# Persistence of *Listeria monocytogenes* in artisanal cheese producing plants

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## Introduction

*Listeria monocytogenes* has been recognized as a human pathogen for decades and is known to be an important foodborne pathogen.

Ubiquitous in nature, *L. monocytogenes* can contaminate foods. Cheese, especially raw milk cheeses, has been implicated in outbreaks of listeriosis worldwide. The risk of these products has led public health officials to recommend that raw milk and diary products prepared from raw milk should not be consumed by susceptible population, particularly pregnant woman.

Therefore, the presence of *L. monocytogenes* is of great concern to the food industry, and tracing isolates within the food chain and the plant environment is of primary importance to evaluate sources and routes of transmission of the pathogen.

The aim of this work was to characterize *L. monocytogenes* isolates recovered between 2004 and 2007 from an artisanal ewe's raw milk cheese producing plant. Discriminative molecular subtyping methods have been used to characterised *L. monocytogenes* in order to understand the routes of contamination in plants.

## Materials and Methods

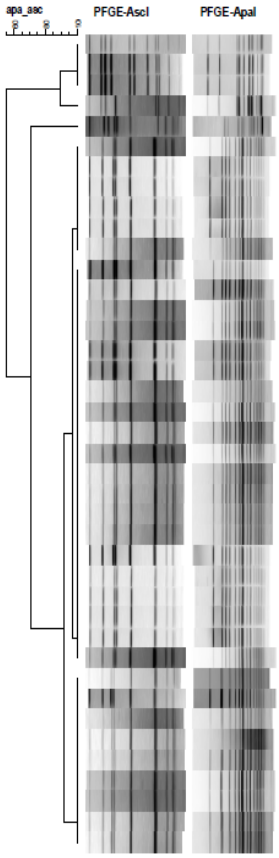
The artisanal cheese manufacturing plant is located in the south of Portugal nearby Lisbon. Soft cheeses (100-250g weight), granted with Protected Origin Denomination (DOP), are manufactured with raw ewe's milk. Between February 2004 and December 2007 in 18 visits to the cheese plant a total of 302 samples (81 cheeses from raw ewe's milk, 90 raw ewe's milk, 3 milk whey and 130 environmental samples) were collected .

Forty isolates were PFGE typed according to the PulseNet protocol (Graves & Swaminathan, 2001) with enzymes AscI and ApaI : 18 from cheese, one from raw milk, 20 from environmental sites, and one from whey.

## Results

The microorganism was recovered in six visits to the factory (33.3%), in 11 environment sites (once in the floor at the plant entrance; twice at the milk reception; four times at the production area - drains and floor; on three occasions in maturation chambers; eight times in cheese washing zone - sink and floor; once in the shipping zone and from the trolley used to carry cheeses between areas), in 13.6% of cheeses, 1.1%% of raw milk, and 33.3% of milk whey.

The combination of profiles obtained with both restriction enzymes patterns (*Apa*I and *Asc*I) yielded a total of six pulsotypes. Isolates MPCR group IIa obtained from cheeses 1 and 2 yielded unique pulsotypes. Pulsotypes 0079 and 0083 show a similarity of 96.9%. Six isolates share pulsotype 0079: from cheeses 7 and 11; and environmental isolates taken from the floor of the shipping area and from the trolley used to carry cheeses from the maturation chambers to the shipping chamber. Twenty isolates share pulsotype 0083, namely isolates from cheeses 3, 5, 6, 8, 9 and 10; from raw ewe's milk; and environmental isolates collected from the production zone (floor or drain); from the cheese washing area (floor or sink), and milk reception floor. Nine Isolates that matched in pulsotype 0027 were from milk whey; cheese 4; and environmental samples from floor factory entrance and maturation chambers; milk reception floor; production area floor and washing cheese's sink area. Pulsotype 0093 was obtained from a single MPCR group IIa isolate from the floor of cheese washing area.




| Isolate code | MPCR | As, Cd biotype | Combined profile | Local | Date |
|---|---|---|---|---|---|
| 1498/2 | IIa | sr | 0095 | Cheese 2 | 23-02-2005 |
| 1499 | IIa | sr | 0095 | Cheese 2 | 23-02-2005 |
| 1500/1 | IIa | sr | 0095 | Cheese 2 | 23-02-2005 |
| 1712/1 | IIa | sr | 0093 | CW zone, floor | 07-12-2005 |
| 929/3 | IIa | ss | 0048 | Cheese 1 | 10-02-2004 |
| 1727 | IVb | ss | 0079 | Shipping zone, floor | 11-01-2006 |
| 1728 | IVb | ss | 0079 | Cheese 7 | 11-01-2006 |
| 1735/2 | IVb | ss | 0079 | Cheese 7 | 11-01-2006 |
| 1777/4 | IVb | ss | 0079 | Cheese 11 | 22-03-2006 |
| 1782/3 | IVb | ss | 0079 | Cheese 11 | 22-03-2006 |
| 1797/1 | IVb | ss | 0079 | trolley | 06-06-2006 |
| 1604/2 | IVb | ss | 0083 | Cheese 3 | 15-06-2005 |
| 1607/2 | IVb | ss | 0083 | Cheese 5 | 15-06-2005 |
| 1635 | IVb | ss | 0083 | Raw milk | 20-07-2005 |
| 1649/1 | IVb | ss | 0083 | CW zone, floor | 20-07-2005 |
| 1653/3 | IVb | ss | 0083 | Cheese 6 | 29-08-2005 |
| 1656/1 | IVb | ss | 0083 | Cheese 6 | 29-08-2005 |
| 1675/1 | IVb | ss | 0083 | CW zone, floor | 12-10-2005 |
| 1676/1 | IVb | ss | 0083 | CW zone, sink | 12-10-2005 |
| 1699/1 | IVb | ss | 0083 | Milk reception, floor | 09-11-2005 |
| 1713/1 | IVb | ss | 0083 | CW zone, sink | 07-12-2005 |
| 1729 | IVb | ss | 0083 | CW zone, floor | 11-01-2006 |
| 1730 | IVb | ss | 0083 | CW zone, sink | 11-01-2006 |
| 1731 | IVb | ss | 0083 | Production zone, floor | 11-01-2006 |
| 1732 | IVb | ss | 0083 | Production zone, drain | 11-01-2006 |
| 1751/1 | IVb | ss | 0083 | Cheese 9 | 01-02-2006 |
| 1752/2 | IVb | ss | 0083 | Cheese 9 | 01-02-2006 |
| 1754/3 | IVb | ss | 0083 | Cheese 10 | 01-02-2006 |
| 1755/1 | IVb | ss | 0083 | Cheese 10 | 01-02-2006 |
| 1757/1 | IVb | ss | 0083 | Cheese 8 | 01-02-2006 |
| 1816/1 | IVb | ss | 0083 | Production zone, drain | 27-09-2006 |
| 1384/1 | IVb | ss | 0027 | Whey | 24-02-2005 |
| 1606 | IVb | ss | 0027 | cheese 4 | 15-06-2005 |
| 1634/1 | IVb | ss | 0027 | Entrance, floor | 20-07-2005 |
| 1636 | IVb | ss | 0027 | Chamber 2, floor | 20-07-2005 |
| 1638/1 | IVb | ss | 0027 | Chamber 1, floor | 20-07-2005 |
| 1648/3 | IVb | ss | 0027 | Chamber 3, floor | 20-07-2005 |
| 1674/1 | IVb | ss | 0027 | Milk reception, floor | 12-10-2005 |
| 1700/3 | IVb | ss | 0027 | Production zone, floor | 09-11-2005 |
| 1701/2 | IVb | ss | 0027 | CW zone, sink | 09-11-2005 |

**Figure 1:** Dendrogram generated by the combination of *AscI* and *ApaI* restriction patterns of isolates of *Listeria monocytogenes obtained from artisanal producer (APC) raw ewe's milk cheese*

## Conclusion

Isolates from raw milk shared the same pulsotype as isolates from six cheeses; furthermore, isolates from the floor at milk reception and another cheese's and milk whey originated the same pulsotype.

Although the contamination in was due on some occasions to transient strains, 20 isolates clustered the same pulsotype indicating that cheeses were contaminated with a resident strain with time of persistence estimated as at least, 14 months.

It is therefore possible to speculate that the source of these cheese isolates was the raw milk itself. Contaminated raw milk can contaminate the plant environment and some strains colonized and persisted for long period.

In the cheese washing area was the place where more positive samples were recovered indicating that at; cheese washing operation non-contaminated cheeses could become contaminated.

Cleaning and disinfection strategies should be designed to target persistent strains that probably have a higher resistance to sanitizers or a higher capacity to attach to surfaces or to form biofilms than sporadic strains.

## Acknowledgments

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