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Tristan Barbeyron, Nolwen Le Duff, Eric Duchaud, François Thomas. *Zobellia alginiliquefaciens* sp. nov., a novel member of the flavobacteria isolated from the epibiota of the brown alga *Ericaria zosteroides* (C. Agardh) Molinari & Guiry 2020. *International Journal of Systematic and Evolutionary Microbiology*, 2023, 73 (6), pp.005924. 10.1099/ijsem.0.005924 . hal-04199452

HAL Id: hal-04199452

<https://hal.sorbonne-universite.fr/hal-04199452>

Submitted on 7 Sep 2023

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1 ***Zobellia alginiliquefaciens* sp. nov., a new member of the flavobacteria isolated from the**
2 **epibiota of the brown alga *Ericaria zosteroides* (C.Agardh) Molinari & Guiry 2020**

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4

5 **Running Title:** *Zobellia alginiliquefaciens* sp. nov.

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12 **Keywords:** *Zobellia alginiliquefaciens*; *Flavobacteriaceae*; Alginate lyase;

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: *Bacteroidota*

17

18 The Genbank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain

19 LLG6346-3.1^T is OQ511313.

20 The Genbank/EMBL/DDBJ accession number for the genome sequence of strain LLG6346-

21 3.1^T is CP119758.

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

24 **Abstract**

25 Strain LLG6346-3.1^T, isolated from the thallus of the brown alga *Ericaria zosteroides*
26 collected in Mediterranean Sea near Bastia in Corsica, France, was characterized using a
27 polyphasic method. Cells were Gram-stain-negative, strictly aerobic, non-flagellated, motile
28 by gliding, rod-shaped and grew optimally at 30-33 °C, at pH 8-8.5 and with 4-5 % NaCl.
29 Strain LLG6346-3.1^T used the seaweed polysaccharide alginic acid as sole carbon source
30 which was vigorously liquefied. Phylogenetic analyses showed that the bacterium is affiliated
31 to the genus *Zobellia* (family *Flavobacteriaceae*, class *Flavobacteriia*). Strain LLG6346-3.1^T
32 exhibited 16S rRNA gene sequence similarity values of 98.5 and 98.3 % to the type strains of
33 *Zobellia russellii* and *Zobellia roscoffensis* respectively, and of 97.4-98.2 % to other species
34 of the genus *Zobellia*. The DNA G+C content of strain LLG6346-3.1^T was determined to be
35 38.3 mol%. Digital DNA-DNA hybridization predictions by the ANI and GGDC methods
36 between strain LLG6346-3.1^T and other members of the genus *Zobellia* showed values of 76-
37 88 %, and below 37 %, respectively. The phenotypic, phylogenetic and genomic analyses
38 show that strain LLG6346-3.1^T is distinct from species of the genus *Zobellia* with validly
39 published names and that it represents a novel species of the genus *Zobellia*, for which the
40 name *Zobellia alginiliquefaciens* sp. nov. is proposed. The type strain is LLG6346-3.1^T (=
41 RCC7657^T = LMG 32918^T).

42

43 The genus *Zobellia* belongs to the family *Flavobacteriaceae* (order *Flavobacteriales*, class
44 *Flavobacteriia*), was proposed by Barbeyron et al. [1] with *Zobellia galactanivorans* as the
45 type species of the genus. At the time of writing, the genus *Zobellia* comprises 8 validly
46 named species, all isolated from marine environments and mostly from macroalgae. For
47 example, *Z. galactanivorans* Dsij^T was retrieved as an epibiont of the red alga *Delesseria*
48 *sanguinea* [2], *Z. russellii* KMM 3677^T and *Z. barbeyronii* 36-CHABK-3-33^T were isolated
49 from the green algae *Acrosiphonia sonderi* and *Ulva* sp. respectively [3, 4], while *Z.*
50 *laminariae* KMM 3676^T originated from the brown alga *Saccharina japonica* [3] and *Z.*
51 *nedashkovskayae* Asnod2-B07-B^T and *Z. roscoffensis* Asnod1-F08^T from the brown alga
52 *Ascophyllum nodosum* [5]. Moreover, metagenomics survey show that the genus *Zobellia* is
53 part of the microbiota of healthy macroalgae [6, 7]. Recent development of *Zobellia*-specific
54 quantitative PCR primers and FISH probes confirmed the presence of the genus on the surface
55 of diverse macroalgal species, with ca. 10^3 - 10^4 16S rRNA copies.cm⁻² [8]. Strain LLG6346-
56 3.1^T was isolated in May 2019 from the surface of *Ericaria zosteroides* (C.Agardh) Molinari
57 & Guiry 2020 thallus during a sampling campaign in the Mediterranean Sea near Negru in
58 Corsica (France, GPS 42.769040 N, 9.333530 E). The algal specimen was collected manually
59 by divers at ca. 20 m depth, before swabbing in the laboratory and inoculation on ZoBell
60 2216E-agar plates [9]. Here, we present a detailed taxonomic investigation of strain
61 LLG6346-3.1^T using a polyphasic approach, including some genomic data deduced from its
62 complete genome and also techniques of whole-genome comparison such as the Average
63 Nucleotide Identity (ANI) and dDDH (digital DNA–DNA hybridization).

64 For comparison, *Zobellia russellii* KMM 3677^T = LMG 22071^T [3] purchased from the
65 Collection de l'Institut Pasteur (CIP; France) and *Zobellia roscoffensis* Asnod1-F08^T = CIP
66 111902^T = RCC6906^T [5] isolated in our laboratory, were used as related type strains. *Z.*
67 *russellii* KMM 3677^T and *Z. roscoffensis* Asnod1-F08^T were studied in parallel with strain

68 LLG6346-3.1^T for all phenotypic tests except for the temperature, pH and NaCl ranges of
69 growth. The three strains were routinely cultivated on ZoBell medium 2216E, either liquid or
70 solidified with 1.5 % (w/v) agar. Pure cultures were stored at -80 °C in the culture medium
71 containing 20 % (v/v) glycerol. All experiments were performed in triplicate. Assays of
72 optimal temperature, pH and NaCl concentration were performed in 24-well plate containing
73 600 µl of medium inoculated with 12 µl of an overnight preculture. Optical density at 600 nm
74 was measured in a Spark Tecan plate reader. The plate lid was pre-treated with 0.05 % Triton
75 X-100 in 20 % ethanol to avoid condensation [10]. Growth was evaluated in ZoBell broth at
76 4, 13, 20, 24, 27, 30, 33, 36, 37, 38 and 40 °C. The optimal pH value for growth was
77 determined at 30 °C in ZoBell broth with pH values adjusted by using 100 mM of the
78 following buffers: MES for pH 5.5; Bis Tris for pH 6.0, 6.5, 7.0 and 7.5; Tris for pH 8.0, 8.5
79 and 9.0; CHES for pH 9.5 and CAPS for pH 10.0, 10.5 and 11.0. The effect of NaCl on
80 growth was determined at 30 °C and at pH 8 in ZoBell broth prepared with distilled water
81 containing 0, 0.5, 1.0, 2.0, 3.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 % NaCl.

82 Cell morphology and motility were investigated on wet mounts of an exponential phase
83 ZoBell broth culture at 20 °C, by using phase-contrast microscopy on an Olympus BX60
84 instrument (Olympus, Tokyo, Japan). The Ryu non-staining KOH method [11] was used to
85 test the Gram reaction.

86 Oxidase activity was assayed using discs impregnated with N,N,N',N'-tetramethyl-p-
87 phenylenediamine dihydrochloride reagent (bioMérieux). Catalase activity was assayed by
88 mixing one colony from a ZoBell agar plate with a drop of hydrogen peroxide (3 %, v/v).
89 Nitrate reductase activity was assayed using ZoBell broth containing 10 g l⁻¹ of sodium
90 nitrate. Nitrate reductase activity was revealed after growth at 20 °C and addition of Griess
91 Reagent. Amylase activity was assayed on 0.2 % (w/v) soluble starch ZoBell agar plates.
92 DNase activity was detected on DNA agar (Difco) prepared with seawater. Amylase and

93 DNase activities were revealed by flooding the plates with Lugol's solution or 1 M HCl,
94 respectively. The degradation of Tween compounds (1 %, v/v) was assayed in ZoBell agar
95 according to Smibert & Krieg [12]. Agarase, κ -carrageenase and ι -carrageenase activities
96 were tested by inoculating ZoBell media solidified with (per litre): 15 g agar (Sigma-Aldrich,
97 ref. A7002), 10 g κ -carrageenin (X-6913; Danisco) or 20 g ι -carrageenin (X-6905; Danisco)
98 respectively. Alginate lyase activity was tested by inoculating ZoBell media solidified with
99 10 g l⁻¹ sodium alginate (Sigma-Aldrich, ref. 180947) according the Draget's method [13].
100 Strains were considered positive when colonies liquefied or produced craters in the solidified
101 substrate. Additional phenotypic characterizations were performed using API 20 E, API 20
102 NE, API 50CH and API ZYM strips according to the manufacturer's instructions
103 (bioMérieux) except that API AUX medium and API 50 CHB/E medium were adjusted to
104 2.5 % NaCl. All strips were inoculated with cell suspensions in artificial seawater and
105 incubated at 20 °C for 72 h. The ability to use carbohydrates as sole carbon and energy
106 sources was also tested in marine minimal medium [14] containing 2.5 g l⁻¹ of the following
107 sugars (all from Sigma-Aldrich unless otherwise stated): D-glucose, D-galactose, D-fructose,
108 L-rhamnose, L-fucose, D-xylose, L-arabinose, D-mannose, sucrose, lactose, maltose, D-
109 mannitol, D-raffinose, amylopectin (Merck), arabinan from sugar beet (Megazyme),
110 arabinoxylan from wheat (Megazyme), xylan from beechwood, pectin from apple, agar,
111 porphyrin from *Porphyra* sp. (home-made extract) laminarin (Goëmar), galactan from gum
112 arabic, galactomannan from carob (Megazyme), glucomannan from konjac (Megazyme),
113 alginic acid from *Laminaria digitata* (Danisco), ι -carrageenin from *Eucheima denticulatum*
114 (Danisco), κ -carrageenin from *Eucheima cottonii*, λ -carrageenin (Dupont), lichenin
115 (Megazyme), ulvin from *Ulva* sp. (Elicityl), xyloglucan from tamarind seed (Megazyme) and
116 sulphated fucoidin from *Ascophyllum nodosum* (kindly provided by Algues et Mer) and
117 *Laminaria hyperborea* (home-made extract).

118 Sensitivity to antibiotics was tested by the disc-diffusion method on ZoBell agar plates and
119 using antibiotic discs (Bio-Rad) containing (μg per disc, unless otherwise stated): penicillin G
120 (10 IU), ampicillin (10), carbenicillin (100), oxacillin (5), streptomycin (500), kanamycin
121 (30), chloramphenicol (30), tetracycline (30), lincomycin (15), bacitracin (130), rifampicin
122 (30), vancomycin (30). The effects of the antibiotics on cell growth were assessed after 24 h
123 of incubation at 20 °C, and susceptibility was scored on the basis of the diameter of the clear
124 zone around the disc.

125 Genomic DNA was extracted from 500 μl of culture of strain LLG6346-3.1^T in Zobell 2216E
126 broth using the Genomic-tip 20/G kit (Qiagen) following the manufacturer's instructions. The
127 Illumina sequencing library was prepared using the Nextera XT DNA kit (Illumina) and
128 sequenced using Illumina MiSeq v3 PE300, resulting in 4,268,034 quality-filtered reads
129 (Table S1, available in the online version of this article). The Nanopore sequencing library
130 was prepared using Ligation Sequencing Kit 1D (SQK-LSK109) and sequenced using
131 MinION flow cell R9.4.1, resulting in 100,270 reads of average length 22,935 nt. Hybrid
132 assembly was performed using unicycler v 0.4.8 in conservative mode and otherwise default
133 settings [15]. The 16S rRNA gene sequence was amplified by PCR using pure genomic DNA
134 as template and primer pairs specific for Bacteria, 8F [16] and 1492R [17]. PCR reactions
135 were typically carried out in a volume of 20 μl containing 10-100 ng template, 0.2 μM each
136 specific primer, 200 μM each dNTP, 1X GoTaq buffer (Promega) and 1.25 U GoTaq DNA
137 polymerase (Promega). PCR conditions were as follows: initial denaturation for 10 min at
138 95°C, followed by 35 cycles of 1 min at 95°C, 30 sec at 50°C, 2 min at 72°C, and final
139 extension of 5 min at 72°C. PCR products were purified using the ExoSAP-IT Express kit
140 according to the manufacturer's protocol (ThermoFisher Scientific) and sequenced by using
141 BigDye Terminator V3.1 (Applied Biosystems) and an ABI PRISM 3130xl Genetic Analyzer
142 automated sequencer (Applied Biosystems/Hitachi). Chargaff's coefficient of the genomic

143 DNA of strain LLG6346-3.1^T was deduced from the complete genome sequence and
144 expressed as the molar percentage of guanine + cytosine. The nucleotide sequence of the 16S
145 rRNA gene deduced from the complete genome sequence of the strain LLG6346-3.1^T and
146 sequences of the 16S rRNA genes from all valid species of the genera *Zobellia*, *Maribacter*
147 and some other related genera of the *Flavobacteriaceae* family were aligned using the
148 software MAFFT version 7 with the L-INS-I strategy [18]. The alignment was then manually
149 refined and phylogenetic analyses, using the neighbour-joining [19], maximum-parsimony
150 [20] and maximum-likelihood [21] methods, were performed using the MEGA 6 package
151 [22]. The different phylogenetic trees were built from a multiple alignment of 50 sequences
152 and 1437 positions. For the neighbour-joining algorithm, the evolutionary model Kimura Two
153 Parameters [23] was used. The maximum-likelihood tree was calculated using the
154 evolutionary model GTR (Generalised Time Reversible) [24] with a discrete Gamma
155 distribution to model evolutionary rate differences among sites (4 categories). This
156 substitution model was selected through submission of the alignment to the online server IQ-
157 TREE (<http://iqtree.cibiv.univie.ac.at/>). The maximum-parsimony tree was obtained using the
158 Subtree-Pruning-Regrafting algorithm [24]. Bootstrap analysis was performed to provide
159 confidence estimates for the phylogenetic tree topologies [25]. A phylogenomic tree was
160 performed on the web server M1CR0B1AL1Z3R [26]. Briefly, a total of 768 conserved
161 orthologous ORFs were detected (identity > 80%, e-value < 0.001). Sequences were aligned
162 using MAFFT [18] and maximum-likelihood [21] phylogeny was reconstructed using RaxML
163 [27] with 100 bootstrap iterations. Pairwise comparisons of 16S rRNA gene sequences were
164 made by using the database EzBioCloud (<https://www.ezbiocloud.net/identify>) [28]. Genomic
165 relatedness was investigated by comparing the strain LLG6346-3.1^T genome sequence with
166 those of the type strains of other *Zobellia* species using the Average Nucleotide Identity (ANI;
167 <http://jspecies.ribohost.com/jspeciesws/#analyse>) [29–31] and the dDDH via the online server

168 Genome to Genome Distance Calculator 2.1 (GGDC; <http://ggdc.dsmz.de/distcalc2.php>) [32].
169 The results from GGDC analysis were obtained from the alignment method Blast+ and the
170 formula 2 (sum of all identities found in HSPs / by overall HSP length) for incomplete
171 genome sequences [33, 34]. Exploration of carbohydrate active enzyme-coding genes in the
172 genomes of the strains LLG6346-3.1^T, *Z. russellii* KMM 3677^T and *Z. roscoffensis* Asnod1-
173 F08^T was carried out via the online server Microscope from the French National Sequencing
174 Center (<http://www.genoscope.cns.fr/agc/microscope/mage>) [35] and the CAZy database
175 (www.cazy.org) [36].

176 The best pairwise comparison score with 16S rRNA gene from the strain LLG6346-3.1^T
177 (1516 bp) were obtained with *Zobellia russellii* KMM 3677^T (98.6 %) (Table S2).
178 Phylogenetic analyses of 16S rRNA genes from species of the family *Flavobacteriaceae*
179 showed that strain LLG6346-3.1^T belongs to the genus *Zobellia* and forms a clade with strains
180 *Z. russellii* KMM 3677^T and *Z. roscoffensis* Asnod1-F08^T (Fig 1, Fig. S1). The 16S rRNA
181 gene sequence similarities between the strain LLG6346-3.1^T and other *Zobellia* species were
182 in the range of 97.4 % with *Z. barbeyronii* 36-CHABK-3-33^T and *Z. nedashkovskayae*
183 Asnod2-B07-B^T [4, 5] to 98.5 % with *Z. galactanivorans* Dsij^T [1] (Table S2). The complete
184 genome of strain LLG6346-3.1^T was composed of 5,066,785 nucleotides and had a Chargaff's
185 coefficient of 38.3 % (Table S1). Analysis of a phylogenomic tree based on 768 proteins from
186 the core genome of sequenced *Zobellia* strains showed that LLG6346-3.1^T formed a clade
187 with *Z. roscoffensis* Asnod1-F08^T (Figure 2). The ANI and GGDC values for strain
188 LLG6346-3.1^T, when compared with other *Zobellia* species, were less than 90 % and less than
189 40 % respectively (88.0 % and 37.1 % with *Z. roscoffensis* Asnod1-F08^T; Table S3). As the
190 normally accepted thresholds of species delineation for ANI and GGDC are 95 % and 70%,
191 respectively [29, 31, 37, 38], these values suggest that strain LLG6346-3.1^T represents a new
192 species of the genus *Zobellia*.

193 Under the microscope, cells of strain LLG6346-3.1^T appeared as rods approximately 0.5 μm
194 in diameter and 2.0–4.0 μm long, attached to the glass of the slide or coverslip and showed
195 gliding motility. Colonies grown on R2A agar at 20 °C showed a weak iridescence. The
196 optimum growth temperature and NaCl concentration for strain LLG6346-3.1^T were higher
197 than for *Z. russellii* KMM 3677^T and *Z. roscoffensis* Asnod1-F08^T (Table 1). This could
198 reflect adaptation to the Mediterranean Sea environment from which strain LLG6346-3.1^T
199 was isolated, where average seawater temperature and salinity are higher than the Pacific
200 Ocean and English Channel from which *Z. russellii* KMM 3677^T and *Z. roscoffensis* Asnod1-
201 F08^T were retrieved, respectively.

202 Growth was observed with some polysaccharides and a few simple sugars allowing
203 differentiation of strain LLG6346-3.1^T from *Z. russellii* KMM 3677^T and *Z. roscoffensis*
204 Asnod1-F08^T (Table 1). The most obvious test to differentiate strain LLG6346-3.1^T from *Z.*
205 *russellii* KMM 3677^T and *Z. roscoffensis* Asnod1-F08^T was hydrolysis of alginic acid. While
206 strain LLG6346-3.1^T hydrolysed to liquefaction and used this marine polysaccharide as the
207 sole carbon source, it was not hydrolysed and therefore not utilized by *Z. roscoffensis*
208 Asnod1-F08^T. For its part, *Z. russellii* KMM 3677^T hydrolysed, used but did not liquefy
209 alginic acid, and only the formation of a crater in the alginic acid, without liquid, was visible
210 (Table 1). Comparative genomics within the genus *Zobellia* suggests that the ability to
211 hydrolyse alginic acid is mainly due to the presence of the alginate lyase-encoding genes
212 *alyA1* and *alyA2* (zgal_1182 and zgal_2618 in genome of *Z. galactanivorans* Dsij^T
213 respectively). The LLG6346-3.1^T strain, which liquefied alginic acid, possesses both genes. *Z.*
214 *russellii* KMM 3677^T that hydrolysed alginic acid without liquefaction, possesses only the
215 *alyA2* gene. By contrast *Z. roscoffensis* Asnod1-F08^T, although possessing homologs of
216 *alyA3*, *alyA4*, *alyA5* and *alyA6* genes from *Z. galactanivorans* Dsij^T, does not possess either
217 the *alyA1* or the *alyA2* genes. These observations suggest that the liquefaction phenotype is

218 linked to the presence of the *alyA1* gene encoding a secreted endo-gulonate lyase [39].
219 Among valid species of *Zobellia*, only *Z. galactanivorans* Dsij^T, *Z. uliginosa* 553(843)^T [1]
220 and *Z. nedashkovskayae* Asnod2-B07-B^T [5] possess the *alyA1* gene and liquefied and utilized
221 alginic acid. However, it is easy to differentiate these species. Unlike *Z. galactanivorans* Dsij^T
222 which is able to hydrolyse all red algal polysaccharides (agars and carrageenins) and *Z.*
223 *nedashkovskayae* Asnod2-B07-B^T which is able to utilize laminarin and fucoidin from
224 *Ascophyllum nodosum*, strain LLG6346-3.1^T did not hydrolyse and use neither agars nor
225 carrageenins (which is consistent with the absence of carrageenase-encoding genes in its
226 genome) nor laminarin nor fucoidin (Table 1). Strains LLG6346-3.1^T and *Z. russellii* KMM
227 3677^T were able to use starch as sole carbon and energy sources and showed a hydrolysis area
228 on soluble starch ZoBell agar plates (Table 1). This suggests that both strains possess a
229 secreted alpha-amylase, consistent with the presence of the amylase-encoding gene *susA* in
230 their genome. As reported previously [5], *Z. roscoffensis* Asnod1-F08^T lacks a *susA* homolog,
231 likely explaining its absence of hydrolysis and use of starch. Finally, the nitrate reductase
232 activity is another discriminant criteria to differentiate the strain LLG6346-3.1^T from *Z.*
233 *roscoffensis* Asnod1-F08^T and *Z. russellii* KMM 3677^T. While the latter two strains showed
234 vigorous nitrate reductase activity after growth in nitrated ZoBell broth, strain LLG6346-3.1^T
235 showed very weak activity under the same conditions.

236 The other physiological features of strain LLG6346-3.1^T compared with *Z. roscoffensis*
237 Asnod1-F08^T and *Z. russellii* KMM 3677^T are listed in Table 1. The three strains were
238 resistant to kanamycin, gentamycin, neomycin, vancomycin, ampicillin, penicillin,
239 carbenicillin, oxacillin, erythromycin, nalidixic acid, trimethoprim/sulfamethoxazole,
240 bacitracin, colistin, polymixin B and chloramphenicol. For streptomycin, whereas *Z.*
241 *roscoffensis* Asnod1-F08^T is sensible, the other two strains are resistant. In the case of
242 lincomycin, whereas the strain LLG6346-3.1^T is sensitive, the other two strains are resistant.

243 Finally, the strain LLG6346-3.1^T, *Z. roscoffensis* Asnod1-F08^T and *Z. russellii* KMM 3677^T
244 were sensitive to rifampicin. In conclusion, phenotypic characterizations and phylogenetic
245 analysis using 16S rRNA gene sequences together with whole-genome pairwise comparisons
246 show that strain LLG6346-3.1^T represents a novel species in the genus *Zobellia*, for which the
247 name *Zobellia alginiliquefaciens* sp. nov. is proposed.

248

249 **DESCRIPTION OF *ZOBELLIA ALGINILIQUEFACIENS* SP. NOV.**

250 *Zobellia alginiliquefaciens* (al.gi.ni.li.que.fa'ci.ens. N.L. pres. part. *liquefaciens*, liquefying;
251 N.L. part. adj. *alginiliquefaciens*, digesting algin, another name for alginic acid).

252 Cells are Gram-stain-negative, aerobic, chemoorganotrophic, heterotrophic and rod-shaped,
253 approximately 0.5 µm in diameter and 2.0–4.0 µm long; a few cells greater than 4 µm long
254 may occur. Flagella are absent. Prosthecae and buds are not produced. Colonies on ZoBell
255 agar are orange-coloured, convex, circular and mucoid in consistency and 2.0–3.0 mm in
256 diameter after incubation for 3 days at 20 °C. Growth in ZoBell 2216E broth occurs from 4 to
257 38 °C (optimum, 30–33°C), at pH 6.5–9.0 (optimum, pH 8–8.5) and in the presence of 2–7%
258 NaCl (optimum, 4–5%). Positive for gliding motility and flexirubin-type pigment production.
259 Nitrate is very weakly reduced. β-Galactosidase-, oxidase- and catalase- positive. Alginic acid
260 is hydrolysed to total liquefaction. DNA, gelatin, starch, aesculin, Tweens 20 and 60 are
261 hydrolysed, but Tween 40, agar, κ-carrageenin and ι-carrageenin are not. D-glucose, D-
262 galactose, D-fructose, L-arabinose, D-mannose, D-xylose, salicin (weakly), sucrose, lactose,
263 D-maltose, melibiose, D-cellobiose, gentiobiose, D-turanose, trehalose, D-mannitol,
264 melezitose, raffinose, N-acetyl-glucosamine, starch, glycogene, inulin (weakly), porphyrin,
265 alginic acid, xylan, galactan (gum arabic), glucomannan, lichenin, 1-O-methyl-D-glucoside
266 and 1-O-methyl-D-mannoside are utilized as carbon and energy sources, but D-arabinose,
267 ribose, L-fucose, D-fucose, D-lyxose, L-rhamnose, L-sorbose, D-tagatose, L-xylose, arbutine,

268 amygdaline, adipic acid, capric acid, malic acid, citric acid, gluconic acid, phenylacetic acid,
269 2 ketogluconate, 5 ketogluconate, 1-O-methyl-D-xyloside, adonitol, D-arabitol, L-arabitol,
270 dulcitol, erythritol, glycerol, inositol, D-sorbitol, xylitol, arabinan, arabinoxylane, pectin
271 (apple), galactomannan, xyloglucan, agar, ι-carrageenin, κ-carrageenin, λ-carrageenin,
272 laminarin, ulvin, fucoïdin (*Ascophyllum nodosum*) and fucoïdin (*Laminaria hyperborea*) are
273 not. Acid is produced from D-glucose, D-galactose, D-tagatose, D-fructose, D-arabinose, L-
274 arabinose, D-mannose, D-fucose, L-fucose, D-lyxose, D-xylose, salicin (weakly), arbutine,
275 sucrose, lactose, D-maltose, melibiose, D-cellobiose, gentiobiose, D-turanose, trehalose,
276 amygdaline, melezitose, raffinose, starch, glycogene, inulin (weakly), D-mannitol, 1-O-
277 methyl-D-glucoside, 1-O-methyl-D-mannoside, and 2 ketogluconate, but not from ribose, L-
278 xylose, L-sorbose, L-rhamnose, glycerol, erythritol, inositol, D-sorbitol, dulcitol, xylitol, D-
279 arabitol, L-arabitol, adonitol, N-acetyl-glucosamine, 5 ketogluconate and 1-O-methyl-D-
280 xyloside. Negative for indole and H₂S production and for arginine dihydrolase, tryptophan
281 deaminase, urease, lysine decarboxylase and ornithine decarboxylase activities. In the API
282 ZYM system, acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8),
283 leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthoL-AS-BI-
284 phosphohydrolase α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-
285 β-glucosaminidase and α-mannosidase activities are present, but lipase (C14), α-
286 chymotrypsin, β-glucuronidase and α-fucosidase activities are absent. The DNA G+C content
287 is 38.3 mol%.

288 The type strain, LLG6346-3.1^T (= RCC7657^T = LMG 32918^T), was isolated from the surface
289 of the brown alga *Ericaria zosteroides* (C.Agardh) Molinari & Guiry 2020.

290 The Genbank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain
291 LLG6346-3.1^T is OQ511313. The Genbank/EMBL/DDBJ accession number for the genome
292 sequence of strain LLG6346-3.1^T is CP119758.

293

294 **Acknowledgements**

295 The LABGeM (CEA/Genoscope and CNRS UMR8030), the France Génomique and French
296 Bioinformatics Institute national infrastructures (funded as part of Investissement d'Avenir
297 program managed by Agence Nationale pour la Recherche, contracts ANR-10- INBS-09 and
298 ANR-11- INBS-0013) are acknowledged for support within the MicroScope annotation
299 platform. We are grateful to the Institut Français de Bioinformatique (ANR-11- INBS-0013)
300 and the Roscoff Bioinformatics platform ABiMS (<http://abims.sb-roscoff.fr>) for providing
301 help for computing and storage resources. We are most grateful to the Genomer platform,
302 Biogenouest genomics and EMBRC France partner core facility for its technical support. We
303 thank Gwenn Tanguy and Erwan Legeay for sequencing. This work has benefited from the
304 CORSICABENTHOS expeditions (PI: Line Le Gall), the marine component of the "*Our*
305 *Planet Reviewed*" programme. The Corsica program is run by Muséum National d'Histoire
306 Naturelle in partnership with Université de Corse Pasquale Paoli and Office de
307 l'Environnement de la Corse (OEC), with the support of Office Français de la Biodiversité
308 (OFB) and Collectivité Territoriale de Corse (CTC). The CORSICABENTHOS 1 expedition
309 took place in May 2019 in collaboration with Parc Naturel Marin du Cap Corse et de l'Agriate
310 ; The organizers are grateful to Madeleine Cancemi, Jean-François Cubells, Jean-Michel
311 Culioli and Jean-Michel Palazzi for their support.

312 **Author contributions**

313 Author contributions following the CRediT taxonomy (<https://casrai.org/credit/>) are as
314 follows: Conceptualization: TB, FT; Formal analysis: TB, FT; Funding acquisition: FT;
315 Investigation: TB, NLD, ED, FT; Project administration: FT; Supervision: TB, FT;
316 Visualization: TB, FT; Writing-original draft: TB; Writing-review and editing: TB, NLD, ED,
317 FT.

318

319 **Funding information**

320 This work was supported by the French ANR project ALGAVOR (grant agreement ANR-18-
321 CE02-0001-01).

322 **Conflicts of interest**

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

324

325 **References**

- 326 1. **Barbeyron T, L'Haridon S, Corre E, Kloareg B, Potin P.** *Zobellia galactanovorans*
327 gen. nov., sp. nov., a marine species of *Flavobacteriaceae* isolated from a red alga, and
328 classification of [*Cytophaga*] *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989
329 as *Zobellia uliginosa* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 2001;51:985–97.
- 330 2. **Potin P, Sanseau A, Le Gall Y, Rochas C, Kloareg B.** Purification and
331 characterization of a new k-carrageenase from a marine *Cytophaga*-like bacterium. *Eur*
332 *J Biochem* 1991;201:241–247.
- 333 3. **Nedashkovskaya OI, Suzuki M, Vancanneyt M, Cleenwerck I, Lysenko AM, et al.**
334 *Zobellia amurskyensis* sp. nov., *Zobellia laminariae* sp. nov. and *Zobellia russellii* sp.
335 nov., novel marine bacteria of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol*
336 2004;54:1643–8.
- 337 4. **Nedashkovskaya O, Otstavnykh N, Zhukova N, Guzev K, Chausova V, et al.**
338 *Zobellia barbeyronii* sp. nov., a New Member of the Family *Flavobacteriaceae*,
339 Isolated from Seaweed, and Emended Description of the Species *Z. amurskyensis*, *Z.*
340 *laminariae*, *Z. russellii* and *Z. uliginosa*. *Diversity* 2021;13:520.

- 341 5. **Barbeyron T, Thiébaud M, Le Duff N, Martin M, Corre E, et al.** *Zobellia*
342 *roscoffensis* sp. nov. and *Zobellia nedashkovskayae* sp. nov., two flavobacteria from
343 the epiphytic microbiota of the brown alga *Ascophyllum nodosum*, and emended
344 description of the genus *Zobellia*. *Int J Syst Evol Microbiol*;71. Epub ahead of print
345 2021. DOI: 10.1099/ijsem.0.004913.
- 346 6. **Miranda LN, Hutchison K, Grossman AR, Brawley SH.** Diversity and abundance of
347 the bacterial community of the red macroalga *Porphyra umbilicalis*: Did bacterial
348 farmers produce macroalgae? *PLoS One* 2013;8:e58269.
- 349 7. **Dogs M, Wemheuer B, Wolter L, Bergen N, Daniel R, et al.** *Rhodobacteraceae* on
350 the marine brown alga *Fucus spiralis* are abundant and show physiological adaptation
351 to an epiphytic lifestyle. *Syst Appl Microbiol* 2017;40:370–382.
- 352 8. **Brunet M, Le Duff N, Fuchs BM, Amann R, Barbeyron T, et al.** Specific detection
353 and quantification of the marine flavobacterial genus *Zobellia* on macroalgae using
354 novel qPCR and CARD-FISH assays. *Syst Appl Microbiol* 2021;44:126269.
- 355 9. **Zobell C.** Studies on marine bacteria I The cultural requirements of heterotrophic
356 aerobes. *J Mar Res* 1941;4:42–75.
- 357 10. **Brewster JD.** A simple micro-growth assay for enumerating bacteria. *J Microbiol*
358 *Methods* 2003;53:77–86.
- 359 11. **Powers EM.** Efficacy of the Ryu nonstaining KOH technique for rapidly determining
360 gram reactions of food-borne and waterborne bacteria and yeasts. *Appl Environ*
361 *Microbiol* 1995;61:3756–3758.
- 362 12. **Smibert R, Krieg N.** General characterization. In: Gerhardt P, Murray R, Costilow R,

- 363 Nester E, Wood W, et al. (editors). *Manual of Methods for General Bacteriology*.
364 Washington, DC., USA: American Society for Microbiology; 1981. pp. 409–443.
- 365 13. **Draget KI, Ostgaard K, Smidsrød O.** Alginate-based solid media for plant tissue
366 culture. *Appl Microbiol Biotechnol* 1989;31:79–83.
- 367 14. **Thomas F, Barbeyron T, Michel G.** Evaluation of reference genes for real-time
368 quantitative PCR in the marine flavobacterium *Zobellia galactanivorans*. *J Microbiol*
369 *Methods*;84. Epub ahead of print 2011. DOI: 10.1016/j.mimet.2010.10.016.
- 370 15. **Wick RR, Judd LM, Gorrie CL, Holt KE.** Unicycler: Resolving bacterial genome
371 assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017;13:1–22.
- 372 16. **Hicks R, Amann R, Stahl D.** Dual staining of natural bacterioplankton with 4',6-
373 diamidino-2-phenylindole and fluorescent oligonucleotide probes targeting kingdom-
374 level 16S rRNA sequences. *Appl Environ Microbiol* 1992;58:2158–2163.
- 375 17. **Kane M, Poulsen L, Stahl D.** Monitoring the enrichment and isolation of sulfate-
376 reducing bacteria by using oligonucleotide hybridization probes designed from
377 environmentally derived 16S rRNA sequences. *Appl Environ Microbiol* 1993;59:682–
378 686.
- 379 18. **Katoh K.** MAFFT: a novel method for rapid multiple sequence alignment based on
380 fast Fourier transform. *Nucleic Acids Res* 2002;30:3059–3066.
- 381 19. **Saitou N, Nei M.** The neighbor-joining method: a new method for reconstructing
382 phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
- 383 20. **Fitch WM.** Toward defining the course of evolution: Minimum change for a specific
384 tree topology. *Syst Biol* 1971;20:406–416.

- 385 21. **Felsenstein J.** Evolutionary trees from DNA sequences: A maximum likelihood
386 approach. *J Mol Evol* 1981;17:368–376.
- 387 22. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** MEGA6: Molecular
388 evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
- 389 23. **Kimura M.** A simple method for estimating evolutionary rates of base substitutions
390 through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
- 391 24. **Nei M, Kumar S.** *Molecular Evolution and Phylogenetics*. New York: Oxford
392 University Press.; 2000.
- 393 25. **Felsenstein J.** Confidence limits on phylogenies: an approach using the bootstrap.
394 *Evolution (N Y)* 1985;39:783–791.
- 395 26. **Avram O, Rapoport D, Portugez S, Pupko T.** M1CR0B1AL1Z3R - a user-friendly
396 web server for the analysis of large-scale microbial genomics data. *Nucleic Acids Res*
397 2019;47:W88–W92.
- 398 27. **Stamatakis A.** RAxML version 8: A tool for phylogenetic analysis and post-analysis
399 of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
- 400 28. **Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, et al.** Introducing EzBioCloud: A
401 taxonomically united database of 16S rRNA gene sequences and whole-genome
402 assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
- 403 29. **Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, et al.**
404 DNA-DNA hybridization values and their relationship to whole-genome sequence
405 similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.

- 406 30. **Figueras MJ, Beaz-Hidalgo R, Hossain MJ, Liles MR.** Taxonomic affiliation of new
407 genomes should be verified using average nucleotide identity and multilocus
408 phylogenetic analysis. *Genome Announc* 2014;2:2–3.
- 409 31. **Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, et al.** Proposed minimal
410 standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol*
411 *Microbiol* 2018;68:461–466.
- 412 32. **Auch AF, von Jan M, Klenk HP, Göker M.** Digital DNA-DNA hybridization for
413 microbial species delineation by means of genome-to-genome sequence comparison.
414 *Stand Genomic Sci* 2010;2:117–134.
- 415 33. **Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M.** Genome sequence-based species
416 delimitation with confidence intervals and improved distance functions. *BMC*
417 *Bioinformatics*;14. Epub ahead of print 2013. DOI: 10.1186/1471-2105-14-60.
- 418 34. **Meier-Kolthoff JP, Göker M, Spröer C, Klenk HP.** When should a DDH experiment
419 be mandatory in microbial taxonomy? *Arch Microbiol* 2013;195:413–418.
- 420 35. **Vallenet D, Belda E, Calteau A, Cruveiller S, Engelen S, et al.** MicroScope - An
421 integrated microbial resource for the curation and comparative analysis of genomic and
422 metabolic data. *Nucleic Acids Res* 2012;41:1–12.
- 423 36. **Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B.** The
424 carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res*
425 2014;42:490–495.
- 426 37. **Richter M, Rosselló-Móra R.** Shifting the genomic gold standard for the prokaryotic
427 species definition. *Proc Natl Acad Sci U S A* 2009;106:19126–19131.

428 38. **Auch AF, Klenk HP, Göker M.** Standard operating procedure for calculating
429 genome-to-genome distances based on high-scoring segment pairs. *Stand Genomic Sci*
430 2010;2:142–148.

431 39. **Thomas F, Lundqvist LCE, Jam M, Jeudy A, Barbeyron T, et al.** Comparative
432 characterization of two marine alginate lyases from *Zobellia galactanivorans* reveals
433 distinct modes of action and exquisite adaptation to their natural substrate. *J Biol*
434 *Chem*;288:23021-23037. DOI: 10.1074/jbc.M113.467217.

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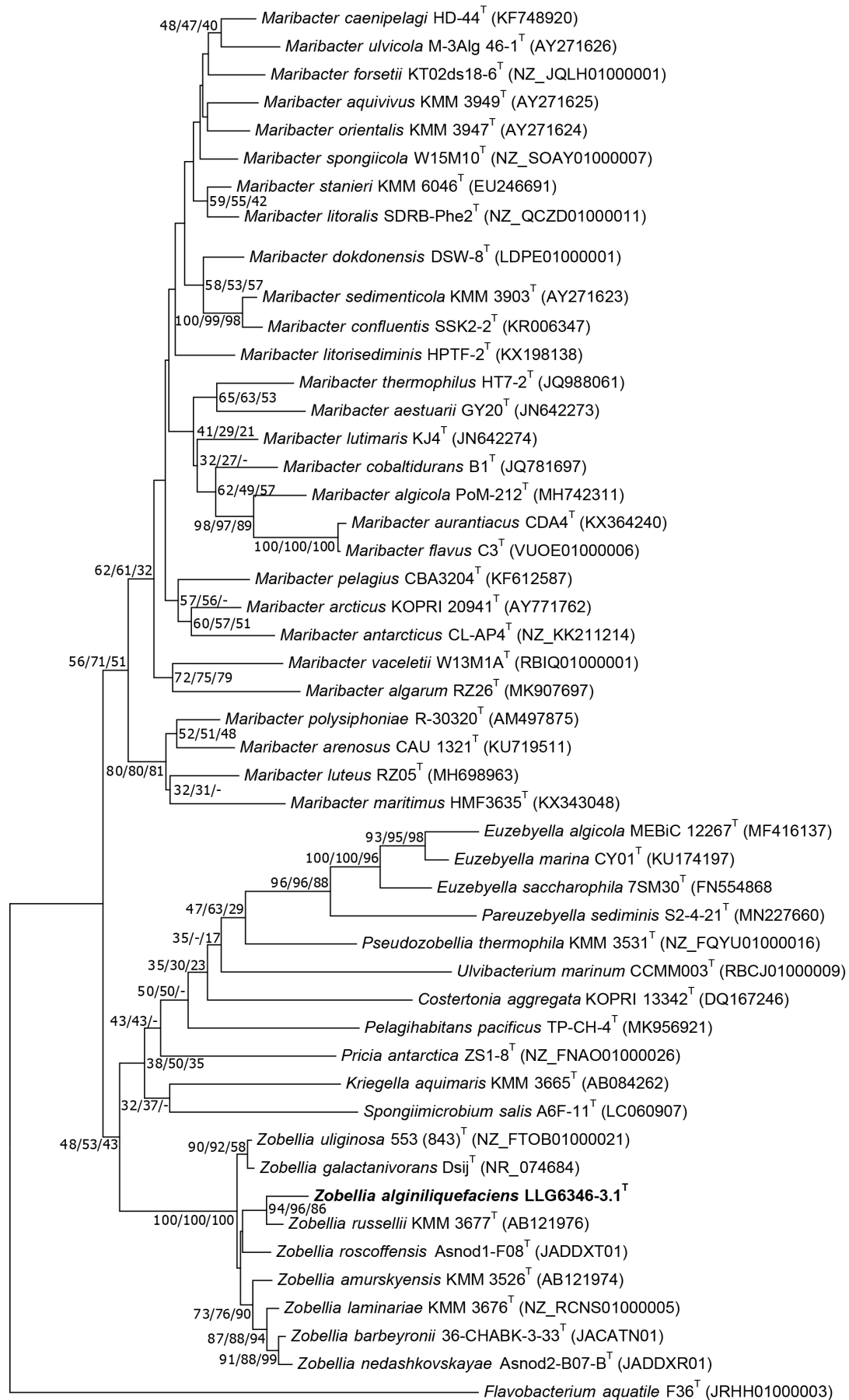


Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between the strain LLG6346-3.1^T and related taxa from the family *Flavobacteriaceae*. Numbers at the nodes indicate bootstrap values (in percentage of 1000 replicates) from neighbour-joining, maximum-likelihood 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%. *Flavobacterium aquatile* F36^T was used as an outgroup. Bar, 0.02 changes per nucleotide position.

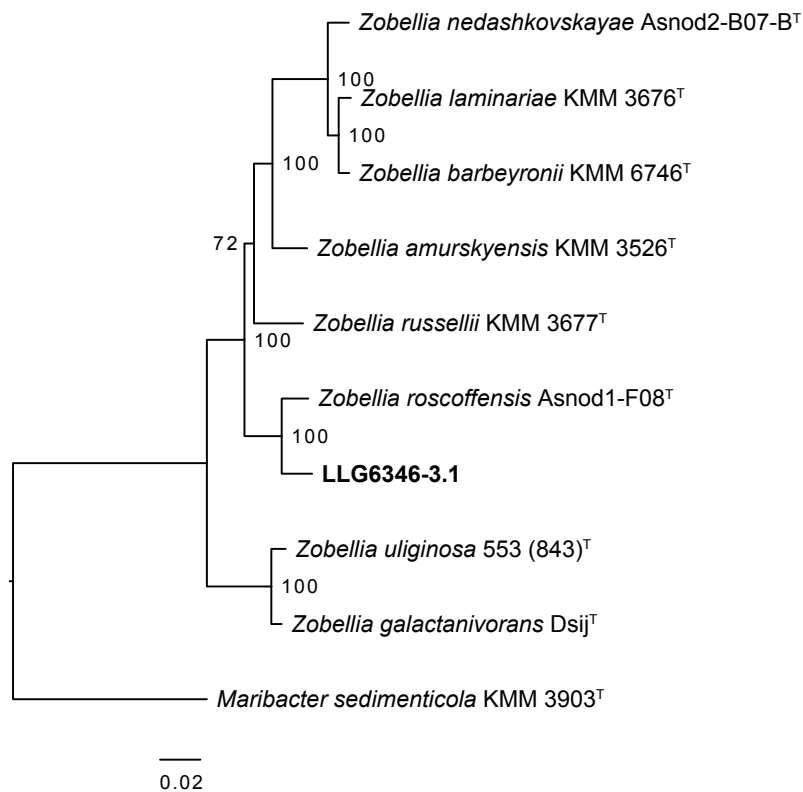


Figure 2

Core proteome phylogenetic analysis of available genomes from *Zobellia* type strains, the newly isolated LLG6346-3.1^T strain and *Maribacter sedimenticola* KMM 3903^T used as an outgroup. Bar, 0.02 substitutions per amino acid position.

Table 1. Phenotypic characteristics of strain LLG6346-3.1^T and of two *Zobellia* species used as related type strains

Strains: 1, LLG6346-3.1^T (*Zobellia alginiliquefaciens* sp. nov.); 2, *Z. roscoffensis* Asnod1-F08^T; 3, *Z. russellii* KMM 3677^T. Cells of all strains share the following characteristics: Gram-negative, aerobic, heterotroph, chemorganotroph, gliding motility, do not form endospores, do not accumulate poly- β -hydroxybutyrate as an intracellular reserve product; require Na⁺ ion or seawater for growth and produce flexirubin-type pigments. All strains are positive for the utilization as a sole carbon source of D-glucose, D-galactose, D-fructose, D-mannose, D-xylose, salicine (weakly), sucrose, D-maltose, lactose, D-cellobiose, gentiobiose, trehalose, raffinose, D-mannitol, N-acetyl-glucosamine, 1-O-methyl-D-glucoside, 1-O-methyl-D-mannoside, lichenin, galactan (gum arabic), glucomannan, xylan and porphyrin; for the hydrolysis of DNA, aesculin, gelatin and Tween 20; for acid and alkaline phosphatase, esterase (C 4), esterase lipase (C 8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol AS-BI-phosphohydrolase, α -glucosidase, β -glucosidase, α -galactosidase, β -galactosidase (PNPG and API ZYM tests), N-acetyl- β -glucosaminidase, α -mannosidase, oxidase and catalase activities; for the acid production from D-glucose, D-galactose, D-fructose, D-mannose, D- and L-arabinose, D-xylose, D-lyxose, D-tagatose, D- and L-fucose, salicine (weakly), D-mannitol, 1-O-methyl-D-glucoside, 1-O-methyl-D-mannoside, D-maltose, lactose, melibiose, sucrose, D-cellobiose, D-turanose, trehalose, amygdaline, raffinose, and arbutine. All strains are negative for indole and H₂S production; for utilization as a sole carbon source of D-arabinose, D-fucose, ribose, L-sorbose, L-xylose, D-lyxose, D-sorbitol, dulcitol, inositol, adonitol, erythritol, xylitol, D- and L-arabitol, arbutine, D-tagatose, gluconic acid, citric acid, capric acid, adipic acid, malic acid, phenylacetic acid, 2 ketogluconate, 5 ketogluconate, arabinan, fucoidin from *Ascophyllum nodosum*, fucoidin from *Laminaria hyperborea*, galactomannan, agar, κ -, ι - and λ -carrageenin, pectin, ulvin and xyloglucan; for the hydrolysis of Tween 40, κ - and ι -carrageenin; for urease, arginine dihydrolase, tryptophan deaminase, lysine decarboxylase, ornithine decarboxylase lipase (C 14), α -chemotrypsine, β -glucuronidase and α -fucosidase activities; for the acid production from L-xylose, glycerol, erythritol, adonitol, dulcitol, D-sorbitol, xylitol, D- and L-arabitol, N-acetyl-glucosamine, gluconic acid and 5 ketogluconate. +, Positive; -, negative; w, weakly positive; na, not available; MMM, Marine Minimum Medium; (liq.), liquefaction.

Characteristic	1	2	3
Growth conditions:			
Temperature range	4-38	4-40*	4-38 [#]
Optimum temperature (°C)	30-33	25-30*	25-28 [#]
pH range	6.5-9	5.5-8.5*	na
Optimum pH	8-8.5	7.5*	na
NaCl range (%)	2-7	2-6*	1-10 [#]
Optimum NaCl (%)	4-5	2*	2-3 [#]
Enzyme:			
Nitrate reductase (nitrated ZoBell medium)	w	+	+
Utilisation of (API20NE):			
L-Arabinose	+	-	+
Utilisation of (API50CH):			
L-Rhamnose	-	+	+

Melezitose	+	-	+
L-Fucose	-	+	+
Glycerol	-	-	+
1-O-methyl-D-Xyloside	-	+	+
D-Turanose	+	+	-
Melibiose	+	+	-
Amygdaline	-	+	-
Starch	+	-	+
Glycogene	+	-	-
Utilisation of (MMM)			
L-Arabinose	W	-	+
L-Rhamnose	-	+	+
Arabinoxylane	-	-	+
Alginate acid	+	-	+
Laminarin	-	+	+
Acid production (API 50CH):			
Ribose	-	-	+
L-Sorbose	-	+	+
L-Rhamnose	-	+	+
Melezitose	+	-	±
1-O-methyl-D-Xyloside	-	-	+
Gentiobiose	+	-	-
Inositol	-	-	+
Starch	+	-	+
Glycogene	+	-	-
2 Ketogluconate	+	+	-
Hydrolysis of:			
Starch (Iugol assay)	+	-	+
Agar (Iugol assay)	-	+	+
Alginate acid	+ (liq.)	-	+
DNA G+C (mol%)	38.3	37.6	39.0

*Data from Barbeyron *et al.* [5]

#Data from Nedashkovskaya *et al.* [3]

