

## Direct Molecular Approach to Monitoring Bacterial Colonization on Vacuum-Packaged Beef

Cecilia Fontana,<sup>1,2</sup> Pier Sandro Cocconcelli,<sup>2,3</sup> and Graciela Vignolo<sup>1\*</sup>

*Centro de Referencia para Lactobacilos (CERELA), CONICET, Chacabuco 145, T4000ILC Tucumán, Argentina<sup>1</sup>; Centro Ricerche Biotecnologiche, Università Cattolica del Sacro Cuore, via Milano 24, 26100 Cremona, Italy<sup>2</sup>; and Istituto di Microbiologia, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, 29100 Piacenza, Italy<sup>3</sup>*

Received 5 January 2006/Accepted 16 May 2006

**Denaturing gradient gel electrophoresis allowed us to monitor total bacterial communities and to establish a pattern of succession between species in vacuum-packaged beef stored at 2 and 8°C for 9 weeks and 14 days. Species-specific PCR was used to confirm the presence of *Lactobacillus sakei* and *Lactobacillus curvatus*. Multiplex PCRs using 16S rRNA-specific primers allowed differentiation between *Leuconostoc* species. These methods provided the desired information about microbial diversity by detecting the main microorganisms capable of colonizing this ecological niche.**

Vacuum packaging under chilled conditions has proved very effective in extending the shelf life of perishable foods, such as fresh meat and meat products, and preventing the growth of food-borne pathogens (8). The oxygen supply will be restricted, depending on the gas permeability of the packaging film, and thus has a selective effect on the microbial population (22). Lactic acid bacteria (LAB), such as *Lactobacillus* spp., *Leuconostoc* spp., *Carnobacterium* spp., and *Brochothrix thermosphacta*, are the main spoilage organisms associated with chilled vacuum-packaged fresh-meat products (6, 18, 21, 26, 33). Shortly after vacuum packaging of meat, LAB populations are usually below the routine detection limit (<10 CFU/g), but they increase during storage (19). Although LAB can cause meat spoilage, a selective growth promotion of LAB capitalizing on their ability to control meat-borne pathogens with a preferential growth of benign strains would minimize their spoilage effects (7, 25, 32, 34).

Methods in molecular microbiology, especially those including the sequencing of genes coding for 16S rRNA, have become a very important tool in the study of bacterial communities in meat samples. The trend is toward culture-independent methods, because they are believed to overcome problems associated with selective cultivation and isolation of bacteria from natural samples. Genetic-fingerprinting techniques provide a profile representing the genetic diversity of a microbial community from a specific environment. Denaturing gradient gel electrophoresis (DGGE) is usually employed to assess the structure and dynamics of microbial communities in food samples without cultivation in response to environmental variations (13, 14, 15, 27). Species-specific PCR is a rapid and reliable molecular technique for the characterization of bacterial communities, and it can be also applied in situ without colony isolation (2). The variations in length and sequences of the 16S/23S rRNA intergenic spacer regions of the rRNA

operon have proved useful for strain and species identification (2, 3, 17). In this work, we describe the application of culture-independent methods to the study of the microbial succession dynamics in vacuum-packaged beef stored at 2 and 8°C for 9 weeks and 14 days, respectively.

**Bacterial control strains and growth conditions.** *Lactobacillus sakei* CRL1463, *Lactobacillus curvatus* CRL 1465, and *Leuconostoc gelidum* CRL 1542 (CERELA culture collection) were used as reference strains. *L. gelidum* and the lactobacilli were cultured in MRS broth (Merck) at 20 and 30°C, respectively. After the bacteria were streaked on appropriate agar plates, DNA extraction was performed using *Microlysis* (LABOGEN, United Kingdom) in accordance with the protocol described by the manufacturer.

**Meat storage and sampling.** A 4-kg cut of lean beef (*Musculus semimembranosus*) was purchased about 48 h after slaughter from two different meat shops. Each refrigerated meat cut was divided into small pieces (each weighing about 10 g) after the superficial meat layer (~0.5 cm) had been removed to restrict contamination. Cutting was carried out at ambient temperature with a sterile knife, and each piece was immediately placed in a sterile plastic bag (Cryovac, Argentina; O<sub>2</sub> transmission rate, 10 to 30 cm<sup>3</sup> m<sup>-2</sup> atm<sup>-1</sup> 24 h<sup>-1</sup> at 25°C and 75% relative humidity) sealed at a final vacuum of 99% using a vacuum-packaging machine (TURBO VAC 320ST; Howden Food Equipment, The Netherlands). Samples were analyzed after 1, 3, 6, and 9 weeks and 4, 6, 9, and 14 days of storage at 2 and 8°C, respectively. All experiments were carried out in duplicate. One-way analysis of variance was used; a *P* value of <0.05 was considered statistically significant.

**Bacterial enumeration and pH measurements.** A 10-g portion of each beef sample was homogenized in 90 ml of 0.1% peptone, pH 7.00 (Difco Laboratories, Detroit, Mich.) in a Stomacher Lab-Blender (model 400; A. J. Seward Laboratory, London, England). Decimal dilutions were prepared, and the following analyses were carried out on agar plates: total aerobic counts on plate count agar (48 h at 30°C); LAB on MRS agar (Merck), pH 6.5 (48 h at 30°C) in an anaerobic jar (Anaerocult C Microphilic gas generator; Merck, Germany);

\* Corresponding author. Mailing address: Centro de Referencia para Lactobacilos, CERELA (CONICET), Chacabuco 145, T4000ILC Tucumán, Argentina. Phone: 54-381-4311720. Fax: 54-381-4005600. E-mail: vignolo@cerela.org.ar.

TABLE 1. Average pH and microbial counts for meat samples during chilled storage

Temp (°C)	Sample	pH ± SD	PCA <sup>a</sup> ± SD	MRS <sup>b</sup> ± SD
2	Time 0	5.57 ± 0.03	0 ± 0.00	2.0 ± 0.00
	Week 1	5.29 ± 0.06	3.3 ± 0.07	4.8 ± 0.00
	Week 3	5.37 ± 0.01	4.2 ± 0.07	5.8 ± 0.07
	Week 6	5.15 ± 0.02	5.2 ± 0.00	6.2 ± 0.07
	Week 9	5.17 ± 0.03	5.3 ± 0.00	6.3 ± 0.07
8	Day 4	5.49 ± 0.05	4.4 ± 0.02	4.5 ± 0.10
	Day 6	5.52 ± 0.02	4.8 ± 0.07	5.3 ± 0.07
	Day 9	5.15 ± 0.10	5.2 ± 0.02	5.4 ± 0.00
	Day 14	5.24 ± 0.02	5.4 ± 0.04	6.6 ± 0.20

<sup>a</sup> Total aerobic counts (log<sub>10</sub> CFU/g).

<sup>b</sup> Lactic acid bacteria (log<sub>10</sub> CFU/g).

*Pseudomonas* on Cetrimide agar (Oxoid) (72 h at 20°C); *Brochothrix thermosphacta* on STAA selective agar (Oxoid) (48 h at 20°C); and total coliforms on McConkey agar (48 h at 37°C). Unless otherwise specified, all media and ingredients were obtained from Britania (Argentina). The pin electrode of a pH meter (692 pH/Ion meter; Metrohm) inserted directly into the sample was used for pH determinations. Three independent measurements were performed for each sample. Bacterial counts and pH changes throughout storage are shown in Table 1. The mean of the pH values decreased during storage at 2 and 8°C to final values of 5.17 ± 0.03 and 5.24 ± 0.02, respectively. LAB counts increased from 10<sup>2</sup> to 10<sup>6</sup> CFU/g after 14 days at 8°C and 6 weeks at 2°C, while total bacterial counts reached 10<sup>5</sup> CFU/g; both populations had a lower growth rate at 2°C. The dominance of LAB in meat is consistent with their ability to multiply under low oxygen concentrations and chilled conditions, with their growth rate accelerating as the temperature increases (20, 30). In this work, LAB counts were rather lower than the ones reported for vacuum-packaged beef stored for 6 to 8 weeks at temperatures between -1.5 and 4°C, with final counts of 10<sup>7</sup> to 10<sup>8</sup> CFU/g (5, 19, 28). *B. thermosphacta* increased from 10<sup>2</sup> CFU/g (time zero) up to 10<sup>4</sup> CFU/g at both temperatures assayed, while *Pseudomonas* and *Enterobacteria* cell counts were below the detection limit (data not shown). The drop in pH values observed in meat samples may be attributed to the selective growth of LAB, which may also have contributed to the inhibition of gram-negative meat-borne organisms (*Pseudomonas* and *Enterobacteria*). Moreover, the lower cell counts obtained in our study can be explained by the removal of the external meat layer during sampling, partially eliminating superficial meat contamination.

**DGGE analysis.** The total microbiota was monitored by DGGE during chilled storage. DNA extraction from meat samples, PCR amplification of V1 and V3 variable regions of the bacterial 16S rRNA gene, and the electrophoresis protocol were performed according to the method of Fontana et al. (16). In this study, different denaturing gradients were assayed for each PCR product in order to obtain the best species discrimination. Denaturing gradients of 35 to 60% and 30 to 50% (100% corresponded to 7 M urea and 40% formamide) were used for V3 and V1 DGGE analyses, respectively. Pure

cultures of the reference strains *L. sakei*, *L. curvatus*, and *L. gelidum* were included in DGGE analysis (Fig. 1A and B). DGGE profiles belonging to the same bacterial species showed the presence of multiple bands with different migration distances, confirming the presence of multiple copies of the 16S rRNA genes as previously described by other authors (9, 13). However, species discrimination and monitoring of bacterial changes during storage could be performed, since no overlapping between PCR fragments was observed. DGGE profiles from the V3 and V1 variable regions showed fluctuations in the microbial populations (Fig. 1). Fragments from both variable regions analyzed that migrated to the same position as those obtained from pure cultures were excised from the gel, reamplified, and sequenced, and their relative identifications were obtained by alignment in GenBank (1). DGGE profiles

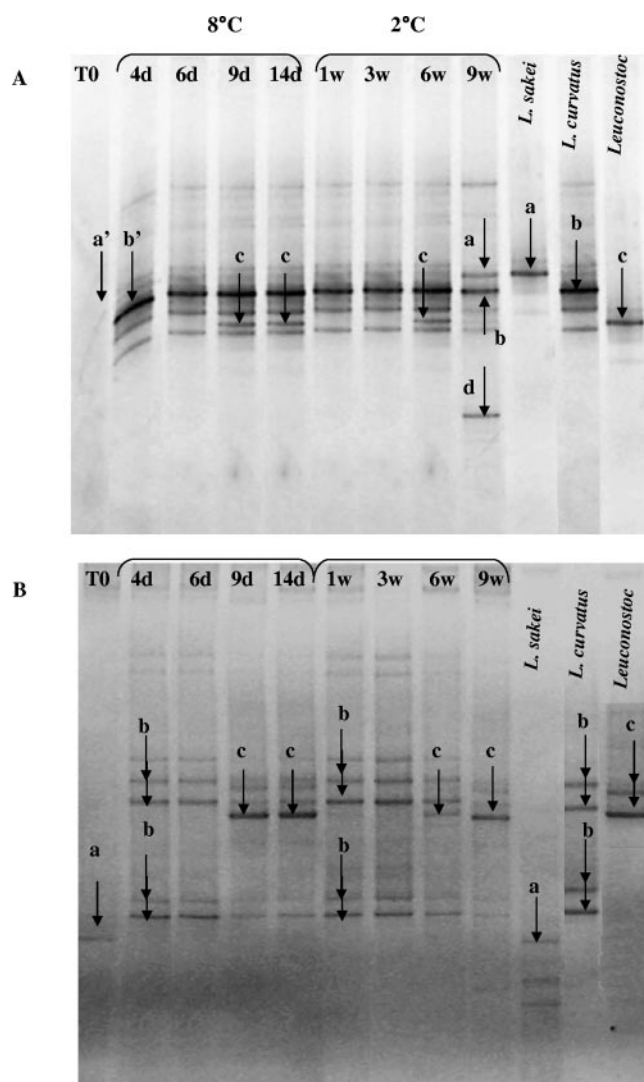


FIG. 1. Vacuum-packaged beef bacterial community DGGE fingerprints. (A) V3 (~200 bp) and (B) V1 (~100 bp) 16S rRNA gene variable-region profiles from meat samples at time zero (T0) and during storage at 8°C (4, 6, 9, and 14 days [d]) and at 2°C (1, 3, 6, and 9 weeks [w]). Bands indicated by letters were excised and subjected to sequencing after reamplification. DNA sequencing was carried out by CRIBI, Università degli Studi, Padova, Italy.

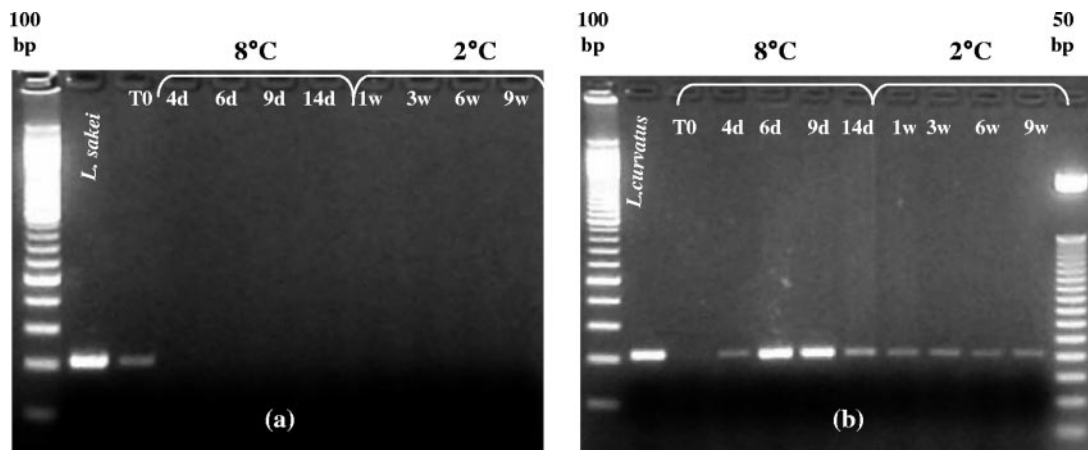


FIG. 2. Electrophoresis on 2% agarose gel of PCR amplification products (~235 bp) obtained using species-specific primers for (a) *L. sakei* (16/Ls) and (b) *L. curvatus* (16/Lc).

showed faintly staining fragments at time zero (bands a) that coincided with the migration distance of the 16S fragment from *L. sakei* (Fig. 1). This band was observed throughout the storage period on the V3 DGGE profile (Fig. 1A). Fragments corresponding to *L. curvatus* (bands b) became evident after 4 days at 8°C and from the first week at 2°C and were detected until 14 days and 9 weeks of storage at 8 and 2°C, respectively (Fig. 1). Other DGGE bands comigrating with the band obtained from *L. gelidum* (band c) were identified as *Leuconostoc* spp. and appeared from day 9 and from week 6 in the samples stored at 8°C and 2°C, respectively (Fig. 1). Band d was detected only at 2°C (week 9) when the V3 bacterial 16S fragment was analyzed (Fig. 1A). The sequence analysis showed that this band was linked to the species *B. thermosphacta*. Although DGGE was used to analyze other meat-based environments (9, 10, 15, 27), as far as we know, this is the first report that used DGGE analysis to rapidly check the bacterial community present on vacuum-packaged meat during chilled storage. In this work, LAB populations were mainly represented by *L. curvatus*, *L. sakei*, and *Leuconostoc* spp. Similar results were obtained using DNA sequence analysis and random amplified polymorphic DNA-PCR to identify strains isolated from vacuum-packaged beef stored at 2°C (36). *B. thermosphacta* was detected by DGGE at the last sampling point at 2°C, having been frequently reported as a major contaminant in raw meat (30).

**Species-specific detection of *Lactobacillus*.** PCR was performed using primers complementary to species-specific sequences in the 16S/23S rRNA gene spacer regions designed by Berthier and Ehrlich (4). The PCR mixture contained 1× Master Mix PCR (Promega, Italy), 1.5 mM MgCl<sub>2</sub>, 0.3 μM of each primer, and ~300 ng of meat DNA as a template. Amplifications consisted of 1 cycle of denaturation for 5 min at 94°C, 20 cycles at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and a final step at 72°C for 7 min. The specificity of the *L. sakei* (16S/Ls) and *L. curvatus* (16S/Lc) primers was demonstrated in two separate PCRs using the respective reference strains as positive controls (Fig. 2). A specific band using 16S/Ls primers was observed only at time

zero (Fig. 2a), while the specific bands obtained with 16/Lc primers (Fig. 2b) were present during the rest of the storage period at both temperatures. The results obtained here are in agreement with previous studies, which underline how *L. sakei*/*L. curvatus* association largely dominates fresh beef, meat, and fish products under vacuum or modified atmospheres (11, 24, 29, 35, 36). However, *Lactobacillus algidus* was reported as the predominant species in chilled vacuum-packaged beef when its sodium dodecyl sulfate-polyacrylamide gel electrophoresis whole-cell protein profile was examined (28). In this study, the use of the 16S/Ls specific primers did not allow the detection of *L. sakei* during the complete storage period, indicating their low sensitivity in species-specific PCR when DNA extracted directly from meat is used as a template. Although this technique is a rapid and reliable molecular tool for the characterization of bacterial communities without colony isolation, its sensitivity can be reduced due to the complexity of the food matrix and the presence of PCR inhibitors (2). Difficulties in recognizing species or genera of LAB when the PCR profiles were complex were also reported in the evaluation of microbial diversity in different types of Mozzarella cheese (12).

TABLE 2. Primers specific to *Leuconostoc* species

Target	Primer name	Sequence	Size <sup>a</sup> (bp)
<i>L. carnosum</i>	Lcar-f	5'-CTTAGTATCGCATGATATC-3'	318
	Lcar-r	5'-CTGGTATGGTACCGTCAGG-3'	
<i>L. citreum</i>	Lcit-f	5'-AAAACCTTAGTATCGCATGATATC-3'	1,298
	Lcit-r	5'-CTTAGACGACTCCCTCCCG-3'	
<i>L. gelidum</i>	Lgel-f	5'-TCGTATCGCATGATACAAG-3'	1,290
	Lgel-r	5'-TAGACGGTCCCTCCTTAC-3'	
<i>L. lactis</i>	Llac-f	5'-AGGCGGCTTACTGGACAAC-3'	742
	Llac-r	5'-CTTAGACGGCTCCTTCCAT-3'	
<i>L. mesenteroides</i>	Lmes-f	5'-AACTTAGTGTGCGATGAC-3'	1,150
	Lmes-r	5'-AGTCGAGTTACAGACTACAA-3'	

<sup>a</sup> Expected size of an amplified PCR fragment using species-specific primer set.

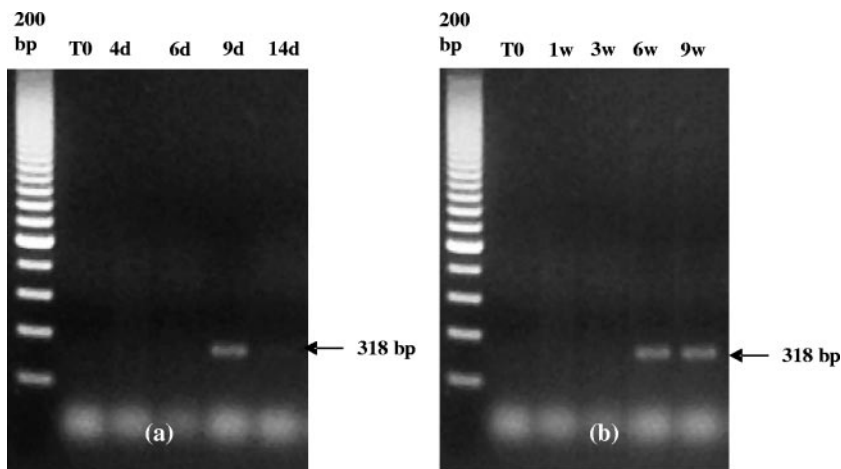


FIG. 3. Multiplex PCR for *Leuconostoc* species differentiation using primer set A from meat samples stored at 8°C (a) and 2°C (b). Agarose gel electrophoresis (2.0%) showed PCR bands of ~318 bp corresponding to *L. carnosum*.

**Multiplex PCR for *Leuconostoc* species detection.** Species-specific primers targeted to the genes encoding 16S rRNA (Table 2) and the PCR amplification conditions described by Lee et al. (23) were used to differentiate between *Leuconostoc* species present during beef chilled storage. The multiplex PCR assay comprises two separate reactions, one using primer set A for *Leuconostoc carnosum*, *Leuconostoc citreum*, and *Leuconostoc mesenteroides* species identification and the other using primer set B for *L. gelidum* and *Leuconostoc lactis*. The specificity of the PCR assay was checked using the corresponding reference strains (data not shown). The PCR mixture (50 µl) contained ~300 ng of meat DNA as a template. When primer set A was used, a specific band corresponding to *L. carnosum* appeared on day 9 (Fig. 3a); it was also detected on day 14 as a very faint band in meat samples stored at 8°C. The same band was observed during weeks 6 and 9 at 2°C (Fig. 3b). When primer set B was used (Fig. 4), a specific band corresponding to *L. gelidum* was detected from day 4 at 8°C (Fig. 4a) and from week 3 at 2°C (Fig. 4b). Specific bands were sequenced to confirm the identities of the *Leuconostoc* species

identified (data not shown). Results from DGGE indicated that *Leuconostoc* constitutes another relevant member of the LAB group present in chilled vacuum-packaged meat, and *L. gelidum* and *L. carnosum* were identified as the representative species by multiplex PCR. *L. gelidum* was previously reported as a common LAB able to dominate beef stored at low temperatures (28, 31, 36).

The molecular techniques used in this study provide a rapid and easy method for the identification of LAB associated with fresh vacuum-packaged beef; this approach is helpful in the tracking of LAB communities developed in raw meat. Even though the molecular bases for the predominance of *L. sakei*, *L. curvatus*, and *L. gelidum* found in this study have not been determined, this information will be valuable when designing and evaluating intervention strategies used to extend the storage life of meat.

**Nucleotide sequence accession numbers.** Nucleotide sequences obtained from DGGE bands a', b', and d (Fig. 1A) were published under the following GenBank accession numbers: DQ336385, *L. sakei* (98% identity); DQ336384, *L. cur-*

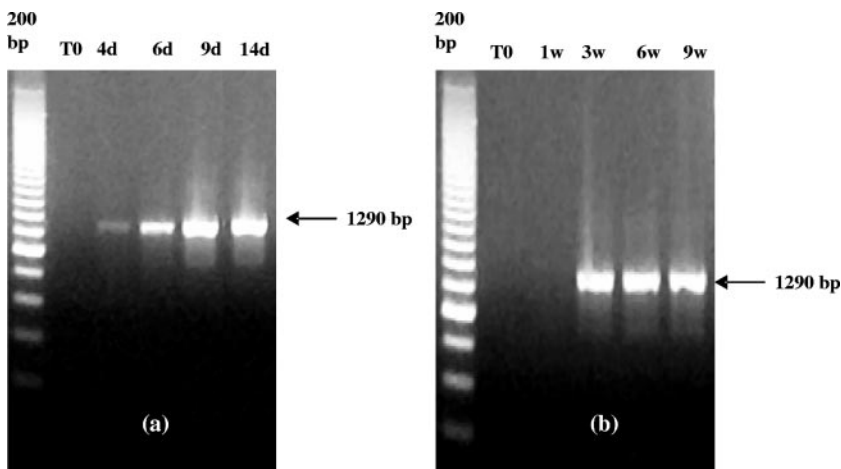


FIG. 4. Multiplex PCR for *Leuconostoc* species differentiation using primer set B from meat samples stored at 8°C (a) and 2°C (b). Agarose gel electrophoresis (1.0%) showed PCR bands of ~1,290 bp corresponding to *L. gelidum*.



vatus (98% identity); and AY946201, *B. thermopacta* (96% identity).

This work was partially supported by grants from CONICET and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), PICT 2003 no. 09-13499, Argentina.

#### REFERENCES

- Altschul, S., T. Madden, A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. Lipman. 1997. Gapped BLAST and PSIBLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**:3389–3402.
- Aymerich, T., B. Martín, M. Garriga, and M. Hugas. 2003. Microbial quality and direct PCR identification of lactic acid bacteria and nonpathogenic staphylococci from artisanal low-acid sausages. *Appl. Environ. Microbiol.* **69**:4583–4594.
- Barry, T., G. Collieran, M. Glennon, L. Dunican, and F. Gannon. 1991. The 16S/23S ribosomal spacer region as a target for DNA probes to identify Eubacteria. *PCR Methods Appl.* **1**:51–56.
- Berthier, F., and S. Ehrlich. 1999. Genetic diversity within *Lactobacillus sakei* and *Lactobacillus curvatus* and design of PCR primers. *Int. J. Syst. Bacteriol.* **49**:997–1007.
- Blixt, Y., and E. Borch. 2002. Comparison of shelf life of vacuum-packed pork and beef. *Meat Sci.* **60**:371–378.
- Borch, E., M. Kant-Muermans, and Y. Blixt. 1996. Bacterial spoilage of meat and cured meat products. A review. *Int. J. Food Microbiol.* **33**:103–120.
- Castellano, P., W. Holzapfel, and G. Vignolo. 2004. The control of *Listeria innocua* and *Lactobacillus sakei* in broth and meat slurry with the bacteriocinogenic strain *Lactobacillus casei* CRL705. *Food Microbiol.* **21**:291–298.
- Church, L., and A. Parsons. 1995. Modified atmosphere packaging technology: a review. *J. Sci. Food Agric.* **67**:143–152.
- Cocolin, L., M. Manzano, C. Cantoni, and G. Comi. 2001. Denaturing gradient gel electrophoresis analysis of the 16S rRNA gene V1 region to monitor dynamic changes in the bacterial population during fermentation of Italian sausages. *Appl. Environ. Microbiol.* **67**:5113–5121.
- Cocolin, L., K. Rantsiou, L. Iacumin, R. Urso, C. Cantoni, and G. Comi. 2004. Study of the ecology of fresh sausages and characterization of populations of lactic acid bacteria by molecular methods. *Appl. Environ. Microbiol.* **70**:1883–1894.
- Comi, G., R. Urso, L. Iacumin, K. Rantsiou, P. Cattaneo, C. Cantoni, and L. Cocolin. 2005. Characterization of naturally fermented sausages produced in the North East of Italy. *Meat Sci.* **69**:381–392.
- Coppola, S., G. Blaiotta, D. Ercolini, and G. Moschetti. 2001. Molecular evaluation of microbial diversity occurring in different types of Mozzarella cheese. *J. Appl. Microbiol.* **90**:414–420.
- Ercolini, D. 2004. PCR-DGGE fingerprinting: novel strategies for detection of microbes in food: a review. *J. Microbiol. Methods* **56**:297–314.
- Ercolini, D., G. Mauriello, G. Blaiotta, G. Moschetti, and S. Coppola. 2004. PCR-DGGE fingerprints of microbial succession during a manufacture of traditional water buffalo mozzarella cheese. *J. Appl. Microbiol.* **96**:263–270.
- Fontana, C., P. Cocconcelli, and G. Vignolo. 2005. Monitoring the bacterial population dynamics during fermentation of artisanal Argentinean sausages. *Int. J. Food Microbiol.* **103**:131–142.
- Fontana, C., G. Vignolo, and P. Cocconcelli. 2005. PCR-DGGE analysis for the identification of microbial populations from Argentinean dry fermented sausages. *J. Microbiol. Methods* **63**:254–263.
- Gürtler, V., and V. Stanisich. 1996. New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. *Microbiology* **141**:3–16.
- Hansen, K., and D. Bautista. 2000. Spoilage problems caused by bacteria, p. 2051–2056. In K. Robinson, C. A. Batt, and P. D. Patel (ed.), *Encyclopedia of food microbiology*, vol. 3. Academic Press, Bath, United Kingdom.
- Jones, R. J. 2004. Observations on the succession dynamics of lactic acid bacteria populations in chill-stored vacuum-packaged beef. *Int. J. Food Microbiol.* **90**:273–282.
- Korkeala, H., R. Ahvenainen, and T. Alanko. 1987. Interrelationship between microbial numbers and other parameters in the spoilage of vacuum-packed cooked sausages. *Int. J. Food Microbiol.* **5**:311–321.
- Korkeala, H., and K. J. Björkroth. 1997. Microbiological spoilage and contamination of vacuum-packaged cooked sausages: a review. *J. Food Prot.* **60**:724–731.
- Labadie, J. 1999. Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Sci.* **52**:299–305.
- Lee, H., Sae-Young Park, and J. Kim. 2000. Multiplex PCR-based detection and identification of *Leuconostoc* species. *FEMS Microbiol. Lett.* **193**:243–247.
- Lyhs, U., J. Björkroth, and H. Korkeala. 2002. Identification of lactic acid bacteria from spoiled, vacuum-packaged “gravad” rainbow trout using ribotyping. *Int. J. Food Microbiol.* **72**:147–153.
- Nissen, H., T. Maugesten, and P. Lea. 2001. Survival and growth of *Escherichia coli* O157:H7, *Yersinia enterocolitica* and *Salmonella enteritidis* on decontaminated and untreated meat. *Meat Sci.* **57**:291–298.
- Nychas, G., and E. Drosinos. 2000. Spoilage of meat, p. 1253–1260. In K. Robinson, C. A. Batt, and P. D. Patel (ed.), *Encyclopedia of food microbiology*, vol. 2. Academic Press, Bath, United Kingdom.
- Rantsiou, K., R. Urso, L. Iacumin, C. Cantoni, P. Cattaneo, G. Comi, and L. Cocolin. 2005. Culture-dependent and -independent methods to investigate the microbial ecology of Italian fermented sausages. *Appl. Environ. Microbiol.* **71**:1977–1986.
- Sakala, R. M., H. Hayashidani, Y. Kato, T. Hirata, Y. Makino, A. Fukushima, T. Yamada, C. Kaneuchi, and M. Ogawa. 2002. Change in the composition of the microflora on vacuum-packaged beef during chiller storage. *Int. J. Food Microbiol.* **74**:87–99.
- Samelis, J., E. Tsakalidou, J. Metaxopoulos, and G. Kalantzopoulos. 1995. Differentiation of *Lactobacillus sakei* and *Lact. curvatus* isolated from naturally fermented Greek dry salami by SDS-PAGE of whole-cell proteins. *J. Appl. Bacteriol.* **78**:157–163.
- Samelis, J., A. Kakouri, and J. Rementzis. 2000. Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial associations of cooked meats at 4°C. *Food Microbiol.* **17**:329–340.
- Shaw, B., and C. Harding. 1989. *Leuconostoc gelidum* sp. nov. and *Leuconostoc carnosum* sp. nov. from chill-stored meats. *Int. J. Syst. Bacteriol.* **39**:217–223.
- Stiles, M. 1996. Biopreservation by lactic acid bacteria. *Antonie Leeuwenhoek* **70**:331–345.
- Taylor, S. A. 1996. Modified atmosphere packing of meat, p. 301–308. In S. A. Taylor, A. Raimundo, M. Severini, and J. M. Smulders (ed.), *Meat quality and meat packaging*. J. M. Eebeamst, Utrecht, The Netherlands.
- Vold, L., A. Holck, Y. Wasteson, and H. Nissen. 2000. High levels of background flora inhibit growth of *Escherichia coli* O57:H7 in ground beef. *Int. J. Food Microbiol.* **56**:219–225.
- Yost, C., and F. Nattress. 2000. The use of multiplex PCR reactions to characterize populations of lactic acid bacteria associated with meat spoilage. *Lett. Appl. Microbiol.* **31**:129–133.
- Yost, C., and F. Nattress. 2002. Molecular typing techniques to characterize the development of a lactic acid bacteria community on vacuum-packaged beef. *Int. J. Food Microbiol.* **72**:97–105.