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Arcobacter-Specific and *Arcobacter butzleri*-Specific 16S rRNA-Based DNA Probes

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The genus *Arcobacter* encompasses gram-negative, aerotolerant, spiral-shaped bacteria formerly designated *Campylobacter cryaerophila*. Two genus-specific 16S rRNA-based oligonucleotide DNA probes (23-mer and 27-mer) were developed. The probes hybridized with strains of *Arcobacter butzleri* ($n = 58$), *Arcobacter cryaerophilus* ($n = 19$), and *Arcobacter skirrowii* ($n = 17$). The probes did not cross-react with any of the reference strains of *Campylobacter*, *Helicobacter*, including "*Flexispira rappini*," or *Wolinella*. The 27-mer hybridized with 61 *Arcobacter* spp. field isolates originating from late-term aborted porcine ($n = 54$) and equine ($n = 2$) fetuses and humans with enteritis ($n = 5$). The species of *Arcobacter* isolates ($n = 56$) recovered from aborted livestock fetuses were determined by ribotyping and were as follows: *A. cryaerophilus* group 1A (11 of 56; 20%), *A. cryaerophilus* group 1B (37 of 56; 66%), *A. butzleri* (5 of 56; 9%), and unknown (3 of 56; 5%). The five human field strains were identified as *A. butzleri*. A species-specific DNA probe (24-mer) for *A. butzleri* was also developed since there is evidence that this organism may be a human pathogen. This probe hybridized with previously characterized strains of *A. butzleri* ($n = 58$), with 10 field strains identified as *A. butzleri* by ribotyping and with 2 strains having an indeterminate ribotype. The *A. butzleri*-specific probe did not cross-react with strains of *A. skirrowii* ($n = 17$) and *A. cryaerophilus* ($n = 19$).

Arcobacter organisms were first isolated from aborted bovine and porcine fetuses as well as from control placentas by Ellis and others in the 1970s (7, 8, 11, 28–31). Subsequent isolations have been made from bulls (10), cows with mastitis (24), poultry (9), water (6, 9, 12, 41), and humans with enteritis (1, 21, 43, 48). Because both the morphology and the mole percent G+C content were similar to those of *Campylobacter* species, *Arcobacter* spp. were originally designated *Campylobacter cryaerophila* (28). However, their aerotolerance, their ability to grow at 30°C (30), and their fatty acid profiles (19) distinguished them from other species of *Campylobacter*.

Phylogenetic analysis showed that *C. cryaerophila* was a genetically distinct, although distantly related, species of *Campylobacter* (45). On the basis of DNA-DNA hybridization studies, Kiehlbauch et al. (16) recognized two subgroups of *C. cryaerophila* and a new species, designated "*C. butzleri*," which was recovered from humans with enteritis. "*C. butzleri*" and *C. cryaerophila* hybridization groups 1A and 1B could also be distinguished by restriction fragment length polymorphism (ribotype) upon hybridization with the cDNA probe for *Escherichia coli* 16S-23S rRNA (16, 17). Catalase activity, growth on MacConkey agar, and resistance to cadmium chloride distinguished "*C. butzleri*" from members of the *C. cryaerophila* complex (15). However, no battery of biochemical assays could differentiate the subgroups of *C. cryaerophila* (15). A proposed serotyping scheme for *C. cryaerophila* also reflected the heterogeneity within the species (2, 23).

In reevaluating the members of the genus *Campylobacter* by immunotyping, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), DNA-rRNA, and DNA-DNA hybridization studies, Vandamme et al. (46) proposed the genus *Arcobacter* for these aerotolerant campylobacter-like organisms and recognized four species: *Arcobacter nitrofigilis* (*Campylobacter nitrofigilis* [26]) from plants and *Arcobacter butzleri*, *Arcobacter skirrowii*, and *Arcobacter cryaerophilus* (49) from animals. A 16S rRNA sequence analysis confirmed that these four species were distinct members of a phylogenetically coherent group that was separate from species of *Campylobacter* and *Helicobacter* (22).

The evidence that *Arcobacter* spp. are pathogenic is based on their more frequent recovery from aborted pig litters and from infertile sows with vaginal discharge than from healthy animals (25). Experimental infection lowered conception rates in sows artificially inseminated with sperm containing *C. cryaerophila* (13) and caused inflammation only in the inoculated udder of a dairy cow (24). *A. butzleri* has been cultured from humans with enteritis who were otherwise healthy (35) and from patients with chronic underlying disease (21, 44). *A. butzleri* may be more prevalent in developing countries with inadequate water supplies since *A. butzleri* accounted for 16% of the *Campylobacter*-like isolates obtained from children with diarrhea in Thailand (43). Although risk factor analysis has implicated cases of human enteritis to the consumption of contaminated water during travel (18), person-to-person transmission may have resulted in an outbreak in a preschool (35, 47, 48). In animals, *A. butzleri* was isolated from diarrheic macaques with colonic lesions; no isolations were made from animals with normal colons (1). Cesarean-delivered, colostrum-deprived piglets experimentally infected with *A. butzleri* shed the organism in their feces until necropsy, at which time the organism was cultured from the ileum, liver, kidney, and brain; no such results were observed in piglets infected with *A. skirrowii* or *A. cryaerophilus* (50).

Because *Arcobacter* spp. may be found in the tissues of aborted livestock fetuses, can grow in microaerobic atmospheres, and are morphologically similar to *Campylobacter*

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spp., diagnostic laboratories may misidentify *Arcobacter* species as *Campylobacter* species. Arcobacters display campylobacter-like motility, do not ferment carbohydrates (28), and hydrolyze indoxyl acetate, a characteristic which is also exhibited by *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter upsaliensis* (34). Glycine tolerance is a major phenotypic feature of *Campylobacter fetus* subsp. *fetus*. However, glycine tolerance was also observed in 58% of strains of *A. butzleri*, in 78% of *A. skirrowii* strains, and in fewer than 25% of *A. cryaerophilus* strains examined in one study (49). Although species of *Arcobacter* are able to grow under microaerobic conditions, they become aerotolerant upon subculture, a characteristic also exhibited by *Campylobacter* species (29). Like *Campylobacter* species, differentiating the species of *Arcobacter* via classical phenotypic testing has produced variable results (49).

Recently our laboratory received several isolates of *Campylobacter*-like organisms from aborted porcine fetuses which were presumptively identified as unusual *C. fetus*-like organisms. Their failure to hybridize with either the probe for *C. fetus* (52) or the genus-specific probe for *Campylobacter* and their subsequent identification as *Arcobacter* spp. on the basis of phenotypic testing provided the impetus to develop specific DNA probes for the rapid identification of these newly described microbes. In addition, since *A. butzleri* has been associated with human enteritis and has been recovered from foods such as poultry (9) and ground pork (5), our goal was to develop a species-specific probe to expedite its identification.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. The American Type Culture Collection (ATCC), Rockville, Md., reference strains evaluated in the present study are listed in Table 1. Well-characterized strains of *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* were also used in the study to verify the specificities of the oligonucleotide probes. The following strains of *A. butzleri* were provided by C. M. Patton from the collection of the Centers for Disease Control and Prevention and have been described earlier (15): D0232, D1106, D1751, D1780, D2197, D2280, D2322, D2446, D2451, D2563, D2568, D2576, D2630, D2638, D2685, D2703, D2709c, D2709t, D2725, D2775, D2776, D2778, D2779, D2780, D2782, D2783, D2784, D2785, D2786, D2787, D2788, D2789, D2790, D2791, D2815, D2828, D2829, D2873, D2885, D2893, D2894, D2895, D2896, D2901, D2900, D2905, D2906, D2907, D2912, D2914, D2916, D2917, D2918, D2919, D2920, and D2921. The following strains of *A. cryaerophilus* were provided by C. M. Patton from the collection of the Center for Disease Control and Prevention and have been described earlier (16): D0460, D2077, D2079, D2080, D2081, D2610, D2639, D2883, D2884, D2887, D2888, D2891, and D2887. *A. cryaerophilus* CCUG 17802 and CCUG 17814 were obtained from the Culture Collection of the University of Goteburg, Goteburg, Sweden. The following strains of *A. skirrowii* were provided by Peter Vandamme from the Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, and have been described previously (49): LMG 8538, LMG 9800, LMG 9801, LMG 9802, LMG 9803, LMG 9911, LMG 9912, LMG 10234, LMG 10236, LMG 10238, LMG 10239, LMG 11072, LMG 11073, LMG 11074, LMG 11076, and LMG11078. *A. cryaerophilus* Neill 02748 was obtained from R. Ellis, Veterinary Research Laboratories, Belfast, Northern Ireland.

Two sets of field isolates of *Arcobacter* were examined in the study. Isolates from the first set were recovered from aborted porcine ($n = 56$) and equine ($n = 2$) fetuses and from humans with enteritis ($n = 5$). The livestock strains were part of the collection of the National Veterinary Services Laboratory, Animal Plant Health Inspection Service, and had been submitted by state laboratories for identification. Phenotypic characterization of these isolates was based on cellular morphology; motility; oxidase activity; hydrolysis of indoxyl acetate; catalase activity; glycine tolerance; growth at 25, 37, and 42°C; growth on MacConkey agar; and growth in 1.5 and 3.5% NaCl. Susceptibility to nalidixic acid and cephalothin was also used in species characterization (unpublished data). The second set of field isolates ($n \simeq 400$) was part of a study to determine the prevalence of *Arcobacter* spp. associated with porcine abortions in Iowa. No attempt was made to phenotype these strains via conventional biochemical methods.

TABLE 1. Results of hybridization of *Arcobacter* sp. probes (23-mer or 27-mer) with strains of *Arcobacter*, *Campylobacter*, *Helicobacter*, and *Wolinella*

Species[a]	Specificity	
	23-mer	27-mer
Arcobacter butzleri ATCC 49616	+	+
Arcobacter cryaerophilus ATCC 43158 group 1A	+	+
Arcobacter cryaerophilus ATCC 49615 group 1B	+	+
Arcobacter cryaerophilus ATCC 43159	+	+
Arcobacter skirrowii CCUG 10374	+	+
Arcobacter nitrofigilis ATCC 33309	+	−
Campylobacter coli ATCC 33559	−	−
Campylobacter concisus ATCC 33237	−	−
Campylobacter fetus subsp. *fetus* ATCC 27374	−	−
Campylobacter fetus subsp. *venerealis* ATCC 19438	−	−
Campylobacter hyointestinalis ATCC 35217	−	−
Campylobacter jejuni ATCC 33560	−	−
Campylobacter lari ATCC 35221	−	−
Campylobacter mucosalis ATCC 43264	−	−
Campylobacter mucosalis NCTC 1101	−	−
Campylobacter mucosalis NCTC 11420	−	−
Campylobacter sputorum biovar *bubulus* ATCC 33562	−	−
Campylobacter sputorum biovar *sputorum* ATCC 35980	−	−
Campylobacter sputorum biovar *fecalis* ATCC 33709	−	−
Campylobacter upsaliensis ATCC 443954	−	−
"*Flexispira rappini*"	−	−
Helicobacter cinaedi ATCC 35683	−	−
Helicobacter fennelliae ATCC 335684	−	−
Helicobacter mustelae ATCC 43772	−	−
Helicobacter pylori ATCC 43504	−	−
Wolinella curva ATCC 35224	−	−
Wolinella rectus ATCC 33238	−	−
Wolinella succinogenes ATCC 29543	−	−

[a] Abbreviations used for culture collections: ATCC, American Type Culture Collection; CCUG, Culture Collection, University of Goteburg; NCTC, National Collection of Type Cultures.

Bacteria were grown on brain heart infusion agar with 10% defibrinated bovine blood and were incubated microaerobically (37°C, 48 h).

16S rRNA sequence analysis. RNA was isolated and partially purified by a modification of the procedure of Lane et al. (20), as described earlier (33). A complete 16S rRNA sequence was determined for *A. cryaerophilus* ATCC 49615 and was compared with previously published sequences of the strains of *Arcobacter*, *Campylobacter*, and *Helicobacter* (32). Programs for data entry, editing, sequence alignment, secondary structure comparison, similarity matrix generation, and phylogenetic tree construction were written in Microsoft Quick BASIC for use on an International Business Machines personal computer (PC-AT) and compatible computers. Our sequence database contains approximately 400 sequences consisting of those determined in our laboratory, published sequences, and those available from the Ribosomal Database Project (32).

Similarity matrices were constructed from the aligned sequences by using only those strains for which 90% of sequence positions were known. The similarity matrices were corrected for multiple base changes by the method of Jukes and Cantor (14). Phylogenetic trees were constructed by the neighbor-joining method of Saitou and Nei (38). Nucleic acid sequences which were selected for use as probes were identified by alignment of 16S rRNA sequences, identification of common bases, and selection of regions where mismatches occurred (33).

GenBank accession numbers. The sequences for *A. cryaerophilus* ATCC 49615, *A. butzleri* CCUG 10370, and reference strains are available for electronic retrieval from the EMBL, GenBank, and DDBJ nucleotide sequence databases under the indicated accession numbers, which are given in parentheses: *A. cryaerophilus* ATCC 49615 (U25805), *A. butzleri* CCUG 10370 (L14262), *A. cryaerophilus* CCUG 17801^T (L14614), *A. nitrogifilis* CCUG 15893 (L14627), *A. skirrowii* CCUG 10374 (L14625), [*Bacteroides*] *gracilis* ATCC 33236^T (L04320), [*Bacteroides*] *ureolyticus* ATCC 33387^T (L04321), *C. coli* CCUG 11283^T (L04312), *Campylobacter concisus* Forsyth Dental Center 484^T (L04322), *Campylobacter curvus* ATCC 35224^T (L04313), *C. fetus* subsp. *fetus* ATCC 27374^T (L04314), *Campylobacter hyointestinalis* ATCC 35217 (M65010), *C. jejuni* CCUG

11284^T (L04315), *C. jejuni* subsp. *doylei* CCUG 24567^T (L14630), *Campylobacter lari* CCUG 23947^T (L04316), *Campylobacter mucosalis* CCUG 6822^T (L06978), *Campylobacter rectus* ATCC 33239^T (L04317), *Campylobacter showae* CCUG 30354 (L06974), *Campylobacter* sp. strain 40-6AT^b (L04318), *Campylobacter* urease positive thermophilic sp. strain CCUG 18267 (L14631), *Campylobacter sputorum* subsp. *bubulus* ATCC 33491 (L04319), *C. upsaliensis* CCUG 14913^T (L14628), *Helicobacter felis* ATCC 49179^T (M37642), *Helicobacter muridarum* CCUG 29262^T (M80205), *Helicobacter mustelae* ATCC 43772 (M35048), *Helicobacter pylori* ATCC 43504T (M88157), and *Wolinella succinogenes* ATCC 29543 (M88159).

Extraction of chromosomal DNA. Bacterial genomic DNA was purified as described previously (50). DNA (2 μg) was digested (3 to 4 h, 37°C) with *Pvu*II in a 20-μl reaction mixture in buffers supplied by the manufacturer (Bethesda Research Laboratories, Gaithersburg, Md.). After digestion, 5 μl of tracking dye (0.1% bromphenol blue, 20% Ficoll type 400) was added to each sample, and DNA fragments were separated on 1.0% agarose (16 h, 60 V) in a horizontal gel bed (120 by 25 cm). Reference strains of *A. butzleri* and *A. cryaerophilus* groups 1A and 1B were included on each gel to facilitate comparison of field strains with these internal standards. A *Hin*dIII digest of bacteriophage lambda was included as a kilobase standard marker. At the completion of electrophoresis, gels were stained (1 h) with ethidium bromide (0.25 mg/ml), visualized with shortwave UV light, and photographed by using a red filter (51).

Nucleic acid hybridization. For slot blot hybridization, purified DNA (2 μg) was applied to each well of a nylon membrane (Gene-Screen; NEN Research, Boston, Mass.) in a Bio-Dot SF microfiltration apparatus (Bio-Rad, Richmond, Calif.). The DNA was denatured (0.5 M NaOH, 1.5 M NaCl), neutralized (1 M Tris, 3 M NaCl [pH 5.5]), and UV irradiated in a UV Stratalinker 1800 (Strategene, La Jolla, Calif.) to covalently bind the DNA to the membrane filters (4). Membranes were prehybridized and hybridized as follows. Prehybridization (3 h at 37°C) was carried out in 6× SSC (0.90 M NaCl plus 0.090 M sodium citrate), 5× Denhardt's solution (0.5% Ficoll, 0.1% polyvinylpyrrolidone, 0.2% bovine serum albumin), and 0.5% SDS with 100 mg of sonicated denatured calf thymus DNA per ml at 37°C (38). The hybridization solution, prepared as described previously (39), contained 10^6 cpm of the ^{32}P-end-labelled oligonucleotide probe. After overnight incubation (37°C), filters were washed once briefly in 2× SSC–0.1% SDS at room temperature; this was followed by two stringency washes (6× SSC, 55°C) of 1 h each. Dried filters were exposed to Kodak X-Omat film with two intensifying screens (−80°C, 2 to 7 days). Films were developed in the X-Omat Film Processor (Kodak, Rochester, N.Y.).

For species identification by ribotyping, *Pvu*II-digested DNA was blotted onto nylon membranes (Gene-Screen; NEN Research) by the method of Southern (40) and was UV cross-linked as described previously (4). To detect the presence of 16S rRNA genes, nylon filters were prehybridized for 3 h and were then hybridized (18 h, 37°C) with the ^{32}P-end-labelled probe for the genus *Arcobacter* (10^7 cpm) and washed as described above.

For dot blot hybridization, 20 to 25 μl of a cell suspension (<0.5 McFarland standard) was added in duplicate to each well of a 96-well Bio-Dot microfiltration apparatus (Bio-Rad). The dried membrane was placed (2 min) on filters moistened with denaturing solution (0.5 M NaOH, 1.5 M NaCl) to lyse the cells. The membrane was then placed on filters saturated with neutralizing solution (1 M Tris, 3 M NaCl [pH 5.5] for 5 min) and UV cross-linked in a UV Stratalinker 1800 (Strategene). Prehybridization, hybridization (2×10^6 cpm per filter), and stringency washings were carried out as described above.

Oligonucleotides were synthesized at the Nucleic Acid Facility of Iowa State University, Ames, and were end-labelled with [γ-^{32}P]ATP by the T4 polynucleotide kinase reaction (36).

RESULTS

Approximately 95% of the total 16S rRNA sequence for *A. cryaerophilus* ATCC 49615 was compared with previously published sequences of the species of *Arcobacter*, *Campylobacter*, and *Helicobacter*. Table 2 shows a similarity matrix derived from 1,427 base comparisons for these strains. A dendrogram constructed from the similarity matrix is shown in Fig. 1. As demonstrated in previous studies (42, 46, 49), the arcobacters do warrant a genus designation separate from those of the campylobacters and helicobacters. The average level of similarity between the arcobacters and campylobacters was approximately 86%, and that between the arcobacters and helicobacters was approximately 85%. The four species of *Arcobacter* form a coherent group, with interspecies levels of similarity of approximately 94% or greater. The two strains of *A. cryaerophilus* were closely related at 99.4% sequence similarity. Further investigation, such as DNA-DNA hybridization among strains representing the two serotypes of *A. cryaerophilus*, is needed to correlate serotype with separate taxonomic status, such as at the subspecies or species level.

***Arcobacter* genus-specific probe.** An *Arcobacter*-specific oligonucleotide DNA probe was designed from the aligned sequences of the two strains each of *A. cryaerophilus*, *A. butzleri*, and *A. skirrowii*. This probe was developed before the sequence of *A. nitrofigilis* was available. This 27-base probe (5′-GAT-ACA-ATA-CAG-GCT-AAT-CTC-TAA-CCA-3′) was derived from a target region, beginning at nucleotide position 219. Subsequently, sequence analysis of *A. nitrofigilis* showed significant base differences beginning at position 1426. Thus, a 23-base oligonucleotide probe (5′-TTT-AGC-ATC-CCC-GCT-TCG-AAT-GA-3′) was designed as the more broadly reactive genus-specific probe for *Arcobacter* species.

Probe specificity was evaluated in a slot blot format against genomic DNAs of reference strains of species and subspecies of *Arcobacter*, *Campylobacter*, *Helicobacter*, and *Wolinella* (Table 1). As predicted, the 27-mer hybridized with *A. butzleri*, *A. cryaerophilus* DNA hybridization groups 1A and 1B, and *A. skirrowii*, but not with *A. nitrofigilis*. The 23-mer reacted with the type strains of *A. butzleri*, *A. cryaerophilus* of hybridization groups 1A and 1B, *A. skirrowii*, and *A. nitrofigilis*. Neither probe reacted with any of the reference strains of the closely related *Campylobacter*, *Helicobacter*, including our laboratory strain of "*Flexispira rappini*," or *Wolinella* species.

The sensitivity of the *Arcobacter* genus-specific probe (27-mer) was further evaluated in a slot blot format by using strains of *A. butzleri* ($n = 58$), *A. skirrowii* ($n = 17$), and *A. cryaerophilus* ($n = 18$). These strains had been identified and characterized previously by DNA hybridization (16, 48). As indicated in Table 1, the 27-mer reacted with all of these characterized strains of *Arcobacter. A. cryaerophilus* ATCC 43159, which had not been verified to the species level by hybridization studies, also reacted with the probe.

The *Arcobacter* probe (27-mer) was used in a cell suspension dot blot format, which did not require extensive DNA extraction. This format was adapted to screen field strains ($n \simeq 400$) that had been recovered during a survey to determine the number of *Arcobacter*-associated porcine abortion cases in Iowa. Isolates were presumptively identified as an *Arcobacter* sp. on the basis of initial growth in P-80 semisolid isolation medium and corkscrew motility under dark-field microscopy. An example is shown in Fig. 2. Cell suspensions of the 3 *Arcobacter* reference strains (positive controls) and 23 of the 24 field strains hybridized with the probe. Bacterial isolates ($n = 3$) which were recovered in P-80 isolation medium but which did not display typical campylobacter- and arcobacter-like morphology under dark-field microscopy (negative controls) did not hybridize with the genus-specific probe.

The 23-mer, *Arcobacter*-specific probe was used in a slot blot format to screen field strains ($n = 67$) isolated in the present study. The probe hybridized with DNA extracted from 63 of the 67 of these field strains of porcine ($n = 56$), equine ($n = 2$), or human ($n = 5$) origin. The *Arcobacter*-specific oligonucleotide hybridized equally well with isolates biochemically identified as *A. butzleri* and *A. cryaerophilus* and with those aerotolerant campylobacters with indeterminate phenotypes. Strains which did not hybridize were subsequently identified as *Pseudomonas* spp. ($n = 2$) or *C. jejuni* ($n = 1$) or were not identified further ($n = 1$).

The species of the 63 field strains which hybridized with the *Arcobacter* probe were determined on the basis of the presence of key restriction fragments detected after hybridization of *Pvu*II-restricted DNA with either the 23-mer or the 27-mer probe. Identical patterns were obtained following hybridization with either probe. The criteria used for species identifica-

TABLE 2. Homology matrix based on 16S rRNA sequences of *A. cryaerophilus*, *A. cryaerophilus* group 1B, *A. butzleri*, *A. skirrowii*, and other closely related bacteria

Strain	% Similarity and % difference compared with[a]:																									
	Acr1	*Acr2*	*Ask*	*Abu*	*Ani*	*Cfe*	*Chy*	6AT	*Cup*	*Cco*	*Cje*	*Cjd*	*Cla*	UPT	*Cmu*	*Ccn*	*Ccu*	*Csp*	*Cre*	*Csh*	*Bgr*	*Bur*	*Hpy*	*Hfe*	*Hmu*	*Hms*	*Wsu*
Arcobacter cryaerophilus		99.4	98.7	97.2	93.8	86.3	86.4	85.5	85.8	86.5	87.1	87.0	86.9	87.2	86.9	87.2	86.3	87.0	85.8	86.1	86.0	86.6	84.5	83.8	85.1	86.2	85.7
Arcobacter cryaerophilus group 1B	0.6		99.1	97.1	94.0	86.3	86.3	85.5	85.7	86.4	87.0	86.9	86.8	87.2	86.8	87.3	86.3	87.0	85.5	86.1	85.8	86.6	84.4	83.7	85.1	86.2	85.7
Arcobacter skirrowi	1.4	0.9		97.0	94.0	86.0	86.0	85.3	85.6	86.4	86.9	86.9	86.6	86.9	86.7	87.3	86.3	87.0	85.2	85.7	85.6	86.4	84.2	83.6	84.8	85.8	85.4
Arcobacter butzleri	2.8	3.0	3.0		94.2	86.2	86.1	85.1	86.1	86.0	86.5	86.5	86.3	86.6	86.5	87.6	87.0	86.8	86.1	86.1	86.6	86.0	85.5	84.6	86.0	86.7	86.2
Arcobacter nitrofigilis	6.5	6.2	6.3	6.0		85.3	85.4	84.8	85.3	85.8	86.3	86.2	86.2	86.6	85.7	86.8	86.4	85.7	84.8	84.7	84.6	85.4	83.4	83.5	84.6	85.1	85.2
Campylobacter fetus subsp. *fetus*	15.2	15.2	15.5	15.2	16.4		98.1	95.5	93.0	95.5	94.5	94.3	94.2	94.3	96.2	95.1	94.3	93.3	93.9	92.7	93.7	92.7	85.2	84.7	85.8	86.0	86.0
Campylobacter hyointestinalis	15.0	15.1	15.5	15.4	16.3	1.9		95.9	92.8	95.7	94.7	94.6	94.4	94.6	94.9	94.1	93.4	92.5	93.0	91.8	92.7	92.8	84.5	83.7	85.3	85.7	85.3
Campylobacter sp. strain 40 6AT	16.1	16.1	16.4	16.6	17.0	4.6	4.2		92.9	96.2	95.2	95.2	94.7	94.8	93.7	93.6	94.0	93.0	92.9	91.6	91.4	92.0	84.3	83.8	85.2	85.5	85.5
Campylobacter upsaliensis	15.8	15.9	16.0	15.4	16.3	7.3	7.6	7.5		94.8	95.8	95.7	95.3	95.3	92.9	92.0	92.0	91.6	91.5	90.6	90.5	91.3	86.0	86.0	86.4	86.7	85.9
Campylobacter coli	14.9	15.0	15.1	15.5	15.7	4.6	4.4	3.9	5.4		98.6	98.5	97.7	97.8	94.2	93.9	93.4	93.0	93.2	92.1	91.5	93.3	85.2	84.5	85.9	86.5	86.2
Campylobacter jejuni	14.2	14.3	14.4	14.9	15.1	5.8	5.5	5.0	4.3	1.4		99.9	98.7	99.3	93.7	93.2	92.7	92.4	92.5	91.4	91.0	92.9	85.4	85.0	85.9	86.8	86.3
Campylobacter jejuni subsp. *doylei*	14.3	14.4	14.4	14.9	15.2	5.9	5.6	5.0	4.4	1.5	0.1		98.5	99.2	93.7	93.1	92.6	92.4	92.3	91.3	91.0	92.8	85.3	84.9	85.9	86.7	86.2
Campylobacter lari	14.4	14.5	14.8	15.2	15.2	6.1	5.8	5.5	4.8	2.4	1.4	1.5		99.3	93.5	93.5	92.2	92.0	92.1	91.1	90.8	92.5	85.6	85.4	86.3	87.3	86.7
Campylobacter sp. UPTC[b]	14.0	14.1	14.3	14.7	14.8	5.9	5.6	5.4	4.9	2.2	0.8	0.8	0.7		96.3	93.5	92.2	92.2	92.2	91.1	91.0	92.6	85.5	85.3	86.4	87.1	86.6
Campylobacter mucosalis	14.4	14.5	14.7	14.9	15.8	3.9	5.3	6.5	7.4	6.0	6.5	6.6	6.8	6.7		95.8	94.7	93.4	94.1	93.3	93.4	92.2	84.9	84.8	85.5	85.6	85.7
Campylobacter concisus	14.0	13.9	13.9	13.5	14.6	5.0	6.2	6.7	8.5	6.4	7.1	7.2	6.8	6.8	4.3		97.0	95.0	95.4	94.4	94.4	91.5	85.1	84.7	86.3	86.1	86.3
Campylobacter curvus	15.1	15.1	15.2	14.3	15.0	5.9	6.9	6.2	8.4	6.9	7.7	7.8	8.2	8.2	5.4	3.1		95.1	96.5	96.2	94.5	90.8	85.1	84.2	85.3	85.8	86.9
Campylobacter sputorum	14.3	14.3	14.3	14.5	15.8	7.0	7.9	7.4	9.0	7.4	8.0	8.1	8.4	8.2	6.9	5.2	5.0		95.0	95.0	94.0	91.9	85.6	85.0	86.6	87.3	86.3
Campylobacter rectus	15.8	16.1	16.5	15.3	16.9	6.4	7.4	7.5	9.0	7.2	8.0	8.1	8.3	8.2	6.1	4.7	3.6	5.1		98.0	95.3	91.7	84.0	83.1	84.8	84.8	86.1
Campylobacter showae	15.4	15.4	15.8	15.4	17.1	7.6	8.7	8.9	10.0	8.3	9.1	9.3	9.5	9.4	7.0	5.8	3.9	5.1	2.0		94.3	91.1	84.3	83.5	85.0	84.9	86.2
[*Bacteroides*] *gracilis*	15.4	15.8	16.0	14.8	17.2	6.6	7.7	9.1	10.1	9.0	9.6	9.6	9.9	9.6	6.9	5.8	5.7	6.2	4.9	5.9		91.4	83.8	82.8	84.0	84.4	85.1
[*Bacteroides*] *ureolyticus*	14.8	14.8	15.0	15.5	16.3	7.6	7.6	8.4	9.2	7.0	7.5	7.6	7.9	7.8	8.3	9.0	9.8	8.6	8.8	9.5	9.2		84.1	83.6	84.8	85.7	85.7
Helicobacter pylori	17.3	17.5	17.7	16.2	18.8	16.5	17.3	17.7	15.5	16.5	16.2	16.4	16.0	16.2	16.9	16.6	16.6	16.0	18.0	17.6	18.2	17.9		95.4	93.0	93.8	90.8
Helicobacter felis	18.3	18.3	18.5	17.3	18.6	17.1	18.4	18.3	15.5	17.3	16.8	16.8	16.2	16.4	17.0	17.1	17.8	16.7	19.1	18.6	19.5	18.4	4.7		92.7	93.5	90.3
Helicobacter muridarum	16.7	16.6	17.0	15.5	17.3	15.8	16.4	16.4	15.0	15.6	15.6	15.6	15.1	14.9	16.1	15.1	16.3	14.8	17.0	16.7	18.0	17.0	7.3	7.6		95.8	93.4
Helicobacter mustelae	15.3	15.3	15.7	14.7	16.6	15.5	15.9	16.1	14.6	14.9	14.5	14.7	14.0	14.2	16.0	15.4	15.7	13.9	17.0	16.9	17.5	15.9	6.5	6.8	4.3		93.8
Wolinella succinogenes	15.9	15.8	16.2	15.2	16.5	15.5	16.3	16.1	15.6	15.2	15.1	15.3	14.6	14.7	15.9	15.1	14.4	15.2	15.4	15.3	16.6	15.9	9.8	10.4	7.0	6.5	

[a] Numbers above the diagonal space represent percentages of similarity, and those below the diagonal space are percentages of difference corrected for multiple base changes. The designations in the column heads (from left to right) represent the strains listed on the left from top to bottom, respectively.

[b] UPTC, urease-positive thermophilic campylobacter.

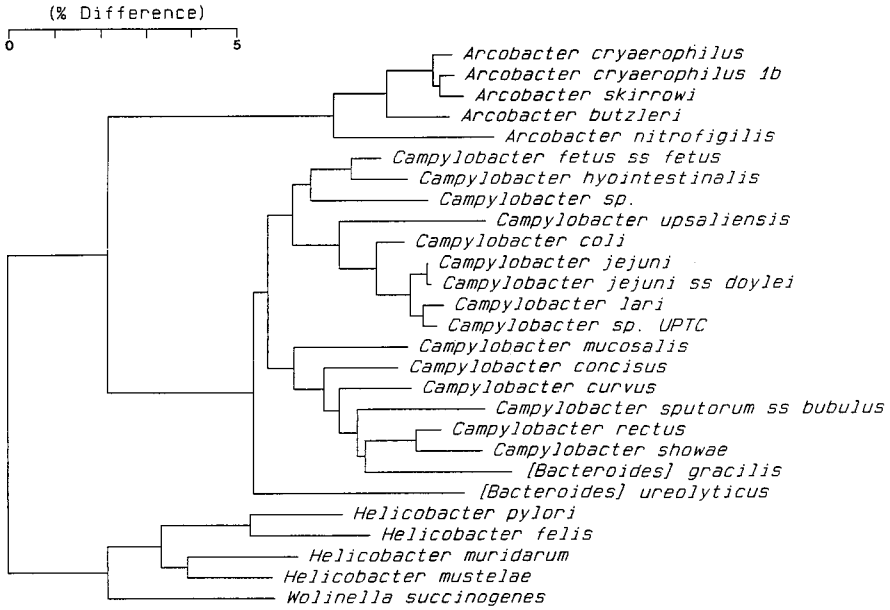

FIG. 1. Phylogenetic tree showing placements of *A. cryaerophilus*, *A. butzleri* and *A. skirrowii* on the basis of 16S rRNA sequence data analysis. The scale bar represents a 5% difference in nucleotide sequence, as determined by measuring the lengths of the horizontal lines connecting any two species. UPTC, urease-positive thermophilic campylobacter.

tion were those described earlier following hybridization with the *E. coli* 16S-23S rRNA probe (18). For *A. butzleri* ATCC 49616, four restriction fragments (~20.8, 15.7, 6.6, and 3.0 kb) hybridized with the 16S rRNA probe. For *A. butzleri* ATCC 43157, a remarkably different pattern was detected, with hybridization occurring with five restriction fragments (~6.5, 5.0, 4.0, 3.4, and 3.0 kb). The ~3.0-kb restriction fragment was detected in 53 of 58 (91%) of the characterized *A. butzleri* strains. Therefore, *A. butzleri* isolates could be identified by the presence of an ~3.0-kb restriction fragment (Fig. 3, lane 1). For *A. cryaerophilus* ATCC 43158 group 1A, five restriction fragments (~8.4, 7.0, 6.2, 4.5, and 3.2 kb) hybridized with the genus-specific probe. Of the characterized strains of *A. cryaerophilus* group 1A (n = 9), six exhibited the ~3.2-kb restriction fragment typical of that group. Isolates could therefore be identified as members of group 1A on the basis of an ~3.2-kb restriction fragment (Fig. 3, lane 2). For *A. cryaerophilus* ATCC 49615 group 1B, five restriction fragments were evident after hybridization (~26.3, 18.4, 3.6, 3.4, and 2.6 kb; Fig. 3, lane 3). Seven of the nine characterized *A. cryaerophilus* group 1B strains exhibited the ~3.2- and 2.6-kb restriction fragments typical of the group. Isolates were therefore identi-

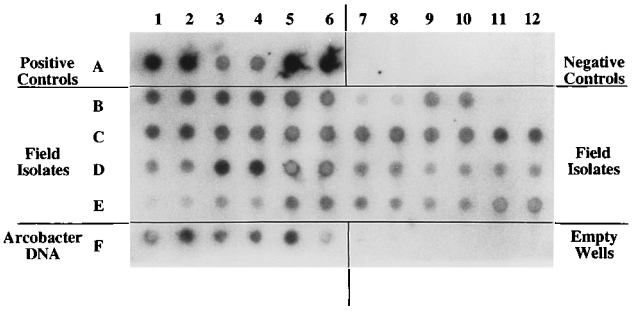

FIG. 2. Cell suspension dot blot hybridization of field strains of *Arcobacter* spp. recovered from aborted porcine fetuses. Each sample was analyzed in duplicate. Note hybridization (positive controls) of the *Arcobacter* probe with cell suspensions of reference strains of *A. butzleri* (wells A1 and A2), *A. cryaerophilus* (wells A3 and A4), and *A. skirrowii* (wells A5 and A6). Cell suspensions of the 23 of 24 field strains (wells B1 through E12), which were presumptively identified as *Arcobacter* spp. on the basis of dark-field microscopy, hybridized with the probe. Note the failure of the probe to hybridize with a field strain (wells B11 and B12). Bacteria which did not display typical *Campylobacter*-like motility (negative controls) failed to hybridize (wells A7 to A12). The probe also hybridized with DNAs of reference strains of *Arcobacter* spp. (wells F1 to F6). Wells F7 to F12 are empty.

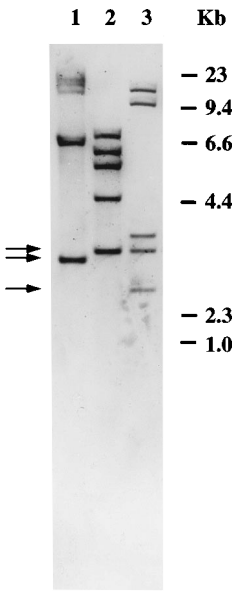

FIG. 3. Patterns of ATCC reference strains following Southern blot hybridization with the *Arcobacter* probe of *Pvu*II-restricted DNA. Note the ~3.0-kb restriction fragment characteristic of *A. butzleri* (middle arrow, lane 1). The ~3.2-kb restriction fragment typical of *A. cryaerophilus* group 1A (upper arrow, lane 2) and the ~3.2- and 2.6-kb restriction fragments characteristic of *A. cryaerophilus* group 1B (upper and lower arrows, lane 3) are indicated.

fied as members of group 1B on the basis of the presence of both the ~3.2- and 2.6-kb restriction fragments. Hybridization patterns therefore indicated that a minimum of four genes encode 16S rRNA.

Next, the species of field isolates of *Arcobacter* were determined on the basis of the presence of key restriction fragments by using the criteria described above for the characterized strains. Reference strains of *A. butzleri* and *A. cryaerophilus* groups 1A and 1B were included as internal standards on each gel to facilitate comparison. On the basis of the presence of the ~3-kb restriction fragment, 10 field strains were identified as *A. butzleri*. These strains were isolated from aborted porcine fetuses ($n = 5$) and humans with enteritis ($n = 5$). The hybridization pattern (ribotype) for each field strain of *A. butzleri* was distinctive. The ~3.2-kb restriction fragment indicative of *A. cryaerophilus* was present in 48 of the 63 probe-positive field strains. These isolates of *A. cryaerophilus* ($n = 48$) were further subtyped on the basis of the absence (hybridization group 1A) or presence (hybridization group 1B) of the additional ~2.6-kb restriction fragment. Eleven isolates, all recovered from aborted porcine fetuses, were identified as *A. cryaerophilus* hybridization group 1A. The remaining 37 isolates were identified as *A. cryaerophilus* hybridization group 1B. The majority of these isolates were from aborted porcine fetuses ($n = 35$); two were recovered from aborted equine fetuses. Three probe-positive isolates from porcine fetuses exhibited patterns atypical of either *A. cryaerophilus* or *A. butzleri*. The biochemical reactions of these three field strains were characteristic of *A. cryaerophilus*. Two strains were lost prior to ribotyping analysis.

***A. butzleri* probe.** Although ribotyping proved useful for species identification, it is laborious and not adaptable to conventional diagnostic laboratories. Furthermore, the ~3-kb restriction fragment which we used to initially identify *A. butzleri* was also detected in 14 of 17 characterized *A. skirrowii* isolates, including the ATCC reference strain. Thus, a cDNA probe complementary to 16S rRNA was designed for unequivocal identification of *A. butzleri* isolates. The *A. butzleri* probe was constructed from a target sequence beginning at nucleotide position 984 (5′-CTA-AAT-CAT-TCT-TAC-TAT-GTC-AAG-3′). The probe hybridized in a slot blot format with *A. butzleri* strains ($n = 58$) previously identified as such by DNA hybridization (15). The probe did not react with characterized strains of *A. cryaerophilus* ($n = 19$), *A. skirrowii* ($n = 17$), or *A. nitrofigilis* ($n = 1$). The validated *A. butzleri* probe was then used in a slot blot format to screen the 63 field strains of *Arcobacter*. Ten of these strains reacted with the *A. butzleri* probe. Of these, eight had been identified as *A. butzleri* by ribotyping with the *Arcobacter* genus-specific probe. The *A. butzleri* probe reacted with two of the field strains which could not be identified by restriction fragment length polymorphism (RFLP) patterns after hybridization with the *Arcobacter* sp. probe. None of the field isolates which exhibited the *A. cryaerophilus* ribotype annealed with the *A. butzleri* probe.

Patterns of *Pvu*II-restricted DNA of the characterized strains of *A. butzleri* were compared following hybridization with the probes for *Arcobacter* sp. and *A. butzleri*. An example is shown in Fig. 4. The patterns exhibited by characterized strains of *A. butzleri* were identical following hybridization with either probe and indicated a minimum copy number of four genes encoding 16S rRNA. The ~3.0-kb restriction fragment typical for *A. butzleri* was evident in 53 of 58 (91%) of the characterized *A. butzleri* strains following hybridization with either probe.

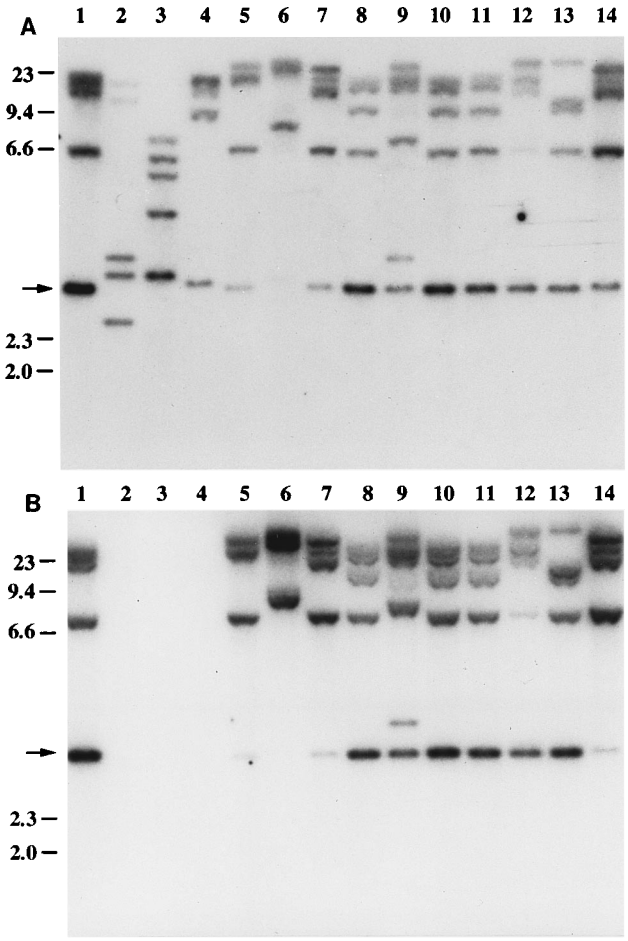

FIG. 4. Strains of *A. butzleri* cleaved with *Pvu*II. Reference strains of *A. butzleri* ATCC 49616 (lanes 1), *A. cryaerophilus* ATCC 43158 group 1A (lanes 3), *A. cryaerophilus* ATCC 49615 group 1B (lanes 2), and *A. skirrowii* ATCC 51132 (lanes 4) are included for comparison. Hybridization patterns are shown for *A. butzleri* 3573 (lanes 5), 3572 (lanes 6), 3571 (lanes 7), 3570 (lanes 8), 3569 (lanes 9), 3568 (lanes 10), 3567 (lanes 11), 3566 (lanes 12), 3565 (lanes 13), and 3564 (lanes 14) from the National Animal Disease Center. Note the ~3.0-kb restriction fragment (arrows) in all strains except strain 3572 from the National Animal Disease Center (lanes 6). (A) Patterns following hybridization with the *Arcobacter* genus-specific probe. (B) Patterns following hybridization with the *A. butzleri*-specific probe. The *A. butzleri* probe hybridizes only with strains of *A. butzleri*. Although faint, the ~3.0-kb fragment is present in lanes 5 and 14. No reaction is seen with *A. cryaerophilus* group 1A (lanes 2), *A. cryaerophilus* group 1B (lanes 3), or *A. skirrowii* (lanes 4). Sizes (in kilobases) were obtained by using *Hin*dIII digest of bacteriophage lambda as the standard.

DISCUSSION

Nucleic acid sequencing of the highly conserved 16S rRNA molecules has allowed phylogenetic appraisal of the *Arcobacter* species. Comparison of approximately 95% of the total 16S rRNA sequence for *A. cryaerophilus* ATCC 49615 with published sequences of the species of *Arcobacter*, *Campylobacter*, and *Helicobacter* confirmed that the arcobacters warrant a separate genus designation (37, 42, 44). The average levels of similarity between the arcobacters and campylobacters and between the arcobacters and helicobacters were approximately 85%. The 16S rRNA sequence data indicated that the species of *Arcobacter* form a coherent group, with interspecies levels of similarity of no less than 93.8%. *A. nitrofigilis*, which is the type strain and is a nitrogen-fixing bacterium (26), was the most distantly related. *A. butzleri*, *A. skirrowii*, and *A. cryaerophilus*,

which have been associated with disease in humans and animals, shared a 97% level of similarity. The two subgroups of *A. cryaerophilus*, which cannot be distinguished by routine laboratory assays but which are differentiated by SDS-PAGE and RFLP analysis, exhibited a 99.4% sequence homology and are the most closely related. Selection of 16S rRNA sequences which differentiate *Arcobacter* organisms from other *Campylobacter*-like organisms has resulted in the design of highly specific nucleic acid probes (23-mer and 27-mer) which are broadly reactive with all species of *Arcobacter*.

Because of the fastidious growth requirements for *Arcobacter* spp., their relative metabolic inertness, and the limited number of reliable phenotyping tests (15, 49), the 16S rRNA probes for *Arcobacter* spp. would be useful in diagnostic laboratories which routinely process *Campylobacter*-like organisms. Correct identification of *Arcobacter* species is required in order to ascertain the host distribution, pathogenicity, and epidemiology of these recently described microbes. For example, in cases of livestock abortion, *Campylobacter* spp. are identified on the basis of motility by dark-field microscopy and evidence of herd infertility; additional phenotyping is limited to determining catalase activity, H_2S production, and glycine tolerance (3). On the basis of these criteria, the percentage of field strains of *Arcobacter* spp. which could be misidentified as species of *Campylobacter* cannot be estimated. However, the impetus for the present study was the receipt of several isolates recovered from aborted livestock fetuses which were sent to us for confirmation as *C. fetus*. These isolates were ultimately identified in our laboratory as *Arcobacter* spp. following hybridization with the *Arcobacter* genus-specific probe.

The genus-specific probe expedited confirmation of *Arcobacter* spp. during a year-long prevalence survey to determine its association with porcine abortions in Iowa. Cell suspension dot blot hybridization, which uses whole cells and bypasses the need for extensive DNA extraction, expedited the screening of the nearly 2,000 fetal tissues from 400 aborted porcine fetuses which were examined (53). A digoxigenin-based chemiluminescent detection system was found to be as sensitive as probing with radiolabelled probes, thus increasing the adaptability of this method to routine laboratory use.

Multiple copies of 16S rRNA genes have been described for the genus *Campylobacter* (22, 27, 37, 52). Hybridization of *Pvu*II-digested *Arcobacter* chromosomal DNA with the genus-specific *Arcobacter* probe identified three to seven genes encoding 16S rRNA for *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*. Furthermore, the presence of key restriction fragments was used in species identification when the criteria described earlier following hybridization with *E. coli* 16S-23S rRNA were used (17). The presence of these distinctive hybridization fragments identified isolates recovered from aborted porcine fetuses as *A. cryaerophilus* group 1B (66%), *A. cryaerophilus* group 1A (20%), and *A. butzleri* (9%), and thus assigned the majority of the isolates to the *A. cryaerophilus* complex.

Sequence analysis of *A. butzleri* 16S RNA indicated its phylogenetic distance from *A. cryaerophilus* and also the relative ease of designing a species-specific probe. The association of *A. butzleri* with enteritis in humans (15, 21, 44, 48) and animals (1) indicated its potential public health importance. The resultant *A. butzleri* probe (24-mer) hybridized only with previously characterized strains of *A. butzleri* and did not react with reference strains of *A. cryaerophilus*, *A. skirrowii*, or *A. nitrofigilis*. The probe verified that 9% of the isolates from aborted porcine fetuses examined in the study were *A. butzleri*, including isolates which exhibited atypical biochemical profiles and unusual patterns following hybridization with the *Arcobacter* genus-specific probe. Cell suspension dot blot hybridization with either the ^{32}P-labelled or the digoxigenin-labelled probe confirmed the presence of *A. butzleri* in 22% of nearly 300 ground pork samples examined (5). This rapid screening method identified *A. butzleri* without the need for extensive phenotypic testing.

RFLP analysis and PCR-based subtyping methods have shown the identities of *Arcobacter* spp. strains in an outbreak (17, 47). The multiple profiles evident upon hybridization with the *Arcobacter* spp. and *A. butzleri* probes indicate the suitability of RFLP as a subtyping method for *Arcobacter* spp.

Arcobacter spp. are included on the list of International Classification of Diseases (ICD-9), although there is limited information regarding their pathogenicities. The 16S rRNA probes for *Arcobacter* spp. and *A. butzleri* will accelerate the identification and elucidate the prevalence of these recently described microbes of potential veterinary and medical significance.

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REFERENCES

1. **Anderson, K. F., J. A. Kiehlbauch, D. C. Anderson, H. M. McClure, and I. K. Wachsmuth.** 1993. *Arcobacter* (*Campylobacter*) *butzleri*-associated diarrheal illness in a nonhuman primate population. Infect. Immun. **61:**2220–2223.
2. **Boudreau, M., R. Higgins, and K. R. Mittal.** 1991. Biochemical and serological characterization of *Campylobacter cryaerophila*. J. Clin. Microbiol. **29:**54–58.
3. **Bryner, J. H.** 1990. Bovine abortion caused by *Campylobacter fetus*, p. 70–81. *In* C. A. Kirkbride (ed.), Laboratory diagnosis of livestock abortion. Iowa State University Press, Ames.
4. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA **81:**1991–1995.
5. **Collins, C. I., I. V. Wesley, and E. A. Murano.** 1994. Incidence of *Arcobacter* spp in ground pork. Dairy, Food Environ. Sanit. **14:**603.
6. **Dhamabutra, N., P. Kamol-Rathanakul, and K. Pienthaweechai.** 1992. Isolation of *Campylobacter* from the canals of Bangkok metropolitan area. J. Med. Assoc. Thailand **75:**350–363.
7. **Ellis, W. A., S. D. Neill, J. J. O'Brien, H. W. Ferguson, and J. Hanna.** 1977. Isolation of Spirillum/Vibrio-like organisms from bovine fetuses. Vet. Rec. **100:**451–452.
8. **Ellis, W. A., S. D. Neill, J. J. O'Brien, and J. Hanna.** 1978. Isolation of spirillum-like organisms from pig fetuses. Vet. Rec. **102:**106.
9. **Festy, B., F. Squinazi, M. Marin, R. Derimay, and H. Lior.** 1993. Poultry meat and waters as the possible sources of *Arcobacter butzleri* associated human disease in Paris, France. Acta Gastro-Enterologica Belg. **56:**35.
10. **Gill, K. P. W.** 1983. Aerotolerant campylobacter strain isolated from a bovine preputial sheath washing. Vet. Rec. **112:**459.
11. **Higgins, R., and R. Degre.** 1979. Isolation of spirillum-like organisms from pig and bovine fetuses. Vet. Rec. **104:**262–263.
12. **Jacob, J., H. Lior, and I. Feuerpfeil.** 1993. Isolation of *Arcobacter butzleri* from a drinking water reservoir in Eastern Germany. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **193:**557–562.
13. **Jahn, B.** 1983. *Campylobacter* im Genitaltrakt des Schweines. Inaugural dissertation. Tieraarztliche Hochschule Hannover, Hannover, Germany.
14. **Jukes, T. H., and C. R. Cantor.** 1969. Evolution of protein molecules, p. 21–132. *In* H. N. Munro (ed.), Mammalian protein metabolism, vol. 3. Academic Press, Inc., New York.
15. **Kiehlbauch, J. A., D. J. Brenner, M. A. Nicholson, C. N. Baker, C. M. Patton, A. G. Steigerwalt, and I. K. Wachsmuth.** 1991. "*Campylobacter butzleri*" sp. nov. isolated from humans and animals with diarrheal illness. J. Clin. Microbiol. **29:**376–385.
16. **Kiehlbauch, J. A., D. N. Cameron, and I. K. Wachsmuth.** 1994. Evaluation of ribotyping techniques as applied to *Arcobacter*, *Campylobacter* and *Helicobacter*. Mol. Cell. Probes **9:**109–116.

17. **Kiehlbauch, J. A., B. D. Plikaytis, B. Swaminathan, D. N. Cameron, and I. K. Wachsmuth.** 1991. Restriction fragment length polymorphisms in the ribosomal genes for species identification and subtyping of aerotolerant *Campylobacter* species. J. Clin. Microbiol. **29:**1670–1676.
18. **Kiehlbauch, J. A., R. V. Tauxe, and K. Wachsmuth.** 1991. Clinical features of *Campylobacter butzleri* associated diarrheal illness. Microb. Ecol. Health Dis. **4**(Suppl.)**:**S92. (Abstract C14-2.)
19. **Lambert, M. A., C. M. Patton, T. J. Barrett, and C. W. Moss.** 1987. Differentiation of *Campylobacter* and *Campylobacter*-like organisms by cellular fatty acid composition. J. Clin. Microbiol. **25:**706–713.
20. **Lane, D. J., B. Pace, G. J. Olsen, D. A. Stahl, M. L. Sogin, and N. R. Pace.** 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci. USA **82:**6955–6959.
21. **Lerner, J., V. Brumberger, and V. Preac-Mursic.** 1994. Severe diarrhea associated with *Arcobacter butzleri*. Eur. J. Clin. Microbiol. Infect. Dis. **13:** 660–662.
22. **Linton, D., F. E. Dewhirst, J. P. Clewley, R. J. Owen, A. P. Burnens, and J. Stanley.** 1994. Two types of 16S rRNA gene are found in *Campylobacter helveticus*: analysis, applications and characterization of the intervening sequence found in some strains. Microbiology **140:**847–855.
23. **Lior, H., and D. Woodward.** 1991. A serotyping scheme for *Campylobacter butzleri*. Abstracts of the VIth International Workshop on *Campylobacter*, *Helicobacter* and related organisms. Microbiol. Ecol. Health Dis. **4:**S93.
24. **Logan, E. F., S. D. Neill, and D. P. Mackie.** 1982. Mastitis in dairy cows associated with an aerotolerant campylobacter. Vet. Rec. **110:**229–230.
25. **Luitjens, W., B. Jahn, and H. H. Schimmelpfennig.** 1986. Studies on the occurrence of *Campylobacter* type Neill in aborted porcine fetuses and in the genital tract of sows, p. 106. *In* Proceedings of the International Pig Veterinary Society 9th Congress. International Pig Veterinary Society, Barcelona, Spain.
26. **McClung, C. R., D. G. Patriquin, and R. E. Davis.** 1983. *Campylobacter nitrofigilis* sp. nov., a nitrogen-fixing bacterium associated with roots of *Spartina alterniflora* Loisel. Int. J. Syst. Bacteriol. **33:**605–612.
27. **Moureau, P., I. Derclaye, D. Gregoire, M. Janssen, and G. R. Cornelis.** 1989. *Campylobacter* species identification based on polymorphism of DNA encoding rRNA. J. Clin. Microbiol. **27:**1514–1517.
28. **Neill, S. D., J. N. Campbell, J. J. O'Brien, S. T. C. Weatherup, and W. A. Ellis.** 1985. Taxonomic position of *Campylobacter cryaerophila* sp. nov. Int. J. Syst. Bacteriol. **35:**342–356.
29. **Neill, S. D., W. A. Ellis, and J. J. O'Brien.** 1978. The biochemical characteristics of *Campylobacter*-like organisms from cattle and pigs. Res. Vet. Sci. **25:**368–372.
30. **Neill, S. D., W. A. Ellis, and J. J. O'Brien.** 1979. Designation of aerotolerant *Campylobacter*-like organisms from porcine and bovine abortions to the genus *Campylobacter*. Res. Vet. Sci. **27:**180–186.
31. **Neill, S. D., J. J. O'Brien, and W. A. Ellis.** 1980. The isolation of aerotolerant campylobacter. Vet. Rec. **106:**152–153.
32. **Olsen, G. J., R. Overbeek, N. Larsen, T. O. Marsh, M. J. McCaughey, M. A. Maciukenas, W.-M. Kuan, T. J. Macke, Y. Xing, and C. R. Woese.** 1992. The ribosomal data base project. Nucleic Acids Res. **20:**2199–2220.
33. **Paster, B. J., and F. E. Dewhirst.** 1988. Phylogeny of campylobacters, wolinellas, *Bacteroides gracilis*, and *Bacteroides ureolyticus* by 16S ribosomal ribonucleic acid sequencing. Int. J. Syst. Bacteriol. **38:**56–62.
34. **Popovic-Uroic, T., C. M. Patton, M. A. Nicholson, and J. A. Kiehlbauch.** 1990. Evaluation of the indoxyl acetate hydrolysis test for rapid differentiation of *Campylobacter*, *Helicobacter*, and *Wolinella* species. J. Clin. Microbiol. **28:**2335–2339.
35. **Pugina, P., G. Benzi, S. Lauwers, R. Van Etterijck, J. P. Butzler, L. Vlaes, and P. Vandamme.** 1991. An outbreak of *Arcobacter* (*Campylobacter*) *butzleri* in Italy. Microb. Ecol. Health Dis. Suppl. **4:**S94.
36. **Richardson, C. C.** 1981. Bacteriophage T4 polynucleotide kinase, p. 299–314. *In* P. D. Boyer (ed.), The enzymes, vol. 14, part A. Academic Press, Inc., New York.
37. **Romaniuk, P. J., and T. J. Trust.** 1987. Identification of *Campylobacter* species by Southern hybridization of genomic DNA using an oligonucleotide probe for 16S rRNA genes. FEMS Microbiol. Lett. **43:**331–335.
38. **Saitou, N., and M. Nei.** 1987. The neighbor-joint method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
39. **Sambrook, J., T. Maniatis, and E. F. Fritsch.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
40. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
41. **Stampi, S., O. Varoli, F. Zanetti, and G. DeLuca.** 1993. *Arcobacter cryaerophilus* and thermophilic campylobacters in a sewage treatment plant in Italy: two secondary treatments compared. Epidemiol. Infect. **110:**633–639.
42. **Stanley, J., D. Linton, A. P. Burnens, F. E. Dewhirst, R. J. Owen, A. Porter, S. L. W. On, and M. Costas.** 1993. *Helicobacter canis* sp. nov., a new species from dogs: an integrated study of phenotype and genotype. J. Gen. Microbiol. **139:**2495–2504.
43. **Taylor, D. N., J. A. Kiehlbauch, W. Tee, C. Pitarangsi, and P. Echeverria.** 1991. Isolation of group 2 aerotolerant *Campylobacter* species from Thai children with diarrhea. J. Infect. Dis. **163:**1062–1067.
44. **Tee, W., R. Baird, M. Dyall-Smith, and B. Dwyer.** 1988. *Campylobacter cryaerophila* isolated from a human. J. Clin. Microbiol. **26:**2469–2473.
45. **Thompson, L. M., R. M. Smibert, J. L. Johnson, and N. R. Krieg.** 1988. Phylogenetic study of the genus *Campylobacter*. Int. J. Syst. Bacteriol. **38:** 190–200.
46. **Vandamme, P., E. Falsen, R. Rossau, B. Hoste, P. Segers, R. Tytgat, and J. De Ley.** 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.
47. **Vandamme, P., B. A. J. Giesendorf, A. VanBelkum, D. Pieraard, S. Lauwers, K. Kersters, J.-P. Butzler, H. Goossens, and W. G. V. Quint.** 1993. Discrimination of epidemic and sporadic isolates of *Arcobacter butzleri* by polymerase chain reaction-mediated DNA fingerprinting. J. Clin. Microbiol. **31:**3317–3319.
48. **Vandamme, P., P. Pugina, G. Benzi, R. Van Etterijck, L. Vlaes, K. Kersters, J.-P. Butzler, H. Lior, and S. Lauwers.** 1992. Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. J. Clin. Microbiol. **30:**2335–2337.
49. **Vandamme, P., M. Vancanneyt, B. Pot, L. Mels, B. Hoste, D. Dewettinck, L. Vlaes, C. Van Den Borre, R. Higgins, J. Hommez, K. Kersters, J.-P. Butzler, and A. Goossens.** 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.
50. **Wesley, I. V., and A. L. Baetz.** 1995. Infection of Cesarean-derived colostrum deprived one-day old piglets with *Arcobacter* spp., abstr. B351, p. 226. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
51. **Wesley, I. V., and J. H. Bryner.** 1989. Antigenic and restriction enzyme analysis of isolates of *Campylobacter fetus* subsp. *venerealis* recovered from persistently infected cattle. Am. J. Vet. Res. **50:**807–813.
52. **Wesley, I. V., R. D. Wesley, M. Cardella, F. E. Dewhirst, and B. J. Paster.** 1991. Oligodeoxynucleotide probes for *Campylobacter fetus* and *Campylobacter hyointestinalis* based on 16S rRNA sequences. J. Clin. Microbiol. **29:**1812–1817.
53. **Wesley, I. V., et al.** 1995. Unpublished data.