PERSISTENCE AND DIVERSITY OF *LISTERIA MONOCYTOGENES* ISOLATES IN NORWEGIAN PROCESSING PLANTS

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Summary. Five salmon processing plants were investigated for periods of eight (plants A and B) and four (plants C, D and E) months for the occurrence of *Listeria monocytogenes.* A total of 226 strains of *L. monocytogenes* were isolated and subtyped. Automated ribotyping with DuPont Qualicon RiboPrinter® system with the enzyme E*co*R1 was used to differentiate and characterize isolates for simple molecular tracking. The isolated strains could be divided into 16 DuPont ribotypes. The ribotypes DUP-1023C, DUP-1039C, DUP-1044E, DUP-1045B, DUP-1046A, DUP-1062B and 1062C were found to be persistent in the plants, as they were found through the whole sampling period. The remaining 9 ribotypes were isolated sporadically. Five of the persistent subtypes (DUP-1023C, DUP-1039C, DUP-1046A, DUP-1062B and 1062C) where isolated in high numbers. Ribotype DUP-1045B and DUP-1039C were found in four of the five investigated plants.
Keywords: *Listeria,* food safety, salmon, ribotyping, lineages, biofilm, cluster analysis.

LISTERIA MONOCYTOGENES PAPLITIMAS IR ĮVAIROVĖ NORVEGIJOS LAŠIŠINIŲ ŽUVŲ APDOROJIMO ĮMONĖSE

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Santrauka. Penkiose Norvegijos lašišinių žuvų apdorojimo įmonėse buvo tiriamas bakterijų *Listeria monocytogenes* paplitimas. Iš viso ištirta ir identifikuota 226 *L. monocytogenes* padermės. Izoliatams atpažinti ir diferencijuoti naudota automatinė „DuPont Qualicon RiboPrinter®“ sistema, pirminėms molekulinėms padermėms nustatyti naudojant E*co*R1 fermentus. Tyrimai parodė, kad subtipai DUP-1023C, DUP-1039C, DUP-1044E, DUP-1045B, DUP-1046A, DUP-1062B ir 1062C tirtose įmonėse buvo visą tyrimų laiką. Iš jų penki (DUP-1023C, DUP-1039C, DUP-1046A, DUP-1062B ir 1062C) buvo randami dažniausiai, o subtipai DUP-1045B ir DUP-1039C aptikti keturiose iš penkių tirtų įmonių. Devyni subtipai buvo izoliuoti pavieniais atvejais.
Raktažodžiai: *Listeria*, maisto sauga, paplitimas, įvairovė, prietaisas „RiboPrinter®“, lašišos.

Introduction. *Listeria monocytogenes* is recognized as an important human pathogen causing sporadic infections and food-borne outbreaks. It may cause invasive disease such as bacteremia, meningitis and severe prenatal infections (Dussurget *et al.*, 2004; Paoli *et al.*, 2005). *L. monocytogenes* is responsible for 15-20 hospitalized cases annually in Norway (MSIS, 2005). Most of these have been considered sporadic and comparatively few outbreaks have been reported worldwide (Jay *et al.*, 2005; Slutsker & Schuchat, 1999). The organism is ubiquitous throughout nature, is regularly isolated from the environment (Beumer *et al.*, 1996), the food processing industry (Fonnesbech *et al.*, 2001; Hoffman *et al.*, 2003; Jacquet *et al.*, 1993; Thimothe *et al.*, 2004) and from the fish processing industry in Norway (Klæboe *et al.*, 2005; Rosef *et al.*, 2002; Rørvik *et al.*, 1995). Post-process contamination of food with *L. monocytogenes* represents a serious problem because of its ability to survive and grow at refrigeration temperature (Farber & Peterkin, 1991). The European Commission has established a general limit of 100 cfu/g *L. monocytogenes* throughout the shelf-life for ready-to-eat foods able to support the growth of the bacterium. Such products include raw or smoked salmon (EC, 2005). This recommendation is based on epidemiological data indicating that *L. monocytogenes* represents a very low risk for all non-predisposed population groups when the concentration is below 100 cfu/g. This EC regulation on *L. monocytogenes* has later been supported by a joint FAO/WHO Codex Alimentarius Commission risk assessment (FAO/WHO, 2004). It is concluded that the consumption of low numbers of *L. monocytogenes* (< 100/g) gives a low probability of causing illness, even among high risk groups. Consequently, control measures that prevent the occurrence of high levels of contamination at the time of consumption would be expected to have the greatest impact on reducing the rates of listeriosis (Chen *et al.*, 2003). Cold-smoked fish products are foods of particular concern due to the lack of a heat inactivation step during processing (Gombas *et al.*,

2003; Thimothe *et al.*, 2004).

Although the presence of *L. monocytogenes* has been demonstrated in many environments, our understanding of the ecology of this organism and its transmission pathways is still limited. The capacity of *L. monocytogenes* to adhere to inert surfaces in the food-processing environment is well known (Hood & Zottola, 1997; Jay *et al.*, 2005; Kalmokoff *et al.*, 2001; Mafu *et al.*, 1990; Paoli *et al.*, 2005). As is the case for micro-organisms in general, *L. monocytogenes* in biofilms are much more resistant to disinfection than their free-living counterparts. Furthermore, thick complex biofilms are more difficult to remove than adhered single cells of the bacteria (Tompkin, 2002).

Ribotyping of *L. monocytogenes* is a tool to subgroup bacteria (Klæboe *et al.*, 2006; Romalde *et al.*, 2002; Wiedmann, 2002). Automated ribotyping provides highly standardized results, which is a prerequisite for the subtyping of large sets of isolates. This characteristic makes it suitable for epidemiological studies. Ribotyping targets conserve chromosomal genetic elements and thus allow for reliable grouping of isolates (Sauders *et al.*, 2003). The purpose of this study was to use the automated ribotyping procedure to characterize and group the isolates of *L. monocytogenes* in order to understand the distribution of the different subtypes in , salmon processing plants.

Material and methods

Sampling and isolate collection. Five salmon processing plants (A, B, C, D and E) were selected for this study. Information on the diversity of the isolates from plants A and B has earlier been published in Klæboe *et al.* 2006, and is included here to give a broader view of the persistence and diversity of *Listeria monocytogenes* in Norway. The sites for sampling were chosen from locations where the risk of cross contamination and growth of *Listeria* were considered high. Plants A and B were only 50 meters apart. Plant B processed fish, delivered fish for consumption, and provided fish for cold smoking in plant A. Plants C, D and E were located far from A and B, and from each other. The samples were collected over periods of eight months (plants A and B) and four months (plant C, D and E). Samples of 25 to 50 grams of salmon were collected from each site. Some of the samples where pieces of fish flesh while others where blended homogenous trimmings from several fish. Traditional cultivation based methods (NMKL, 2004) were used for the isolation of *L. monocytogenes*. The number of isolates of *L. monocytogenes* obtained from plants A, B, C, D and E were 75, 35, 60, 31 and 25 respectively.

Automated ribotyping. The 226 *L. monocytogenes* isolates were subtyped using the enzyme *Eco*R1 and the Automated DuPont Qualicon RiboPrinter®, following the manufacturer's instructions (Qualicon, 1998). In brief, cells were grown overnight on blood agar plates at 37°C and colony material was transferred to a lysis buffer where it was disrupted by incubation for 10 minutes at 90°C and placed in the RiboPrinter®. The process performed a restriction digestion of chromosomal DNA, separated the restriction fragments by agarose gel electrophoresis, transferred the fragments to a nylon membrane, probed the membrane with a chemiluminescent ribosomal *E. coli* rrnB rRNA operon probe and recorded the image produced. Comparison and classification of the riboprint patterns were carried out using pattern-matching software included in the RiboPrinter® system. Each *Eco*R1 pattern was compared to a library of ribotype patterns supplied by Qualicon (the DUP-ID library) and placed in a group defined by pattern similarity ≥ 85% (e.g. DUP-1023). If the DuPont identification number included more than one distinct ribotype pattern, which generally differed by position of only one weak band, the samples were placed in different subgroups. The DuPont identification number was then designated with an additional alphabetized letter suffix (e.g. DUP-1023C). The ribotypes were used to assign the isolates into the three *L. monocytogenes* lineages (I, II and III) (Sauders *et al.*, 2006)**.** Subtypes isolated through the entire sampling period are defined as "persistent" and the others "sporadic".

Clustering of subtypes. Images generated by the RiboPrinter® were saved in TIFF format and transferred to the GelCompar® II software (Applied Maths, Saint-Martens-Laten, Belgium) for computer analysis. Similarity between the isolates was determined by Pearson correlation using an optimization of 1,56 % and a position tolerance of 0,50 %. The dendrogram was generated by the unweighted pair group method with arithmetic averages (UPGMA).

Results. The 226 *L. monocytogenes* isolates could be divided into 16 DuPont ribotypes (Table 1). Seven of the ribotypes showed persistence in the plants. DUP-1023C persisted in plant A and B, with 32 of 74 isolates. In plant C, DUP-1039C, DUP-1046A and DUP-1062C persisted with 8, 39 and 13 of the 60 isolates respectively. In plant D, DUP-1039C, DUP-1045B and DUP-1044E persisted with 17, 5 and 3 of 31 isolates respectively. In plant E the DUP-1062B was persistent with 24 of 25 samples. The remaining 9 ribotypes were found sporadically. Both DUP-1045B and DUP-1039C were found in four of the plants. The similarities of isolates are shown in Figure 1. The dendrogram did not reveal any clustering of the persistent ribotypes or the human pathogen Lineage I isolates (Fig.1). This indicates that there is no clear connection between the *E.co*R1 ribotypes shown in the dendrogram and the genetic properties that are associated with persistence or pathogenesis.

Discussion. The incidence of *L. monocytogenes* in cold-smoked salmon and heat treated fish products has been reported to range from 4.3% to 36% (Embarek, 1994; Gombas *et al.*, 2003). A risk assessment performed by the US Food and Drug Administration (FDA) and the U.S. Department of Agriculture estimated that 15% of all smoked fish products are contaminated with *L. monocytogenes* (FDA/FSIS, 2003). According to the annual reports on trends and sources of zoonoses and zoonotic agents in Norway (www.zoonose.no), the detection rate in similar products has varied from 5-15% the last 10 years.

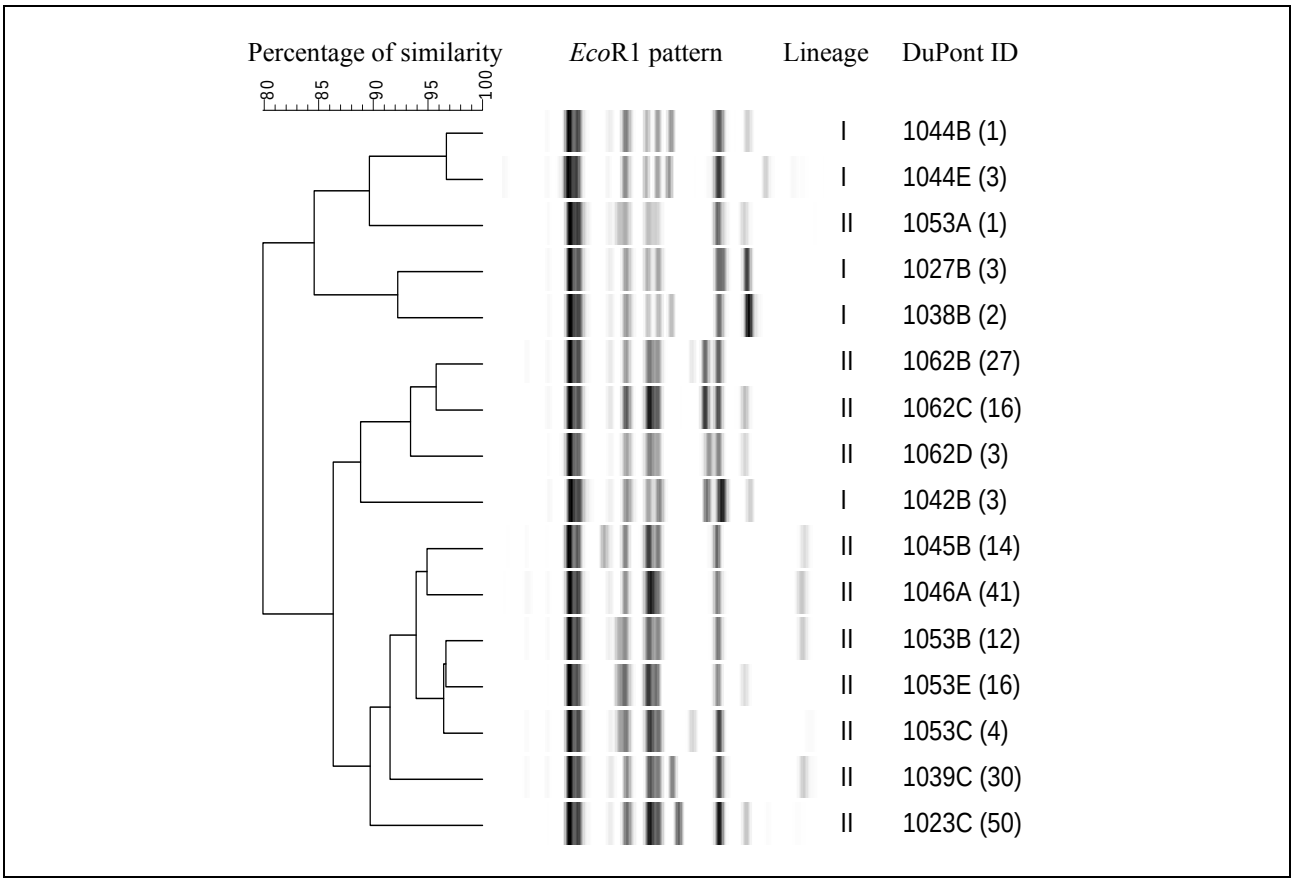

Figure 1. **The diversity of 16 *Eco*R1 subtypes. The DuPont ID refers to the DuPont identification number. The numbers of isolates are shown in parentheses**

Table 1: **Distribution of 16 *Listeria monocytogenes* ribotypes isolated in salmon processing plants in Norway**

	Plant					
DuPont-ID	A*	B*	C	D	E	No. of isolates
DUP-1023C	32	18	-	-	-	50
DUP-1027B	2	1	-	-	-	3
DUP-1038B	2	-	-	-	-	2
DUP-1039C	3	2	8	17	-	30
DUP-1042B	-	3	-	-	-	3
DUP-1044B	-	-	-	1	-	1
DUP-1044E	-	-	-	3	-	3
DUP-1045B	6	2	-	5	1	14
DUP-1046A	-	-	39	2	-	41
DUP-1053A	1					1
DUP-1053B	12	-	-	-	-	12
DUP-1053C	3	1	-	-	-	4
DUP-1053E	8	8	-	-	-	16
DUP-1062B	2	1	-	-	24	27
DUP-1062C	-	-	13	3	-	16
DUP-1062D	3	-	-	-	-	3
No. in each plant	74	36	60	31	25	*n = 226*

*Published in Klæboe et al. 2006.

From the environments in plants A and B, *L. monocytogenes* was isolated in 81% and 50% of the samples respectively (Klæboe *et al.*, 2005). It should be noted that the sampling focused on spots considered to represent high risk areas with respect to *L. monocytogenes* contamination. *L. monocytogenes* has also been isolated

from the surface of fresh salmon at other sites in Norway (Rosef *et al.*, 2002). Although raw materials may directly contribute to *L. monocytogenes* contamination of the finished cold-smoked fish products (Eklund *et al.*, 1995), it is more likely that raw materials introduce *L. monocytogenes* into the processing plant environment and may thus contribute indirectly to finished product contamination. Most authors appear to agree that post processing cross contamination from the production environment represents an important source for contamination of the final product (Autio *et al.*, 1999; Farber, 1991; Rørvik *et al.*, 1995; Rørvik *et al.*, 1997). Our data support the hypothesis that *L. monocytogenes* subtypes are spread throughout the plants over time by the movement of equipment such as fish smoking racks and possibly by employees (Tompkin *et al*., 1999).

Limited information is available about transmission from different environmental sources and raw materials to the finished products. To determine the contamination patterns in the production environment, molecular methods are necessary. In the present study we used automated ribotyping and found 16 subtypes using the enzyme *Eco*R1 (Table 1). This shows the broad spectrum of variants among the species in the fish industry environment and the ability for specific strains to persist in the plants. In spite of the high level of contamination, *L. monocytogenes* infection in humans traced to fish products is rare, even though a few cases are reported in the literature (Ericsson *et al.,* 1997; Miettinen *et al.*, 1999). There are, however, strong indications that human and food isolates represent distinct but overlapping populations (Gray *et al.*, 2004). Wiedmann and co-workers showed a correlation between alleles for *Act*A, *hly* and *inl*A, and based on these virulence genes they could separate *L. monocytogenes* into three genetic lineages (Wiedmann *et al.*, 1997; Sauders *et al.*, 2006). In general, lineage I represented strains isolated during epidemic outbreaks of listeriosis. Lineage II represented both industrial, animal isolates and sporadic human isolates (Norton *et al.*, 2001). Very few human isolates were classified into lineage III, and Wiedmann et al. (1997) suggested that at least some *L. monocytogenes* subtypes may have limited human pathogenic potential. Multiple methods, i.e. PFGE (Brosch *et al*. 1994), *act*A sequencing (Zhou *et al*. 2005), sequence data for *sig*B (Moorhead *et al*. 2003) or *fla*A, *iap* and *hly*. (Rasmussen *et al*. 1995) can be applied to classify *L. monocytogenes* into the three lineages. In our survey, ribotype information was used because it is reliable and correlates well with more time-consuming methods, e.g. analyzing different alleles. All the persistent strains except from DUP-1044E are classified as lineage II, which is an environmental adapted genetic lineage. DUP-1044E was only found three times in the total sample material. This indicates that most of the strains isolated from the salmon processing plants have limited human pathogenic potential.

With the automated riboprinting procedure, human pathogenic strains from clinical specimens can easily be compared with strains isolated from ready-to-eat food. It is, however, rare to find human cases after consumption of fish, and only sporadic cases of listeriosis have occurred after consumption of seafood. But both DUP-1027B, DUP-1038B, DUP-1039C, DUP-1042B, DUP-1045B and DUP-1046A are associated with human listeriosis in Norway the last 10 years with 4, 22, 14, 13, 7 and 1 case respectively (out of 137 cases) (unpulished results).

Listeria species have the ability to make biofilm and colonize the processing environment, and may survive this way for several years (Bremer *et al.*, 2001; Djordjevic *et al.*, 2002). The distribution of the DUP ribotypes indicated that specific subtypes of *L. monocytogenes* can exist in the environment for long periods. They probably establish themselves in specific niches of the plant, and are not eliminated by the cleaning and sanitizing procedures used (Tompkin, 2002). It is likely that these strains play an important role in the formation of biofilm. Future studies are necessary to reveal if these strains have special qualities that make them more effective biofilm-makes than the sporadically found strains.

The presence of the organism is generally of major concern in the food production sector because of the high mortality of listeriosis among predisposed persons. An improved understanding of the ecology of *L. monocytogenes* and other *Listeria* spp. in food processing plant environments will be required to understand the epidemiology of *Listeria* in the fish industry and the possible linkage to human listeriosis.

References

1. 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. *Appl Environ Microbiol* 65, 150-155.

2. Beumer, R. R., te Giffel, M. C., Spoorenberg, E. & Rombouts, F. M. (1996). *Listeria* species in domestic environments. *Epidemiol Infect* 117, 437-442.

3. Bremer, P. J., Monk, I. & Osborne, C. M. (2001). Survival of *Listeria monocytogenes* attached to stainless steel surfaces in the presence or absence of *Flavobacterium* spp. *J Food Prot* 64, 1369-1376.

4. Chen, Y. H., Ross, W. H., Scott, V. N. & Gombas, D. E. (2003). *Listeria monocytogenes*: low levels equal low risk. *J Food Prot* 66, 570-577.

5. Djordjevic, D., Wiedmann, M. & McLandsborough, L. A. (2002). Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. *Appl Environ Microbiol* 68, 2950-2958.

6. Dussurget, O., Pizarro-Cerda, J. & Cossart, P. (2004). Molecular Determinants of *Listeria Monocytogenes* Virulence. *Annu Rev Microbiol*. Jun 16.

7. EC (2005). Commission Regulation on

Microbiological Criteria for Foodstuffs. Official Journal of the European Union.

8. Eklund, M. W., Poysky, F. T., Paranjpye, R. N., Lashbrook, L. C., Peterson, M. E. & Pelroy, G. A. (1995). Incidence and Sources of *Listeria Monocytogenes* in Cold-Smoked Fishery Products and Processing Plants. *Journal of Food Protection* 58, 502-508.

9. Embarek, B. P. K. (1994). Presence, detection and growth of Listeria monocytogenes in seafoods: a review. *Int J Food Microbiol* 23, 17-34.

10. Ericsson, H., Eklow, 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. *J Clin Microbiol* 35, 2904-2907.

11. FAO/WHO (2004). Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: Interpretative summary. In *Microbiological Risk Assessment Series 4*.

12. Farber, J. M. (1991). *Listeria monocytogenes* in fish products. *Journal of Food Protection* 54, 922-924.

13. Farber, J. M. & Peterkin, P. I. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiol Rev* 55, 476-511.

14. FDA/FSIS (2003).Quantitative assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods: US Food and Drug Administration / Food Safaty and Inspection.

15. Fonnesbech, V. B., Huss, H. H., Ojeniyi, B., Ahrens, P. & Gram, L. (2001). Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. *Appl Environ Microbiol* 67, 2586-2595.

16. Gombas, D. E., Chen, Y., Clavero, R. S. & Scott, V. N. (2003). Survey of *Listeria monocytogenes* in ready-to-eat foods. *J Food Prot* 66, 559-569.

17. Gray, M. J., Zadoks, R. N., Fortes, E. D. & other authors (2004). *Listeria monocytogenes* isolates from foods and humans form distinct but overlapping populations. *Appl Environ Microbiol* 70, 5833-5841.

18. Hoffman, A. D., Gall, K. L., Norton, D. M. & Wiedmann, M. (2003). *Listeria monocytogenes* contamination patterns for the smoked fish processing environment and for raw fish. *J Food Prot* 66, 52-60.

19. Hood, S. K. & Zottola, E. A. (1997). Adherence to stainless steel by foodborne microorganisms during growth in model food systems. *Int J Food Microbiol* 37, 145-153.

20. Jacquet, C., Rocourt, J. & Reynaud, A. (1993). Study of *Listeria monocytogenes* contamination in a dairy plant and characterization of the strains isolated. *Int J Food Microbiol* 20, 13-22.

21. Jay, J. M., Loessner, M. J. & Golden, A. D. (2005). *Modern Food Microbiology*, 7. ed. Food Science Text Series. Springer, 606-617.

22. Kalmokoff, M. L., Austin, J. W., Wan, X. D., Sanders, G., Banerjee, S. & Farber, J. M. (2001). Adsorption, attachment and biofilm formation among isolates of *Listeria monocytogenes* using model conditions. *J Appl Microbiol* 91, 725-734.

23. Klæboe, H., Rosef, O. & Sæbø, M. (2005). Longitudinal studies on *Listeria monocytogenes* and other *Listeria* species in two salmon processing plants. *Int J Environ Health Res* 15, 71-77.

24. Klæboe, H., Rosef, O., Fortes, E. & Wiedmann, M. (2006). Ribotype diversity of *Listeria monocytogenes* isolates from two salmon processing plants in Norway. *Int J Environ Health Res* 16, 375-383.

25. Mafu, A. A., Roy, D., Goulet, J. & Magny, P. (1990). Attachment of *Listeria monocytogenes* to Stainless-Steel, Glass, Polypropylene, and Rubber Surfaces after Short Contact Times. *Journal of Food Protection* 53, 742-746.

26. Miettinen, M. K., Siitonen, A., Heiskanen, P., Haajanen, H., Bjorkroth, K. J. & Korkeala, H. J. (1999). Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. *J Clin Microbiol* 37, 2358-2360.

27. MSIS (2005). Listeriosis in Norway 1995-2004. *MSIS-report no15, 2005*. The National Institute for Public Health. Oslo, Norway (www.msis.no).

28. NMKL 136 (2004). 3.ed. *Listeria monocytogenes*, detection in foods. *Nordic Commitee on Food Analysis*. Veterinarian Institute. Oslo, Norway.

29. Norton, D. M., Scarlett, J. M., Horton, K., Sue, D., Thimothe, J., Boor, K. J. & Wiedmann, M. (2001). Characterization and pathogenic potential of *Listeria monocytogenes* isolates from the smoked fish industry. *Appl Environ Microbiol* 67, 646-653.

30. Paoli, G. C., Bhunia, A. K. & Bayles, D. O. (2005). *Foodborne Pathogens*: Caister Academic Press.

31. Qualicon (1998). Riboprinter system data analysis user guide.

32. Romalde, J. L., Castro, D., Magarinos, B., Lopez-Cortes, L. & Borrego, J. J. (2002). Comparison of ribotyping, randomly amplified polymorphic DNA, and pulsed-field gel electrophoresis for molecular typing of *Vibrio tapetis*. *Syst Appl Microbiol* 25, 544-550.

33. Rosef, O., Djupvik, J. & Kolberg, M. (2002). Listeria bakterier i to eksportslakterier for laks.

Listeria in salmon slaughterhouses. *Nor Vet Tidsskr* 114, 293-296.

34. Rørvik, L. M., Caugant, D. A. & Yndestad, M. (1995). Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughterhouse and smoked salmon processing plant. *Int J Food Microbiol* 25, 19-27.

35. Rørvik, L. M., Skjerve, E., Knudsen, B. R. & Yndestad, M. (1997). Risk factors for contamination of smoked salmon with *Listeria monocytogenes* during processing. *Int J Food Microbiol* 37, 215-219.

36. Sauders, B. D., Fortes, E. D., Morse, D. L., Dumas, N., Kiehlbauch, J. A., Schukken, Y., Hibbs, J. R. & Wiedmann, M. (2003). Molecular subtyping to detect human listeriosis clusters. *Emerg Infect Dis* 9, 672-680.

37. Sauders, B. D., Schukken, Y., Kornstein, L. & other authors (2006). Molecular epidemiology and cluster analysis of human listeriosis cases in three U.S. states. *J Food Prot* 69, 1680-1689.

38. Slutsker, L. & Schuchat, A. (1999). Listeriosis in Humans. In *Listeria,* listeriosis and food

39. Safety.. Ed. E. T. Ryser & E. H. Marth: Marcel Dekker: 75-95.

40. Thimothe, J., Nightingale, K. K., Gall, K., Scott, V. N. & Wiedmann, M. (2004). Tracking of *Listeria monocytogenes* in smoked fish processing plants. *J Food Prot* 67, 328-341.

41. Tompkin, R. B., Scott, V. N., Bernard, D. T., Sveum, W. H. & Gombas, K. S. (1999). Guidelines to prevent post-processing contamination from *Listeria monocytogenes. Dairy Food Environ Sanit*, 551-562.

42. Tompkin, R. B. (2002). Control of *Listeria monocytogenes* in the food-processing environment. *J Food Prot* 65, 709-725.

43. Wiedmann, M., Bruce, J. L., Keating, C., Johnson, A. E., McDonough, P. L. & Batt, C. A. (1997). Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. *Infect Immun* 65, 2707-2716.

44. Wiedmann, M. (2002). Molecular subtyping methods for *Listeria monocytogenes*. *J AOAC Int* 85, 524-531.

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