Arcobacter mytili sp. nov., an indoxyl acetate-hydrolysis-negative bacterium isolated from mussels

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Three *Arcobacter* isolates, recovered from mussels (genus *Mytilus*), and one isolate from brackish water in Catalonia (north-east Spain) showed a novel pattern using a recently described identification method for members of the genus *Arcobacter*, 16S rRNA gene RFLP. Enterobacterial repetitive intergenic consensus PCR fingerprinting demonstrated that the three isolates from mussels belonged to two genotypes and that the fourth isolate from water belonged to a third genotype. Analysis of the 16S rRNA and *rpoB* gene sequences showed that the new isolates formed a separate lineage within the genus *Arcobacter*. This was also confirmed by the low DNA–DNA relatedness values (16–30 %) of the isolates with the type strains of recognized *Arcobacter* species. Hydrolysis of indoxyl acetate, a characteristic trait for all species of the genus *Arcobacter*, was negative for the novel isolates. The susceptibility of the novel isolates to cefoperazone, together with the lack of urease production and nitrate reduction, further enabled them to be differentiated from recognized *Arcobacter* species based on physiological characteristics. Genotypic and phenotypic characteristics indicated that the new isolates represent a novel species of the genus *Arcobacter*, for which the name *Arcobacter mytili* sp. nov. is proposed, with the type strain F2075^T (=CECT 7386^T =LMG 24559^T). The DNA G+C content of strain F2075^T was 26.9 mol%.

In 1991, Vandamme and colleagues reclassified the 'aerotolerant campylobacters' in the genus *Arcobacter*, including *Arcobacter nitrofigilis* and *Arcobacter cryaerophilus* (Vandamme *et al.*, 1991). The genus was emended a year later with the addition of *Arcobacter butzleri* and *Arcobacter skirrowii* (Vandamme *et al.*, 1992). Two more novel species have since been described, *Arcobacter cibarius*, isolated from broiler carcasses in Belgium (Houf *et al.*, 2005) and *Arcobacter halophilus*, isolated from a hypersaline lagoon in Hawaii (Donachie *et al.*, 2005) and the genus currently comprises six species. Moreover, an autotrophic, obligate microaerophilic sulfide-oxidizing bacteria named '*Candidatus* Arcobacter sulfidicus' of marine origin was described in 2002 as a possible additional novel taxon (Wirsen *et al.*, 2002).

The type species of the genus, *A. nitrofigilis*, was first recovered from the roots of *Spartina alterniflora*, a salt marsh plant (McClung *et al.*, 1983), and since then there have been few reports on this species (Figueras *et al.*, 2008). *A. butzleri* is the most common species in environmental water, food and clinical samples (Ho *et al.*, 2006). In fact, this species was ranked as the fourth most common campylobacterium isolated from human faeces in two independent studies performed in Belgium and France (Vandenberg *et al.*, 2004; Prouzet-Mauléon *et al.*, 2006). Recently, this species was considered to be a serious hazard to human health by the International Commission on Microbiological Specification for Foods (ICMSF, 2002). *A. cryaerophilus*, the second most commonly isolated species of the genus, has been recovered from cases of diarrhoea and bacteraemia in humans, as well as from the meat of several animals (Ho *et al.*, 2006). This species was also recovered from faeces of 1.4 % of healthy people (Houf & Stephan, 2007). *A. skirrowii* is usually isolated from

Abbreviations: ERIC-PCR, Enterobacterial repetitive intergenic consensus PCR; m-PCR, multiplex PCR.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and *rpoB* sequences of strain F2075^T are EU669904 and EU669901, respectively.

Supplementary figures showing the ERIC-PCR patterns and a phylogenetic tree based on *rpoB* gene sequences for species of the genus *Arcobacter* are available with the online version of this paper.

preputial fluids of bulls and faeces of animals, including sheep and cattle (Vandamme *et al.*, 2005). It has also been associated with chronic diarrhoea in an old man (Wybo *et al.*, 2004) and has been recently detected in humans with and without diarrhoea in South Africa (Samie *et al.*, 2007). *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* are frequently isolated from animal faeces (Van Driessche *et al.*, 2003) and have recently been associated with faecally polluted environmental waters (Collado *et al.*, 2008). Although the epidemiology of *Arcobacter* species is not clear, it has been suggested that water and foods are the transmission route of arcobacters to humans (Ho *et al.*, 2006).

As part of a study on the prevalence of arcobacters in meat and shellfish products in Catalonia (north-east Spain), three isolates (F2026, F2075^T and F2076) were recovered from mussels (genus *Mytilus*) in 2006 and an additional strain (T234) was isolated from brackish water in 2008. Using a recently proposed *Arcobacter* species identification method, 16S rRNA gene RFLP, these isolates showed a specific pattern that was different from those defined for the six recognized species of the genus (Figueras *et al.*, 2008). In the present study, a polyphasic approach was used to establish the taxonomic position of these novel isolates. For this purpose, phylogenetic analyses of the 16S rRNA and *rpoB* gene sequences, DNA–DNA hybridization experiments, DNA G+C content determination, enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) genotyping and phenotypic analysis were performed. Based on the results obtained, we propose the allocation of these isolates to a novel species of the genus *Arcobacter*.

Mussel samples were taken from the Ebro river delta (north-east Spain) on 29 July (one sample) and 21 September 2006 (two samples) and were processed by homogenizing 10 g of mussel flesh with 90 ml *Arcobacter* enrichment broth (Oxoid) supplemented with CAT comprising (mg l^{-1}) cefoperazone (8), amphotericin B (10) and teicoplanin (4). After incubation for 48 h at 30 °C under aerobic conditions, 200 µl broth was inoculated on a blood agar (BA) plate following the procedure described by Collado *et al.* (2008). Small, colourless or beige-to-off-white, translucent colonies, the characteristic form of colonial growth for members of the genus *Arcobacter*, were selected from each sample for genetic identification. This was performed using a multiplex PCR (m-PCR) for simultaneous detection of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* (Houf *et al.*, 2000) and a 16S rRNA gene RFLP method, which consists of an amplification of 1026 bp of the 16S rRNA gene and posterior digestion with *Mse*I endonuclease to obtain restriction patterns that enable characterization of the six recognized species (Figueras *et al.*, 2008). Three mussel isolates, one recovered from the July sample (F2026) and one from each of the two September samples (F2075^T and F2076), were identified as *A. skirrowii* with the m-PCR method due to the presence of a 653 bp band on the agarose gel that could not be differentiated from the 641 bp band that is typical of *A. skirrowii*. However, the isolates showed a novel RFLP pattern (650/143/138 bp) that did not correspond to any of the recognized *Arcobacter* species (Figueras *et al.*, 2008). One additional isolate (T234) was recovered (June 2008) from brackish water (salinity 14.4 ‰) of the Ebro river delta using the isolation procedure described by Collado *et al.* (2008). This isolate was also identified as *A. skirrowii* with m-PCR and showed the same RFLP pattern as the mussel isolates.

To investigate the genetic relatedness of the four isolates, ERIC-PCR (Houf *et al.*, 2002) was performed. Isolates F2075^T and F2076, despite having been isolated from different samples, were considered to have the same genotype on the basis of sharing the same ERIC-PCR pattern. This pattern was clearly different from those of isolates F2026 and T234 (see Supplementary Fig. S1, available in IJSEM Online).

The 16S rRNA genes of the four isolates (around 1460 bp) were amplified according to Martínez-Murcia *et al.* (1992) and sequenced in both directions using a BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems) with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems), according to the manufacturer's instructions. Sequence assembly was performed with AUTO ASSEMBLER (Applied Biosystems). In addition, the *rpoB* genes of the four novel isolates and 11 *Arcobacter* strains [two strains for each recognized species of the genus *Arcobacter*, with the exception of *A. halophilus* (see Supplementary Table S1)], were amplified using the PCR primers CamrpoB-L and RpoB-R and the conditions that have been established for use with the genus *Campylobacter* (Korczak *et al.*, 2006). The expected PCR product size (524 bp) was obtained for all strains, with additional unexpected bands in some cases. The bands of the expected size were purified from the agarose gel with the GFX PCR DNA and Gel Band Purification kit (GE Healthcare) according to the manufacturer's instructions. The PCR products were sequenced in both directions and in duplicate. The *rpoB* gene sequences were used to calculate the percentage nucleotide substitutions for a continuous stretch of 487 bp (positions 1552–2039 according to *Escherichia coli* numbering).

Both sets of sequence data, for the 16S rRNA and *rpoB* genes, from strains F2075^T and F2076 were identical and confirmed the ERIC-PCR results. Therefore, strain F2076 was not subjected to further phylogenetic or phenotypic analysis.

Using sequences obtained from this study and from GenBank, separate alignments of 16S rRNA gene sequences (1409 bp) and *rpoB* sequences (487 bp) were performed with CLUSTAL W (Thompson *et al.*, 1994). Genetic distances were obtained using Kimura's two-parameter model (Kimura, 1980) and evolutionary trees were constructed by the neighbour-joining method with the MEGA4 program (Tamura *et al.*, 2007). The stability of each relationship was assessed by bootstrap analysis (1000 replicates).

In both of the phylogenetic trees derived from the 16S rRNA and *rpoB* gene sequences (Fig. 1 and Supplementary

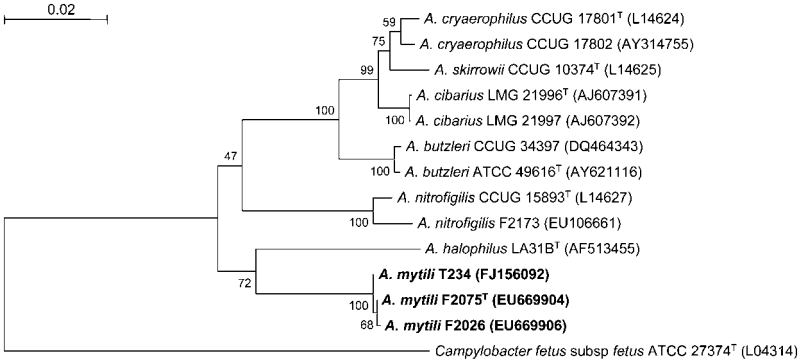

Fig. 1. Neighbour-joining phylogenetic tree showing the relationship of *Arcobacter mytili* sp. nov. with other *Arcobacter* species on the basis of 1409 nucleotides from the 16S rRNA gene. Numbers at nodes represent percentage bootstrap values (1000 replications). Bar, 2 substitutions per 100 nt.

Fig. S2), three of the novel strains (F2026, F2075^T and T234) formed a distinct clade with *A. halophilus* LA31B^T. The 16S rRNA gene sequence of strain F2075^T was compared with the sequences of type strains deposited in GenBank using both BLASTN (Altschul *et al.*, 1990) and EZTAXON (Chun *et al.*, 2007) and it showed the highest similarity values with the type strains of the six *Arcobacter* species: *A. halophilus*, 94.8 %; *A. nitrofigilis*, 93.8 %; *A. butzleri*, 93.6 %; *A. cibarius*, 93.3 %, *A. cryaerophilus*, 93.1 %, and *A. skirrowii*, 92.8 %. The levels of similarity to *Campylobacter* species were below 87.9 %. The 16S rRNA gene sequence of strain F2075^T showed 99.9 and 99.8 % sequence similarities with those of strains F2026 and T234, respectively. The *rpoB* gene sequence similarities between strain F2075^T and each *Arcobacter* type strain were 88.0 %, 86.7 %, 85.8 %, 83.0 %, 82.1 % and 81.9 % for *A. halophilus*, *A. butzleri*, *A. nitrofigilis*, *A. cryaerophilus*, *A. cibarius* and *A. skirrowii*, respectively. The inter-species rate of nucleotide substitutions for the *rpoB* gene was over 10.7 %, while the intra-species variation ranged from 0.2 to 5.3 %, with the sequences from *A. skirrowii*, *A. butzleri* and the novel isolates being at the lower range of intra-species variability (see Supplementary Fig. S2). In a recent study that analysed the relationship between *rpoB* gene sequence similarity and DNA–DNA hybridization for 230 bacteria, a DNA–DNA relatedness value of more than 70 % correlated with a *rpoB* gene sequence similarity of 97.7 %, and this was proposed as the cut-off value for species delineation (Adékambi *et al.*, 2008). The *rpoB* gene sequence similarities between strain F2075^T and strains F2026 and T234 were 99.8 and 99.6 %, respectively, which are clearly above this cut-off value.

For DNA–DNA hybridization experiments and for the determination of DNA G+C content, genomic DNA was prepared according to the procedure of Wilson (1987) with the modification by Cleenwerck *et al.* (2002). DNA–DNA hybridizations were performed at 32 °C according to a modification (Goris *et al.*, 1998; Cleenwerck *et al.*, 2002) of the method described by Ezaki *et al.* (1989). Reciprocal reactions were performed for every comparison pair and the variation found was within the limits of this method (Goris *et al.*, 1998). The DNA–DNA relatedness values reported are the means of a minimum of four hybridizations. The DNA–DNA relatedness value obtained for strain F2075^T with *A. halophilus* LA31B^T, the most closely related species on the basis of the 16S rRNA and *rpoB* gene sequences, was 30 %, while values with all other recognized *Arcobacter* species were 23 % or below (Table 1). Although these DNA–DNA hybridization values may seem low, they are not low in comparison with those recently published in the description of *A. halophilus*, which ranged between 4 and 12 % with the other recognized *Arcobacter* species (Donachie *et al.*, 2005). The G+C content of each DNA sample was determined by three independent analyses using the HPLC technique (Mesbah *et al.*, 1989). The DNA G+C content of strain F2075^T was 26.9 mol%, which is within the previously defined range of 26.8–35 mol% for the genus (Donachie *et al.*, 2005; Houf *et al.*, 2005; Vandamme *et al.*, 2005).

Phenotypic characterization of strains F2026, F2075^T and T234 was performed using the biochemical identification scheme of Vandamme *et al.* (2005) (Table 2). NaCl tolerance and susceptibility to cefoperazone were tested on nutrient broth no. 2 (Oxoid) supplemented with 5 % whole sheep blood and 1.5 % agar. The indoxyl acetate hydrolysis test was performed according to Mills & Gherna (1987) and confirmed using indoxyl acetate diagnostic tablets (IAC)-DIETABS (Rosco Diagnostica). All tests were conducted at least twice. The novel isolates were biochemically different from the recognized species of the genus

Table 1. DNA–DNA relatedness between strain F2075^T and the type strains of other *Arcobacter* species

Results are expressed as the mean ± standard deviation.

Strain	DNA–DNA relatedness with strain F2075^T (%)
A. halophilus LA31B^T	30±12
A. butzleri LMG 10828^T	23±1
A. skirrowii LMG 6621^T	19±8
A. nitrofigilis CECT 7204^T	16±2
A. cibarius CECT 7203^T	16±6
A. cryaerophilus LMG 9904^T	16±7

Table 2. Differential characteristics of *Arcobacter mytili* sp. nov. and other species of the genus *Arcobacter*

Species: 1, *A. mytili* sp. nov. (*n*=3, data from this study); 2, *A. nitrofigilis* (*n*=4, this study); 3, *A. halophilus* (*n*=1); 4, *A. cibarius* (*n*=15); 5, *A. cryaerophilus* (*n*=19; four strains were retested in this study); 6, *A. butzleri* (*n*=12); 7, *A. skirrowii* (*n*=9). Data taken from previous studies (On *et al.*, 1996; Donachie *et al.*, 2005; Houf *et al.*, 2005) unless otherwise indicated. +, >95 % Strains positive; −, <11 % strains positive; v, 12–94 % strains positive.

Characteristic	1	2	3	4	5	6	7
Growth condition							
Air at 25 °C	+	+	+	v	+	+	+
MacConkey agar	+	−	−	+	v	+	−
Minimal media	−	−	−	+	−*	+	−
NaCl 4 % (w/v)	+	+	+	−	−	−	+
Cefoperazone (64 mg l^{-1})	−	−	−	+	+	+	+
Enzyme activity							
Oxidase	+	+	+	+	+	+	+
Catalase	+	+	−	v	+	v	+
Urease	−	+	−	−	−	−	−
Nitrate reduction	−	+	+	−	+†	+	+
Indoxyl acetate hydrolysis	−	+	+	+	+	+	+

*Two of the four strains tested in this study (LMG 7537 and LMG 10241) were positive.
†Two of the four strains tested in this study (LMG 9904^T and LMG 9065) were negative.

Arcobacter in that they did not hydrolyse indoxyl acetate (Table 2). Since the studies of On *et al.* (1996) demonstrated that *A. nitrofigilis* was able to hydrolyse indoxyl acetate, which contradicted previous data (Mills & Gherna, 1987), all species of the genus *Arcobacter* have been considered to be indoxyl-acetate-hydrolysis-positive. In the present study, using four strains of *A. nitrofigilis* (CECT 7204^T, LMG 7547, F2173 and F2176), the results of On *et al.* (1996) were corroborated as, despite the reactions being slower and weaker than that observed for the other species, they were clearly positive. The susceptibility to cefoperazone (64 mg l^{-1}) differentiated the novel isolates from *A. butzleri*, *A. cibarius*, *A. cryaerophilus* and *A. skirrowii*. The lack of urease activity enabled the novel strains to be differentiated from *A. nitrofigilis*. The growth in MacConkey agar and the inability to reduce nitrate to nitrite differentiated the novel strains from *A. halophilus*. In addition, strains F2026, F2075^T and T234 differed from the other *Arcobacter* speicies by their fast growth in BA (growth was observable after 24 h incubation in aerobic conditions).

Motility was observed in young cultures by examining wet mounts in nutrient broth no. 2 by phase-contrast microscopy. Cell size, morphology and presence of flagella (Fig. 2) were determined with transmission electron microscopy (JEOL 1011) after negative staining with 2 % (w/v) phosphotungstic acid solution (pH 6.9) for 1 min and with scanning electron microscopy after fixing pieces of agar containing cells of

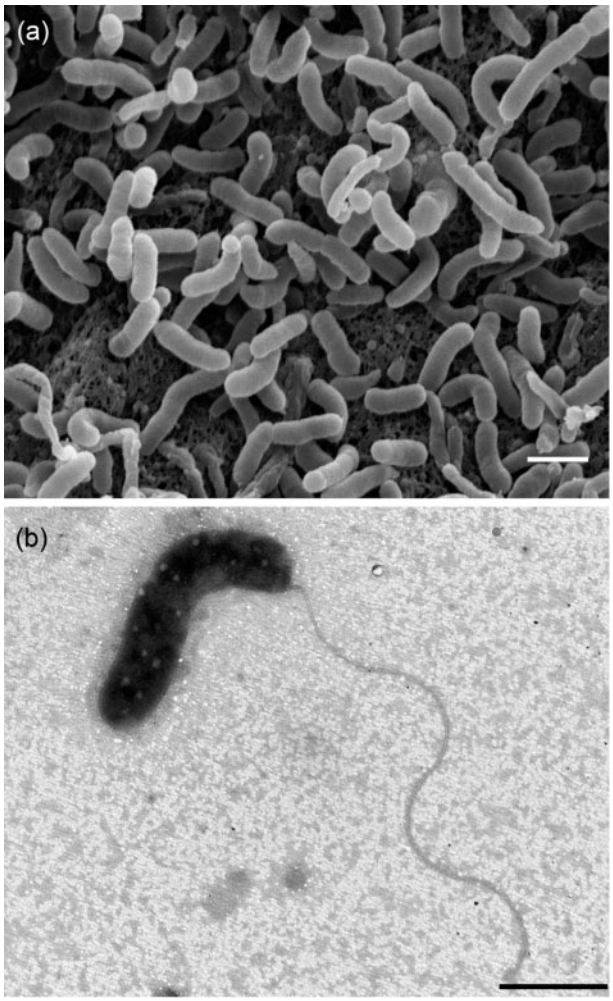

Fig. 2. Images of cells of strain F2075^T as observed with scanning electron microscopy (a) and transmission electron microscopy, negatively stained (b). Bars, 1 μm.

growing strain F2075^T in 2.5 % glutaraldehyde in phosphate buffer for 24 h. Subsequently, the samples were post-fixed in 1 % osmium tetroxide for 2 h. After dehydration and critical-point drying, specimens were mounted and coated with a thin layer of gold before examination with a JEOL JSM 6400 scanning electron microscope.

The data presented here support the suggestion that the four novel strains belong to a previously unrecognized species of the genus *Arcobacter*, for which the name *Arcobacter mytili* sp. nov. is proposed.

The recently described 16S rRNA gene RFLP *Arcobacter* identification protocol (Figueras *et al.*, 2008) is currently the only fast method that enables the differentiation of these four novel strains from the rest of the species of the genus on the basis of specific restriction patterns. In contrast, the m-PCR method (Houf *et al.*, 2000) misidentifies the four novel strains as *A. skirrowii*.

Description of *Arcobacter mytili* sp. nov.

Arcobacter mytili (my′ti.li. L. gen. n. *mytili* of a mussel, from the genus name *Mytilus*, from which the species was first isolated).

Cells are Gram-negative, non-encapsulated, non-spore-forming, slightly curved rods, some S-shaped, 0.4–0.6 μm wide and 1–3 μm long. Motile by means of a single polar flagellum. Colonies on BA incubated in aerobic conditions at 30 °C for 48 h are 2–4 mm in diameter, beige to off-white, circular with entire margins, convex and non-swarming. Pigments are not produced. All strains grow on BA at room temperature (18–22 °C) and at 30 and 37 °C under aerobic or microaerobic culture conditions with no significant differences. Under aerobic conditions, all strains grow at 30 °C on MacConkey agar and on media containing 2.0–4.0 % (w/v) NaCl. No growth is obtained on casein, minimal medium or media containing 64 mg cefoperazone l^{-1}. Weak growth is obtained in anaerobic conditions at 30 °C and in aerobic conditions at 42 °C, and no growth is observed at 4 °C. Oxidase-positive and weakly catalase-positive. Strains are not haemolytic and do not hydrolyse indoxyl acetate. Urease is not produced and nitrate is not reduced. Hydrogen sulfide is not produced in triple-sugar iron agar medium.

The type strain, F2075^T (=CECT 7386^T=LMG 24559^T), was isolated from mussels from Catalonia, Spain. The DNA G+C content of the type strain is 26.9 mol%.

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References

Adékambi, T., Shinnick, T. M., Raoult, D. & Drancourt, M. (2008). Complete *rpoB* gene sequencing as a suitable supplement to DNA–DNA hybridization for bacterial species and genus delineation. *Int J Syst Evol Microbiol* **58**, 1807–1814.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

Cleenwerck, I., Vandemeulebroecke, K., Janssens, D. & Swings, J. (2002). Re-examination of the genus *Acetobacter*, with descriptions of *Acetobacter cerevisiae* sp. nov. and *Acetobacter malorum* sp. nov. *Int J Syst Evol Microbiol* **52**, 1551–1558.

Collado, L., Inza, I., Guarro, J. & Figueras, M. J. (2008). Presence of *Arcobacter* spp. in environmental waters correlates with high levels of fecal pollution. *Environ Microbiol* **10**, 1635–1640.

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

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.

Figueras, M. J., Collado, L. & Guarro, J. (2008). A new 16S rDNA-RFLP method for the discrimination of the accepted species of *Arcobacter*. *Diagn Microbiol Infect Dis* **62**, 11–15.

Goris, J., Suzuki, K., De Vos, P., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA-DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1148–1153.

Ho, H. T. K., Lipman, L. J. & Gaastra, W. (2006). *Arcobacter*, what is known about a potential foodborne zoonotic agent! *Vet Microbiol* **115**, 1–13.

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 and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. *FEMS Microbiol Lett* **193**, 89–94.

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., Stephen, L., On, 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.

ICMSF (2002). *Microorganisms in Foods 7 – Microbiological Testing in Food Safety Management*. International Commission on Microbiological Specifications for Foods. New York: Kluwer Academic/Plenum.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Korczak, B. M., Stieber, R., Emler, S., Burnens, A. P., Frey, J. & Kuhnert, P. (2006). Genetic relatedness within the genus *Campylobacter* inferred from *rpoB* sequences. *Int J Syst Evol Microbiol* **56**, 937–945.

Martínez-Murcia, A. J., Benlloch, S. & Collins, M. D. (1992). Phylogenetic interrelationships of members of the genera *Aeromonas* and *Plesiomonas* as determined by 16S ribosomal DNA sequencing: lack of congruence with results of DNA-DNA hybridizations. *Int J Syst Bacteriol* **42**, 412–421.

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., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Mills, C. K. & Gherna, R. L. (1987). Hydrolysis of indoxyl acetate by *Campylobacter* species. *J Clin Microbiol* **25**, 1560–1561.

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

Prouzet-Mauléon, V., Labadi, L., Bouges, N., Menard, A. & Megraud, F. (2006). *Arcobacter butzleri*: underestimated enteropathogen. *Emerg Infect Dis* **12**, 307–309.

Samie, A., Obi, C. L., Barrett, L. J., Powell, S. M. & Guerrant, R. L. (2007). Prevalence of *Campylobacter* species, *Helicobacter pylori* and *Arcobacter* species in stool samples from the Venda region, Limpopo, South Africa: Studies using molecular diagnostic methods. *J Infect* **54**, 558–566.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

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 (1992). 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., Dewhirst, F. E., Paster, B. J. & On, S. L. W. (2005). Genus II. *Arcobacter* Vandamme, Falsen, Rossau, Segers, Tytgat and De Ley 1991a, 99^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, pp. 1161–1165. Edited by D. J. Brenner, N. P. Kreig, J. T. Staley & G. M. Garrity. New York: Springer.

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.

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.

Wilson, K. (1987). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 241–245. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Greene Publishing and Wiley Interscience.

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.

Wybo, I., Breynaert, J., Lauwers, S., Lindenburg, F. & Houf, K. (2004). Isolation of *Arcobacter skirrowii* from a patient with chronic diarrhea. *J Clin Microbiol* **42**, 1851–1852.