Antonie van Leeuwenhoek (2015) 107:209–216
DOI 10.1007/s10482-014-0318-x

ORIGINAL PAPER

Phaeobacter marinintestinus sp. nov., isolated from the intestine of a sea cucumber (*Apostichopus japonicus*)

Mi-Hwa Lee · Eun-Ji Song · Myung-Ji Seo · Dong-Wook Hyun · Jin-Woo Bae · So-Young Lee · Seong Woon Roh · Young-Do Nam

Received: 28 April 2014 / Accepted: 24 October 2014 / Published online: 30 October 2014
© Springer International Publishing Switzerland 2014

Abstract A Gram-negative, strictly aerobic, non-motile, and rod-shaped bacterial strain designated UB-M7^T was isolated from the intestine of a sea cucumber (*Apostichopus japonicus*) collected from Pohang in South Korea. Strain UB-M7^T displayed optimal growth at 25 °C, pH 7.0–7.5, and with 2.0–3.0 % (w/v) NaCl. Phylogenetic analyses based on the 16S rRNA gene sequences showed that strain UB-M7^T clustered with *Phaeobacter arcticus* DSM 23566^T, *Phaeobacter inhibens* DSM 16374^T, *Phaeobacter gallaeciensis* BS107^T, and *Phaeobacter leonis* 306^T, exhibiting 16S rRNA gene sequence similarity values of 96.8, 96.6, 96.4, and 96.2 %, respectively. Strain UB-M7^T was found to exhibit the highest *gyrB* sequence similarity value of 80.6 % to the type strain of *P. arcticus*. The major respiratory quinone of strain UB-M7^T was found to be ubiquinone 10 (Q-10). The major cellular fatty acids (>5 % of the total fatty acids) are summed features 8 (comprising $C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$), 11-methyl $C_{18:1}$ $\omega7c$, and cyclo $C_{19:0}$ $\omega8c$. The DNA G+C content was found to be 58.5 mol% and DNA–DNA relatedness value with *P. arcticus* JCM 14644^T was 17.2 ± 2.4 %. The major polar lipids of strain UB-M7^T were identified as phosphatidylethanolamine, phosphatidylglycerol, di-phosphatidylglycerol, phosphatidylcholine; one phospholipid, and three other lipids remain unidentified. Based on its phenotypic, phylogenetic, and chemotaxonomic properties it is concluded that strain UB-M7^T represents a novel species in the genus *Phaeobacter*, for which the name *Phaeobacter marinintestinus* sp. nov. is proposed. The type strain is UB-M7^T (=KCCM 43045^T = JCM 19926^T).

Electronic supplementary material The online version of this article (doi:10.1007/s10482-014-0318-x) contains supplementary material, which is available to authorized users.

M.-H. Lee · E.-J. Song · S.-Y. Lee · Y.-D. Nam (✉)
Fermentation Research Group, Korea Food Research Institute (KFRI), Sungnam 463-746, Republic of Korea
e-mail: youngdo98@kfri.re.kr

E.-J. Song · S. W. Roh (✉) · Y.-D. Nam
Korea University of Science and Technology, Daejeon 305-350, Republic of Korea
e-mail: seong18@gmail.com

M.-J. Seo
Division of Bioengineering, University of Incheon, Inchon 406-772, Republic of Korea

D.-W. Hyun · J.-W. Bae
Department of Life and Nanopharmaceutical Sciences and Department of Biology, Kyung Hee University, Seoul 130-701, Republic of Korea

S. W. Roh
Jeju Center, Korea Basic Science Institute, Jeju 690-140, Republic of Korea

Keywords Novel species · *Phaeobacter marinintestinus* · Polyphasic taxonomy · Sea cucumber

Introduction

The genus *Phaeobacter* in the class Alphaproteobacteria was first proposed by Martens et al. (2006) based on the reclassification of *Roseobacter gallaeciensis* as *Phaeobacter gallaeciensis*. At present, the genus *Phaeobacter* comprises seven species with validly published names: *P. gallaeciensis*, *P. inhibens* (Martens et al. 2006), *P. daeponensis* (Yoon et al. 2007), *P. arcticus* (Zhang et al. 2008), *P. caeruleus* (Vandecandelaere et al. 2009), *P. leonis* (Gaboyer et al. 2013), and *P. aquaemixtae* (Park et al. 2014). All of the described members of this genus are Gram-negative, obligately aerobic, chemoheterotrophic, rod-shaped bacteria. They show various colony colors, i.e., white, beige, yellow, blue, or brown series on marine agar 2,216 (MA, Becton–Dickinson) and have Q-10 as the major respiratory quinone (Gaboyer et al. 2013; Martens et al. 2006; Vandecandelaere et al. 2009; Zhang et al. 2008). In particular, *Phaeobacter* species were reported as fish probiotic bacteria with anti-*Vibrio* activity by production of tropodithietic acid (TDA), which contributed to growing a biotechnological interest (Porsby et al. 2008). During the screening of bacteria with polysaccharide-degrading activities from marine invertebrates, a bacterial strain (designated UB-M7^{T}) was isolated from the intestine of a sea cucumber (*Apostichopus japonicus*) collected from Pohang on the east coast of South Korea. The comparative 16S rRNA gene sequence analysis showed that strain UB-M7^{T} was closely affiliated to members of the genus *Phaeobacter*. The aim of the present study was to determine the exact taxonomic position of strain UB-M7^{T} using a polyphasic characterization approach, which included phenotypic and chemotaxonomic properties, and a detailed phylogenetic analysis based on 16S rRNA and *gyrB* gene sequences.

Materials and methods

Bacterial strains and culture conditions

Strain UB-M7^{T} was isolated from the intestine of a sea cucumber using the standard dilution plating technique on marine agar (MA, Becton–Dickinson, BD, USA) at 25 °C and cultivated routinely in the same conditions. Strain UB-M7^{T} was deposited in the Korean Culture Center of Microorganisms (KCCM) as KCCM 43045^{T} and in the Japan Collection of Microorganisms (JCM) as JCM 19926^{T}. *P. arcticus* JCM 14644^{T} was obtained from the JCM (Japan), and *P. inhibens* DSM 16374^{T}, *P. leonis* DSM 25627^{T}, and *P. gallaeciensis* DSM 26640^{T} were obtained from Deutsche Sammlung von Mikroorganisem und Zellkulturen (DSMZ, Germany), which were used as a reference strain in the phenotypic characterization and fatty acid analysis. Strain UB-M7^{T} was cultivated for 5 days at 25 °C in marine broth 2216 (MB, Becton–Dickinson, BD, USA) to obtain the cell biomass required for DNA extraction, the isoprenoid quinone and polar lipid analyses. For the cellular fatty acids analysis, the cell masses of strain UB-M7^{T} and the reference type strains were harvested from MA plates after cultivation for 5 days at 25 °C. The isolate was preserved at −80 °C in 20 % (w/v) glycerol suspension for long-term storage.

Phenotypic and biochemical characterization

The cellular morphology and flagellation were examined using light microscopy (Eclipse 80i, Nikon) and transmission electron microscopy (SUPRA 55VP, Carl Zeiss), respectively. For transmission electron microscopy, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and examined after being air dried. Gram staining was performed using a Biomérieux Gram stain kit, according to the manufacturer's instructions. Growth in anaerobic conditions was determined after incubation on MA in an airtight jar (AnaeroPack Rectangular Jar, Mitsubishi Gas Chemical Company Inc.) using the GasPak EZ anaerobe pouch system (Becton–Dickinson). The obligately anaerobic bacterium *Bifidobacterrium breve* KCTC 3419 was used as a positive control. Growth was tested at various temperatures (4, 10, 15, 20, 23, 25, 27, 30, 37, 40, and 45 °C) using MA. The pH range for growth was determined using MA at pH values ranging from 5.0 to 11.0 (increments of 0.5 pH units), which was adjusted by adding the following buffers (final concentration, 50 mM): acetate buffer, pH 5.0–6.0; Tris buffer, pH 7.0–9.0; and glycine buffer, pH 10.0–11.0. Growth in the absence of NaCl and in the presence of 0, 0.5, 1.0 and 2.0 % (w/v) NaCl

was investigated using trypticase soy broth, which was prepared according to the formula for the BD medium, except NaCl was excluded and 0.45 % (w/v) $MgCl_2\cdot6H_2O$ or 0.06 % (w/v) KCl was added. Growth in the presence of 2.0–10.0 % (w/v) NaCl (w/v, increments of 1.0 %) was investigated using MB. Growth in liquid medium was examined daily over a period of 20 days with shaking incubation at 25 °C, which was determined by measuring the absorbance at 600 nm and using fresh MB as the blank. Additionally, growth on solid medium was determined by observing the development of colonies from same quadrant. The catalase and oxidase activities were determined as described by Cowan and Steel (1965). Hydrolysis of casein, starch, hypoxanthine, L-tyrosine, and xanthine were tested on MA using the substrate concentrations stated by Cowan and Steel (1965). Nitrate-reduction activity of strain UB-M7^T with *Shewanella litorisediminis* SMK1-12^T as a positive control was investigated in sulphate-depleted medium supplemented with 10 mM of potassium nitrate as a terminal electron acceptor and 10 mM of succinate as a carbon source (electron donor). The hydrolysis of aesculin and Tween 20, 40, 60, and 80 were tested as described previously (Lányí 1987), except artificial seawater was used to prepare the media. The artificial seawater contained (l^{-1} distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g $MgCl_2\cdot6H_2O$, 5.94 g $MgSO_4\cdot7H_2O$, and 1.3 g $CaCl_2\cdot2H_2O$ (Bruns et al. 2001). The utilization of various substrates as sole carbon and energy sources was tested using GN2 Microplates (Biolog). The activities of various enzymes were determined using the API ZYM system (bioMérieux), which was incubated at 25 °C for 8 h. Acid production from different carbohydrates was determined using API 50CH (bioMérieux), according to the manufacturer's instructions. Biochemical tests using commercial microtest systems (API galleries and GN2 Microplates) were performed in duplicate.

Molecular characterization

Cell biomass of strain UB-M7^T for DNA extraction was obtained from cultures grown in MB for 5 days at 25 °C. Genomic DNA was extracted and purified using a G-spin DNA extraction kit (iNtRON Biotechnology). The 16S rRNA gene and DNA gyrase B subunit gene (*gyrB*) were amplified using two universal primer pairs (9F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1512R (5′-ACGGTTACCTTGTTACGACTTC-3′) for amplification of 16 s rRNA gene; UP-1 (5′-CAYGCNGGNGGNAARTTYGA-3′) and UP-2r (5′-CCRTCNACRTCNGCRTCNGTCAT-3′) for amplification of *gyrB* gene) and the following PCR reaction: 1cycle of 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 60 °C for 45 s, 72 °C for 1 min; followed by a final extension step of 72 °C for 7 min (Yamamoto and Harayama 1995; Yoon et al. 1998). Sequencing of the amplified genes and phylogenetic analysis were performed as described previously (Lee and Yoon 2012). The 16S rRNA and *gyrB* gene sequences of strain UB-M7^T and closely related type strains were aligned using CLUSTAL_W (Thompson et al. 1994). Gaps at both ends of the alignment were omitted from further analysis. Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981), and maximum-parsimony methods (Fitch 1971) with the PHYLIP package (Felsenstein 2005). Bootstrap analysis was performed using 1,000 replication (Felsenstein 1985). The similarities of 16S rRNA and *gyrB* genes between strain UB-M7^T and closely related type strains were calculated by the PairPro2 pairwise alignment program. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki et al. (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded, and the means of the remaining three values were quoted as DNA–DNA relatedness values. The DNA G+C content was determined using the method of Tamaoka and Komagata (1984) with minor modification of reaction with nuclease P1 (Sigma). The nucleotides were eluted using a mixture of 0.55 M $NH_4H_2PO_4$ (pH 4.0)/acetonitrile (40:1, v/v) with a flow rate of 1 ml min^{-1} at room temperature and detected based on the UV absorbance at 270 nm. *Escherichia coli* DNA was used as a standard.

Chemotaxonomic characterization

Isoprenoid quinones were extracted according to the method of Komagata and Suzuki (1987) and analyzed using a reversed-phase HPLC system, which was equipped with a YMC ODS-A column (250 × 4.6 mm). The isoprenoid quinones were eluted using a mixture of chloroform/methanol (2:1, v/v) with a flow rate of 1 ml min^{-1} at room

Table 1 Differential phenotypic characteristics of *Phaeobacter marinintestinus* UB-M7[T] and close related species of the genus *Phaeobacter*

Characteristic	1	2	3	4	5
Catalase activity	−	−	+	+	+
Hydrolysis of					
Aesculin	+	−	−	−	−
Gelatin	+	−	−	−	−
Tyrosine	−	+	+	−	+
Tween 80	+	+	+	−	−
Hypoxanthine	−	+	+	+	+
Utilization[a]					
L-Arabinose	+	+	−	−	+
D-Glucose	+	+	+	+	−
D-Galactose	−	−	+	+	+
D-Cellobiose	+	−	+	+	−
D-Trehalose	+	−	+	−	+
D-Xylose	−	−	+	+	−
Maltose	+	+	+	−	−
Sucrose	−	−	+	−	+
Acetate	−	−	+	+	−
Citrate	−	−	+	+	+
Lactate	+	−	+	+[b]	−
Succinate	+	−	+	+	+
L-Glutamate	+	−	+	−	−
L-Leucine	−	−	+	+[b]	−
L-Serine	+	−	+	−[b]	+
Enzyme activity (API ZYM)					
Alkaline phosphatase	+	+	−	+	+
Esterase (C 4)	+	+	−	+	+
Esteraselipase (C 8)	+	+	−	+	+
Acid phosphatase	+	+	+	−	+
α-Glucosidase	−	−	+	−	−
Susceptibility to antibiotics					
Ampicillin (10 μg)	−	+	+	+	+
Stereptomycin (50 μg)	−	+	−	−	+
Novobiocin (5 μg)	+	−	−	−	−
Cephalothin (30 μg)	−	−	+	+	+
Kanamycin (30 μg)	+	+	−	−	−
Tetracyclin (30 μg)	−	+	+	+	−
Carbenicillin (100 μg)	−	+	+	+	+
Penicillin G (20 U)	−	−	+	+	+
Chloramphenicol (100 μg)	−	+	+	+	+
Lincomycin (15 μg)	+	−	−	−	−
G+C content (mol%)	58.5	59.6	55.7	57.6–58	58.8

All data were obtained from this study except for utilization of substrates and DNA G+C content. All strains were positive for the following: oxidase activity; utilization of D-fructose and D-mannose; susceptibility to neomycin, gentamicin, and polymyxin B; and leucine arylamidase activity. All strains were negative for the following: Gram stain; anaerobic growth; nitrate reduction; indole and H_2S production; urease activity; hydrolysis of casein, starch, and xanthine; and presence of arginine dihydrolase, lysine decarboxylase, lipase (C 14), ornithine decarboxylase, tryptophan deaminase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, Naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-Mannosidase, and α-fucosidase

Strains 1 Phaeobacter marinintestinus UB-M7[T] sp. nov., *2 P. arcticus* JCM 14644[T], *3 P. inhibens* DSM 16374[T], *4 P. gallaeciensis* DSM 26640[T], *5 P. leonis* DSM 25627[T]. + Positive, – negative

[a] Utilization test for *P. arcticus* (Zhang et al. 2008) and *P. gallaeciensis* (Park et al. 2014) was determined with API 20E/20NE kit and Biolog GN2 Microplate, respectively. These characteristics for *P. inhibens* (Martens et al. 2006) and *P. leonis* (Gaboyer et al. 2013) were investigated in ASW medium with vitamin solution and mineral medium of MB, respectively

[b] Data from Gaboyer et al. (2013)

temperature and detected based on the UV absorbance at 275 nm. Cellular fatty acids were saponified, methylated, and extracted according to the standard MIDI protocol (Sherlock Microbial Identification System, version 6.2). The fatty acids were analyzed by GC (Hewlett Packard 6890) and identified using the TSBA database of the Microbial Identification System (Sasser 1990). Polar lipids were extracted according to the procedures described by Minnikin et al. (1977) and separated by 2-dimensional TLC using chloroform/methanol/water (65:25:3.8, v/v/v) for the first dimension and chloroform/methanol/acetic acid/water (40:7.5:6:1.8, v/v/v/v) for the second dimension (Minnikin et al. 1977). Individual polar lipids were identified by spraying with ethanolic

molybdophosphoric acid, molybdenum blue, ninhydrin, (Sigma) α-naphthol reagents (Komagata and Suzuki 1987; Minnikin et al. 1984), and Dragendorff's reagent (Sigma).

Results and discussion

Phenotypic characteristics

Strain UB-M7^T is Gram-negative, obligately aerobic, non-motile, and rod-shaped. The temperature range for growth was found to be between 10 and 30 °C, with optimum growth at 25 °C. Strain UB-M7^T exhibited growth at pH 7.0–9.0, with optimum growth at pH 7.0–7.5. It grows in the presence of 2.0–6.0 % (w/v) NaCl, with an optimum of approximately 2.0–3.0 % (w/v) NaCl. Mg^{2+} ions are not required for growth. Strain UB-M7^T was found to exhibit oxidase activity but not catalase and urease activities, nitrate is not reduced to nitrite. Strain UB-M7^T is able to hydrolyze aesculin, gelatin, and Tween 20, 40, 60 and 80, but not casein, starch, tyrosine, xanthine, and hypoxanthine. Other morphological, physiological, and biochemical properties of strain UB-M7^T are presented in Table 1 and species description. While strain UB-M7^T shares many similar phenotypic features with recognized species of the genus *Phaeobacter,* it can be easily distinguished from other *Phaeobacter* species by its hydrolitic activity and the susceptibility to antibiotics (Table 1).

Phylogenetic analysis

The 16S rRNA gene sequence of strain UB-M7^T comprised 1,389 nucleotides. After alignments, gaps at both ends were omitted and final 1,356 bp of 16S rRNA gene sequence was used for the further phylogenetic analysis. In the phylogenetic tree constructed with the neighbor-joining algorithm, strain UB-M7^T joined the cluster comprising *Phaeobacter* species at a bootstrap resampling value of 86.5 % (Fig. 1). Strain UB-M7^T was found to exhibit 16S rRNA gene sequence similarities of 96.8, 96.6, 96.4, and 96.2 % to the type strains of *P. arcticus*, *P. inhibens*, *P. gallaeciensis*, and *P. leonis*, respectively, and 16S rRNA gene sequence similarities of <96 % to type strains of the other validly named *Phaeobacter* species. The *gyr*B sequences of strain UB-M7^T and closely related reference strains of *Phaeobacter* species except *P. gallaeciensis* NBRC 16654^T, *P. arcticus* JCM 14644^T, *P. inhibens* DSM 16374^T and *P. leonis* DSM 25627^T, were determined in this study (Supplementary file). The neighbor-joining phylogenetic tree based on *gyr*B sequences showed that strain UB-M7^T clustered with the genus *Phaeobacter* with a bootstrap resampling value of 88.0 % and exhibited the highest *gyr*B sequence similarity (80.6 %) to *P. arcticus* JCM 14644^T and *gyr*B sequence similarities of 78.6–80.1 % to other members of the genus *Phaeobacter* included in the analysis (Supplementary Fig. S1). Similar topology maintained in trees constructed using the maximum-likelihood and maximum-parsimony algorithms based on 16S rRNA and *gyr*B genes sequences (Fig. 1; Supplementary Fig. S1). DNA–DNA relatedness between strain UB-M7^T and to *Phaeobacter arcticus* JCM 14644^T was found to be 17.2 ± 2.4 %.

Chemotaxonomic characteristics

The predominant isoprenoid quinone detected in strain UB-M7^T is ubiquinone-10 (Q-10), which is a characteristic ubiquinone for bacteria of the genus *Phaeobacter*. Table 2 shows the cellular fatty acid profiles of strain UB-M7^T and closely related type strains of *Phaeobacter* species, which were grown and analyzed in identical conditions. The major fatty acids that comprised >5 % of the total fatty acids in strain UB-M7^T are summed features 8 (60.9 %, $C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$), 11-methyl $C_{18:1}$ $\omega7c$ (9.9 %), and cycle $C_{19:0}$ $\omega8c$ (9.3 %) (Table 2). The fatty acid profiles of strain UB-M7^T and other species of the genus *Phaeobacter* are similar although some differences in the fatty acids proportions are noted with cycle $C_{19:0}$ $\omega8c$ as major fatty acid (Table 2). The major polar lipids in strain UB-M7^T were identified as phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylcholine (PC), one phospholipid (PL), and three lipids (L1, L2, and L3) remains to be identified (Supplementary Fig. S2). The polar lipid profile of strain UB-M7^T is similar to that of other members of the genus *Phaeobacter* including PE, PG, DPG, PC, and L1–3 as major components, and an unidentified aminolipid was found to be absent amongst the polar lipids of the organism (Yoon et al. 2007). The DNA G+C content of strain UB-M7^T was found to be 58.5 mol%, which is similar to that of other members of the genus *Phaeobacter* (Table 1).

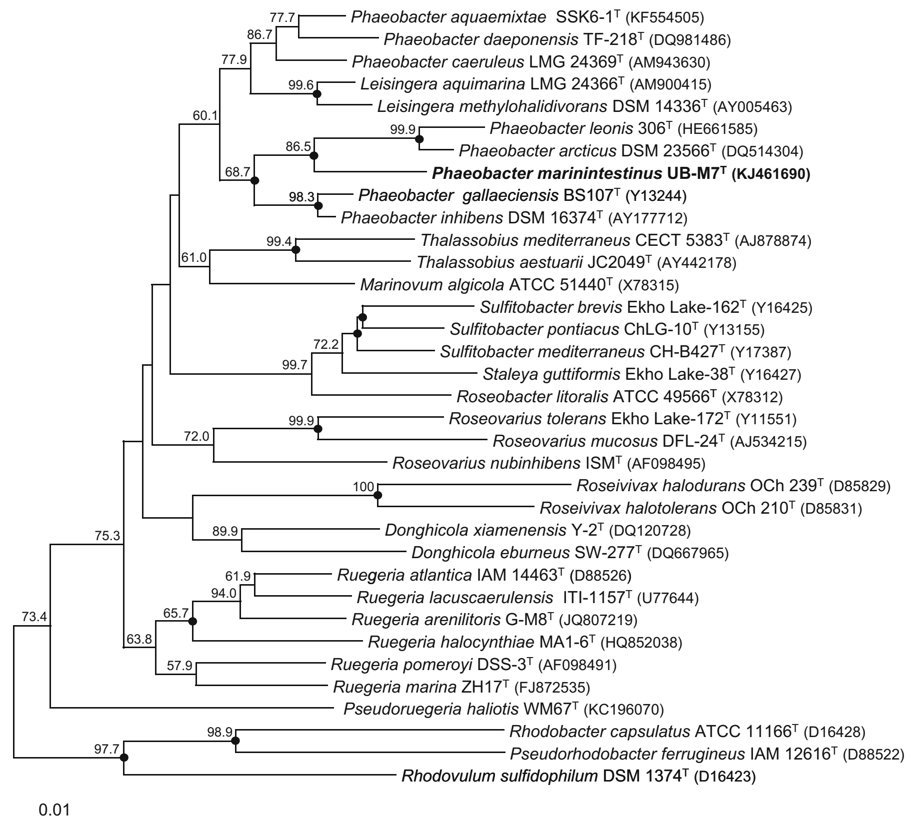

Fig. 1 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, which shows the positions of *Phaeobacter marinintestinus* UB-M7 ^{T}, other *Phaeobacter* species, and some representatives of related taxa. Bootstrap values (expressed as percentages of 1,000 replicates) >50 % are shown at the branching points. The *filled circles* indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Stappia stellulata* IAM 12621^{T} (GenBank accession number, D88525) was used as an outgroup (not shown). *Bar* 0.1 substitutions per nucleotide position

Thus the phylogenetic and chemotaxonomic properties provide evidence that it will be appropriate to classify strain UB-M7^{T} as a member of the genus *Phaeobacter*. Strain UB-M7^{T} is easily distinguishable from other validly named species of the genus *Phaeobacter*, by several different phenotypic characteristics, as shown in Table 1. These differential phenotypic and genetic properties and its phylogenetic distinctiveness are sufficient to suggest distinct taxonomic standing of strain UB-M7^{T} within *Phaeobacter* genus (Stackebrandt and Goebel 1994; Wayne et al. 1987). Based on the results obtained in this study, we propose that UB-M7^{T} represents a novel species in the genus *Phaeobacter*, for which the name *Phaeobacter marinintestinus* sp. nov. is proposed.

Description of *Phaeobacter marinintestinus* sp. nov

Phaeobacter marinintestinus (ma.rin.in.tes.ti'nus. L. adj. *marinus*, of the sea; L. adj. *intestinus*, inside; N.L. masc. adj. *marinintestinus*, inside (an organism) from the sea; this refers to the isolation of the strain from the intestine of a sea animal, i.e., sea cucumber).

Cells are Gram-negative, obligately aerobic, non-motile, and rod-shaped, measuring approximately 0.8–1.4 μm in diameter and 2.4–4.6 μm in length after incubation on MA at 25 °C for 7 days. On MA, colonies are circular to slightly irregular, smooth, glistening, yellowish white in color, and 1.0–3.5 mm in diameter after incubation for 20 days at 25 °C. Anaerobic growth does not occur on MA. The optimal growth temperature is 25 °C and growth occurs at 10–30 °C but not at 4 and 37 °C. The optimal pH for growth is between 7.0 and 7.5, but growth occurs at pH 7.0–9.0. Growth occurs in the presence of 2.0–6.0 % (w/v) NaCl with optimal growth in the presence of 2.0–3.0 % (w/v) NaCl. Mg^{2+} ions are not required for growth. Oxidase positive. Catalase and urease negative. H_2S is not produced. Nitrate is not reduced to nitrite. Aesculin, gelatin, and Tween 20, 40, 60, and 80 are hydrolyzed, but not casein, starch, tyrosine, xanthine, and hypoxanthine. *N*-acetylglucosamine, L-arabinose, D-cellobiose, D-fructose, D-glucose,

Table 2 Cellular fatty acid compositions (%) of *Phaeobacter marinintestinus* UB-M7 T and closely related type strains of the genus *Phaeobacter*

Fatty acid	1	2	3	4	5
Straight-chain fatty acid					
$C_{16:0}$	4.6	8.3	7.0	8.1	7.3
$C_{17:0}$	1.8	–	0.3	0.2	0.5
$C_{18:0}$	2.0	1.4	2.8	4.1	3.4
Unsaturated fatty acid					
$C_{17:1}$ $\omega 7c$	0.5	1.4	0.4	–	0.2
Hydroxy fatty acid					
$C_{10:0}$ 3-OH	0.2	3.9	2.0	2.3	1.5
$C_{12:0}$ 3-OH	3.9	–	2.2	2.5	–
$C_{16:0}$ 2-OH	3.1	10.1	3.3	3.9	1.3
$C_{18:1}$ 2-OH	1.7	3.2	0.1	–	–
11-methyl $C_{18:1}$ $\omega 7c$	**9.9**	**17.4**	**10.2**	**11.0**	**2.7**
Cyclo $C_{19:0}$ $\omega 8c$	9.3	–	–	–	–
Summed features[a]					
3	–	1.3	–	–	–
8	**60.9**	**50.7**	**70.4**	**66.6**	**81.9**

The predominant fatty acids amounting to more than 5% in all strains were highlighted in bold

All data were obtained from this study. Fatty acids that represented <1.0 % in all strains were omitted

Strains 1 P. marinintestinus UB-M7^T sp. nov., *2 P. arcticus* JCM 14644^T *3 P. inhibens* DSM 16374^T, *4 P. gallaeciensis* DSM 26640^T, *5 P. leonis* DSM 25627^T. – Not detected

[a] Summed features represent groups of two or three fatty acids that could not be separated by GLC using the MIDI system. Summed features 3 comprised $C_{16:1}$ $\omega 7c$/$C_{16:1}$ $\omega 6c$ and/or $C_{16:1}$ $\omega 6c$/$C_{16:1}$ $\omega 7c$; summed features 8 comprised $C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$

gluconate, L-glutamate, glycerol, lactose, maltose, D-mannitol, D-mannose, propionate, succinate, trehalose, adonitol, L-alanine, and L-serine are utilized as carbon and energy sources, but not D-galactose, D-xylose, sucrose, sorbitol, acetate, citrate, L-malate, malonate, D-ribose, *myo*-inositol, salicin, L-rhamnose, formate, D-melezitose, sorbitol, L-histidine, and L-leucine. Acid is produced from aesculin, fructose, mannitol, melibiose, glucose, mannose, sucrose, maltose, trehalose, raffinose, D-turanose, 5-ketogluconate, adonitol, salicin, and glycerol, but not from sorbitol, inositol, L-xylose, ribose, D-arabinose, melezitose, galactose, rhamnose, cellobiose, lactose, L-fucose, and sorbose. Assays using the API ZYM system indicate the presence of alkaline phosphatase, esterase (C 4), esterase lipase (C 8), leucine arylamidase, and acid phosphatase, but the absence of lipase (C 14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. The predominant ubiquinone is Q-10. The major fatty acids are summed features 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$), 11-methyl $C_{18:1}$ $\omega 7c$, and cyclo $C_{19:0}$ $\omega 8c$. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, one unidentified phospholipid, and three unidentified lipids. The DNA G+C content of the type strain is 58.5 mol%.

The type strain, UB-M7^T (=KCCM 43045^T = JCM 19926^T), was isolated from the intestine of a sea cucumber (*Apostichopus japonicus*). The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UB-M7^T is KJ461690.

References

Bruns A, Rohde M, Berthe-Corti L (2001) *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. Int J Syst Evol Microbiol 51:1997–2006

Cowan ST, Steel KJ (1965) Manual for the identification of medical bacteria. Cambridge University Press, London

Ezaki T, Hashimoto Y, Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int J Syst Bacteriol 39:224–229

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

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

Felsenstein J (2005) PHYLIP (Phylogeny Inference Package), version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416

Gaboyer F, Tindall BJ, Ciobanu MC, Duthoit F, Le Romancer M, Alain K (2013) *Phaeobacter leonis* sp. nov., an alphaproteobacterium from Mediterranean Sea sediments. Int J Syst Evol Microbiol 63:3301–3306

Komagata K, Suzuki K (1987) Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–207

Lányí B (1987) Classical and rapid identification methods for medically important bacteria. Methods Microbiol 19:1–67

Lee MH, Yoon JH (2012) Shewanella litorisediminis sp. nov., a gammaproteobacterium isolated from a tidal flat sediment. Antonie Van Leeuwenhoek 102:591–599

Martens T, Heidorn T, Pukall R, Simon M, Tindall BJ (2006) Brinkhoff TReclassification of Roseobacter gallaeciensis Ruiz-Ponte,1998 as Phaeobacter gallaeciensis gen. nov., comb. nov., description of Phaeobacter inhibens sp. nov., reclassification of Ruegeria algicola (Lafay et al. 1995) Uchino et al. 1999 as Marinovum algicola gen. nov., comb. nov., and emended descriptions of the genera Roseobacter, Ruegeria and Leisingera. Int J Syst Evol Microbiol 56:1293–1304

Minnikin DE, Patel PV, Alshamaony L, Goodfellow M (1977) Polar lipid composition in the classification of Nocardia and related bacteria. Int J Syst Bacteriol 27:104–117

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

Park S, Park DS, Bae KS, Yoon JH (2014) Phaeobacter aquaemixtae sp. nov., isolated from the junction between the ocean and a freshwater spring. Int J Syst Evol Microbiol 64:1378–1383

Porsby CH, Nielsen KF, Gram L (2008) Phaeobacter and Ruegeria species of the Roseobacter clade colonize separate niches in a Danish Turbot (Scophthalmus maximus)-rearing farm and antagonize Vibrio anguillarum under different growth conditions. Appl Environ Microbiol 74:7356–7364

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101. MIDI Inc, Newark

Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849

Tamaoka J, Komagata K (1984) Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microbiol Lett 25:125–128

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Vandecandelaere I, Segaert E, Mollica A, Faimali M, Vandamme P (2009) *Phaeobacter caeruleus* sp. nov., a blue-coloured, colony-forming bacterium isolated from a marine electroactive biofilm. Int J Syst Evol Microbiol 59:1209–1214

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGEea (1987) International committee on systematic bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Yamamoto S, Harayama S (1995) PCR amplification and direct sequencing of gyrB genes with universal primers and their application to the detection and taxonomic analysis of Pseudomonas putida strains. Appl Environ Microbiol 61:1104–1109

Yoon JH, Lee ST, Park YH (1998) Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rDNA sequences. Int J Syst Bacteriol 48(Pt 1):187–194

Yoon JH, Kang SJ, Lee SY, Oh TK (2007) *Phaeobacter daeponensis* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. Int J Syst Evol Microbiol 57:856–861

Zhang DC, Li HR, Xin YH, Liu HC, Chi ZM, Zhou PJ, Yu Y (2008) *Phaeobacter arcticus* sp. nov., a psychrophilic bacterium isolated from the Arctic. Int J Syst Evol Microbiol 58:1384–1387