Reinekea marinisedimentorum gen. nov., sp. nov., a novel gammaproteobacterium from marine coastal sediments

Lyudmila A. Romanenko,1 Peter Schumann,2 Manfred Rohde,3 Valery V. Mikhailov1 and Erko Stackebrandt2

1Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch, Russian Academy of Sciences, 690022 Vladivostok, Prospekt 100 Let Vladivostoku, 159, Russia
2DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany
3GBF – Gesellschaft für Biotechnologische Forschung GmbH, D-38124 Braunschweig, Germany

A Gram-negative, oxidase- and catalase-positive, rod-shaped bacterium, designated strain KMM 3655T, was isolated from a coastal marine sediment sample. The novel bacterium required sodium ions for growth and grew between 0–5 and 5 % NaCl and at 4–37 °C, but not at 40 °C. It reduced nitrate, formed acids from glucose under aerobic and anaerobic conditions, utilized a limited spectrum of organic substrates and did not produce gelatinase, caseinase, amylase or chitinase. The major isoprenoid quinone was Q8. Polar lipids consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and an unknown phospholipid. Fatty acid analysis of strain KMM 3655T revealed C16 : 0,C16 : 1v7c and C18 : 1v7c as predominant components. The G+C content of the DNA was 51.1 mol%. Phylogenetic analysis of the 16S rDNA sequence placed the new isolate within the c-Proteobacteria as a separate deep branch, with about 90 % sequence similarity to representatives of the genus Oceanospirillum and other remotely related genera. Combined phylogenetic and physiological data show that the new marine sediment isolate, KMM 3655T, represents a novel genus and species, for which the name Reinekea marinisedimentorum gen. nov., sp. nov. is proposed. The type strain is KMM 3655T (= DSM 15388T).

During a survey of the biodiversity of micro-organisms that are associated with marine sediments, the Gram-negative, rod-shaped, non-pigmented bacterium KMM 3655T was isolated from a marine coastal sediment sample and characterized by using phenotypic and phylogenetic analyses.

Sediment samples were collected from the coastal sea water area of Reineke Island, Peter the Great Bay, Sea of Japan, Russia, in June 2002. A sediment sample was obtained at a depth of 0–2 m by using a sterile tube and was serially diluted with sterile sea water. An aliquot of each dilution was spread on marine agar 2216 (MA; Difco) and incubated at 28 °C for 7 days. The bacterium was cultivated aerobically in marine broth (MB; Difco) and on MA or half-strength MA supplemented with yeast extract (0.5 %, w/v) and NaCl (1.5 %, w/v) at 35–37 °C and stored at −80 °C in liquid medium supplemented with 30 % (v/v) glycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain KMM 3655T is AJ561121.
Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of PCR products were carried out as described by Rainey et al. (1996). Purified PCR products were sequenced directly by using a Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems), according to the manufacturer’s instructions. An Applied Biosystems 310 DNA genetic analyser was used for electrophoresis of sequence reaction products. The 16S rDNA sequence of strain KMM 3655T was aligned manually with nucleotide sequences that were obtained from GenBank and EMBL. The method of Jukes & Cantor (1969) was used to calculate evolutionary distances. Various algorithms, provided by Felsenstein (1993), were used to locate the phylogenetic position of the sequence. GenBank accession numbers of the reference strains used in phylogenetic analysis are shown in Fig. 2.

Cultural and metabolic properties are indicated in Table 1 and in the taxa descriptions. Analysis of quinone compounds revealed Q8 to be the major isoprenoid quinone. Polar lipids included phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, and an unknown phospholipid. Predominant cellular fatty acids of strain KMM 3655T were C16:0, C16:1ω7c and C18:1ω7c. A more detailed fatty acid composition is given in the genus description. The DNA G+C content of strain KMM 3655T was 51.1 mol%.

The chemotaxonomic traits of strain KMM 3655T (ubiquinone Q8 and high amounts of the fatty acids C16:0, C16:1ω7c and C18:1ω7c) are similar to those found for other marine γ-Proteobacteria: Alteromonas (Baumann et al., 1984b), Glaciecola (Bowman et al., 1998), Marinobacter (Nguyen et al., 1999), Alcanivorax borkumensis SK27 (Yakimov et al., 1998), Oleiphilus messсинensis ME102 (Golyshin et al., 2002) and Oceanobacter (Satomi et al., 2002). The DNA G+C content of 51.1 mol% fell into the range of those of members of the genus Shewanella (39–55 mol%), A. borkumensis SK27 (53.4 mol%), Oleiphilus messсинensis ME102 (49.0 mol%) and the genus Oceanospirillum (45–50 mol%) (Satomi et al., 2002) (Table 1).

Comparative 16S rDNA gene sequence analysis data confirmed the affiliation of the new isolate to the γ-Proteobacteria (Fig. 2). Strain KMM 3655T stands phylogenetically isolated, but is related remotely to members of a broad range of taxa (Oceanospirillum, Oleiphilus, Marinobacter, Alcanivorax and Vibrionaceae) (<90% sequence similarity). Slightly different phylogenetic trees were obtained by applying different algorithms and outgroup reference organisms (not shown), but in no case was the branching pattern supported by high bootstrap values. Highest sequence similarity of strain KMM 3655T was obtained to a partial sequence (1000 bp, GenBank no. AY172302) of an as yet uncultured organism, clone 18, which was recovered from DNA from a standard Petri dish (S. Epstein, K. Lewis and T. Kaeberlein, unpublished data) (Fig. 2).
The distinct phylogenetic position of strain KMM 3655\textsuperscript{T} is supported by its physiological and biochemical properties. The new isolate differed from marine, Gram-negative, strictly aerobic, non-fermentative, heterotrophic and haloophilic γ-Proteobacteria that belong to the genera *Alteromonas*, *Pseudoalteromonas* (Baumann et al., 1972, 1984b; Gauthier et al., 1995), *Marinomonas* (Van Landschoot & De Ley, 1983), *Glaciecola* (Bowman et al., 1998), *Idiomarina* (Ivanova et al., 2000) and *Thalassomonas* (Macián et al., 2001) in the ability to ferment glucose anaerobically, maximum and minimum growth temperatures, narrow salinity range for growth and a limited spectrum of compounds utilized. In addition, members of the genus *Idiomarina* are characterized by iso-C\textsubscript{15}:0 and iso-C\textsubscript{17}:0 as major fatty acids and members of the genus *Thalassomonas* differ by abundance of the fatty acids C\textsubscript{15}:0, C\textsubscript{16}:0 and C\textsubscript{17}:1\textsubscript{9c}. The major phenotypic differences between strain KMM 3655\textsuperscript{T} and facultative anaerobes of the genus *Shewanella* [*Shewanella algae* and *Shewanella amazonensis* (Venkateswaran et al., 1998, 1999)], were as follows: sodium

Table 1. Main phenotypic characteristics that differentiate strain KMM 3655\textsuperscript{T} from some marine members of the γ-Proteobacteria

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Psychrophilic growth</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth at/in: 40 °C</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>10% NaCl</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Facultatively anaerobic</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin hydrolysis</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>D-Glucose utilization</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Na\textsuperscript{+} required for growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Major fatty acids</td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textsubscript{7c}}</td>
<td>C\textsubscript{18:1\textsubscript{7c}}</td>
<td>ND</td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textsubscript{7c}}</td>
<td>iso-C\textsubscript{15:0}, iso-C\textsubscript{15:0}</td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textsubscript{7c}}</td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textsubscript{7c}}</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>51-1</td>
<td>45-50</td>
<td>49-0</td>
<td>54</td>
<td>52</td>
<td>52</td>
<td>44-47</td>
</tr>
</tbody>
</table>
chloride concentration required for growth, minimal and maximal growth temperatures, inability to hydrolyse many compounds, assimilation pattern and fatty acid composition (Table 1). Strain KMM 3655T is phylogenetically distant from members of the genera Vibrio (Baumann et al., 1984a), Photobacterium (Baumann & Baumann, 1984) and Aeromonas (Popoff, 1984). Representatives of these genera are able to ferment glucose, reduce nitrate and produce acid from carbohydrates, but they can be differentiated from strain KMM 3655T by arginine dihydrolase production, broad spectrum of assimilation compounds, fatty acid composition and low DNA G+C contents for Vibrio and Photobacterium (38–51 mol% and 40–44 mol%, respectively) or high DNA G+C contents (57–63 mol%) for Aeromonas. Characteristics that differentiate strain KMM 3655T from other marine gammaproteobacteria are listed in Table 1. Based on phenotypic, morphological and physiological dissimilarities and significant distance in 16S rRNA gene sequence, strain KMM 3655T could not be assigned to any known species or genus within the γ-Proteobacteria; it is therefore proposed to classify strain KMM 3655T as the type strain of the type species of a novel genus, Reinekea gen. nov., as Reinekea marinisedimentorum sp. nov.

Description of Reinekea gen. nov.

Reinekea (Rei.ne.kea’). N.L. fem. n. Reinekea derived from Reineke, geographical name of Reineke Island, Peter the Great Bay, Sea of Japan, Russia, the place where the bacterium was first isolated).

Gram-negative, heterotrophic, oxidase- and catalase-positive, rod-shaped and motile. Sodium ions are essential for growth. Growth occurs in 0·5–5 % NaCl and between 4 and 37 °C. No growth is observed in >5 % NaCl or at 40 °C. Facultatively anaerobic; acid is produced from some carbohydrates under anaerobic and aerobic conditions. Predominant isoprenoid quinone is Q8. Polar lipids include phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine and an unknown phospholipid. Major fatty acids are C16 : 0, C16 : 1ω7c and C18 : 1ω7c. Represents a main phylogenetic sublineage within the γ-Proteobacteria, showing remote relatedness to other marine and non-marine members of this class. The type species is Reinekea marinisedimentorum.

Description of Reinekea marinisedimentorum sp. nov.

Reinekea marinisedimentorum (ma.ri.ni.se.di.me.to’rum. L. adj. marinus of the sea; L. n. sedimentum settings, sediment; N.L. gen. pl. n. marinisedimentorum from marine sediments).

In addition to properties given in the genus description, the species is characterized as follows. Cells are 0·4–0·5 μm in diameter and 1·5–1·7 μm in length. Motile by single polar flagella. Cells grown on agar slants occur singly or as cell aggregates without flagella. Gelatinase, caseinase, amylase and chitinase are not produced. Aerobic formation of acid occurs from glucose, sucrose, lactose, maltose, galactose, glycerol and mannitol; no acid is formed aerobically from arabinose, rhamnose or N-acetylglucosamine. Under anaerobic conditions, acid is produced from glucose and maltose, but not from acetate, citrate, peptone, ethanol or glycerol. Negative for arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase activities. Positive for nitrate reduction, PNPG (β-nitrophenyl β-D-glucopyranoside) test and utilization of D-glucose, D-mannitol and maltose, according to the API 20NE system (bioMérieux); negative for indole production, arginine dihydrolase, urease production and utilization of ascinul, gelatin, L-arabinose, D-mannose, N-acetylglucosamine, D-gluconate, caprate, adipate, L-malate, citrate and phenylacetic. Positive for D-mannitol utilization in Biolog GN tests; weakly positive for utilization of Tween 40 and 80, L-arabinose, psicose, D-sorbitol, sucrose, α-ketobutyric acid and L-alanomycolic acids; other organic substrates included in the Biolog GN substrate panel are not utilized. Positive for alkaline phosphatase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase, according to API ZYM tests; negative for esterase C4, esterase lipase C8, lipase C14, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, z-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, z-mannosidase and x-fucosidase. Major fatty acid methyl esters are C16 : 0 (31·61 %), C16 : 1ω7c (26·72 %) and C18 : 1ω7c (19·04 %); C15 : 0 (4·11 %), C17 : 0 (5·94 %), C14 : 0 (1·99 %), C14 : 0 3-OH (2·35 %), C17 : 1ω9c (1·29 %), C17 : 1ω6c (1·89 %), C16 : 0 N alcohol (1·21 %) and C16 : 1ω7c alcohol (1·09 %) are present as minor acids; C17 : 0 10-methyl, C18 : 0 and C12 : 0 ALDE are detected at a level of <1 %. The DNA G+C content of the type strain is 51·1 mol%.

The type strain is KMM 3655T (=DSM 15388T). Isolated from marine coastal sediments offshore from Reineke Island, Peter the Great Bay, Sea of Japan, Russia.

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References


