EPIDEMIOLOGY AND BEHAVIOUR OF *LISTERIA MONOCYTOGENES* STRAINS ALONG THE FISH CHAIN PRODUCTION

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Introduction

The microbial quality is one of the most important aspect of the quality of food from animal origin. The lack of applying this concept can result in a rapid deterioration or in the presence of potentially human pathogens at a high level in the product. In this field, *Listeria monocytogenes* is one of the major problem due to the severity of human listeriosis. Actually listeriosis is a rare but very serious foodborne disease affecting preferentially pregnant women, new-born infants and immunocompromised patients (Rocourt *et al.,* 2000). Nevertheless the relationship between *Listeria monocytogenes* and fishes and fish products still remains enigmatic. In fact, *Listeria monocytogenes* occurs widely in different environments including fish production farms and slaughter plants and consequently can contaminate the processing line and finished products (Johansson *et al.,* 1999). But in an other hand, in recent years, fishes and fish products were not really associated with human listeriosis cases. Only 2 cases in relation with vacuum-packed gravad and cold-smoked rainbow trout preparations were described (Ericsson *et al.,* 1997, Miettinen *et al.,* 1999 and Tham *et al.,* 2000). During the production, fishes are submitted to different environmental conditions, from a rearing period in an opened place in the water, to a slaughter then a processing plants where products could be salted and smoked. All these steps can promote, or not, the presence of bacteria and specially of *Listeria monocytogenes.*

In this context it seemed interesting to follow the epidemiology and the behaviour of strains isolated in different steps.

Material and Methods

Two fish farms (A and B) and one processing plant (C) situated along a river were investigated for the presence of *Listeria monocytogenes*. In total, 7 series of sampling (swabbing equipment, water from upper and down river, fishes…) were realised as follow : one on site A and 3 on sites B and C. All samples (102) were analysed for *Listeria monocytogenes* detection using an enrichment (Fraser broth) and isolation (ALOA medium) procedures, then typical colonies were identified using Microgen-Listeria-ID test. *Listeria monocytogenes* strains were characterised by Pulsed Field Gel Electrophoresis (PFGE) technique using *Apa*1 and *Asc*1 enzymes.

Results and Discussion

Listeria monocytogenes was present in different sites (table 1), not only on the surface of fishes but also on the equipment and in the water sampled in the river and in farms. The environment of premises and notably where conditions are in favour of creating biofilms can justify this presence of *Listeria monocytogenes.*

During the first series of sampling in the slaughtering plant, it can be noted a very high level of contamination, specially on the equipment (conveyor belt, boxes….). Nevertheless the occurrence was lower during the second and the third series, due to the improvement of the cleaning and disinfecting procedures and of the hygienic conditions applied during slaughtering.

Combination of *Apa*1 and *Asc*1 results allows classifying isolates into PFGE types. Seven types were identified among 44 strains tested (table 2). Two types are predominant : the first one (type 15) corresponding to the serotype 4b, was present in the 2 farms, not only on the surface and in the intestines of fishes, but also in the environment and in the water. The second one (type 18) was present only in the processing plant. These results confirm that strains present during the primary production do not get over the other during the processing step (Dauphin *et al.,* 2001). The presence of the same genotype 18 in the processing plant after several weeks and whether the improvement of hygienic procedures confirms the ability of different resident strains to survive and colonize surfaces of equipment.

It was interesting to compare the behaviour of these strains, and others isolated in different fish productions environment (salmon and trout), specially with different concentration of salt (NaCl) in the medium. These results confirm that the "resident" strain (type 18) seems to be very well adapted to a high concentration of salt (10 %) and that the growth of strain isolated from a marine environment is not very strong in the absence of salt. In general the different phases of microbial growth (lag. time and growth rate) were affected by the concentration of salt added in the medium. Interestingly it can be noted that there is no relationship between the behaviour of these strains and the genotype obtained by PFGE.

Conclusion

The characterisation of *Listeria monocytogenes* strains isolated in different places along a river, confirms the diversity of these bacteria in the fish farms and the ability of one type to colonise the equipment of the processing plant, despite the improvement of hygienic conditions. Therefore the study of these strains in contact with different concentrations of salt shows difference during the growth phase and no relationship between the genotype and this behaviour.

References

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Table 1 : Results of *L. monocytogenes* from different sites and samples.

Sites	A		B			C	
Series	1	2	3	4	5	6	7
Fishes*	8/17	1/4	1/4	0/4	2/7	2/3	0/10
	(4/4)	(1/1)	(1/1)	(0/1)	(1/2)	(1/1)	(0/3)
Environment	5/9	2/2	1/1	1/2	9/12	1/15	2/11
Water	1/4	1/2	0/1	1/4	-	-	-
Total	14/30	4/8	2/6	2/10	11/19	3/18	2/21

* ( ) : Number of positive fishes out of number of analysed samples

Table 2 : Number of isolates of *L. monocytogenes* according to PFGE types.

PFGE Types	N° of isolates
11	1
15	10
17	5
18	22
19	1
30	2
32	3

Table 3 : PFGE types of *L. monocytogenes* isolated from different sites.

Sites	A		B			C	
Series	1	2	3	4	5	6	7
Fishes	11-15-30-32	15	15-19	-	18	18	-
Environment	15-17-30-32	15-17	17	15	18	18	18
Water	30	15	-	15	-	-	-