International Food Research Journal 23(6): 2702-2709 (December 2016)

Journal homepage: *http://www.ifrj.upm.edu.my*

International FOOD RESEARCH Journal

Detection of *Listeria monocytogenes* in foods using monoclonal antibody by dot-ELISA and multiplex-PCR

[1]Prommajan, K., [1,2]Palaga, T. and [1,2*]Rengpipat, S.

[1]*Program in Biotechnology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*

[2]*Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*

Article history

Received: 8 December 2015
Received in revised form: 23 February 2016
Accepted: 8 March 2016

Keywords

Listeria monocytogenes
Listeria spp
Monoclonal antibody
Dot-ELISA
Multiplex-PCR

Abstract

Monoclonal antibodies (MAbs) against *Listeria monocytogenes* were raised using the NCTC 11994 and EGD-e isolates of *L. monocytogenes* to immunize BALB/c mice. The antigenic protein of 65 kDa from *L. monocytogenes* EGD-e was found to react with MAb LMF3-238. This MAb characterized as IgG1 specifically reacted with 11 out of 12 different strains of *L. monocytogenes*, covering serotypes 1/2a, 1/2b, 1/2c, 4a and 4b that are virulent markers. MAb LMF3-238 detected *L. monocytogenes* EGD-e after inoculation into pork meat and storage at 4°C for 36 hours at a limit of detection of ~10^6 colony forming units/ml by dot-ELISA. From 311 *Listeria* spp. colonies, isolated from a total of 50 different fresh and processed foods and randomly selected from PALCAM agar after enrichment, *L. monocytogenes* was identified by MAb LMF3-238 using dot-ELISA with relative accuracy, relative sensitivity and relative specificity of 95.2%, 92.5% and 98.0%, respectively, compared to the multiplex-PCR method.

© All Rights Reserved

Introduction

The genus *Listeria* is comprised of seventeen species (Euzéby, 1997) of small Gram-positive facultative anaerobic and non-spore forming rod bacteria (Jadhav *et al*., 2012). All *Listeria* species are widely found in nature as well as in many food industry-related applications. The species of most concern in the food industry is *Listeria monocytogenes*, which may appear coccoid or in pairs. They motile at 20°C and can grow and survive in extreme conditions, including at a temperature range of 1–45°C, pH 4.3–9.6 and high salt concentrations (Roberts and Wiedmann, 2003). Recently *L. monocytogenes* has become an important food-borne pathogen, causing human listeriosis with a 17% case-fatality rate (Scallan *et al*., 2011). After ingestion of contaminated food, *L. monocytogenes* disseminates from the intestinal lumen to the central nervous system and the fetoplacental unit. The clinical features of listeriosis include meningitis, septicemia, meningoencephalitis, perinatal infections, gastroenteritis and spontaneous abortions in humans. It can cause severe symptoms in certain groups of people, such as pregnant woman, neonates and elderly people. The bacteria can be transmitted in ready-to-eat foods as well as raw retail food products. Implicated food associated outbreaks of listeriosis have included coleslaw, pasteurized milk and soft cheese (Linnan *et al*., 1988; Swaminathan and Gerner-Smidt, 2007). Other horizontal transmission routes of listeriosis are from human to human through contact with infected urine, feces, conjunctival and nasal secretions, epidermal pus and blood. Its presence in the environment can lead to food contamination during processing on the belt line in the processing plant (Blatter *et al*., 2010), since *L. monocytogenes* exists in soil and water and can survive in processed food for a long time after contamination of the food.

Thailand is one of the world's top ten producers and exporters of food, including processed food products. According to the National Food Institute of Thailand, the total food exported in 2013 was valued at US$ 30.3 billion, with over US$ 7 billion during the first quarter of 2014 (Thailand Board of Investment, 2014). Examination of raw material, in-process and finished goods for *L. monocytogenes*, including environmental samples in the food chain, have to be performed routinely in order to reduce the chance of contaminated foods reaching the public. Thus, a simple and rapid test for the isolation and identification of *L. monocytogenes* is required. In this study specific monoclonal antibodies (MAbs) against *L. monocytogenes* were produced and their accuracy, specificity and sensitivity in the identification of *L. monocytogenes* by dot enzyme-linked

*Corresponding author.
Email: *srengpipat@gmail.com*

immunosorbent assay (dot-ELISA) were evaluated in comparison with the multiplex polymerase chain reaction (multiplex-PCR) approach.

Materials and Methods

Media and reagents

Tryptic soy broth (TSB), tryptic soy agar (TSA), polymyxin-acriflavine-lithium-chloridecftazidime-aesculin-mannitol medium (PALCAM) and UVM modified *Listeria* enrichment broth (UVM-LEB) were from Difco Laboratories (Franklin Lakes, NJ, USA). Complete and incomplete Freund's adjuvants, 3, 3´-diaminobenzidine tetrahydrochloride (DAB), polyethylene glycol (PEG) and phosphate buffer saline (PBS) were from Sigma-Aldrich (St. Louis, MO, USA). Goat-anti-mouse IgG-horseradish peroxidase (GAM-HRP) was from Jackson Immuno Research Laboratories (West Grove, PA, USA). The Bradford protein assay and nitrocellulose membrane was from Bio-Rad (Hercules, CA, USA). Myeloma cells P3X63Ag8 (ATCC TIB-9) were purchased from the American Type Culture Collection (Manassas, VA, USA).

Bacterial strains

The bacterial sources and strains in this study were: *Listeria monocytogenes, Listeria ivanovii, Listeria innocua, Listeria grayi, Listeria seeligeri, Listeria welshimeri, Bacillus cereus* ATCC 1729, *Rhodococcus equi* DMST 17256, *Escherichia coli* ATCC 25922 and *Salmonella* Typhimurium ATCC 13311 as indicated in Table 2.

Antigen preparation and immunization

The *L. monocytogenes* NCTC 11994 and EGD-e strains were prepared as antigens for immunization in the form of heat-killed plus sonicated and heat-killed plus sodium dodecyl sulfate (SDS)-mercaptoethanol treated forms, respectively as previously described (Khamjing *et al*., 2011). All of the antigens were divided into several aliquots and stored at –20°C until use.

A group of 7 eight-week-old female BALB/c mice (National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand) were injected intra-peritoneally with 100 µl of the antigen mixture in an equal volume of Complete Freund's adjuvant using *L. monocytogenes s*train NCTC 11994 for mice numbers 1 and 2 and strain EGD-e for mice numbers 3–7. Subsequently, immunization was performed three more times at two-week intervals with the same antigen mixed in incomplete Freund's adjuvant. The antisera titer was determined by dot-ELISA and Western blotting. All procedures involving laboratory animals were approved and conducted in accordance with the guidelines of the Institutional Animal Care and Use Committees, Institute of Biotechnology and Genetic Engineering at Chulalongkorn University, Bangkok, Thailand (Protocol No. 1161001).

Hybridoma production

The cell fusion protocols were performed as previously reported (Khamjing *et al.*, 2011). Briefly, the P3X63Ag8 myeloma cells were fused with spleen cells using 50% (w/v) PEG. Hybridoma culture supernatants from each clone were screened for antibodies against *L. monocytogenes* by dot-ELISA. Positive cultures were cloned by limiting dilution until monoclones were obtained. The isotypes of the mouse immunoglobulins produced by the hybridoma cells were determined by sandwich ELISA using Sigma's mouse MAb isotyping kit (Sigma-Aldrich) according to the manufacturer's instructions.

Dot enzyme-linked immunosorbent assay (dot-ELISA)

Bacteria were cultured in TSB at 37°C for 16–18 hours, harvested by centrifugation (6,230 g for 10 minutes at 4°C), and then washed three times in PBS. The cell pellets were resuspended in PBS and adjusted to an optical density of 1.0 at 600 nm (1 cm light path). An aliquot of the cell suspension was heat-killed at 60°C for 60 minutes to inactivate the bacteria. Heat-killed bacteria were used for screening and the cross-reactivity test. Each bacterial sample (1 µl/spot) was spotted onto a nitrocellulose membrane, dried at 37°C for 30 minutes, blocked in PBST (PBS with 0.05% (v/v) Tween 20) containing 5% (w/v) skim milk (PBST-M) and then probed with the hybridoma supernatant of each MAb at a 1:10 dilution in PBST-M for 3 hours or overnight at 4°C. After washing in PBST, the membrane was incubated with GAM-HRP at a 1:3,000 dilution for 2 hours, washed and developed in DAB substrate solution. The positive result was appeared as dark spot.

For the sensitivity test, two-fold serial dilutions of heat-killed *L. monocytogenes* strains NCTC 11994 or EGD-e (from ~10^5 to ~10^9 colony forming units (CFU)/ml) were prepared in PBS and 1 µl of each dilution was assayed by dot-ELISA. The lowest bacterial cell concentration that gave a positive reaction was defined as the limit of detection (LOD). Data are representative of three repeat experiments.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis

The protein concentrations were measured using the Bradford protein assay (Bio-Rad). Thirty µg of the total protein in supernatant from either *L. monocytogenes* NCTC 11994 or *L. monocytogenes* EGD-e cell suspension after sonication and centrifugation at 9,820 g for preparation was separated by 8% SDS-PAGE as previously described (Laemmli, 1970). The electrophoresis was performed at 100 volts for 90 minutes. The proteins were transferred onto a nitrocellulose membrane, blocked in PBST-M and probed with the respective hybridoma culture supernatant (1:10 dilution). Then washed and developed with DAB substrate solution.

Artificially L. monocytogenes*-inoculated pork samples*

Pork meat, purchased from a local supermarket, was cleaned thoroughly with three times of sterile water and then 25 g of sample was homogenized in 215 ml of UVM-LEB. The *L. monocytogenes* EGD-e suspension 10 ml was inoculated into stomacher bags to a final concentration of 0 (negative control) and ~10^5 to ~10^9 CFU/g, a total of six bags and then incubated at 4°C for 48 hours. One ml of sample was removed at 0, 12, 24, 36 and 48 hours after inoculation and transferred to microtubes. After incubation at 60°C for 60 minutes and centrifugation at 9,730 g for 10 minutes, the cell debris was washed three times with PBS and resuspended in an equal volume of PBS prior to spotting 1 µl on to a nitrocellulose membrane. The membrane was then blocked and probed with MAb LMF3-238 for the dot-ELISA as mentioned above. Data are representative of three repeat experiments.

Multiplex polymerase chain reaction (multiplex-PCR)

Genomic DNA of bacteria was extracted using the boiled-cell method (Chai *et al*., 2007; Marian *et al*., 2012). The PCR assay was performed by following the procedures from previous report (Doumith *et al*., 2004) with the gene fragment specific oligonucleotide primer pairs including forward primer (F): 5′-AGGATGCATCTGCATTCAA-3′ and reverse primer (R): 5′-CGCCACACTTGAGATAT-3′ to amplify a 818 bp of *hly* gene (Thomas *et al*., 1991; Koo and Jaykus, 2000), F: 5′-GCTACAATTACACAAGATACTCC-3′ and R: 5′-GCTTCTTTTGAATTATAAGG-3′ to amplify a 2,189 bp of *inlA* gene (this study) and F: 5′-GCTGAAGAGATTGCGAAAGAAG-3′ and R: 5′-CAAAGAAACCTTGGATTTGCGG-3′ to amplify a 370 bp of *prs* gene (Doumith *et al*., 2004). Amplification was performed at 95ºC for 5 minutes, followed by 35 cycles of 95ºC for 60 seconds, 56ºC for 60 seconds and 72ºC for 150 seconds; and then a final 72ºC for 10 minutes. The PCR products were electrophoresed at 100 volts for 30 minutes in 2% (w/v) agarose-TAE gel, stained with ethidium bromide and visualized by UV-transillumination.

Isolation and identification of L. monocytogenes *from foods*

A total of 20 fresh vegetables and 30 processed meat products, representing fresh and processed foods, respectively, were purchased from Thai local supermarket in Bangkok, Thailand. The samples were cleaned with sterile water three times. Isolation of *Listeria* spp. was then performed following the standard method of ISO11290–1:1996. *Listeria* spp. colonies on PALCAM agar were randomly selected and subcultured to clonality and then screened for the presence of *L. monocytogenes* using MAb LMF3-238 by dot-ELISA and multiplex-PCR as described previously. The relative accuracy, relative specificity and relative sensitivity between the two methods were compared and see details in Table 3.

Results and Discussion

Characterization of MAbs against L. monocytogenes

In this study *L. monocytogenes* EGD-e (Garner *et al*., 2006) and *L. monocytogenes* NCTC 11994 (Bannister, 1987), a virulent strain that can cause severe symptoms in humans, were used as antigens for immunization. The obtained fourteen MAbs (Table 1) could be categorized into five groups based upon their cross reactivity pattern by dot-ELISA with all MAbs produced (Prommajan, 2015) and Western blot analysis (Figure 1). MAbs in group 1 consisted of one clone, namely LMF3-238, which reacted specifically to some strains of all *L. monocytogenes* tested and did not cross react with *Listeria* spp. and the other bacteria tested. MAb in group 2 (LMF1-3), MAbs in group 3 (LMF2-1, LMF2-2 and LMF2-3), MAbs group 4 (LMF7-129) and MAbs in group 5 (LMF5-39, LMF5-67, LMF5-116, LMF5-201, LMF5-260, LMF5-342, LMF6-22 and LMF6-64) reacted with some strains of *Listeria* spp. and did not cross react with other Gram-positive and Gram-negative bacteria tested. This confirmed our previous study (Prommajan, 2015) that illustrated the non-reactivity of these MAbs on several Gram-positive and Gram-negative bacteria of 5 genus, 17 genus with 25 species, respectively. However, the specificity

Table 1. Grouping and characteristics of MAbs against *L. monocytogenes*

Group	MAb	Immunogens/ Treatment	Isotype	LOD (CFU/ml)	Antigen Recognized (kDa)
1	**LMF3-238**	EGD-e/HSDS	IgG_1	~1.65×10^9	65
2	**LMF1-3**	NCTC11994/HS	IgG_{2a}	~4.40×10^8	10
3	**LMF2-1**, 2, 3	NCTC11994/HS	IgG_{2a}	~4.40×10^9	10
4	**LMF7-129**	EGD-e/HSDS	IgG_1	~1.65×10^9	65
5	LMF5-39, 342, LMF6-22	EGD-e/HSDS	IgG_1	~1.65×10^9	65
	LMF5-67, LMF6-64	EGD-e/HSDS	IgM	~8.25×10^8	65
	LMF5-116, 201, 260	EGD-e/HSDS	IgG_{2a}	~1.65×10^9	65

EGD-e/HSDS = *L. monocytogenes* EGD-e in heat-killed plus sodium dodecyl sulfate (SDS)-mercaptoethanol forms, NCTC11994/HS = *L. monocytogenes* in heat-killed plus sonicated forms, Limit of detection (LOD) are from only the representative MAb from each group (shown in bold type).

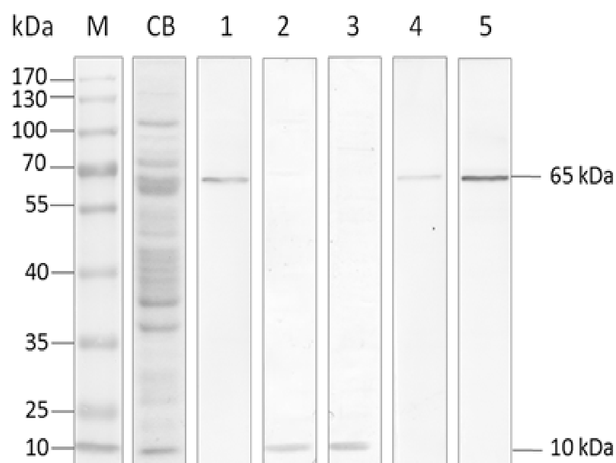

Figure 1. SDS-PAGE and Western blot analysis of antigens. The cell lysates of *L. monocytogenes* EGD-e (lanes CB; 1, 4 and 5) and *L. monocytogenes* NCTC11994 (lanes 2 and 3), at 30 µg protein/lane, were resolved by 8% (w/v) SDS-PAGE, stained with Coomassie brilliant blue and transferred to nitrocellulose membrane and then probed with a representative MAb from each of the five groups (see Table 1), as shown in lanes 1–5, respectively. M is a protein molecular weight marker. Blots shown are representative of three repeat experiments.

depended on individual MAbs (Table 2). Isotypes of MAbs were characterized as IgG2a for MAbs in groups 2, 3 and 5. IgG1 of some MAbs in group 1, 4 and 5, were detected. While IgM was the isotype found in MAbs of group 5 (Table 1). The LOD for *L. monocytogenes* was about ~10^8–10^9 CFU/ml (Table 1), which is in the same range as that using dot-ELISA for the detection of *Vibrio harveyi* (Phianphak *et al.*, 2005),*Vibrio parahaemolyticus* (Prompamorn *et al.*, 2013), and *Yersinia enterocolytica* (Khamjing *et al.*, 2011). Therefore, use of purified MAbs combined with the other detection methods such as nano-beads captured (Wang *et al.*, 2011) or tagging with fluorescence signal (Bruno *et al.*, 2015) are recommended to improve the sensitivity in *L. monocytogenes* detection.

Specificity of MAbs against L. monocytogenes

MAb groups 1, 4 and 5 reacted with an antigenic protein of 65 kDa while MAb group 2 and 3 detected a 10 kDa protein (Figure 1). The results indicate that ten out of fourteen MAbs recognize a 65 kDa protein band as specific to *L. monocytogenes.* This protein may have an epitope shared in *L. monocytogenes* and some *Listeria* spp. of which 66 kDa cell surface protein reacted with MAbs C11E9 and EM-7G1 as reported previously (Bhunia *et al.*, 1991; Bhunia and Johnson, 1992; Heo *et al.*, 2007). MAbs group 2 and 3 recognized only low molecular weight protein with very light smear ladder-liked pattern bands by Western blot. In addition, this protein gave a negative reactivity with MAbs group 2 and 3 by dot-ELISA while a whole cell of *L. monocytogenes* did positive (Prommajan, 2015). This evidence could be explained that these MAbs group 2 and 3 possibly recognized only conformational or native epitopes. In addition these MAbs group 2 and 3 were also obtained from mice No. 1–2 immunized with heat-killed plus sonicated forms which were not treated with chemical reagents. Moreover, these epitopes may be altered under the denaturing conditions (treated with 2-mercaptoethanol, DTT; dithiothreitol, SDS, and boil) from SDS-PAGE, therefore after transblotted to nitrocellulase membrane reactivity from those MAbs could not occur (data not shown). This confirmed the same results as reported previously (Goding, 1996; Lin *et al.*, 2006). Their specificity for the detection of different *L. monocytogenes* strains and different *Listeria* spp. are shown in Table 2. Only MAb LMF3-238 group 1 was specific for *L. monocytogenes,* and reacted with 11 of the 12 tested *L. monocytogenes* strains from serotypes 1/2a, 1/2b, 1/2c, 4a and 4b

Table 2. Specificity of MAbs against *Listeria* spp. and their genes

Bacterial strain (serotype)	MAb group					Multiplex PCR		
	1	2	3	4	5	*prs*	*hly*	*inlA*
L. monocytogenes EGD-e (1/2a)	+++	+	–	–	+++	+++	+++	++
L. monocytogenes V7 (1/2a)	+	+++	+++	++	+	+++	+++	+++
L. monocytogenes F4233 (1/2b)	+	+++	+	–	+	+++	+++	++
L. monocytogenes ATCC19112 (1/2c)	++	+++	+	++	++	+++	+++	+++
L. monocytogenes ATCC19114 (4a)	+	+++	+	+	–	+++	+++	+++
L. monocytogenes ScottA (4b)	+++	+	+	+	++	+++	+++	+++
L. monocytogenes F4393 (4b)	+	+	–	+	–	+++	+++	+++
L. monocytogenes NCTC11994 (4b)*	+++	++	+	++	+++	+++	+++	+++
L. monocytogenes ATCC19116 (4c)	–	+	–	++	–	+++	+++	+++
L. monocytogenes ATCC7644 (1/2c)	++	+++	++	+++	++	+++	+++	+++
L. monocytogenes DMST17303mu*	+++	++	+	+	+++	+++	+++	+++
L. monocytogenes DMST17303cu*	+++	++	++	+	+++	+++	+++	+++
L. ivanovii ATCC19119	–	+	+	–	+++	+++	–	–
L. ivanovii ATCC19119mu*	–	–	–	–	+	+++	–	–
L. ivanovii DMST9012*	–	+	+	–	+++	+++	–	–
L. innocua ATCC33090	–	++	–	+	+++	+++	–	–
L. innocua NCTC11288	–	+++	+++	+++	++	+++	–	–
L. innocua SLCC2745	–	++	+	–	+	+++	–	–
L. innocua DMST9011*	–	++	+	+	+++	+++	–	–
L. grayi LM37	–	+++	++	–	–	+++	–	–
L. seeligeri ATCC35967	–	+++	++	–	++	+++	–	–
L. welshimeri ATCC35897	–	–	–	+	++	+++	–	–
L. welshimeri 143	–	–	–	–	++	+++	–	–
B. cereus ATCC1729*	–	–	–	–	–	–	–	–
R. equi DMST17256*	–	–	–	–	–	–	–	–
E. coli ATCC25922*	–	–	–	–	–	–	–	–
S. Typhimurium ATCC13311*	–	–	–	–	–	–	–	–

+++ = intense staining, ++ = moderate staining, + = light staining, – = not detected, *prs* = phosphoribosyl pyrophosphate synthetase gene, *hly* = listeriolysin O gene and *inlA* = internalin A gene

Bacteria species codes are given in the text.

Note: Source of bacteria were given by Center for Food Safety, Department of Food Science, University of Arkansas, AR, USA except *that were from the Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

but not the one tested strain from *L. monocytogenes* serotype 4c. However, these *L. monocytogenes* serotypes detected by MAb LMF3-238 are the major virulent markers found in raw meat, meat products and fresh fish (Chaiyo, 2014; Obaidat *et al.*, 2015). In addition, MAbs from whole cell antigens from hybridoma production from previous report (Heo *et al.*, 2007) also showed some specificity with *L. monocytogenes* serotypes, but not reacted with 4c or 4e. Rare case of 4c and 4e serotypes of *L. monocytogenes* has been detected in food. Only 4c serotype of *L. monocytogenes* in fresh ham food (1%) was reported previously (Prencipe *et al.*, 2012). MAbs in groups 2–5 cross-reacted with other *Listeria* spp. Though the same sized protein was detected in groups 1, 4 and 5, the MAbs clearly detected a different antigen that, for groups 4 and 5, was shared across *Listeria* spp. (Bhunia and Johnson, 1992; Lin *et al.*, 2006; Heo *et al.*, 2007). The multiplex-PCR method confirmed the presence of *Listeria* spp. (*prs* gene) and specifically *L. monocytogenes* through the *hly* and *inlA* genes (Table 2) that code for listeriolysin O and internalin A, respectively (Thomas *et al.*, 1991; Koo and Jaykus, 2000) (Table 2). The prs gene codes for phosphoribosyl pyrophosphate synthetase and is commonly detected in all *Listeria* spp. (Doumith *et al.*, 2004). All three gene amplifications in this study therefore, could be used to identify *L. monocytogenes* and *Listeria* spp. by multiplex-PCR as standard techniques.

Table 3. Detection of natural *L. monocytogenes* contaminations in fresh and processed food, as determined by dot-ELISA and multiplex-PCR methods

Dot-ELISA	Multiplex-PCR	
	Positive	Negative
Positive	149 (a)	3 (b)
Negative	12 (c)	147 (d)

Relative accuracy= [(a+d)/n] × 100 = 95.2%; Relative sensitivity = [a/(a+c)]×100 = 92.5%; Relative specificity= [d/(b+d)] × 100 = 98.0%; n = 311 (modified from ISO16140:2003)

Detection of L. monocytogenes *in artificially contaminated pork samples*

Only MAb LMF3-238 was selected for use in the detection of *L. monocytogenes* in food samples due to its apparent specificity for *L. monocytogenes.* Chilled foods have become increasingly popular and represent a growing source of potential *L. monocytogenes* contamination that needs to be screened. Here, different *L. monocytogenes* inoculum levels were added into pork samples and incubated at 4°C to create artificially contaminated pork of known initial bacterial levels. Then, at every 12 hours from 0 to 48 hours after inoculation, samples were collected for detection using MAb LMF3-238 by dot-ELISA. The LOD for *L. monocytogenes* EGD-e in the homogenized pork sample was ~10^9 CFU/ml at the time of inoculation and decreased down to an equivalent initial inoculum level of ~10^6 CFU/ml after 36 hours culture of the homogenized sample in UVM-LEB (Figure 2a). The density pattern measured by Quality One Software from blot also confirmed the same LOD as shown in Figure 2b. By following ISO 11290–1:1996 standard method for *Listeria* spp. and *L. monocytogenes* identification it will require a considerably longer time (4 to 7 days) to obtain results than does by dot-ELISA (within 2 days). Primary and secondary enrichment steps with 24 then 48 hours, respectively are required to recover Listeria colonies on selective agar. More 48-72 hours on confirmation step is then performed for identification of *Listeria* spp. (ISO 11290–1:1996). In this study only one step with primary enrichment broth after inoculated was used, and then skipped forward to give results within 5 hours after primary enrichment step.

Detection of naturally L. monocytogenes*-contaminated foods*

Fresh vegetables and processed meat products were purchased from a local supermarket and screened for the presence of *L. monocytogenes* by following

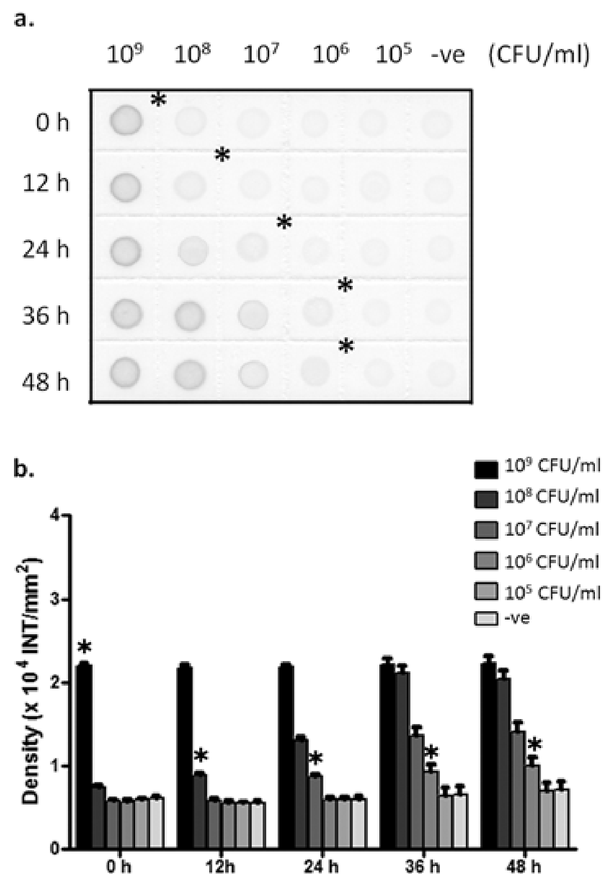

Figure 2. The detection of *L. monocytogenes* in pork meat artificially-contaminated with *L. monocytogenes* EGD-e using MAb LMF3-238 by dot-ELISA. (a) Representative blots are from triplicate trials. (b) The average ± 1SD density of the blots (three replicate blots), as measured using Quantity One Software (Bio-Rad, USA). h is hour and the asterisk indicates the LOD.

the ISO11290–1:1996 standard procedure. Specific appearance of *Listeria* spp. colonies were detected from 5 of 20 fresh vegetables (25%) and 10 of 30 processed meat products (33.33%) samples. From a total of 311 *Listeria* spp. colonies randomly selected from PALCAM agar and subcultured to clonality, a 95.2%, 92.5% and 98.0% relative accuracy, relative sensitivity and relative specificity, respectively, of *L. monocytogenes* detection was noted between the dot-ELISA using MAb LMF3-238 and muliplex PCR methods (Table 3). It implies that there is an acceptable accordance between the two methods. Dot-ELISA is simple, relatively cheap, consumes only 5 hours of time for detection and does not require a skilled person. However, using MAb LMF3-238 by dot-ELISA could only detect 11/12 tested strains, in contrast to the multiplex-PCR method, so this needs further examination. In addition, more verification and validation of the method has to be performed before applicable use.

Conclusions

We developed MAb LMF3-238 specific for pathogenic *L. monocytogenes*. It could be applied in dot-ELISA for *L. monocytogenes* detection in artificially contaminated pork meat. In this report MAbs to *L. monocytogenes* were produced and evaluated for their specific detection of isolated *L. monocytogenes* from fresh and processed foods using dot-ELISA with high relative accuracy, relative sensitivity and relative specificity compared to the multiplex-PCR method. In addition, dot-ELISA will be preliminarily helpful for large-scale screening for the qualitative, simple and rapid detection of *L. monocytogenes* or any other applications.

Acknowledgements

The authors sincerely thank Robert Butcher for revising the manuscript and providing many suggestions. This research was supported by grants from the Agricultural Research Development Agency (Public Organization) and the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund).

References

Bannister, B.A. 1987. *Listeria monocytogenes* meningitis associated with eating soft cheese. Journal of Infection 15: 165-168.

Bhunia, A.K., Ball, P.H., Fuad, A.T., Kurz, B.W., Emerson, J.W. and Johnson, M.G. 1991. Development and characterization of a monoclonal antibody specific for *Listeria monocytogenes* and *Listeria innocua.* Infection and Immunity 59: 3176-3184.

Bhunia, A.K. and Johnson, M.G. 1992. Monoclonal antibody specific for *Listeria monocytogenes* associated with a 66-kilodalton cell surface antigen. Applied and Environmental Microbiology 58: 1924-1929.

Blatter, S., Giezendanner, N., Stephan, R. and Zweifel, C. 2010. Phenotypic and molecular typing of *Listeria monocytogenes* isolated from the processing environment and products of a sandwich-producing plant. Food Control 21: 1519-1523.

Bruno, J.G., Phillips, T., Montez, T., Garcia, A., Sivils, J.C., Mayo, M.W. and Greis, A. 2015. Development of a fluorescent enzyme-linked DNA aptamer-magnetic bead sandwich assay and portable fluorometer for sensitive and rapid *Listeria* detection. Journal of Fluorescence 25: 173-183.

Chai, L.C., Robin, T., Ragavan, U.M., Gunsalam, J.W., Bakar, F.A., Ghazali, F.M., Radu, S. and Kumar, M.P. 2007. Thermophilic *Campylobacter* spp. in salad vegetables in Malaysia. International Journal of Food Microbiology 117: 106-111.

Chaiyo, S., 2014. Prevalence of *Listeria monocytogenes* in raw meat and meat products in some areas of Chiang Mai province. Chiang Mai, Thailand: Chiang Mai University, MSc thesis.

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C. and Martin, P. 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. Journal of Clinical Microbiology 42: 3819-3822.

Euzéby, J.P. 1997. List of bacterial names with standing in nomenclature: a folder available on the internet, Genus *Listeria*. 47: 590-592.

Garner, M.R., Njaa, B.L., Wiedmann, M. and Boor, K.J. 2006. Sigma B contributes to *Listeria monocytogenes* gastrointestinal infection but not to systemic spread in the guinea pig infection model. Infection and Immunity 74: 876-886.

Goding, J.W. 1996. Monoclonal antibodies: Principles and Practice. 3rd ed. London: Academic Press.

Heo, S.A., Nannapaneni, R., Story, R.P. and Johnson, M.G. 2007. Characterization of new hybridoma clones producing monoclonal antibodies reactive against both live and heat-killed *Listeria monocytogenes*. Journal of Food Science 72: M008-015.

ISO 11290-1:1996. Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* -- Part 1: Detection method. International Organization for Standardization, Geneva, Switzerland.

ISO 16140:2003. Microbiology of food and animal feeding stuffs -- Protocol for the validation of alternative methods. International Organization for Standardization, Geneva, Switzerland.

Jadhav, S., Bhave, M. and Palombo, E.A. 2012. Methods used for the detection and subtyping of *Listeria monocytogenes*. Journal of Microbiological Methods 88: 327-341.

Khamjing, W., Khongchareonporn, N. and Rengpipat, S. 2011. Detection by using monoclonal antibodies of *Yersinia enterocolitica* in artificially-contaminated pork. Microbiology and Immunology 55: 605-615.

Koo, K. and Jaykus, L.A. 2000. Selective amplification of bacterial RNA: use of a DNA primer containing mismatched bases near its 3' terminus to reduce false-positive signals. Letters in Applied Microbiology 31: 187-192.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Lin, M., Todoric, D., Mallory, M., Luo, B.S., Trottier, E. and Dan, H. 2006. Monoclonal antibodies binding to the cell surface of *Listeria monocytogenes* serotype 4b. Journal of Medical Microbiology 55: 291-299.

Linnan, M.J., Mascola, L., Lou, X.D., Goulet, V., May, S., Salminen, C., Hird, D.W., Yonekura, M.L., Hayes, P., Weaver, R., Audurier, A., Plikaytis, B.D., Fannin, S.L., Kleks, A. and Broome, C.V. 1988. Epidemic listeriosis associated with Mexican-style cheese. The New England Journal of Medicine 319: 823-828.

Marian, M.N., Sharifah Aminah, S.M., Zuraini, M.I., Son, R., Maimunah, M., Lee, H.Y., Wong, W.C.

and Elexson, N. 2012. MPN-PCR detection and antimicrobial resistance of *Listeria monocytogenes* isolated from raw and ready-to-eat foods in Malaysia. Food Control 28: 309-314.

Obaidat, M.M., Bani Salman, A.E., Lafi, S.Q. and Al-Abboodi, A.R. 2015. Characterization of *Listeria monocytogenes* from three countries and antibiotic resistance differences among countries and *Listeria monocytogenes* serogroups. Letters in Applied Microbiology 60: 609-614.

Phianphak, W., Rengpipat, S., Rukpratanporn, S., Longyant, S., Chaivisuthangkura, P., Sithigorngul, W. and Sithigorngul, P. 2005. Production of monoclonal antibodies for detection of *Vibrio harveyi*. Diseases of Aquatic Organisms 63: 161-168.

Prencipe, V.A., Rizzi, V., Acciari, V., Iannetti, L., Giovannini, A., Serraino, A., Calderone, D., Rossi, A., Morelli, D., Marino, L., Migliorati, G. and Caporale, V. 2012. *Listeria monocytogenes* prevalence, contamination levels and strains characterization throughout the Parma ham processing chain. Food Control 25: 150-158.

Prommajan, K., 2015. Production and characterization of monoclonal antibodies againt *Listeria monocytogenes.* Bangkok, Thailand: Chulalongkorn University, PhD thesis.

Prompamorn, P., Longyant, S., Pengsuk, C., Sithigorngul, P. and Chaivisuthangkura, P. 2013. Rapid identification and differentiation of *Vibrio parahaemolyticus* from *Vibrio* spp. in seafood samples using developed monoclonal antibodies. World Journal of Microbiology and Biotechnology 29: 721-731.

Roberts, A.J. and Wiedmann, M. 2003. Pathogen, host and environmental factors contributing to the pathogenesis of listeriosis. Cellular and Molecular Life Sciences 60: 904-918.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L. and Griffin, P.M. 2011. Foodborne illness acquired in the United States--major pathogens. Emerging Infectious Diseases 17: 7-15.

Swaminathan, B. and Gerner-Smidt, P. 2007. The epidemiology of human listeriosis. Microbes and Infection 9: 1236-1243.

Thailand Board of Investment 2014. Thailand: Feeding the World. 24: 5-7.

Thomas, E.J., King, R.K., Burchak, J. and Gannon, V.P. 1991. Sensitive and specific detection of Listeria monocytogenes in milk and ground beef with the polymerase chain reaction. Applied and Environmental Microbiology 57: 2576-2580.

Wang, H., Li, Y., Wang, A. and Slavik, M. 2011. Rapid, sensitive, and simultaneous detection of three foodborne pathogens using magnetic nanobead-based immunoseparation and quantum dot-based multiplex immunoassay. Journal of Food Protection 74: 2039-2047.