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TAXONOMIC DESCRIPTION

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Arenimonas halophila sp. nov., isolated from soil

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Abstract

A Gram-staining-negative, aerobic, non-motile, rod-shaped bacterium, designated CAU 1453^T, was isolated from soil and its taxonomic position was investigated using a polyphasic approach. Strain CAU 1453^T grew optimally at 30 °C and at pH 6.5 in the presence of 1 % (w/v) NaCl. Phylogenetic analysis based on the 16S rRNA gene sequences revealed that CAU 1453^T represented a member of the genus *Arenimonas* and was most closely related to *Arenimonas donghaensis* KACC 11381^T (97.2 % similarity). CAU 1453^T contained ubiquinone-8 (Q-8) as the predominant isoprenoid quinone and iso-$C_{15:0}$ and iso-$C_{16:0}$ as the major cellular fatty acids. The polar lipids consisted of diphosphatidylglycerol, a phosphoglycolipid, an aminophospholipid, two unidentified phospholipids and two unidentified glycolipids. CAU 1453^T showed low DNA–DNA relatedness with the most closely related strain, *A. donghaensis* KACC 11381^T (26.5 %). The DNA G+C content was 67.3 mol%. On the basis of phenotypic, chemotaxonomic and phylogenetic data, CAU 1453^T represents a novel species of the genus *Arenimonas*, for which the name *Arenimonas halophila* sp. nov. is proposed. The type strain is CAU 1453^T (=KCTC 62235^T=NBRC 113093^T).

The genus *Arenimonas*, a member of the family *Xanthomonadaceae* in the class *Gammaproteobacteria* was proposed by Kwon *et al.* [1]. Members of the genus *Arenimonas* are Gram-stain-negative, rod-shaped, aerobic and non-spore forming, have Q-8 as the major isoprenoid quinone and iso-$C_{15:0}$ and iso-$C_{16:0}$ as the predominant cellular fatty acids [1–5]. At the time of writing, this genus includes 12 species with validly published names; *A. donghaensis* [1], *A. oryziterrae* [2], *A. daejeonensis* [3], *A. metalli* [4], *A. aestuarii* [5], *A. malthae* [6], *A. composti* [7], *A. taoyuanensis* [8], *A. daechungensis* [9], *A. maotaiensis* [10], *A. subflava* [11] and *A. soli* [12] and also '*A. alkanexedens*' [13]. They have been isolated from soil (seashore sand, oil-contaminated soil, rice-field soil, estuary sediment, frozen soil and saline–alkaline soil), compost, iron mine and fresh water. During the screening of bacteria from soil samples, a bacterial strain, designated CAU 1453^T, was isolated from a soil sample collected in Modo (37° 32′ 12.28″ N, 126° 24′ 51.47″ E) in the Republic of Korea. The objective of this study was to assess the taxonomic position of this bacterial strain using a polyphasic taxonomy that included the phenotypic, chemotaxonomic and genotypic properties and 16S rRNA gene sequences analysis.

CAU 1453^T was isolated from soil by the dilution plating method using marine agar 2216 (MA; Difco) [14]. After 7 days of incubation under aerobic conditions at 30 °C, a single colony was purified by subculturing on MA and maintained at −80 °C in marine broth 2216 (MB; Difco) supplemented with 25 % (v/v) glycerol. The type strains of the closely related species *A. donghaensis* KACC 11381^T (=HO3 R19^T), *A. aestuarii* KACC 18504^T (=S2-21^T) and *A. daejeonensis* KCTC 12667^T (=T7-07^T) were obtained from the Korean Collection for Type Cultures (KCTC, Jeongeup, Republic of Korea) and the Korean Agricultural Culture Collection (KACC; Suwon, Republic of Korea) and used as reference strains in physiological, biochemical and fatty acid analysis. *A. donghaensis* KACC 11381^T was used for DNA–DNA hybridization.

Genomic DNA was isolated from biomass using a genomic DNA extraction kit (Intron). PCR amplification of 16S rRNA gene was performed following established procedures [15]. The amplified 16S rRNA gene fragment was sequenced using a 3730 automatic DNA sequencer (Applied Biosystems). Multiple sequences were analyzed using the EzBioCloud database (http://www.ezbiocloud.net) [16] and the CLUSTAL_X 2.1 software [17] to calculate

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Abbreviations: API, Analytical Profile Index; APL, aminophospholipid; DPG, diphosphatidylglycerol; GL, glycolipid; KACC, Korean Agricultural Culture Collection; KCTC, Korean Collection for Type Cultures; NBRC, Biological Resource Center; PGL, phosphoglycolipid; PL, phospholipid; Q-8, ubiquinone-8.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU 1453^T is MG214546.

Three supplementary figures are available with the online version of this article.

sequence similarity. Evolutionary distance was determined by the neighbor-joining method described by Jukes and Cantor [18]. Phylogenetic trees were reconstructed using the neighbor-joining [19], least-squares [20], maximum-parsimony [21] and maximum-likelihood [22] algorithms in the PHYLIP package [23]. Branch support in the neighbor-joining tree was estimated by the bootstrap resampling method [24] with 1000 replicates of the neighbor-joining dataset with the SEQBOOT and CONSENSE programs from the PHYLIP package.

Comparative analysis based on 16S rRNA gene sequences of CAU 1453^T (1547 bp) with the related strains' sequences in the GenBank database (access October 2017) revealed that the strain represented a member of the genus *Arenimonas* and was most closely related to *A. donghaensis* KACC 11381^T (97.2 % similarity) followed by *A. aestuarii* KACC 18504^T (96.5 % similarity), *A. daejeonensis* KCTC 12667^T (96.4 % similarity) and other species of the genus *Arenimonas* (93.99–96.39.0 %) (Fig. 1). In addition, the phylogenetic inference was similar to that of the neighbor-joining tree with the least-squares, maximum-parsimony and maximum-likelihood algorithms (Fig. S1, available in the online version of this article).

The DNA G+C content of CAU 1453^T was determined using HPLC, as described by Mesbah *et al.* [25]. The DNA G+C content of CAU 1453^T was 67.3 mol%, a value in the range reported for species within the genus *Arenimonas* [8, 10]. The extent of DNA–DNA relatedness between CAU 1453^T and the most closely related, phylogenetic neighbour, *A. donghaensis* KCTC 12667^T was determined using the fluorometric microplate method described by Ezaki *et al.* [26], as modified by Goris *et al.* [27]. The DNA–DNA relatedness between CAU 1453^T and the most closely related strain, *A. donghaensis* KCTC 12667^T was 26.5±0.5 %. The value is below the 70 % cut-off point suggested by Wayne *et al.* [28] for the purpose of defining genomic species, supporting the hypothesis that CAU 1453^T represents a distinct species.

For the investigation of morphological, physiological and biochemical characteristics, CAU 1453^T was cultivated routinely on MA at 30 °C [29], except for spore formation, which was examined on nutrient sporulation medium [30]. Cell morphology of the isolate was examined under light microscopy (DM 1000; Leica). The presence of flagella on cells from an exponentially growing culture was determined using transmission electron microscopy (JEM 1010; JEOL). Gram staining was performed using the bioMérieux Gram staining kit (bioMérieux). Cell motility was assessed by using stab cultures in semisolid agar tubes and observing the turbidity [31]. The growth at different temperatures (4, 10, 20, 30, 37 and 45 °C) was evaluated on MA by incubating the cultures in an aerobic incubator (MIR-253; Sanyo) and in an anaerobic chamber (Bactron; Sheldon) for 72 h. The pH range and NaCl tolerance were tested in MB at 30 °C for 7 days by measuring the turbidity. The pH of MB was adjusted to pH 4.0 to 11.0 at 0.5 pH-unit intervals by adding 1 M HCl or 1 M NaOH after sterilization. The NaCl tolerance at various concentrations of NaCl (0–15 %; w/v, at increments of 1.0 %) was examined by culturing in MB prepared according to the BD Difco formula except that NaCl was excluded and that 0.45 % (w/v) $MaCl_2.6H_2O$ and 0.06 % (w/v) KCl was added.

Oxidase activity was determined from the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine [32] and catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. Hydrolysis of casein, starch, gelatin and urea and nitrate reduction were assessed as described previously [33]. The API ZYM, API 20NE and API 50CH systems (bioMérieux) were used to determine enzyme activities after incubation at 37 °C and utilization of carbohydrates after incubation at 30 °C, according to the manufacturer's recommendation.

The cultural, biochemical and physiological characteristics of CAU 1453^T are listed in Table 1 and in the species description. Cells were Gram-staining-negative, rod-shaped, non-motile, non-flagellated, non-spore forming and aerobic, 1.3–2.2 µm in length and 0.2–0.4 µm in width. After incubation for 72 h at 30 °C, colonies on MA were opaque, white, circular, smooth and slightly convex. Flagella were not observed (Fig. S2). CAU 1453^T could grow at 10–37 °C with optimal growth at 30 °C. The pH range for growth was 5.5–9.5 with optimal pH 6.5. Growth occurred in the presence of 0–7 % (w/v) NaCl with optimal growth at 1 %. CAU 1453^T showed positive reactions for catalase and oxidase, hydrolyzed gelatin, casein and urea and displayed activities of alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin and naphtol-AS-BI-phosphohydrolase. However, several characteristics of CAU 1453^T, such as positive reactions for inositol and D-tagatose utilization and negative reaction for acid phosphatase, distinguished CAU 1453^T from the closely related species, *A. donghaensis* KACC 11381^T, *A. aestuarii* KACC 18504^T and *A. daejeonensis* KCTC 12667^T.

For the determination of fatty acid composition, cell masses of CAU 1453^T and three reference strains, *A. donghaensis* KACC 11381^T, *A. aestuarii* KACC 18504^T and *A. daejeonensis* KCTC 12667^T, were grown in MB at 30 °C to the mid-exponential phase of growth and were harvested according to a protocol of standard MIDI (Sherlock Microbial Identification System version 6.1). Cellular fatty acid methyl esters were separated using a gas chromatograph (6890N; Agilent) and were identified using the Microbial Identification software package (MOORE library ver. 5.0; MIDI database TSBA6). The polar lipids of CAU 1453^T was extracted using a chloroform/methanol system and identified using two-dimensional thin-layer chromatography according to the procedures of Minnikin *et al.* [34]. The plates were sprayed with 10 % ethanolic molybdatophosphoric acid, molybdenum blue, ninhydrin and α-naphthol/sulphuric acid reagent (Sigma-Aldrich) to detect total lipid,

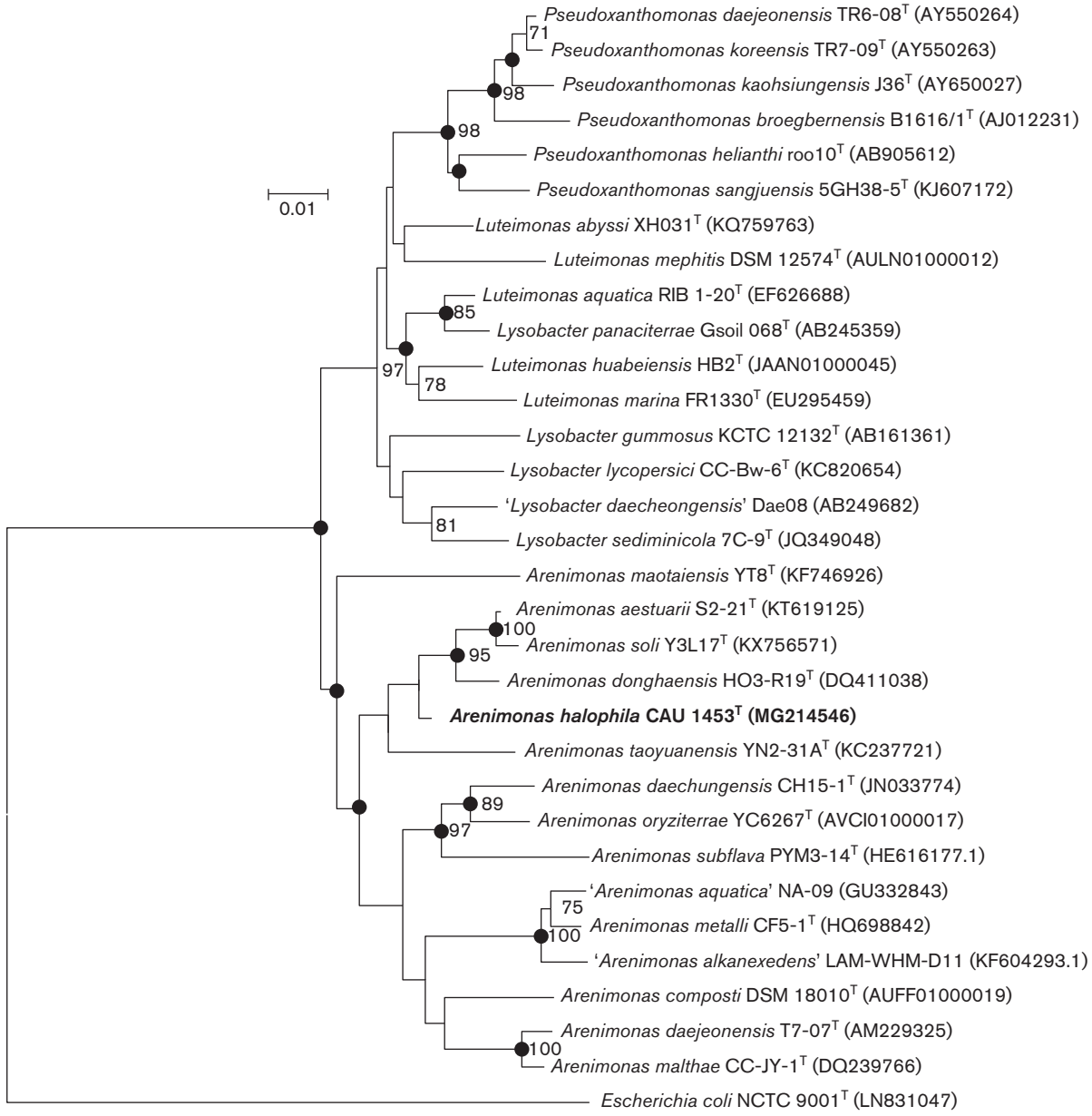

Fig. 1. Neighbor-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the phylogenetic relationships of CAU 1453^T and the related strain of species of the genus *Arenimonas* and other related genera. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the least-squares, maximum-likelihood, and maximum-parsimony algorithms. Bootstrap values >70 %, based on a neighbor-joining analysis of 1000 resampled datasets, are shown at nodes. *Escherichia coli* NCTC 9001^T (LN831047) is used as an outgroup organism.

phospholipids, aminolipids, and glycolipids, respectively. Isoprenoid quinone was analyzed by high performance liquid chromatography (Alliance 2690; Waters) as described by Komagata and Suzuki [35]. Isoprenoid quinone was eluted with methanol/isopropyl ether (3 : 1, v/v)] at a flow rate of 1 ml min^{-1}.

The predominant respiratory quinone detected in CAU 1453^T was Q-8. This quinone was present in the reference strains *A. donghaensis* KACC 11381^T, *A. aestuarii* KACC 18504^T and *A. daejeonensis* KCTC 12667^T and other members of the genus *Arenimonas* [1–13]. The major polar lipids of CAU 1453^T were diphosphatidylglycerol, a phosphoglycolipid, an aminophospholipid, two unidentified phospholipids and two unidentified glycolipids (Fig. S3). This polar lipids pattern was similar to those of other species of the genus *Arenimonas* [1, 3, 5, 8] but this strain differs from

Table 1. Phenotypic and chemotaxonomic characteristics that distinguish CAU 1453^T from the type strains of the most closely related species of the genus *Arenimonas*

Strains: 1, CAU 1453^T; 2, *A. donghaensis* KACC 11381^T; 3, *A. aestuarii* KACC 18504^T; 4, *A. daejeonensis* KCTC 12667^T. All data were obtained during this study unless indicated.

Characteristic	1	2	3	4
Temperature range (°C)	10–37	4–37	15–40	15–37
Optimum	30	30	30	30
pH range	5.5–9.5	7.0–9.0	7.0–8.5	5.5–9.0
Optimum	6.5	7.5	7.5	7.5
NaCl tolerance (%)	0–7	0–3	0–2	0–3
Optimum	1	1	0	1
Motility	−	+	−	+
Hydrolysis of:				
Casein	+	+	+	−*
Gelatin	+	+	+	−
Starch	−	−	−	+*
Urea	+	−	+*	+
Aesculin	−	−	+	−
Acid production from:				
Inositol	+	−	−	−
Aesculin ferric citrate	W	−	+	−
Salicin	−	−	W	−
Inulin	−	W	−	−
Turanose	W	+	−	−
D-Tagatose	+	−	−	−
Enzyme activity:				
Lipase (C14)	−	−	+	−
Leucine arylamidase	+	−	+	+
Valine arylamidase	W	−	+	−
Cystine arylamidase	−	−	+	−
Acid phosphatase	−	+	+	+
Arginine dihydrolase	W	−	−	+
DNA G+C content (mol%)	67.3	65.0†	62.2‡	68.3§

*These data were different from those reported in previous studies.
†Data from Kwon *et al.* [1].
‡Data from Jeong *et al.* [5].
§Data from Jin *et al.* [3].

Table 2. Cellular fatty acid profile (percentages) of CAU 1453^T and the type strains of the most closely related species of the genus *Arenimonas*

Strains: 1, CAU 1453^T; 2, *A. donghaensis* KACC 11381^T; 3, *A. aestuarii* KACC 18504^T; 4, *A. daejeonensis* KCTC 12667^T. All data were obtained during this study. All strains were cultivated with the same medium and conditions. Values are percentages of the total fatty acids. −, not detected, TR, tract amount (<1 %).

Fatty acid	1	2	3	4
Saturated:				
$C_{14:0}$	TR	1.1	2.1	TR
$C_{16:0}$	2.2	2.0	3.5	1.5
Unsaturated:				
$C_{15:1}\omega 9c$	−	−	−	3.2
$C_{16:1}\omega 7c$ alcohol	−	−	−	1.9
Branched:				
iso-$C_{10:0}$	1.1	TR	TR	TR
iso-$C_{11:0}$	3.9	3.6	6.8	4.1
iso-$C_{14:0}$	7.1	6.1	4.3	2.7
iso-$C_{15:0}$	30.7	29.4	30.5	28.0
iso-$C_{15:1}$F	2.4	3.2	3.0	2.9
anteiso-$C_{15:0}$	TR	1.3	TR	1.3
iso-$C_{16:0}$	16.5	25.9	11.3	18.4
iso-$C_{16:1}$H	1.1	TR	1.3	TR
iso-$C_{17:0}$	1.3	1.1	1.3	1.3
anteiso-$C_{17:0}$	TR	−	8.8	TR
Hydroxy:				
iso-$C_{11:0}$ 3-OH	9.1	8.0	14.1	9.3
iso-$C_{12:0}$ 3-OH	1.1	1.0	TR	TR
Summed feature:				
1	2.1	1.2	TR	2.0
2	TR	−	1.3	−
3	2.7	4.4	TR	1.5
9	17.7	11.5	11.2	18.9

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1, iso-$C_{15:1}$H and/or $C_{13:0}$ 3-OH; summed feature 2, $C_{12:0}$ aldehyde and/or unknown; summed feature 3, $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$; summed feature 9, iso-$C_{17:1}\omega 9c$ and/or $C_{16:0}$ 10-methyl.

other species by having unidentified aminophospholipid and unidentified phosphoglycolipid. CAU 1453^T contained saturated, unsaturated, branched and hydroxyl fatty acids (Table 2). The major cellular fatty acids (>10 %) was iso-$C_{15:0}$ (30.7 %) and iso-$C_{16:0}$ (16.5 %). The overall fatty acid profile of CAU 1453^T was similar to those of type strains of species of the genus *Arenimonas*. However, the presence of slightly higher amounts of iso-$C_{10:0}$ and iso-$C_{12:0}$ 3-OH than in other strains were distinguishing characteristics between CAU 1453^T and other related species of the genus *Arenimonas*.

On the basis of phenotypic, chemotaxonomic, phylogenetic and genotypic data, CAU 1453^T represents a novel species of genus *Arenimonas* for which the name *Arenimonas halophila* is proposed.

DESCRIPTION OF *ARENIMONAS HALOPHILA* SP. NOV.

Arenimonas halophila sp. nov. (ha.lo′phi.la. Gr. n. *hals* salt; Gr. adj. *philos* loving; N.L. fem. adj. *halophila* salt-loving).

Cells are Gram-reaction-negative, strictly aerobic, non-motile, non-spore-forming and rod-shaped, approximately 1.3–2.2 µm in length and 0.2–0.4 µm in width. Colonies on MA incubated at 30 °C for 3 days are white pigmented, opaque, circular, smooth and convex and have entire edges. Optimal growth occurs at 30 °C (range 10–37 °C), p 6.5

(range pH 5.5–9.5), and 1 % (w/v) NaCl (range 0–7 %). Catalase and oxidase activities are positive. In API 20E and 20NE, lysine decarboxylase, arginine dihydrolase (weakly), hydrolysis of casein, gelatin and urea are positive but nitrate reduction, indole production, glucose fermentation, hydrolysis of starch and aesculin and β-galactosidase and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are negative. In API ZYM, activities of alkaline phosphatase, esterase lipase (C8), leucine arylamidase, trypsin and naphthol-AS-BI-phosphate are positive and esterase (C4), valine arylamidase and α-chymotrypsin are weakly positive but lipase (C14), cystine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. In API 50CH, acid is produced from inositol, D-tagatose and potassium 5-ketogluconate; and D-fructose, L-sorbose, aesculin ferric citrate, turanose and D-lyxose are weakly acid-producing. The polar lipids contain diphosphatidylglycerol, a phosphoglycolipid, an aminophospholipid, two unidentified phospholipids and two unidentified glycolipids, and the major qunoine is Q-8. The major cellular fatty acids are iso-$C_{15:0}$ and anteiso-$C_{16:0}$.

The type strain is CAU 1453^T (=KCTC 62235^T=NBRC 113093^T), isolated from soil collected from Modo in the Republic of Korea. The DNA G+C content of the type strain is 67.3 mol%.

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

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