

Avrilella dinanensis gen. nov., sp. nov., a novel bacterium of the family Flavobacteriaceae isolated from human blood

Caroline Leyer, Mohamed Sassi, Frédéric Gourmelen, Agnès Burel, Racha Beyrouthy, Samer Kayal, Richard Bonnet, Vincent Cattoir

▶ To cite this version:

Caroline Leyer, Mohamed Sassi, Frédéric Gourmelen, Agnès Burel, Racha Beyrouthy, et al.. Avrilella dinanensis gen. nov., sp. nov., a novel bacterium of the family Flavobacteriaceae isolated from human blood. Systematic and Applied Microbiology, 2020, 43 (5), pp.1-8. 10.1016/j.syapm.2020.126124 . hal-02930737

HAL Id: hal-02930737 https://hal.science/hal-02930737

Submitted on 15 Sep 2020 $\,$

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1	Avrilella dinanensis gen. nov., sp. nov., a novel bacterium of the family Flavobacteriaceae
2	isolated from human blood
3	
Λ	Punning title: A diagnansis from human blood
4	Kulling title. A. ununensis from human blood
5	
6	Caroline Leyer ^{a#¶} , Mohamed Sassi ^{b#} , Frédéric Gourmelen ^c , Agnès Burel ^d , Racha Beyrouthy ^{e,f,g} ,
7	Samer Kayal ^a , Richard Bonnet ^{e,f,g} , Vincent Cattoir ^{a,b,h} *
8	
9	[#] These authors contributed equally to this work.
10	
11	^a CHU de Rennes, Service de Bactériologie-Hygiène hospitalière, Rennes, France
12	^b Université de Rennes 1, Unité Inserm U1230, Rennes, France
13	^c CH de Saint-Malo, Laboratoire de Biologie médicale, Saint-Malo, France
14	^d Univ Rennes, MRic TEM, CNRS, Inserm, BIOSIT - UMS 3480, US_S 018, Rennes, France
15	^e CHU Clermont-Ferrand, Laboratoire de Bactériologie & CNR de la Résistance aux Antibiotiques, Clermont-Ferrand, France
16	^f CNR de la Résistance aux Antibiotiques (laboratoire associé « Entérobactéries : résistance aux C3G et colistine »), Clermont-
17	Ferrand, France
18	^g Université Clermont Auvergne, UMR INSERM 1071 USC INRA2018, Clermont-Ferrand, France.
19	^h CNR de la Résistance aux Antibiotiques (laboratoire associé « Entérocoques »), Rennes, France
20	
21	[¶] Present address: Laboratoire de Microbiologie, CH de Cornouaille, Quimper, France.
22	
23	*Corresponding author: Prof. Vincent Cattoir, CHU de Rennes, Service de Bactériologie-
24	Hygiène hospitalière, 2 rue Henri Le Guilloux, 35033 Rennes Cedex, France. +33-2-99-28-98-
25	28, Fax: +33-2-99-28-41-59, E-mail: vincent.cattoir@chu-rennes.fr.
26	

- 27 Word count: Abstract: 228 words; Text = 2,451 words; 5 Tables; 3 Figures; 35 References; 6
- 28 Supplemental materials.
- 29
- 30 Keywords: A. dinanensis; Flavobacteriaceae; Flavobacterium; Gram-negative rod;
- 31 bacteremia.

32 Abstract

Polyphasic taxonomic analysis was performed on a novel bacterium, designated UR159^T, isolated in 33 2016 from human blood of a septic patient hospitalized in France. Preliminary 16S rRNA gene 34 sequence-based phylogenetic analysis indicated that strain UR159^T belonged to the family 35 Flavobacteriaceae, forming a distinct phyletic line distantly related (<94% sequence similarity) to 36 known species of the family. Further phenotypic, chemotaxonomic and genomic analyses were 37 performed. Cells were non-motile, oxidase-negative, catalase-positive Gram-negative rod. It was 38 39 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}09c (8.8%). Whole 40 genome sequencing revealed a 2.3-Mbp genome encoding a total of 2,262 putative genes with a 41 42 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 43 44 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) 45 percentages were also estimated and were lower than 65% (cut-off proposed for genus delineation 46 for uncultivated prokaryotes) in all cases, except for F. marinum that was just at the limit (65.1%). 47 Based on these findings, we propose it as a new genus and species, Avrilella dinanensis gen. nov., sp. 48 nov. (type strain $UR159^{T} = CIP \ 111616^{T} = DSM \ 105483^{T}$). 49

50 Introduction

51 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 52 genera with a validly published and correct name are part of the *Flavobacteriaceae* family 53 (www.bacterio.net). Members of this family are Gram-negative non-spore-forming rods 54 widely described from diverse environmental habitats, especially in the marine environment 55 [2]. Many species are pathogenic to animals, especially flavobacterial infections in fish [3]. 56 Even though very rarely isolated in humans, some species are considered as opportunistic 57 pathogens, especially in immunocompromised patients and those with severe underlying 58 comorbidities (e.g., cancer, diabetes mellitus and chronic hematological, hepatic or kidney 59 disorders) [4]. Besides Capnocytophaga spp., the most frequently found species isolated 60 61 from clinical materials belong to the following genera: Bergeyella (B. zoohelcum), Chryseobacterium (C. indologenes), Elizabethkingia (E. menigoseptica), Myroides (M. 62 odoratus, M. odoratimimus), Sphingobacterium (S. multivorum, S. spitivorum) and Weeksella 63 (W. virosa) [5]. Most case reports report these organisms from blood, urine, wounds and 64 respiratory secretions [4,5]. 65

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.

71 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.

79 Materials and methods

80 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% CO₂ 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).

87 *Cell morphology and growth conditions*

Cell morphology was observed using both light microscopy and transmission electron 88 microscopy (TEM). For light microscopy, Gram staining was performed using cells grown on 89 90 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 91 92 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 93 same buffer. Cells were post-fixed in 1% OsO₄ for 60 min at 4 °C, rinsed in cacodilate buffer 94 and embedded in 2% OmniPur[®] agarose, low melting (Calbiochem, Merck). After the 95 96 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 97 sections using ultramicrotome LEICA UC7 (Leica, Wetzlar, Germany). These sections were 98 mounted on copper grids and stained. Grids were observed using a JEM-1400 Electron 99 Microscope (JEOL Ltd, Tokyo, Japan) at an accelerating voltage of 120 kV and equipped with 100 101 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 foreach 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, 48hour and 5-day incubation at 35°C while CO₂ requirement was tested in an incubator supplied with 5% CO₂. 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].

112 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 H₂O₂ 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.

125 Chemotaxonomic analysis

126 The fatty acid methyl ester (FAME) analysis was performed at the Belgian Coordinated 127 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 128 under aerobic conditions on LMG medium 304. Inoculation and harvesting of the cells, and 129 the extraction and analysis conformed to the recommendations of the commercial MIDI 130 microbial identification system (Microbial IDentification Inc.). The whole-cell fatty acid 131 composition was determined by gas chromatography on an Agilent Technologies 6890N gas 132 chromatograph. The peak naming table MIDI TSBA 5.0 was used. 133

134 DNA extraction and 16S rRNA phylogenetic analysis

DNA was isolated from UR159^T using the using the Quick-DNA fungal/bacterial miniprep kit 135 (Zymo Research, Irvine, CA) according to the manufacturer's recommendations. The 16S 136 rRNA gene of UR159^T was amplified by PCR and sequenced using the universal primers 27F 137 and 1541R, corresponding to base positions 8-27 and 1541-1525 of the 16S rRNA gene of 138 139 *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 140 141 [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 142 143 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-144 likelihood (ML) methods [13]. Branch positions were statically tested with a bootstrap of 145 1,000 replicates. 146

147 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_FNXE0000000.1) as the reference genome sequence.

Genome annotation was performed by the NCBI Prokaryotic Genome Annotation Pipeline 158 (PGAP) (www.ncbi.nlm.nih.gov/genome/annotation prok/). For mobile genetic elements, 159 prophages were predicted using PHAST [19] completed by the Actinobacteriophage 160 161 database (https://phagesdb.org) and plasmids were searched using the online plasmid search tool (https://plasmid.med.harvard.edu/PLASMID/). Functional analysis 162 was performed using RPSBLAST program on COG database implemented in WebMGA 163 (http://weizhongli-lab.org/webMGA). 164

165 Comparative genomics and phylogenomic analysis

To confirm the status as a new taxon of UR159^T, Average Nucleotide Identity (ANI), in silico 166 DNA-DNA hybridization (isDDH), Average Amino Acid Identity (AAI) values were determined. 167 The calculation of the ANI between strain UR159^T and 343 genomes of *Flavobacteriaceae*-168 related species performed using BLASTN and Pyani v0.2.10 module 169 was (https://pypi.org/project/pyani/) [20], following the algorithm described by Goris et al. [21]. 170 According to Pyani and 16S rRNA gene similarities, 25 available Flavobacteriaceae genomes 171 172 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 173

estimation of AAI was determined using the tool AAI calculator (http://enve-174 175 omics.ce.gatech.edu/) [23]. The isDDH values were calculated with the same data set for ANI Genome-to-Genome calculation using the Distance calculator (GGDC 2.1; 176 https://ggdc.dsmz.de/) with the formula 2. Pangenomic analysis of the 26 genomes was 177 conducted using PIRATE software [24]. To calculate phylogenomic relations between strain 178 U159^T and phylogenetic neighbours, a core-genome tree was constructed as follows. All 179 predicted protein-coding genes annotated from each available genome were compared 180 using the all-versus-all BLAST search [25]. Only proteins for which coding sequences with 181 >50% amino acid identity and >70% sequence coverage were considered as orthologous. The 182 nucleotide sequences of core gene set were aligned using MUSCLE software and MEGA6 183 software [26] was used for the phylogenomic tree reconstruction using the neighbour-184 185 joining method with Jukes-Cantor correction.

186 Sequence accession numbers

¹⁸⁷ The 16S rRNA sequence generated was submitted to GenBank with the accession number ¹⁸⁸ MF278923. The draft genome sequence of UR159^T was deposited in NCBI under the ¹⁸⁹ accession number NZ_NIPO00000000.

191 **Results and discussion**

Strain UR159^T was not identified by the MALDI-TOF mass spectrometry using the Bruker 192 database (version 2.3) but the profile was reproducible (Figure S1). The almost-complete 193 sequence of the 16S rRNA gene was obtained (1,516 bp) and used for sequence comparison 194 and phylogenetic analysis. Strain UR159^T exhibited the highest level of nucleotide pairwise 195 similarity (93.1%) with *Flavobacterium marinum* SW105^T [27]. Sequence similarity was lower 196 with the next closest relatives (Flavobacterium spp., Myroides spp.) with percentages of 16S 197 rRNA gene sequence similarity in all cases lower than 92.9%. The ML and NJ phylogenetic 198 trees were almost identical and both confirmed the affiliation of $UR159^{T}$ to the 199 *Flavobacteriaceae* (Figure 1). The sequence similarity between the 16S rRNA gene of UR159^T 200 and the closest phylogenetic neighbors, which was distinctly below the predetermined cut-201 202 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 203 microorganism requiring further characterization. 204

205 Phenotypic properties

Strain UR159^T grew optimally on BA after 24-h incubation at 35°C under ambient air, yielding 206 ~1-3-mm, yellow-pigmented, circular, convex, smooth colonies (Figure S2). No growth was 207 observed when incubated anaerobically and it was demonstrated UR159^{T} was strictly 208 aerobic (Figure S2). It failed to grow on Drigalski, cetrimide and Hektoen agar (data not 209 shown). It was a non-motile, oxidase-negative, catalase-positive Gram-negative rod (Figure 210 2A) able to grow between 15°C and 37°C. TEM confirmed that cells of UR159^T were rod-211 shaped bacteria measuring an average size of 0.35 \pm 0.02 μ m wide and 1.12 \pm 0.16 μ m long 212 (Figure 2B). Biochemically, it was metabolically rather inert since almost all reactions were 213 negative (especially indole production, Voges-Proskaeur, nitrate reduction, H₂S production 214

and urease tests) except of the production of gelatinase, and presented some differences 215 with species of closely-related genera (Table 1) [27,30-33]. Furthermore, strain UR159^T 216 appeared susceptible to amoxicillin-clavulanate (MIC <2/2 mg/L), ciprofloxacin (MIC = 0.25 217 mg/L), cotrimoxazole (MIC = 2/38 mg/L) and tigecycline (MIC = 0.5 Mg/L) whereas MICs 218 were higher for piperacillin-tazobactam (MIC = 8 mg/L), cefotaxime (MIC >4 mg/L), 219 ceftazidime (MIC = 128 mg/L), cefepime (MIC = 32 mg/L), imipenem (MIC = 4 mg/L), 220 meropenem (MIC = 8 mg/L), gentamicin (MIC >4 mg/L), amikacin (MIC >16 mg/L), 221 fosfomycin (MIC >64 mg/L) and colistin (MIC >8 mg/L). 222

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}@9c (8.8%) iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1}@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].

228 Genomic features

The draft genome size of UR159^T was 2,331,799 bp long (3 scaffolds with a final coverage of 229 124×) (Table 3 and Figure S3), which is smaller than those of most other members of the 230 Flavobacteriaceae family (Table 4). Based on whole genome sequence, the genomic DNA 231 G+C content was determined at 37.6 mol%, which is consistent with those of the type strains 232 of closely related genera (31.4-44.2 mol%) (Tables 3 and 4). The genome encoded a total of 233 2,262 putative genes, including 2,176 protein-coding genes and 61 RNAs (50 tRNAs, 7 rRNAs 234 235 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 236 putative function, and 773 genes were annotated as hypothetical proteins (35.5%) (Table 237 S1). Whereas no extrachromosomal element was found, a 6.5-kb prophage-like element was 238

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

241 *Comparative genomic analysis*

Phylogenomic analysis revealed a lack of relatives at the species or genus level, with ANI 242 values between strain UR159^T and the most closely related members of the 243 Flavobacteriaceae between 69.7 and 74.0% (alignment coverage from 7.0% to 29.0%) while 244 those of isDDH ranged from 17.9 to 38.7% (Table 4 and Figures S4). These calculated 245 similarity values were below the proposed and generally accepted species boundaries for 246 ANI and isDDH that are 95-96% and 70%, respectively [8,35]. These results indicate that 247 strain UR159^T constitutes a novel taxon at least at the species level, not related at this level 248 to any of the closest phylogenetic neighbors, which can be visualized with the core-genome 249 250 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, 251 should be used instead. Thus, AAI percentages were also estimated with a threshold AAI 252 value of 65%, which has been proposed for genus delineation for uncultivated prokaryotes 253 [29]. AAI values for strain UR159^T and the most closely related species were in all cases 254 lower than 65% (Table 4), except for F. marinum that is just at the limit (65.1%) (Table 4 and 255 Figure S5). By pangenomic analysis, only 472 orthologous genes (20.8%) were determined to 256 be encoded in all genomes whereas 514 genes (22.7%) were predicted to be unique to strain 257 UR159^T compared to other genomes tested. The complete genome of UR159^T shared 258 between 761 (35.5%) to 1403 (65.4%) common gene families with Weeksella virosa and F. 259 *marinum* genomes, respectively (Table 4). 260

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

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

267

268 Acknowledgments

269 We have no relevant financial disclosures or funding to declare.

271 References

- 272 [1] Bernardet, J.F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K., Vandamme, P. (1996) Cutting a Gordian knot: 273 emended classification and description of the genus *Flavobacterium*, emended description of the family
- 274 Flavobacteriaceae, and proposal of Flavobacterium hydatis nom. nov. (basonym, Cytophaga aquatilis Strohl and Tait

275 1978). Int. J. Syst. Bacteriol. 46, 128-148.

- [2] Jooste, P.J., Hugo, C.J. (1999) The taxonomy, ecology and cultivation of bacterial genera belonging to the family
 Flavobacteriaceae. Int. J. Food Microbiol. 53, 81-94.
- 278 [3] Loch, T.P., Faisal, M. (2015) Emerging flavobacterial infections in fish: A review. J. Adv. Res. 6, 283-300.
- Steinberg, J.P., Burd, E.M. (2010) Other Gram-negative and Gram-variable bacilli. In: Mandell, G.L., Bennett, J.E., Dolin,
 R. (Eds.), Principles and practice of infectious diseases. Seventh Edition, Churchill Livingstone Elsevier, Philadelphia,
- 281 pp. 3015-3033.
- Vaneechoutte, M., Nemec, A., Kämpfer, P., Cools, P., Wauters, G. (2015) *Acinetobacter, Chryseobacterium, Moraxella*,
 and other nonfermentative Gram-negative rods. In: Jorgensen, J.H., Pfaller, M.A. (Eds.), Manual of clinical
 microbiology. 15th Edition, ASM press, Washington DC, pp. 813-837.
- 285 [6] Bernardet, J.F., Nakagawa, Y., Holmes, B., Subcommittee On The Taxonomy Of, F., Cytophaga-Like Bacteria Of The 286 International Committee On Systematics Of, P. (2002) Proposed minimal standards for describing new taxa of the 287 family *Flavobacteriaceae* and emended description of the family. Int. J. Syst. Evol. Microbiol. 52, 1049-1070.
- Ramasamy, D., Mishra, A.K., Lagier, J.C., Padhmanabhan, R., Rossi, M., Sentausa, E., Raoult, D., Fournier, P.E. (2014) A
 polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. Int. J. Syst.
 Evol. Microbiol. 64, 384-391.
- 291 [8] Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D.R., da Costa, M.S., Rooney, A.P., Yi, H., Xu, X.W., De Meyer,
- S., Trujillo, M.E. (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int.
 J. Syst. Evol. Microbiol. 68, 461-466.
- Winker, S., Woese, C.R. (1991) A definition of the domains Archaea, Bacteria and Eucarya in terms of small subunit
 ribosomal RNA characteristics. Syst. Appl. Microbiol. 14, 305-310.
- [10] Flandrois, J.P., Perrière, G., Gouy, M. (2015) leBIBI^{QBPP}: a set of databases and a webtool for automatic phylogenetic
 analysis of prokaryotic sequences. BMC Bioinformatics. 16, 251.
- [11] National Center for Biotechnology Information (NCBI) [Internet]. Bethesda (MD): National Library of Medicine (US),
 National Center for Biotechnology Information; [1988] [cited 2017 Apr 06]. Available from:
 https://www.ncbi.nlm.nih.gov/.
- 301 [12] Madeira, F., Park, Y.M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., Basutkar, P., Tivey, A.R., Potter, S.C., Finn, R.D.,
- 302 Lopez, R. (2019) The EMBL-EBI search and sequence analysis tools APIs in 2019. Nucleic. Acids Res. 47, W636–W641.

- Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018) MEGA X: Molecular evolutionary genetics analysis across
 computing platforms. Mol. Biol. Evol. 35, 1547-1549.
- 305 [14] Bolger, A.M., Lohse, M., Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics
 306 30, 2114-2120.
- 307 [15] Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S.,
- Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A. (2012) SPAdes: a new
 genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455-477.
- Antipov, D., Hartwick, N., Shen, M., Raiko, M., Lapidus, A., Pevzner, P.A. (2016) plasmidSPAdes: assembling plasmids
 from whole genome sequencing data. Bioinformatics 32, 3380-3387.
- 312 [17] Dias, Z., Dias, U., Setubal, J.C. (2012) SIS: a program to generate draft genome sequence scaffolds for prokaryotes.
 313 BMC Bioinformatics 13, 96.
- 314 [18] Boetzer, M., Pirovano, W. (2012) Toward almost closed genomes with GapFiller. Genome Biol. 13, R56.
- 315 [19] Zhou, Y., Liang, Y., Lynch, K.H., Dennis, J.J., Wishart, D.S. (2011) PHAST: a fast phage search tool. Nucleic Acids Res. 39,
- 316 347-352.
- Pritchard, L., Glover, R.H., Humphris, S., Elphinstone, J.G., Toth, I.K. (2016) Genomics and taxonomy in diagnostics for
 food security: soft-rotting enterobacterial plant pathogens. Anal Methods. 8, 12-24.
- Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P., Tiedje, J.M. (2007) DNA-DNA hybridization
 values and their relationship to whole-genome sequence similarities. Int. J. Syst. Evol. Microbiol. 57, 81-91.
- 321 [22] Seemann, T. (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics. 30, 2068-2069.
- Rodriguez-R, L.M., Konstantinidis, K.T. (2014) Bypassing cultivation to identify bacterial species. ASM Microbe 9, 111 118.
- Bayliss, S.C., Thorpe, H.A., Coyle, N.M., Sheppard, S.K., Feil, E.J. (2019) PIRATE: A fast and scalable pangenomics
 toolbox for clustering diverged orthologues in bacteria. Gigascience 8.
- [25] Alam, I., Nadeem, S.A., Brooke, J.M. (2008) avaBLAST: A fast way of doing all versus all BLAST. *In* 2008 Cairo
 International Biomedical Engineering Conference, CIBEC 2008 | Cairo Int. Biomed. Eng. Conf., CIBEC.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis
 version 6.0. Mol. Biol. Evol. 30, 2725-2729.
- 330 [27] Song, L., Liu, H., Huang, Y., Dai, X., Zhou, Y. (2013) *Flavobacterium marinum* sp. nov., isolated from seawater. Int. J.
- 331 Syst. Evol. Microbiol. 63, 3551-3555.
- Kim, M., Oh, H.S., Park, S.C., Chun, J. (2014) Towards a taxonomic coherence between average nucleotide identity and
 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int. J. Syst. Evol. Microbiol. 64, 346-351.
- 334 [29] Konstantinidis, K.T., Rossello-Mora, R., Amann, R. (2017) Uncultivated microbes in need of their own taxonomy. ISME

- 335 J. 11, 2399-2406.
- [30] Lata, P., Lal, D., Lal, R. (2012) *Flavobacterium ummariense* sp. nov., isolated from hexachlorocyclohexane contaminated soil, and emended description of *Flavobacterium ceti* Vela et al. 2007. Int. J. Syst. Evol. Microbiol. 62,
 2674-2679.
- [31] Tomova, A., Tomova, I., Vasileva-Tonkova, E., Lazarkevich, I., Stoilova-Disheva, M., Lyutskanova, D., Kambourova, M.
 (2013) *Myroides guanonis* sp. nov., isolated from prehistoric paintings. Int. J. Syst. Evol. Microbiol. 63, 4266-4270.
- [32] Liu, H., Lu, P., Zhu, G. (2017) *Flavobacterium cloacae* sp. nov., isolated from waste water. Int. J. Syst. Evol. Microbiol.
 67, 659-663.
- [33] Li, G., Chen, X., Li, Y., Shi, S., Jiang, L., Jiang, Y., Han, L., Huang, X., Jiang, C. (2019) *Flavobacterium viscosus* sp. nov. and
 Flavobacterium tangerina sp. nov., from primates feces. Curr. Microbiol. 76, 818-823.
- 345 [34] Cheng, L.F., Chen, H.M., Zheng, T., Fu, G.H., Shi, S.H., Wan, C.H., Huang, Y. (2012) Complete genomic sequence of the
 346 virulent bacteriophage RAP44 of *Riemerella anatipestifer*. Avian Dis. 56, 321-327.
- 347 [35] Mahato, N.K., Gupta, V., Singh, P., Kumari, R., Verma, H., Tripathi, C., Rani, P., Sharma, A., Singhvi, N., Sood, U., Hira,
- 348 P., Kohli, P., Nayyar, N., Puri, A., Bajaj, A., Kumar, R., Negi, V., Talwar, C., Khurana, H., Nagar, S., Sharma, M., Mishra,
- 349 H., Singh, A.K., Dhingra, G., Negi, R.K., Shakarad, M., Singh, Y., Lal, R. (2017) Microbial taxonomy in the era of OMICS:
- 350 application of DNA sequences, computational tools and techniques. Antonie Van Leeuwenhoek 110, 1357-1371.

352 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 \times 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.



0.10

0.020









Vaginella massiliensis Marseille-P2517^T (FLMR0000000) Weeksella virosa DSM 16922^T (CP002455) *Capnocytophaga canimorsus* Cc2^T (CDOJ0100000) *Capnocytophaga cynodegmi* DSM 19736^T (ARAI0000000) *Capnocytophaga granulosa* ATCC 51502^T (ADDA0000000) *Capnocytophaga haemolytica* CCUG 32990^T (CP014227) *Capnocytophaga ochracea* DSM 7271^T (CP001632) Joostella marina DSM 19592^T (AJUG00000000) Aequorivita sublithincola DSM 14238^T (CP003280) Aequorivita viscosa DSM 26349^T (FQYV01000000) Myroides quanonis DSM 26642^T (FORU01000000) Myroides odoratus CIP 103059^T (AHKQ0000000) Avrilella dinanensis UR159^T (NIPO00000000) Flavobacterium unmariense DS-12^T (FOVI01000000) *Flavobacterium marinum* CGMCC 1-10825^T (FNXE01000000) Flavobacterium beibuense F44-8^T (JRLV0100000) Flavobacterium rivuli WB 3-3-2^T (JRLX0100000) Flavobacterium columnare ATCC 49512^T (FORU00000000) *Flavobacterium indicum* GPTSA100-9^T (HE774682) Flavobacterium cauense R2A-7^T (AVBI0000000) Flavobacterium filum DSM 17961^T (AUDM0000000) Flavobacterium psychrophilum DSM 3660^T (FMVE0100000) Flavobacterium chilense DSM 24724^T (FRBT0100000) Flavobacterium johnsoniae DSM 2064^T (FRAO01000000) Flavobacterium gilvum EM1308^T (CP017479) Flavobacterium succinicans DSM 4002^T (FOUT01000000)

Characteristic	Avrilella	Flavobacterium	Flavobacterium	Myroides
	dinanensis	marinum	unmariense	guanonis
	UR159 ^T	$SW105^{T}$	$DS-12^{T}$	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	-	-	+	+
lpha-galactosidase	-	-	-	nd
β-galactosidase	-	-	-	-
β-glucosidase	-	-	-	nd
Citrate utilization	-	w	-	+
Indole production	-	-	-	-
Acetoin production		nd	nd	
(Voges-Proskaeur reaction)	-	nu	nu	-
Nitrate reduction	-	-	-	-
H ₂ S production	-	-	nd	-

Table 1.	Differential	characteristics o	f strain UR159	and type strains of	f closely relate	d genera	[20,23-26]

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

Fatty acid (%)	<i>A. dinanensis</i> UR159 ^T	<i>F. marinum</i> 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} <i>@</i> 9c	8.8	13.1					
iso-C _{15:0} 3-OH	-	2.5					
iso-C _{16:0} 3-OH	1.4	-					
C _{18:1} <i>@</i> 9 <i>c</i>	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	-					

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

The fatty acids amounting to <1% of the total fatty acids are not shown. Summed feature 3 comprises ($C_{16:1}\omega7c$ 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).

Characteristic	A. dinanensis UR159 ^T
Genbank accession no.	NZ_NIPO0000000
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 3. Genomic characteristics of Avrilella dinanensis UR159^T gen. nov. sp.

 nov.

<u> </u>	- Conhank accossion		%						
Strain	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 $14238^{^{\intercal}}$	CP003280.1	3,520,671	36.2	70.7	11.2	23.5	54.5	68.0	47.2
Aequorivita viscosa DSM 26349 ^{T}	FQYV0100000.1	3,526,954	36.5	70.5	10.5	38.7	54.6	69.0	48.1
Capnocytophaga canimorsus $Cc2^{^{ m T}}$	CDOJ01000000.1	2,504,412	36.1	71.4	10.7	20.5	54.4	58.1	39.6
Capnocytophaga cynodegmi DSM 19736 $^{^{\intercal}}$	ARAI00000000.1	2,657,104	34.4	71.5	10.2	21.5	54.5	57.9	39.7
Capnocytophaga granulosa $ATCC 51502^{^{T}}$	ADDA0000000.1	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 $^{^{\intercal}}$	CP001632.1	2,612,925	39.6	70.8	8.6	25.9	53.5	56.1	37.4
Flavobacterium beibuense F44-8 $^{^{\mathrm{T}}}$	JRLV01000000.1	3,800,758	37.7	71.2	14.8	19.2	58.3	73.5	54.1
Flavobacterium cauense R2A-7 ^T	AVBI0000000.1	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 $49512^{^{T}}$	FORU0000000.1	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 $^{^{T}}$	FRAO0100000.1	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 $DS extsf{-}12^{^{T}}$	FOVI01000000.1	3,493,887	34.7	73.1	25.9	19.8	63.8	80.0	64.3
Joostella marina DSM 19592 ^T	AJUG0000000.1	4,508,243	33.6	71.1	10.8	19.6	55.2	68.0	46.9
Myroides guanonis DSM 26542^{T}	FORU0100000.1	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 $^{^{T}}$	FLMR0000000.1	2,434,476	38.2	72.0	9.6	22.5	51.7	61.9	36.9
Weeksella virosa DSM 16922 $^{^{\intercal}}$	CP002455.1	2,272,954	35.9	71.9	9.8	26.4	51.1	63.9	35.5

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.

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. suffella. 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	Gram-negative, aerobic rods that grow as vellow-	Non-motile, oxidase-negative, catalase-positive Gram-negative
traits	pigmented, circular, convex, smooth colonies.	rod able to grow between 15°C and 37°C. Strictly aerobic
	Strains are positive for catalase and oxidase, and	growing optimally on blood agar after 24-h incubation at 35°C
	metabolically rather inert except for a gelatinase	under ambient air, vielding ~1-3-mm, vellow-pigmented, circular.
	activity. Major fatty acids are iso-branched fatty	convex, smooth colonies. Metabolically rather inert, except the
	acids predominantly iso-C15.0 iso-C17.1@9c and	production of gelatinase. Susceptibility to amoxicillin-clavulanate
	iso-C17:0 3-OH. The genus is affiliated to the family	(MIC, 0.5 mg/L), cefoxitin (MIC, 1.5 mg/L), ertapenem (MIC, 1.5
	<i>'Elavobacteriaceae'</i> order 'Elavobacteriales' class	mg/L) imipenem (MIC. 2 mg/L), meropenem (MIC. 2 mg/L).
	'Elavobacterija'.	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/I) aminoglycosides (MICs >16 mg/I) 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 NIPO0000000
Genome size (bp)		2,331,799
GC mol%		37.6
Number of strains in the study		1
, Information related to the Nagova protocol		Not applicable
Designation of the type strain		UR159 [†]
Strain collection numbers		$CIP \ 111616^{T} = DSM \ 105483^{T}$

Code	% of total	% of unique	Description
	genes	genes	
J	9,54	0,84	Translation, ribosomal structure and biogenesis
К	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
т	3,42	0,00	Signal transduction mechanisms
М	9,40	12,97	Cell wall/membrane/envelope biogenesis
Ν	0,42	0,61	Cell motility
U	2,42	0,90	Intracellular trafficking, secretion, and vesicular transport
0	5,26	6,59	Posttranslational modification, protein turnover, chaperones
С	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
н	5,30	4,05	Coenzyme transport and metabolism
I	5,29	1,67	Lipid transport and metabolism
Р	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

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



Figure S1. Mass spectrum from *Avrilella dinanensis* UR159[⊤] 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.