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A Centrifugation-plating Method for the Rapid Enumeration and Detection of *L. monocytogenes* in Meat Products

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Introduction

Listeria monocytogenes is a high-risk foodborne pathogen that is frequently associated with meat products [5]. Methods for its assay generally involve cultural enrichment, followed by selective plating, biochemical confirmation, ELISA and PCR procedures [2;4]. These methods give presence or absence data, but do not provide information about the populations of the organism in the product.

Quantitative data are important to properly evaluate the degree of risk. Standard cultural methods may not provide complete or accurate ecological information about the occurrence of *Listeria* strains in samples because of the selective bias of enrichment procedures. This paper reports a novel centrifugation-plating method that detects and enumerates *Listeria monocytogenes* in meat products within 24-48 hours. Strain diversity isolated by this method was compared with that obtained by the standard cultural method.

Methodology

Food samples, purchased at retail outlets, were rinsed (chickens), or macerated in a Stomacher (salami and meat minces) with 225ml of Buffered Peptone Water (BPW). Particles of meat mince in macerates

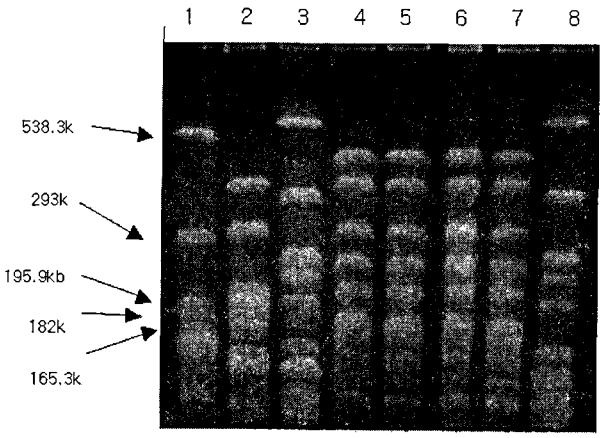

Fig. 1 Pulsed Field Gel Electrophoresis profiles of some *Listeria* isolates from chicken carcasses
Lanes: *L. monocytogenes* (LM-2) was used as a molecular size marker; 2 : isolate 40-3 (*L. monocytogenes*); 3 : isolate 47-10 (*L. welshimeri*); 4: isolate 48-2 (*L. monocytogenes*); 5: isolate 48-3 (*L. monocytogenes*); 6: isolate 48-4 (*L. monocytogenes*); 7:isolate 48-7 (*L. monocytogenes*); 8: isolate 48-6 (*L. welshimeri*);

were solubilized by incubation at 37°C for 30min with a mixture of proteolytic enzymes (1%) and Tween 80 (1%). Rinses or macerates of products were centrifuged (10,000rpm, 10min, and 10°C) to sediment and concentrate microbial cells. The concentrated biomass was spread-inoculated over large plates (150X20mm) of PALCAM agar, and incubated anaerobically at 37°C for 24 - 48hr. Confirmation of suspicious colonies as *L. monocytogenes* or *Listeria* species was performed by a multiplex PCR reaction according to the protocol of Bansal [1]. Confirmed *Listeria* isolates were subtyped by Pulsed Field Gel Electrophoresis (PFGE), based on the method of Destro et al. [3], with minor modifications. Some samples were also analyzed for *L. monocytogenes* by the Australian Standard method [6]. The protocol used is summarized in Figure 1.

Result & Discussion

Comparison of detection and enumeration of *L. monocytogenes* and *Listeria* species in minced meat products by the centrifugation-plating and standard cultural methods showed that the centrifugation-plating method gave a higher frequency of detection of *L. monocytogenes* and *Listeria* species, as well as population data, which ranged between 0 - 60 cfu/25g. The standard cultural method detected *L. monocytogenes* in two (10%) of the 20 samples tested. In contrast, centrifugation plating detected *L. monocytogenes* in five (25%) of the samples. *Listeria* species were found in 8 (40%) of the samples by the culture method, and 9 (45%) of the samples by centrifugation plating. However, there was one sample (No. 9) where *L. monocytogenes* was detected by the standard method but not by centrifugation plating.

Table 1 summarizes the populations of *L. monocytogenes* and other *Listeria* species found in chicken, salami, and meat mince samples. Of the 50 chicken carcasses examined, *L. monocytogenes* and other *Listeria* species were found on approximately 30% of samples. Most of the positive samples had counts in the range 1- 100 cfu/carcass, with about 10% in the range of 101 - 500 cfu/carcass. No chickens were found with *L. monocytogenes* or *Listeria* species counts exceeding 500cfu/carcass. Of the 50 samples of salami examined, only 1 (2%) showed the presence of *L. monocytogenes* at 172cfu/25g. However, 6% of the samples were positive for other *Listeria* spp. with counts of about 12 cfu/25g. Twenty five percent of mince meat samples contained *L. monocytogenes* - all in the range 1-100 cfu/25g. A higher proportion (45%) was positive for other *Listeria* spp., with 5% in the range 100-500 cfu/25g. No meat samples tested gave *L.*

Table 1. Populations of *Listeria* in meat samples determined by the centrifugation-plating method

Population (cfu) of *Listeria*[a]	Chicken (/carcass)		Salami (/25g)		Meat mince (/25g)	
	LM[b]	L[c]	LM	L	LM	L
None	34 (68%)	35 (70%)	49 (98%)	47 (94%)	15 (75%)	11 (55%)
1-100	10 (20%)	10 (20%)	0 (0%)	3 (6%)	5 (25%)	8 (40%)
101 -500	6 (12%)	5 (10%)	1 (2%)	0 (0%)	0 (0%)	1 (5%)
501 - 1000	0 (0%)	0 (0%)	0 (0 %)	0 (0%)	0 (0%)	0 (0%)

[a]: Data represent cfu/chicken carcass and cfu/25g sample of salami or meat mince.
[b]: *L. monocytogenes*
[c]: other *Listeria* species

monocytogenes and *Listeria* species with over 500 cells per carcasses or 25g.

More than 150 strains of *L. monocytogenes*, isolated from various food samples, were grouped into 43 pulsotypes using PFGE. Figure 1 shows an example of the banding patterns obtained from PFGE of the isolates. Generally, most isolates gave 8-14 bands, but usually 10 major bands were obtained in the range 100kb to 400kb.

Table 2 gives the diversity of *L. monocytogenes* strains isolated from samples that showed positive results by both the centrifugation-plating and Australian standard cultural methods. For the 3 samples examined, both methods gave similar strains (pulsotypes).

Table. 2 Diversity (pulsotypes) of *L. monocytogenes* strains isolated from foods by centrifugation-plating and Australian standard cultural methods

Sample	Pulsotype	
	Centrifugation-plating method	Australian standard cultural method
Minced meat	A, B	A, B
Whole chicken	C	C
Whole chicken	D	D

Conclusion

The novel centrifugation-plating method combined with PCR confirmation of isolated colonies could successfully detect and enumerate *L. monocytogenes* and other *Listeria* species from meat samples. Generally, quantitative, population data could be obtained within 48 hours, which is faster than the standard cultural method that gives data in 4-5 days. The qualitative recovery of *Listeria* from these products was comparable or better than that obtained by standard cultural methods. Pulsed field gel electrophoresis revealed significant heterogeneity in strains of *L. monocytogenes* recovered from meat products.

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