

Arcobacter lanthieri sp. nov., isolated from pig and dairy cattle manure

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A study was undertaken to determine the prevalence and diversity of species of the genus *Arcobacter* in pig and dairy cattle manure, which led to the identification of strains AF1440^T, AF1430 and AF1581. Initially identified as *Arcobacter butzleri* based on colony morphology and initial PCR-confirmation tests, analyses of 16S rRNA gene sequences of these strains confirmed that they belonged to the genus *Arcobacter* and were different from all known species of the genus. The isolates formed a distinct group within the genus *Arcobacter* based on their 16S rRNA, *gyrB*, *rpoB*, *cpn60*, *gyrA* and *atpA* gene sequences and fatty acid profiles. Their unique species status was further supported by physiological properties and DNA–DNA hybridization that allowed phenotypic and genotypic differentiation of the strains from other species of the genus *Arcobacter*. The isolates were found to be oxidase, catalase and esterase positive and urease negative; they grew well at 30 °C under microaerophilic conditions and produced nitrite and acetoin. Based on their common origin and various physiological properties, it is proposed that the isolates are classified as members of a novel species with the name *Arcobacter lanthieri* sp. nov. The type strain is AF1440^T (=LMG 28516^T=CCUG 66485^T); strains AF1430 (=LMG 28515=CCUG 66486) and AF1581 (=LMG 28517=CCUG 66487) are reference strains.

The genus *Arcobacter* was described by Vandamme *et al.* (1991). Since then, the genus has extended considerably, and currently encompasses 20 species (Levicán *et al.*, 2015) with validly published names, namely *Arcobacter nitrofigilis*, *A. cryaerophilus*, *A. butzleri*, *A. skirrowii*, *A. cibarius*, *A. marinus*, *A. trophiarum*, *A. molluscorum*, *A. ellisii*, *A. thereius*, *A. halophilus*, *A. mytili*, *A. defluvii*,

A. bivalviorum, *A. venerupis*, *A. cloacae*, *A. suis*, *A. anaerophilus*, *A. ebronensis* and *A. aquimarinus*. Several of these species have been associated with diseases in humans and animals such as gastroenteritis, mastitis, bacteraemia, reproductive disorders in livestock and abortion (Ho *et al.*, 2006). Species of the genus *Arcobacter* inhabit a wide variety of ecological niches and have been isolated from various hosts including humans, livestock, poultry and shellfish (Figueras *et al.*, 2011; Houf *et al.*, 2005; Kabeya *et al.*, 2003; On *et al.*, 1995). The most studied species including *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* have been increasingly recognized in recent years as emergent pathogens and as potential zoonotic agents (Ho *et al.*, 2006; Houf & Stephan, 2007). Routes of transmission of members of the genus *Arcobacter* may possibly

Abbreviation: ANI, average nucleotide identity

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *gyrB*, *rpoB*, *cpn60*, *gyrA* and *atpA* genes and whole-genome sequences of strains AF1440^T, AF1430 and AF1581 are detailed in Table S1.

Three supplementary tables and five supplementary figures are available with the online Supplementary Material.

include food and water (Lee *et al.*, 2012); however, direct contact with carriers of the emerging pathogen may also be a route (Fera *et al.*, 2009; Van Driessche *et al.*, 2005).

In this study, a polyphasic and genomic taxonomic characterization (Prakash *et al.*, 2007) of three isolates belonging to the genus *Arcobacter* recovered in 2010 from pig ($n=2$) and dairy cattle ($n=1$) manure tanks, collected from Canadian farms near Ottawa, Ontario, was performed in order to determine their taxonomic position. With the sequencing and phylogenetic analysis of the 16S rRNA gene, the isolates were identified taxonomically as belonging to the genus *Arcobacter*. This potential novel species was characterized by phenotypic and phylogenetic analyses of five housekeeping genes (*gyrB*, *rpoB*, *cpn60*, *gyrA* and *atpA*) and whole-genome sequences. Based on the phylogenetic analyses, whole-genome sequence and DNA–DNA hybridization data, these isolates revealed significant differences compared with known species of the genus *Arcobacter* including *A. butzleri*, *A. cryaerophilus*, *A. cibarius*, *A. anaerophilus* and *A. nitrofigilis*. Many of the phenotypic characteristics of the strains corresponded to those of previously described species of the genus *Arcobacter* isolated from animal sources.

For initial isolation of arcobacters, 1 g faecal sample collected from pig or dairy cattle manure tank was diluted in 9 ml peptone water (PW) using a tenfold serial dilution approach and 100 μ l suspension plated directly on *Arcobacter* selective isolation agar (ASIA) (Oxoid) containing antibiotic supplements (fluorouracil, amphotericin B, cefoperazone, novobiocin and trimethoprim). The plates were incubated at 30 °C under microaerophilic conditions (85% N₂, 10% CO₂ and 5% O₂) for 3–6 days as described by Houf *et al.* (2001). The putative arcobacters were further purified by repetitive streaking to obtain single colonies on modified agarized *Arcobacter* medium (m-AAM; Oxoid) containing selective antibiotic supplements (cefoperazone, amphotericin B and teicoplanin), and the plates were incubated under the conditions indicated above. Isolates with small, translucent, beige to whitish colonies were recovered. The isolates were further identified to the genus-level by amplification of an expected PCR product of 1223 bp in an *Arcobacter* genus-specific PCR assay (Harmon & Wesley, 1996). In addition, the 16S rRNA and 23S rRNA gene-based multiplex-PCR assay for *A. butzleri*, *A. skirrowii* and *A. cryaerophilus* was applied to identify these isolates to the species-level using the oligonucleotide primer pairs and PCR protocol described by Houf *et al.* (2000). The isolates showed similarity to *A. butzleri*, with a PCR fragment size of 401 bp. However, two other amplified PCR products (198 and 1125 bp) were also observed that did not correspond to the fragment sizes specific for *A. skirrowii* (641 bp) and *A. cryaerophilus* (257 bp). Additionally, the strains were subjected to the multiplex-PCR assay specific for *A. butzleri*, *A. cibarius*, *A. cryaerophilus*, *A. skirrowii* and *A. thereius* (Doudah *et al.*, 2010), in which no PCR amplification was observed. Therefore, sequence-based phylogenetic analysis and

whole-genome sequencing were performed for taxonomic classification of these isolates within the genus *Arcobacter*.

For phylogenetic analysis, genomic DNA was prepared by boiling a single colony at 100 °C for 10 min in 100 μ l nuclease-free sterile water. The cell lysates were centrifuged and 1 μ l supernatant containing genomic DNA was used to amplify the 16S rRNA, *gyrB*, *rpoB* and *cpn60* genes by PCR using previously reported oligonucleotide primers and protocols (Bruce *et al.*, 1992; Collado *et al.*, 2011; Hill *et al.*, 2006; Korczak *et al.*, 2006). Amplified PCR products were further purified using the Qiaquick PCR purification kit (Qiagen) and electrophoresed on a 1.5% agarose gel to check for purity and band intensity. Sequencing reactions were performed as described previously (Tambong *et al.*, 2006) using BigDye terminator chemistry, and the amplified products were sequenced on an ABI 3130xL Genetic Analyzer (Applied Biosystems) according to the manufacturer's recommendation. In addition, *gyrA* and *atpA* gene sequences were obtained from the whole-genome sequence (Adam *et al.*, 2014). Table S1 (available in the online Supplementary Material) shows the GenBank accession numbers for the 16S rRNA (1439 bp), *gyrB* (359 bp), *rpoB* (486 bp), *cpn60* (555 bp), *gyrA* (2691 bp) and *atpA* (1518 bp) gene sequences generated in this study. For phylogenetic inference, six different alignments were created corresponding to the alignment of the sequences of each gene, in order to define the phylogenetic position of the novel species. The sequences for the three novel strains were further analysed and compared by multiple sequence alignment and phylogenetic analysis with known reference species of the genus *Arcobacter*. Multiple sequence alignment was performed by using the MUSCLE program using the MEGA software version 6.0 (Tamura *et al.*, 2011). The phylogeny based on the maximum-likelihood (Felsenstein, 1981), neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) algorithms was assessed by performing bootstrap analyses with 1000 replications for the reliability of tree topologies. In phylogenetic trees reconstructed using the 16S rRNA gene sequence and five housekeeping gene sequences, the three new isolates clustered next to *A. cryaerophilus*, *A. cibarius*, *A. butzleri*, *A. thereius* and *A. skirrowii*. This association was supported by bootstrap values (>70%) no matter whether the maximum-likelihood (Fig. 1), maximum-parsimony or neighbour-joining (Figs. S1 and S2) algorithm was used to determine phylogeny. The maximum-likelihood tree was computed by the general time-reversible (GTR) model of nucleotide substitution with five discrete gamma rate categories (Lanave *et al.*, 1984). The 16S rRNA gene sequence showed close similarities between these strains and the type strains of species of the genus *Arcobacter*, the $\geq 97\%$ threshold that is generally recognized as delineating a genus-species (Stackebrandt & Goebel, 1994). The phylogenetic analysis showed close relatedness of the three strains to *A. cryaerophilus* CCUG 17801^T (97.1%), followed by *A. cibarius* LMG 21996^T (96.6%), *A. butzleri* ATCC 49616^T (95.7%), *A. thereius* 16389^T (95.4%) and *A. skirrowii*

CCUG 10374^T (95.2%). Similarly, the multilocus phylogenetic analysis reconstructed with the concatenated sequences of five housekeeping genes (*gyrB*, *rpoB*, *cpn60*, *gyrA* and *atpA*) from all the type strains also confirmed the close relatedness of these three faecal strains as a novel phylogenetic group within the genus *Arcobacter* (Fig. S3).

For further analysis, whole-genome sequencing of purified genomic DNA was performed using the UltraClean Tissue and Cells DNA Isolation kit (Mo-Bio) following the manufacturer's instructions using paired-end sequencing on an Illumina HiSeq2500 instrument with TrueSeq V3 chemistry at the National Research Council Canada (Saskatoon, SK, Canada). The whole-genome sequence project has been deposited at GenBank/EMBL/DDJB under the accession numbers presented in Table S1 (Adam *et al.*, 2014). Furthermore, average nucleotide identity (ANI) analysis of conserved and shared genes was performed among the three strains, along with available genome sequences of other members of the genus *Arcobacter* including *A. butzleri* ED-1, *A. cibarius* LMG 21996^T, *A. anaerophilus* IR-1 and *A. nitrofigilis* DSM 7299^T, based on pairwise genome comparisons, with support of the tetranucleotide frequency correlation coefficient (TETRA), in order to reveal their total similarities in terms of percentage sequence identity. We used the JSpecies software (Richter & Rosselló-Móra, 2009), which is based on the BLAST alignment algorithm (Altschul *et al.*, 1997), to calculate ANI values, and on the algorithm described by Teeling *et al.* (2004) for TETRA values. ANI calculations for each sequence pair showed similarity ranging from 98.21 to 99.98% among the three strains and 72.33–79.87% similarity between the three strains and other members of the genus *Arcobacter*, while the corresponding TETRA values were 0.99745–0.99932 and 0.77302–0.97971, respectively.

DNA–DNA hybridization was carried out to determine the genomic DNA relatedness between strains AF1440^T, AF1430 and AF1581 and their closest relatives (the type strains of *A. cryaerophilus*, *A. cibarius* and *A. butzleri*). Genomic DNA from the isolates and reference strains was extracted and purified using a Wizard Genomic DNA purification kit (Promega) according to the manufacturer's instructions. DNA–DNA hybridization was carried out following the DNA microarray analysis coupled to reverse genome probing technique, as described previously (Ramisse *et al.*, 2003) with some modifications. Briefly, DNA (1 µg) extracts were sonicated, diluted (50 ng) and denatured by boiling for 10 min. Random-primed DNA labelling with digoxigenin–dUTP and hybridization were performed using a DIG High Prime DNA Labelling and Detection Starter kit II (Roche Applied Science) in accordance with the manufacturer's instructions. Genomic DNA (50 ng) was denatured by the alkaline method and immobilized on a nylon membrane (Hybond-N+; Amersham) using a V&P 384s solid Multi-blot Replicator (V&P Scientific Inc.) as indicated by Tambong *et al.* (2006). DNA preparations were labelled and hybridization performed

after pre-hybridizing the spotted membrane at 55 °C for 2 h. Hybridization was carried out with the different labelled DNAs (50 ng ml⁻¹) at 55 °C for 16 h, followed by stringency washes and detection as recommended by the manufacturer (Roche Applied Science). Chemiluminescence detection was based on the CSPD reaction at room temperature for 5 min and performed in accordance with the manufacturer's protocol (Roche Applied Science). The membranes were then exposed to autoradiography film (Hyperfilm-ECL; Amersham) for 10 s, and the signal intensities were scanned using an HP Scanjet 5590 (Hewlett Packard) and quantified by using ImageJ version 1.48v (Schneider *et al.*, 2012). The signal produced by self-hybridization of the probe with homologous target DNA was taken as 100%, and percentage relatedness values were calculated for the four replicate samples. High levels (≥85%) of DNA–DNA relatedness were observed between the three novel isolates, AF1440^T, AF1430 and AF1581. DNA relatedness values were based on a minimum of four hybridizations for both direct and reciprocal reactions (Table S2). These results indicate that strains AF1440^T, AF1430 and AF1581 represent a novel species, given the recommendation of a threshold value of 70% DNA–DNA relatedness for species definition as indicated by Wayne *et al.* (1987).

The DNA G+C contents of strains AF1440^T, AF1430 and AF1581 were determined from the whole-genome DNA sequence (Adam *et al.*, 2014). The DNA G+C contents of AF1440^T, AF1430, and AF1581 were 26.6, 26.4 and 26.8 mol%, respectively, confirming the previously defined low range of 26.8–35 mol% for the genus *Arcobacter* (Vandamme *et al.*, 2005).

The isolates were further genotyped by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) using previously described primers and conditions (Houf *et al.*, 2002) to recognize potential clones. Three different genotypes, based on patterns that differed by one or more bands, were obtained among the pig and dairy cattle manure isolates (Fig. S4). This result indicates that different genotypes may persist in the same or different faecal source.

Growth of the isolates was checked by culturing on Campylobacter blood-free charcoal cefoperazone deoxycholate agar (CCDA) and MacConkey agar (Oxoid) at 25, 30, 37 and 42 °C under aerobic and microaerophilic conditions, as well as on trypticase soy agar (TSA; BD) containing 1% glycine or 4% (w/v) NaCl (Houf *et al.*, 2009; Kim *et al.*, 2010), and incubated for up to 6 days. Oxidase activity was assessed by using 1% (w/v) tetramethyl *p*-phenylenediamine (bioMérieux), and catalase activity was determined from bubble production in 3% (v/v) hydrogen peroxide solution. All strains were able to grow on CCDA, MacConkey agar and TSA containing glycine, as well as under aerobic and microaerophilic conditions at 25, 30 and 37 °C, but not at 42 °C or on TSA with 4% NaCl. The isolates produced oxidase and catalase, hydrolysed

indoxyl acetate and reduced nitrate, but no α -haemolysis was observed on blood agar. Enzyme activities, utilization of various carbon sources and acid production from substrates were tested with commercial API Campy and API 20E biochemical assay kits (bioMérieux) according to the manufacturer's protocols. The assays were performed at 36 °C for 24 h under microaerophilic and aerophilic conditions, respectively. The strains did not produce acid from the tested carbohydrates, including amygdalin, arabinose, D-glucose, inositol, melibiose, mannitol, rhamnose, sorbitol and sucrose, in common with other species of the genus *Arcobacter*. In addition, the strains showed no enzyme activity, similar to other species of the genus *Arcobacter*, for hippurate hydrolysis, γ -glutamyltransferase, pyrrolidonyl arylamidase, L-arginine arylamidase, L-aspartate arylamidase, gelatin hydrolysis, H₂S production, β -galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, tryptone deaminase and indole production. The most relevant phenotypic characteristics that differentiated the novel species from other taxa within the genus are summarized in Table 1.

Susceptibility to a range of antibiotics including azithromycin, cefoperazone, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, nalidixic acid and tetracycline was tested on Mueller–Hinton agar (Oxoid) using the agar dilution method (Van den Bulck *et al.*, 2005; CLSI, 2010). These tolerance test preparations were incubated for 3 days under microaerophilic conditions at 30 °C. Interpretative criteria were in accordance with the CLSI (2011). The novel isolates were susceptible to all antimicrobials tested, as indicated by low MIC values (ranging from 4 to 64 $\mu\text{g ml}^{-1}$), except cefoperazone (64 $\mu\text{g ml}^{-1}$) and clindamycin (8 $\mu\text{g ml}^{-1}$).

For scanning electron microscopy, bacterial strains were prepared according to a previously described procedure (Greco-Stewart *et al.*, 2012). Briefly, cells were fixed in 2.8% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 6.8), dehydrated in an ethanol series (20–100% ethanol), critical-point dried and coated in gold. The gold-coated specimens were examined and imaged using a Philips XL-30 ESEM scanning electron microscope operated at 7.5 kV accelerating voltage. In addition, cell morphology was observed by transmission electron

Table 1. Differential physiological characteristics of the isolated strains and related species of the genus *Arcobacter*

Strains/species: 1, AF1440^T; 2, AF1430; 3, AF1581; 4, *A. nitrofigilis*; 5, *A. cryaerophilus*; 6, *A. butzleri*; 7, *A. skirrowii*; 8, *A. cibarius*; 9, *A. halophilus*; 10, *A. mytili*; 11, *A. thereius*; 12, *A. marinus*; 13, *A. trophiarum*; 14, *A. defluvi*; 15, *A. molluscorum*; 16, *A. ellisii*; 17, *A. bivalviorum*; 18, *A. venerupis*; 19, *A. cloacae*; 20, *A. suis*; 21, *A. anaerophilus*; 22, *A. ebronensis*; 23, *A. aquimarinus*. Data for strains and reference species in columns 1–17 were obtained and analysed in this study by using API Campy and API 20E biochemical assay kits (bioMérieux); data for species in columns 18–23 were obtained from Sasi Jyothsna *et al.* (2013) and Levican *et al.* (2015). ND, Not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Growth in/on:																							
Air at 37 °C	+	+	+	-	+	+	+	-	+	+	-	+	-	+	+	+	+	-	+	-	-	-	+
CO ₂ at 37 °C	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	-	-	+
CO ₂ at 42 °C	-	-	-	-	-	+	-	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-
4% (w/v) NaCl	-	-	-	+	-	-	+	-	+	+	-	+	-	-	+	-	+	-	-	-	+	-	-
1% (w/v) Glycine	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-
MacConkey agar	+	+	+	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	ND	-	-
CCDA	+	+	+	-	+	+	+	-	-	-	-	-	+	+	-	+	+	+	+	-	ND	-	+
Enzyme activity:																							
Urease	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-
Catalase	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-	-	+
Esterase	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	ND	ND	ND	ND	ND	ND
Hippurate hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	ND	ND	ND	ND	ND	ND
Alkaline phosphatase	-	-	-	-	+	+	-	-	-	+	-	+	-	-	+	-	+	ND	ND	ND	ND	ND	ND
Voges–Proskauer test (acetoin production)	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	ND	ND	ND	ND	ND	ND
Sodium succinate assimilation	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	ND	ND	ND	ND	ND	ND
Nitrate reduction	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+
Nitrite production	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND
TTC reduction	+	+	+	-	-	+	-	+	+	+	-	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND
Indoxyl acetate hydrolysis	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+
Resistance to cefoperazone (64 mg l ⁻¹)	+	+	+	-	+	+	+	+	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-

microscopy after fixing the culture strain on glow discharge parlodion and carbon-coated copper EM grids and negatively stained using a 2% solution of phosphotungstic acid (pH 7.2) as described by Hayat (1989). Cells were observed and high-resolution transmission electron micrographs were obtained using a Hitachi H7000 TEM operating at 75 kV. Cells of strain AF1440^T were rods, 0.2–0.4 µm wide and 1.4–2.0 µm long, with a single polar flagellum at one end of the cell (Fig. S5).

The total cellular fatty acid profiles of the three novel strains, along with strains of other species including *A. butzleri*, *A. cibarius*, *Escherichia coli* and *Pseudomonas aeruginosa* as controls, were determined by using a modified bacterial fatty acid extraction procedure from the Microbial Identification System (MIDI Inc.) as described by Chao *et al.* (2010). The control strains of *A. butzleri* and *A. cibarius* were subcultured on m-AAM media and incubated at 30 °C under microaerophilic conditions; whereas, *E. coli* and *P. aeruginosa* were subcultured on TSA media at 37 °C under aerobic conditions. All samples were freeze-dried for 1 day at –62 °C under vacuum prior to fatty acid extraction. The resulting whole-cell fatty acid methyl esters were analysed with a Varian CP-3800 gas chromatograph with a flame-ionization detector (GC-FID). The data were analysed with the Varian Galaxie software (version 1.9.3). Whole-cell fatty acid analysis revealed that C_{16:0}, C_{16:1n7c}, C_{16:1n7t} and C_{18:1n7c} were the predominant fatty acids, with minor amounts of C_{12:0}, C_{12:0} 3-OH, C_{14:0}, C_{23:0}, C_{17:0}, C_{18:1n7t}, C_{18:0}, C_{15:0}, C_{14:0} 2-OH and C_{14:0} 3-OH. The novel strains differed from their nearest phylogenetic neighbours by the absence of C_{18:2n6} from the reference strains (Table S3).

On the basis of our results, by the application of a polyphasic approach including analyses of 16S rRNA, *gyrB*, *rpoB*, *cpn60*, *gyrA* and *atpA* gene sequences, whole-genome sequences, DNA–DNA hybridization values, biochemical properties, growth patterns, antibiotic resistance, electron microscopy and fatty acid composition, a novel species, *Arcobacter lanthieri* sp. nov., is described to accommodate strains AF1440^T, AF1430 and AF1581.

Description of *Arcobacter lanthieri* sp. nov.

Arcobacter lanthieri (lan.thi.e'ri. N.L. masc. gen. n. *lanthieri* named in honour of the late Dr Martin Lanthier, a Canadian microbiologist, who initiated this research study to identify emerging bacterial pathogens in the agriculture sector).

Cells are Gram-negative, microaerobic, slightly curved, rod-shaped, 0.2–0.4 µm wide and 1.4–2.0 µm long, and motile with one polar flagellum. After growth on m-AAM medium at 30 °C for 3 days, colonies are small, translucent and beige to whitish. Cells grow well at 25–37 °C (optimum 30 °C) on m-AAM, ASIA, CCDA, MacConkey agar and TSA plus 1% glycine. Positive for nitrate

reduction, triphenyl tetrazolium chloride (TTC) reduction and the Voges–Proskauer test. Strains are positive for oxidase, catalase, esterase, nitrite production and indoxyl acetate hydrolysis, but show no activity for gelatin hydrolysis, H₂S production, sodium succinate assimilation, hippurate hydrolysis, γ-glutamyltransferase, pyrrolidonyl arylamidase, L-arginine arylamidase, L-aspartate arylamidase, alkaline phosphatase, β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, tryptone deaminase and indole production. In addition, D-glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, arabinose are not fermented. The major fatty acids (>6%) are C_{16:0}, C_{16:1n7c}, C_{16:1n7t} and C_{18:1n7c}; C_{18:2n6} is present in this species, but not in closely related species. The DNA G+C content of the type strain is 26.6 mol%.

The type strain, AF1440^T (=LMG 28516^T=CCUG 66485^T), and strain AF1430 (=LMG 28515=CCUG 66486) were isolated from pig manure, while AF1581 (=LMG 28517=CCUG 66487) was isolated from dairy cattle manure taken from the South Nation River basin near Ottawa, Ontario, Canada.

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References

- Adam, Z., Whiteduck-Léveillé, K., Cloutier, M., Tambong, J. T., Chen, W., Lewis, C. T., Lévesque, C. A., Topp, E., Lapen, D. R. & other authors (2014). Draft genome sequences of three *Arcobacter* strains of pig and dairy cattle manure origin. *Genome Announc* 2, e00377–e00314.
- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped blast and psi-blast: a new generation of protein database search programs. *Nucleic Acids Res* 25, 3389–3402.
- Bruce, K. D., Hiorns, W. D., Hobman, J. L., Osborn, A. M., Strike, P. & Ritchie, D. A. (1992). Amplification of DNA from native populations of soil bacteria by using the polymerase chain reaction. *Appl Environ Microbiol* 58, 3413–3416.
- Chao, J., Wolfaardt, G. M. & Arts, M. T. (2010). Characterization of *Pseudomonas aeruginosa* fatty acid profiles in biofilms and batch planktonic cultures. *Can J Microbiol* 56, 1028–1039.
- CLSI (2010). *Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated fastidious bacteria*; Approved Guideline, 2nd edn. CLSI document M45-A2. Wayne, PA: Clinical and Laboratory Standards Institute.
- CLSI (2011). *Performance standards for antimicrobial susceptibility testing*, 21st Informational Supplement. CLSI M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute.
- Collado, L., Levican, A., Perez, J. & Figueras, M. J. (2011). *Arcobacter defluvii* sp. nov., isolated from sewage samples. *Int J Syst Evol Microbiol* 61, 2155–2161.

- Doudidah, 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.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- 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.
- Figueras, M. J., Collado, L., Levican, A., Perez, J., Solsona, M. J. & Yustes, C. (2011). *Arcobacter molluscorum* sp. nov., a new species isolated from shellfish. *Syst Appl Microbiol* **34**, 105–109.
- Greco-Stewart, V. S., Brown, E. E., Parr, C., Kalab, M., Jacobs, M. R., Yomtavian, R. A. & Ramirez-Arcos, S. M. (2012). *Serratia marcescens* strains implicated in adverse transfusion reactions form biofilms in platelet concentrates and demonstrate reduced detection by automated culture. *Vox Sang* **102**, 212–220.
- Harmon, K. M. & Wesley, I. V. (1996). Identification of *Arcobacter* isolates by PCR. *Lett Appl Microbiol* **23**, 241–244.
- Hayat, M. A. (1989). *Principles and Techniques of Electron Microscopy: Biological Applications*, 3rd edn. Boca Raton, FL: CRC Press.
- 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.
- Ho, H. T. K., Lipman, L. J. & Gaastra, W. (2006). *Arcobacter*, what is known and unknown 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., 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., On, S. L., 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., On, S. L., 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.
- Kabeya, H., Maruyama, S., Morita, Y., Kubo, M., Yamamoto, K., Arai, S., Izumi, T., Kobayashi, Y., Katsube, Y. & Mikami, T. (2003). Distribution of *Arcobacter* species among livestock in Japan. *Vet Microbiol* **93**, 153–158.
- Kim, H. M., Hwang, C. Y. & Cho, B. C. (2010). *Arcobacter marinus* sp. nov. *Int J Syst Evol Microbiol* **60**, 531–536.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- 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.
- Lanave, C., Preparata, G., Saccone, C. & Serio, G. (1984). A new method for calculating evolutionary substitution rates. *J Mol Evol* **20**, 86–93.
- Lee, C., Agidi, S., Marion, J. W. & Lee, J. (2012). *Arcobacter* in Lake Erie beach waters: an emerging gastrointestinal pathogen linked with human-associated fecal contamination. *Appl Environ Microbiol* **78**, 5511–5519.
- Levican, A., Rubio-Arcos, S., Martinez-Murcia, A., Collado, L. & Figueras, M. J. (2015). *Arcobacter ebronensis* sp. nov. and *Arcobacter aquimarinus* sp. nov., two new species isolated from marine environment. *Syst Appl Microbiol* **38**, 30–35.
- On, S. L., Stacey, A. & Smyth, J. (1995). Isolation of *Arcobacter butzleri* from a neonate with bacteraemia. *J Infect* **31**, 225–227.
- Prakash, O., Verma, M., Sharma, P., Kumar, M., Kumari, K., Singh, A., Kumari, H., Jit, S., Gupta, S. K. & other authors (2007). Polyphasic approach of bacterial classification – an overview of recent advances. *Indian J Microbiol* **47**, 98–108.
- Ramisse, V., Balandreau, J., Thibault, F., Vidal, D., Vergnaud, G. & Normand, P. (2003). DNA-DNA hybridization study of *Burkholderia* species using genomic DNA macro-array analysis coupled to reverse genome probing. *Int J Syst Evol Microbiol* **53**, 739–746.
- Richter, M. & Rosselló-Móra, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* **106**, 19126–19131.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasi Jyothsna, T. S., Rahul, K., Ramaprasad, E. V. V., Sasikala, Ch. & Ramana, Ch. V. (2013). *Arcobacter anaerophilus* sp. nov., isolated from an estuarine sediment and emended description of the genus *Arcobacter*. *Int J Syst Evol Microbiol* **63**, 4619–4625.
- Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* **9**, 671–675.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tambong, J. T., de Cock, A. W. A. M., Tinker, N. A. & Lévesque, C. A. (2006). Oligonucleotide array for identification and detection of *Pythium* species. *Appl Environ Microbiol* **72**, 2691–2706.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.
- Teeling, H., Meyerdierks, A., Bauer, M., Amann, R. & Glöckner, F. O. (2004). Application of tetranucleotide frequencies for the assignment of genomic fragments. *Environ Microbiol* **6**, 938–947.
- Van den Bulck, K., Decostere, A., Baele, M., Driessen, A., Debongnie, J. C., Burette, A., Stolte, M., Ducatelle, R. & Haesebrouck, F. (2005). Identification of non-*Helicobacter pylori* spiral organisms in gastric samples from humans, dogs, and cats. *J Clin Microbiol* **43**, 2256–2260.
- Van Driessche, E., Houf, K., Vangroenweghe, F., De Zutter, L. & Van Hoof, J. (2005). Prevalence, enumeration and strain variation of *Arcobacter* species in the faeces of healthy cattle in Belgium. *Vet Microbiol* **105**, 149–154.

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., 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. 2C. pp. 1161–1165. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology, Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.