Arcobacter thereius sp nov., isolated from pigs and ducks

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Arcobacter trophiarum sp. nov., isolated from fattening pigs

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In the course of a longitudinal study elucidating the dynamics of *Arcobacter* populations in pigs, 16 isolates of Gram-reaction-negative, rod-shaped, slightly curved, non-spore-forming bacteria were grouped by amplified fragment length polymorphism analysis into a distinct phenon within the genus *Arcobacter*. Fragments were generated for all isolates in a genus-specific PCR assay, but no amplicon was obtained in a species-specific multiplex-PCR test. Numerical analysis of the whole-cell protein profiles also showed that all isolates clustered in a single group that was distinct from related members of the genus *Arcobacter*. DNA–DNA hybridizations between two representative strains, designated 64^T and 122, of the isolates obtained exhibited a mean DNA–DNA relatedness of 72 %. DNA–DNA hybridizations between strains 64^T and 122 and reference strains of other animal-related bacteria of the genus *Arcobacter* revealed binding values of 47 % or less. The DNA G+C contents of the two representative strains were 28.5 and 28.4 mol%, respectively, and analysis of three marker genes identified *Arcobacter cryaerophilus*, *A. thereius*, *A. cibarius* and *A. skirrowii* as their closest phylogenetic neighbours. Strains 64^T and 122 could be distinguished from other members of the genus *Arcobacter* by means of biochemical tests for catalase and urease activities, nitrate reduction, indoxyl acetate hydrolysis, lack of growth at 37 °C, growth in 2 % (w/v) NaCl, growth on 0.1 % sodium deoxycholate and non-supplemented *Campylobacter* charcoal-deoxycholate base medium and resistance to cephalothin (32 mg l^{-1}) and cefoperazone (64 mg l^{-1}). Additionally, a PCR assay was developed for the detection and identification of strains 64^T and 122, which represent a novel species of the genus *Arcobacter*, for which the name *Arcobacter trophiarum* sp. nov. is proposed. The type strain is strain 64^T (=LMG 25534^T =CCUG 59229^T).

In the late 1970s, Ellis *et al.* (1977, 1978) reported on the isolation of *Campylobacter*-like organisms from the internal organs of aborted porcine and bovine fetuses. These bacteria differed from previously described species of the genus *Campylobacter* in their ability to grow in air and at lower temperatures (Neill *et al.*, 1979; Vandamme *et al.*, 1992b). In 1991, the genus *Arcobacter* was proposed as a second genus within the family *Campylobacteraceae* to encompass those bacteria formerly known as aerotolerant campylobacters (Vandamme *et al.*, 1991). At the time of writing, nine species have been described in this genus, four of which are apparently only present in environmental matrices with the remaining five species being human or animal associated.

Arcobacter nitrofigilis, *A. halophilus*, *A. mytili*, *A. marinus* and a number of as-yet unclassified organisms, including '*Candidatus* Arcobacter sulfidicus', have been isolated from sources such as coastal seawater, shellfish, sea sediment, salt-water lakes, water from underground cavities of oil wells and various kinds of sludge (Collado *et al.*, 2009; Donachie *et al.*, 2005; Kim *et al.*, 2010; McClung *et al.*, 1983; Wirsen *et al.*, 2002). *A. butzleri*, *A. cryaerophilus* and

Abbreviation: AFLP, amplified fragment length polymorphism.

The GenBank/EBML/DDBJ accession numbers for the partial sequences of the 16S rRNA, 23S rRNA and *hsp60* genes of strains 64^T and 122 are FN650333, FN650334 and FN650337 and FN650332, FN650335 and FN650336, respectively.

Five supplementary figures and one supplementary table are available with the online version of this paper.

A. skirrowii have been implicated in human illnesses, such as enteritis and septicaemia, and in reproductive disorders in livestock, as was described recently for the species *Arcobacter thereius* (Houf *et al.*, 2009). However, isolation of these bacteria from clinically healthy animals has also been frequently reported (van Driessche *et al.*, 2003). So far, the species *Arcobacter cibarius* has only been isolated from broiler carcasses and piggery effluent (Chinivasagam *et al.*, 2007; Houf *et al.*, 2005).

A. butzleri is the species that has been most predominantly associated with human infection, whereas *A. cryaerophilus* also seems to occur in humans but without clinical symptoms (Houf & Stephan, 2007; Vandenberg *et al.*, 2004). Routes of infection are still unclear but are assumed to be food- and waterborne. Contact with infected pets and person-to-person transmission have also been identified as potential routes of human infection (Fera *et al.*, 2009; Houf *et al.*, 2008; Petersen *et al.*, 2007; Vandamme *et al.*, 1992a).

In this study, we present the polyphasic taxonomic characterization of 16 *Arcobacter* isolates recovered from faecal samples taken rectally from 12 pigs on two Belgian farrow-to-finish farms during a longitudinal study of *Arcobacter* epidemiology within fattening pigs (Table 1). Samples (5 g) of faeces were enriched in 45 ml *Arcobacter*-selective broth containing (l^{-1}) 24 g *Arcobacter* broth CM 0965 (Oxoid), 50 ml lysed defibrinated horse blood (E&O Laboratories) and a selective supplement, previously developed by Houf *et al.* (2001), comprising (l^{-1}) 100 mg 5-fluorouracil, 10 mg amphotericin B, 16 mg cefoperazone, 32 mg novobiocin and 64 mg trimethoprim (Sigma). This was followed by inoculation on *Arcobacter*-selective agar plates containing (l^{-1}) 24 g *Arcobacter* broth, 12 g Agar Technical no. 3 LP 0013 (Oxoid) and the selective supplement described above, from which 12 isolates were recovered. Four isolates were recovered after direct plating of faecal material on the selective agar plates mentioned above. All incubations were performed at 28 °C in a microaerobic atmosphere and plates were checked every 24 h up to 72 h of incubation. Following incubation, small, colourless, translucent colonies were visible on *Arcobacter*-selective and blood agar plates. Typical bluish colonies were observed on these plates during examination with Henry transillumination, as previously reported for species of *Arcobacter* (Houf *et al.*, 2009; Houf & Stephan, 2007).

Table 1. Origin and identity of novel strains isolated during this study

All isolates were obtained from samples of pig faeces taken at farms in Belgium.

Strain number*	Host no. and age (in weeks)
A1 (A/1/3 enr)	Pig 24 (12)
31 (B/2/2 enr)	Pig 41 (14)
32 (B/2/2 enr)	Pig 42 (14)
36 (B/2/2 enr)	Pig 43 (14)
48 (B/2/2 enr)	Pig 44 (14)
64^T =LMG 25534^T (B/2/2 d5)	Pig 45 (14)
65 (B/2/2 d7)	Pig 45 (14)
74 (B/2/2 enr)	Pig 48 (14)
85 (B/2/2 enr)	Pig 49 (14)
92 (B/2/2 enr)	Pig 50 (14)
95 (B/2/2 d1)	Pig 50 (14)
101 (B/2/2 d7)	Pig 50 (14)
108 (B/3/1 enr)	Pig 33 (16)
119 (B/3/1 enr)	Pig 36 (16)
122 =LMG 25535 (B/3/1 enr)	Pig 38 (16)
132 (B/3/2 enr)	Pig 43 (16)

*Code: farm / sampling occasion / pen number ; d, isolated after quantitative analysis; enr, isolated after enrichment.

For molecular-based identification and characterization, genomic DNA from the 16 isolates was extracted using the guanidine thiocyanate method (Pitcher *et al.*, 1989). The concentration of each DNA template was determined spectrophotometrically (BioPhotometer; Eppendorf) at 260 nm and adjusted to 50 ng μl^{-1}. The DNA templates were stored at −20 °C. An expected 1223 bp fragment was generated for all isolates using a genus-specific PCR assay (Harmon & Wesley, 1996), but no fragments were obtained in a multiplex-PCR assay specific for *A. butzleri*, *A. cibarius*, *A. cryaerophilus*, *A. skirrowii* and *A. thereius* (Douidah *et al.*, 2010).

Numerical analysis of amplified fragment length polymorphism (AFLP) profiles using an *Hha*I–*Hin*dIII-based protocol (Debruyne *et al.*, 2010) was performed to identify and characterize the isolates and distinguish them from species of *Campylobacter*. This analysis identified 10 different AFLP profiles (data not shown) and clustered the 16 isolates together in a phenon distinct from characterized members of the genus *Arcobacter*.

Cluster analysis of DNA-banding patterns, obtained by a modified enterobacterial repetitive intergenic consensus PCR (Houf *et al.*, 2002), confirmed the heterogeneity of the isolates. Based on criteria defined in previous studies (Houf *et al.*, 2003; van Driessche *et al.*, 2004), four main genotypes could be identified (see Supplementary Fig. S1, available in IJSEM Online).

Two strains, designated 64^T and 122, were selected for further genomic analysis. DNA–DNA hybridizations were performed with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989), using a HTS7000 Bio Assay Reader (PerkinElmer) to measure fluorescence. DNA–DNA hybridization experiments, performed at 30 °C, showed that strains 64^T and 122 exhibited a mean DNA binding level of 72 %. Mean DNA–DNA relatedness between strains 64^T and 122 and type strains of related *Arcobacter* species were as follows: 47 % (*A. cryaerophilus* LMG 9904^T), 38 % (*A. skirrowii* LMG 6621^T), 38 % (*A. thereius* LMG 24486^T), 36 % (*A. cibarius* LMG 21996^T) and 31 % (*A. butzleri* LMG 10828^T). The SD was 7 %.

The DNA G+C contents of strains 64^T and 122 were determined by enzymic degradation of DNA into nucleosides as described by Mesbah & Whitman (1989). The mixture of nucleosides obtained was then separated by HPLC using a Waters Symmetry Shield C8 column thermostatted at 37 °C. The solvent used was 0.02 M ammonium phosphate (pH 4) with 1.5 % acetonitrile. Non-methylated λ-phage DNA (Sigma) was used as the calibration reference. The DNA G+C contents of 64^T and 122 were 28.5 and 28.4 mol%, respectively, confirming previously reported low G+C values for members of the family *Campylobacteraceae* (Vandamme *et al.*, 1992a).

To determine the phylogenetic position of strains 64^T and 122, the partial sequences of the 16S rRNA, 23S rRNA and heat-shock protein 60 (*hsp60*) genes were determined as described previously (Debruyne *et al.*, 2010; Houf *et al.*, 2009; Vandamme *et al.*, 2006). Sequences were assembled using BioNumerics version 4.61 (Applied Maths) and aligned using the CLUSTAL_X software package (Thompson *et al.*, 1997). Clustering was performed using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood and maximum-parsimony methods using the BioNumerics version 4.61 software package. Unknown bases were discarded from the analysis and bootstrap values were determined using 500 replicates. Although the general topology of the reconstructed trees was not always identical, the different branching algorithms consistently revealed the same closely related species. Based on the 16S rRNA gene sequences, searches using FASTA revealed that the closest phylogenetic neighbours to strains 64^T and 122 were the type strains of *A. cryaerophilus* (98.2 %), *A. thereius* (98.1 %), *A. cibarius* (97.8 %) and *A. skirrowii* (97.4 %) (Supplementary Fig. S2). Analysis of 23S rRNA gene sequences revealed that the nearest phylogenetic neighbour was *A. thereius* (98.2 %) (Fig. 1). Analysis of the *hsp60* gene sequences were performed as described previously (Debruyne *et al.*, 2010; Hill *et al.*, 2006) and FASTA searches revealed that the nearest phylogenetic neighbours were the type strains of *A. thereius* (92.4 %), *A. skirrowii* (91.3 %) and *A. cryaerophilus* (91.0 %) (Supplementary Fig. S3). These results suggest that *hsp60* gene sequence analysis gives higher resolution compared with using rRNA gene sequences.

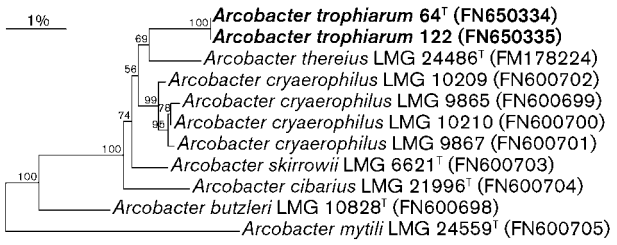

Fig. 1. Neighbour-joining phylogenetic tree based on 23S rRNA gene sequences of strains 64^T and 122 and related members of the genus *Arcobacter*. Bootstrap values are shown as percentages of 500 replicates. Bar, 1 % sequence divergence.

Whole-cell protein profiles were determined to further differentiate the novel isolates from related species of the genus *Arcobacter*. SDS-PAGE was performed using preparations of whole-cell proteins as described by Pot *et al.* (1994). The isolates were grown on Mueller–Hinton agar plates CM0337 (Oxoid) supplemented with 5 % (v/v) defibrinated horse blood and incubated microaerobically at 30 °C. Whole-cell protein profiles of *Arcobacter* reference strains and reference strains of species of the genera *Campylobacter* and *Helicobacter* were available from previous studies (Houf *et al.*, 2009; Vandamme *et al.*, 1992a, b). Densitometric analysis, normalization and interpolation of the protein profiles and numerical analysis were performed using the GelCompar version 4.6.1 software package (Applied Maths). The similarity between all pairs of traces was expressed using Pearson's product–moment correlation coefficient and values were presented as percentages of similarity. The results of a numerical analysis of the protein profiles of the 16 isolates and *Arcobacter* reference strains are shown in Supplementary Fig. S4. All 16 of the novel isolates were grouped in a single cluster with a similarity levels above 84 % and were clearly distinct from the reference strains of species of the genus *Arcobacter* (see Supplementary Fig. S4).

Over 20 phenotypic characteristics were tested in representatives of 10 species of the genus *Arcobacter* by using an extensive biochemical identification scheme for species of the genus *Arcobacter* and related bacteria as described by On *et al.* (1996). Comparison of these test results demonstrated several phenotypic differences between taxa. The relevant phenotypic characteristics that differentiated the novel strains from other taxa are given in Table 2. The inability to reduce nitrate differentiates the novel isolates from species with validly published names of the genus *Arcobacter*, except for *A. cibarius* and *A. mytili*. The inability to grow at 37 °C under aerobic conditions differentiates the novel isolates from *A. butzleri*, *A. skirrowii*, *A. mytili*, *A. halophilus* and *A. marinus*. The inability to grow at 37 °C under microaerobic conditions distinguished the novel isolates from *A. cibarius*. Furthermore, the novel isolates could be differentiated from *A. mytili* based on their ability to grow on medium containing 64 mg cefoperazone l^{-1} and to hydrolyse indoxyl acetate. In contrast to *A. halophilus* and *A. nitrofigilis*, the novel isolates were able to grow on non-supplemented *Campylobacter* charcoal-deoxycholate base medium and were resistant to cephalothin (32 mg l^{-1}) and cefoperazone (64 mg l^{-1}). The novel isolates could be distinguished from *A. halophilus* by the presence of catalase activity and growth on medium containing 0.1 % sodium deoxycholate. Furthermore, their inability to show urease activity differentiated the novel isolates from *A. nitrofigilis*. Apart from the inability to grow at 37 °C and to reduce nitrate to nitrite, the novel isolates can be distinguished from *A. marinus* by the ability to show catalase activity.

Table 2. Characteristics that differentiate the novel isolates from representatives of other members of the genus *Arcobacter*

Taxa: 1, strains of *Arcobacter trophiarum* (*n*=10); 2, *A. butzleri* (*n*=12); 3, *A. cryaerophilus* (*n*=19); 4, *A. skirrowii* (*n*=9); 5, *A. cibarius* (*n*=15) (data from this study); 6, *A. thereius* (*n*=8); 7, *A. mytili* (data from Collado *et al.*, 2009 unless indicated; *n*=3); 8, *A. nitrofigilis* (*n*=2); 9, *A. halophilus* (*n*=1); 10, *A. marinus* (*n*=1) (Kim *et al.*, 2010). Data from this study unless otherwise indicated. Values are percentages of strains that tested positive. CCDA, *Campylobacter* charcoal-deoxycholate base media. NT, Not tested.

Characteristic	1	2	3	4	5	6	7	8	9	10
Catalase activity	100	33	100	100	54	100	100	100	0	0
Urease activity	0	0	0	0	0	0	0	100	0	0
Nitrate reduction	0	100	100	100	0	100	0	100	100	100
Indoxyl acetate hydrolysis	100	100	100	100	100	100	0	100	100	100†/0‡
Alpha-haemolysis	0	0	0	100	0	30	0	0	NT	0
Growth at 37 °C †	0	100	58	100	100	0	100	0	100	100
Growth at 42 °C †	0	25	0	11	0	0	0*	0	0	NT
Growth in:										
Air at 25 °C	100	100	100	100	31	100	100	100	100	100
Air at 37 °C	0	100	50	100	0	0	100	50	100	100
2 % (w/v) NaCl	100	92	84	100	0	88	100	100	100	100
4.0 % (w/v) NaCl	40	0	0	100	0	0	100	100	100	100
Growth on media containing:										
0.1 % Sodium deoxycholate	100	100	70	67	100	25	100*	50	0	NT
0.05 % Safranin	90	100	100	100	100	100	0*	0	NT	NT
Cephalothin (32 mg l^{-1})	100	100	100	100	100	100	100*	0	0	NT
Cefoperazone (64 mg l^{-1})	100	100	100	100	100	100	0	0	0	NT
Growth on:										
Non-supplemented CCDA	100	100	100	100	60	75	100*	0	0	NT
MacConkey agar	80	83	16	0	100	63	100	0	0	NT

*Data from this study using *A. mytili* LMG24559^T.
†Cells grown in microaerobic conditions.
‡Cells grown in aerobic conditions.

For electron microscopic analysis, strain 64^T was grown on blood agar plates for 48 h at 28 °C under microaerobic conditions and the bacteria were harvested and fixed in HEPES buffer with 2.5 % glutaraldehyde for 24 h. The samples were post-fixed in 1 % osmium tetroxide for 2 h at room temperature. The fixed samples were dehydrated through ascending grades of ethanol and transferred to a critical point drier CPD 030 (Bal-Tec). The dried tissues were mounted on a metal stub and sputter-coated with platinum using a JFC-1300 auto fine coater (JEOL). Examination of the samples was performed on a JEOL JSM 5600 LV scanning electron microscope. Cells of strain 64^T appeared as slender rods, about 0.3–0.5 μm wide and 1.4–2.0 μm long with a single unsheathed polar flagellum (Fig. 2).

For the identification of strains 64^T and 122, a PCR assay with primers targeting the *hsp60* gene was developed and the sensitivity and specificity were evaluated to conform to previous studies (Houf *et al.*, 2000; Douidah *et al.*, 2010). Reference strains of species of the genus *Arcobacter* and other closely related bacteria were obtained from the BCCM/LMG and CIP bacteria collections (see

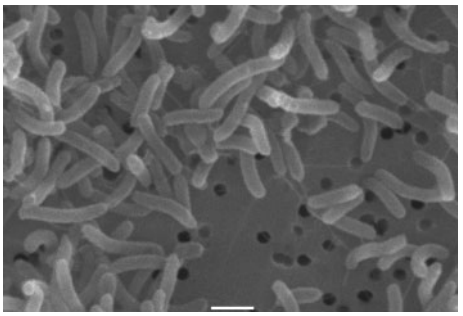

Fig. 2. Scanning electron micrograph of cells of strain 64^T. Bar, 1 μm.

Supplementary Table S1). *Arcobacter* reference strains were grown on Mueller–Hinton agar plates supplemented with 5 % defibrinated horse blood for 48 h at 30 °C and under microaerobic conditions obtained by evacuating 80 % of the normal atmosphere and introducing a gas mixture of 8 % CO_2, 8 % H_2 and 84 % N_2 into the jar. Cultivation of closely related organisms was performed according to their specific needs. PCRs were performed in a reaction mixture (50 µl final volumes) composed of sterile water (Sigma), 2 µl bacterial DNA (50 ng μl^{-1}), 5 µl 10× PCR buffer (Invitrogen), 1.4 mmol l^{-1} $MgCl_2$ (Invitrogen), 2 U *Taq* DNA polymerase (Invitrogen), a dNTP mixture with each dNTP at a final concentration of 10 mmol l^{-1} (Invitrogen) and 50 pmol of forward hsp60F (5′-TTGAACTTAAAAA-AGCTTCGAG-3′) and reverse hsp60R (5′-TCCATCAAC-ATCTTCAGCTAC-3′) primers (Invitrogen). A PerkinElmer Gene-Amp System 9700 thermocycler was used (Applied Biosystems). Prior to cycling, samples were heated at 94 °C for 3 min. The PCR protocol involved 30 cycles of denaturation (94 °C for 45 s), primer annealing (58 °C for 45 s) and chain extension (72 °C for 45 s), followed by a final elongation step at 72 °C for 5 min. The PCR products (10 µl) were size separated by electrophoresis at 100 V for 90 min in 1 % agarose gels using 0.5 TBE as a buffer. A Track-It 100 bp ladder (Invitrogen) was used as molecular mass marker. Gels were stained with ethidium bromide (1 µg ml^{-1}) and DNA fragments were visualized by UV transillumination and photographed. The selected primers amplified a 383 bp fragment of the *hsp60* gene of the novel isolates (see Supplementary Fig. S5). No PCR product was generated for closely related species of the genera *Arcobacter*, *Campylobacter* or *Helicobacter*, nor for strains of *Escherichia coli* and *Salmonella enterica* (not shown).

The occurrence of these novel isolates in the intestinal tract of healthy fattening pigs needs further attention and is of general interest for reasons of public health. Although the pathogenic potential of these strains is unknown, it is noteworthy that several related species (*A. butzleri*, *A. cryaerophilus*, *A. cibarius* and *A. skirrowii*) of the emerging pathogen *Arcobacter* were isolated from food of animal origin and have been implicated as sources of human infection. Moreover, the lack of growth at 37 °C of strains 64^T and 122 and *A. thereius* (Houf *et al.*, 2009) under laboratory conditions that allow cultivation of other host-associated arcobacters is remarkable as they were all isolated from pigs. Recently, reliable and fast PCR-based methods for the detection of animal-related species of the genus *Arcobacter* have been developed. For example, a multiplex-PCR that can identify species *A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, *A. cibarius* and *A. thereius* was described by Douidah *et al.* (2010). During the present study, a PCR assay for the detection and identification of a novel species of the genus *Arcobacter* has been developed. Although there is no evidence for the colonization of *Arcobacter trophiarum* sp. nov. in pigs, one pig continued to excrete the same strain for more than 2 weeks.

On the basis of their phylogenetic and phenotypic properties strains 64^T and 122 represent a novel species of the genus *Arcobacter*, for which the name *Arcobacter trophiarum* sp. nov. is proposed.

Description of *Arcobacter trophiarum* sp. nov.

Arcobacter trophiarum (tro.phi.ar′um. Gr. n. *trophias* fattened animal or animal kept in stable; hence N.L. gen. pl. n. *trophiarum* of/from fattened animals or animals kept in stables).

Cells are slightly curved, Gram-reaction-negative rods, 1.4–2.0 × 0.3–0.5 µm. They form whitish, slightly convex, non-swarming, smooth, rounded colonies with entire margins of about 2 mm in diameter on blood agar after 48 h of incubation at 28 °C under microaerobic conditions and translucent to opaque smooth-rounded colonies 1–2 mm in diameter on *Arcobacter* selective agar. In a microaerobic atmosphere, growth is observed after 2 days of incubation at room temperature (18–22 °C) and 30 °C but not at 37 or 42 °C. No growth occurs at 37 °C in aerobic conditions but does occur at 25 and 30 °C on blood agar. Produces oxidase and catalase and hydrolyses indoxyl acetate but does not show urease activity or reduction of nitrate. No alpha haemolysis is seen on blood agar. Grows under microaerobic conditions on non-supplemented *Campylobacter* charcoal-deoxycholate base media and on media containing 2 % NaCl, 0.1 % sodium deoxycholate, 32 mg cephalothin l^{-1} and 64 mg cefoperazone l^{-1}. The majority (~80 %) of known isolates grow on MacConkey agar and ~90 % of known isolates grow on media containing 0.05 % safranin.

The type strain, 64^T (=LMG 25534^T =CCUG 59229^T), was isolated from faeces of a 14-week-old piglet at a Belgian farm in 2007.

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References

Chinivasagam, H. N., Corney, B. G., Wright, L. L., Diallo, I. S. & Blackall, P. J. (2007). Detection of *Arcobacter* spp. in piggery effluent and effluent-irrigated soils in southeast Queensland. *J Appl Microbiol* **103**, 418–426.

Collado, L., Cleenwerck, I., Van Trappen, S., De Vos, P. & Figueras, M. J. (2009). *Arcobacter mytili* sp. nov., an indoxyl acetate-hydrolysis-negative bacterium isolated from mussels. *Int J Syst Evol Microbiol* **59**, 1391–1396.

Debruyne, L., Houf, K., Douidah, L., De Smet, S. & Vandamme, P. (2010). Reassessment of the taxonomy of *Arcobacter cryaerophilus*. *Syst Appl Microbiol* **33**, 7–14.

Donachie, S. P., Bowman, J. P., On, S. L. W. & Alam, M. (2005). *Arcobacter halophilus* sp. nov., the first obligate halophile in the genus *Arcobacter*. *Int J Syst Evol Microbiol* **55**, 1271–1277.

Douidah, L., De Zutter, L., Vandamme, P. & Houf, K. (2010). Identification of five human and mammal associated *Arcobacter* species by a novel multiplex-PCR assay. *J Microbiol Methods* **80**, 281–286.

Ellis, W. A., Neill, S. D., O'Brien, J. J., Ferguson, H. W. & Hanna, J. (1977). Isolation of *Spirillum*/*Vibrio*-like organisms from bovine fetuses. *Vet Rec* **100**, 451–452.

Ellis, W. A., Neill, S. D., O'Brien, J. J. & Hanna, J. (1978). Isolation of spirillum-like organisms from pig fetuses. *Vet Rec* **102**, 106.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Fera, M. T., La Camera, E., Carbone, M., Malara, D. & Pennisi, M. G. (2009). Pet cats as carriers of *Arcobacter* spp. in Southern Italy. *J Appl Microbiol* **106**, 1661–1666.

Harmon, K. M. & Wesley, I. V. (1996). Identification of *Arcobacter* isolates by PCR. *Lett Appl Microbiol* **23**, 241–244.

Hill, J. E., Paccagnella, A., Law, K., Melito, P. L., Woodward, D. L., Price, L., Leung, A. H., Ng, L. K., Hemmingsen, S. M. & Goh, S. H. (2006). Identification of *Campylobacter* spp. and discrimination from *Helicobacter* and *Arcobacter* spp. by direct sequencing of PCR-amplified *cpn60* sequences and comparison to cpnDB, a chaperonin reference sequence database. *J Med Microbiol* **55**, 393–399.

Houf, K. & Stephan, R. (2007). Isolation and characterization of the emerging foodborn pathogen *Arcobacter* from human stool. *J Microbiol Methods* **68**, 408–413.

Houf, K., Tutenel, A., De Zutter, L., Van Hoof, J. & Vandamme, P. (2000). Development of a multiplex PCR assay for the simultaneous detection of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. *FEMS Microbiol Lett* **193**, 89–94.

Houf, K., Devriese, L. A., De Zutter, L., Van Hoof, J. & Vandamme, P. (2001). Development of a new protocol for the isolation and quantification of *Arcobacter* species from poultry products. *Int J Food Microbiol* **71**, 189–196.

Houf, K., De Zutter, L., Van Hoof, J. & Vandamme, P. (2002). Assessment of the genetic diversity among arcobacters isolated from poultry products by using two PCR-based typing methods. *Appl Environ Microbiol* **68**, 2172–2178.

Houf, K., De Zutter, L., Verbeke, B., Van Hoof, J. & Vandamme, P. (2003). Molecular characterization of *Arcobacter* isolates collected in a poultry slaughterhouse. *J Food Prot* **66**, 364–369.

Houf, K., On, S. L. W., Coenye, T., Mast, J., Van Hoof, J. & Vandamme, P. (2005). *Arcobacter cibarius* sp. nov., isolated from broiler carcasses. *Int J Syst Evol Microbiol* **55**, 713–717.

Houf, K., De Smet, S., Baré, J. & Daminet, S. (2008). Dogs as carriers of the emerging pathogen *Arcobacter*. *Vet Microbiol* **130**, 208–213.

Houf, K., On, S. L. W., Coenye, T., Debruyne, L., De Smet, S. & Vandamme, P. (2009). *Arcobacter thereius* sp. nov., isolated from pigs and ducks. *Int J Syst Evol Microbiol* **59**, 2599–2604.

Kim, H. M., Hwang, C. Y. & Cho, B. C. (2010). *Arcobacter marinus* sp. nov. *Int J Syst Evol Microbiol* **60**, 531–536.

McClung, C. R., Patriquin, D. G. & Davis, R. E. (1983). *Campylobacter nitrofigilis* sp. nov., a nitrogen-fixing bacterium associated with roots of *Spartina alterniflora* Loisel. *Int J Syst Bacteriol* **33**, 605–612.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* **479**, 297–306.

Neill, S. D., Ellis, W. A. & O'Brien, J. J. (1979). Designation of aerotolerant *Campylobacter*-like organisms from porcine and bovine abortions to the genus *Campylobacter*. *Res Vet Sci* **27**, 180–186.

On, S. L. W., Holmes, B. & Sackin, M. J. (1996). A probability matrix for the identification of campylobacters, helicobacters and allied taxa. *J Appl Bacteriol* **81**, 425–432.

Petersen, R. F., Harrington, C. S., Kortegaard, H. E. & On, S. L. W. (2007). A PCR-DGGE method for detection and identification of *Campylobacter*, *Helicobacter*, *Arcobacter* and related Epsilobacteria and its application to saliva samples from humans and domestic pets. *J Appl Microbiol* **103**, 2601–2615.

Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.

Pot, B., Vandamme, P. & Kersters, K. (1994). Analysis of electrophoretic whole-organism protein fingerprints. In *Chemical Methods in Prokaryotic Systematics*, pp. 493–521. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

van Driessche, E., Houf, K., Van Hoof, J., De Zutter, L. & Vandamme, P. (2003). Isolation of *Arcobacter* species from animal feces. *FEMS Microbiol Lett* **229**, 243–248.

van Driessche, E., Houf, K., Vangroenweghe, F., Nollet, N., De Zutter, L., Vandamme, P. & Van Hoof, J. (2004). Occurrence and strain diversity of *Arcobacter* species isolated from healthy Belgian pigs. *Res Microbiol* **155**, 662–666.

Vandamme, P., Falsen, E., Rossau, R., Hoste, B., Segers, P., Tytgat, R. & De Ley, J. (1991). Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov. *Int J Syst Bacteriol* **41**, 88–103.

Vandamme, P., Vancanneyt, M., Pot, B., Mels, L., Hoste, B., Dewettinck, D., Vlaes, L., Van Den Borre, C., Higgins, R. & other authors (1992a). Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *Int J Syst Bacteriol* **42**, 344–356.

Vandamme, P., Pugina, P., Benzi, G., Vanetterijck, R., Vlaes, L., Kersters, K., Butzler, J. P., Lior, H. & Lauwers, S. (1992b). Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. *J Clin Microbiol* **30**, 2335–2337.

Vandamme, P., Holmes, B., Bercovier, H. & Coenye, T. (2006). Classification of Centers for Disease Control Group Eugonic Fermenter (EF)-4a and EF-4b as *Neisseria animaloris* sp. nov. and *Neisseria zoodegmatis* sp. nov., respectively. *Int J Syst Evol Microbiol* **56**, 1801–1805.

Vandenberg, O., Dediste, A., Houf, K., Ibekwem, S., Souayah, H., Cadranel, S., Douat, N., Zissis, G., Butzler, J. P. & Vandamme, P. (2004). *Arcobacter* species in humans. *Emerg Infect Dis* **10**, 1863–1867.

Wirsen, C. O., Sievert, S. M., Cavanaugh, C. M., Molyneaux, S. J., Ahmad, A., Taylor, L. T., Delong, E. F. & Taylor, C. D. (2002). Characterization of an autotrophic sulfide-oxidizing marine *Arcobacter* sp. that produces filamentous sulfur. *Appl Environ Microbiol* **68**, 316–325.