APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1992, p. 3443–3447
0099-2240/92/113443-05$02.00/0

Vol. 58, No. 11

Discrimination of *Listeria monocytogenes* from Other *Listeria* Species by Ligase Chain Reaction

MARTIN WIEDMANN,[1] JOHN CZAJKA,[1] FRANCIS BARANY,[2] AND CARL A. BATT[1]*

Department of Food Science, Cornell University, Ithaca, New York 14853,[1] and Department of Microbiology, Cornell University Medical College, New York, New York 10021[2]

Received 8 June 1992/Accepted 5 July 1992

A ligase chain reaction assay based on a single-base-pair difference in the V9 region of the 16S rRNA gene (16S rDNA) was developed to distinguish between *Listeria monocytogenes* and other *Listeria* species. For this purpose, two pairs of primers were designed, with one primer of each pair being radioactively labeled. The ligated product was separated from the primers by denaturing polyacrylamide gel electrophoresis and then detected by autoradiography. To achieve a higher sensitivity, the 16S rDNA was initially amplified by polymerase chain reaction prior to the ligase chain reaction. The ligase chain reaction was tested on 19 different *Listeria* species and strains and proved to be a highly specific diagnostic method for the detection of *L. monocytogenes*.

Listeria spp. are gram-positive bacilli, and while most species within the genus are nonpathogenic to humans, *L. monocytogenes* has the potential to cause listeriosis (12). It is primarily a livestock pathogen, but it has been seen sporadically in humans for many years. It is increasingly clear that *L. monocytogenes* is the predominant (and perhaps only) pathogenic species found in food (10). The selective detection of *L. monocytogenes* is of great importance, as the ecology of *Listeria* spp. is not well understood and the detection of *Listeria* spp. other than *L. monocytogenes* may not be an accurate indicator of a food's safety (10).

Various systems for detecting *L. monocytogenes* by using either monoclonal antibodies or nucleic acid probes alone (reviewed in reference 12) or nucleic acid probes in conjunction with the polymerase chain reaction (PCR) have been reported (7, 8, 11, 13, 17, 21, 22).

The use of 16S rRNA as a distinct signature for bacteria has become the method of choice for identifying and differentiating microorganisms when no other nucleic acid sequence constitutes a unique desired target (24). A DNA probe based upon a 16S rRNA sequence (rDNA), which detects all *Listeria* spp., has been developed, although the sequences that discriminate it from other genera were not reported (14). Also, a 16S rRNA-based oligonucleotide probe specific for *L. monocytogenes* has been designed and was used in a hybridization assay (19).

The 16S rRNA nucleotide sequence from a number of *Listeria* spp. has been determined either after reverse transcription (9) or from PCR-amplified genomic rDNA (5). Differences in single base pairs between *L. monocytogenes* and *L. innocua* were described for the V2 and the V9 regions (5, 9, 19).

The ligase chain reaction (LCR) has been shown to be a highly sensitive and specific method for discriminating between DNA sequences differing in only a single base pair (2; reviewed in reference 3). LCR is based on the principle of ligation of two adjacent synthetic oligonucleotide primers which hybridize uniquely to one strand of the target DNA. The junction of the two primers is positioned so that the nucleotide at the 3′ end of the upstream primer coincides with a potential single-base-pair difference in the targeted sequence, which defines different alleles or species. If the base pair at that site matches the nucleotide at the 3′ end of the upstream primer, the two adjoining primers can be covalently joined by the ligase. In the LCR, a second pair of primers complementary to the first pair are present, again with the nucleotide at the 3′ end of the upstream primer denoting the sequence difference. In a cycling reaction with a thermostable *Thermus aquaticus* DNA ligase, the ligated product can then serve as a template for the next reaction cycle, leading to an exponential amplification process analogous to PCR amplification. If there is a mismatch at the primer junction, it will be discriminated by the thermostable ligase, and the primers will not be ligated. The absence of the ligated product therefore indicates at least a single-base-pair change in the target sequence (3). This was demonstrated by using LCR to discriminate between normal β^A- and sickle β^S-globin genotypes in humans (2).

We describe the use of the LCR to discriminate *L. monocytogenes* from other, nonpathogenic *Listeria* spp. A primary PCR amplification of the 16S rDNA was combined with a secondary LCR amplification to yield a highly sensitive and specific method for identifying *L. monocytogenes*.

(The results presented in this article are part of M. Wiedmann's thesis, which will be submitted to the Ludwig-Maximilians-Universität, Munich.)

MATERIALS AND METHODS

Preparation of genomic DNA and PCR of 16S rDNA. Listeriae were grown in 5 ml of Listeria enrichment broth (BBL, Becton Dickinson Microbiology Systems) at 37°C on a shaker at 150 rpm. Cells from an 8-h culture (approx. 10^9 cells) of *Listeria* spp. were pelleted, resuspended in 100 μl of distilled water, and then placed in a boiling-water bath for 10 min. A 1-μl aliquot of this lysate was used for PCR amplification of the V2 region with two primers for the 16S rDNA (5, 20), 16S-5 (5′CCGAATTCGTCGACAACAGAGTTTGATCATGG3′) and mp19-INT (5′CGGAAACCCCCTAACACTTA3′), or for amplification of the V9 region with two universal primers for the 16S rDNA (5, 20), 16S-3 (5′CCCGGGATCCAAGCTTTAACCTTGTTACGACTT3′) and

* Corresponding author.

TABLE 1. Sequences and melting temperatures of primers used in the LCR

Primer	Sequence (5'→3')	T_m (°C)
V2 region-specific pairs		
Lm 1 (labeled)	AGTGTGGCGCATGCCAGCTCTTTTGA	78
Lm 3	GGACCGGGGCTAATACCGAATGATAA	70
Lm 2R (labeled)	TATCATTCGGTATTAGCCCCGGTTT	72
Lm 4R	GGAAAAGCGTGGCATGCGCCACACTT	76
V9 region-specific pairs		
Lm 1a (labeled)	GTACAAAGGGTCGCGAAGCCG	68
Lm 3a	AAGCTACACACGTGCTACAATGGATA	70
Lm 2Ra (labeled)	ATCCATTGTAGCACGTGTGTAGC	68
Lm 4Ra	AACGGCTTCGCGACCCTTTGTACT	70

mp18-INT (5'ATTAGATACCCTGGTAGTCC3'). The amplified DNA was phenol-chloroform extracted, ethanol precipitated, and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) by standard procedures (18). Alternatively, the amplified DNA was used directly in the LCR. These preparations were analyzed for the presence of the expected 16S rDNA fragment of 840 bp for the V2 region or of 720 bp for the V9 region by electrophoresis in a 1.5% agarose gel in TBE buffer (89 mM Tris-borate, 2 mM EDTA [pH 8.2]). The DNA concentration was estimated by visual comparison with a standard DNA preparation.

Primers for LCR and LDR. For the LCR, two sets (Lm 1, Lm 2R, Lm 3, and Lm 4R for the V2 region and Lm 1a, Lm 2Ra, Lm 3a, and Lm 4Ra for the V9 region) of four different primers were used. The primer sequences are shown in Table 1. For the ligase detection reaction (LDR) of the V9 region, either the primer pair Lm 1a plus Lm 3a or the pair Lm 2Ra plus Lm 4Ra was used.

Primers Lm 1, 1a, 2R, and 2Ra were 5'-end labeled with [γ-^{32}P]ATP (Amersham) as described previously (2).

LCR and LDR conditions. The LCR and LDR were performed as described previously (2, 23) with minor modifications. One set of primers (50 fmol of each primer) were incubated in the presence of target 16S rDNA (5 fmol) in 50 μl of reaction buffer (50 mM Tris-HCl, 100 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 10 mM dithiothreitol, 1 mM NAD^+, 0.01% Triton X-100, 20 μg of salmon sperm DNA) with 75 nick-closing units of *T. aquaticus* DNA ligase (purified as described previously [4]) and overlaid with 50 μl of mineral oil. The reaction cycle was 1 min at 94°C followed by 4 min at 65°C, and this cycle was repeated 25 times. Annealing temperatures of 70 and 74°C were also tested in the LCR performed with the primers for the V2 region. The reaction was stopped by adding 40 μl of formamide containing 10 mM EDTA, 0.2% bromophenol blue, and 0.2% xylene cyanole. Alternatively, bacterial lysates (prepared as described above) were also used directly in the LCR.

As described previously, only one pair of primers was used in the LDR, whereas two pairs of primers were used in the LCR.

To evaluate the overall sensitivity of the PCR-coupled LCR, serial dilutions of an 8-h *L. monocytogenes* culture were used to prepare boiled lysates and tested with the primers for the V9 region. These samples were analyzed for the PCR product after PCR amplification, and then 5 μl of the PCR product was used for the second LCR step.

Electrophoresis. LCR samples were heated to 90°C for 5 min, and 10 μl was loaded on a 16% polyacrylamide minigel (8 by 7.3 cm in Mini-PROTEAN II Electrophoresis Cell; Bio-Rad) containing 7 M urea. Electrophoresis was carried out in TBE buffer (89 mM Tris-borate, 2 mM EDTA [pH 8.2]) at 175 V constant voltage for 1 h. Gels were autoradiographed on Kodak X-Omat AR film at −20°C for 12 h.

RESULTS

L. monocytogenes and *L. innocua* show single-base-pair differences at nucleotide 193 (V2 region) and at nucleotide 1259 (V9 region) in the 16S rDNA (5, 9, 19). All other *Listeria* spp. are far more divergent in that region (9). These two sites were used to design primers for an LCR assay (Fig. 1 and 2).

An LCR assay based on those single-base-pair differences was developed which is successful in distinguishing *L.*

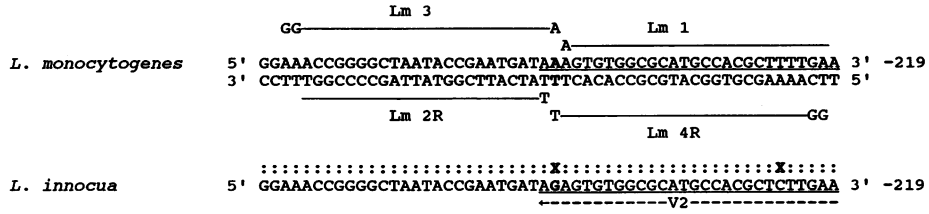

FIG. 1. Nucleotide sequence of the V2 region site in *L. monocytogenes* and in *L. innocua* used for LCR and locations of primers Lm 1, Lm 2R, Lm 3, and Lm 4R. Oligonucleotides Lm 3 and Lm 4R contained 2-nucleotide tails to prevent ligation of the 3' end. The primers were 25 (Lm 1), 25 (Lm 2R), 26 (Lm 3), and 26 (Lm 4R) nucleotides long. X's mark differences between *L. monocytogenes* and *L. innocua*. Nucleotides that are identical in *L. monocytogenes* and *L. innocua* are shown as colons. The target nucleotide for LCR is shown in boldface. Primers are noted by a bar when the nucleotides are identical to those in *L. monocytogenes*; the nucleotide at the ligation junction is also depicted for clarity. The sequences of each primer (in the 5'→3' orientation) are shown in Table 1.

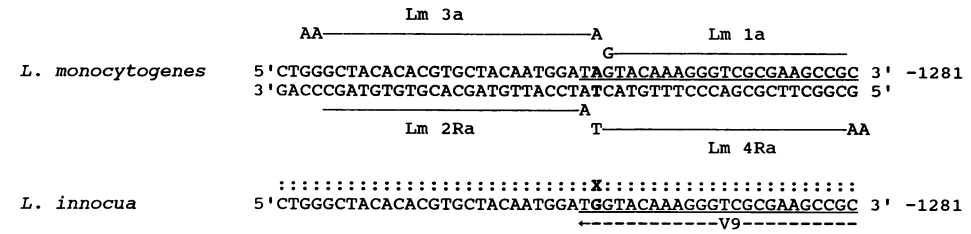

FIG. 2. Nucleotide sequence of the V9 region site in *L. monocytogenes* and in *L. innocua* used for LCR and locations of primers Lm 1a, Lm 2Ra, Lm 3a, and Lm 4Ra. Oligonucleotides Lm 3a and Lm 4Ra contained 2-nucleotide tails to prevent ligation of the 3' end. Primers were 21 (Lm 1a), 23 (Lm 2Ra), 26 (Lm 3a), and 24 (Lm 4Ra) nucleotides long. Other details are explained in the legend to Fig. 1.

monocytogenes from other *Listeria* spp. This method was tested on 19 different *L. monocytogenes* strains and other *Listeria* spp. (Table 2). Even species closely related to *L. monocytogenes*, such as *L. innocua*, could be reliably distinguished.

When crude bacterial lysates were used without prior PCR in the LCR assay with the V9 primers, a ligation product was detectable with a 24-h autoradiogram exposure (data not shown). PCR amplification of either the V2 or the V9 region of the 16S rDNA was successful in generating DNA fragments which were suitable for use in the LCR. There was no significant difference in sensitivity or specificity whether phenol-chloroform-extracted or unpurified DNA from the PCR step was used for the LCR (data not shown).

The two complementary pairs of primers designed for the V9 region had a low variation in melting temperature (68 to 70°C). When this set of primers was used in an LCR assay (as well as in LDR) (Fig. 3), clear differentiation between *L. monocytogenes* and other *Listeria* spp. was possible. In Fig. 4, representative results of the LCR for 13 *Listeria* spp. with the primers for the V9 region are shown. On the autoradiogram, the two labeled primers are visible at the bottom of the gel; the lower band is primer Lm 1a (21 nucleotides), and the higher one is primer Lm 2Ra (23 nucleotides). The ligated products for primer pairs Lm 1a plus 3a and Lm 2Ra plus 4Ra are both 47 bases long, and therefore only one band appears on the autoradiogram.

TABLE 2. *Listeria* strains used in the LCR

Species and strain	Serotype[a]	Source or reference[b]	LCR product
L. monocytogenes			
A/F2380	ND	USDA-ERRC	+
C/ATCC 15313	NT	USDA-ERRC	+
D/LCDC91-329	1/2a	USDA-ERRC	+
E	ND	USDA-ERRC	+
SLCC 53	1/2a	UVM	+
SLCC 2371	1/2a	UVM	+
SLCC 5779	1/2a	UVM	+
ATCC 19112	1/2c	UVM	+
Scott A	4b	Univ. of Minnesota	+
L. innocua			
SA 3 VT	ND	USDA	−
SH3V	ND	Univ. of Minnesota	−
P5V5	ND	Univ. of Minnesota	−
L. ivanovii			
KC 1714	ND	USDA	−
L 29/R1-7	ND	UVM/FDA	−
L 30/R1-8	ND	UVM/FDA	−
L. seeligeri	ND	Univ. of Minnesota	−
L. welshimeri			
H2.V.G.	ND	USDA	−
CCK9LG	ND	Univ. of Minnesota	−
L. grayi KC 1773 (ATCC 19120)	ND	USDA	−

[a] Information about the serotypes was provided from the laboratories that provided the strains. ND, not determined; NT, not typeable.

[b] USDA, U.S. Department of Agriculture; ERRC, Eastern Regional Research Center; UVM, University of Vermont, FDA, Food and Drug Administration.

The addition of 0.01% Triton X-100 to the reaction mixture gave higher yields of ligation product with both LDR and LCR (Fig. 3). This was quantified by excising the bands from gels after a 25-cycle LCR and measuring the specific activities of the ligation product and the unligated primers by liquid scintillation counting. With 5 fmol of target DNA, a slightly higher amount of false-positive ligation was detected when 0.01% Triton X-100 was used than in LCR reactions without Triton X-100.

When 0.01% Triton X-100 was used, approximately 80% of the starting primers were ligated after 25 LCR cycles at a concentration of 5 fmol of target DNA (data not shown). Since only one pair of primers was used in the LDR, only one band for the unligated labeled primer is seen (primer Lm 2Ra, 23 nucleotides), while in the autoradiogram of the LCR, two bands of unligated primers are seen (see above for explanation).

When *L. innocua* served as a target in the LCR with the V9 primer set, a very small amount of ligation product was detected only when the gel was autoradiographed for more than 20 h. In species distantly related to *L. monocytogenes* (e.g., *L. seeligeri* and *L. grayi*), no ligation product was observed (Fig. 4) even when the gel was autoradiographed for more than 36 h.

When different dilutions of target cells were used for the PCR-coupled LCR, small numbers of target cells (150 to 15,000 CFU) gave no detectable PCR product in ethidium bromide-stained gels, but the second LCR step revealed the presence of *L. monocytogenes*.

The LCR primers for the V2 region exhibited a high degree of ligation in the absence of target DNA. Since the melting temperature of those primers ranged from 70 to 78°C, cycles with higher annealing temperatures (70 or 74°C) than originally described were tested. At an annealing temperature of 74°C, the amount of ligated product decreased drastically, not only in the absence but also in the presence of target; however, target-independent ligation products were still observed (data not shown).

DISCUSSION

The ability to accurately identify *L. monocytogenes* is important in guaranteeing the safety of food products. Discrimination between the closely related *L. innocua* and *L. monocytogenes* is very difficult and time-consuming when

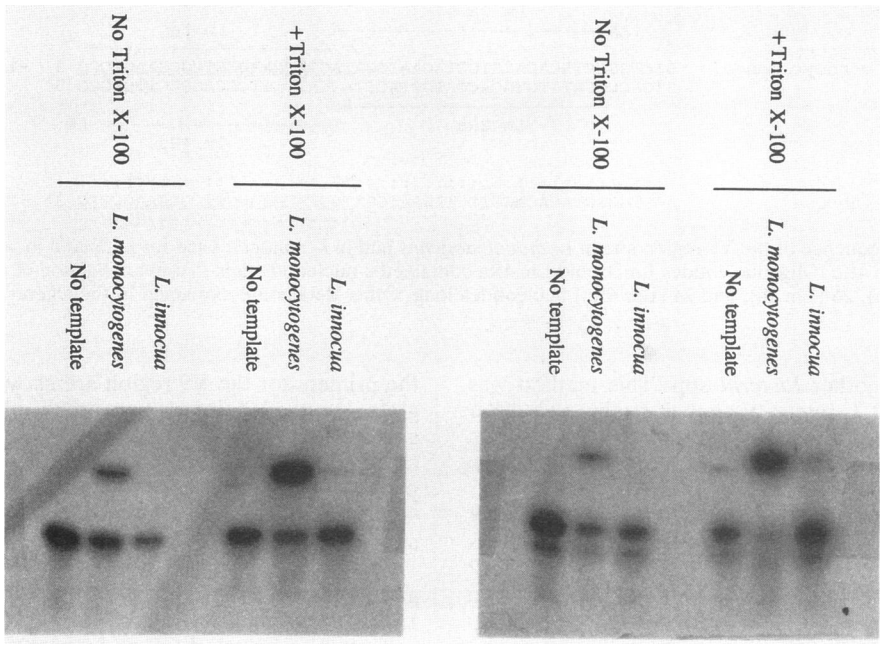

FIG. 3. Autoradiogram of LCR and LDR products for *L. monocytogenes* and *L. innocua*. (Left) LDR with Lm 2Ra and 4Ra; (right) LCR with Lm 3a, 1a, 2Ra, and 4Ra. The primers Lm 1a, 2Ra, 3a, and 4Ra were specific for the single-base-pair difference at nucleotide 1259 of the V9 region 16S rDNA. Radioactive primers for LCR are 21 and 23 nucleotides long, while the LCR products are 47 nucleotides long, respectively. The radioactive primer for LDR is 23 nucleotides long, and the LDR product is 47 nucleotides long. The higher ligation rate seen when the buffer was supplemented with 0.01% Triton X-100 is apparent in the comparison of the first three lanes with the last three lanes of each LCR and LDR.

common microbiological methods are used. In this study, we showed that LCR is capable of distinguishing *L. monocytogenes* from *L. innocua* and also from all other *Listeria* spp. tested.

When LCR was used for detection of the β^A-globin gene, the sensitivity was reported to be limited to about 200 target molecules (2). PCR has been shown to have a much greater sensitivity, although it cannot always easily discriminate between two targets differing in only a single base pair. For example, Bej et al. (6) were able to detect fewer than 5 coliform bacteria in 100 ml of water by using PCR amplification. The PCR primers for the V2 region allowed the amplification of an 840-bp fragment, whereas the universal PCR primers for the V9 region amplified a 720-bp fragment. In the PCR amplification, no difference was observed in the results obtained with crude bacterial cell lysates and purified DNA.

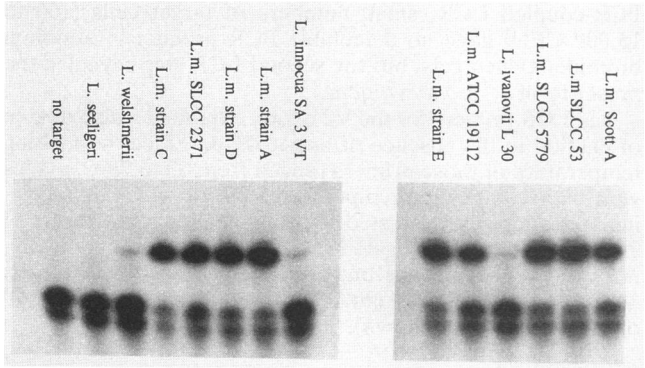

FIG. 4. Autoradiogram showing LCR for a selected number of different *Listeria* species and strains. The primers Lm 1a, 2Ra, 3a, and 4Ra, specific for the single-base-pair difference at nucleotide 1259 of the V9 region 16S rDNA, were used for this LCR. The autoradiogram was exposed for 22 h. For further explanations, see the legend to Fig. 3. L.m., *L. monocytogenes*.

When using the PCR to amplify the target region for the LCR, it is possible to combine the high sensitivity of the PCR with the high specificity of the LCR. Furthermore, our experiments showed that the second LCR step can increase the sensitivity of the PCR step for the detection of *L. monocytogenes* when ethidium bromide staining of the agarose gels is used to detect the PCR products. One primer of an LCR primer pair can be radioactively labeled, and the ligation product can be detected by gel electrophoresis and subsequent autoradiography of the gel. This approach was shown to be highly sensitive, so that even minimal amounts of ligation products could be detected (2). Primers containing a mismatch with the target DNA yielded from <0.2 to 1.3% of the ligation product obtained with perfect complementarity, depending on the target nucleotides involved (2). *L. monocytogenes* has an A · T base pair while *L. innocua* has the base pair G · C at nucleotide 1259 in the V9 target region (Fig. 2). Based on our earlier data with G · T and C · A mismatches, a 1.3% background signal with *L. innocua* would be expected. Since this ratio was estimated for 10 amol of target DNA, a somewhat greater amount of false-positive ligation product would be expected for our experiments, in which 5 fmol of target DNA was used.

The inclusion of 0.01% Triton X-100 in the LCR reaction mixture increased LCR efficiency, but it also intensified false-positive ligation when 5 fmol of target DNA was used and the gel was exposed for longer than 12 h. This problem

might be eliminated by using either shorter exposure times or smaller amounts of target DNA.

One possible reason for the unsatisfactory results observed with the V2 region primer set could be that the melting temperatures of the four primers were too different, resulting in extensive primer-primer ligations. Once an initial ligation product is formed, it will then serve as a target in subsequent cycles. Therefore, the use of primer pairs with very similar melting temperatures seems to be important (2). It is also possible that one of the primers of this set serves transiently as a template for the other primers, even though a comparison of the sequences of these primers does not reveal any obvious complementarity. Since the melting temperatures of the V2 primers were higher than those for the V9 primers, we used higher annealing temperatures for the LCR with the V2 primers to decrease the amount of annealing at noncomplementary targets. However, this approach did not solve the problems with this primer set. Furthermore, self-annealing of one of the primers could be a reason for the formation of a ligation product in the absence of target DNA. Secondary structure in the target may also account for a smaller amount of ligation product.

In future studies, the use of a PCR-coupled LCR for the specific detection of *L. monocytogenes* could be simplified by using a nonradioactive reporter. The use of biotin for labeling one primer and of a suitable nonisotopic reporter group for the second primer should allow product capture and detection in a manner amenable to automation (15, 16).

One of the main advantages of the use of PCR-coupled LCR is that even if the target sequence for the LCR can only be amplified to the attomolar range (because of competition from other bacteria in the original sample, whose 16S rDNA is also amplified by the universal PCR primers), this amount of DNA is still sufficient to be detected by LCR (2, 3).

The use of PCR primers that specifically amplify the 16S rDNA of all *Listeria* spp. will allow the detection of any *Listeria* spp. in the first step. The LCR as the second reaction could then specifically confirm the presence of *L. monocytogenes*.

The test presented in this article has the potential to become an alternative to the commonly used, time-consuming culture procedures for the diagnosis of *L. monocytogenes* in food and environmental samples. Further refinement of this PCR-coupled LCR, especially in the presence of competitive microflora, is needed to reach the final goal of a valuable method for detecting *L. monocytogenes* in food.

ACKNOWLEDGMENTS

We are very grateful to E. Winn-Deen from Applied Biosystems for suggesting the use of Triton X-100 in the LCR.

This work was supported by the Northeast Dairy Foods Research Center; a grant from the Cornell Biotechnology Program, which is sponsored by the New York State Science and Technology Foundation, a consortium of industries, the U.S. Army Research Office, and the National Science Foundation, to C.B.; and by a grant from the National Institutes of Health (GM 41337-03) to F.B. M.W. was supported by a stipend of the Gottlieb Daimler- und Carl Benz-Stiftung (2.92.04).

REFERENCES

1. **American Type Culture Collection.** 1985. Catalogue of bacteria, phages, rDNA vectors. American Type Culture Collection, Rockville, Md.
2. **Barany, F.** 1991. Genetic disease detection and DNA amplification using cloned thermostable ligase. Proc. Natl. Acad. Sci. USA **88**:189–193.
3. **Barany, F.** 1991. The ligase chain reaction (LCR) in a PCR world. PCR Methods Applications **1**:5–16.
4. **Barany, F., and D. Gelfand.** 1991. Cloning, overexpression and nucleotide sequence of a thermostable DNA ligase-encoding gene. Gene **109**:1–11.
5. **Batt, C. A., N. Bsat, J. Czajka, M. Piani, K. Sultana, M. Wiedmann, and R. Whitaker.** Submitted for publication.
6. **Bej, A. K., R. J. Steffan, J. DiCesare, L. Haff, and R. M. Atlas.** 1990. Detection of coliform bacteria in water by polymerase chain reaction and gene probes. Appl. Environ. Microbiol. **56**:307–314.
7. **Bessesen, M. T., Q. Luo, H. A. Rotbart, M. J. Blaser, and R. T. Ellison III.** 1990. Detection of *Listeria monocytogenes* by using the polymerase chain reaction. Appl. Environ. Microbiol. **56**:2930–2932.
8. **Border, P. M., J. J. Howard, G. S. Platsow, and K. W. Siggens.** 1990. Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. Lett. Appl. Microbiol. **11**:158–162.
9. **Collins, M. D., S. Wallbanks, D. J. Lane, J. Shah, R. Nietupski, J. Smida, M. Dorsch, and E. Stackebrandt.** 1991. Phylogenetic analysis of the genus *Listeria* based on reverse transcriptase sequencing of 16S rRNA. Int. J. Syst. Bacteriol. **41**:240–246.
10. **Cox, L. J., T. Kleiss, J. L. Cordier, C. Cordellana, P. Konkel, C. Pedrazzini, R. Beumer, and A. Siebenga.** 1989. *Listeria* spp. in food processing, non-food and domestic environments. Food Microbiol. **6**:49–61.
11. **Deneer, H. G., and I. Boychuk.** 1991. Species-specific detection of *Listeria monocytogenes* by DNA amplification. Appl. Environ. Microbiol. **57**:606–609.
12. **Gavalchin, J., K. Landy, and C. A. Batt.** 1992. Rapid methods for the detection of *Listeria*, p. 189–204. *In* D. Bhatnagar and T. E. Cleveland (ed.), Molecular approaches to improving food quality and safety. Van Nostrand Reinhold, New York.
13. **Golsteyn Thomas, E. J., R. K. King, J. Burchak, and V. P. J. Gannon.** 1991. Sensitive and specific detection of *Listeria monocytogenes* in milk and ground beef with the polymerase chain reaction. Appl. Environ. Microbiol. **57**:2576–2580.
14. **Klinger, J. D., A. Johnson, D. Croan, P. Flynn, K. Whippie, M. Kimball, J. Lawrie, and M. Curiale.** 1988. Comparative studies of nucleic acid hybridization assay for *Listeria* in foods. J. Assoc. Off. Anal. Chem. **7**:669–673.
15. **Landegren, U., R. Kaiser, J. Sanders, and L. Hood.** 1988. A ligase-mediated gene detection method. Science **241**:1077–1080.
16. **Nickerson, D. A., R. Kaiser, S. Lappin, J. Stewart, L. Hood, and U. Landegren.** 1990. Automated DNA diagnostics using an ELISA-based oligonucleotide ligation assay. Proc. Natl. Acad. Sci. USA **87**:8923–8927.
17. **Niederhauser, C., U. Candrian, C. Höfelein, M. Jermini, H.-P. Bühler, and J. Lüthy.** 1992. Use of polymerase chain reaction for detection of *Listeria monocytogenes* in food. Appl. Environ. Microbiol. **58**:1564–1568.
18. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Wang, R.-F., W.-W. Cao, and M. G. Johnson.** 1991. Development of a 16S rRNA-based oligomer probe specific for *Listeria monocytogenes*. Appl. Environ. Microbiol. **57**:3666–3670.
20. **Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane.** 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173**:697–703.
21. **Wernars, K., K. J. Heuvelman, T. Chakraborty, and S. H. W. Notermans.** 1991. Use of the polymerase chain reaction for direct detection of *Listeria monocytogenes* in soft cheese. J. Appl. Bacteriol. **70**:121–126.
22. **Wernars, K., K. Heuvelman, S. Notermans, E. Domann, M. Leimeister-Wächter, and T. Chakraborty.** 1992. Suitability of the *prfA* gene, which encodes a regulator of virulence genes in *Listeria monocytogenes*, in the identification of pathogenic *Listeria* spp. Appl. Environ. Microbiol. **58**:765–768.
23. **Winn-Deen, E. S., and D. M. Iovannisci.** 1991. Sensitive fluorescence method for detecting DNA ligation amplification products. Clin. Chem. **37**:1522–1523.
24. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51**:221–271.