CHARACTERISATION OF *Listeria monocytogenes* ISOLATED FROM TRADITIONAL FERMENTED MEAT PRODUCTS

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INTRODUCTION

Listeria monocytogenes **is commonly isolated from various types of processed meats from many countries and grows well in high pH cooked meat and poultry products. In Portugal there is a scarceness of information regarding prevalence and incidence of this bacterium in food products.**

One of the most appreciated traditional smoked fermented sausages in the north of Portugal is the *alheira*, which contains a variety of comminuted meats (poultry, duck, turkey, partridge, game, pork or calf), bread, olive oil, fat and spices. Previous studies showed that *L. monocytogenes* was the most prevalent microbiological hazard (75%) in *alheiras*.

AP-PCR (Arbitrarily Primed Polymerase Chain Reaction) has been used as a rapid and relatively simple technique for epidemiological subtyping of *L. monocytogenes* isolates that can produce reproducible and useful results.

The aim of this study was to perform a cluster analysis of the DNA fingerprints generated by AP-PCR of 128 randomly selected *L. monocytogenes* isolates from *alheiras* sampled from different producers.

MATERIALS AND METHODS

DNA was extracted from 128 *L. monocytogenes* isolates grown overnight in BHI at 37ºC. Cells were ressuspended in sterile deionized water after two washing cycles in 0.9% saline solution (centrifugation at 13700 rpm for 5 minutes). Subsequently, cells were boiled at 100ºC for 15 minutes and absorbance was read at 600 nm. Suspensions were diluted in order to obtain a 200 µl aliquot with 0.75 absorbance.

PCR mixtures contained 1X Taq buffer, 2.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate, 0.1 mM primer (D8635), 2 Taq units (Fermentas) per 25 µl reaction and 1 µl of isolated *L. monocytogenes* DNA.

Reaction mixes were cycled through the following temperature profiles: 1 cycle of 94ºC for 2 min; 35 cycles of 94ºC for 1 min, 46.9ºC for 1 min and 72ºC for 1.5 min; and then 1 cycle at 72ºC for 10 min. The amplified products were resolved by electrophoresis on 1.2 % agarose gels in TAE buffer containing 0.5 µg of ethidium bromide per ml. EZ load 100 bp PCR molecular ruler (bioRad) was included as a molecular weight standard.

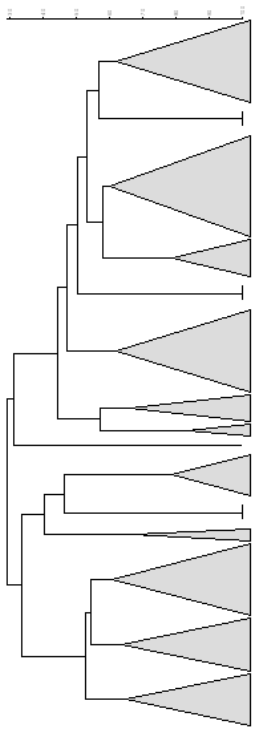
Cluster analysis of the AP-PCR fingerprints was accomplished using software GelCompar II version 4.0 (Applied Maths).

RESULTS AND DISCUSSION

The number of *L. monocytogenes* subtyping profiles detected in products of a single producer varied from one to five whilst the same isolate type was present in one to six producers.

Simpson's numerical index of diversity (Hunter and Gaston, 1988) was used to evaluate the discriminatory capacity of AP-PCR analysis with primer D8635 reaching 90%.

It had been previously found that the discriminatory capacity of the combination of serogrouping by multiplex PCR, arsenic, cadmium and tetracycline sensitivities reached 85.9%.



Cluster	Number of isolates	Number of producers
1	17	4
2	2	1
3	21	6
4	8	1
5	2	1
6	17	5
7	6	2
8	3	2
9	1	1
10	9	1
11	2	1
12	3	1
13	15	2
14	11	4
15	11	3

Figure 1 – Dendrogram (UPGMA clustering based on Jaccard similarity coefficient) of *L.monocytogenes* AP-PCR profiles with primer D8635 for isolates collected from traditional fermented meat products (n=128). Clusters were recognized based on similarity values higher than 60%.

CONCLUSION

- **AP-PCR and cluster analysis of the profiles obtained of *L.monocytogenes* revealed 15 clusters.**
- **Further investigation of the recognized clusters will be accomplished by molecular typing with AP-PCR using other primers.**
- **Subsequently, a composite data analysis will be performed and isolates with representative patterns of each cluster will be selected for thermal inactivation studies.**
- **These studies will aim at evaluating if temperatures reached during cooking, grilling, roasting, microwaving or frying of *alheiras* are able to efficiently inactivate this foodborne pathogen.**

REFERENCES

Hunter P.H. and Gaston M.A. 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J. Clin. Microbiol.* 26 (11): 2465-2466.

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