Chryseobacterium caeni sp. nov., isolated from bioreactor sludge

Zhe-Xue Quan,¹ Kwang Kyu Kim,² Myung-Kyum Kim,² Long Jin² and Sung-Taik Lee²

Correspondence

Sung-Taik Lee

e_stlee@kaist.ac.kr

¹Department of Microbiology and Microbial Engineering, School of Life Sciences, Fudan University, Shanghai 200433, People's Republic of China

²Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea

A Gram-negative, non-spore-forming, yellow-pigmented bacterium, strain N4^T, was isolated from a nickel-complexed cyanide-degrading bioreactor and subjected to a polyphasic taxonomic study. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain N4^T is affiliated to the genus *Chryseobacterium* of the family *Flavobacteriaceae*. The levels of 16S rRNA gene sequence similarity between strain N4^T and the type strains of all known *Chryseobacterium* species were 93.2–95.8%, suggesting that strain N4^T represents a novel species within the genus *Chryseobacterium*. The strain contained iso-C_{15:0} and summed feature 4 as the major fatty acids and menaquinone MK-6 as the predominant respiratory quinone. The G + C content of the genomic DNA was 38.2 mol%. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain N4^T represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium caeni* sp. nov. is proposed. The type strain is N4^T (=KCTC 12506^T = CCBAU 10201^T = DSM 17710^T).

The genus *Chryseobacterium* of the family *Flavobacteriaceae* was created by Vandamme *et al.* (1994) to accommodate six species formerly classified within the genus *Flavobacterium*. At present, the genus *Chryseobacterium* comprises 17 species: *Chryseobacterium gleum* (type species), *C. balustinum*, *C. indologenes*, *C. indoltheticum* and *C. scophthalmum* (Vandamme *et al.*, 1994) and the recently described species *C. defluvii* (Kämpfer *et al.*, 2003), *C. joostei* (Hugo *et al.*, 2003), *C. daecheongense* (Kim *et al.*, 2005a), *C. formosense* (Young *et al.*, 2005), *C. shigense* (Shimomura *et al.*, 2005), *C. taichungense* (Shen *et al.*, 2005), *C. vrystaatense* (de Beer *et al.*, 2005), *C. hispanicum* (Gallego *et al.*, 2006), *C. piscium* (de Beer *et al.*, 2006), *C. soldanellicola* (Park *et al.*, 2006), *C. taeanense* (Park *et al.*, 2006) and *C. wanjuae* (Weon *et al.*, 2006). '*Chryseobacterium proteolyticum*' was described by Yamaguchi & Yokoe (2000), but this name has not yet been validly published. Two former *Chryseobacterium* species, *C. meningosepticum* (Vandamme *et al.*, 1994) and *C. miricola* (Li *et al.*, 2003), have been transferred to the novel genus *Elizabethkingia* (Kim *et al.*, 2005b).

During a study of bacterial communities associated with the sludge of metal-complexed cyanide treatment bioreactors

(Quan *et al.*, 2006), conducted using a culture-dependent approach, a number of bacterial strains were isolated. Strain N4^T was isolated from a nickel-complexed cyanide treatment bioreactor. A comparative analysis of 16S rRNA gene sequences indicated that strain N4^T was a member of the clade representing the genus *Chryseobacterium*. In order to determine the precise taxonomic position of strain N4^T, a polyphasic taxonomic study was carried out.

Strain N4^T was cultivated on R2A agar (Difco) at 28 °C for 48 h. Cell biomass for quinone analysis and for DNA extraction was obtained directly from agar plates. For fatty acid methyl ester analysis, strain N4^T was cultivated on tryptic soy agar (Difco) at 28 °C for 24 h for direct comparison with reference strains. Cell morphology was examined under a phase-contrast microscope (1000× magnification; Nikon). A Gram reaction was performed as described by Gerhardt *et al.* (1994). Flexirubin-type pigments were detected according to the method of Fautz & Reichenbach (1980). Catalase activity was determined by means of bubble production in a 3% (v/v) hydrogen peroxide solution. Oxidase activity was determined from the oxidation of 1% *p*-aminodimethylaniline oxalate. The hydrolysis of starch was tested on starch agar (Difco). Additional enzyme activities, acid production from carbohydrates and the utilization of various substrates as sole carbon sources were determined by using API ZYM, API 20E, API 20NE and API 32GN galleries according to the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain N4^T is DQ336714.

The API ZYM profiles of strain N4^T and other *Chryseobacterium* species are available as supplementary material in IJSEM Online.

manufacturer's instructions (bioMérieux). The effects of NaCl concentration (0–5 %), pH (5–10) and temperature (5–42 °C) on growth were determined on R2A agar.

Respiratory quinones were analysed as described by Komagata & Suzuki (1987), using reversed-phase HPLC. For quantitative analysis of the cellular fatty acid composition, a loop of cell mass was harvested and the fatty acids were then saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). Fatty acids were analysed by a gas chromatograph (model 6890; Hewlett Packard) and identified by using the Microbial Identification software package (Sasser, 1990). Chromosomal DNA was isolated and purified using a Cell Culture DNA Midi kit (Qiagen) according to the manufacturer's protocol. For the determination of G + C content, DNA was degraded enzymically into nucleotides and analysed by reversed-phase HPLC as described by Mesbah

et al. (1989). Non-methylated λ -phage DNA (Sigma) was used as a calibration reference.

The 16S rRNA gene was amplified by a PCR using two universal primers (Quan *et al.*, 2005). The PCR product was purified using a QIAquick PCR purification kit (Qiagen). The 16S rRNA gene sequence was determined directly using the PCR-amplified DNA as a sequencing template. A sequencing PCR was performed with forward and reverse primers (Quan *et al.*, 2005) using a DNA Analyzer (3730X1; Applied Biosystems). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed using the CLUSTAL_X program (Thompson *et al.*, 1997) and gaps were edited in the BioEdit program (Hall, 1999). Phylogenetic trees were constructed on the basis of three tree-making algorithms: neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1992) and maximum-parsimony

Table 1. Phenotypic characteristics that differentiate strain N4^T from other species of the genus *Chryseobacterium*

Taxa: 1, strain N4^T (data from this study); 2, *C. gleum* (n=2); 3, *C. balustinum*; 4, *C. indologenes* (n=7); 5, *C. indoltheticum*; 6, *C. scopthalmum* (n=2); 7, *C. defluvii* (Kämpfer *et al.*, 2003); 8, *C. joostei* (n=11); 9, *C. daecheongense* (Kim *et al.*, 2005a); 10, *C. formosense* (Young *et al.*, 2005); 11, *C. taichungense* (Shen *et al.*, 2005); 12, *C. shigense* (Shimomura *et al.*, 2005); 13, *C. vrystaatense* (n=36; de Beer *et al.*, 2005); 14, *C. soldanellicola* (Park *et al.*, 2006); 15, *C. taeanense* (Park *et al.*, 2006); 16, *C. piscium* (n=4; de Beer *et al.*, 2006); 17, *C. hispanicum* (Gallego *et al.*, 2006); 18, *C. wanjuae* (Weon *et al.*, 2006). Data for taxa 2–6 and 8 are taken from Hugo *et al.* (2003). Number of strains examined, when greater than 1, is shown in parentheses. +, Positive; W, weakly positive; –, negative; V, variable; D, delayed; NA, data not available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Growth on/at:																		
MacConkey agar	–	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	W
5 °C	+	–	D	–	+	D	–	+	–	–	–	+	+	+	+	+	D	+
37 °C	+	+	+	–	–	–	+	–	+	–	+	–	–	+	+	–	–	+
42 °C	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Urease activity	+	–	–	–	–	+	–	V	–	–	–	–	+	–	–	+	–	–
Nitrate reduction	–	+	+	–	–	–	–	–	+	–	–	–	–	–	–	+	+	–
Nitrite reduction	–	+	–	–	–	–	+	–	–	NA	NA	–	+	–	–	–	+	NA
Hydrolysis of:																		
Tween 80	–	+	+	+	+	+	NA	+	NA	–	NA	NA	+	–	+	V	–	NA
Starch	–	+	–	+	–	–	+	+	+	NA	NA	+	–	NA	NA	–	+	+
Production of:																		
Hydrogen sulphide	–	–	–	–	+	–	–	–	–	–	–	+	V	–	–	–	–	NA
Indole	–	+	+	+	+	–	+	+	–	+	W	+	+	–	–	+	W	–
Acid production from:																		
L-Arabinose	–	+	–	–	–	–	–	–	–	–	–	–	NA	–	–	NA	+	–
D-Fructose	–	+	+	D	–	–	+	+	+	NA	NA	+	NA	–	–	NA	+	–
Glycerol	–	+	–	D	–	–	+	V	+	NA	NA	–	NA	–	–	NA	+	–
Lactose	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	–	NA	–	–
D-Maltose	–	+	–	+	+	–	+	+	+	–	+	–	NA	–	–	NA	+	–
D-Mannitol	–	–	–	–	–	–	–	V	–	–	–	–	NA	–	–	NA	–	–
Trehalose	–	+	–	+	–	+	+	+	+	+	+	–	NA	–	–	NA	–	+
D-Xylose	–	+	–	–	–	–	–	–	–	W	+	–	NA	–	–	NA	+	–
DNA G + C content (mol%)*	38.2	38.0	33.1	38.5	33.8	34.2	38.8	36.8	36.6	NA	NA	36.6	37.1	28.8	32.1	33.6	34.3	37.8

*DNA G + C contents shown are for type strains.

Table 2. Cellular fatty acid profiles (%) of strain N4^T and species of the genus *Chryseobacterium*

Taxa: 1, strain N4^T; 2, *C. gleum* ($n=5$; data from Hugo *et al.*, 1999); 3, *C. balustinum*; 4, *C. indologenes* ($n=45$; Hugo *et al.*, 1999); 5, *C. indoltheticum*; 6, *C. scopthalmum* ($n=7$); 7, *C. defluvii* (Kämpfer *et al.*, 2003); 8, *C. joostei* ($n=11$; Hugo *et al.*, 2003); 9, *C. daecheongense* (Kim *et al.*, 2005a); 10, *C. formosense* (Young *et al.*, 2005); 11, *C. taichungense* (Shen *et al.*, 2005); 12, *C. shigense* (A. Hiraishi, personal communication); 13, *C. vrystaatense* ($n=7$; de Beer *et al.*, 2005); 14, *C. soldanellicola* (Park *et al.*, 2006); 15, *C. taeenense* (Park *et al.*, 2006); 16, *C. piscium* ($n=4$; de Beer *et al.*, 2006); 17, *C. hispanicum* (Gallego *et al.*, 2006); 18, *C. wanjuae* (Weon *et al.*, 2006). Data for taxa 3, 5 and 6 are taken from Mudarris *et al.*, 1994. Number of strains examined, when greater than 1, is shown in parentheses. For all strains studied, fatty acids that account for <1% of the total fatty acid content are not shown so the percentages do not add up to 100%. Means $\pm$ SD are given. tr, Trace (<1%); ND, not detected; ECL, equivalent chain-length (i.e. the identity of the fatty acid is unknown).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
iso-C _{13:0}	1.0	tr	tr	tr	ND	tr	2.8	tr	1.6	3.6	tr	2.6	1.1 $\pm$ 0.4	tr	tr	0.9 $\pm$ 0.6	2.5	tr
Unknown, ECL 13.566	2.6	1.2 $\pm$ 0.4	1.6	2.1 $\pm$ 0.7	tr	3.3 $\pm$ 0.5	tr	1.1 $\pm$ 0.2	1.5	tr	6.7	2.4	1.4 $\pm$ 0.3	2.3	3.1	tr	1.0	2.9
iso-C _{15:0}	26.4	35.4 $\pm$ 2.9	32.3	34.3 $\pm$ 4.9	29.5	34.9 $\pm$ 0.5	58.5	34.6 $\pm$ 2.0	51.2	52.2	35.4	39.7	41.8 $\pm$ 1.4	41.8	36.1	38.3 $\pm$ 5.0	26.1	40.0
iso-C _{15:0} 3-OH	3.2	2.5 $\pm$ 0.1	2.7	2.6 $\pm$ 0.2	2.2	2.9 $\pm$ 0.1	2.6	2.9 $\pm$ 0.3	2.0	1.8	4.3	4.1	2.7 $\pm$ 0.3	2.7	3.1	2.4 $\pm$ 0.3	4.0	3.7
anteiso-C _{15:0}	4.3	tr	tr	tr	4.9	tr	3.2	tr	1.0	2.1	tr	tr	1.7 $\pm$ 0.7	1.9	1.1	2.7 $\pm$ 1.9	3.6	tr
C _{16:0}	13.5	1.3 $\pm$ 0.4	1.6	tr	tr	1.1 $\pm$ 0.1	1.3	tr	1.8	1.5	1.3	tr	1.1 $\pm$ 0.4	1.4	1.8	1.1 $\pm$ 0.2	2.4	tr
C _{16:0} 3-OH	6.7	1.1 $\pm$ 0.1	1.4	1.0 $\pm$ 0.2	tr	1.3 $\pm$ 0.1	tr	1.2 $\pm$ 0.2	tr	tr	2.6	1.3	1.3 $\pm$ 0.3	1.3	tr	1.3 $\pm$ 0.3	4.4	ND
iso-C _{16:0} 3-OH	ND	ND	tr	ND	1.2	tr	tr	ND	tr	1.1	1.4	tr	tr	tr	tr	tr	ND	tr
Unknown, ECL 16.580	ND	1.7 $\pm$ 0.1	1.3	1.7 $\pm$ 0.2	1.3	1.7 $\pm$ 0.2	tr	1.6 $\pm$ 0.1	1.0	1.0	1.7	1.0	1.2 $\pm$ 0.2	1.5	1.4	1.2 $\pm$ 0.2	1.9	ND
iso-C _{17:0}	ND	1.6 $\pm$ 0.6	1.0	tr	tr	1.1 $\pm$ 0.1	2.0	tr	3.0	2.3	tr	tr	tr	tr	1.1	1.2 $\pm$ 0.2	ND	2.9
iso-C _{17:0} 3-OH	9.8	21.8 $\pm$ 0.3	16.8	19.2 $\pm$ 1.8	15.4	17.1 $\pm$ 0.2	14.1	20.1 $\pm$ 1.2	15.7	10.9	22.4	19.6	15.4 $\pm$ 1.8	17.7	18.8	16.2 $\pm$ 3.1	17.6	21.9
iso-C _{17:1} ω 9c	ND	20.2 $\pm$ 3.9	27.1	24.2 $\pm$ 3.1	24.6	21.3 $\pm$ 0.4	3.8	22.9 $\pm$ 1.9	7.6	4.3	8.9	11.9	19.7 $\pm$ 2.3	14.6	15.8	18.7 $\pm$ 2.8	1.3	11.7
Summed feature 4*	32.4	11.8 $\pm$ 0.8	9.2	11.1 $\pm$ 1.3	12.0	11.8 $\pm$ 0.6	6.5	12.1 $\pm$ 1.3	8.4	6.5	13.8	12.4	9.1 $\pm$ 0.9	9.7	11.2	10.8 $\pm$ 1.3	26.7	11.0

*Summed feature 4 contains iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c/t.

(Swofford, 1993) by using the MEGA3 program (Kumar *et al.*, 2004), with bootstrap values based on 1000 replications (Felsenstein, 1985). Evolutionary distances were calculated using the method of Jukes & Cantor (1969).

Details of the cultural, physiological and biochemical characteristics of strain N4^T are given in the species description and in Table 1. The results from the API ZYM galleries are available in Supplementary Table S1 in IJSEM Online. The cellular fatty acid profile of strain N4^T was characterized by the predominance of summed feature 4 (iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c/t), iso-C_{15:0} and C_{16:0}, and was similar to those of other *Chryseobacterium* species. However, N4^T differed from other *Chryseobacterium* species (except *C. hispanicum*) in containing a very large amount of summed feature 4 and lacking iso-C_{17:1} ω 9c (Table 2). The predominant respiratory quinone was menaquinone MK-6. The DNA G + C content was 38.2 mol%.

The almost-complete 16S rRNA gene sequence (1483 bp) of strain N4^T was determined. The levels of 16S rRNA gene sequence similarity between N4^T and the type strains of other *Chryseobacterium* species ranged from 95.8% (with *C. joostei*) to 93.2% (with *C. balustinum*). Sequence similarities with all other species of the family *Flavobacteriaceae* included in the phylogenetic analysis were below 93.4%. In the phylogenetic tree based on the neighbour-joining algorithm, strain N4^T clustered with species of the genus *Chryseobacterium* (Fig. 1). Similar tree topologies were also

found in the trees generated with the maximum-parsimony and minimum-evolution algorithms (data not shown).

The above-mentioned chemotaxonomic features, together with the morphological, physiological and biochemical characteristics, strongly support the classification of strain N4^T within the genus *Chryseobacterium*. The phylogenetic distinctiveness was sufficient to categorize strain N4^T as representing a novel species within the genus *Chryseobacterium* (Stackebrandt & Goebel, 1994). The strain could also be distinguished from other *Chryseobacterium* species by means of some important phenotypic characteristics (summarized in Table 1): growth conditions, enzyme activities, acid production from carbohydrates, fatty acid profile (Table 2) and API ZYM profile (see Supplementary Table S1 in IJSEM Online). On the basis of the results obtained, strain N4^T is sufficiently distinct from all known *Chryseobacterium* species to be recognized as representing a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium caeni* sp. nov. is proposed.

Description of *Chryseobacterium caeni* sp. nov.

Chryseobacterium caeni (ca.e'ni. L. gen. n. *caeni* of sludge).

Cells are aerobic, non-spore-forming, non-motile rods. Gram-negative, oxidase- and catalase-positive. Good growth is observed on R2A, tryptic soy and nutrient agars, but not on MacConkey agar. Colonies are translucent and shiny with entire edges, becoming mucoid after 3 days

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of *Chryseobacterium caeni* N4^T and nearest neighbours based on 16S rRNA gene sequences. Species of some genera within the family *Flavobacteriaceae* were used to define the root. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at the branching points. Bar, 2 substitutions per 100 nucleotide positions.

incubation. Bright yellow flexirubin-type pigments are produced. Growth occurs at 5–37 °C, but not at 42 °C; the optimum temperature for growth is between 28 and 30 °C. The pH range for growth is 6.0–10.0, with an optimum at between pH 6.5 and 8.0. Cells grow in the presence of 0–3 % NaCl, but not with 5 % NaCl. The major fatty acids are summed feature 4 (iso-C_{15:0} 2-OH and/or C_{16:1} ω7*t*), iso-C_{15:0} and C_{16:0}. Menaquinone MK-6 is the predominant respiratory quinone. The G+C content of the genomic DNA is 38.2 mol%. Acid is not produced from amygdalin, L-arabinose, D-fructose, D-glucose, glycerol, inositol, lactose, D-melibiose, D-maltose, D-mannitol, L-rhamnose, D-sorbitol, D-sucrose, trehalose or D-xylose. The following substrates are utilized as sole carbon sources: D-glucose, L-arabinose, D-sucrose, D-maltose and glycogen. The following substrates are not utilized as sole carbon sources: D-mannitol, D-melibiose, L-fucose, D-sorbitol, propionate, caprate, valerate, citrate, histidine, 2-ketogluconate, 3-hydroxybutyrate,

4-hydroxybenzoate, L-proline, L-rhamnose, N-acetylglucosamine, D-ribose, inositol, itaconate, suberate, malonate, acetate, DL-lactate, L-alanine, 5-ketogluconate, 3-hydroxybenzoate, L-serine, D-mannose, gluconate, caprate, adipate, malate or phenyl acetate. Positive for urease and β-glucosidase activities, but negative for indole production. Nitrate and nitrite are not reduced. Results from the API ZYM test are given in Supplementary Table S1 in IJSEM Online.

The type strain, N4^T (=KCTC 12506^T=CCBAU 10201^T=DSM 17710^T), was isolated from the sludge of a nickel-complexed cyanide treatment bioreactor.

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