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Short communication

Arcobacter cloacae sp. nov. and *Arcobacter suis* sp. nov., two new species isolated from food and sewage☆

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ABSTRACT

Three strains recovered from mussels (F26), sewage (SW28-13^T) and pork meat (F41^T) were characterized as *Arcobacter*. They did not appear to resemble any known species on the basis of their 16S rDNA-RFLP patterns and the *rpoB* gene analyses. However, strains F26 and SW28-13^T appeared to be the same species. The 16S rRNA gene sequence similarity of strains SW28-13^T and F41^T to the type strains of all other *Arcobacter* species ranged from 94.1% to 99.6% and 93.4% to 98.8%, respectively. Phenotypic characteristics and the DNA–DNA hybridization (DDH) results showed that they belonged to 2 new *Arcobacter* species. A multilocus phylogenetic analysis (MLPA) with the concatenated sequences of 5 housekeeping genes (*gyrA*, *atpA*, *rpoB*, *gyrB* and *hsp60*) was used for the first time in the genus, showing concordance with the 16S rRNA gene phylogenetic analysis and DDH results. The MALDI-TOF mass spectra also discriminated these strains as two new species. The names proposed for them are *Arcobacter cloacae* with the type strain SW28-13^T (=CECT 7834^T = LMG 26153^T) and *Arcobacter suis* with the type strain F41^T (=CECT 7833^T = LMG 26152^T).

The genus *Arcobacter*, created by Vandamme et al. in 1991 [29], belongs to the family *Campylobacteraceae* and embraces Gram-negative, motile and oxidase positive, slightly curved, rod-shaped bacteria [28]. It currently includes 15 species, nine of which were isolated from environmental samples: *Arcobacter nitrofigilis*, from the roots of *Spartina alterniflora* [21]; *Arcobacter halophilus*, from a hypersaline lagoon [7]; *Arcobacter mytili*, *Arcobacter molluscorum*, *Arcobacter ellisii*, *Arcobacter bivalviorum* and *Arcobacter venerupis*, from shellfish [2,11,13,20]; *Arcobacter marinus*, isolated from both seawater and starfish [18]; and *Arcobacter defluvii*, from sewage [5]. The other six species have been described from human or animal sources: *Arcobacter butzleri* from human faeces, *Arcobacter cryaerophilus*, *Arcobacter skirrowii* and *Arcobacter trophiarum* from animal faeces [6,17,22,30]; *Arcobacter cibarius* from chicken meat [14] and *Arcobacter thereius* from porcine abortion [15]. The taxonomy of this genus has changed substantially in recent years and nine of the species have been described since 2009 [3,20]. The analysis of the 16S rRNA gene sequences deposited in GenBank indicates that there are many potentially new *Arcobacter* species that have yet to be characterized [31].

In a study that investigated the prevalence of *Arcobacter* spp. in different kinds of food [4], two strains, one from pork meat (F41^T) and the other from mussels (F26), did not resemble any *Arcobacter* species known at that time on the basis of their 16S rDNA Restriction Fragment Length Polymorphism patterns (16S rDNA-RFLP) [10]. The *rpoB* gene of both strains was sequenced and provided further evidence that they belonged to two new *Arcobacter* species but had not been described while waiting for new strains to be isolated. No other strains with the characteristics of F41^T has since been found, but another strain (SW28-13^T) isolated from sewage of a Waste Water Treatment Plant (WWTP) was found to be similar to strain F26 on the basis of its *rpoB* gene and 16S rDNA-RFLP pattern. The aim of this study was to use a polyphasic approach in order to characterize the strains F41^T, F26 and SW28-13^T as belonging to two new *Arcobacter* species.

Strain (F41^T) was recovered from pork meat purchased from a retail market, the mussel strain (F26) was collected from the Ebro river delta (both in March 2008), and the sewage strain (SW28-13^T) was isolated in March 2009 from a WWTP in the city of Reus, Spain. All strains had the expected colony morphology for *Arcobacter* species, i.e., small, translucent, beige to off-white on blood agar, and were characterized as Gram-negative, slightly curved, motile rods that produce oxidase activity [2,5,11,13,20,27]. Strains were identified by two different m-PCRs [8,16] and by the 16S rDNA-RFLP

☆ The GenBank/EMTBL/DDBJ accession numbers of the sequences of strain SW28-13^T, F26 and F41^T, for the 16S rRNA gene are HE565360, HE565361 and FJ573216, respectively, while the *gyrA*, *atpA*, *rpoB*, *gyrB* and *hsp60* genes of all *Arcobacter* strains included are JF802986 to JF803234 and HE997169 to HE997171.

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Table 1
Differential characteristics of *Arcobacter cloacae* sp. nov. and *Arcobacter suis* sp. nov. from other members of the genus.

Characteristics	1	2	3	4	5	6	7	8[a]	9	10	11[a]	12	13	14	15	16	17
Growth in/on																	
Air at 37 °C	+	–	V(–)	V(+)	+	+	–	+	+	–	+	–	+	+	+	+	–
CO_2 at 37 °C	+	–	–	V(+)	+	+	+	+	+	–	+	–	+	+	+	+	+
CO_2 at 42 °C	–	–	–	–	V(+)	–	–	–	+	–	–	–	+	+	+	–	–
0.5% (w/v) NaCl	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+	+
4% (w/v) NaCl	–	–	+	–	–	+	–	+	+	–	+	–	–	+	–	+	–
1% (w/v) glycine	–	–	–	–	–	–	–	+	+	+	+	V(–)	–	–	–	–	–
0.05% safranin	+	–	–	+	+	+	+	–	–	+	+	V(+)	+	+	–	–	–
0.1% sodium deoxycholate	+	+	V(–)	V(+)	+	+	+	–	+	V(–)	–	+	+	+	+[b]	–	–
1% (w/v) oxgall	+	–	–	+	V(+)	+	+	–	+	–	–	+	+	+	–	–	–
0.04% TTC	–	–	–	+	+	V(–)	V(–)	–	–	V(–)	–	+	–	–	–	–	–
0.01% TTC	+	+	–	+	+	+	+	–	–	+	–	+	+	+	–	–	–
Minimal medium	V(+)	+	–	–[c]	+	–	+	–	–	+	–	–[d]	+	–	+	–	+
MacConkey	+	+	–	V(–)	+	–	+	–	+	V(+)	–	V(+)[e]	+	+	V(+)	–	+
CCDA	+	–	–	+	+	+	V(–)	–	–	V(–)	–	+	+	–	+[b]	–	+
Resistance to																	
Cefoperazone (64 mg l^{-1})	–	–	–	+	+	+	+	–	–	+	–	+	V(+)	+	–	–	–
Enzyme activity																	
Catalase	+	+	+	+	V(+)	+	V(–)	–	+[f]	+	–	+	+[f]	+	+	+	+
Urease	–	–	+	–	–	–	–	–	–	–	–	–	+	–	V(–)	–	+
Nitrate reduction	+	+	+	+[g]	+	+	–	+	+[h]	+	+	–	+	+[i]	+	–	+
Indoxyl acetate hydrolysis	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+

Taxa: 1, *Arcobacter cloacae* (*n* = 2); 2, *Arcobacter suis* (*n* = 1); 3, *A. nitrofigilis* (*n* = 4) [2,23]; 4, *A. cryaerophilus* (*n* = 19) [2,23]; 5, *A. butzleri* (*n* = 12) [23]; 6, *A. skirrowii* (*n* = 9) [23]; 7, *A. cibarius* (*n* = 15) [14]; 8, *A. halophilus* (*n* = 1) [7,13]; 9, *A. mytili* (*n* = 3) [2]; 10, *A. thereius* (*n* = 8) [15]; 11, *A. marinus* (*n* = 1) [13,18]; 12, *A. trophiarum* (*n* = 11) [6,13]; 13, *A. defluvii* (*n* = 8) [5]; 14, *A. molluscorum* (*n* = 3) [11]; 15, *A. ellisii* (*n* = 3) [13]; 16, *A. bivalviorum* (*n* = 3) [20]; 17, *A. venerupis* (*n* = 1) [20]. The specific responses for type strains were coincidental or otherwise expressed in brackets. Unless otherwise indicated: +, ≥95% strains positive; –, ≤11% strains positive; V, 12–94% strains positive; CO_2 indicates microaerobic conditions. TTC: 2,3,5-triphenyl tetrazolium chloride, CCDA: Campylobacter Charcoal Deoxycholate Agar.

[a] For these strains, testing was carried out on media supplemented with 2% NaCl, with the exception of 0.5 and 4% (w/v) NaCl, catalase and indoxyl acetate hydrolysis [13].
[b] All strains grew weakly after 5 days of incubation.
[c] Two (LMG 7537 and LMG 10241) of the four strains tested were positive [2].
[d] Test not evaluated by De Smet et al. [6] but by Figueras et al. [13].
[e] Strains LMG 25534^T, LMG 25535 of *A. trophiarum* and strain FE2 (CECT 7650) of this species identified in our laboratory all grew on MacConkey agar, contrary to 80% of the strains described for this species [6].
[f] Weak reaction [2,5].
[g] Two (LMG 9904^T and LMG 9065) of the four strains tested were negative [2].
[h] Nitrate reduction was positive for the 3 strains of *A. mytili* [11], contrary to our previously published data [2].
[i] Nitrate is reduced after 72 h and 5 days for all strains under microaerobic and aerobic conditions, respectively [13].

[10] specific for the genus, but discordant results were obtained with all three methods. Briefly, all strains (SW28-13^T, F26 and F41^T) produced an amplicon of the size described for *A. cryaerophilus* with the m-PCR of Houf et al. [16]; while with the m-PCR of Douidah et al. [8], strains SW28-13^T and F26 showed no amplification and strain F41T produced the expected amplicon for *A. butzleri*. Strains SW28-13^T and F26, on the other hand, produced a 16S rDNA-RFLP pattern that was different from any previously described for other *Arcobacter* spp. [10,12] (Figs. S1 and S2), and strain F41^T produced an RFLP pattern the same as the recently described species *A. defluvii* [5]. Nevertheless, with the newly proposed 16S rDNA-RFLP *Arcobacter* identification method that uses the *Bfa*I endonuclease, strain F41^T showed a species-specific RFLP pattern (580, 175, 169, and 87 bp.) different to *A. defluvii* (405, 184, 175, 93, 87 and 83 bp.) [12] (Fig. S2).

Strains SW28-13^T, F26 and F41^T were motile under the phase contrast microscope and a single polar flagellum could be seen under the transmission electron microscope (data not shown), which was also used to measure the cell size and to define the morphology of the strains, as in previous studies [2]. All strains were phenotypically characterized in parallel with the type strains of all *Arcobacter* species using the tests recommended for the family *Campylobacteraceae* and the genus *Arcobacter* [27], including those used in previous studies [2,5,11,23]. Table 1 shows the key traits that differentiate strains SW28-13^T, F26 and F41^T from other *Arcobacter* spp. The pork meat strain F41^T is unable to grow under aerobic and microaerobic conditions at 37 °C, a characteristic only previously observed for species *A. thereius* and *A. trophiarum*, despite both species having been isolated from warm blooded animals, such as pigs and ducks [6,15]. This could therefore be considered as the first discriminating trait for this species.

The 16S rRNA (1401 bp) and *gyrB* (618 bp) genes were sequenced and analysed as previously described [2,5], and the *gyrA* (686 bp), *atpA* (622 bp), *rpoB* (621 bp), *hsp60* (587 bp) as described elsewhere (L. Collado, M.J. Figueras, A. Levican & A.J. Martínez-Murcia, in preparation). EzTaxon software was used for similarities [1] and MEGA software version 5 [26] and CLUSTAL W [19] for alignments, for calculating genetic distances and for clustering using the neighbour-joining, maximum parsimony and maximum likelihood algorithms [26]. The 16S rRNA gene sequence similarity of strains SW28-13^T and F26 was 99.8% and the former was chosen as the type strain. The similarity of strain SW28-13^T to all *Arcobacter* spp., including strain F41^T, ranged from 94.1% (common to *A. halophilus* and *A. mytili*) to 99.6% (*A. ellisii*), while similarity of the strain F41^T with the other species ranged from 93.4% (*A. mytili*) to 98.9% (*A. defluvii*). In the maximum parsimony phylogenetic tree produced from the 16S rRNA gene (1401 bp) (Fig. 1), and also using other algorithms (Figs. S3 and S4), strains SW28-13^T and F26 grouped close to the species *A. ellisii* and *A. defluvii* but formed an independent phylogenetic line, as did the strain F41^T (Fig. 1).

Direct and reverse DNA–DNA hybridization (DDH) experiments were carried out for the new strains and those that showed a 16S rRNA gene sequence similarity of 97% or higher (Table 2) using the methodology described in a previous study [5], and all results were under 70% (Table 2), thus corroborating that the strains SW28-13^T and F41^T represented two new species. Furthermore, DDH experiments were carried out for strains SW28-13^T and F26 and results confirmed that they belonged to the same species (Table 2).

A multilocus phylogenetic analysis (MLPA) was carried out by concatenating 5 housekeeping genes (*gyrA, atpA*, *rpoB, gyrB* and *hsp60*, 3134 bp) (Fig. 2), as recommended by the "ad hoc committee