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Research Note

Raw and Processed Fish Show Identical *Listeria monocytogenes* Genotypes with Pulsed-Field Gel Electrophoresis

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ABSTRACT

A total of 257 raw fish samples at two different sites were examined for the presence of *Listeria monocytogenes*. The prevalence of *L. monocytogenes* was 4%. From 11 positive samples, nine different *L. monocytogenes* pulsed-field gel electrophoresis genotypes were recovered. From nine pulsotypes recovered from raw fish and 32 pulsotypes shown by 101 fish product isolates, two raw fish and fish product pulsotypes were indistinguishable from each other. Although the prevalence of *L. monocytogenes* in raw fish is low, the range of *L. monocytogenes* strains entering the processing plant in large amounts of raw material is wide. This indicates that the raw material is an important initial contamination source of *L. monocytogenes* in fish processing plants. This postulation is supported by the identical pulsotypes recovered from both raw and processed fish. Some *L. monocytogenes* strains entering a plant may thus contaminate and persist in the processing environment, causing recurrent contamination of the final products via processing machines.

Ready-to-eat fish products, among many other food items, are vehicles of listeriosis, a life-threatening disease caused by *Listeria monocytogenes*. Gravad and cold-smoked rainbow trout have been linked to the listeriosis epidemics in 1994 and 1999, respectively *(9, 22)*. Although the vehicles of most sporadic listeriosis cases remain unknown, fish products have occasionally been linked to an illness of one or two persons *(10, 23)*. Reported human listeriosis cases are not the only evidence of fish being an important source of listeriosis. In a Finnish retrospective study, a *L. monocytogenes* type recovered from several sporadic listeriosis cases during an 8-year follow-up *(19)* turned out to be identical to an epidemic strain that originated in fish *(22)*.

L. monocytogenes has been isolated from several fish products *(5, 7, 11, 17, 18, 20, 27)*. The contamination rate of fish rises sharply during processing, particularly during brining and slicing *(2, 8, 27)*. The source of product contamination is thought to be the processing environment *(2, 8, 12, 13, 27)*.

L. monocytogenes contamination is believed to enter the processing plant from multiple sources *(2, 4, 13, 27)*. The roles of, for example, raw material, personnel, transport vehicles, and air-mediated contamination have been discussed. Even though Rørvik et al. *(27)*, Autio et al. *(2)*, and Hoffman et al. *(13)* concluded that raw material is not a main source of the contamination of final products, in several studies the possibility that the raw product is one of the sources of *L. monocytogenes* in a processing plant has not been excluded *(2, 12, 13)*. The purpose of this study was to clarify the role of raw fish as an initial source of fish processing plant and product contamination by *L. monocytogenes* by showing identical genotypes in raw fish and fish product isolates using pulsed-field gel electrophoresis (PFGE).

MATERIALS AND METHODS

Sampling. A total of 257 raw rainbow trout (*Oncorhynchus mykiss*) samples were examined between 1998 and 2001. Fish were farmed in brackish water, open sea farms on the west coast of Finland. One sample contained slime, skin, gills, and fins from one to five fish heads. Heads were picked at two different sites (Table 1). Heads were examined fresh or after freezing at −20°C (Table 2). Fresh heads were stored in ice until the examination, which was conducted within 4 h of sampling. Frozen heads were examined immediately after melting to room temperature.

Bacterial strains. All 41 raw fish isolates of *L. monocytogenes* (19 isolates from fresh samples and 22 isolates from frozen samples) that were recovered during this study. A total of 101 isolates recovered from the fish products (Table 3) of 21 nonidentified fish processing plants from 1996 to 2002, and belonging to the culture collection of the Department of Food and Environmental Hygiene were included.

Bacteriological analysis. Raw fish samples were homogenized in a stomacher blender for 30 s with half-Fraser broth (Oxoid, Basingstoke, UK). *L. monocytogenes* was isolated using a two-step enrichment method according to the guidelines of the

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TABLE 1. *Prevalence of* Listeria monocytogenes *in raw fish sampled at two different sites*

Sampling site	No. of samples	No. (%) of *L. monocytogenes*–positive samples
Slaughterhouse, during slaughtering	45	2 (4)
Processing factory, before processing	212	9 (4)
Total	257	11 (4)

International Organization for Standardization *(1)*. Samples were spread from Fraser enrichment broth (Oxoid) to PALCAM (Oxoid) and Oxford (Oxoid) agars. In addition, *L. monocytogenes* blood agar *(15)* (Trypticase soy agar base [Difco, Becton Dickinson, Sparks, Md.], 10 g/liter of lithium chloride [Merck KgaA, Darmstadt, Germany], 10 mg/liter of polymyxine B sulfate [Sigma, St. Louis, Mo.], 20 mg/liter of ceftazidime [Abtek Biologicals Ltd., Liverpool, UK], and 5% sterile defibrinated sheep blood) was used. Five typical colonies from each selective plate were identified by hemolytic activity, Gram staining, catalase reaction, and an API *Listeria* kit (bioMérieux, Inc., Marcy l'Etoile, France).

In situ DNA isolation and PFGE. In situ DNA isolation and PFGE typing were performed as described by Autio et al. *(3)*. Restriction enzymes *Asc*I (New England Biolabs, Beverly, Mass.) and *Apa*I (Boehringer Mannheim, Mannheim, Germany) were used for the digestion of DNA.

PFGE pattern analysis. The numerical analysis of macrorestriction patterns (MRPs) and clustering was performed using commercial BioNumerics software 2.5 (Applied Maths, Kortrijk, Belgium). For each isolate, fragments yielded by the *Apa*I restriction enzyme that were bigger than 48.5 kb were included in the analysis. The similarity between restriction patterns, based on band position, was expressed with Dice coefficient correlation. Position tolerance was optimal when set at 1.0% for the total length of both *Apa*I and *Asc*I patterns with no increase. The clustering and construction of dendrograms were performed by the unweighted pair-group method with arithmetic averages.

Serotyping. One to two representative isolates from each pulsotype were serotyped with the commercial *Listeria* antisera (Denka Seiken, Tokyo, Japan) as described by the manufacturer. All isolates representing an identical pulsotype were interpreted as belonging to the same serotype.

RESULTS

The prevalence of *L. monocytogenes* in raw fish was 4% (11 of 257). No statistically significant differences were present in the prevalence of *L. monocytogenes*–positive fish samples between the two sampling sites (Table 1) or between fresh and frozen samples (Table 2) (χ^2, $P > 0.05$).

TABLE 2. *Prevalence of* Listeria monocytogenes *in fresh and frozen raw fish*

Type of sample	No. of samples	No. (%) of *L. monocytogenes*–positive samples
Fresh	140	7 (5)
Frozen	117	4 (3)
Total	257	11 (4)

TABLE 3. *Number of* Listeria monocytogenes *isolates originating from different fish products*

Fish product	No. of products	No. of isolates
Rainbow trout (*Oncorhynchus mykiss*)		
Cold smoked		
Whole or fillet	19	23
Cut or sliced	14	14
Gravad		
Whole or fillet	11	11
Cut or sliced	25	26
Other	7	7
Other fish[a]		
Cold smoked	1	1
Gravad	1	1
Other	12	16
Roe product	2	2
Total	92	101

[a] Other fish samples include seven whitefish (*Coregonus albula*), three salmon (*Salmo salar*), two squids (*Coleoidea*), one herring (*Clupea harengus*), and one coalfish (*Pollachius firens*).

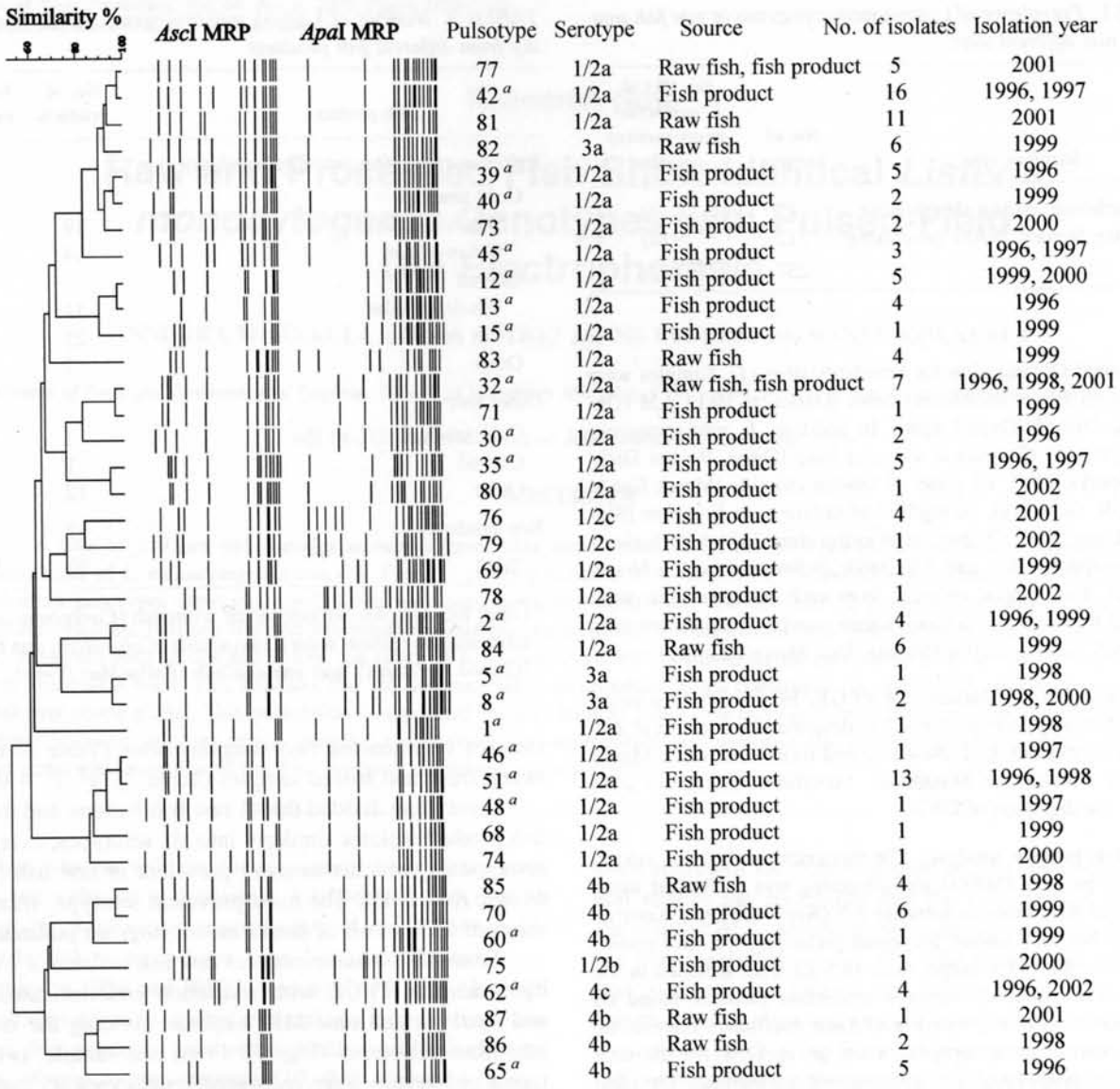
Serotyping divided the 41 raw fish isolates and the 101 fish product isolates similarly into six serotypes, except for serotype 3a, which was more prevalent in raw fish (Table 4) (χ^2, $P < 0.05$). The most prevalent serotype, 1/2a, represented 73% of all of the *L. monocytogenes* isolates.

From 41 *L. monocytogenes* raw fish isolates of 11 positive samples, PFGE with restriction endonucleases *Asc*I and *Apa*I yielded nine MRPs apiece, dividing the isolates into nine pulsotypes (Fig. 1). From one sample, two different pulsotypes were recovered (pulsotypes 85 and 86). The same pulsotype was recovered from four samples (pulsotype 81). From 101 fish product isolates, *Asc*I and *Apa*I yielded 32 and 23 MRPs, respectively, resulting in 32 different pulsotypes (Fig. 1).

Two raw fish pulsotypes were indistinguishable from fish product pulsotypes (Fig. 1). Pulsotype 32 was recovered from gravad fish produced by two different producers in 1996 and 1998 and from raw fish in 2001. Pulsotype 77

TABLE 4. *Distribution of* Listeria monocytogenes *isolates recovered from raw and processed fish according to serotype*

Serotype	No. (%) of *L. monocytogenes* isolates		
	Raw fish	Processed fish	Total
1/2a	28 (68)	76 (75)	104 (73)
4b	7 (17)	12 (12)	19 (13)
3a	6 (15)	3 (3)	9 (6)
1/2c	0 (0)	5 (5)	5 (4)
4c	0 (0)	4 (4)	4 (3)
1/2b	0 (0)	1 (1)	1 (1)
Total no. of isolates	41 (100)	101 (100)	142 (100)



Pulsotype	Serotype	Source	No. of isolates	Isolation year
77	1/2a	Raw fish, fish product	5	2001
42[a]	1/2a	Fish product	16	1996, 1997
81	1/2a	Raw fish	11	2001
82	3a	Raw fish	6	1999
39[a]	1/2a	Fish product	5	1996
40[a]	1/2a	Fish product	2	1999
73	1/2a	Fish product	2	2000
45[a]	1/2a	Fish product	3	1996, 1997
12[a]	1/2a	Fish product	5	1999, 2000
13[a]	1/2a	Fish product	4	1996
15[a]	1/2a	Fish product	1	1999
83	1/2a	Raw fish	4	1999
32[a]	1/2a	Raw fish, fish product	7	1996, 1998, 2001
71	1/2a	Fish product	1	1999
30[a]	1/2a	Fish product	2	1996
35[a]	1/2a	Fish product	5	1996, 1997
80	1/2a	Fish product	1	2002
76	1/2c	Fish product	4	2001
79	1/2c	Fish product	1	2002
69	1/2a	Fish product	1	1999
78	1/2a	Fish product	1	2002
2[a]	1/2a	Fish product	4	1996, 1999
84	1/2a	Raw fish	6	1999
5[a]	3a	Fish product	1	1998
8[a]	3a	Fish product	2	1998, 2000
1[a]	1/2a	Fish product	1	1998
46[a]	1/2a	Fish product	1	1997
51[a]	1/2a	Fish product	13	1996, 1998
48[a]	1/2a	Fish product	1	1997
68	1/2a	Fish product	1	1999
74	1/2a	Fish product	1	2000
85	4b	Raw fish	4	1998
70	4b	Fish product	6	1999
60[a]	4b	Fish product	1	1999
75	1/2b	Fish product	1	2000
62[a]	4c	Fish product	4	1996, 2002
87	4b	Raw fish	1	2001
86	4b	Raw fish	2	1998
65[a]	4b	Fish product	5	1996

FIGURE 1. *A combined dendrogram of* AscI *and* ApaI *MRPs of* L. monocytogenes *strains isolated from raw fish and fish products. One representative strain of each pulsotype was included. The cophonetic correlation of the dendrogram is 85%. Serotypes, numbers of isolates, and isolation years have also been presented.* [a]*Strains and pulsotypes have previously been described by Autio et al. (3).*

was also recovered from raw fish in 2001. In the same year, an identical pulsotype was found in one gravad fish sample.

DISCUSSION

The prevalence of *L. monocytogenes* in raw fish was 4%. In previous studies, the prevalence of *L. monocytogenes* has varied from 0 to 50% *(2, 5, 8, 12, 13, 24, 25, 27, 28),* with an average overall prevalence of 9% (190 of 2,073). The differences in the prevalence of *L. monocytogenes* in raw fish may at least be partly due to water quality *(5).* Moreover, slaughtered raw fish from certain slaughterhouses may frequently be contaminated with *L. monocytogenes* because of contamination during slaughtering *(26).*

The most prevalent serotype in both raw and processed fish was 1/2a. In a previous study, the most prevalent serotype in raw fish was 4b *(14).* Serotype 1/2a has predominated in fish products *(14, 16)* and in fish processing plants *(6, 16).*

A total of nine different pulsotypes were recovered from 11 *L. monocytogenes*–positive raw fish samples, indicating high genetic diversity of the bacteria in raw fish. Although the prevalence of *L. monocytogenes* in raw fish is low, the range of *L. monocytogenes* strains entering the processing plant with large amounts of raw material is wide. This supports our hypothesis that the raw material is a source of *L. monocytogenes* contamination in fish processing plants. Further support is found in the same pulsotypes being recovered from both raw fish and fish product isolates of *L. monocytogenes* (Fig. 1). An explanation for the identical pulsotypes may be that the strain originating from raw fish has entered and persisted in a processing plant, contaminating the final products via the processing environment. Alternatively, the *L. monocytogenes* strain in raw fish may have survived a nonlistericidal process, resulting in contamination of the final product. In both cases, the initial source of the contamination of the final product

is the raw material. Our findings are in accord with those of Eklund et al. *(8),* Norton et al. *(25),* and Fonnesbech Vogel et al. *(12).* Eklund et al. *(8)* reported raw fish as the source of *L. monocytogenes* contamination in a processing plant. However, they based their conclusion solely on prevalence studies, with no further typing of *L. monocytogenes* isolates being done. Fonnesbech Vogel et al. *(12)* found the same *L. monocytogenes* random amplified polymorphic DNA type from raw fish, the processing environment, and final products sampled at a smokehouse on several occasions. Norton et al. *(25)* concluded that both raw fish and the processing environment serve as potential sources of final product contamination. The most probable source sometimes being raw material, and other times, the processing environment. Rørvik et al. *(27)* and Autio et al. *(2)* reported that raw fish is not a source of final product contamination, concluding the main source to be the processing environment. Autio et al. *(2)* did not, however, exclude the possibility that the initial source of plant contamination is the raw material. Strains recovered from samples during a single sampling period may not necessarily represent all of the strains present in the plant *(21).* Moreover, *L. monocytogenes* strains have been suggested to be able to persist in the fish processing environment for years *(2, 9, 12, 13, 25).* We therefore conclude that raw material is an important initial source of *L. monocytogenes* in fish processing plants. Certain *L. monocytogenes* raw fish strains entering plants may contaminate the processing environment and persist there, causing recurrent contamination of the final products via processing machines.

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