Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
International Journal of Food Microbiology 84 (2003) 285–297
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Genetic variability among isolates of *Listeria monocytogenes* from food products, clinical samples and processing environments, estimated by RAPD typing

Iciar Martinez[a,b,*], Liv-Marit Rørvik[c], Vigdis Brox[b,1], Jørgen Lassen[d], Marit Seppola[b], Lone Gram[e], Birte Fonnesbech-Vogel[e]

[a] *SINTEF Fisheries and Aquaculture Ltd., N-7465 Trondheim, Norway*
[b] *Norwegian Institute of Fisheries and Aquaculture Ltd., 9291 Tromsø, Norway*
[c] *Norwegian School of Veterinary Science, Postboks 8146 Dep, 0033 Oslo, Norway*
[d] *Division of Infectious Disease Control, Norwegian Institute of Public Health, PO Box 4404 Nydalen, N-0403 Oslo, Norway*
[e] *Danish Institute for Fisheries Research, Department of Seafood Research, c/o Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark*

Received 3 May 2002; received in revised form 6 September 2002; accepted 9 September 2002

Abstract

RAPD analysis with four primers was used to examine the genetic relationship among 432 strains of *Listeria monocytogenes* isolated from clinical and veterinarian cases of listeriosis, dairy, vegetable, meat- and fish-based food items, environmental samples and samples collected from one transport terminal, one poultry-processing company and four Atlantic salmon-processing plants. The purpose of the study was to determine whether clinical isolates belonged to a specific genetic group, whether links could be made between food groups and clinical cases and whether specific genetic groups were associated with specific food products or processing units. There was great genetic variability among the isolates, which produced a total of 141 RAPD composites based on the RAPD analysis with four primers. The RAPD composites divided in two major clusters and clinical isolates were evenly distributed in both of them. None of the isolates from food products had the same RAPD composite as isolates from human patients, thus, no particular food commodity could be linked to clinical cases. Each food-processing environment was contaminated with more than one RAPD composite and the genetic variability found within each company was, in most cases, of approximately the same magnitude as the variability found when considering all the samples. In each plant, one or a few types persisted over time, indicating the presence of an established in-house flora. Our results indicate that most of the analysed cases of listeriosis were sporadic and, further, that these cases cannot be traced to a few specific food sources. We also found that no particular RAPD composite was better suited for survival in specific food types or food-processing environments, indicating that although differences may be found in virulence properties of individual strains, all *L. monocytogenes* must be treated as potentially harmful.

Keywords: *Listeria monocytogenes*; RAPD; Composite

* Corresponding author. SINTEF Fisheries and Aquaculture Ltd., N-7465 Trondheim, Norway. Tel.: +47-735-96-384; fax: +47-735-96-363.
E-mail address: iciar.martinez@sintef.no (I. Martinez).
[1] Present address: Department of Medical Genetics, Regional Hospital in Tromsø, 9038 Tromsø, Norway.

0168-1605/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0168-1605(02)00423-3

1. Introduction

The importance of *Listeria monocytogenes* as a foodborne human pathogen has been recognised since the 1980s (Farber and Peterkin, 1991; Gellin et al., 1991), and it is now acknowledged that 85–95% of the listeriosis cases are foodborne (Buzby et al., 1996). The main products associated with listeriosis are ready-to-eat food products, typically with long shelf life at refrigerated temperature. Especially, soft cheese, dairy and meat products (Farber and Peterkin, 1991) have been involved in outbreaks of listeriosis, whereas seafood products have less frequently been considered the cause of the disease. *L. monocytogenes* has been found regularly in poultry products in several screenings. Frequencies of 41–84% have been reported from broiler carcasses and raw chicken products, and 0–61% from processed poultry products (Franco et al., 1995; Lawrence and Gilmour, 1994; Uyttendale et al., 1999).

L. monocytogenes has often been isolated from seafood, including ready-to-eat products such as shrimp, cold and hot-smoked salmon and trout (Ben Embarek, 1994): between 9% and 28% of the samples of ready-to-eat shrimp, crab and smoked fish analysed in several surveys contained *L. monocytogenes* (Ben Embarek, 1994; Dillon et al., 1994; Farber and Peterkin, 1991; Rocourt and Bille, 1997; Rørvik and Yndestad, 1991). In cold-smoked salmonids, it has been typically detected in 10–40% of the surveyed samples (Autio et al., 1999; Jørgensen and Huss, 1998). Shellfish and raw fish are thought to have played a role in an outbreak in Auckland, New Zealand, in 1980 (Lennon et al., 1984), and cold-smoked rainbow trout caused five cases of febrile gastroenteritis in Finland (Miettinen et al., 1999). In Sweden, *L. monocytogenes* isolated from two clinical cases and from two fish products (vacuum-packed smoked rainbow trout and gravad salmon) were indistinguishable by phage-typing, and restriction endonuclease analysis (REA) followed by pulsed-field gel electrophoresis (PFGE), and the fish products were likely sources of the illness (Ericsson et al., 1997; Loncarevic et al., 1998). Rørvik et al. (2000) used multilocus enzyme electrophoresis (MEE) and REA typing to determine that isolates from vacuum-packed smoked salmon, a processing plant and clinical isolates of sporadic listeriosis in Norway were indistinguishable.

Although present in the environment and on food raw materials, one of the major sources of *L. monocytogenes* in food products is the processing environment itself. *L. monocytogenes* can colonise the processing environment, utensils, brines, etc. (Destro et al., 1996; Fonnesbech Vogel et al., 2001a,b; Giovannacci et al., 1999; Lawrence and Gilmour, 1995; Rørvik et al., 1995, 1997, 2000; Wenger et al., 1990). This colonisation (Giovannacci et al., 1999; Johansson et al., 1999; Rørvik et al., 1995, 1997), as well as job rotation among departments (Rørvik et al., 1997), have been established as primary mechanisms for contamination of the final products in some processing lines. In most cases, the contamination of the final product is believed to have occurred during processing because the strains found in the incoming raw materials are different from the strains found in the final product (Autio et al., 1999; Destro et al., 1996; Fonnesbech Vogel et al., 2001a,b; Rørvik et al., 1995). In other cases, when the strains of *L. monocytogenes* contaminating the raw materials were indistinguishable from those isolated from the final product, the contaminated incoming raw materials have been regarded to be the source of the continuous contamination (Fonnesbech Vogel et al., 2001a; Giovannacci et al., 1999; Lawrence and Gilmour, 1995).

Typing of *L. monocytogenes* is important in epidemiological studies for investigation of foodborne outbreaks (i.e., comparing clinical and food isolates) and, in the food-processing environment, to identify the source or sources of contamination and routes of spreading. The World Health Organization (WHO) sponsored an international collaborative study to evaluate methods for subtyping of *L. monocytogenes* (Bille and Rocourt, 1996), including random amplification of polymorphic DNA (RAPD) typing (Welsh and McClelland, 1990; Williams et al., 1990). RAPD typing had a high discriminatory power, but intra- and interlaboratory problems of the reproducibility were found. RAPD requires a careful methodological standardisation (Fonnesbech Vogel et al., 2001b) and has been successfully used to characterise *L. monocytogenes* by several authors (Boerlin et al., 1995; Farber and Addison, 1994; Fonnesbech Vogel et al., 2001a,b; Kerr et al., 1995; Lawrence et al., 1993; MacGowan et al., 1993; Mazurier and Wernars, 1992; O'Donoghue et al., 1995; Rocourt, 1994; Wagner et al., 1996).

The aim of this work was to determine whether clinical isolates from Norwegian cases of listeriosis belonged to a specific genetic group, if links could be made between food groups and clinical cases and if specific patterns emerged as to specific genetic groups being associated with specific food products or processing units. The novelty of the present work consists of the fact that we were able to draw conclusions from an unusually large collection of isolates: we are not aware of previous works addressing these issues with the number of isolates and spreading both in time and in origin that we show here. We have chosen random amplification of polymorphic DNA (RAPD) to discriminate among strains. The standardised protocols described by Fonnesbech Vogel et al. (2001a,b) were used to minimise intralaboratory variations. Interlaboratory comparisons were performed by typing a random subset of strains in two of the laboratories.

2. Materials and methods

2.1. Isolates of L. monocytogenes

A total of 432 strains of *L. monocytogenes* were analysed. The strains were isolated from clinical cases, food products, environmental sources and food-processing environments (Table 1). The procedures for sampling, isolation, identification and serotyping of *L. monocytogenes* are described by Rørvik et al. (1995, 2000). The isolates from human patients had been collected from different Norwegian hospitals and sent to the Norwegian Institute of Public Health for further analysis.

2.2. DNA isolation

L. monocytogenes isolates were preserved frozen at − 80 °C in Microbank™ vials (Pro-Lab Diagnostics,

Table 1
Source and description of 432 isolates of *L. monocytogenes* used in the present work

Source	Description	No.	Collected in:
Human patients		51	1986–1996
Diseased animals	cow suffering mastitis	1	1988
	stillborne pig	1	1994
Seawater		2	1994
Food items	raw meats	4	1988, 1989 and 1996
	meat-based products	17	1988, 1989, 1996 and 1997
	plants and vegetables	4	1990 and 1997
	milk and cheese	3	1997
	raw fish	5	1989, 1992 and 1997
	seafood	25	1989, 1990, 1992–1994, 1996, 1997 and 1998
Company AC (chicken processing)	received alive chicken, slaughtered them and produced raw chicken as final product	113	1988, 1989, 1995 and 1996
Company A (Atlantic salmon)	received alive salmon into its slaughterhouse and produced smoked salmon in a separated smoking house	45	1991–1993
Company B (Atlantic salmon)	took in alive salmon and slaughtered it, slaughtered salmon being the final product	33	1993
Company G (Atlantic salmon)	received alive salmon and slaughtered it, the finals products being slaughtered salmon and raw fish products	91	1996, 1997 and 1998
Company AI (Atlantic salmon)	received slaughtered Atlantic salmon and produced smoked salmon (final product)	19	1996
Transport terminal (fish)	fish in boxes were temporarily stored and reloaded for further shipment	18	1996

More detailed data are given in Tables 2–4. Of the examined companies, only the company AI was not Norwegian.

Ontario, Canada). One bead was transferred to 1 ml of Brain–Heart Infusion medium (Difco) and incubated overnight at 27 °C. To standardise the intralaboratory conditions (Fonnesbech Vogel et al., 2001a), DNA isolation was carried out with the Dynabeads DNA Direct Universal™ kit (Dynal ASA, Oslo, Norway) following the instructions of the manufacturer. The isolated DNA was resuspended in 40–80 μl of resuspension buffer.

2.3. RAPD analysis

RAPD analysis was carried out with READY To Go™ RAPD Analysis Beads (Amersham Pharmacia Biotech, Uppsala Sweden) according to the instructions of the manufacturer: 23.5 μl of sterile Milli-Q H_2O containing 0.5 μM primer were added to each bead followed by 1.5 μl of the extracted DNA, which gave a final concentration of between 0.4 and 2 ng DNA/μl. This amount of DNA was chosen after a titration of the extracts to ensure that the patterns were dependant on the DNA sequences and not on the DNA concentration (Welsh and McClelland, 1990).

The primers used were Eric-2 (5′-AAG TAA GTG ACT GGG GTG AGC G-3′) (Vila et al., 1996), M-01 (5′-GTT GGT GGC T-3′) (Fonnesbech Vogel et al., 2001b; Lawrence et al., 1993), M-13 (5′-GAG GGT GGC GGT TCT-3′) (Gräser, et al., 1993) and P-2 (5′-GTT TCG CTC C-3′). Primers Eric-2, M-01 and M-13 were purchased from MedProbe (Oslo, Norway). Primer P-2 was provided with the READY To Go™ RA PD Analysis Beads kit.

The reaction mixture was overlaid with 20 μl of Chill Out Wax (MJ Research, Waltham, MA, USA) and amplifications were performed on a PTC-100 programmable thermal controller (MJ Research). The thermal program for amplification was 94 °C for 2 min, followed by 40 cycles of 94 °C for 20 s, 35 °C for 20 s, 72 °C for 2 min with a final step at 72 °C for 7 min. Each amplification contained four controls: three positive (two *E. coli* strains—C1a and BL21—provided with the kit and one *L. monocytogenes*, CCUG 1452) and one negative (containing H_2O instead of DNA).

When the amplifications were finished, 7.5 μl of loading dye (15% Ficoll, 0.05% bromophenol blue, 0.05% xylene cyanol) were added to each tube and 13 μl of this mixture were loaded into the wells of 14 × 20 cm (length × width) 2% Nusieve/Seakem LE (1:3) FMC (Philadelphia, PA, USA) agarose gels. A 100-bp ladder (Gibco BRL, Life Technologies Invitrogen, San Diego, CA) was loaded every fourth or eighth sample for normalisation of the gels. Gel and electrophoresis buffer was 0.5 × TBE (Sambrook et al., 1989) and electrophoresis was carried out at 5 V/cm for about 3.5–4 h. The gels were stained for 15 min in 0.5 × TBE containing 0.5 μg/ml ethidium bromide, destained for another 15 min in the same buffer without ethidium bromide and photographed under UV light with a Polaroid (Cambridge, MA, USA) camera and film 55.

Seven isolates from the company A, four from AC and five from AI were typed both at the Norwegian Institute of Fisheries and Aquaculture (Tromsø, Norway) as described above, and at the Danish Institute for Fisheries Research (Lyngby, Denmark) as described by Fonnesbech Vogel et al. (2001b).

2.4. Data analysis

The Polaroid photographs of the gels were scanned using a CanonScan FB 320Pscanner (Tokyo, Japan) and analysed with the software GelCompar II, version 2.0 (Applied Maths, Kortrijk, Belgium). A total of 92 polymorphic markers, of sizes varying from about 200 to 2300 bp, were used in the analysis: 23 markers generated by ERIC-2, 34 by M-01, 18 by M-13 and 17 by P-02. Cluster analysis was performed on a composite data set containing the 92 markers using the Dice correlation and cluster analysis by the unweighted pair group method using arithmetic averages (UPGMA) (Fonnesbech Vogel et al., 2001a). This analysis always produces two major clusters, which have been called A and B in this work. Cluster analysis was performed on all the 141 RC composites, as well as on each of the subgroups of RC-types found in the following subsets of samples: (i) clinical and veterinarian isolates; (ii) from environmental samples and food products, (iii) from the chicken-processing company A; (iv–vii) from each of the Atlantic salmon-processing companies A, AI, B and G and (viii) from the transport terminal T.

3. Results

3.1. RAPD profiles and genetic diversity

Fig. 1 shows the patterns obtained with each primer on four randomly selected isolates of *L. monocyto-*

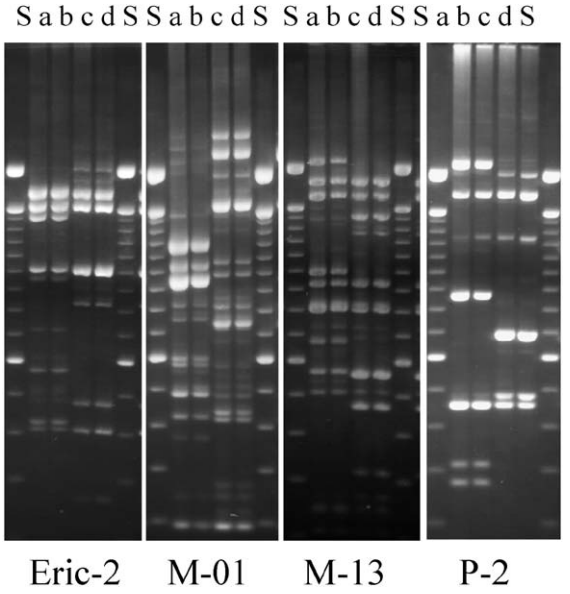

Fig. 1. RAPD analysis of isolates (a) 1455, (b) 1458, (c) 1475, and (d) 1476 from the chicken-processing company AC with the four primers indicated in the photograph. Isolates 1455 and 1458 had the composite RAPD-type RC-59 and isolates 1475 and 1476 were composite RAPD-type RC-32.

genes. Although primer P-02 produced the lowest number of polymorphic bands, we recommend it for the clarity of the patterns. The combinations of the RA PD-types obtained with the four primers produced a total of 141 different composite RAPD profiles. Data treatment is based on these composite RAPD profiles that were named RC-1–RC-141. The numbers are arbitrary: closer numbers does not mean closer genetic relationship.

The 141 RCs were grouped in two clusters, diverging at a percentage of similarity of 42.2 ± 7.2% (Fig. 2). The percentage of similarity within cluster A, which comprised 173 isolates belonging to 53 RCs, was 59.7 ± 2.7%. Within cluster B, with 259 isolates that belonged to 88 RCs, the percentage of similarity was 61.3 ± 2.5%. Serotypes 1 and 4 were represented in both clusters. Each RC-type was classified as belonging to either cluster A or to cluster B and this classification was unchanged regardless of whether all the RC-type were included in the analysis or whether the cluster analysis was performed on subsets of samples indicated in Section 2.4.

3.2. Strains from clinical and veterinarian isolates

The 53 isolates of *L. monocytogenes* from Norwegian clinical and veterinarian cases during the period of 1986–1996 belonged to 34 different RCs (Table 2). The two most frequent types were represented by five isolates each, the cases with RC-18 are considered to have had a common origin: an outbreak in the city of Trondheim caused by vacuum-packed meat sausage (Hellesnes et al., 1992). However, the isolates typed as RC-26 came from different years and were, therefore, considered to be sporadic. Cluster analysis of these strains gave two clusters diverging at a 41.6 ± 7.0% similarity, about 68% of the isolates belonged to cluster A and 32% to cluster B.

3.3. Isolates from environmental samples and food products

There was great genetic variability among the 60 isolates from environmental and food samples (Table 3). They belonged to 48 different RC-types and cluster analysis of these strains gave two clusters diverging at a 40.4 ± 7.7% similarity. No RC-type was present in all four types of food products: meat-, milk- and fish-based products and vegetables. Only two RC-types were found in more than one group of food types: the same RC-type was present in sliced cheese and in the silo sample (RC-139), and the RC-type from the imported cheese was indistinguishable from one found in shrimps (RC-38). The most abundant types were represented only by three strains each, and those were isolated in different years.

Approximately 27% of the strains isolated from seafood belonged to cluster A, in which serovars 1 and 4 were found, and about 73% to cluster B, with only serovars 1. These figures were very similar in the meat-based products (26% and 74% belonging to cluster A and B, respectively) and they were almost inverted in the isolates from diseased humans and animals: about 68% belonged to cluster A and 32% to cluster B.

3.4. Isolates from processing environments

3.4.1. Chicken-processing company

One hundred and thirteen isolates from the chicken-processing company AC, obtained during an 8-year

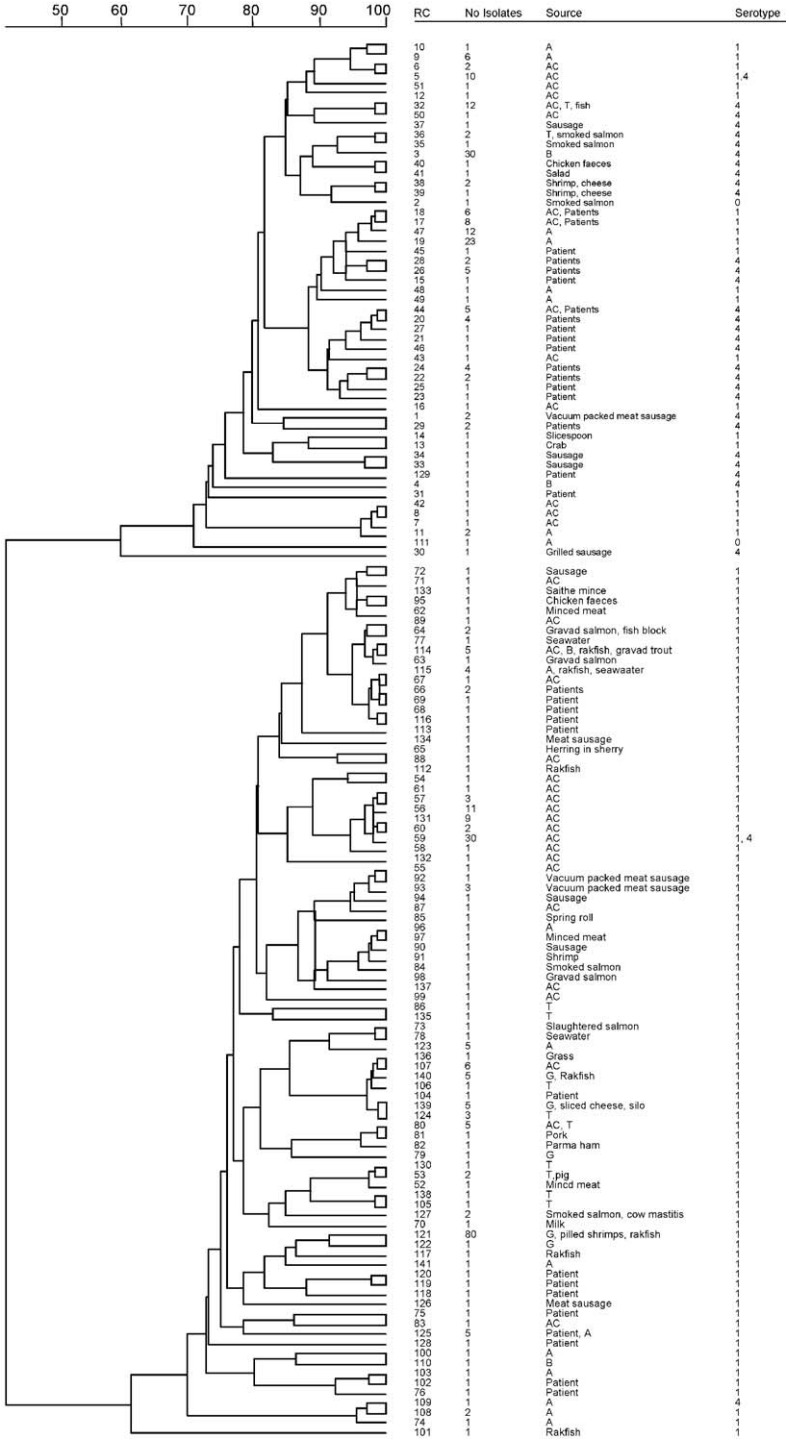
50
60
70
80
90
100
RC
No Isolates
Source
Serotype
10 1 A 1
9 6 A 1
6 2 AC 1
5 10 AC 1,4
51 1 AC 1
12 1 AC 1
32 12 AC, T, fish 4
50 1 AC 4
37 1 Sausage 4
36 2 T, smoked salmon 4
35 1 Smoked salmon 4
3 30 B 4
40 1 Chicken faeces 4
41 1 Salad 4
38 2 Shrimp, cheese 4
39 1 Shrimp, cheese 4
2 1 Smoked salmon 0
18 6 AC, Patients 1
17 8 AC, Patients 1
47 12 A 1
19 23 A 1
45 1 Patient 1
28 2 Patients 4
26 5 Patients 4
15 1 Patient 4
48 1 A 1
49 1 A 1
44 5 AC, Patients 4
20 4 Patients 4
27 1 Patient 4
21 1 Patient 4
46 1 Patient 4
43 1 AC 1
24 4 Patients 4
22 2 Patients 4
25 1 Patient 4
23 1 Patient 4
16 1 AC 1
1 2 Vacuum packed meat sausage 4
29 2 Patients 4
14 1 Slicespoon 1
13 1 Crab 1
34 1 Sausage 4
33 1 Sausage 4
129 1 Patient 4
4 1 B 4
31 1 Patient 1
42 1 AC 1
8 1 AC 1
7 1 AC 1
11 2 A 1
111 1 A 0
30 1 Grilled sausage 4
72 1 Sausage 1
71 1 AC 1
133 1 Saithe mince 1
95 1 Chicken faeces 1
62 1 Minced meat 1
89 1 AC 1
64 2 Gravad salmon, fish block 1
77 1 Seawater 1
114 5 AC, B, rakfish, gravad trout 1
63 1 Gravad salmon 1
115 4 A, rakfish, seawaater 1
67 1 AC 1
66 2 Patients 1
69 1 Patient 1
68 1 Patient 1
116 1 Patient 1
113 1 Patient 1
134 1 Meat sausage 1
65 1 Herring in sherry 1
88 1 AC 1
112 1 Rakfish 1
54 1 AC 1
61 1 AC 1
57 3 AC 1
56 11 AC 1
131 9 AC 1
60 2 AC 1
59 30 AC 1, 4
58 1 AC 1
132 1 AC 1
55 1 AC 1
92 1 Vacuum packed meat sausage 1
93 3 Vacuum packed meat sausage 1
94 1 Sausage 1
87 1 AC 1
85 1 Spring roll 1
96 1 A 1
97 1 Minced meat 1
90 1 Sausage 1
91 1 Shrimp 1
84 1 Smoked salmon 1
98 1 Gravad salmon 1
137 1 AC 1
99 1 AC 1
86 1 T 1
135 1 T 1
73 1 Slaughtered salmon 1
78 1 Seawater 1
123 5 A 1
136 1 Grass 1
107 6 AC 1
140 5 G, Rakfish 1
106 1 T 1
104 1 Patient 1
139 5 G, sliced cheese, silo 1
124 3 T 1
80 5 AC, T 1
81 1 Pork 1
82 1 Parma ham 1
79 1 G 1
130 1 T 1
53 2 T,pig 1
52 1 Minced meat 1
138 1 T 1
105 1 T 1
127 2 Smoked salmon, cow mastitis 1
70 1 Milk 1
121 80 G, pilled shrimps, rakfish 1
122 1 G 1
117 1 Rakfish 1
141 1 A 1
120 1 Patient 1
119 1 Patient 1
118 1 Patient 1
126 1 Meat sausage 1
75 1 Patient 1
83 1 AC 1
125 5 Patient, A 1
128 1 Patient 1
100 1 A 1
110 1 B 1
103 1 A 1
102 1 Patient 1
76 1 Patient 1
109 1 A 4
108 2 A 1
74 1 A 1
101 1 Rakfish 1

Table 2
Data on the clinical and veterinarian isolates of *L. monocytogenes* used in the present study: year of isolation, RAPD composite (RC)-type, serotypes and cluster

Source	Year	No. of isolates	RC no. (no. of strains if more than 1)	Serotype	Cluster
Patients	1986	2	17 (2)	1	A
	1987	2	22, 28	4	A
	1988	3	24, 25, 26	4	A
	1992	14	18 (5), 24 (3), 31, 44, 75, 76, 102, 116	1, 4, 1, 4, 1, 1, 1, 1	A
	1993	2	20, 45	4, 1	A
	1993	3	113, 119, 120	1	B
	1994	4	15, 22, 23, 27	4	A
	1994	2	69, 128	1	B
	1995	7	20, 26 (2), 28, 29 (2), 46	4	A
	1995	4	66 (2), 68, 118	1	B
	1996	6	20 (2), 21, 26 (2), 129	4	A
	1996	2	106, 125	1	B
Mastitis	1988	1	127	1	B
Stillborne pig	1994	1	53	1	B

period, belonged to 37 RCs. The most common RC-type was represented with 30 isolates, followed by RCs with 11, 10 and 9 isolates each (Table 4).

The final product (raw chicken) was contaminated with a variety of strains that belonged to 18 different RCs. Two RC-types, RC-5 and RC-59, seemed to be particularly common or persistent, since they were identified in 1988–1989 and 1995 and in 1988, 1995 and 1996, respectively. RC-59, in particular, was present in samples from the eviscerating area, in several locations along processing and in the final product. The RC-types found in samples of faeces, on the other hand, seemed to be limited to these samples and they were not identified in other specimens. A unique strain of *L. monocytogenes* was identified in the sink where workers washed their hands. The two clusters formed by analysis of the RC from this company diverged at 43.5 ± 6.1% similarity.

3.4.2. Atlantic salmon-processing companies

No common RC-type was found to be predominant in the Atlantic salmon-processing companies A, B, G and AI taken as a whole (Table 4). Each company had its characteristic composition of strains of *L. monocytogenes*, and in each company most of the isolates of *L. monocytogenes* belonged to one (companies B, G and AI) or two (company A) RC-types. In companies A and G, which were sampled in several years, the respective predominant RC-types were found to be recurrent during 2 or 3 years. Six isolates from seawater samples taken around two of the companies (A and B) were identified as *L. monocytogenes*. Each of them had a different RC-type, and three of them were indistinguishable from isolates identified in the company. Cluster analysis of *L. monocytogenes* strains from these companies revealed B to be the company with the highest genetic variability (clusters dividing at a 31.3 ± 1% similarity), followed by the transport company (40.3 ± 4.6%) and AI (46.2 ± 2.9%). The lowest genetic variability was registered within company G with clusters A and B diverging at 73.2 ± 1% similarity.

In the fish transport terminal, we found 17 isolates of *L. monocytogenes* that belonged to 11 different RCs and the most abundant RC-type was represented by only four isolates. One of the isolates from this location had the same RC-type than the isolate from the diseased pig, two other RC-types were common to types found in food products, two other were common to RC-types from the chicken-processing company and one was indistinguishable from a RC-type found in the salmon-processing company G. Taking all the processing environments (AC, A, B, G, AI and the transport company T) into consideration, only three RCs were found in more than one of them. The distribution of the strains of *L. monocytogenes* isolated in these processing environments

Fig. 2. Dendogram of the RAPD composites of 141 isolates (one for each RC) obtained after UPGMA analysis of the Dice coefficient. The numbers on the top left represent the percent similarity. Clusters A and B diverged at a 42.3 ± 7.2% similarity. The columns on the right are (as indicated on the top), from left to right: the RC-type, the total number of isolates belonging to the RC, the source from which the RC-types were isolated and the serotype to which they belonged. A, AI and G: Atlantic salmon processing companies; T: fish transport terminal; AC: poultry slaughterhouse, as described in Table 1.

Table 3
Isolates of *L. monocytogenes* from environmental sources and isolates from food items: year of isolation, RC-type, serotypes and cluster

Source	Year	No. of isolates	RC no.	Serotype	Cluster
Vacuum-packed meat sausage	1988	2	1 (2)	4	A
		1	37	4	A
		2	92, 93	1	B
	1989	2	93, 126	1	B
Minced meat	1988	1	62	1	B
	1989	1	97	1	B
	1996	1	52	1	B
Meat sausages and sliced meat sausages	1988	1	94	1	B
	1996	1	30	4	A
	1996	4	72, 90, 93, 134	1	B
Sliced meat sausage	1996	2	33, 34	4	A
Pork meat	1996	1	81	1	B
Parma ham	1997	1	82	1	B
Grass	1990	1	123	1	B
Silo	1990	1	139	1	B
Salat	1997	1	41	4	A
Spring roll	1997	1	85	1	B
Milk	1997	1	70	1	B
Cheese, imported	1997	1	38	4	A
Cheese sliced	1997	1	139	1	B
Seawater, Elnesvågen Romsdal	1994	2	77, 78	1	B
Minced saithe fillet	1989	1	133	1	B
Haddock fillets	1997	1	32	4	A
Haddock block	1997	1	64	1	B
Cod block	1997	1	32	4	A
Slaughtered salmon	1992	1	73	1	B
Smoked salmon	1989	1	2	0	A
	1989	2	35, 36	4	A
	1989	1	84	1	B
	1994	1	127	1	B
Gravad salmon	1993	1	63	1	B
	1994	1	98	1	B
	1996	1	64	1	B
Gravad trout	1997	1	114	1	B
Rakfish	1993	5	112, 114, 115, 117, 121	1	B
	1994	5	101, 107, 114, 115 (2)	1	B
Slicespoon	1997	1	14	1	A
Shrimps	1989	2	38, 39	4	A
	1989	1	91	1	B
Peeled shrimps	1989	1	121	1	B
Cooked frozen crab	1990	1	13	1	A
Sherry herring	1992	1	65	1	B

Table 4
L. monocytogenes RAPD composites found in different processing environments. Company AC processed chicken, and companies A, B, G and AI processed Atlantic salmon. TRA was a fish transport terminal. In parentheses are the numbers of isolates that belong to the given RC

Source	Year	No. of isolates	RAPD composite type				
			Seawater	Faeces	Raw material	Processing[a]	Final product
AC	1988	11					5 (6), 6, 12, 59, 60 (2)
	1989	2					5 (2)
	1995	73		40, 87[b], 89, 95, 114		16, 17 (4), 32, 44 (3), 50, 56 (11), 57 (3),67, 71, 83, 131 (3), 132, 136, 137	5, 17 (2), 18, 32 (7), 43, 44, 51, 59 (13), 61, 99, 131 (6)
	1996	27				5, 6, 32, 42, 58, 59 (11), 80, 88	7, 8, 54, 55, 59 (5)
A	1991	29	125[c]		103	19 (22), 125 (3)	11, 19
	1992	15	11, 115			9 (5), 10, 100, 111, 123 (4), 141	
	1993	1					9
B	1993	33	3, 110, 114			3 (22), 4	3 (7)
G	1996	55				79, 104, 107 (3), 121 (29), 122, 139 (2), 140	107 (2), 121 (13), 139, 140
	1997	29				121 (26)	121 (3)
	1998	7				121 (4)	121 (3)
AI	1996	19				47 (8), 48, 96, 108	47 (4), 49, 74, 108, 109
TRA	1996	18				32, 36, 53, 80 (4), 86, 105, 124 (3), 130, 135, 138, 140 (3)	

[a] Processing environment and product during processing.
[b] Faeces and feathers from the floor in the receiving area.
[c] Seawater from a well-boat transporting living salmon.

according to the cluster they belonged to was as follows: 64% of the strains from the chicken-processing company AC, 89% of the strains from the transport terminal T and 100% of the strains from the processing plant G belonged to cluster B. In the slaughter and smoking companies A, B and AI, strains belonging to cluster A were predominant: 71%, 94% and 74%, respectively.

Table 5
RAPD profiling from two different laboratories (FF, Lyngby, Denmark; and NIFA, Tromsø, Norway) of 16 strains of *L. monocytogenes* isolates

Company	Isolate	RAPD-type with primers								RAPD composite	
		Eric-2		M-01		M-13		P-02		FF	NIFA
		FF	NIFA	FF	NIFA	FF	NIFA	FF	NIFA		
A	1	a	1	–	21	a	36	a	1	a	19
A	2	a	1	a	21	a	36	a	1	a	19
A	3	a	1	a	21	a	36	a	1	a	19
A	4	a	1	a	21	a	36	a	1	a	19
A	5	a	1	a	21	a	36	a	1	a	19
A	6	b	28	b	68	b	14	b	105	b	125
A	7	b	28	b	68	b	14	b	105	b	125
AC	8	c	1	c	225	c	37	c	2	c	44
AC	9	c	1	c	225	c	37	c	2	c	44
AC	10	c	1	c	225	c	37	c	2	c	44
AC	11	c	1	c	**215**	c	37	c	2	c	**43**
AI	12	d	10	d	72	d	31	d	145	d	74
AI	13	d	**23**	d	72	d	31	d	145	d	**108**
AI	14	e	2	e	21	e	35	e	1	e	47
AI	15	e	2	e	21	e	35	e	1	e	47
AI	16	f	23	f	72	f	31	f	15	f	109

In bold are the two isolates where disagreement was found.

3.5. Comparison of the results obtained at two different laboratories

As mentioned, a subset of strains were RAPD-typed at two laboratories: Lyngby (Denmark) and Tromsø (Norway) with each of the four primers (Table 5). In general, good agreement in terms of genetic clustering was seen; however, two isolates gave incongruent results: isolates no. 11 (interlaboratory difference due to primer M-01) and no. 13 (difference due to primer Eric-2), which gives a 12.5% of incompatible results.

4. Discussion

The issue of the reproducibility and usefulness of the RAPD fingerprinting technique has been widely discussed by many authors (Destro et al., 1996; Ellsworth, et al., 1993; Farber and Addison, 1994; Fonnesbech Vogel et al., 2001a,b; Kerr et al., 1995; Lawrence and Gilmour, 1995; MacGowan et al., 1993; MacPherson et al., 1993; Mazurier et al., 1992). We have followed the protocol described by Fonnesbech Vogel et al. (2001b), and recommend it to future users because it is very easy to perform and decreases the intralaboratory variability, specially regarding the quality of the DNA, which is one of the most relevant variables (Kerr et al., 1995; Micheli et al., 1994). In the present work, two primers gave different results at different labs with some isolates. Both primers (M-01 and Eric-2) gave complex patterns and that, combined with the use of different equipment for amplification and electrophoresis, almost certainly explains the different results (Martinez et al., 2001). Comparisons based on the results from typing with primers that produce clearer and less polymorphic patterns, such as P-02, may increase the interlaboratory congruency, although probably at the expense of reduction in the discriminatory power. Kerr et al. (1995) suggested the use of least three primers for typing in studies where RAPD was the only technique applied. The need to use several primers is illustrated here by comparison of the results obtained at two different laboratories. Our results also show that the use of several primers increases the interlaboratory congruency of the analysis even if different primers are used, and confirm previous works (Kerr et al., 1995; Mazurier and Wernars, 1992) showing that different serotypes of *L. monocytogenes* may share the same RAPD profile (Fig. 2).

In addition to the amplification reaction, the fingerprint patterns are strongly influenced by the separation technique used, such as gel size, composition and migration conditions when gel electrophoresis is used. It is interesting to note that we chose the four primers used after an initial screening of a total of 13. Primers tested and rejected for producing either too weak or too few bands, or difficult to score polymorphic bands under our given amplification conditions were DAF-4, REP-1 and REP-2 (Vila et al., 1996); UBC-127, UBC-155 and UBC-156 (Farber and Addison, 1994), HLWL-74 (Mazurier and Wernars, 1992); and LURP-1 and LURP-2 (Kerr et al., 1995). The fact that some of these primers have been successfully used for RAPD typing of *L. monocytogenes* by other authors stresses the need for strict standardisation conditions to make interlaboratory comparisons of the RAPD patterns. Under the present conditions, it is our opinion that for full identification of RAPD patterns, one should analyse in a batch, and preferably simultaneously, all the samples under scrutiny (Martinez et al., 2001).

None of the food products analysed in this work showed the same RC-types as the clinical and veterinarian isolates, and may, in principle, be dismissed as the source of the strains isolated from the patients. There did not seem to be one RC-type predominant among human patients during the 10 years in which the isolates were collected although some RC-types occurred more frequently than others. Thus, these cases of listeriosis may theoretically have constituted an outbreak with a common source, as was the mentioned Trondheim outbreak. Unfortunately, we did not have available an isolate of *L. monocytogenes* from the food product that caused it and can, therefore, not trace it back to a food product in the present work. The genetic variability found among the strains isolated from seafood supports previous studies (Boerlin et al., 1997) indicating that these products do not constitute a particular microenvironment in which only some strains survive. In contrast, the same genetic type of a strain may be found in different food samples, and vice versa, i.e., one given kind of product may harbour genetically different strains of *L. monocytogenes*. Our results indicate that the same applies to meat-based

products. Some RC-types were common to strains isolated from human listeriosis and from the chicken-processing company AC but while the strains from AC were isolated in 1995, those from diseased subjects were isolated in 1989 and 1992. One RC-type, RC-125, was isolated from company A in 1991 and from a listeriosis case in 1996. Thus, due to the difference in time, it is not possible to attribute to any of these companies the human cases of listeriosis. However, these data indicate that strains of *L. monocytogenes* contaminating different processing environments (chicken and fish) in principle can cause human listeriosis. We found as well, confirming previous works (Destro et al., 1996; Fonnesbech Vogel et al., 2001a,b; Lawrence and Gilmour, 1995; Rørvik, 2000; Rørvik et al., 2000), that several types of *L. monocytogenes*, may be found in one food-processing environment, including the processing line and the final product.

Lawrence and Gilmour (1995) found 18 different RAPD profiles (using only primer M-01) contaminating poultry products and the processing environment. One of the RAPD-types contaminating the incoming birds, and persistent during the 6-month period of their study, was also one of the types found in the final product (cooked poultry). Therefore, the raw material was considered to be an important source of contamination of the final product. In the present study, only two RC-types were found in the slaughter area and in the final product, but these were not found in the samples of faeces of the birds, in spite of the fact that these were contaminated with at least four different RC-types that were not identified in other samples. Therefore, during the time span covered by this study, the incoming birds did not seem to be the source of contamination in this company. However, the persistence of RC-59 during the 8 years of the study indicates that the initial contamination may have taken place very long time ago.

In all the examined salmon-processing companies, only one or a few RC-types seemed to be predominant in the plant, but no plant was contaminated with only one RC-type and no single RC-type was predominant in more than one company. Our data, thus, support previous works on contamination patterns of *L. monocytogenes* in Atlantic salmon and trout smoking industries (Autio et al., 1999; Fonnesbech Vogel et al., 2001a,b; Rørvik et al., 1995, 2000): a few strains, which initially, at some time, may have been introduced with the raw material, seem to have found a niche, from where they are constantly shed during processing thus contaminating the products and cross-contaminating and recontaminating equipment, utensils and workers' hands. In the Atlantic salmon-processing companies A and B, the seawater around the companies was contaminated by *L. monocytogenes*, and two strains were detected in the processing environment of the companies 18 and 10 months before they were isolated from the seawater, respectively. The third common strain was identified from a well-boat for living salmon at the same day as it was isolated from smoked salmon, and not later. Therefore, we, as Rørvik et al. (2000), consider it more likely that the companies were the sources of the contamination to the seawater, and not that the seawater was the source of the contamination in the companies.

For the processing conditions examined in this work, it is impossible to have critical control points type 1, i.e., steps in the production lines at which it is possible to achieve a complete control over a hazard (International Commission on Microbial Specifications for Food, 1988). Therefore, especially for products such as smoked salmon intended to be consumed without further heating, strict observance of hygienic rules, as well as cleaning and disinfecting procedures, are of the outmost importance. The long-time prevalence of the same *L. monocytogenes* strains, together with the genetic variability of the strains found, seem to indicate that a continuous contamination and cross-contamination of the production facilities, and everything in contact with them, including the hands of the workers (as shown by the *L. monocytogenes* isolated from a sink used by the workers) are taking place in these premises due to either deficient or incorrect hygienic procedures.

In summary, we do not find that strains of *L. monocytogenes* isolated from cases of human listeriosis form a genetically separated or even homogeneous cluster. Similarly, our results do not support that certain genetically related strains are better adapted to particular food products, or to a particular type of processing line. On the other hand, very different (according to RAPD typing) strains may contaminate the same type of food product or the same processing line and cause disease in humans or animals. Thus, our data indicate that most of the cases of listeriosis analysed here are

indeed sporadic random events caused by single food items.

Acknowledgements

The financial support of the Norwegian Research Council (project NFR 121801/112) and the Danish Ministry for Food, Agriculture and Fisheries (project 93s-2459-a98-00003) are gratefully acknowledged.

References

Autio, T., Hielm, S., Miettinen, M., Sjoberg, A.M., Aarnisalo, K., Bjorkroth, J., Mattila Sandholm, T., Korkeala, H., 1999. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. Applied and Environmental Microbiology 65, 150–155.

Ben Embarek, P.K., 1994. Presence, detection and growth of *Listeria monocytogenes* in seafoods, a review. International Journal of Food Microbiology 23, 17–34.

Bille, J., Rocourt, J., 1996. WHO International Multicenter *Listeria monocytogenes* subtyping study—rationale and set-up of the study. International Journal of Food Microbiology 32, 251–262.

Boerlin, P., Bannerman, E., Ischer, F., Rocourt, J., Bille, J., 1995. Typing *Listeria monocytogenes*—a comparison of random amplification of polymorphic DNA with 5 other methods. Research Microbiology 146, 35–49.

Boerlin, P., Boerlin-Petzold, F., Bannerman, E., Bille, J., Jemmi, T., 1997. Typing isolates of *Listeria monocytogenes* isolates from fish products and human listeriosis cases. Applied and Environmental Microbiology 63, 1338–1342.

Buzby, J.C., Roberts, T., Lin, C.-T.J., MacDonald, J.M., 1996. Bacterial foodborne disease. Medical cost and productivity losses. Agricultural Economic Report, vol. 741. Food and Consumer Economics Division, Economic Research Service, U.S. Department of Agriculture, Washington, DC.

Destro, M.T., Leitao, M.F.F., Farber, J.M., 1996. Use of molecular typing methods to trace the dissemination of *Listeria monocytogenes* in a shrimp processing plant. Applied and Environmental Microbiology 62, 705–711.

Dillon, R., Patel, T., Ratman, S., 1994. Occurrence of *Listeria* in hot and cold smoked seafood products. International Journal of Food Microbiology 22, 73–77.

Ellsworth, D.L., Rittenhouse, K.D., Honeycutt, R.L., 1993. Artifactual variation in randomly amplified polymorphic DNA banding patterns. Biotechniques 4, 214–217.

Ericsson, H., Eklöw, A., Danielsson-Tham, M.L., Loncarevic, S., Mentzing, L.O., Persson, I., Unnerstad, H., Tham, W., 1997. An outbreak of listeriosis suspected to have been caused by rainbow trout. Journal of Clinical Microbiology 35, 2904–2907.

Farber, J.M., Addison, C.J., 1994. RAPD typing for distinguishing species and strains in the genus *Listeria*. Journal of Applied Bacteriology 77, 242–250.

Farber, J.M., Peterkin, P.I., 1991. *Listeria monocytogenes*, a foodborne pathogen. Microbiological Reviews 55, 476–511.

Fonnesbech Vogel, B., Huss, H.H., Ojeniyi, B., Ahrens, P., Gram, L., 2001a. Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Applied and Environmental Microbiology 67, 2586–2595.

Fonnesbech Vogel, B., Jorgensen, L.V., Ojeniyi, B., Huss, H.H., Gram, L., 2001b. Diversity of *Listeria monocytogenes* isolates from cold-smoked salmon produced in different smokehouses as assessed by random amplified polymorphic DNA analyses. International Journal of Food Microbiology 65, 83–92.

Franco, C.M., Quinto, E.J., Fente, C., Rodriguez-Otero, J.L., Dominguez, L., Cepeda, A., 1995. Determination of the principal sources of *Listeria* spp. contamination in poultry meat and a poultry processing plant. Journal of Food Protection 12, 1320–1325.

Gellin, B.G., Broome, C.V., Bibb, W.F., Weaver, R.E., Gaventa, S., Mascola, L., 1991. The epidemiology of listeriosis in the United States—1986. American Journal of Epidemiology 133, 392–401.

Giovannacci, I., Ragimbeau, C., Quenguiner, S., Salvat, G., Vendeuvre, J.-L., Carlier, V., Ermel, G., 1999. *Listeria monocytogenes* in pork slaughtering and cutting plants. Use of RAPD, PFGE and PCR-REA for tracing and molecular epidemiology. International Journal of Food Microbiology 53, 127–140.

Gräser, Y., Klare, I., Halle, E., Gantenberg, R., Buchholz, P., Jacobi, H.D., Presber, W., Schönian, G., 1993. Epidemiological study of an *Acitenobacter baumannii* outbreak by using polymerase chain reaction fingerprinting. Journal of Clinical Microbiology 31, 2417–2420.

Hellesnes, I., Blystad, H., Bevanger, L., 1992. An outbreak of listeriosis in Trøndelag. Norwegian Infectious Diseases Notification System 20, 43.

ICMSF (International Commission on Microbial Specifications for Foods), 1988. Microorganisms in Foods: 4. Application of the Hazard Analysis and Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality. Blackwell Scientific Publications, Oxford.

Johansson, T., Rantala, L., Palmu, L., Honkanen-Buzalski, T., 1999. Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. International Journal of Food Microbiology 47, 111–119.

Jørgensen, L.V., Huss, H.H., 1998. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. International Journal of Food Microbiology 42, 127–131.

Kerr, K.G., Kite, P., Heritage, J., Hawkey, P.M., 1995. Typing of epidemiologically associated environmental and clinical strains of *Listeria monocytogenes* by random amplification of polymorphic DNA. Journal of Food Protection 58, 609–613.

Lawrence, L.M., Gilmour, A., 1994. Incidence of *Listeria* spp. and *Listeria monocytogenes* in a poultry processing environment and poultry products and their rapid confirmation by multiplex PCR. Applied and Environmental Microbiology 60, 4600–4604.

Lawrence, L.M., Gilmour, A., 1995. Characterization of *Listeria*

monocytogenes isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. Applied and Environmental Microbiology 61, 2139–2144.

Lawrence, L.M., Harvey, J., Gilmour, A., 1993. Development of a random amplification of polymorphic DNA typing method for *Listeria monocytogenes*. Applied and Environmental Microbiology 59, 3117–3119.

Lennon, D., Lewis, B., Mantell, C., Becroft, D., Dove, B., Farmer, K., Tonkin, S., Yeates, N., Stamp, R., Mickelson, K., 1984. Epidemic perinatal listeriosis. Pediatry and Infectious Diseases 3, 30–34.

Loncarevic, S., Danielsson-Tham, M.-L., Gerner-Schidt, P., Sahlström, L., Tham, W., 1998. Potential sources of human listeriosis in Sweden. Food Microbiology 15, 65–69.

MacGowan, A.P., O'Donaghue, K., Nicholls, S., McLauchlin, J., Bennett, P.M., Reeves, D.S., 1993. Typing of *Listeria* spp. by random amplified polymorphic DNA (RAPD) analysis. Journal of Medical Microbiology 38, 322–327.

MacPherson, J.M., Eckstein, P.E., Scoles, G.J., Gajadhar, A.A., 1993. Variability of the random amplified polymorphic DNA assay among thermal cyclers, and effects of primer and DNA concentration. Molecular and Cellular Probes 7, 293–299.

Martinez, I., Jakobsen Friis, T., Seppola, M., 2001. Requirements for the application of protein sodium dodecyl sulfate-polyacrylamide gel electrophoresis and randomly amplified polymorphic DNA analyses to product speciation. Electrophoresis 22, 1526–1533.

Mazurier, S.-I., Wernars, K., 1992. Typing of *Listeria* strains by random amplification of polymorphic DNA. Research Microbiology 143, 499–505.

Mazurier, S.-I., Audurier, A., Marquet-Van der Mee, N., Notermans, S., Wernars, K., 1992. A comparative study of randomly amplified polymorphic DNA analysis and conventional phage typing for epidemiological studies of *Listeria monocytogenes* isolates. Research Microbiology 143, 507–512.

Micheli, M.R., Bova, R., Pascale, E., D'Ambrosio, E., 1994. Reproducible random amplified polymorphic DNA (RAPD) method. Nucleic Acids Research 22, 1921–1922.

Miettinen, M.K., Siitonen, A., Heiskanen, P., Haajanen, H., Björkroth, K.J., Korkeala, H.J., 1999. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold smoked rainbow trout. Journal of Clinical Microbiology 37, 2358–2360.

O'Donoghue, K., Bowker, K., Mclauchlin, J., Reeves, D.S., Bennett, P.M., Macgowan, A.P., 1995. Typing of *Listeria monocytogenes* by random amplified polymorphic DNA (RAPD) analysis. International Journal of Food Microbiology 27, 245–252.

Rocourt, J., 1994. *Listeria monocytogenes*, the state of the science. Dairy Food and Environmental Sanitation 14, 70–82.

Rocourt, J., Bille, J., 1997. Foodborne listeriosis. World Health Statistics Quarterly 50, 67–73.

Rørvik, L.M., 2000. *Listeria monocytogenes* in the smoked salmon industry. International Journal of Food Microbiology 62, 183–190.

Rørvik, L.M., Yndestad, M., 1991. *Listeria monocytogenes* in foods in Norway. International Journal of Food Microbiology 13, 97–104.

Rørvik, L.M., Caugant, D.A., Yndestand, M., 1995. Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughterhouse and smoked salmon processing plant. International Journal of Food Microbiology 25, 19–27.

Rørvik, L.M., Skjerve, E., Knudsen, B.R., Yndestad, M., 1997. Risk factors for contamination of smoked salmon with *Listeria monocytogenes* during processing. International Journal of Food Microbiology 37, 215–219.

Rørvik, L.M., Aase, B., Alvestad, T., Caugant, D., 2000. Molecular epidemiological survey of *Listeria monocytogenes* and seafood-processing plants. Applied and Environmental Microbiology 66, 4779–4784.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press.

Uyttendale, M., de Troy, P., Debevere, J., 1999. Incidence of *Salmonella*, *Campylobacter jejuni*, *Campylobacter coli* and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgian retail market. Journal of Food Protection 62, 735–740.

Vila, J., Marcos, M.A., Jimenez de Anta, T., 1996. A comparative study of different PCR-based DNA fingerprinting techniques for typing of the *Acitenobacter calcoaceticus–A. baumannii* complex. Journal of Medical Microbiology 44, 482–489.

Wagner, M., Maderner, A., Brandl, E., 1996. Random amplification of polymorphic DNA for tracing and molecular epidemiology of *Listeria* contamination in a cheese plant. Journal of Food Protection 59, 384–389.

Welsh, J., McClelland, M., 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18, 7213–7218.

Wenger, J.D., Swaminathan, B., Hayes, P.S., Green, S.S., Pratt, M., Pinner, R.W., Schuchat, A., Broome, C.V., 1990. *Listeria monocytogenes* contamination of turkey franks—evaluation of a production facility. Journal of Food Protection 53, 1015–1019.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful genetic markers. Nucleic Acids Research 18, 6531–6535.