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Running title: *A. dinanensis* from human blood

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Abstract

Polyphasic taxonomic analysis was performed on a novel bacterium, designated UR159^T, isolated in 2016 from human blood of a septic patient hospitalized in France. Preliminary 16S rRNA gene sequence-based phylogenetic analysis indicated that strain UR159^T belonged to the family *Flavobacteriaceae*, forming a distinct phyletic line distantly related (<94% sequence similarity) to known species of the family. Further phenotypic, chemotaxonomic and genomic analyses were performed. Cells were non-motile, oxidase-negative, catalase-positive Gram-negative rod. It was strictly aerobic yielding yellow-pigmented colonies, and was metabolically rather inert. Major fatty acids were iso-branched fatty acids, predominantly iso-$C_{15:0}$ (55.5%) and iso-$C_{17:1}\omega 9c$ (8.8%). Whole genome sequencing revealed a 2.3-Mbp genome encoding a total of 2,262 putative genes with a genomic DNA G+C content at 37.6 mol%. The average nucleotide identity (ANI) and in silico DNA-DNA hybridization (isDDH) values between strain UR159^T and the most closely related members of the *Flavobacteriaceae* were <75% and <39%, respectively, much below the established cut-offs for ANI (<95-96%) and isDDH (<70%) for species and genus delineation. Average Amino Acid Identity (AAI) percentages were also estimated and were lower than 65% (cut-off proposed for genus delineation for uncultivated prokaryotes) in all cases, except for *F. marinum* that was just at the limit (65.1%). Based on these findings, we propose it as a new genus and species, *Avrilella dinanensis* gen. nov., sp. nov. (type strain UR159^T = CIP 111616^T = DSM 105483^T).

Introduction

The *Flavobacteriaceae* family, belonging to the phylum Bacteroidetes, was described in 1992 [1]. Since this date, it has much evolved with many new taxa allocated and currently 153 genera with a validly published and correct name are part of the *Flavobacteriaceae* family (www.bacterio.net). Members of this family are Gram-negative non-spore-forming rods widely described from diverse environmental habitats, especially in the marine environment [2]. Many species are pathogenic to animals, especially flavobacterial infections in fish [3]. Even though very rarely isolated in humans, some species are considered as opportunistic pathogens, especially in immunocompromised patients and those with severe underlying comorbidities (e.g., cancer, diabetes mellitus and chronic hematological, hepatic or kidney disorders) [4]. Besides *Capnocytophaga* spp., the most frequently found species isolated from clinical materials belong to the following genera: *Bergeyella* (*B. zoohelcum*), *Chryseobacterium* (*C. indologenes*), *Elizabethkingia* (*E. menigoseptica*), *Myroides* (*M. odoratus*, *M. odoratimimus*), *Sphingobacterium* (*S. multivorum*, *S. spitivorum*) and *Weeksella* (*W. virosa*) [5]. Most case reports report these organisms from blood, urine, wounds and respiratory secretions [4,5].

In the present study, we characterized a new isolate (designated UR159^T) and determined its taxonomic position using a detailed phenotypic and genotypic analysis (polyphasic approach) [6-8]. Our data suggest that this strain was not related to any previous taxon and thus, we propose it as a new genus and species, *Avrilella dinanensis* gen. nov., sp. nov., within the *Flavobacteriaceae* family.

Patient

An unknown Gram-negative rod was isolated in 2016 from two different aerobic blood culture bottles in a 93-year-old female patient hospitalized in the hospital of Dinan (Brittany,

France). The patient suffered from a sepsis (with a temperature at 39°C) after a red blood cell transfusion for chronic anemia (Hb <8 g/dl) in the context of a myelodysplastic syndrome. Although the association between infection and transfusion was not clearly proven, the patient was empirically treated by amoxicillin-clavulanate (1 g tid, 10 days) and became afebrile after 2 days of antimicrobial therapy.

Materials and methods

Strain isolation

Strain UR159^T was recovered from blood cultures (Bactec™ Plus/aerobic bottles; Becton Dickinson) of the patient. Positive bottles were used for Gram staining and inoculation onto blood and chocolate agar plates incubated at 35°C aerobically and in a 5% CO_2 atmosphere, respectively. Strain UR159^T was routinely cultivated aerobically at 35°C on blood agar (BA; ThermoFisher) and preserved for long term at -80°C as a suspension in brain-heart infusion (BHI; BD Difco™) supplemented with 20% glycerol (v/v).

Cell morphology and growth conditions

Cell morphology was observed using both light microscopy and transmission electron microscopy (TEM). For light microscopy, Gram staining was performed using cells grown on BA at 35°C for 24 hours. For TEM, bacterial cells from an exponential culture were collected after an overnight incubation, transferred to Eppendorf tubes and washed three times in cacodilate buffer (0.15 M, pH 7.4). Fractions of bacterial suspension were fixed at 4 °C for 60 min with 2% glutaraldehyde in 0.15 M cacodilate buffer to be washed three times in the same buffer. Cells were post-fixed in 1% OsO_4 for 60 min at 4 °C, rinsed in cacodilate buffer and embedded in 2% OmniPur® agarose, low melting (Calbiochem, Merck). After the dehydration series in acetone, the cells samples were embedded in conventional Epon (EMS, 1420) and then polymerized at 60 °C for 48 h. Resins blocks were sectioned into 80-nm sections using ultramicrotome LEICA UC7 (Leica, Wetzlar, Germany). These sections were mounted on copper grids and stained. Grids were observed using a JEM-1400 Electron Microscope (JEOL Ltd, Tokyo, Japan) at an accelerating voltage of 120 kV and equipped with an Orious® SC1000 camera (Gatan-Ametek, Pleasanton, USA). The cell size measurements

were carried out by TEM on two batches of culture and on 50 different bacterial cells for each culture.

Morphology, size and pigmentation of colonies were observed under optimal growth conditions on BA after 24 hours of incubation at 35°C under ambient air. Ability to grow under aerobic, microaerophilic and anaerobic conditions was assessed after 24-hour, 48-hour and 5-day incubation at 35°C while CO_2 requirement was tested in an incubator supplied with 5% CO_2. Note that microaerophilic and anaerobic conditions were obtained using the Anoxomat Anaerobic Cultivation System (Mart Microbiology, Drachten, The Netherlands). The growth ability was examined on blood agar plates incubated at 15, 30, 37 and 42°C. Gliding motility was tested as previously described [6].

Biochemical and antimicrobial susceptibility testing

Identification was attempted by using the MALDI-TOF mass spectrometry (Microflex™ LT/SH 60 Hz; Bruker Daltonics, Bremen, Germany) according to manufacturer's instructions for routine. Oxidase activity was determined with the MASTDISCS® ID Oxidase Discs (Mast Diagnostics) and catalase was detected using a 3% (v/v) aqueous H_2O_2 solution. Enzyme and carbon use profiles were generated with the API 20E and Vitek2 GN card microtest systems (bioMérieux) according to the manufacturer's instructions.

The antimicrobial susceptibility profile of the isolate was assessed by determining minimum inhibitory concentrations (MICs) using a commercial assay (Sensititre Gram-negative MIC plate, Thermo Scientific) according to manufacturer's instructions. The following antibiotics were tested: amoxicillin-clavulanate, piperacillin-tazobactam, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, gentamicin, amikacin, ciprofloxacin, cotrimoxazole, fosfomycin, tigecycline and colistin.

Chemotaxonomic analysis

The fatty acid methyl ester (FAME) analysis was performed at the Belgian Coordinated Collections of Microorganisms/Laboratory for Microbiology of the Faculty of Sciences of the Ghent University (BCCM/LMG), Gent, Belgium. In brief, cells were grown for 24 hours at 35°C under aerobic conditions on LMG medium 304. Inoculation and harvesting of the cells, and the extraction and analysis conformed to the recommendations of the commercial MIDI microbial identification system (Microbial IDentification Inc.). The whole-cell fatty acid composition was determined by gas chromatography on an Agilent Technologies 6890N gas chromatograph. The peak naming table MIDI TSBA 5.0 was used.

DNA extraction and 16S rRNA phylogenetic analysis

DNA was isolated from UR159^T using the using the Quick-DNA fungal/bacterial miniprep kit (Zymo Research, Irvine, CA) according to the manufacturer's recommendations. The 16S rRNA gene of UR159^T was amplified by PCR and sequenced using the universal primers 27F and 1541R, corresponding to base positions 8-27 and 1541-1525 of the 16S rRNA gene of *Escherichia coli*, respectively [9]. Both LeBibi-QBPP and NCBI nucleotide collection (nr/nt) databases were used to identify the nearest neighbor taxa with validly published names [10,11]. A sequence alignment using 16S rRNA gene sequences was constructed with the MUSCLE software [12]. All sequences with less than 95% coverage were eliminated, resulting in a final dataset of 139 sequences (consensus sequence = 1,255 bp). The phylogenetic tree was constructed using MEGA X version 10.7.1 with neighbor-joining (NJ) and maximum-likelihood (ML) methods [13]. Branch positions were statically tested with a bootstrap of 1,000 replicates.

Genome sequencing, assembly and annotation

After mechanical DNA shearing (Covaris® ultrasonicator), the DNA libraries were prepared using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs) and

sequenced as paired-end reads (2 x 300 bp) using the Illumina MiSeq platform and MiSeq reagent kit v3.

The Illumina reads were trimmed using Trimmomatic v0.32 [14], quality filtered with the Fastx-toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and assembled using SPAdes v3.14.0 that includes plasmidSPAdes [15,16]. Note that contigs <1,000 bp were discarded from the assembly and chromosomal contigs were then scaffolded using the SIS [17] and GapFiller v1.10 [18] softwares with *Flavobacterium marinum* CGMCC 1.10825^T (GenBank accession number NZ_FNXE00000000.1) as the reference genome sequence.

Genome annotation was performed by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (www.ncbi.nlm.nih.gov/genome/annotation_prok/). For mobile genetic elements, prophages were predicted using PHAST [19] completed by the Actinobacteriophage database (https://phagesdb.org) and plasmids were searched using the online plasmid search tool (https://plasmid.med.harvard.edu/PLASMID/). Functional analysis was performed using RPSBLAST program on COG database implemented in WebMGA (http://weizhongli-lab.org/webMGA).

Comparative genomics and phylogenomic analysis

To confirm the status as a new taxon of UR159^T, Average Nucleotide Identity (ANI), in silico DNA-DNA hybridization (isDDH), Average Amino Acid Identity (AAI) values were determined. The calculation of the ANI between strain UR159^T and 343 genomes of *Flavobacteriaceae*-related species was performed using BLASTN and Pyani v0.2.10 module (https://pypi.org/project/pyani/) [20], following the algorithm described by Goris *et al.* [21]. According to Pyani and 16S rRNA gene similarities, 25 available *Flavobacteriaceae* genomes were selected and downloaded from GenBank for comparative genomic analysis. The protein coding sequences were predicted for all genomes by using Prokka v1.14.5 [22]. The

estimation of AAI was determined using the tool AAI calculator (http://enve-omics.ce.gatech.edu/) [23]. The isDDH values were calculated with the same data set for ANI calculation using the Genome-to-Genome Distance calculator (GGDC 2.1; https://ggdc.dsmz.de/) with the formula 2. Pangenomic analysis of the 26 genomes was conducted using PIRATE software [24]. To calculate phylogenomic relations between strain U159^T and phylogenetic neighbours, a core-genome tree was constructed as follows. All predicted protein-coding genes annotated from each available genome were compared using the all-versus-all BLAST search [25]. Only proteins for which coding sequences with >50% amino acid identity and >70% sequence coverage were considered as orthologous. The nucleotide sequences of core gene set were aligned using MUSCLE software and MEGA6 software [26] was used for the phylogenomic tree reconstruction using the neighbour-joining method with Jukes-Cantor correction.

Sequence accession numbers

The 16S rRNA sequence generated was submitted to GenBank with the accession number MF278923. The draft genome sequence of UR159^T was deposited in NCBI under the accession number NZ_NIPO00000000.

Results and discussion

Strain UR159^T was not identified by the MALDI-TOF mass spectrometry using the Bruker database (version 2.3) but the profile was reproducible (Figure S1). The almost-complete sequence of the 16S rRNA gene was obtained (1,516 bp) and used for sequence comparison and phylogenetic analysis. Strain UR159^T exhibited the highest level of nucleotide pairwise similarity (93.1%) with *Flavobacterium marinum* SW105^T [27]. Sequence similarity was lower with the next closest relatives (*Flavobacterium* spp., *Myroides* spp.) with percentages of 16S rRNA gene sequence similarity in all cases lower than 92.9%. The ML and NJ phylogenetic trees were almost identical and both confirmed the affiliation of UR159^T to the *Flavobacteriaceae* (Figure 1). The sequence similarity between the 16S rRNA gene of UR159^T and the closest phylogenetic neighbors, which was distinctly below the predetermined cut-off levels in order to classify bacterial isolates as novel taxa at the genus (<95%) and (<97-98.65%) species levels [7,8,28,29], suggested that the strain was a potentially undescribed microorganism requiring further characterization.

Phenotypic properties

Strain UR159^T grew optimally on BA after 24-h incubation at 35°C under ambient air, yielding ~1-3-mm, yellow-pigmented, circular, convex, smooth colonies (Figure S2). No growth was observed when incubated anaerobically and it was demonstrated UR159^T was strictly aerobic (Figure S2). It failed to grow on Drigalski, cetrimide and Hektoen agar (data not shown). It was a non-motile, oxidase-negative, catalase-positive Gram-negative rod (Figure 2A) able to grow between 15°C and 37°C. TEM confirmed that cells of UR159^T were rod-shaped bacteria measuring an average size of 0.35 ± 0.02 µm wide and 1.12 ± 0.16 µm long (Figure 2B). Biochemically, it was metabolically rather inert since almost all reactions were negative (especially indole production, Voges-Proskaeur, nitrate reduction, H_2S production

and urease tests) except of the production of gelatinase, and presented some differences with species of closely-related genera (Table 1) [27,30-33]. Furthermore, strain UR159^T appeared susceptible to amoxicillin-clavulanate (MIC <2/2 mg/L), ciprofloxacin (MIC = 0.25 mg/L), cotrimoxazole (MIC = 2/38 mg/L) and tigecycline (MIC = 0.5 Mg/L) whereas MICs were higher for piperacillin-tazobactam (MIC = 8 mg/L), cefotaxime (MIC >4 mg/L), ceftazidime (MIC = 128 mg/L), cefepime (MIC = 32 mg/L), imipenem (MIC = 4 mg/L), meropenem (MIC = 8 mg/L), gentamicin (MIC >4 mg/L), amikacin (MIC >16 mg/L), fosfomycin (MIC >64 mg/L) and colistin (MIC >8 mg/L).

The cellular fatty acids of strain UR159^T mainly comprised iso-branched fatty acids, predominantly iso-$C_{15:0}$ (55.5%), iso-$C_{17:1}\omega 9c$ (8.8%) iso-$C_{17:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}\omega 9c$ and/or iso-$C_{15:0}$ 2-OH) (Table 2). Strain UR159^T and *F. marinum* SW105^T shared similar fatty acid profiles, with minor differences in the respective proportions of the components (Table 2) [27].

Genomic features

The draft genome size of UR159^T was 2,331,799 bp long (3 scaffolds with a final coverage of 124×) (Table 3 and Figure S3), which is smaller than those of most other members of the *Flavobacteriaceae* family (Table 4). Based on whole genome sequence, the genomic DNA G+C content was determined at 37.6 mol%, which is consistent with those of the type strains of closely related genera (31.4-44.2 mol%) (Tables 3 and 4). The genome encoded a total of 2,262 putative genes, including 2,176 protein-coding genes and 61 RNAs (50 tRNAs, 7 rRNAs and 4 ncRNAs) and 25 pseudogenes (Table 3). A total of 1,645 genes (75.5%) were assigned to a COG functional category with 1406 genes (64.5% of the total genes) with known putative function, and 773 genes were annotated as hypothetical proteins (35.5%) (Table S1). Whereas no extrachromosomal element was found, a 6.5-kb prophage-like element was

found (Figure S3), showing sequence homology to the phage RAP44 of *Riemerella anatipestifer* [34].

Comparative genomic analysis

Phylogenomic analysis revealed a lack of relatives at the species or genus level, with ANI values between strain UR159^T and the most closely related members of the *Flavobacteriaceae* between 69.7 and 74.0% (alignment coverage from 7.0% to 29.0%) while those of isDDH ranged from 17.9 to 38.7% (Table 4 and Figures S4). These calculated similarity values were below the proposed and generally accepted species boundaries for ANI and isDDH that are 95-96% and 70%, respectively [8,35]. These results indicate that strain UR159^T constitutes a novel taxon at least at the species level, not related at this level to any of the closest phylogenetic neighbors, which can be visualized with the core-genome tree (Figure 3). Additionally, species that share less than 80% ANI are too divergent to be compared only based on this parameter, and AAI, which provides a more robust resolution, should be used instead. Thus, AAI percentages were also estimated with a threshold AAI value of 65%, which has been proposed for genus delineation for uncultivated prokaryotes [29]. AAI values for strain UR159^T and the most closely related species were in all cases lower than 65% (Table 4), except for *F. marinum* that is just at the limit (65.1%) (Table 4 and Figure S5). By pangenomic analysis, only 472 orthologous genes (20.8%) were determined to be encoded in all genomes whereas 514 genes (22.7%) were predicted to be unique to strain UR159^T compared to other genomes tested. The complete genome of UR159^T shared between 761 (35.5%) to 1403 (65.4%) common gene families with *Weeksella virosa* and *F. marinum* genomes, respectively (Table 4).

In conclusion, taking into account all the findings from phenotypic, chemotaxonomic, phylogenetic, and genomic analysis, strain UR159^T cannot be allocated to any genus and

263 species previously described. Hence, this strain represents a novel taxon within the
264 *Flavobacteriacae* family, for which the name *Avrilella dinanensis* gen. nov., sp. nov. is
265 proposed. The descriptions of the *Avrilella* gen. nov. and *Avrilella dinanensis* sp. nov. are
266 given in Table 5.
267
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270

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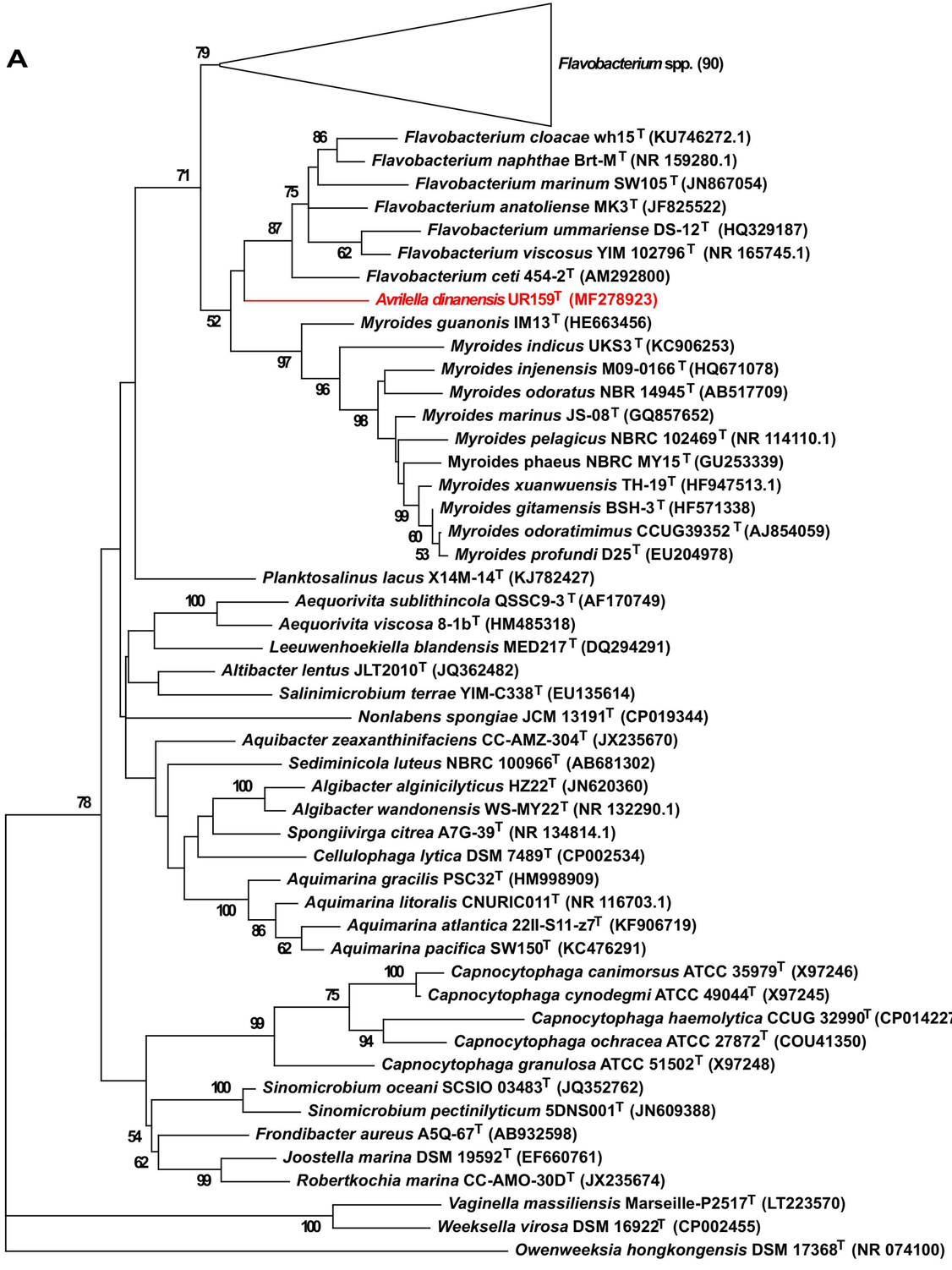
Legends of the figures

Figure 1. Maximum-likelihood (A) and neighbor-joining (B) trees based on the alignment of 1,255 bp of 16S rRNA gene sequences, showing the phylogenetic position of strain U159^T among the *Flavobacteriaceae* family. Boostrap values higher than 50% are indicated at branch points. The strain *Owenweeksia hongkongensis* DSM 17368^T was used as an outgroup. Sequence accession numbers are shown in parentheses. Scale bars: 0.1 or 0.02 substitutions per nucleotide position.

Figure 2. (A) Gram staining image (at × 1000 magnification) and (B) transmission electron microscopy (scale bar = 0.5 µm) of *Avrilella dinanensis* UR159^T.

Figure 3. Neighbour-joining core gene phylogenetic tree including genomes of 26 members *Flavobacteriaceae* family. Bootstrap values higher than 60% are indicated at indicated at branch points. Scale bar: 0.01 substitution per nucleotide position.

A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86
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Flavobacterium naphthae Brt-MT (NR 159280.1)
71
Flavobacterium marinum SW105T (JN867054)
75
Flavobacterium anatoliense MK3T (JF825522)
87
Flavobacterium ummariense DS-12T (HQ329187)
62
Flavobacterium viscosus YIM 102796T (NR 165745.1)
Flavobacterium ceti 454-2T (AM292800)
Avrilella dinanensis UR159T (MF278923)
52
Myroides guanonis IM13T (HE663456)
97
Myroides indicus UKS3T (KC906253)
Myroides injenensis M09-0166T (HQ671078)
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Myroides odoratus NBR 14945T (AB517709)
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Myroides marinus JS-08T (GQ857652)
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Myroides phaeus NBRC MY15T (GU253339)
Myroides xuanwuensis TH-19T (HF947513.1)
99
Myroides gitamensis BSH-3T (HF571338)
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Myroides odoratimimus CCUG39352T (AJ854059)
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Myroides profundi D25T (EU204978)
Planktosalinus lacus X14M-14T (KJ782427)
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Aequorivita viscosa 8-1bT (HM485318)
Leeuwenhoekiella blandensis MED217T (DQ294291)
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Salinimicrobium terrae YIM-C338T (EU135614)
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Spongiivirga citrea A7G-39T (NR 134814.1)
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Aquimarina gracilis PSC32T (HM998909)
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Aquimarina litoralis CNURIC011T (NR 116703.1)
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Aquimarina atlantica 22II-S11-z7T (KF906719)
62
Aquimarina pacifica SW150T (KC476291)
100
Capnocytophaga canimorsus ATCC 35979T (X97246)
75
Capnocytophaga cynodegmi ATCC 49044T (X97245)
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Capnocytophaga haemolytica CCUG 32990T (CP014227)
94
Capnocytophaga ochracea ATCC 27872T (COU41350)
Capnocytophaga granulosa ATCC 51502T (X97248)
100
Sinomicrobium oceani SCSIO 03483T (JQ352762)
Sinomicrobium pectinilyticum 5DNS001T (JN609388)
54
Frondibacter aureus A5Q-67T (AB932598)
62
Joostella marina DSM 19592T (EF660761)
99
Robertkochia marina CC-AMO-30DT (JX235674)
Vaginella massiliensis Marseille-P2517T (LT223570)
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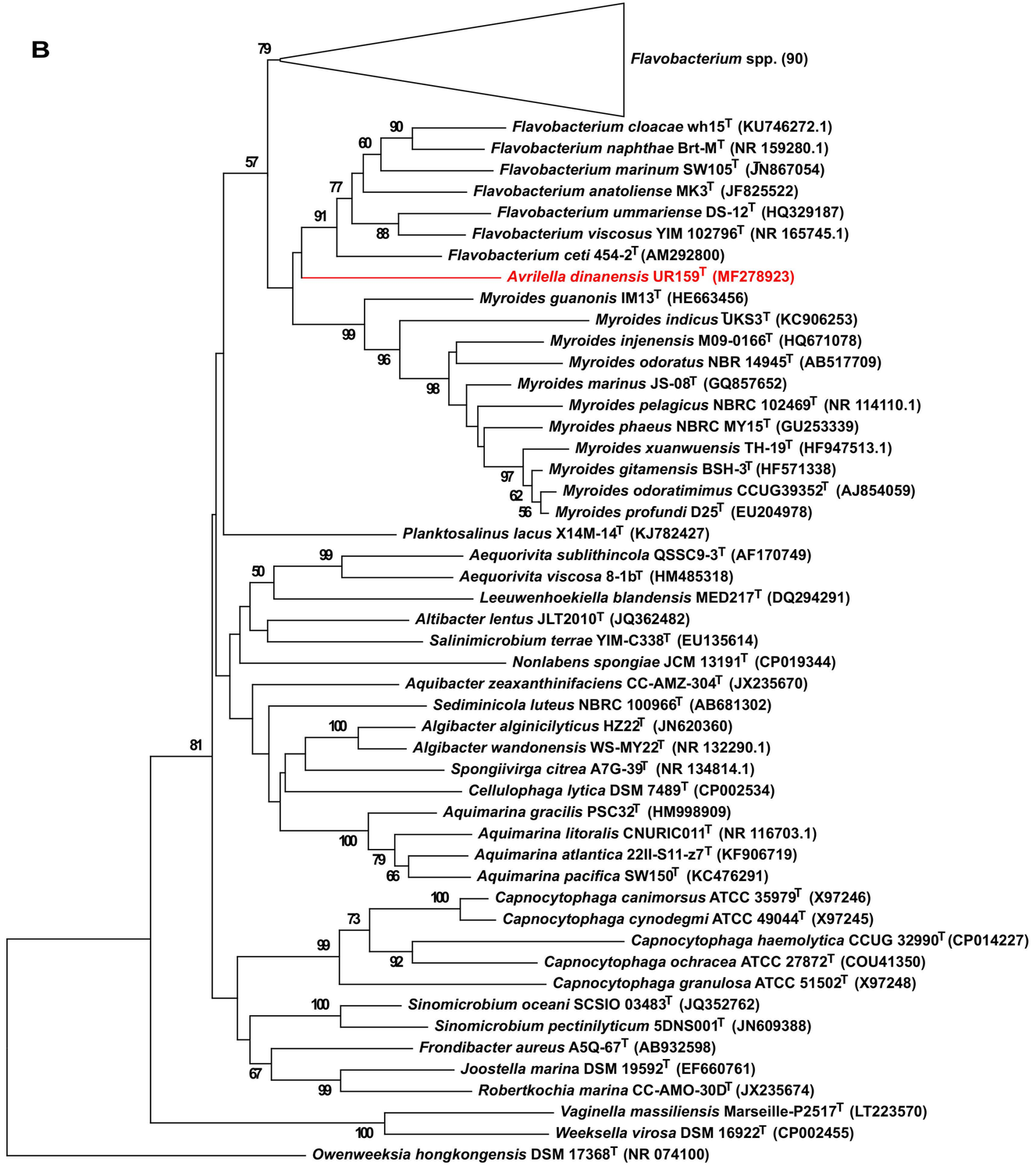
B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Flavobacterium anatoliense MK3T (JF825522)
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Flavobacterium ummariense DS-12T (HQ329187)
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Flavobacterium viscosus YIM 102796T (NR 165745.1)
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Avrilella dinanensis UR159T (MF278923)
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Myroides indicus UKS3T (KC906253)
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Myroides odoratus NBR 14945T (AB517709)
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Myroides xuanwuensis TH-19T (HF947513.1)
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Planktosalinus lacus X14M-14T (KJ782427)
99
Aequorivita sublithincola QSSC9-3T (AF170749)
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Aequorivita viscosa 8-1bT (HM485318)
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Salinimicrobium terrae YIM-C338T (EU135614)
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Aquibacter zeaxanthinifaciens CC-AMZ-304T (JX235670)
Sediminicola luteus NBRC 100966T (AB681302)
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Capnocytophaga canimorsus ATCC 35979T (X97246)
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Capnocytophaga ochracea ATCC 27872T (COU41350)
Capnocytophaga granulosa ATCC 51502T (X97248)
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Sinomicrobium oceani SCSIO 03483T (JQ352762)
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Vaginella massiliensis Marseille-P2517T (LT223570)
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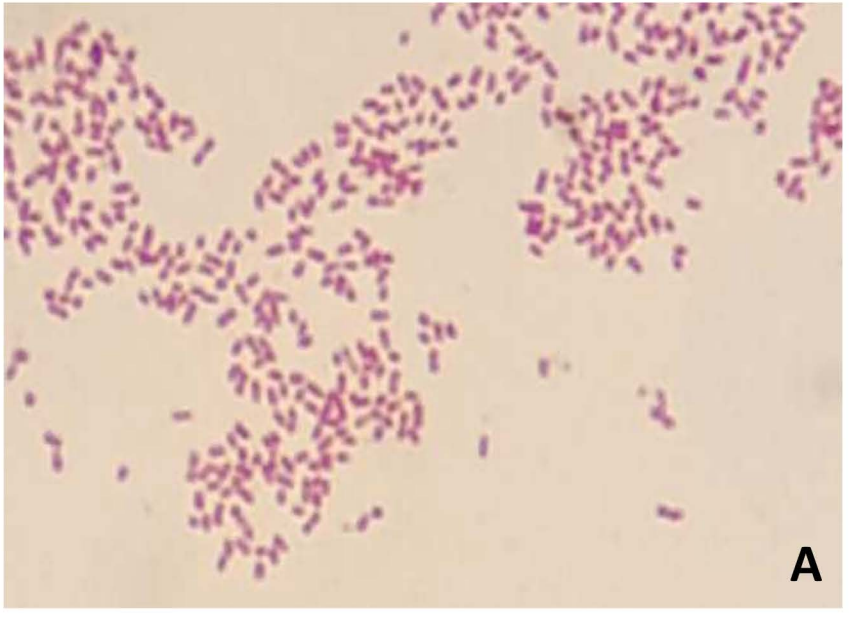
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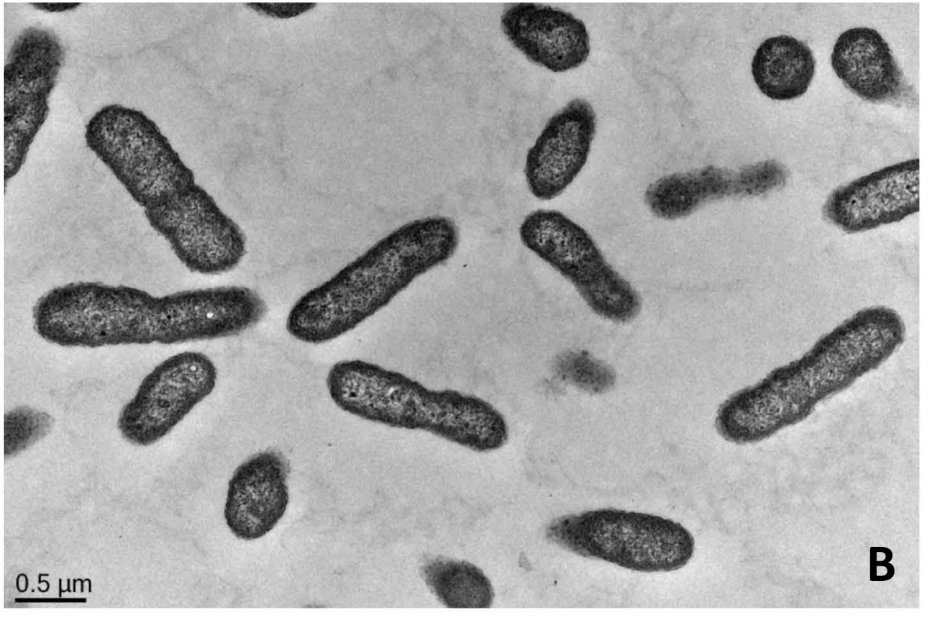
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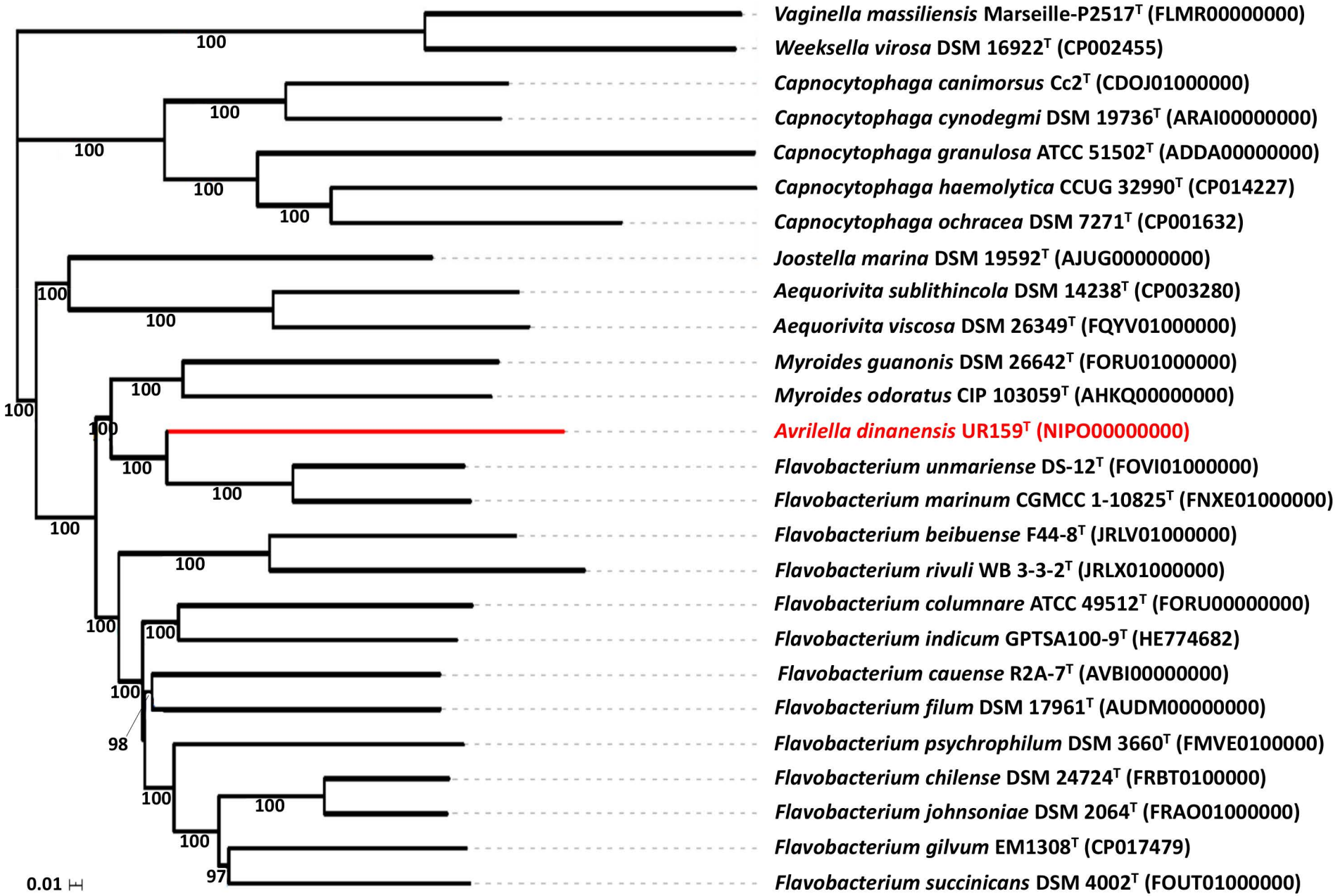
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Flavobacterium filum DSM 17961T (AUDM00000000)
Flavobacterium psychrophilum DSM 3660T (FMVE0100000)
Flavobacterium chilense DSM 24724T (FRBT0100000)
Flavobacterium johnsoniae DSM 2064T (FRAO01000000)
Flavobacterium gilvum EM1308T (CP017479)
Flavobacterium succinicans DSM 4002T (FOUT01000000)
100
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98
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100
97
0.01

Table 1. Differential characteristics of strain UR159^T and type strains of closely related genera [20,23-26].

Characteristic	***Avrilella dinanensis* UR159^T**	*Flavobacterium marinum* SW105^T	*Flavobacterium unmariense* DS-12^T	*Myroides guanonis* IM13^T
Colony pigmentation	Yellow	Yellow	Yellow	Yellow
Growth at:				
15°C	+	+	+	+
30°C	+	+	+	+
37°C	+	+	+	+
42°C	-	+	-	-
Gliding motility	-	-	-	-
Respiratory metabolism	Strictly aerobic	Strictly aerobic	Strictly aerobic	Strictly aerobic
Oxidase	+	+	+	+
Catalase	+	+	+	+
Substrates utilized:				
D-glucose	-	-	-	-
D-mannose	-	+	+	-
Maltose	-	+	+	-
L-arabinose	-	+	-	-
D-Mannitol	-	+	-	-
D-Sorbitol	-	-	-	-
Enzyme activity :				
Arginine dihydrolase	-	-	-	-
Lysine decarboxylase	-	-	nd	-
Ornithine decarboxylase	-	-	nd	-
Gelatinase	+	-	-	+
Urease	-	-	+	+
α-galactosidase	-	-	-	nd
β-galactosidase	-	-	-	-
β-glucosidase	-	-	-	nd
Citrate utilization	-	w	-	+
Indole production	-	-	-	-
Acetoin production (Voges-Proskaeur reaction)	-	nd	nd	-
Nitrate reduction	-	-	-	-
H_2S production	-	-	nd	-

-, negative; nd: not determined; +, positive; w, weakly positive.

Table 2. Cellular fatty acid compositions of *Avrilella dinanensis* UR159^T gen. nov. sp. nov. and *Flavobacterium marinum* SW105^T [20]

Fatty acid (%)	***A. dinanensis* UR159^T**	***F. marinum* SW105^T**
iso-$C_{14:0}$	-	-
iso-$C_{15:0}$	55.5	52.7
$C_{16:0}$	2.2	-
iso-$C_{15:0}$ 3-OH	3.2	-
iso-$C_{16:0}$	-	1.1
iso-$C_{17:1}$ $\omega 9c$	8.8	13.1
iso-$C_{15:0}$ 3-OH	-	2.5
iso-$C_{16:0}$ 3-OH	1.4	-
$C_{18:1}$ $\omega 9c$	1.5	-
iso-$C_{17:0}$ 3-OH	4.0	11.4
Unknown 13.565	8.9	-
Unknown 16.582	2.5	-
Summed feature 3	4.9	15.1
Summed feature 4	1.7	-

The fatty acids amounting to <1% of the total fatty acids are not shown. Summed feature 3 comprises ($C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2-OH) and summed feature 4 comprises (iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B). -, not detected or Trace (<1% of total).

Table 3. Genomic characteristics of *Avrilella dinanensis* UR159^{T} gen. nov. sp. nov.

Characteristic	***A. dinanensis* UR159^{T}**
Genbank accession no.	NZ_NIPO00000000
Genome size	2,331,799
No. scaffolds (sizes)	3 (2,273,643 bp; 5,646 bp; 52,510 bp)
G+C content (%)	37.6
Total genes	2,262
Protein-coding genes	2,176
No. hypothetical proteins	1,183
rRNAs	7
tRNAs	50
ncRNAs	4
Pseudogenes	25
Extrachromosomal elements	0

Table 4. Relatedness of the sequenced genome of *Avrilella dinanensis* UR159^T gen. nov. sp. nov. to whole genome sequences of types strains of closely related genera based on genome size, G+C content, ANI, in silico DDH, AAI and orthologous proteins.

Strain	Genbank accession no.	Size	%						
			G+C	Mean ANI identity	Mean ANI coverage	In silico DDH	Mean AAI identity	Mean AAI coverage	Orthologous proteins
Avrilella dinanensis UR159^T	NZ_NIPO00000000.1	2,331,799	37.7	100.0	100.0	100.0	100.0	100.0	100.0
Aequorivita sublithincola DSM 14238^T	CP003280.1	3,520,671	36.2	70.7	11.2	23.5	54.5	68.0	47.2
Aequorivita viscosa DSM 26349^T	FQYV01000000.1	3,526,954	36.5	70.5	10.5	38.7	54.6	69.0	48.1
Capnocytophaga canimorsus Cc2^T	CDOJ01000000.1	2,504,412	36.1	71.4	10.7	20.5	54.4	58.1	39.6
Capnocytophaga cynodegmi DSM 19736^T	ARAI00000000.1	2,657,104	34.4	71.5	10.2	21.5	54.5	57.9	39.7
Capnocytophaga granulosa ATCC 51502^T	ADDA00000000.1	2,746,278	41.4	69.7	7.3	19.7	52.2	57.5	37.2
Capnocytophaga haemolytica CCUG 32990^T	CP014227.1	2,688,484	44.2	70.1	7.0	27.9	53.4	54.7	37.3
Capnocytophaga ochracea DSM 7271^T	CP001632.1	2,612,925	39.6	70.8	8.6	25.9	53.5	56.1	37.4
Flavobacterium beibuense F44-8^T	JRLV01000000.1	3,800,758	37.7	71.2	14.8	19.2	58.3	73.5	54.1
Flavobacterium cauense R2A-7^T	AVBI00000000.1	3,110,902	38.2	72.4	18.4	17.9	60.2	71.1	54.9
Flavobacterium chilense DSM 24724^T	FRBT0100000.1	6,113,833	33.7	72.0	13.4	20.3	57.4	76.2	55.5
Flavobacterium columnare ATCC 49512^T	FORU00000000.1	3,162,432	31.5	72.0	15.7	21.8	58.8	65.6	51.0
Flavobacterium filum DSM 17961^T	AUDM00000000.1	3,192,093	33.8	72.6	16.4	20.2	59.3	66.6	50.6
Flavobacterium gilvum EM1308^T	CP017479.1	4,402,594	35.2	72.5	13.3	21.3	57.7	69.0	50.7
Flavobacterium indicum GPTSA100-9^T	HE774682.1	2,993,089	31.4	72.2	16.8	18.9	58.1	68.4	51.5
Flavobacterium johnsoniae DSM 2064^T	FRAO01000000.1	6,052,484	34.0	71.9	13.2	19.9	57.6	76.7	55.9
Flavobacterium marinum CGMCC 1-10825^T	FNXE01000000.1	2,977,689	35.4	74.0	29.0	22.5	65.1	80.1	65.4
Flavobacterium psychrophilum DSM 3660^T	FMVE0100000.1	2,626,144	32.3	71.7	16.5	19.0	59.0	64.7	50.6
Flavobacterium rivuli WB 3-3-2^T	JRLX01000000.1	4,479,972	39.6	70.7	12.1	18.2	57.3	73.6	54.0
Flavobacterium succinicans DSM 4002^T	FOUT01000000.1	3,664,904	35.5	71.8	14.0	18.8	57.5	68.8	51.0
Flavobacterium ummariense DS-12^T	FOVI01000000.1	3,493,887	34.7	73.1	25.9	19.8	63.8	80.0	64.3
Joostella marina DSM 19592^T	AJUG00000000.1	4,508,243	33.6	71.1	10.8	19.6	55.2	68.0	46.9
Myroides guanonis DSM 26542^T	FORU01000000.1	3,140,132	33.3	71.3	19.6	18.1	60.5	73.5	58.2
Myroides odoratus CIP 103059T	AHKQ00000000.1	4,227,641	35.5	71.8	16.4	19.9	58.8	75.6	56.8
Vaginella massiliensis Marseille-P2517^T	FLMR00000000.1	2,434,476	38.2	72.0	9.6	22.5	51.7	61.9	36.9
Weeksella virosa DSM 16922^T	CP002455.1	2,272,954	35.9	71.9	9.8	26.4	51.1	63.9	35.5

Table 5. Protologue for *Avrilella dinanensis*.

Genus name	*Avrilella*	
Species name		*Avrilella dinanensis*
Genus status	gen. nov.	
Genus etymology	[A.vril.el'la. N.L. dim. suff. -ella. *Avrilella*, named after the French bacteriologist and writer Jean-Loup Avril, Rennes, France]	
Type species of the genus	*Avrilella dinanensis*	
Specific epithet	-	*dinanensis*
Species status	-	sp. nov.
Species etymology	-	*Avrilella dinanensis* (di.nan.en'sis. N.L. fem. adj. *dinanensis*, of Dinan, after the name of the city in Brittany where the strain was originally isolated)
Description of the new taxon and diagnostic traits	Gram-negative, aerobic rods that grow as yellow-pigmented, circular, convex, smooth colonies. Strains are positive for catalase and oxidase, and metabolically rather inert except for a gelatinase activity. Major fatty acids are iso-branched fatty acids, predominantly iso-C15:0, iso-C17:1ω9*c* and iso-C17:0 3-OH. The genus is affiliated to the family '*Flavobacteriaceae'*, order 'Flavobacteriales', class 'Flavobacteriia'.	Non-motile, oxidase-negative, catalase-positive Gram-negative rod able to grow between 15°C and 37°C. Strictly aerobic growing optimally on blood agar after 24-h incubation at 35°C under ambient air, yielding ~1-3-mm, yellow-pigmented, circular, convex, smooth colonies. Metabolically rather inert, except the production of gelatinase. Susceptibility to amoxicillin-clavulanate (MIC, 0.5 mg/L), cefoxitin (MIC, 1.5 mg/L), ertapenem (MIC, 1.5 mg/L) imipenem (MIC, 2 mg/L), meropenem (MIC, 2 mg/L), ciprofloxacin (MIC, 0.19 mg/L), cotrimoxazole (MIC 0.094 mg/L) and tigecycline (MIC, 0.5 Mg/L) but resistant to cefotaxime (MIC >32 mg/L), ceftazidime (MIC >32 mg/L), cefepime (MIC, 16 mg/L), aminoglycosides (MICs >16 mg/L), fosfomycin (MIC >64 mg/L) and colistin (MIC >8 mg/L).
Country of origin		France
Region of origin		Brittany
Date of isolation		01/09/2016
Source of isolation		Human blood
Sampling date		01/09/2016
Latitude		48° 27' 0'' N
Longitude		2° 2' 49.2'' W
Altitude (meters above sea level)		76 m
16S rRNA gene accession number		MF278923
Genome accession number		NZ_NIPO00000000
Genome size (bp)		2,331,799
GC mol%		37.6
Number of strains in the study		1
Information related to the Nagoya protocol		Not applicable
Designation of the type strain		UR159^T
Strain collection numbers		CIP 111616^T = DSM 105483^T

Table S1. Proportions of genes associated with general COG functional categories.

Code	% of total genes	% of unique genes	Description
J	9,54	0,84	Translation, ribosomal structure and biogenesis
K	5,48	10,56	Transcription
L	5,92	7,69	Replication, recombination and repair
D	1,25	0,76	Cell cycle control, cell division, chromosome partitioning
V	2,23	1,75	Defense mechanisms
T	3,42	0,00	Signal transduction mechanisms
M	9,40	12,97	Cell wall/membrane/envelope biogenesis
N	0,42	0,61	Cell motility
U	2,42	0,90	Intracellular trafficking, secretion, and vesicular transport
O	5,26	6,59	Posttranslational modification, protein turnover, chaperones
C	6,24	1,23	Energy production and conversion
G	3,18	2,86	Carbohydrate transport and metabolism
E	8,13	5,06	Amino acid transport and metabolism
F	3,49	1,01	Nucleotide transport and metabolism
H	5,30	4,05	Coenzyme transport and metabolism
I	5,29	1,67	Lipid transport and metabolism
P	6,38	7,85	Inorganic ion transport and metabolism
Q	2,13	1,68	Secondary metabolites biosynthesis, transport and catabolism
R	14,52	21,00	General function prediction only
S	8,29	15,16	Function unknown

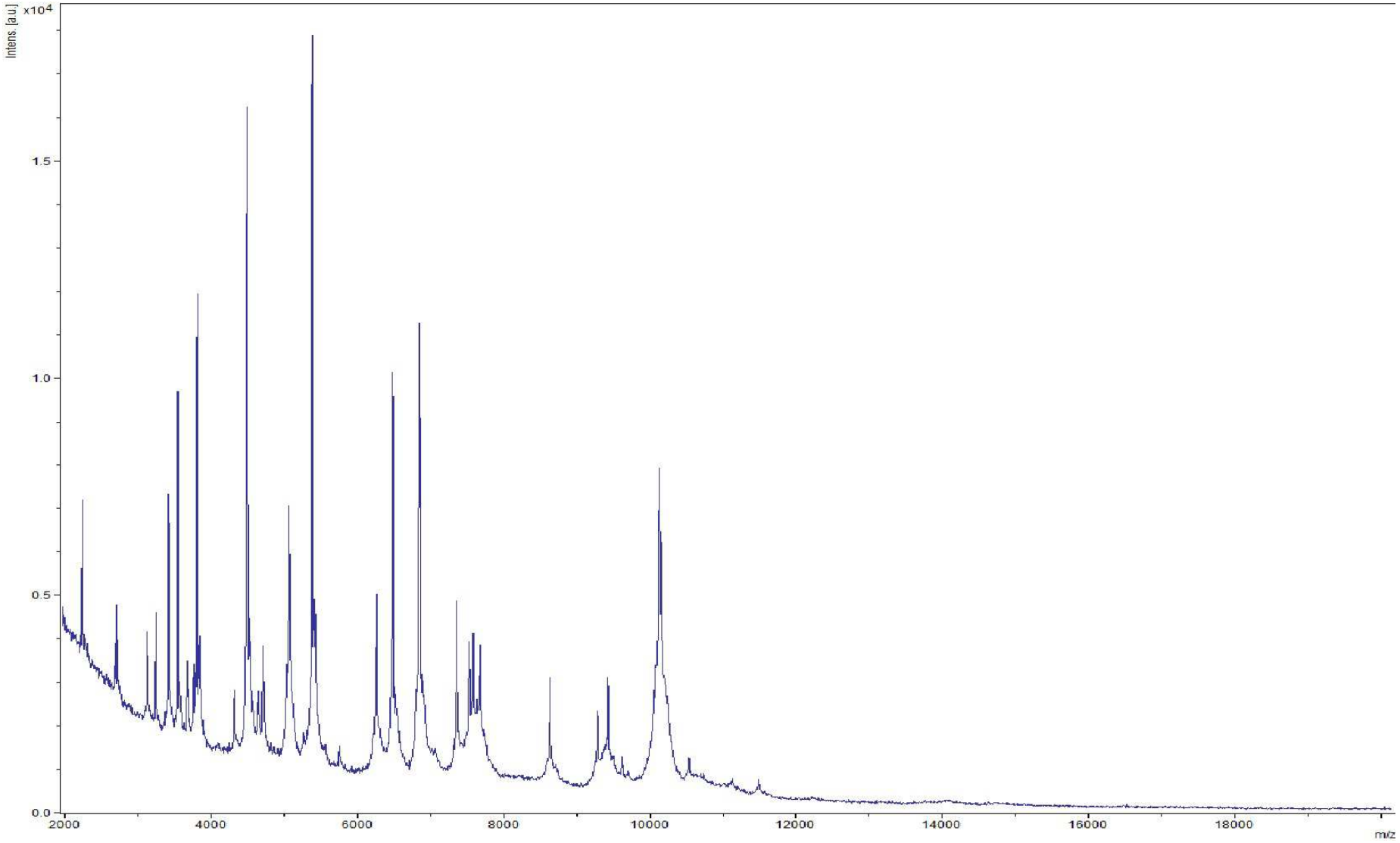

Figure S1. Mass spectrum from *Avrilella dinanensis* UR159^{T} obtained with the Microflex MALDI-TOF instrument (Bruker Daltonics).

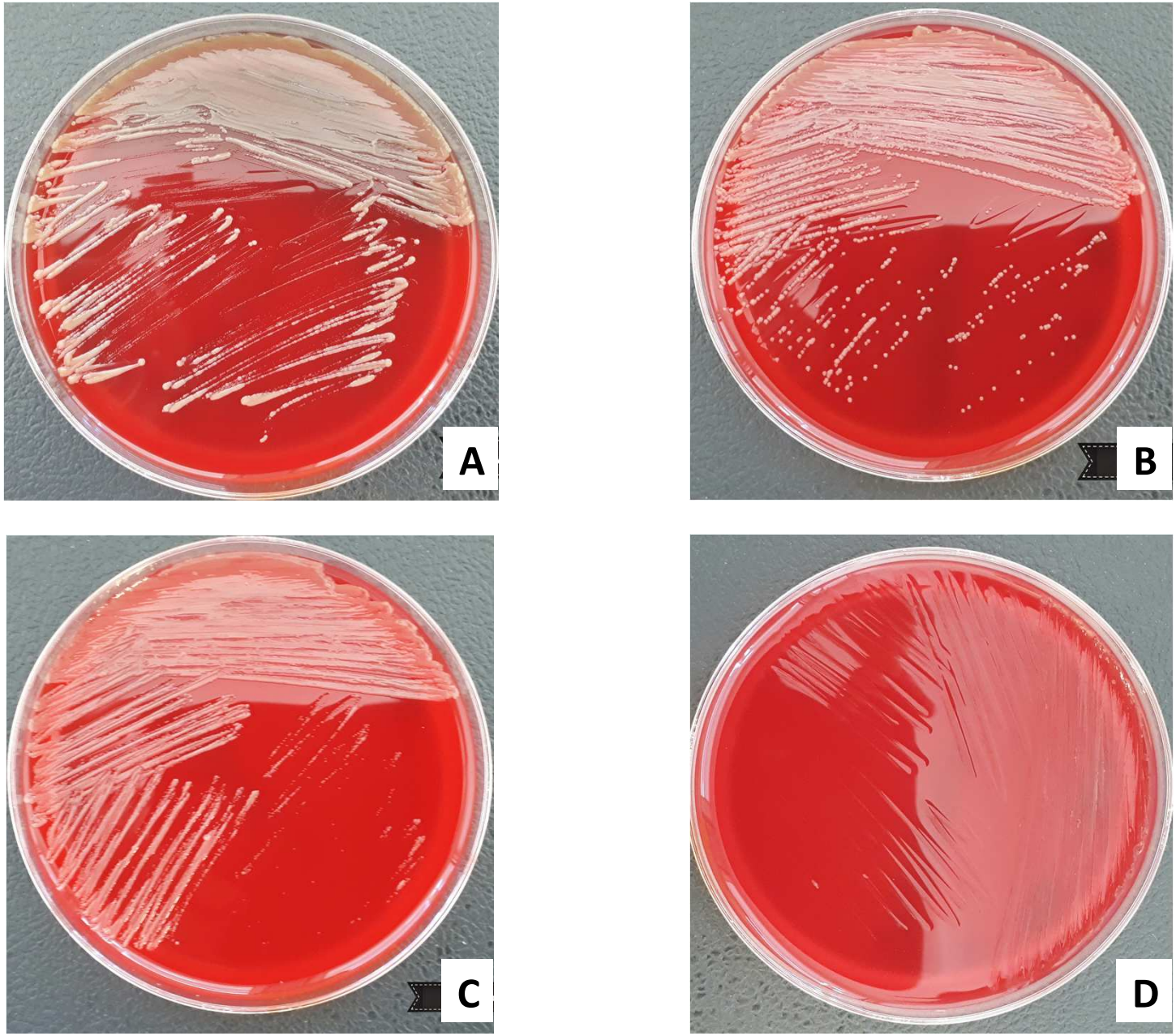

Figure S2. Growth of strain UR159^{T} on blood agar after 24-h incubation at 35°C under (A) ambient air, 5% CO_2 atmosphere (B), microaerophily (C), and anaerobiosis (D).

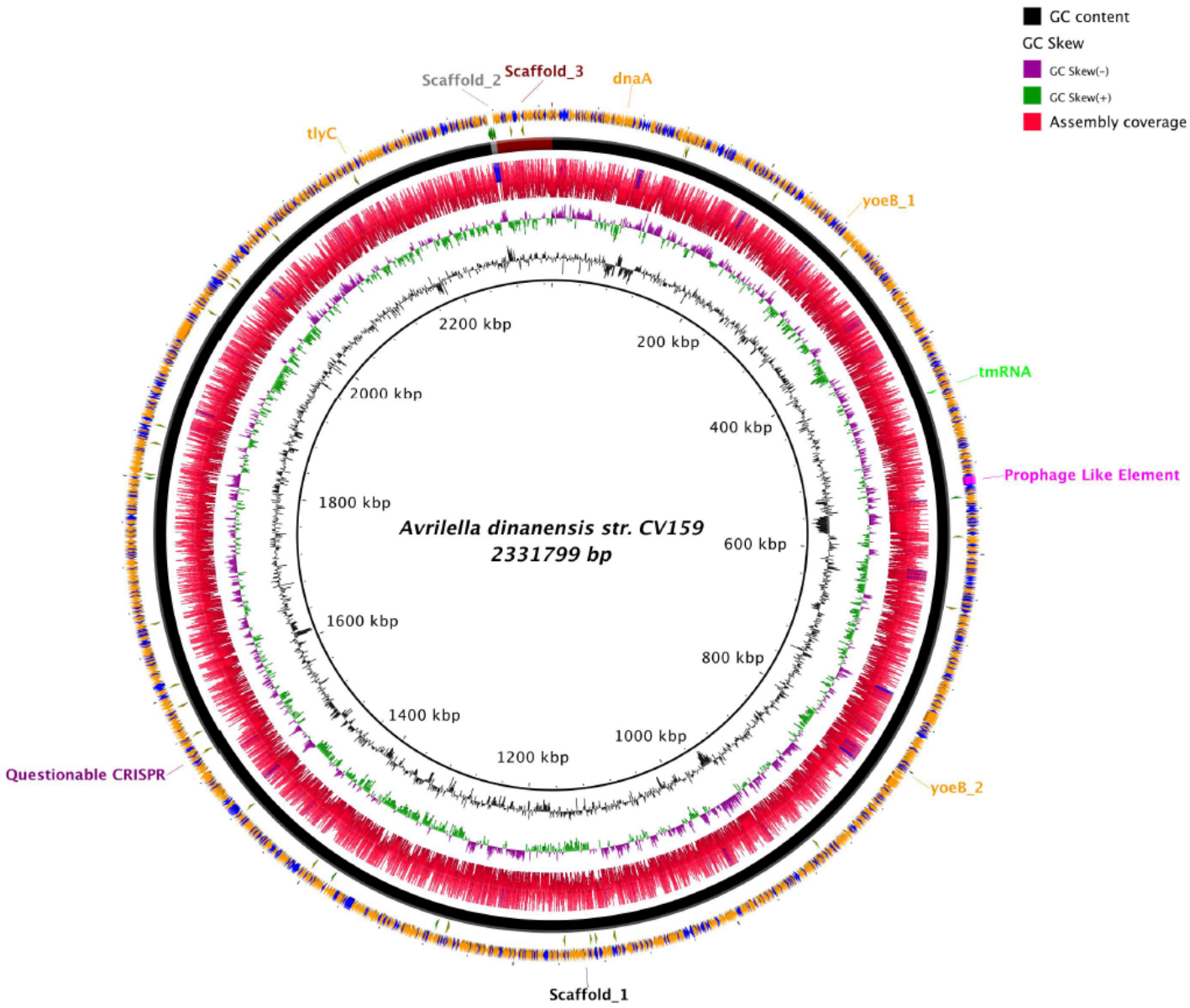

Figure S3. Graphical circular map of UR159^{T} genome. Seven rings, outwards: (1) nucleotide positions, (2) G+C %mol content, (3) G+C skew, (4) Assembly coverage, (5) scaffolds, (6) RNA sequences, and (7) annotated genes (orange) versus unknown proteins (blue)

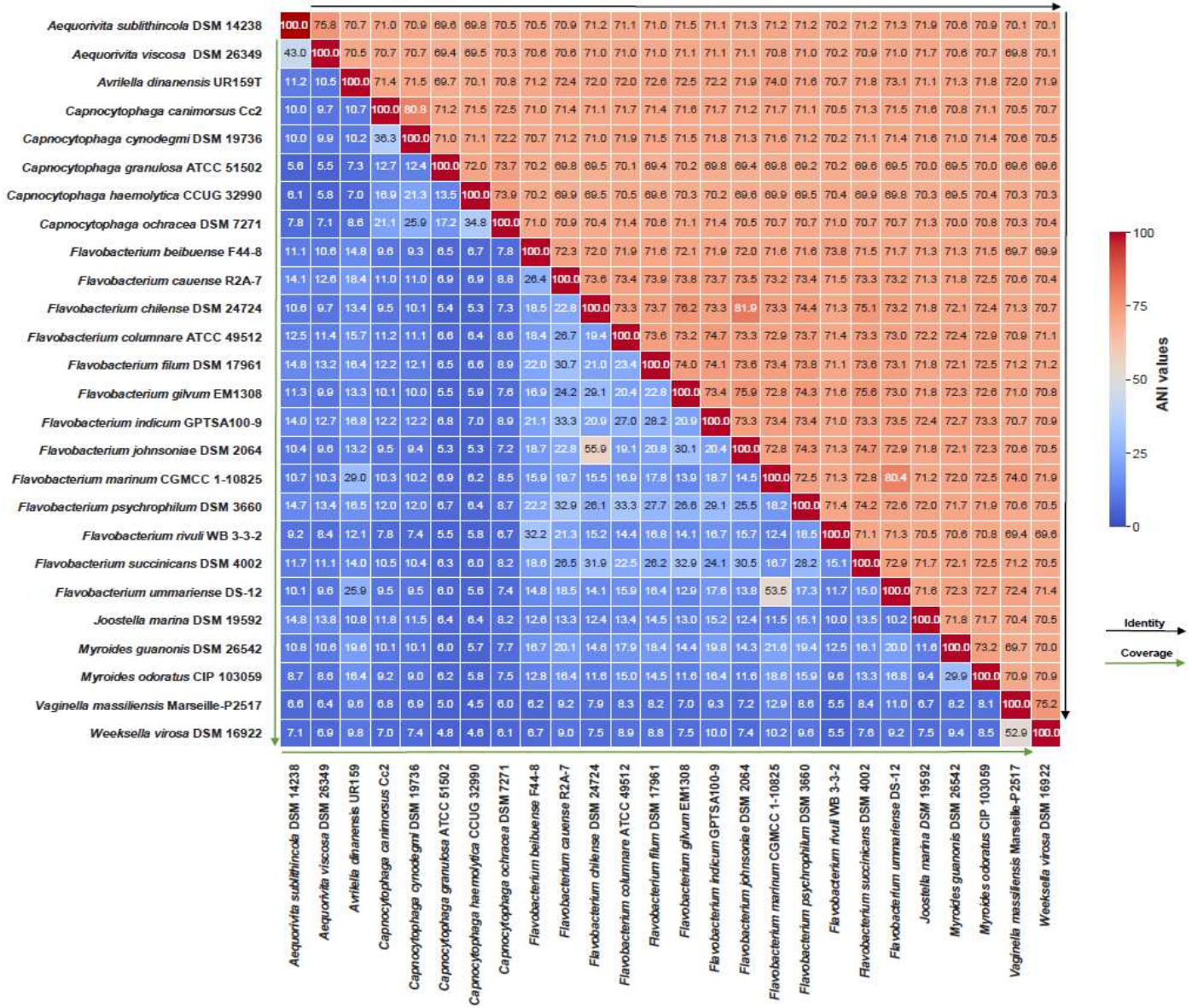

Figure S4. Average nucleotide identity (ANI) and average nucleotide coverage (ANC) percentages between strain UR159^{T} and the most closely related members of the *Flavobacteriaceae* family. ANI (black arrows) and ANC (green arrows) are represented by heat maps, where similarity percentages are represented by the color key histograms on the right panel.

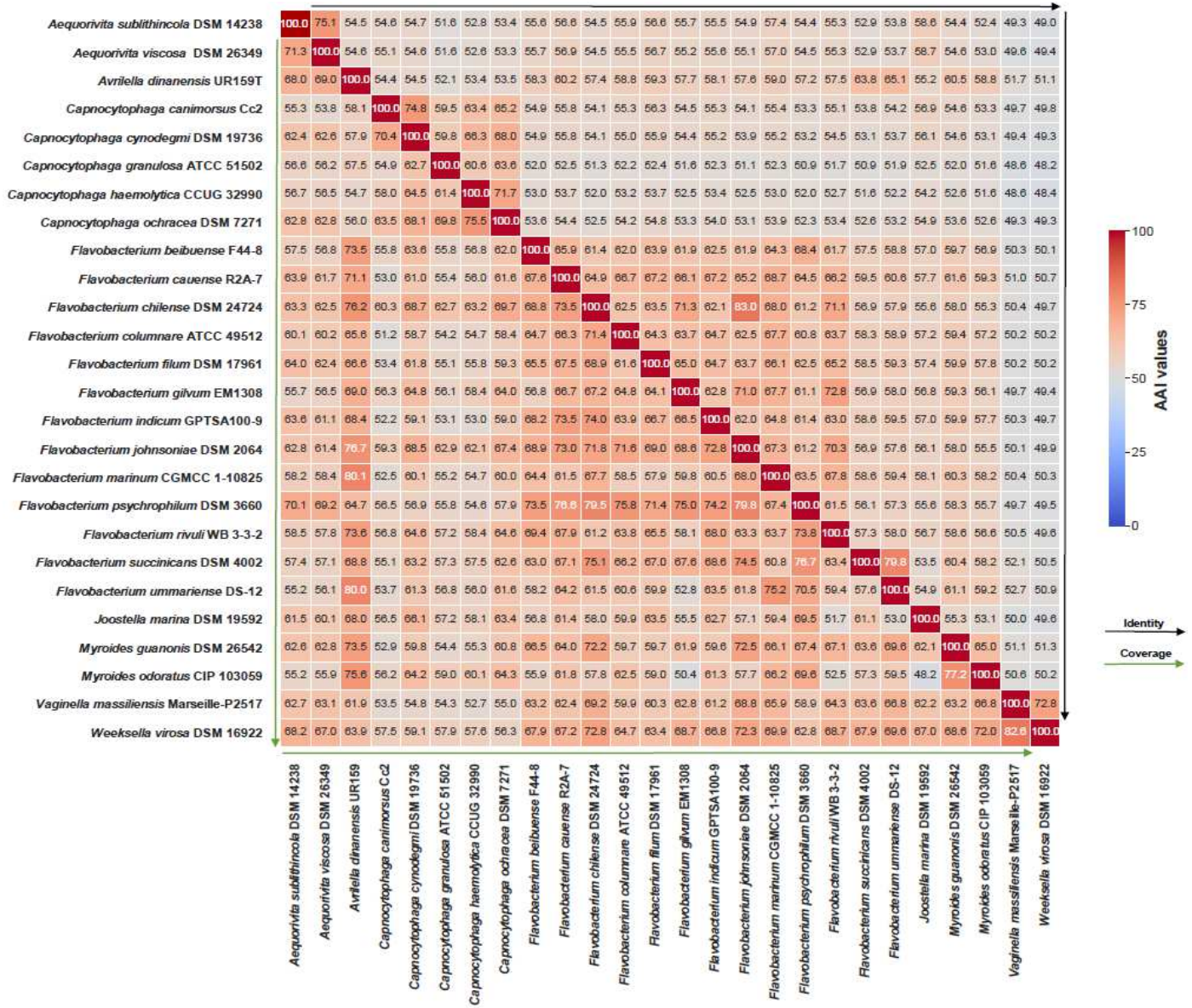

Figure S5. Average amino acid identity (AAI) and average amino acid coverage (AAC) percentages between strain UR159^{T} and the most closely related members of the *Flavobacteriaceae* family. AAI (black arrows) and AAC (green arrows) are represented by heat maps, where similarity percentages are represented by the color key histograms on the right panel.