Caenimonas koreensis gen. nov., sp. nov., isolated from activated sludge

Seung Hyun Ryu,3 Dae Sung Lee,2 Minjeong Park,1 Qian Wang,1 Ho Hee Jang,1 Woojun Park3 and Che Ok Jeon1,4

Correspondence
Che Ok Jeon
cojeon@gnu.ac.kr

1Division of Applied Life Science (BK21 Program), EB-NCRC, PMBBRC, Gyeongsang National University, Jinju 660-701, Republic of Korea
2Department of Environmental Engineering, Kyungpook National University, Daegu 702-701, Republic of Korea
3Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-701, Republic of Korea
4Department of Life Science, Chung-Ang University, Seoul 156-756, Republic of Korea

A Gram-negative, rod-shaped bacterium, designated strain EMB320T, was isolated from activated sludge performing enhanced biological phosphorus removal in a sequencing batch reactor. The isolate was strictly aerobic and non-motile. Growth was observed between 10 and 35 °C (optimum 30 °C) and between pH 6.0 and 9.0 (optimum pH 7.0–8.0). The predominant cellular fatty acids of strain EMB320T were C16:0, C18:1ω7c and summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH). The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Strain EMB320T contained ubiquinone-8 (Q-8) as the major respiratory quinone system and 2-hydroxyputrescine and putrescine as the major polyamines, which suggests that it belongs to the Betaproteobacteria. The G+C content of the genomic DNA was 62.7 mol%. Comparative 16S rRNA gene sequence analysis showed that strain EMB320T formed a phyletic lineage distinct from other genera within the family Comamonadaceae. On the basis of chemotaxonomic data and molecular properties, strain EMB320T represents a novel genus and species within the family Comamonadaceae, for which the name Caenimonas koreensis sp. nov. is proposed. The type strain of Caenimonas koreensis is EMB320T (=KCTC 12616T =DSM 17982T).

The family Comamonadaceae belonging to the Betaproteobacteria (Stackebrandt et al., 1988) was described to include the genera Comamonas, Acidovorax, Hydrogenophaga, Xylophilus and Variovorax (described to include the former Alcaligenes paradoxus), as well as phylogenetically misnamed [Aquaspirillum] and phytopathogenic [Pseudomonas] species (Willems et al., 1991). Since then, several species have been transferred to other genera or reclassified as members of novel genera by the application of molecular and improved phenotypic approaches (Willems et al., 1992; Hiraishi, 1994; Wen et al., 1999; Ding & Yokota, 2004), and new genera such as Rhodoferax, Xenophilus, Polaromonas, Alicycliphilus and Ramlibacter have been added to the family (Hiraishi et al., 1991; Irgens et al., 1996; Blümel et al., 2001; Heulin et al., 2003; Mechichi et al., 2003).

Activated sludge processes with cyclic changes of anaerobic and aerobic conditions have been used to remove phosphate from wastewater and are becoming more important for reducing eutrophication of lakes. An understanding of the microbial community responsible for phosphorus removal is a prerequisite for understanding the mechanism of enhanced biological phosphorus removal (EBPR). Therefore, efforts to isolate bacteria performing EBPR have been made in our laboratory (Lu et al., 2006) and here we describe the taxonomic characterization of a strain isolated from activated sludge that represents a novel genus belonging to the family Comamonadaceae.

Strain EMB320T was isolated from activated sludge performing EBPR in a lab-scale sequencing batch reactor (SBR). Sodium acetate was supplied as a sole carbon source.

Abbreviation: EBPR, enhanced biological phosphate removal.

*These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EMB320T is DQ349098.

A transmission electron micrograph of cells and results of TLC of polar lipids of strain EMB320T and maximum-likelihood and maximum-parsimony 16S rRNA gene sequence-based phylogenetic trees are available as supplementary material with the online version of this paper.
and the operation of the SBR has been described elsewhere (Jeon et al., 2003). A sludge sample was diluted serially with 1% (w/v) saline solution and spread on R2A agar (Difco) and incubated at 20 °C for 7 days for isolation. Subcultivation was done on R2A agar at 30 °C for 5 days. The type strains of some other related taxa, Rhodoferax ferrireducens DSM 15236^T, Curvibacter delicatus DSM 11558^T, Xylophilus amplus DSM 7250^T, Ramlhibacter tataouinensis DSM 14655^T, Variorovax paradoxus KCTC 12459^T and Polaromonas naphthalenivorans CJ2^T, were used as reference strains for biochemical tests.

Gram staining was performed using the bioMérieux Gram stain kit according to the manufacturer’s instructions. Cell morphology and motility were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JOEL) as described by Jeon et al. (2005). Physiological characteristics of strain EMB320^T were examined by growing the isolate on R2A medium at different temperatures and pH. R2A medium was prepared with different pH values as described previously (Gomori, 1955). Oxidase activity was tested by oxidation of 1% (w/v) tetramethyl-p-phenylenediamine (Merck) and catalase activity was evaluated by production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution. The hydrolysis of compounds was assessed on R2A agar after 5 days of incubation according to methods described previously (Lányi, 1987; Smibert & Krieg, 1994). Nitrate reduction was performed according to the method of Lányi (1987) and acid production from carbohydrates was tested as described by Leifson (1963). Utilization of thiosulfate was tested in R2A broth supplemented with 10 mM Na_2S_2O_3·5H_2O as described by Spring et al. (2004) and the concentration of sulfate, the end product of thiosulfate oxidation, was quantified in spent R2A medium using anion chromatography (Dionex; ICS-1000). Carbon source utilization was tested in mineral medium as described by Kämpfer et al. (1991). The utilization of D-fructose, D-glucose, glycerol, malonate, D-mannitol, maleate and D- and L-tryptophan was tested. Chemolithoautotrophic growth of strain EMB320^T with hydrogen gas was tested on medium 81 (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) agar under the conditions described by Malik & Schlegel (1981). More enzyme activities and biochemical features were determined by using API kits (API ZYM, API 20E and API 20NE) as recommended by the manufacturer (bioMérieux).

Strain EMB320^T on R2A agar formed white, glistening, translucent, slightly sticky and slightly raised circular colonies when grown at 30 °C for 5 days. Growth was observed at temperatures between 10 and 35 °C, with an optimum growth temperature of 30 °C. The strain grew at the range of pH 6.0–9.0 with optimum growth between pH 7.0 and 8.0. Cells of the isolate were non-motile rods (0.4–0.6 μm wide and 0.8–2.0 μm long) without flagella (Supplementary Fig. S1, available in IJSEM Online), Gram-negative and oxidase- and catalase-positive. The isolate did not grow on R2A agar with the removal of yeast extract and peptone under aerobic conditions, but it grew well following the addition of vitamin mixture (Wolin et al., 1963), meaning that it was possible to replace these complex nutrients (yeast extract and peptone) with vitamins. Anaerobic growth was not observed after incubation for 10 days at 30 °C on R2A agar or on R2A agar containing 10 mM nitrate.

Analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI Inc.) after cultivation for 5 days on R2A agar at 30 °C. Analyses of polar lipids and isoprenoid quinones were carried out using the methods described by Komagata & Suzuki (1987). Polyamines were analysed using an HPLC (Shimadzu LC-10A) equipped with a fluorescence detector (Shimadzu RF-10AXL) and a reversed-phase column [Akzo Nobel; Kromasil ODS (250 × 4.6 mm)] as described previously (Busse & Auling, 1988; Busse et al., 1997). The DNA G+C content of strain EMB320^T was determined using an HPLC fitted with a reversed-phase column [GROM; GROM-SIL 100 ODS-2FE (250 × 4.6 mm)] according to the method of Tamaoka & Komagata (1984). The major respiratory lipoquinone of strain EMB320^T was ubiquinone-8 (Q-8). The cellular fatty acids of the strain were summed feature 3 (C_{16:0}ω7c and/or iso C_{15:0} 2-OH; 41.8%), C_{16:0} (25.9%), C_{18:1ω7c} (15.8%), C_{18:1ω7c} 11-methyl (4.9%), C_{10:0} 3-0H (3.5%), C_{17:0} (2.0%), C_{15:0} (1.9%), C_{14:0} (1.5%), C_{12:0} (0.9%), C_{18:0} (0.9%) and summed feature 7 (C_{19:0} cyclo ω10c and/or C_{19:1}ω6c; 0.9%). The strain exhibited a polar lipid profile consisting of major lipid phosphatidylethanolamine, moderate amounts of phosphatidylglycerol and diphosphatidylglycerol and a small amount of an unknown amino group-containing lipid (Supplementary Fig. S2). The presence of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol and the lack of glycolipids were in agreement with the polar lipid characteristics reported for the related species Polaromonas naphthalenivorans, Polaromonas aquatica and Xenophilus azovorans. Strain EMB320^T was characterized by the presence of the betaproteobacteria-specific diaminie 2-hydroxyputrescine [55 μmol (g dry weight)^{-1}] and putrescine [16 μmol (g dry weight)^{-1}]. The content of 2-hydroxyputrescine was a little higher than in any other species analysed so far, such as P. naphthalenivorans and P. aquatica (Busse & Auling, 1988; Kämpfer et al., 2006). Other polyamines were only detected in trace amounts. The G+C content of the genomic DNA of strain EMB320^T was 62.7 mol%. The phenotypic characteristics of strain EMB320^T are presented in the genus and species descriptions and are compared with those of closely related taxa in Table 1.

Sequencing and assembly of the 16S rRNA gene were carried out as described previously (Lane, 1991). The resultant 16S rRNA gene sequence (1457 nucleotides) of strain EMB320^T was compared with available 16S rRNA gene sequences from GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) to determine an approximate phylogenetic affiliation and was aligned with closely related members using the CLUSTAL W software.
Table 1. Characteristics of strain EMB320\textsuperscript{T} (Caenimonas koreensis gen. nov., sp. nov.) and related members of the family Comamonadaceae


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain EMB320\textsuperscript{T}</th>
<th>Ramlibacter</th>
<th>Variovorax</th>
<th>Rhodoferax</th>
<th>Curvibacter</th>
<th>Polaromonas</th>
<th>Xylophilus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Rods</td>
<td>Rods or</td>
<td>Rods</td>
<td>Curved rods</td>
<td>Curved rods</td>
<td>Rods or</td>
<td>Rods</td>
</tr>
<tr>
<td>Flagella</td>
<td>None</td>
<td>None</td>
<td>Peritrichous</td>
<td>One polar</td>
<td>None or</td>
<td>One polar</td>
<td>One polar</td>
</tr>
<tr>
<td>Oxidase*</td>
<td>+</td>
<td>None</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Urease*</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduction*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pigments on R2A*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 4 °C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Chemolithothrophic growth with H\textsubscript{2}</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucose fermentation*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β-Galactosidase*</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hydrolysis of:*</td>
<td>Aesculin</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of:*</td>
<td>d-Glucose, l-arabinose, d-mannose, d-mannitol</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gluconate, adipic acid</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quinone(s)</td>
<td>Q-8</td>
<td>NA</td>
<td>Q-8</td>
<td>Q-8, RQ-8</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8, RQ-8</td>
</tr>
<tr>
<td>Major cellular fatty acids</td>
<td>16:0, 16:07c, iso-15:0 2-OH, 18:1</td>
<td>16:0, 16:1, 16:0, 16:1, 16:0, 16:1, 16:1</td>
<td>16:0, 16:10c, 18:0, 17:0 7c</td>
<td>16:0, 16:10c, 18:0, 17:0 7c</td>
<td>16:0, 16:10c, 18:0, 17:0 7c</td>
<td>16:0, 16:10c, 18:0, 17:0 7c</td>
<td>16:0, 16:10c, 18:0, 17:0 7c</td>
</tr>
<tr>
<td>Major 3-OH acid</td>
<td>10:0</td>
<td>10:0</td>
<td>10:0</td>
<td>8:0</td>
<td>8:0</td>
<td>10:0</td>
<td>10:0</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>62.7</td>
<td>66-70</td>
<td>66-69</td>
<td>59-62</td>
<td>62-66</td>
<td>52-63</td>
<td>68-69</td>
</tr>
</tbody>
</table>

*Data obtained in this study for Ramlibacter tataouinensis TTB310\textsuperscript{T}, Variovorax paradoxus IAM 12373\textsuperscript{T}, Rhodoferax ferrireducens T118\textsuperscript{T}, Curvibacter delicatus LMG 4328\textsuperscript{T}, Polaromonas naphthalenivorans CJ2\textsuperscript{T} and Xylophilus amplus DSM 7250\textsuperscript{T}. All of these strains and strain EMB320\textsuperscript{T} were negative for indole and arginine dihydrolase production and assimilation of N-acetylglucosamine, maltose, capric acid, malic acid, trisodium citrate and phenylacetic acid.

†C_{17:0} cyclo is present only in P. aquatica CCG 39402\textsuperscript{T} (37.3 %) and P. naphthalenivorans CJ2\textsuperscript{T} (7.2 %) (Jeon et al., 2004; Sizova & Panikov, 2007).

‡Only P. hydrogenivorans DSM 17735\textsuperscript{T} and P. naphthalenivorans CJ2\textsuperscript{T} contain C_{10:0} 3-OH as the major hydroxylated fatty acid (Jeon et al., 2004; Sizova & Panikov, 2007).

(Thompson et al., 1994). Phylogenetic trees were constructed using three different methods, the neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms, which are available in the PHYLIP software, version 3.6 (Felsenstein, 2002). Sequence similarity values were computed using Similarity Matrix version 1.1 (Ribosomal Database Project II; http://35.8.164.52/html/) (Cole et al., 2003). A bootstrap analysis was performed according to the algorithm of Kimura’s two-parameter model (Kimura, 1980) of the NJ method in the PHYLIP package. Comparative analysis of 16S rRNA gene sequences showed that the isolate was closely related to Variovorax paradoxus IAM 12373\textsuperscript{T}, Curvibacter delicatus LMG 4328\textsuperscript{T}, Curvibacter gracilis 7-1\textsuperscript{T}, Xylophilus amplus DSM 7250\textsuperscript{T} and Ramlibacter tataouinensis TTB310\textsuperscript{T}, with similarities of 96.8, 96.8, 96.8, 96.7 and 96.0 %, respectively. However, the phylogenetic analysis indicated that strain EMB320\textsuperscript{T} formed a phyletic lineage with the genera Curvibacter, Rhodoferax and Polaromonas within the family Comamonadaceae with a low bootstrap value (44 %), meaning that the phylogenetic topology is not stable (Fig. 1). Additional phylogenetic analyses also showed that the phylogenetic topologies varied remarkably by the addition or removal of just one species. The topologies of phylogenetic trees built using the ML and MP algorithms also supported the notion that there is no genus group that shows a clear phylogenetic relationship with strain EMB320\textsuperscript{T} in the family Comamonadaceae (Supplementary Fig. S3), which was confirmed using the Ribosomal Database Project Classifier program (Wang et al., 2007).
Fig. 1. Neighbour-joining tree showing phylogenetic relationships based on 16S rRNA gene sequences of strain EMB320T and related taxa. Bootstrap values are shown as percentages of 1000 replicates when greater than 50%.

Description of Caenimonas gen. nov.

Caenimonas (Cae’ni.mo’nas. L. n. caenum mud, sludge; N.L. fem. n. from Gr. fem. n. monas a unit, monad; N.L. fem. n. Caenimonas monad isolated from sludge).

Cells are Gram-negative, strictly aerobic, non-motile rods, 0.4–0.6 µm wide and 0.8–2.0 µm long, at 30 °C on R2A agar. Catalase- and oxidase-positive. Nitrate is reduced to nitrite and thiosulfate is oxidized to sulfate. No aerobic chemoautotrophic growth with hydrogen as substrate.

The DNA G+C content of the type strain of the type species is 62.7 mol% (HPLC). Phylogenetically, the genus belongs to the family Comamonadaceae. The type species is Caenimonas koreensis.

Description of Caenimonas koreensis sp. nov.

Caenimonas koreensis (ko.re.en’sis. N.L. fem. adj. koreensis pertaining to Korea, where the type strain was isolated).

Displays the following properties in addition to those given in the genus description. Colonies are white, glistening, translucent, slightly sticky, raised and circular on R2A agar. Growth occurs optimally at 30 °C and pH 7.0–8.0. Negative for the production of indole, H₂S and acetoin and negative for citrate utilization (API 20E). Hydrolyses urea, gelatin and Tween 20 but does not hydrolyse casein, Tween 80, tyrosine, ascesulin or starch. Acid is produced from raffinose, myo-inositol, D-lactose, L-arabinobiose and D-fructose, but not from D-glucose, sorbitol, sucrose, rhamnose, mygalamin, melibiose, D-galactose, D-mannose, D-mannitol, arbutin or salicin. Negative for assimilation of D-glucose, D-mannose, D-mannitol, N-acetylgalactosamine, maltose, L-arabinose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phylolylactic acid (API 20NE). Utilizes D-fructose, D-glucose, malonate and maleate as sole carbon sources, but does not utilize glycerol, D-mannitol or D- or L-tryptophan. Produces alkaline phosphatase, leucine arylamidase and urease, but not lipase (C14), cystine arylamidase, trypsin, x-chymotrypsin, x-galactosidase, β-galactosidase, β-glucuronidase, z-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, z-mannosidase, β-fucosidase, arginine dihydrolase, lysine decarboxylase or ornithine decarboxylase. Weak activity is observed for esterase (C4), esterase lipase (C8), valine arylamidase, acid phosphatase, naphthol-AS-Bl-phosphohydrolase and triptophan deaminase.

The type strain is EMB320T (=KCTC 12616T =DSM 17982T), isolated from activated sludge that performed EBPR.

Acknowledgements

These efforts were supported by grants from the MOST/KOSEF to the Environmental Biotechnology National Core Research Center (grant R15-2003-012-02002-0) and to the 21C Frontier Microbial Genomics and Application Center Program (grant MG05-0104-4-0), Ministry of Science & Technology, Korea. S.H.R., M.P. and Q.W. were supported by scholarships from the BK21 program, the Ministry of Education and Human Resources Development in Korea.

References


automated 16S rDNA sequencing.


