

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

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**International Journal of Systematic Evolutionary Microbiology**

DOI:

[10.1099/ijsem.0.002111](https://doi.org/10.1099/ijsem.0.002111)

Published: 01/09/2017

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

*Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):*

Chernikova, T., Dallimore, J., Lunsdorf, H., Heipieper, H. J., & Golyshin, P. (2017).

Monaibacterium marinum, gen. nov, sp. nov, a new member of the Alphaproteobacteria isolated from seawater of Menai Straits, Wales, UK. *International Journal of Systematic Evolutionary Microbiology*, 67, 3310-3317. <https://doi.org/10.1099/ijsem.0.002111>

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

3

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

23

24 New taxa - *Proteobacteria*

25

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

28

29 The GenBank/EMBL/DDBJ accession number of the partial 16S rRNA gene sequence of  
30 strain C7<sup>T</sup>: KP797887

31

32 **Abstract**

33

34 The novel Gram-negative, aerobic, non-motile, non-spore-forming, short rod bacterium,  
35 strain C7<sup>T</sup>, was isolated from the seawater sample of Menai Straits (Wales, UK) and  
36 characterised. Phylogenetic analysis of 16S rRNA gene sequences showed that this strain  
37 represented a distinct lineage within the *Roseobacter* clade of family *Rhodobacteracea*  
38 within *Alphaproteobacteria*. The members of the genera *Pontivivens* (*P. insulae* GYSW-  
39 23<sup>T</sup>), *Celeribacter* (*C. manganoxidans* DY2-5<sup>T</sup>), *Donghicola* (*D. eberneus* SW-277<sup>T</sup>),  
40 *Roseovarius* (*R. halotolerans* HJ50<sup>T</sup> and *R. pacificus* 81-2<sup>T</sup>), *Cribrihabitans* (*C. marinus*  
41 CZ-AM5<sup>T</sup>) and *Aestuariahabitans* (*A. beolgyonensis* BB-MW15<sup>T</sup>) were the closest  
42 relatives with 16S rRNA gene sequence identities between 93.4 % and 95.6 %. The strain  
43 C7<sup>T</sup> could utilize a restricted number of complex substrates with a preference for yeast  
44 extract and tryptone, consistently with earlier observations that peptides may serve as an  
45 important energy and carbon source for bacteria from the *Roseobacter* clade. Growth  
46 occurred in the absence of sodium ions. The isolate C7<sup>T</sup> is a mesophilic bacterium that  
47 optimally grows at 20 °C. The strain can grow under microaerophilic conditions. The  
48 major fatty acid was C<sub>18:1</sub> *cis* d11. The only detected ubiquinone was Q10. The polar  
49 lipids of C7<sup>T</sup> strain were phosphatidylglycerol, two unknown aminolipids and three  
50 unknown lipids. The DNA G+C content of the strain was 60.0 mol%. Based on the  
51 results of the morphological, physiological and phylogenetic analyses, the new genus,  
52 *Monaibacterium* gen. nov., to include the new species *Monaibacterium marinum* sp.  
53 nov., is proposed. Strain C7<sup>T</sup> (=DSM 100241<sup>T</sup>, =LMG 28800<sup>T</sup>) is the type and only strain  
54 of *M. marinum*.

55

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

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

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

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

92 Gram staining, amylase, oxidase, catalase, lipase and gelatinase activities were tested as  
93 described by Smibert & Krieg [9]. Tween 80 (Sigma) was used in the lipase test medium.  
94 Nitrate reduction and accumulation of poly- $\beta$ -hydroxybutyrate were determined using the  
95 standard methods of Baumann & Baumann [10]. Production of hydrogen sulfide was  
96 monitored using Hydrogen Sulfide test strips (Sigma-Aldrich). Motility of the cells was  
97 examined by a phase contrast light microscopy with a Zeiss Axioplan 2 imaging  
98 microscope (Carl Zeiss, Germany) and by the soft agar stabbing method (tube method) in  
99 ONR7a agar medium with 0.025% (w/v) yeast extract. Analysis of utilization of different  
100 carbon sources was done using BIOLOG GN2 test according to the manufacturer. For  
101 this test, the growth of the strain C7<sup>T</sup> was estimated after incubation at 20 °C for 72 h and  
102 96 h. Utilization of organic substrates as sole carbon and energy sources was tested at  
103 concentrations of 25 mM in liquid ONR7a medium supplemented with 1 ml l<sup>-1</sup> trace  
104 element solution SL-10 [11] and 10 ml l<sup>-1</sup> Kao and Michayluk vitamin solution (100x)  
105 (Sigma-Aldrich). The ONR 7a medium without added carbon sources and uninoculated  
106 ONR 7a medium were used as controls.

107 Antibiotic susceptibility was analysed using Antimicrobial Susceptibility Testing  
108 methods with the following application disks (Thermo Scientific Oxoid<sup>TM</sup>): ampicillin  
109 (25 mg), kanamycin (30mg), streptomycin (25 mg), tetracycline (10 mg), nalidixic acid  
110 (30 mg), neomycin (30 mg), vancomycin (30 mg), erythromycin (5 mg), gentamicin (30  
111 mg), trimethoprim (2.5 mg), rifampicin (30 mg), spectinomycin (25 mg),  
112 chloramphenicol (30 mg), oxacillin (5 mg), novobiocin (30 mg).

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

116 Strain C7<sup>T</sup> appeared catalase- and oxidase-positive. Cells were tested negative in  
117 reduction of nitrate, production of hydrogen sulfide and indole and in hydrolysis of

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

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

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

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

150 The pH range for growth was assessed at pH 4.5-9.5 (at intervals of 0.5 pH units) using  
151 the following buffers: citric acid/sodium citrate for pH 4.5 – 5.0; 2-(N-  
152 morpholino)ethanesulfonic acid (MES) for pH 5.5-6.5; 3-[N-  
153 Tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (TAPSO) for pH 7.0-  
154 8.0; Tris base/Tris-HCl for pH 8.5-9.5. The results have revealed that strain C7<sup>T</sup> grew  
155 well within the range of pH of 5.5- 9.0. The optimal pH for growth was found to be at  
156 7.5.

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

162 Anaerobic growth of the strain C7<sup>T</sup> was tested on ONR7a agar plates in anaerobic jar in  
163 oxygen-free atmosphere created by Anaerocult A (Merck, Germany) as well as in the  
164 liquid ONR7a medium with headspace of the vials filled with a sterile mixture of  
165 N<sub>2</sub>/CO<sub>2</sub>/H<sub>2</sub> (80/10/10). Elemental sulfur (S<sup>0</sup>, 1 g l<sup>-1</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) as a sodium salt (2  
166 mM) were tested as electron acceptors for anaerobic growth. Resazurin (1 mg l<sup>-1</sup>) and  
167 Na<sub>2</sub>S (1 mM) as indicator to monitor anaerobic conditions and reducing agent,  
168 respectively, were added. The growth of the strain C7 in anaerobic conditions was  
169 monitored for 4 weeks. Anaerobic growth of strain C7<sup>T</sup> was not observed. However,  
170 strain C7<sup>T</sup> could grow in microaerophilic conditions when CampyGen (Oxoid) was used  
171 for generation of microaerophilic conditions with 5% O<sub>2</sub>, 10% CO<sub>2</sub> and 85% N<sub>2</sub>.

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

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

179 yeast extract at 20 °C and harvested at the late exponential growth phase. Extraction and  
180 separation of respiratory quinones were performed by methods described by Tindall [15,  
181 16]. Polar lipids were extracted by the method modified after Bligh and Dyer [17] and  
182 separated according to Tindall *et al.* [18]. Analysis of quinones for strain C7<sup>T</sup> has shown  
183 that Q10 was the only detected ubiquinone, which is a common feature for the organisms  
184 from the class *Alphaproteobacteria* [19]. The polar lipids detected in C7<sup>T</sup> strain were  
185 phosphatidylglycerol, two unknown aminolipids and three unknown lipids  
186 (Supplementary Fig. S2, available in the Supplementary materials). The polar lipids  
187 pattern of new strain C7<sup>T</sup> showed noticeable differences with those of the representatives  
188 of phylogenetically related genera *Pontivivens*, *Celeribacter*, *Roseovarius*, *Cribrihabitans*  
189 and *Aestuariahabitans*. These differences included the absence in the strain C7<sup>T</sup> of  
190 phosphatidylcholine that was present in the lipids' profiles of the type strains of *P.*  
191 *insulae* GYSW-23<sup>T</sup>, *C. manganoxidans* DY2-5<sup>T</sup>, *R. halotolerans* HJ50<sup>T</sup>, *C. marinus* CZ-  
192 AM5<sup>T</sup> and *A. beolgyonensis* BB-MW15<sup>T</sup> as well as the absence of diphosphatidylglycerol  
193 and phosphatidylethanolamine, which were present in *R. halotolerans* HJ50<sup>T</sup> and *C.*  
194 *marinus* CZ-AM5<sup>T</sup> [21-27]. Furthermore, comparative analysis of strain C7<sup>T</sup> with *P.*  
195 *insulae* GYSW-23<sup>T</sup> showed the absence of phosphatidylcholine (PC) and other  
196 phospholipids (PL) in the former (indicated in the Supplementary Fig. S2 as PC, PL1 and  
197 PL2).

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

210 CZ-AM5<sup>T</sup> and *A. beolgyonensis* BB-MW15<sup>T</sup> were mainly represented by C<sub>18:1 cis</sub> d11  
211 that comprised more than 80% of total fatty acids content in some species. The profiles of  
212 fatty acids that were obtained for strain C7<sup>T</sup> and *P. insulae* GYSW-23<sup>T</sup> grown under the  
213 same conditions showed the difference in the proportions of two out of three principal  
214 fatty acids in these two strains. The comparison of the fatty acid profiles of strain C7<sup>T</sup>  
215 that was grown at 4 °C and 20 °C showed a different degree of saturation that expresses  
216 the fluidity of the cell membrane (Supplementary Table S1, available in the  
217 Supplementary materials).

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

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

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

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

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

282

### 283 **Description of *Monaibacterium* gen. nov.**

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

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

293 The type species is *Monaibacterium marinum*.

294

### 295 **Description of *Monaibacterium marinum* sp. nov.**

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

297 Cells are non-motile, aerobic short rods with a size of 1.7 µm (± 0.2 µm) in length and  
298 600 nm (± 76 nm) in width. Colonies are 2-3 mm in diameter. Catalase- and oxidise-  
299 positive. Negative for nitrate reduction and indole production. The temperature range for  
300 growth was 4-31 °C with the optimum at 20 °C. The pH range for growth was 5.5- 9.0  
301 with the optimum at 7.5. Growth occurs in the absence of Na<sup>+</sup> ions, optimally grows at  
302 NaCl concentrations between 1-7 % (w/v). Strain can tolerate concentration of NaCl up

303 to 9 % (w/v). Yeast extract (0.025 % (w/v)) is the preferable substrate for growth. The  
304 major fatty acid is C<sub>18:1 cis</sub> d11. The polar lipids are phosphatidylglycerol, two unknown  
305 aminolipids and three unknown lipids. The detected ubiquinone are Q10. The DNA G+C  
306 content of the type strain is 60.0 mol%. The type strain, C7<sup>T</sup> (=DSM 100241<sup>T</sup>, =LMG  
307 28800<sup>T</sup>), was isolated from seawater of Menai Straits (Wales, UK).

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309

### 310 **Acknowledgements**

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

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### 320 **Conflicts of interest**

321 Authors declare that there are no conflicts of interest.

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### 323 **References**

- 324 1. **Buchan A, Gonzales J, Moran MA.** Overview of the marine *Roseobacter* lineage.  
325 *Appl Environ Microbiol* 2005;71:5665-5677.
- 326 2. **Wagner-Döbler I, Biebl H.** Environmental biology of the marine *Roseobacter*  
327 lineage. *Ann Rev Microbiol* 2006;60:255–280.
- 328 3. **Brinkhoff T, Giebel H-A, Simon M.** Diversity, ecology, and genomics of the  
329 *Roseobacter* clade: a short overview. *Arch Microbiol* 2008;189:531-539.
- 330 4. **Slightom RN, Buchan A.** Surface colonization by marine roseobacters: integrating  
331 genotype and phenotype. *Appl Environ Microbiol* 2009;75:6027–6037.
- 332
- 333
- 334
- 335

- 336 5. **Shiba T.** *Roseobacter litoralis* gen. nov., sp. nov., and *Roseobacter dentrificans* sp.  
337 nov., aerobic pink-pigmented bacteria which contain bacteriochlorophyll a. *Syst Appl*  
338 *Microbiol* 1991;14:140–145.  
339
- 340 6. **Garrity GM, Bell JA, Lilburn T.** Taxonomic outline of the prokaryotes. *Bergey's*  
341 *Manual of Systematic Bacteriology*. 2004. Release 5.0.  
342 [http://www.bergeys.org/outlines/bergeysoutline\\_5\\_2004.pdf](http://www.bergeys.org/outlines/bergeysoutline_5_2004.pdf)  
343
- 344 7. **Young A.** The Menai Strait – A proposed marine nature reserve. British Marine Life  
345 Study Society (Vernal Glaucus). 1995. [www.glaucus.org.uk/Menai.htm](http://www.glaucus.org.uk/Menai.htm) [accessed: 19  
346 April 2012].  
347
- 348 8. **Dyksterhouse SE, Gray JP, Herwig RP, Lara JC, Staley JT.** *Cycloclasticus*  
349 *pugetti* gen. nov., sp. nov., an aromatic hydrocarbon-degrading bacterium from  
350 marine sediments. *Int J Syst Evol Microbiol* 1995;45:116-123.  
351
- 352 9. **Smibert, R.M. & Krieg, N.R.** General characterization. In: Gerhardt P, Murray  
353 RGE, Costilow RN, Nester EW, Wood WA, Krieg NR, Phillips GB (editors). *Manual*  
354 *of Methods for General Bacteriology*, Washington, DC: American Society for  
355 Microbiology; 1981. pp.409-443.  
356
- 357 10. **Baumann, P. & Baumann, L.** The marine Gram-negative eubacteria; genera  
358 *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas* and *Alcaligenes*. In: Starr MP,  
359 Stolp H, Truper HG, Balows A, Schlegel HG (editors). *The Prokaryotes*. Berlin:  
360 Springer; 1981. pp. 1302-1330.  
361
- 362 11. **Widdel F, Kohring G, Mayer F.** Studies in sulfate-reducing bacteria that decompose  
363 fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen.  
364 nov. sp.nov. and *Desulfonema magnum* sp. nov. *Arch Microbiol* 1983;134:286-294.  
365
- 366 12. **Golyshina OV, Pivovarova TA, Karavaiko GI, Kondratéva TF, Moore ER et al.**  
367 *Ferroplasma acidiphilum* gen. nov., sp. nov., an acidophilic, autotrophic, ferrous-  
368 iron- oxidizing, cell-wall-lacking, mesophilic member of the *Ferroplasmaceae* fam.  
369 nov., comprising a distinct lineage of the *Archaea*. *Int J Syst Evol Microbiol* 2000;  
370 50:997-1006.  
371
- 372 13. **Mesbah M, Premachandran U, Whitman WB.** Precise measurement of the G+C  
373 content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J*  
374 *Syst Bacteriol* 1989; 39:159-167.  
375
- 376 14. **Tamaoka J, Komagata K.** Determination of DNA base composition by reversed-  
377 phase high-performance liquid chromatography. *FEMS Microbiol Letters* 1984;25:  
378 125-128.  
379
- 380 15. **Tindall BJ.** A comparative study of the lipid composition of *Halobacterium*  
381 *saccharovorum* from various sources. *Syst Appl Microbiol* 1990;13:128-130

382  
383  
384  
385  
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422  
423  
424  
425

16. **Tindall BJ.** Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Letts* 1990;66:199-202
17. **Bligh EG, Dyer WJ.** A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911-917.
18. **Tindall BJ, Sikorski J, Smibert RM, Kreig NR.** Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf G, Schmidt TM, Snyder LR (editors). *Methods for General and Molecular Microbiology*. Washington, DC: American Society for Microbiology; 2007. pp. 330-393.
19. **Tindall BJ, Rossello-Mora R, Busse HJ, Ludwig W, Kampf P.** Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 2010;60:249-266.
20. **Morrison WR, Smith LM.** Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J Lipid Res* 1964;5:600-608.
21. **Park S, Won W-M, Park J-M, Jung Y-T, Yoon J-H.** *Pontivivens insulae* gen. nov., sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* 2015; 65:2896-2902.
22. **Wang L, Liu Y, Wang Y, Dai X, Zhang X-H.** *Celeribacter manganoxidans* sp. nov., a manganese-oxidizing bacterium isolated from deep-sea sediment of a polymetallic nodule province. *Int J Syst Evol Microbiol* 2015;65:4180-4185.
23. **Yoon J-H, Kang S-J, Oh T-K.** *Donghicola eburneus* gen. nov., sp. nov., isolated from seawater of the East Sea in Korea. *Int J Syst Evol Microbiol* 2007;57:73-76.
24. **Wang B, Tan T, Shao Z.** *Roseovarius pacificus* sp. nov., isolated from deep-sea sediment. *Int J Syst Evol Microbiol* 2009;59:1116-1121.
25. **Oh Y-S, Lim H-J, Cha I-T, Im W-T, Yoo J-S et al.** *Roseovarius halotolerans* sp. nov., isolated from deep seawater. *Int J Syst Evol Microbiol* 2009;59:2718-2723.
26. **Chen Z, Liu Y, Liu L-Z, Zhong Z-P, Liu Z-P et al.** *Cribrihabitans marinus* gen. nov., sp. nov., isolated from a biological filter in a marine recirculating aquaculture system. *Int J Syst Evol Microbiol* 2014;64:1257-1263.
27. **Yoon J-H, Park S, Jung Y-T.** *Aestuariihabitans beolguensis* gen. nov., sp. nov., a novel alphaproteobacterium isolated from tidal flat sediment. *Antonie van Leeuwenhoek* 2013;104:217-224.

- 426 28. **Lane DJ.** 16S/23S sequencing. In: Stackebrandt E, Goodfellow M. (editors). *Nucleic*  
427 *Acid Techniques in Bacterial Systematics*. NY: John Willey and Sons; 1991. pp.148-  
428 163.  
429
- 430 29. **Wright ES, Yilmaz LS, Noguera DR.** DECIPHER, A search-based approach to  
431 chimera identification for 16S rRNA sequences. *Appl Environ Microbiol* 2012;78:  
432 3717-3725.  
433
- 434 30. **Hall TA.** Biological sequence alignment editor for Win95/98/NT/SK/XP. *Nucl Acids*  
435 *Symp Ser* 1999;41:95-98.  
436
- 437 31. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** Basic local alignment  
438 search tool. *J Mol Biol* 1990;215:403-410.  
439
- 440 32. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** MEGA6: Molecular  
441 Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013;30:2725-2729.  
442
- 443 33. **Nei M, Kumar S.** *Molecular Evolution and Phylogenetics*. New York: Oxford  
444 University Press; 2000.  
445
- 446 34. **Felsenstein J.** Evolutionary tree from DNA sequences: a maximum likelihood  
447 approach. *J Mol Evol* 1981;17:368-376.  
448
- 449 35. **Evans GL, Hardman-Mountford NJ, Hartnoll RG, Kennington K, Mitchelson-**  
450 **Jacob EG et al.** Long-term environmental studies in the Irish Sea: a review.  
451 Scientific Report No. 02. 2003. 17th November Defra Contract CDEP 84/5/311.  
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453 **Legends to figures**

454

455 **Fig 1. Ultrastructure of *Monaibacterium marinum* C7<sup>T</sup> cells.**

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

463 Bars, white underlaid in (a) and (b): 2.5  $\mu\text{m}$ .

464

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

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

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

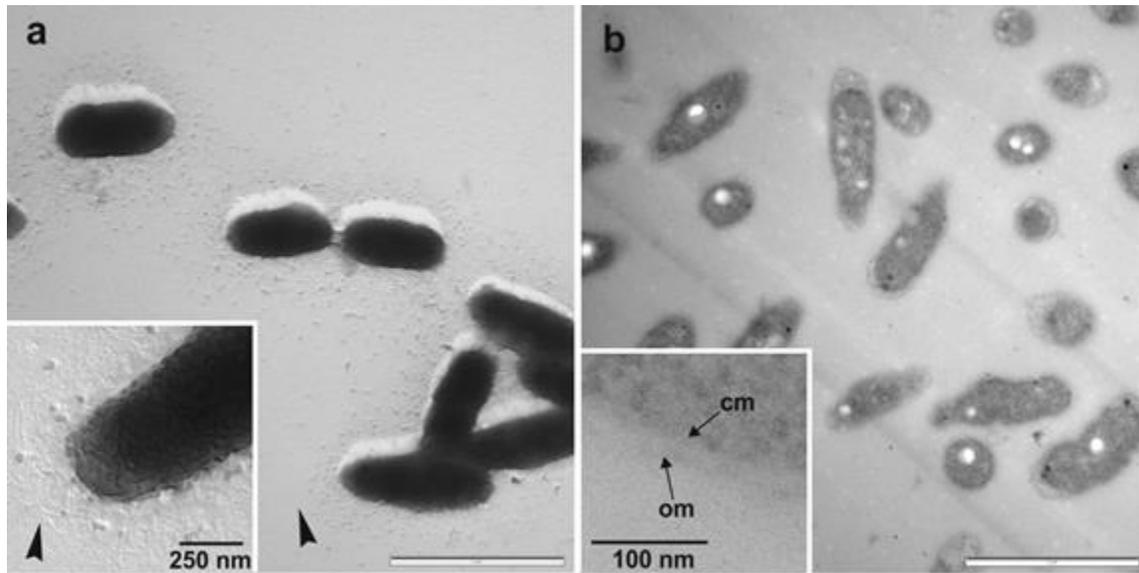
\*Strains: C7<sup>T</sup> (this study); *Pontivivens insulae* GYSW-23<sup>T</sup> (this study); *Celeribacter manganoxidans* DY2-5<sup>T</sup> (Wang *et al.*, 2015); *Donghicola eberneus* SW-277<sup>T</sup> (Yoon *et al.*, 2007); *Roseovarius pacificus* 81-2<sup>T</sup> (Wang *et al.*, 2009); *Roseovarius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



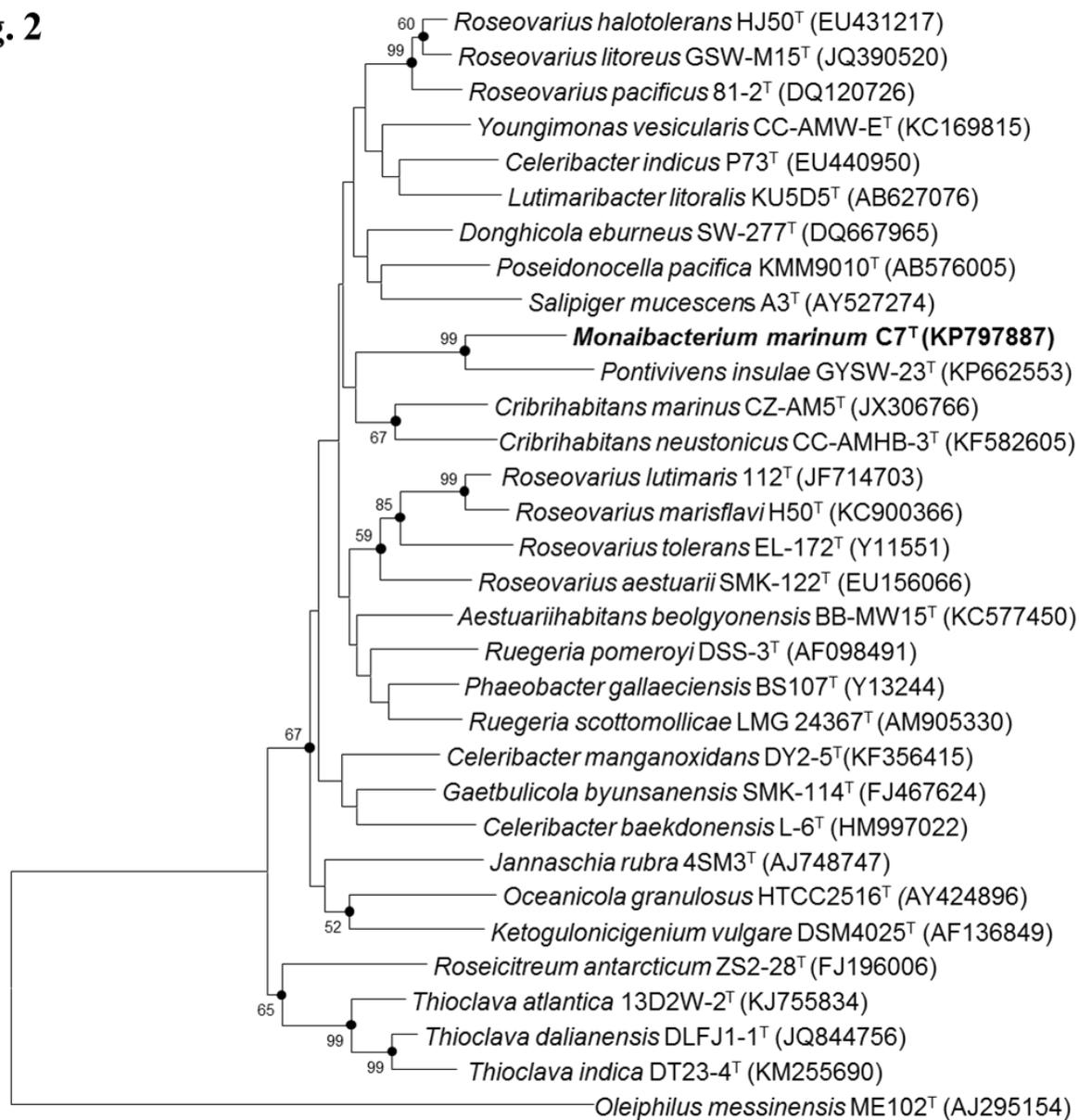
Tween 80	-	-	-	+	-	nd	-	+
Gelatine	-	-	-	-	-	+	-	w
Indole production	-	nd	nd	-	-	-	-	nd
Utilization of:								
L-Arabinose	-	-	-	+	-	+	nd	-
D-Mannose	-	-	+	+	nd	+	+	-
Accumulation of PHB	+	-	-	-	-	-	-	-
DNA G+C content (mol%)	60.0	60.6	64.8	59.7	62.3	59.0±0.1	60.4	62.7

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

**Fig. 1**



**Fig. 2**



**Fig. 2**

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