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Using RAPD-PCR as molecular assessment on the performance of CHROMAgar™ *Listeria* and PALCAM agar on isolation of *Listeria* spp. and *L. monocytogenes* from foods

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Abstract: In this study, *Listeria* spp. were isolated from naturally contaminated samples of beef burger, minced beef and sliced cheese using PALCAM and CHROMagar™. Samples were enriched with FDA-BAM method and plated on PALCAM and CHROMagar™ *Listeria* before confirmation using PCR on *hlyA* and LLO toxin genes specific to *L. monocytogenes*. Identification of isolates showed a total of 45 isolates of *Listeria* spp. and two *L. monocytogenes*. All the 47 isolates were then subjected to RAPD analysis using two oligomers (OPA14 and OPA15) and fingerprint clustering was able to cluster the *L. monocytogenes* from *Listeria* spp. based on isolation from agar types as well as *L. monocytogenes* from *Listeria* spp. Studies showed that OPA14 and OPA15 are useful for rapid discrimination of *Listeria* spp. and *L. monocytogenes*. The differences observed on the isolation of *Listeria* spp. from PALCAM and CHROMagar™ *Listeria* that may have an impact on epidemiological studies.

Keywords: *L. monocytogenes*, RAPD fingerprinting, PALCAM agar, CHROMagar™ *Listeria*

Introduction

Listeria monocytogenes is a major concern in food safety because it can cause human listeriosis with mortality rate of up to 25% in susceptible individuals (Farber and Peterkin, 1991). *L. monocytogenes* is Gram positive, rod-shaped, with 13 serovar designations (Seeliger, 1958; Donker-Voet, 1972) which are 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7. Although the 13 serovars have been associated with outbreaks, serovar 1/2a, 1/2b and 4b are the causative agents for most human listeriosis (Farber and Peterkin, 1991). In *L. monocytogenes*, three lineages have been established which are lineage 1 (serotypes 1/2b, 3b, 4b, 4d and 4c), lineage II (1/2a, 1/2c, 3a and 3c) and lineage III (4a and 4c) (Nadon *et al*., 2001) which indicate the diversity of strain as well as the potential competitive fitness among strains.

L. monocytogenes is ubiquitous in environment and it is found in silage, sewage and farms (Farber and Peterkin, 1991; Hoff, 2003) but human listeriosis has been associated with food safety because of contamination in various types of foods especially ready-to-eats (Sahilah *et al.*, 2010; Yousr *et al*., 2010). This is because such products are not subjected to thermal treatments and can be kept refrigerated that allow opportunities for the pathogen to grow to a dangerous level.

The increasing frequency of literature on evaluation of the existing protocols and methods for isolation and detection of *L. monocytogenes* (Busch and Donnelly, 1992; Ryser *et al*., 1996; Bruhn *et al*., 2005) underline the importance of detection and isolation method for this bacterium in foods regardless for food safety or epidemiological studies purposes. Identification and isolation of *L. monocytogenes* have been traditionally isolated on various agars such as PALCAM (polymyxin-acriflavine-lithium-chloride-ceftazidime-aesculin-mannitol) agar, modified Oxford agar, ALOA™, CHROMagar™ *Listeria*, blood agar and etc. Identification of isolates on agar is dependent on the substrate utilization such as cleavage of PI-PLC of L-α-phosphatidyl-inositol to form a white precipitation zone around colony in combination of chromogenic substrate 5-bromo-4-chloro-3-indoxyl-β-D-glucopyranoside applied in CHROMagar™ *Listeria*, and cleavage of aesculin and breakdown of the product, aesculitin with ferric ion to form grayish-greenish colonies with brown-black halo center in PALCAM agar. The differences in the substrate utilization and selectivity of the agar can also play

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a role in differentiating the genotypic characteristics and can be traced via molecular methods.

Randomly amplified polymorphic DNA (RAPD) is a rapid and highly promising tool for discrimination of *L. monocytogenes* strains (Boerlin *et al*., 1995). Application of RAPD in epidemiology studies have been widely used (Giovannacci *et al*., 1999) and extended into other studies for validation or comparison of methods for the reproducibility of published primers for *L. monocytogenes* detection (Aznar and Alarcon, 2002). Other than that, investigations on the association of isolates in foods and clinical incidences showed the diversity of *L. monocytogenes* was unable to cluster the causative agent to the source (Martinez *et al*., 2003; Aurora *et al*., 2009). The epidemiological tracing may be affected by enrichment media or agar as found in some studies. For example, studies by Gracieux *et al*. (2003), showed that selective agars such as PALCAM, Oxford, Rapid *L. mono*(RLM) and ALOA *Listeria* has higher count of virulent *L. monocytogenes* compared to the nonvirulent strains while Bruhn *et al*. (2005) showed the bias in the Universiti of Vermont broth enrichment in Lineage 2 *L. monocytogenes*. Due to the potential bias that may exist in enrichment broth, this study extends the knowledge to investigate the potential bias on PALCAM and CHROMagar™ *Listeria*. To the best of our knowledge, the exact profile on the preferences of *Listeria* spp. isolation based on these two agars have not been carried out and this represent the first study on differentiating the *Listeria* spp. and *L. monocytogenes* isolated on PALCAM and CHROMagar from the same sample via molecular fingerprinting. Therefore, the objective of the study is to conduct the assessment on the preference of PALCAM and CHROMagar™ *Listeria* in isolation of *Listeria* spp. in food samples using RAPD fingerprinting.

Materials and Methods

Sampling and enrichment

Total of twenty samples (4 cheeses, 8 beef burgers and 8 minced beefs) were purchased randomly from supermarkets for isolation and detection of *L. monocytogenes*. Sample processing was carried out based on FDA-BAM method. Briefly, 10 g of sample was homogenized with Buffered *Listeria* Enrichment broth (BLEB) for 4 h at 30°C followed by the addition of selective supplements (50 mg/L cycloheximide, 15 mg/L acrifilavine HCl, 40 mg nalidixic acid) and further incubated at 30°C up to 48 h. Each sample was then streaked on PALCAM and CHROMagar™ *Listeria* before incubated at 30°C and 37°C for 48 and 24 h respectively. Single isolates were selected from the plates and further streaked on similar agar to ensure pure single colony.

DNA extraction, PCR, RAPD and gel electrophoresis

Single colonies obtained from the agar were streaked on TSAyeast (0.6% yeast) and incubated overnight in 37°C for 12 – 16 h. Colonies grown on the TSA were collected in sterile 1.5 mL microcentrifuge tube with 1 mL of sterile distilled water. Tubes were centrifuged at 12,000 rpm for 3 min with a washing step. Pellets were collected and resuspended with 400 µL of sterile distilled water and homogenized with vortex until no clumps were observed. The tubes were transferred into a dry bath at 100°C for 15 min and quickly transferred into -20°C for 15 min for crude cell lysis method for DNA extraction (Lee *et al*., 2009; Ponniah *et al*., 2010). Tubes were then subjected to cenfrifugation at 10,000 rpm for 5 min and supernatant was transferred into new sterile tubes and kept at 4°C to be used while preparation of PCR cocktail was carried out.

Identification of *Listeria* spp. and *L. monocytogenes* was carried out using primers: L1-U1 (*Listeria* spp.), LM1-LM2 (*L. monocytogenes*) as multiplex identification and multiplex toxin genes detection in *L. monocytogenes* (IAP1-IAP2) and (LMA-LMB) primers. PCR profiles and cocktails were prepared as shown in Table 1. Twenty five oligomers were screened for RAPD and the primers with most bands were selected to be used for RAPD fingerprinting. OPA 14 and OPA 15 provided best band on *L. monocytogenes* ATCC 19112 (serovar 1/2c from human) control culture therefore these two primers were used for RAPD fingerprinting of the isolates.

Ten µL of RAPD-PCR products was pipetted into the agarose gel (1.0%) using 0.5× TBE buffer and subjected to gel electrophoresis for 1 h 52 min at 80 V and 1 kB ladder (PROMEGA, USA) was used as the DNA marker reference. Gel viewing was carried out using GeneSnap (SYNgene, USA) and gel pictures were saved as TIFF file for further analysis on GelCompar II (Applied Maths, Belgium).

Analysis of RAPD fingerprinting

Gel image was carried out based on manual by GelCompar II (Applied Maths, Belgium). The dendogram was constructed using Pearson's correlation and UPGMA on similarities of bands. Individual dendogram was constructed based on OPA 14 and OPA 15 and a combination of two oligomers for composite fingerprint profiling.

Calculation of discriminatory index (*D*) was

Table 1. Details of PCR primers, profiles and sequences used for identification and RAPD fingerprinting analysis of *Listeria* spp. and *L. monocytogenes*

Target	For	Sequence 5'-3'	Product size (bp)	PCR cocktail (25 µL per tube)	PCR profile	Reference
16sRNA	Identification of *Listeria* spp.	U1 – CAG CMG CCG CGG TAA TWC L1 - CTC CAT AAA GGT GAC CCT	916	1× GoTaq™ Green buffer 1.5 mM $MgCl_2$ 0.5 nmol primer (LM1, LM2) 1.0 nmol primer (U1, L1) Unit Taq Polymerase 4 µL DNA	94°C – 5' 30 cycles 94°C – 30 s 53°C – 45 s 72°C – 1' 30 s Final extension 72°C – 5'	Border *et al*., 1990
hly A	Identification of *L. monocytogenes*	LM1- CCT AAG ACG CCA ATC GAA LM2-AAG CGC TTG CAA CTG CTC	702			Border *et al*., 1990
Invasion-associated protein	*L. monocytogenes iap* toxin	IAP1 - ACA AGC TGC ACC TGT TGC AG IAP2 - TGA CAG CGT GTG TAG TAG CA	131	1× GoTaq™ Green buffer 1.5 mM $MgCl_2$ 2 mM dNTP 0.5 nmol each primer 0.5 Unit Taq Polymerase 4 ul DNA	94°C – 2' 30 cycles 94°C – 30 s 58°C – 45 s 72°C – 1' 30 s Final extension 72°C – 5'	Kohler *et al*., 1990
Listeriolysin O (–α haemolysis, listeriolysin O)	*L. monocytogenes LLO* toxin	LMA – CGG AGG TTC CGC AAA AAA TG LMB – CCT CCA CAG TGA TCG ATG TT	234			Furrer *et al*, 1991
DNA	RAPD	OPA14 - TGT GTG CTG G		1× GoTaq™ Green buffer 4.5 mM $MgCl_2$ 5 mM dNTP 50mM primer 0.5 Unit Taq Polymerase 4 ul DNA	94°C – 4' 45 cycles 94°C – 1' 36°C – 1' 72°C – 2' Final extension 72°C – 4'	This study
DNA	RAPD	OPA 15 - TTC CGA ACC C				

based on the following formula according to Hunter and Gaston (1998):

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} nj\ (nj-1)$$

where D is the discrimination index, N is the total number of strains in sample population, *s* is total number of types described, and n_j is the number of strain belonging to the *j*th type.

Results and Discussion

In present study, a total of 20 samples were appraised for detection of *L. monocytogenes* using microbiological plating method. With regards to the small number of sample, it should be noted that the emphasis of study was placed on the differences in the isolation preference based on the two selective agars, PALCAM and CHROMagar. Prevalence of *L. monocytogenes* in the samples was 5% (1/20) in PALCAM agar and none detected in CHROMagar.

During the isolation procedure, more single colonies were observed on PALCAM agar compared to CHROMagar (data not shown). This could be attributed to several factors such as the additional selective agents (nalidixic acid to suppress gram-negative bacteria and acriflavin for suppression of other gram positive bacteria) in PALCAM that reduces the competing flora in the food matrix while CHROMagar is known to be highly sensitive to differentiate *L. monocytogenes* from *L. innocua* with formation of white halo around the colony. The description of the identified 47 isolates were summarized in Table 2 to indicate reference key, source, type of samples, identification and agar used for isolation.

Three dendrograms were generated from the RAPD fingerprinting; OPA14 (Figure 1) and OPA15 (Figure 2) individually and combination of OPA14 and OPA15 (Figure 3). The discriminatory index for OPA14 was 0.492 and OPA15 at 0.930. The dendogram generated showed similarity of 30% to 100% for combination of primers, 40% to 98% for OPA 15 and 30 % to 100% for OPA14. Increasing the number of primers or combination of fingerprinting methods such as PFGE or restriction enzymes can enhance the discriminatory index between the strains (Chiu *et al*., 2010; Sahilah *et al*., 2010; Yousr *et al*., 2010; Sujaya *et al*., 2010). However, in current study; high discriminatory index was achieved with single oligomer OPA15 as it was able to distinguish between the two isolates of *L. monocytogenes* and resulted in 18 types of patterns. When comparing the population of *Listeria* spp. in samples, the isolates from the cheese samples were more heterogenous compared to the beef samples as the isolates were not clustered together although they were isolated with the same agar from the sample.

Differences in the isolation of *Listeria* spp. and *L. monocytogenes* from the agar can be due to many factors such as the various serotypes or strains, types of cell surface antigens and physiological differences (recovery from temperature stress) (Buncic *et al*.,

Table 2. Descriptions on the key, reference sample, type of sample, identification of isolates, agar for isolation and pattern types obtained in RAPD fingerprinting of *Listeria* spp. and *L. monocytogenes*

Key	Reference	Brand	ID	Agar	Pattern type	
					OPA14	OPA15
1	2	Cheese – A	*Listeria* spp.	PALCAM	II	VII
2	2	Cheese – A	*Listeria* spp.	PALCAM	I	VII
3	2	Cheese – A	*Listeria* spp.	PALCAM	I	VIII
4	2	Cheese – A	*Listeria* spp.	PALCAM	I	VII
5	11	Beef burger - B	*Listeria* spp.	PALCAM	I	X
6	11	Beef burger - B	*Listeria* spp.	PALCAM	I	X
7	11	Beef burger - B	*Listeria* spp.	PALCAM	I	X
8	11	Beef burger - B	*Listeria* spp.	PALCAM	I	VII
9	11	Beef burger - B	*Listeria* spp.	PALCAM	I	XI
10	12	Beef burger - B	*Listeria* spp.	PALCAM	I	IV
11	12	Beef burger - B	*Listeria* spp.	PALCAM	I	IX
12	12	Beef burger - B	*Listeria* spp.	PALCAM	I	XVI
13	12	Beef burger - B	*Listeria* spp.	PALCAM	I	XVII
14	13	Beef burger - B	*Listeria* spp.	PALCAM	I	XVII
15	13	Beef burger - B	*Listeria* spp.	PALCAM	I	XVII
16	13	Beef burger - B	*Listeria* spp.	PALCAM	I	XVIII
17	13	Beef burger - B	*Listeria* spp.	PALCAM	I	XVIII
18	14	Beef burger - B	*Listeria* spp.	PALCAM	I	IX
19	14	Beef burger - B	*Listeria* spp.	PALCAM	IV	VI
20	15	Beef burger - B	*L. monocytogenes*	PALCAM	VI	XII
21	15	Beef burger - B	*L. monocytogenes*	PALCAM	VII	I
30	16	Beef burger - B	*Listeria* spp.	PALCAM	II	X
31	16	Beef burger - B	*Listeria* spp.	PALCAM	II	X
32	16	Beef burger - B	*Listeria* spp.	PALCAM	II	XVIII
33	16	Beef burger - B	*Listeria* spp.	PALCAM	I	X1
34	17	Beef burger - B	*Listeria* spp.	PALCAM	I	XIV
35	17	Beef burger - B	*Listeria* spp.	PALCAM	I	X
36	17	Beef burger - B	*Listeria* spp.	PALCAM	I	XIII
37	17	Beef burger - B	*Listeria* spp.	PALCAM	I	XIII
38	19	Beef burger - B	*Listeria* spp.	PALCAM	I	XIII
39	19	Beef burger - B	*Listeria* spp.	PALCAM	III	VII
40	19	Minced beef – C	*Listeria* spp.	PALCAM	III	XV
41	19	Minced beef – C	*Listeria* spp.	PALCAM	III	VII
42	19	Minced beef – C	*Listeria* spp.	PALCAM	III	VII
43	19	Minced beef – C	*Listeria* spp.	PALCAM	III	VII
44	20	Minced beef – C	*Listeria* spp.	PALCAM	III	XV
45	20	Minced beef – C	*Listeria* spp.	PALCAM	V	XV
I	12	Beef burger - B	*Listeria* spp.	CHROMagar™ *Listeria*	I	VI
J	12	Beef burger - B	*Listeria* spp.	CHROMagar™ *Listeria*	I	III
Q	15	Beef burger - B	*Listeria* spp.	CHROMagar™ *Listeria*	I	II
R	15	Beef burger - B	*Listeria* spp.	CHROMagar™ *Listeria*	I	II
S	15	Beef burger - B	*Listeria* spp.	CHROMagar™ *Listeria*	I	II
U	15	Beef burger - B	*Listeria* spp.	CHROMagar™ *Listeria*	I	II
V	15	Beef burger - B	*Listeria* spp.	CHROMagar™ *Listeria*	I	II
W	15	Beef burger - B	*Listeria* spp.	CHROMagar™ *Listeria*	I	V
X	15	Beef burger - B	*Listeria* spp.	CHROMagar™ *Listeria*	I	V
Y	15	Beef burger - B	*Listeria* spp.	CHROMagar™ *Listeria*	I	V

2001). Nonetheless, studies by Gorski *et al*. (2006) indicated that the competitive fitness between strains were also dependent on the isolation sources such as enrichment procedure containing antibiotics that facilitates isolation of *Listeria* spp. from samples. It has been postulated that the differences in genotypic and phenotypic characteristics are highly related to the serotypes, however, Gorski *et al*. (2006) concluded in their studies that competitiveness between the serotype tested 1/2a and 4b were not correlated to serotype. To overcome the variability in exposure to different types of enrichment media, this study used only FDA-BAM method of BLEB. Therefore, the RAPD fingerprinting represents the differences of colonies isolated from each selective agar.

Finding from this study suggests that PALCAM and CHROMagar may play a role in the unequal representation of *Listeria* spp. and possibly *L. monocytogenes* during isolation. The bias that exists in protocols and the media used can lead to hindrance in epidemiological investigations particularly during outbreaks in food contamination. Data also supplements the important findings from Bruhn *et al*. (2005) that the selectivity of broths may misrepresent the actual subset of *Listeria* spp. in foods, bias in selectivity of agar will magnify the misrepresentation.

Human listeriosis is a severe consequence due to the consumption of contaminated foods and implementations of appropriate protocols should be carefully reviewed. Suggestion on the improvement to reduce the bias may be done by plating on more than one agar for each sample particularly during an investigation for outbreaks.

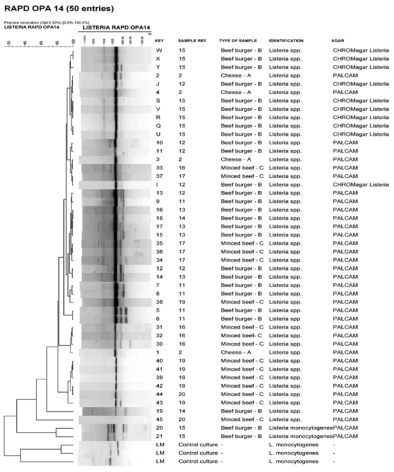

Figure 1. Dendogram clustering by RAPD OPA14 using Pearson correlation

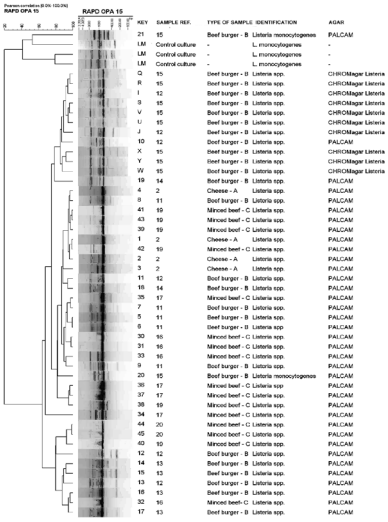

Figure 2. Dendogram clustering for RAPD OPA15 using Pearson correlation

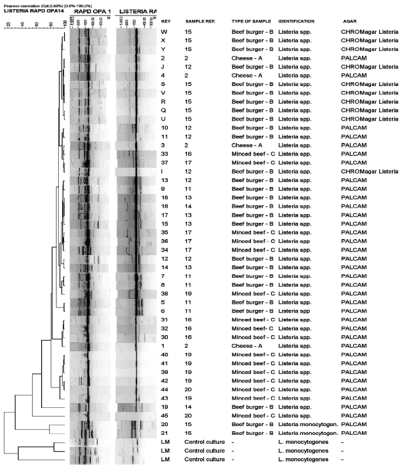

Figure 3. Dendogram clustering combination of RAPD OPA14 and OPA15 for *L. monocytogenes* isolates from PALCAM and CHROMagar™ *Listeria*

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References

Aurora, R., Prakash, A. and Prakash, S. 2009. Genotypic characterization of *Listeria monocytogenes* isolated from milk and ready-to-eat indegenous milk products. Food Control 20: 835-839.

Aznar, R. and Alarcon, B. 2002. On the specificity of PCR detection of *Listeria monocytogenes* in foods: a comparison of published primers. Systematic and Applied Microbiology 25: 109-119.

Boerlin, P., Bannerman, E., Ischer, F., Rocourt, J. and Bille, J. 1995. Typing *Listeria monocytogenes*: a comparison of random amplification on polymorphic DNA with 5 other methods.Research in Microbiology 146: 35- 49.

Border, P. M., Howard, J. J., Plastow, G. S., Siggens, K., W. 1990. Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. Letters in Applied Microbiology 11: 158 – 162.

Buncic, S., Avery, S. M., Rocourt, J. and Dimitrijevic, M. 2001. Can food-related environemtal factors induce different behavior in two key serovars, 4b and 1/2a, of *Listeria monocytogenes*? International Journal of Food Microbiology 65: 201-212.

Busch, S. V. and Donnelly, C. W. 1992. Development of a repair-enrichment broth for resuscitation of heat-injured *Listeria monocytogenes* and *Listeria innocua*. Applied and Environmental Microbiology 58(1): 14-20.

Chiu, T,-H., Pang, J, -C., Chen, M,-H. and Tsen, H,-Y. 2010. Improvement of strain discrimination by combination of RAPD with PFGE for the analysis of swine isolates of *Salmonella enterica* serovar Choleraesuis. World Journal of Microbiology and Biotechnology. DOI:10.1007/s11274-010-0467-7.

Donker-Voet, J. 1972. *Listeria monocytogenes*: some biochemical and serological aspects. Acta Microbiologica Academiae Scientiarium Hungaricae 19: 287-291.

Farber, J. M. and Peterkin, P. I. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbioy Review 55: 476-511.

Furrer, B., Candrian, U., Hoefelein, C. and Luethy, J. 1991. Detection and identification of *Listeria monocytogenes* in cooked sausage products and milk by *in vitro* amplification of haemolysin gene fragments. Journal of Applied Bacterioloy 70: 372 –379.

Giovannacci, I., Ragimbeau, C., Queguiner, S., Salvat, G., Vendeuvre, J. –L., Carler, V. and Ermel, G. 1999. *Listeria monocytogenes* in pork slaughtering and cutting plants use of RAPD, PFGE and PCR-REA for tracking and molecular epidemiology. International Journal of Food Microbiology 53: 127-140.

Gorski, L., Flaherty, D. and Mandrell, R. E. 2006. Strains in mixed cultures with and without food in the U.S. Food and Drug Administration enrichment protocol. Applied and Environmental Microbiology 72(1): 776-783.

Gracieux, P., Roche, S., M., Pardon, P. and Velge, P. 2003. Hypovirulent *Listeria monocytogenes* strains are less frequently recovered than virulent strains on PALCAM and Rapid' *L. mono* media. International Journal of Food Microbiology 83: 133-145.

Hof, H. 2003. History and epidemiology of listeriosis. FEMS Immunology and Medical Microbiology 35: 199-202.

Hunter, P. R. and Gaston, M. A. 1988. Numerical index of the discriminatory ability of typing system: an application of Simpson's index of diversity. Journal of Clinical Microbiology 26: 2465 – 2466.

Kohler, S., Leimeister-Wachter, M., Chakraborty, T., Lottspeich, F. and Goebel, W. 1990. The gene coding for protein p60 of *Listeria monocytogenes* and its use as a specific probe for *Listeria monocytogenes*.

Infections and Immunity 58: 1943 –1950.

Lee, H. Y., Chai, L. C., Tang, S. Y., Selamat, J., Mohd Ghazali, F., Nakaguchi, Y., Nishibuchi, M. and Son, R.2009. Application of MPN-PCR for biosafety of *Bacillus cereus s.l.* in ready-to-eat cereals. Food Control 20: 1068-1071.

Martinez, I., Rorvik, L,-M., Brox, V., Lassen, J., Seppola, M., Gram, L. and Fonnesbech-Vogel, B. 2003. Genetic variability among isolates of *Listeria monocytogenes* from food products, clinical samples and processing environments, estimated by RAPD typing. International Journal of Food Microbiology 84: 285-297.

Nadon, C. A., Woodward, D. L., Young, C., Rodgers, F. G. and Wiedmann, M. 2001. Correlations between molecular subtyping and serotyping of *Listreia monocytogenes*. Journal of Clinical Microbiology 39: 2704-2707.

Ponniah, J., Tunung, R., Paie, M. S., Son, R., M. Ghazali, F., Cheah, Yk K., Nishibuchi, M., Nakaguchi, N. and Malakar, P. K. 2010. *Listeria monocytogenes* in raw salad vegetables sold at retail level in Malaysia. Food Control 21(5): 774-778.

Ryser, E. T., Arimi, S. M., Bunduki, M. C. and Donelly, C. W. 1996. Recovery of different *Listeria* ribotypes from naturally contaminated, raw refrigerated meat and poultry products with two primary enrichment media. Applied and Environmental Microbiology 62: 1781-1787.

Sahilah, A. M., Audrey, L. Y. Y., Ong, S. L., Wan Sakeenah, W. N., Safiyyah, S., Norrakiah, A. S., Aminah, A. and Ahmad Azuhairi, A. 2010. DNA profiling among egg and beef meats isolates of *Escherichia coli* by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) and random amplified polymorphic DNA-PCR (RAPD-PCR). International Food Research Journal 17: 853-866.

Seeliger, H. P. R. 1958. Listeriosen. Springer-Verlakg KG. Berlin.

Sujaya, I. N., Nocianitri, K. A. and Asano, K. 2010. Diversity of bacterial flora of Indonesian *ragi tape* and their dynamics during the *tape* fermentation as determined by PCR-DGGE. International Food Research Journal 17: 239-245.

Yousr, A. H., Napis, S., Rusul, G. R. A., Alitheen, N. B. and Son, R. 2010. Molecular typing of *Aeromonas* species using RAPD and ERIC-PCR fingerprinting. International Food Research Journal 17: 691-698.