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International Journal of Systematic Evolutionary Microbiology

DOI:
10.1099/ijsem.0.002111

Published: 01/09/2017

Peer reviewed version

Monaibacterium marinum, gen. nov, sp. nov, a new member of the *Alphaproteobacteria* isolated from seawater of Menai Straits, Wales, UK

Tatyana N. Chernikova[1*], James Dallimore[1], Heinrich Lünsdorf[2], Hermann J. Heipieper[3] and Peter N. Golyshin[1,4]

[1]School of Biological Sciences, Bangor University, Deiniol Road, Gwynedd LL57 2UW, UK

[2]Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany

[3]Helmholtz Centre for Environmental Research – UFZ, Permoserstrasse 15, 04318 Leipzig, Germany

[4]Immanuel Kant Baltic Federal University, Universitetskaya 1, 36040 Kaliningrad, Russia

*Author for correspondence:

t.chernikova@bangor.ac.uk

Tel.: +44(0)1248 382456

Running title: *Monaibacterium marinum* gen. *nov*., sp. *nov*.

New taxa - *Proteobacteria*

Key words: *Monaibacterium marinum*, seawater, *Alphaproteobacteria, Roseobacter* clade, Menai Straits

The GenBank/EMBL/DDBJ accession number of the partial 16S rRNA gene sequence of strain C7^T: KP797887

Abstract

The novel Gram-negative, aerobic, non-motile, non-spore-forming, short rod bacterium, strain C7^T, was isolated from the seawater sample of Menai Straits (Wales, UK) and characterised. Phylogenetic analysis of 16S rRNA gene sequences showed that this strain represented a distinct lineage within the *Roseobacter* clade of family *Rhodobacteracea* within *Alphaproteobacteria*. The members of the genera *Pontivivens* (*P. insulae* GYSW-23^T), *Celeribacter* (*C. manganoxidans* DY2-5^T), *Donghicola* (*D. eberneus* SW-277^T), *Roseovarius* (*R. halotolerans* HJ50^T and *R. pacificus* 81-2^T), *Cribrihabitans* (*C. marinus* CZ-AM5^T) and *Aestuariihabitans* (*A. beolgyonensis* BB-MW15^T) were the closest relatives with 16S rRNA gene sequence identities between 93.4 % and 95.6 %. The strain C7^T could utilize a restricted number of complex substrates with a preference for yeast extract and tryptone, consistently with earlier observations that peptides may serve as an important energy and carbon source for bacteria from the *Roseobacter* clade. Growth occurred in the absence of sodium ions. The isolate C7^T is a mesophilic bacterium that optimally grows at 20 ºC. The strain can grow under microaerophilic conditions. The major fatty acid was $C_{18:1}$ *cis* d11. The only detected ubiquinone was Q10. The polar lipids of C7^T strain were phosphatidylglycerol, two unknown aminolipids and three unknown lipids. The DNA G+C content of the strain was 60.0 mol%. Based on the results of the morphological, physiological and phylogenetic analyses, the new genus, *Monaibacterium* gen. nov., to include the new species *Monaibacterium marinum* sp. nov., is proposed. Strain C7^T (=DSM 100241^T, =LMG 28800^T) is the type and only strain of *M. marinum*.

Organisms from the *Roseobacter* clade within *Rhodobacteracea* (*Alphaproteobacteria)* are a physiologically and morphologically diverse and abundant group of bacteria thriving in a variety of marine habitats [1-4]. Since 1991, when the first strain of this clade was described by Shiba [5], the numbers of genera belonging to this group grew continuously and currently account for more than three dozens [6]. Research into the physiology, morphology and metabolic versatility of the members of this clade has revealed that they possess various features such as phototrophy, CO oxidation, degradation of aromatic compounds, lithoheterotrophy (sulfite or thiosulfate oxidation), methylotrophy, mixotrophy, DMSP demethylation, production of secondary metabolites, rosette formation, gas vacuoles, poly-β-hydroxybutyrate granules [1, 2]. These characteristics in combination with different lifestyles and isolation sources might reflect an adaptation of these organisms to a large variety of marine environmental niches.

This study was conducted to investigate the microbial diversity in superficial seawater from Menai Straits (Wales, UK). This site has been proposed as a Marine Nature Reserve and is characterised by a unique range of flora and fauna making it an interesting study case for diversity of indigenous marine bacteria [7].

In this paper, the results of isolation and physiological characterisation of a new strain $C7^T$ are presented. Strain $C7^T$ was isolated from seawater collected from Menai Straits (St. George's Pier, 53°13'31.3"N; 4°09'33.3"W, Menai Bridge, North Wales, UK) using initial enrichment culture with hydrocarbons. Following sampling, the seawater samples were transported to the laboratory and processed immediately. For initial enrichment, 250 ml of seawater were placed to 1 l Erlenmeyer flask and supplemented with 5 mM NH_4Cl and 0.2% (v/v) crude oil (Arabian light) and incubated with shaking (150 rpm) for 20 days at 20 ºC. Later, the aliquots of the enrichment culture were serially diluted and used to inoculate agar plates with ONR7a mineral medium [8]. Bacto agar BD (15 g l^{-1}) was used for preparation of solid media. Bacteria were grown for 7 days at room temperature in vapours of *n*-alkane mixture containing C_{12}, C_{14}, C_{16} in equal ratios, which was added on Whatman filter paper pads placed on the lids of inverted Petri dishes. Individual colonies of different morphology were transferred onto fresh ONR7a agar plates for purification. One of the isolates, designated $C7^T$, was selected for further characterization. *Pontivivens insulae* GYSW-23^T was used as a reference strain for

analysis of fatty acid and polar lipids and was obtained from DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). Cell biomass of *P. insulae* GYSW-23^T for fatty acid and polar lipids analysis was collected from cultures grown at the same growth conditions as for the strain C7^T, unless otherwise stated.

Gram staining, amylase, oxidase, catalase, lipase and gelatinase activities were tested as described by Smibert & Krieg [9]. Tween 80 (Sigma) was used in the lipase test medium. Nitrate reduction and accumulation of poly-β-hydroxybutyrate were determined using the standard methods of Baumann & Baumann [10]. Production of hydrogen sulfide was monitored using Hydrogen Sulfide test strips (Sigma-Aldrich). Motility of the cells was examined by a phase contrast light microscopy with a Zeiss Axioplan 2 imaging microscope (Carl Zeiss, Germany) and by the soft agar stabbing method (tube method) in ONR7a agar medium with 0.025% (w/v) yeast extract. Analysis of utilization of different carbon sources was done using BIOLOG GN2 test according to the manufacturer. For this test, the growth of the strain C7^T was estimated after incubation at 20 ºC for 72 h and 96 h. Utilization of organic substrates as sole carbon and energy sources was tested at concentrations of 25 mM in liquid ONR7a medium supplemented with 1 ml l^{-1} trace element solution SL-10 [11] and 10 ml l^{-1} Kao and Michayluk vitamin solution (100x) (Sigma-Aldrich). The ONR 7a medium without added carbon sources and uninoculated ONR 7a medium were used as controls.

Antibiotic susceptibility was analysed using Antimicrobial Susceptibility Testing methods with the following application disks (Thermo Scientific Oxoid^{TM}): ampicillin (25 mg), kanamycin (30mg), streptomycin (25 mg), tetracycline (10 mg), nalidixic acid (30 mg), neomycin (30 mg), vancomycin (30 mg), erythromycin (5 mg), gentamicin (30 mg), trimethoprim (2.5 mg), rifampicin (30 mg), spectinomycin (25 mg), chloramphenicol (30 mg), oxacillin (5 mg), novobiocin (30 mg).

For the ultrastructural analysis of C7^T cells, the mid-log grown cells were fixed with glutaraldehyde and prepared for electron microscopic analysis, as it has been described in details by Golyshina *et al*. [12].

Strain C7^T appeared catalase- and oxidase-positive. Cells were tested negative in reduction of nitrate, production of hydrogen sulfide and indole and in hydrolysis of

gelatin and Tween 80. They stained Gram negative and were non-motile. Cells contained small poly-β-hydroxybutyrate inclusions. Results from BIOLOG GN2 test revealed that strain C7^T showed no oxidation response to any carbon sources tested under BIOLOG conditions. Among substrates tested as sole carbon and energy sources, strain C7^T was able to grow on yeast extract and tryptone. A weak growth was observed on maltose, Na-lactate, Na-citrate dihydrate. Although this strain was isolated from enrichment with *n*-alkane mixture, it was not able to grow in liquid culture on tested aliphatic hydrocarbons with chain length between C_{10} and C_{20}, but most likely utilised some organic impurities from the solidified agar medium. The full list of substrates tested is available in Supplementary Materials. Strain C7^T was susceptible to ampicillin, streptomycin, erythromycin, gentamicin, rifampicin, spectinomycin, chloramphenicol, oxacillin and novobiocin, but not to nalidixic acid, tetracycline, trimethoprim, kanamycin, neomycin and vancomycin.

Strain C7^T formed colonies (0.5 – 1.5 mm in diameter) on a solid ONR7a medium after 3 days of incubation. Colonies appeared as circular, white-coloured, flat and smooth, with even margins. The ultrastructural analysis of C7^T cells is shown in Fig.1. Electron microscopy analysis of shadow-cast and ultrathin-sectioned samples showed short-rod-shaped cells of the strain C7 and Gram-negative cell architecture with an outer membrane (Fig. 1 (b)). Cells of C7 were 1.7 µm (± 0.2 µm) in length and when cross-sectioned they were 600 nm (± 76 nm) in width (Fig.1 (a, b)). Cells did not show flagellation and a thin low-density slime matrix could be observed, which occasionally – dependent on the cell's physiological state - contained nanoscale granules (Fig. 1 (a)). The cytoplasm contained electron-translucent polyhydroxyalkanoate (PHA) storage granules. The periplasmic space often appeared dilated in the polar region and – based on the specific chemistry – membrane contrast was rather weak, which made it difficult to clearly outline outer and cytoplasmic membranes (Fig. 1 (b)).

The ability of strain C7^T to grow at various temperatures, pH and salinity ranges was determined in ONR7a supplemented with 0.025 % (w/v) yeast extract. The temperature range for growth of strain C7^T was examined at 0, 1, 2, 4, 10, 15, 20, 25 and 30-35 ºC (at intervals of 1 ºC) using spectrophotometric absorbance measurements at 600 nm. Growth

occurred at temperatures 4-31 ºC, with an optimum at 20 ºC. No growth was observed at temperature lower than 4 ºC and at temperatures higher than 32 ºC.

The pH range for growth was assessed at pH 4.5-9.5 (at intervals of 0.5 pH units) using the following buffers: citric acid/sodium citrate for pH 4.5 – 5.0; 2-(N-morpholino)ethanesulfonic acid (MES) for pH 5.5-6.5; 3-[N-Tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (TAPSO) for pH 7.0-8.0; Tris base/Tris-HCl for pH 8.5-9.5. The results have revealed that strain $C7^T$ grew well within the range of pH of 5.5- 9.0. The optimal pH for growth was found to be at 7.5.

The impact of salinity on growth of strain $C7^T$ was tested within the NaCl concentration range of 0-12% (w/v) at intervals of 1%. The results of this examination showed that the strain did not require presence of Na-ions for growth and was able to grow at NaCl concentrations between 0 to 9% (with a broad optimum between 1-7 % (w/v) NaCl). No growth occurred at the salinity higher than 9 % (w/v).

Anaerobic growth of the strain $C7^T$ was tested on ONR7a agar plates in anaerobic jar in oxygen-free atmosphere created by Anaerocult A (Merck, Germany) as well as in the liquid ONR7a medium with headspace of the vials filled with a sterile mixture of $N_2/CO_2/H_2$ (80/10/10). Elemental sulfur (S^0, 1 g l^{-1}) and nitrate (NO_3^-) as a sodium salt (2 mM) were tested as electron acceptors for anaerobic growth. Resazurin (1 mg l^{-1}) and Na_2S (1 mM) as indicator to monitor anaerobic conditions and reducing agent, respectively, were added. The growth of the strain C7 in anaerobic conditions was monitored for 4 weeks. Anaerobic growth of strain $C7^T$ was not observed. However, strain $C7^T$ could grow in microaerophilic conditions when CampyGen (Oxoid) was used for generation of microaerophilic conditions with 5% O_2, 10% CO_2 and 85% N_2.

The DNA G+C content of the isolate was determined using the HPLC method described previously [13, 14]. Purified non-methylated lambda phage DNA (Sigma-Aldrich) was used as a standard. The G+C content of strain $C7^T$ is of 60.0 mol%.

Analyses of respiratory quinones and polar lipids were carried out by the Identification Service, Leibniz-Institute DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). For these analyses, the biomass of strain $C7^T$ was obtained from the culture grown in ONR7a supplemented with 0.025 % (w/v)

yeast extract at 20 ºC and harvested at the late exponential growth phase. Extraction and separation of respiratory quinones were performed by methods described by Tindall [15, 16]. Polar lipids were extracted by the method modified after Bligh and Dyer [17] and separated according to Tindall *et al*. [18]. Analysis of quinones for strain C7^T has shown that Q10 was the only detected ubiquinone, which is a common feature for the organisms from the class *Alphaproteobacteria* [19]. The polar lipids detected in C7^T strain were phosphatidylglycerol, two unknown aminolipids and three unknown lipids (Supplementary Fig. S2, available in the Supplementary materials). The polar lipids pattern of new strain C7^T showed noticeable differences with those of the representatives of phylogenetically related genera *Pontivivens, Celeribacter, Roseovarius, Cribrihabitans* and *Aestuariihabitans.* These differences included the absence in the strain C7^T of phosphatidylcholine that was present in the lipids' profiles of the type strains of *P. insulae* GYSW-23^T, *C. manganoxidans* DY2-5^T, *R. halotolerans* HJ50^T, *C. marinus* CZ-AM5^T and *A. beolgyonensis* BB-MW15^T as well as the absence of diphosphatidylglycerol and phosphatidylethanolamine, which were present in *R. halotolerans* HJ50^T and *C. marinus* CZ-AM5^T [21-27]. Furthermore, comparative analisys of strain C7^T with *P. insulae* GYSW-23^T showed the absence of phosphatidylcholine (PC) and other phospholipids (PL) in the former (indicated in the Supplementary Fig. S2 as PC, PL1 and PL2).

Analysis of FAME in hexane was performed using a GC-FID System (HP5890, Hewlett & Packard, Palo Alto, USA) and a CP-Sil 88 capillary column (Chrompack, Middelburg, The Netherlands; length, 50 m; inner diameter, 0.25 mm; 0.25 µm film) according to the standard protocols [17, 20]. For fatty acid analysis, cell biomass of strain C7^T was grown in marine broth 2216 (BD Difco) at its optimal growth temperature, 20 ºC, and harvested in the late exponential phase (as recommended by the MIDI protocol) in order to allow a direct comparison of the obtained data with the that reported for other type species of *P. insulae, C. manganoxidans, D. eberneus, R. pacificus, R. halotolerans, C. marinus* and *A. beolgyonensis* grown at their optimal growth temperatures [21-27]. The fatty acid composition of strain C7^T is shown in Table 1. The fatty acid profiles of strain C7^T and those of phylogenetically related species of *P. insulae* GYSW-23^T, *C. manganoxidans* DY2-5^T, *D. eberneus* SW-277^T, *R. halotolerans* HJ50^T , *R. pacificus* 81-2^T, *C. marinus*

CZ-AM5^T and *A. beolgyonensis* BB-MW15^T were mainly represented by $C_{18:1}$ *cis* d11 that comprised more than 80% of total fatty acids content in some species. The profiles of fatty acids that were obtained for strain C7^T and *P. insulae* GYSW-23^T grown under the same conditions showed the difference in the proportions of two out of three principal fatty acids in these two strains. The comparison of the fatty acid profiles of strain C7^T that was grown at 4 ºC and 20 ºC showed a different degree of saturation that expresses the fluidity of the cell membrane (Supplementary Table S1, available in the Supplementary materials).

For analysis of 16S rRNA gene sequence, total genomic DNA was isolated from the strain C7^T using the QIAGEN Blood & Cell Culture DNA kit (QIAGEN, Germany) according to the manufacturer's protocol. PCR amplification of 16S rRNA gene was done using the forward primer 16F27 (5' AGAGTTTGATCMTGGCTCAG-3') and reverse primer R1492 (5'-TACGGYTACCTTGTTACGACTT-3') [28]. The PCR product was cloned into the pCR-2.1 vector (Invitrogen) and sequenced with standard primers (M13 and rM13). Sequencing of amplified 16S rRNA gene was performed at Macrogen (South Korea). Vector contamination was analyzed with VecScreen: Screen a Sequence for Vector Contamination available at http://www.ncbi.nlm.nih.gov/tools/vecscreen/. Chimera formation was checked using DECIPHER web tool (http://decipher.cee.wisc.edu/FindChimeras.html) [29]. The nearly full-length 16S rRNA gene sequence (1416 bp) of strain C7^T was assembled using the BioEdit program [30]. The 16S rRNA gene sequences of reference strains with validly published names were obtained from the GenBank database after the BLASTn [31] search of SSU rRNA subset of the GenBank. Multiple alignments and construction of a phylogenetic tree was performed using MEGA6 software [32]. The evolutionary distances were calculated using a neighbour-joining Tamura-Nei method and bootstrap analysis with 1000 replicates [33]. The maximum-likelihood [34] method was also used to reconstruct the phylogenetic tree. The analysis of 16S rRNA gene sequence of strain C7^T revealed that the isolate occupied a distinct position within *Roseobacter* clade, clustering with *Pontivivens insulae* GYSW-23^T (Fig. 2; Supplementary Fig. S1, available in the Supplementary materials). Pairwise comparison of 16S rRNA gene sequences showed that the new strain had 95.6 %, 94.5 %, 93.7%, 93.5%, 93.6%, 93.4% and 93.7%

sequence identity with the closest organisms, *Pontivivens insulae* GYSW-23^T, *Celeribacter manganoxidans* DY2-5^T, *Donghicola eberneus* SW-277^T, *Roseovarius halotolerans* HJ50^T, *Roseovarius pacificus* 81-2^T, *Cribrihabitans marinus* CZ-AM5^T and *Aestuariihabitans beolgyonensis* BB-MW15^T, respectively, which suggests that the strain likely represents a separate genus, which was further supported by its phenotypic and chemotaxonomic properties distinguishing it from phylogenetically closest neighbours. Strain C7^T seems to differ from phylogenetically related organisms with validly published names within the *Roseobacter* clade: inhabiting the marine environment with the maximal temperature below 20 ºC [35], this strain is confined to an upper temperature limit of 31 ºC. This temperature is lower than the optima of 35 ºC and 45 ºC identified for other mesophilic members of genera *Pontivivens*, *Celeribacter*, *Donghicola*, *Roseovarius*, *Cribrihabitans* and *Aestuariihabitans*. Another distinct feature is that strain does not require sodium chloride for growth, however it can tolerate up to 9 % (w/v) NaCl. Additionally, differences were found in the inability of strain C7^T to utilize the majority of carbon sources used by the strains of genera *Pontivivens*, *Celeribacter*, *Donghicola*, *Roseovarius*, *Cribrihabitans* and *Aestuariihabitans*: L-malate, pyruvate, D-glucose, L-arabinose, L-rhamnose, sucrose, D-mannose, D-sorbitol, propionate. The growth experiments with addition of growth factors such as vitamins and trace elements did not support the growth of the new strain on these carbon sources. Growth occurred on yeast extract and weakly on tryptone, which is in accordance with the observation that peptides are an important energy and carbon source for bacteria belonging to the *Roseobacter* clade [3]. Other differential phenotypic characteristics of the strain C7^T with those in representatives of *Roseobacter* clade are listed in the Table 2.

In relation with the most closely related phylogenetic neighbour from the genus *Pontivivens*, with which the strain C7^T shares 95.6 % of SSU rRNA gene sequence identity, which is a bordercase for distinguishing a separate genus, their physiological differences are overwhelming and include (to refer to the most contrasting ones to *Pontivivens* spp., as indicated in, but not limited to the, Table 2): (1) the inability of C7 to grow above 31 ºC and a lower temperature growth optimum, (2) inability of C7^T to grow at any sugar monomers utilisable by *Pontivivens* spp. and its ability to utilise citrate, (3) independence of C7^T from sodium and its broader optimum for Na^+ concentrations for

growth, (4) a distinct ability in C7^T to accumulate of polyhydroxyalkanoic acid polymers, (5) no nitrate reduction in C7^T, (6) very distinct cell morphologies and colours of colonies, (8) non-coinciding antibiotic susceptibility patterns, and finally, (9) as referred in the section on chemotaxonomy, marked differences in polar lipids compositions of C7^T with *Pontivivens insulae* GYSW-23^T.: the absence of phosphatidylcholine and phospholipids in the former.

Above facts collectively suggest that the new marine strain C7^T cannot be affiliated to any recognized bacterial genus and species and can be considered to represent a novel genus and a novel species, for which the name *Monaibacterium marinum* gen. nov., sp. nov. is proposed.

Description of *Monaibacterium* gen. nov.

Monaibacterium gen. nov. (Mo.na.i.bac.te'ri.um. L. n. *Mona* the Latin name of the Isle of Anglesey, -i-, connecting vowel; Gr. n. *bakterion* small rod; N.L. neut. n. *Monaibacterium,* a bacterium from nearby of Isle of Anglesey from which the type strain was isolated).

Gram-negative, non-motile short rods. Mesophilic bacterium. Aerobic, can grow in microaerophilic conditions. Cells contain Q10 as the only detected ubiquinone and $C_{18:1}$ *cis* d11as the major fatty acid. The major components of polar lipids are phosphatidylglycerol and two unknown aminolipids. The DNA G+C content is 60.0 mol%. Isolated from superficial seawater.

The type species is *Monaibacterium marinum.*

Description of *Monaibacterium marinum* sp. nov.

Monaibacterium marinum (ma.ri'num, L. neut. adj. *marinum* inhabiting the sea)

Cells are non-motile, aerobic short rods with a size of 1.7 µm (± 0.2 µm) in length and 600 nm (± 76 nm) in width. Colonies are 2-3 mm in diameter. Catalase- and oxidise-positive. Negative for nitrate reduction and indole production. The temperature range for growth was 4-31 ºC with the optimum at 20 ºC. The pH range for growth was 5.5- 9.0 with the optimum at 7.5. Growth occurs in the absence of Na^+ ions, optimally grows at NaCl concentrations between 1-7 % (w/v). Strain can tolerate concentration of NaCl up

to 9 % (w/v). Yeast extract (0.025 % (w/v)) is the preferable substrate for growth. The major fatty acid is $C_{18:1}$ *cis* d11. The polar lipids are phosphatidylglycerol, two unknown aminolipids and three unknown lipids. The detected ubiquinone are Q10. The DNA G+C content of the type strain is 60.0 mol%. The type strain, C7^T (=DSM 100241^T, =LMG 28800^T), was isolated from seawater of Menai Straits (Wales, UK).

Acknowledgements

The skillful work of Mrs. Inge Kristen (HZI, Central Unit for Microscopy, Braunschweig, Germany) in electron microscopic sample preparation is gratefully acknowledged. We are very thankful to Prof. Dr. Bernhard Schink for his help with Latin names for the new genus and species. This work was supported by the European Commission FP7 grants MAGICPAH (FP7-KBBE-2009-245226) and KILLSPILL (FP7-KBBE-2012-312139), EU Horizon 2020 research and innovation program grant INMARE (634486) and ERA Net IB2 Project MetaCat through UK Biotechnology and Biological Sciences Research Council (BBSRC) Grant BB/M029085/1.

Conflicts of interest

Authors declare that there are no conflicts of interest.

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Legends to figures

Fig 1. Ultrastructure of *Monaibacterium marinum* C7^T cells.

Representative overviews are shown in the micrographs. (a) Inset of shadow-casted cells shows short rod cells surrounded by a halo of a slime matrix, interspersed with granular substances (inset: arrow). Arrowheads in (a) indicate the direction of PtC-shadowing. (b) Ultrathin section view of cells, which show intracellular polyhydroxyalkanoate storage granules electron translucent inclusions. Polyphosphate granules are shown as black inclusions. Inset: Detail view of the cell wall, cytoplasmic (cm) and outer (om) membranes, which is of Gram-negative construct.

Bars, white underlaid in (a) and (b): 2.5 µm.

Fig.2. Neighbour-joining phylogenetic tree of 16S rRNA gene sequences of *Monaibacterium marinum* C7^T and related type strains. Bootstrap values >50% are shown at nodes. SSU rRNA gene sequence of *Oleiphilus messinensis* ME102^T was used as the outgroup. GenBank sequence accession numbers are shown in brackets. Scale bar represents 0.02 substitutions per nucleotide position.

Table 1. Fatty acid profiles of strain C7^T in comparison to other related strains of *Roseobacter* clade. Values are given as percentage of total fatty acids.

Fatty acid*	**Strain C7^T**	***Pontivivens insulae* GYSW-23^T**	***Celeribacter manganoxidans* DY2-5^T**	***Donghicola eberneus* SW-277^T**	***Roseovarius pacificus* 81-2^T**	***Roseovarius halotolerans* HJ50^T**	***Cribrihabitans marinus* CZ-AM5^T**	***Aestuariihabitans beolgyonensis* BB-MW15^T**
$C_{10:0}$ 3-OH						0.7		3.8
$C_{12:0}$					4.2	5.9		
$C_{12:0}$ 3-OH				4.9	4.6	5.6	4.5	
$C_{12:1}$ 3-OH						2.7		
$C_{14:0}$				1.4				
iso-$C_{15:0}$ 2-OH				0.9				7.7
$C_{16:0}$	4.0	2.8	10.6	13.6	6.2	10.4	4.1	6.0
$C_{16:0}$ 2-OH							1.5	8.0
$C_{16:0}$ 3-OH						0.9		
$C_{16:1}$ 2-OH								1.5
$C_{16:1}$ *cis* d9				0.5				
$C_{16:1}$ *cis* d7			2.9		1.4			
$C_{17:0}$				1.3				
$C_{17:0}$ 2-OH								1.7
$C_{17:1}$ *cis* d9							1.9	
$C_{18:0}$	0.6	0.2	1.6	9.2	3.8	2.9	1.5	2.0
$C_{18:1}$ *cis* d9								
$C_{18:1}$*cis* d11	95.4	96.9	72.6	61.6	73.9	52.6	80.3	48.9
11-Methyl$C_{18:1}$ d11			3.8	5.2			2.6	3.0
$C_{19:0}$ *cyclo* d11			7.3			9.2		2.5

*Strains: C7^T (this study); *Pontivivens insulae* GYSW-23^T (this study); *Celeribacter manganoxidans* DY2-5^T (Wang *et al.*, 2015); *Donghicola eberneus* SW-277^T (Yoon *et al*., 2007); *Roseovarius pacificus* 81-2^T (Wang *et al*., 2009); *Roseovarius halotolerans* HJ50^T (Oh *et al*., 2009), *Cribrihabitans marinus* CZ-AM5^T (Chen *et al*., 2014), *Aestuariihabitans beolgyonensis* BB-MW15^T (Yoon *et al*., 201

Table 2. Differential phenotypic characteristics of strain C7^T and type strains of related species within the *Roseobacter* clade.
+, Positive; -, negative; w, weak; nd, not determined

Characteristic*	**Strain C7^T**	***Pontivivens insulae* GYSW-23^T**	***Celeribacter manganoxidans* DY2-5 ^{T}**	***Donghicola eburneus* SW-277^T**	***Roseovarius pacificus* 81-2^T**	***Roseovarius halotolerans* HJ50^T**	***Cribrihabitans marinus* CZ-AM5^T**	***Aestuariihabitans beolgyonensis* BB-MW15^T**
Cell morphology	Short rods	Coccoid, ovoid or rod-shaped	Rod-shaped	Cocci or rods	Ovoid to rods	Ovoid to rods	Rods	Rods
Colony colour	White	Grayish-yellow	Cream	Ivory	Faintly pink	Faintly pink	Opaque cream	Grayish-yellow
Motility	-	-	-	-	+	-	+	-
Requirement of Na^+ ions	-	+	+	+	+	+	-	+
Growth in NaCl (%):								
Range	0-9	1-8	1-11	0.5-11	1-15	0.5-20	0-12	1.5-8
Optimum	2-7	2-3	3-4	2	2-12	3-4	4	2-3
Growth temperature (ºC):								
Range	4-31	15-35	0-37	10-42	4-45	10-45	15-40	4-35
Optimum	20	25	28	37	25	35	30-35	30
Growth at:								
4 ºC	+	-	+	-	w	-	-	+
37 ºC	-	-	+	+	+	+	+	-
pH Optimum	7.5	7.0-8.0	7.0-7.5	7.0-8.0	6.2-8.5	7.5	6.5-7.5	7.0-8.0
Reduction of nitrate to nitrite	-	+	-	+	-	-	-	-
Hydrolysis of:								

Tween 80	-	-	-	+	-	nd	-	+
Gelatine	-	-	-	-	-	+	-	w
Indole production	-	nd	nd	-	-	-	-	nd
Utilization of:								
L-Arabinose	-	-	-	+	-	+	nd	-
D-Mannose	-	-	+	+	nd	+	+	-
Accumulation of PHB	+	-	-	-	-	-	-	-
DNA G+C content (mol%)	60.0	60.6	64.8	59.7	62.3	59.0±0.1	60.4	62.7

*Strains: Strain C7^{T} (this study); *Pontivivens insulae* GYSW-23^{T} (Park *et al*., 2015); *Celeribacter manganoxidans* DY2-5 ^{T} (Wang *et al*., 2015); *Donghicola eburneus* SW-277^{T} (Yoon *et al*., 2007); *Roseovarius pacificus* 81-2^{T} (Wang *et al*., 2009); *Roseovarius halotolerans* HJ50^{T} (Oh *et al*., 2009); *Cribrihabitans marinus* CZ-AM5^{T}(Chen *et al*., 2014); *Aestuariihabitans beolgyonensis* BB-MW15^{T} (Yoon *et al*., 2013).

Fig. 1

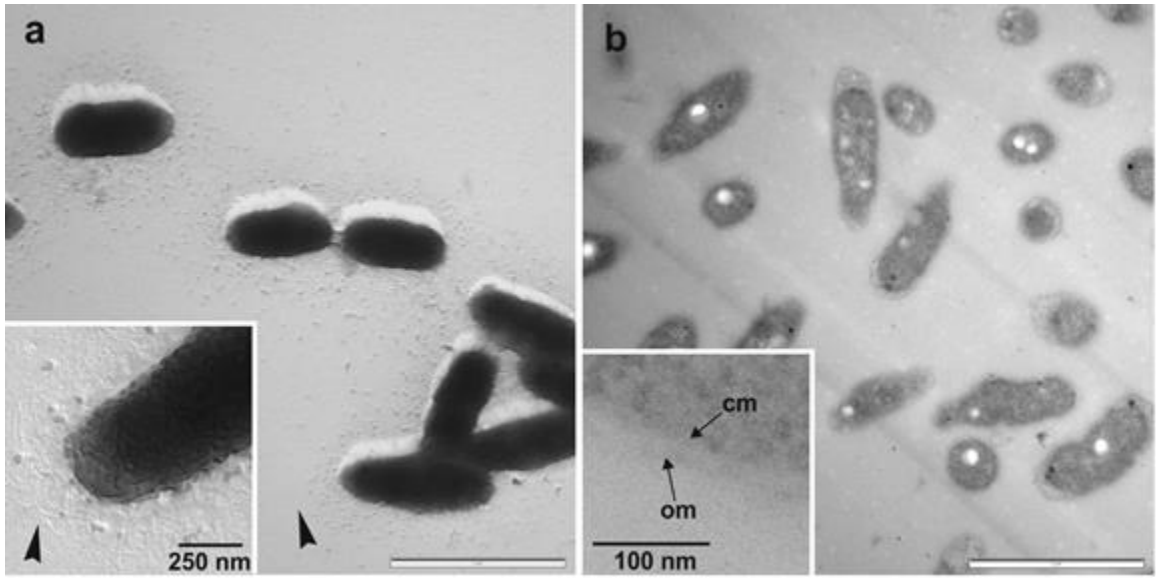

Fig. 2

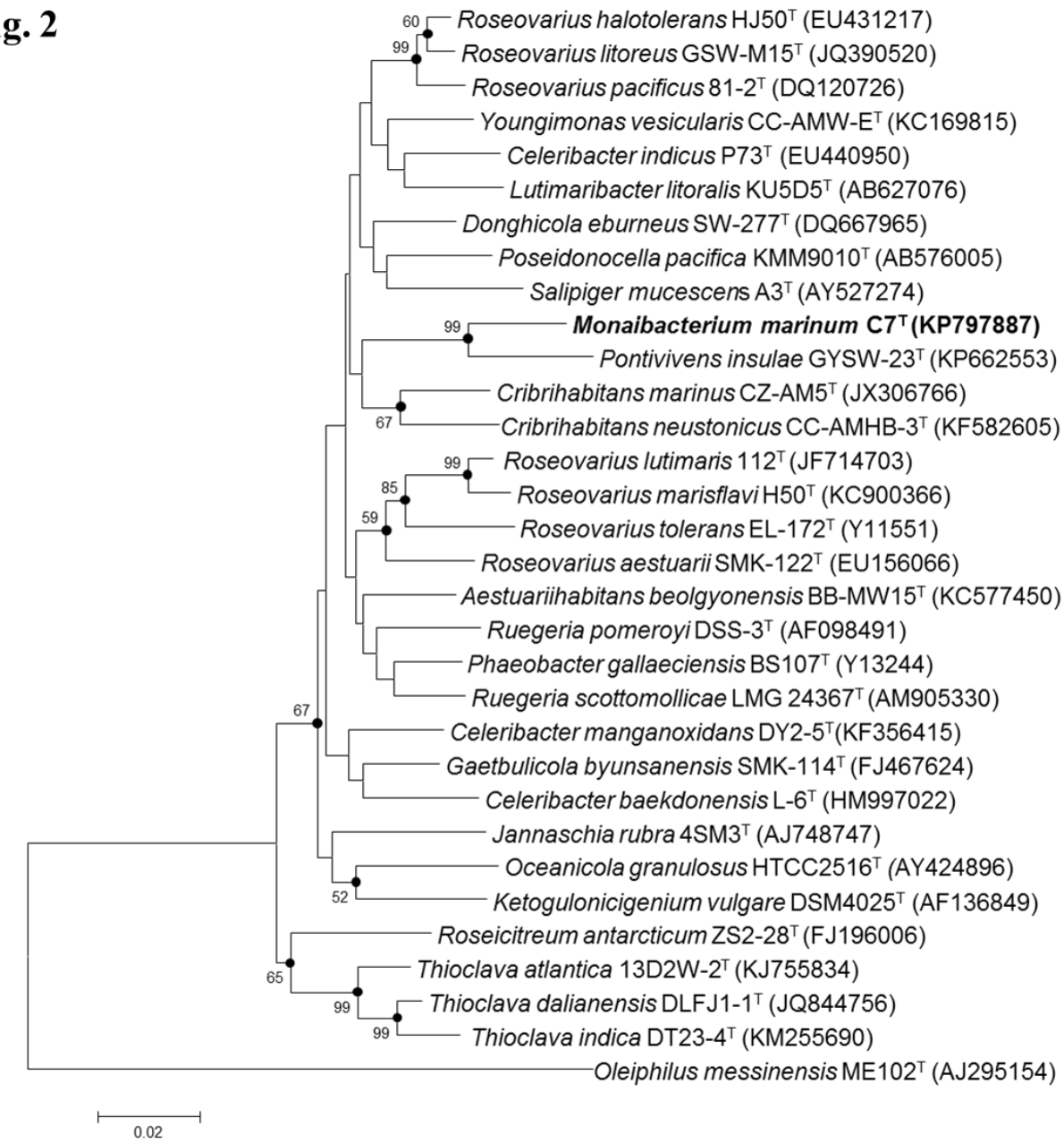
60
99
Roseovarius halotolerans HJ50T (EU431217)
Roseovarius litoreus GSW-M15T (JQ390520)
Roseovarius pacificus 81-2T (DQ120726)
Youngimonas vesicularis CC-AMW-ET (KC169815)
Celeribacter indicus P73T (EU440950)
Lutimaribacter litoralis KU5D5T (AB627076)
Donghicola eburneus SW-277T (DQ667965)
Poseidonocella pacifica KMM9010T (AB576005)
Salipiger mucescens A3T (AY527274)
99
Monaibacterium marinum C7T (KP797887)
Pontivivens insulae GYSW-23T (KP662553)
Cribrihabitans marinus CZ-AM5T (JX306766)
67
Cribrihabitans neustonicus CC-AMHB-3T (KF582605)
99
Roseovarius lutimaris 112T (JF714703)
85
Roseovarius marisflavi H50T (KC900366)
59
Roseovarius tolerans EL-172T (Y11551)
Roseovarius aestuarii SMK-122T (EU156066)
Aestuariihabitans beolgyonensis BB-MW15T (KC577450)
Ruegeria pomeroyi DSS-3T (AF098491)
Phaeobacter gallaeciensis BS107T (Y13244)
Ruegeria scottomollicae LMG 24367T (AM905330)
67
Celeribacter manganoxidans DY2-5T(KF356415)
Gaetbulicola byunsanensis SMK-114T (FJ467624)
Celeribacter baekdonensis L-6T (HM997022)
Jannaschia rubra 4SM3T (AJ748747)
Oceanicola granulosus HTCC2516T (AY424896)
52
Ketogulonicigenium vulgare DSM4025T (AF136849)
Roseicitreum antarcticum ZS2-28T (FJ196006)
65
Thioclava atlantica 13D2W-2T (KJ755834)
99
Thioclava dalianensis DLFJ1-1T (JQ844756)
99
Thioclava indica DT23-4T (KM255690)
Oleiphilus messinensis ME102T (AJ295154)
0.02

Fig. 2