Journal of Food Protection, Vol. 72, No. 7, 2009, Pages 1491–1495
Copyright ©, International Association for Food Protection

Research Note

Specific PCR Detection of *Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii*, and *Arcobacter cibarius* in Chicken Meat

DANIELA PENTIMALLI,[1] NICOLETTE PEGELS,[2] TERESA GARCÍA,[2] ROSARIO MARTÍN,[2] AND ISABEL GONZÁLEZ[2]*

[1]*Dipartimento di Sanità Pubblica, Facoltà di Agraria, Università degli studi di Parma, Parma, Italy; and* [2]*Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain*

MS 08-536: Received 23 October 2008/Accepted 28 December 2008

ABSTRACT

An enrichment PCR assay using species-specific primers was developed for the detection of *Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii,* and *Arcobacter cibarius* in chicken meat. Primers for *A. cryaerophilus, A. skirrowii,* and *A. cibarius* were designed based on the *gyrA* gene to amplify nucleic acid fragments of 212, 257, and 145 bp, respectively. The *A. butzleri*–specific primers were designed flanking a 203-bp DNA fragment in the 16S rRNA gene. The specificity of the four primer pairs was assessed by PCR analysis of DNA from a panel of *Arcobacter* species, related *Campylobacter, Helicobacter* species, and other food bacteria. The applicability of the method was then validated by testing 42 fresh retail-purchased chicken samples in the PCR assay. An 18-h selective preenrichment step followed by PCR amplification with the four *Arcobacter* primer sets revealed the presence of *Arcobacter* spp. in 85.7% of the retail chicken samples analyzed. *A. butzleri* was the only species present in 50% of the samples, and 35.7% of the samples were positive for both *A. butzleri* and *A. cryaerophilus. A. skirrowii* and *A. cibarius* were not detected in any of the chicken samples analyzed. The enrichment PCR assay developed is a specific and rapid alternative for the survey of *Arcobacter* contamination in meat.

The genus *Arcobacter* belongs to the rRNA superfamily VI of the Proteobacteria and was created in 1991 to accommodate organisms initially regarded as aerotolerant campylobacters *(37).* Although the high prevalence of *Campylobacter* in foods of animal origin has been documented as the main source of human gastrointestinal infection *(6),* data for *Arcobacter* species are much more limited. However, arcobacters are increasingly found on meats and have been isolated from people with diarrhea, which enhances the significance of these bacteria as a potential food safety concern *(20, 39, 41). Arcobacter butzleri, Arcobacter cryaerophilus* (with two subgroups), *Arcobacter skirrowii,* and *Arcobacter cibarius* are the *Arcobacter* species potentially associated with human disease *(23, 39).* Among these, mainly *A. butzleri,* but also *A. cryaerophilus* and *A. skirrowii,* have been isolated from raw meats, with the highest prevalence in poultry followed by pork, beef, and lamb *(6, 26, 34).* These organisms also have been recovered from untreated water, meat processing equipment surfaces, and environmental samples *(17, 40). A. cibarius* was isolated in 2005 from the skin of broiler chicken carcasses *(23)* and, more recently, from piggery effluents *(10).*

Conventional culture and phenotypic protocols may provide recovery and differentiation of arcobacters from related organisms, but these techniques are cumbersome to perform, time-consuming, and highly limited in specificity. *Arcobacter* species are fastidious in growth requirements, relatively biochemically inert, and morphologically similar to campylobacters, factors that may contribute to incorrect detection and identification of these organisms when relying on agar plating or phenotypic tests *(33).* In view of culture failure and misidentification, nucleic acid approaches, particularly PCR-based methods, are increasingly being considered for detection, identification, and monitoring of arcobacters in foods. These methods include simplex and multiplex PCR assays with species-specific primers *(7, 15, 16, 19, 24, 30),* PCR plus restriction fragment length polymorphism analyses *(31),* PCR plus random amplification of polymorphic DNA *(5, 22),* PCR plus DNA sequencing *(27),* PCR plus enzyme-linked immunosorbent assays *(3),* and real-time PCR assays *(1, 8).*

The objective of the present investigation was to develop a rapid enrichment PCR assay for detection of the four emerging pathogenic *Arcobacter* species in poultry meat: *A. butzleri, A. cryaerophilus, A. skirrowii,* and *A. cibarius.* The assay utilizes species-specific primers designed from *gyrA* and 16S rRNA gene sequences of these species and was applied to screen for the presence of arcobacters in 42 fresh retail-purchased chicken samples.

* Author for correspondence. Tel: 34-91-3943751; Fax: 34-91-3943743; E-mail: gonzalzi@vet.ucm.es.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and DNA extraction. The bacterial strains used in this study are listed in Table 1. *A. butzleri, A. cryaerophilus,* and *A. skirrowii* were grown on Mueller-Hinton agar supplemented with 5 to 10% defibrinated horse blood (Oxoid, Basingstoke, UK). *A. cibarius* was grown on brain heart infusion agar (Pronadisa, Madrid, Spain). All *Arcobacter* strains were incubated aerobically for 48 to 72 h at 30°C. *Campylobacter* and *Helicobacter* spp. were grown in Mueller-Hinton blood agar (5 to 10%) and incubated for 48 h at 37°C microaerobically. After recovery of bacteria from pure cultures, DNA was extracted following the method proposed by Sanz et al. *(35).*

Primer design. Sequences from *gyrA* and 16S rRNA genes of *Arcobacter, Campylobacter,* and *Helicobacter* strains available in the National Center for Biotechnology Information database were aligned and compared, and regions containing species-specific nucleotide differences were examined for the design of primers for *A. butzleri, A. cryaerophilus, A. skirrowii,* and *A. cibarius.* A fifth oligonucleotide primer pair was used as a control to avoid false-negative results in the PCRs. The sequences and description of every primer used in this study and the length of the generated amplicons are listed in Table 2. EMBOSS software package version 2.2.0 and Primer Express 2.0 software (Perkin-Elmer, Applied Biosystems Division, Foster City, CA) were used for primer design.

PCR setup. After a series of preliminary experiments, optimal amplification conditions were established as follows. Each reaction (25 μl) was set up to contain 2 μl of extracted bacterial DNA, 2 mM $MgCl_2$, 200 μM concentrations of each deoxynucleoside triphosphate, 15 pmol of primers 16SArcobutz, GyrArcocry, GyrArcoski, and 16Bact, 25 pmol of primers GyrArcocib, and 1 U of *Thermus thermophilus* DNA polymerase (Biotools, Madrid, Spain) in a reaction buffer containing 75 mM Tris-HCl, pH 9.0, 50 mM KCl, 20 mM $(NH_4)_2SO_4$, and 0.001% bovine serum albumin. The cycling conditions were 94°C for 3 min for denaturation, 40 cycles (*Arcobacter* primers 16SArcobutz, GyrArcocry, GyrArcoski, and GyrArcocib) or 25 cycles (bacterial primers 16SBact) of amplification at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final extension step at 72°C for 7 min.

Analysis of artificially contaminated chicken samples. Batches of 40-g *Arcobacter*-free chicken muscle samples were aseptically seeded with pure cultures of *A. butzleri, A. cryaerophilus, A. skirrowii,* and *A. cibarius* to obtain bacterial counts of approximately 10, 10^2, 10^3, and 10^4 CFU/g. Negative uninoculated controls were included in each experiment. Inoculated and control samples were homogenized in a stomacher with 120 ml (1:4 dilution) of *Arcobacter* broth (Oxoid), and 10-ml aliquots of each homogenate were supplemented with 0.008 g/liter cefoperazone, 0.01 g/liter amphotericin, and 0.004 g/liter teicoplanin (CAT; Oxoid) before performing DNA extraction and microbiological analysis.

Bacterial DNA was extracted and purified from inoculated chicken samples as follows. A 0.5-ml portion of each homogenate was digested in 0.5 ml of an extraction buffer, pH 8.0 (10 mM Tris, 150 mM NaCl, 2 mM EDTA, and 1% sodium dodecyl sulfate), 100 μl of 5 M guanidine hydrochloride, and 40 μl of 20 mg/ml proteinase K (Boehringer Mannheim GmbH, Mannheim, Germany). Digests were incubated for 3 h at 55°C with shaking at 60 rpm and then left to cool at room temperature. Five hundred microliters of chloroform (Sigma-Aldrich, St. Louis, MO) was added to the lysate before centrifugation at 13,000 rpm for 10 min. The clear aqueous supernatant obtained after centrifugation (500 μl) was used to purify the DNA using the Wizard DNA cleanup system kit (Promega Corp., Madison, WI) with a vacuum manifold according to the manufacturer's instructions.

TABLE 1. *Bacterial strains used in this study*

Species	Strain[a]
Arcobacter butzleri	LMG 9910
A. butzleri	LMG 10828[T]
A. cryaerophilus subgroup 1	LMG 9863
A. cryaerophilus subgroup 2	LMG 7537
A. skirrowii	LMG 6621[T]
A. skirrowii	LMG 8538
A. cibarius	CECT 7203
A. cibarius	LMG 21997
Campylobacter jejuni	Clinical isolate
C. jejuni subsp. *jejuni*	LMG 6444[T]
C. coli	Clinical isolate
C. fetus subsp. *fetus*	LMG 6442[T]
C. lari	LMG 8845
Helicobacter pylori	LMG 18041[T]
H. pullorum	LMG 16318
Escherichia coli	CECT 515
Salmonella Enteritidis	CECT 4300
Yersinia enterocolitica	CECT 559
Listeria innocua	CIP 103575
Pseudomonas fluorescens	B52[b]

[a] LMG, Laboratory for Microbiology, Gent, Belgium; CECT, Spanish Type Culture Collection, Valencia, Spain; CIP, Institute Pasteur Collection, Paris, France.
[b] Isolate from milk origin supplied by Food Research Centre of Ottawa, Ottawa, Ontario.

Growth and populations of *Arcobacter* were determined by incubation of seeded chicken samples in modified cefsulodin-irgasan-novobiocin (mCIN)–CAT agar at 30°C for 3 to 4 days under aerobic conditions. The mCIN-CAT agar is a variant of the mCIN agar developed by Collins et al. *(11).* Unseeded samples were also analyzed to confirm the absence of *Arcobacter* colonies.

Analysis of retail chicken samples. Chilled fresh chicken samples ($n = 42$) were purchased from several local supermarkets and retail shops. Forty-gram portions (drumsticks, leg quarters, and breasts with the skin) were homogenized in a stomacher with 120 ml (1:4 dilution) of *Arcobacter* broth, and 10-ml aliquots of each homogenate were supplemented with CAT. A subsequent enrichment step was performed by incubation of the samples at 30°C for 18 h under microaerophilic conditions. In every experiment, control meat portions artificially contaminated with approximately 5 cells per g of each *Arcobacter* species were included. DNA extraction and PCR were carried out before and after the enrichment step following the procedure described above for the artificially contaminated samples.

Selective recovery of *Arcobacter* spp. from naturally contaminated meats and seeded controls was accomplished in mCIN-CAT agar before and after enrichment. The plates were incubated aerobically at 30°C for 3 to 4 days and then examined for typical *Arcobacter* colonies.

RESULTS

Specificity of the four *Arcobacter* primer sets was verified by PCR testing of heterologous *Arcobacter* species, closely related *Campylobacter* and *Helicobacter* species, and other bacterial strains (Table 1). Specific 203-, 212-,

TABLE 2. *Oligonucleotide primers used in this study*

Primer name	Sequence (5′ to 3′)	Target gene	Target species	Amplicon size (bp)
16SArcobutzFw	AGTTGTTGTGAGGCTCCAC	16S rRNA	*A. butzleri*	203
16SArcobutzRv	GCAGACACTAATCTATCTCTAAATCA			
GyrArcocryFw	TGCTAAAATTGCAGATGTACCA	*gyrA*	*A. cryaerophilus*	212
GyrArcocryRv	AATTCCTTTTTCAGAAACTGTACG			
GyrArcoskiFw	GAGACAACTTTTGGAACTATTCTATGA	*gyrA*	*A. skirrowii*	257
GyrArcoskiRv	GAAGATAGATTAACTTTTGCTTGTTG			
GyrArcocibFw	TGGAAATATTGTTGGTGAAGTTCAG	*gyrA*	*A. cibarius*	145
GyrArcocibFw	ATCTACATTTACAATACTTACTCCCGAA			
16SBactFw	CAGCAGCCGCGGTAATA	16S rRNA	Bacteria	290
16SBactRv	TGGACTACCAGGGTATCTAAT			

257-, and 145-bp amplicons were successfully generated from *A. butzleri, A. cryaerophilus, A. skirrowii,* and *A. cibarius,* respectively. To discard possible false-negative PCR results, samples were simultaneously analyzed with a positive amplification control designed to amplify a 290-bp conserved 16S rRNA bacterial region. The sensitivity of *Arcobacter* primers was then evaluated through PCR analysis of chicken samples artificially seeded with *Arcobacter* species in the range of 10 to 10^4 CFU/g, obtaining a detection limit of approximately 10^3 CFU/g of chicken meat. A similar sensitivity pattern was achieved with the four species-specific primer pairs (results not shown).

The applicability of the PCR assay was assessed by testing 42 retail-purchased chicken samples with the four *Arcobacter* primer sets for the presence or absence of *Arcobacter* spp. A selective 18-h microaerobic preenrichment step was performed for subsequent PCR detection of small numbers of *Arcobacter* in chicken. Using this protocol, *Arcobacter* spp. were detected in 85.7% (36 of 42) of retail-purchased chicken samples analyzed. *A. butzleri* was the only species present in 50% (21) of the samples, whereas 35.7% (15) of the samples were positive for both *A. butzleri* and *A. cryaerophilus.* In contrast, none of the chicken samples yielded PCR signals with the *A. skirrowii* and *A. cibarius* primers (Table 3). Control meat portions artificially seeded with 5 CFU/g of each *Arcobacter* species produced a positive amplification result after enrichment. In all experiments, bacterial control primers confirmed the presence of amplifiable DNA in the samples (results not shown).

A close correlation between the enrichment PCR technique and the conventional plating procedure was achieved. Every PCR-positive sample produced visible characteristic *Arcobacter* colonies on mCIN-CAT agar, and no *Arcobacter* growth was detected in samples that were PCR negative.

DISCUSSION

A crucial requirement for successfully detecting specific microorganisms with a PCR assay is to choose adequate genetic markers during the primer design process that allow a high degree of specificity *(36).* The *gyrA* gene encoding a subunit of DNA gyrase has been used as an alternative support for ribosomal markers in bacterial phylogenetic and identification studies *(1, 8).* The *gyrA* gene is essential for bacterial viability and has been recently sequenced in the genus *Arcobacter (2, 29).* Thus, the *gyrA* gene was the tool of choice for primer design in our study. Analysis of *gyrA* sequence alignment allowed the design of primers for *A. cryaerophilus, A. skirrowii,* and *A. cibarius* complementary to regions exhibiting sufficient species-specific nucleotide differences. However, areas searched along the *gyrA* sequences did not show optimal specificity for *A. butzleri.* Because the 16S rRNA gene has a mosaic structure of phylogenetically conserved and variable regions that is adequate for species-specific discrimination of particular microorganisms *(42),* this gene was used to attempt specific detection of *A. butzleri.* Results obtained indicated that *A. butzleri* 16S rRNA primers consistently amplified the targeted 203-bp fragment of *A. butzleri,* whereas primers directed toward the *gyrA* gene yielded the desired 212-, 257-, and 145-bp fragments of *A. cryaerophilus, A. skirrowii,* and *A. cibarius,* respectively, indicating a high degree of specificity.

The PCR is an inherently highly specific and sensitive technique, but when dealing with food matrices of complex constituents, a decrease in assay sensitivity may occur because of PCR inhibition. Preenrichment of bacteria to a certain level combined with the use of effective DNA extraction techniques that minimize the presence of inhibitors in the samples are further requirements for successful detection of low numbers of organisms in foods when using PCR techniques *(28, 36).*

Various broth and plating formulations have been developed, most of them dependent on enrichment media supplemented with antibiotics and incubated at lower temperatures than those used for closely related *Campylobacter* species *(11, 13, 14, 18, 21, 25).* Using these procedures, variable isolation rates reaching values as high as 95% *(4, 34)* have been documented for *Arcobacter.* Results of preliminary assays indicated that the detection threshold of the direct PCR assay (without any enrichment) was approximately 10^3 CFU of *Arcobacter* per gram of chicken meat. For improved sensitivity when screening naturally contaminated poultry samples, the amplification assay was combined with an 18-h incubation step in an *Arcobacter* enrichment medium widely used in previous studies *(16, 24, 32, 34).* After enrichment and subsequent PCR processing with the four *Arcobacter* primer sets, the presence of *Arcobacter,* particularly *A. butzleri,* was detected in 85.7% of the retail chicken samples analyzed. The enrichment PCR assay also detected the presence of *A. cryaerophilus* and *A. butzleri* together in 35.7% of the samples. These results are

TABLE 3. *PCR and microbiological results obtained after analysis of 42 fresh retail-purchased chicken samples*

Sample no.	*Arcobacter* population (CFU/g)[a]		Detection by PCR (18 h)[b]			
	Before enrichment	After 18 h	Abutz	Acry	Aski	Acib
1	ND	4×10^4	+	−	−	−
2	ND	3×10^4	+	−	−	−
3	ND	4.8×10^4	+	−	−	−
4	ND	ND	−	−	−	−
5	8	2.4×10^4	+	−	−	−
6	ND	UC	+	+	−	−
7	4	3×10^5	+	−	−	−
8	ND	3.4×10^4	+	−	−	−
9	ND	ND	−	−	−	−
10	ND	3.6×10^4	+	−	−	−
11	ND	UC	+	+	−	−
12	ND	1.6×10^5	+	−	−	−
13	ND	3.1×10^5	+	−	−	−
14	ND	2.4×10^4	+	+	−	−
15	ND	1.1×10^5	+	−	−	−
16	16	1.2×10^6	+	+	−	−
17	ND	UC	+	−	−	−
18	ND	UC	+	−	−	−
19	ND	3.9×10^5	+	+	−	−
20	8	1.8×10^4	+	−	−	−
21	ND	2×10^4	+	−	−	−
22	ND	3.4×10^5	+	−	−	−
23	ND	UC	+	−	−	−
24	64	UC	+	+	−	−
25	ND	2.4×10^4	+	−	−	−
26	ND	6.8×10^4	+	−	−	−
27	ND	ND	−	−	−	−
28	8	UC	+	+	−	−
29	16	UC	+	+	−	−
30	ND	2×10^4	+	−	−	−
31	ND	ND	−	−	−	−
32	8	1×10^6	+	−	−	−
33	ND	1.9×10^6	+	+	−	−
34	4	1.8×10^6	+	+	−	−
35	4	1.4×10^6	+	+	−	−
36	ND	2.4×10^5	+	+	−	−
37	ND	5.6×10^5	+	+	−	−
38	ND	ND	−	−	−	−
39	ND	9.6×10^5	+	+	−	−
40	ND	ND	−	−	−	−
41	ND	8.8×10^4	+	−	−	−
42	ND	3.6×10^5	+	+	−	−

[a] ND, not detected; UC, uncountable.
[b] All samples were negative before enrichment. Abutz, *A. butzleri*; Acry, *A. cryaerophilus*; Aski, *A. skirrowii*; Acib, *A. cibarius*.

consistent with those of studies in which *A. butzleri* has been found to be the prevailing species in poultry products, followed by *A. cryaerophilus* and *A. skirrowii (12, 21).* In agreement with our observations, concurrent detection in foods of more than one *Arcobacter* species, particularly *A. butzleri* and *A. cryaerophilus,* is not uncommon *(22, 38).* None of the naturally contaminated chicken samples tested produced amplification signals for *A. skirrowii* or *A. cibarius,* indicating the absence of these species in these samples. Within this context, the fact that other authors have failed to detect *A. skirrowii* in chicken suggests that the low recovery rates reported for this species may be attributed to its higher susceptibility (compared with other arcobacters) to the antimicrobial agents used in selective media or to growth competition favoring other bacteria *(9, 21).* With respect to *A. cibarius,* only one previous study has documented its isolation from broiler carcasses *(23).* The pathogenic potential of *A. cibarius* is unknown, but because other closely related arcobacters have been implicated as agents of foodborne human disease, detection procedures also should include this species. To our knowledge, this is the first report of the possibility of detecting *A. cibarius* in poultry by means of a species-specific PCR assay.

DNA-based techniques currently available for *Arcobacter* detection and identification are undoubtedly valuable for monitoring these organisms in food. However, some of these methods are focused on the genus *Arcobacter* or target only *A. butzleri (7, 16, 19),* whereas others lack specificity for a particular species within this genus *(1, 24)* or require postprocessing of the PCR products either by endonuclease or sequencing procedures *(27, 31).* The *Arcobacter*-specific enrichment PCR assay developed in the present study offers some significant advantages over phenotypic tests and other PCR-based methods: (i) it is rapid and straightforward, (ii) it is sensitive and specific, and (iii) it has discriminatory ability for the four emerging pathogenic *Arcobacter* species—*A. butzleri, A. cryaerophilus, A. skirrowii,* and *A. cibarius.* A limitation of the assay is that the fragment lengths of the *gyrA* and 16S rRNA PCR products selected (from 145 to 257 bp) impeded multiplex detection of the four *Arcobacter* species in the same reaction. However, this *Arcobacter*-specific PCR assay meets the demand for future adaptation to a real-time PCR–based format that does not require reagent addition and gel separation and allows quantification of the bacterial load in the samples. In this context, two real-time PCR assays using either Taqman *(8)* or FRET-based *(1)* technology have been reported recently for *Arcobacter* detection. However, the assay of Brightwell et al. *(8)* is directed to only *A. butzleri* and *A. cryaerophilus* and that of Abdelbaqi et al. *(1)* failed to discriminate *A. skirrowii.* The enrichment PCR assay developed in this work could be useful for the routine detection and identification of the four *Arcobacter* species currently associated with human disease caused by consumption of contaminated poultry and other meat commodities.

ACKNOWLEDGMENTS

This work was financed by funds from project PR34/07-15902 from Santander Central Hispano–Universidad Complutense de Madrid (Madrid, Spain) and by the Programa de Vigilancia Sanitaria S-0505/AGR/000265 of the Comunidad de Madrid.

REFERENCES

1. Abdelbaqi, K., A. Buissonniere, V. Prouzet-Mauléon, J. Gresser, I. Wesley, F. Megraud, and A. Menard. 2007. Development of a real-time fluorescence resonance energy transfer PCR to detect *Arcobacter* species. *J. Clin. Microbiol.* 45:3015–3021.
2. Abdelbaqi, K., A. Menard, V. Prouzet-Mauléon, F. Bringaud, P. Le-

hours, and F. Megraud. 2007. Nucleotide sequence of the *gyrA* gene of *Arcobacter* species and characterization of human ciprofloxacin-resistant clinical isolates. *FEMS Immunol. Med. Microbiol.* 49:337–345.

3. Antolín, A., I. González, T. García, P. E. Hernández, and R. Martín. 2001. *Arcobacter* spp. enumeration in poultry meat using a combined PCR-ELISA assay. *Meat Sci.* 59:169–174.
4. Atabay, H. I., F. Aydin, K. Houf, M. Sahin, and P. Vandamme. 2003. The prevalence of *Arcobacter* spp. on chicken carcasses sold in retail markets in Turkey, and identification of the isolates using SDS-PAGE. *Int. J. Food Microbiol.* 81:21–28.
5. Atabay, H. I., D. D. Bang, F. Aydin, H. M. Erdogan, and M. Madsen. 2002. Discrimination of *Arcobacter butzleri* isolates by polymerase chain reaction–mediated DNA fingerprinting. *Lett. Appl. Microbiol.* 35:141–145.
6. Atabay, H. I., and J. E. L. Corry. 1997. The prevalence of campylobacters and arcobacters in broiler chickens. *J. Appl. Microbiol.* 83: 619–626.
7. Bastyns, K., D. Cartuyvels, S. Chappelle, P. Vandamme, H. Gossens, and R. De Watcher. 1995. A variable 23S rDNA region is a useful discriminating target for genus-specific and species-specific PCR amplification in *Arcobacter* species. *Syst. Appl. Microbiol.* 18:353–356.
8. Brightwell, G., E. Mowat, R. Clemens, J. Boerema, D. J. Pulford, and S. L. On. 2007. Development of a multiplex and real time PCR assay for the specific detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus. J. Microbiol. Methods* 68:318–325.
9. Cervenka, L. 2007. Survival and inactivation of *Arcobacter* spp., a current status and future prospect. *Crit. Rev. Microbiol.* 33:101–108.
10. Chinivasagam, H. N., B. G. Corney, L. L. Wright, I. S. Diallo, and P. J. Blackall. 2007. Detection of *Arcobacter* spp. in piggery effluent and effluent-irrigated soils in southeast Queensland. *J. Appl. Microbiol.* 103:418–426.
11. Collins, C. I., I. V. Wesley, and E. A. Murano. 1996. Detection of *Arcobacter* spp. in ground pork by modified plating methods. *J. Food Prot.* 59:448–452.
12. Corry, J. E. L., and H. I. Atabay. 2001. Poultry as a source of *Campylobacter* and related organisms. *J. Appl. Microbiol.* 90:96S–114S.
13. de Boer, E., J. J. H. C. Tilburg, D. L. Woodward, H. Lior, and W. M. Johnson. 1996. A selective medium for the isolation of *Arcobacter* from meats. *Lett. Appl. Microbiol.* 23:64–66.
14. Eifert, J. D., R. M. Castle, F. W. Pierson, C. T. Larsen, and C. R. Hackney. 2003. Comparison of sampling techniques for detection of *Arcobacter butzleri* from chickens. *Poult. Sci.* 82:1898–1902.
15. González, A., S. Botella, R. M. Montes, Y. Moreno, and M. A. Ferrús. 2007. Direct detection and identification of *Arcobacter* species by multiplex PCR in chicken and wastewater samples from Spain. *J. Food Prot.* 70:341–347.
16. González, I., A. Antolín, T. García, P. E. Hernández, and R. Martín. 2000. Development of a combined PCR-culture technique for the rapid detection of *Arcobacter* spp. in chicken meat. *Lett. Appl. Microbiol.* 30:207–212.
17. Gude, A., T. J. Hillman, C. R. Helps, V. M. Allen, and J. E. Corry. 2005. Ecology of *Arcobacter* species in chicken rearing and processing. *Lett. Appl. Microbiol.* 41:82–87.
18. Hamill, S., S. D. Neill, and R. H. Madden. 2008. A comparison of media for the isolation of *Arcobacter* spp. from retail packs of beef. *J. Food Prot.* 71:850–854.
19. Harmon, K. M., and I. V. Wesley, 1997. Multiplex PCR for the identification of *Arcobacter* and differentiation of *Arcobacter butzleri* from other arcobacters. *Vet. Microbiol.* 58:215–227.
20. Ho, H. T., L. J. Lipman, and W. Gaastra. 2006. *Arcobacter,* what is known and unknown about a potential foodborne zoonotic agent! *Vet. Microbiol.* 115:1–13.
21. 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.
22. Houf, K., L. De Zutter, J. Van Hoof, and P. Vandamme. 2002. Assessment of the genetic diversity among arcobacters isolated from poultry products by using two PCR-based typing methods. *Appl. Environ. Microbiol.* 68:2172–2178.
23. Houf, K., S. L. On, T. Coenye, J. Mast, J. Van Hoof, and P. Vandamme. 2005. *Arcobacter cibarius* sp. nov., isolated from broiler carcasses. *Int. J. Syst. Evol. Microbiol.* 55:713–717.
24. 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.
25. Johnson, L. G., and E. A. Murano. 1999. Development of a new medium for the isolation of *Arcobacter* spp. *J. Food Prot.* 62:456–462.
26. Kabeya, H., Y. Kobayashi, S. Maruyama, and T. Mikami. 2003. One step polymerase chain reaction based typing of *Arcobacter* species. *Int. J. Food Microbiol.* 81:163–168.
27. Kärenlampi, R. I., T. P. Tolvanen, and M.-L. Hänninen. 2004. Phylogenetic analysis and PCR–restriction fragment length polymorphism identification of *Campylobacter* species based on partial *groEL* gene sequences. *J. Clin. Microbiol.* 42:5731–5738.
28. Lantz, P.-G., B. Hahn-Hagerdal, and P. Rådström. 1994. Sample preparation methods in PCR-based detection of food pathogens. *Trends Food Sci. Technol.* 5:384–389.
29. Menard, A., F. Dachet, V. Prouzet-Mauléon, M. Oleastro, and F. Mégraud. 2005. Development of a real-time fluorescence resonance energy transfer PCR to identify the main pathogenic *Campylobacter* spp. *Clin. Microbiol. Infect.* 11:281–287.
30. Neubauer, C., and M. Hess. 2006. Detection and identification of food-borne pathogens of the genera *Campylobacter, Arcobacter* and *Helicobacter* by multiplex PCR in poultry and poultry products. *J. Vet. Med.* 53:376–381.
31. Neubauer, J., V. Jauk, and W. Szolgyenyi. 2003. Phenotypic and genotypic differentiation of *Campylobacter* spp. isolated from Austrian broiler farms: a comparison. *Avian Pathol.* 32:33–37.
32. Pejchalová, M., E. Dostalíková, M. Slámová, I. Brozková, and J. Vytrasová. 2008. Prevalence and diversity of *Arcobacter* spp. in the Czech Republic. *J. Food Prot.* 71:719–727.
33. Prouzet-Mauléon, V., L. Labadi, N. Bouges, A. Menard, and F. Megraud. 2006. *Arcobacter butzleri*: underestimated enteropathogen. *Emerg. Infect. Dis.* 12:307–309.
34. Rivas, L., N. Fegan, and P. Vanderlinde. 2004. Isolation and characterisation of *Arcobacter butzleri* from meat. *Int. J. Food Microbiol.* 91:31–41.
35. Sanz, A., R. Martín, M. B. Mayoral, P. E. Hernández, I. González, and T. García. 2005. Development of a PCR-culture technique for rapid detection of yeast species in vacuum packed ham. *Meat Sci.* 71:230–237.
36. Scheu, P., M. K. Berghof, and U. Stahl. 1998. Detection of pathogenic and spoilage microorganisms in food with the polymerase chain reaction. *Food Microbiol.* 15:13–31.
37. Vandamme, O., E. Falsen, R. Rossau, B. Hoste, P. Segers, R. Tygat, and J. Delay. 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.
38. Van Driessche, E., and K. Houf. 2007. Characterization of the *Arcobacter* contamination on Belgian pork carcasses and raw retail pork. *Int. J. Food Microbiol.* 118:20–26.
39. Vandenberg, O., A. Dediste, K. Houf, S. Ibekwem, H. Souayah, S. Cadranel, N. Douat, G. Zissis, J. P. Butzler, and P. Vandamme. 2004. *Arcobacter* species in humans. *Emerg. Infect. Dis.* 10:1863–1867.
40. Vytrasová, J., M. Pejchalová, and K. Harsová. 2003. Isolation of *Arcobacter butzleri* and *A. cryaerophilus* in samples of meats and from meat-processing plants by a culture technique and detection by PCR. *Fol. Microbiol.* 48:227–232.
41. Wesley, I. V. 1997. *Helicobacter* and *Arcobacter*: potential human foodborne pathogens? *Trends Food Sci. Technol.* 8:293–299.
42. Wilson, K. H., R. B. Blitchingson, and R. C. Green. 1990. Amplification of bacterial 16S ribosomal RNA with polymerase chain reaction. *J. Clin. Microbiol.* 28:1942–1946.