Maribacter arcticus sp. nov., isolated from Arctic marine sediment

Kyeung Hee Cho,[1] Soon Gyu Hong,[1] Hyun Hee Cho,[1] Yoo Kyung Lee,[1] Jongsik Chun[2] and Hong Kum Lee[1]

Correspondence
Hong Kum Lee
hklee@kopri.re.kr

[1]Polar BioCenter, Korea Polar Research Institute, KORDI, Songdo Techno Park, Songdo-dong 7-50, Yeonsu-gu, Incheon 406-840, Republic of Korea

[2]School of Biological Sciences and Institute of Microbiology, Seoul National University, 56-1 Shillim-dong, Kwanak-gu, Seoul 151-742, Republic of Korea

A Gram-negative, non-motile, aerobic bacterium, designated strain KOPRI 20941^T, was isolated from a sample of marine sediment from Ny-Ålesund, Spitsbergen, Norway. A phylogenetic analysis based on 16S rRNA gene sequences revealed that the Arctic isolate nested within the genus *Maribacter* and showed the highest sequence similarity (98.1 %) with respect to *Maribacter orientalis* KMM 3947^T. Chemotaxonomic data (DNA G+C content of 36 mol%; MK-6 as the major respiratory quinone and iso-$C_{17:0}$ 3-OH, $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH and iso-$C_{15:0}$ as the major fatty acids) supported the affiliation of strain KOPRI 20941^T to the genus *Maribacter*. The results of phylogenetic analyses, physiological and biochemical tests and a DNA–DNA reassociation test (<54 % relatedness) allowed genotypic and phenotypic differentiation of the strain from the recognized species of the genus *Maribacter*. Therefore strain KOPRI 20941^T represents a novel species of the genus *Maribacter*, for which the name *Maribacter arcticus* sp. nov. is proposed. The type strain is KOPRI 20941^T (=KCTC 22053^T=JCM 14790^T).

The genus *Maribacter* was proposed by Nedashkovskaya *et al.* (2004) for isolates collected from green alga, seawater and marine sediment, and at the time of writing, comprises *Maribacter aquivivus*, *Maribacter orientalis*, *Maribacter sedimenticola*, *Maribacter ulvicola* (Nedashkovskaya *et al.*, 2004) and *Maribacter dokdonensis* (Yoon *et al.*, 2005). In this study, an Arctic bacterial isolate was subjected to a polyphasic analysis and was identified as a novel member of the genus *Maribacter*.

Strain KOPRI 20941^T was isolated from a sample of sediment collected at Ny-Ålesund, Spitsbergen Islands, Norway. Isolation was carried out using ZoBell's marine agar (ZoBell, 1946) at 10 °C. The isolate was maintained routinely on ZoBell's marine agar and was preserved as a glycerol suspension (10 %, v/v) at −80 °C.

The 16S rRNA gene was PCR amplified from a single colony using the universal primers described by Lane (1991). An almost-complete 16S rRNA gene sequence (1486 bp) of strain KOPRI 20941^T was obtained. The sequence was aligned manually with type strains of the genus *Maribacter* and related species, obtained from the GenBank database. Secondary structural information implemented in the jPHYDIT program (Jeon *et al.*, 2005) was used for accurate multiple alignment. A phylogenetic tree was constructed using the parsimony method with the exhaustive search option implemented in the PAUP 4.10 program (Swofford, 2002). The resultant phylogenetic tree was assessed by means of bootstrap analysis (Felsenstein, 1985) based on 1000 resampled datasets. The 16S rRNA gene sequence of *Zobellia galactanivorans* Dsij^T (GenBank accession no. AF208293) was used as an outgroup. Sequence comparisons with 16S rRNA gene sequences from the EzTaxon database (Chun *et al.*, 2007) revealed that strain KOPRI 20941^T had the highest similarity with *M. orientalis* KMM 3947^T (98.1 %), followed by *M. aquivivus* KMM 3949^T (97.1 %), *M. dokdonensis* DSW-8^T (96.3 %), *M. sedimenticola* KMM 3903^T (96.0 %) and *M. ulvicola* KMM 3951^T (95.8 %). Phylogenetic analysis showed that strain KOPRI 20941^T is nested within the genus *Maribacter* (Fig. 1).

Genomic relatedness between strain KOPRI 20941^T and the type strains of *M. orientalis* and *M. aquivivus* was examined using DNA–DNA reassociation as described by De Ley *et al.* (1970) and Huß *et al.* (1983) using a UV/VIS-spectrophotometer equipped with a temperature controller (Cary 300 Bio; Varian). The DNA–DNA relatedness values between strain KOPRI 20941^T and *M. aquivivus* KMM 3949^T and *M. orientalis* KMM 3947^{T}were 43.5 % and 53.8 %, respectively.

For the determination of chemotaxonomic characteristics, strain KOPRI 20941^T was grown routinely on marine

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KOPRI 20941^T is AY771762.

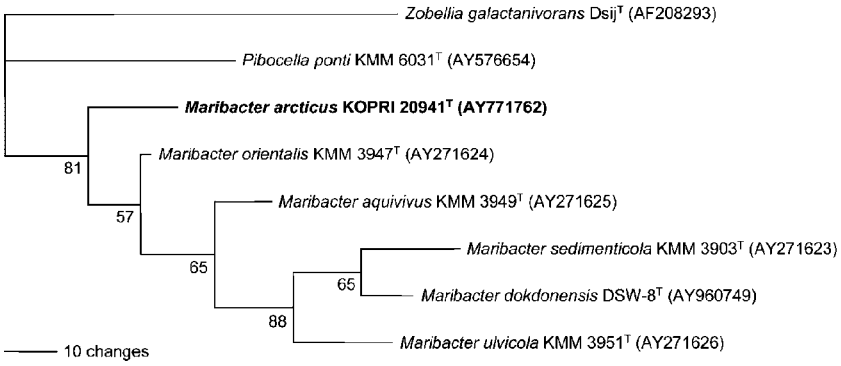

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain KOPRI 20941^T within the genus *Maribacter*. The tree was constructed using the exhaustive search option with the parsimony criterion. Bootstrap percentages (>50 %) are shown at nodes. *Z. galactanivorans* Dsij^T was used as an outgroup. Bar, 10 nucleotide changes.

2216E agar (MA; Difco) at 20 °C. Isoprenoid quinones were extracted from 2-day-old cells according to the method of Minnikin *et al.* (1984). Analysis by HPLC (Collins, 1985) revealed MK-6 as the predominant isoprenologue. Cellular fatty acid methyl esters were analysed by GC (model 6890A apparatus; Hewlett-Packard) using the Microbial Identification (MIDI) system. The cellular fatty acid profile was as follows: iso-$C_{17:0}$ 3-OH (19.1 %), $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH (14.8 %), iso-$C_{15:0}$ (12.5 %), anteiso A-$C_{15:0}$ (9.0 %), iso G-$C_{15:1}$ (8.0 %), iso-$C_{17:1}\omega9c$ (7.9 %), iso-$C_{16:0}$ 3-OH (4.0 %), $C_{15:0}$ (3.9 %), $C_{17:0}$ 2-OH (3.8 %), iso-$C_{15:0}$ 3-OH (2.8 %), $C_{16:0}$ 3-OH (1.5 %), iso-$C_{16:0}$ (1.0 %) and an unknown fatty acid (sum of equivalent chain-lengths 13.565 and 16.582; 2.8 %). The DNA G+C content was 36 mol%, as determined using the thermal melting method (Marmur & Doty, 1962; Mandel *et al.*, 1970; Johnson, 1985). The above characteristics of strain KOPRI 20941^T are consistent with its assignment to the genus *Maribacter*.

Temperature (4–50 °C) and pH (pH 5–10, using increments of 1 pH unit) ranges for growth were determined in a temperature gradient incubator (TVS126MA; Advantec) using MA medium. Growth at different salinities was determined in modified ZoBell's marine broth (0.5 % Bacto peptone, 0.1 % yeast extract, 0.01 % ferric citrate in distilled water) by adding different amounts (0–10 %, w/v) of sea salts (Sigma). Requirements for Ca^{2+}, K^+ and Mg^{2+} ions were examined as described by Bae *et al.* (2007). Growth under anaerobic conditions was determined in an anaerobic chamber. For this experiment, an MA plate was incubated under $N_2/CO_2/H_2$ (80 : 10 : 10) for 7 days at 20 °C. Enzyme activities, the assimilation and fermentation of sole carbon sources, nitrate reduction and indole production were determined using API 20E, API 20NE, API 50 CHB and API ZYM tests (bioMérieux). Morphological examinations were performed by using scanning and transmission electron microscopy on cells grown on an MA plate at 20 °C. Gliding motility was observed according to the methods of Bernardet *et al.* (2002) and Bowman (2000). Other routine phenotypic tests were performed using previously described methods (Sohn *et al.*, 2004). Detailed results from these experiments are given in the species description. Morphological and physiological characteristics that serve to differentiate strain KOPRI 20941^T from the recognized species (at the time of writing) of the genus *Maribacter* are given in Table 1.

Strain KOPRI 20941^T can be readily differentiated from the recognized members of the genus *Maribacter* on the basis of a number of phenotypic characteristics (see Table 1): optimum growth, optimum pH, acetoin production, hydrolysis of agar, gelatin, starch and Tweens 40 and 80, nitrate reduction and carbon-source assimilation and fermentation. Therefore, we conclude that strain KOPRI 20941^T represents a novel species of the genus *Maribacter*, for which the name *Maribacter arcticus* sp. nov. is proposed.

Description of *Maribacter arcticus* sp. nov.

Maribacter arcticus (arc′ti.cus. L. masc. adj. *arcticus* northern, pertaining to the Arctic, the geographical origin of the type strain).

Gram-negative, oxidase-positive, catalase-positive, strictly aerobic and mesophilic. Cells are irregular, non-motile rods (1.2–3.1 × 0.3–0.5 μm). Colonies are circular, convex with entire edges, glistening, yellow in colour and about 1.0 mm in diameter after incubation for 8 days on ZoBell's marine agar at 20 °C. Diffusible pigments are not produced. Growth occurs at 4–30 °C (optimum, 20–22 °C), pH 5–10 (optimum, pH 6) and with 1–7 % sea salts (optimum, 2–4 %, corresponding to 1.6–3.1 % NaCl). Growth is observed only in the presence of Ca^{2+} or Mg^{2+} ions in addition to NaCl. Acid is not produced from the following: *N*-acetylglucosamine, adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, aesculin, fructose, D-fucose, L-fucose, galactose, gentiobiose, gluconate, glucose, glycerol, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-lyxose, maltose, mannitol, mannose, melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-D-xyloside, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, starch, sucrose, D-tagatose, trehalose, turanose, xylitol, D-xylose and L-xylose (API 50 CHB). Acetoin and cytochrome oxidase are produced, but indole,

Table 1. Characteristics that differentiate strain KOPRI 20941^T from closely related species of the genus *Maribacter*

Strains: 1, KOPRI 20941^T; 2, *M. aquivivus* KMM 3949^T; 3, *M. dokdonensis* DSW-8^T; 4, *M. orientalis* KMM 3947^T; 5, *M. sedimenticola* KMM 3903^T; 6, *M. ulvicola* KMM 3951^T. Data were taken from this study and from Nedashkovskaya *et al.* (2004) and Yoon *et al.* (2005). +, Positive; −, negative; w, weakly positive.

Characteristic	1	2	3	4	5	6
Optimum growth temp.	20–22	21–23	30	21–23	22–24	21–23
Optimum growth pH	6	7.5–8.5	7–8	7.5–8.5	7.5–8.5	7.5–8.5
Acetoin production	+	−	−	−	−	−
Hydrolysis of:						
Agar	−	+	+	−	+	+
Gelatin	−	+	−	+	−	−
Starch	−	−	−	−	+	−
Tween 40	−	+	+	+	+	+
Tween 80	−	+	+	−	+	+
Nitrate reduction	+	+	−	−	+	−
Acid from:						
L-Arabinose	−	−	+	+	+	−
Cellobiose	−	−	+	+	−	+
D-Galactose	−	−	+	+	−	−
D-Glucose	−	−	+	+	−	+
Lactose	−	−	+	+	−	+
Maltose	−	−	+	+	−	+
D-Mannitol	−	−	+	−	−	−
Melibiose	−	−	w or +	+	−	−
Raffinose	−	−	w or +	−	−	−
L-Rhamnose	−	−	−	−	−	+
Sucrose	−	−	+	+	−	+
D-Xylose	−	−	+	+	−	−
Utilization of:						
L-Arabinose	−	−	−	+	−	−
D-Glucose	−	+	+	+	−	+
Mannose	−	+	+	+	−	+

H_2S, urease and gelatinase are not produced (API 20E). Nitrate is reduced to nitrite. *N*-Acetylglucosamine, adipate, arabinose, caprate, citrate, gluconate, glucose, malate, maltose, mannitol, mannose and phenyl acetate are not assimilated (API 20NE test). *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, esterase lipase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase are present, but α-chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, α-mannosidase, lipase and trypsin are not present (API ZYM). The predominant cellular fatty acids are iso-$C_{17:0}$ 3-OH, $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH and iso-$C_{15:0}$. The DNA G+C content of the type strain is 36 mol%.

The type strain, KOPRI 20941^T (=KCTC 22053^T= JCM 14790^T), was isolated from a sample of marine sediment collected at Ny-Ålesund, Spitsbergen, Norway.

Acknowledgements

This work was supported by a grant (PE07050) from KOPRI, Republic of Korea.

References

Bae, S. S., Kwon, K. K., Yang, S. H., Lee, H.-S., Kim, S.-J. & Lee, J.-H. (2007). *Flagellimonas eckloniae* gen. nov., sp. nov., a mesophilic marine bacterium of the family *Flavobacteriaceae*, isolated from the rhizosphere of *Ecklonia kurome*. *Int J Syst Evol Microbiol* **57**, 1050–1054.

Bernardet, J.-F., Nakagawa, Y. & Holmes, B. (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.

Bowman, J. P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 1861–1868.

Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequence. *Int J Syst Evol Microbiol* **57**, 2259–2261.

Collins, M. D. (1985). Analysis of isoprenoid quinones. *Methods Microbiol* **18**, 329–366.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Jeon, Y. S., Chung, H., Park, S., Hur, I., Lee, J. H. & Chun, J. (2005). jPHYDIT: a JAVA-based integrated environment for molecular phylogeny of ribosomal RNA sequences. *Bioinformatics* **21**, 3171–3173.

Johnson, J. L. (1985). Determination of DNA base composition. *Methods Microbiol* **18**, 1–31.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Mandel, M., Lgambi, L., Bergendahl, J., Dodson, M. L. & Scheltgen, E. (1970). Correlation of melting temperature and cesium chloride buoyant density of bacterial deoxyribonucleic acid. *J Bacteriol* **101**, 333–338.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Lysenko, A. M., Rohde, M., Rhee, M. S., Frolova, G. M., Falsen, E., Mikhailov, V. V. & Bae, K. S. (2004). *Maribacter* gen. nov., a new member of the family *Flavobacteriaceae*, isolated from marine habitats, containing the species *Maribacter sedimenticola* sp. nov., *Maribacter aquivivus* sp. nov., *Maribacter orientalis* sp. nov. and *Maribacter ulvicola* sp. nov. *Int J Syst Evol Microbiol* **54**, 1017–1023.

Sohn, J. H., Kwon, K. K., Kang, J.-H., Jung, H.-B. & Kim, S.-J. (2004). *Novosphingobium pentaromativorans* sp. nov., a high-molecular-mass polycyclic aromatic hydrocarbon-degrading bacterium isolated from estuarine sediment. *Int J Syst Evol Microbiol* **54**, 1483–1487.

Swofford, D. L. (2002). PAUP*: Phylogenetic analysis using parsimony (and other methods), version 4. Sunderland, MA: Sinauer Associates.

Yoon, J.-H., Kang, S.-J., Lee, S.-Y., Lee, C.-H. & Oh, T.-K. (2005). *Maribacter dokdonensis* sp. nov., isolated from sea water off a Korean island, Dokdo. *Int J Syst Evol Microbiol* **55**, 2051–2055.

ZoBell, C. E. (1946). *Marine Microbiology: a Monograph on Hydrobacteriology*. Waltham, MA: Chronica Botanica