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Short communication

Arcobacter ellisii sp. nov., isolated from mussels☆

Maria José Figueras[a,*], Arturo Levican[a], Luis Collado[a,b], Maria Isabel Inza[c], Clara Yustes[c]

[a] Unitat de Microbiologia, Departament de Ciències Mediques Bàsiques, Facultat de Medicina i Ciències de la Salut, IISPV, Universitat Rovira i Virgili, Reus, Spain
[b] Institute of Microbiology, Faculty of Sciences, Universidad Austral de Chile, Valdivia, Chile
[c] Agencia de Protecció de la Salut, Laboratori de Salut Pública de Tarragona, Spain

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ABSTRACT

As part of a study carried out for detecting *Arcobacter* spp. in shellfish, three mussel isolates that were Gram-negative slightly curved rods, non-spore forming, showed a new 16S rDNA-RFLP pattern with a specific identification method for the species of this genus. Sequences of the 16S rRNA gene and those of the housekeeping genes *rpoB, gyrB* and *hsp60* provided evidence that these mussel strains belonged to an unknown genetic lineage within the genus *Arcobacter*. The similarity between the 16S rRNA gene sequence of the representative strain (F79-6^T) and type strains of the other *Arcobacter* species ranged between 94.1% with *A. halophilus* and 99.1% with the recently proposed species *A. defluvii* (CECT 7697^T). DDH results between strain F79-6^T and the type strain of the latter species were below 70% (53 ± 3.0%). Phenotypic characteristics together with MALDITOF mass spectra differentiated the new mussel strains from all other *Arcobacter* species. All the results indicate that these strains represent a new species, for which the name *Arcobacter ellisii* sp. nov. with the type strain F79-6^T (=CECT 7837^T = LMG 26155^T) is proposed.

The genus *Arcobacter* is included in the family *Campylobacteraceae*, together with the genera *Campylobacter* and *Sulfurospirillum* and embraces a group of bacteria characterized for being aerotolerant and growing at lower temperatures than members of the genus *Campylobacter* [4,41]. The first isolation of bacteria of this genus is attributed to Ellis et al. [14] who recovered spirillum/vibrio-like microorganisms from internal organs of naturally aborted bovine foetuses. These and other isolates, were later described as *Campylobacter cryaerophila* by Neill et al. [28]. However, this species and another with similar characteristics (*Campylobacter nitrofigilis*) were allocated to the new genus *Arcobacter* in 1991 by Vandamme et al. [39] with the names *Arcobacter cryaerophilus* and *Arcobacter nitrofigilis*. The latter is the type species of the genus and is a nitrogen-fixing bacterium recovered originally from roots of the salt marsh plant *Spartina alterniflora* [27]. The amendment to the genus in 1992 by Vandamme et al. [40] included the reclassification of *Campylobacter butzleri* isolated from humans and animals with diarrhoea [23] as *Arcobacter butzleri*, and the description of the new species *Arcobacter skirrowii* isolated from the faeces of lambs with diarrhoea, aborted porcine, ovine, and bovine foetuses, and the prepuce of bulls.

Since then, the genus has expanded with the addition of several new species: *Arcobacter cibarius* from chicken meat [21], *Arcobacter halophilus* from an hypersaline lagoon [11], *Arcobacter mytili* from mussels [6], *Arcobacter thereius* from porcine abortions [22], *Arcobacter marinus* from a mixture of seawater, seaweeds and a starfish [24], *Arcobacter trophiarum* from faeces of fattening pigs [10], *Arcobacter defluvii* from sewage water [8] and finally *Arcobacter molluscorum*, from mussels and oysters [16].

The species *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, have been associated with gastrointestinal disease and bacteraemia in humans, *A. butzleri* being the most commonly isolated species [4]. The latter was the fourth most common *Campylobacter*-like organisms isolated from the stools of patients with diarrhoea in two separate studies carried out in Belgium and France [30,42]. *Arcobacter* species have been implicated in animal diseases including abortion, septicaemia, mastitis, gastritis and enteritis [4,17,18], and are frequently isolated from meat, mainly from poultry, followed by pork and beef [4,7,18,43]. The abundant presence of the microbes in drinking water and in food of animal origin suggests that these are the transmission routes of these bacteria [4,17].

Species of this genus have been isolated from environmental waters, where it was demonstrated that their presence correlated with that of the indicators of faecal pollution [5]. In fact, it was

☆ The GenBank/EMBL/DDBJ accession numbers of the sequences of strain F79-6^T (=CECT 7837^T = LMG 26155^T) for the 16S rRNA, the *rpoB* the *gyrB* and the *hsp60* genes are FR717550, FR717542, FR717545, FR717548, respectively. The 16S rRNA, the *rpoB* the *gyrB* and the *hsp60* genes sequences of strains F79-2 (FR717551, FR717543, FR717546, FR717549, respectively) and F79-7 (FR717552, FR717544, FR717547, FR717553, respectively) have also been deposited.

* Corresponding author. Tel.: +34 977759321; fax: +34 977759322.
E-mail address: mariajose.figueras@urv.cat (M.J. Figueras).

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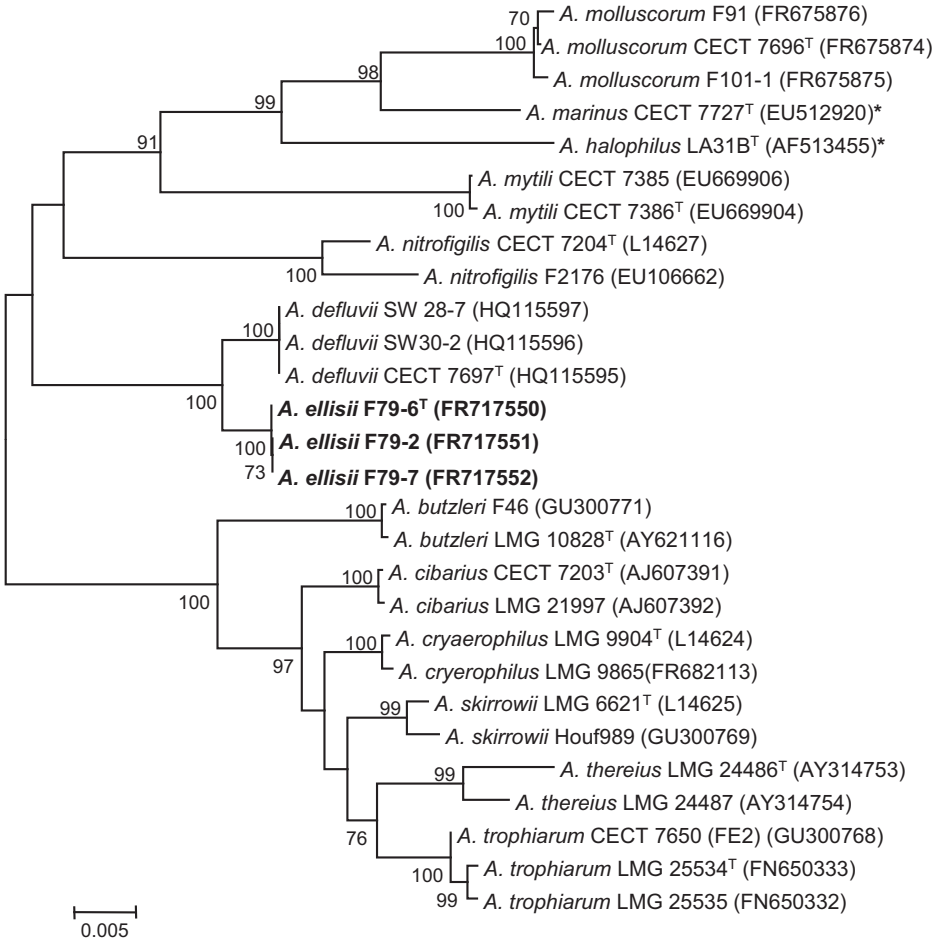

Fig. 1. Neighbour-joining tree based on 16S rRNA sequences showing the phylogenetic position of *Arcobacter ellisii* sp. nov. within the genus *Arcobacter*. Bootstrap values (>70%) based on 1000 replications are shown at the nodes of the tree. Bar, 5 substitutions per 1000 nt. *Only the type strain is available so far.

shown that *Arcobacter* spp. entered the seawater together with faecally contaminated freshwater [5].

In a study that investigated the occurrence of arcobacters in shellfish, a high prevalence and diversity of these bacteria has been demonstrated in clams and mussels [7]. In that survey, the species *A. mytili* [6], was discovered. Very recently, another new species *A. molluscorum* has also been isolated from mussels and oysters [16].

As part of a new ongoing survey carried out for detecting *Arcobacter* in shellfish using the same isolation protocol that has been described previously [7,16], three isolates recovered from mussel samples (F79-2, F79-6 and F79-7) proved to belong to the genus on the basis of their colony morphology on blood agar (small, translucent colourless or beige to off-white), and phenotypic characteristics (Gram-negative motile slightly curved rods positive for oxidase). Molecular identification was carried out using the restriction fragment length polymorphism (16S rDNA-RFLP) designed for this genus [15] and two different multiplex PCR (m-PCR) methods [13,19]. With 16S rDNA-RFLP, the three strains showed a common pattern different from those previously described (Fig. S1), while with the two m-PCR they showed discrepant results. With the m-PCR described for the identification of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* [19], an amplicon was obtained similar to the one expected for *A. cryaerophilus* (Fig. S2). However, with the recent m-PCR designed for the identification of five *Arcobacter* species associated with humans and other mammals, the three new strains showed an amplicon similar to the one expected for *A. butzleri* [13]. However, an additional, less intense, band similar to that expected for *A. cryaerophilus* was produced by the strains F79-2 and F79-6 (Fig. S2). The new RFLP pattern observed and the contradictory results obtained by the m-PCR methods suggested that the three isolates might belong to a potential new *Arcobacter* species and required further investigation.

The three isolates were genotyped using the enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), as described by Houf et al. [20], in order to find out if they were different strains. Results showed that each isolate had a different ERIC-PCR pattern, indicating that they indeed represent different strains (Fig S3) and strain F79-6^T was chosen as the type.

The 16S rRNA, *rpoB*, *gyrB* and *hsp60* genes of the three isolates were amplified and sequenced using primers and conditions previously described [6,8,9] with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The obtained sequences were assembled using SEQMAN software and the phylogenetic analyses were carried out using sequences of all type strains and other strains of all species obtained in previous studies and deposited in the GenBank. The similarity of the 16S rRNA gene sequences was determined by using EzTaxon software [3]. Independent alignments of 16S rRNA (1405 nt), *rpoB* (487 nt), *gyrB* (665 nt) and *hsp60* (555 nt) gene sequences were carried out using CLUSTAL W software [37]. Genetic distances were obtained using Kimura's two-parameter model [25] and phylogenetic trees were constructed with the neighbour-joining [32] and maximum likelihood, both using MEGA software version 4 [36], and with maximum parsimony, using PAUP software [35].

The independently obtained neigbour joining phylogenetic trees for these genes (16S rRNA, *rpoB*, *gyrB* and *hsp60*) showed that these mussel strains belonged to an unknown genetic lineage within the genus *Arcobacter* (Fig. 1, Figs. S4–S6) and this was even