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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

Tristan Barbeyron[1], Nolwen Le Duff[1], Eric Duchaud[2] and François Thomas[1,*]

Abstract

Strain LLG6346-3.1^T, isolated from the thallus of the brown alga *Ericaria zosteroides* collected from the Mediterranean Sea near Bastia in Corsica, France, was characterised using a polyphasic method. Cells were Gram-stain-negative, strictly aerobic, non-flagellated, motile by gliding, rod-shaped and grew optimally at 30–33 °C, at pH 8–8.5 and with 4–5% NaCl. LLG6346-3.1^T used the seaweed polysaccharide alginic acid as a sole carbon source which was vigorously liquefied. The results of phylogenetic analyses indicated that the bacterium is affiliated to the genus *Zobellia* (family *Flavobacteriaceae*, class *Flavobacteriia*). LLG6346-3.1^T exhibited 16S rRNA gene sequence similarity values of 98.6 and 98.3% to the type strains of *Zobellia russellii* and *Zobellia roscoffensis*, respectively, and of 97.4–98.5% to members of other species of the genus *Zobellia*. The DNA G+C content of LLG6346-3.1^T was determined to be 38.3 mol%. Digital DNA–DNA hybridisation predictions by the average nucleotide identity (ANI) and genome to genome distance calculator (GGDC) methods between LLG6346-3.1^T and other members of the genus *Zobellia* showed values of 76–88% and below 37%, respectively. The results of phenotypic, phylogenetic and genomic analyses indicate that LLG6346-3.1^T is distinct from species of the genus *Zobellia* with validly published names and that it represents a novel species of the genus *Zobellia*, for which the name *Zobellia alginiliquefaciens* sp. nov. is proposed. The type strain is LLG6346-3.1^T (= RCC7657^T = LMG 32918^T).

The genus *Zobellia*, part of to the family *Flavobacteriaceae* (order *Flavobacteriales*, class *Flavobacteriia*), was proposed by Barbeyron *et al.* [1] with *Zobellia galactanivorans* as the type species of the genus. At the time of writing, the genus *Zobellia* comprises eight species with validly published names, all isolated from marine environments and mostly from macroalgae. For example, *Zobellia galactanivorans* Dsij^T was retrieved as an epibiont of the red alga *Delesseria sanguinea* [2], *Zobellia russellii* KMM 3677^T and *Zobellia barbeyronii* 36-CHABK-3-33^T were isolated from the green algae *Acrosiphonia sonderi* and *Ulva* sp. respectively [3, 4], while *Zobellia laminariae* KMM 3676^T originated from the brown alga *Saccharina japonica* [3] and *Zobellia nedashkovskayae* Asnod2-B07-B^T and *Zobellia roscoffensis* Asnod1-F08^T from the brown alga *Ascophyllum nodosum* [5]. Moreover, the results of metagenomics surveys indicate that members of the genus *Zobellia* are part of the microbiota of healthy macroalgae [6, 7]. Recent development of *Zobellia*-specific quantitative PCR primers and fluorescence *in situ* hybridisation (FISH) probes confirmed the presence of members of the genus on the surfaces of diverse macroalgal species, with approximately 10^3–10^4 16S rRNA copies cm^{-2} [8]. LLG6346-3.1^T was isolated in May 2019 from the surface of *Ericaria zosteroides* (C. Agardh) Molinari & Guiry 2020 thallus during a sampling campaign in the Mediterranean Sea near Negru in Corsica (France, GPS 42.769040 N, 9.333530 E). The algal specimen was collected manually by divers at approximately 20 m depth, before swabbing in the laboratory and inoculation on ZoBell 2216E-agar plates [9]. Here, we present a detailed taxonomic investigation of LLG6346-3.1^T using a polyphasic approach, including some genomic data deduced from its complete genome and also techniques of whole-genome comparison, such as average nucleotide identity (ANI) and digital DNA–DNA hybridisation (dDDH) analyses.

Author affiliations: [1]Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff (SBR), 29680 Roscoff, Brittany, France; [2]INRAE VIM-UR0892 Molecular Immunology and Virology, research group of Infection and Immunity of Fish, Research Center of Jouy-en-Josas, F-78352 Jouy-en-Josas, Ile-de-France, France.
***Correspondence:** François Thomas, fthomas@sb-roscoff.fr
Keywords: *Zobellia alginiliquefaciens*; Flavobacteriaceae; Alginate lyase.
Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridisation; GGDC, Genome-Genome Distance Calculator; ML, maximum-likelihood; MP, maximum parsimony; NJ, neighbour-joining.
The Genbank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LLG6346-3.1^T is OQ511313. The Genbank/EMBL/DDBJ accession number for the genome sequence of strain LLG6346-3.1^T is CP119758.
One supplementary figure and three supplementary tables are available with the online version of this article.

For comparison, *Zobellia russellii* KMM 3677^T = LMG 22071^T [3] purchased from the Collection de l'Institut Pasteur (CIP; France) and *Zobellia roscoffensis* Asnod1-F08^T = CIP 111902^T = RCC6906^T [5] isolated in our laboratory, were used as related type strains. *Z. russellii* KMM 3677^T and *Z. roscoffensis* Asnod1-F08^T were studied in parallel with LLG6346-3.1^T for all phenotypic tests except for the temperature, pH and NaCl ranges of growth. The three strains were routinely cultivated on ZoBell medium 2216E, either liquid or solidified with 1.5% (w/v) agar. Pure cultures were stored at −80 °C in the culture medium containing 20% (v/v) glycerol. All experiments were performed in triplicate. Assays of optimal temperature, pH and NaCl concentration were performed in 24-well plates containing 600 µl of medium inoculated with 12 µl of an overnight preculture. OD_{600} was measured in a Spark Tecan plate reader. The plate lids was pre-treated with 0.05% Triton X-100 in 20% ethanol to avoid condensation [10]. Growth was evaluated in ZoBell broth at 4, 13, 20, 24, 27, 30, 33, 36, 37, 38 and 40 °C. The optimal pH value for growth was determined at 30 °C in ZoBell broth with pH values adjusted by using 100 mM of the 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 and 9.0; CHES for pH 9.5 and CAPS for pH 10.0, 10.5 and 11.0. The effect of NaCl on growth was determined at 30 °C and at pH 8 in ZoBell broth prepared with distilled water 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.

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

Oxidase activity was assayed using discs impregnated with N,N,N′,N′-tetramethyl-*p*-phenylenediamine dihydrochloride reagent (bioMérieux). Catalase activity was assayed by mixing one colony from a ZoBell agar plate with a drop of hydrogen peroxide (3%, v/v). Nitrate reductase activity was assayed using ZoBell broth containing 10 g l^{-1} of sodium nitrate. Nitrate reductase activity was revealed after growth at 20 °C and addition of Griess Reagent. Amylase activity was assayed on 0.2% (w/v) soluble starch ZoBell agar plates. DNase activity was detected on DNA agar (Difco) prepared with seawater. Amylase and DNase activities were revealed by flooding the plates with Lugol's solution or 1 M HCl, respectively. The degradation of Tween compounds (1%, v/v) was assayed in ZoBell agar according to the methods of Smibert and Krieg [12]. Agarase, κ-carrageenase and ι-carrageenase activities were tested by inoculating ZoBell media solidified with (per litre): 15 g agar (ref. A7002; Sigma-Aldrich), 10 g κ-carrageenin (X-6913; Danisco) or 20 g ι-carrageenin (X-6905; Danisco) respectively. Alginate lyase activity was tested by inoculating ZoBell media solidified with 10 g l^{-1} sodium alginate (ref. 180947; Sigma-Aldrich) according the method of Draget's *et al.* [13]. Strains were considered positive when colonies liquefied or produced craters in the solidified substrate. Additional phenotypic characterisations were performed using API 20 E, API 20 NE, API 50CH and API ZYM strips according to the manufacturer's instructions (bioMérieux) except that API AUX medium and API 50 CHB/E medium were adjusted to 2.5% NaCl. All strips were inoculated with cell suspensions in artificial seawater and incubated at 20 °C for 72 h. The ability to use carbohydrates as sole carbon and energy sources was also tested in marine minimal medium [14] containing 2.5 g l^{-1} of the following sugars (all from Sigma-Aldrich unless otherwise stated): glucose, D-galactose, D-fructose, L-rhamnose, L-fucose, D-xylose, L-arabinose, D-mannose, sucrose, lactose, maltose, D-mannitol, raffinose, amylopectin (Merck), arabinan from sugar beet (Megazyme), arabinoxylan from wheat (Megazyme), xylan from beechwood, pectin from apple, agar, porphyrin from *Porphyra* sp. (home-made extract) laminarin (Goëmar), galactan from gum arabic, galactomannan from carob (Megazyme), glucomannan from konjac (Megazyme), alginic acid from *Laminaria digitata* (Danisco), ι-carrageenin from *Euchema denticulatum* (Danisco), κ-carrageenin from *Euchema cottonii*, λ-carrageenin (Dupont), lichenin (Megazyme), ulvin from *Ulva* sp. (Elicityl), xyloglucan from tamarind seed (Megazyme) and sulphated fucoidin from *Ascophyllum nodosum* (kindly provided by Algues et Mer) and *Laminaria hyperborea* (home-made extract).

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

Genomic DNA was extracted from 500 µl of culture of LLG6346-3.1^T in Zobell 2216E broth using the Genomic-tip 20 G^{-1} kit (Qiagen) following the manufacturer's instructions. The Illumina sequencing library was prepared using the Nextera XT DNA kit (Illumina) and sequenced using MiSeq v3 PE300 (Illumina), resulting in 4268034 quality-filtered reads (Table S1, available in the online version of this article). The Nanopore sequencing library was prepared using Ligation Sequencing Kit 1D (SQK-LSK109) and sequenced a using MinION flow cell R9.4.1, resulting in 100270 reads of average length 22935 nt. Hybrid assembly was performed using unicycler v 0.4.8 in conservative mode and otherwise default settings [15]. The 16S rRNA gene sequence was amplified by PCR using pure genomic DNA as template and primer pairs specific for bacteria, 8F [16] and 1492R [17]. PCR reactions were typically carried out in a volume of 20 µl containing 10–100 ng template, 0.2 µM each specific primer, 200 µM each dNTP, 1× GoTaq buffer (Promega) and 1.25 U GoTaq DNA polymerase (Promega). PCR conditions were as follows: initial denaturation for 10 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 30 s at 50 °C and 2 min at 72 °C and final extension of 5 min at 72 °C. PCR products were purified using the ExoSAP-IT Express kit according to the manufacturer's protocol (ThermoFisher Scientific) and sequenced by using BigDye Terminator V3.1 (Applied Biosystems) and an ABI PRISM 3130xl Genetic Analyzer automated sequencer (Applied Biosystems/Hitachi). Chargaff's coefficient of the genomic DNA of LLG6346-3.1^T was deduced

from the complete genome sequence and expressed as the molar percentage of guanine+cytosine. The nucleotide sequence of the 16S rRNA gene deduced from the complete genome sequence of LLG6346-3.1^T and sequences of the 16S rRNA genes from all species of the genera *Zobellia*, *Maribacter* and some other related genera of the family *Flavobacteriaceae* with validly published names were aligned using the software MAFFT version 7 with the L-INS-I strategy [18]. The alignment was then manually refined and phylogenetic analyses, using the neighbour-joining [19], maximum-parsimony [20] and maximum-likelihood [21] methods, were performed using the MEGA 6 package [22]. The different phylogenetic trees were reconstructed from a multiple alignment of 50 sequences and 1437 positions. For the neighbour-joining algorithm, the Kimura two parameters evolutionary model [23] was used. The maximum-likelihood tree was reconstructed using the generalised time reversible (GTR) evolutionary model [24] with a discrete gamma distribution to model evolutionary rate differences among sites (four categories). This substitution model was selected through submission of the alignment to the online server IQ-TREE (http://iqtree.cibiv.univie.ac.at/). The maximum-parsimony tree was obtained using the subtree-pruning-regrafting algorithm [24]. Bootstrap analysis was performed to provide confidence estimates for the phylogenetic tree topologies [25]. A phylogenomic tree was reconstructed using the web server M1CR0B1AL1Z3R [26]. Briefly, a total of 768 conserved orthologous ORFs were detected (identity>80%, e-value<0.001). Sequences were aligned using MAFFT [18] and maximum-likelihood [21] phylogeny was reconstructed using RaxML [27] with 100 bootstrap iterations. Pairwise comparisons of 16S rRNA gene sequences were made by using the database EzBioCloud (https://www.ezbiocloud.net/identify) [28]. Genomic relatedness was investigated by comparing the genome sequence of LLG6346-3.1^T with those of the type strains of other species of the genus *Zobellia* using the average nucleotide identity (ANI; http://jspecies.ribohost.com/jspeciesws/#analyse) [29–31] and the dDDH via the online server Genome to Genome Distance Calculator 2.1 (GGDC; http://ggdc.dsmz.de/distcalc2.php) [32]. The results from GGDC analysis were obtained from the alignment method BLAST+ and formula 2 [sum of all identities found in high-scoring segment pairs (HSPs) / by overall HSP length] for incomplete genome sequences [33, 34]. Exploration of carbohydrate active enzyme-coding genes in the genomes ofLLG6346-3.1^T, *Z. russellii* KMM 3677^T and *Z. roscoffensis* Asnod1-F08^T was carried out via the online server Microscope from the French National Sequencing Centre (http://www.genoscope.cns.fr/agc/microscope/mage) [35] and the CAZy database (www.cazy.org) [36].

The best pairwise comparison score with 16S rRNA gene from LLG6346-3.1^T (1516 bp) was obtained with *Zobellia russellii* KMM 3677^T (98.6%) (Table S2). The results of phylogenetic analyses of 16S rRNA genes from species of the family *Flavobacteriaceae* indicated that LLG6346-3.1^T represents a member of the genus *Zobellia* and forms a clade with *Z. russellii* KMM 3677^T and *Z. roscoffensis* Asnod1-F08^T (Figs 1 and S1). The 16S rRNA gene sequence similarities between LLG6346-3.1^T and other species of the genus *Zobellia* were in the range of 97.4% with *Z. barbeyronii* 36-CHABK-3-33^T and *Z. nedashkovskayae* Asnod2-B07-B^T [4, 5] to 98.5% with *Z. galactanivorans* Dsij^T [1] (Table S2). The complete genome of LLG6346-3.1^T was composed of 5066785 nucleotides and had a Chargaff's coefficient of 38.3% (Table S1). The results of analysis of a phylogenomic tree based on 768 proteins from the core genomes of sequenced strains of members of the genus *Zobellia* indicated that LLG6346-3.1^T formed a clade with *Z. roscoffensis* Asnod1-F08^T (Fig. 2). The ANI and GGDC values for LLG6346-3.1^T, when compared with other species of the genus *Zobellia*, were less than 90% and less than 40% respectively (88.0% and 37.1% with *Z. roscoffensis* Asnod1-F08^T; Table S3). As the normally accepted thresholds of species delineation for ANI and GGDC are 95% and 70%, respectively [29, 31, 37, 38], these values indicate that LLG6346-3.1^T represents a novel species of the genus *Zobellia*.

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

Growth was observed with some polysaccharides and a few simple sugars, allowing differentiation of LLG6346-3.1^T from *Z. russellii* KMM 3677^T and *Z. roscoffensis* Asnod1-F08^T (Table 1). The most obvious test to differentiate LLG6346-3.1^T from *Z. russellii* KMM 3677^T and *Z. roscoffensis* Asnod1-F08^T was hydrolysis of alginic acid. While LLG6346-3.1^T hydrolysed to liquefaction and used this marine polysaccharide as a sole carbon source, it was not hydrolysed and therefore not utilised by *Z. roscoffensis* Asnod1-F08^T. For its part, *Z. russellii* KMM 3677^T hydrolysed and used, but did not liquefy, alginic acid and only the formation of a crater in the alginic acid, without liquid, was visible (Table 1). The results of comparative genomic analysis within the genus *Zobellia* indicates that the ability to hydrolyse alginic acid is mainly due to the presence of the alginate lyase-encoding genes *alyA1* and *alyA2* (zgal_1182 and zgal_2618 in the genome of *Z. galactanivorans* Dsij^T, respectively). The LLG6346-3.1^T strain, which liquefied alginic acid, possesses both genes. *Z. russellii* KMM 3677^T, which hydrolysed alginic acid without liquefaction, possesses only the *alyA2* gene. In contrast *Z. roscoffensis* Asnod1-F08^T, although possessing homologues of *alyA3, alyA4, alyA5* and *alyA6* genes from *Z. galactanivorans* Dsij^T, does not possess either the *alyA1* or the *alyA2* gene. These observations indicates that the liquefaction phenotype is linked to the presence of the *alyA1* gene encoding a secreted endo-guluronate lyase [39]. Among species of the genus *Zobellia* with validly published names, only *Z. galactanivorans* Dsij^T, *Z. uliginosa* 553(843)^T [1] and *Z. nedashkovskayae* Asnod2-B07-B^T [5] possessed the *alyA1* gene and liquefied and utilised alginic acid. However, it is easy to differentiate these species. Unlike *Z. galactanivorans* Dsij^T, which is able to hydrolyse all red algal polysaccharides

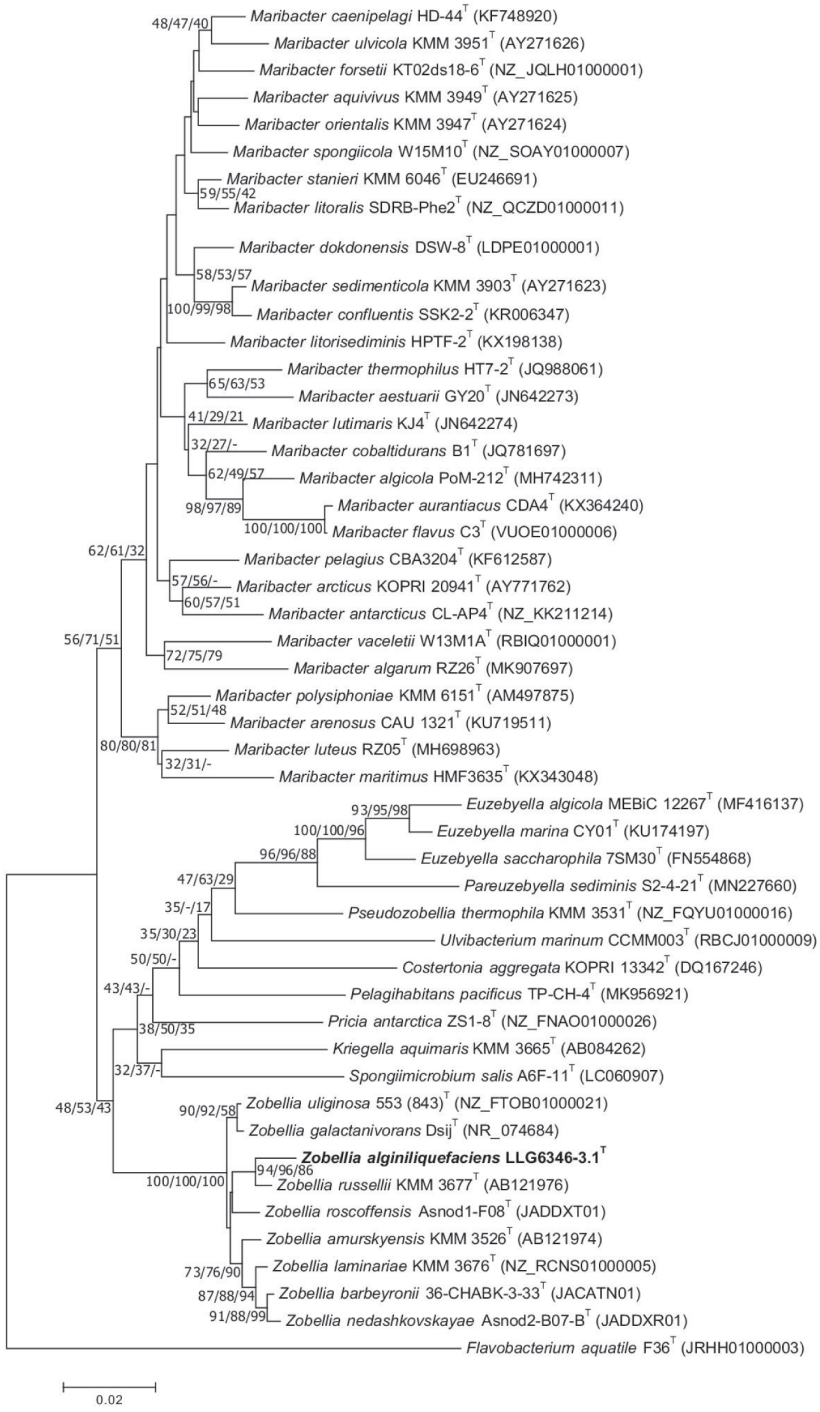

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between LLG6346-3.1^T and related taxa from the family *Flavobacteriaceae*. Numbers at the nodes indicate bootstrap values (as percentages of 1000 replicates) from neighbour-joining, maximum-likelihood 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 values are not conserved in other trees and are below 70%. *Flavobacterium aquatile* F36^T was used as an outgroup. Bar, 0.02 changes per nucleotide position.

(agars and carrageenins), and *Z. nedashkovskayae* Asnod2-B07-B^T, which is able to utilise laminarin and fucoidin from *Ascophyllum nodosum*, LLG6346-3.1^T did not hydrolyse and use agars or carrageenins (which is consistent with the absence of carrageenase-encoding genes in its genome) nor laminarin or fucoidin (Table 1). LLG6346-3.1^T and *Z. russellii* KMM 3677^T were able to use starch as sole carbon and energy sources and showed a hydrolysis area on soluble starch ZoBell agar plates (Table 1).

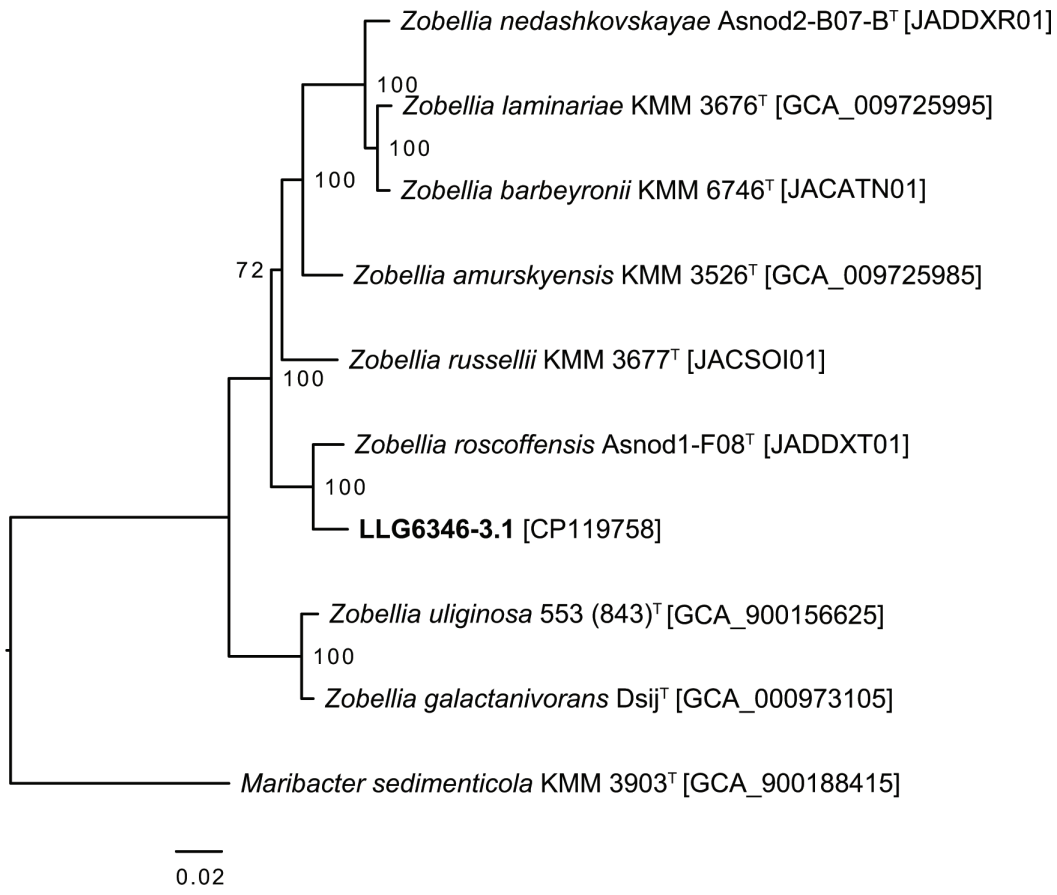

Fig. 2. Core proteome phylogenetic analysis of available genomes from type strains of species of the genus *Zobellia*, the newly isolated LLG6346-3.1^T and *Maribacter sedimenticola* KMM 3903^T used as an outgroup. Bar, 0.02 substitutions per amino acid position.

This indicates that both strains possess a secreted alpha-amylase, consistent with the presence of the amylase-encoding gene *susA* in their genomes. As reported previously [5], *Z. roscoffensis* Asnod1-F08^T lacks a *susA* homologue, probably explaining the absence of hydrolysis and use of starch. Finally, nitrate reductase activity is another discriminant criteria to differentiate LLG6346-3.1^T from *Z. roscoffensis* Asnod1-F08^T and *Z. russellii* KMM 3677^T. While the latter two strains showed vigorous nitrate reductase activity after growth in nitrated ZoBell broth, LLG6346-3.1^T showed very weak activity under the same conditions.

The other physiological features of LLG6346-3.1^T compared with *Z. roscoffensis* Asnod1-F08^T and *Z. russellii* KMM 3677^T are listed in Table 1. The three strains were resistant to kanamycin, gentamycin, neomycin, vancomycin, ampicillin, penicillin, carbenicillin, oxacillin, erythromycin, nalidixic acid, trimethoprim/sulfamethoxazole, bacitracin, colistin, polymixin B and chloramphenicol. For streptomycin, whereas *Z. roscoffensis* Asnod1-F08^T is sensitive, the other two strains are resistant. In the case of lincomycin, whereas LLG6346-3.1^T is sensitive, the other two strains are resistant. Finally, LLG6346-3.1^T, *Z. roscoffensis* Asnod1-F08^T and *Z. russellii* KMM 3677^T were sensitive to rifampicin. In conclusion, the results of phenotypic characterisations and phylogenetic analysis using 16S rRNA gene sequences together with whole-genome pairwise comparisons indicate that strain LLG6346-3.1^T represents a novel species of the genus *Zobellia*, for which the name *Zobellia alginiliquefaciens* sp. nov. is proposed.

DESCRIPTION OF *ZOBELLIA ALGINILIQUEFACIENS* SP. NOV.

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

Cells are Gram-stain-negative, aerobic, chemoorganotrophic, heterotrophic and rod-shaped, approximately 0.5 µm in diameter and 2.0–4.0 µm long; a few cells greater than 4 µm long may occur. Flagella are absent. Prosthecae and buds are not produced. Colonies on ZoBell agar are orange, convex, circular and mucoid in consistency and 2.0–3.0 mm in diameter after incubation for 3 days at 20 °C. Growth in ZoBell 2216E broth occurs from 4 to 38 °C (optimum, 30–33 °C), at pH 6.5–9.0 (optimum, pH 8–8.5) and in the presence of 2–7% NaCl (optimum, 4–5%). Positive for gliding motility and flexirubin-type pigment production. Nitrate is very weakly

Table 1. Phenotypic characteristics of LLG6346-3.1^T and of two related type strains of species of the genus *Zobellia*

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 are Gram-reaction-negative, aerobic, heterotrophic, chemorganotrophic, display gliding motility, do not form endospores, do not accumulate poly-*β*-hydroxybutyrate as an intracellular reserve product; require Na^+ ions or seawater for growth and produce flexirubin-type pigments. All strains are positive for the utilisation as a sole carbon source of D-glucose, D-galactose, D-fructose, D-mannose, D-xylose, salicin (weakly), sucrose, maltose, lactose, 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 All strains are positive 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 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, salicin (weakly), D-mannitol, 1-*O*-methyl-D-glucoside, 1-*O*-methyl-D-mannoside, maltose, lactose, melibiose, sucrose, cellobiose, turanose, trehalose, amygdalin, raffinose and arbutin. All strains are negative for indole and H_2S production; utilisation 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, arbutin, 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-chymotrypsin, *β*-glucuronidase and α-fucosidase activities; 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	–	+	+
Turanose	+	+	–
Melibiose	+	+	–
Amygdalin	–	+	–
Starch	+	–	+
Glycogen	+	–	–
Utilisation of (MMM)			
L-Arabinose	W	–	+
L-Rhamnose	–	+	+
Arabinoxylane	–	–	+
Alginic acid	+	–	+
Laminarin	–	+	+

Continued

Table 1. Continued

Characteristic	1	2	3
Acid production from (API 50CH):			
Ribose	–	–	+
L-Sorbose	–	+	+
L-Rhamnose	–	+	+
Melezitose	+	–	±
1-*O*-Methyl-D-xyloside	–	–	+
Gentiobiose	+	–	–
Inositol	–	–	+
Starch	+	–	+
Glycogen	+	–	–
2-Ketogluconate	+	+	–
Hydrolysis of:			
Starch (lugol assay)	+	–	+
Agar (lugol assay)	–	+	+
Alginic acid	+ (liq.)	–	+
DNA G+C content (mol%)	38.3	37.6	39.0

*Data from Barbeyron *et al.* [5].
†Data from Nedashkovskaya *et al.* [3].

reduced. *β*-Galactosidase-, oxidase- and catalase-positive. Alginic acid is hydrolysed to total liquefaction. DNA, gelatin, starch, aesculin, Tweens 20 and 60 are hydrolysed but Tween 40, agar, κ-carrageenin and ι-carrageenin are not. D-glucose, D-galactose, D-fructose, L-arabinose, D-mannose, D-xylose, salicin (weakly), sucrose, lactose, maltose, melibiose, cellobiose, gentiobiose, turanose, trehalose, D-mannitol, melezitose, raffinose, *N*-acetyl-glucosamine, starch, glycogen, inulin (weakly), porphyrin, alginic acid, xylan, galactan (gum arabic), glucomannan, lichenin, 1-*O*-methyl-D-glucoside and 1-*O*-methyl-D-mannoside are utilised as carbon and energy sources but D-arabinose, ribose, L-fucose, D-fucose, D-lyxose, L-rhamnose, L-sorbose, D-tagatose, L-xylose, arbutin, amygdalin, adipic acid, capric acid, malic acid, citric acid, gluconic acid, phenylacetic acid, 2-ketogluconate, 5-ketogluconate, 1-*O*-methyl-D-xyloside, adonitol, D-arabitol, L-arabitol, dulcitol, erythritol, glycerol, inositol, D-sorbitol, xylitol, arabinan, arabinoxylan, pectin (apple), galactomannan, xyloglucan, agar, ι-carrageenin, κ-carrageenin, λ-carrageenin, laminarin, ulvin, fucoidin (*Ascophyllum nodosum*) and fucoidin (*Laminaria hyperborea*) are not. Acid is produced from D-glucose, D-galactose, D-tagatose, D-fructose, D-arabinose, L-arabinose, D-mannose, D-fucose, L-fucose, D-lyxose, D-xylose, salicin (weakly), arbutin, sucrose, lactose, maltose, melibiose, cellobiose, gentiobiose, turanose, trehalose, amygdalin, melezitose, raffinose, starch, glycogen, inulin (weakly), D-mannitol, 1-*O*-methyl-glucoside, 1-*O*-methyl-D-mannoside and 2-ketogluconate but not from gluconic acid, ribose, L-xylose, L-sorbose, L-rhamnose, glycerol, erythritol, inositol, D-sorbitol, dulcitol, xylitol, D-arabitol, L-arabitol, adonitol, *N*-acetyl-glucosamine, 5-ketogluconate and 1-*O*-methyl-D-xyloside. Negative for indole and H_2S production and for arginine dihydrolase, tryptophan deaminase, urease, lysine decarboxylase and ornithine decarboxylase activities. In the API ZYM system, acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase α-galactosidase, *β*-galactosidase, α-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase and α-mannosidase activities are present, but lipase (C14), α-chymotrypsin, *β*-glucuronidase and α-fucosidase activities are absent.

The type strain, LLG6346-3.1^T (= RCC7657^T = LMG 32918^T), was isolated from the surface of the brown alga *Ericaria zosteroides* (C.Agardh) Molinari & Guiry 2020.The DNA G+C content of the type strain is 38.3 mol%. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LLG6346-3.1^T is OQ511313. The GenBank/EMBL/DDBJ accession number for the genome sequence of strain LLG6346-3.1^T is CP119758.

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Author contributions
Author contributions following the CRediT taxonomy (https://casrai.org/credit/) are as follows: conceptualisation: T.B., F.T.; formal analysis: T.B., F.T.; funding acquisition: F.T.; investigation: T.B., N.L.D., E.D., F.T.; project administration: F.T.; supervision: T.B., F.T.; visualisation: T.B., F.T.; writing–original draft: T.B.; writing–review and editing: T.B., N.L.D., E.D., F.T.

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

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