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Aureimonas glaciistagni sp. nov., isolated from a melt pond on Arctic sea ice

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A Gram-staining-negative, motile, aerobic and rod-shaped bacterial strain, PAMC 27157^T, was isolated from a melt pond on sea ice in the Chukchi Sea. Phylogenetic analysis of the 16S rRNA gene sequence of strain PAMC 27157^T revealed an affiliation to the genus *Aureimonas* with the closest sequence similarity (96.2 %) to that of *Aureimonas phyllosphaerae*. Strain PAMC 27157^T grew optimally at 30 °C and pH 7.0 in the presence of 3.5 % (w/v) NaCl. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylmonomethylethanolamine, sulfoquinovosyldiacylglycerol and an unidentified aminolipid. The major cellular fatty acid was summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$, 83.1 %) and the major respiratory quinone was Q-10. The genomic DNA G+C content was 69.1 mol%. The combined phylogenetic, phenotypic and chemotaxonomic data showed that strain PAMC 27157^T could be clearly distinguished from species of the genus *Aureimonas* with validly published names. Thus, strain PAMC 27157^T should be classified as representing a novel species in the genus *Aureimonas*, for which the name *Aureimonas glaciistagni* sp. nov. is proposed. The type strain is PAMC 27157^T (=KCCM 43049^T=JCM 30183^T).

The family *Aurantimonadaceae* in the order *Rhizobiales* (Kuykendall, 2005) contains four genera, which are *Aurantimonas* (Denner *et al.*, 2003), *Aureimonas* (Rathsack *et al.*, 2011), *Fulvimarina* (Cho & Giovannoni, 2003) and *Martelella* (Rivas *et al.*, 2005). Despite the difficulty in defining distinct taxonomic groups in the family *Aurantimonadaceae* due to the lack of consistency in 16S rRNA gene sequence analysis (Lee *et al.*, 2005), attempts have been made to elucidate the taxonomic positions of the previously described species in the family *Aurantimonadaceae*. Rathsack *et al.* (2011) transferred the former three species in the genus *Aurantimonas* to a new genus *Aureimonas*, proposing that the genus *Aureimonas* is distinguishable by the presence of the glycolipid sulfoquinovosyldiacylglycerol (SQDG) as well as 16S rRNA gene sequence analyses.

Abbreviation: SQDG, sulfoquinovosyldiacylglycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PAMC 27157^T is KM273177.

One supplementary table and one supplementary figure are available with the online Supplementary Material.

The genus *Aureimonas* comprised seven recognized species at the time of writing (List of Prokaryotic Names with Standing in Nomenclature; http://www.bacterio.net/aureimonas.html). The members of the genus *Aureimonas* have been isolated from various terrestrial environments such as a plant leaf, cave, iron plate, water-cooling system and urban air (Jurado *et al.*, 2006; Weon *et al.*, 2007; Kim *et al.*, 2008; Rathsack *et al.*, 2011; Lin *et al.*, 2013; Madhaiyan *et al.*, 2013). Here we describe a novel strain belonging this genus, which was isolated from a freshwater melt pond on Arctic sea ice.

Sampling of melt pond water (salinity of 0.2‰) was performed on sea ice in the Chukchi Sea (82° 10′ 45″ N, 171° 52′ 6″ W), during the ARAON expedition in August 2012. Aliquots (100 µl) of melt pond water were spread onto marine agar (MA; Difco) and nutrient agar (NA; Difco) to isolate halotolerant and freshwater bacteria, respectively. After incubation at 20 °C under aerobic conditions for 5 days, single colonies were streaked onto the respective plates and then subcultured more than four times to isolate pure strains. Strain PAMC 27157^T first originated from a colony grown on MA. After salinity and

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temperature optima were determined, strain PAMC 27157^T was routinely cultured onto MA and in marine broth (MB; Difco) at 30 °C. The strain was preserved in MB with 30 % (v/v) glycerol at −80 °C.

Aureimonas phyllosphaerae KACC 16231^T, *Aureimonas jatrophae* KACC 16230^T, *Aureimonas ureilytica* KACC 11607^T and *Aureimonas altamirensis* KCTC 22106^T were used as reference strains in morphological, physiological and biochemical analyses. Growth of each strain was tested on MA, R2A (Difco) and tryptic soy agar (TSA; Difco) under various temperature and salinity conditions. All morphological and physiological characteristics described hereafter were based on cultures grown for 4 days on their optimal media (R2A for *Aureimonas phyllosphaerae* KACC 16231^T, *Aureimonas jatrophae* KACC 16230^T and *Aureimonas ureilytica* KACC 11607^T; TSA for *Aureimonas altamirensis* KCTC 22106^T) at 30 °C, unless otherwise specified.

For 16S rRNA gene amplification by PCR, DNA was extracted from a single colony by the boiling method (Englen & Kelley, 2000). The crude extracts were used as DNA template for PCRs, which included 27F and 1492R primers (Lane, 1991) and *Taq* DNA polymerase (Takara). The PCR product was purified by treatment with shrimp alkaline phosphatase and exonuclease I (USB). Direct sequencing of the purified PCR product was performed using sequencing primers (27F, 518F, 800R and 1492R; Lane, 1991; Anzai *et al.*, 1997) in an Applied Biosystems sequencer (ABI 3730XL) at Cosmo Genetech (Seoul, Korea). The almost complete 16S rRNA gene sequence (1469 bp) of strain PAMC 27157^T was compared against sequences in the GenBank and EzTaxon-e databases using BLASTN (Altschul *et al.*, 1997; Kim *et al.*, 2012). The 16S rRNA gene sequences of closely related taxa obtained from the GenBank database were aligned using the RDP aligner (Cole *et al.*, 2014) based on secondary structures. Phylogenetic analysis was performed using the program MEGA 6 (Tamura *et al.*, 2013). Distance matrices were calculated according to the Jukes and Cantor model (Jukes & Cantor, 1969). Phylogenetic trees were inferred using the neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1992) and maximum-likelihood methods (Felsenstein, 1981) using bootstrap analyses based on 1000 replications.

Morphological and physiological tests were performed as follows. Gram staining was performed using the method of Smibert & Krieg (1994). Cell motility of the strain was observed by the hanging drop method (Suzuki *et al.*, 2001). Cells grown for 3 days with shaking (100 r.p.m.) at 30 °C in MB were observed by epifluorescence microscopy and transmission electron microscopy (EX2; JEOL) to examine cell morphology. Anaerobic growth was tested on MA agar using the GasPak anaerobic system (BBL) at 30 °C for 15 days. Catalase activity was determined by bubble formation after adding 3 % (v/v) H_2O_2 and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine (Cappuccino & Sherman, 2002). The temperature range for growth was determined by assessing changes in the OD_{600} over time in optimal broth media for each strain (2 °C and 5–45 °C at increments of 5 °C) for 14 days. The pH range for growth was also determined by assessing changes in the OD_{600} in optimal broth media adjusted to different pH (pH 5.0–12.0, at increments of 0.5 pH units), incubating at 30 °C over 10 days. Buffers were used for pH range experiments [citrate phosphate buffer for pH 5.0–5.5, MES for pH 6.0–6.5, MOPS for pH 7.0–7.5, 2-amino-2-methyl-1,3-propanediol (AMPD) for pH 8.0–9.5 and CAPS for pH 10.0–12.0, each at a final concentration of 50 mM; Hwang & Cho, 2008; Cho *et al.*, 2013; Jang *et al.*, 2013]. The pH of a subsample of autoclave-sterilized media was measured before inoculation of cells to check pH change. There were no significant pH changes of media after autoclave sterilization. Salt tolerance was tested on the basis of changes in the OD_{600} in synthetic ZoBell medium (per litre distilled water: 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate) supplemented with 0–20 % NaCl or sea salts (Sigma) in increments of 0.5 % (w/v) from 0 to 10 % and at 12, 15, 18 and 20 % at 30 °C over 10 days.

Hydrolysis of starch and Tweens 20, 40, 60 and 80 was tested according to the method of Hansen & Sørheim (1991) after 7 days of incubation. Decomposition of hypoxanthine was determined by the method of Smibert & Krieg (1994). Carbon source utilization was tested in basal medium including 0.4 % (w/v) carbon source (Bruns *et al.*, 2001) for strain PAMC 27157^T. The basal medium was slightly modified by excluding NaCl and $CaCl_2$ for strains *Aureimonas phyllosphaerae* KACC 16231^T, *Aureimonas jatrophae* KACC 16230^T, *Aureimonas ureilytica* KACC 11607^T and *Aureimonas altamirensis* KCTC 22106^T due to their slow growth in moderately saline medium. Carbon utilization was determined as negative when growth was equal to, or less than, that in the negative control with no carbon source. Growth was measured by monitoring changes in the OD_{600} during 14 days of incubation at 30 °C. In addition, other biochemical activities of strain PAMC 27157^T and the reference strains were determined by using API ZYM, API 20NE and API 50CH kits (bioMérieux) according to the manufacturer's instructions.

For the chemotaxonomic analyses of isoprenoid quinones, fatty acids and polar lipids, strain PAMC 27157^T and the reference strains cultured on their optimal media at 30 °C for 4 days were used. Polar lipids were extracted by the method described by Minnikin *et al.* (1984), examined by two-dimensional TLC and identified by spraying with detection reagents (Komagata & Suzuki, 1987). All TLC plates were scanned immediately after the end of reaction with detection reagents. The fatty acid methyl esters in whole cells of strain PAMC 27157^T and the reference strains were analysed by gas chromatography according to the instructions of the Microbial Identification System

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(version 6.2; MIDI) with the RTSBA6 database at the Korean Culture Center of Microorganisms (KCCM) in Seoul, Korea. Isoprenoid quinone composition was determined according to the method of Minnikin *et al.* (1984) and analysed by HPLC at the KCCM as described by Collins (1985). The DNA G+C content of strain PAMC 27157^T was also analysed by HPLC at the KCCM.

Phylogenetic analyses based on 16S rRNA gene sequence showed that strain PAMC 27157^T belonged to the genus *Aureimonas*. Strain PAMC 27157^T was most closely related to the type strain *Aureimonas phyllosphaerae* KACC 16231^T with 16S rRNA gene sequence similarity of 96.2 %. The 16S rRNA gene sequence similarity between strain PAMC 27157^T and the other related species of the genus *Aureimonas* were 94.7–95.8 %. The phylogenetic tree analyses showed that strain PAMC 27157^T formed a robust clade comprising *Aureimonas jatrophae*, *Aureimonas phyllosphaerae*, *Aureimonas ureilytica*, *Aureimonas rubiginis* and *Aureimonas ferruginea* in the genus *Aureimonas* (Fig. 1). Thus, low similarity values (i.e. <97 %) with recognized species of the genus *Aureimonas* and the phylogenetic position of strain PAMC 27157^T showed that the strain could be assigned to a novel species in the genus *Aureimonas*. The DNA G+C content of strain PAMC 27157^T was 69.1 mol%.

The results of morphological, physiological and biochemical characteristics for strain PAMC 27157^T are given in the species description and Table 1. The major isoprenoid quinone of strain PAMC 27157^T was Q-10, which is a consistent result in the class *Alphaproteobacteria* (Rathsack *et al.*, 2011). The fatty acid profile was dominated by summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; 83.1 %; Table S1 available in the online Supplementary Material). Previous studies have shown that $C_{18:1}\omega7c$ is predominant in all members of the genus *Aureimonas* (Weon *et al.*, 2007; Rathsack *et al.*, 2011; Madhaiyan *et al.*, 2013). Distinct differences have also been observed among members of the genus. It is notable that the proportion of fatty acid $C_{18:0}$ in strain PAMC 27157^T (6.2 %) was higher than those in other type strains (1.0–2.8 %; Table S1). The polar lipids of strain PAMC 27157^T were composed of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylmonomethylethanolamine, an unidentified aminolipid and SQDS (Fig. S1). The polar lipid analyses showed similar compositions to those of other species of the genus *Aureimonas* (Madhaiyan *et al.*, 2013). Especially, our result confirmed the presence of SQDG, which is a specific feature of the genus *Aureimonas*, not detected in members of the genera *Fulvimarina* and *Aurantimonas* in the family *Aurantimonadaceae* (Rathsack *et al.*, 2011). Furthermore, other phenotypic characteristics can obviously differentiate strain PAMC 27157^T from phylogenetically related species of the genus *Aureimonas*, for example optimal salinity for growth, certain enzyme activities, acid production from some carbohydrates and utilization of sole carbon sources (Table 1).

In conclusion, based on the phylogenetic, physiological and chemotaxonomic data described above, we suggest that strain PAMC 27157^T represents a novel species of the genus *Aureimonas*, for which the name *Aureimonas glaciistagni* sp. nov. is proposed.

Description of *Aureimonas glaciistagni* sp. nov.

Aureimonas glaciistagni (gla.ci.i.stag′ni. L. fem. n. *glacies* ice; L. gen. n. *stagni* of/from a pond; N.L. gen. n. *glaciistagni* from/of an ice pond).

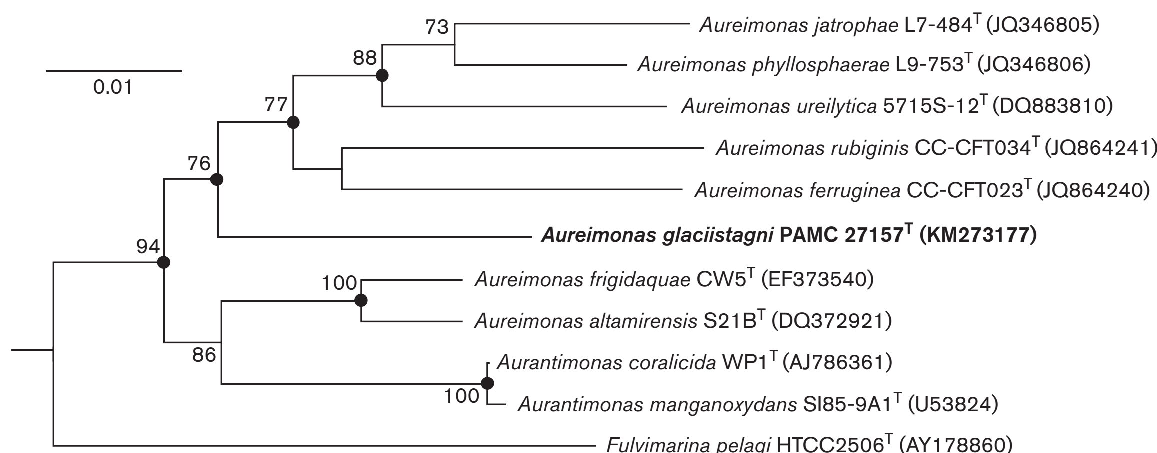

Fig. 1. Neighbour-joining tree showing the phylogenetic position of strain PAMC 27157^T and related species of the family *Aurantimonadaceae*, based on 16S rRNA gene sequences. Bootstrap values >60 % (based on 1000 replications) are given at branch points. Filled circles indicate that the corresponding nodes were also obtained in both the maximum-likelihood and the minimum-evolution trees. Bar, 0.01 substitutions per nucleotide position.

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Table 1. Selected physiological and biochemical characteristics of strain PAMC 27157^T and closely related species of the genus *Aureimonas*

Strains: 1, PAMC 27157^T; 2, *Aureimonas phyllosphaerae* KACC 16231^T; 3, *Aureimonas jatrophae* KACC 16230^T; 4, *Aureimonas ureilytica* KACC 11607^T; 5, *Aureimonas altamirensis* KCTC 22106^T. Data were obtained in this study, unless otherwise indicated. +, Positive; w, weakly positive; −, negative.

Characteristic	1	2	3	4	5
Temperature range for growth (°C) (optimum)	5−40 (30)	10−40 (30)	10−35 (30)	10−40 (30)	5−40 (30)
NaCl tolerance range for growth (%, w/v) (optimum)	0−5.0 (3.5)	0−1.5 (0)	0−3.5 (0)	0−5 (1.5)	0−5 (0−2)
Hydrolysis of:					
Tweens 20, 60	+	−	+	+	+
Tweens 40, 80	+	−	+	−	+
API ZYM tests					
Cystine arylamidase	−	+	−*	−	−
Trypsin	w	−	+	+	+
Valine arylamidase	w	+	+	+	−
API 20NE tests					
Arginine dihydrolase	−	−	−	−	+
Fermentation of glucose	−	−	+*	−	+
β-Galactosidase	−	−	+	+*	−
β-Glucosidase	+	−	−*	+	−
Acid production (API 50CH)					
D-Adonitol	−	−	+	+	w
Erythritol	−	−	+	+	+
Aesculin	−	+	+	+	−
D-Fructose	+	−	+	+	w
Glycogen	−	−	−	+	−
Methyl β-D-xylopyranoside	−	−	+	−	−
Raffinose	+	−	−	+	−
D-Sorbitol	−	−	+	−	−
Utilization as sole carbon source					
L-Arabinose	+	−	−	−	−
Cellobiose	+	−	+	−	−
Inositol	−	+	−	−	−
D-Mannose	+	−	−	−	−
L-Ornithine	−	−	+	−	−
L-Proline	+	+	+	−	+
Trehalose	+	−	−	−	−
DNA G+C content (mol%)†	69.1	69.4	66.1	67.0	71.8

*Result opposite to that of each original study.
†Data for strains 2–3, 4 and 5 were from Madhaiyan *et al.* (2013), Weon *et al.* (2007) and Jurado *et al.* (2006), respectively.

Gram-staining-negative, aerobic and motile. Short rods of size 0.5–0.6 μm in width and 1.1–2.1 μm in length. After 7 days of incubation on MA at 30 °C, colonies are circular, convex, entire, opaque and yellow in colour, and 1.0–1.5 mm in diameter. Growth occurs at 5 to 40 °C (optimum 30 °C) and pH 6.0 to 10.5 (optimum pH 7.0). Growth occurs in ZoBell broth with 0 to 5.0 % NaCl (optimum 3.5 %). Positive for catalase and oxidase activities. Hypoxanthine and starch are not hydrolysed, but Tweens 20, 40, 60 and 80 are hydrolysed. According to the API ZYM test, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14; weakly positive), leucine arylamidase, valine arylamidase (weakly positive), trypsin (weakly positive), acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for cystine arylamidase, α-chymotrypsin, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. According to the API 20NE test, positive for urease and β-glucosidase activities, but negative for arginine dihydrolase, fermentation of glucose, β-galactosidase, indole production, nitrate reduction and protease activities. According to the API 50CH test, positive for acid production from D-arabinose, L-arabinose, cellobiose (weakly positive), D-fructose, D-fucose, D-galactose, gentiobiose (weakly positive), D-glucose, L-rhamnose, lactose (weakly positive), D-lyxose (weakly positive), D-mannose, melibiose, raffinose, D-ribose, sucrose, D-xylose and L-xylose, but negative for *N*-acetylglucosamine, D-adonitol,

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amygdalin, D-arabitol, L-arabitol, arbutin, dulcitol, erythritol, aesculin, L-fucose, glycerol, glycogen, inositol, inulin, D-mannitol, maltose, melezitose, methyl-α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, trehalose, turanose and xylitol. L-Arabinose, DL-aspartate, cellobiose, D-fructose, glucose, maltose, D-mannitol, D-mannose, L-proline, pyruvate, raffinose, sucrose and trehalose are utilized as the sole carbon source, but acetate, citrate, formate, L-glutamate, glycerol, inositol, melibiose, L-ornithine and L-rhamnose are not. The only isoprenoid quinone is Q-10. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylmonomethylethanolamine, an unidentified aminolipid and SQDS.

The type strain is PAMC 27157^T (=KCCM 43049^T=JCM 30183^T), isolated from a melt pond on sea ice in the Chukchi Sea. The genomic DNA G+C content of the type strain is 69.1 mol%.

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