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

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The family Comamonadaceae belonging to the Beta-proteobacteria (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, Alicyclobifilus 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.
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, *Rhodolobacter ferrireducens* DSM 15236^T^, *Curvibacter delicatus* DSM 11558^T^, *Xylophilus amelinus* DSM 7250^T^, *Ramlibacter tataouinensis* DSM 14655^T^, *Variorovax paradoxus* KCTC 12459^T^ and *Polaromonas napthalenivorans* 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; JEOL) 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) tetramethylphenylenediamine (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 (Lánya, 1987; Smibert & Krieg, 1994). Nitrate reduction was performed according to the method of Lánya (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$_2$S$_2$O$_3$.5H$_2$O 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 C$_{15:0}$ 2-OH; 41.8\%), C$_{16:0}$ (25.9\%), C$_{18:1}$ω9cω7c (15.8\%), C$_{18:1}$ω7cC11-methyl (4.9\%), C$_{10:0}$ω3-0H (3.5\%), C$_{17:0}$ (2.0\%), C$_{15:0}$ (1.9\%), C$_{14:0}$ (1.5\%), C$_{12:0}$ (0.9\%), C$_{18:1}$ω9c (0.9\%) and summed feature 7 (C$_{19:0}$ cyclo ω10c and/or C$_{19:1}$ω6cω9c 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 napthalenivorans*, *Polaromonas aquatica* and *Xenophilus azovorans*. Strain EMB320^T^ was characterized by the presence of the betaproteobacteria-specific diamin 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. napthalenivorans* 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 EMB320T (Caenimonas koreensis gen. nov., sp. nov.) and related members of the family Comamonadaceae


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain EMB320T</th>
<th>Ramlbacter</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 cysts</td>
<td>Rods</td>
<td>Curved rods</td>
<td>Curved rods</td>
<td>Rods or cocci</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 polar</td>
<td>None or polar</td>
<td>One polar</td>
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<tr>
<td>Oxidase*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>-</td>
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<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>
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<tr>
<td>Pigments on R2A*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Growth at 4°C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Chemolithotrophic growth with H2*</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Glucose fermentation*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>β-Galactosidase*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>Hydrolysis of:*</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Quinone(s)</td>
<td>Q-8</td>
<td>NA</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8, RQ-8</td>
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<tr>
<td>Major cellular fatty acids</td>
<td>16:0, 16:0Δ7c/iso-15:0 2-OH, 18:1</td>
<td>16:0, 16:1</td>
<td>16:0, 16:1</td>
<td>16:0, 16:1</td>
<td>16:0, 16:1</td>
<td>16:0, 16:1</td>
<td>16:1:06c, 18:1:07c, 17:0 cyclo†</td>
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<td>Major 3-OH acid</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>
<td>NA</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>62.7</td>
<td>67–70</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 Ramlbacter tataouinensis TTB310T, Variovorax paradoxus IAM 12373T, Rhodoferax ferrireducentis T118T, Curvibacter delicatus LMG 4328T, Polaromonas naphthalenivorans CJ2T and Xylophilus amphilinus DSM 7250T. All of these strains and strain EMB320T were negative for indole and arginine dihydrolase production and assimilation of N-acetylglucosamine, maltose, capric acid, malic acid, trisodium citrate and phenylacetic acid.

†C17:0 cyclo is present only in P. aquatica CCUG 39402T (37.3 %) and P. naphthalenivorans CJ2T (7.2 %) (Jeon et al., 2004; Sizova & Panikov, 2007).

‡Only P. hydrogenivorans DSM 17735T and P. naphthalenivorans CJ2T contain C10:0 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://ribosomal-database-project-II.github.io/ribosomal-database-project-II.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 12373T, Curvibacter delicatus LMG 4328T, Curvibacter gracilis 7-1T, Xylophilus amphilinus DSM 7250T and Ramlbacter tataouinensis TTB310T, with similarities of 96.8, 96.8, 96.8, 96.7 and 96.0 %, respectively. However, the phylogenetic analysis indicated that strain EMB320T 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 EMB320T in the family Comamonadaceae (Supplementary Fig. S3), which was confirmed using the Ribosomal Database Project Classifier program (Wang et al., 2007).
Neighbour-joining tree showing phylogenetic relationships

Fig. 1.

Description of Caenimonas gen. nov.

Caenimonas (Caenimonas n. g.; Caenimonas n. sp. nov.)

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.

References


Acknowledgements

These efforts were supported by grants from the MOST/ KOSEF to the Environmental Biotechnology National Core Research Center (grant R18-2003-012-02002-0) 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.

Description of Caenimonas koreensis sp. nov.

Caenimonas koreensis (KO2216(R2A)T = DSM 17892T), isolated from activated sludge that performed EBPR.

The type strain is EMB320T (KCTC 12616T = DSM 17892T), isolated from activated sludge that performed EBPR.


