Arcobacter cibarius sp. nov., isolated from broiler carcasses

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Twenty Gram-negative, rod-shaped, slightly curved, non-spore-forming bacteria that gave a negative result in Arcobacter species-specific PCR tests but that yielded an amplicon in an Arcobacter genus-specific PCR test were isolated from 13 unrelated broiler carcasses. Numerical analysis of the profiles obtained by SDS-PAGE of whole-cell proteins clustered all isolates in a single group distinct from the other Arcobacter species. DNA–DNA hybridization among four representative strains exhibited DNA binding values above 91 %. DNA–DNA hybridization with reference strains of the current four Arcobacter species revealed binding levels below 47 %. The G + C contents ranged between 26·8 and 27·3 mol%. Pairwise comparison of 16S rRNA gene sequences revealed the mean values for similarity to the type strain of Arcobacter cryaerophilus (97·5 %), Arcobacter butzleri (96·5 %), Arcobacter skirrowii (96·0 %) and Arcobacter nitrofigilis (95·0 %). The levels of similarity to Campylobacter and Helicobacter species were below 88 and 87 %, respectively. The isolates could be distinguished from other Arcobacter species by the following biochemical tests: catalase, oxidase and urease activities; reduction of nitrate; growth at 25 and 37 °C under aerobic conditions; growth on 2–4 % (w/v) NaCl media; and susceptibility to cephalothin. These data demonstrate that the 20 isolates represent a single novel Arcobacter species, for which the name Arcobacter cibarius sp. nov. is proposed, with LMG 21996T (=CCUG 48482T) as the type strain.

The genus Arcobacter encompasses bacteria formerly known as aerotolerant campylobacters (Vandamme & De Ley, 1991) and belongs to the family Campylobacteraceae. Arcobacter presently contains four species. Arcobacter butzleri, Arcobacter cryaerophilus and Arcobacter skirrowii are associated with reproductive disorders (On et al., 2002), mastitis (Logan et al., 1982) and gastric ulcers (Suarez et al., 1997) in livestock and have also been isolated from human clinical samples, including diarrhoea and blood (Mansfield & Forsythe, 2000). Arcobacter nitrofigilis is a free-living, nitrogen-fixing bacterium associated with the roots of Spartina alterniflora, a salt-marsh plant (McClung et al., 1983). Furthermore, a number of potentially novel species have been described from environments such as oilfields, sea water and coral surfaces; one of these hitherto uncultured organisms of marine origin has been designated ‘Candidatus Arcobacter sulfidicus’ (Wirsen et al., 2002).

In the present study, we report on the polyphasic taxonomic characterization of 20 Arcobacter isolates that were recovered from the skins of 13 unrelated broiler carcasses. During a long-term study of Arcobacter contamination on poultry carcasses, arcobacters were isolated using the selective direct isolation method developed by Houf et al. (2001). Isolates were analysed by multiplex PCR for species-level identification (Houf et al., 2000) and were characterized at strain level by modified enterobacterial repetitive intergenic consensus PCR (Houf et al., 2002).

The 20 isolates from the present study were recovered from broiler skin and appeared as colourless, translucent, small colonies on Arcobacter selective agar plates. No amplicons were generated in the Arcobacter species-specific multiplex-PCR assay. However, an Arcobacter genus-specific 1223 bp fragment was generated for all isolates in a genus-specific
PCR assay (Harmon & Wesley, 1996). Cluster analysis of the DNA-banding patterns obtained after modified enterobacterial repetitive intergenic consensus PCR (Houf et al., 2002) revealed a large degree of genotypic heterogeneity. On the basis of strain delineation, as defined in a previous study (Houf et al., 2002), 16 genotypes could be identified.

Preparation of whole-cell proteins and SDS-PAGE were performed as described by Pot et al. (1994). For each genotype, one representative isolate was grown microaerobically on Mueller–Hinton (Oxoid) blood-agar plates (5 %, v/v, defibrinated horse blood) and incubated microaerobically at 37 °C. Whole-cell protein profiles of Arcobacter reference strains and of type and reference strains of Campylobacter and Helicobacter species were available from previous studies (Vandamme et al., 1992). Densitometric analysis, normalization and interpolation of the protein profiles, and numerical analysis, were performed using the GelCompar software package (version 4.2; Applied Maths). The similarity between all pairs of traces was expressed by using the Pearson product moment correlation coefficient presented as percentages of similarity. A numerical analysis of the protein profiles of the 16 isolates and of Arcobacter reference strains is shown in Fig. 1. All 16 isolates grouped in a single cluster above a similarity level of 75 % and were clearly distinct from the other Arcobacter species (Fig. 1).

Four isolates, LMG 21996T, LMG 21997, LMG 21998 and R-16100, representing four distinct genotypes were selected at random for further genomic analyses. DNA was prepared as described by Pitcher et al. (1989). The nearly complete sequences of the 16S rRNA genes of strains LMG 21996T and LMG 21997 were amplified by PCRs using conserved primers (5′-AGAGTTTGATCCTGGCTGAG-3′ and 5′-AAGGAGGTGATCCAGCCGCA-3′) (Coenye et al., 1999). The PCR products were purified using a QIAquick PCR purification kit (Qiagen) according to the manufacturer’s instructions. Sequence analysis was performed with an Applied Biosystems 310 DNA sequencer and the protocols of the manufacturer (Perkin-Elmer) using the ABI Prism Dye Terminator cycle sequencing ready reaction kit. Sequence assembly was performed using the program AutoAssembler (Perkin-Elmer). Approximately 1460 bases were used and unknown bases were excluded from the calculations. Pairwise comparison of the 16S rRNA gene sequences obtained with those of neighbouring taxa retrieved from the EMBL database revealed the following mean similarity values: 97–5 % for the type strain of

![Fig. 1. Dendrogram derived from the numerical analysis of the whole-cell protein profiles of A. cibarius and Arcobacter reference strains. A variable dense band region was omitted from the numerical analysis as described previously (Vandamme et al., 1992).](image-url)
A. cryaerophilus; 96.5% for A. butzleri; 96.0% for A. skirrowii; and 95.0% for Arcobacter nitrofigilis. The levels of similarity to Campylobacter and Helicobacter species were below 88 and 87%, respectively.

DNA–DNA hybridizations were performed with photo-biotin-labelled probes in microplate wells as described by Ezaki et al. (1989), using an HTS7000 Bio Assay Reader (Perkin-Elmer) for the fluorescence measurements. The hybridization temperature was 30 °C. DNA–DNA hybridization experiments showed that the four representative strains exhibited DNA binding levels above 91% (data not shown). DNA binding levels with reference strains of A. butzleri, A. cryaerophilus, A. skirrowii and A. nitrofigilis were below 47% (data not shown).

The G + C contents of the four strains were determined by enzymically degrading DNA into nucleosides, as described by Mesbah et al. (1989). The nucleoside mixture obtained was then separated by HPLC using a Waters Symmetry Shield C8 column kept at 37 °C. The solvent was 0.02 M NH₄H₂PO₄ (pH 4.0) with 1.5% (w/v) acetonitrile. Non-methylated λ phage DNA (Sigma) was used as the calibration reference. The G + C contents ranged between 26-8 and 27-3 mol%. The G + C contents of the four reference strains ranged between 27-2 and 28-2 mol%, confirming previously reported values (Vandamme et al., 1992).

The phenotypes of 15 of the 16 distinct genotypes were determined by using an extensive biochemical identification scheme for arcobacters and related bacteria, as described by On et al. (1996). Strains LMG 21996T, LMG 21997 and LMG 21998 were examined for motility by suspending 3-day-old cultures in nutrient broth no. 2 (Oxoid) and examining one drop of the suspension under a cover-slip using dark-field microscopy at 40× magnification with a Laborlux S microscope (Ernst Leitz). Cells of each of the three strains examined demonstrated motility under the conditions described. Most of the cells appeared to be poorly motile but a few cells clearly exhibited a rapid, darting motility. Strains LMG 21996T, LMG 21997 and LMG 21998 were examined by transmission electron microscopy (TEM208S; FEI) after negative staining with 2% (w/v) uranylacetate as described by Imberechts et al. (1996) but with the modification that the grids were treated before staining with 1% (w/v) alcian blue 8G to render them more hydrophilic. Cells of each of the three strains examined were rods, about 0.5 μm wide and 1.5 μm long, with a single polar un sheathed flagellum at one end of the cell (Fig. 2).

The ability of the strains to hydrolyse indoxyl acetate and their ability to grow at room temperature (approx. 18–25 °C) under microaerobic conditions were characteristics typical of other Arcobacter species. Nonetheless, the strains could be distinguished from all other extant species (Table 1). These data demonstrate that the 20 isolates represent a single novel Arcobacter species, for which the name Arcobacter cibarius sp. nov. is proposed, with LMG 21996T (=CCUG 48482T) as the type strain.

Although the pathogenic potential of A. cibarius is unknown, it is noteworthy that the closely related species A. butzleri and A. cryaerophilus have been implicated as agents of human disease, principally gastroenteritis (Mansfield & Forsythe, 2000). As with A. cibarius, these species also occur in poultry (Atabay et al., 1998), which is a well-established source of foodborne zoonotic disease. However, isolation of Arcobacter species requires specific conditions, and current methods are not optimal for all species (Houf et al., 2001). Although the role of arcobacters in human disease has yet to be fully determined, the

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Table 1. Characteristics that differentiate A. cibarius (n=15) from A. butzleri (n=12), A. cryaerophilus (n=19), A. skirrowii (n=9) and A. nitrofigilis (n=2)
widespread occurrence of these organisms in foods of animal origin justifies further studies to develop effective detection methods for accurately assessing their prevalence and significance in human disease.

**Description of Arcobacter cibarius sp. nov.**

*Arcobacter cibarius* (ci.ba’ri.us. L. adj. *cibarius* pertaining to food).

Cells are slightly curved, Gram-negative rods that are 1·5 μm long and 0·5 μm wide. They form whitish, low convex, non-swarming, smooth-rounded colonies with entire margins that are about 2 mm in diameter on blood agar after 72 h incubation at 28 °C under microaerobiotic conditions. Strains form translucent to opaque smooth-rounded colonies that are 1–2 mm in diameter on *Arcobacter* selective agar. In microaerobic conditions, growth is observed at room temperature (18–22 °C) and at 37 °C, but not at 42 °C. Weak growth is obtained in anaerobic conditions on both unsupplemented 5 % blood agar and blood agar containing 0·1 % (w/v) trimethylamine N-oxide. No growth is obtained at 37 °C in aerobic conditions; some (31 %) strains grow at 25 °C aerobically. No haemolysis is seen on blood agar. Strains produce oxidase and hydrolyse indoxyl acetate; some (54 %) strains show catalase activity. Alkaline phosphatase, urease, DNase and hippuricase activities are not detected. Nitrate, selenite and triphenyltetrazolium chloride are not reduced. Hydrogen sulfide is not produced in triple-sugar iron agar medium. Under microaerobic conditions, all strains grow on unsupplemented nutrient, minimal and potato-starch media and on media containing 0·02–0·05 % (w/v) safranin, 32 mg cephalothin litre−1, 32 mg carbenicillin litre−1, 64 mg ceftazidime litre−1, 0·032 % (w/v) methyl orange and 0·1 % (w/v) sodium deoxycholate. No growth is obtained on cassein medium or in media containing 1·0 % (w/v) glycine, 2·0–4·0 % (w/v) NaCl or 0·2 % (w/v) pyronin. Strains vary in their ability to grow on MacConkey, lecithin, tyrosine and *Campylobacter* charcoal-deoxycholate base media and on media containing 0·04 % (w/v) triphenyltetrazolium chloride, 0·1 % (w/v) potassium permanganate, 0·001 % (w/v) sodium arsenite, 32 mg nalidixic acid litre−1, basic fuchsin, crystal violet, Janus green and sodium fluoride.

The type strain, LMG 21996T, was isolated from the skin of a broiler carcass in Belgium in 2002. *A. cibarius* strains LMG 21996T, LMG 21997 and LMG 21998 have been deposited in the BCCM/LMG (Laboratorium voor Microbiologie, Gent, Belgium) culture collection. The type strain is also available from the CCUG (University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden) culture collection as CCUG 48482T.

**References**


