Assessment of the Genetic Diversity among Arcobacters Isolated from Poultry Products by Using Two PCR-Based Typing Methods

Kurt Houf,[1]* Lieven De Zutter,[1] Jan Van Hoof,[1] and Peter Vandamme[2]

Department of Veterinary Food Inspection, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke,[1] and Department of Microbiology, Faculty of Sciences, Ghent University, 9000 Ghent,[2] Belgium

In this study, enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and randomly amplified polymorphic DNA PCR (RAPD-PCR) were optimized for characterization of *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii*. In addition, a simple and rapid DNA extraction method was tested for use in both typing procedures. Both methods had satisfactory typeability and discriminatory power, but the fingerprints generated with ERIC-PCR were more reproducible and complex than those obtained with RAPD-PCR. The use of nondiluted boiled cell suspensions as DNA templates was found to be very useful in ERIC-PCR. Characterization of large numbers of *Arcobacter* isolates is therefore preferably performed by the ERIC-PCR procedure. Isolates for which almost identical ERIC fingerprints are generated may subsequently be characterized by RAPD-PCR, although adjustment and standardization of the amount of the DNA template are necessary. In the second part of this study, the genotypic diversity of arcobacters present on broiler carcasses was assessed by using both typing methods. A total of 228 cultures from 24 samples were examined after direct isolation and enrichment. The isolates were identified by using a multiplex PCR as *A. butzleri* (*n* = 182) and *A. cryaerophilus* (*n* = 46). A total of 131 types (91 *A. butzleri* types and 40 *A. cryaerophilus* types) were discerned without discordance between the two typing techniques. The analysis of the poultry isolates showed that poultry products may harbor not only more than one species but also multiple genotypes. All genotypes were confined to one poultry sample, and only three genotypes were found after simultaneous enrichment and direct isolation. These results demonstrate that different outcomes can be obtained in epidemiological studies depending on the isolation procedure used and the number of isolates characterized.

The genus *Arcobacter* belongs to rRNA superfamily VI of the *Proteobacteria* and currently includes four species (23). Two species, *Arcobacter butzleri* and *A. cryaerophilus*, have been associated with human diarrheal illness (11, 21) and bacteremia (7, 16, 30). They have also been isolated from farm animals with clinical symptoms of mastitis (12), diarrhea (9), and reproduction abnormalities (3, 20), as well as from clinically healthy farm animals (28) and poultry (29). A third species, *A. skirrowii*, has been recovered from fecal samples from experimentally infected poultry (29) and pigs (27) and from lambs with diarrhea (24). Additional isolates have been obtained from the internal organs of porcine, ovine, and bovine aborted fetuses and from preputial sheath washes of bulls. This organism has also been recovered from two broiler carcasses (1). Association of *A. skirrowii* with human infection has not been reported. The fourth *Arcobacter* species, *A. nitrofigilis*, is a nitrogen-fixing bacterium associated with the roots of *Spartina alterniflora*, a salt marsh plant (14). This organism requires an elevated salt level for growth and has never been associated with animal or human infection. Therefore, this species was not considered in this study.

Examination of human and veterinary clinical specimens for the presence of *Arcobacter* species is rarely performed, and in most cases suboptimal procedures are used (6).

In addition, little is known about the risk factors for human infection. However, the high rates of *Arcobacter* contamination of raw poultry products (1, 5) and the distribution of the same biotypes (2; H. Lior and D. L. Woodward, abstract from the International Workshop on Campylobacter, Helicobacter, and Related Organisms, 21 to 23 September 1993, Brussels, Belgium, Acta Gastro-Enterol. Belg. Suppl. **6:**28, 1993) and serotypes (H. Lior and D. L. Woodward, Acta Gastro-Enterol. Belg. Suppl. **6:**29, 1993) among human and poultry isolates indicate that handling of raw poultry and consumption of undercooked poultry products may be routes of infection. As the precise contribution of poultry to human infection is still unclear, characterization of *Arcobacter* isolates can contribute to our knowledge of the epidemiological behavior of these organisms. However, few typing studies have been described so far. Methods such as biotyping (Lior and Woodward, Acta Gastro-Enterol. Belg. Suppl. **6:**28, 1993), serotyping (Lior and Woodward, Acta Gastro-Enterol. Belg. Suppl. **6:**29, 1993), and comparison of cellular protein profiles (24) have been shown to have limited discriminatory capabilities and to rely on phenotypic characteristics that may be unstable and poorly reproducible. Molecular typing methods, such as the randomly amplified polymorphic DNA (RAPD) assay (H. Lior and G. abstract from the International Workshop on Campylobacter, Helicobacter, and Related Organisms, 21 to 23 September 1993, Brussels, Blegium, Wang, Acta Gastro-Enterol Belg. Suppl. **6:**29, 1993), ribotyping (10), and repetitive-motif-based polymorphic DNA analysis (22), have been performed with limited numbers of arcobacters, but there has not been a profound evaluation of the performance and discriminatory abilities of these methods for the three *Arcobacter* species exam-

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* Corresponding author. Mailing address: Department of Veterinary Food Inspection, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium. Phone: 32 09 264 74 51. Fax: 32 09 264 74 91. E-mail: kurt.houf@rug.ac.be.

TABLE 1. *Arcobacter* strains used in this study

Species	Isolate	Reference no.[a]	Source	Country of origin
A. butzleri	1	LMG 6620	Human, blood	United Kingdom
	2	LMG 9869	Piglet, liver	United Kingdom
	3	LMG 9906	Porcine fetus, eye	Northern Ireland
	4	LMG 10220	Pig, feces	Canada
	5	LMG 10223	Bovine, liver	Canada
	6	LMG 10240	Horse, feces	Canada
	7	LMG 10828[T]	Human, feces	United States
	8	LMG 11118	Human, feces	Italy
	9	LMG 11119	Human, feces	Italy
	10	LMG 11120	Human, feces	Italy
	11	LMG 14714	Human, feces	Greece
	12	LMG 15918	Human, feces	Hungary
A. cryaerophilus	13	LMG 7536[T]	Ovine fetus	Northern Ireland
	14	LMG 9066	Porcine fetus, eye	Northern Ireland
	15	LMG 9905	Bovine fetus, kidney	Northern Ireland
	16	LMG 10210	Bovine fetus	Canada
	17	LMG 9861	Bovine fetus, peritoneal	Northern Ireland
	18	LMG 9864	Porcine fetus, eye	Northern Ireland
	19	LMG 9867	Equine fetus, spleen	Northern Ireland
	20	LMG 10209	Bovine fetus	Canada
	21	LMG 10217	Porcine fetus, placenta	Canada
A. skirrowii	22	LMG 6621[T]	Lamb, feces	United Kingdom
	23	LMG 9802	Bull, preputial fluid	Belgium
	24	LMG 9912	Bovine fetus, thoracal	Northern Ireland
	25	LMG 10234	Porcine fetus, lung	Canada
	26	LMG 10239	Bovine, feces	Canada
	27	LMG 14983	Bull, preputial fluid	United States

[a] T = type strain. LMG, Bacteria Collection Laboratory, Ghent University, Ghent, Belgium.

ined in this study. It has been shown that pulsed-field gel electrophoresis of macrorestriction fragments (8; Lior and Wang, Acta Gastro-Enterol. Belg. Suppl. **6**:29, 1993) is a well-suited method with respect to its discriminatory power for *A. butzleri* and *A. cryaerophilus*, but this procedure is complex, costly, and time-consuming (15).

In this study, we optimized enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and RAPD-PCR for genetic characterization of *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* and assessed the performance and discriminatory abilities of these methods by using a well-defined strain collection consisting of members of these three *Arcobacter* species. In addition, the usefulness of a simple and fast DNA preparation method was evaluated. The genotypic diversity of arcobacters present on broiler carcasses was studied, and the impact of the isolation procedure and the number of isolates characterized on epidemiological investigation results was determined.

MATERIALS AND METHODS

Bacterial strains and growth conditions. For optimization and evaluation of the typeability, reproducibility, and discriminatory power of the two typing methods, 24 geographically and epidemiologically unrelated *Arcobacter* strains were obtained from the BCCM/LMG Bacteria Collection (Ghent University, Ghent, Belgium). These collection strains were isolated from humans and animals and are listed in Table 1. The collection used also included three *A. butzleri* isolates from a well-documented outbreak in a nursery and primary school in Italy (25). The strains were grown on Mueller-Hinton agar plates (CM 337; Oxoid, Basingstoke, United Kingdom) and were incubated for 48 h at 29°C under microaerobic conditions by evacuating 80% of the normal atmosphere and introducing a gas mixture consisting of 8% CO_2, 8% H_2, and 84% N_2 into each jar. For each strain, a cell suspension was prepared in 10 ml of sterile water with an optical density at 660 nm of about 0.074 ± 0.002, which corresponded to a concentration of approximately 10^8 CFU/ml.

DNA preparation. Template DNA was extracted from a 1.0-ml cell suspension of each strain by two methods. Before extraction, all of the cell suspensions were centrifuged for 5 min at 13,000 rpm (Eppendorf model 5417-R centrifuge) to pellet the cells and the supernatants were discarded. In the first method, DNA extracts were prepared by resuspending the cell pellets in 500 μl of sterile distilled water and boiling the suspensions for 10 min. After a quick spin to pellet the cell debris, the supernatants were used as DNA templates for subsequent amplifications. In the second method, the bacterial pellets were resuspended in 100 μl of Tris-EDTA buffer and genomic DNA was extracted by the guanidinium thiocyanate method described by Pitcher et al. (18).

Five microliters of each DNA preparation was size separated by electrophoresis to analyze the integrity of the DNA extracted. The concentration of each DNA template was determined spectrophotometrically at A_{260} and adjusted to 25 ng μl^{-1}. The DNA templates obtained by both methods were stored at $-25°C$.

Conditions used for RAPD-PCR. All PCR amplifications were performed by using 25 pmol of primer with Ready-To-Go RAPD analysis beads (Amersham Pharmacia Biotech, Uppsala, Sweden) containing premixed, predispensed Ampli*Taq* DNA polymerase, as well as all necessary buffer ingredients and nucleotides. The cycling parameters were as follows: denaturing at 95°C for 30 s, annealing at 36°C for 1 min, and extension at 72°C for 2 min for a total of 45 cycles. Prior to cycling, samples were heated at 94°C for 5 min. For each strain, 25, 50, and 100 ng of DNA, obtained by both extraction methods, were tested. In addition, 1 μl of each nondiluted boiled cell suspension was analyzed. For the initial screening, RAPD fingerprints were generated for all 27 strains by using the six arbitrary 10-mer primers included in the RAPD analysis primer set (Amersham Pharmacia Biotech). All amplifications were performed in 25-μl mixtures by using a Perkin-Elmer GeneAmp System 9700 PCR thermocycler.

Conditions used for the ERIC-PCR assay. Each 50-μl PCR mixture was composed of autoclaved deionized water, 5 μl of 10× PCR buffer (Invitrogen, GmbH), 5 U of *Taq* polymerase (Invitrogen, GmbH), and a deoxynucleoside triphosphate mixture containing each deoxynucleoside triphosphate (Invitrogen, GmbH) at a final concentration of 0.2 mM. Several concentrations of primers, $MgCl_2$, and DNA templates were tested. Annealing was performed at different temperatures, as indicated below. The ERIC motifs 1R (5′-ATGTAAGCTCC TGGGGATTCAC-3′) and 2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′) designed by Versalovic et al. (26) were each used at concentrations of 25, 50, and 100 pmol/50 μl. The effects of $MgCl_2$ concentrations of 2.5, 3.0, 3.5, and 4.0 mM were evaluated. The DNA concentrations that were used in the RAPD assays, including 1-μl portions of the boiled cell suspensions, were tested. The PCR consisted of 35, 38, 40, or 42 cycles of 94°C for 1 min, 25, 40, or 50°C for 1 min, and 72°C for 2 min. Prior to cycling, samples were heated at 94°C for 5 min.

Reproducibility. In order to assess the reproducibility of the two typing methods, genomic DNA of the 27 collection strains was extracted by the two extraction procedures and amplified by the two PCR-based typing methods on three nonconsecutive days, and the fingerprints generated were compared.

Poultry isolates. Neck skin samples from 24 broiler carcasses were collected in four different Belgian poultry slaughterhouses and examined for the presence of *Arcobacter*. Each sample was taken on a different day and from a different poultry flock. Samples were obtained immediately after chilling and were examined by using the protocol developed previously, including isolation with and without a primary enrichment step (5). Briefly, 10 g of neck skin was added to 10 ml of *Arcobacter* enrichment broth consisting of 28 g of *Arcobacter* broth (CM 965; Oxoid) per liter, 5% lysed horse blood, and the previously developed selective supplement (5) and homogenized in a stomacher blender for 1 min at the normal speed. One hundred microliters of each neck skin homogenate was inoculated by the spiral plating method onto an *Arcobacter* selective agar plate containing 28 g of *Arcobacter* broth per liter, 12 g of Agar Technical no. 3 (L13; Oxoid Ltd., Basingstoke, Hampshire, England) per liter, and the previously developed selective supplement (5) and incubated at 28°C for 24 to 72 h. To detect low *Arcobacter* contamination levels, the neck skin homogenates were incubated for 48 h at 28°C in a microaerobic atmosphere and then inoculated onto *Arcobacter* selective agar plates. Following incubation, all colonies obtained by direct isolation were examined. In addition, 2 to 10 randomly sampled colonies, obtained after enrichment, were picked from the agar plates. Subsequently, all isolates were identified by using a species-specific multiplex PCR assay (4) and were characterized further by the two typing methods.

Analysis of PCR products. The PCR products were size separated by electrophoresis of 8-μl portions of the reaction mixtures in ethidium bromide-stained 2% agarose gels with 1× Tris-borate-EDTA buffer for 2.5 h at 100 V. The DNA profiles were visualized by UV transillumination and photographed. The gels were analyzed both by visual inspection and by computer-aided methods. DNA patterns that differed in one or more DNA fragments were considered patterns

A

M 1 2 3 M 4 5 6 M 7 8 9 M 10 11 12 M13 14 M M 15 16 17M 18 19 20 M 21 22 23 M 2425 26 M 27 M

2072-
1500-

600-

100-

B

1 2 3 M 4 5 6 M 7 8 9 M 10 11 12 M 1314 15 16 17 18 M 19 20 21 M 22 23 24 M 25 26 27
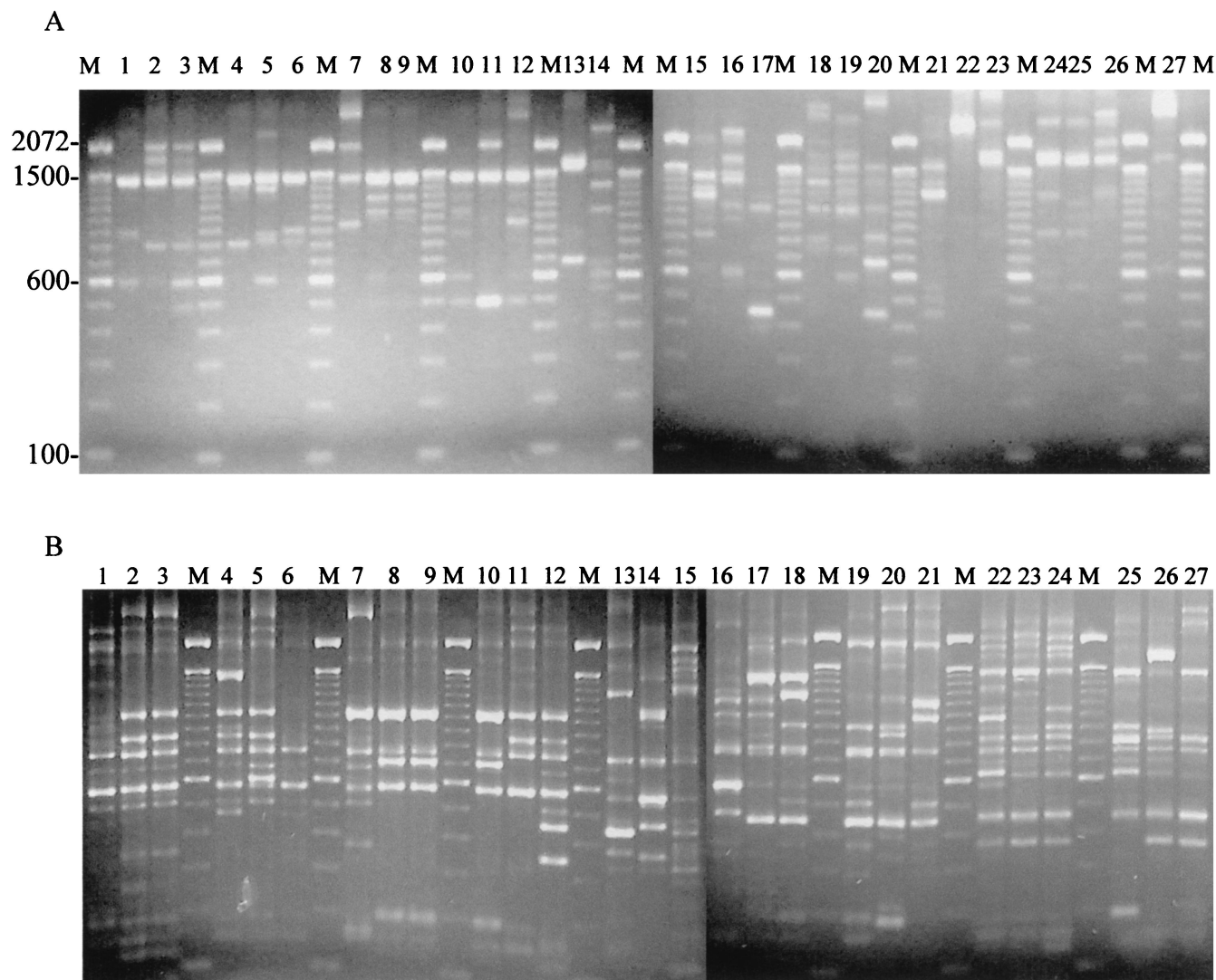
FIG. 1. RAPD (A) and ERIC (B) fingerprints of the 27 *Arcobacter* collection strains. The lane numbers correspond to the isolate numbers shown in Table 1. Lanes M contained a reference marker.

that represented different types. Whenever type differences relied on only one band, a repeat analysis was performed (including a repeat DNA extraction) to confirm the reproducibility of the fingerprint. To interpret the profiles of the poultry isolates, computer-based normalization and interpolation of the DNA profiles and numerical analysis using the Pearson product moment correlation coefficient with 1% position tolerance were performed using the GelCompar 4.2 software package (Applied Maths, Kortrijk, Belgium). Dendrograms were constructed by using the unweighted pair group linkage analysis method. For convenience, the correlation levels were expressed as percentages of similarity.

RESULTS

DNA preparation. DNA templates of all 27 *Arcobacter* collection strains were prepared on three occasions by using both extraction procedures. DNA was detected in all extracts; the concentrations ranged from 35 to 600 ng of DNA μl^{-1}, and there was no apparent effect of extraction procedure, *Arcobacter* species, or day of extraction. When the integrity of the DNA extracts was checked on agarose gels, clear-cut DNA

bands were observed when the extraction procedure of the Pitcher et al. (18) was used. When boiled lysates were analyzed, smears of DNA appeared in the agarose gel.

RAPD-PCR. Of the six random primers tested, primer 1 (5′-GGTGCGGGAA-3′) gave the clearest and most distinctive band pattern for each of the 27 strains examined. When 25 pmol of this primer was used, unique profiles were obtained for all of the *Arcobacter* strains except the three outbreak isolates (Fig. 1A). The profiles were composed of two to nine fragments with different intensities. The sizes of the DNA fragments generated by the amplification process and used for determination ranged from 300 to 2,072 bp. When 100 ng of DNA or 1 μl of nondiluted boiled cell suspension was used, few or no DNA fragments were amplified, while nondiscriminatory profiles consisting of two or three fragments were obtained when 50 ng of DNA was utilized (data not shown). When the fingerprints generated with 25-ng DNA templates obtained by the two extraction methods were compared, sim-

TABLE 2. Numbers of *Arcobacter* isolates analyzed

Abattoir	Broiler	After enrichment						Direct isolation					
		A. butzleri			*A. cryaerophilus*			*A. butzleri*			*A. cryaerophilus*		
		No. of colonies	No. of ERIC types	No. of RAPD types	No. of colonies	No. of ERIC types	No. of RAPD types	No. of colonies	No. of ERIC types	No. of RAPD types	No. of colonies	No. of ERIC types	No. of RAPD types
A	1	0[a]	—	—	5	4	3	0	—	—	9	7	7
A	2	0	—	—	5	5	5	0	—	—	0	—	—
A	3	5	4	4	0	—	—	0	—	—	0	—	—
A	4	5	2	2	0	—	—	0	—	—	0	—	—
A	5	5	2	2	0	—	—	0	—	—	9	9	9
B	6	0	—	—	0	—	—	0	—	—	0	—	—
B	7	2	1	1	0	—	—	21	8	6	2	2	2
B	8	2	1	1	0	—	—	7	3	3	2	2	2
B	9	0	—	—	0	—	—	0	—	—	0	—	—
B	10[b]	2	1	1	0	—	—	7	3	3	1	1	1
C	11	2	1	1	0	—	—	14	7	6	1	1	1
C	12	2	2	2	0	—	—	1	1	1	2	2	2
C	13	2	2	2	0	—	—	0	—	—	0	—	—
C	14	0	—	—	0	—	—	0	—	—	0	—	—
C	15	10	4	4	0	—	—	0	—	—	0	—	—
C	16	0	—	—	0	—	—	0	—	—	0	—	—
C	17[b]	10	5	5	0	—	—	8	7	7	2	1	1
D	18	6	4	4	0	—	—	5	4	4	4	3	2
D	19	10	3	3	0	—	—	0	—	—	0	—	—
D	20	4	1	1	0	—	—	0	—	—	0	—	—
D	21	10	3	3	0	—	—	0	—	—	0	—	—
D	22	10	3	3	0	—	—	0	—	—	0	—	—
D	23	10	3	3	0	—	—	6	6	6	1	1	1
D	24	10	7	7	0	—	—	3	3	3	3	2	3
Total		110	49	49	10	9	8	72	42	40	36	31	31

[a] 0, no arcobacters isolated; —, not applicable.
[b] One of the *A. butzleri* types was found both after enrichment and after direct isolation.

ilar degrees of discrimination were observed, although the profiles generated were not identical (data not shown).

ERIC-PCR. To enhance the discriminatory power of ERIC-PCR fingerprinting, the optimal annealing temperature and optimal concentrations of $MgCl_2$, DNA template, and primers were determined. The Mg^{2+} concentration and the annealing temperature were observed to have profound effects on the complexity of the band pattern (data not shown). Only the use of 4 mM $MgCl_2$ and an annealing temperature of 25°C produced strain-specific band patterns. While an $MgCl_2$ concentration of 2.5 or 3 mM and annealing at 25°C resulted in poor amplification, higher annealing temperatures generated species-specific profiles with all $MgCl_2$ concentrations tested. The fingerprints, however, did not vary significantly when different amounts of DNA template or primers were used (irrespective of the DNA preparation procedure), although slight differences in band intensity were noticed.

An annealing temperature of 25°C with 4 mM $MgCl_2$ and 25 pmol of each primer generated reproducible fingerprints with a sufficient number of amplimers for typing purposes. The profiles were specific for unrelated strains and identical for the three outbreak *Arcobacter* isolates (Fig. 1B). The number of amplimers in the ERIC-PCR profiles ranged from 3 to 15. The band patterns used to determine the ERIC-PCR types comprised DNA fragments between 100 and 2,072 bp long. For optimal results, 40 amplification cycles were used, as fewer cycles resulted in fewer amplimers and a poor yield of some fragments (data not shown).

Reproducibility. Twenty-five nanograms of DNA of each collection strain, obtained by the two extraction methods, was examined with both typing methods in triplicate. One microliter of nondiluted boiled cell suspension was tested only with the ERIC-PCR, as using 1 μl of a nondiluted boiled lysate in RAPD-PCR resulted in amplification of few or no DNA fragments.

Excellent reproducibility was obtained for ERIC-PCR with all three types of DNA templates and for RAPD-PCR with 25-ng DNA templates obtained by the guanidinium thiocyanate or boiled cell method. In some fingerprints, the major bands were less intense and the minor bands were difficult to visualize, but the presence or absence of DNA fragments was very consistent.

Poultry isolates. Of the 24 broiler neck skin samples, 20 and 9 obtained after enrichment and by direct isolation, respectively, were found to be positive for the presence of *Arcobacter* strains. The numbers of colonies randomly collected from the agar plates after enrichment and the total numbers of colonies obtained after direct isolation are shown in Table 2. For ERIC-PCR and multiplex PCR, the poultry isolates were examined by using boiled cell suspensions as DNA templates. Based on the results obtained by comparing different amounts of DNA from the collection strains, a portion of each supernatant was adjusted so that the DNA concentration was 25 ng $μl^{-1}$, and 1 μl was used as the DNA template in RAPD-PCR.

A total of 228 subcultures were identified by multiplex PCR as *A. butzleri* (182 isolates) or *A. cryaerophilus* (46 isolates) and
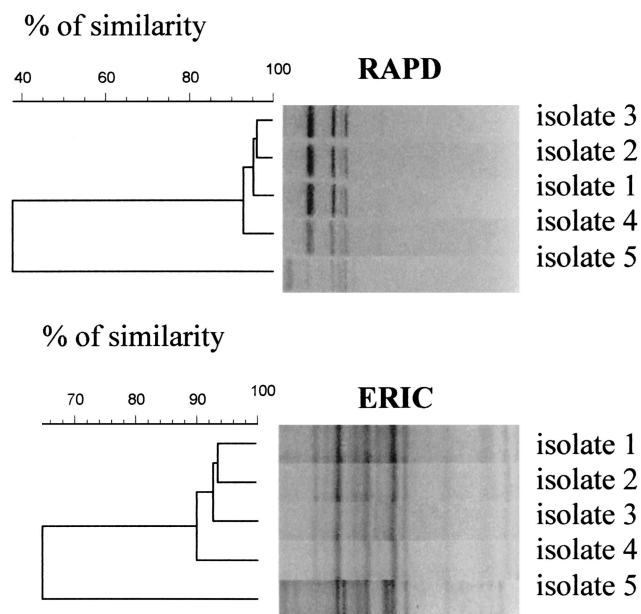
% of similarity

% of similarity

FIG. 2. Dendrograms based on RAPD and ERIC fingerprints for *A. butzleri* isolates obtained after enrichment from broiler 4.

were characterized further by the two typing methods. When direct isolation was used, both *Arcobacter* species were isolated simultaneously from nine broiler samples. When enrichment was used, *A. cryaerophilus* was isolated only in the absence of *A. butzleri*. *A. cryaerophilus* was detected in 11 broiler samples by direct isolation but in only 2 samples by enrichment culture. Furthermore, discordance in the *Arcobacter* species isolated was observed for broiler 5, from which *A. butzleri* was isolated by enrichment culture and *A. cryaerophilus* was isolated by direct isolation.

All 228 isolates were examined by both typing methods. ERIC-PCR generated fingerprints for 197 isolates; DNA smears appeared for the remaining 31 isolates. The supernatants of these 31 isolates were diluted 1/10 in sterile water, and amplification was performed again by using 1-μl portions of the diluted supernatants as the DNA templates. After this second analysis, only three isolates (one *A. butzleri* isolate and two *A. cryaerophilus* isolates) remained uncharacterized. RAPD fingerprints were obtained for all but 12 isolates. Genomic DNA was reextracted, the concentration was adjusted to 25 ng μl^{-1}, and 1 μl was used in a new RAPD-PCR. When this was done, six isolates (three *A. butzleri* isolates and three *A. cryaerophilus* isolates) remained uncharacterized. DNA were extracted by the guanidinium thiocyanate method from the three isolates that were not characterized by ERIC-PCR and the six isolates that were not characterized by RAPD-PCR, and these DNA were tested by using both PCR methods. ERIC and RAPD fingerprints were obtained for all isolates. However, the latter patterns were not included in the subsequent analysis because of the different DNA extraction method used.

Computer analysis of the patterns allowed rapid comparison of the different gel tracks and construction of dendrograms. For both methods, groups consisting of identical fingerprints or fingerprints with minor differences in band intensity were characterized by Pearson correlation coefficients of 90% or higher.

Pearson correlation coefficients lower than 78% were obtained for band patterns that differed by the presence of two or more distinct DNA fragments. For some isolates (for example, isolates 1 and 2 from broiler 1 [Fig. 2]), ERIC fingerprints differed in only one fragment. Such fingerprints were characterized by Pearson correlation coefficients ranging from 78 to 90%. When the corresponding RAPD profiles were analyzed, different patterns (and thus different types) were observed, which were characterized by Pearson correlation coefficients lower than 78%. Cluster analysis of the DNA patterns of all of the isolates tested revealed great heterogeneity among the *Arcobacter* isolates obtained after direct isolation, as well as among the isolates obtained after enrichment. Ninety-one *A. butzleri* types and 40 *A. cryaerophilus* types were determined by ERIC-PCR, and 89 *A. butzleri* types and 39 *A. cryaerophilus* types could be distinguished by RAPD-PCR analysis (Table 2). The discriminatory powers of the two methods were identical. Differences in the total number of types obtained by the two typing methods (Table 2) were due to the presence of some isolates for which no fingerprint was obtained by one of the methods used. The same type was never detected in more than one poultry sample, and only three identical *A. butzleri* types were found after direct isolation and after enrichment in the same sample.

DISCUSSION

In the first part of this study, ERIC-PCR and RAPD-PCR were optimized for differentiation of *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* strains. In addition, a simple and rapid DNA extraction method was tested for use with both PCR methods, in order to obtain maximum resolution with a minimum of effort. In the second part of the study, both typing methods were used to characterize 228 arcobacters isolated from 24 broiler carcasses with and without prior enrichment.

The usefulness of the two typing methods was evaluated by using the three main criteria for typing schemes, namely, typeability, reproducibility, and discriminatory power (19). To do this, a well-defined collection of three related *Arcobacter* isolates and 24 unrelated *Arcobacter* isolates that represented the three animal-associated species in this genus was used. With both methods, satisfactory typeability and reproducibility were obtained, as a reproducible profile was obtained for each strain tested. The two typing methods generated identical and unique fingerprints for the outbreak and unrelated strains, respectively, illustrating the discriminatory power of the methods and their suitability for use in epidemiological investigations. The amount of DNA used in the PCR-based methods and the method of DNA extraction seemed to have important effects on the results obtained. Particularly in the RAPD-PCR assay, variations in the quality and amount of DNA template had a major impact on the results obtained. For this assay, the best typing results were obtained with DNA template standardized at a concentration of 25 ng μl^{-1} and extracted by the guanidinium thiocyanate or boiled cell method. The use of nondiluted boiled cell extract as the DNA template generated useful fingerprints only in ERIC-PCR.

When the 228 *Arcobacter* poultry isolates were analyzed, 86 and 95% of the isolates could be characterized by ERIC and RAPD fingerprints, respectively. No discordance between the typing results obtained by the two typing methods was ob-

served. Dilution of the DNA templates of 31 isolates for which DNA smears instead of band patterns were obtained after ERIC-PCR increased the typeability of the method to 98%. There was great variation among the DNA patterns of *Arcobacter* poultry isolates. A total of 131 types (91 *A. butzleri* types and 40 *A. cryaerophilus* types) from 24 neck skin samples from 24 broilers were discerned. The diversity of patterns was especially interesting because a particular genotype never occurred in more than one poultry sample. Moreover, only three genotypes were detected after enrichment and after direct isolation. These findings have major implications for epidemiological studies of arcobacters. In order to characterize the source of *Arcobacter* contamination by genotyping, it is necessary to examine a large number of isolates, and both direct isolation and enrichment should be used in order to recover as many different strains as possible.

The presence of considerable heterogeneity among arcobacters in one animal specimen was suggested previously by biotyping (2; Lior and Woodward, Acta Gastro-Enterol. Belg. Suppl. **6:**28, 1993) and serotyping (2; Lior and Woodward, Acta Gastro-Enterol. Belg. Suppl. **6:**29, 1993) studies. When DNA-based methods were used, genotypic variation was demonstrated among *Arcobacter* isolates from a farrow-to-finish swine facility (8) and among *A. butzleri* isolates from mechanically separated turkey samples (13). The possible explanations for the large amount of heterogeneity include multiple sources of contamination, the presence of multiple parent genotypes for both species in a single animal, and a high degree of genomic recombination among the progeny of parent genotypes. The ability to undergo genomic rearrangements has been demonstrated in *Campylobacter* species (17) but not in members of the genus *Arcobacter*. Genomic rearrangements were not detected among 14 outbreak isolates of *A. butzleri*, as all isolates belonged to the same serotype and had identical phenotypic characteristics and protein and ERIC-PCR profiles (22). Furthermore, in vitro cultivation of *Arcobacter* isolates over a period of time did not induce genomic rearrangements, as identical fingerprints were obtained in the reproducibility experiments in this study.

In conclusion, ERIC-PCR and RAPD-PCR are both valuable techniques for characterizing *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* isolates. Both methods have satisfactory typeability and discriminatory power. One-band differences between two fingerprints generated by one method reliably represented different types, as confirmed by the results of the other typing method. The fingerprints generated with ERIC-PCR were more reproducible and complex than the fingerprints generated with RAPD-PCR. Moreover, the use of nonstandardized amounts of boiled cell suspensions was possible only with ERIC-PCR. Therefore, for characterization of a large number of *Arcobacter* isolates, ERIC-PCR performed with boiled cell suspensions as the DNA templates is the first choice for a characterization method. Isolates for which almost identical ERIC fingerprints are generated may subsequently be characterized by RAPD-PCR, although adjustment and standardization of the amount of DNA template will be necessary. Our analysis of poultry isolates showed that poultry products may harbor not only more than one species but also multiple genotypes of the species. Depending on the isolation procedure used, different *Arcobacter* species can be isolated. Moreover, a large number of isolates should be characterized in order to recover as many different strains as possible.

REFERENCES

1. **Atabay, H. I., J. E. L. Corry, and S. L. W. On.** 1998. Diversity and prevalence of *Arcobacter* spp. in broiler chickens. J. Appl. Microbiol. **84:**1007–1016.
2. **de Boer, E., J. Tilburg, D. Woodward, H. Lior, and W. Johnson.** 1996. A selective medium for the isolation of *Arcobacter* from meats. Lett. Appl. Microbiol. **23:**64–66.
3. **Fernandez, H., X. Rojas, and T. Gajardo.** 1995. First isolation in Chile of *Arcobacter cryaerophilus* from a bovine abortion. Arch. Med. Vet. **27:**111–114.
4. **Houf, K., A. Tutenel, L. De Zutter, J. Van Hoof, and P. Vandamme.** 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.
5. **Houf, K., L. A. Devriese, L. De zutter, J. Van Hoof, and P. Vandamme.** 2001. Development of a new protocol for the isolation and quantification of *Arcobacter* species from poultry products. Int. J. Food Microbiol. **71:**189–196.
6. **Houf, K., L. A. Devriese, L. De Zutter, J. Van Hoof, and P. Vandamme.** 2001. Susceptibility of *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* to antimicrobial agents used in selective media. J. Clin. Microbiol. **39:**1654–1656.
7. **Hsueh, P., L. Teng, P. Yang, S. Wang, S. Chang, S. Ho, W. Hsieh, and K. Luh.** 1997. Bacteremia caused by *Arcobacter cryaerophilus* 1B. J. Clin. Microbiol. **35:**489–491.
8. **Hume, M. E., R. B. Harvey, L. H. Stanker, R. E. Droleskey, T. L. Poole, and H. B. Zhang.** 2001. Genotypic variation among *Arcobacter* isolates from a farrow-to-finish facility. J. Food Prot. **64:**645–651.
9. **Kiehlbauch, J. A., D. Brenner, M. Nicholson, C. Baker, C. Patton, A. Steigerwalt, and I. Wachsmuth.** 1991. *Campylobacter butzleri* sp. nov. isolated from humans and animals with diarrheal illness. J. Clin. Microbiol. **29:**376–385.
10. **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 **8:**109–116.
11. **Lerner, J., V. Brumberger, and V. Preac-Mursic.** 1994. Severe diarrhea associated with *Arcobacter butzleri*. Eur. J. Clin. Microbiol. Infect. Dis. **13:**660–662.
12. **Logan, E., S. Neill, and D. Mackie.** 1982. Mastitis in dairy cows associated with an aerotolerant *Campylobacter*. Vet. Rec. **110:**229–230.
13. **Manke, T. R., I. V. Wesley, J. S. Dickson, and K. M. Harmon.** 1998. Prevalence and genetic variability of *Arcobacter* species in mechanically separated turkey. J. Food. Prot. **61:**1623–1628.
14. **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.
15. **Olive, D. M., and P. Bean.** 1999. Principles and applications of methods for DNA-based typing of microbial organisms. J. Clin. Microbiol. **37:**1661–1669.
16. **On, S. L. W., A. Stacey, and J. Smyth.** 1995. Isolation of *Arcobacter butzleri* from a neonate with bacteraemia. J. Infect. **31:**225–227.
17. **On, S. L. W.** 1998. *In vitro* genotypic variation of *Campylobacter coli* documented by pulsed-filed gel electrophoresis DNA profiling: implications for epidemiological studies. FEMS Microbiol. Lett. **165:**341–346.
18. **Pitcher, D. G., N. A. Saunders, and R. J. Owen.** 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. **8:**151–158.
19. **Power, E. G. M.** 1996. RAPD typing in microbiology—a technical review. J. Hosp. Infect. **34:**247–265.
20. **Shroeder-Tucker, L., I. V. Wesley, J. A. Kiehlbauch, D. J. Larson, L. A. Thomas, and G. A. Erickson.** 1996. Phenotypic and ribosomal RNA characterization of *Arcobacter* species isolated from porcine aborted fetuses. J. Vet. Diagn. Investig. **8:**186–195.
21. **Tee, W., R. Baird, M. Dyall-Smith, and B. Dwyer.** 1988. *Campylobacter cryaerophila* isolated from a human. J. Clin. Microbiol. **26:**2469–2473.
22. **Vandamme, P., B. A. Giesendorf, A. Van Belkum, D. Pierard, S. Lauwers, K. Kesters, J. P. Butzler, H. Goossens, and W. G. Quint.** 1993. Discrimination of epidemic and sporadic isolates of *Arcobacter butzleri* by polymerase chain reaction-mediated DNA fingerprinting. J. Clin. Microbiol. **31:**3317–3319.
23. **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.
24. **Vandamme, P., M. Vancanneyt, B. Pot, L. Mels, B. Hoste, D. Dewettinck, L.**

Vlaes, C. Van Den Borre, R. Higgens, J. Hommez, K. Kesters, J. P. Butzler, and H. 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.

25. Vandamme, P., P. Pugina, G. Benzi, R. Van Etterijck, L. Vlaes, K. Kesters, 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.

26. Versalovic, J., T. Koeuth, and J. R. Lupski. 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res. **19:**6823–6831.

27. Wesley, I. V., A. L. Baetz, and D. J. Larson. 1996. Infection of cesarean-derived colostrum-deprived 1-day-old piglets with *Arcobacter butzleri, Arcobacter cryaerophilus*, and *Arcobacter skirrowii*. Infect. Immun. **64:**2295–2299.

28. Wesley, I. V., S. J. Wells, K. M. Harmon, A. Green, L. Schroeder-Tucker, M. Glover, and I. Siddique. 2000. Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. Appl. Environ. Microbiol. **66:**1994–2000.

29. Wesley, I. V., and A. L. Baetz. 1999. Natural and experimental infections of *Arcobacter* in poultry. Poultry Sci. **78:**536–545.

30. Yan, J. J., W. C. Ko, A. H. Huang, H. M. Chen, Y. T. Jin, and J. J. Wu. 2000. *Arcobacter butzleri* bacteremia in a patient with liver cirrhosis. J. Formosan Med. Assoc. **99:**166–169.