

MOLECULAR TYPING OF *LISTERIA MONOCYTOGENES* ISOLATED FROM COLD-SMOKED MEAT PRODUCTS BY PULSED- FIELD GEL ELECTROPHORESIS

LISTERIA MONOCYTOGENES MOLEKULĀRĀ TIPIZĒŠANA AUKSTI KŪPINĀTOS GAĻAS PRODUKTOS AR PULSĒJOŠĀ LAUKA GĒLA ELEKTROFORĒZI.

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ABSTRACT

The aim of this work was to investigate genetic diversity of *L. monocytogenes* isolated from cold-smoked sliced vacuum packaged pork in the one meat processing plant over 1-year period. A total 48 *Listeria monocytogenes* isolates were detected in eight cold-smoked sliced vacuum packaged meat production lots from January 2003 until January 2004. Molecular typing of all isolates was applied to determine strain genetic homology by pulsed-field gel electrophoresis (PFGE). Genomic *AscI* macrorestriction pattern (MRP) yielded 12 different PFGE types, which showed large genetic heterogeneity among isolates within one meat processing plant. Two persistent and ten nonpersistent PFGE types were detected. This study showed severe and continuous *L. monocytogenes* contamination in the meat processing plant on cold-smoked sliced vacuum packaged meat production line over a whole 1-year period.

KEYWORDS: *Listeria monocytogenes*, cold-smoked meat products, pulsed-field gel electrophoresis.

INTRODUCTION

Listeria monocytogenes is world-wide known food-borne pathogen which has caused several outbreaks of listeriosis in many countries (Schlech, 1983; Farber and Peterkin, 1991). Listeriosis is atypical and rare food-borne disease however it is of concern because of high mortality rate. Most often it causes sepsis, meningitis, and miscarriage in susceptible hosts that is high risk groups of human population, including pregnant women, neonates and immunocompromised adults (Slutsker et al., 1999). Some authors (Miettinen et al. 1999; Dalton et al. 1997; Heitmann et al. 1997; Riedo et al. 1994) show that *Listeria monocytogenes* can also cause febrile gastroenteritis or other diarrhea associated disease. It is now widely recognized that the consumption of contaminated food is an important route of transmission of listeriosis and a wide range of food products have been showed to be associated with both outbreaks and sporadic cases (EC, Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on *Listeria monocytogenes*, 1999).

As for many food-borne diseases, food of animal origin is the main source or vehicle of the listeriosis. *L. monocytogenes* is psychrotrophic bacterium able to grow at very low temperatures (2° to 4°C) and, especially, in vacuum packaged products. Most often contamination with *L. monocytogenes* in meat and fish industry is possible in different processing areas such as brining, post-brining, slicing, vacuum packaging and other areas. Brining equipment and brining solution have been shown to be a source of *L. monocytogenes* contamination in cold-smoked meat and fish products (Autio et al. 1999; Greer et al. 2003). Ready-to-eat (RTE) meat products that have received brining and smoking procedures before packing in vacuum provide conducive environment for multiplication of *L. monocytogenes* because of the reduced competitive flora and high salt tolerance of the organism. Long storage time in vacuum package at temperature between 2° to 6°C facilitate the growth of *L. monocytogenes* in the product. Therefore cold-smoked vacuum packaged meat products should be considered as risk product for susceptible groups of population.

Serotyping has been widely used as auxiliary instrument in epidemiological studies of *Listeria monocytogenes* (Filice et al. 1978), but some years after (Schwartz and Cantor, 1984) pulsed-field gel electrophoresis (PFGE) method was developed, which is now used also for epidemiological subtyping of *Listeria monocytogenes* by many scientists around the world. In order to trace the source of this organism, for example, on processed cold-smoked rainbow trout, Autio et al. (1999) did PFGE analyses on several *Listeria monocytogenes* isolates and found that some isolates in the final product were associated with brining and slicing procedures and corresponding equipment. This experience can be used with certainty to trace the source of this organism in cold-smoked meat processing plants as well as used to determine continuous contamination with persistent and nonpersistent *L. monocytogenes* strains.

Aim of the study. The aim of this work was to investigate genetic diversity of *L. monocytogenes* isolated from cold-smoked sliced vacuum packaged pork in the one meat processing plant by pulsed-field gel electrophoresis. Following tasks were set up:

- 1) to study the prevalence of *L. monocytogenes* in cold-smoked sliced vacuum packaged pork produced in the meat processing plant over a 1-year period;
- 2) to evaluate genetic heterogeneity of *L. monocytogenes* isolates by pulsed-field gel electrophoresis;
- 3) to determine whether the meat processing plant has persistent or nonpersistent *L. monocytogenes* contamination over the study period.

MATERIALS AND METHODS

Samples and sampling. Sampling was performed at retail shops over a 12 months period during the years 2003 and 2004. A total 95 samples of RTE cold-smoked sliced vacuum packaged pork products were collected. All products were produced at the same meat processing plant. During sampling temperature was taken at all shop counters where products were stored before sale. The vacuum packages of samples were transported to the laboratory with plastic ice packs in isolated boxes. The products were stored in constant conditions in incubators at 6°C until laboratory tests were started.

Detection and identification of *L. monocytogenes*. Isolation of *L. monocytogenes* was performed according to the conventional ISO 11290-1:1996 method (Anonymous, 1996) with modification by Johansson et al. (1998) using *Listeria monocytogenes* blood agar (LMBA) instead of Oxford agar. Method included two-step enrichment and plating on selective agars. Identification was based on β -hemolysis, Gram staining and catalase reaction. API-Listeria® kits (BioMérieux, France) were used for further identification of *Listeria* sp. All *L. monocytogenes* isolates were preserved by freezing at -70°C until molecular typing by PFGE.

Isolation of genomic DNA of *L. monocytogenes* and molecular typing by PFGE. After freezing all 48 *L. monocytogenes* strains were recovered and grown on sheep blood agar for 24 h at 37°C. One colony was transferred into tripticase soy broth [TSB] (Difco Laboratories, Michigan) and grown overnight at 37°C. DNA isolation and PFGE were performed as described by Autio et al. (1999). *AscI* enzymes (New England BioLabs) were used for restriction endonuclease digestion. Pulsed-field gel electrophoresis was performed in 1.0% agarose gel (Seakem Gold, FMC Bioproducts, Maine) at 200 V at 8°C in a Gene Navigator system with a hexagonal electrode (Pharmacia, Uppsala). Pulsetime for *AscI* was 1 s to 35 s for 18 h. Low range PFG markers (New England BioLabs) were used for fragment size determination. After pulsed-field gel electrophoresis gels were stained with ethidium bromide. Gels were photographed with an Alpha Imager 2000 system (Alpha Innotech, California).

Analysis of *AscI* macrorestriction pattern. *AscI* patterns were analyzed by BioNumerics software (Applied Maths, Kortrijk, Belgium). Similarity based on the position

of bands was expressed as Dice coefficient correlation. The clustering and construction of the dendrogram was performed by unweighted pair group method using arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

Of the cold-smoked sliced vacuum packaged pork products 50% (48 of 95) were positive for *L. monocytogenes*. A total 48 *L. monocytogenes* isolates were detected in eight meat production lots in the one meat processing plant over a 1-year period. Overall pulsed-field gel electrophoresis results are shown in the dendrogram (Figure 1).

Genomic *AscI* macrorestriction pattern (MRP) yielded 12 different PFGE types, which showed large genetic heterogeneity among isolates. We have isolated two persistent *L. monocytogenes* strains during the study period in six out of eight production lots.

Our study shows continuous *L. monocytogenes* contamination with both persistent and nonpersistent strains in the meat processing plant on cold-smoked sliced vacuum packaged meat production line. It means that there are some processing steps where continuous persistent *L. monocytogenes* contamination is possible.

High prevalence of *L. monocytogenes* in cold-smoked meat products has been shown already in previous studies (Bērziņš II *et al.* 2004) in several meat processing plants. There are studies where is proved that meat contamination with *L. monocytogenes* occurs usually from meat processing environment and equipment rather than from animals (Nesbakken *et al.*, 1996; Heir *et al.*, 2004).

Brining injections and recirculating brine used in the production have been proved as one of the important contamination sites (Autio *et al.*, 1999, Bērziņš I *et al.*, 2004, Greer *et al.*, 2004). There are some characteristic sources of *L. monocytogenes* contamination from equipment used in meat processing industry as well as there is possible transfer of persistent *L. monocytogenes* strains from one plant to another with processing equipment (Lunden *et al.* 2002).

Therefore there is a need for contamination and intervention studies in the meat processing plant to prevent *L. monocytogenes* contamination during cold-smoked vacuum packaged pork production in different processing steps what is the following part of our studies.

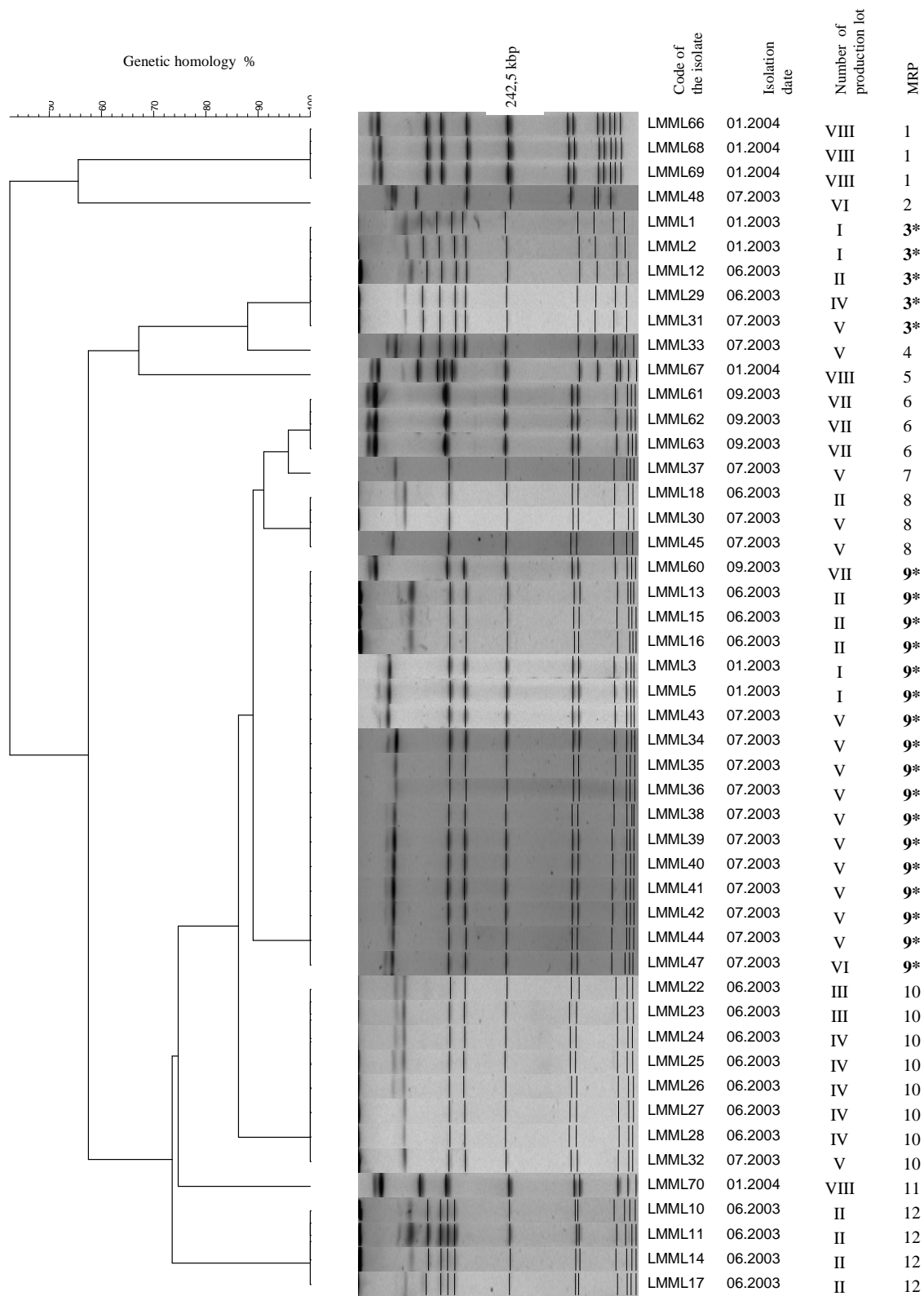


Figure 1. Dendrogram of *AscI* macrorestriction patterns (MRP) of *L. monocytogenes* isolated from cold-smoked sliced vacuum packaged meat products of the meat processing plant over 1-year period. Similarity analysis was performed using the Dice coefficient and clustering was performed by UPGMA (position tolerance 1.0%).

*MRP showing persistent strains.

CONCLUSIONS

1. There is a high prevalence of *L. monocytogenes* in cold-smoked sliced vacuum packaged pork produced in the meat processing plant.
2. Continuous *L. monocytogenes* contamination is severe problem in the meat processing plant on cold-smoked sliced vacuum packaged meat production line.
3. The meat processing plant has a continuous *L. monocytogenes* contamination with both persistent and nonpersistent strains.
4. As shelf-life of the cold-smoked sliced vacuum packaged pork products is long with high prevalence of *L. monocytogenes* in the product there is a need to carry out immediate proper contamination studies in the meat processing plant to prevent continuous *L. monocytogenes* contamination.

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