Rubricoccus marinus gen. nov., sp. nov., of the family 'Rhodothermaceae', isolated from seawater

Sanghwa Park,¹ Susumu Yoshizawa,² Kazuhiro Kogure² and Akira Yokota¹

Correspondence

Sanghwa Park
psh214@hotmail.com

¹Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-Ku, Tokyo 113-0032, Japan

²Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8564, Japan

A coccoid- and amorphous-shaped, non-gliding, reddish bacterium, designated SG-29^T, was isolated from seawater in the western North Pacific Ocean near Japan. The strain was Gram-stain-negative, obligately aerobic, heterotrophic and catalase-positive. Nitrate was reduced to nitrogen and acid was produced from aesculin, turanose, 2-keto-gluconate and arabinose. Growth occurred with 1–5 % NaCl (optimum 2 % NaCl) and at 5–37 °C (optimum 20–30 °C). The G+C content of genomic DNA was 68.9 mol% (HPLC). The predominant isoprenoid quinone was MK-7 and the major cellular fatty acids (>10 %) were iso-C_{17:1}ω9c, C_{17:1}ω8c and iso-C_{17:0}. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain SG-29^T belonged to the phylum *Bacteroidetes*. The most closely related strain was *Rhodothermus obamensis* JCM 9785^T, with which the isolate exhibited 86.1 % 16S rRNA gene sequence similarity. On the basis of phenotypic and phylogenetic features, strain SG-29^T is classified in a novel genus and species within the family 'Rhodothermaceae', for which the name *Rubricoccus marinus* gen. nov., sp. nov. (=NBRC 107124^T =KCTC 23197^T) is proposed.

The family 'Rhodothermaceae' of the class 'Cytophagia' in phylum *Bacteroidetes* (Ludwig *et al.*, 2008) includes three genera at the time of writing: *Rhodothermus* (Alfredsson *et al.*, 1988), *Salinibacter* (Antón *et al.*, 2002) and the recently proposed genus *Salisaeta* (Vaisman & Oren, 2009). Members of these genera have been isolated from extreme environments and exhibit extreme thermophilic or halophilic characteristics: *Rhodothermus marinus* was isolated from submarine hot springs in Iceland, *Salinibacter ruber* was isolated from hypersaline crystallizer ponds in Spain and *Salisaeta longa* was isolated from the Dead Sea (Israel/Jordan). At present, the name 'Rhodothermaceae' has not been validly described.

An aerobic, red-pigmented bacterium was isolated from seawater samples obtained from the western North Pacific Ocean (30° 40' N 138° 00' E; depth 50 m) during voyage KT-09-11 of RV *Tansei Maru* on 2–6 July 2009 (Ocean Research Institute, The University of Tokyo and Japan Agency for Marine-Earth Science and Technology). Samples (200 µl) were inoculated onto medium G (containing l⁻¹ 80 % aged seawater: 0.5 g peptone, 0.1 g yeast extract, 5 g Gelrite) and incubated at 15 °C for 30 days. The isolated strain was routinely cultivated and maintained on half-strength marine agar 2216 (1/2 MA; containing l⁻¹: 4.4 g MgCl₂, 2.5 g peptone, 1.62 g Na₂SO₃, 0.9 g CaCl₂,

0.5 g yeast extract, 0.27 g KCl, 0.8 g NaHCO₃, 0.5 g ferric citrate, 0.04 g KBr, 0.015 g SrCl₂, 0.01 g H₃BO₃, 4 mg Na₂HPO₄, 2 mg Na₂SiO₃, 1.2 mg NaF, 0.8 mg NH₄NO₃, 15 g agar; Difco) with 2 % NaCl at 20 °C.

Growth at 5, 10, 15, 20, 25, 30, 37 and 45 °C, at pH 5–10 and with 0–15 % NaCl was determined on 1/2 MA containing 2 % NaCl unless otherwise specified. Gram-staining was performed as described by Murray *et al.* (1994). Cell morphology and motility were observed using light microscopy (BX60; Olympus) and transmission electron microscopy (JEOL 1011 TEM). Growth under anaerobic conditions was determined after incubation for 4 weeks in an AnaeroPack (Mitsubishi Gas Chemical Co). Catalase activity was determined by bubble formation in a 3 % H₂O₂ solution. Oxidase activity was determined using cytochrome oxidase test paper (Nissui Pharmaceutical). API 20 E, API 20 NE, API 50 CH and API ZYM strips (bioMérieux) were used to determine physiological and biochemical characteristics. The suspension medium for the API tests contained 2 % (w/v) NaCl. Results of API 20 E, API 20 NE and API 50 CH were recorded after 5 days and those of API ZYM were recorded after 2 days at 20 °C. Determination of the respiratory quinone was carried out as described by Xie & Yokota (2003). For analysis of fatty acid methyl esters, cells were prepared for 3 weeks at 20 °C and fatty acids were extracted and prepared according to the standard protocols provided by

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the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990).

Genomic DNA was prepared according to the method of Marmur (1961) and the DNA base composition was determined using the HPLC method of Mesbah *et al.* (1989). A fragment of approximately 1450 bp of the 16S rRNA gene was amplified from extracted DNA using universal bacterial primers (27F and 1492R; Lane, 1991; Weisburg *et al.*, 1991). To ascertain the phylogenetic position of strain SG-29^T, the 16S rRNA gene sequence was compared with sequences obtained from GenBank (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov>) using FASTA. Multiple alignments of sequences were performed using CLUSTAL X version 1.83 (Thompson *et al.*, 1997) and gaps and ambiguous bases were omitted for sequence comparison. Aligned sequences were analysed using MEGA version 4 (Tamura *et al.*, 2007). Evolutionary distances were calculated using distance options according to the Kimura two-parameter model (Kimura, 1983). Clustering with the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods was performed using PAUP version 4 (Swofford, 2002). Bootstrap values (Felsenstein, 1985) were calculated from 1000 replications. Similarities were calculated using the same software values.

Cells of strain SG-29^T after 3 weeks at 20 °C were coccoid and amorphous, reddish pigmented and approximately 0.3–0.5 µm in diameter. Spores were not observed (Fig. 1). A reddish pigment was extracted from cells by using acetone after freeze drying and was analysed by spectrophotometry (Du-530; Beckman). The pigment showed a UV-visible spectrum with absorption maxima at 474 nm and 446 nm. No growth was observed under anaerobic conditions; growth occurred only under aerobic conditions. The temperature range for growth was 5–37 °C. Optimum conditions for growth were 20–30 °C and pH 5–9. The isolate required sodium ions for growth and grew with 1–5 % NaCl. The cultural, physiological and biochemical characteristics of strain SG-29^T were compared with *Salisaeta longa* S4-4^T, *Salinibacter ruber* M31^T and *Rhodothermus marinus* DSM 4252^T (Table 1). The major cellular fatty acids of strain

Fig. 1. Transmission electron micrograph of cells of strain SA-29^T, showing coccoid and amorphous morphology. Bar, 500 nm.

Table 1. Differential characteristics of strain SG-29^T and related members of the phylum *Bacteroidetes*

Strains: 1, *Rubricoccus marinus* sp. nov. SG-29^T; 2, *Salisaeta longa* S4-4^T (data from Vaisman & Oren, 2009); 3, *Salinibacter ruber* M31^T (Antón *et al.*, 2002); 4, *Rhodothermus marinus* DSM 4252^T (Sako *et al.*, 1996). All strains are catalase-positive.

Characteristic	1	2	3	4
Pigment	Reddish	Red	Red	Reddish
Cell dimensions (µm)	0.3–0.5	15–30	0.4–2.6	2–2.5
Temperature for growth (°C)				
Range	5–37	37–46	32–47	54–77
Optimum	20–30	37–46	37–47	65
NaCl for growth (% w/v)	1–5	5–20	15–30	2–6
DNA G + C content (mol%)	68.85	62.9	66.5	64.4
Oxidase	–	+	+	+

SG-29^T were iso-C_{17:1}ω9c (21.9 %), C_{17:1}ω8c (16.9 %), iso-C_{17:0} (14.5 %) and C_{16:0} (7.6 %), which distinguished the isolate from the three genera of the phylum *Bacteroidetes* (Table 2).

An almost-complete 16S rRNA gene sequence for strain SG-29^T was determined and showed that the isolate belonged to the phylum *Bacteroidetes*. Analysis using 1278 bases showed that all of the type strains of species with validly described names exhibited <87 % 16S rRNA gene sequence similarity with strain SG-29^T: 86.1 % with *Rhodothermus obamensis* JCM 9785^T, 85.9 % with *Rhodothermus marinus* DSM 4252^T (the type strain of the type species of genus *Rhodothermus*), 85.4 % with *Salisaeta longa* S4-4^T, 85.3 % with *Rhodothermus profundus* PRI 2902^T and 84.8 % with *Salinibacter ruber* M31^T. Phylogenetic trees obtained using the neighbour-joining method (Fig. 2) and maximum-likelihood method (data not shown) revealed clear affiliations between strain SG-29^T and the cluster

Table 2. Major cellular fatty acid composition of strain SG-29^T and related members of the phylum *Bacteroidetes*

Strains: 1, *Rubricoccus marinus* sp. nov. SG-29^T; 2, *Salisaeta longa* S4-4^T (data from Vaisman & Oren, 2009); 3, *Salinibacter ruber* M31^T (Bardavid *et al.*, 2007); 4, *Rhodothermus marinus* DSM 4252^T (Marteinsson *et al.*, 2010). –, Not detected; ND, no data available.

Fatty acid (%)	1	2	3	4
iso-C _{15:0}	3.9	8.5	25	6
anteiso-C _{15:0}	0.2	3.8	3.9	12.3
C _{16:0}	7.6	ND	9.8	4.1
iso-C _{16:0}	2.2	26–30	–	27.8
C _{16:1} ω9c	0.5	35–38	29	ND
iso-C _{17:0}	14.5	ND	0.9	20.7
anteiso-C _{17:0}	0.4	ND	1.1	13.9
C _{17:1} ω8c	16.9	ND	ND	ND
iso-C _{17:1} ω9c	21.9	ND	ND	ND
C _{18:1} ω11c	–	ND	24.4	ND

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain SG-29^T among related members of the phylum Bacteroidetes. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes (neighbour-joining/maximum-likelihood); –, <50%. Filled circles indicate nodes recovered with >50% bootstrap support in the maximum-likelihood tree. *Chlorobium limicola* 6330^T and *Verrucomicrobium spinosum* DSM 4136^T were used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

containing *Salisaeta longa* S4-4^T, *Salinibacter ruber* M31^T and *Rhodothermus marinus* DSM 4252^T, and strain SG-29^T was placed on a separate branch within this cluster.

Phenotypic, chemotaxonomic and genotypic differences between strain SG-29^T and members of related genera showed that strain SG-29^T is sufficiently different to be assigned to a novel genus in the family 'Rhodothermaceae'. The name *Rubricoccus marinus* gen. nov., sp. nov. is proposed.

Description of *Rubricoccus* gen. nov.

Rubricoccus (Ru.bri.coc'cus. L. adj. *ruber* reddish; N.L. n. *coccus* from Gr. masc. n. *kokkos* a berry, unit; N.L. masc. n. *Rubricoccus* reddish coccus).

Cells are reddish, coccoid and amorphous, non-gliding, Gram-stain-negative and obligately aerobic. The major respiratory quinone is MK-7. The G+C content of the genomic DNA is 68.9 mol%. The predominant cellular fatty acids (>10%) are iso-C_{17:1}ω9c, C_{17:1}ω8c and iso-C_{17:0}. The type species is *Rubricoccus marinus*.

Description of *Rubricoccus marinus* sp. nov.

Rubricoccus marinus (ma.ri'nus. L. masc. adj. *marinus* of the sea, marine).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.3–0.5 μm in diameter. Grows at 5–37 °C (optimum 20–30 °C), but not at 45 °C, at pH 5–9 and with 1–5% (w/v) NaCl. Catalase-positive and oxidase-negative. Positive for esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin,

acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for alkaline phosphatase, *N*-acetyl-β-glucosaminidase, α- and β-galactosidases, α- and β-glucosidases, β-glucuronidase, α-mannosidase and α-fucosidase. Produces acetoin, reduces nitrate to nitrogen and weakly hydrolyses gelatin. Acid is produced from aesculin, 2-ketogluconate, turanose and arabinose. The major cellular fatty acids are iso-C_{17:1}ω9c, C_{17:1}ω8c, iso-C_{17:0} and C_{16:0}. The DNA G+C content of the type strain is 68.9 mol%.

The type strain, SG-29^T (=NBRC 107124^T =KCTC 23197^T), was isolated from the western North Pacific Ocean near Japan.

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