INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Kweon *et al.*, *Int. J. Syst. Evol. Microbiol.* 2021;71:004759
DOI 10.1099/ijsem.0.004759

MICROBIOLOGY SOCIETY

Cupriavidus cauae sp. nov., isolated from blood of an immunocompromised patient

Oh Joo Kweon[1]†, Wenting Ruan[2]†, Shehzad Abid Khan[2], Yong Kwan Lim[1], Hye Ryoun Kim[1], Che Ok Jeon[2,*] and Mi-Kyung Lee[1,*]

Abstract

A novel Gram-stain-negative, facultative aerobic and rod-shaped bacterium, designated as MKL-01^T and isolated from the blood of immunocompromised patient, was genotypically and phenotypically characterized. The colonies were found to be creamy yellow and convex. Phylogenetic analysis based on 16S rRNA gene and whole-genome sequences revealed that strain MKL-01^T was most closely related to *Cupriavidus gilardii* LMG 5886^T, present within a large cluster in the genus *Cupriavidus*. The genome sequence of strain MKL-01^T showed the highest average nucleotide identity value of 92.1% and digital DNA–DNA hybridization value of 44.8% with the closely related species *C. gilardii* LMG 5886^T. The genome size of the isolate was 5750268 bp, with a G+C content of 67.87 mol%. The strain could grow at 10–45 °C (optimum, 37–40 °C), in the presence of 0–10% (w/v) NaCl (optimum, 0.5%) and at pH 6.0–10.0 (optimum, pH 7.0). Strain MKL-01^T was positive for catalase and negative for oxidase. The major fatty acids were $C_{16:0}$, summed feature 3 ($C_{16:1}\omega7c/C_{16:1}\omega6c$ and/or $C_{16:1}\omega6c/C_{16:1}\omega7c$) and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$). The polar lipid profile consisted of phosphatidylglycerol, phosphatidylethanolamine, two unidentified phospholipids and one unidentified polar lipid. Moreover, strain MKL-01^T contained ubiquinone Q-8 as the sole respiratory quinone. Based on its molecular, phenotypic and chemotaxonomic properties, strain MKL-01^T represents a novel species of the genus *Cupriavidus*; the name *Cupriavidus cauae* sp. nov. is proposed for this strain. The type strain is MKL-01^T.

The genus *Cupriavidus* is characterized by Gram-stain-negative, rod-shaped bacteria that are obligate aerobes (chemoorganotrophs or chemolithotrophs) [1]. The genus *Cupriavidus* was first proposed in 1987 by Makkar and Casida, who described a non-obligate bacterial predator in soil, named *Cupriavidus necator* N-1^T [2]. The genus *Cupriavidus* includes 19 validly published taxa, with *C. necator* reported as the type species [3], including the most recently described species, *Cupriavidus agavae* [4]. *Cupriavidus* species are found in the soil, rhizosphere and in human clinical specimens, particularly in samples obtained from debilitated patients [1, 4–9]. Of all the *Cupriavidus* species, *Cupriavidus gilardii* has been most frequently isolated from clinical specimens, including blood, throat, abscess and/or stool specimens [7–9]. In the present study, a presumably novel bacterium belonging to the genus *Cupriavidus* isolated from human blood was taxonomically characterized using a polyphasic approach; the name *Cupriavidus cauae* sp. nov. is proposed for this strain. The type strain is MKL-01^T.

ISOLATION AND ECOLOGY

Strain MKL-01^T was isolated from the blood sample of a 26-year-old female patient who was receiving chemotherapy for pre-diagnosed acute myeloid leukaemia. Blood cultures were performed using blood drawn from five different sites (two peripheral veins and three lumens of a Hickman line). The results of blood cultures performed in aerobic bottles (BacT/Alert FA Plus, bioMérieux) after approximately 13–15 h of incubation at 35 °C using the BacT/Alert 3D blood culture system (bioMérieux) were positive. Blood from the positive bottles was initially cultured on blood agar plates (BAPs; Synergy Innovation Co.) and incubated at 37 °C under 5% CO_2. Colonies on BAPs were light grey, translucent and 1 mm

Author affiliations: [1]Department of Laboratory Medicine, Chung-Ang University College of Medicine, Seoul, Republic of Korea; [2]Department of Life Science, Chung Ang University, Seoul, Republic of Korea.
***Correspondence:** Mi-Kyung Lee, cpworld@cau.ac.kr; Che Ok Jeon, cojeon@cau.ac.kr
Keywords: *Cupriavidus cauae*; new taxa; phylogenetic analysis; taxonomy.
Abbreviations: ANI, average nucleotide identity; BAP, blood agar plate; DDH, digital DNA-DNA hybridization; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbor-joining; TLC, thin-layer chromatography; UBCG, up-to-date bacterial core gene; WGS, whole-genome sequencing.
The GenBank accession numbers for the 16S rRNA gene and genome sequences of strain MKL-01^T are MN453488 and VWRN00000000, respectively.
†These authors contributed equally to this work
Four supplementary figures are available with the online version of this article.

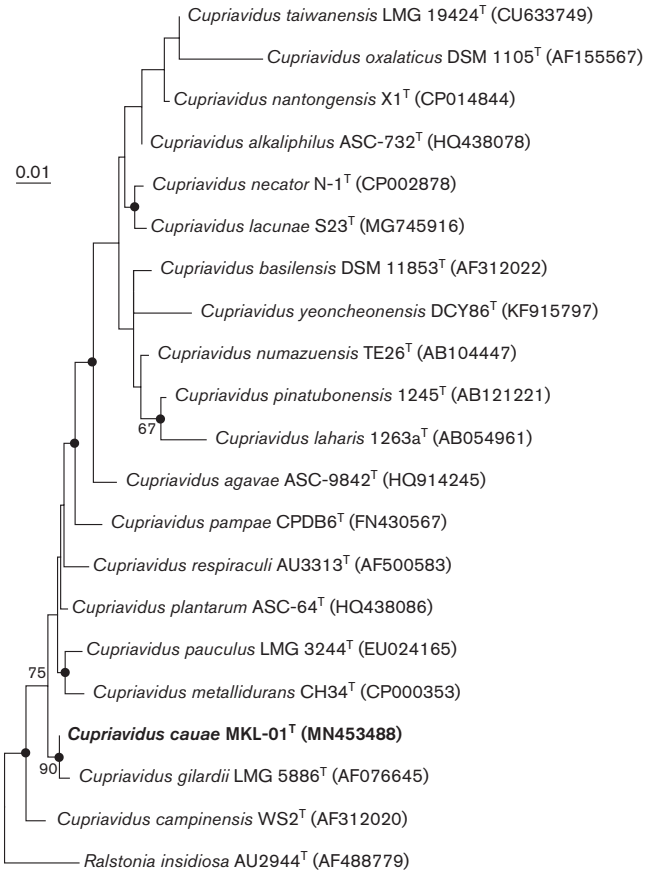

Fig. 1. A maximum-likelihood tree showing the phylogenetic relationships between strain MKL-01^T and its closely related taxa based on 16S rRNA gene sequences. Bootstrap values above 60% are shown on nodes as percentages of 1000 replicates. Filled circles (●) indicate the corresponding nodes that were also recovered in trees generated using the neighbour-joining and maximum-parsimony algorithms. *Ralstonia insidiosa* AU2944^T (AF488779) was used as an outgroup. The scale bar equals 0.01 changes per nucleotide position. GenBank accession numbers are indicated in parentheses.

in size. Strain MKL-01^T was the only isolate that grew on BAPs without any accompanying bacteria. Further investigations to determine the exact taxonomic status of MKL-01^T were conducted using this isolate.

16S rRNA PHYLOGENY

For 16S rRNA gene sequencing, the genomic DNA of strain MKL-01^T was extracted using the Wizard Genomic DNA Purification Kit (Promega). The 16S rRNA gene of strain MKL-01^T was amplified using universal primers [10]. Direct sequencing of the amplicon was performed using the Applied Biosystems 3500 Dx Genetic Analyzer (Thermo Fisher Scientific). The 16S rRNA gene sequence of strain MKL-01^T was compared with those of all other reported type strains using the Nucleotide Similarity Search program in the EzBioCloud server (www.ezbiocloud.net/identify) [11] and BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) [12]. The 16S rRNA gene sequences of closely related species of the genus *Cupriavidus* were obtained from GenBank (www.ncbi.nlm.nih.gov/genbank/) [13]. Phylogenetic trees based on the 16S rRNA gene sequences were reconstructed using the maximum-likelihood (ML), neighbour-joining (NJ) and maximum-parsimony (MP) algorithms in the MEGA7 software [14]. Bootstrap values were determined based on 1000 replications.

The comparison of 16S rRNA gene sequences between strain MKL-01^T and other type strains showed that strain MKL-01^T was most closely related to *C. gilardii* LMG 5886^T (99.72% similarity), followed by *Cupriavidus plantarum* LMG 26296^T (99.24%) and *Cupriavidus taiwanensis* R-1^T (98.97%). Phylogenetic analysis using the ML algorithm showed that strain MKL-01^T was grouped with *C. gilardii* LMG 5886^T, contained within a large cluster within the genus *Cupriavidus* (Fig. 1). Phylogenetic analysis using the NJ and MP algorithms also revealed *C. gilardii* LMG 5886^T as the most closely related species (Fig. S1, available in the online version of this article).

GENOME FEATURES

Genomic DNA was prepared using the Wizard Genomic DNA Purification Kit (Promega). The DNA concentration was measured using the Quat-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific). The DNA library was constructed using the TruSeq Nano DNA LT Library Prep Kit (Illumina), according to the manufacturer's instructions. The library was quantified by Bioanalyzer 2100 (Agilent Technologies) using the DNA 7500 Kit (Agilent Technologies). Whole-genome sequencing (WGS) was performed on the Illumina MiSeq platform (2×300 bp; Illumina). Any possible contamination of genomic data with other organisms was screened using the ContEst16S algorithm (ChunLab), in which 16S rRNA gene fragments are screened to determine whether the genome assembly is contaminated [15]. For genome-based phylogenomic analysis, the up-to-date bacterial core gene (UBCG) pipeline (www.ezbiocloud.net/tools/ubcg) [16] was used to extract 92 core housekeeping genes from the genomes of strain MKL-01^T and related taxa. An ML tree with bootstrap values (100 replications) based on the concatenated 92 core housekeeping genes was reconstructed using MEGA7 software [14]. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (DDH) values between strain MKL-01^T and its most closely related species were determined using the EzTaxon-e server (www.ezbiocloud.net/tools/ani) [17] and the server-based Genome-to-Genome Distance Calculator version 2.1 (http://ggdc.dsmz.de/distcalc2.php), respectively [18].

The genome size of the isolate obtained by WGS was 5750268 bp. Moreover, the G+C content was 67.87 mol%, average sequencing depth was 294.57, total number of contigs was 76 and the N50 value was 163605 bp. The genome sequence of strain MKL-01^T showed the highest ANI value of 92.1% and DDH value of 44.8% with the closely related species *C. gilardii* JZ4^T; these values were lower than the thresholds (ANI 95% and DDH 70%) for prokaryotic species delineation (Table 1) [19]. A phylogenetic tree based on the 92 housekeeping core genes showed that strain MKL-01^T formed a close phylogenetic lineage with *C. gilardii* JZ4^T

Table 1. Genome sequencing summaries and general features of strain MKL-01^T and related taxa belonging to the genus *Cupriavidus**

Strains: 1, MKL-01^T (this study); 2, *Cupriavidus gilardii* JZ4^T; 3, *Cupriavidus metallidurans* NDB4MOL1^T; 4, *Cupriavidus necator* PHE3-6^T; 5, *Cupriavidus plantarum* MA1-4a^T.

Genomic feature		1	2	3	4	5
Genome size (kb)		5749.937	5760.01	6953.11	3870	6278.407
Coverage		294.57×	656.77×	–	–	145.0×
No. of contigs		76	115	04	04	19
G+C content (mol%)		64.9	67.4	63.54	66.5	66.0
No. of genes		5140	5198	6378	3676	5712
No. of protein coding genes		4901	5014	6163	3502	5561
No. of pseudogenes		178	108	136	104	88
Total tRNA genes		53	61	63	57	52
GenBank accession number		VWRN00000000	LVXY00000000	FYAX00000000	LVWN00000000	RCCX00000000
				DDH value (%)†		
	1	–	44.8	18.9	25.8	24.7
	2	92.1	–	20.1	25.1	24.3
ANI value (%)‡	3	79.8	80.1	–	23.8	23.7
	4	81.4	80.9	79.7	–	23.9
	5	77.5	79.3	80.1	79.8	–

*The bioinformatic analysis of the genomes was carried out using the NCBI prokaryotic genome annotation pipeline www.ncbi.nlm.nih.gov/genome/annotation_prok/.
†DDH, digital DNA–DNA hybridization.
‡ANI, average nucleotide identity.

(Fig. S2). In conclusion, phylogenetic analysis based on the 16S rRNA gene and whole-genome sequences of strain MKL-01^T indicated that strain MKL-01^T represents a novel species belonging to the genus *Cupriavidus*.

PHYSIOLOGY AND CHEMOTAXONOMY

For comparing the phenotypic properties, cells of strain MKL-01^T, *C. gilardii* DSM 17292^T, *C. metallidurans* KACC 15166^T, *C. necator* KACC 12221^T, *C. respiraculi* LMG 21510^T and *C. plantarum* LMG 26296^T were harvested during their exponential growth phases (optical density of approximately 0.8 at 600 nm) at their optimal temperatures in Reasoner's 2A medium (R2A; Becton, Dickinson and Company).

The growth of strain MKL-01^T was evaluated at 37 °C for 3 days on several bacteriological agar media, including marine agar (MA; Becton, Dickinson and Company), Luria–Bertani (LB) agar (ecton, Dickinson and Company), R2A agar, nutrient agar (NA; Becton, Dickinson and Company) and tryptic soy agar (TSA; Becton, Dickinson and Company). To determine the optimal growth conditions, strain MKL-01^T was cultured at different temperatures (0–55 °C at 5 °C intervals) on R2A medium. In addition, it was cultured at different pH values (pH 3.0–11.0 at intervals of 1.0 pH unit). The pH values of the R2A broth were adjusted using the following buffers: for pH 3.0–5.0, citrate buffer; for pH 6.0–7.0, Na_2HPO_4–NaH_2PO_4; for pH 8.0–9.0, Tris–HCl buffer; and for pH 10.0–11.0, sodium carbonate buffer. If necessary, the pH values were adjusted again after autoclaving for 15 min at 121 °C. The NaCl tolerance was tested in R2A broth [0–20% (w/v) at intervals of 1%] [20]. Gram staining (bioMérieux) was performed according to the manufacturer's instructions. The gliding motility of strain MKL-01^T was assessed on R2A agar medium containing 0.3% agar. The cells of strain MKL-01^T were cultured for 2–3 days at 37 °C on R2A agar and their morphology was assessed under a phase-contrast microscope (Carl Zeiss) after flagellar staining with Ryu staining solution [21] and under a transmission electron microscope (LEO906, Zeiss) after negative staining with phosphotungstic acid stain. In addition, flagella-encoding genes within the genome of strain MKL-01^T were identified using BLASTn searches against respective gene sequences retrieved from Uniprot. To assess anaerobic growth, strain MKL-01^T was streaked on R2A agar and incubated at 30 °C for 21 days under the anaerobic condition prepared by the GasPak Plus system (BBL). Oxidase and catalase tests were performed according to standard methods [22]. The hydrolysis of casein, Tween 20, Tween 80, aesculin, tyrosine and starch was tested according

Table 2. Differential phenotypic and physiological characteristics of strain MKL-01^T and related taxa belonging to the genus *Cupriavidus*

Strains: 1, MKL-01^T (this study); 2, *Cupriavidus gilardii* DSM 17292^T; 3, *Cupriavidus metallidurans* KACC 15166^T; 4, *Cupriavidus necator* KACC 12221^T; 5, *Cupriavidus respiraculi* LMG 21510^T; 6, *Cupriavidus plantarum* LMG 26296^T. All strains were negative for lipase (C14), α-galactosidase, *β*-glucuronidase, α-mannosidase and α-fucosidase activities; indole production; and casein, aesculin and starch hydrolysis. All strains were positive for catalase, esterase (C4), leucine arylamidase, valine arylamidase, arginine dihydrolase and urease activities and D-mannose and potassium gluconate assimilation. Symbols: +, positive; –, negative; NA, not available.

Characteristics	**1**	**2**	**3**	**4**	**5**	**6**
Isolation source*	Human	Various clinical sources	Industrial locations	Soil	Human	*Agave* rhizosphere
Cell size (μm)*	1.36–2.40×0.59–1.29	NA	0.8×1.2–2.2	NA	NA	NA
Flagellation*	–	+	+	+	+	+
Range for growth:*						
Temperature (°C)	10–45	30–42	20–41	15–50	28–37	15–42
pH	6.0–10.0	–	NA	5.5–9.2	NA	4.5–11.0
NaCl (%)	0–10	0.5–1.5	NA	0–2.0	NA	0–1.5
Anaerobic growth*	+	–	NA	–	NA	–
Oxidase*	–	+	+	+	+	+
Hydrolysis of:†						
Tween 20, Tween 80	–	–	–	+	–	–
Tyrosine	+	+	–	+	–	–
Enzyme activity of (API ZYM):†						
Alkaline phosphatase	+	+	+	–	–	+
Esterase lipase (C8)	+	+	+	+	+	–
Crystine arylamidase	–	+	–	+	–	–
Trypsin	+	+	–	–	–	–
α-Chymotrypsin	–	+	–	+	+	+
Acid phosphatase	+	–	+	+	+	+
Naphthol-AS-BI-phosphohydrolase	–	+	+	+	+	+
β-Galactosidase	+	–	–	–	+	+
α-Glucosidase	+	+	+	–	+	+
β-Glucosidase	–	+	+	–	+	+
N-Acetyl-*β*-glucosaminidase	–	+	–	+	+	–
Reduction of nitrate to nitrite (API 20NE)†	+	+	–	+	+	+
Glucose fermentation (API 20NE)†	–	–	+	–	+	+
Assimilation of (API 20NE):†						
D-Glucose	–	+	+	+	+	+
L-Arabinose	–	+	+	–	+	+
D-Mannitol	+	+	–	–	+	+
N-Acetyl glucosamine, maltose, capric acid, adipic acid	+	+	–	+	+	+

Continued

Table 2. Continued

Characteristics	1	2	3	4	5	6
Malic acid, trisodium citrate	+	–	+	–	–	+
Phenylacetic acid	–	–	–	+	–	–

*Data from previous studies [1, 2, 5, 25–27].
†Regarding the characteristics of closely related strains, the results of hydrolysis, enzyme activity, and assimilation, were obtained in the present study.

to previously described methods [23]. Additional biochemical and enzymatic tests for identifying the abilities of strain MKL-01^T and its closely related reference strains to assimilate and convert different substrates were performed using the API 20NE and API ZYM kits (bioMérieux) according to the manufacturer's instructions. Antibiotic susceptibility was examined on Mueller–Hinton agar using different antibiotic discs containing ticarcillin/clavulanic acid (75/10 µg), piperacillin (30 µg), gentamicin (10 µg), piperacillin/tazobactam (100/10 µg), ceftazidime (30 µg), cefepime (30 µg), ceftriaxone (30 µg), imipenem (10 µg), meropenem (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg) and trimethoprim/sulfamethoxazole (1.25/23.75 µg). Plates were incubated for 18 h at 35 °C under 5% CO_2. The zone diameters were read and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) document M100 [24]. Regarding the phenotypic and physiological characteristics of closely related reference strains, the results of hydrolysis, enzyme activity, assimilation and antibiotic resistance were obtained in the present study and the remaining data were from previous studies [1, 2, 5, 25–27].

Strain MKL-01^T grew well on R2A agar. It could also grow on MA, TSA, NA and LB agar. The colonies of strain MKL-01^T on R2A agar after incubation for 48 h at 37 °C were circular, light yellow, convex, and 1.5 mm in diameter. Strain MKL-01^T was a Gram-stain-negative and rod-shaped bacterium. Although the genes *flg*, *fli* and *flh* necessary for the synthesis of flagella were grouped within gene clusters in the whole-genome sequence of strain MKL-01^T, cells of strain MKL-01^T were found to be non-motile and non-flagellated on a low-melting agar plate and under a phase-contrast microscope and transmission electron microscope. Moreover, the strain was found to grow under anaerobic conditions, suggesting that it was a facultative aerobe. Cells were 0.59–1.29 µm wide and 1.36–2.40 µm long (Fig. S3). The strain could grow at 10–45 °C in R2A medium, with the optimal temperature being 37–40 °C. The NaCl concentration range for growth was 0–10% (w/v) in R2A medium, with the optimal concentration being 0.5%. Moreover, it could grow in a pH range of 6.0–10.0 in R2A medium, with the optimal pH being 7.0. Strain MKL-01^T showed a catalase-positive activity, similar to the five closest related reference strains *C. gilardii* DSM 17292^T, *C. metallidurans* KACC 15166^T, *C. necator* KACC 12221^T, *C. respiraculi* LMG 21510^T and *C. plantarum* LMG 26296^T. The abilities of strain MKL-01^T and closely related reference strains to assimilate and convert different substrates are listed in Table 2. Strain MKL-01^T and closely related reference strains were found to be negative for lipase (C14), α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase activities; indole production and casein, aesculin and starch hydrolyses. All the strains were positive for the activities of esterase (C_4), leucine arylamidase, valine arylamidase, nitrate reduction, arginine dihydrolase, urease, D-mannose and potassium gluconate assimilation. However, strain MKL-01^T had many different phenotypic characteristics from its related type strains, including different growth conditions and several other enzymatic and assimilation activities (Table 2).

Strain MKL-01^T was resistant to meropenem (10 µg) and gentamicin (10 µg); intermediately susceptible to ticarcillin/clavulanic acid (75/10 µg) and ceftazidime (30 µg); and susceptible to piperacillin (30 µg), piperacillin/tazobactam (100/10 µg), cefepime (30 µg), ceftriaxone (30 µg), imipenem (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg).

For analysing the fatty acid composition, strain MKL-01^T and its closely related reference strains were cultured in R2A broth under their optimal growth conditions and harvested during their exponential growth phases (optical density at 600 nm, 0.8). The fatty acid composition was analysed using previous methods [28], including the following steps: saponification, methylation and fatty acid extraction from the harvested cells, followed by gas chromatography for the assessment of fatty acid methyl esters. The results were analysed using the RTSBA6 database of the Microbial Identification System (Sherlock version 6.0B) [28]. Moreover, the polar lipids of strain MKL-01^T were analysed by two-dimensional thin-layer chromatography (TLC) following the approach previously described by Minnikin *et al.* [29]. The TLC plate was developed using the solvent system consisting of chloroform–methanol–water (65:25:4, v/v) for the first dimension and chloroform–methanol–acetic acid–water (80:12:15:4, v/v) for the second dimension. Polar lipids were detected using the following reagents: 10% ethanolic molybdophosphoric acid specific to total polar lipids, ninhydrin specific to aminolipids, Dittmer–Lester reagent specific to phospholipids and α-naphthol/sulfuric acid specific to glycolipids. Four TLC plates were prepared for each sample. The respiratory quinones of strain MKL-01^T were analysed according to the method of Minnikin *et al.* [30] using the LC-20A HPLC System (Shimadzu) equipped with a diode

Table 3. Cellular fatty acid compositions (%) of strain MKL-01^T and related taxa belonging to the genus *Cupriavidus*

Strains: 1, MKL-01^T (the present study); 2, *Cupriavidus gilardii* DSM 17292^T; 3, *Cupriavidus metallidurans* KACC 15166^T; 4, *Cupriavidus necator* KACC 12221^T; 5, *Cupriavidus respiraculi* LMG 21510^T; 6, *Cupriavidus plantarum* LMG 26296^T. All data were obtained from this study. Data are expressed as percentages of the total fatty acids. Fatty acids amounting to less than 0.5% in all strains are not shown. Symbols: TR, trace amount (<0.5%); –, not detected.

Characteristics	1	2	3	4	5	6
Saturated:						
$C_{12:0}$	TR	–	TR	TR	1.28	1.12
$C_{14:0}$	8.40	3.7	2.62	3.98	5.67	5.95
$C_{16:0}$	26.57	9.20	9.78	10.46	14.22	11.74
$C_{18:0}$	0.52	0.96	TR	TR	0.58	–
Unsaturated:						
$C_{14:1}$ $\omega 5c$	0.58	–	2.42	TR	–	–
$C_{16:1}$ $\omega 5c$	0.83	0.61	TR	–	TR	TR
$C_{16:1}$ $\omega 11c$	–	0.52	0.62	1.00	TR	–
alcohol $C_{16:1}$ $\omega 7c$	–	–	TR	1.57	–	–
Branched:						
iso-$C_{10:0}$	0.69	1.31	2.88	2.06	2.43	0.97
iso-$C_{14:0}$	–	2.80	2.21	6.26	TR	0.78
iso-$C_{15:0}$	–	18.09	16.78	8.20	–	–
iso-$C_{16:0}$	TR	2.21	1.42	3.43	0.98	1.36
iso-$C_{19:0}$	1.37	–	–	–	4.52	4.64
anteiso-$C_{14:0}$	TR	1.17	0.80	0.58	0.62	TR
anteiso-$C_{17:0}$	TR	2.25	1.71	1.11	TR	1.07
Hydroxy:						
$C_{8:0}$3-OH	TR	1.31	1.02	0.73	0.89	TR
$C_{12:0}$3-OH	–	TR	TR	TR	TR	TR
$C_{14:0}$2-OH	TR	TR	TR	4.71	1.30	2.42
iso-$C_{17:0}$3-OH	–	1.85	1.32	1.18	1.85	–
Summed features:*						
2	7.27	6.70	7.50	6.92	9.46	5.24
3	15.97	7.31	6.42	7.14	9.10	12.46
8	14.51	–	5.27	3.94	6.58	12.09

*Summed features represent groups of two or three fatty acids that cannot be separated by gas–liquid chromatography using the MIDI system. Summed feature 2 corresponds to an unidentified fatty acid with an equivalent chain length value of 10.9525, 12:0 ALDE or any combination of these fatty acids; summed feature 3, $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; summed feature 8, $C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$.

array detector (SPD-M20A, Shimadzu) and a reversed-phase column (250×4.6 mm; Kromasil).

The predominant fatty acids of strain MKL-01^T included the saturated fatty acid $C_{16:0}$, summed feature 3 ($C_{16:1}$ $\omega 7c$/$C_{16:1}$ $\omega 6c$ and/or $C_{16:1}$ $\omega 6c$/$C_{16:1}$ $\omega 7c$) and summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$). The overall fatty acid composition of strain MKL-01^T was quite similar to those of the closely related type strains used in the present study (Table 3). However, some clear differences were observed. For instance, $C_{16:1}$ $\omega 11c$, iso-$C_{14:0}$, iso-$C_{15:0}$ and iso-$C_{17:03}$-OH were present in the closely related type strains but were not detected in strain MKL-01^T. Moreover, summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$) was present as a predominant fatty acid in strain MKL-01^T but not detected in its most closely related strain, *C. gilardii* DSM

17292^T. The polar lipid profile of strain MKL-01^T consisted of phosphatidylglycerol, phosphatidylethanolamine, two unidentified phospholipids and one unidentified polar lipid (Fig. S4). The polar lipids phosphatidylglycerol and phosphatidylethanolamine have been commonly detected in members of the genus *Cupriavidus* [6, 31]. Isoprenoid quinone analysis revealed that strain MKL-01^T contained ubiquinone Q-8 as the sole respiratory quinone, similar to other reference strains belonging to the genus *Cupriavidus* [31].

Based on the results of the phylogenetic analyses using 16S rRNA gene and whole-genome sequences; the difference in morphology, fatty acids and polar lipids; and some distinguishable phenotypic characteristics, strain MKL-01^T can be differentiated from closely related type strains belonging to the genus *Cupriavidus*. Therefore, strain MKL-01^T can be considered to represent a novel species belonging to the genus *Cupriavidus*, for which the name *Cupriavidus cauae* sp. nov. is proposed.

DESCRIPTION OF *CUPRIAVIDUS CAUAE* SP. NOV.

Cupriavidus cauae (cau′ae. N.L. gen. n. *cauae* of CAU referring to Chung-Ang University or Chung-Ang University Hospital, Seoul, Republic of Korea, where the type strain was isolated).

Cells are Gram-stain-negative, facultatively aerobic, non-spore-forming, rod-shaped (0.59–1.29×1.36–2.40 µm) and non-motile without a flagellum. Gliding motility is absent. Colonies on R2A agar after incubation for 48 h at 37 °C are circular, light yellow, convex and 1.5 mm in diameter. In R2A broth, growth is observed at 10–45 °C (optimum, 37–40 °C), in the presence of 0–10% (w/v) NaCl (optimum, 0.5%) and at pH 6.0–10.0 (optimum, pH 7.0). The strain is positive for catalase and negative for oxidase. Nitrate reduction is positive, while indole production and glucose fermentation are negative. Moreover, tyrosine hydrolysis is positive, but hydrolysis of Tween 20, Tween 80, casein, aesculin and starch is negative. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, α-glucosidase, β-galactosidase, arginine dihydrolase and urease activities are positive, but lipase (C14), crystine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are negative. It can assimilate D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid and trisodium citrate. However, it cannot assimilate D-glucose, L-arabinose and phenylacetic acid. The major fatty acids (>10% of total) are $C_{16:0}$, summed feature 3 ($C_{16:1}\omega7c$/$C_{16:1}\omega6c$ and/or $C_{16:1}\omega6c$/$C_{16:1}\omega7c$) and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$). The polar lipid profile consists of phosphatidylglycerol, phosphatidylethanolamine, two unidentified phospholipids and one unidentified polar lipid. The strain contains ubiquinone Q-8 as the sole respiratory quinone.

The type strain is MKL-01^T. It was isolated from the blood of immunocompromised patient in the Republic of Korea. The genomic DNA of this strain has 67.87 mol% G+C content.

Funding information
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No.2020R1A5A1018052). The funders did not have any additional role in the study design, data acquisition and analysis, decision to publish, or preparation of the manuscript.

Author contributions
O. J. K., data curation, formal analysis, investigation, methodology and writing (original draft). W. R., data curation, investigation, methodology, visualization and writing (original draft). S. A. K., data curation, investigation, methodology, software and visualization. Y. K. L., investigation, software and visualization. H. R. K., investigation and methodology. C. O. J., conceptualization, writing (review and editing), project administration, supervision and validation. M. L., conceptualization, writing (review and editing), funding acquisition, project administration, supervision and validation.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Because the patient had expired before the time of bacteria identification, an Institutional Review Board (IRB) review of the study and the need for obtaining informed consent from the patient for the publication were waived according to the Chung-Ang University Hospital IRB policy (IRB No. 2001-001-19296).

References
1. Vandamme P, Coenye T. Taxonomy of the genus *Cupriavidus*: a tale of lost and found. *Int J Syst Evol Microbiol* 2004;54:2285–2289.
2. Makkar NS, Casida LE. *Cupriavidus necator* gen. nov., sp. nov.; a nonobligate bacterial predator of bacteria in soil. *Int J Syst Evol Microbiol* 1987;37:323–326.
3. LPSN. List of prokaryotic names with standing in nomenclature 2019.
4. Arroyo-Herrera I, Maldonado-Hernández J, Rojas-Rojas FU, Meza-Radilla G, Larios-Serrato V *et al*. *Cupriavidus agavae* sp. nov., a species isolated from Agave L. rhizosphere in northeast Mexico. *Int J Syst Evol Microbiol* 2020;70:4165–4170.
5. Estrada-de los Santos P, Solano-Rodríguez R, Matsumura-Paz LT, Vásquez-Murrieta MS, Martínez-Aguilar L. *Cupriavidus plantarum* sp. nov., a plant-associated species. *Arch Microbiol* 2014;196:811–817.
6. Estrada-de los Santos P, Martínez-Aguilar L, López-Lara IM, Caballero-Mellado J. *Cupriavidus alkaliphilus* sp. nov., a new species associated with agricultural plants that grow in alkaline soils. *Syst Appl Microbiol* 2012;35:310–314.
7. Zhang Z, Deng W, Wang S, Xu L, Yan L *et al*. First case report of infection caused by *Cupriavidus gilardii* in a non-immunocompromised Chinese patient. *IDCases* 2017;10:127–129.
8. Tena D, Losa C, Medina MJ, Saez-Nieto JA. Muscular abscess caused by *Cupriavidus gilardii* in a renal transplant recipient. *Diagn Microbiol Infect Dis* 2014;79:108–110.
9. Karafin M, Romagnoli M, Fink DL, Howard T, Rau R *et al*. Fatal infection caused by *Cupriavidus gilardii* in a child with aplastic anemia. *J Clin Microbiol* 2010;48:1005–1007.
10. CLSI. *Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing*, 2nd ed. CLSI standard MM18 2nd ed. ed. Wayne, PA: Clinical and Laboratory Standards Institutes; 2018.
11. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y *et al*. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.

12. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990;215:403–410.
13. Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. *Nucleic Acids Res* 2016;44:D67–D72.
14. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
15. Lee I, Chalita M, Ha S-M, Na S-I, Yoon S-H *et al*. ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. *Int J Syst Evol Microbiol* 2017;67:2053–2057.
16. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007;73:5261–5267.
17. Yoon SH, Ha S-min, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek* 2017;110:1281–1286.
18. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
19. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR *et al*. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466.
20. Gomori G. Preparation of buffers for use in enzyme studies. *Handbook of Biochemistry and Molecular Biology*. CRC Press; 2010. pp. 721–724.
21. Kodaka H, Armfield AY, Lombard GL, Dowell VR. Practical procedure for demonstrating bacterial flagella. *J Clin Microbiol* 1982;16:948–952.
22. Gerhardt P. *Methods for General and Molecular Bacteriology*. Washington, D.C.: American Society for Microbiology; 1994.
23. Mac Faddin JF. *Biochemical Tests for Identification of Medical Bacteria*. Williams & Wilkins Co; 1976.
24. CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*, 13th Ed. CLSI standard M100, 13th ed. ed. Wayne, PA: Clinical and Laboratory Standards Institutes; 2018.
25. Coenye T, Falsen E, Vancanneyt M, Hoste B, Govan JR. Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. *International journal of systematic bacteriology* 1999;49:405–413.
26. Goris J, De Vos P, Coenye T, Hoste B, Janssens D *et al*. Classification of metal-resistant bacteria from industrial biotopes as *Ralstonia campinensis* sp. nov., *Ralstonia metallidurans* sp. nov. and *Ralstonia basilensis* Steinle et al. 1998 emend. *Int J Syst Evol Microbiol* 2001;51:1773–1782.
27. Coenye T, Vandamme P, LiPuma JJ. *Ralstonia respiraculi* sp. nov., isolated from the respiratory tract of cystic fibrosis patients. *Int J Syst Evol Microbiol* 2003;53:1339–1342.
28. Sasser M. MIDI technical note 101. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*. Newark. DE: MIDI; 1990. pp. 1–7.
29. Minnikin DE, Patel PV, Alshamaony L, Goodfellow M. Polar lipid composition in the classification of Nocardia and related bacteria. *Int J Syst Evol Microbiol* 1977;27:104–117.
30. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al*. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
31. Feng T, Kim KH, Oh J, Jeon CO. *Cupriavidus lacunae* sp. nov., isolated from pond-side soil. *Antonie van Leeuwenhoek* 2019;112:543–551.