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TAXONOMIC DESCRIPTION
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Arthrobacter paludis sp. nov., isolated from a marsh

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Abstract

A novel Gram-stain-positive, strictly aerobic, non-endospore-forming bacterium, designated CAU 9143^T, was isolated from a hydric soil sample collected from Seogmo Island in the Republic of Korea. Strain CAU 9143^T grew optimally at 30 °C, at pH 7.0 and in the presence of 1 % (w/v) NaCl. The phylogenetic trees based on 16S rRNA gene sequences revealed that strain CAU 9143^T belonged to the genus *Arthrobacter* and was closely related to *Arthrobacter ginkgonis* SYP-A7299^T (97.1 % similarity). Strain CAU 9143^T contained menaquinone MK-9 (H_2) as the major respiratory quinone and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two glycolipids and two unidentified phospholipids as the major polar lipids. The whole-cell sugars were glucose and galactose. The peptidoglycan type was A4*a* (L-Lys–D-Glu2) and the major cellular fatty acid was anteiso-$C_{15:0}$. The DNA G+C content was 64.4 mol% and the level of DNA–DNA relatedness between CAU 9143^T and the most closely related strain, *A. ginkgonis* SYP-A7299^T, was 22.3 %. Based on phenotypic, chemotaxonomic and genetic data, strain CAU 9143^T represents a novel species of the genus *Arthrobacter*, for which the name *Arthrobacter paludis* sp. nov. is proposed. The type strain is CAU 9143^T (=KCTC 13958^T,=CECT 8917^T).

The genus *Arthrobacter*, a member of the family *Micrococcaceae*, was established by Conn and Dimmick [1] with the description of *Arthrobacter globiformis* as the type species of the genus. Currently, the genus comprises 49 recognized species with validly published names (www.bacterio.net/arthrobacter.html). The genus *Arthrobacter* consists of aerobic, rod–coccus-shaped, Gram-stain-positive, catalase-positive bacteria that are characterized by the presence of A3*a* and A4*a*, respectively, as the major peptidoglycan and MK-9 (H_2) or MK-8/MK-9 as the major menaquinones [2–4]. Members of the genus *Arthrobacter* have been isolated from various environments, such as sediment [5], soil environment [6–11], pond [12], sea water [2], Antarctica [13] and rhizosphere of *Ginkgo biloba* L. [3]. A bacterial strain, designated CAU 9143^T, was isolated from a hydric soil sample beside reservoir in Seogmo Island (37° 40′ 54.84″ N, 126° 22′ 15.14″ E) in the Republic of Korea. In this study, a bacterial strain, CAU 9143^T, was characterized that was phylogenetically closely related to the genus *Arthrobacter*.

Isolation was performed according to Gordon and Mihm [14] using marine agar 2216 (MA; Difco). The soil sample was crushed, serially diluted and incubated under aerobic conditions at 30 °C for 7 days. Strain CAU 9143^T was purified by subculture and maintained at −70 °C in marine broth 2216 (MB; Difco) supplemented with 25 % (v/v) glycerol. Strain CAU 9143^T has been deposited in the Korean Collection for Type Cultures (KCTC; Jeongeup, Republic of Korea) and the Spanish Type Culture Collection (CECT; Valencia, Spain) as KCTC 13958^T and CECT 8917^T, respectively. The type strains of the most closely related species, *Arthrobacter ginkgonis* SYP-A7299^T (=KCTC 39 592^T), *Arthrobacter oryzae* KV-651^T (=KCTC 29628^T) and *Arthrobacter halodurans* JSM 078085^T (=KCTC 19430^T) were obtained from the Korean Collection for Type Cultures (KCTC; Jeongeup, Republic of Korea) and used as reference strains.

Genomic DNA of strain CAU 9143^T was extracted and purified using a genomic DNA extraction kit (Intron). The the 16S rRNA gene of strain CAU 9143^T was amplified by PCR using the universal primers 8F/1525R following established procedures [15]. The amplicon of the 16S rRNA gene was sequenced directly using a 3730 automatic DNA sequencer (Applied Biosystems). Multiple alignments and calculation of sequence similarity levels with 16 recognized members of the genus *Arthrobacter* and representative members of the family *Micrococcaceae* were carried out by using the CLUSTAL_X 2.1 software [16] and EzTaxon-e

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Abbreviations: CECT, Spanish Type Culture Collection; DPG, diphosphatidylglycerol; FAMEs, fatty acid methyl esters; GL, two glycolipids; KCTC, Korean Collection for Type Cultures; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, two unknown phospholipids.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU 9143^T is JN176140.
Three supplementary figures are available with the online version of this article.

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(www.ezbiocloud.net/). Phylogenetic trees were generated by using three algorithms: neighbour-joining [17], maximum-likelihood [18] and maximum-parsimony [19]. The distance matrix was produced on the basis of the Jukes–Cantor model [20]. The tree topology was evaluated by the bootstrap resampling method [21] with 1000 replicates of the neighbour-joining dataset with the SEQBOOT and CONSENSE programs from the PHYLIP 3.66 package. The G+C content of the genomic DNA was determined using high-performance liquid chromatography (HPLC) by using the method of Tamaoka and Komagata [22]. The extent of DNA–DNA relatedness between CAU 9143^T and the most closely related strain, *A. ginkgonis* SYP-A7299^T, was determined by following Goris *et al.* [23].

The 16S rRNA gene sequence of strain CAU 9143^T (1497 bp) was determined and compared with the available reference sequences in the GenBank database (access September 2017). Phylogenetic analysis indicated that the strain fell into the genus *Arthrobacter*. The neighbour-joining tree is shown in Fig. 1. The trees reconstructed by the maximum-likelihood and maximum-parsimony algorithms showed a similar topology (Fig. S1, available in the online version of this article). Pairwise analysis showed that *A. ginkgonis* SYP-A7299^T (97.1 %), *A. oryzae* NRRL B-24478^T (96.7 %) and *A. halodurans* JSM 078085^T (96.7 %) were the most closely related species to strain CAU 9143^T. The G+C content of the DNA of strain CAU 9143^T was 64.4 mol %, which was close to the values observed for other species of the genus *Arthrobacter*. The DNA–DNA relatedness between CAU 9143^T and the most closely phylogenetic neighbour, *A. ginkgonis* SYP-A7299^T, was 22.3 %. The value is below the 70 % cut-off point suggested by Wayne *et al.* [24] for the determination of genomic species, supporting

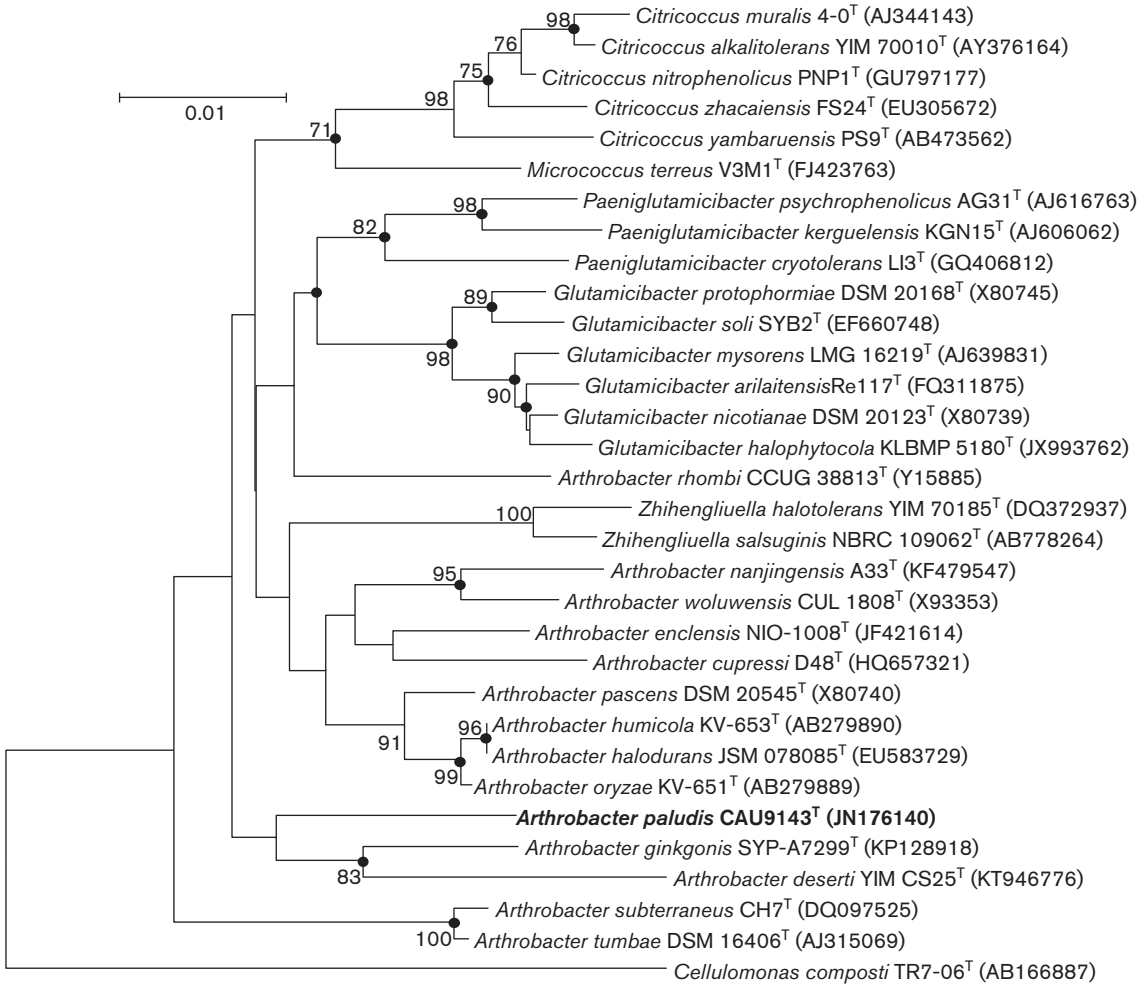

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain CAU 9143^T and the type strains of recognized *Arthrobacter* species. Dots indicate that the corresponding nodes were also recovered in the trees generated with maximum-likelihood and maximum-parsimony algorithms. The numbers at the nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values >70 % are given. Bar, 0.01 substitutions per nucleotide position. *Cellulomonas composti* TR7-06^T (AB166887) is used as an outgroup.

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the proposal that strain CAU 9143^T represents a separate species.

Strain CAU 9143^T was cultivated routinely on an MA plate at 30 °C to investigate all morphological, physiological and biochemical characteristics [25]. The spore formation was tested on nutrient sporulation medium [26]. After 7 days of growth, endospore formation was determined by staining with malachite green as described previously by Conn *et al.* [27]. Cell morphology was examined by DM 1000 light microscopy (Leica). JEM 1010 transmission electron microscopy (JEOL) was used to determine the presence of flagella from an exponentially growing culture. Gram-staining was conducted by using the bioMérieux Gram-staining kit. Gliding motility was tested as described by Bowman [28]. The temperature range for growth of strain CAU 9143^T at 4, 10, 20, 25, 30, 37, 40 and 45 °C was determined by measuring the turbidity of MB after 72 h incubation in an MIR-253 aerobic incubator (Sanyo) and a Bactron anaerobic chamber (Sheldon). Growth was tested at 30 °C in MB adjusted to pH 4.5–11.0 at 0.5 pH unit intervals. The pH values of <6, 6–9 and >9 were carried out by using sodium acetate/acetic acid, Tris/HCl and Na_2CO_3 buffers, respectively. The salinity range for growth was assessed in MB by excluding NaCl [29] and adding 0–15 % NaCl (w/v) as described by Rodríguez-Valera *et al.* [30]. Oxidase activity was determined was examined using 1 % (w/v) tetramethyl-*p*-phenylenediamine [31]. Catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. Hydrolysis of casein, gelatin and aesculin, and nitrate reduction were examined according to Smibert and Krieg [32]. Acid production from carbohydrates and other biochemical and physiological features were investigated using the API 20E, API 20NE and API 50CH systems (bioMérieux). Enzyme activities were tested using the API ZYM kit according to the manufacturer's instructions. API 20E, API 20NE and API 50CH test strips were read after incubation for 48 h at 30 °C and the API ZYM test strip was read after incubation for 24 h at 30 °C.

Detailed phenotypic features of strain CAU 9143^T are given in Table 1 and in the species description. Strain CAU 9143^T was Gram-stain-positive and strictly aerobic. Endospores were not observed. Cells were rod–coccus-shaped and approximately 0.8–1.0 µm in diameter and 1.4–1.6 µm in length. Colonies were cream-coloured and circular. Flagella were not observed (Fig. S2). Strain CAU 9143^T grew at temperatures between 20 and 37 °C (optimum 30 °C) and at NaCl concentrations between 0 and 8 % (optimum 1 %). Visible growth occurred at pH 6–9 and strain CAU 9143^T showed the highest visible growth at pH 7.0. Strain CAU 9143^T hydrolysed casein and gelatin, and had enzyme activities for alkaline phosphate, esterase (C4), leucine arylamidase, naphtol-AS-BI-phosphohydrolase and α- glucosidase. Strain CAU 9143^T had negative reactions for amygdalin, D-adonitol, cellobiose, D-fucose, D-galactose, lactose, D-mannitol, D-mannopyranoside, melibiose, D-sorbitol, *N*-acetylglucosamine, sucrose, glycogen, L-arabinose, L-arabitol, L-xlyose and methyl-α *N*-acetylglucosamine, which are representative characteristic of closely related species: *A. ginkgonis* SYP-A7299^T, *A. halodurans* JSM 078085^T and *A. oryzae* KV-651^T. However, strain CAU 9143^T differed from the closely related species by its positive reaction for D-arabinose, trehalose, D-arabitol and xylitol. These differences are sufficient to suggest that strain CAU 9143^T is different from other *Arthrobacter* species.

Table 1. Differential properties of strain CAU 9143^T and the type strains of the most closely related *Arthrobacter* species

Strains: 1, CAU 9143^T; 2, *A. ginkgonis* SYP-A7299^T; 3, *A. oryzae* KV-651^T; 4, *A. halodurans* JSM 078085^T. Data were taken from this study unless indicated. +, Positive; –, negative

Characteristic	1	2	3	4
Colony colour	Cream	Yellow	Cream	Yellow
Motility	+	–[a]	+[b]	–[c]
Temperature (°C) range	20–37	20–35[a]	4–34[b]	4–35[c]
pH range	6.0–9.0	6–10[a]	6.0–11.0[b]	6.0–9.5[c]
NaCl (%, w/v) range	0–8	0–4[a]	0–2[b]	0–12[c]
Nitrate reduction	+	–	+	–
Oxidase	–	–	+	+
Hydrolysis				
Aesculin	–	–	+	+
Gelatine	+	–	+	–
Utilization of carbon				
D-Arabinose	+	–	–	–
Potassium gluconate	–	+	+	–
Trisodium citrate	–	+	–	–
Maltose	+	+	+	–
D-Arabitol	+	–	–	–
Trehalose	+	–	–	–
Xylitol	+	–	–	–
Enzyme activities				
Alkaline phosphate	+	+	–	–
Esterase (C4)	–	+	+	+
β-Galactosidase	–	–	+	–
DNA G+C content (mol%)	66.4	68.9[a]	67[b]	63.3[c]

*Data taken from: *a*, Cheng *et al.* [3]; *b*, Kageyama *et al.* [4]; *c*, Chen *et al.* [2].

For ascertainment of fatty acids, the cell mass of strain CAU 9143^T and the type strains of the most closely related *Arthrobacter* species, *A. ginkgonis* SYP-A7299^T, *A. halodurans* JSM 078085^T and *A. oryzae* KV-651^T, were harvested from MA at late-exponential growth phase after cultivation for 3 days at 30 °C according to a standard MIDI protocol (Sherlock Microbial Identification System version 6.1). Fatty acid methyl esters (FAMEs) were obtained as described previously [33], and separated by using a 6890 N automated gas chromatography system (Agilent Technologies). The peaks were identified by using the Microbial Identification software package (MOORE library version 5.0; MIDI database TSBA6). Determination of the cell-wall peptidoglycan type

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of strain CAU 9143^T was carried out by the Identification Service of the DSMZ (Braunschweig, Germany). Polar lipids were determined using two-dimensional thin-layer chromatography (TLC; silica gel 60 F254; Merck) by the method of Minnikin *et al.* [34] and identified by two-dimensional TLC and spraying with appropriate detection reagents [35]. Whole-cell sugars and isoprenoid quinones were analysed as described Komagata and Suzuki [36] using TLC and a reversed-phase HPLC (Waters), respectively. The quinones were eluted by an isocratic solvent system [methanol/isopropyl ether (3 : 1, v/v)] using a flow rate of 1 ml min^{-1}.

The fatty acids of strain CAU 9143^T and a detailed comparison of the fatty acid profiles among the three reference strains are listed in Table 2. Strain CAU 9143^T contained saturated and branched-chain fatty acids; anteiso-$C_{15:0}$ (54.7 %), iso-$C_{16:0}$ (15.6 %), iso-$C_{15:0}$ (12.4 %) and anteiso-$C_{17:0}$ (10.4 %). The fatty acid anteiso-C15 : 0 was a major element in strain CAU 9143^T and closely related species, although there were differences in the amounts of some fatty acids. The total hydrolysate of the peptidoglycan contained the amino acids lysine, alanine, glutamic acid and small amounts of glycine in the molar ratio of approximately 1.0 : 2.7 : 3.0 : 0.3, and the partial hydrolysate of the peptidoglycan contained the peptides L-Ala–D-Glu and L-Lys–D-Ala. From these data, the cell-wall peptidoglycan was of the type A4*a* L-Ala–D-Glu2. The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two glycolipids and two unidentified phospholipids (Fig. S3). Strain CAU 9143^T contained glucose and galactose as whole-cell sugars, and menaquinone MK-9 (H_2) as a predominant respiratory quinone. This character is representative of numerous species of the genus *Arthrobacter*.

Therefore, the data from the phenotypic, chemotaxonomic and phylogenetic studies provide sufficient evidence to recognize strain CAU 9143^T as a novel species of the genus *Arthrobacter*, for which the name *Arthrobacter paludis* sp. nov. is proposed.

Table 2. Cellular fatty acid compositions (%) of strain CAU 9143^T and the type strains of the most closely related *Arthrobacter* species

Strains: 1, CAU 9143^T; 2, *A. ginkgonis* SYP-A7299^T; 3, *A. oryzae* KV-651^T; 4, *A. halodurans* JSM 078085^T. Data were obtained in this study. –, Not detected.

Fatty acids	1	2	3	4
Saturated				
$C_{14:0}$	0.4	1.8	–	0.9
$C_{16:0}$	1.6	4.0	3.1	1.2
Branched chain				
Anteiso-$C_{15:0}$	54.7	52.8	50.5	44.8
Anteiso-$C_{17:0}$	10.4	12.7	12.5	5.9
Iso-$C_{14:0}$	1.0	0.8	–	1.3
Iso-$C_{15:0}$	12.4	6.6	6.4	7.3
Iso-$C_{16:0}$	15.6	4.9	4.4	7.6
Iso-$C_{16:1}$H	0.7	1.6	1.6	10.1
Iso-$C_{17:0}$	1.0	0.9	–	1.1
Summed feature 3	0.7	4.9	8.4	6.2

*Summed features represent groups of two or three fatty acids that could not be separated by gas–liquid chromatography with the MIDI system. Summed feature 3 contained $C_{16:1}\omega7c/\omega6c$.

DESCRIPTION OF *ARTHROBACTER PALUDIS* SP. NOV.

Arthrobacter paludis (pa.lu′dis. L. gen. n. *paludis* of a marsh).

Cells are Gram-stain-positive, aerobic and rod–coccus-shaped, and approximately 0.8–1.0 µm in diameter and 1.4–1.6 µm in length. Colonies on MA are cream-coloured and circular after 3 days of incubation at 30 °C. Growth occurs at 20–37 °C (optimum, 30 °C) and at pH 6.0–8.5 (optimum, 7.0) in the presence of 0–8 % NaCl (w/v) (optimum 1 %). Catalase activity is present. Gelatin, casein and aesculin are hydrolysed. In the API 50CH strip test, reactions for D-arabinose, D-arabitol, D-glucose, maltose, trehalose, turanose and xylitol are positive, while reactions for amygdalin, D-ribose, cellobiose, D-xylose, methyl-β D-xylopyranoside, D-fructose, lactose, melibiose, erythritol, glycerol, inositol, L-rhamnose and L-sorbose are negative. In the API ZYM strip test, α-glucosidase, alkaline phosphate, esterase (C4), leucine arylamidase and naphtol-AS-BI-phosphohydrolase are found to be present. Additionally, strain CAU 9143^T has positive reactions for D-sorbitol, D-mannose, D-mannitol, maltose, inositol, malic acid and phenylacetic acid, and negative reactions for 4-nitrophenyl-β D-galactopyranoside, adipic acid, capric acid, sucrose, melibiose, amygdalin, L-arabinose, L-tryptophane, *N*-acetyl-glucosamine, sodium pyruvate and trisodium citrate in the API 20E and 20NE strips. The peptidoglycan type was A4*a* (L-Lys–D-Glu2). Glucose and galactose are the whole-cell sugars. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two glycolipids and two unidentified phospholipids. The major cellular fatty acid is anteiso-$C_{15:0}$ and the major respiratory quinone is MK-9 (H_2).

The type strain, CAU 9143^T (=KCTC 13958^T, =CECT 8917^T), was isolated from marsh on Seogmo Island in the Republic of Korea. The DNA G+C content is 64.4 mol%.

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

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