Altererythrobacter sediminis sp. nov., isolated from lagoon sediments

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A Gram-stain-negative, non-motile, non-spore-forming, ovoid rod-shaped bacterium, designated strain CAU1172^T, was isolated from lagoon sediments along the east coast of the Republic of Korea. Strain CAU1172^T formed a yellow pigment on marine agar. Growth occurred at 20– 37 °C (optimum, 30 °C), at pH 6.5–10 (optimum, 7.5) and in the presence of 0–4 % (w/v) NaCl (optimum, 1%). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain CAU1172^T formed a separate lineage within the genus *Altererythrobacter*, and was most closely related to *Altererythrobacter gangjinensis* KJ7^T (96.1 % similarity). Ubiquinone 10 (Q-10) was the predominant respiratory quinone. The dominant fatty acids were C_{18:1} ω 7c, C_{17:1} ω 6c and summed feature 3 (comprising C_{16:1} ω 7c and/or C_{16:1} ω 6c). The polar lipids were composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, sphingoglycolipid, phospatidylcholine and four unidentified lipids. The DNA G+C content of strain CAU1172^T represents a novel species of the genus *Altererythrobacter*, for which the name *Altererythrobacter sediminis* sp. nov. is proposed. The type strain is CAU1172^T (=KCTC 42453^T=NBRC 110917^T).

The genus Altererythrobacter was first proposed by Kwon et al. (2007) and the description was emended by Xue et al. (2012). At the time of writing, the genus Altererythrobacter includes 16 species with validly published names (Parte, 2014; http:// www.bacterio.net/palleronia.html). These species have been isolated from seawater (Lai et al., 2009; Seo & Lee, 2010; Park et al., 2011; Jung et al., 2014; Lei et al., 2014; Yang et al., 2014), sea sediment (Kwon et al., 2007; Matsumoto et al., 2011; Wu et al., 2014), tidal flats (Fan et al., 2011; Jeong et al., 2013), sea urchin (Nedashkovskaya et al., 2013), rhizosphere of wild rice (Kumar et al., 2008) and desert sand (Xue et al., 2012). Two additional Altererythrobacter species, Altererythrobacter rigui (Kang et al., 2016) and Altererythrobacter confluentis (Park et al., 2016), have been proposed and published online in this journal. Members of the genus Altererythrobacter can be characterized by yellow colonies, $C_{18:1}\omega7c$ as the main fatty acid and ubiquinone 10 (Q-10) as the major respiratory quinone (Kwon et al., 2007). In this study, a Gram-stain-negative bacterium, designated strain CAU1172^T, was isolated from lagoon sediments near the east coast in the Republic of Korea. These lagoons are a coastal wetland environment

created by the combined influence of rivers and the ocean, and study of their ecosystems has focused on plankton and water quality (Lee *et al.*, 2010). Accordingly, the aim of this study was to determine the exact taxonomic position of strain CAU1172^T by using a polyphasic characterization including the determination of phenotypic properties and detailed phylogenetic analysis based on the 16S rRNA gene sequence.

Strain CAU1172^T was isolated from a sediment sample of a lagoon along the east coast of Korea (38° 13' 07.1" N 128° 34' 51.9" E), using the dilution plating method described by Gordon & Mihm (1962). The sample was plated on marine agar 2216 (MA; Difco) and incubated at 30 °C for 10 days under aerobic conditions. A single colony was purified through subculture plating and preserved at -80° C in marine broth (MB; Difco) containing 25 % (v/v) glycerol. Strain CAU1172^T was deposited in the Korean Collection for Type Cultures (KCTC) and the Biological Resource Center, National Institute of Technology and Evaluation (NITE) (NBRC) as KCTC 42420^T and NBRC 110917^T, respectively. The type strains of the most closely related species of the genus Altererythrobacter, namely Altererythrobacter gangjinensis KACC 16190^T, A. aestiaquae KCTC 42006^T, A. luteolus KCTC 12311^T and A. indicus KACC 13863^T, were purchased from the Korean Agricultural Culture Collection (KACC) and KCTC and used as reference strains.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU1172^T is KP779619.

Two supplementary figures are available with the online Supplementary Material.

Genomic DNA of strain CAU1172^T was obtained by the method of Marmur (1961). The 16S rRNA gene was amplified by PCR using the universal primers 27F and 1525R (Lane, 1991) and the conditions described by Nam et al. (2004). The amplified PCR product was purified via a PCR purification kit (Bioneer) according to the manufacturer's instructions. The amplified 16S rRNA gene was sequenced directly using a BigDye Terminator Cycle Sequencing Kit and a 3730 automatic DNA sequencer (Applied Biosystems Life Technologies). Sequences of related species were obtained via the EzTaxon-e.EzBioCloud.net program (http://www.ezbiocloud.net/eztaxon) (Kim et al., 2012). The sequence data were aligned with closely related Altererythrobacter species using CLUSTAL X 2.1 software (Larkin et al., 2007). Evolutionary distance matrices were determined using the neighbour-joining method described by Jukes & Cantor (1969). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) algorithms in the PHYLIP package (Felsenstein, 1989). Tree topology was evaluated by bootstrap analysis based on 1000 replicates (Felsenstein, 1985) of the neighbour-joining dataset with the SEQBOOT and CONSENSE programs from the PHYLIP package. The G+C content of the genomic DNA was determined as described by Tamaoka & Komagata (1984) using HPLC.

The nearly full-length 16S rRNA gene sequence of strain CAU1172^T (1457 bp) was obtained. Phylogenetic analysis of strain CAU1172^T based on 16S rRNA gene sequences showed that it belonged to the genus *Altererythrobacter* (Fig. 1). Strain CAU1172^T shared highest 16S rRNA gene sequence similarity with *A. gangjinensis* KJ7^T (96.1 %) based on the EzTaxon-e results; levels of similarity to the type strains of other recognized *Altererythrobacter* species were 93.2–95.4 %. The DNA G+C content of strain CAU1172^T was 63.2 mol%, a value in the range described for *Altererythrobacter* species (Wu *et al.*, 2014).

Strain CAU1172^T was cultivated routinely on MA at 30 $^{\circ}$ C to examine all morphological, physiological and biochemical characteristics (Bernardet et al., 2002). Cell morphology and motility were examined using a DM 1000 light microscope (Microsystems) and a JEM 1010 transmission electron microscope (JEOL). The presence of flagella was determined by transmission electron microscopy using cells from an exponentially growing culture after negatively staining of cells with 1 % (w/v) phosphotungstic acid. Gliding motility was examined in an MB culture for 72 h using the hanging-drop method (Bowman, 2000). The presence of flexirubin-type pigments on MA was determined as described by Bernardet et al. (2002) and pigment analysis was examined with methanol on MA according to the method of Rainey et al. (2003) using a UV-visible spectrophotometer (Tecan). Gram staining was carried out using the bioMérieux Gram stain kit. The optimal growth temperature was determined at different temperatures (4, 10, 20, 30, 37, 40 and 45 °C) on MA in a MIR-253 aerobic incubator (Sanyo) and in a Bactron anaerobic chamber

(Sheldon). Growth was examined at 30 °C in MB adjusted to pH 4.5-11.5 at increments of 0.5 pH unit intervals. The pH values of 4.5-6, 6-9 and 9-11.5 were obtained using sodium acetate/acetic acid, Tris/HCl and Na₂CO₃ buffers, respectively. Growth in the absence of NaCl and in the presence of 1-15.0% (w/v) NaCl (at increments of 1%) was investigated at 30 °C in MB prepared according to the formula of the Difco medium, except that NaCl was excluded and that 0.45 % (w/v) MgCl₂.6H₂O and 0.06 % (w/v) KCl was added. Catalase and oxidase production tests were examined according to Cappuccino & Sherman (2002). Hydrolysis of starch, gelatin, casein, aesculin and citrate was tested as described by Lányí (1987) and Smibert & Krieg (1994). Additionally, biochemical characterizations were examined by using the API 20E, API 20NE and API 50CH kits (bioMérieux). Enzyme activities were determined using the API ZYM kit (bioMérieux) according to the manufacturer's instructions. API 20E, API 20NE and API ZYM strips were read after 24 h and API 50CH strips after 48 h. Antibiotic susceptibility was tested on MA at 30 °C by using BD BBL Sensi-Disc antimicrobial susceptibility test discs (BD Diagnostics) (µg per disc unless stated otherwise): amoxicillin (20), ampicillin (10), carbenicillin (100), cefoxitin (30), cephalothin (30), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), penicillin (10 U), nalidixic acid (30), polymyxin B (300 U), rifampin (5), streptomycin (10), tetracycline (30), tobramycin (10) and trimethoprim/sulfamethoxazole (1.25/23.75).

Detailed phenotypic characteristics of strain CAU1172^T are given in Table 1 and in the species description. Cells of strain CAU1172^T were Gram-stain-negative, aerobic, non-gliding and rod-shaped. Endospores and flagella were not observed (Fig. S1, available in the online Supplementary Material). Strain CAU1172^T grew at temperatures between 20 and 37 °C (optimum 30°C) and NaCl concentrations between 0 and 4% (w/v) (optimum 1%). Visible growth occurred at pH 6.5-10 (optimum pH 7.5). The oxidase test was negative, but catalase test was positive. Strain CAU1172^T hydrolysed aesulin and had enzyme activities for alkaline phosphate, esterase (C4), esterase lipase (C8), α -chymotrypsin and naphthol-AS-BI-phosphohydrolase. However, strain CAU1172^T differed from its closest relatives, A. gangjinensis KJ7^T, A. aestiaquae HDW-31^T, A. luteolus SW-109^T and A. indicus MSSRF26^T, by its utilization of D-arabinose and D-ribose, and enzyme activities of esterase (C4) and valine arylamidase. Strain CAU1172^T was susceptible to amoxicillin, ampicillin, carbenicillin, cefoxitin, cephalothin, chloramphenicol, erythromycin, kanamycin, nalidixic acid, penicillin, rifampin, trimethoprim/sulfamethoxazole and tobramycin, but resistant to polymyxin B and streptomycin. Overall, the results obtained in this study are almost co-parallel with previously published data for the four reference Altererythrobacter species (Yoon et al., 2005; Kumar et al., 2008; Jeong et al., 2013; Jung et al., 2014).

Chemotaxonomic characteristics, including fatty acids and respiratory quinone, of strain CAU1172^T were compared under the same culture conditions used in previous *Altererythrobacter* studies (Yoon *et al.*, 2005; Kumar *et al.*, 2008;

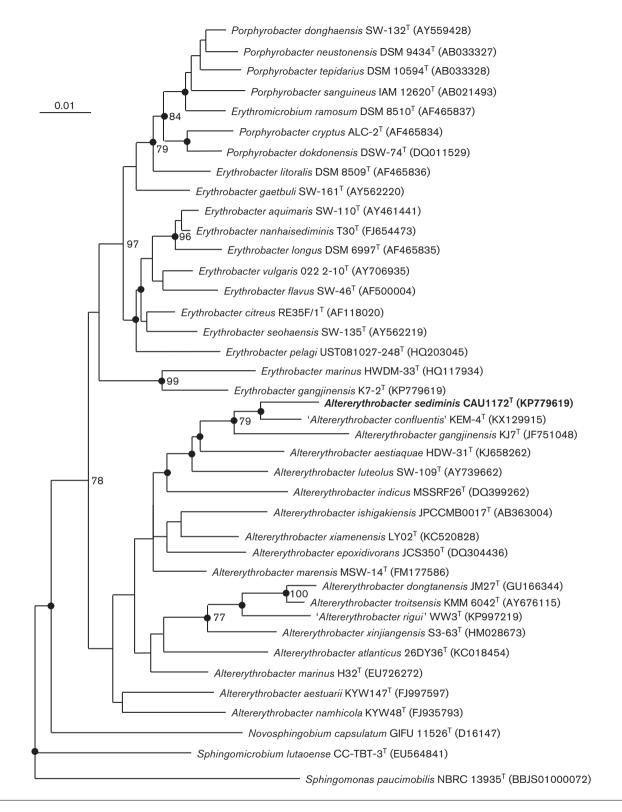


Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain CAU1172^T, the type strains of recognized *Altererythrobacter* species and some other related taxa. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and least-squares algorithms. The numbers at 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. *Sphingomonas paucimobilis* NBRC 13935^T (U37337) was used as the outgroup organism.

Table 1. Differential properties between strain CAU1172^T and the type strains of the most closely related *Altererythrobacter* species

Strains: 1, CAU1172^T; 2, *A. gangjinensis* KACC 16190^T; 3, *A. aestiaquae* KCTC 42006^T; 4, *A. indicus* KACC 13863^T; 5, *A. luteolus* KCTC 12311^T. Data were obtained in this study unless indicated otherwise. All strains were positive for hydrolysis of aesculin and activity of alkaline phosphatase. All strains were negative for hydrolysis of urea, and activity of lipase (C14), α -galactosidase, β -glucuronidase, α -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5
Cell shape*	Ovoid rod	Rod ^a	Rod^b	Rod ^c	Rod^d
Growth:*					
Temperature range	20-37	5–35 ^a	$10-40^{b}$	4-42 ^c	20–40 ^d
(optimum) (°C)	(30)	(25)	(30)	(25-30)	(35)
pH range	6.5-10.0	6.0–9.5 ^a	$5.0 - 11.0^{b}$	ND	6.0–8.5 ^d
(optimum)	(7.5)	(7.0)	(7.0)	$(7.0)^{c}$	(6.5)
NaCl concentration	0-4	0–9 ^a	$0-6^{b}$	0-12 ^c	$0-6^{d}$
(optimum) (%, w/v)	(1)	(2)	(1-2)	(2)	(2)
Hydrolysis of:					
Gelatin	_	_	_	_	+
Starch	_	-	_	_	+
Utilization of:					
D-Arabinose	+	-	_	_	_
L-Arabinose	+	-	_	+	_
D-Ribose	+	-	_	_	_
D-Xylose	+	-	_	+	_
Enzyme activity (API ZYM):					
Esterase (C4)	+	-	_	_	_
Esterase lipase (C8)	+	+	_	+	_
Leucine arylamidase	_	+	+	+	_
Valine arylamidase	_	+	+	+	+
Crystine arylamidase	_	+	+	_	+
Trypsin	_	+	+	_	_
lpha-Chymotrypsin	+	+	+	_	_
Acid phosphatase	_	+	-	_	_
Naphthol-AS-BI-phosphohydrolase	+	+	-	_	_
β -Galactosidase	_	+	-	_	_
β -Glucosidase	_	_	+	_	_
DNA G+C content (mol%)*	63.2	60.2^{a}	67.2 ^b	66.8 ^c	60.3^{d}

*Data taken from: *a*, Jeong *et al.* (2013); *b*, Park *et al.* (2011); *c*, Kumar *et al.* (2008); *d*, Kwon *et al.* (2007).

Jeong *et al.*, 2013; Jung *et al.*, 2014). The cell mass of strain CAU1172^T was harvested from MA in late-exponential growth phase after cultivation for 3 days at 30 °C according to the standard MIDI protocol (Sherlock Microbial Identification System version 6.1). Fatty acid methyl esters were obtained as described by Minnikin *et al.* (1980), and separated using a 6890N automated gas chromatography system (Agilent Technologies). The peaks were identified by using the Microbial Identification software package (MOORE library ver. 5.0; MIDI database TSBA6). Polar lipids were obtained from cells of strain CAU1172^T grown on MA for 3 days at 37 °C. The polar lipids of strain CAU1172^T were identified by two-dimensional TLC (Silica gel 60 F 254, 20×20 cm; Merck) as described by Minnikin *et al.*

(1984). Isoprenoid quinones were extracted according to Komagata & Suzuki (1987) and analysed by HPLC.

The only isoprenoid quinone of strain CAU1172^T was Q-10. This characteristic is in agreement with members of the genus *Altererythrobacter*. The fatty acid profile of strain CAU1172^T is shown Table 2. The main fatty acids (present at >10%) were $C_{18:1}\omega7c$ (31.91%), $C_{17:1}\omega6c$ (17.80%) and summed feature 3 (12.18%). This fatty acid profile is similar to that of *A. gang-jinensis* in that unsaturated fatty acids $C_{18:1}\omega7c$ and $C_{17:1}\omega6c$ were predominant, but there were differences between strain CAU1172^T and other *Altererythrobacter* species in the percentages of some other fatty acids. However, strain CAU1172^T could be distinguished from other *Altererythrobacter* species by the presence of the branched-chain fatty acid iso- $C_{17:1}\omega9c$.

Table 2. Cellular fatty acid compositions (%) of strain CAU1172^T and the type strains of the most closely related *Altererythrobacter* species

Strains: 1, CAU1172^T; 2, *A. gangjinensis* KACC 16190^T; 3, *A. aestia-quae* KCTC 42006^T; 4, *A. indicus* KACC 13863^T; 5, *A. luteolus* KCTC 12311^T. All data were obtained in this study. Fatty acids amounting to <1.0 % in all strains are not shown. TR, Trace amount (<1.0 %); -, not detected.

Fatty acid	1	2	3	4	5
Saturated					
C _{14:0}	-	-	-	1.5	-
C _{16:0}	8.4	6.3	11.4	8.0	4.9
C _{17:0}	1.3	TR	1.3	-	-
C _{18:0}	-	_	-	1.2	-
Unsaturated					
С _{18:1} ω7с 11-methyl	9.3	4.7	2.3	-	10.4
$C_{18:1}\omega 5c$	1.2	1.0	-	1.2	2.6
$C_{16:1}\omega 5c$	-	1.0	-	-	2.1
$C_{17:1}\omega 6c$	17.8	16.2	2.5	5.7	5.4
$C_{17:1}\omega 8c$	2.9	4.0	3.5	1.5	-
$C_{18:1}\omega7c$	31.9	47.9	40.6	67.6	45.8
Branched-chain					
iso-C _{17:1} <i>ω</i> 9 <i>c</i>	1.5	-	-	-	-
iso-C _{18:1} H	1.1	-	-	-	-
iso-C _{16:0} 3-OH	-	-	-	-	1.1
Hydroxy					
C _{14:0} 2-OH	5.4	1.2	4.9	10.4	-
C _{15:0} 2-OH	4.1	1.2	5.2	1.6	-
C _{16:0} 2-OH	1.4	1.1	2.7	1.0	2.4
С _{16:1} 2-ОН	-	2.2	-	-	1.4
Summed features*					
3	12.2	11.2	22.3	-	17.2

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

The polar lipid composition of strain CAU1172^T indicated diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, sphingoglycolipid and phospatidylcholine as major polar lipids; four unidentified polar lipids were also detected (Fig. S2). The profile of major polar lipids of strain CAU1172^T was similar that of other *Altererythrobacter* species such as *Altererythrobacter indicus* (Kumar *et al.*, 2008), *A. aestuarii* (Park *et al.*, 2011), *A. dongtanensis* (Fan *et al.*, 2011), *A. gangjinensis* (Jeong *et al.*, 2013), *A. aestiaquae* (Jung *et al.*, 2014), *A. namhicola* (Park *et al.*, 2011) and *A. oceanensis* (Yang *et al.*, 2014). Methanol extracts of strain CAU1172^T showed absorption maxima at 450 and 476 nm, indicating the presence of carotenoid-like pigments.

Therefore, these data provide appropriate evidence to recognize the proposal to identify strain CAU1172^T based on phylogenetic, phenotypic, biochemical and chemotaxonomic analysis as representing a novel species of the genus Altererythrobacter, for which the name Altererythrobacter sediminis sp. nov. is proposed.

Description of *Altererythrobacter* sediminis sp. nov.

Altererythrobacter sediminis sp. nov. (se.di'mi.nis. L. gen. n. sediminis of sediment).

Cells are 0.2–0.5 µm in diameter and 1.5–3.3 µm in length. Colonies on MA are yellow, circular and convex after 3 days of incubation at 30 °C. Cells do not contain flexirubin-type pigments on MA but carotenoid pigments are detected at wavelengths between 450 and 476 nm. Cells are Gram-stainnegative, aerobic, non-spore forming, ovoid rods. Growth occurs at 20-37 °C (optimum, 30 °C), at pH 6.5-10.0 (optimum, 7.5) and in the presence of 0-4 % (w/v) NaCl (optimum, 1%). Catalase is present but oxidase is absent. Gelatin, casein, starch and urea are not hydrolysed. H₂S is not produced. Citrate is not utilized. D-Arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, D-galactose, Lsorbose, L-rhamnose, dulcitol, inositol, D-sorbitol and potassium 5-ketogluconate are utilized as sole carbon and energy source. In 20NE strips, L-arginine and aesculin are positive, but potassium nitrate, L-tryptophan, 4-nitrophenyl- β -D-galactopyranoside, L-arabinose, D-mannise, D-mannitol, Nacetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are negative. In API ZYM strips, alkaline phosphatase, esterase (C4), esterase lipase (C8), α -chymotrypsin and naphthol-AS-BI-phosphohydrolase are positive, but lipase (C14), leucine arylamidase, valine arylamidase, crystine arylamidase, trypsin, acid phosphatase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α fucosidase are negative. The only respiratory quinone is Q-10. The major fatty acids (>10 % of the total) are $C_{18:1}\omega 7c$, $C_{17:1}\omega 6c$ and summed feature 3 (comprising $C_{16:1}\omega 7c$ and/ or $C_{16:1}\omega_{6c}$).

The type strain, CAU 1172^{T} (=KCTC 42453^{T} =NBRC 110917^{T}), was isolated from a sample of lagoon sediment from along the east coast of Korea. The DNA G+C content of the type strain is 63.2 mol%.

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