Chryseoglobus frigidaquae gen. nov., sp. nov., a novel member of the family *Microbacteriaceae*

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A motile, rod-shaped, yellow-pigmented bacterium, designated strain CW1^T, was isolated from a water-cooling system in the Republic of Korea. Cells were Gram-stain-positive, aerobic, catalase-positive and oxidase-negative. Strain CW1^T formed slender rods with unusual bulbous protuberances. The major fatty acids were iso-C_{16:1} (33.7%), anteiso-C_{15:0} (27.2%), iso-C_{14:0} (13.3%) and C_{16:0} (10.8%). The cell-wall peptidoglycan was of type B2β, containing lysine as the diamino acid. The respiratory quinones were menaquinones with 12, 13 and 14 isoprene units. A phylogenetic tree based on 16S rRNA gene sequences showed that strain CW1^T formed an evolutionary lineage within the radiation enclosing members of the family *Microbacteriaceae* and was related to, but distant from, members of the genera *Microcella* and *Yonghaparkia*. On the basis of the evidence presented, strain CW1^T is considered to represent a novel species of a new genus in the family *Microbacteriaceae*, for which the name *Chryseoglobus frigidaquae* gen. nov., sp. nov. is proposed. The type strain of *Chryseoglobus frigidaquae* is CW1^T (=KCTC 13142^T =JCM 14730^T).

The family *Microbacteriaceae* (Park *et al.*, 1993; Stackebrandt *et al.*, 1997) comprises actinobacteria that are irregularly shaped, aerobic, non-motile, non-spore-forming and Gram-positive and have G+C-rich genomic DNA. At the time of writing, the family *Microbacteriaceae* comprised 31 recognized genera, members of which originated from natural and artificial habitats, such as distilled water, freshwater, groundwater, plants, seawater, sewage and soil (An *et al.*, 2008; Evtushenko *et al.*, 2000; Han *et al.*, 2003; Männistö *et al.*, 2000; Sheridan *et al.*, 2003; Tiago *et al.*, 2005, 2006; Vaz-Moreira *et al.*, 2008; Yoon *et al.*, 2006). Cell walls of microbacteria are characterized by the presence of diamino acids such as lysine, ornithine and diaminobutyric acid, the distribution of which is one of the key chemotaxonomic markers for generic differentiation (Schleifer & Kandler, 1972). Menaquinones with 9–12 isoprene units (MK-9 to -12) are the predominant isoprenoid quinones, and straight or branched, saturated fatty acids constitute the major membrane lipid components (Jones & Collins, 1986; Collins & Bradbury, 1992). Some psychrophilic bacteria of the *Microbacteriaceae*, namely *Frigoribacterium faeni* and *Cryobacterium psychrophilum*, contain a certain amount of

unsaturated fatty acids when grown at lower temperatures (Suzuki *et al.*, 1997; Kämpfer *et al.*, 2000). The cell-wall peptidoglycan is of type B *sensu* Schleifer & Kandler (1972); no mycolic acids are found.

During the course of a study on the microbial diversity of a water-cooling system, a novel bacterium, designated strain CW1^T, was isolated and was the subject of a taxonomic investigation. On the basis of evidence from the present polyphasic study, strain CW1^T is considered to represent a novel species of a new genus in the family *Microbacteriaceae*.

Strain CW1^T was isolated from a cooled water sample from an oxygen-producing plant in the Republic of Korea during February 2005 by using the standard dilution plating technique. Isolation was achieved by using plate count agar (PCA; Difco) at 25 °C for 5 days. The isolate was routinely cultured on trypticase soy agar (TSA; Difco) and was maintained at –80 °C as a suspension in trypticase soy broth (TSB; Difco) containing glycerol (20%, w/v).

Bacterial DNA preparation and PCR amplification and sequencing of the 16S rRNA gene were carried out as described by Chun & Goodfellow (1995). The resultant 16S rRNA gene sequence of strain CW1^T was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were inferred from the regions available for all sequences (positions 22–1459 according to the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CW1^T is EF373534.

A table giving the complete fatty acid profile of strain CW1^T is available as supplementary material with the online version of this paper.

Escherichia coli numbering system) by 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 bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out by using the PHYDIT program (available at <http://plaza.snu.ac.kr/~jchun/phydit/>) and PAUP 4.0 (Swofford, 1998) as described by Chun *et al.* (2000).

Preliminary sequence comparison with 16S rRNA gene sequences held in GenBank indicated that strain CW1^T was related closely to members of the family *Microbacteriaceae*. The newly determined sequence was then aligned manually against representatives of the family *Microbacteriaceae*. Strain CW1^T showed highest 16S rRNA gene sequence similarity to *Microcella putealis* CV-2^T (97.6 %), followed by *Microcella alkaliphila* AC4r^T (97.0 %) and *Yonghaparkia alkaliphila* KSL-113^T (96.6 %). To elucidate the phylogenetic relationship between the novel isolate and other species within the family *Microbacteriaceae*, phylogenetic trees were constructed by using two tree-making algorithms. The neighbour-joining tree (Fig. 1) showed that strain

CW1^T formed a distinct branch with the clade comprising the genera *Microcella* and *Yonghaparkia*; this topology was also recovered in the Fitch–Margoliash tree (not shown). On the basis of 16S rRNA gene sequence similarity data and the results of phylogenetic analysis, it is clear that strain CW1^T represents a novel species of a new genus within the family *Microbacteriaceae*.

Growth on various standard bacteriological media was tested by using nutrient agar (NA; Difco), TSA and R2A agar (Difco) according to the manufacturer's instructions. Cells grown at 30 °C for 2 days were observed via scanning electron microscopy (S-4800; Hitachi). Cells of strain CW1^T grown on TSA at 30 °C for 3 days were used for physiological and biochemical tests. Motility was examined by observing cells grown in wet mounts by using phase-contrast microscopy (TMS-F; Nikon). Growth at various NaCl concentrations (0–10 %, w/v, at increments of 1.0 %) was investigated in 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 that was adjusted to pH 4–12 (in increments of 1 pH unit) by the addition of HCl or NaOH. Growth temperature (4–42 °C) and growth in an anaerobic chamber (CO₂/H₂/N₂, 10:10:80; Sheldon Manufacturing) were checked by

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the relationship between strain CW1^T and members of the family *Microbacteriaceae*. Numbers at nodes are levels of bootstrap support (percentages) based on neighbour-joining analyses of 1000 resampled datasets. Bar, 0.01 substitutions per nucleotide position.

incubation on TSA for up to 1 week. Catalase and oxidase activities were determined by using 3 % (v/v) hydrogen peroxide and Kovács' reagent (Kovács, 1956), respectively. 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 Kovács' indole reagent in 1 % tryptone broth. H₂S production was determined on Kligler iron agar (Difco). The absorption spectrum of pigments extracted with ethanol (Gosink *et al.*, 1998) was analysed by 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 by using the API 20E, API 20NE and API 50CH systems (bioMérieux). Enzyme activities were tested by using an API ZYM kit (bioMérieux) following the manufacturer's instructions. Antibiotic resistance was determined with the disc diffusion method by using commercial antibiotic-impregnated discs (BBL Becton Dickinson). Results were interpreted according to guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2003).

Strain CW1^T formed smooth, raised (convex), entire, translucent, non-slimy, yellowish colonies (approximately 1 mm in diameter) after 3 days at 30 °C (pH 7) on TSA. Strain CW1^T grew aerobically between 20 and 40 °C (optimally at 30 °C) and between pH 6 and 11 (optimally at pH 7–8). Strain CW1^T tolerated up to 3 % NaCl, but optimal growth was observed in the absence of NaCl. Strain CW1^T produced diffusible yellow pigments. Growth did not occur on MacConkey agar. Microscopic observations showed that cells of the novel strain were motile, Gram-positive, non-spore-forming, pleomorphic, short, slender rods, about 0.15–0.2 µm wide and 1.5–2.0 µm long. The cells frequently appeared swollen at the ends or in the middle, as is often observed for members of the genus *Rhodoglobus*. Scanning electron microscopy revealed extremely slender cells with enlarged, bulbous protuberances that were approximately 0.6 µm in diameter (Fig. 2). Some of the cells were slightly curved, but lacked the typical fragmented shapes often seen with members of the genus *Arthrobacter*. Strain CW1^T had morphological features consistent with its placement within the family *Microbacteriaceae*. The detailed results of physiological and biochemical analyses are given in the species description, and Table 1 provides several phenotypic characteristics that can be used to separate strain CW1^T from phylogenetically related genera.

Cellular fatty acids of strain CW1^T grown on TSA for 2 days at 30 °C were prepared (in duplicate) and were analysed as methyl esters by GLC according to the instructions of the Microbial Identification System (MIDI, 1999). Phospholipids were analysed by using standard procedures (Minnikin *et al.*, 1984). Quantitative analysis of amino acids was performed after derivatization by GC and GC/MS (320-MS Quadrupole GC/MS; Varian) according to MacKenzie (1987). Determination of the

Fig. 2. Scanning electron micrograph of cells of strain CW1^T. Cells were grown on TSA at 25 °C for 2 days. Bar, 2 µm.

peptidoglycan structure of strain CW1^T was carried out as described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose was applied instead of paper chromatography. The cell-wall acyl type was determined by the method of Uchida *et al.* (1999). Respiratory quinones were extracted (from 300 mg freeze-dried cells) and purified according to the method of Minnikin *et al.* (1984) and were analysed by HPLC as described by Kroppenstedt (1985). The G+C content of the genomic DNA was calculated by using the formula described by Mandel *et al.* (1970). The DNA sample was prepared in duplicate and the G+C content was determined by thermal denaturation (Marmur & Doty, 1962).

Major fatty acids of strain CW1^T were iso-C_{16:0} (33.7 %), anteiso-C_{15:0} (27.2 %), iso-C_{14:0} (13.3 %) and C_{16:0} (10.8 %); the complete fatty acid profile is given in Supplementary Table S1, available in IJSEM Online. Straight-chain saturated, anteiso-methyl-branched and iso-methyl-branched fatty acids were the predominant components (>10 % of the total fatty acids), as with most members of the family *Microbacteriaceae*, except the genera *Microcella*, *Mycetocola*, *Subtercola* and *Yonghaparkia*. The major polar lipids detected in strain CW1^T were diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. The total hydrolysate (4 M HCl, 16 h, 100 °C) of the peptidoglycan contained the amino acids lysine, glutamic acid, homoserine (Hsr), alanine and glycine in a molar ratio of approximately 1.0:1.0:0.6:0.8:1.4. The partial hydrolysate (4 M HCl, 0.75 h, 100 °C) of the peptidoglycan contained (in addition to the amino acids) the peptides Gly→D-Glu and Lys→D-Ala. From these data it was concluded that strain CW1^T has peptidoglycan of type B2β ({Gly} [L-Hsr] D-Glu→D-Lys, where { } and [] indicate positions 1 and 3 of the peptide subunit). This peptidoglycan structure has previously been reported only for *Frigoribacterium faeni* (Kämpfer *et al.*, 2000). The respiratory quinones found in strain CW1^T were menaquinones MK-13 (44 %), MK-14 (35 %) and MK-12

Table 1. Phenotypic characteristics that differentiate strain CW1^T from its phylogenetic neighbours in the family *Microbacteriaceae*

Data for reference genera were taken from An *et al.* (2008), Evtushenko *et al.* (2000), Han *et al.* (2003), Reddy *et al.* (2008), Sheridan *et al.* (2003), Tiago *et al.* (2005, 2006), Yoon *et al.* (2006) and the present study. +, Positive; –, negative; v, variable; NR, not reported; tr, trace amount.

Characteristic	Strain CW1 ^T	<i>Microcella</i>	<i>Yonghaparkia</i>	<i>Salinibacterium</i>	<i>Rhodoglobus</i>	<i>Leifsonia</i>	<i>Schumannella</i>
Colony colour	Yellow	Yellow	Yellow	NR	Red	Yellow or white	Yellow
Cell shape	Slender rods, swollen	Slender rods	Slender rods	Irregular rods	Pleomorphic, short, slender rods, swollen	Irregular rods or filaments	Short rods
Motility	+	–	–	–	+	v	–
Growth temperature range (°C)	20–40	15–40	10–37	4–37	–2 to 21	0–42	8–35
Cell-wall diamino acid(s)*	Lys	Lys, Orn	DAB	Lys, Orn	Orn	DAB	DAB
Presence of homoserine	+	–	–	–	tr	–	–
Major fatty acids (>10 % of total)	i-C _{16:0} , ai-C _{15:0} , i-C _{14:0} , C _{16:0}	i-C _{16:0} , ai-C _{15:0} , i-C _{14:0} , i-C _{15:0}	ai-C _{15:0} , i-C _{16:0} , ai-C _{17:0}	ai-C _{15:0} , i-C _{16:0} , i-C _{14:0}	ai-C _{15:0} , ai-C _{17:0} , i-C _{16:0}	ai-C _{15:0} , ai-C _{17:0} , i-C _{16:0}	ai-C _{15:0} , i-C _{16:0}
Major menaquinone(s)	12, 13, 14	12, 13, 14	12	11	11, 12	10, 11, 12	10, 11
DNA G+C content (mol%)	68.3	67.1–68.8	71.1	61.0	62.0	64.0–73.4	58.7

*DAB, Diaminobutyric acid; Lys, lysine; Orn, ornithine.

(21 %). The DNA G+C content of strain CW1^T was 68.3 ± 0.4 mol%, within the range of values reported for members of the family *Microbacteriaceae*.

The taxonomic relationship between strain CW1^T and the type strains of *Microcella putealis*, *Microcella alkaliphila* and *Yonghaparkia alkaliphila* was further examined based on DNA–DNA hybridization experiments. Genomic DNA–DNA relatedness was determined by using a membrane filter technique with DIG High Prime DNA Labelling and Detection Starter kit II (Roche Applied Science) according to the manufacturer's instructions and Lee *et al.* (2003).

Strain CW1^T shared 22, 29 and 16 % DNA–DNA relatedness with *Microcella putealis* KCTC 19625^T, *Microcella alkaliphila* KCTC 19624^T and *Yonghaparkia alkaliphila* KCTC 19126^T, respectively, demonstrating that it represents a novel species (Wayne *et al.*, 1987).

Phylogenetic, genomic, chemotaxonomic and phenotypic data clearly indicate that strain CW1^T represents a novel species of a new genus within the family *Microbacteriaceae*, for which the name *Chryseoglobus frigidaquae* gen. nov., sp. nov. is proposed.

Description of *Chryseoglobus* gen. nov.

Chryseoglobus (Chry.se.o.glo'bus. Gr. adj. *chryseos* golden; L. masc. n. *globus* ball; N.L. masc. n. *Chryseoglobus* yellow ball).

Cells are Gram-positive, aerobic, non-sporulating, motile, catalase-positive, oxidase-negative, pleomorphic, short, slender rods. Bulbous protuberances are formed at the ends or in the middle of cells. The cell-wall peptidoglycan contains D-lysine as a diamino acid. The glycan moiety of the peptidoglycan contains acetyl residues. The respiratory quinones are MK-12, MK-13 and MK-14. Mycolic acids are not present. Diphosphatidylglycerol, phosphatidylglycerol and an unknown glycolipid are detected in polar lipid extracts. Branched saturated components are the predominant fatty acids. The type species is *Chryseoglobus frigidaquae*.

Description of *Chryseoglobus frigidaquae* sp. nov.

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

Has the following characteristics in addition to those given for the genus. Colonies on TSA agar are yellow, raised, entire, translucent, circular, smooth and approximately 1 mm in diameter after 3 days at 30 °C (pH 7). Produces diffusible yellow pigments. Grows well on TSA and NA. Growth occurs in the presence of 0–3 % (w/v) NaCl (optimum 0 %), at pH 6–11 (optimum pH 7–8) and at 20–40 °C (optimum 30 °C). Produces β -galactosidase, α -

glucosidase, aesculin hydrolase and leucine arylamidase. Nitrate is not reduced. Does not produce lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, gelatinase, arginine dihydrolase, indole or urease. Does not produce alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glycosaminidase, α -mannosidase or α -fucosidase. Utilizes L-arabinose, ribose and aesculin as sole carbon and energy sources, but not adonitol, glucose, mannose, mannitol, *N*-acetylglucosamine, fructose, maltose, gluconate, caprate, adipate, malate, citrate, phenylacetate, glycerol, erythritol, D-arabinose, D-xylose, L-xylose, methyl β -D-xyloside, galactose, sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, amygdalin, arbutin, salicin, cellobiose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, β -gentiobiose, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. Major fatty acids are iso-C_{16:0}, anteiso-C_{15:0}, iso-C_{14:0} and C_{16:0}. The DNA G+C content of the type strain is 68.3 mol%.

The type strain, CW1^T (=KCTC 13142^T =JCM 14730^T), was isolated from a water-cooling system at Gwangyang, Republic of Korea.

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