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Roseomonas aerofrigidensis sp. nov., isolated from an air conditioner

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Abstract

A Gram-stain-negative, strictly aerobic bacterium, designated HC1^T, was isolated from an air conditioner in South Korea. Cells were orange, non-motile cocci with oxidase- and catalase-positive activities and did not contain bacteriochlorophyll *a*. Growth of strain HC1^T was observed at 10–45 °C (optimum, 30 °C), pH 4.5–9.5 (optimum, pH 7.0) and 0–3 % (w/v) NaCl (optimum, 0 %). Strain HC1^T contained summed feature 8 (comprising $C_{18:1}\omega7c$/$C_{18:1}\omega6c$), $C_{16:0}$ and cyclo-$C_{19:0}\omega8c$ as the major fatty acids and ubiquinone-10 as the sole isoprenoid quinone. Phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and an unknown aminolipid were detected as the major polar lipids. The major carotenoid was hydroxyspirilloxanthin. The G+C content of the genomic DNA was 70.1 mol%. Phylogenetic analysis, based on 16S rRNA gene sequences, showed that strain HC1^T formed a phylogenetic lineage within the genus *Roseomonas*. Strain HC1^T was most closely related to the type strains of *Roseomonas oryzae*, *Roseomonas rubra*, *Roseomonas aestuarii* and *Roseomonas rhizosphaerae* with 98.1, 97.9, 97.6 and 96.8 % 16S rRNA gene sequence similarities, respectively, but the DNA–DNA relatedness values between strain HC1^T and closely related type strains were less than 70 %. Based on phenotypic, chemotaxonomic and molecular properties, strain HC1^T represents a novel species of the genus *Roseomonas*, for which the name *Roseomonas aerofrigidensis* sp. nov. is proposed. The type strain is HC1^T (=KACC 19097^T=JCM 31878^T).

The genus *Roseomonas* was first proposed by Rihs *et al.* [1] as a member of the family *Acetobacteraceae* of the class *Alphaproteobacteria* and the phylum *Proteobacteria*. Many members of the genus *Roseomonas* were initially isolated from clinical samples such as blood, wounds and genitourinary specimens, probably indicating their association with human infectious diseases [1–5]. However, recently members of the genus *Roseomonas* have been isolated from many other non-clinical environmental samples such as activated sludge, a water-cooling system, water, air, phyllosphere, Arctic tundra soil, rhizosphere soil and a lagoon sediment [6–14]. Members of the genus *Roseomonas* feature Gram-stain-negative, motile or non-motile, plump cocci or short rods that contain ubiquinone-10 (Q-10) as the predominant isoprenoid quinone and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine as the polar lipids. In addition, some species of the genus *Roseomonas* produce bacteriochlorophyll-*a* and carotenoids of the spirilloxanthin series [1, 13–17]. In the present study, a novel species of the genus *Roseomonas*, designated strain HC1^T, was isolated from a room air conditioner and its taxonomic properties were characterized using a polyphasic approach.

Strain HC1^T was isolated from a room air conditioner mounted on a wall of a laboratory office in Seoul, South Korea. Briefly, a sample mixed with dust and condensed water was obtained from the evaporation fins of the air conditioner where heat-exchange occurs, and resuspended in PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 2 mM KH_2PO_4, pH 7.2). The resuspended sample was serially diluted in PBS buffer, spread on R2A agar (BD) and incubated at 25 °C for 3 days. The 16S rRNA genes from colonies grown on R2A agar were PCR-amplified using the universal primers F1 (5′-AGA GTT TGA TCM TGG CTC AG-3′) and R13 (5′-TAC GGY TAC CTT GTT ACG ACT T-3′). The PCR amplicons were sequenced using the universal primers 340F (5′-CCT ACG GGA GGC AGC AG-3′), 518R (5′-ATT ACC GCG GCT GCT GG-3′) and 805F (5′-GAT TAG ATA CCC TGG TAG TC-3′) to obtain almost complete 16S rRNA gene sequences. The resulting 16S rRNA gene sequences were compared with those of validated type strains using the Nucleotide Similarity Search

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Keywords: *Roseomonas aerofrigidensis*; new taxon; species novel; air conditioner.
Abbreviations: DDH, DNA–DNA hybridization; LB, Luria-Bertani; ML, maximum-likelihood; MP, maximum-parsimony; NA, nutrient agar; NJ, neighbour-joining; Q-10, ubiquinone-10; RDP, Ribosomal Database Project; TSA, tryptic soy agar.
The 16S rRNA gene sequence of strain HC1^T has been deposited in GenBank under the accession number KY126356.
One supplementary table and three supplementary figures are available with the online Supplementary Material.

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program in the EzTaxon-e server [18]. From the 16S rRNA gene sequence analysis, a putative novel strain belonging to the genus *Roseomonas* of the family *Acetobacteraceae*, designated strain HC1^T, was selected for further phenotypic and phylogenetic analyses. Strain HC1^T was routinely cultured on R2A agar at 30 °C for 3 days. Strain HC1^T was stored in R2A broth containing 15 % (v/v) glycerol at –80 °C for long-term preservation. The type strains of *Roseomonas oryzae* (KCTC 42542^T), *Roseomonas rubra* (KEMB 563-468^T), *Roseomonas aestuarii* (KACC 16549^T), *Roseomonas rhizosphaerae* (KACC 17225^T), *Roseomonas cervicalis* (KACC 11686^T) and *Roseomonas gilardii* subsp. *gilardii* (KACC 11652^T) were used as reference strains for comparison of phenotypic properties and cellular fatty acid compositions.

The 16S rRNA gene sequences of strain HC1^T and closely related type strains were aligned using the fast secondary-structure aware Infernal aligner, which is available in the Ribosomal Database Project (RDP) [19]. Phylogenetic trees, based on the neighbour-joining (NJ) and maximum-parsimony (MP) algorithms were reconstructed using PHYLIP software (ver. 3.695) [20], and their tree topologies were evaluated through a bootstrap analysis based on a 1000-resampled dataset. A phylogenetic tree with bootstrap values based on the maximum-likelihood (ML) algorithm was also reconstructed using RAxML-HPC BlackBox (version 8.2.4) of the Cyber-Infrastructure for Phylogenetic Research project (CIPRES, www.phylo.org; [21]). An additional taxonomic analysis was performed using the RDP naïve Bayesian rRNA Classifier tool at an 80 % confidence threshold (http://rdp.cme.msu.edu/classifier; [22]). DNA–DNA relatedness between strain HC1^T and the type strains of *R. oryzae*, *R. rubra*, *R. aestuarii* and *R. rhizosphaerae* was evaluated by DNA–DNA hybridization (DDH) using a genome-probing microarray method [23]. The DDH experiments were performed by reciprocally interchanging DDH between probe and target DNA.

An NJ algorithm-based phylogenetic tree displayed that strain HC1^T formed a phylogenetic lineage with *R. oryzae* JC288^T within the genus *Roseomonas* (Fig. 1). Phylogenetic trees based on the ML and MP algorithms also supported strain HC1^T forming a phylogenetic lineage within the genus *Roseomonas* (Fig. S1, available in the online Supplementary Material). Taxonomic analysis, using the RDP Classifier tool, also showed that strain HC1^T was classified as a member of the genus *Roseomonas*. Comparative analysis, based on 16S rRNA gene sequences, revealed that strain HC1^T was most closely related to *R. oryzae* JC288^T, *R. rubra* S5^T, *R. aestuarii* JC17^T, *R. rhizosphaerae* YW11^T and *R. cervicalis* ATCC 49957^T with 98.1, 98.1, 97.9, 97.6 and 96.8 % sequence similarities, respectively. The levels of DNA–DNA relatedness between strain HC1^T and *R. oryzae*, *R. rubra*, *R. aestuarii* and *R. rhizosphaerae* were 48.5±2.4, 38.8±4.1, 45.1±2.9 and 33.2±5.8 % respectively, values which are well below the 70 % threshold generally accepted for species delineation [24, 25].

Growth of strain HC1^T was tested on R2A agar (BD), laboratory prepared Luria-Bertani (LB) agar, nutrient agar (NA, BD) and tryptic soy agar (TSA, BD) at 30 °C for 3 days. Growth of strain HC1^T at different temperatures (4, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C) and pH values (4.0–10.0 at 0.5 pH unit intervals) was evaluated on R2A agar and in R2A broth, respectively, after 3 days of incubation. R2A broth media below pH 7.0 and at pH 7.5–10.0 were prepared using Na_2HPO_4-NaH_2PO_4 and Tris-HCl buffers, respectively [26]. The pH values of R2A broth media were checked again after sterilization (121 °C for 15 min) and adjusted if necessary. Growth of strain HC1^T at different NaCl concentrations (0, 1, 2, 3, 4, 5, 6 and 7 %, w/v) was evaluated in R2A broth prepared in the laboratory based on the BD formula. Gram-staining was investigated using the bioMérieux Gram stain kit, according to the manufacturer's instructions. Oxidase activity was evaluated by the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck), and catalase activity was tested by the production of oxygen bubbles in 3 % (v/v) aqueous H_2O_2 [27]. Cell morphology was investigated using phase-contrast microscopy (AXIO Scope A1; ZEISS) and transmission electron microscopy (JEM-1010; JEOL) with cells in the exponential phase grown on R2A agar at 30 °C. Anaerobic growth was assessed on R2A agar under anaerobic conditions (with 4–10 % CO_2) using the GasPak Plus system (BBL) at 30 °C for 21 days. The following phenotypic properties of strain HC1^T and six reference strains were investigated under the same conditions in parallel: the hydrolysis of casein, starch, aesculin, tyrosine, urea, gelatin, Tween 20 and Tween 80 was tested on R2A agar [27, 28]; nitrate reduction was assessed according to a method described previously [28]; additional enzymatic activities, biochemical features and oxidation of various carbon sources were evaluated using API ZYM, API 20NE (bioMérieux) and GN2 MicroPlates (Biolog), respectively, according to the manufacturer's instructions, except that the test strains were incubated at their optimal growth temperatures.

Strain HC1^T grew well on R2A agar, LB agar, NA and TSA (optimum, R2A agar). Cells of strain HC1^T were Gram-stain-negative and non-motile cocci (1.1–1.3 µm in diameter) (Fig. S2). Anaerobic growth of strain HC1^T was not observed after 21 days of incubation at 30 °C. Although some phenotypic properties (including enzyme activity of catalase and oxidase, esterase (C4), leucine arylamidase and naphthol-AS-BI-phosphohydrolase and assimilation of malic acid) were similar to those of the reference strains of the genus *Roseomonas*, many other phenotypic properties (such as nitrate reduction to nitrite, hydrolysis of casein, aesculin, Tween 20 and urea and activities of alkaline phosphatase, esterase lipase (C8), lipase (C14), valine arylamidase, crystine arylamidase, β-glucosidase, α-chymotrypsin, acid phosphatase, β-glucuronidase, α-glucosidase and *N*-acetyl-β-glucosaminidase) differentiated strain HC1^T from the reference species of the genus *Roseomonas*. Additional physiological and biochemical characteristics of strain HC1^T are described in the species description, and compared with those of related members of the genus *Roseomonas* in Tables 1 and S1.

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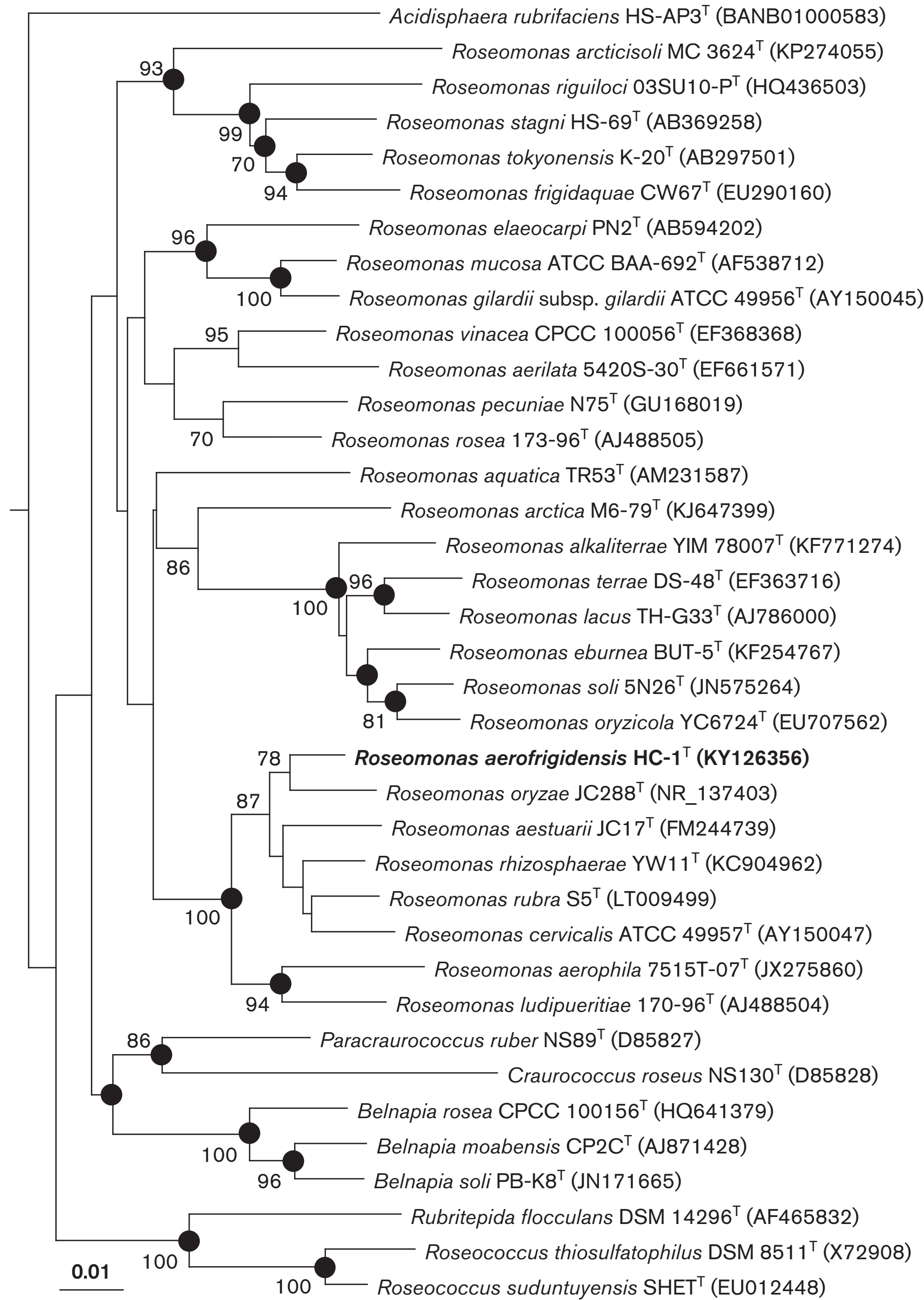

Fig. 1. A neighbor-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic relationships of strain HC1^T and related taxa. Bootstrap values are shown on nodes as percentages of 1000 replicates (only values over 70 %). Filled circles (●) indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Erythrobacter longus* DSM6997^T (JMIW01000006) was used as an outgroup (not shown). The scale bar equals 0.01 changes per nucleotide position.

The DNA G+C content of strain HC1^T was determined by the fluorometric method [29] using SYBR Green I and a real-time PCR thermocycler (Bio-Rad). Isoprenoid quinones were extracted according to a previously described method [30] and analyzed using a model LC-20A HPLC system (Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu) and a reversed-phase column (250 Í 4.6 mm, Kromasil; Akzo Nobel), as previously described [31]. For cellular fatty acid analysis, strain HC1^T and six reference strains were cultivated in R2A broth at 30 °C and microbial cells were harvested at the same growth stage (exponential phase, optical density, OD_{600}=0.8). The cellular

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Table 1. Comparison of phenotypic characteristics of strain HC1^T and the type strains of closely related species of the genus *Roseomonas*

Taxa: 1, strain HC1^T (present study); 2, *R. oryzae* KCTC 42542^T [13]; 3, *R. rubra* KEMB 563-468^T [14]; 4, *R. aestuarii* KACC 16549^T [15]; 5, *R. rhizosphaerae* KACC 17225^T [16]; 6, *R. cervicalis* KACC 11686^T [1]; 7, *R. gilardii* subsp. *gilardii* KACC 11652^T [1]. All data were obtained from this study, except for the DNA G+C contents. All strains were positive for the following characteristics: catalase, oxidase, activity of esterase (C4), leucine arylamidase and naphthol-AS-BI-phosphohydrolase and assimilation of malic acid. All strains were negative for the following characteristics: hydrolysis of starch, tyrosine, gelatin and Tween 80, reduction of nitrate to nitrogen, indole production, fermentation of glucose, enzyme activity of arginine dihydrolase, trypsin, α-galactosidase, β-galactosidase, α-mannosidase and α-fucosidase, and the assimilation of D-glucose, *N*-acetyl-glucosamine, maltose and capric acid. +, Positive; –, negative.

Characteristic	1	2	3	4	5	6	7
Ranges for growth							
Temperature (°C)	10–45	4–45	10–45	20–40	15–40	20–40	15–45
pH	4.5–9.5	6.0–7.5	6.5–9.0	6.0–8.0	5.5–8.0	5.5–9.0	5.0–8.0
NaCl (%, w/v)	0–3.0	0–6.0	0–3.0	0–1.0	0–5.0	0–1.0	0–6.0
Reduction of nitrate to nitrite	–	+	+	+	–	–	–
Hydrolysis of:							
Casein, aesculin	–	–	+	–	–	–	–
Tween 20	+	+	–	+	–	–	+
Urea	+	–	+	+	+	+	+
Enzyme activity (API ZYM)							
Alkaline phosphatase	+	–	+	+	+	+	+
Esterase lipase (C8)	+	+	+	+	+	+	–
Lipase (C14)	–	–	–	–	+	+	+
Valine arylamidase	–	–	+	–	–	+	–
Crystine arylamidase, β-glucosidase	–	–	+	–	–	–	–
α-Chymotrypsin	+	+	–	+	+	+	+
Acid phosphatase	+	+	+	–	+	+	+
β-Glucuronidase	–	+	+	–	–	–	–
α-Glucosidase	–	+	+	–	–	–	+
N-Acetyl-β-glucosaminidase	–	+	–	–	–	–	–
Assimilation (API 20NE) of:							
L-Arabinose	–	–	–	–	–	+	+
D-Mannose	–	–	+	–	–	–	–
D-Mannitol, phenylacetic acid	–	–	–	–	–	–	+
Potassium gluconate	–	–	+	+	–	+	–
Adipic acid	+	–	+	+	+	+	–
Trisodium citrate	–	–	+	–	+	+	+
DNA G+C content (mol%)	70.1	67.5	73.5	66.2	69.6	70.4	67.6

fatty acids of microbial cells were saponified and methylated using the standard MIDI protocol. The fatty acid methyl esters were analyzed by gas chromatography (Hewlett Packard 6890) and identified by using the TSBA6 database of the Microbial Identification System (Sherlock ver. 6.0B; [32]). The polar lipids of strain HC1^T were chromatographically analyzed by TLC using cells harvested during the exponential growth phase, following the procedure described previously [33]. The following reagents were used to detect different polar lipids: 10 % (v/v) ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (for aminolipids), Dittmer-Lester reagent (for phospholipids), α-naphthol (for glycolipids) and Dragendorff reagent (for choline). Pigments of cell extracts were analyzed using an HPLC system (Shimadzu) equipped with a C_{18}-column, according to the method described previously [34].

The genomic DNA G+C content of strain HC1^T was approximately 70.1 mol%, which was in the range of the DNA G+C contents of species of the genus *Roseomonas* (Table 1). The isoprenoid quinone of strain HC1^T was Q-10, which is identical to those detected in other members of the genus *Roseomonas* [1, 13–17]. The major cellular fatty acids (>5 % of the total fatty acids) of strain HC1^T were summed feature 8 (comprising $C_{18:1}\omega7c$/$C_{18:1}\omega6c$, 73.4 %), $C_{16:0}$ (13.2 %) and cyclo-$C_{19:0}\omega8c$ (6.7 %). The overall fatty acid profile of strain HC1^T was similar to those of members of the genus *Roseomonas*, but there were some differences in the respective proportions of some fatty acid components such as cyclo-$C_{19:0}\omega8c$, $C_{18:1}$ 2-OH and summed feature 3 ($C_{16:1}\omega7c$/$C_{16:1}\omega6c$) (Table 2). The major polar lipids of strain HC1^T were phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and an unknown aminolipid (Fig. S3). Diphosphatidylglycerol, two unknown aminolipids and two

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Table 2. Cellular fatty acid compositions (as a percentage of the total) of strain HC1^T and the type strains of closely related species of the genus *Roseomonas*

Taxa: 1, strain HC1^T; 2, *R. oryzae* KCTC 42542^T; 3, *R. rubra* KEMB 563-468^T; 4, *R. aestuarii* KACC 16549^T; 5, *R. rhizosphaerae* KACC 17225^T; 6, *R. cervicalis* KACC 11686^T; 7, *R. gilardii* subsp. *gilardii* KACC 11652^T. All data were obtained from this study. Major components (>5.0 %) are highlighted in bold; –, Not detected.

Fatty acid	1	2	3	4	5	6	7
Saturated							
$C_{10:0}$	–	–	–	–	–	–	0.8
$C_{12:0}$	–	2.3	–	1.4	–	–	–
$C_{14:0}$	–	–	3.5	–	0.5	0.4	–
$C_{16:0}$	**13.2**	**13.2**	**18.8**	**13.8**	**11.2**	**11.2**	**28.1**
$C_{18:0}$	–	–	–	–	–	0.4	–
Unsaturated							
$C_{13:1}$ at 12–13	1.2	1.7	–	–	–	–	1.7
$C_{14:1}\omega5c$	–	–	–	–	–	–	4.7
$C_{16:1}\omega5c$	–	–	2.1	2.5	2.4	2.0	–
$C_{17:1}\omega6c$	–	–	–	–	–	0.7	–
$C_{18:1}\omega5c$	–	–	–	–	–	0.8	–
cyclo-$C_{19:0}\omega8c$	**6.7**	–	–	2.1	–	2.0	**13.5**
$C_{20:2}\omega6,9c$	–	–	–	–	–	–	2.6
Hydroxy							
$C_{16:0}$ 3-OH	–	–	–	–	–	0.6	–
$C_{18:1}$ 2-OH	2.7	**10.0**	–	4.3	4.3	**5.5**	3.2
Branched							
iso-$C_{10:0}$	–	–	–	–	–	–	**9.1**
iso-$C_{17:0}$ 3-OH	–	–	–	–	–	–	2.3
Summed features*							
2	–	–	–	–	–	0.3	–
3	3.0	**10.2**	**10.0**	**13.0**	**6.9**	2.8	2.1
8	**73.2**	**62.6**	**65.6**	**62.9**	**74.7**	**73.3**	**31.9**

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 2, $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I; summed feature 3, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 8, $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

unknown glycolipids were also detected as the minor polar lipids. The profile of the polar lipids of strain HC1^T was in good accordance with those of members of the genus *Roseomonas* [1, 13–17], except for some minor components. Hydroxyspirilloxanthin was detected as the major carotenoid in strain HC1^T, but bacteriochlorophyll *a* was not detected. In conclusion, the physiological and chemotaxonomic features and the phylogenetic inference of strain HC1^T support the proposition that it represents a novel species of the genus *Roseomonas*, for which the name *Roseomonas aerofrigidensis* sp. nov. is proposed.

DESCRIPTION OF *ROSEOMONAS AEROFRIGIDENSIS* SP. NOV.

Roseomonas aerofrigidensis (ae.ro.fri.gi.den′sis. Gr. fem. n. *aer* air; L. adj. *frigidus* cold, cool, chilled; L. suff. *-ensis -is -e* suffixes used in the sense of 'belonging to' or 'coming from'; N.L. fem. adj. *aerofrigidensis* pertaining to cooling air, as the strain was isolated from an air conditioner).

Cells are Gram-stain-negative, strictly aerobic, non-motile cocci (1.1–1.3 µm in diameter). Bacteriochlorophyll *a* is not produced. Colonies on R2A agar are pink and circular. Growth occurs at 10–45 °C (optimum, 30 °C), pH 4.5–9.5 (optimum, pH 7.0) and 0–3 % (w/v) NaCl (optimum, 0 %). Indole production and fermentation of glucose do not occur. Oxidase- and catalase-positive. Nitrate reduction is not observed. Hydrolyzes Tween 20 and urea, but not starch, casein, tyrosine, aesculin, Tween 80 and gelatin. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are positive, but lipase (C14), valine arylamidase, crystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase and arginine dihydrolase activities are negative. Assimilation of adipic acid and malic acid is positive, but assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid is negative. Major cellular fatty acids are summed feature 8 (comprising $C_{18:}\omega7c$/$C_{18:1}\omega6c$), $C_{16:0}$, and cyclo-$C_{19:0}\omega8c$. The isoprenoid quinone is Q-10. The major polar lipids are phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and an unknown aminolipid. The major carotenoid is hydroxyspirilloxanthin.

The type strain is HC1^T (=KACC 19097^T=JCM 31878^T), isolated from a room air conditioner in South Korea.The G+C content of the genomic DNA of the type strain is 70.1 mol%.

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

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