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Tristan Barbeyron, Erwann Zonta, Sophie Le Panse, Éric Duchaud, Gurvan Michel. *Alteromonas fortis* sp. nov., a non-flagellated bacterium specialized in the degradation of iota-carrageenan, and emended description of the genus *Alteromonas*. *International Journal of Systematic and Evolutionary Microbiology*, 2019, 69 (8), pp.2514-2521. 10.1099/ijsem.0.003533 . hal-02353973

HAL Id: hal-02353973

<https://hal.science/hal-02353973v1>

Submitted on 7 Nov 2019

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1 ***Alteromonas fortis* sp. nov., a non-flagellated bacterium specialized in the degradation**
2 **of iota-carrageenan, and emended description of the genus *Alteromonas***

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4

5 **Running Title:** *Alteromonas fortis* sp. nov.

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12 **Keywords:** *Alteromonas fortis*; *Alteromonadaceae*; iota-carrageenase;

13 **Abbreviations:** ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization;
14 GGDC, Genome-Genome Distance Calculator; ML, maximum-likelihood; MP, maximum-
15 parsimony; NJ, neighbour-joining.

16 **Subject category:** New Taxa: *Proteobacteria*

17

18 The Genbank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 1^T is
19 MK007076.

20 The Genbank/EMBL/DDBJ accession number for the genome sequence of strain 1^T is
21 ERS2860007.

22 Four supplementary figures and two supplementary tables are available in the online version
23 of this article.

24

25 **Abstract**

26 Strain 1^T, isolated in the seventies from the thallus of the carrageenophytic red algae *Eucheuma*
27 *spinosum* collected in Hawaii, USA, was retrospectively characterized using phenotypic,
28 phylogenetic and genomic methods. Bacterial cells were Gram-stain-negative, strictly aerobic,
29 non-flagellated, coccoid, ovoid or rod-shaped, and grew at 10-42 °C (optimum 20-25 °C), at
30 pH 5.5-10 (optimum pH 6-9) and with 2-12 % NaCl (optimum 2-4 %). Strain 1^T grew on the
31 seaweed polysaccharides ι-carrageenan, laminarin and alginic acid as sole carbon sources. The
32 major fatty acids (>10 %) were C_{16:0}, C_{18:1 ω7c} and summed feature 3 (C_{16:1 ω7c} and/or iso-C_{15:0}
33 2OH) and significant amounts of C_{16:0} N alcohol (6.7 %) and 10 methyl C_{17:0} (8.6 %) were also
34 present. The only respiratory quinone was Q-8, and major polar lipids were
35 phosphatidylethanolamine, phosphatidylglycerol and an unknown aminolipid. Phylogenetic
36 analyses based on 16S rRNA gene sequence comparisons showed that the bacterium is affiliated
37 to the genus *Alteromonas* (family *Alteromonadaceae*, class *Gammaproteobacteria*). Strain 1^T
38 exhibits 16S rRNA gene sequence similarity values of 98.8-99.2 % to the type strains of
39 *Alteromonas mediterranea* and *Alteromonas australica* respectively, and of 95.7-98.6 % to
40 those of the other species of the genus *Alteromonas*. The DNA G+C content of strain 1^T is 43.9
41 mol%. Based on the genome sequence of strain 1^T, DNA-DNA hybridization predictions by the
42 average nucleotide identity (ANI) and Genome-to-Genome Distance Calculations (GGDC)
43 between strain 1^T and other members of the genus *Alteromonas* showed values of 70-80 %, and
44 below 26 %, respectively. The phenotypic, phylogenetic and genomic analyses show that strain
45 1^T is distinct from species of the genus *Alteromonas* with validly published names and that it
46 represents a novel species of the genus *Alteromonas*, for which the name *Alteromonas fortis* sp.
47 nov. is proposed. The type strain is 1^T (= ATCC 43554^T = CIP XXXX).

48

49 The genus *Alteromonas*, the type genus of the family *Alteromonadaceae* (order *Alteromonadales*,
50 class *Gammaproteobacteria*), was proposed by Baumann et al. [1] with *Alteromonas macleodii*
51 as the type species of the genus. At the time of writing, the genus *Alteromonas* comprises 21
52 validly named species, all isolated from marine environments. In the late seventies, Pr. W.
53 Yaphe and its co-workers (McGill University, Montréal, Quebec, Canada) collected samples of
54 the red alga *Eucheuma spinosum* J. Agardh 1847 (Rhodophyta, Solieriaceae) from Hawaii
55 (USA) in order to isolate carrageenolytic bacterial strains. Strain 1^T was isolated from the
56 surface of *Eucheuma spinosum* thalli [2] for its capacity to produce a ι-carrageenase activity
57 useful to determine the structure of carrageenans [3]. Strain 1^T was deposited in the American
58 Type Culture Collection (ATCC 43554) and its iota-carrageenase was also intensively studied
59 at the molecular, biochemical and structural levels, [4-9] and used as an enzymatic tool for
60 carrageenan structural analyses [10]. Despite all these studies, no taxonomic study of strain 1^T
61 has been undertaken yet. Here, we present a detailed taxonomic investigation of strain 1^T using
62 a polyphasic approach, including some genomic data deduced from its complete genome and
63 also techniques of whole-genome comparison such as the Average Nucleotide Identity (ANI)
64 and dDDH (digital DNA–DNA hybridization) that have been shown to yield data similar to
65 those of traditional DNA–DNA hybridization method [11, 12, 13].

66 For comparison, *Alteromonas australica* H17^T = CIP 109921^T [14] and *Alteromonas*
67 *mediterranea* DE^T = CIP 110805^T [15] were purchased from the Collection de l'Institut Pasteur
68 (CIP; France) and used as reference strains; they were grown and studied in parallel with strain
69 1^T in all phenotypic tests and in quinone, fatty acid and polar lipid analyses. The three strains
70 were routinely cultivated on ZoBell medium 2216E [16], either liquid or solidified with 1.5 %
71 (w/v) agar. Pure cultures were stored at -80 °C in the culture medium containing 20 % (v/v)
72 glycerol. All experiments were performed in triplicate. Growth was evaluated in ZoBell broth
73 at 4, 9, 11, 12, 13, 15, 17, 18, 20, 22, 24, 30, 37, 40, 42, 45 and 48 °C. The optimal pH value

74 for growth was determined at 20 °C in ZoBell broth which pH values had been adjusted by
75 using 50 mM of the following buffers: MES for pH 5.5; Bis Tris for pH 6.0, 6.5, 7.0 and 7.5;
76 Tris for pH 8.0, 8.5 and 9.0; CHES for pH 9.5 and CAPS for pH 10.0, 10.5 and 11.0. The effect
77 of NaCl on growth was determined at 20 °C and at pH 8 in ZoBell broth prepared with distilled
78 water containing 0, 0.5, 1.0, 2.0, 3.0, 6.0, 8.0, 10, 12, 15, 20, 25 and 30 % NaCl. To test the
79 influence of other salts on growth, the same NaCl range was used in ZoBell broth prepared with
80 artificial seawater without NaCl but containing 6.3 g MgSO₄ l⁻¹, 4.2 g MgCl₂ l⁻¹, and 0.7 g KCl
81 l⁻¹.

82 Cell morphology and motility were investigated on wet mounts of an exponential phase ZoBell
83 broth culture at 20 °C, by using phase-contrast microscopy Olympus BX60 (Olympus, Tokyo,
84 Japan). The presence of flagella was determined using transmission electron microscopy. For
85 negative staining, samples were sedimented onto formvar grids for 10 minutes. They were
86 stained 2 minutes in uranyl acetate 2 % and washed in distilled water. Grids were dried and
87 examined using a JEOL JEM61400 transmission electron microscopy (Jeol, Tokyo, Japan). The
88 Gram Stain Set S kit (BD Difco) and the Ryu non-staining KOH method [17] were used for
89 testing the Gram reaction.

90 Oxidase activity was assayed using small pieces of 3MM paper (Whatman) soaked of the
91 reagent N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (BioMérieux). Catalase
92 activity was assayed by mixing one colony from a ZoBell agar plate with a drop of hydrogen
93 peroxide (3 %, v/v). Amylase activity was assayed on 0.2 % (w/v) soluble starch ZoBell agar
94 plates. DNase activity was detected on DNA agar (Difco) prepared with seawater. Amylase and
95 DNase activities were revealed by flooding the plates with Lugol's solution or 1 M HCl,
96 respectively. Tween compounds (1 %, v/v) was assayed in ZoBell agar according to Smibert &
97 Krieg [18]. Alginate lyase, agarase, κ-carrageenase and ι-carrageenase activities were tested by
98 inoculating ZoBell media solidified with (per litre): 50 g sodium alginate (Sigma-Aldrich, ref.

99 180947), 15 g agar (Sigma-Aldrich, ref. A7002), 10 g κ -carrageenan (X-6913; Danisco) or 20
100 g ι -carrageenan (X-6905; Danisco) respectively. Strains were considered positive when
101 colonies liquefied or produced craters in the solidified substrate. Additional phenotypic
102 characterizations were performed using API 20 E, API 20 NE, API 50CH and API ZYM strips
103 according to the manufacturer instructions (bioMérieux) except that API AUX medium and
104 API 50 CHB/E medium were adjusted to 2.5 % NaCl. All strips were inoculated with cell
105 suspensions in artificial seawater and incubated at 25 °C for 48 h. The ability to use
106 carbohydrates as sole carbon and energy sources was also tested in marine minimal medium
107 [19] supplemented with 250 mg casaminoacid l⁻¹ and containing 5 g l⁻¹ of the following sugars
108 (all from Sigma-Aldrich unless otherwise stated): D-glucose, D-galactose, D-fructose, L-
109 rhamnose, L-fucose, D-xylose, sucrose, lactose, maltose, melibiose, mannitol, glycerol, starch
110 (Fluka), amylopectin (Merck), pullulan, arabinan, dextran, sulfate dextran, xylan, xanthan,
111 pectin (apple), agar, agarose (Eurogentec), laminarin (Goëmar), alginic acid, ν -carrageenan
112 (Danisco), ι -carrageenan, μ -carrageenan (Goëmar), κ -carrageenan, λ -carrageenan (Danisco),
113 sulphated fucoidan (extracted from *Pelvetia canaliculata*) and ulvan (kindly provided by Pr.
114 Bruno Moerschbacher, University of Münster, Germany).

115 Sensitivity to antibiotics was tested by the disc-diffusion method on ZoBell agar plates and
116 using antibiotic discs (Bio-Rad) containing (μ g per disc, unless otherwise stated): ampicillin
117 (10), penicillin G (10 IU), gentamicin (10), kanamycin (30), streptomycin (10) neomycin (30
118 IU), tetracycline (30), erythromycin (15), rifampicin (5), chloramphenicol (30) and nalidixic
119 acid (30). The effects of the antibiotics on cell growth were assessed after 3 days incubation at
120 20 °C, and susceptibility was scored on the basis of the distance from the edge of the clear zone
121 to the disc.

122 Analysis of respiratory quinones [20, 21], fatty acids [22, 23] and polar lipids [24; 25] of strain
123 1^T, *A. australica* H17^T and *A. mediterranea* DE^T were carried out by the Identification Service
124 and Dr. Brian Tindall, DSMZ, Braunschweig, Germany.

125 Genomic DNA from strain 1^T was extracted as described by Barbeyron et al. [26] and purified
126 on a caesium chloride gradient. The genome of strain 1^T was sequenced using Illumina
127 (GAIIx, 2 x 74 paired-end reads with 300 bp insert size) reads. The 22,629,503 sequence
128 reads were assembled in 37 contigs (>1 kb, coverage > 70X) using Velvet [27]. Contigs were
129 ordered using an optical map (Argus system, OpGene, Maryland, USA) and the *SpeI*
130 restriction enzyme [28], and most of the gaps were filled using PCR and Sanger sequencing.
131 The Velvet contigs and the Sanger reads were assembled using the whole-genome shotgun
132 assembler Phrap and the assembly was visualized with the interface Consed [29]. The final
133 assembly consists of 1 scaffold (7 ordered contigs) validated using the optical map. The 16S
134 rRNA gene sequence was amplified by PCR using pure genomic DNA as template and the
135 couple of primers specific for Bacteria, 8F [30] and 1492R [31]. PCR reactions were typically
136 carried out in a volume of 25 µl containing 10-100 ng template, 0.4 µM each specific primer,
137 250 µM each dNTPs, 0.1 mg BSA, 1X GoTaq buffer (Promega) and 1.25 U GoTaq DNA
138 polymerase (Promega). The different polymerization steps were as previously described [32].
139 PCR products were purified using the Exostar kit according to the manufacturer's protocol
140 (GE Healthcare) and sequenced by using BigDye Terminator V3.1 (Applied Biosystems), and
141 an ABI PRISM 3130xl Genetic Analyzer automated sequencer (Applied Biosystems/Hitachi).
142 Chargaff's coefficient of the genomic DNA, expressed as the molar percentage of guanine +
143 cytosine, of strain 1^T were deduced from the complete genome sequence. The nucleotide
144 sequence of 16S rRNA gene deduced from the complete genome sequence of the strain 1^T and
145 sequence of 16S rRNA genes from all valid species of the genus *Alteromonas* were aligned
146 using the software MAFFT version 7 with the L-INS-I strategy [33] and the alignment was

147 then manually refined. Phylogenetic analysis using the neighbour-joining [34], maximum-
148 parsimony [35] and maximum-likelihood [36] methods were applied, as implemented in the
149 MEGA 5.1 package [37]. The different phylogenetic trees were built from a multiple
150 alignment of 55 sequences and 1444 positions. For the neighbour-joining algorithm, the
151 evolutionary model Kimura Two Parameters [38] was used. The maximum-likelihood tree
152 was calculated using the evolutionary model GTR (Generalised Time Reversible) [39] with a
153 discrete Gamma distribution to model evolutionary rate difference among sites (4 categories).
154 This substitution model was selected among other models submitting the alignment to the
155 online server IQ-TREE (<http://iqtree.cibiv.univie.ac.at/>). The maximum-parsimony tree was
156 obtained using the Subtree-Pruning-Regrafting algorithm [39]. Bootstrap analysis was
157 performed to provide confidence estimates for the phylogenetic tree topologies [40]. Pairwise
158 comparisons of 16S rRNA gene sequences were made by using the database EzBioCloud
159 (<https://www.ezbiocloud.net/identify>) [41] and the FASTA software [42]. Genomic
160 relatedness was investigated by comparing the strain 1^T genome sequence with those of the
161 type strains of other *Alteromonas* species using the Average Nucleotide Identity (ANI;
162 <http://jspecies.ribohost.com/jspeciesws/#analyse>) [11, 12, 13] and the dDDH via the online
163 server Genome to Genome Distance Calculator 2.1 (GGDC;
164 <http://ggdc.dsmz.de/distcalc2.php>) [43]. The results from GGDC analysis were obtained from
165 the alignment method Blast+ and the formula 2 (sum of all identities found in HSPs / by
166 overall HSP length) for incomplete genome sequences [44, 45]. Exploration of carbohydrate
167 active enzyme-coding genes in the genomes of the strains 1^T, *A. australica* H17^T and *A.*
168 *mediterranea* DE^T was carried out via the online server Microscope from the French National
169 Sequencing Center (<http://www.genoscope.cns.fr/agc/microscope/mage>) and the database
170 CAZy (www.cazy.org) [46].

171 The best pairwise comparison scores with 16S rRNA gene from the strain 1^T (1521 bp) were
172 obtained with *Alteromonas mediterranea* DE^T (99.2 %) and *A. australica* H17^T (98.8 %; Table
173 S1, available in the online version of this article). Phylogenetic analyses of 16S rRNA genes
174 from species of the family *Alteromonadaceae* showed that strain 1^T was only distantly related
175 to *Alteromonas* species and that *A. australica* H17^T and *A. mediterranea* DE^T formed a clade
176 with strain 1^T (Figs 1 and S1). The 16S rRNA gene sequence similarities between the strain 1^T
177 and other *Alteromonas* species were in the range of 95.2 % with *A. pelagimontana* 5.12^T [47]
178 and 98.6 % with *A. hispanica* F-32^T [48] (Table S1). The complete genome of strain 1^T was
179 sequenced to evaluate its potentiality for degradation. The genome was composed of 4,664,520
180 nucleotides. The ANI and GGDC values for strain 1^T when compared with other *Alteromonas*
181 species were less than 83 % and less than 30 % respectively (82.9 % and 27.2 % with *A.*
182 *macleodii* 107^T; Table S2). As the normally accepted thresholds of species delineation for ANI
183 and GGDC are 95 % and 70%, respectively [11, 13, 49, 50], these values suggest that strain 1^T
184 represents a new species of the genus *Alteromonas*. In addition, a pairwise genome comparison
185 with other *Alteromonas* species showed that *A. stellipolaris* ANT 69a^T [51] and *A. addita*
186 R10SW13^T [52] may represent the same species since ANI and GGDC (formula 2) values
187 between them were 98.7 and 90.1 %, respectively (Table S2).

188 Cells of strain 1^T were Gram-stain-negative, irregular-shaped short rods or coccoids (Fig S2).
189 In contrast with *Alteromonas australica* H17^T and *A. mediterranea* DE^T which showed an active
190 motility with one polar flagellum (Fig. S2), strain 1^T was non-motile and devoid of flagella
191 (Fig. S2). The absence of flagella and of motility was already reported for *A. confluentis*
192 DSSK2-12^T [53]. So far, strain 1^T and *A. confluentis* DSSK2-12^T are the only two *Alteromonas*
193 species that share this characteristic.

194 Growth was observed with many carbohydrates and a few sugars allowed to differentiate strain
195 1^T from *A. australica* H17^T and *A. mediterranea* DE^T. However, some polysaccharides from

196 marine environments, such as ι -carrageenan were exclusively hydrolysed by strain 1^T (Table 1,
197 and Fig. S3). This observation was consistent with the absence of any gene encoding a family
198 82 of glycoside hydrolases (GH82) having ι -carrageenase activity in the genomes of *A.*
199 *australica* H17^T and *A. mediterranea* DE^T, whereas the genome of the strain 1^T contains three
200 GH82-encoding genes. Moreover, some marine polysaccharides could be used as sole carbon
201 source by strain 1^T, such as ν -, ι -, and λ -carrageenans (Table 1) while μ - and κ -carrageenans
202 were not used. None of the three strains studied hydrolyzed κ -carrageenan. Alginic acid was
203 liquefied by the strain 1^T and by *A. australica* H17^T but not by *A. mediterranea* DE^T (Table 1
204 and Fig. S3). This result is consistent with the observations that the genomes of strain 1^T and
205 *A. australica* H17^T possess 7 and 6 genes encoding for alginate lyases respectively, whereas
206 that of *A. mediterranea* DE^T has only 3 genes. Finally, more common polysaccharides such as
207 xylan, pectin, lichenin and glycogen were only used by strain 1^T (Table 1). Although the three
208 strains were able to use starch as sole carbon and energy sources, only strain 1^T showed a
209 hydrolysis area on soluble starch ZoBell agar plates (Fig. S3). The absence of secreted amylase
210 from *A. mediterranea* DE^T was already reported [15]. In contrast, we were not able to confirm
211 the presence of an amylase previously reported from *A. australica* H17^T [14]. Analysis of the
212 genomes of *A. australica* H17^T and *A. mediterranea* DE^T revealed that among the genes
213 encoding for GH13 enzymes involved in the hydrolysis of α -1,4 glucans, no α -amylase-
214 encoding gene was present, whereas strain 1^T genome contains a gene encoding for a secreted
215 α -amylase. The utilisation of starch as sole carbon source by *A. australica* H17^T and *A.*
216 *mediterranea* DE^T could be due to the presence of a pullulanase-encoding gene present in their
217 genome and whose enzyme product is predicted as an outer-membrane-linked lipoprotein.

218 The other physiological growth features of strain 1^T compared with *A. australica* H17^T and *A.*
219 *mediterranea* DE^T are listed in Table 1. The three strains shared the same antibiotics sensitivity
220 profile. The major fatty acids (>10 % of the total fatty acids) of strain 1^T were summed feature

221 3 (21.3 %; C_{16:1}ω7c and/or iso-C_{15:0} 2OH), C_{16:0} (15.2 %) and C_{18:1}ω7c (14.5 %) (Table 2). The
222 fatty acid profile of strain 1^T was similar to those of *A. australica* H17^T and *A. mediterranea*
223 DE^T (Table 2) and conform to previous results [54]; however, strain 1^T showed some
224 differences in its fatty acid composition. The presence of significant amounts of 10 methyl C_{17:0}
225 (8.6 %) and of the fatty alcohols C_{16:0} N alcohol (6.7 %) and C_{16:1}ω7c alcohol (3.3 %) appears
226 to be specific to the strain 1^T since *A. australica* H17^T and *A. mediterranea* DE^T, contain only
227 1 % or less of these components (Table 2). The respiratory quinone of strain 1^T was ubiquinone-
228 8 (Q-8) in line with *A. australica* H17^T and *A. mediterranea* DE^T; the latter strains also contain
229 1 % of Q-7. The major polar lipids of strain 1^T were phosphatidylethanolamine,
230 phosphatidylglycerol and an unknown aminolipid (Fig. S4), in line with *A. australica* H17^T and
231 *A. mediterranea* DE^T and with previously published results for the genus *Alteromonas* [14; 54].
232 In conclusion, phenotypic characterizations and phylogenetic analysis using 16S rRNA gene
233 sequences together with whole-genome pairwise comparisons show that strain 1^T represents a
234 novel species in the genus *Alteromonas*, for which the name *Alteromonas fortis* sp. nov. is
235 proposed.

236 **EMENDED DESCRIPTION OF THE GENUS *ALTEROMONAS* BAUMANN *ET AL.***
237 **1972 (APPROVED LISTS 1980) EMEND. GAUTHIER *ET AL.* 1995, VAN TRAPPEN**
238 ***ET AL.* (2004)**

239 The description of the genus is as given by Gauthier et al. [55] and by Van Trappen et al. [51]
240 with the following additional morphological feature. Motile by means of a single unsheathed
241 polar flagellum or non-motile and non-flagellated.

242 **DESCRIPTION OF *ALTEROMONAS FORTIS* SP. NOV.**

243 *Alteromonas fortis* (for'tis. L. masc. adj. *fortis*, strong).

244 Cells are Gram-reaction-negative, aerobic, chemoorganotrophic, heterotrophic, non-motile and
245 ovoid or rod-shaped, approximately 0.8–1.4 µm in diameter and 0.8-2.5 µm in length; a few
246 cells greater than 4 µm in length may occur. Flagella are absent. Prosthecae and buds are not
247 produced. Colonies on ZoBell agar are cream-coloured, convex, circular and mucoid in
248 consistency and 2.0–3.0 mm in diameter after incubation for 24 hours at 30 °C. Growth in
249 ZoBell broth occurs at 10 and 42 °C (optimum, 25 °C), at pH 5.5 to 10 (optimum pH 6.0-9.0)
250 and in the presence of 2 to 12 % NaCl (optimum, 2-4 %) In the presence of Mg and KCl, the
251 growth also occurs with 0 and 1 % NaCl. Nitrate is not reduced. Oxidase- and catalase-positive.
252 DNA, aesculin, gelatin, starch, ι-carrageenan, alginic acid and Tween 20, 40, 60 and 80 are
253 hydrolysed, but agar and κ-carrageenan are not. D-glucose, D-galactose, D-fructose, D-ribose,
254 sucrose, D-turanose, D-maltose, D-lactose, melibiose, cellobiose, raffinose, glycerol, D-
255 mannitol, methyl-α-D-glucoside, laminarin, starch, amylopectin, pullulan, glycogen, galactan
256 (gum arabic), pectin (apple), xanthan, ν-carrageenan, ι-carrageenan, arbutin and salicin are
257 utilized as carbon and energy sources, but L-rhamnose, L-sorbose, D-arabinose, L-arabinose,
258 D-xylose, L-xylose, D-fucose, L-fucose, D-lyxose, D-tagatose, melezitose, erythritol, D-
259 arabitol, L-arabitol, dulcitol, inositol, D-sorbitol, xylitol, adonitol, methyl-α-D-mannoside,
260 methyl-β-D-xyloside, *N*-acetyl-glucosamine, inulin, dextran, arabinan, agar, agarose, μ-
261 carrageenan, κ-carrageenan, ulvan, sulfated fucoidan and gluconate are not. Acid is produced
262 from D-glucose (weakly), D-galactose, D-fructose, D-maltose, D-lactose, melibiose, sucrose,
263 D-mannitol, methyl-α-D-glucoside, salicin, D-cellobiose, starch, glycogen, but not from D-
264 mannose, L-sorbose, L-rhamnose, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-
265 lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, glycerol, erythritol, dulcitol,
266 inositol, sorbitol, adonitol, xylitol, methyl-α-D-mannoside, methyl-β-D-xyloside, *N*-acetyl-
267 glucosamine, D-turanose, melezitose, raffinose and inulin. Negative for indole and H₂S
268 production and for arginine dihydrolase, tryptophan deaminase, urease, lysine decarboxylase,

269 ornithine decarboxylase activities. In the API ZYM system, activity from acid phosphatase,
270 alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine
271 arylamidase, trypsin, α -chymotrypsin, naphthol-AS-BI- phosphohydrolase, α -galactosidase, β -
272 galactosidase, α -glucosidase, and β -glucosidase activities are present. but activity from α -
273 mannosidase, α -fucosidase, β -glucuronidase, and N-acetyl- β -glucosaminidase activities are
274 absent. Resistant to (mg per disc) gentamycin (10), kanamycin (30), penicillin (6), ampicillin
275 (10) and tetracycline (30). The only ubiquinone detected is Q-8. The major fatty acids (>10 %
276 of the total fatty acids) are summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2OH), C_{16:0} and
277 C_{18:1} ω 7c. Significant amounts of 10 methyl C_{17:0}, C_{16:0} N alcohol and C_{16:1} ω 7c alcohol are also
278 present. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and an
279 unknown aminolipid. The DNA G+C content of the type strain 1^T is 43.9 mol%.

280 The type strain is 1^T (=ATCC 43554^T), isolated in the late seventies from decomposing marine
281 red algae *Eucheuma spinosum* J. Agardh 1847 in Hawaii, USA.

282 **Acknowledgements**

283 This work has benefited from the facilities and expertise of the high-throughput sequencing
284 platforms of i2bc (Centre de Recherche de Gif-sur-Yvette, <http://www.i2bc.paris-saclay.fr/>)
285 and we especially wish to acknowledge Ghislaine Magdelenat and Valérie Barbe of the
286 CEA/GENOSCOPE (<http://www.genoscope.cns.fr/>) for the optical map. We acknowledge the
287 LABGeM (CEA/Genoscope & CNRS UMR8030), the France Génomique and French
288 Bioinformatics Institute national for support within the MicroScope annotation platform.
289 Finally, the authors wish to express their gratitude to Dr Simon Dittami for the 16S rRNA gene
290 amplification and Dr. Jean-François Bernardet and Dr. Elisabeth Ficko-Blean for advice and
291 critical reading of the manuscript.

292 **Funding information**

293 This work benefited from the support of France Génomique and French Bioinformatics Institute
294 national infrastructures and from the French Ministry of Research via programs “IDEALG” and
295 “Blue Enzymes”, funded as part of Investissement d’Avenir program by the contracts ANR-10-
296 INBS-09, ANR-11-INBS-0013, ANR-10-BTBR-04 and ANR-14-CE19-0020-01 managed by
297 the National Research Agency.

298 **Conflicts of interest**

299 The authors declare that there are no conflicts of interest.

300

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450

Table 1. Phenotypic characteristics of strain 1^T and of two *Alteromonas* species used as reference strains

1, Strain 1^T (*Alteromonas fortis* sp. nov.); 2, *Alteromonas australica* H17^T; 3, *Alteromonas mediterranea* DE^T. The three strains share the following characteristics: Gram-negative, aerobic, heterotroph, chemorganotroph with respiratory metabolism; do not form endospores, do not accumulate poly- β -hydroxybutyrate as an intracellular reserve product; require Na⁺ ion or seawater for growth. All strains are positive for the utilization of D-glucose (*A. fortis* 1^T weakly positive), D-galactose, D-fructose, sucrose, maltose, lactose, melibiose, cellobiose, starch, pullulan, laminarin, glycerol, mannitol, and aesculine; for the hydrolysis of Tweens 20, 40, 60 and 80; and for DNase, gelatinase, alkaline and acid phosphatases, lipase esterase (C8), leucine, valine and cystine arylamidases, trypsin, α -glucosidases, β -galactosidases, oxidase and catalase activities All strains are negative for the utilization of L-rhamnose, L-sorbose, D- and L-arabinose, D- and L-xylose, D- and L-fucose, erythritol, lyxose, tagatose, arabitol, dulcitol, inositol, D-sorbitol, xylitol, adonitol and gluconate; for the hydrolysis of melezitose, inulin, arabinan, dextran, sulfate dextran, agar, agarose and μ - and κ -carrageenan; for α -mannosidase, α -fucosidase, arginine dihydrolase, lysine and ornithine decarboxylases, tryptophan deaminase, urease, β -glucuronidase, and *N*-acetyl- β -glucosaminidase activities; and for indole reaction and H₂S production. +, Positive; -, negative; W, weakly positive.

Characteristic	1	2	3
Growth			
Temp optimum, (°C)	25	NA	NA
Temp range, (°C)	10-42	4-37*	4-40†
pH optimum	6-9	7.5-8*	7.5-8†
pH range	5.5-10	6-10*	6-11†
NaCl, range (%)	2-12	1-10*	1-15†
Flagellar motility	-	+	+
Nitrate reduction	-	+	+
Hydrolysis of			
Alginic acid	+	+	-
ι -Carrageenan	+	-	-
Utilization of			
D-Mannose	+	+	-
Glycogene	+	-	-
Lichenin	+	-	-
Alginic acid	+	+	-
Pectin	+	-	-
Xylan	+	-	-
ν -Carrageenan	+	-	-
ι -Carrageenan	+	-	-
λ -Carrageenan	+	-	-
Enzymes (API ZYM)			
Lipase (C14)	-	+	+
α -Chymotrypsin	+	w	w
α -Galactosidase	+	w	+
Production of diffusible amylase	+	-	-
DNA G+C mol %	43.9	43*	44.9†

*Data from Ivanova *et al.* [14]

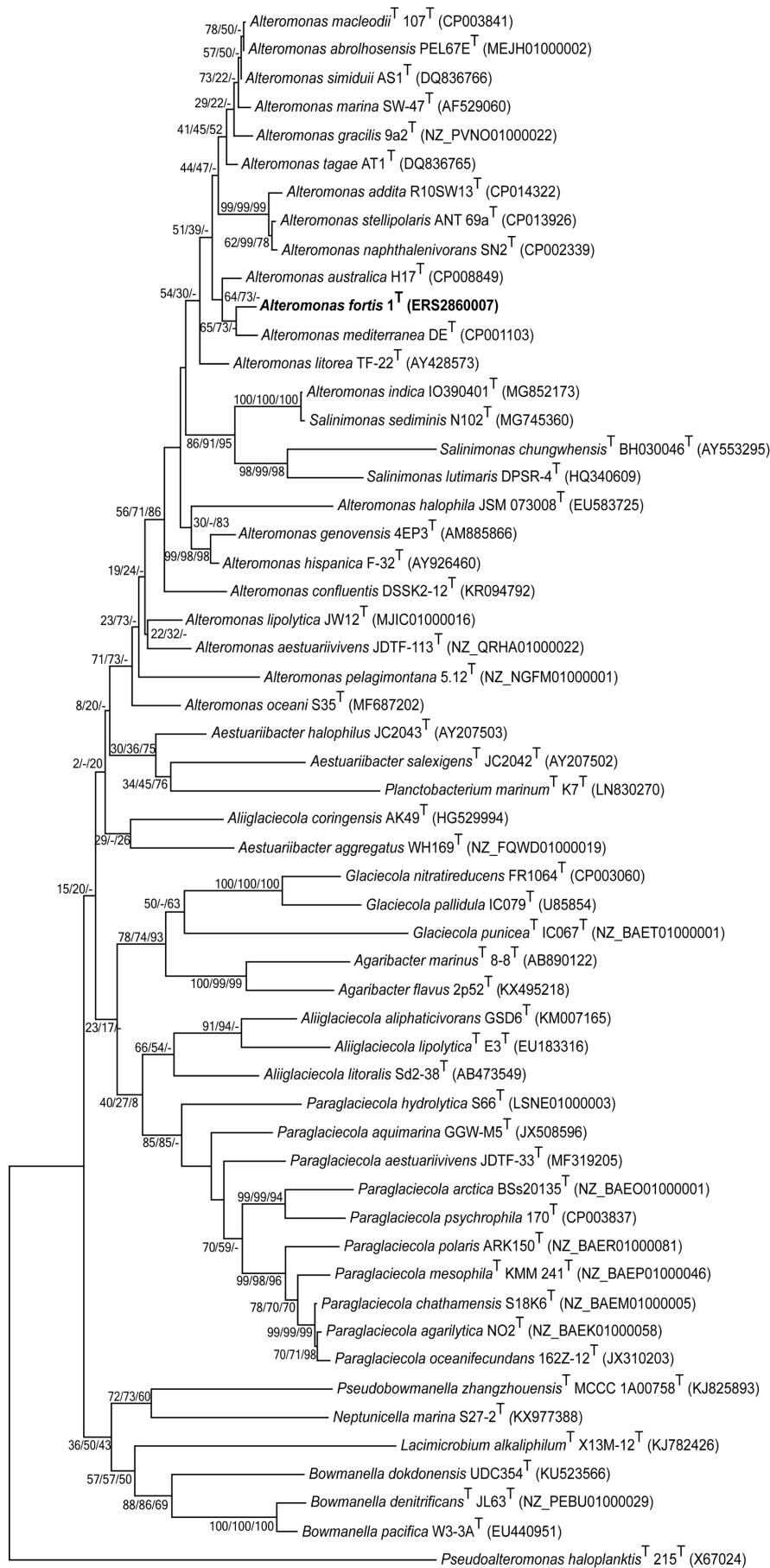
†Data from Ivanova *et al.* [15]

Table 2. Cellular fatty acid composition of strain 1^T and the type strains of two reference strains.

Strains: 1, 1^T (*Alteromonas fortis* sp. nov.); 2, *A. australica* H17^T; 3, *A. mediterranea* DE^T. Data are percentages of the total fatty acids. Fatty acids that represented <1.0 % in the three strains were omitted. Fatty acids that represented >10.0 % are indicated in bold. -, Fatty acids not detected or below 1 %.

Fatty acid	1	2	3
Straight-chain			
C _{12:0}	1.7	1.9	1.9
C _{14:0}	1.8	1.6	1.6
C _{15:0}	2.2	2.8	2.7
C _{16:0}	15.2	15.8	15.7
C _{16:0} N alcohol	6.7	-	-
C _{17:0}	3.3	5.5	5.5
C _{18:0}	-	1.0	1.2
Branched chain			
iso-C _{16:0}	1.0	-	-
anteiso-C _{17:0}	1.0	-	-
10 methyl C _{17:0}	8.6	1.1	1.1
Unsaturated			
C _{15:1} ω8 <i>c</i>	1.7	1.6	1.5
C _{16:1} ω7 <i>c</i> alcohol	3.3	-	-
C _{17:1} ω8 <i>c</i>	7.5	12.7	12.5
C _{18:1} ω7 <i>c</i>	14.5	18.6	18.7
Hydroxy			
C _{10:0} 3-OH	1.1	1.0	1.1
C _{11:0} 3-OH	1.0	1.3	1.3
Summed features*			
2	2.7	2.8	2.9
3	21.3	21.9	21.7

*Summed feature 2 contained C_{14:0} 3OH and/or iso-C_{16:1} I; summed feature 3 contained C_{16:1} ω7*c* and/or iso-C_{15:0} 2OH



0.01

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain 1^T and related taxa from the family *Alteromonadaceae*. Numbers at the nodes refer to the bootstrap values (1000 replicates) in neighbour-joining, maximum-likelihood and maximum-parsimony analyses respectively, while dashes instead of numbers indicate that the node was not observed in the corresponding analysis. For nodes conserved in at least two trees, all bootstrap values are shown. Nodes without bootstrap value are not conserved in other trees and < 70 %. The T letter in superscript after the species epithet indicate the type species of the genus. *Pseudoalteromonas haloplanktis* 215^T was used as an outgroup. Bar, 0.02 changes per nucleotide position.

Supplementary Table S1

Pairwise nucleotide comparisons (in percentage of similarity sequence) between *Alteromonas* species 16S rRNA gene sequences.

	1																						
<i>Alteromonas fortis</i> 1 ^T	1	100																					
<i>A. australica</i> H17 ^T	2	98.8	100																				
<i>A. mediterranea</i> DE ^T	3	99.2	98.8	100																			
<i>A. abrolhosensis</i> PEL67E ^T	4	98.5	99.0	98.1	100																		
<i>A. addita</i> R10SW13 ^T	5	97.4	97.7	97.8	98.1	100																	
<i>A. aestuariivivens</i> JDTF-113 ^T	6	97.6	96.9	97.7	96.9	96.7	100																
<i>A. confluentis</i> DSSK2-12 ^T	7	97.7	96.6	97.7	96.9	96.6	97.5	100															
<i>A. genovensis</i> 4EP3 ^T	8	98.5	97.2	98.0	97.2	97.2	97.2	97.2	100														
<i>A. gracilis</i> 9a2 ^T	9	98.1	98.7	98.3	99.4	98.0	96.9	97.1	97.4	100													
<i>A. halophila</i> JSM 073008 ^T	10	96.8	95.5	96.2	95.4	95.5	96.2	96.2	96.7	95.5	100												
<i>A. hispanica</i> F-32 ^T	11	98.6	97.6	98.3	97.5	97.6	97.3	97.4	99.1	97.6	97.0	100											
<i>A. indica</i> IO390401 ^T	12	96.1	97.0	96.5	96.7	97.4	95.8	95.4	96.2	97.1	94.8	96.5	100										
<i>A. lipolytica</i> JW12 ^T	13	98.0	97.0	97.8	97.1	96.5	98.5	98.4	97.2	97.3	96.0	97.2	95.5	100									
<i>A. litorea</i> TF-22 ^T	14	98.3	97.7	98.7	98.3	97.5	97.7	98.1	98.1	98.5	96.4	98.1	96.8	97.8	100								
<i>A. macleodii</i> 107 ^T	15	98.1	98.7	97.9	99.8	98.2	96.9	97.0	97.2	99.4	95.4	97.4	96.8	97.2	98.2	100							
<i>A. marina</i> SW-47 ^T	16	98.3	98.5	98.3	99.6	97.9	97.1	97.4	97.5	99.1	95.7	97.7	96.5	97.4	98.6	99.2	100						
<i>A. naphthalenivorans</i> SN2 ^T	17	97.5	97.9	97.5	98.4	99.4	96.4	96.3	97.4	98.1	95.3	97.7	97.2	96.3	97.4	98.2	98.1	100					
<i>A. oceani</i> S35 ^T	18	97.4	97.0	97.8	96.7	96.6	98.1	97.2	96.5	96.9	95.3	96.9	95.7	98.6	97.3	96.8	96.9	96.3	100				
<i>A. pelagimontana</i> 5.12 ^T	19	95.2	94.3	95.0	94.3	94.4	95.7	95.1	94.8	94.7	94.4	95.2	95.4	95.6	95.0	94.0	94.3	94.1	96.5	100			
<i>A. simiduii</i> AS1 ^T	20	97.1	97.0	96.7	98.0	96.6	95.3	95.9	96.2	97.6	94.6	96.3	94.9	96.1	97.0	98.1	98.2	96.9	96.1	93.8	100		
<i>A. stellipolaris</i> ANT 69a ^T	21	97.5	97.8	97.8	98.3	99.7	96.6	96.7	97.2	98.0	95.5	97.7	97.3	96.7	97.7	98.1	98.1	99.7	96.6	94.4	98.4	100	
<i>A. tagae</i> AT1 ^T	22	96.9	96.6	97.0	97.7	96.5	96.1	96.3	96.3	97.2	94.7	96.4	94.0	96.1	97.5	97.2	98.0	96.8	94.6	93.6	97.8	96.8	100

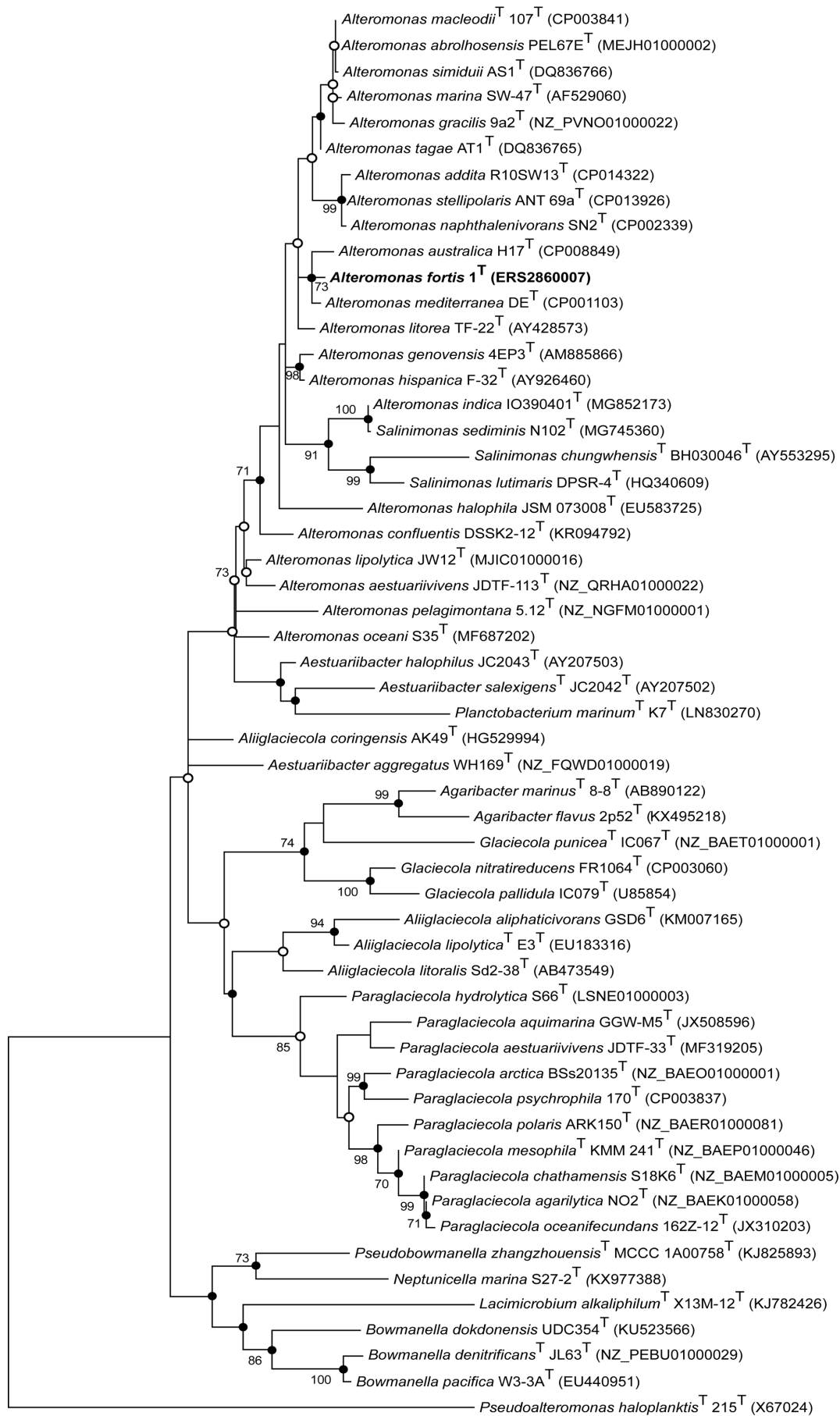
Supplementary Table S2

Whole genome relatedness analysis (in percentage) of strain 1^T and *Alteromonas* species which whole genome sequences are available.

Average nucleotide identities (ANI) are shown below the diagonal and genome-to-genome distance calculations (GGDC) are shown below the diagonal. GGDC values were calculated online (<http://ggdc.dsmz.de/distcalc2.php>) and the results from formula 2 were used. ANI values were calculated with an online program (<http://jspecies.ribohost.com/jspeciesws/#analyse>). The GenBank accession numbers for the whole genome sequences are given in the top row.

		GGDC												
		<i>Alteromonas fortis</i> 1 ^T	<i>A. australica</i> H17 ^T	<i>A. mediterranea</i> DE ^T	<i>A. abrolhosensis</i> PEL67E ^T	<i>A. addita</i> R10SW13 ^T	<i>A. aestuariivivens</i> JDTF-113 ^T	<i>A. confluentis</i> DSSK2-12 ^T	<i>A. gracilis</i> 9a2 ^T	<i>A. lipolytica</i> JW12 ^T	<i>A. macleodii</i> 107 ^T	<i>A. naphthalenivorans</i> SN2 ^T	<i>A. pelagimontana</i> 5.12 ^T	<i>A. stellipolaris</i> ANT 69a ^T
Accession number		ERS2860007	CP008849	CP001103	MEJH01000002	CP014322	QRHA00000000	MDHN01000001	PVNO00000000	MJIC01000001	CP003841	CP002339	NGFM01000001	CP013926
ANI	<i>Alteromonas fortis</i> 1 ^T		22.10	24.60	26.10	21.30	20.30	20.80	23.90	21.70	27.20	21.30	19.80	21.50
	<i>A. australica</i> H17 ^T	73.88		23.50	21.90	20.30	19.80	19.20	21.60	21.70	22.00	20.50	18.20	20.50
	<i>A. mediterranea</i> DE ^T	80.91	74.65		24.30	21.30	20.60	21.80	23.60	23.30	24.30	22.20	22.30	21.20
	<i>A. abrolhosensis</i> PEL67E ^T	82.18	73.87	80.31		20.80	20.40	21.10	24.40	21.60	36.30	21.20	19.50	20.80
	<i>A. addita</i> R10SW13 ^T	73.44	73.31	73.47	73.49		20.90	21.30	21.20	22.40	21.00	39.30	19.40	90.10
	<i>A. aestuariivivens</i> JDTF-113 ^T	69.92	69.55	70.0	69.78	69.52		19.50	21.40	20.20	20.80	20.70	18.90	20.90
	<i>A. confluentis</i> DSSK2-12 ^T	69.99	69.46	70.40	69.93	69.72	70.49		21.20	21.30	21.80	20.90	18.60	21.40
	<i>A. gracilis</i> 9a2 ^T	80.26	73.69	80.00	80.52	73.35	70.09	69.96		24.00	24.80	21.20	20.30	21.20
	<i>A. lipolytica</i> JW12 ^T	69.61	69.12	69.60	69.50	69.19	69.95	69.90	69.40		22.50	24.30	20.00	23.00
	<i>A. macleodii</i> 107 ^T	82.93	73.64	80.41	88.40	73.38	69.90	69.92	80.71	69.47		21.20	19.90	21.10
	<i>A. naphthalenivorans</i> SN2 ^T	73.59	73.59	73.92	73.59	89.62	69.59	69.68	73.25	69.13	73.48		21.00	39.20
	<i>A. pelagimontana</i> 5.12 ^T	69.92	69.51	70.44	69.72	69.81	70.49	70.05	69.81	69.22	69.72	70.17		19.30
	<i>A. stellipolaris</i> ANT 69a ^T	73.59	73.54	73.52	73.56	98.74	69.68	69.73	73.26	69.21	73.48	89.64	69.75	

The percentages in bold indicate the values above the thresholds of species boundary (95 % for ANI and 70 % for GGDC).

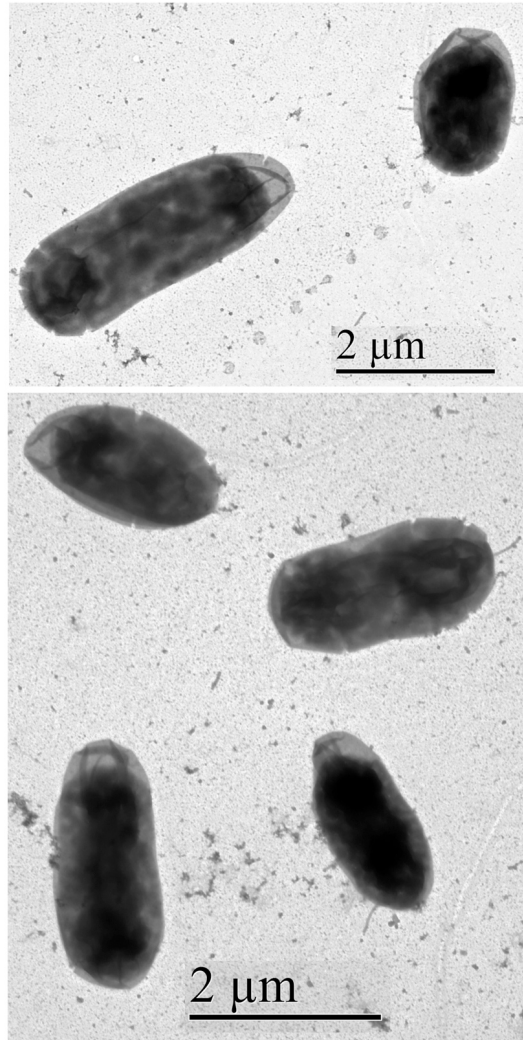


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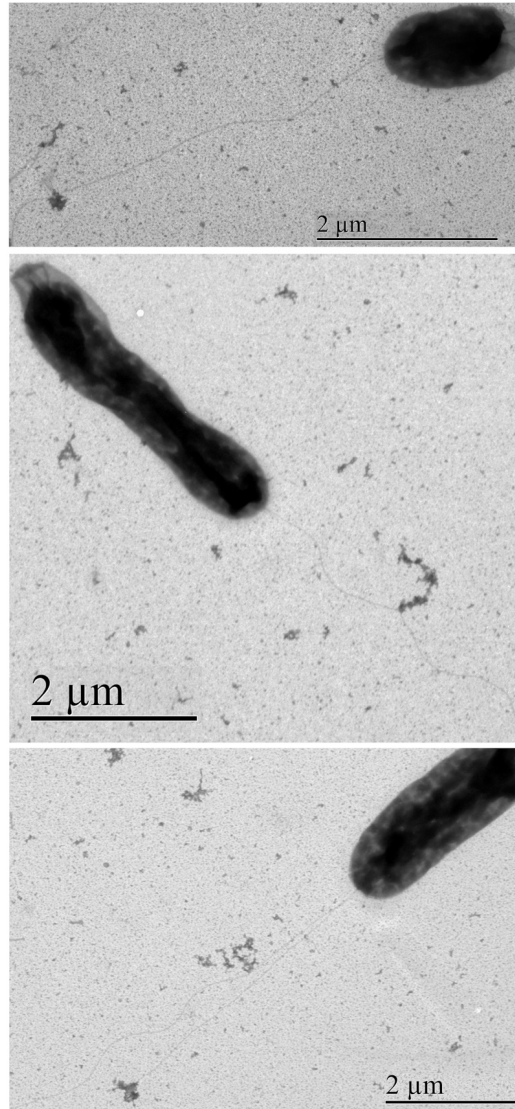
Supplementary Fig. S1

Maximum-likelihood (ML) tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain 1^T and related taxa from the family *Alteromonadaceae*. Numbers at nodes are bootstrap values shown as percentages of 1000 replicates; only values >70 % are shown. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the neighbour-joining (NJ) and maximum-parsimony algorithms, while open circles indicate that the nodes were only recovered in the tree generated with the NJ and ML algorithms. The T letter in superscript after the species epithet indicate the type species of the genus. *Pseudoalteromonas haloplanktis* 215^T was used as an outgroup. Bar, 0.02 changes per nucleotide position.

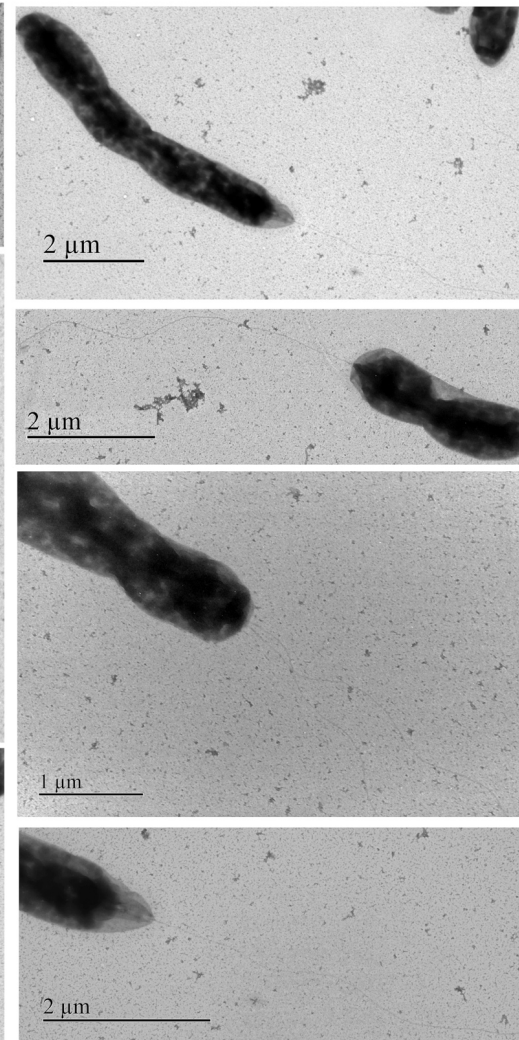
Alteromonas fortis 1^T



Alteromonas australica H17^T

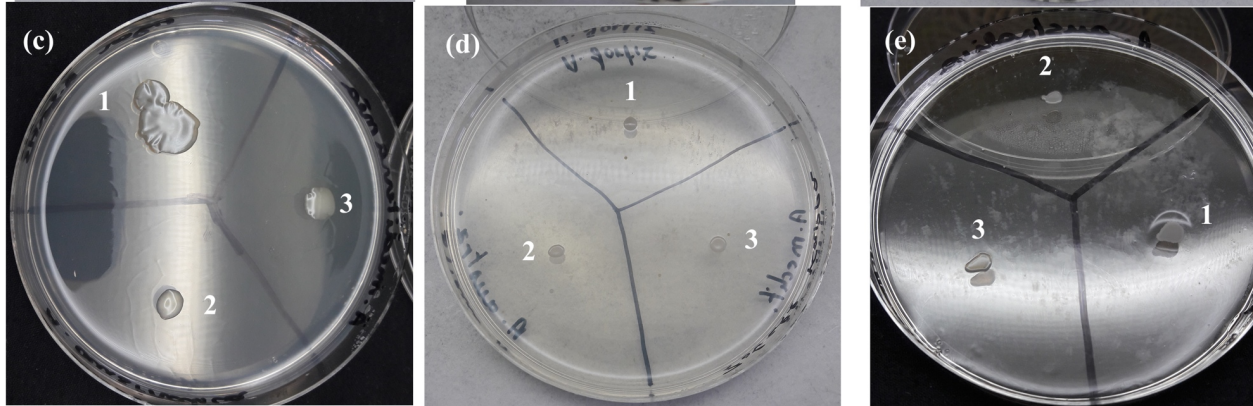
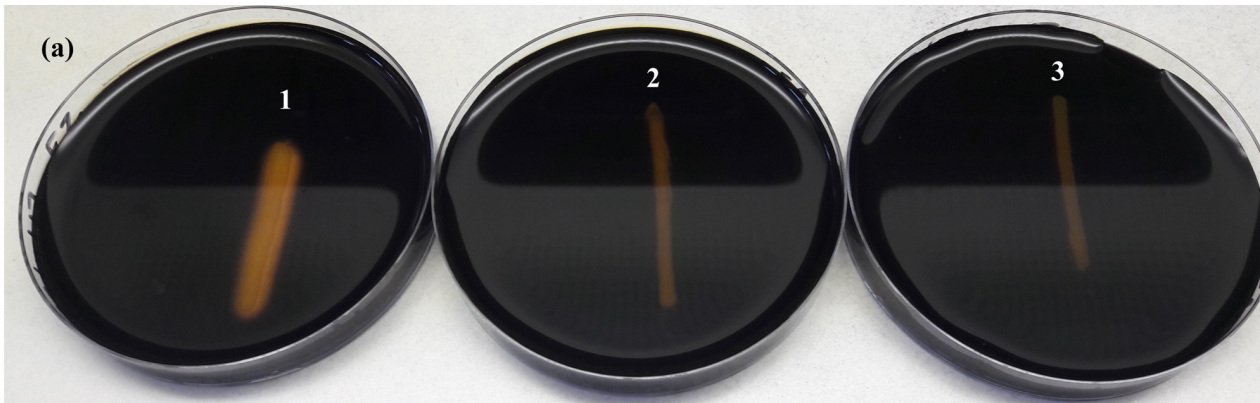


Alteromonas mediterranea DE^T



Supplementary Fig. S2

Transmission electron micrographs of negatively stained cells of strain 1^T (*Alteromonas fortis* sp. nov.), *Alteromonas australica* H17^T and *A. mediterranea* DE^T. Cells of *A. fortis* 1^T are devoid of flagella whereas cells of *A. australica* H17^T and *A. mediterranea* DE^T display one or two polar flagella.



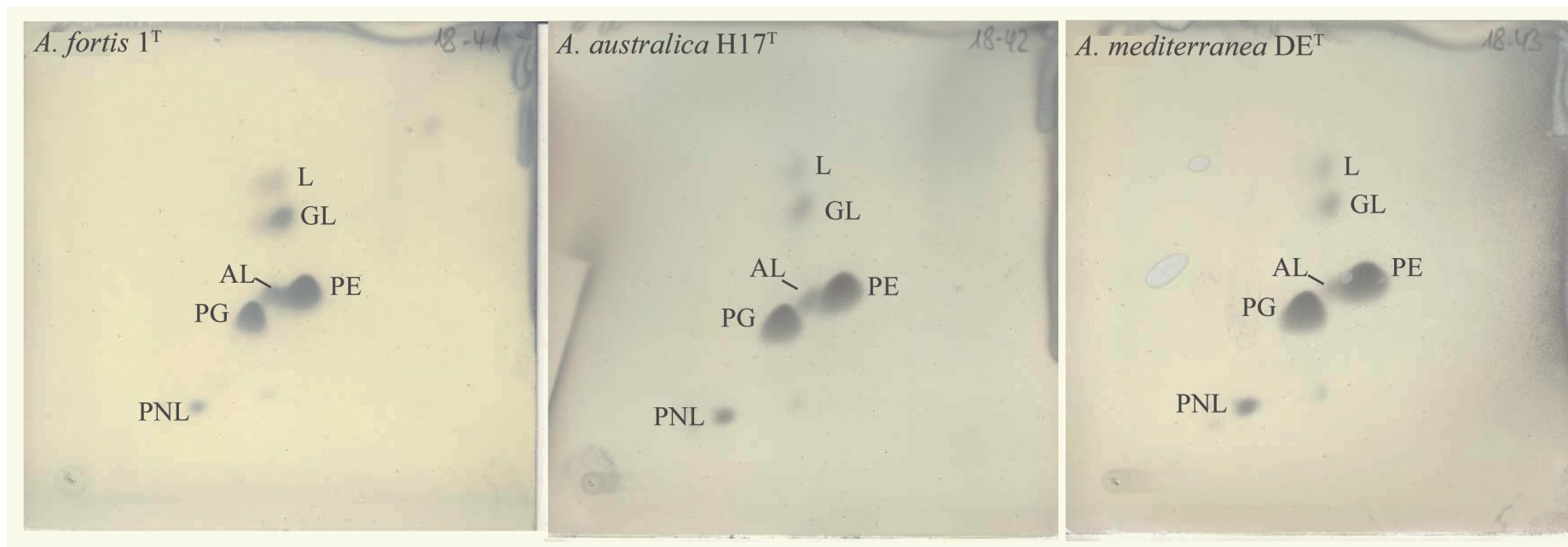
Supplementary Fig. S3

Hydrolytic activities on polysaccharides by 1, strain 1^T (*Alteromonas fortis* sp. nov.); 2, *A. australica* H17^T; and 3, *A. mediterranea* DE^T.

(a), Lugol-flooded ZoBell agar plates containing 0.2 % soluble starch showing the presence of a halo of starch hydrolysis around the culture of strain 1^T. No hydrolysis is visible around the cultures of *A. australica* H17^T and *A. mediterranea* DE^T.

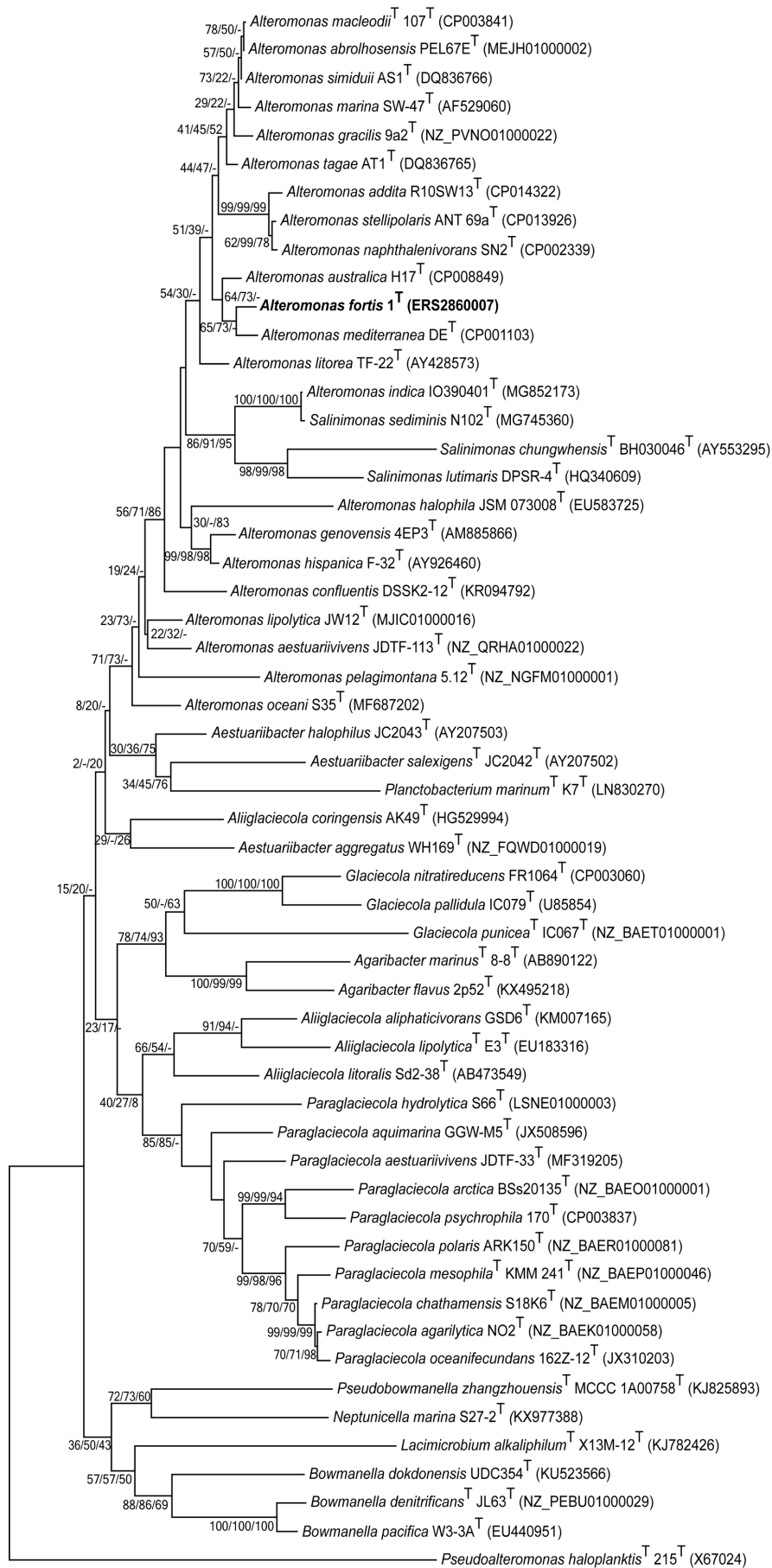
(b), ZoBell alginate plates showing the degradation and liquefaction of the alginate gel by strain 1^T and *A. australica* H17^T, in contrast with *A. mediterranea* DE^T.

Bacterial growth on (c), ZoBell agar; (d), ZoBell κ-carrageenin; (e), ZoBell ι-carrageenin showing the absence of degradation of agar and κ-carrageenan by the three strains and hydrolysis of ι-carrageenan by strain 1^T only.

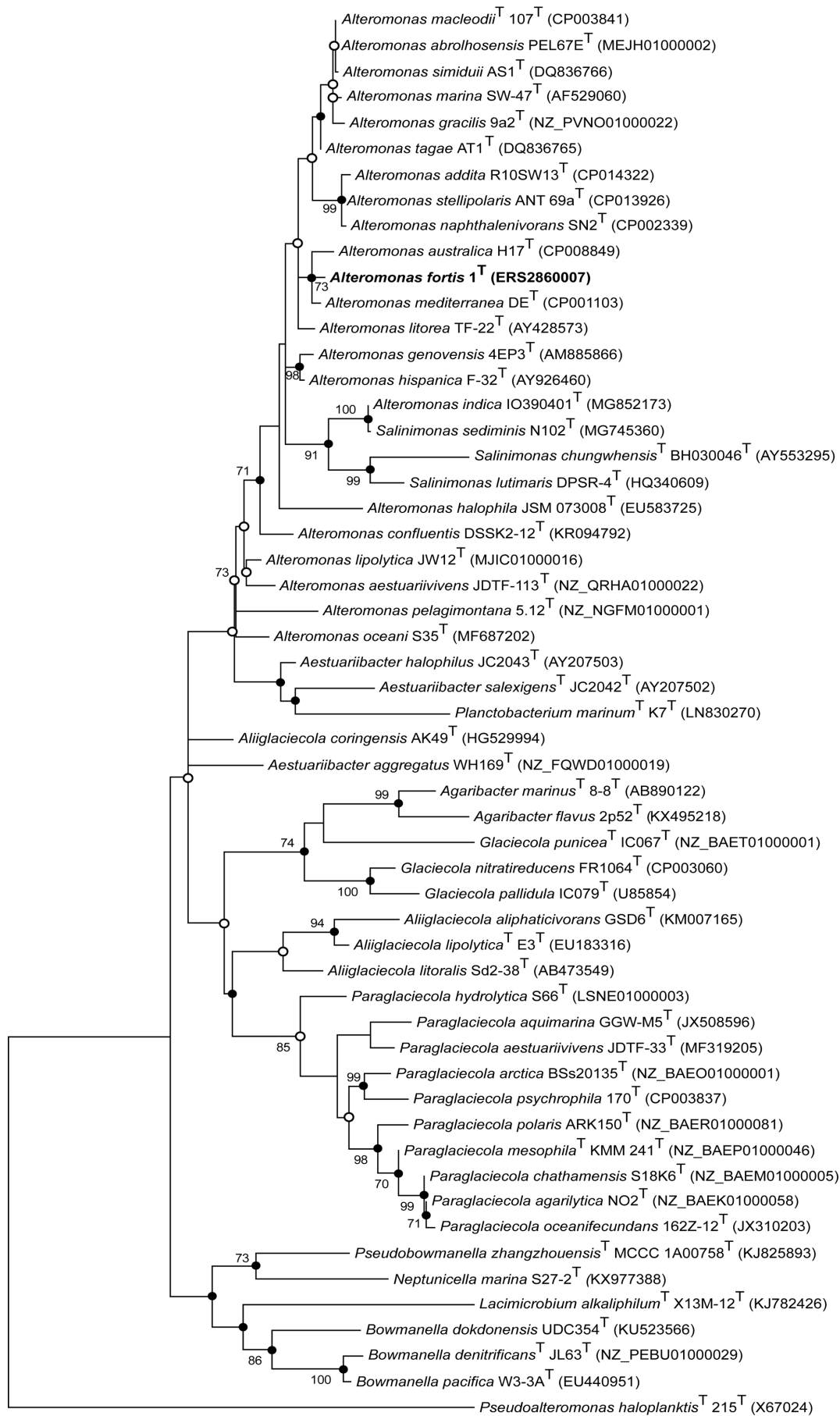


Supplementary Fig. S4

Two-dimensional thin layer chromatography of the polar lipids of strain 1^T (*Alteromonas fortis* sp. nov.), *Alteromonas australica* H17^T and *A. mediterranea* DE^T. L, unidentified lipid; AL, aminolipid; GL, glycolipid; PG, phosphatidylglycerol; PE, phosphatidylethanolamine, PNL, phosphoaminolipid.

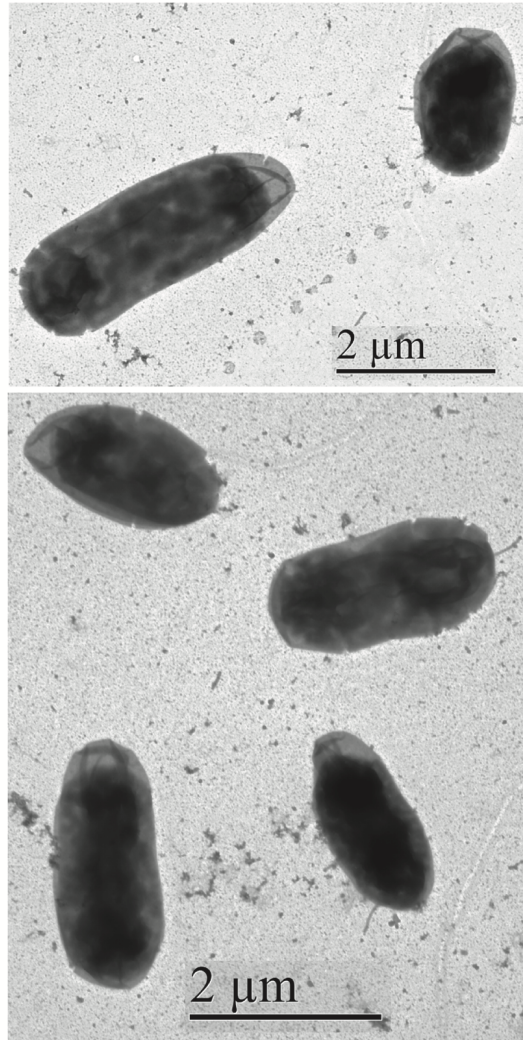


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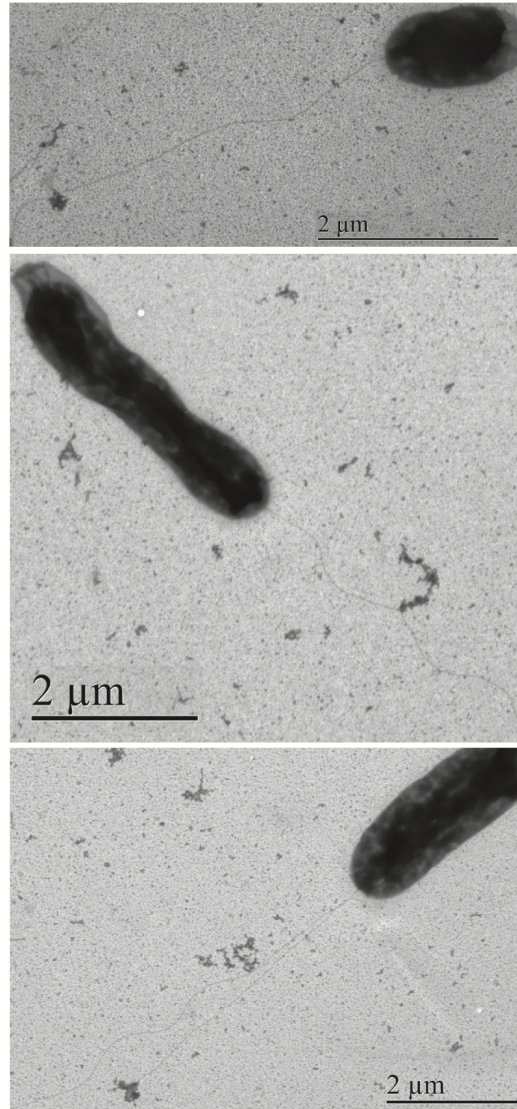


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Alteromonas fortis 1^T



Alteromonas australica H17^T



Alteromonas mediterranea DE^T

