjusted to pH 4.0–12.0 (at intervals of 0.5 pH unit) prior to autoclaving using citrate/NaH2PO4 buffer, 0.1 M (for pH range 4.0–5.3), phosphate buffer, 0.1 M (for pH range 6–7.5), Tris buffer, 0.2 M (for pH range 8–10) [27], and 5 M NaOH (for pH range 10.5–12.0). Testing of pH after autoclaving revealed only minor changes. Anaerobic growth was tested on R2A agar incubated at 28 °C for 14 days by using a BBL anaerobic jar with a GasPak EZ gas generating container (Becton Dickinson). Endospore formation was examined according to the Schaeffer–Fulton method by staining bacterial cells with malachite green [26]. Catalase and oxidase tests were performed as described previously [10]. The indole test was conducted by adding Kovac’s reagent, and hydrogen sulphide production was tested by observing the presence or absence of blackening in SIM medium (Oxoid). The methyl red/Voges–Proskauer test was performed in MR–VP broth and incubated at 28 °C for 48 h [28]. The DNA degradation test was performed by flooding growth on a DNA agar plate with 1 M HCl [29]. Starch hydrolysis was performed as described by Tindall et al. [30], and Tween 40, Tween 80 and aesculin hydrolysis tests were conducted according to the methods of Smibert and Krieg [31]. Gelatin and casein hydrolysis tests were assessed according to the procedure described by Cowan and Steel [32]. Hydrolysis tests for carboxymethylcellulose, tyrosine and chitin were conducted using R2A media supplemented with carboxymethylcellulose (1 %, w/v), tyrosine (0.1 %, w/v) and chitin (1 %, w/v) [29]. Other physiological, biochemical and enzymic activities were assessed using API 20NE, API ID 32GN and API ZYM test kits (bioMérieux) according to the manufacturer’s instructions.

The polar lipids were analysed from freeze-dried cells as described by Minnikin et al. [33]. Appropriate detection reagents [33, 34] were used to visualize the spots: phosphomolybdic acid reagent, 5 % (w/v) solution in ethanol (Sigma–Aldrich), was used to detect total polar lipids; ninhydrin reagent (0.2 %, w/v, solution; Sigma Life Science) was used to detect amino acids; Zinzadze reagent (molybdenum blue spray reagent, 1.3 %; Sigma Life Sciences) was used to detect phospholipids; and α-naphthol reagent was used to detect glycolipids. The respiratory quinones were extracted with chloroform/methanol (2 : 1; v/v), and analysed by HPLC [35, 36]. For the analysis of fatty acids, strain DKC-1 T and its reference strains were cultured on R2A agar plates at 28 °C for 3 days. Biomass of all strains was harvested after the same growth phase (late exponential phase) and subjected to saponification and methylation, and was extracted using the
standard MIDI protocol (Sherlock Microbial Identification System, version 6.0B). Analysis of fatty acids was performed with a gas chromatograph (HP 6890 Series GC System; Hewlett Packard), and the fatty acids were identified using the TSBA6 database of the Microbial Identification System (MIDI) [37].

**Fig. 1.** Maximum-likelihood tree based on nearly complete 16S rRNA gene sequences showing the phylogenetic position of strain DKC-1T among closely related taxa. Filled circles indicate nodes recovered in neighbour-joining and maximum-parsimony trees. The numbers at the nodes indicate the percentage of 1000 bootstrap replicates; only values >50 % are shown. GenBank accession numbers are given in parentheses. *Brevundimonas diminuta* DSM 7234T was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.
The measurement of the G+C content of the chromosomal DNA for strain DKC-1T was performed according to the procedure described by Mesbah et al. [38]. DNA–DNA hybridization was performed between strain DKC-1T and five reference strains (D. japonica KACC 11396T, R. aciditrophicus KCTC 42660T, R. koreensis KACC 17650T, D. kyungheensis KACC 16981T and R. terrae KACC 11761T) according to the method described by Ezaki et al. [39].

### Table 1. Differentiating characteristics of strain DKC-1T and related species belonging to the family Xanthomonadaceae

<table>
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<tr>
<th>Characteristic</th>
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<td>Colour</td>
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<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
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<td>pH range for growth</td>
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<td>5.5–8.0</td>
<td>3.5–9.0</td>
<td>6.0–8.0</td>
<td>5.5–9.5</td>
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<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Highest NaCl tolerance (% w/v)</td>
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<td>2.0</td>
<td>1.5</td>
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<td>++</td>
<td>–/+</td>
<td>++</td>
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<td>Hydrolysis of:</td>
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<tr>
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<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
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<td>–</td>
<td>–</td>
<td>+</td>
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<td>+</td>
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<tr>
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<td>+</td>
<td>w</td>
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<td>+</td>
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<td>+</td>
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<td>Enzyme activity</td>
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<td>Cystine arylamidase</td>
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<td>α-Glucosidase</td>
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<tr>
<td>β-Glucosidase</td>
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<td>w</td>
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<td>+</td>
<td>+</td>
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<td>N-Acetyl-β-glucosaminidase</td>
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<td>+</td>
<td>–</td>
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<tr>
<td>α-Mannosidase</td>
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<td>α-Fucosidase</td>
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<tr>
<td>d-Mannose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>w</td>
<td>–</td>
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<tr>
<td>Maltose</td>
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<td>+</td>
<td>+</td>
<td>w</td>
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<td>+</td>
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<td>Malic acid</td>
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<td>–</td>
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<td>Assimilation from API ID 32 GN</td>
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</tr>
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<td>Glycogen</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
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<tr>
<td>L-Serine</td>
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<td>–</td>
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<td>Salicin</td>
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<td>–</td>
<td>w</td>
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<td>–</td>
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<td>Propionic acid</td>
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<td>–</td>
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<td>L-Proline</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>63.1</td>
<td>(63.4)</td>
<td>(69.2)</td>
<td>(65.2)</td>
<td>(64.8)</td>
<td>(62.5)</td>
</tr>
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</table>

Strains: 1, DKC-1T; 2, D. japonica KACC 11396; 3, R. aciditrophicus KCTC 42660; 4, R. koreensis KACC 17650; 5, D. kyungheensis KACC 16981T; 6, R. terrae KACC 11761T. All data are from the present study, except for those indicated in parentheses, which were taken from the literature [1, 2, 40, 42, 43]. +, Positive; –, negative; w, weakly positive.
Salmon sperm was used as the negative control, and photobiotin was used as a probe to label DNA of strain DKC-1T. The values of DNA–DNA relatedness were determined fluorometrically in microplate wells using a 1420 Multilabel Counter (Perkin Elmer). Additionally, for reverse hybridization, each reference strain was labelled with photobiotin and used as a probe to strain DKC-1T. All the assays were carried out in triplicate.

The light-yellow-coloured colonies of strain DKC-1T grew well on R2A, TSA and NA. A transmission electron photomicrograph (Fig. S1, available with the online Supplementary Material) of a cell of strain DKC-1T showed a rod-shaped structure containing a single, polar, attached flagellum. Growth was observed at temperatures of 20–37°C and at pH 4.5–9.0. Strain DKC-1T could tolerate up to 3% (w/v) NaCl concentration. Strain DKC-1T hydrolysed carboxymethylcellulose, hydrolysed ascorbic acid and tyrosine weakly, and could not hydrolyse casein, starch, gelatin, DNA or chitin. Other phenotypic characteristics of strain DKC-1T are given in the species description and illustrated along with those of other closely related species in Table 1.

The only isoprenoid quinone of strain DKC-1T was ubiquinone 8 (Q-8), as found in all known members of the genus Dyella [1, 2]. Strain DKC-1T contained phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidyl-N-methylethanolamine as the major polar lipids. Additionally, two unidentified aminophospholipids, one unidentified phospholipid and two unidentified lipids could not be separated using the MIDI system. Summed feature 3 contained iso-C15:0 (22.1%), iso-C16:0 (19.1%), summed feature 9 contained iso-C17:1ω9c and/or C16:0 10-methyl.

Table 2. Cellular fatty acid profiles of DKC-1T and closely related reference strains of the family Xanthomonadaceae

<table>
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<th>Fatty acid</th>
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<th>6</th>
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<tbody>
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<td>Saturated</td>
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<tr>
<td>C14:0</td>
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<td>–</td>
<td>–</td>
<td>0.2</td>
<td>0.4</td>
<td>–</td>
</tr>
<tr>
<td>C16:0</td>
<td>2.7</td>
<td>2.4</td>
<td>3.1</td>
<td>3.6</td>
<td>1.8</td>
<td>1.0</td>
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<tr>
<td>C18:0</td>
<td>0.2</td>
<td>–</td>
<td>0.4</td>
<td>0.3</td>
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<td>0.7</td>
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<tr>
<td>anteiso-C15:0</td>
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<td>2.1</td>
<td>9.8</td>
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<td>1.0</td>
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<td>0.8</td>
<td>0.4</td>
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<tr>
<td>iso-C11:0</td>
<td>4.1</td>
<td>4.5</td>
<td>3.9</td>
<td>6.7</td>
<td>5.9</td>
<td>7.8</td>
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<td>iso-C14:0</td>
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<tr>
<td>iso-C17:0</td>
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<td>iso-C18:0</td>
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<tr>
<td>iso-C19:0</td>
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<tr>
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<td>–</td>
<td>2.0</td>
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<tr>
<td>iso-C11:0 3-OH</td>
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<tr>
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<td>–</td>
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<td>iso-C13:0 3-OH</td>
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<tr>
<td>iso-C17:0 3-OH</td>
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<td>0.9</td>
<td>–</td>
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<td>0.8</td>
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<td>Summed features*</td>
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<td>22.6</td>
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*Summed features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 3 contained C16:1ω7c and/or C16:1ω6c; summed feature 9 contained iso-C17:1ω9c and/or C16:0 10-methyl.
C_{11:0} 3-OH (4.5 %) and iso-C_{11:0} (4.1 %). The fatty acid profile of strain DKC-1T was similar to that of other members of the family Xanthomonadaceae, indicating that strain DKC-1T is a member of the family Xanthomonadaceae [1–4]. However, the presence of some qualitative and quantitative differences in the contents of the fatty acids clearly distinguished strain DKC-1T from other closely related reference strains (Table 2).

The DNA G+C content of strain DKC-1T was 63.1 mol%, which falls within the range of 62.0 to 64.0 mol% for the genus Dyella [1, 2]. DNA–DNA hybridization values between strain DKC-1T and five reference strains (D. japonica KACC 11396T, R. aciditrophus KCTC 42660T, R. koreensis KACC 17650T, D. kyungheensis KACC 16981T and R. terrae KACC 11761T) were 52.3±1.5 % (reciprocal, 47.3±1.2 %), 44.7±2.1 % (reciprocal, 45.7±0.6 %), 38.7±1.5 % (reciprocal, 32.7±2.1 %), 40.9±1.0 % (reciprocal, 50.7±2.5 %) and 32.7±4.9 % (reciprocal, 30.0±2.6 %), respectively. These values were lower than the threshold value of 70 %, which clearly demonstrated that strain DKC-1T differs genetically from other members of the family Xanthomonadaceae at the species level [41].

Based on the distinct phylogenetic, phenotypic, biochemical and chemotaxonomic data mentioned above, strain DKC-1T represents a novel species within the genus Dyella, for which the name Dyella agri sp. nov. is proposed.

**DESCRIPTION OF DYELLA AGRI SP. NOV.**

*Dyella agri* (a’gri. L. gen. n. agri of grassland).

Cells are Gram-staining negative, aerobic, motile with a polar flagellum, non-spore forming and rod-shaped. The size of the cells is 0.5 to 0.8 µm wide and 1.5 to 2.0 µm long. The size of the colonies is 1.0–2.0 mm in diameter after incubation on R2A agar at 28 °C for 5 days. Colonies on R2A are light yellow, circular, smooth, convex and opaque with entire margin. Cells grow on R2A, TSA and NA; grow weakly on LB and brain heart infusion agar; and do not grow on MacConkey agar or marine agar. Cell growth can be observed at temperatures of 20–37 °C (optimum, 25–35 °C) and at pH 4.5–9.0 (optimum, pH 6.0–8.0). The cells grow optimally in the absence of NaCl but can tolerate 3.0 % NaCl in R2A broth. Positive for catalase and oxidase tests but negative for H₂S production, indole production, methyl red and Voges–Proskauer tests. Hydrolyses casein, Tween 40, Tween 80, starch, gelatin, DNA or chitin. Positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, acid phosphatase, β-galactosidase, α-mannosidase and α-fucosidase; weakly positive for α-chymotrypsin, naphthol-AS-BI-phosphohydrolase and α-glucosidase; and negative for esterase (C4), lipase (C14), trypsin, α-galactosidase, β-gluconidase, β-glucosidase and N-acetyl-β-glucomaminidase. Positive for 4-nitrophenyl-β-D-galactopyranoside; weakly positive for fermentation of glucose and aesculin ferric citrate; and negative for nitrate reduction, L-tryptophan, L-arginine, urea and gelatin in the API 20NE test. The cells assimilate D-glucose, N-acetylglucosamine and maltose; and cannot assimilate L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate, malic acid or phenylactic acid. Can utilize N-acetylglucosamine, maltose, L-serine, D-glucose, L-fucose, propionic acid, and L-histidine; utilize sodium acetate and L-proline weakly; and cannot utilize L-arginine, D-ribose, inositol, sucrose, itaconic acids, suberic acid, sodium malonate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, D-mannitol, salicin, melibiose, D-sorbitol, L-arabinose, capric acid, valeric acid, trisodium citrate, potassium 2-ketogluconate, 3-hydroxybutyric acid or 4-hydroxybenzoic acid. Q-8 is the only respiratory quinone. The major polar lipids present are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and phosphatidyl-N-methylethanolamine. Two unidentified aminophospholipids, one unidentified phospholipid and two unidentified lipids are present as the minor polar lipids. The major fatty acids are iso-C_{16:0}, summed feature 9 (iso-C_{17:0}ω9c and/or C_{16:0}10-methyl), iso-C_{17:0}, iso-C_{11:0} 3-OH and iso-C_{11:0}.

The type strain, DKC-1T (=KEMB 9005-571T=KACC 19176T=JCM 31925T), was isolated from reclaimed grassland soil, Hwaseong, South Korea. The DNA G+C content of the type strain is 63.1 mol%.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**Ethical statement**
This study does not describe any experimental work related to humans.

**References**


