Journal of Food Protection, Vol. 68, No. 7, 2005, Pages 1467–1471

Research Note

Rapid Quantitative Detection of *Listeria monocytogenes* in Salmon Products: Evaluation of Pre–Real-Time PCR Strategies

DAVID RODRÍGUEZ-LÁZARO,[1] ANNA JOFRÉ,[2] TERESA AYMERICH,[2] MARGARITA GARRIGA,[2] AND MARIA PLA[1]*

[1]*Institute of Food and Agricultural Technology (INTEA), University of Girona, Campus Montilivi (edif. Politècnica1), E-17071 Girona, Spain; and*
[2]*Institute for Food and Agricultural Research and Technology (IRTA), Meat Technology Center, Granja Camps i Armet E-17121 Monells, Spain*

MS 04-459: Received 29 September 2004/Accepted 5 February 2005

ABSTRACT

The spread and persistence of *Listeria monocytogenes* in smoked fish products and seafood processing factories are big concerns. Thus, the corresponding quality assurance programs must include adequate microbiological control measures. We evaluated eight different pre-PCR sample processing strategies to be coupled with a previously developed real-time PCR assay for the quantitative detection of *L. monocytogenes* in salmon products. The optimal pre-PCR procedure involved filtration and DNA purification with the use of a commercial kit. This strategy could detect 10 CFU of *L. monocytogenes* per g of smoked salmon and could quantify 1,000 CFU/g with excellent accuracy compared with the standard plate count method. Thus, this method could be a promising alternative for the quantitative detection of *L. monocytogenes* in smoked fish products and processing factories. This method could also detect the bacterium in raw salmon.

Listeria monocytogenes is a human bacterial pathogen that is frequently found in food products *(17, 18).* Ingestion of foods contaminated with this bacterium can result in listeriosis, a severe infectious disease characterized by meningoencephalitis, abortion, septicemia, and a high rate of fatality (20 to 30%) *(34).* Seafood products are considered to be at risk for human listeriosis *(31)* because of the frequent occurrence of *L. monocytogenes* in raw, frozen, and smoked seafoods *(7, 9, 16, 26). L. monocytogenes* can survive the cold-smoking process *(11, 23),* making *L. monocytogenes* contamination a great concern for the smoked fish industry.

The standard method for the identification of *L. monocytogenes* involves growth in preenrichment and selective media, followed by a battery of confirmatory biochemical and serological tests *(4).* However, it can take up to 10 days to obtain valid data *(10).* The disadvantages of conventional culturing methods have prompted the development of alternative rapid molecular-based methods such as the real-time PCR (RTi-PCR). RTi-PCR is highly specific, sensitive, and efficient and can also be used for quantification *(19).* Furthermore, the absence of post-PCR steps reduces the risk of cross-contamination and allows high throughput and automation.

One of the major drawbacks of RTi-PCR is the inhibitory effect of a variety of substances that are very often present in food samples and that can be coextracted with the target DNA. These substances can lead to the underestimation of bacterial loads or false negative results *(32, 35).* Because food samples vary in composition and associated microbiota, pre-PCR strategies need to be optimized for each food matrix to produce PCR-compatible samples *(22, 27, 35).*

Various RTi-PCR assays have been described for the specific quantitative detection of *L. monocytogenes.* These assays have been applied to several food matrices, including milk *(12, 21, 24),* water *(24),* vegetables *(14),* and meat and meat products *(28).* However, they have never been used to analyze raw salmon or salmon-derived products. We present a rapid and sensitive method for the reliable quantitative identification of *L. monocytogenes* in smoked salmon. This method is based on a simple sample processing procedure, coupled with a previously developed RTi-PCR assay *(29).* It can be easily combined with standard microbiological methods and can also be used to detect *L. monocytogenes* in fresh salmon samples.

MATERIALS AND METHODS

Artificial contamination. Smoked and raw salmon slices were obtained at the local market and immediately processed. They were spiked with decreasing amounts of *L. monocytogenes* CTC 1010 (serotype 1/2c, from meat origin; 1×10^6, 1×10^5, 1×10^4, 1×10^3, 1×10^2, 1×10^1, and 1 CFU/g, and 1 CFU/25 g) grown in Trypticase soy broth plus 0.6% yeast extract (Difco, Becton Dickinson, Sparks, Md.) at 37°C with shaking. The percentage of fat in the analyzed fish products was evaluated by the standard protocol ISO 1443 *(3).* Spiked salmon samples (25 g) were diluted 10-fold with 0.1% peptone and 0.85% NaCl and homogenized for 1 min in stomacher bags with filters (125-μm pore size; Biochek, Foster City, Calif.) in a Stomacher Labblender device (model 400, Cooke Laboratory Products, Alexandria, Va.). *L. monocytogenes* counts were determined as described in ISO 11290-2 *(5)* after plating on Palcam agar (Merck, Darmstadt, Germany) and incubating at 30°C for 48 h. Low *L. monocytogenes* levels (1×10^2, 1×10^1, and 1 CFU/g, and 1 CFU/25 g) were

* Author for correspondence. Tel: +34-972 419852; Fax: +34-972 418399; E-mail: maria.pla@udg.es.

TABLE 1. *Detection of* L. monocytogenes *by RTi-PCR with the use of different pre-PCR strategies*[a]

Initial inoculum (CFU/g)[b]	Chelex-based DNA purification				Wizard-based DNA purification			
	No trypsin or Triton X-100		Trypsin and Triton X-100		No trypsin or Triton X-100		Trypsin and Triton X-100	
	F	Non-F	F	Non-F	F	Non-F	F	Non-F
1×10^6	+	+	+	+	+	+	+	+
1×10^5	+	+	+	+	+	+	+	+
1×10^4	+	+	+	+	+	+	+	+
1×10^3	6/9	1/9	−	−	+	+	+	+
1×10^2	−	−	−	−	+	+	5/9	4/9
1×10^1	−	−	−	−	6/9	3/9	−	−

[a] F, filtration; +, amplification in all nine replicates performed in three independent experiments. Signal ratios indicate positive/total reactions.

[b] Approximate CFU/g as measured by the plate count method.

also enumerated by the five-tube most-probable-number technique. After incubation at 37°C for 48 h in Half Fraser broth, 0.5 ml of each 10-ml tube was centrifuged for 5 min at 12,000 × *g* and resuspended in 50 μl of water. One microliter of the product was used for RTi-PCR.

Pre-PCR treatments. Homogenized salmon samples were subjected to a combination of the following pre-PCR treatments (see Table 1). (i) Filtration: 5 ml of homogenized salmon samples were filtered through a 22- to 25-μm-pore-size Miracloth filter (Calbiochem, San Diego, Calif.), and 2 ml of filtrate was used for subsequent purification steps. (ii) Detergent and enzymatic pretreatment: 240 μl of Triton X-100 was added to 2 ml of homogenized salmon solution (either after filtration or not). It was mixed vigorously, and 1% (wt/vol) powdered trypsin was added before incubation at 37°C for 30 min. Cells were centrifuged at 2,500 × *g* for 30 min. The resulting pellet was resuspended in 1 ml of nuclease-free water and subjected to DNA extraction. (iii) Two alternative DNA extraction protocols were used. (a) Chelex-based DNA purification: 2 ml of sample was centrifuged for 5 min at 12,000 × *g* and 4°C. The supernatant was carefully discarded, and the pellet was resuspended in 100 μl of 6% Chelex 100 chelating ion exchange resin (Bio-Rad, Munich, Germany) suspension and incubated at 56°C for 20 min. The sample was then vortexed at high speed for 10 s and placed in a 100°C heat block for 8 min. The sample was vortexed again and immediately chilled on ice. Finally, it was centrifuged for 5 min at 16,000 × *g* and 4°C, and the supernatant was carefully transferred to a fresh tube. One microliter was used directly in RTi-PCR assays. (b) Wizard genomic DNA purification: 2 ml of sample was purified with the Wizard Genomic DNA Purification kit (Promega, Madison, Wis.) according to the manufacturer's instructions.

All of these procedures were repeated independently on 3 different days.

RTi-PCR conditions. RTi-PCRs were performed as previously described *(29)*. Briefly, TaqMan PCR core reagents (Applied Biosystems, Foster City, Calif.) were used in a 20-μl reaction volume containing 1× PCR TaqMan buffer A (includes carboxy-X-rhodamine as a passive reference dye); 6 mM $MgCl_2$; 200 μM each of dATP, dCTP, and dGTP; 400 μM dUTP; 50 nM *hlyAQF*/*R* primers; 100 nM *hlyAQP* probe; 1 U of Ampli*Taq* Gold DNA polymerase; 0.2 U of AmpErase uracil *N*-glycosylase; and 1 μl of sample solution. Reactions were run in an ABI PRISM 7700 Sequence Detection System device (Applied Biosystems) with the following program: 2 min at 50°C, 10 min at 95°C, and 50 cycles of 15 s at 95°C and 1 min at 63°C. Reactions were analyzed with Sequence Detection System software 1.9.1 (Applied Biosystems). Quantification was performed by comparison with a standard regression curve of C_T values generated from samples of known concentrations. RTi-PCR assays with $C_T \geq 40$ were considered negative. Nontemplate controls and nonspiked salmon samples were included in all PCR runs and were negative. Unless otherwise stated, all reactions were performed in triplicate. The efficiency of the reactions was calculated as described in Knutsson et al. *(20)*.

Relative accuracy of the method. Relative accuracy is defined as the closeness of the agreement between the results obtained by an accepted method and the results obtained by an alternative method *(6)*. The accuracy of our methods was calculated relative to the standard plate count technique (ISO 11290:1998). Three salmon samples were artificially contaminated with each serial dilution of a *L. monocytogenes* suspension, then bacteria were enumerated by the plate count method and our RTi-PCR–based methods. The results obtained with each RTi-PCR–based method are expressed as a percentage of the results obtained with the plate count method.

RESULTS AND DISCUSSION

Comparison of different pre-PCR strategies for *L. monocytogenes* detection in smoked salmon samples. We compared two different pre-PCR DNA purification strategies that were based on (i) the nonproprietary Chelex 100 resin that is widely used as a pre-PCR step in conventional PCR analyses of food microorganisms and (ii) the commercially available Wizard Genomic DNA Purification kit. Because salmon is rich in fat (10.0%), which can inhibit PCR, we also studied the effect of prior treatment with trypsin and Triton X-100. Finally, we tested the convenience of an additional filtration step through a filter (22- to 25-μm pore size) that should not retain *L. monocytogenes (33)* but should remove larger particles.

Qualitative RTi-PCR results obtained from all possible combinations of pre-PCR treatments (Table 1) showed that the Wizard-based DNA purification step gave better sensitivity than the Chelex-based DNA purification (around two orders of magnitude). The enzymatic and detergent treat-

TABLE 2. *Quantification capacity of the RTi-PCR combined with the different pre-PCR strategies*[a]

	Chelex-based DNA purification				Wizard-based DNA purification			
	No Trypsin or Triton X-100		Trypsin and Triton X-100		No Trypsin or Triton X-100		Trypsin and Triton X-100	
	F	Non-F	F	Non-F	F	Non-F	F	Non-F
Range[b]	1×10^7–1×10^4	1×10^7–1×10^4	1×10^7–1×10^5	1×10^7–1×10^5	1×10^7–1×10^3	1×10^7–1×10^3	1×10^7–1×10^4	1×10^7–1×10^4
LOQ	1×10^4	1×10^4	NA	NA	1×10^3	1×10^3	1×10^4	1×10^4
R^2	0.98 ± 0.01	0.95 ± 0.03	0.88 ± 0.05	0.41 ± 0.38	1.00 ± 0.01	0.99 ± 0.01	0.97 ± 0.02	0.93 ± 0.11
E	0.87 ± 0.22	1.10 ± 0.03	0.59 ± 0.31	2.26 ± 3.21	0.93 ± 0.09	1.01 ± 0.06	1.33 ± 0.21	0.82 ± 0.11

[a] F, filtration; NA, not applicable.

[b] Range designates the number of *L. monocytogenes* inoculum levels along which linearity persisted and thus was used to calculate LOQ (limit of quantification; calculated only in quantifiable samples), R^2, and E (PCR efficiency obtained from $E = 10^{-1/s} - 1$, where s is the slope) *(13, 20)*.

ment decreased (around one order of magnitude) the sensitivity of the assay, probably because of the additional handling of samples. Finally, filtration before any other treatment slightly increased the detection probability of low concentrations of *L. monocytogenes* in smoked salmon samples (Table 1). The best qualitative results were thus obtained with a combination of filtration and DNA purification with the Wizard kit. This combination allowed consistent detection of 1×10^2 *L. monocytogenes* cells per g of smoked salmon (i.e., a detection limit of 1×10^2 cells per g) and detection of 10 CFU/g with 66% probability. This detection limit is compatible with the currently recommended levels for *L. monocytogenes (25).* The qualitative results obtained with this pretreatment protocol were similar to those obtained with genomic DNA purified from *L. monocytogenes* liquid cultures *(29)*, suggesting that they produced high yields of DNA suitable for amplification.

Comparison of different pre-PCR strategies for *L. monocytogenes* quantification in smoked salmon samples. We evaluated the ability of the methods to enumerate *L. monocytogenes* in smoked salmon. For each combination of pre-PCR treatments, we calculated regression curves of the RTi-PCR C_T values versus *L. monocytogenes* inocula (Table 2). Several pre-PCR treatments led to C_T values that were not fully suitable for building appropriate regression curves because only three inoculum concentrations produced positive results (Table 1). For many pre-PCR treatments, mainly those including trypsin or Triton X-100, the linearity of the regression curves was poor, as indicated by R^2 values below 0.97 with high standard deviations. The efficiencies of these reactions were also far from 1 (which is the optimal value) and presented high standard deviation values. As expected, these pre-PCR treatments were associated with the highest detection limit (Table 1).

In contrast, the use of Wizard led to highly efficient RTi-PCR (E close to 1; see Table 2) and allowed accurate quantification of *L. monocytogenes* (as indicated by R^2 values above 0.99) over a range of at least five orders of magnitude. The confidence intervals based on the standard deviations of C_T values did not overlap each other down to 10^3 *L. monocytogenes* CFU per g of sample (data not shown), indicating that reliable quantification was possible above this limit. These values were similar regardless of whether the smoked salmon samples had been filtered through a 22- to 25-μm-pore-size Miracloth filter. Both methods (Wizard with or without filtration) were appropriate for quantitative detection of *L. monocytogenes* in samples of smoked salmon, although the method with a filtration step is recommended because it was more sensitive (Table 1).

TABLE 3. *Relative accuracy of the selected RTi-PCR method for the quantification of* L. monocytogenes *in smoked salmon samples*

Initial inoculum (CFU/g)[a]	Relative accuracy[b]	
	Filtration	No filtration
1×10^6	114.87 ± 13.96	105.79 ± 12.75
1×10^5	80.98 ± 15.20	96.68 ± 22.99
1×10^4	111.69 ± 33.43	95.11 ± 23.76
1×10^3	101.51 ± 19.55	105.60 ± 17.85

[a] Approximate CFU per gram.

[b] Relative accuracy was calculated as a percentage of CFU per gram obtained in the plate count (standard) method *(6)*. Standard deviations were <10% between triplicates in the same experiment.

Relative quantification accuracy. Relative accuracy is a critical parameter for the implementation of an alternative microbiological diagnostic method. A relative accuracy of 100% indicates total agreement between the alternative method and the reference method (here ISO 11290:1998). The mean relative accuracy of the selected methods varied from 81 to 115% (Table 3) along the whole quantitative range tested (i.e., down to 10^3 CFU/g), which fits with the ISO 16140:2003 recommendations *(6)*. The Wizard protocol produced similar results with and without a prior filtration step, indicating that they can both be used.

The International Commission on Microbiological Specification for Foods concluded that 100 CFU of *L. monocytogenes* per g of food at the time of consumption is acceptable for consumers who are not at risk *(15, 25)*. Therefore, the selected methods fulfill these requirements. In addition, because foods are complex matrices, most available detection systems require selective enrichment steps to overcome the problem of potential PCR inhibitors,

especially for low pathogen concentrations *(1, 2, 8, 32)*. Our method does not require any culture steps, meaning that results can be obtained considerably quicker. Thus, this method could be used as a quick, routine technique for the quantification of *L. monocytogenes* down to 1,000 CFU/g and for detection of down to 10 CFU/g in smoked salmon. The same performance in terms of relative accuracy and limits of detection and quantification has been reported for various meat samples with the same RTi-PCR assay in combination with optimized pre–RTi-PCR treatments *(30)*.

In addition, if levels below 100 CFU/g are to be analyzed, our method can be combined with the most-probable-number technique with final confirmation by the RTi-PCR assay here used, thus generating reliable results in only 48 h (data not shown). This is a considerable advantage over the time-consuming and laborious confirmation procedure based on isolation of *L. monocytogenes* on PALCAM media and subsequent confirmation by biochemical and morphological tests *(4)*.

Application to raw salmon. We next looked at the possibility of applying the selected RTi-PCR–based method to raw salmon samples (containing 12.9% fat). Reliable sensitivity was achieved at concentrations down to 1×10^4 CFU/g, and it was possible to detect 1×10^3 CFU/g in 33% of the nine replicates performed in three independent experiments. These values are considerably higher than those obtained for smoked salmon (around two orders of magnitude). In addition, the C_T values obtained were not linearly correlated with the initial inoculum sizes (producing a regression curve of $E > 2.5$ and $R^2 < 0.5$), making quantification impossible, perhaps because of the presence of PCR inhibitors in raw salmon samples that are copurified during our pre-PCR procedure. To assess this possibility, we used a previously described RTi-PCR assay developed for *Mycobacterium avium* subsp. *paratuberculosis (28)*, with 10^5 copies of the *M. avium* subsp. *paratuberculosis* internal amplification control in the presence of 1 μl of processed raw salmon samples as substrate. We obtained the same C_T values (standard deviation = 0.18 cycles) both in the presence and absence of processed raw salmon samples. These results show the absence of PCR inhibitors and suggest that the pre-PCR steps result in major losses of *L. monocytogenes* cells.

We report a reliable RTi-PCR–based method for the fast quantitative detection of *L. monocytogenes* in smoked salmon. It has an excellent quantification capacity, as proved by its linearity (mean R^2, >0.99), PCR efficiency (mean E, >0.93), quantification limit (<100 CFU/g), and wide dynamic quantification range (at least five orders of magnitude). Moreover, it showed an adequate relative accuracy with respect to the standard plate count technique. It can be combined easily with the most-probable-number technique for enumeration of low loads of *L. monocytogenes*, thus offering a promising alternative to traditional microbiological methods. In addition, it is suitable for the detection of this bacterium in raw salmon.

ACKNOWLEDGMENT

This work was supported by the Spanish Ministerio de Ciencia y Tecnología (INIA, CAL01-058-C2-2 and CICYT AGL2002-03496).

REFERENCES

1. Al-Soud, W., and P. Rådström. 2000. Effects of amplification facilitators on diagnostic PCR in the presence of blood, feces, and meat. *J. Clin. Microbiol.* 38:4463–4470.
2. Al-Soud, W., and P. Rådström. 2001. Purification and characterization of PCR-inhibitory components in blood cells. *J. Clin. Microbiol.* 39:485–493.
3. Anonymous. 1973. Meat and meat products—determination of total fat content. International Organization for Standardization ISO 1443: 1973.
4. Anonymous. 1996. Microbiology of food and animal feeding stuffs—horizontal method for the detection and enumeration of *Listeria monocytogenes*—part 1: detection method. International Organization for Standardization ISO 11290-1:1996.
5. Anonymous. 1998. Microbiology of food and animal feeding stuffs—horizontal method for the detection and enumeration of *Listeria monocytogenes*—part 2: enumeration method. International Organization for Standardization ISO 11290-2:1998.
6. Anonymous. 2003. Microbiology of food and animal feeding stuffs—protocol for the validation of alternative methods. International Organization for Standardization ISO 16140:2003.
7. Ben Embarek, P. K. 1994. Presence, detection and growth of *Listeria monocytogenes* in seafoods: a review. *Int. J. Food Microbiol.* 23: 17–34.
8. Bhagwat, A. A. 2003. Simultaneous detection of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* strains by real-time PCR. *Int. J. Food Microbiol.* 84:217–224.
9. Eklund, M. W., F. T. Poysky, R. N. Paranjpye, L. C. Lashbrook, M. E. Peterson, and G. A. Pelroy. 1994. Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. *J. Food Prot.* 58:502–508.
10. Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev.* 55:476–511.
11. Guyer, S., and T. Jemmi. 1991. Behavior of *Listeria monocytogenes* during fabrication and storage of experimentally contaminated smoked salmon. *Appl. Environ. Microbiol.* 57:1523–1527.
12. Hein, I., D. Klein, A. Lehner, A. Bubert, E. Brandl, and M. Wagner. 2001. Detection and quantification of the *iap* gene of *Listeria monocytogenes* and *Listeria innocua* by a new real-time quantitative PCR assay. *Res. Microbiol.* 152:37–46.
13. Higuchi, R., C. Fockler, G. Dollinger, and R. Watson. 1993. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Biotechnology* 11:1026–1030
14. Hough, A. J., S. A. Harbison, M. G. Savill, L. D. Melton, and G. Fletcher. 2002. Rapid enumeration of *Listeria monocytogenes* in artificially contaminated cabbage using real-time polymerase chain reaction. *J. Food Prot.* 65:1329–1332.
15. International Commission on Microbiological Specification for Foods. 1996. Establishment of sampling plans for microbiological safety criteria for foods in international trade including recommendations for control of *Listeria monocytogenes*, *Salmonella enteriditis*, *Campylobacter* and enterohemorragic *E. coli*. Codex Committee on Food Hygiene, 29th session, 21 to 25 October 1996, Agenda item 11, CX/FH 96/91-16. Codex Alimentarius Commission, Rome.
16. Jørgensen, L. V., and H. H. Huss. 1998. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. *Int. J. Food Microbiol.* 42:127–31.
17. Kathariou, S. 2000. Pathogenesis determinants of *Listeria monocytogenes*, p. 295–314. *In* J. W. Cary, J. Linz, and D. Bhatnagar (ed.), Microbial foodborne diseases: mechanisms of pathogenesis and toxin synthesis. Technomic Publishing Co., Inc., Lancaster, Pa.
18. Kathariou, S. 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *J. Food Prot.* 65:1811–1829.
19. Klein, D. 2002. Quantification using real-time PCR technology: applications and limitations. *Trends Mol. Med.* 8:257–260.
20. Knutsson, R., C. Löfström, H. Grage, J. Hoorfar, and P. Rådström. 2002. Modeling of 5′ nuclease real-time responses for optimization of a high-throughput enrichment PCR procedure for *Salmonella enterica*. *J. Clin. Microbiol.* 40:50–62.
21. Koo, K., and L. A. Jaykus. 2003. Detection of *Listeria monocyto-*

genes from a model food by fluorescence resonance energy transfer–based PCR with an asymmetric fluorogenic probe set. *Appl. Environ. Microbiol.* 69:1082–1088.
22. Lantz, P. G., W. Abu al-Soud, R. Knutsson, B. Hahn-Hagerdal, and P. Rådström. 2000. Biotechnical use of PCR for microbiological analysis of biological samples. *Biotechnol. Annu. Rev.* 5:87–130.
23. Macrae, M., and D. M. Gibson. 1990. Survival of *Listeria monocytogenes* in smoked salmon, p. 3-168–3-174. *In* P. Zeuthen, J. C. Cheftel, C. Eriksson, T. R. Gormley, P. Linko, and K. Paulus (ed.), Processing and quality of foods. 3. Chilled foods: the revolution in freshness. Proceedings of the Final COST 91 bis Final Seminar, 2 to 5 October 1989, Gøteborg. Elsevierg, London.
24. Nogva, H. K., K. Rudi, K. Naterstad, A. Holck, and D. Lillehaug. 2000. Application of 5′-nuclease PCR for quantitative detection of *Listeria monocytogenes* in pure cultures, water, skim milk, and unpasteurized whole milk. *Appl. Environ. Microbiol.* 66:4266–4271.
25. Nørrung, B. 2000. Microbiological criteria for *Listeria monocytogenes* in foods under special consideration of risk assessment approaches. *Int. J. Food Microbiol.* 62:217–221
26. Norton, D. M., M. A. McCamey, K. L. Gall, J. M. Scarlett, K. J. Boor, and M. Wiedmann. 2001. Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish processing industry. *Appl. Environ. Microbiol.* 67:198–205.
27. Rådström, P., R. Knutsson, P. Wolfs, M. Dahlenborg, and C. Löfström. 2003. Pre-PCR processing of sampling, p. 31–50. *In* K. Sachse and J. Frey (ed.), Methods in molecular biology: PCR detection of microbial pathogens. Humana Press, Totowa, N.J.
28. Rodríguez-Lázaro, D., M. D'Agostino, A. Herrewegh, M. Pla, N. Cook, and J. Ikonomopoulos. Real-time PCR-based methods for quantitative detection of *Mycobacterium avium* subsp. *paratuberculosis* in water and milk. *Int. J. Food Microbiol.*, in press.
29. Rodríguez-Lázaro, D., M. Hernández, M. Scortti, T. Esteve, J. A. Vázquez-Boland, and M. Pla. 2004. Quantitative detection of *Listeria monocytogenes* and *Listeria innocua* by real-time PCR: assessment of *hly*, *iap* and *lin02483* targets and AmpliFluor™ technology. *Appl. Environ. Microbiol.* 70:1366–1377.
30. Rodríguez-Lázaro, D., A. Jofré, T. Aymerich, M. Hugas, and M. Pla. 2004. Rapid quantitative detection of *Listeria monocytogenes* in meat products by real-time PCR. *Appl. Environ. Microbiol.* 70: 6299–6301.
31. Rørvik, L. M. 2000. *Listeria monocytogenes* in the smoked salmon industry. *Int. J. Food Microbiol.* 20:183–90.
32. Rossen, L., P. Norskov, K. Holmstrom, and O. F. Rasmussen. 1992. Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. *Int. J. Food Microbiol.* 17:37–45.
33. Seeliger, H. P. R., and D. Jones. 1986. Listeria, p. 1235–1245. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, Md.
34. Vázquez-Boland, J. A., M. Kuhn, P. Berche, T. Chakraborty, G. Domínguez-Bernal, W. Goebel, B. González-Zorn, J. Wehland, and J. Kreft. 2001. *Listeria* pathogenesis and molecular virulence determinants. *Clin. Microbiol. Rev.* 14:584–640.
35. Wilson, I. G. 1997. Inhibition and facilitation of nucleic acid amplification. *Appl. Environ. Microbiol.* 63:3741–3751.