

Chryseobacterium mulctrae sp. nov., isolated from raw cow's milk

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Abstract

A Gram-stain-negative bacterial strain, designated CA10^T, was isolated from bovine raw milk sampled in Anseong, Republic of Korea. Cells were yellow-pigmented, aerobic, non-motile bacilli and grew optimally at 30 °C and pH 7.0 on tryptic soy agar without supplementation of NaCl. Phylogenetic analysis based on the 16S rRNA gene sequences revealed that strain CA10^T belonged to the genus *Chryseobacterium*, family *Flavobacteriaceae*, and was most closely related to *Chryseobacterium indoltheticum* ATCC 27950^T (98.75 % similarity). The average nucleotide identity and digital DNA–DNA hybridization values of strain CA10^T were 94.4 and 56.9 %, respectively, relative to *Chryseobacterium scophthalmum* DSM 16779^T, being lower than the cut-off values of 95–96 and 70 %, respectively. The predominant respiratory quinone was menaquinone-6; major polar lipid, phosphatidylethanolamine; major fatty acids, iso-C_{15:0}, summed feature 9 (iso-C_{17:1}ω9c and/or C_{16:0} 10-methyl), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) and iso-C_{17:0} 3-OH. The results of physiological, chemotaxonomic and biochemical analyses suggested that strain CA10^T is a novel species of genus *Chryseobacterium*, for which the name *Chryseobacterium mulctrae* sp. nov. is proposed. The type strain is CA10^T (=KACC 21234^T=JCM 33443^T).

The genus *Chryseobacterium* of the family *Flavobacteriaceae* was first described by Vandamme *et al.* [1] and later emended by Kämpfer *et al.* [2]. As of June 2019, 112 species of the genus *Chryseobacterium* have been described (www.bacterio.net/chryseobacterium.html), with *Chryseobacterium gleum* being the type species. Members of the genus *Chryseobacterium* have been isolated from various environments, including fresh water, glaciers, soil, sea urchins, raw chicken, fish, mosquito, plants and beverages [3–11]. Members of the genus *Chryseobacterium* are typically non-motile, yellow-pigmented, Gram-stain-negative bacilli, typically containing menaquinone-6 (MK-6) as the predominant respiratory quinone, phosphatidylethanolamine (PE) as the major polar lipid and branched chain fatty acids (iso-C_{15:0}, iso-C_{17:1}ω9c and iso-C_{17:0} 3-OH) as the major fatty acids [12].

The taxonomic, morphological, physiological and biochemical characteristics of strain CA10^T, isolated from raw milk, were examined. Furthermore, 16S rRNA gene sequencing, average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) analyses were conducted. Strain CA10^T is proposed herein as the type strain of the novel species of the genus *Chryseobacterium*.

ISOLATION AND ECOLOGY

Strain CA10^T was isolated from bovine raw milk at a Chung-Ang University-affiliated farm (Anseong, Republic of Korea). The sample was serially diluted and plated on skim milk agar (SMA; 5 % skim milk with 1.5 % agar, w/v). Yellow-pigmented colonies were observed on the plate with 10⁻² dilutions of raw milk after 10 days of incubation at 10 °C among which proteolytic bacteria were screened on the basis of a zone of clearance. Strain CA10^T was routinely cultured on tryptic soy agar (TSA) at 30 °C for 2 days and preserved in 10 % skim milk supplemented with 25 % (v/v) glycerol at –80 °C for further use.

16S rRNA gene phylogeny

Genomic DNA of strain CA10^T was extracted using QIAamp PowerFecal DNA Kit (Qiagen). The 16S rRNA gene was amplified via PCR using the universal bacterial primers 27F (5-AGAGTTTGATCMTGGCTCAG-3) and 1492R (5-TACGGYTACCTTGTACGACTT-3) [13]. The PCR product was purified using a PCR purification kit (Qiagen). The sequence of the purified PCR product was determined at SolGent Co. Ltd. (Daejeon, Republic of Korea). For consensus sequences, universal primers 27F, 1492R, 785F

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Keywords: *Chryseobacterium* strain CA10^T; *Chryseobacterium mulctrae*; bovine raw milk.

Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; MK, menaquinone; MLSA, multilocus sequence analysis; PE, phosphatidylethanolamine; SMA, skim milk agar; TSA, tryptic soy agar; TSB, tryptic soy broth.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence is MK896290, for the genome it is VAJL00000000, and those for the *rpoB*, *gyrB* and *groEL* genes are MK904810, MK904811 and MK904812, respectively.

Two supplementary tables and seven supplementary figures are available with the online version of this article.

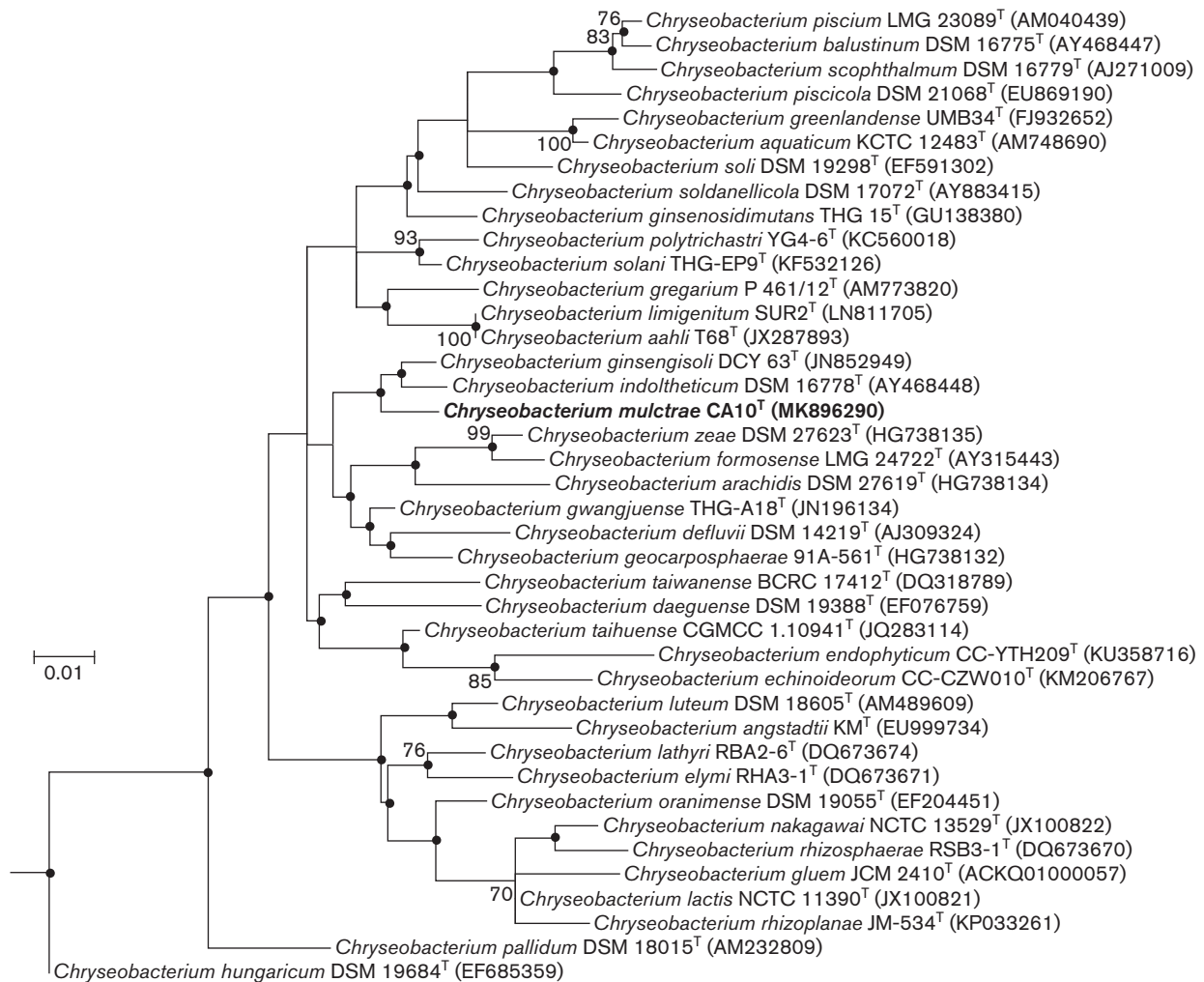


Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the relationship of CA10^T with closely related type strains and type species of the genus *Chryseobacterium*. Bootstrap values (expressed as the percentage of 1000 replications) are shown at the branch position: only values greater than 70 % are shown at nodes. Filled circles indicate the same branches found in the maximum-likelihood and neighbour-joining phylogenetic trees. The tree was rooted using *Flavobacterium haoranii* LQY-7^T (GQ988780) as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

(5-GGATTAGATACCCTGGTA-3) and 805R (5-GAC TACCAGGGTATCTAATC-3) were used. The 16S rRNA gene sequence (1478 bp) of strain CA10^T was aligned with those of related type strains and the type species of genus *Chryseobacterium* from the EzTaxon-e server (www.ezbiocloud.net/) [14] using CLUSTAL_W software [15]. Phylogenetic trees were reconstructed by using the neighbour-joining and maximum-likelihood methods [16, 17] and -MEGA-X software [18]. The Jukes–Cantor model was used to determine evolutionary distances [19]. Bootstrap analysis with 1000 replications was performed to assess the topology of both trees [20]. Analysis of its 16S rRNA gene sequence revealed that strain CA10^T belonged to the genus *Chryseobacterium* and was most similar to

Chryseobacterium indoltheticum ATCC 27950^T (98.75 %), *Chryseobacterium scophthalmum* DSM 16779^T (98.47 %), *Chryseobacterium balustinum* DSM 16775^T (98.12 %) and *Chryseobacterium ginsengisoli* DCY 63^T (98.03 %). According to the maximum-likelihood phylogenetic tree, supported by the neighbour-joining tree, strain CA10^T is clustered with the members of the genus *Chryseobacterium*, and closest to *C. indoltheticum* ATCC 27950^T and *C. ginsengisoli* DCY 63^T (Fig. 1). Therefore, we selected four type strains, *C. indoltheticum* KCTC 2920^T, *C. ginsengisoli* KCTC 237 60^T, *C. scophthalmum* KACC 16926^T and *C. balustinum* KACC 11660^T, based on the 16S rRNA gene sequence similarity as well as the phylogenetic tree, and the strain of the type species, *C. gluem* JCM 2410^T, as reference strains.

Table 1. Differential characteristics of CA10^T and related species and type species of the genus *Chryseobacterium*

Strains: 1, CA10^T; 2, *Chryseobacterium indoltheticum* KCTC 2920^T; 3, *Chryseobacterium ginsengisoli* KCTC 23760^T; 4, *Chryseobacterium scopthalmum* KACC 16926^T; 5, *Chryseobacterium balustinum* KACC 11660^T; 6, *Chryseobacterium gleum* JCM 2410^T. All data are from this study. +, Positive; w, weakly positive; –, negative

Characteristic	1	2	3	4	5	6
Growth at 10 °C	+	+	+	+	+	–
Growth at 40 °C	–	–	–	–	–	+
Optimum temperature for growth (°C)	30	30	30	30	25	37
pH for growth:						
Range	5.0–9.0	5.0–9.0	5.0–8.0	5.0–10.0	6.0–9.0	6.0–10.0
Optimum	7.0	8.0	7.0	8.0	7.0	7.0
NaCl for growth (% w/v)	0–4	0–4	0–2	0–4	0–2	0–4
Hydrolysis of:						
Starch	–	–	+	–	–	+
Cellulose	–	–	+	–	–	+
DNase	–	–	–	+	+	–
Urea	–	–	–	+	–	+
Indole production	+	+	–	+	+	+
Nitrate reduction	–	–	–	–	+	+
β-Galactosidase activity	–	–	–	–	–	+
Assimilation of:						
Arabinose	–	–	–	–	–	+
Potassium gluconate	–	–	w	–	–	w
Trisocium citrate	–	–	–	–	–	+
Suberate	–	+	+	+	+	+
L-Alanine	+	+	+	–	+	+
3-Hydroxy-benzonate	–	+	+	+	+	+
L-Serine	–	–	–	+	–	–
Mannitol	–	+	+	+	+	+
Caprate	+	–	–	–	–	–
Valerate	+	+	–	–	–	+
Histidine	+	+	+	–	+	+
4-Hydroxy-benzonate	–	–	+	+	+	+
DNA G+C content (mol%)	33.8	33.8*	31.6†	33.5*	33.6*	36.8*

*The DNA G+C contents of the reference strains were calculated using the R package SeqinR based on the genome sequences.

†Data from Nguyen et al. [38]

GENOME FEATURES

The complete genome of strain CA10^T was sequenced by ChunLab Whole Genome Analysis Service (Seoul, Republic of Korea) using the PacBio RS II platform and assembled using PacBio SMRT Analysis software version 2.3.0. The genome size was 4 868 651 bp with a 204.01× coverage and four contigs. In total, 4610 coding sequences (CDSs), 18 rRNA genes, and 75 tRNA genes were identified. The DNA G+C content of strain CA10^T was determined, using the R-package SeqinR [21], to be 33.8 mol %, which is within the range for the genus *Chryseobacterium* [1]. The complete genome sequence was deposited in GenBank under the accession number VAJL00000000.

Since the 16S rRNA gene of strain CA10^T displayed >98.7 % similarity to that of *C. indoltheticum* ATCC 27950^T [22, 23],

further genomic analysis was conducted. For *in silico* genomic comparison, ANI and dDDH analyses were performed [24]. Strain CA10^T was compared with the closest strain, an additional 27 related type strains and the type species of genus *Chryseobacterium*. The genomes were retrieved from the EzTaxon-e server. The ANI values were determined using Orthologous Average Nucleotide Identity Tool Software (OAT, implemented at www.ezbiocloud.net) [25]. The ANI value of strain CA10^T relative to that of *C. indoltheticum* ATCC 27950^T was 88 % and that relative to closely related strains was <94.4 % (Table S1, available in the online version of this article), both being lower than the cut-off value of 95–96 % [26]. The Genome-to-Genome Distance Calculator (version 2.1; <http://ggdc.dsmz.de/distcalc2.php>) was used to determine dDDH values [27]. The dDDH value of strain CA10^T relative to that of the most closely related

Table 2. Cellular fatty acid compositions (%) of strain CA10^T and related species and type species of the genus *Chryseobacterium*

Strains: 1, CA10^T; 2, *Chryseobacterium indoltheticum* KCTC 2920^T; 3, *Chryseobacterium ginsengisoli* KCTC 23760^T; 4, *Chryseobacterium scophthalmum* KACC 16926^T; 5, *Chryseobacterium balustinum* KACC 11660^T; 6, *Chryseobacterium gleum* JCM 2410^T. All data are from this study. TR, Trace amount (<1 %)

Fatty acid	1	2	3	4	5	6
iso-C _{13:0}	1.1	1.7	TR	TR	TR	–
iso-C _{14:0}	1.4	1.5	TR	TR	TR	–
iso-C _{15:0}	37.7	42.4	35.3	28.7	26.7	30.5
iso-C _{15:0} 3-OH	3.5	TR	3.1	3.3	2.5	2.6
anteiso-C _{15:0}	6.0	10.0	3.1	3.7	3.0	TR
C _{16:0}	1.6	1.8	2.5	1.2	1.9	1.8
C _{16:0} 3-OH	TR	TR	1.5	1.1	1.1	1.3
iso-C _{16:0}	1.9	5.8	1.5	1.5	6.9	–
iso-C _{16:0} 3-OH	3.7	5.5	3.1	2.3	4.2	TR
iso-C _{16:1} h	TR	TR	TR	TR	2.0	–
iso-C _{17:0}	TR	TR	1.6	TR	1.2	1.2
iso-C _{17:0} 3-OH	10.9	5.9	18.4	15.9	14.2	18.3
C _{17:0} 2-OH	TR	1.3	1.2	1.9	1.9	TR
C _{18:0} 3-OH	–	–	–	–	–	7.1
Summed feature 3*	11.1	5.4	8.6	14.6	9.3	12.5
Summed feature 9*	15.0	14.0	15.5	21.2	21.1	21.9

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of iso-C_{15:0} 2-OH and/or C_{16:1ω7c}; summed feature 9 consisted of iso-C_{17:1ω9c} and/or C_{16:0} 10-methyl as indicated by Montero-Calasanz et al. [35].

strain was 44.6 % and rest of strains was <57 % (Table S1). Values less than the threshold of 70 % are appropriate for species delineation [28]. Unlike the results of 16S rRNA gene analysis, the ANI and dDDH values of strain CA10^T were higher than those of *C. scophthalmum* DSM 16779^T, followed by *Chryseobacterium piscium* CCUG 51923^T, *C. balustinum* DSM 16775^T, and *C. indoltheticum* ATCC 27950^T. These results indicate that strain CA10^T represents a novel species of the genus *Chryseobacterium*.

Furthermore, housekeeping genes encoding RNA polymerase β subunit (*rpoB*), DNA gyrase subunit B (*gyrB*) and a 60 kD chaperonin (*cpn60*, *groEL*) were assessed to determine genetic uniqueness [24]. The sequences of the 28 related strains and type species of genus *Chryseobacterium* were obtained from their whole-genome sequences. The sequence similarities of the *rpoB* and *gyrB* genes of strain CA10^T were the highest to those of *C. scophthalmum* DSM 16779^T (98.3 and 97.2 %, respectively), and for *groEL*, sequence similarity was the highest for *Chryseobacterium elymi* RHA3-1^T (95.4 %; Table S2) and 92.3 % to *C. scophthalmum* DSM 16779^T. A maximum-likelihood phylogenetic tree for all genes was reconstructed using MEGA-X software with the Jukes-Cantor model and 1000 bootstrap replicates [17–20]. The phylogenetic tree based on *rpoB* and *gyrB* genes revealed that

strain CA10^T formed a cluster with *C. scophthalmum* DSM 16779^T and was closely related to *C. indoltheticum* ATCC 27950^T, *C. piscium* CCUG 51923^T and *C. balustinum* DSM 16775^T. Based on the *groEL* phylogenetic tree, strain CA10^T formed a cluster with *Chryseobacterium angstadtii* KM^T and closely related to *C. elymi* KCTC 22547^T and *Chryseobacterium lathyri* KCTC 22544^T (Figs S1, S2 and S3). Multilocus sequence analysis (MLSA) was performed using *rpoB*, *gyrB*, *groEL* and 16S rRNA genes (Fig. S4). MLSA trees obtained from the concatenated sequences of four genes were reconstructed under the same conditions as above. As inferred from the MLSA phylogenetic tree, strain CA10^T falls within the genus *Chryseobacterium* with *C. scophthalmum* DSM 16779^T, *C. balustinum* DSM 16775^T, *C. indoltheticum* ATCC 27950^T and *C. piscium* CCUG 51923^T as the next related species. The *rpoB*, *gyrB* and *groEL* sequences were deposited in GenBank under the accession numbers MK904810, MK904811, and MK904812, respectively.

Comparative genomic analysis was performed for strain CA10^T and four closely related strains selected on the basis of ANI and dDDH values. The Venn diagram shows that 2718 CDSs are shared by all five strains and strain CA10^T contains unique 827 CDSs (Fig. S5).

PHYSIOLOGY AND CHEMOTAXONOMY

For phenotypic comparison, reference strains *C. indoltheticum* KCTC 2920^T, *C. ginsengisoli* KCTC 23760^T, *C. scophthalmum* KACC 16926^T, and *C. balustinum* KACC 11660^T were obtained from the Korean Collection for Type Collection (KCTC) and the Korean Agricultural Culture Collection (KACC). *Chryseobacterium gleum* JCM 2410^T was obtained from the Japan Collection of Microorganisms (JCM). These strains were reactivated in tryptic soy broth (TSB; BD Difco) and subcultured on TSA (BD Difco) at 30 °C for 48 h. For long-term maintenance, cultures were preserved in TSB supplemented with 25 % (v/v) glycerol at –80 °C. Each strain was cultured in TSB at 30 °C for 48 h before use.

Gram-staining was performed using a Gram stain kit (BD Difco). Motility was assessed in motility agar (10 g l⁻¹ tryptose, 5 g l⁻¹ NaCl and 5 g l⁻¹ agar). Catalase activity was analysed using 3 % (v/v) H₂O₂, and oxidase activity was assessed using an oxidase strip (Sigma Aldrich). Flexirubin-type pigment was detected using 20 % (w/v) KOH [29]. Growth on different culture media was assessed using TSA, nutrient agar (NA; BD Difco), LB broth (BD Difco) supplemented with 1.5 % (w/v) agar (LBA), MacConkey agar (MA; BD Difco) and R2A agar (BD Difco). Growth at different temperatures was assessed on TSA at 4, 10, 25, 30, 37 and 40 °C and at pH 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. TSB was used, and the pH of the medium was adjusted before autoclaving with 0.1 M citrate–phosphate buffer (pH 5.0–7.0) and 0.1 M carbonate–bicarbonate buffer (pH 8.0–10.0) [30]. TSB devoid of NaCl was prepared to determine growth at 0 % of NaCl. Salinity tolerance was examined using TSB without or with 1, 2, 3, 4 and 5 % (w/v) NaCl as a final

concentration. Anaerobic growth was observed after 10 days incubation at 30 °C on TSA plates, using an anaerobic jar with a GasPak EZ anaerobe Container System (BD Difco). Starch and cellulose hydrolyses were assessed using starch agar (3 g l⁻¹ beef extract, 10 g l⁻¹ soluble starch and 12 g l⁻¹ agar) and cellulose agar [0.5 g l⁻¹ (NH₄)₂SO₄, 0.5 g l⁻¹ L-asparagine, 1 g l⁻¹ KH₂PO₄, 0.2 g l⁻¹ MgSO₄, 0.1 g l⁻¹ CaCl₂, 0.5 g l⁻¹ yeast extract, 10 g l⁻¹ cellulose, and 20 g l⁻¹ agar], respectively, and detected by flooding the plate with Gram's iodine after 2 days culturing [31, 32]. Casein hydrolysis was assessed using SMA. DNase agar (Oxoid) was used for the DNA hydrolysis test. For Tween 20 and Tween 80 hydrolyses, Tween agar (10 g l⁻¹ bacto-peptone, 5 g l⁻¹ NaCl, 0.1 g l⁻¹ CaCl₂·H₂O and 15 g l⁻¹ agar with 1 % Tween 20 or 80, v/v) was used [33]. Furthermore, physiological and biochemical features and enzymatic activities were assessed using API ID 32GN and API 20NE kit (bioMérieux) in accordance with the manufacturer's instructions. Strain CA10^T, *C. indoltheticum* KCTC 2920^T, *C. ginsengisoli* KCTC 23760^T, *C. scophthalmum* KACC 16926^T, *C. balustinum* KACC 11660^T and *C. gleum* JCM 2410^T were assessed under the same laboratory conditions.

Cell morphology was determined using transmission electron microscopy (TEM). In preparation for TEM, cells were grown on TSA for 48 h at 30 °C and suspended in distilled water (DW). A grid was placed on the suspension for a minute and then negative stained with phosphotungstic acid. It was washed twice with DW and dried. Cell was observed under microscope (JEM-1010, JEOL).

Cells of strain CA10^T were Gram-stain-negative, catalase- and oxidase-positive, aerobic bacilli and produced a flexirubin-type pigment (Fig. S6). Casein and Tween 80 were hydrolysed, while Tween 20 and DNA were not. Cells grew at 10–37 °C (optimum, 30 °C) at pH 5.0–9.0 (pH 7.0) and in TSB containing 0–4 % NaCl (0 % NaCl). Growth was optimum on TSA, observed on NA, LBA and R2A agar, but not observed on MA. The phenotypic characteristics of strain CA10^T and the reference strains are described in Table 1.

Respiratory quinones and polar lipids were extracted from freeze-dried cells using the integrated method [34]. Quinones were identified via reverse-phase HPLC (Supercosil LC-18-S; 250×4.6 mm, 5 μm). A mixture of methanol and 2-propanol (4:1, v/v) was used as a solvent with a flow rate of 1.0 ml min⁻¹. The predominant quinone was MK-6. Polar lipids were analysed via two-dimensional thin-layer chromatography (silica gel plates; Merck), followed by spreading of appropriate reagents [35, 36]. Phosphomolybdic acid hydrate was used to detect total lipids and for aminolipids, phospholipids and glycolipids, ninhydrin, molybdenum blue spray reagent and α-naphthol, respectively, were used. The major lipid of strain CA10^T was PE, along with three unidentified aminolipids and four unidentified phospholipids (Fig. S7).

To assess fatty acids, strain CA10^T and the reference strains were cultured on R2A agar plates at 30 °C for 2 days. All strains were harvested at the exponential phase. After saponification and methylation, fatty acids were extracted using a standard protocol and the Sherlock Microbial Identification System (MIDI) [37]. Fatty acid methyl esters were analysed using Microbial Identification software with Sherlock system 6.3 and the Sherlock aerobic bacterial database (TSBA 6.21; MIDI). Fatty acid analysis was performed using Ace EMzyme (Anseong, Republic of Korea). The major fatty acids of the novel strain were iso-C_{15:0} (37.7 %), summed feature 9 (iso-C_{17:1}ω9c and/or C_{16:0} 10-methyl; 15.0 %), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; 11.1 %), and iso-C_{17:0} 3-OH (10.9 %). The differences in fatty acid content between strain CA10^T and related species of genus *Chryseobacterium* are shown in Table 2.

Based on the results of our taxonomic and morphological analyses, strain CA10^T shares the characteristics of major menaquinone MK-6, PE as the major polar lipid and iso-C_{15:0} as the major fatty acid with described species in the genus *Chryseobacterium*. However, on the basis of its phylogenetic distance from known *Chryseobacterium* species, genomic features having lower than the cut-off values of ANI and dDDH, and a combination of unique phenotypic characteristics (as shown in Table 1), strain CA10^T represents a novel species in the genus *Chryseobacterium*, for which the name *Chryseobacterium mulctrae* sp. nov. is proposed.

DESCRIPTION OF *CHRYSEOBACTERIUM MULCTRAE* SP. NOV.

Chryseobacterium mulctrae (mulc'trae. L. gen. n. *mulctrae* of a milk pail).

Cells are Gram-stain-negative, aerobic, non-sporulating and non-motile bacilli. Colonies are yellow, circular with an entire margin, and smooth on TSA after 2 days of incubation at 30 °C. Growth occurs at 10–37 °C (optimum, 30 °C) and at pH 5.0–9.0 (pH 7.0). Cell growth is optimal in TSB not supplemented with NaCl; however, it displays tolerance to up to 4 % NaCl. The strain is catalase- and oxidase-positive, and flexirubin-type pigments are produced. Cells hydrolyse casein and Tween 80, but not Tween 20, starch, cellulose and DNA. The API 20NE test reveals that strain CA10^T is negative for nitrite reduction, glucose fermentation, L-arginine, urea and 4-nitrophenyl-β-D-galactopyranoside. Positive for indole production, β-glucosidase and gelatin hydrolysis. The strain assimilates D-glucose and D-mannose, but not L-arabinose, D-mannitol, N-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. In the API ID 32GN test strip, all substrates, except for suberate, 3-hydroxy-benzonate, L-serine, mannitol and 4-hydroxy-benzonate, are assimilated. Cells contain MK-6 as the sole respiratory quinone, PE, three unidentified aminolipids and four unidentified lipids as the major lipids, and iso-C_{15:0}, summed feature 9 (iso-C_{17:1}ω9c and/or C_{16:0} 10-

methyl), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω7c) and iso-C_{17:0} 3-OH as the major fatty acids.

The type strain, CA10^T (=KACC 21234^T=JCM 33443^T), was isolated from raw cow's milk sampled in Anseong, Republic of Korea. The DNA G+C content of the type strain is 33.8 mol %.

Funding information

This study was supported by the Chung-Ang University Graduate Research Scholarship in 2018. We thank Professor Aharon Oren for his expert suggestion concerning the species epithet and Latin etymology.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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