Aurantimonas frigidaquae sp. nov., isolated from a water-cooling system

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A motile, short rod-shaped and yellow-pigmented bacterium, designated strain CW5^T, was isolated from a water-cooling system at Gwangyang, Republic of Korea. Cells were Gram-negative, facultatively anaerobic and catalase- and oxidase-positive. The major fatty acids were C_{18:1ω7c} (64.7 %) and C_{16:0} (14.1 %). The DNA G + C content was 63.9 mol%. A phylogenetic tree based on 16S rRNA gene sequence comparison showed that strain CW5^T clustered within the *Aurantimonas* lineage and is closely related to the type strains of *Aurantimonas altamirensis* (98.5 % sequence similarity) and *Aurantimonas corallicida* (95.7 %). The phenotypic characteristics and DNA–DNA hybridization data indicate that strain CW5^T could be distinguished from the phylogenetic relatives *A. altamirensis* and *A. corallicida*. On the basis of the evidence presented in this study, strain CW5^T represents a novel species of the genus *Aurantimonas*, for which the name *Aurantimonas frigidaquae* sp. nov. is proposed. The type strain is CW5^T (=KCTC 12893^T =JCM 14755^T).

The genera *Aurantimonas* (Denner *et al.*, 2003), *Fulvimarina* (Cho & Giovannoni, 2003) and *Marteella* (Rivas *et al.*, 2005) constitute the three members of the recently described family ‘*Aurantimonadaceae*’ within the order *Rhizobiales* and take up a distinct phylogenetic position separable from the other families within the order *Rhizobiales*. The genus *Aurantimonas* was first described by Denner *et al.* (2003) and, at the time of writing, the genus comprises the two species *Aurantimonas corallicida* and *Aurantimonas altamirensis*, respectively isolated from diseased coral (*Dichocoenia stokesi*) (Denner *et al.*, 2003) and subterranean soil (Jurado *et al.*, 2006) (<http://www.bacterio.cict.fr/a/aurantimonas.html>).

In the course of our study on the microbial diversity of a water-cooling system, a yellow-pigmented bacterium, designated CW5^T, was isolated and was the subject of a taxonomic investigation. On the basis of evidence from a polyphasic study, strain CW5^T represents a novel species in the genus *Aurantimonas*.

Strain CW5^T was isolated from a cooled water sample from an oxygen-producing plant in the Republic of Korea during February 2005 using the standard dilution-plating

technique. Isolation was achieved using plate count agar (PCA; Difco) at 25 °C for 7 days. The isolate was routinely cultured on tryptic soy agar (TSA; Difco) and maintained as a glycerol suspension (20 %, w/v) at –80 °C.

Bacterial DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). The resultant sequence of strain CW5^T (1378 nt) was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were inferred from the regions available for all sequences (positions 22–1450; *Escherichia coli* numbering system) using the Fitch–Margoliash (Fitch & Margoliash, 1967) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by means of bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the jPHYDIT program and PAUP 4.0 (Swofford, 1998), as described previously (Chun *et al.*, 2000).

Preliminary sequence comparison with 16S rRNA gene sequences held in GenBank indicated that our isolate was related closely to the genus *Aurantimonas*. The newly determined sequence was then aligned manually against

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CW5^T is EF373540.

representatives of the genus *Aurantimonas*. Strain CW5^T showed the highest 16S rRNA gene sequence similarity with the type strain of *A. altamirensis* (98.5 %) followed by the type strain of *A. corallicida* (95.7 %). To elucidate the phylogenetic relationship between the novel isolate and other species of the genus *Aurantimonas*, phylogenetic trees were constructed by using two different tree-making algorithms. The neighbour-joining tree (Fig. 1) showed that strain CW5^T was closely related to the type strain of *A. altamirensis* with 99 % bootstrap support.

Growth on various standard bacteriological media was tested by using nutrient agar (NA), tryptic soy agar (TSA), R2A agar, MacConkey agar and marine agar (MA) (all from Difco) according to the manufacturer's instructions. Cells of strain CW5^T grown on TSA at 25 °C for 3–7 days were used for physiological and biochemical tests. Motility was examined by observing cells grown in wet mounts using phase-contrast microscopy (TMS-F; Nikon). Growth at various NaCl concentrations (0–10 %, w/v, in increments of 1.0 %) was investigated in tryptic soy broth (TSB) prepared according to the formula of the Difco medium except that no NaCl was used. The pH range for growth was determined in TSB (Difco) that was adjusted to various pH values (pH 3–12, in increments of 1 pH unit) by the addition of HCl and NaOH. Growth temperature (4–42 °C) and growth in an anaerobic chamber (CO₂/H₂/N₂, 10:10:80; Sheldon Manufacturing) were checked using TSA, using incubation times of up to 1 week. Catalase and oxidase activities were determined using 3 % (v/v) hydrogen peroxide and Kovacs' reagent (Kovacs, 1956), respectively. Acid production from sugars was tested as described by Yamaguchi & Yokoe (2000). Nitrate reduction was tested on nitrate broth containing 0.2 % KNO₃ (Skerman, 1967). Citrate utilization was tested on Simmons' citrate agar (Sigma). Indole production was determined with Kovacs' indole reagent in 1 % tryptone broth. H₂S production was determined on Kligler iron agar (Difco). L-Phenylalanine deamination was examined with

the method of Richard & Kiredjian (1995). Urease activity was tested on Christensen's medium (Oxoid). Hydrolysis of casein, starch and L-tyrosine was examined on NA containing 2 % skimmed milk, 0.2 % soluble starch and 0.5 % L-tyrosine (Barrow & Feltham, 1993), respectively. Precipitation on egg yolk was determined on NA containing 10 % egg yolk (Barrow & Feltham, 1993). DNase activity was determined with DNase test agar (Difco). Methyl red and Voges–Proskauer tests were analysed as described by Smibert & Krieg (1994). The absorption spectrum of pigment extracted by ethanol (Gosink *et al.*, 1998) was analysed using an Ultrospec 2100 pro UV/visible spectrophotometer (Amersham Biosciences). The presence of flexirubin-type pigments was examined as described by Bernardet *et al.* (2002). Other physiological and biochemical tests were performed using API 20E, API 20NE and API 50CH strips (bioMérieux). Enzyme activities were tested using the API ZYM kit (bioMérieux) following the manufacturer's instructions. Antibiotic resistance was determined with the disc diffusion method using commercial antibiotic-impregnated discs (BBL Becton Dickinson). The results were interpreted according to the guidelines set forth by the CLSI (2003) (formerly the NCCLS).

Cells of strain CW5^T were facultatively anaerobic, Gram-negative, motile, short rods. Colonies grown on TSA plates for 5 days at 25 °C were circular, convex with entire margins, smooth, opaque, yellow in colour and approximately 3.0 mm in diameter. The novel strain grew well on TSA, PCA, R2A and NA, but grew weakly on MA. On TSA, strain CW5^T was able to grow at 15–37 °C, but not at 42 °C. Detailed results of physiological and biochemical analyses are given in Table 1 and the species description. Pigment extracts of strain CW5^T showed an absorption maximum at 450 nm and a shoulder at 477 nm. Flexirubin-type pigments were absent. It is evident from Table 1 that there are several phenotypic characters that readily separate strain CW5^T from the phylogenetically related species *A. corallicida* and *A. altamirensis*.

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain CW5^T, members of the genus *Aurantimonas* and related members of the order *Rhizobiales*. Percentages at nodes are levels of bootstrap support based on a neighbour-joining analyses of 1000 resampled datasets. The sequence of *Rhodospirillum rubrum* ATH 1.1.1^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 1. Characteristics that differentiate strain CW5^T from other *Aurantimonas* species

Data for *A. coralicida* DSM 14790^T and *A. altamirensis* LMG 23375^T are from Denner *et al.* (2003) and Jurado *et al.* (2006). All three strains are positive for catalase, oxidase, urease, esterase (C4), esterase lipase (C8), acid phosphatase, leucine arylamidase and utilization of glucose, malate, L-arabinose, D-xylose, galactose and mannose. All three strains are negative for Gram stain, sporulation, hydrolysis of gelatin, lipase (C14), cystine arylamidase, α -chymotrypsin, *N*-acetyl- β -glucosaminidase, α -fucosidase, α -glucosidase, β -glucuronidase, α -mannosidase and utilization of adipate, caprate, citrate and phenylacetate. +, Positive; –, negative; w, weakly positive.

Characteristic	Strain CW5 ^T	<i>A. coralicida</i> DSM 14790 ^T	<i>A. altamirensis</i> LMG 23375 ^T
Colony colour	Yellow	Golden orange	Yellow
Temperature range (°C)	15–37	4–40	10–40
Motility	+	+	–
Cell shape	Short rod	Rod	Short rod
Arginine dihydrolase	–	+	–
Acetoin production	+	–	–
Nitrate reduction	+	–	–
Indole production	+	–	–
Hydrolysis of aesculin	+	–	–
Enzyme activity (API ZYM)			
Alkaline phosphatase	–	+	+
Trypsin	–	–	+
Naphthol-AS-BI-phosphohydrolase	–	+	+
α -Galactosidase	+	–	–
β -Galactosidase	+	–	–
β -Glucosidase	+	–	–
Utilization of (API 50CH):			
L-Arabinose	–	+	+
L-Fucose	+	w	w
Gentiobiose	w	w	–
Maltose	–	–	+
Rhamnose	–	+	+
Ribose	w	+	+
L-Xylose	–	+	–
Erythritol	+	–	+
Mannitol	+	–	+
<i>N</i> -Acetyl- β -glucosamine	+	–	+
Gluconate	w	+	+
DNA G + C content (mol%)	63.9	66.3	71.8

Cellular fatty acids of strain CW5^T were determined under growth conditions identical to those described by Jurado *et al.* (2006). Cells of strain CW5^T grown on NA supplemented with 2% (w/v) NaCl for 2 days at 28 °C were prepared and analysed by GLC according to the instructions of the Microbial Identification System (MIDI, 1999). The G + C content of the DNA was determined by using the thermal denaturation method of Marmur & Doty (1962). The major fatty acids of strain CW5^T are shown in Table 2. The predominant fatty acid was 7-octadecenoic acid (C_{18:1}ω7c), as was the case for *A. coralicida* and *A. altamirensis*. However, strain CW5^T and these species showed significant differences in the content of C_{16:0}, C_{18:0} and C_{19:0}ω8c cyclo, as shown in Table 2.

The DNA G + C content of strain CW5^T was 63.9 mol%, a figure lower than the values reported for the type strains of

A. coralicida (66.3 mol%; Denner *et al.*, 2003) and *A. altamirensis* (71.8 mol%; Jurado *et al.*, 2006).

The taxonomic relationship between strain CW5^T and phylogenetic relatives was further examined using DNA–DNA hybridization. Genomic relatedness was determined using a membrane filter technique (Seldin & Dubnau, 1985) according to the method described by Baik *et al.* (2006). DNA relatedness of strain CW5^T with *A. altamirensis* KACC 11704^T and *A. coralicida* KACC 12050^T was 45 and 28 %, respectively. It is clear from 16S rRNA gene sequence and DNA–DNA hybridization data that strain CW5^T represents a novel species in the genus *Aurantimonas* (Wayne *et al.*, 1987).

In addition, a number of physiological and chemotaxonomic characters clearly distinguish the isolate from other

Table 2. Fatty acid compositions of strain CW5^T and type strains of other *Aurantimonas* species

Data for reference strains were obtained from Jurado *et al.* (2006). Values are percentages of total fatty acids. tr, Trace amount (<1 %); –, not detected.

Fatty acid	Strain CW5 ^T	<i>A. coralicida</i> DSM 14790 ^T	<i>A. altamirensis</i> LMG 23375 ^T
C _{16:0}	14.1	6.2 ± 0.0	11.3 ± 0.1
iso-C _{16:0}	–	–	1.4 ± 0.0
C _{18:0}	5.8	2.0 ± 0.0	tr
C _{18:0} 3-OH	1.1	–	–
C _{18:1} 2-OH	5.6	3.2 ± 0.0	3.5 ± 0.1
C _{18:1} ω7c	64.7	69.4 ± 0.0	74.4 ± 0.2
C _{19:0} ω8c cyclo	6.5	13.2 ± 0.0	–
C _{20:1} ω7c	–	tr	–
Summed feature 2*	1.4	–	–
Summed feature 3*	tr	–	–

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 2 contained C_{14:0} 3-OH and/or iso-C_{16:1} I, and summed feature 3 contained iso-C_{15:0} 2-OH and/or C_{16:1} ω7c.

phylogenetically related species (Tables 1 and 2). Therefore, strain CW5^T represents a novel species within the genus *Aurantimonas*, for which the name *Aurantimonas frigidaquae* sp. nov. is proposed.

Description of *Aurantimonas frigidaquae* sp. nov.

Aurantimonas frigidaquae (fri.gi.da'quae. L. adj. *frigidus* cold; L. fem. n. *aqua* water; N.L. gen. n. *frigidaquae* from/of cold water, because the type strain was isolated from a water-cooling system).

Cells are short rods (0.6–0.8 × 0.8–1.2 μm). Gram-negative, motile, facultatively anaerobic and yellow-pigmented bacterium. Grows best on media such as TSA, NA and R2A. Grows on MacConkey medium. Colonies on TSA agar are convex, circular, smooth, opaque with entire margins, approximately 3 mm in diameter after 5 days at 25 °C (pH 7). Growth occurs in 0–7 % (w/v) NaCl (optimum growth in the absence of NaCl). Growth occurs at pH 5–11 (optimum pH 7) and at 15–37 °C (optimum 25 °C). Oxidase and catalase activities are present, but lysine decarboxylase, ornithine decarboxylase, L-phenylalanine arylamidase and valine arylamidase activities are absent. Aesculin is hydrolysed, but casein, DNA, gelatin, starch, Tween 80 and tyrosine are not. Citrate is not utilized. Acid is produced from 0.5 % (w/v) glucose, but not from maltose. Negative for methyl red and Voges–Proskauer reactions. No precipitate is formed on egg-yolk agar. Does not produce H₂S. In API 50CH tests, acid is produced from cellobiose, D-fucose, fructose, raffinose, sucrose, adonitol, D- and L-arabitol, inositol, mannitol, N-acetylglucosamine, amygdalin, arbutin, aesculin and salicin, but not from lactose, melezitose, D-tagatose, dulcitol, glycerol, methyl β-D-xyloside, methyl α-D-mannoside, methyl α-D-glucoside, inulin, glycogen, starch, gluconate, 2-ketogluconate or

5-ketogluconate. In the API ZYM gallery, esterase (C4), esterase lipase (C8), leucine arylamidase and acid phosphatase activities are present, but lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Cells are sensitive to (μg per disc, unless indicated otherwise): ampicillin (10), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), polymyxin B (300 U), streptomycin (10) and tetracycline (30), but resistant to bacitracin (10), penicillin (10 U) and vancomycin (30). Other physiological and biochemical characteristics are given in Table 1. Major fatty acids are C_{18:1} ω7c (64.7 %) and C_{16:0} (14.1 %); the complete fatty acid composition of the type strain is given in Table 2. The DNA G + C content of the type strain is 63.9 mol%.

The type strain is CW5^T (=KCTC 12893^T =JCM 14755^T), isolated from a water-cooling system at Gwangyang, Republic of Korea.

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