Virulence genes in strains of *L. monocytogenes* isolated from cheeses.

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Introduction

Listeriosis is a severe infection caused by *Listeria monocytogenes,* particularly among the elderly, very young and immunocompromized individuals. This disease is also associated with late-term miscarriages in pregnant women. Dairy products have been involved in several cases of listeriosis, and, in particular, the role of cheese in transmitting this bacteria has been much studied. The species *L. monocytogenes* comprises a diversity of strains with varying virulence, whilst many have pathogenic potential and can result in disease, others have no, or limited virulence (1).

The genetic typing or characterization of *L. monocytogenes* is, normally performed in strains isolated using quality control procedures or pathology laboratory methods. Whether these methods pre-select certain genetically differentiable sub-groups or not, has not, to our knowledge, been a subject of interest.

In this study different isolation procedures were used to isolate *L. monocytogenes* from retail purchased cheeses. Where cheeses proved positive for *L. monocytogenes*, randomly selected representative colonies were sub-cultured from each isolation method. The resultant isolates were screened for specific virulence genes using Real Time PCR.

Materials and Methods

70 raw milk cheeses were purchased from local retail establishments (Porto, Portugal) during the period December 2005 - March 2006. Analysis by Most Probable Number, Vidas LMO2 and direct count was performed. From the 7 positive cheeses, 64 colonies were identified and studied for the virulence genes *hly*, *inlA*, *inlB*, *plcA*, *plcB*, *act*A (2) and *lmo2821* (3) using Real Time PCR.

Cells were cultured overnight at 30°C in brain heart infusion broth, washed in saline (0.9% NaCl [wt/ vol]), centrifuged (15 min at 4,000 x g), and the supernatant discarded. Cells were resuspended in 50 µl of sterile distilled water. A 100-fold dilution of the cells was lysed by boiling at 100°C for 10 min (4).

The PCR mix contained 1µl of *Listeria* template DNA and 2.5µl of each primer pair (100 nM), 12,5µl of IQIM Sybr® Green Supermix and 9 µl of water. Control tubes containing primers without template DNA, template DNA from *L. innocua* and from *L. monocytogenes* Scott A were included.

In the beginning the template DNA was denatured at 94ºC for 3 min followed of 44 cycles of amplification (each cycle consisted of denaturation at 94º C for 1 min, annealing at 63ºC for 2 min and elongation at 72 ºC for 1 min) using a Chromo 4 Real Time PCR (Bio-Rad).

Results

Figures 1 to 7 show the results obtained for the 64 strains isolated from the 7 positive cheeses.

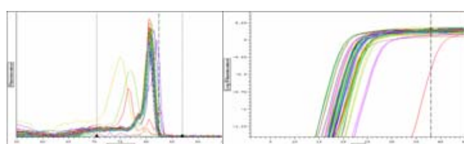

Figure 1 - Detection of *Hly*A.

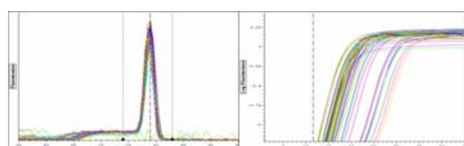

Figure 2 - Detection of *Inl*A.

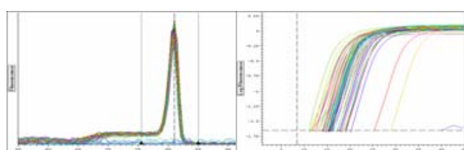

Figure 3 - Detection of *Inl*B.

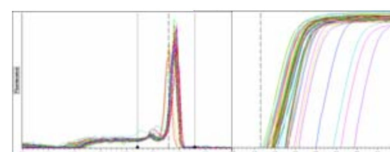

Figure 4 - Detection of *Act*B.

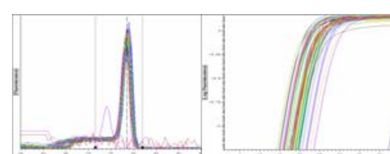

Figure 5 - Detection of *Plc*A.

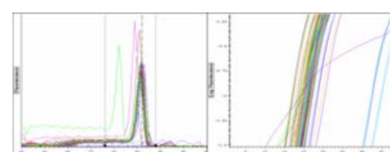

Figure 6 - Detection of *Plc*B.

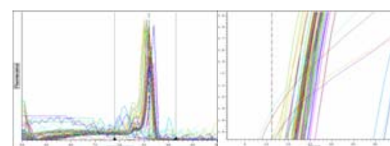

Figure 7 - Detection of *Inl*J.

Conclusion

The methods of isolation/detection employed, yielded isolates which were not distinguishable in terms of the presence of the virulence genes screened for.

Real Time PCR can be used to genetically characterize strains of *L.* monocytogenes with significant savings in terms of manpower and materials. The interpretation of curves requires some care, as can be seen in figures 1, 5, 6 and 7 where an electrophoresis of the PCR products was required to confirm the results. The use of other *Listeria* species and blanks with the DNA template without primers or primers without DNA template are essential to withdraw conclusions. In the present study all virulence genes tested were present in all strains isolated from cheeses demonstrating their pathogenic potential.

References

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Acknowledgments

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