Aurantimonas ureilytica sp. nov., isolated from an air sample

Hang-Yeon Weon,¹ Byung-Yong Kim,² Seung-Hee Yoo,² Jae-Ho Joa,³ Ki Hwan Lee,⁴ Yong-Seon Zhang,⁵ Soon-Wo Kwon² and Bon-Sung Koo²

Correspondence

Soon-Wo Kwon

swkwon@rda.go.kr

¹Applied Microbiology Division, National Institute of Agricultural Science and Technology, Rural Development Administration (RDA), Suwon 441-707, Korea

²Korean Agricultural Culture Collection, Microbial Genetics Division, National Institute of Agricultural Biotechnology, RDA, Suwon 441-707, Korea

³National Institute of Subtropical Agriculture, RDA, Jeju 690-150, Republic of Korea

⁴Taeon Lily Experimental Station, Chungnam Provincial Agricultural Research and Extension Services, Taeon 357-952, Republic of Korea

⁵National Institute of Highland Agriculture, RDA, Pyongchang 232-955, Republic of Korea

A novel strain, designated 5715S-12^T, was isolated from an air sample collected from Suwon region, Republic of Korea, using R2A medium. The cells were strictly aerobic, Gram-negative, motile, short rods. Comparison of the 16S rRNA gene sequence of strain 5715S-12^T showed the highest sequence similarities to *Aurantimonas altamirensis* S21B^T (95.9 %) and *Aurantimonas coralicida* WP1^T (95.4 %). Phylogenetic trees indicated that the strain formed a cluster with members of the family *Aurantimonadaceae* (*A. altamirensis*, *A. coralicida* and *Fulvimarina pelagi*). The major fatty acid was C_{18:1}ω7c. The predominant isoprenoid quinone was ubiquinone 10 (Q-10). Diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylglycerol, phosphatidylcholine and four unknown lipids were found as the polar lipid components. The DNA G + C content was 67.0 mol%. On the basis of the phenotypic and phylogenetic features studied, we propose that strain 5715S-12^T be assigned to a novel species of the genus *Aurantimonas*, for which the name *Aurantimonas ureilytica* sp. nov. (type strain 5715S-12^T = KACC 11607^T = DSM 18598^T) is proposed.

The genus *Aurantimonas*, with *Aurantimonas coralicida* as the type species, was proposed for an isolate from a diseased colony of the scleractinian coral *Dichocoenia stokesi* (Denner *et al.*, 2003). A second species, *Aurantimonas altamirensis*, was proposed recently for an isolate from a subterranean environment (Jurado *et al.*, 2006). With the genus *Fulvimarina*, isolated from marine environments (Cho & Giovannoni, 2003), this genus took up a distinct phylogenetic position separable from the other families within the order *Rhizobiales*. Thus, these two genera were classified into an eleventh family within the order *Rhizobiales* of the *Alphaproteobacteria*, the family *Aurantimonadaceae*.

Abbreviation: PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5715S-12^T is DQ883810.

A transmission electron micrograph of a cell of strain 5715S-12^T, polar lipid profiles of strain 5715S-12^T and related strains and a 16S rRNA gene sequence-based maximum-parsimony tree are available as supplementary material with the online version of this paper.

In the course of the study of bacterial strains isolated from air samples, we isolated a yellow-coloured bacterium, 5715S-12^T. The air samples were collected with an MAS-100 air sampler (Merck) (single-stage multiple-hole impactor) in Suwon city, Korea, on 15 July 2005. The sampler contained Petri dishes with R2A agar (BBL) amended with 200 µg cycloheximide ml⁻¹ (Sigma). After sampling, plates were incubated at 28 °C for 5 days.

For phenotypic characterization, R2A medium was used as the basal medium. For transmission electron microscopy, cells were grown for 48 h on R2A medium, negatively stained with 0.5 % (w/v) uranyl acetate and examined with an LEO model 912AB electron microscope. Gram staining, catalase, oxidase, poly-β-hydroxybutyrate (PHB) formation and hydrolysis of casein, DNA, pectin and starch were determined by the method of Smibert & Krieg (1994). Hydrolysis of CM-cellulose (0.1 %, w/v), chitin (1 %, w/v) and tyrosine (0.5 %, w/v) was tested by the appearance of clear zones around colonies. Temperature, salinity and pH ranges for growth were tested over the ranges of 5–45 °C,

0–10 % (w/v) NaCl and pH 4.0–10.0. Pigment analysis was performed as reported by Denner *et al.* (2003). Further biochemical characteristics were determined using the API 20NE, API ID 32GN and API ZYM systems, following the instructions of the manufacturer (bioMérieux).

Isoprenoid quinones were extracted from lyophilized cells and analysed by HPLC as described previously (Groth *et al.*, 1996). Polar lipid profiles were determined according to the methods of Minnikin *et al.* (1984). Whole-cell fatty acids were analysed according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990) after cultivation on marine agar 2216 (MA; Difco) for 3 days at 28 °C. The DNA G+C content was measured according to Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18S; Supelco).

The 16S rRNA gene of the isolate was amplified by a PCR and sequenced directly using an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The closest known relatives of the novel isolate were determined by performing GenBank/EMBL/DDBJ database searches. The software package MEGA version 3.1 (Kumar *et al.*, 2004) was used for

all analyses. Distances (using distance options according to Kimura's two-parameter model) and clustering using the neighbour-joining and maximum-parsimony methods were determined by using bootstrap values based on 1000 replicates.

Strain 5715S-12^T grew on R2A, MA, nutrient agar (Difco) and tryptic soy agar (Difco), but did not grow on MacConkey agar (Difco). Colonies were yellow, round and convex with clear margins after 3 days on R2A. Cells were short rods with more than one polar flagellum (Supplementary Fig. S1 available in IJSEM Online). Strain 5715S-12^T yielded carotenoid pigments with peaks in the absorption spectrum at 447 and 470–471 nm and with a slight inflexion at 424–427 nm. Detailed phenotypic properties are summarized in Table 1 and the species description.

Strain 5715S-12^T contained ubiquinones that consisted mainly of ubiquinone 10 (Q-10). Strain 5715S-12^T revealed a complex polar lipid pattern, including diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylmethylethanolamine,

Table 1. Phenotypic characteristics of strain 5715S-12^T and the type strains of *Aurantimonas* species and *F. pelagi*

Strains: 1, strain 5715S-12^T (data from this study); 2, *A. coralicida* DSM 14790^T (unless indicated, data from Denner *et al.*, 2003); 3, *A. altamirensis* S21B^T (Jurado *et al.*, 2006); 4, *F. pelagi* DSM 15513^T (Cho & Giovannoni, 2003). All strains are strictly aerobic and positive for catalase, oxidase and urease. All strains are negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and hydrolysis of aesculin and gelatin. All strains are positive for alkaline phosphatase, esterase (C4), leucine arylamidase and naphthol-AS-BI-phosphohydrolase and negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. All strains except *F. pelagi* DSM 15513^T assimilate D-glucose, D-mannose and malic acid and do not assimilate capric acid, adipic acid or trisodium citrate (not reported for *F. pelagi* DSM 15513^T). +, Positive; w, weak; –, negative; ND, no data available.

Characteristic	1	2	3	4
Source	Air	Marine	Cave	Marine
Flagellation	>1, polar	>1, polar	–	–
Colony colour	Yellow	Golden orange	Yellow	Brownish yellow
Growth in the absence of NaCl	+	–	+	w
Growth at 10 % (w/v) NaCl	–	–	–	+
PHB formation	+	+*	+*	–
Assimilation of:				
L-Arabinose	+	+*	+*	–
D-Mannitol	+	–*	–*	–
N-Acetylglucosamine	–	–*	+*	+
D-Maltose	–	–*	+*	+
Potassium gluconate	–	+*	+*	ND
Phenylacetic acid	–	–*	–*	+
Activity of:				
Esterase lipase (C8)	+	+*	–*	+*
Acid phosphatase	+	–*	+*	–*
α -Glucosidase	–	–*	–*	+*
DNA G+C content (mol%)	67.0	66.3	71.8	57.6–59.9

*Data from this study.

Table 2. Cellular fatty acid compositions of strain 5715S-12^T and the type strains of *Aurantimonas* species and *F. pelagi*

Strains: 1, strain 5715S-12^T; 2, *A. coralicida* DSM 14790^T; 3, *A. altamirensis* S21B^T; 4, *F. pelagi* DSM 15513^T. Data were obtained in this study and are percentages of total fatty acids. Fatty acids representing less than 1.0 % in all strains were omitted.

Fatty acid	1	2	3	4
C _{16:0}	9.0	5.2	8.0	2.6
C _{16:0} 2-OH	—	2.9	—	—
C _{16:0} 3-OH	1.3	—	—	—
C _{18:1} ω7c	62.2	61.8	72.9	80.1
C _{18:1} ω5c	—	2.1	—	—
C _{18:0}	1.1	3.2	1.0	11.2
C _{18:1} 2-OH	12.7	3.2	5.7	—
C _{18:0} 3-OH	1.4	1.2	1.1	—
C _{19:0} cyclo ω8c	1.6	11.0	7.8	3.1
C _{20:1} ω7c	—	1.0	—	2.1
Summed feature 2*	3.9	—	2.1	—
Summed feature 3*	6.9	—	1.0	—
Summed feature 5*	—	5.8	—	—

*Summed feature 2 comprises C_{14:0} 3-OH and/or iso-C_{16:1} I, summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1} ω7c and summed feature 5 comprises C_{18:2} ω6,9c and/or anteiso-C_{18:0}.

phosphatidylglycerol, phosphatidylcholine and four unknown lipids (Supplementary Fig. S2 available in IJSEM Online). Its polar lipid pattern was generally similar to those of *A. coralicida* DSM 14790^T and *Fulvimarina pelagi* DSM 15513^T except for some minor unknown lipids. Strains 5715S-12^T and *A. coralicida* DSM 14790^T can be differentiated from *F. pelagi* DSM 15513^T by the presence of the unknown lipid L3 and a larger amount of phosphatidylmonomethylethanolamine. The major fatty acid was C_{18:1} ω7c (62.2 %), and moderate amounts of C_{18:1} 2-OH (12.7 %), C_{16:0} (9.0 %) and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω7c) (6.9 %) were also detected

(Table 2). The fatty acid profile of strain 5715S-12^T was similar to that of *A. altamirensis* S21B^T except for the presence of a small amount (1.3 %) of C_{16:0} 3-OH in the former strain (Table 2). The DNA G+C content was 67.0 mol%.

A total of 1398 nucleotides from the 16S rRNA gene sequence of strain 5715S-12^T were used in the phylogenetic analysis. Comparison of the 16S rRNA gene sequence of strain 5715S-12^T with homologous sequences of some members of the order *Rhizobiales* demonstrated that strain 5715S-12^T was closely related to *A. altamirensis* S21B^T (95.9 % sequence similarity), *A. coralicida* WP1^T (95.4 %), *Ensifer fredii* LMG 6217^T (93.6 %), *Mycoplana dimorpha* IAM 13154^T (93.3 %), *Mesorhizobium loti* LMG 6125^T (92.9 %) and *F. pelagi* HTCC2506^T (92.8 %). A neighbour-joining tree (Fig. 1) shows that strain 5715S-12^T forms a cluster with the two *Aurantimonas* species and *F. pelagi*. According to the maximum-parsimony tree (Supplementary Fig. S3 available in IJSEM Online), strain 5715S-12^T was grouped with *A. altamirensis* S21B^T within the genus *Aurantimonas* clade. Based on these results, it is concluded that strain 5715S-12^T represents a novel species of the genus *Aurantimonas*, for which the name *Aurantimonas ureilytica* sp. nov. is proposed.

Description of *Aurantimonas ureilytica* sp. nov.

Aurantimonas ureilytica (ur.ei.ly'ti.ca. N.L. n. urea urea; N.L. fem. adj. lytica from Gr. adj. lutikos dissolving; N.L. fem. adj. ureilytica urea-dissolving).

Cells are motile, Gram-negative, non-spore-forming, short rods (1.0–1.5 µm wide and 1.5–2.5 µm long). Strictly anaerobic and catalase- and oxidase-positive. The ranges (optima) of temperature, pH and NaCl concentration for growth are 4–37 °C (25–30 °C), pH 4–9 (pH 7–8) and 0–3 %. Negative for nitrate reduction, indole production, glucose fermentation and arginine dihydrolase. Cells hydrolyse starch, Tween 80 and urea, but not aesculin,

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain 5715S-12^T and related taxa based on 16S rRNA gene sequences. Numbers at nodes are levels of bootstrap support (%) based on neighbour-joining analyses of 1000 resampled datasets; only values above 60 % are given. Bar, 0.02 substitutions per nucleotide position.

casein, chitin, CM-cellulose, DNA, gelatin, pectin or tyrosine. According to API 20NE and API ID 32GN test strips, D-glucose, L-arabinose, D-mannose, D-mannitol, malic acid, L-rhamnose, D-ribose, inositol, sucrose, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, D-melibiose, L-fucose, D-sorbitol, propionic acid, valeric acid, potassium 2-ketogluconate, 3-hydroxybutyric acid and 4-hydroxybenzoic acid are assimilated. Does not assimilate *N*-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, phenylacetic acid, itaconic acid, suberic acid, sodium malonate, 3-hydroxybenzoic acid, L-serine, salicin, L-histidine or L-proline. According to API ZYM test strips, positive for activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative for activities of lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. The major fatty acid is C_{18:1} ω 7c. The predominant isoprenoid quinone is Q-10. The DNA G + C content of the type strain is 67.0 mol%.

The type strain is 5715S-12^T (=KACC 11607^T =DSM 18598^T), isolated from air in the Republic of Korea.

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