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NOTE

Caenimonas terrae sp. nov., Isolated from a Soil Sample in Korea, and Emended Description of the Genus *Caenimonas* Ryu *et al.* 2008

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A white-coloured bacterium, SGM1-15^T, was isolated from a paddy soil sample from Suwon, Republic of Korea. The cells were strictly aerobic, Gram-negative and curved rod-shaped. A phylogenetic analysis based on 16S rRNA gene sequences revealed that strain SGM1-15^T was closely related to *Curvibacter delicatus* LMG 4328^T (97.6% similarity) and *Caenimonas koreensis* EMB320^T (97.5% similarity). The major respiratory quinone system was Q-8 and the predominant cellular fatty acids were $C_{16:0}$ (39.9%), summed feature 3 ($C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH; 24.3%) and $C_{17:0}$ cyclo (22.7%). The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The major polyamines were 2-hydroxypurescine, purescine and spermidine. The DNA G+C content was 68.7 mol%. On the basis of the phylogenetic, physiologicl and chemotaxonomic data, stain SGM1-15^T represents a novel species of the genus *Caenimonas*, for which the name *Caenimonas terrae* sp. nov. is proposed. The type strain of *Caenimonas terrae* is SGM1-15^T (=KACC 13365^T =NBRC 106341^T).

Keywords: 16S rRNA gene sequence, *Caenimonas terrae*, taxonomy

The genus *Caenimonas* was proposed for an isolate from activated sludge obtained after performing enhanced biological phosphate removal (EBPR) in a lab-scale sequencing batch reactor (Ryu *et al.*, 2008). This genus was a member of the family *Comamonadaceae* belonging to the *Beta-proteobacteria* (Stackebrandt *et al.*, 1988). It was characterized as Gram-negative, strictly aerobic, non-motile rods. Chemotaxonomically, the genus contained ubiquinone-8 as the predominant isoprenoid quinone and $C_{16:1}\omega7c$ and/ or iso-$C_{15:0}$ 2-OH, $C_{16:0}$, and $C_{18:1}\omega7c$ as the major fatty acids. Major polar lipids were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Until the current study, this genus comprised only one species with *Caenimonas koreensis* (Ryu *et al.*, 2008).

During the course of a study on bacterial diversity in Korea, a number of novel bacterial strains were isolated from a field soil sample collected from a green house (37° 16′ 29″ N 126° 59′ 32″ E) in Suwon, Republic of Korea. The soil sample was serially diluted with 0.85% (w/v) NaCl, and suitable 10-fold dilutions were plated onto R2A agar (Difco, USA). The plates were incubated at 28°C for five days. Strain SGM1-15^T appeared on the R2A agar, distinguished by its white-colored colony, and was subjected to taxonomic investigation. Routine cultivation was conducted at 30°C with R2A agar. Maintenance in glycerol served as a medium-term preservation method. Cultures were preserved in liquid N_2 and freeze-dried. Phenotypic, chemotaxonomic and phylogenetic analyses revealed that strain SGM1-15^T was a member of the genus *Caenimonas*. On the basis of the substantial evidence obtained from polyphasic taxonomic studies, we propose SGM1-15^T as the type strain of a novel species of the genus *Caenimonas*, namely *Caenimonas terrae*.

Phenotypic characteristics, including Gram-staining, catalase activity, oxidase activity, and hydrolysis of carboxymethylcellulose (CM-cellulose), casein, chitin, hypoxanthine, tyrosine, Tween 80, starch, and xanthine, were performed using the methods of Smibert and Krieg (1994). The pH range (pH 4.0–10.0 at intervals of 1.0 pH units) for growth was determined in R2A broth that was buffered with citrate/phosphate buffer or Tris/hydrochloride buffer (Breznak and Costilow, 1994). Temperature tolerance was tested by growing cells at 5, 10, 15, 20, 25, 28, 30, 35, 37, 40, and 45°C. Salt tolerance was tested on liquid R2A medium supplemented with 0–5% NaCl (0, 0.5, 1, 1.5, 2, 3, 4, and 5%, w/v) after seven days of incubation at 30°C. Utilization of thiosulfate was tested in R2A broth supplemented with 10 mM $Na_2S_2O_3{\cdot}5H_20$ as described by Ryu *et al.* (2008). Carbon utilization tests were performed in mineral medium with microtire plates, as described by Kämpfer *et al.* (1991). Additional enzyme activities, and biochemical features were tested by using the API 20NE and API ZYM systems (bioMérieux, France) according to the manufacturer's instructions. The API ZYM test strip was read after 4 h incubation at 30°C, while the other API strips were examined after seven days at 30°C. Cell morphology was observed by transmission electron micro-

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strain SGM1-15^T is GU181268.

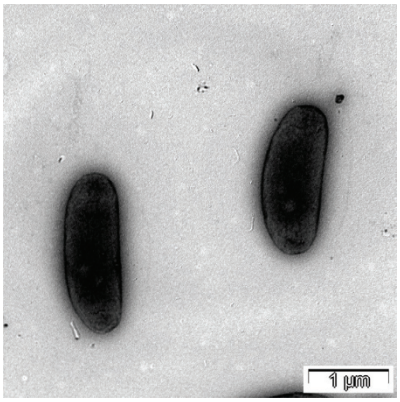

Fig. 1. Transmission electron micrograph of cells of strain SGM1-15^T after growth for three days on R2A agar. Bar, 1 µm.

scopy (912AB; LEO, Germany) and phase-contrast microscopy (AXIO; Zeiss, Germany) using cells grown on R2A agar at 30°C for three days.

The cells of strain SGM1-15^T were white, strictly aerobic, Gram-negative, and curved rod-shaped (Fig. 1). Strain SGM1-15^T grew well on nutrient agar (NA; Difco), but did not grow on Luria-Bertani (LB; Difco), trypticase soy agar (TSA; Difco), or MacConkey agar (Difco). According to the carbon utilization test in mineral medium on microtire plates for 14 days at 30°C, strain SGM1-15^T utilized some substrates. The strain did not assimilate all the substrates embedded in API 20NE and API ID32 GN test strips (up to 21 days incubation). The phenotypic characteristics of the strains are summarized and compared with those of a type strain of a closely related taxon in Table 1.

The 16S rRNA gene sequence of strain SGM1-15^T was determined as described previously (Weon *et al.*, 2006) and analyzed, using mega version 5 as well as arb (version December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 106 database (release April 2011; Pruesse *et al.*, 2007). The aligned nucleotide positions with 30 and 50% conservation filters and without filters were used for phylogenetic analysis. Phylogenetic trees were inferred using neighbor-joining with the Kimura two-parameter model, maximum parsimony and maximum likelihood algorithms in MEGA 5 software. The EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007) was used for determining the sequence similarities among strains. A neighbor-joining tree (without filters) is shown in Fig. 2.

Strain SGM1-15^T showed the highest 16S rRNA gene sequence similarity with respect to *Curvibacter delicatus* LMG 4328^T (97.6%) and *Caenimonas koreensis* EMB320^T (97.5%). In the phylogenetic tree, strain SGM1-15^T formed a cluster with 75% bootstrap support and this cluster grouped with the type strain of the genus *Caenimonas* (Fig. 1). The tree was constructed using maximum parsimony and maximum likelihood algorithms also supported the affiliation of this strain with the genus *Caenimonas*.

Cellular fatty acid methyl esters were prepared in R2A agar for five days at 30°C, and analyzed using gas chromatography according to the instructions of the Microbial Identification System (MIDI). Isoprenoid quinones were analyzed by high performance liquid chromatography (HPLC), as described by Groth *et al.* (1996). The G+C content (mol%) was determined by HPLC analysis of deoxyribonucleosides, as described by Mesbah *et al.* (1989), using a reverse-phased column (Supelcosil LC-18-S; Supelco, USA). DNA-DNA hybridization was carried out in triplicate using the filter hybridization method described by Seldin and Dubnau (1985). Probe labeling was conducted using the nonradioactive DIG-High prime system (Roche, Switzerland); hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA-DNA relatedness was quantified using a densitometer (Bio-Rad, USA). Extraction and analysis of polar lipids by two-dimensional thin layer chromatograpgy (TLC), was performed according to Minnikin *et al.* (1984). Polyamines were analyzed by HPLC, as described previously (Busse *et al.*, 1997; Busse and Auling, 1998).

The predominant fatty acids of strain SGM1-15^T were $C_{16:0}$ (39.9%), summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH, 24.3%), and $C_{17:0}$ cyclo (22.7%) (Table 2). Strain SGM1-15^T had ubiquinone 8 as the predominant isoprenoid quinone.

Table 1. Differential characteristics among strain SGM1-15^T and other *Caenimons* species

Strains: 1, SGM1-15^T; 2, *Caenimonas koreensis* KACC 13431^T. Data are from this study unless indicated. Both strains are strictly aerobic and Gram-negative. Both strains do not hydrolyse casein, starch, xanthine, hypoxanthine, CM-cellulose, chitin and Tween 80. Both strains are negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease aesculin hydrolysis and β-galactosidase, but positive for gelatin hydrolysis (API 20 NE test strips). Both strains do not assimilate rhamnose, inositol, itaconic acid, sodium malonate, lactate, potassium 5-ketogluconate, salicin, fucose, mannose, adipate and phenyl-acetate, but assimilate *N*-acetylglucosamine, saccharose, 3-hydroxybutyrate, proline and potassium gluconate. According to the API ZYM, both strains are positive activities for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase, but negative for 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 and α-fucosidase. +, positive; –, negative.

Characteristic	1	2
Catalase/oxidase	-/+	+/+
Tyrosine hydrolysis	-	+
Assimilation		
L-Arabinose	+	-
Ribose	+	-
Maltose	+	-
Mannitol	-	+
Sorbitol	+	-
Glycogen	+	-
Glucose	+	-
Melibiose	-	+
Sodium acetate	+	-
Propionic acid	-	+
Trisodium citrate	+	-
Histidine	-	+
Malate	+	-
Potassium 2-ketogluconate	+	-
Enzymatic activities (API ZYM):		
Acid phosphatase	-	+
DNA G+C content (mol%)	68.7	62.7[a]

[a] Data from Ryu *et al.* (2008).

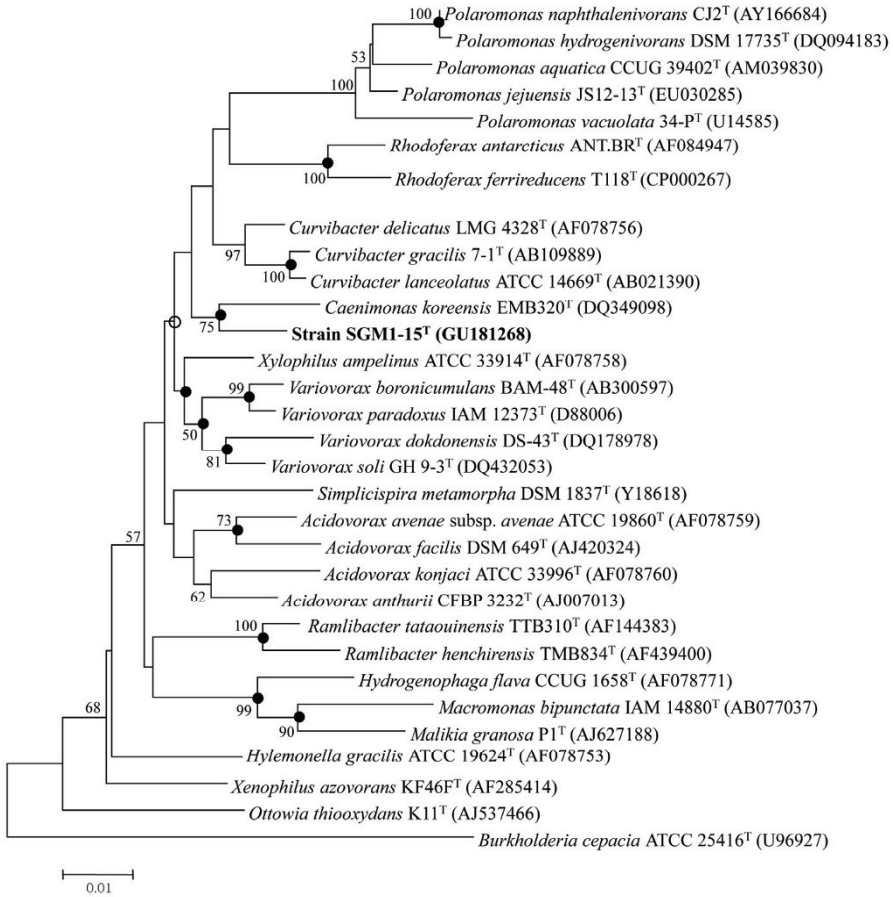

Fig. 2. Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain SGM1-15^T and closely related species. Filled circles indicate that the corresponding branches were also recovered in the maximum-likelihood and maximum-parsimony algorithms. Open circle indicates that the corresponding nodes were also recovered in trees generated with the maximum-likelihood algorithm. The bootstrap values below 50% were not indicated. Bar, 0.01 changes per nucleotide position.

The DNA G+C content was 68.7 mol%. Strain SGM1-15^T showed DNA-DNA hybridization values of 27±3, 18±3, 56±4, 26±2, and 39±3% towards *Curvibacter delicatus* LMG 4328^T, *Caenimonas koreensis* EMB320^T, *Variovorax paradoxus* IAM 12373^T, *Variovorax soli* KACC 11579^T, and *Variovorax boronicumulans* NBRC 103145^T, respectively. These values are below the threshold value (70%) suggested for species delineation (Wayne *et al.*, 1987), indicating that strain SGM1-15^T represents a novel species of the genus *Caenimonas*. This strain contained quinone with eight isoprene units (Q-8) as the predominant quinone. Strain SGM1-15^T had diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as the major polar lipids (Fig. 3). The polyamine pattern exhibited characteristics of members of

Table 2. Cellular fatty acid compositions (%) of SGM1-15^T and other *Caenimons* species

Strains: 1, SGM1-15^T; 2, *Caenimonas koreensis* KACC 13431^T.
All strains were cultivated on R2A medium at 30°C before harvesting cell mass. -, not detected or <1%.

Fatty acid	1	2
$C_{10:0}$ 3-OH	1.8	1.4
$C_{12:0}$	-	1.6
$C_{14:0}$	1.1	-
$C_{15:1}$ ω6c	-	1.5
$C_{16:0}$	39.9	34.4
$C_{17:0}$	-	1.7
$C_{17:0}$ cyclo	22.7	-
$C_{18:1}$ ω7c	4.9	3.4
$C_{18:1}$ ω9c	4.1	-
Summed feature 3[a]	24.3	51.3
Summed feature 7[b]	-	1.1

[a] Summed feature 3 comprises $C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH
[b] Summed feature 7 comprises $C_{19:1}$ ω6c, $C_{19:0}$ cyclo and/or an unknown fatty acid with an equivalent chain length of 18.846.

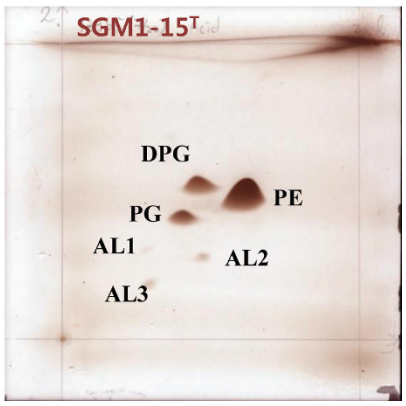

Fig. 3. Two-dimensional thin-layer chromatograph of the polar lipids of strain SGM1-15^T. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; phosphatidylethanolamine, PE; AL, unknown aminolipid.

Beta-proteobacteria (μmol/g dry weight; 2-hydroxyputrescine, 6.5; putrescine, 20.5; cadaverine, 0.9; spermidine, 7.4).

Strain SGM1-15^T can be differentiated from *Caenimonas koreensis* EMB320^T based on the absence of traits such as the production of catalase, tyrosine hydrolysis, and acid phosphatase activity. The fatty acid composition of SGM1-15^T was different from that of *Caenimonas koreensis* EMB320^T. The common major fatty acids of strain SGM1-15^T and *Caenimonas koreensis* EMB320^T were $C_{16:0}$ and summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH). However, strain SGM1-15^T had $C_{17:0}$ cyclo as another major fatty acid whereas *Caenimonas koreensis* EMB320^T did not.

Based on the data from polyphasic studies including analyses of physiological traits, fatty acid composition, 16S rRNA gene sequence and DNA-DNA hybridization, strain SGM1-15^T represents a novel species of the genus *Caenimonas*, for which the name *Caenimonas terrae* sp. nov. is proposed.

Emended description of the genus *Caenimonas* Ryu *et al.* 2008

The description of *Caenimonas* is as given by Ryu *et al.* (2008), with the following changes. This genus is catalase-positive or negative. Nitrate reduction is positive or negative. The predominant cellular fatty acids are $C_{16:0}$, summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH), $C_{18:1}$ ω7c or $C_{17:0}$ cyclo. The DNA G+C content is 62.7–68.7 mol%.

Description of *Caenimonas terrae* sp. nov.

Caenimonas terrae (ter'rae. L.gen. n. *terrae* of the soil)

Cells are strictly aerobic, Gram-negative, and curved rod-shaped (0.6–0.7 μm in width and 1.4–2.3 μm in length). Catalase is negative and oxidase is positive. Strain grows on R2A and NA, but not LB, TSA, or MacConkey. Colonies are white-coloured, butter-like in texture, and circular. Strain grows in the range of 10–40°C (optimum, 28–30°C) and pH 5–8 (optimum, pH 7). It does not tolerate concentrations of salt above 0.5%. Strain does not hydrolyze casein, starch, xanthine, hyposanthine, tyrosine, CM-cellulose, chitin, or Tween 80. Strain is negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease aesculin hydrolysis and β-galactosidase, but positive for gelatin hydrolysis. Strain is positive for thiosulfate oxidation. Strain does not assimilate rhamnose, inositol, itaconic acid, sodium malonate, lactate, potassium 5-ketogluconate, mannitol, melibiose,salicin, fucose, mannose, propionate, adipate, or phenyl-acetate, but does assimilate *N*-acetylglucosamine, ribose, maltose, saccharose, glycogen, glucose sorbitol, L-arabinose, sodium acetate, trisodium citrate, potassium 2-ketogluconate, malate, 3-hydroxybutyrate, proline and potassium gluconate. Strain is positive (by API ZYM strips) for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Major fatty acids are $C_{16:0}$ (39.9%), summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH, 24.3%) and $C_{17:0}$ cyclo (22.7%) (>10% of the total fatty acids). Ubiquinone-8 is the predominant quinone. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine. The major polyamines are 2-hydroxypurescine, purescine and spermidine. The DNA G+C content of the type strain is 68.7 mol%.

The type strain, SGM1-15^T (=KACC 13365^T =NBRC 106341^T), was isolated from a field soil sample in Suwon Republic of Korea.

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