Aliarcobacter vitoriensis sp. nov., isolated from carrot and urban wastewater

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Running title:

Description of the new species *Aliarcobacter vitoriensis*

Abstract

Two isolates, one recovered from a carrot and another one from urban wastewater, were characterized using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequences revealed that both isolates clustered together, and were most closely related to *Aliarcobacter lanthieri*. Multilocus phylogenetic analysis (MLPA) using the concatenated sequences of five housekeeping genes (*atpA, gyrA, gyrB, hsp60* and *rpoB*) suggested that these isolates formed a distinct phylogenetic lineage among the genera derived from the former genus *Arcobacter*. Whole-genome sequence, *in silico* DNA-DNA hybridization (*is*DDH) and the average nucleotide identity (ANI) value between the genome of strain F199^T and those of related species confirmed that these isolates represent a novel species. These strains can be differentiated from its phylogenetically closest species *A. lanthieri* by its inability to growth on 1% glycine and by their enzyme activity of esterase lipase (C8) and acid phosphatase. Our results, by the application of a polyphasic analysis, confirmed that these two isolates represent a novel species of the genus *Aliarcobacter*, for which the name *Aliarcobacter vitoriensis* sp. nov. is proposed. The type strain is F199^T (=CECT 9230^T= LMG 30050^T).

Keywords:

Arcobacter

Aliarcobacter vitoriensis sp. nov

Carrot

Wastewater

Abbreviations:

m-PCR, multiplex polymerase chain reaction; ERIC-PCR, enterobacterial repetitive intergenic consensus polymerase chain reaction; MLPA, multilocus phylogenetic analysis; *is*DDH, *in silico* DNA-DNA hybridization; ANI, average nucleotide identity; CRISPR, clustered regularly interspaced short palindromic repeats.

Introduction

The genus *Arcobacter* belongs to the family *Campylobacteraceae* and was first described in 1991 by Vandamme *et al*. [44]. The genus comprises at least 31 recognized species, and 12 of them have been described in the last 4 years: *A. ebronensis* [22], *A. aquimarinus* [22], *A. lanthieri* [47], *A. pacificus* [49], *A. faecis* [48], *A. acticola* [31], *A. lekithochrous* [8], *A. haliotis* [42], *A. canalis* [32], *A. lacus* and *A. caeni* [33] and *A. peruensis* [5]. However, the taxonomy of *Arcobacter* species has been recently reassessed as a result of the division of the genus into at least six different genera: *Arcobacter*, *Aliarcobacter* gen. nov., *Pseudarcobacter* gen. nov., *Halarcobacter* gen. nov., *Malaciobacter* gen. nov., and *Poseidonibacter* gen. nov [34, 35].

In 2002, the International Commission on Microbiological Specifications for Foods classified *Arcobacter (*now *Aliarcobacter) butzleri*, the most prevalent species among arcobacters, as a serious hazard to human health [17]. *Arcobacter* species have been isolated from environmental waters, shellfish, and food of animal sources. Some members have been related to gastrointestinal diseases and sometimes bacteraemia in humans [45, 1, 46] or mastitis and abortions in animals [13]. Consumption of contaminated foods of animal origin or non-treated water is considered the major transmission route [40].

The aim of this study was to characterize, using a polyphasic approach, one isolate (F199^T) from a carrot sample and one isolate (FW-59) from wastewater in order to determine their taxonomic position within the former genus *Arcobacter*. These strains were isolated in an earlier study conducted in Vitoria-Gasteiz, Spain, which aimed to determine the occurrence of *Arcobacter* species in different food products and surface waters.

Materials and Methods

Bacterial Isolation and Identification. Wastewater samples were collected from the Crispijana wastewater treatment plant located approximately 6 km West of Vitoria-Gasteiz (North Spain). Five hundred mL of wastewater were sequentially filtered through membrane filters with decreasing pore size: 20-0.22 µm filters (Millipore). The 0.45 µm and 0.22 µm filters were enriched in 10 mL *Arcobacter* broth (Oxoid) supplemented with cefoperazone, amphotericin B and teicoplanin (CAT, Oxoid) and incubated aerobically at 30 °C for 24 hours. After enrichment, the passive membrane filtration technique was applied using 0.45 µm membrane filters. The Columbia blood agar plates (Oxoid) were incubated at 30 °C for a maximum of 7 days under aerobic conditions.

The strain F199 was isolated from a carrot purchased in a local farmers' market in Vitoria-Gasteiz, Spain. For *Arcobacter* isolation, 10 g of sample was homogenized with 90 mL (1:10 wt/vol) of Arcobacter-CAT broth (Oxoid) in a stomacher bag. The sample was then incubated aerobically at 30 °C for 48 h. After enrichment, 0.2 mL of the broth were inoculated by passive filtration with 0.45-µm nitrocellulose membrane filters (Millipore) onto blood agar plates (Columbia agar supplemented with 5% sheep blood, Oxoid) and incubated for 48-72 h at 30°C under aerobic conditions, as previously reported by Nieva-Echevarria *et al*. [26]. Bacterial DNA was isolated from broth cultures using the PrepMan™ Ultra reagent (Applied Biosystems) according to the manufacturer's specifications. The concentration of each DNA extraction was determined spectrophotometrically (NanoDrop 2000, Thermo Fisher Scientific), diluted to 20 ng/µL and stored at −20 °C. Assignation to the genus *Arcobacter* was carried out by using a genus-specific PCR [4]. The identification of the isolates to the species level was accomplished by two multiplex PCRs (m-PCR) for *Arcobacter* spp. [14, 10]. To discard clonality, isolates were genotyped by enterobacterial repetitive intergenic consensus

PCR (ERIC-PCR) as described by Houf *et al.* [15]. Patterns that differed by one or more bands were considered different genotypes.

Phylogenetic analysis. To analyse the phylogenetic position of the strains, amplification of the 16S rRNA gene of the two isolates was carried out using the primers 27F and 1492R as previously described [20]. Amplicons were purified using NucleoSpin® Gel and PCR Clean up (Macherey-Nagel) according to the manufacturer's instructions. The amplicons were sequenced bidirectionally by Sistemas Genómicos (Valencia, Spain). Additionally, a phylogenetic analysis was constructed using the 16S rRNA gene sequence of both F199^T and FW-59 strains and those of all type strains deposited in the GenBank except LMG 28652^T (heterotypic synonym of CECT 8942^T) [9]. Sequences were aligned using ClustalW [21] and the phylogenetic tree was constructed using the Neighbor Joining algorithm [19; 37] with MEGA 6.0 software [41]. Similarities of the 16S rRNA genes were calculated with the software MegAling version 7.0.0 (DNASTAR®). In addition, the *rpoB* gene of each strain was sequenced using primers and conditions described by Collado *et al*. [6]. Sequences were aligned and the phylogenetic tree was constructed using the Maximum Likelihood method [25] using MEGA 6.0 software. In order to complete the phylogenetic analysis of the two strains, a phylogenomic analysis was also held. For this purpose the genomes from the proposed new species and the other ones from the former *Arcobacter* genus were annotated using Prokka v1.2 [39]. The core genome of the 30 analyzed genomes was obtained using Roary software [30] with a 80% cutoff for the BLASTp analysis. The phylogeny was inferred using SplitsTree version 4.14.2 [16] following conditions describe previously [38] with a neighbor net drawing and Jukes-Cantor correction [3, 16].

Genome analysis. The genomic DNA of the strains F199^T and FW-59 was obtained using a NucleoSpin® Tissue kit (Macherey-Nagel) in accordance with the manufacturer's protocol.

The whole-genome sequence was obtained using MiSeq platform of Illumina and assembled with SPAdes 3.12.0 software [27]. Genome was annotated with Rapid Annotation Subsystems Technology (RAST) [29]. A genome comparison using the Average Nucleotide Identity (ANI) and the *in silico* DNA-DNA hybridization (*is*DDH) values was performed between the strains F199^T (PDKB01), FW-59 (PDKA01) and the GenBank obtained genomes of *Aliarcobacter (Arcobacter) lanthieri* strain LMG 28516^T (JARU01), *A. (Arcobacter) faecis* LMG 28519^T (JARS01), A. *(Arcobacter) butzleri* RM4018^T (NC_009850), *A. (Arcobacter) skirrowii* LMG 6621^T (NXIC00), *A. (Arcobacter) thereius* LMG 24486^T (LLKQ01) and *A. (Arcobacter) trophiarum* LMG 25534^T (PDKD00) [35]. These species were selected according to the results obtained with the m-PCRs or 16S rRNA sequence homology. The ANI value was calculated with JSpeciesWS [36]. The *is*DDH was calculated with the genome-to-genome calculator (GGDC2.0 software) using results obtained with the formula 2, as recommended by the software developers [2, 23].

As *Arcobacter* species are considered emerging zoonotic pathogens associated with human gastroenteritis, the two isolates were screened for the presence of virulence genes. The presence of ten putative virulence genes was determined by PCR. The primers and PCR protocols used for partial amplification of *cadF, ciaB, cj1349, hecA, hecB, irgA, mviN, pldA* and *tlyA* were according to Douidah *et al*. [11], and *iroE* gene detection was performed using primers and PCR protocol according to Karadas *et al.* [18].

Phenotypic Characterization. Phenotypic characteristics were determined by a set of classical and specific tests recommended for the description of novel species in the Family *Campylobacteraceae* [43, 22, 28] including: cell morphology and motility, Gram staining, catalase and oxidase activity, acid production from glucose by oxidation and fermentation,

143 nitrate reduction, Voges-Proskauer, indole, urea, hydrolysis of indoxyl acetate, and hydrogen
144 sulphide production in triple-sugar iron agar.
145 Cell size, bacterial morphology and the presence of flagella of the strain chosen as the
146 representative strain (F199^{T}) were determined by transmission electron microscopy (JEOL
147 1400 Plus). Cells were grown on blood agar (Oxoid) for 24 h at 30 °C and fixed with 2%
148 glutaraldehyde in Sorensen's phosphate buffer 0,1M for 1 h at room temperature. Fixed cells
149 were mounted in a glow-discharge carbon coated grid and stained with 2% uranyl acetate
150 for 1 min. Motility was determined in young cultures by hanging drop preparations in
151 *Arcobacter* broth (Oxoid). Colony morphology was assessed on Columbia blood agar (Oxoid)
152 incubated at 30 °C for 48 h under aerobic conditions.
153 Growth at 25, 30, 37 and 42 °C was determined on Nutrient broth nº 2 (Oxoid)
154 supplemented with 5% sheep blood (Thermo Scientific) and 1.5% agar under aerobic and
155 microaerophilic conditions, the latter in a jar system with GENbag microaer system
156 (bioMérieux). The ability to grow on different growth media was assayed by culturing on
157 non-supplemented *Campylobacter* charcoal deoxycholate agar (CCDA; Oxoid), MacConkey
158 agar (Scharlau), Davis Minimal medium (Fluka), and Nutrient broth nº 2 (Oxoid)
159 supplemented with 5% sheep blood (Thermo Scientific) and 1.5% agar containing 1% glycine,
160 2 and 4% (w/v) NaCl, 1% oxgall, 0.1% sodium deoxycholate, 64 mg L^{-1} cefoperazone, 0.05%
161 safranin, 0.0005% crystal violet; 0.005% basic fuchsine, 0.001% brilliant green, and 0.01%,
162 0.04% and 0.1% triphenyl tetrazolium chloride (TTC) at 30 °C incubation under aerobic
163 conditions for up to 48 hours.
164 Oxidase activity was assessed by using Bactident Oxidase strips (Merck), and catalase activity
165 by ID Color Catalase reactive (bioMerieux). The indoxyl acetate hydrolysis test was
166 performed according to Mills and Gherna [24], meanwhile hippurate hydrolysis was

determined by using a Hippurate Strips kit (Sigma). In addition, enzyme activities, utilization of various carbon sources and acid production from substrates were tested with API 20E, 20NE and API ZYM biochemical kits (BioMerieux) according to the manufacturer's procedure; the assays were performed at 30 °C for 48 h under aerobic conditions. All tests were conducted at least twice and appropriate positive and negative controls were also tested.

Antimicrobial susceptibility to six antibiotics (ampicillin, amoxicillin-clavulanic acid, ciprofloxacin, erythromycin, tetracycline, and gentamicin) was determined using MIC Test Strips® (Liofilchem, Werfen) following the manufacturer's instructions and Mueller Hinton Agar with 5% horse blood and 20 mg/l ß-NAD (MHF, Biomerieux). After 48 h of incubation at 30°C under aerobic conditions, the minimum inhibitory concentration (MIC) was determined. Interpretative criteria were based upon CASFM/EUCAST breakpoints for *Campylobacter* [12].

In addition to the classical phenotypic analysis a whole-cell matrix-assisted laser-desorption time-of-flight mass spectrometry (MALDI-TOF MS) fingerprint analysis was also performed at the Microbiology Service of the Hospital Universitario de Álava (Vitoria-Gasteiz, Spain). The MALDI-TOF MS profiles of the strains F199^T and FW-59 and of the most related species of *Arcobacter* were obtained using a Microflex LT spectrometer (Bruker Daltonics) with the flexAnalysis version 3.4 software. The profiles obtained for each strain was analyzed and compared, and the corresponding dendrogram was constructed by considering the average value of the triplicates for each strain.

Results and Discussion

A polyphasic approach was carried out in order to characterize two *Arcobacter* isolates; one of them (F199^T) was obtained from a carrot sample and the other one (FW-59) from wastewater collected at a wastewater treatment plant.

Bacterial strain identification. All isolates produced an amplicon of the expected size described for *Arcobacter butzleri* (401 bp) with the m-PCR of Houf *et al.* [14]; however, with the m-PCR of Douidah *et al*. [10] these isolates gave two amplicons of the same size expected for *A. butzleri* (2061 bp) and *A. skirrowii* (198 bp) (Supplementary Fig. S1). The results obtained by ERIC-PCR showed that each isolate had a different band pattern, indicating that they represented different strains (Supplementary Fig. S2).

Phylogeny. Due to the discordant or incongruent results obtained with both m-PCR in the species identification of the isolates, a sequence-based phylogenetic analysis was performed. The sequences of the 16S rRNA gene of the two isolates showed a similarity of 99.93% among themselves. The phylogenetic analysis of the 16S rRNA gene of the strain F199^T and FW-59 presented a separated branch from the *Aliarcobacter (Arcobacter) lanthieri* type strain LMG 28516^T (Fig. 1). The similarity of the 16S rRNA gene between the candidate new species represented by the strain F199^T and the strain *A. lanthieri* LMG 25816^T was 99.2%, while that similarity with the other described species of the former genus ranged from 98.5% with *Aliarcobacter (Arcobacter) faecis* LMG 28519^T to 91.0% with *Halarcobacter* (*Arcobacter) bivalviorum* CECT 7835^T [34]. The phylogenetic analysis of the *rpoB* gene showed that the two isolates (F199^T and FW-59) grouped in a cluster with a separated branch from *A. lanthieri* (Supplementary Fig. S3). The phylogenomic analysis based on the core genome made up of 61 genes (Fig. 2 and Supplementary Table S1) of the type strains of the former genus *Arcobacter* showed a cluster formed by the two *A. vitoriensis* sp. nov. strains in a separated branch, with the type strain of *A. lanthieri* LMG 28516^T as the nearest

214 species, as evidenced the previous analysis of the *rpoB* and the 16S rRNA genes. In all cases
215 the new candidate species grouped in a cluster with a separated branch from *A. lanthieri.*
216 **Genome features.** The genome of F199^T and FW-59 isolates were analysed in order to
217 confirm that the cluster represented by these strains belongs to a new species. Table 1
218 summarizes the features of both sequenced genomes, F199^T and FW-59, which were
219 assembled in 66 and 144 contigs, respectively. The obtained values of ANI (<96%) and *is*DDH
220 (<70%) confirmed that strains F199^T and FW-59 represented a new species (Table 2).
221 Moreover, the G+C content of both genomes, 27.0% for F199^T and 27.4% for FW-59, were
222 within the ones described for the genus (26.6% to 28.2%) [49, 7]. Both genomes studied
223 were annotated using RAST [29]. While the genome sequence of the strain F199^T showed
224 2,353 protein-coding sequences and 47 RNA coding ones, that of FW-59 showed 2,570 and
225 53, respectively. None of the studied genomes contained clustered regularly interspaced
226 short palindromic repeats (CRISPR). Genes related with the synthesis of polar lipids were also
227 screened for. Both genomes carried genes related with the synthesis of phosphatidylglycerol
228 (PG) i. e. phosphatidylglycerolphosphatase A (*pspA*, EC3.1.3.27) and phosphatidase
229 cytidylyltransferase (*cdsA*, EC 2.7.7.41); and with the synthesis of phosphatidylethanolamine
230 (PE) i. e. the phosphatidylserine descarboxilase gene (*psd*, EC4.1.1.65). However, none of the
231 genomes possessed the *pspB* gene (phosphatidylglycerolphosphatase B (EC 3.1.3.27)),
232 phenomenon not occurred in other published species [31, 49] with the exception of the
233 genome of *A. faecis* LMG 28519^T. When the annotated genome of the strain F199^T was
234 compared against that of *A. lanthieri* LMG 28516^T, RAST showed 89 differences consisting on
235 54 genes only present in *A. vitoriensis* sp. nov. strain F199^T and 36 genes only present in *A.*
236 *lanthieri* LMG 28516^T (Supplementary Table S2).

About the putative virulence genes, PCR detection revealed the presence of *ciaB* (encodes *Campylobacter jejuni* invasion antigen B that contributes to host cell invasion) and *mviN* (encodes virulence factor, inner membrane protein required for peptidoglycan biosynthesis) genes in both isolates; additionally, the *hecA* gene (encodes a protein member of the filamentous haemagglutinin family (FHA) and promotes adherence of bacteria to host cells) was detected in FW-59 strain. The presence of virulence-associated genes indicate that this new species could pose a health risk to humans and animals.

Phenotype. Cells were Gram-negative and motile rods under the light microscope, and the strain F199^T possessed a single polar flagellum that was observed under the transmission electron microscopy (Supplementary Fig. S5). The cells formed small, beige to off-white, convex colonies with regular margins of ~2-4 mm in diameter. No β-haemolysis was observed. The most relevant phenotypic characteristics of the novel species are summarized in Table 3. No phenotypic differences were observed between isolates F199^T and FW-59. Overall, the determining phenotypic tests when differentiating the novel species from the most closely related *Aliarcobacter* species, are: growth on different media (MacConkey, non-supplemented CCDA and minimal medium), in presence of 4% NaCl and 1% glycine, and at different incubation conditions (37 and 42 °C, air and microaerobic conditions); cefoperazone susceptibility, acetoin production, triphenyl tetrazolium chloride (TTC) reduction and nitrate reduction. In the API 20NE assimilation tests, all substrates gave negative results. In the API 20E tests, both isolates presented a positive result for acetoin production (Voges-Prosakauer) and nitrate reduction; the remaining reactions gave negative results. Regarding enzymatic activities tested with the API ZYM system, both *A. vitoriensis* sp. nov. strains (F199^T and FW-59) showed the same enzymatic profile with the presence of acid phosphatase, esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase activity.

Strains F199^T and FW-59 were resistant to ampicillin (MIC, 32 and 12 μg/mL, respectively) and to tetracycline (4 μg/mL); moreover, isolate F199^T was resistant to amoxicillin-clavulanic acid (12 μg/mL). Both isolates were susceptible to ciprofloxacin, erythromycin, and gentamicin.

The dendrogram (Supplementary Fig. S4) representing the distances calculated from the fingerprint profiles obtained by MALDI-TOF MS showed a clearly distinct group formed by the two strains (F199^T and FW-59) separated from other *Aliarcobacter* type species analyzed. Our results, by the application of a polyphasic analysis, support the identity of these two isolates representing a novel species of the genus *Aliarcobacter* [35], previously *Arcobacter,* for which the name *Aliarcobacter vitoriensis* sp. nov. is proposed. The type strain is F199^T (=CECT 9230^T= LMG 30050^T), and Table 4 shows the protologue.

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Tables

Table 1.

Summarized genome features of both *Aliarcobacter vitoriensis* sp. nov. strains used in this study.

Strain	GenBank Accession no.	Genome features							
		Size (Mbp)	Contings	G+C (%)	CDS (No.)	tRNAs (No.)	ncRNAs (No.)	rRNAs (No.)	CRISPs (No.)
F199^{T}	PDKB01	2.43	66	27.0	2,353	42	2	1 x 5S and 16S, 3 x 23S	0
FW-59	PDKA01	2.58	144	27.4	2,570	46	2	1 x 5S-16S-23S	0

CDS stands for protein-coding sequence
tRNA stands for transfer RNA
ncRNA stands for non-coding RNA
rRNA stands for ribosomal RNA

Table 2.

Results (percentages) of *is*DDH and ANI between the genome of *Aliarcobacter vitoriensis* sp. nov. and those of the closely related species; values below 70 and 96%, respectively, indicate that the genomes belong to different species.

Strain	Genome sequence analysis	Sequence similarity of related species with isolates *Aliarcobacter vitoriensis* sp. nov. F199^T and FW-59					
		A. lanthieri **LMG 28516^T**	***A. faecis*** **LMG 28519^T**	***A. butzleri*** **RM4018^T**	***A. skirrowii*** **LMG 6621^T**	***A. thereius*** **LMG 24486^T**	***A. trophiarum*** **LMG 25534^T**
F199^T	*is*DDH (%)	33.20	23.70	23.30	22.30	21.40	22.40
	ANI (%)	86.72	80.47	80.34	79.47	78.61	79.97
FW-59	*is*DDH (%)	33.20	23.80	23.40	22.40	21.50	22.50
	ANI (%)	86.83	80.87	80.40	79.37	78.51	79.93

448 **Table 3.**

449 Differential characteristics between *Aliarcobacter vitoriensis* sp. nov. and the most closely related species of the genus *Aliarcobacter*. Taxa: 1, *A.*

450 *vitoriensis* sp. nov. (n=2); 2, *A. lanthieri* LMG 28516^T; 3, *A. faecis* LMG 28519^T; 4, *A. butzleri* CCUG 30485^T; 5, *A. thereius* CCUG 56902^T; 6, *A.*

451 *skirrowii* CECT 8223^T; 7, *A. trophiarum* CCUG 59229^T. All the data were obtained in this work.

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Growth in/on:							
Air at 37 °C	**+**	**+**	**+**	**+**	**-**	**+**	**-**
CO_2 at 37 °C	**+**	**+**	**+**	**+**	**-**	**+**	**-**
CO_2 at 42 °C	-	**-**	**-**	**+**	**-**	**-**	**-**
4% (w/v) NaCl	-	**-**	**-**	**-**	**-**	**+**	**-**
1% (w/v) Glycine	**-**	**+**	**-**	**-**	**+**	**-**	**-**
MacConkey agar	**+**	**+**	+	+	+	-	+
CCDA	**+**	**+**	+	+	-	+	+
Minimal medium	-	**-**	-	+	+	-	-
Resistance to cefoperazone (64 mg l^{-1})	**+**	**+**	-	+	+	+	+
Enzyme activity:							
Catalase	**+**	**+**	+	+	+	+	+
Voges–Proskauer test	**+**	**+**	+	-	-	-	-
Nitrate reduction	**+**	**+**	+	+	+	+	-
TTC reduction	**+**	**+**	+	+	-	-	-
Alkaline phosphatase	**-**	**-**	**+**	+	-	-	-
Acid phosphatase	**+**	**-**	**-**	**+**	**-**	nd	nd
Esterase lipase (C8)	**+**	**-**	**-**	**-**	**-**	nd	nd
Naphtol-AS-BI-phsphohydrolase	**+**	**+**	**-**	**+**	**+**	nd	nd

452 +, Positive; -, negative; nd, not determined; w, weak positive reaction. CO_2 indicates microaerobic conditions.

453

454

Table 4.

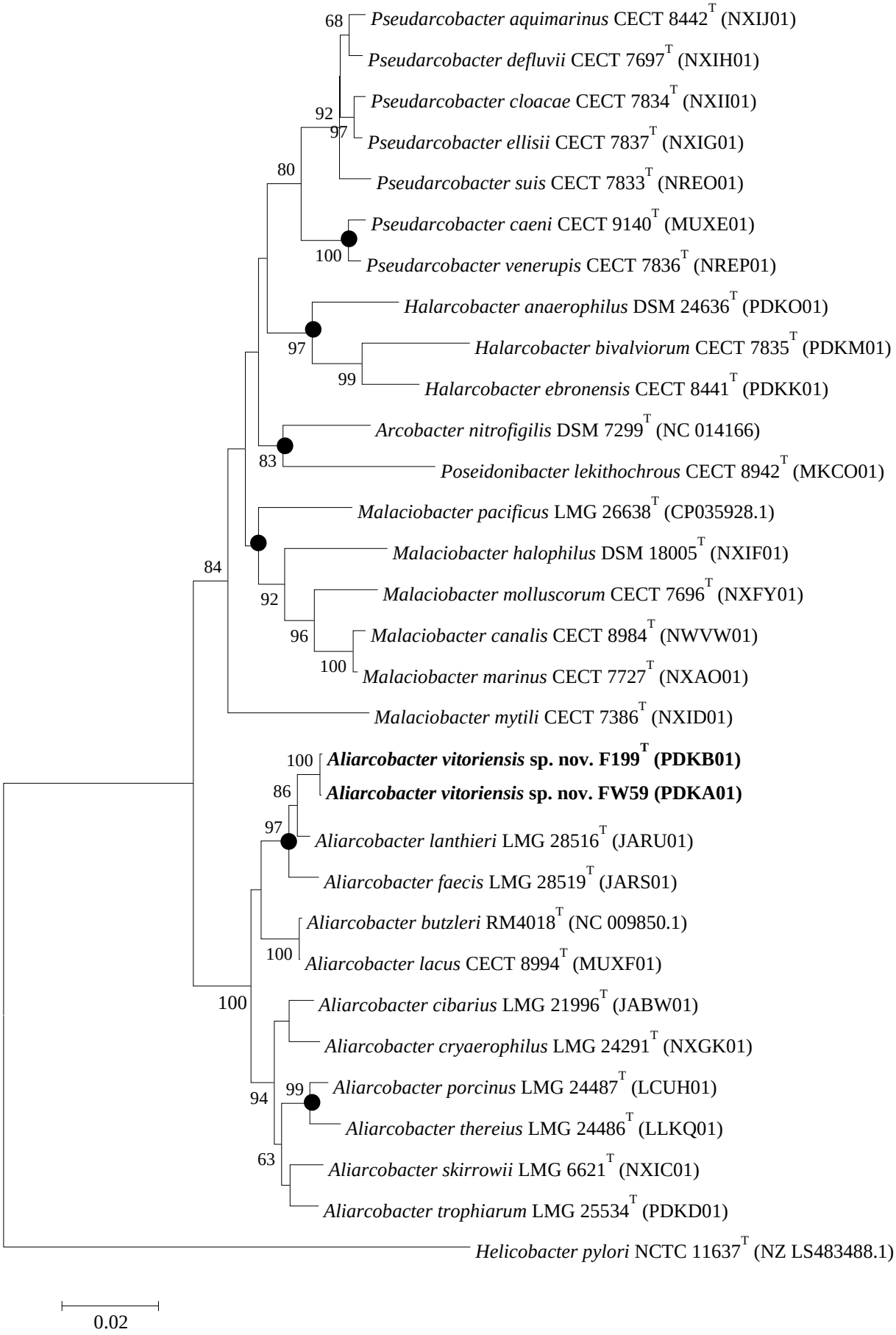
Protologue for *Aliarcobacter vitoriensis* sp. nov.

Genus name	***Aliarcobacter***
Species name	*Aliarcobacter vitoriensis*
Specific epithet	*vitoriensis*
Species status	sp. nov.
Species etymology	(vi.to.ri.en'sis, N.L. masc. adj. *vitoriensis*, pertaining to the city of Vitoria, Spain, the geographical origin of the species)
Description of the new taxon and diagnostic traits	Gram-negative slightly curved rods, non-encapsulated, 0.4–0.5 µm wide and 1.3–1.8 µm long. They are motile by a single polar flagellum. Colonies on blood agar incubated in aerobic conditions at 30 °C for 48 h are 2–4 mm in diameter, beige to off-white, circular with entire margins, and convex. No alpha haemolysis is observed on blood agar. Pigments are not produced. Cells grow well under both aerobic and microaerobic conditions with no significant differences at 25, 30, and 37 °C on nutrient medium supplemented with 5% sheep blood, but not at 42 °C. Under aerobic conditions at 30 °C the strain grows on MacConkey, non-supplemented campylobacter charcoal deoxycholate agar (CCDA) and on nutrient medium supplemented with 5% sheep blood also containing 2% (w/v) NaCl; 0.1% sodium deoxycholate; 1% oxgall; 0.04% 2,3,5-triphenyl tetrazolium chloride (TTC); 64 mg l−1 cefoperazone; 0.05% safranin; 0.0005% crystal violet; 0.005% basic fuchsine or 0.001% brilliant green. No growth occurs on Davis minimal agar, nor on nutrient medium supplemented with 5% sheep blood containing 4% (w/v) NaCl, 1% glycine or 0.1% TTC. Positive for oxidase, catalase, acid phosphatase, esterase lipase (C8) and naphtol-AS-BI-phosphohydrolase activities, nitrate and triphenyl tetrazolium chloride (TTC) reduction, the Voges–Proskauer (acetoin production) test and indoxyl acetate hydrolysis. Negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptone deaminase, alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase and cysteine arylamidase activities, hydrogen sulphide in triple-sugar iron agar medium, hippurate hydrolysis and indole and citrate utilization tests. D-glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, arabinose are not fermented or oxidized. The genome contains genes related to the synthesis of the polar lipids phosphatidylglycerol (PG) and phosphatidylethanolamine (PE).
Country of origin	Spain
Region of origin	Vitoria-Gasteiz

Date of isolation	09/2015
Source of isolation	A carrot purchased in a local farmers' market
Sampling date	16/09/2015
Latitude	42°50'28.79"N
Longitude	2°40'21.04"W
Altitude (meters above sea level)	525 m
16S rRNA gene accession nr.	GenBank: KX913922
Genome accession number [RefSeq; EMBL; …]	GenBank: PDKB00000000
Genome status	Complete
Genome size	2,427 Kbp
GC mol%	27.0
Number of strains in study	2
Source of isolation of non-type strains	Wastewater
Information related to the Nagoya Protocol	-
Designation of the Type Strain	F199^{T}
Strain Collection Numbers	F199^{T}=CECT 9230^{T}= LMG 30050^{T}

457

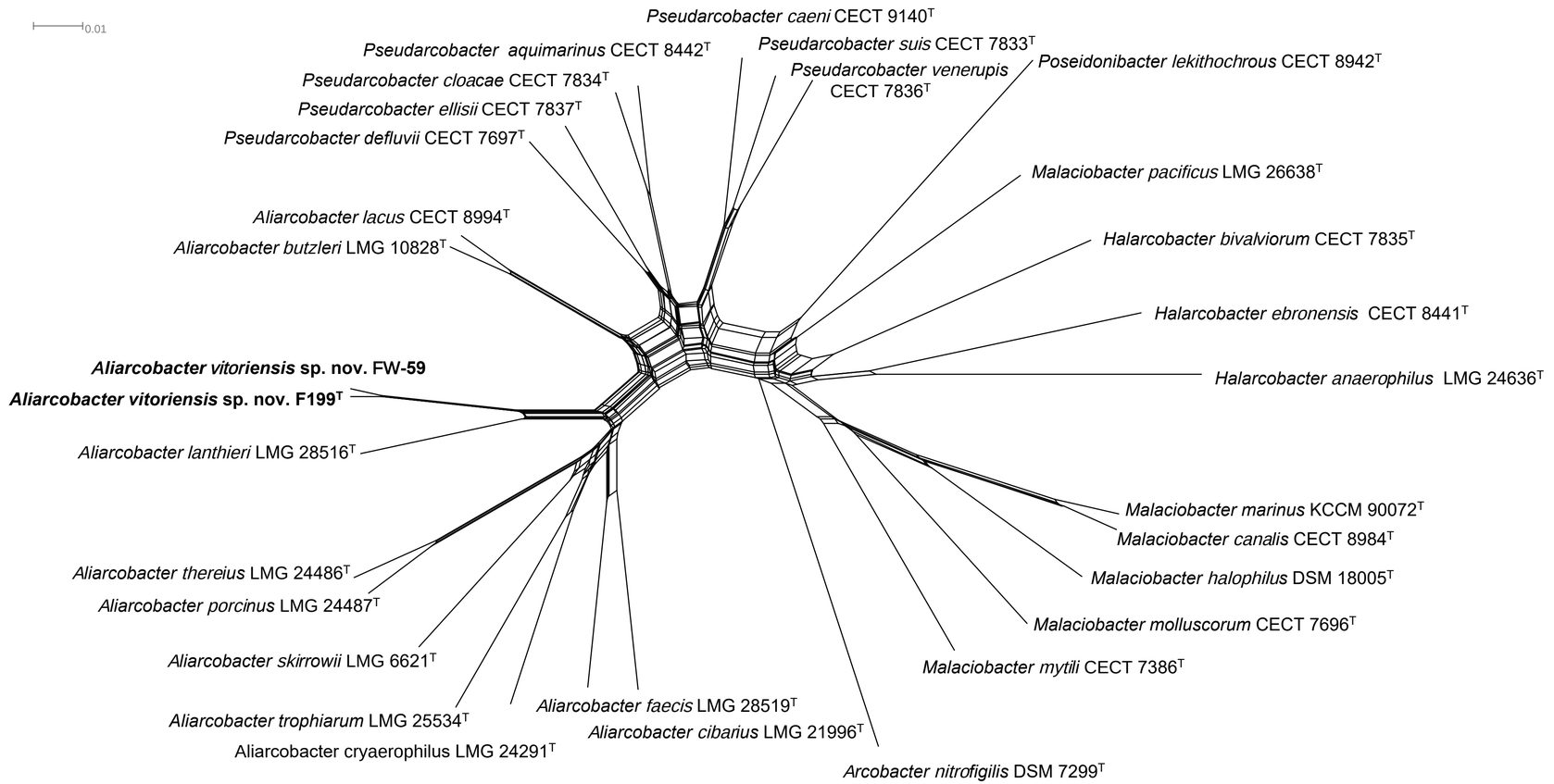
Figure 1


68
Pseudarcobacter aquimarinus CECT 8442T (NXIJ01)
Pseudarcobacter defluvii CECT 7697T (NXIH01)
92
97
Pseudarcobacter cloacae CECT 7834T (NXII01)
Pseudarcobacter ellisii CECT 7837T (NXIG01)
80
Pseudarcobacter suis CECT 7833T (NREO01)
Pseudarcobacter caeni CECT 9140T (MUXE01)
100
Pseudarcobacter venerupis CECT 7836T (NREP01)
Halarcobacter anaerophilus DSM 24636T (PDKO01)
97
Halarcobacter bivalviorum CECT 7835T (PDKM01)
99
Halarcobacter ebronensis CECT 8441T (PDKK01)
Arcobacter nitrofigilis DSM 7299T (NC 014166)
83
Poseidonibacter lekithochrous CECT 8942T (MKCO01)
Malaciobacter pacificus LMG 26638T (CP035928.1)
Malaciobacter halophilus DSM 18005T (NXIF01)
84
92
Malaciobacter molluscorum CECT 7696T (NXFY01)
96
Malaciobacter canalis CECT 8984T (NWVW01)
100
Malaciobacter marinus CECT 7727T (NXAO01)
Malaciobacter mytili CECT 7386T (NXID01)
100
Aliarcobacter vitoriensis sp. nov. F199T (PDKB01)
86
Aliarcobacter vitoriensis sp. nov. FW59 (PDKA01)
97
Aliarcobacter lanthieri LMG 28516T (JARU01)
Aliarcobacter faecis LMG 28519T (JARS01)
Aliarcobacter butzleri RM4018T (NC 009850.1)
100
Aliarcobacter lacus CECT 8994T (MUXF01)
100
Aliarcobacter cibarius LMG 21996T (JABW01)
Aliarcobacter cryaerophilus LMG 24291T (NXGK01)
99
94
Aliarcobacter porcinus LMG 24487T (LCUH01)
Aliarcobacter thereius LMG 24486T (LLKQ01)
63
Aliarcobacter skirrowii LMG 6621T (NXIC01)
Aliarcobacter trophiarum LMG 25534T (PDKD01)
Helicobacter pylori NCTC 11637T (NZ LS483488.1)
0.02

Caption Figure 1

Figure 1. Neighbour-joining tree based on 16S rRNA gene sequences (1512 bp) showing the phylogenetic position of the strains of *A. vitoriensis* sp. nov. F199^{T} and FW-59 within the former genus *Arcobacter*. Bootstrap values (>50 %) based on 1000 replications are shown at the nodes of the tree. Bar, 2 substitutions per 100 nt. Closed circles indicate concordance between Neighbour-joining and Maximun likelihood methods.

Figure 2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Pseudarcobacter caeni CECT 9140T
Pseudarcobacter aquimarinus CECT 8442T
Pseudarcobacter suis CECT 7833T
Pseudarcobacter venerupis CECT 7836T
Poseidonibacter lekithochrous CECT 8942T
Pseudarcobacter cloacae CECT 7834T
Pseudarcobacter ellisii CECT 7837T
Pseudarcobacter defluvii CECT 7697T
Malaciobacter pacificus LMG 26638T
Aliarcobacter lacus CECT 8994T
Aliarcobacter butzleri LMG 10828T
Halarcobacter bivalviorum CECT 7835T
Halarcobacter ebronensis CECT 8441T
Aliarcobacter vitoriensis sp. nov. FW-59
Aliarcobacter vitoriensis sp. nov. F199T
Halarcobacter anaerophilus LMG 24636T
Aliarcobacter lanthieri LMG 28516T
Malaciobacter marinus KCCM 90072T
Malaciobacter canalis CECT 8984T
Aliarcobacter thereius LMG 24486T
Malaciobacter halophilus DSM 18005T
Aliarcobacter porcinus LMG 24487T
Malaciobacter molluscorum CECT 7696T
Aliarcobacter skirrowii LMG 6621T
Malaciobacter mytili CECT 7386T
Aliarcobacter trophiarum LMG 25534T
Aliarcobacter faecis LMG 28519T
Aliarcobacter cibarius LMG 21996T
Aliarcobacter cryaerophilus LMG 24291T
Arcobacter nitrofigilis DSM 7299T

Caption Figure 2

Figure 2. Split decomposition network constructed with the concatenated sequences of 61 core genes (41,878 bp) showing the position of *Aliarcobacter vitoriensis* sp. nov. whitin the former genus *Arcobacter*. Scale bar, base substitutions per site.