Genetic diversity of *Listeria monocytogenes* isolated from Portuguese cheeses

G. Almeida, R. Magalhães, I. Santos, J. Barbosa, T. Hogg, P.Teixeira

CBQF/ Universidade Católica Portuguesa, Escola Superior de Biotecnologia
Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
***Corresponding author:** pcteixeira@esb.ucp.pt

Introduction

Listeria species are Gram-positive, ubiquitous bacteria widely distributed in the environment. *Listeria monocytogenes* is the etiological agent of human and animal listeriosis.

Consumption of contaminated foods is probably the primary source of human listeriosis. Meat, milk and vegetables represent important sources of contamination by *L. monocytogenes.* To reduce the incidence of listeriosis it would be necessary to reduce the contamination level at production and at retail or to inhibit the growth of *L. monocytogenes* in high risk foods. Typing of bacterial isolates reveals the relationship among isolates.
The aim of this work was the characterization of isolates obtained from cheeses at market or ready-for-market and isolates obtained from cheeses analysed at the Portuguese Food Safety Authority Laboratories.

Materials and Methods

Cheeses were purchased from retail establishments around Porto during the period February 2004 - June 2007 or were taken from the producer's premises (15 cheese producers) as ready-for-market during the period January 2004 – December 2007 and were analysed, within their assigned shelf lives. It was also requested to other food control laboratories to supply cheese isolates.

Genoserotyping was performed as described by Doumith *et al.* (2004).
PFGE was performed according to the PulseNet protocol (Graves & Swaminathan, 2001) with enzymes *Asc*I and *Apa*I.

Results

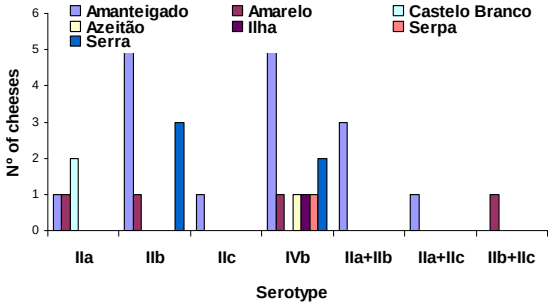

Figure 1. Results of genoserotyping of isolates from cheeses collected at market.

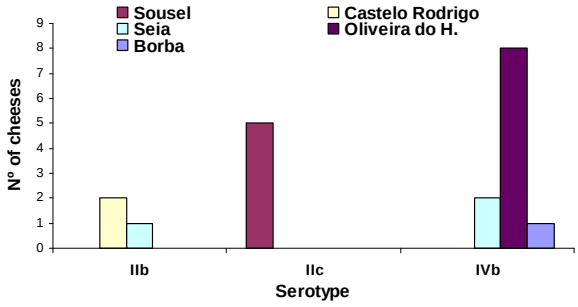

Figure 2. Results of genoserotyping of isolates from cheeses ready-for-market.

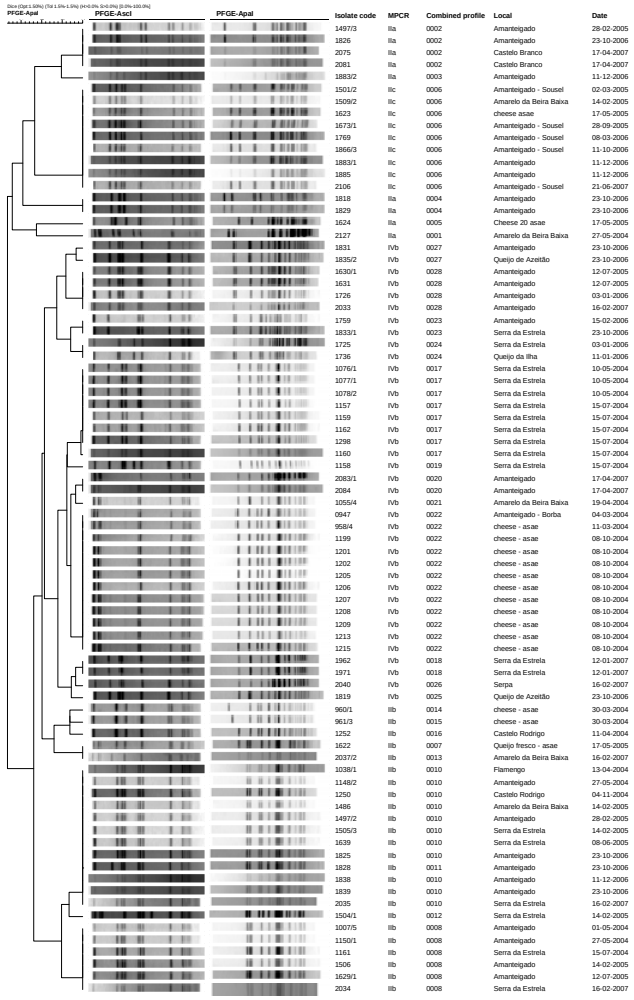

Figure 3. Dendrogram generated by the combination of AscI and ApaI restriction patterns of isolates of *Listeria monocytogenes* obtained from purchased and ready-for-market cheeses.

The combination of profiles obtained with both restriction enzymes patterns (ApaI and AscI) yielded a total of 27 pulsotypes, 14 strains originated unique pulsotypes.

Conclusion

The same pulsotype was found in cheeses from different producers that were purchased in pieces in the same supermarket on the same day, suggesting the possibility of contamination at retail level.
It is important to note that some of the strains that have been implicated in listeriosis are still present in cheeses at retail and might be implicated in new sporadic cases. Moreover, one of the most famous Portuguese cheese with a Protected Origin Denomination carried pulsotypes that have been implicated in human cases of listeriosis.

References

Graves, L. and B. Swaminathan (2001). "PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis." International Journal of Food Microbiology **65**: 55-62.

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