Systematic and Applied Microbiology 38 (2015) 30–35

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: www.elsevier.de/syapm

Arcobacter ebronensis sp. nov. and *Arcobacter aquimarinus* sp. nov., two new species isolated from marine environment

Arturo Levican[a,b,c], Sara Rubio-Arcos[a], Antonio Martinez-Murcia[d], Luis Collado[e], María José Figueras[a,*]

[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] *Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Facultad de Ciencias Biológicas Universidad Andrés Bello, Viña del Mar, Chile*
[c] *Interdisciplinary Center for Aquaculture Research (INCAR) , Concepción, Chile*
[d] *Area de Microbiología, EPSO, Universidad Miguel Hernández, Orihuela, Alicante 03312, Spain*
[e] *Institute of Biochemistry and Microbiology, Faculty of Sciences, Universidad Austral de Chile, Valdivia, Chile*

ARTICLE INFO

Article history:
Received 18 May 2014
Received in revised form 28 October 2014
Accepted 31 October 2014

Keywords:
Arcobacter
A. ebronensis
A. aquimarinus
MLPA
16S rRNA
MALDI-TOF

ABSTRACT

Two strains recovered from mussels (F128-2^T) and sea water (W63^T) were characterized as *Arcobacter* sp., but they could not be assigned to any known species using the molecular identification methods specific for this genus (16S rDNA-RFLP and m-PCR) and *rpoB* gene analysis. The 16S rRNA gene sequence similarity to the type strains of all *Arcobacter* species ranged from 92.2% to 96.7% with strain F128-2^T, and from 94.1% to 99.4% with strain W63^T, the most similar being *A. bivalviorum* (CECT 7835^T) and *A. defluvii* (CECT 7697^T), respectively. The phylogenetic analyses of 16S rRNA, and the concatenated sequences of *gyrB, gyrA, rpoB, atpA* and *hsp60* genes confirmed that strains F128-2^T and W63^T belonged to two new lineages within the genus *Arcobacter*. Moreover, both strains showed differential phenotypic characteristics and MALDI-TOF mass spectra from all other *Arcobacter* species. Therefore, it has been demonstrated the existence of two new *Arcobacter* species and the proposed names are *Arcobacter ebronensis* (type strain F128-2^T = CECT 8441^T = LMG 27922^T), and *Arcobacter aquimarinus* (type strain W63^T = CECT 8442^T = LMG 27923^T).

Members of the genus *Arcobacter* (Ar'co.bac.ter. L. n. *arcus*, bow; Gr. n. *bacter*, rod; M. L. masc. n. *Arcobacter*, bow-shaped rod) are Gram negative, slightly curved rods positive for oxidase and usually motile and belong to the Epsilonproteobacteria and family *Campylobacteraceae* [4,38]. These bacteria had previously been classified as *Campylobacter* spp. because of their similar morphology, despite they differed from the latter genus because they are aerotolerant and able to grow at lower temperatures [4,38]. In fact, based on the latter characteristics Vandamme et al. created the genus *Arcobacter* in 1991 with 2 species formerly known as campylobacters, *Arcobacter nitrofigilis* and *Arcobacter cryaerophilus* [39]. Currently this genus includes 18 species that have been recovered from different hosts and environments [4,33]. Moreover, the analysis of the 16S rRNA gene sequences deposited in GenBank indicates that many other potential new *Arcobacter* species remain to be characterized [40].

Some *Arcobacter* spp. have been linked with gastroenteritis and bacteraemia in humans, and with abortions, mastitis and diarrhoea in animals, and they are considered as potential water and food-borne pathogens [4,5,17]. In this sense, it has been demonstrated that the presence of *Arcobacter* in water increases with the levels of faecal pollution [6]. It was suggested that *Arcobacter* spp, entered seawater with the contaminated inputs of freshwater despite some species could be autochthonous of the marine environment [6]. So far *Arcobacter* was recovered either from the water or from the stools of the patients in 3 drinking water outbreaks, 2 of them in USA and 1 in Slovenia [18,23,32]. However, in none of them the implication of this microbe was completely proven. *Arcobacter* spp. have also been found in association with food of animal origin, mainly meat products, but also in shellfish [4,5,28]. Shellfish could be an important reservoir and source of infection of these bacteria, as suggested in recent studies [5,28].

In a recent study on the prevalence of *Arcobacter* in different types of shellfish collected from the Ebro river delta [28], one strain recovered from mussels (F128-2^T) could not be assigned to any known species. The same occurred for another strain of our collection (W63^T) obtained from a seawater sample. The objective of the present study was to establish the taxonomic position of both strains (F128-2^T and W63^T) using a polyphasic approach.

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

http://dx.doi.org/10.1016/j.syapm.2014.10.011
0723-2020/

Strain F128-2^T was obtained from mussels collected from the Ebro delta, Catalonia (northeast Spain) in June 2011, while strain W63^T was isolated from a seawater sample at the Garraf beach also in Catalonia, in September 2008. Both strains showed the typical colony morphology of arcobacters on blood agar (small, translucent, beige to off-white) and were Gram negative, slightly curved rods that produce oxidase activity, as previously described for the species of this genus [4,39].

The characterization of the strains was initially attempted using specific *Arcobacter* identification methods i.e. two Multiplex-PCR (m-PCR) [10,21] and the restriction fragment length polymorphism of the 16S rRNA gene (16S rDNA-RFLP) [12,15]. Both strains (F128-2^T and W63^T) produced an amplicon of the expected size described for *A. cryaerophilus* with the m-PCR of Houf et al. [21]. However, with the m-PCR of Douidah et al. [10] strain W63^T produced the expected amplicon for *Arcobacter butzleri* while strain F128-2^T, produced no amplification. On the other hand, with the recently updated16S rRNA RFLP identification method [15] strain F128-2^T produced a new pattern after digestion with the endonuclease *MseI* (719, 138, 81, 52, 34 and 3 bp; Fig. S1), while W63^T produced the same pattern described for *Arcobacter defluvii* when digested with *MseI* [7] or *BfaI* endonucleases [15] (Fig. S1). Considering these contradictory results, the *rpoB* (621 bp) and the 16S rRNA (1401 bp) genes of both strains (F128-2^T and W63^T) were sequenced and analysed as described previously [3,25] and the constructed phylogenetic trees indicated that both strains formed independent phylogenetic lines within the genus (Fig. 1 and Fig. S2). In the 16S rRNA gene tree, strain W63^T clustered with the species *A. defluvii, Arcobacter cloacae* and *Arcobacter ellisii*, whereas the most closest species for strain F128-2^T were *A. bivalviorum* and *A. anaerophilus* (Fig. 1).

The 16S rRNA gene similarity, calculated with the EzTaxon software [2], between strains F128-2^T and W63^T was 95.7%. Similarities between strain F128-2^T and other *Arcobacter* spp. ranged from 92.2%, with the type strain of *A. cryaerophilus* (LMG 9904^T), to 96.7%, with the type strain of *A. bivalviorum* (CECT 7835^T). The similarities of strain W63^T with other arcobacters ranged from 94.1%, with the type strain of *Arcobacter mytili* (CECT 7386^T), to 99.4%, with the type strain of *A. defluvii* (CECT 7697^T). These similarities were all within the range from 91.1% (for *A. cryaerophilus* and *A. bivalviorum*) to 99.6% (for *A. cloacae* and *A. ellisii)* described for the genus [16,25].

In the definition of several *Arcobacter* species the use of the concatenated sequences of housekeeping genes (*gyrA*, *atpA*, *rpoB*, *gyrB* and *hsp60*) has shown a better resolution, than DDH results [3,7,8,13,16,25,26]. In fact, the "ad hoc committee for the re-evaluation of the species definition in bacteriology" has suggested that this approach could be used as an alternative to DDH if correlation with the latter method was demonstrated [34] as has recently been done for the genus *Arcobacter* [7,13,25,26]. This approach has been named multilocus sequence analysis (MLSA) or multilocus phylogenetic analysis (MLPA) [14,25,36]. In the *Arcobacter* studies, the MLPA has also provided a more robust overall phylogenetic relatedness (bootstrap values of 100% for all the species clusters) than 16S rRNA gene [3,7,8,13,16,25,26]. For strains F128-2^T and W63^T, apart from the *rpoB*, the sequences of the 4 remaining genes i.e. *gyrB* (618 bp), *gyrA* (686 bp), *atpA* (622 bp), and *hsp60* (595 bp) were obtained as described previously [7,25]. In addition, in order to complete the MLPA with all currently accepted species, the sequences of the 5 genes (*gyrA, atpA*, *rpoB, gyrB* and *hsp60*) from the strain DSM 24636^T of the recently described species *A. anaerophilus* [33] were obtained. Alignments were performed using the MEGA software version 5 [35] and CLUSTAL W [24] and clustering, using the neighbour-joining, maximum parsimony and maximum likelihood algorithms. The phylogenetic tree obtained with the sequences of the 5 concatenated genes (3142 bp), using different the neighbour joining (Fig. 2) and other algorithms (data not shown) confirmed initial *rpoB* results that both strains belonged to two independent and unknown phylogenetic lines within the genus.

Both strains (F128-2^T and W63^T) were characterized as motile under the phase contrast microscope, and under the transmission electron microscope, they showed a single polar flagellum (Fig. S3). Further characterization was carried out using the test recommended in the minimal standards for the family *Campylobacteraceae* [37], as well as others tests previously used in the description of other new *Arcobacter* spp. [25]. All tests were carried out at least twice for the 2 new strains and for all the type strains of *Arcobacter* species with the exception of *A. anaerophilus* that could not be maintained alive despite repeated efforts using different culture conditions. The test that were able to differentiate strains F128-2^T and W63^T between them and also from all other *Arcobacter* spp. are shown in Table 1. Interestingly, strain F128-2^T showed growth on media with 4% NaCl, as also did other species isolated in association with shellfish or starfish like *A. nitrofigilis, Arcobacter skirrowii, Arcobacter halophilus, A. mytili, Arcobacter marinus, Arcobacter molluscorum* and *A. bivalviorum*. However, F128-2^T could be easily differentiated from all of these species by 7 to 12 tests (Table 1). On the other hand, the closest phylogenetic species to F128-2^T was *A. anaerophilus* (Figs. 1 and 2). However, they could be differentiated by at least 3 tests (Table 1) and by the fact that *A. anaerophilus* is strictly anaerobe. The most similar species to strain W63^T, was *A. cloacae* but they could be differentiated because, in contrast to W63^T, the later species is able to grow on MacConkey but not on blood agar at 30 °C under anaerobic conditions [25].

Additional characterization of the mussels (F128-2^T) and seawater (W63^T) strains included the analysis of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). This analysis was carried out in parallel with the type strains of all *Arcobacter* species as previously described [16], with the only exception, as commented, of strain DSM 24636^T of *A. anaerophilus* which could not be kept alive in our laboratory to carry out the MALDI-TOF analysis. The obtained MALDI-TOF profiles of strains were hierarchically clustered in a dendrogram using the SPECLUST web tool (http://bioinfo.thep.lu.se/speclust.html) [1]. In the obtained dendrogram, strain F128-2^T clustered close to the type strain of *A. halophilus* (LA31B^T) and W63^T, close to the type strain of *A. cloacae*, CECT 7834^T (Fig. S4 and Table S1).

Considering the origin of the studied strains (shellfish and water), they both were tested for the presence of 5 putative virulence genes (*ciaB*, *irgA*, *hecA*, *cj1349* and *cadF*) as previously described [11]. Strains F128-2^T and W63^T possessed the *ciaB* gene that codifies for a major invasine protein in genus *Campylobacter* [11,30], while W63^T also possessed the *cj1349* gene, which codifies for a fibronectin binding protein in *Campylobacter jejuni*. Despite of this, strain W63^T was not able to adhere or invade the human intestinal Caco-2 cell lines in a previous study [27]. These results warrant future studies on the potential pathogenic role of F128-2^T and W63^T for humans.

In this study it has been demonstrated the existence of two new *Arcobacter* species, for which the names *Arcobacter ebronensis* (type strain F128-2^T = CECT 8441^T = LMG 27922^T), and *Arcobacter aquimarinus* (type strain W63^T = CECT 8442^T = LMG 27923^T) are proposed.

Description of *Arcobacter ebronensis* sp. nov.

Arcobacter ebronises (e.bro.nen'sis. N.L. masc. adj. *ebronensis*, of or belonging to Ebro river delta Spain, where shellfish sample harbouring strain F128-2^T was collected)