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Use of PFGE typing for tracing contamination with *Listeria monocytogenes* in three cold-smoked salmon processing plants

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Abstract

The sites of *Listeria monocytogenes* contamination in three cold-smoked salmon (*Salmo salar*) processing plants were detected by sampling salmon and the plant's environment and equipment at different production stages. Of the 141 samples collected from three processing plants, 59 (42%) were contaminated with *L. monocytogenes*. The rates of contamination varied as to the plant and the sample source. *L. monocytogenes* isolates from 17 various contaminated seafood products (fresh, frozen and smoked fishes, cooked mussels) were also studied. A total of 155 isolates from the three plants and the various seafoods were characterized by genomic macrorestriction using *Apa*I and *Sma*I with pulsed-field gel electrophoresis (PFGE) and 82 isolates were serotyped. Macrorestriction yielded 20 pulsotypes and serotyping yielded four serovars: 1/2a, 1/2b, 1/2c, 4b (or e), with 77 (93%) belonging to serovar 1/2a. One clone of *L. monocytogenes* predominated and persisted in plant I and was the only pulsotype detected in the final product although it was not isolated from raw salmon. No *L. monocytogenes* was detected in the smoked skinned salmon processed in plant II, even though 87% of the raw salmon was contaminated. All the smoked salmon samples collected in plant III were contaminated with a unique clone of *L. monocytogenes*, which may have occurred during slicing. In the three plants, the contamination of final products did not seem to originate from the *L. monocytogenes* present on raw salmon, but from the processing environment.

Keywords: Cold-smoked salmon processing plant; *L. monocytogenes*; PFGE typing

1. Introduction

Listeria monocytogenes has been recognized as a foodborne pathogen since the 1980s. Milk and dairy products, vegetables and meat products are the most frequently implicated foods for transmission of listeriosis. Even though their implication in human listeriosis is often only suspected, a variety of seafoods have also been found contaminated with *L. monocytogenes*, particularly cold-smoked salmon and ready-to-eat seafoods (Ben Embarek, 1994; Dillon et al., 1994). The microorganism can survive

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the cold-smoking process and the smoked products are often consumed without further heating. Furthermore these products are mainly vacuum-packed, which ensures a long shelf life and potentially enables *L. monocytogenes* to grow during storage (Guyer and Jemmi, 1991). However, the sources of *L. monocytogenes* in the final products and the routes of contamination are still not clearly defined.

The general incidence of *L. monocytogenes* in smoked salmon varies between 0 and 75%, with an average of 10% (Ben Embarek, 1994; Dillon et al., 1994; Loncarevic et al., 1996a). During recent years smoked fish have been identified as potential sources of listeriosis for humans. However the link between cases of human listeriosis and smoked fish has only recently been demonstrated (Ericsson et al., 1997; Loncarevic et al., 1997). The former reported an outbreak with nine cases of human listeriosis suspected to have been caused by smoked and 'gravad' trout. It is therefore of major importance to prevent the contamination of fishery products, especially ready-to-eat products such as vacuum-packed cold-smoked fish. Prevention implies that we identify the potential sources of contamination of smoked fish and trace the dissemination of *L. monocytogenes* in processing plants. It may be impossible to control completely the presence of *L. monocytogenes* on the final product (Jemmi, 1993; Ben Embarek, 1994; Huss et al., 1995; Autio et al., 1999). However, as the contamination seems to take place during processing of salmon, it should be possible to reduce this contamination to a low level by adherence to good manufacturing practices (Huss et al., 1995).

Strain characterization by pulsed-field gel electrophoresis (PFGE) has been successfully applied to typing of *L. monocytogenes* in epidemiological investigations. Genomic macrorestriction using rare cutting endonucleases such as *Sma*I or *Apa*I, followed by PFGE exhibits good discriminatory power and reproducibility for comparing strains of *L. monocytogenes* belonging to identical or different serovars (Brosch et al., 1991; Destro et al., 1996; Kerouanton et al., 1998). PFGE has been used to assess the distribution of *L. monocytogenes* strains within various food processing environments (Jacquet et al., 1995; Destro et al., 1996; Unnerstad et al., 1996; Ericsson et al., 1997; Giovannacci et al., 1999).

In this study, PFGE typing was applied to *L. monocytogenes* isolates collected from three smoked salmon processing plants and from various seafoods processed in different plants. This study was aimed at trying to trace *L. monocytogenes* dissemination through three cold-smoked salmon plants.

2. Materials and methods

2.1. Salmon processing

The three plants (plants I to III) were located in the same industrial area but were physically separate. They used whole or ready-filleted fresh salmon (*Salmo salar*) as raw material, mostly imported from Norway and Scotland. Whole salmon were filleted, and all fillets were salted with dry salt, rinsed, smoked (maximum temperature 28°C), skinned, sliced and vacuum-packed. All plants produced smoked herring as well.

2.2. Sampling

One hundred and forty-one samples were collected in the three processing plants, among which 83 (58%) originated from plant I. The first series of samples was collected in plant I from salmon and from the processing environment at different production stages during September–October 1998. Samples from the processing environment were collected by swabbing 450 cm^2 of working surfaces, equipment and floors. The skin of raw salmon was swabbed and salmon flesh was also sampled during and after production by collecting 25 g of smoked flesh. Most samples were collected during production, but some were taken after cleaning and disinfection. The second series of sampling was carried out in the three plants between February and May 1999, and focused both on the raw salmon entering the plant (still without any contact with the processing environment) and on the final product. Seventeen samples from various seafoods, already known to be contaminated and processed in other plants were included in this study. Details of samples distribution are given in Table 1.

2.3. Bacteriological analysis

Examination for *L. monocytogenes* was carried out according to the EN ISO 11290 method for detection of *L. monocytogenes* (Anonymous, 1996), by a two-

Table 1
Incidence of *L. monocytogenes* in the samples obtained from three processing plants and various seafoods and results of serotyping and PFGE typing with *Apa*I and *Sma*I

Sampling site and sample source	No. of positive samples (total no. of samples)	No. of isolates	Restriction pattern		Pulsotype	No. of isolates per pulsotype	Serovar and no. of isolates per serovar	
			*Apa*I	*Sma*I				
Plant 1								
Raw salmon surfaces	2 (18)	4	A9	S9	XII	4	1/2a	3
Environment:								
In contact with salmon	5 (7)	13	A1	S1	I	8	1/2a	4
			A1	S1c	IV	3	1/2a	4
			A2	S2	V	1	1/2a	1
			A1b	S1	III	1	1/2a	1
Without contact	7 (8)	22	A1	S1	I	15	1/2a	1
			A1	S1c	IV	2	1/2a	1
			A2	S2	V	1	1/2a	1
			A1b	S1	III	1	1/2a	1
			A6	S6	IX	1	1/2a	1
			A1	S1b	II	1	1/2a	1
			A6	S6	IX	1	1/2a	1
Environment after cleaning-disinfection:								
In contact with salmon	2 (4)	6	A1	S1	I	6	1/2a	3
Without contact	4 (5)	11	A1	S1	I	4	1/2a	2
			A1b	S1	III	1	1/2a	1
			A3	S3	VI	1	4b or e	1
			A4	S4	VII	2	1/2a	1
			A5	S5	VIII	1	1/2a	1
			A6	S6	IX	2	1/2a	2
Swabbing worker's hands								
Transport boxes	1 (1)	2	A9	S9	XII	2	1/2a	1
	2 (5)	4	A1	S1	I	3	1/2a	1
Salmon during processing			A2	S2	V	1	1/2a	1
	9 (14)	27	A1	S1	I	23	1/2a	11
			A1b	S1	III	2	1/2a	1
			A1	S1c	IV	1	1/2a	1
			A3	S3	VI	1	4b or e	1
Smoked salmon (final product)	2 (21)	6	A1	S1	I	6	1/2a	3
Plant 2								
Raw salmon surface:								
From Scotland (whole)	7 (8)	7	A6	S6	IX	5	1/2a	2
			A7	S7	X	2	1/2c	1
From Norway (fillets)	6 (7)	7	A7	S7	X	6	1/2c	2
			A8	S8	XI	1	1/2a	1
Skin of smoked salmon (Norwegian)	1 (2)	4	A8	S8	XI	4	1/2a	3
Plant 3								
Floor (after rinsing of salmon)	2 (2)	5	A11	S11	XIV	5	1/2a	2
Salmon during processing	1 (6)	2	A11	S11	XIV	2	1/2a	1
Flesh of smoked salmon	11 (11)	18	A10	S10	XIII	18	1/2a	8
Other plants:								
Smoked tuna	1	1	A15	S15	XVIII	1	1/2a	1
Smoked coalfish	1	1	A15	S15	XVIII	1	1/2a	1
	1	1	A14	S14	XVII	1	1/2a	1
Cut raw salmon	1	1	A6	S6	IX	1	1/2a	1
Cod fillet	4	4	A9	S9	XII	4	1/2a	1
Coalfish fillet	2	2	A9	S9	XII	2	1/2a	1
Frozen herring	5	5	A16	S16	XIX	4	1/2a	2
			A17	S17	XX	1	1/2a	1
Shelled cooked mussels	2	2	A12	S12	XV	1	1/2b	1
			A13	S13	XVI	1	1/2b	1

step enrichment procedure in Fraser broth (Oxoid, Basingstoke, UK) and identification of colonies both from Palcam and Oxford agar (Oxoid). Three suspect colonies were streaked both from Oxford and Palcam agar onto Trypticase Soy Agar (Difco Laboratories, Detroit, MI, USA). The *L. monocytogenes* isolates were selected by Rapid'L.mono agar (Sanofi Diagnostic Pasteur, Paris, France) and species was confirmed according to biochemical criteria described in the EN ISO 11290 method (Anonymous, 1996). Five *L. monocytogenes* isolates per smoked salmon sample and three pure isolates per other type of sample were kept at room temperature in the bacterial strain storage medium 63683 (Sanofi Diagnostic Pasteur).

2.4. DNA preparation for macrorestriction

Prior to each DNA preparation, a loopful of culture taken from the storage medium was streaked onto Trypticase Soy Agar plates and incubated overnight at 37°C. Bacterial lawns obtained from overnight cultures were harvested, washed twice in 2 ml sterile 0.01 M Tris–HCl, pH 7.6, 1 M NaCl (TN buffer) and pelleted by centrifugation at $10\,000 \times g$ (Biofuge Pico, Hereaus) for 1 min.

L. monocytogenes DNA preparation for genomic macrorestriction prior to PFGE was based on the principle of lysis of whole cells embedded in agarose according to a slightly modified procedure of Moore and Datta (1994). The absorbance of washed cells was read at 600 nm and adjusted to 4 with TN buffer. Bacterial suspensions were mixed with an equal volume of 1% agarose (Agarose standard, Eurobio) solution in TN buffer. The mixture was dispensed in plug molds of 100 μl volume (Bio-Rad Laboratories). Agarose plugs were incubated in a lysis solution (1 ml per agarose plug) containing 6 mM Tris–HCl, pH 7.6, 0.1 M EDTA, pH 8, 1% (w/v) lauryolsarcosine (Sigma, St. Louis, MO), NaCl 5 M solution, 10 g l^{-1} lysozyme (Boehringer-Mannheim, Germany) for at least 4 h at 37°C. After removing the lysis solution, plugs were incubated in a 0.5 M EDTA, pH 9, 1% (w/v) lauryolsarcosine and 1 g l^{-1} proteinase K (Boehringer) solution for at least 40 h at 50°C under soft agitation. In order to inactivate proteinase K, agarose plugs were washed twice with 10 mM Tris–HCl, pH 7.6, 2 mM EDTA (TE buffer) for 30 min at room temperature and then incubated with 2 mM aminoethyl-benzenesulfonyl fluoride (Pefabloc®, Boehringer) at 37°C for 2 h under soft agitation. Finally the Pefabloc® solution was removed and plugs were washed twice with TE buffer for 30 min at room temperature. Agarose plugs were then cut into 25 μl mini-plugs and stored in TE buffer at 4°C.

2.5. DNA Macrorestriction and PFGE analysis

Before cleavage with *Apa*I and *Sma*I, each 25-μl mini-plug was incubated overnight at 4°C in a 100-μl solution containing A buffer (Boehringer). DNA within each agarose plug was cleaved with 60 U of *Apa*I and with 40 U of *Sma*I (Boehringer) for 5 h at 25°C, according to the manufacturer's instructions.

Macrorestriction fragments were resolved by the PFGE technique of Contour Clamped Homogeneous Electric Field (CHEF-DRIII Bio-Rad Laboratories, Richmond, USA). *Apa*I and *Sma*I macrorestricted samples were loaded in the wells of 1.2% agarose (Agarose standard, Eurobio, France) gel in 0.5× TBE buffer (44.5 mM Tris, 44.5 mM boric acid, 1.25 mM EDTA, pH 8.3). Fragments restricted with *Apa*I were resolved at 250 V in two steps: pulse times from 15 to 35 s over 7 h and 2 to 20 s over 13 h. Fragments restricted with *Sma*I were resolved at 250 V with pulse times from 2 to 20 s over 21 h. The electrophoresis buffer was 0.5× TBE and its temperature was maintained at 14°C. The lambda DNA concatemers (Boehringer) were used as molecular weight size standards.

Gels were stained with ethidium bromide and images were captured under U.V. illumination by a video system (Gel Doc 2000 system, Bio-Rad Laboratories). Pulsed-field electrophoretic patterns were compared by means of Molecular Analyst software fingerprinting (Bio-Rad Laboratories). Similarities between profiles, based on bands positions, were derived from the Dice correlation coefficient with a maximum position tolerance of 1.2%. *L. monocytogenes* strains were clustered by the technique of the unweighted pair group method using arithmetic averages (UPGMA) and a dendrogram was constructed to reflect the genetic distance between them. The pulsotypes were clustered at a similarity level of 85%. Five human strains were inserted into the dendrogram: four strains collected from the French outbreak of 1992, originating from pork tongue in

aspic (Salvat et al., 1995) and one strain collected from a French listeriosis case in 1999 (originating from a delicatessen).

2.6. Serotyping

One to 11 *L. monocytogenes* randomly chosen to represent each of the 20 pulsotypes were serotyped using O and H antisera according to the manufacturer's instructions (Eurobio). A total of 82 isolates were serotyped.

3. Results

3.1. Quantitative results

The overall proportion of samples contaminated with *L. monocytogenes* was 42% (59/141) (Table 1). In plant I, 64% of the salmon sampled during processing and 84% of the plant environment samples were contaminated. The rates of smoked, skinned salmon contamination varied by plant: 0% (plant II), 9.5% (plant I) and 100% (plant III). Eleven and 87% of the raw salmon samples tested were positive in plant I and II, respectively. Only two of the positive samples contained more than 10 CFU/g: one salmon sampled during salting in plant I (200 CFU/g) and one smoked salmon skin sampled from plant II (75 CFU/g).

3.2. PFGE analysis

Among 155 *L. monocytogenes* isolates characterized by PFGE, 138 originated from the salmon and the plants' environments and 17 from the various seafoods. Each restriction enzyme yielded 17 unique restriction patterns, named A1–A17 for patterns distinguished by *Apa*I and S1–S17 for patterns distinguished by *Sma*I (Table 1), dividing the isolates into 20 different pulsotypes (listed from I to XX). The results obtained from *Apa*I and *Sma*I showed only slight differences.

For each isolate, macrorestriction fragments greater or equal to 48.5 kb were recorded for analysis with the Molecular Analyst software fingerprinting (Bio-Rad Laboratories). Figs. 1 and 2 show dendrograms of the isolates based on *Apa*I and *Sma*I digestions, respectively. Agglomerative hierarchical cluster analysis led to the discrimination of *L. monocytogenes* isolates into seven clusters (Table 2 and Figs. 1 and 2). Seventy-seven *L. monocytogenes* isolates grouped into the major cluster (cluster E). These isolates, of which 66 (86%) belonged to pulsotype I, were all collected in plant I: at all processing stages (including final product) except from raw salmon. The four isolates collected from the French outbreak (1992) formed a separate cluster (less than 60% similarity with all the other isolates). The human isolate collected in 1999 only clustered with an isolate (pulsotype XVI) which originated from cooked mussels (85% similarity). The other isolate (pulsotype XV) collected from cooked mussels did not cluster with any other isolate. Clusters D, F, G contained both isolates from the plants and from various fishes (and origins). Every fish isolate from various origins clustered with isolates collected from the plants.

Each plant was contaminated with its own *L. monocytogenes* pulsotypes. The only exception was pulsotype IX, which contaminated both the raw salmon in plant II and the processing environment after cleaning and disinfection in plant I. Pulsotype IX was common between raw packed salmon (among the products obtained from various plants) and raw salmon sampled in plant II. Pulsotype XII was isolated both from raw salmon in plant I and from coalfish and cod fillets. Finally the same pulsotype XVIII contaminated both smoked coalfish and smoked tuna, which had been processed in the same plant (different from plants I, II, III) during the same time period.

Most of the samples collected in the plants were contaminated with only one *L. monocytogenes* pulsotype. Three pulsotypes at most could be detected in a single sample. In plant I, eight of 34 samples were contaminated with two different pulsotypes and three samples with three different pulsotypes.

3.3. Serotyping

Of 82 *L. monocytogenes* isolates representative of the 20 pulsotypes characterized in this study, 75 (91%) belonged to serovar 1/2a (Table 1). Three isolates of the same pulsotype (X) and which formed cluster C, belonged to serovar 1/2c. Two isolates of the same pulsotype (VI) and genomically distant from all the other isolates (less than 60% similarity),

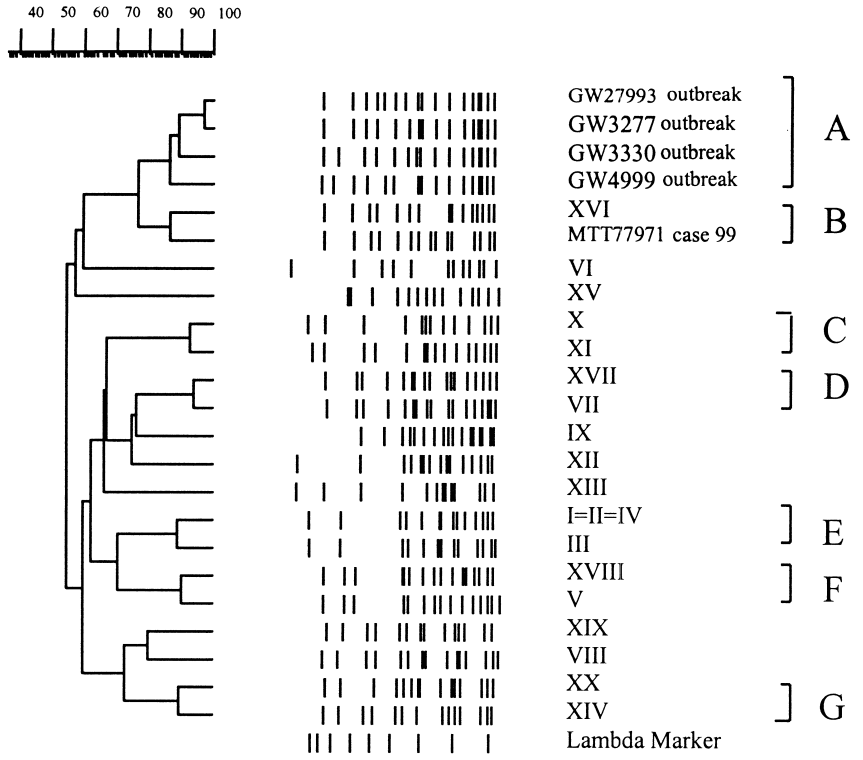

Fig. 1. Dendrogram obtained by *Apa*I demonstrating the genetic relationships of the 20 PFGE types observed from 155 seafood isolates and human cases.

belonged to serovar 4b or e. Finally, the two isolates collected from cooked mussels (pulsotypes XV and XVI) and genetically distant from the other isolates, were serovar 1/2b.

4. Discussion

4.1. Typing methods

PFGE typing based on the restriction enzymes *Apa*I and *Sma*I yielded 20 genomically distinct groups (pulsotypes) among 155 isolates collected from three plants and various seafoods. Ten pulsotypes were distinguished among 95 isolates collected in plant I, which shows a good discriminatory power. The PFGE process we applied is very time-consuming compared to newly rapid PFGE methods for typing *L. monocytogenes*, which should now be preferred (Katsuda et al., 2000). Serotyping of 82 isolates representative of the 20 pulsotypes revealed the presence of four serovars of which 91% (77/82) belonged to serovar 1/2a. Previous findings have shown that *L. monocytogenes* strains isolated from smoked salmon belong mostly to serogroup 1/2 (Rorvik et al., 1995; Loncarevic et al., 1996a) particularly to serovar 1/2a (Guyer and Jemmi, 1991; Johansson et al., 1999). However, the *L. monocytogenes* strain responsible for the only reported human listeriosis linked to Rainbow trout belonged to serovar 4b (Ericsson et al., 1997).

Genomic variability of *L. monocytogenes* strains was noticed in single samples and up to three different PFGE patterns were detected. This is in agreement with previous findings (Loncarevic et al., 1996b; Ericsson et al., 1997; Autio et al., 1999): several isolates from the same sample should be typed in epidemiological surveys since the finding of several types in a single sample may be due to multiple sources of contamination at a single site.

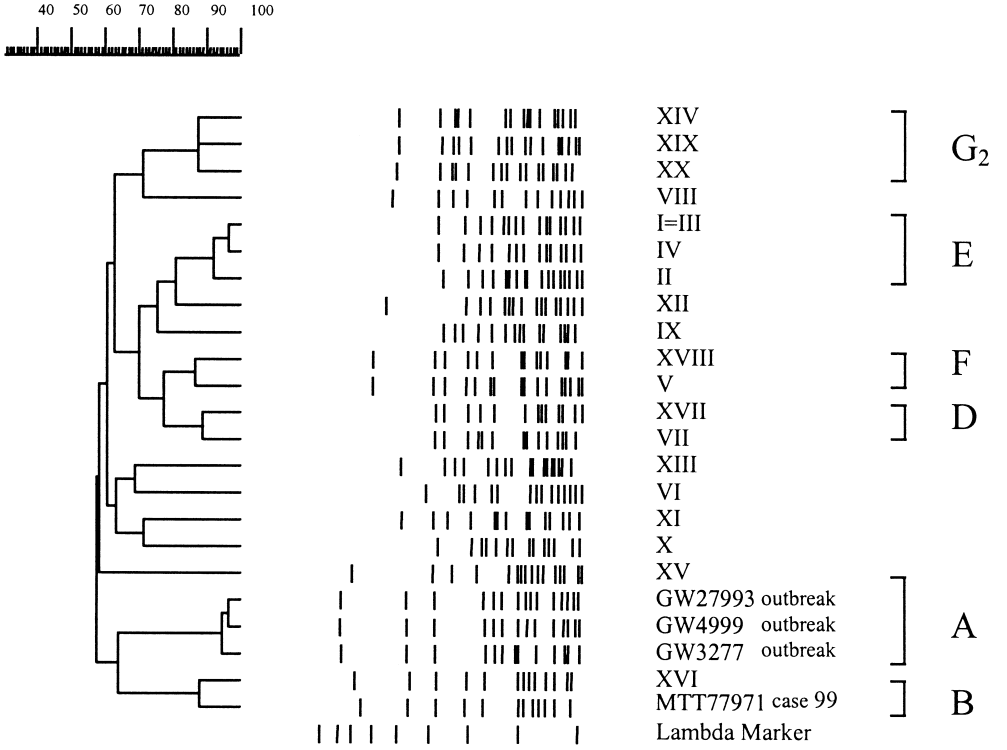

Fig. 2. Dendrogram obtained by *Sma*I demonstrating the genetic relationships of the 20 PFGE types observed from 155 seafood isolates and human cases.

Table 2
Clustering obtained with PFGE of *L. monocytogenes* isolates with more than 85% similarity, collected from three processing plants and various seafoods

Cluster	Pulsotypes	Isolate origin
A	Epidemic isolates	The four epidemic isolates (4b or e) of the French outbreak in 1993
B	XVI and human isolate	Shelled cooked mussels — listeriosis in 1999
C	X[a], XI[a]	Plant II: raw Scottish and Norwegian salmon; smoked salmon skin
D	VII, XVII	Smoked coalfish Plant I: floor between filleting and salting areas after cleaning and disinfection
E	I, II, III, IV	Plant I: at all production stages (except raw salmon)
F	V, XVIII	Smoked coalfish and tuna Plant I: equipment and floor of the filleting area
G/G2	XIV, XX, XIX[b]	Plant III: floor and salmon in the filleting area Frozen raw herring

[a] Clustered with *Apa*I only.
[b] Clustered with *Sma*I only.

4.2. Sources and routes of contamination

In plant I, 70% of the isolates belonged to a major and ubiquitous pulsotype (I). The final product was only contaminated with this predominant clone, while this clone was not isolated from raw salmon. This clone was also persistent since it was isolated on six different sampling dates in a 2-month interval. Rorvik et al. (1995) noticed by MEE (multilocus enzyme electrophoresis) that an electrophoretic type had colonized a plant and was the only type isolated from the smoked salmon. Loncarevic et al. (1996a) isolated the same pulsotype from smoked trout and 'gravad' salmon processed in the same plant after a 4-year period. The persistence of a predominant clone seems to be common in the food industry (Lawrence and Gilmour, 1995; Rorvik et al., 1995; Ojeniyi et al., 1996; Unnerstad et al., 1996; Loncarevic et al., 1997; Autio et al., 1999; Johansson et al., 1999). A recent study isolated a persistent pulsotype both in a pork cutting plants and in live animals (Giovannacci et al., 1999).

Persistent clones may originate from raw fish, employees or external environment of the plant. They may be capable of colonizing and multiplying in a food processing environment, because of their ability to attach to surfaces and form biofilms, giving them added protection against biocidal agents (Mafu et al., 1990). Persistent clones may represent a hazard for human health: in the only outbreak of listeriosis suspected to have been caused by smoked/gravad trout, the human isolate and the persistent type in the concerned plant were of the same clonal type (Ericsson et al., 1997). Brett et al. (1998) also identified, by five different typing methods, a common strain from three human samples, from mussels consumed by the same three patients and from the corresponding mussel processing environment (sampled in 1990 and 1993).

Three pulsotypes (II, III, IV) were closely related genetically to the major pulsotype I: one restriction pattern (obtained with one restriction enzyme) in common and the other one very similar to pulsotype I. We can suppose that they all derived from the pulsotype I, or conversely that the main type derived from the other ones and became predominant because of its better adaptation to the processing environment. An alteration of the genome may also have occurred during culturing of the isolates.

The efficiency of cleaning and disinfecting was assessed in plant I: 6/9 samples were contaminated with *L. monocytogenes* after cleaning and disinfection, with 10/19 isolates belonging to the major pulsotype. Certain strain types were present on the floor only after cleaning and disinfection and were not isolated during processing. These types may have originated from the herring smoking chain, located in the same area as the salmon processing chain. Water flowing from the cleaning of the herring chain may have mixed with water from the salmon chain. The herring processing chain should be sampled for *L. monocytogenes* strains to detect if they can be spread from one chain to another. Autio et al. (1999) also pointed out the difficulty in effective cleaning and disinfecting in a plant. In their survey, after a thorough cleaning with hot steam, hot air and hot water, none of the environment and smoked salmon samples were contaminated with *L. monocytogenes* for 5 months. Hence plant I should improve their cleaning and disinfection procedure in order to prevent colonization of persistent *L. monocytogenes* clones in the plant environment.

In plant I some pulsotypes appeared sporadically on salmon during processing and were not detected later (pulsotypes V, VI, VIII, IX), as also observed in the studies of Destro et al. (1996) in a shrimp processing plant, and Rorvik et al. (1995) and Johansson et al. (1999) in salmon processing plants. These strains may be well adapted to the processing environment but may be bad competitors and not able to persist on the product. Among these sporadic pulsotypes, pulsotype VI was very different: genetically distant from all the isolates and of serovar 4b or e, distinct from the major serovar. Pulsotype VI was represented by only two isolates collected at 10-day intervals first from salmon during salting and then from a plastic apron in the filleting area after cleaning and disinfection. Johansson et al. (1999) suggest the presence of high salt tolerance strains, since they isolated most of the sporadic strains from the salting unit. We cannot explain why this clone is very different from all the *L. monocytogenes* strains typed in this study, but it seems to have persisted through salting, cleaning and disinfecting.

The hands of a worker in the filleting area were contaminated with the same pulsotype as on raw fish (plant I), suggesting that his hands had been contaminated by the raw salmon. According to Destro et

al. (1996) and Autio et al. (1999), the role of employees in fish contamination is minor but their role can become major in the case of rotation of assigned duties (Rorvik et al., 1997). This idea is also related to the importance of applying good manufacturing practices.

In plant I, the *L. monocytogenes* pulsotypes isolated from swabbing of the raw salmon skin, were not isolated later from the flesh of smoked salmon. In plant II, where 87% of raw salmon surfaces were contaminated, no *L. monocytogenes* was found in the flesh of smoked salmon. Salmon bred in Scotland and Norway were contaminated both with one common pulsotype and also with their own unique pulsotypes. The four isolates collected from the skin (which is removed before packaging) of Norwegian salmon after smoking all belonged to the same pulsotype as the one isolated exclusively from the same batch of Norwegian raw salmon. Hence, some *L. monocytogenes* initially present on surface of raw salmon seem able to persist through processing, but without being spread on the smoked flesh. Good manufacturing practices, that-is-to-say no contact between skin and flesh during processing may explain part of this observation. Moreover, the *L. monocytogenes* naturally present on raw salmon may also be more adapted to skin than flesh and therefore be less able to persist on flesh during processing. In previous studies, high rates of surface contamination (skin and mucus) of trout and salmon were also found and smoked fish were always less frequently contaminated than raw fish (Jemmi and Keutsch, 1994; Eklund et al., 1995; Rorvik et al., 1995; Autio et al., 1999). Eklund et al. (1995) concluded that external surfaces of raw fish were the initial source of contamination of final product, and that contamination was later spread by equipment and workers. Their conclusion was based only on a proportion of contaminated samples at each stage of processing and not on qualitative comparisons. Rorvik et al. (1995) and Autio et al. (1999) found by molecular typing that the pulsotypes present on raw fish were no longer present either in the plant environment or on smoked fish. Ojeniyi et al. (1996) came to the similar conclusions in the study of seven poultry slaughterhouses that live animals may contribute poorly to the global slaughterhouse contamination. Hence it seems that the raw salmon skin is a vehicle for *L. monocytogenes,* but these strains are not present later on the smoked salmon flesh. It would be of interest to identify the origin of contamination of raw salmon. According to previous studies, only a low proportion and most often none of the salmon sampled in slaughterhouses and breeding farms were found contaminated with *Listeria* spp. (Huss et al., 1995; Rorvik et al., 1995; Ben Embarek et al., 1997; Autio et al., 1999; Johansson et al., 1999).

In plant III the proportion of contaminated final products was 100%, while one of six samples collected during processing was contaminated. Five salmon skins collected after smoking and just before slicing were not contaminated, although the flesh was contaminated. Moreover, the genomic typing distinguished only two pulsotypes: one was associated both with the floor of the salting and rinsing area and with one salmon after rinsing and the other one was associated with two different batches of smoked salmon (produced at 2-month intervals). From both quantitative and qualitative results in plant III, we can suspect that contamination may have occurred during slicing and was spread by the slicing machine. This type of machine is difficult to clean efficiently and it was not completely disinfected every day. Johansson et al. (1999) pointed to the slicing and salting machines as the most critical hazard points in such plants; the application of HACCP, focused on these points, resulted in negative samples in the whole plant. Even though *L. monocytogenes* might be naturally more adapted to salmon skin than flesh, a contamination of the flesh can easily occur during processing if good manufacturing practices are not respected.

In this study, raw salmon was contaminated with various pulsotypes (five pulsotypes from 15 raw salmon samples in plants I and II). Furthermore, we found common pulsotypes from raw salmon in plants I and II and from various raw fish of other origins. We also noticed that all isolates of various seafoods origins clustered with isolates collected in the three plants. Hence, the *L. monocytogenes* strains isolated from salmon in the plants were not specific to salmon. This observation is supported by Ben Embarek (1994) and Rorvik et al. (1995) who sampled various final products in several plants. It is likely that some clones are well adapted to fish, and become common in the fish industry. Conversely, the two isolates originating from cooked mussels were genetically distant from all the fish isolates. This

seafood was both the only mollusc and the only cooked product among the collected samples. The mussels may have been contaminated after cooking with *L. monocytogenes* strains that have no link with the raw product. Pulsotype XVI clustered with the human isolate (collected in 1999), which suggests that cooked mussels can be implicated in human listeriosis. Listeriosis outbreaks associated with mussels have already been reported (Mitchell, 1991; Brett et al., 1998).

5. Conclusion

This study showed coherent results in the three plant studies. *L. monocytogenes* seems ubiquitous in smoked salmon processing plants. Raw material most certainly represents a constant entry of *L. monocytogenes* into the plant, but that entry does not seem to be a major contamination factor of the final product. Moreover, certain clones of *L. monocytogenes* may be persistent in the processing environment (floors, equipment) and may contaminate salmon during processing. The efficiency of cleaning and disinfection is uncertain against the persistent and ubiquitous pulsotypes. Certain points of the processing chain, such as the machines, are particularly susceptible to contamination, because of the difficulties in efficient cleaning. These conclusions are in agreement with those given by Autio et al. (1999) and Destro et al. (1996) in fish industry, and also with studies carried out in other food industries.

Sampling of other fish species may be relevant, since species are either hot- or cold-smoked and either wild or farm bred (e.g., herring, mackerel). More studies should be focused on persistent clones in plants environments. Our study has also shown that analysis of contamination points is specific to each plant environment. Particular attention must be paid to the detection of critical points through application of hazard analysis critical control points (HACCP). Then, production of smoked salmon with low levels of contamination seems possible.

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