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Typing *Listeria monocytogenes* Isolates from Fish Products and Human Listeriosis Cases

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Seventy-two *Listeria monocytogenes* isolates originating from 10 different fish products of 12 producers and 47 isolates from human listeriosis cases were typed by serotyping and multilocus enzyme electrophoresis. Seventy-five of these isolates were further subtyped by restriction analysis of genomic DNA with the enzyme *Xho*I and by pulsed-field gel electrophoresis using the enzymes *Apa*I and *Sma*I. The results show that several *L. monocytogenes* clones identified by multilocus enzyme electrophoresis are frequently found in fish products of different origins. One of these clones is the same as another previously shown to be frequently associated with meat and meat products. The epidemic-associated electrophoretic type 1 was only rarely found in fish products. No association was found between any type of fish product and a particular lineage of *L. monocytogenes*. Both long-term persistence of a strain and simultaneous presence of several clearly distinct strains in the products of single producers were observed. The comparison of *L. monocytogenes* isolates from human clinical listeriosis cases in Switzerland and those from imported fish products by use of multilocus enzyme electrophoresis showed that they do not form two clearly distinct lineages but nevertheless belong to two separate populations. None of the 48 subtypes distinguished by the combination of all four typing methods could be found in both populations of human origin and those of fish origin.

Listeriosis is a rare but severe illness with a mortality rate of approximately 30% (9). This disease is caused by the ubiquitous bacterium *Listeria monocytogenes*, and recent studies have shown that both sporadic and epidemic cases of listeriosis in humans are mainly of food-borne origin (9, 19, 22). Several kinds of food have been implicated in or suspected to be the origin of human listeriosis. These are mainly soft cheese, milk and milk products, meat and meat products, vegetables, and seafood (9). Fish products have been shown to be often contaminated with *L. monocytogenes* (2). In addition, *L. monocytogenes* can grow to significant levels under storage conditions on such products, even in prepackaged food (12, 13, 20). Since some fish products, such as smoked fish, are often eaten raw or undercooked, they may represent a risk factor for susceptible persons. Thus, it is surprising that fish products have not been more frequently implicated in human listeriosis (8, 10, 15).

The aim of the present work was to examine the diversity of *L. monocytogenes* strains from fish products and to compare them to isolates of human origin. For this purpose, *L. monocytogenes* isolates recovered from imported fish products in Switzerland were typed by serotyping, multilocus enzyme electrophoresis (MEE), microrestriction enzyme analysis (REA), and macrorestriction enzyme analysis (pulsed-field gel electrophoresis [PFGE]). The results were used to examine whether some individual strains or groups of strains are more frequently associated with fish products than others or could be associated with particular types of fish products. The diversity and persistence of strains in products of single producers were examined. Finally, the isolates from fish products were compared to isolates from human clinical listeriosis cases recovered in Switzerland in order to determine whether human and fish isolates represent two distinct populations of *L. monocytogenes* or belong to the same genetic pool of strains.

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MATERIALS AND METHODS

Description of *L. monocytogenes* isolates from fish products and isolation procedure. Seventy-two *L. monocytogenes* isolates originating from 10 different imported fish products (salmon terrine, salmon gravad, smoked salmon, smoked trout, smoked buckling, smoked halibut, smoked mackerel, smoked herring, schillerlocken, and tarama) of 12 producers (in Denmark [2], France [3], Germany [1], Great Britain [4], Norway [1], and New Zealand [1]) were examined. Sixty-five isolates were recovered during routine microbiological testing of imported food in Switzerland between January 1993 and December 1994. Seven isolates were recovered from imported products of a single producer between January and July of 1995. *L. monocytogenes* was isolated from the fish products as previously described (14). Only one *L. monocytogenes* isolate per package or piece of fish product was used for typing.

***L. monocytogenes* isolates from humans.** Forty-seven *L. monocytogenes* isolates from human clinical listeriosis cases that occurred in Switzerland between January 1993 and December 1994 were used for the present study. These represent more than 90% of the listeriosis cases registered in the country during this period.

Serotyping. All the isolates were serotyped by the method of Seeliger and Höhne (23).

MEE. All the isolates studied were typed by MEE as described elsewhere (5, 24). Twenty-one specific enzyme loci were studied. The following enzymes were examined after electrophoresis in buffer system A (24): aconitase, alanine dehydrogenase, α-naphthyl-propionate esterase, β-naphthyl-propionate esterase, glutamate-oxalate transaminase, glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, mannose-phosphate isomerase, nucleoside phosphorylase, phenylalanyl-leucine peptidase, leucyl-glycyl-glycine peptidase, phosphoglucose isomerase, and 6-phosphogluconate dehydrogenase. The following enzymes were examined after electrophoresis in buffer system F (24): acid phosphatase, adenylate kinase, catalase, fumarase, glutamate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, indophenyl oxidase, and lactate dehydrogenase. Staining procedures were the same as described previously (5). Each unique combination of migration patterns (equated with alleles at the corresponding gene loci) for the 21 enzymes studied is called an electrophoretic type (ET). The ETs were numbered arbitrarily, except for ET1, which corresponds to the ET1 described earlier (5, 17, 18). For the ETs which could be split into further subtypes by one or several of the other typing methods, a letter was added to the ET designation in order to identify each subtype. The genetic distance between pairs of ETs was defined as the proportion of enzyme loci at which dissimilar alleles occurred. A dendrogram was built using a matrix of pairwise genetic distances and the average-linkage method of clustering. *L. monocytogenes* pop-

ulations of human and those of fish origin were compared by means of the Wright's fixation index, F_{st} (25). Departure from 0 for F_{st} was tested per locus, as well as overall, by a permutation procedure (11). In the absence of clinical and epidemiological data on the human listeriosis cases, they were all considered to be epidemiologically unrelated, sporadic listeriosis cases, and all the corresponding isolates available were used for this analysis. To avoid a bias related to any link between fish isolates obtained from the same producer, only one representative isolate per subtype per producer determined by the four typing methods was used for the F statistics.

REA. When isolates from several fish products were shown by MEE to belong to the same ET, they were subtyped by REA (7) unless they were isolated from the same product of the same producer on the same date. In the latter case, only one isolate was subtyped by REA. When isolates of human and those of fish origin were shown to belong to the same ET, they were also subtyped by REA. Genomic DNA of the isolates was extracted by the method of Nocera et al. (16). Eight micrograms of DNA from each isolate was digested with 40 U of the restriction enzyme *Xho*I (Boehringer GmbH, Mannheim, Germany) for 5 to 6 h under conditions recommended by the manufacturer. Electrophoresis was done in 0.6% standard agarose gels (Eurobio, Les Ulis, France) with recirculated 1× TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) at 15°C until the bromphenol blue marker had migrated 22 cm. The gels were stained in an ethidium bromide solution (1 μg/ml) and photographed under shortwave UV light. Migration patterns were compared visually without knowledge of the results obtained with the other typing methods.

PFGE. The isolates typed by REA were also typed by PFGE as follows. The preparation of the DNA and restriction with the enzymes *Apa*I and *Sma*I were performed as described by Brosch and collaborators (6). Field inversion gel electrophoresis with 1.1% Fast Lane agarose gels (FMC BioProducts, Rockland, Maine) was used to separate the DNA fragments after restriction. The gels were run in recirculated 0.5× TBE buffer at 10°C and 10 V/cm. A Switchback pulse controller (model PC500; Hoefer Scientific Instruments, San Francisco, Calif.) was used for generating the pulsations. For the fragments obtained with *Apa*I, electrophoresis for 20 h with pulsation from 1 to 15 s (linear ramp, forward-to-reverse ratio of 3:1) was followed by a 5-h electrophoresis with pulsation from 0.5 to 5 s (linear ramp, forward-to-reverse ratio of 3:1). For the fragments obtained with *Sma*I, a 22-h electrophoresis with pulsation from 0.9 to 7 s (linear ramp, forward-to-reverse ratio of 3:1) was used. The gels were stained in an ethidium bromide solution (1 μg/ml) and photographed under shortwave UV light. The migration patterns were read visually without knowledge of the results obtained with the other methods.

RESULTS

***L. monocytogenes* serovars.** The *L. monocytogenes* isolates examined were of the following serovars: 1/2a (47 fish isolates and 17 human isolates), 1/2b (2 fish isolates and 8 human isolates), 1/2c (4 fish isolates), 3a (2 fish isolates), 4b (1 fish isolate and 21 human isolates), 7 (12 fish isolates), and atypical serovar 7 without the C flagellar antigen (4 fish isolates). In addition, one human isolate was nontypeable.

MEE types in fish products. Nine ETs were found among the 72 *L. monocytogenes* isolates from fish products. The genetic relationships between these ETs are represented in Fig. 1. Five of the nine ETs (ET4, ET9, ET11, ET22, and ET26) were found in products of several producers. The same five ETs were found in more than one fish product. No association was shown between a specific product and any particular ET or group of closely related ETs (Fig. 1).

Subtyping of isolates from fish products by REA and PFGE. Most fish isolates of the same ET and the same serovar were also subtyped by REA and PFGE. Isolates from fish products of different producers that belonged to the same ET were differentiated on the basis of both REA and PFGE, except for a few isolates of ET9 and ET26, which were distinguished only by PFGE (or, in the case of some isolates of ET9, were not differentiated at all) (Fig. 1).

Follow-up of products from four producers. *L. monocytogenes* isolates from products of producers A, C, I, and L were recovered over periods of 563, 101, 22, and 609 days, respectively. They were all compared to one another by serotyping and MEE, and most of them were also compared by REA and PFGE if not distinguishable by means of the first two methods. The results are reported in Table 1. The products of three of the four producers contained several subtypes of *L. monocytogenes*. However, the majority of isolates from the products of three of the four producers belonged to only one major subtype each. In two cases, the major subtypes were detected on several different days in different batches of different products (Table 1, producers A and C).

MEE types in *L. monocytogenes* isolates of human origin and comparison with isolates from fish products. Twenty ETs were found among the 47 isolates of human origin examined. Three ETs (ET1, ET4, and ET26) were common to human and fish products (Fig. 1); however, these three ETs were not common among isolates from fish products. One of these three ETs (ET1) was the same as the epidemic-associated ET1 described by Piffaretti and collaborators (18). Grouping of the ETs by the average-linkage method of clustering did not separate the population studied into distinct fish- and human-associated lineages (Fig. 1). However, an analysis of allele frequencies by means of F statistics showed that the *L. monocytogenes* population of human origin and the population from fish products under study belonged to two separate pools of strains. The genetic diversity and the F_{st} are reported for each locus, as well as overall, in Table 2. An F_{st} of 0 means that there is no subdivision between populations, whereas an F_{st} of 1 means that the populations are completely isolated genetically. The overall F_{st} value was significantly different from 0. The F_{st} values for all the polymorphic loci except for aconitase, alanine dehydrogenase, β-naphthyl-propionate esterase, and 6-phosphogluconate dehydrogenase were also different from 0.

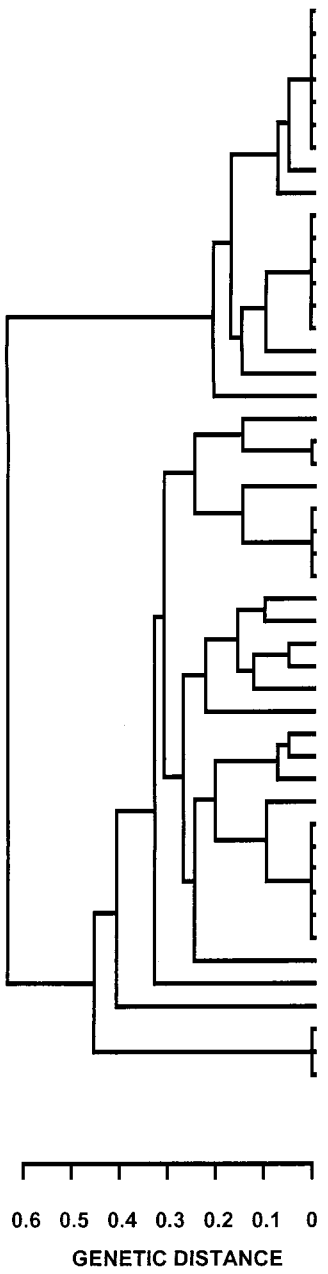
Comparison of isolates from human and fish products by REA and PFGE. Most of the isolates of fish or human origin belonging to the same ET could be distinguished from one another by either REA or PFGE. For some ET4 isolates and for all of the ET26 isolates, only PFGE allowed isolates of different origins to be distinguished.

Agreement between the four typing methods. A total of 75 isolates were typed by all four methods. With six serovars, plus one atypical variant of serovar 7 and one nontypeable strain, and six ETs distinguished for these 75 isolates, serotyping and MEE were clearly the less-discriminating methods. REA was more efficient in distinguishing 17 subtypes, and PFGE was the most powerful technique with 25 subtypes. In spite of their low level of discrimination among the isolates studied, serotyping and MEE did not always give concordant results. However, the distinctions made by either serotyping or MEE were always confirmed by the results of REA or PFGE. The only exception to this rule is represented by the atypical isolates of serovar 7 mentioned above.

All the subtypes distinguished by REA were confirmed by PFGE; furthermore, PFGE split 6 otherwise homogeneous types into 13 subtypes (Fig. 1).

DISCUSSION

Several methods have been used in the past for typing *L. monocytogenes*. Some of them, like serotyping, are not very powerful in terms of discrimination (4) and usually only perform a first, broad differentiation of strains. Other methods, such as PFGE, are very efficient in distinguishing strains of *L. monocytogenes* but may be more prone than other techniques to some instability of the characteristics used for typing (1, 3). MEE and REA take an intermediate position in this classification (4). They are based on more stable markers and present a satisfactory power of discrimination among strains. In addition, MEE facilitates assessment of the genetic relationships among isolates and can be used to compare populations to one another in terms of population genetics (24). In the present study, we used four different typing techniques covering all



ET	Serovar	*Xho*I	*Apa*I	*Sma*I	Subtype	Human	Fish	Producer	Product
1	4b	1	1	1	1a	-	+ (1)	K	SmS
1	4b	2	2	2	1b	+ (2)	-		
1	4b	3	3	3	1c	+ (1)	-		
1	4b	4	4	4	1d	+ (1)	-		
1	4b	4	5	5	1e	+ (1)	-		
1	4b	5	3	4	1f	+ (1)	-		
1	4b	5	6	6	1g	+ (6)	-		
2	4b	nd	nd	nd	2	+ (1)	-		
3	4b	nd	nd	nd	3	+ (2)	-		
4	1/2b	6	7	7	4a	-	+ (1)	M	SmHe
4	1/2b	6	8	8	4b	+ (1)	-		
4	1/2b	7	9	9	4c	-	+ (1)	A	Tar
4	1/2b	7	10	7	4d	+ (2)	-		
4	1/2b	8	9	10	4e	+ (2)	-		
4	1/2b	9	9	11	4f	+ (1)	-		
5	4b	nd	nd	nd	5	+ (6)	-		
6	1/2b	nd	nd	nd	6	+ (1)	-		
7	1/2b	nd	nd	nd	7	+ (1)	-		
8	1/2a	nd	nd	nd	8	+ (2)	-		
9	1/2a	10	11	12	9a	-	+ (2)	C, L,	SmT, SGr, SmB, SmS, SmHa
9	1/2a	10	12	13	9b	-	+ (3)	C, F, H	SmT, SmS, SGr
10	1/2a	nd	nd	nd	10	+ (1)	-		
11	1/2c	11	13	14	11a	-	+ (1)	C	Schl
11	1/2c	12	14	15	11b	-	+ (1)	A	Tar
11	1/2c	13	15	16	11c	-	+ (1)	B	Tar
11	1/2c	14	16	17	11d	-	+ (1)	D	SmS
12	1/2a	nd	nd	nd	12	+ (2)	-		
13	1/2a	nd	nd	nd	13	+ (1)	-		
14	1/2a	nd	nd	nd	14	+ (1)	-		
15	1/2a	nd	nd	nd	15	+ (1)	-		
16	1/2a	nd	nd	nd	16	-	+ (1)	G	SmS
17	1/2a	nd	nd	nd	17	-	+ (1)	E	SmS
18	1/2a	nd	nd	nd	18	+ (1)	-		
19	1/2a	nd	nd	nd	19	+ (2)	-		
20	1/2a	nd	nd	nd	20	+ (1)	-		
21	1/2a	nd	nd	nd	21	+ (1)	-		
22	7	15	17	18	22a	-	+ (1)	A	SmS
22	1/2a	15	17	19	22b	-	+ (1)	A	SmT
22	7	16	18	20	22c	-	+ (1)	A	SmT, SmS
22	7 atyp	16	18	20	22d	-	+ (1)	A	SmT, SmS
22	3a	16	18	21	22e	-	+ (1)	A	SmT, STr
22	1/2a	16	19	22	22f	-	+ (1)	C	SmS
23	NT	nd	nd	nd	23	+ (1)	-		
24	1/2a	nd	nd	nd	24	+ (1)			
25	1/2a	nd	nd	nd	25	-	+ (1)	L	SmT
26	1/2a	17	20	23	26a	-	+ (1)	I	SmS
26	1/2a	17	20	24	26b	-	+ (1)	L	SmT
26	1/2a	17	21	25	26c	+ (3)	-		

FIG. 1. Genetic relationships among and characteristics of *L. monocytogenes* isolates from human clinical listeriosis and fish products. The dendrogram was obtained by the average-linkage method of clustering using a matrix of pairwise genetic distances based on MEE results at 21 genetic loci. Columns: *Xho*I, subtypes distinguished by REA analysis using the restriction enzyme *Xho*I; *Apa*I and *Sma*I, subtypes distinguished by PFGE using the enzymes *Apa*I and *Sma*I, respectively; Subtype, combined subtype based on several typing methods; Human, numbers in parentheses indicate the number of isolates from different clinical cases of each subtype; Fish, numbers in parentheses indicate the number of isolates of each subtype used for the F statistics; Product, fish products in which the corresponding *L. monocytogenes* subtypes have been isolated. Abbreviations: NT, not typeable; nd, not determined; SmS, smoked salmon; SmHe, smoked herring; Tar, tarama; SmT, smoked trout; SGr, salmon gravad; SmB, smoked buckling; SmHa, smoked halibut; Schl, schillerlocken; STr, salmon terrine; +, subtypes found in human or fish isolates; −, subtypes not found in human or fish isolates.

three categories of methods to study the diversity of *L. monocytogenes* isolates from different fish products and the persistence of strains in products of some producers. *L. monocytogenes* isolates of human clinical origin isolated in Switzerland in 1993 and 1994 were compared by MEE with isolates originating from fish products imported into this country during the same period.

Our results show that several *L. monocytogenes* ETs can be found in fish products from more than one producer. Two of these ETs (ET9 and ET11) have even each been found in products of four different producers. However, except for ET9 (Fig. 1), the genetically related isolates from fish products of different producers can be distinguished from one another by means of more discriminating methods, like REA or PFGE. In

TABLE 1. *L. monocytogenes* isolates obtained during follow-up cultures of fish products of four producers[a]

Producer	Fish product	Day[b]	Serovar	ET	REA[f] with *Xho*I	PFGE[f] with:		Subtype[c]
						*Apa*I	*Sma*I	
A	Tarama	27	1/2b	4	7	9	9	4c
	Tarama	34	1/2c	11	12	14	15	11b
	Smoked salmon	457, 475	7	22	15	17	18	22a
	Smoked trout	338 ($n = 2$)	1/2a	22	15	18	19	22b
	Smoked trout	316, 337 ($n = 2$), 358, 517	7	22	16	19	20	22c
	Smoked salmon	338, 485, 493, 500, 563	7	22	16	19	20	22c
	Smoked salmon	352 ($n = 2$)	7 atyp[d]	22	16	19	20	22d
	Smoked trout	352 ($n = 2$)	7 atyp	22	16	19	20	22d
	Salmon terrine	0	3a	22	16	19	21	22e
	Smoked trout	358	3a	22	16	19	21	22e
C	Smoked trout	0, 3, 27 ($n = 2$), 28, 30, 41, 44, 53	1/2a	9	10	11	12	9a
	Salmon gravad	3, 10, 28	1/2a	9	10	11	12	9a
	Smoked salmon	30	1/2a	9	10	11	12	9a
	Smoked buckling	34	1/2a	9	10	11	12	9a
	Smoked halibut	50	1/2a	9	10	11	12	9a
	Smoked trout	10, 27, 34	1/2a	9	10	12	13	9b
	Salmon gravad	34	1/2a	9	10	12	13	9b
	Smoked trout	10 ($n = 2$), 28 ($n = 2$), 30 ($n = 3$), 34 ($n = 2$), 41 ($n = 2$), 53	1/2a	9	ND[e]	ND	ND	ND
	Salmon gravad	28, 34	1/2a	9	ND	ND	ND	ND
	Smoked mackerel	53	1/2a	9	ND	ND	ND	ND
	Schillerlocken	50	1/2c	11	11	13	14	11a
	Smoked salmon	101	1/2a	22	16	20	22	22f
I	Smoked salmon	0 ($n = 2$)	1/2a	26	17	21	23	26a
		22	1/2a	26	17	21	23	26a
L	Smoked trout	0	1/2a	9	10	11	12	9a
		201	1/2a	26	17	21	24	26b
		609	1/2a	25	ND	ND	ND	25

[a] The isolates are classified by producer and subtype.
[b] Day, the number of days between the first positive sample (day 0) and the following positive samples. The numbers in parentheses represent the number of identical isolates obtained from a given product type on the same date.
[c] Subtypes are defined by a combination of the serotyping, MEE, REA, and PFGE results.
[d] 7 atyp, atypical serovar 7 missing the flagellar C antigen (i.e., O-XII/XIII, H-A, H-B).
[e] ND, not determined.
[f] The numbers given for REA and PFGE represent the types distinguished by these two methods and are the same as in Fig. 1.

addition, Jemmi and Keusch (14) as well as Rørvik and coworkers (21) have previously shown that *L. monocytogenes* strains contaminating fish products may not be the same as those found on the raw material at the beginning of the production chain. Thus, a direct epidemiological link between genetically closely related isolates, which could explain their presence in products of different origin, is very unlikely. These results suggest, as has been observed in meat and meat products, that some clones (particularly those marked by ET9, ET11, and ET22) are widespread in fish products. In fact, one of these recurrent clones from fish products (ET11) is identical to ET19 of Boerlin and Piffaretti (5) and has also been shown to occur very frequently in meat products in Switzerland (5). Such clones may be adapted to survival and growth in fish and meat products or may be very common in the processing environment, thereby contaminating fish and meat products along the production chain.

The recovery of genetically unrelated *L. monocytogenes* isolates from smoked salmon of different origins, from smoked trout from different producers, and from different tarama preparations suggests that these types of products are not each contaminated by specifically adapted clones (Fig. 1). This was expected, since no single genetic lineage of *L. monocytogenes* could be associated with specific types of food in the past (5, 17).

The follow-up of products from single producers showed that under certain circumstances, the same strain may contaminate the production of a plant for several months (up to 247 days for producer A). This is in agreement with the findings of Rørvik and collaborators (21), who found a strain that persisted for over 8 months in the products and in the factory of a smoked-salmon producer. Our results show that in the case of a persisting strain, cross-contamination between different products of a single producer may also take place (Table 1). This is not unexpected when different products are manufactured on the same production line or in the same production area, which may be colonized by a particular *L. monocytogenes* clone. As observed by Rørvik and collaborators in one smoked salmon factory (21), we showed that in the majority of cases with several isolates per producer, distinct and genetically unrelated clones could also be recovered in their products. This suggests that a renewal of strains within a plant and the persistence of one major strain may coexist. The dominance of one pattern or the other probably depends on the hygienic measures taken in the plant and perhaps on the presence of a clone adapted to the plant environment.

No genetic lineage of *L. monocytogenes* could be specifically associated with one origin or the other (Fig. 1). However, the majority of isolates from fish products belonged to genomic group II of Piffaretti and collaborators (18), and only three

TABLE 2. Genetic diversity and F_{st} values in *L. monocytogenes* populations from humans and those from fish products

Locus[a]	Number of alleles	Genetic diversity[b]	F_{st}[c]	Significance[d]
ACO	2	0.028	−0.017	Not significant
ACP	4	0.524	0.303	Significant
ADK	2	0.496	0.357	Significant
ALDH	2	0.320	0.054	Not significant
CAT	7	0.700	0.121	Significant
EST1	4	0.632	0.202	Significant
EST2	9	0.690	0.041	Not significant
FUM	4	0.711	0.133	Significant
GD2	1	0	Not applicable	Not applicable
GOT	3	0.182	0.145	Significant
GP1	4	0.577	0.312	Significant
G6P	3	0.630	0.181	Significant
IDH	5	0.633	0.274	Significant
IPO	1	0	Not applicable	Not applicable
LDH	1	0	Not applicable	Not applicable
MPI	4	0.648	0.299	Significant
NSP	1	0	Not applicable	Not applicable
PEP1	3	0.564	0.306	Significant
PEP2	5	0.730	0.232	Significant
PG1	1	0	Not applicable	Not applicable
6PG	2	0.490	0.050	Not significant
Overall	Not applicable	0.4074	0.210	Significant

[a] Abbreviations: ACO, aconitase; ACP, acid phosphatase; ADK, adenylate kinase; ALDH, alanine dehydrogenase; CAT, catalase; EST1, α-naphthyl-propionate esterase; EST2, β-naphthyl-propionate esterase; FUM, fumarase; GD2, glutamate dehydrogenase; GOT, glutamate-oxalate transaminase; GP1, glyceraldehyde-3-phosphate dehydrogenase; G6P, glucose-6-phosphate dehydrogenase; IDH, isocitrate dehydrogenase; IPO, indophenyl oxidase; LDH, lactate dehydrogenase; MPI, mannose-phosphate isomerase; NSP, nucleoside phosphorylase; PEP1, phenylalanine-leucine peptidase; PEP2, leucyl-glycyl-glycine peptidase; PGI, phosphoglucose isomerase; 6PG, 6-phosphogluconate dehydrogenase.

[b] The genetic diversity was calculated with the following formula: $D = 1 - 1/K \sum_{L=1}^{K} \sum_{i=1}^{a} p_i^2$, where K is the number of loci and p is the frequency of the ith allele.

[c] F_{st} values were estimated by the method of Weir and Cockerham (25).

[d] Statistical significance indicates whether the F_{st} value obtained is significantly different from 0 (11).

belonged to serovar 4b- and 1/2b-associated group I. This is in contrast with the isolates of human origin, which were equally distributed in both genomic groups (Fig. 1). In addition, a significant difference between *L. monocytogenes* strains from humans and those from fish is clearly supported by the results of the F statistics analysis. There may be several explanations for the observation of these two separate populations. First, despite the precautions taken, one cannot exclude a bias related to the sampling of the isolates used for the comparison. Second, the *L. monocytogenes* isolates chosen from imported products collected at the border may not be entirely representative of products already on the market in Switzerland. However, most of the fish and fish products eaten in Switzerland are imported, and the inland production represents the smaller part of the market. Finally, there is probably a true difference between the *L. monocytogenes* populations from humans and those from fish products eaten in Switzerland. Despite a growing consumption of fish in this country, these products seem to be only rarely implicated in clinical listeriosis in human. Other types of food are more likely to be of epidemiological significance. The situation may, however, be different in countries where much more fish is eaten than in Switzerland. In Denmark, Nørrung and Skovgaard (17) found that a significant proportion of isolates from fish and fish products belonged to the same ETs as isolates from human listeriosis cases. However, these authors did not confirm the identities of the human and fish isolates by methods like REA or PFGE. Further studies should be conducted in countries where the population consumes large amounts of seafood, with more discriminating methods than MEE being used to clarify this point.

As found in a previous comparison of *L. monocytogenes* isolates from humans and meat products (5), the relative frequency of some very restricted and genetically unrelated clones (ET9, ET11, and ET22 being mainly in fish products and ET1 being mainly in humans) may differ considerably from one population to the other. This is probably due to some specific properties of these clones not associated with their general genetic backgrounds. These differences between clones may also explain why fish products are still only rarely associated with clinical listeriosis in humans despite the frequent contamination and growing consumption of ready-to-eat foods like smoked salmon. The most frequently isolated strains in fish products may differ substantially from those found in humans with regard to some important pathogenicity-associated characteristics. Interestingly, the epidemic-associated serovar 4b clone (marked by ET1), which has been suspected to present a higher pathogenic potential than other clones (5, 17, 18), was found only once in a fish product in the course of the present study. A screening of 22 additional serovar 4b isolates from other fish products received at our center during the period 1993 to 1994 without detailed anamnestic data yielded only 2 ET1 isolates. These were received on the same date and originated from the same laboratory. They were therefore very likely to be epidemiologically related (data not shown). In another screening performed in our laboratory on 50 isolates from fish products from Switzerland and abroad, none was found to belong to ET1 (4a). This confirms the low frequency of the ET1 clone in fish products in Switzerland. These results seem to contrast with data from Denmark (17) in which 3 out of 20 isolates from fish belonged to ET1. However, in the latter study, no detailed data were available on the origins of the corresponding fish products, and an epidemiological link between the three ET1 isolates cannot be excluded.

As expected, PFGE was the most discriminating typing method, followed by REA, MEE, and serotyping. The less-discriminating methods did not split types distinguished by the more discriminating techniques into further subtypes (except for the atypical serovar 7 isolates found in products of producer A). Differences based on only one among several methods for the differentiation of subtypes point to a very recent divergence of lineages and may not necessarily represent reliable markers for epidemiological tracing (1). In the case of the present study, such minor differences were only rarely of interest for the analysis of the data presented. On only two occasions did the differentiation between two subtypes found in products of a single producer rely on a single characteristic. In the first case, two subtypes (subtypes 22c and 22d, producer A) could be distinguished by a subtle difference in serovars. In the second case (subtypes 9a and 9b, producer C), the difference observed in PFGE patterns suggested that an insertion of approximately 20 kb in the genome of a strain could have led to the appearance of the second subtype (data not shown). Thus, a single genetic event could be the origin of the presence of two very closely related subtypes in both cases. However, the data at our disposal do not allow determination of when this genetic event took place, the exact epidemiological significance of the small differences observed remains difficult to assess.

In conclusion, our data show that a few particular *L. monocytogenes* clones are widespread among fish products from sev-

eral different countries. No association between a type of fish product and a particular genetic group of *L. monocytogenes* was observed. Patterns of continuous change in the contaminating strains and persistence of a major strain with cross-contamination were observed among fish products of the same origin. Finally, the *L. monocytogenes* isolates of human origin and those from fish products formed two significantly different populations.

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