Chelatococcus daeguensis sp. nov., isolated from wastewater of a textile dye works, and emended description of the genus *Chelatococcus*

Jung-Hoon Yoon,¹ So-Jung Kang,¹ Wan-Taek Im,² Sung-Taik Lee² and Tae-Kwang Oh¹

Correspondence

Jung-Hoon Yoon
jhyoon@kribb.re.kr

¹Korea Research Institute of Bioscience and Biotechnology (KRIIBB), PO Box 115, Yusong, Daejeon, Republic of Korea

²Department of Biological Science, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea

A Gram-negative, non-spore-forming, rod-shaped bacterial strain, K106^T, was isolated from wastewater collected from a textile dye works in Korea. Strain K106^T grew optimally at pH 7.0–7.5 and 30–37 °C in the presence of 0–1.0 % (w/v) NaCl. A phylogenetic tree based on 16S rRNA gene sequences showed that strain K106^T joined the type strain of *Chelatococcus asaccharovorans* with a bootstrap resampling value of 99.9 %. The predominant ubiquinone of strain K106^T was Q-10. The fatty acid profile of strain K106^T was similar to that of *C. asaccharovorans* DSM 6462^T. Major polar lipids of strain K106^T and *C. asaccharovorans* DSM 6462^T were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, two aminolipids and two unidentified phospholipids. *sym*-Homospermidine, spermidine and putrescine were major polyamines. The DNA G + C content was 68.3 mol%. Strain K106^T exhibited 16S rRNA gene sequence similarity of 96.6 % to the type strain of *C. asaccharovorans*. DNA–DNA relatedness data and differential phenotypic properties, particularly differences in cell morphology and the ability to utilize nitrilotriacetate, demonstrated that strain K106^T can be differentiated from *C. asaccharovorans*. On the basis of phenotypic, phylogenetic and genetic data, strain K106^T represents a novel species of the genus *Chelatococcus*, for which the name *Chelatococcus daeguensis* sp. nov. is proposed. The type strain is K106^T (=KCTC 12979^T =CCUG 54519^T).

The genus *Chelatococcus* was proposed by Auling *et al.* (1993) with *Chelatococcus asaccharovorans* as the sole recognized species of the genus. Phylogenetic analysis based on 16S rRNA gene sequences showed that the genus *Chelatococcus* falls within the *Alphaproteobacteria* (Auling *et al.*, 1993; Lee *et al.*, 2005). In this study, we describe a bacterial strain, K106^T, which was isolated from wastewater of a textile dye works in Korea. 16S rRNA gene sequence comparison indicated that this strain is most closely related phylogenetically to the genus *Chelatococcus*. However, cells of strain K106^T were rod-shaped, unlike those of *C. asaccharovorans*. Accordingly, the aim of the present study was to determine the exact taxonomic position of strain K106^T by a polyphasic characterization that included determination of phenotypic and chemotaxonomic properties, detailed phylogenetic analysis based on 16S rRNA gene sequences and genetic analysis.

Abbreviation: NTA, nitrilotriacetate.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain K106^T is EF584507.

Strain K106^T was isolated by means of the standard dilution-plating technique on trypticase soy agar (TSA; Difco) at 30 °C. *C. asaccharovorans* DSM 6462^T, which was used as a reference strain for DNA–DNA hybridization and chemotaxonomic and phenotypic analyses, was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. The morphological properties of strain K106^T and *C. asaccharovorans* DSM 6462^T were investigated on TSA, R2A agar (Difco) and nutrient agar (NA; Difco). The physiological and biochemical characteristics of strain K106^T were investigated using routine cultivation on TSA at 37 °C. Cell morphology was examined by light microscopy (Nikon E600) using cells from exponentially growing cultures. The Gram reaction was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–55 °C) was measured on TSA. The pH range for growth was determined in nutrient broth (Difco) adjusted, prior to sterilization, to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units) by the addition of HCl or Na₂CO₃.

Growth in the absence of NaCl and at various NaCl concentrations (0.5 %, w/v, and 1.0–7.0 %, w/v, at intervals of 1.0 %) was investigated using trypticase soy broth (TSB) prepared according to the formula of the Difco medium except that NaCl was excluded from the medium formula. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and on TSA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction were studied as described previously (Lányi, 1987). Susceptibility to antibiotics was tested on TSA plates using discs containing the following antibiotics in the amounts indicated: polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; carbenicillin, 100 µg. Utilization of various substrates, enzyme activities and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50CH and API ZYM systems (bioMérieux). The cells were suspended in AUX medium according to the manufacturer's instructions to inoculate the API 50CH system. Utilization of nitrilotriacetate (NTA) was tested with a synthetic medium as described by Egli *et al.* (1988); *C. asaccharovorans* DSM 6462^T was used as a positive control.

Cell biomass of strain K106^T and *C. asaccharovorans* DSM 6462^T for DNA extraction and for the analyses of isoprenoid quinones, polar lipids and polyamines was obtained from cultivation in TSB (Difco) at 37 and 30 °C, respectively. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For cellular fatty acid analysis, cell mass of strain K106^T and *C. asaccharovorans* DSM 6462^T was harvested from TSA plates after incubation for 5 days at 30 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987).

The presence of phosphatidylcholine was identified by spraying with Dragendorff's reagent (Sigma). Polyamines were extracted and analysed as described by Busse & Auling (1988) and Schenkel *et al.* (1995). The DNA G + C content was determined by the method of Tamaoka & Komagata (1984) with the modification that the DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded, and the means of the remaining three values are quoted as DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain K106^T are given in the species description or are shown in Table 1. Cells of strain K106^T were rods on TSA, R2A agar and NA, whereas cells of *C. asaccharovorans* DSM 6462^T were cocci on these media. The almost-complete 16S rRNA gene sequence of strain K106^T determined in this study comprised 1442 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain K106^T joined the lineage comprising the type strain of *C. asaccharovorans* at a bootstrap resampling value of 99.9 % (Fig. 1). The relationship between strain K106^T and the type strain of *C. asaccharovorans* was maintained in trees based on the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain K106^T exhibited 16S rRNA gene sequence similarity of 96.6 % to the type strain of *C. asaccharovorans* and of less than 93.6 % to other species included in the phylogenetic analysis. The predominant isoprenoid quinone detected in strain K106^T was ubiquinone-10 (Q-10), at a peak area ratio of approximately 83 %; a significant amount of Q-8 (approx. 15 %) was also detected. Strain K106^T had a cellular fatty acid profile that contained large amounts of unsaturated, cyclo and hydroxy fatty acids; the major fatty acids (>10 % total fatty acids) were C_{18:1}ω7c and C_{19:0} cyclo ω8c (Table 2). This fatty acid profile was similar to that of *C. asaccharovorans* DSM 6462^T analysed in this study, although there were differences in the proportions of some fatty acids (Table 2). Major polar lipids found in strain K106^T were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, two aminolipids and two unidentified phospholipids. The major polar lipids of *C. asaccharovorans* DSM 6462^T analysed in this study were the same. Major polyamines detected in this study were sym-homospermidine, spermidine and putrescine, as also found in *C. asaccharovorans* (Auling *et al.*, 1993). The DNA G + C content of strain K106^T was 68.3 mol%.

Strain K106^T differed from *C. asaccharovorans* in that its cells were rod-shaped. However, there were no distinct phenotypic, particularly chemotaxonomic, properties to differentiate strain K106^T from *C. asaccharovorans*, as

Table 1. Differential phenotypic characteristics of strain K106^T and *C. asaccharovorans* DSM 6462^T

Data were obtained in this study. +, Positive; –, negative; w, weakly positive. Both strains are positive for catalase, oxidase, acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, nitrate reduction, hydrolysis of aesculin, utilization of D- and L-arabinose, ribose, D-xylose, glucose, mannose, rhamnose, D-lyxose, D- and L-fucose, gluconate, 2-ketogluconate, 5-ketogluconate, adipate and malate and susceptibility to gentamicin, carbenicillin and tetracycline, and negative for H₂S production, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, 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, α -fucosidase, hydrolysis of casein, gelatin, starch, hypoxanthine, xanthine, L-tyrosine and Tweens 20, 40, 60 and 80, utilization of erythritol, methyl β -D-xyloside, dulcitol, mannitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, *N*-acetylglucosamine, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, turanose, D-tagatose and D- and L-arabitol, acid production from mannitol, sorbitol, sucrose, melibiose and amygdalin and susceptibility to ampicillin, cephalothin, lincomycin, novobiocin, oleandomycin, polymyxin B and penicillin G.

Characteristic	<i>C. daeguensis</i> K106 ^T	<i>C. asaccharovorans</i> DSM 6462 ^T
Cell morphology	Rods	Cocci to oval
Urease	–	+
Arginine dihydrolase	+	–
Utilization of:		
NTA, phenylacetate	–	+
Glycerol, adonitol, galactose, fructose, inositol, amygdalin, arbutin, aesculin, salicin, cellobiose, xylitol, gentiobiose, citrate	+	–
L-Xylose	+	w
Sorbose	w	–
Acid production from:		
Glucose, inositol, arabinose	+	–
Rhamnose	w	–
Susceptibility to:		
Streptomycin, chloramphenicol	+	–
Kanamycin, neomycin	–	+

shown by Auling *et al.* (1993) and in this study. Accordingly, it seems reasonable that strain K106^T is classified as a member of the genus *Chelatococcus*. Strain

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Chelatococcus daeguensis* sp. nov. K106^T and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) >50% are shown at branching points. Dots indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Sphingomonas paucimobilis* GIFU 2395^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

K106^T was distinguishable from *C. asaccharovorans* by differences in cell morphology and several phenotypic characteristics (Table 1). The mean level of DNA–DNA relatedness between strain K106^T and *C. asaccharovorans* DSM 6462^T was 13% when their DNAs were used individually as labelled DNA probes for cross-hybridization. The phylogenetic distinctiveness, differential phenotypic properties and DNA–DNA relatedness data are sufficient to categorize strain K106^T as a representative of a species that is separate from *C. asaccharovorans* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain K106^T represents a novel species of the genus *Chelatococcus*, for which the name *Chelatococcus daeguensis* sp. nov. is proposed.

Description of *Chelatococcus daeguensis* sp. nov.

Chelatococcus daeguensis (dae.gu.en'sis. N.L. masc. adj. *daeguensis* of Daegu, Korea, from where the type strain was isolated).

Cells are Gram-negative, non-spore-forming rods (0.3–0.6 × 1.0–4.0 µm). Colonies on TSA are circular, convex, smooth, glistening, moderate yellow in colour and 0.7–1.0 mm in diameter after incubation for 3 days at 37 °C. Optimal temperature for growth is 30–37 °C. Growth occurs at 20 and 51 °C, but not at 15 or 52 °C. Optimal pH for growth is 7.0–7.5; growth occurs at pH 5.5 and 10.0,

Table 2. Cellular fatty acid compositions of *C. daeguensis* K106^T and *C. asaccharovorans* DSM 6462^T

Values are percentages of total fatty acids. Fatty acids that represented <0.5% in both strains were omitted. ECL, Equivalent chain length. Data were obtained in this study.

Fatty acid	<i>C. daeguensis</i> K106 ^T	<i>C. asaccharovorans</i> DSM 6462 ^T
Straight-chain		
C _{16:0}	2.2	8.5
C _{17:0}	0.9	0.4
C _{18:0}	1.7	0.9
Unsaturated		
C _{18:1} ω7c	64.4	47.4
C _{20:1} ω7c	0.4	1.4
Hydroxy		
C _{18:1} 2-OH	3.7	0.9
C _{18:0} 3-OH	3.4	0.8
Cyclo		
C _{17:0} cyclo	0.3	3.2
C _{19:0} cyclo ω8c	16.7	29.6
Unknown		
ECL 14.959	0.8	0.2
Summed features*		
2	3.4	4.0
3	0.7	1.1

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

but not at pH 5.0 or 10.5. Growth occurs in the presence of 0–5% (w/v) NaCl, with optimal growth at 0–1.0% (w/v) NaCl. Anaerobic growth does not occur on TSA, but occurs on TSA supplemented with nitrate. The predominant ubiquinone is Q-10. The major fatty acids (>10% of total fatty acids) are C_{18:1} ω7c and C_{19:0} cyclo ω8c. Major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, two aminolipids and two unidentified phospholipids. *sym*-Homospermidine, spermidine and putrescine are major polyamines. The DNA G+C content of the type strain is 68.3 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, K106^T (=KCTC 12979^T =CCUG 54519^T), was isolated from wastewater of a textile dye works at Daegu, Korea.

Emended description of the genus *Chelatococcus* Auling *et al.* 1993

The description of the genus *Chelatococcus* is as given by Auling *et al.* (1993) with the following amendments. Cells are cocci or rods. Utilization of NTA is positive or negative. The major fatty acids are C_{18:1} ω7c and C_{19:0} cyclo ω8c.

Major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, two aminolipids and two unidentified phospholipids. *sym*-Homospermidine, spermidine and putrescine are major polyamines. The DNA G+C content is 63.3–68.3 mol%.

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