Maribacter thermophilus sp. nov., isolated from an algal bloom in an intertidal zone, and emended description of the genus *Maribacter*

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A novel facultatively anaerobic, Gram-stain-negative bacterium, designated strain HT7-2^T, was isolated from *Ulva prolifera* collected from the intertidal zone of Qingdao sea area, China, during its bloom. Cells were rod-shaped (1.9–3.5×0.4–0.6 μm), non-sporulating and motile by gliding. Strain HT7-2^T was able to grow at 4–50 °C (optimum 40–42 °C), pH 5.5–8.5 (optimum pH 7.0), 0–8 % (w/v) NaCl (optimum 2–3 %) and 0.5–10 % (w/v) sea salts (optimum 2.5 %). The genomic DNA G+C content was 38.8 mol%. The phylogenetic analysis based on 16S rRNA gene sequences revealed that strain HT7-2^T belonged to the genus *Maribacter* with sequence similarity values of 94.5–96.6 %, and was most closely related to *Maribacter aestuarii* GY20^T (96.6 %). Chemotaxonomic analysis showed that the main isoprenoid quinone was MK-6 and the major fatty acids were iso-$C_{15:0}$ and unknown equivalent chain-length 13.565. The polar lipids of strain HT7-2^T consisted of one phosphatidylethanolamine, four unidentified lipids and one unidentified aminolipid. On the basis of the phenotypic, phylogenetic and chemotaxonomic characteristics, strain HT7-2^T (=CGMCC 1.12207^T=JCM 18466^T) is concluded to represent a novel species of the genus *Maribacter*, for which the name Maribacter *thermophilus* sp. nov. is proposed. An emended description of the genus *Maribacter* is also proposed.

The genus *Maribacter*, belonging to the family *Flavobacteriaceae*, was initially proposed by Nedashkovskaya *et al.* (2004). At the time of writing, the genus consists of twelve species with validly published names, including *Maribacter aquivivus*, *Maribacter orientalis*, *Maribacter sedimenticola*, *Maribacter ulvicola* (Nedashkovskaya *et al.*, 2004), *Maribacter dokdonensis* (Yoon *et al.*, 2005), *Maribacter polysiphoniae* (Nedashkovskaya *et al.*, 2007), *Maribacter arcticus* (Cho *et al.*, 2008), *Maribacter forsetii* (Barbeyron *et al.*, 2008), *Maribacter antarcticus* (Zhang *et al.*, 2009), *Maribacter stanieri* (Nedashkovskaya *et al.*, 2010), *Maribacter chungangensis* (Weerawongwiwat *et al.*, 2013) and *Maribacter aestuarii* (Lo *et al.*, 2013). In this study, a novel species of the genus *Maribacter*, isolated from *Ulva prolifera*, previously known as *Enteromorpha prolifera* (Hayden *et al.*, 2003), is proposed based on the phenotypic, phylogenetic and chemotaxonomic characteristics.

During the bloom of *Ulva prolifera* spreading in Qingdao sea area in summer of 2010, fresh seaweeds were sampled and stored at 4 °C before use. Strain HT7-2^T was isolated and purified using the dilution-plating method on modified marine 2216 plates (Liu *et al.*, 2014) at 28 °C. The strain was preserved at −80 °C in modified marine broth (MB) supplemented with 20 % (v/v) glycerol. Unless otherwise stated, the strain was incubated in modified MB at 41 °C.

Cell morphology and gliding motility were observed using transmission electron microscopy (JEM-1230; JEOL) and light microscopy (BX40; Olympus) when cells were in the exponential phase of growth (Bernardet *et al.*, 2002). The temperature range for growth was determined at 4, 15, 20, 25, 28, 30, 35, 38, 39, 40, 41, 42, 43, 44, 45, 50, 52 and 55 °C. To measure the pH range for growth, the initial pH of modified MB was adjusted in 0.5 pH unit steps and buffered with agents MES (pH 5.0–6.5), BIS/TRIS/propane

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Abbreviations: ECL, equivalent chain-length.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of HT7-2T is JQ988061.

Three supplementary figures are available with the online Supplementary Material.

Table 1. Phenotypic characteristics of strain HT7-2^T and the type strains of the genus *Maribacter*

Strains: 1, HT7-2^T; 2, *M. aestuarii* GY20^T; 3, *M. sedimenticola* KMM 3903^T; 4, *M. polysiphoniae* KMM 6151^T. All strains are positive for the following characteristics: catalase and oxidase activity; hydrolysis of aesculin; utilization of fructose, galactose, lactose, mannitol, mannose and xylose; acid production from arabinose, fructose, glucose and mannose; resistance to kanamycin, polymyxin B and streptomycin. All strains are negative in the following characteristics: nitrate reduction, hydrolysis of agar, casein, starch, chitin and CM-cellulose; methyl red and Voges–Proskauer tests. All strains are sensitive to chloramphenicol and tetracycline. +, Positive; −, negative; w, weakly positive.

Characteristic	1	2	3	4
Colony colour*	Y	YO	Y	Y
Type of metabolism†	F	A	A	A
Requirement for Ca^{2+} or Mg^{2+} for growth	+	+	−‡	−‡
Temperature for growth (°C)				
Range	4.0–50.0	10.0–30.0	4.0–33.0	4.0–41.0
Optimum	40.0–42.0	25.0	22.0–24.0	30.0–32.0
NaCl concentration for growth (%)				
Range	0.5–10.0	1.0–5.0	1.0–6.0	1.0–8.0
Optimum	2.0–3.0	2.0–3.0	1.5–2.0	1.5–2.0
pH for growth				
Range	5.5–8.5	6.5–10.5	5.5–10.0	5.5–10.0
Optimum	7.0	7.0–8.0	7.5–8.5	7.5–8.5
Production of:				
H_2S	+	−	−	+
Flexirubin	+	−	−	−
β-Galactosidase activity	+	+	−	+
Hydrolysis of gelatin	+	−	−	+
Utilization of:				
L-Arabinose	+	−	+	+
D-Glucose	+	−	+	+
D-Sorbitol	−	+	+	+
Acid production from:				
D-Galactose	+	+	−	+
Lactose	+	−	+	+
D-Mannitol	+	−	w	+
Raffinose	+	−	+	+
D-Sorbitol	−	+	+	+
D-Xylose	+	−	+	+
Antibiotic susceptibility				
Ampicillin	−	+	−	−
Benzylpenicillin	−	+	+	−
Carbenicillin	−	+	−	−
Novobiocin	+	+	−	−
Lincomycin	+	+	−	−
API 20NE				
D-Glucose	+	−	−	+
Gelatin	+	−	−	−
API ZYM				
Esterase lipase (C8)	+	w	−	+
Cystine arylamidase	w	−	−	w
Trypsin	+	+	−	w
α-Chymotrypsin	w	+	w	−
Naphthol-AS-BI-phosphohydrolase	+	+	w	+
α-Galactosidase	+	−	−	−
β-Galactosidase	+	+	+	−
α-Glucosidase	+	+	+	−
β-Glucosidase	+	+	w	−
α-Mannosidase	+	w	−	+
DNA G+C content (mol%)	38.8 %	40.1 %	35.6 %	39.5 %

*Y, yellow; YO, yellow–orange; O, orange.

†F, Facultatively anaerobic; A, aerobic.

‡Data were taken from Nedashkovskaya *et al.* (2010).

(pH 6.5–9.0) and CAPSO (pH 9.0–10.0) at 30 mM. Salt tolerance was tested in modified MB with 0–10 % (w/v) NaCl and in 0–12 % (w/v) sea salts (Sigma Aldrich) solution supplemented with 5 g trypticase l^{-1}, 5 g peptone (BD) l^{-1} and 1 g yeast extract (BD) l^{-1}. Growth under anaerobic conditions was checked at 41 °C in modified MB medium supplemented with 0.5 g cysteine l^{-1} and 1 mg resazurin l^{-1} using Hungate tubes with a gas phase of 100 % N_2. The requirement of Ca^{2+} or Mg^{2+} for growth was tested as described by Nishijima *et al.* (2009). Production of H_2S, nitrate reduction, and catalase and oxidase activities were tested as described by Mata *et al.* (2002) and Wu *et al.* (2010). Hydrolysis of aesculin, agar, casein, chitin, CM-cellulose, starch, Tweens 20 and 80 and tyrosine were tested on agar plates, as described by Mata *et al.* (2002), Yoon *et al.* (2005) and Kawasaki *et al.* (2002). Single carbon source utilization tests were performed using the medium described by Nedashkovskaya *et al.* (2004). Acid production was tested using modified MOF medium which contained (per litre distilled water): 5 g carbohydrates, 20 g NaCl, 5 g $MgCl_2.6H_2O$, 2 g $MgSO_4.7H_2O$, 1 g KCl, 0.5 g $CaCl_2$, 0.001 g $FeSO_4$, 0.5 g $(NH_4)_2SO_4$, 1 g trypticase peptone (BD), 0.1 g yeast extract (BD), 0.5 g Tris and 0.01 g phenol red (pH 7.5) (Leifson, 1963). Flexirubin pigments were tested using 15 % KOH (Fautz & Reichenbach, 1980). Strains were also tested using API 20NE and API ZYM systems (bioMérieux) according to the manufacturer's instructions except that the cells were suspended in 2 % (w/v) NaCl solution. Susceptibility to antibiotics was tested on agar plates using discs containing the following antibiotics (concentration per disc): ampicillin (10 μg), benzylpenicillin (10 IU), carbenicillin (100 μg), chloramphenicol (30 μg), kanamycin (30 μg), lincomycin (2 μg), novobiocin (30 μg), polymyxin B (300 IU), streptomycin (10 μg) and tetracycline (30 μg).

After incubation in modified MB for 36 h, cells in the exponential phase of growth were harvested for chemotaxonomic analysis. Fatty acids methyl esters (FAMEs) were obtained and analysed as described by Zhang *et al.* (2010). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987), and analysed using a liquid chromatography-MS system (Tindall, 1990). Polar lipids were extracted and separated by two-dimensional TLC on silica gel 60 F_{254} plates (Merck) (Minnikin *et al.*, 1977), and analysed as described by Xu *et al.* (2011) and Zhang *et al.* (2013).

Genomic DNA was extracted using a Quick Bacteria Genomic DNA Extraction kit (DongSheng Biotech). The 16S rRNA gene was amplified by PCR using the primer pair 27F (5′-AGAGTTTGATCCTGGCT-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) and cloned into pMD 19-T vector (TaKaRa) for sequencing. Pairwise alignment of sequences was performed by the ExTaxon-e service (Kim *et al.*, 2012). Multiple sequences alignment and evolution tree reconstruction were accomplished by using the MEGA5 package (Tamura *et al.*, 2011). Evolutionary distances, used in the neighbour-joining tree, were calculated following the algorithm of Kimura's two-parameter model (Kimura, 1980). The genomic DNA G+C content was determined by reverse-phase HPLC as described by Mesbah & Whitman (1989).

A bacterium isolated from *Ulva prolifera* in this study, designated strain HT7-2^T, was Gram-stain-negative, rod-shaped (Fig. S1, available in the online Supplementary Material), motile by gliding and non-sporulating. Strain HT7-2^T grew at 4–50 °C (optimum 40–42 °C), pH 5.5–8.5 (optimum pH 7.0), 0–8 % (w/v) NaCl (optimum 2–3 %) and 0.5–10 % (w/v) sea salts (optimum 2.5 %). Weak growth was observed under anaerobic conditions. Detailed results of physiological and biochemical examinations are summarized in Table 1 and in the species description.

The fatty acid profiles of strain HT7-2^T and the reference strains are shown in Table 2. The major fatty acids of strain HT7-2^T were iso-$C_{15:0}$ (42.6 %), unknown equivalent chain-length (ECL) 13.565 (20.6 %), iso-$C_{15:1}$ G (5.2 %) and iso-$C_{17:1}\omega9c$ (5.4 %), which is similar to those of reference strains of the genus *Maribacter*; only limited differences in the proportions of some components were found. The main respiratory quinone detected in strain

Table 2. Fatty acid compositions of strain HT7-2^T and the type strains of related species of the genus *Maribacter*

Strains: 1, HT7-2^T; 2, *M. aestuarii* GY20^T; 3, *M. sedimenticola* KMM 3903^T; 4, *M. polysiphoniae* KMM 6151^T. Values are percentages of total fatty acids; those that were present at <1 % in all strains are not shown. ECL, equivalent chain-length; tr, trace (<1 %); ND, not detected. Major components (≥5 %) are highlighted in bold type. All data were from this study.

Fatty acid	1	2	3	4
Unknown ECL 11.543	4.6	3.1	4.0	4.7
$C_{12:1}$ at 11–2	ND	ND	ND	1.4
$C_{13:1}$ at 12–13	tr	tr	2.6	tr
Unknown ECL 13.565	**20.6**	**18.4**	**20.0**	**7.4**
iso-$C_{14:0}$	ND	tr	ND	1.7
$C_{14:0}$	tr	tr	1.1	tr
iso-$C_{15:1}$ G*	**5.2**	**13.6**	**14.1**	**9.0**
iso-$C_{15:0}$	**42.6**	**23.0**	**31.0**	**14.4**
anteiso-$C_{15:0}$	tr	1.8	2.6	**5.8**
$C_{15:1}\omega6c$	tr	tr	ND	4.8
$C_{15:0}$	2.9	**8.2**	4.5	**13.1**
iso-$C_{16:1}$ H	ND	tr	ND	2.5
iso-$C_{16:0}$	tr	2.0	tr	1.4
$C_{16:0}$	1.2	1.6	2.6	2.3
iso-$C_{15:0}$ 3-OH	2.0	1.5	1.5	2.5
iso-$C_{17:1}\omega9c$	**5.4**	4.7	2.0	2.7
iso-$C_{16:0}$ 3-OH	4.2	3.0	0.7	**7.3**
iso-$C_{17:0}$ 3-OH	4.8	4.7	4.4	1.7
Summed Feature 3†	1.7	**6.6**	3.6	**6.4**

*The position and configuration of the double bond was not known.
†Summed feature 3 contained $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

HT7-2^T was MK-6, which is in agreement with other species of the genus *Maribacter*. The major polar lipids of strain HT7-2^T were one phosphatidylethanolamine and three unidentified lipids (L2–4), the minor components were one unidentified lipid (L1) and one unidentified aminolipid (Fig. S2), which is similar to the reference strains.

Pairwise alignment of 16S rRNA gene sequences showed that strain HT7-2^T was related to the genus *Maribacter* with sequence similarity values of 94.5–96.6 %, and was most closely related to *M. aestuarii* GY20^T (96.6 %). Conformably, phylogenetic analysis based on the multiple sequences alignment indicated that strain HT7-2^T belonged to the genus *Maribacter* by clustering with *M. aestuarii* GY20^T and showing the shortest evolutionary distance to it in both the neighbour-joining tree (Fig. 1) and the maximum-likelihood tree (Fig. S3). The genomic DNA G+C content of strain HT7-2^T was 38.8 mol% as determined by HPLC.

Phylogenetic, genomic and chemotaxonomic analysis clearly indicated that strain HT7-2^T belonged to genus *Maribacter*. However, several phenotypic differences could be easily found among strain HT7-2^T and the type strains of species belonging to the genus *Maribacter* (summarized in Table 1). For example, utilization of D-sorbitol, acid production from D-sorbitol and production of flexirubin were positive in strain HT7-2^T while negative in all reference trains. As for the major fatty acids, the content of iso-$C_{15:0}$ was higher in the novel isolate (42.6 %) than those in *M. aestuarii* GY20^T (23.0 %), *M. sedimenticola* KMM 3903^T (31.0 %) and *M. polysiphoniae* KMM 6151^T (14.4 %). Moreover, the optimal and maximal temperatures for growth of strain HT7-2^T were notably higher than those of all other type strains of the genus *Maribacter*, which were considered as typical phenotypic characteristics of strain HT7-2^T.

Therefore, based on the characteristics from the polyphasic taxonomic study described above, strain HT7-2^T is considered to represent a novel species of the genus *Maribacter*, for which the name Maribacter *thermophilus* sp. nov. is proposed.

Emended description of the genus *Maribacter* Nedashkovskaya *et al.* 2004, emend. Barbeyron *et al.* 2008, emend. Nedashkovskaya *et al.* 2010, emend. Weerawongwiwat *et al.* 2013, emend. Lo *et al.* 2013

The description is the same as that given by Nedashkovskaya *et al.* (2004) and emended by Barbeyron *et al.* (2008),

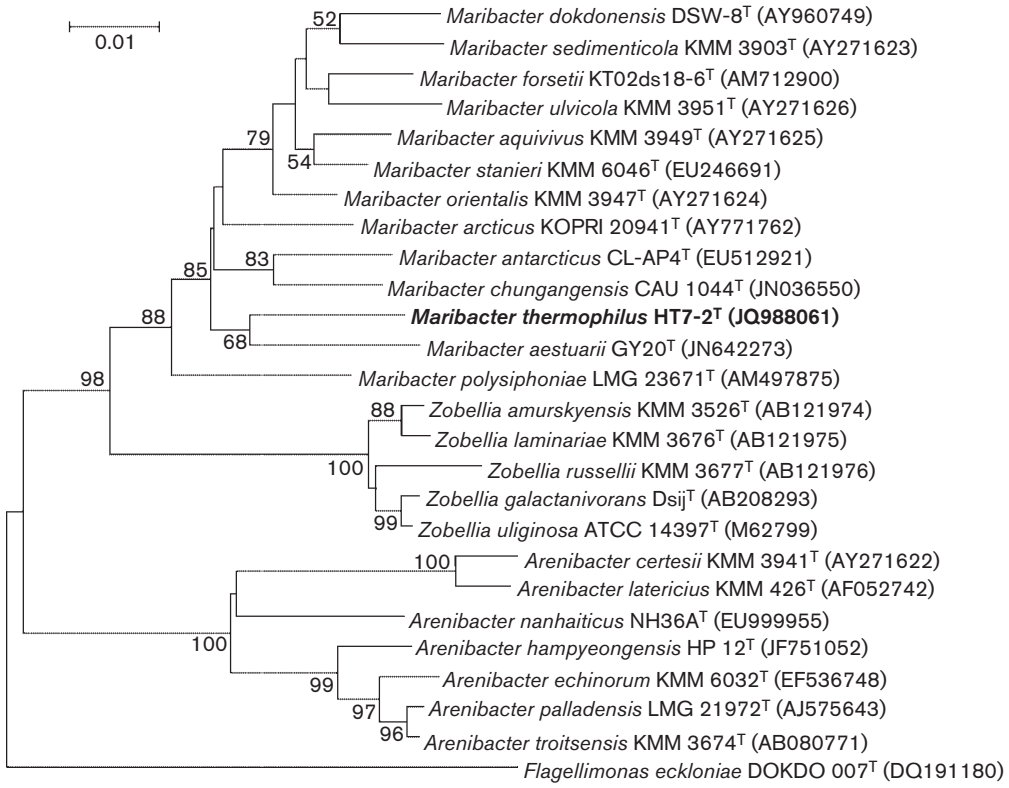

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationship among strain HT7-2^T, the type strains of species belonging to the genus *Maribacter* and representatives of some other related taxa. *Flagellimonas eckloniae* DOKDO 007^T was used as an outgroup. Bootstrap values were based on 1000 replicates; only values >50 % are shown at the branching points. Bar, 0.01 substitutions per nucleotide position.

Nedashkovskaya *et al.* (2010), Weerawongwiwat *et al.* (2013) and Lo *et al.* (2013) except for the following amendments. The type of metabolism is aerobic or facultatively anaerobic. Flexirubin pigments can be produced by certain strains.

Description of Maribacter *thermophilus* sp. nov.

Maribacter *thermophilus* [ther.mo′phi.lus. Gr. n. *thermê* hot; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. masc. adj. *thermophilus* heat-loving].

Cells are rod-shaped, 1.9–3.5 × 0.4–0.6 μm, Gram-stain-negative, facultatively anaerobic, motile by gliding and non-sporulating. Produce non-diffusible yellow pigments and flexirubin pigments. Colonies are circular, yellow in colour, shiny, convex and 1.5–3.0 mm in diameter after incubation for 2 days on modified MB agar plates at 41 °C. Weak growth is observed under anaerobic conditions. Growth occurs at 4–50 °C (optimum 40–42 °C), pH 5.5–8.5 (optimum pH 7.0), 0–8 % (w/v) NaCl (optimum 2–3 %) and 0.5–10 % (w/v) sea salts (optimum 2.5 %). Requires Ca^{2+} or Mg^{2+} for growth. Positive for catalase, oxidase and H_2S production. Positive for hydrolysis of aesculin, Tweens 20 and 80 and tyrosine. Utilizes L-arabinose, D-fructose, D-galactose, D-glucose, lactose, D-mannitol, D-mannose, raffinose and D-xylose. Positive for acid production from L-arabinose, D-fructose, D-galactose, D-glucose, lactose, D-mannitol, D-mannose, raffinose and D-xylose. Sensitive to chloramphenicol, lincomycin, novobiocin and tetracycline. Negative for hydrolysis of agar, casein chitin, CM-cellulose and starch. No utilization of D-sorbitol. No acid production from D-sorbitol. Resistant to ampicillin, benzylpenicillin, carbenicillin, kanamycin, polymyxin B and streptomycin. In the API 20NE system, tests for fermentation of D-glucose, hydrolysis of aesculin and gelatin, β-galactosidase activity, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, adipic acid, malic acid and phenylacetic acid are positive. Tests for nitrate reduction, indole production, arginine dihydrolase activity, hydrolysis of urea, and assimilation of capric acid and trisodium citrate are negative. In the API ZYM system, tests for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β- glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase activities are positive. Tests for lipase (C14), β-glucuronidase and α-fucosidase activities are negative. Tests for cystine arylamidase and α-chymotrypsin activities are weakly positive. The major fatty acids (⩾5 % of the total fatty acids) are iso-$C_{15:0}$, unknown ECL 13.565, iso-$C_{15:1}$ G and iso-$C_{17:1}\omega 9c$. The main respiratory quinone is MK-6. The polar lipids consist of phosphatidylethanolamine, four unidentified polar lipids and one unidentified aminolipid.

The type strain, HT7-2^T (=CGMCC 1.12207^T=JCM 18466^T), was isolated from *Ulva prolifera* collected from the intertidal zone of Qingdao sea area, China, during its bloom. The genomic DNA G+C content of the type strain is 38.8 mol% (HPLC).

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