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ORIGINAL ARTICLE

A loop-mediated isothermal amplification method for rapid detection of *Vibrio parahaemolyticus* in seafood

Xiaohong Sun · Qian Xu · Yingjie Pan · Weiqing Lan · Yong Zhao · Vivian C. H. Wu

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Abstract *Vibrio parahaemolyticus* is an important human pathogen responsible for foodborne gastroenteritis worldwide. In this paper, a loop-mediated isothermal amplification (LAMP) method was developed for detection of *V. parahaemolyticus* in seafood. A set of four primers, two outer and two inner, was designed specifically to recognize the thermolabile hemolysin gene (*tlh*) of *V. parahaemolyticus*. The LAMP assay was capable of detecting a minimum of 900 fg test tube^{-1} for *V. parahaemolyticus* genomic DNA and 2.4×10^2 CFU mL^{-1} for pure cultures. The detection limit for the seeded seafood samples was 8.9×10^2 CFU g^{-1}. In addition, 42 shares of natural seafood samples were tested and 8 samples were recorded positive for *V. parahaemolyticus*, while 6 were positive by conventional culture methods. In conclusion, the LAMP assay is an effective and low-cost method with high specificity and sensitivity for rapid detection and identification of *V. parahaemolyticus* both in culture isolates and seafood samples.

Keywords *Vibrio parahaemolyticus* · Loop-mediated isothermal amplification (LAMP) · Thermolabile hemolysin gene (*tlh*) · Seafood

X. Sun · Y. Pan · W. Lan · Y. Zhao
College of Food Science and Technology,
Shanghai Ocean University,
Shanghai 201306, People's Republic of China

Q. Xu
Shanghai International Airport Entry-Exit
Inspection and Quarantine Bureau,
Shanghai 201202, People's Republic of China

V. C. H. Wu (✉)
Department of Food Science and Human Nutrition,
The University of Maine,
5735 Hitchner Hall,
Orono, ME 04469–5735, USA
e-mail: vivian.wu@umit.maine.edu

Introduction

Vibrio parahaemolyticus is a Gram-negative, halophilic bacterium that is distributed worldwide in the estuarine and coastal environments, especially in fish, shellfish, and seafood products. It is a crucial foodborne pathogen that can cause acute gastroenteritis in humans. Foodborne illness caused by this pathogen is generally a result of consumption of raw or undercooked seafood contaminated with the pathogen. Clinical manifestations of *V. parahaemolyticus* infections include diarrhea, headache, vomiting, nausea, abdominal cramps, low fever, and chills, with the incubation period ranging from 4 to 96 h (Wong et al. 2000; Drake et al. 2007). Foodborne outbreaks and isolated cases of *V. parahaemolyticus* have occurred throughout the world. It causes approximately half the food poisoning outbreaks in Taiwan, Japan, and several Southeast Asian countries and is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States (Wong et al. 2000; Su and Liu 2007) and in China(Liu 2004). Due to these facts, early, rapid and accurate detection of this pathogen is necessary and would provide both the seafood industry and the consumer with an early warning of potential health risks associated with potentially contaminated seafood and allow appropriate measures to prevent disease outbreak to be swiftly undertaken.

Conventional microbiological methods for detection of *V. parahaemolyticus* are costly in labor, materials, and time. Loop-mediated isothermal amplification (LAMP) was reported as DNA amplification with high specificity, efficiency, and rapidity under isothermal conditions (Notomi et al. 2000). It relies on autocycling strand displacement DNA synthesis performed by a DNA polymerase with high strand displacement activity and a set of four specially designed primers (two inner and two outer)

that recognize a total of six distinct sequences on the target DNA. The reaction is complete within 1 h at temperatures ranging from 60 to 65°C. Therefore, the LAMP method can achieve high specificity in pathogen detection in a short time without expensive equipment. The method has been successfully applied to detect *Salmonella*, *Shigella,* verotoxin-producing *Escherichia coli* and *Vibrio vulnificus* (Hara-Kudo et al. 2005; Ohtsuka et al. 2005; Song et al. 2005; Hara-Kudo et al. 2007; Ren et al. 2009).

In the present study, we applied the LAMP method for the rapid detection of *V. parahaemolyticus* in seafood. The specific primers were designed to target the thermolabile hemolysin gene (*tlh*) of *V. parahaemolyticus*. The conditions of the assay were optimized and the specificity and sensitivity of the primers in the LAMP assay for detection of *V. parahaemolyticus* were determined. Finally, the assay was applied to detect the bacterium in artificially contaminated and natural seafood.

Materials and methods

Bacterial strains and samples

The bacterial strains used in this study were obtained from the Institute of Microbiology of Chinese Academy of Science, Guangdong Institute of Microbiology of China, and previously isolated from food, water and sediment samples (Table 1). Sixteen strains of *V. parahaemolyticus*, 6 strains of other *Vibrio* spp. and 9 strains of 7 bacterial species other than *Vibrio* spp. were used in the study. The strains of *Vibrio* were grown on tryptic soy agar (TSA; Land Bridge, China) or tryptic soy broth containing 3% NaCl for 18 h at 37°C. The other bacteria were cultured on TSA at 37°C or in Luria broth for 18 h. In addition, thiosulfate citrate bile salt sucrose agar (TCBS; Haibo, China) was used to confirm *V. parahaemolyticus*. Forty-two seafood samples including shrimp, granulated ark shell, venus clam, constricted tagelus, surf clam, Manila clam, oyster, and hard clam were collected from seafood stores in Shanghai and Ningbo, China (Table 2). Seafood samples were collected during the months between September and December.

DNA preparation

Two methods were applied in the preparation of DNA for LAMP. In method I, DNA was extracted by using EZ Spin Column Bacterial Genomic DNA Isolation Kit UNIQ-10 (SBETS, Shanghai, China) according to the manufacturer's protocol. In method II, DNA was extracted using the boiling method. One milliliter of *V. parahaemolyticus* overnight cultured in tryptic soy broth containing 3% NaCl and non-*V. parahaemolyticus* in Luria broth were centrifuged at 12,000 *g* for 5 min. Bacteria resuspended in 100 μL of sterile double distilled water were boiled at 100°C for 10 min and immediately ice incubated for 2 min. After centrifugation at 12,000 *g* for another 5 min, the supernatant was used as temple DNA.

Design of LAMP primers

A set of species-specific LAMP primers comprised of two outer and two inner primers was designed to target the thermolabile hemolysin (*tlh*) gene of *V. parahaemolyticus* (GenBank accession number M36437) using Primer Pre-

Table 1 List of bacterial strains used in this study and specificity results of the LAMP primers

Bacterial species	Source	Number of strains tested	LAMP result
Vibrio parahaemolyticus ATCC 33846	IMCAS[a]	1	+
V. parahaemolyticus ATCC 33847	IMCAS[a]	1	+
V. parahaemolyticus ATCC 17802	IMCAS[a]	1	+
V. parahaemolyticus	SHOU[b], EI[c]	13	+
Vibrio campbellii ATCC 33864	IMCAS[a]	1	−
Vibrio harveyi ATCC 33842	IMCAS[a]	1	−
Vibrio fluvialis ATCC 33809	IMCAS[a]	1	−
Vibrio cholerae	SHOU[b], EI[c]	3	−
Staphylococcus aureus subsp. *aureus* 1.1361	GDIM[d]	1	−
Salmonella spp. 1.1552	GDIM[d]	1	−
Listeria innocua 1.230	GDIM[d]	1	−
Listeria welshimeri 1.231	GDIM[d]	1	−
Listeria monocytogenes 1.228	GDIM[d]	1	−
Listeria monocytogenes 1.229	GDIM[d]	1	−
Escherichia coli O157:H7	IMCAS[a]	1	−
Shigella spp.	IMCAS[a]	2	−

+ Positive reaction, − negative reaction

[a] Institute of Microbiology of Chinese Academy of Sciences

[b] Shanghai Ocean University

[c] Environmental isolate

[d] Guangdong Institute of Microbiology

Table 2 The result of detection of *V. parahaemolyticus* in natural seafood

Sample type	No. of samples	No. of samples positive for *V. parahaemolyticus* by LAMP and conventional PCR	No. of samples positive for *V. parahaemolyticus* by the conventional method
Granulated ark shell	4	0	0
Venus clam	8	2	2
Constricted tagelus	12	4	2
Surf clam	2	0	0
Manila clam	2	0	0
Oyster	10	2	2
Hard clam	4	0	0
Total	42	8	6

mier 5.0 Software (Premier, Canada) (Table 3). The forward inner primer (FIP) consisted of the F1c sequence (complementary to F1), a TTTT spacer and the F2 sequence. The backward inner primer (BIP) consisted of the B1c sequence (complementary to B1), a TTTT spacer and the B2 sequence. The outer primers consisted of the forward outer primer F3 and the backward outer primer B3. The LAMP primers sequences and positions are shown in Fig. 1.

Optimization of LAMP reaction

For optimization of the reaction, purified DNA from *V. parahaemolyticus* strains ATCC 33846 was used. One milliliter of the overnight culture at 10^8 CFU mL^{-1} was used to extract DNA using the EZ Spin Column Bacterial Genomic DNA Isolation Kit UNIQ-10. The initial extracted DNA concentration was 45 ng μL^{-1}. Two microliters of DNA template was added to the LAMP reaction mixture. The LAMP reaction was carried out in a 25-μL reaction mixture containing the following reagents and the optimal concentrations of each reagent were determined using the following conditions: 2–18 mM $MgSO_4$ (SBETS), 0–1.8 mM dNTPs (SBETS), 0–1.2 M betaine (Sigma, USA), the ratios of outer primers and inner primers ranging from 1:1 to 1:8, 1 U *Bst* DNA polymerase large fragment (New England Biolabs, USA), and 1× ThermoPol buffer (New England Biolabs, USA) per reaction mixture. Sterile doubl-distilled water was used to adjust the volume of each reaction mixture to 25 μL. In addition, the LAMP reaction time varied from 30 to 60 min and the temperatures varied from 55 to 65°C to optimize the LAMP procedures. Aliquots of 3 μL of the products were analyzed by 2% agarose gel electrophoresis.

Specificity of LAMP assay

The specificity of the set of LAMP primers for the *tlh* gene of *V. parahaemolyticus* was determined by LAMP amplification of the genomic DNA (extracted by method I) from 16 *V. parahaemolyticus* strains and 15 bacterial strains other than *V. parahaemolyticus* listed in Table 1. The optimal reaction conditions obtained from the previous procedures were used.

Sensitivity of LAMP assay

The sensitivity of the assay was determined using *V. parahaemolyticus* ATCC 33846 DNA extracted by method I and II separately.

Vibrio parahaemolyticus ATCC 33846 was cultivated overnight and extracted using method I. The DNA concentration was estimated by UV absorption spectrophotometry at a wavelength of 260 nm using Ultrospec 1100 pro (GE Healthcare, USA). The DNA was then 10-fold serially diluted in sterile double-distilled water. Aliquots of each 2 μL dilution were amplified by LAMP using the

Table 3 Sequences of the LAMP primers

Name	Sequence (5′→3′)
FIP (Forward inner primer)	GCCCATTCCCAATCGGTCG-TTTT-CTATGTTTCGCTGTTGGTATCG
BIP (Backward inner primer)	GTTCTACACCAACACGTCGCA-TTTT-TCGCCAAATCTAATGTTGCTTC
F3 (Forward outer primer)	CAGCACGCAAGAAAACCA
B3 (Backward outer primer)	ATTGTCAGCGGCGAAGAA

Fig. 1 **a** Schematic diagram showing the positions at which the primers attach for amplification of the *tlh* gene. **b** Locations and nucleotide sequences of the *tlh* gene of *V. parahaemolyticus* used for designing the inner and outer primers. The DNA sequences of primer sites are *underlined*. Oligonucleotide sequences in *bold* and listed at the bottom were used as LAMP primers

optimum reaction condition. A LAMP reaction mixture containing 2 μL of double-distilled water instead of genomic DNA was used as a negative control.

To determine the sensitivity of detection for pure cultures, overnight cultivated *V. parahaemolyticus* ATCC 33846 was quantitated by direct plating. After 10-fold serially dilution, a total of 2 μL template DNA (extracted by method II) of each dilution were amplified by LAMP using the optimum reaction condition. A LAMP reaction mixture containing 2 μL of double-distilled water instead of template DNA was used as a negative control.

Detection of *V. parahaemolyticus* in seeded shrimp

The shrimp used in this study were purchased from a local seafood store (Shanghai, China). The shrimp were stored in a sterile beaker on ice and exposed to ultraviolet (UV) light to eliminate any naturally occurring *V. parahaemolyticus* strains. Overnight cultivated *V. parahaemolyticus* ATCC 33846 were serially diluted in alkaline peptone water (APW). The number of cells in each dilution was quantitated by direct plating. Each of the *V. parahaemolyticus* cultures (1 mL) were used separately to inoculate 10 g of UV-treated shrimp. Subsequently, the seeded shrimp were placed in a sterile stomacher bag containing 90 mL of APW and were homogenized using a Stomacher (MIX2; AES Laboratire, France) for 3 min. Aliquots of 1 mL of the solution were treated using method I to extract DNA. For LAMP amplification, 2 μL of template DNA were used in a 25-μL reaction mixture under the optimal reaction condition as previously developed. DNA extracted from unseeded shrimp homogenate was used as a negative control.

Detection of *V. parahaemolyticus* in natural seafood

Forty-two shares of natural seafood samples were tested in the study (Table 2). Under aseptic condition, 25 g of the muscles of the seafood samples were separated from the shells, homogenized in 225 mL APW for 1 min in a homogenizer (Binrong, Shanghai, China) and incubated at

37°C for 10 h. A loopfull of enrichment broth was streaked on TCBS agar (Haibo) and incubated overnight at 37°C. Biochemical tests were done to identify *V. parahaemolyticus* (FDA 2004). Simultaneously, 1 mL of the enrichment broth was treated with DNA extraction method I and II. For LAMP assay, 2 μL of the DNA were used in a 25-μL reaction mixture under the optimal reaction condition as previously developed. Meanwhile, the other 2 μL was used for conventional PCR, which was carried out in a final volume of 25 μL as follows: prior denaturation at 94°C for 3 min, then 25 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, elongation at 72°C for 2 min; finally, elongation at 72°C for 3 min (FDA 2004).

Results

Optimization of LAMP assay

The LAMP reaction conditions were optimized using various concentrations of $MgSO_4$, dNTPs, betaine and primers, as well as different amplification temperature and reaction time. The results indicated that the reaction could be carried out when the Mg^{2+} concentration is between 6 and 10 mM and the optimal concentration was 8 mM (Fig. 2a). As shown in Fig. 2b, the ladder-like bands could be obtained with dNTPs ranging from 1.0 to 1.4 mM while 1.0 mM is the best. The reaction could be carried out when the betaine concentration is higher than 0.6 M and the optimal concentration was 0.8 M (Fig. 2c). Figure 2d demonstrates that the target gene was amplified in a primer ratio ranging from 1:2 to 1:8 while the ratio at 1:6 (0.2 μM each of F3 and B3, 1.2 μM each of FIP and BIP) gave the best amplification. As shown in Fig. 2e, f, the amplification could be detected initially at 45 min and the optimal time was 1 h while the clearest pattern could be obtained at 60° C. Based on the above results, the optimal LAMP assay condition in a 25-μL reaction volume is 8 mM $MgSO_4$, 1.0 mM dNTP, 0.8 M betaine, 1.2 μM each of FIP and BIP, 0.2 μM each of F3 and B3, 1 U *Bst* DNA polymerase large fragment, and 1× ThermoPol buffer with 2 μL total DNA as template per 25 μL reaction mixture. Besides this, the best results were obtained with the following procedures: incubation at 60°C for 1 h and inactivation at 80°C for 10 min.

Specificity of LAMP assay

In order to evaluate the specificity of LAMP, 31 bacteria strains were tested for the LAMP assay. The LAMP assay correctly detected 16 *V. parahaemolyticus* strains. The 6 other *Vibrio* species and 9 non-*Vibrio* bacterial species had

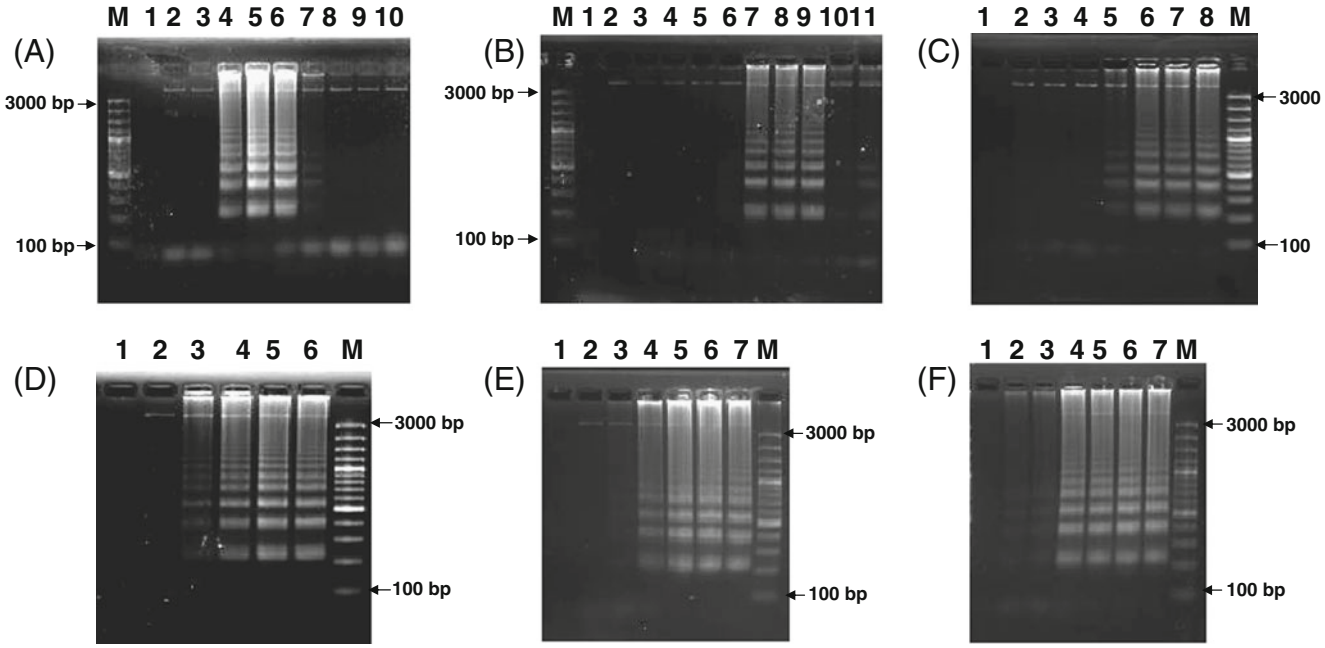

Fig. 2 Optimization of the LAMP reaction for the detection of *V. parahaemolyticus*. *M*: 100 bp marker; *1*: negative control. **a** Effect of Mg2+ concentrations on the LAMP reaction: *2–10* Mg2+ was 2, 4, 6, 8, 10, 12, 14, 16 and 18 mM, respectively. **b** Effect of dNTPs concentrations on the LAMP reaction: *2–11* dNTP was 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 mM, respectively. **c** Effect of betaine concentrations on the LAMP reaction: *2–8* betaine was 0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 M, respectively. **d** Effect of the ratio of outer and inner primers on the LAMP reaction: *2–6* the ratio was 1:1, 1:2, 1:4, 1:6 and 1:8, respectively. **e** Effect of temperature on the LAMP reaction: *2–7* the temperature was 53, 55, 58, 60, 63 and 65°C, respectively. **f** Effect of reaction time on the LAMP reaction: *2–7* the amplification time was 15, 30, 45, 60, 75 and 90 min, respectively

negative results (Table 1). LAMP reaction was also negative for distilled water (negative control). The result indicated that the LAMP assay was highly specific to *V. parahaemolyticus* strains.

Sensitivity of LAMP assay

The detection limit of the LAMP assay for *V. parahaemolyticus* ATCC 33846 was determined using 10-fold serial dilutions of *V. parahaemolyticus* extracted DNA and culture suspension, and 2% agarose gel electrophoresis was used to analyze the results. The initial concentration of bacterial inoculum was 2.39×10^{8} CFU mL^{-1}. The initial DNA concentration was 45 ng μL^{-1}. The amplification products showed a typical ladder-like pattern on gel electrophoresis which indicated that stem-loop DNA with inverted repeats was formed (Notomi et al. 2000). Template DNA ranging from 9 ng to 9 fg was used in the LAMP reaction. The result showed that the minimum DNA concentration required for amplification was 9 pg DNA $tube^{-1}$ (Fig. 3). In addition, 10-fold dilution of pure culture was directly used to test the LAMP assay. Reactions remained positive up to 2.4×10^{2} CFU mL^{-1} (Fig. 4). Furthermore, the detection limit of *V. parahaemolyticus* in seafood was studied. The sensitivity of the LAMP assay for the direct detection of *V. parahaemolyticus* in seeded shrimp was 8.9×10^{2} CFU g^{-1} (Fig. 5). We also compared the detection limit between conventional PCR and LAMP. The sensitivity of LAMP was found to be at least 10 times more than that of the conventional PCR assay in the detection of pure culture and 1.000 times more in the detection of seeded shrimp (Fig. 6a-c).

In addition, our results showed that both DNA extraction methods were suitable for LAMP detection of *V. parahaemolyticus*. Since the boiling method was simple and rapid, total detection time was reduced.

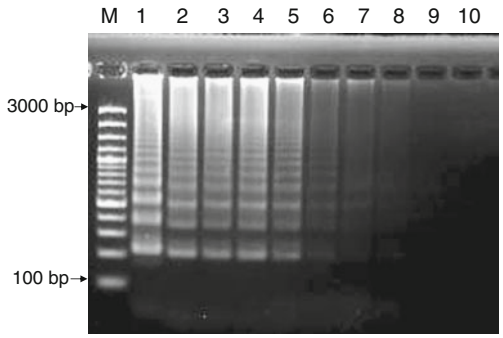

Fig. 3 The sensitivity of LAMP for detection of *V. parahaemolyticus* 33846 genomic DNA. *M* 100 bp marker; *1* positive control; *2* 9 ng test $tube^{-1}$; *3* 900 pg test $tube^{-1}$; *4* 90 pg test $tube^{-1}$; *5* 9 pg test $tube^{-1}$; *6* 900 fg test $tube^{-1}$; *7* 90 fg test $tube^{-1}$; *8* 9 fg test $tube^{-1}$; *9* 0.9 fg test $tube^{-1}$; *10* negative control

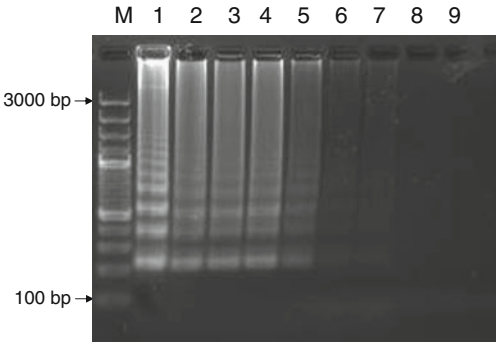

Fig. 4 The sensitivity of LAMP for detection *V. parahaemolyticus* ATCC 33846 in pure cultures. *M* 100 bp marker; *1* positive control; *2* 2.39×10^{5} CFU mL^{-1}; *3* 2.39×10^{4} CFU mL^{-1}; *4* 2.39×10^{3} CFU mL^{-1}; *5* 2.39×10^{2} CFU mL^{-1}; *6* 2.39×10^{1} CFU mL^{-1}; *7* 2.39 CFU mL^{-1}; *8* 2.39×10^{-1} CFU mL^{-1}; **9** negative control

Detection of *V. parahaemolyticus* in natural seafood

To determine whether *V. parahaemolyticus* could be detected in natural samples with LAMP assay, we examined natural seafood. Of 42 collected seafood samples tested in the study, 8 were detected as positive using LAMP assay, indicating that *V. parahaemolyticus* was present in these samples. We also used conventional PCR assay to amplify the *tlh* gene in the natural samples. The same result that 8 were positive was obtained. Meanwhile, 6 out of 42 samples were positive for *V. parahaemolyticus* using the conventional culture method according to the FDA protocol. Two constricted tagelus were detected positive using LAMP and conventional PCR but negative using conventional culture method (Table 2).

Discussion

Proper primer design is crucial for performing LAMP amplification. It is known that *tdh* (thermostable direct hemolysin) and *trh* (tdh-related hemolysin) genes are two major virulence factors for the pathogenesis of *V. parahaemolyticus*. However, most of the *V. parahaemolyticus* that are isolated from the environmental and seafood samples are reported to be *tdh* negative, *trh* negative, or both *tdh* and *trh* negative. Therefore, a species-specific gene was chosen to detect the total *V. parahaemolyticus*. The gene for *tlh* encoding the thermolabile hemolysin (TLH) has been shown to be observed in all *V. parahaemolyticus* strains (Bej et al. 1999; Taniguchi et al. 1985, 1986). This gene is therefore a useful target for the detection of total *V. parahaemolyticus* (McCarthy et al. 1999; Dileep et al. 2003; Kaufman et al. 2004; Ward and Bej 2006; Su and Liu 2007). In this study, a set of four specific primers was designed to recognize six distinct regions on the target *tlh* gene of *V. parahaemolyticus*

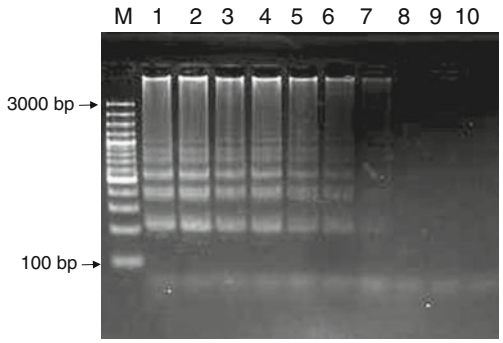

Fig. 5 The sensitivity of LAMP for *V. parahaemolyticus* ATCC 33846 in seeded shrimp samples. *M* 100 bp marker; *1* positive control; *2* 8.9×10^{6} CFU g^{-1}; *3* 8.9×10^{5} CFU g^{-1}; *4* 8.9×10^{4} CFU g^{-1}; *5* 8.9×10^{3} CFU g^{-1}; *6* 8.9×10^{2} CFU g^{-1}; *7* 8.9×10^{1} CFU g^{-1}; *8* 8.9×100 CFU g^{-1}; *9* 8.9×10^{-1} CFU g^{-1}; *10* negative control

(Fig. 1). The specificity of these primers was tested using *Vibrio parahaemolyticus*, *V. campbellii, V. harveyi, V. fluvialis, V. cholerae, Salmonella, Staphylococcus aureus, Listeria innocua, L. welshimeri, L. monocytogenes, E. coli,* and *Shigella*. The LAMP reaction was positive for all the *V. parahaemolyticus* strains and negative for the other species tested (Table 1), demonstrating that these primers were specific for identification of *V. parahaemolyticus*. The specificity of LAMP in detection of *V. parahaemolyticus* was in conformity with other studies in the bacterial detection (Han and Ge 2008; Nemoto et al. 2009; Li et al. 2009).

In our study, the sensitivity of LAMP was found to be 10 times more than that of the conventional PCR assay. Our *tlh*-based LAMP assay was able to detect about one *V. parahaemolyticus* cell per reaction tube, in contrast to ten cells using *tlh*-based PCR. Similarly, the detection limit of *tdh*-based LAMP assay for *V. parahaemolyticus* was reported to one cell per reaction tube and 10-fold more sensitive than PCR (Nemoto et al. 2009). Additionally, several studies on the detection of other vibrios also found that LAMP was 10-fold more sensitive than PCR (Han and Ge 2008; Fall et al. 2008). This was also in accordance with the results reported for the detection of vt-producing *E. coli* (Yano et al. 2007) and *Salmonella* (Wang et al. 2008). It was also reported that LAMP was 100 times more sensitive than PCR in detecting *Yersinia pseudotuberculosis* (Horisaka et al. 2004) and *Bordetella pertussis* (Kamachi et al. 2006). The greater sensitivity was due to the high amplification efficiency of the LAMP method. There is no time loss for thermal change under isothermal conditions in LAMP (Ren et al. 2009).

Our LAMP method used for the detection of *V. parahaemolyticus* in seeded shrimp was found to be highly sensitive because it could detect *V. parahaemolyticus* up to 8.9×10^{2} CFU g^{-1}, whereas the detection limit of *V. parahaemolyticus* was only 8.9×10^{5} CFU g^{-1} by conventional PCR. Our result was comparable with the result of *V. vulnificus* detection in raw oysters (Han and Ge 2008). Food components such as organic and phenolic compounds, glycogen, fats, and Ca^{2+} were reported to inhibit

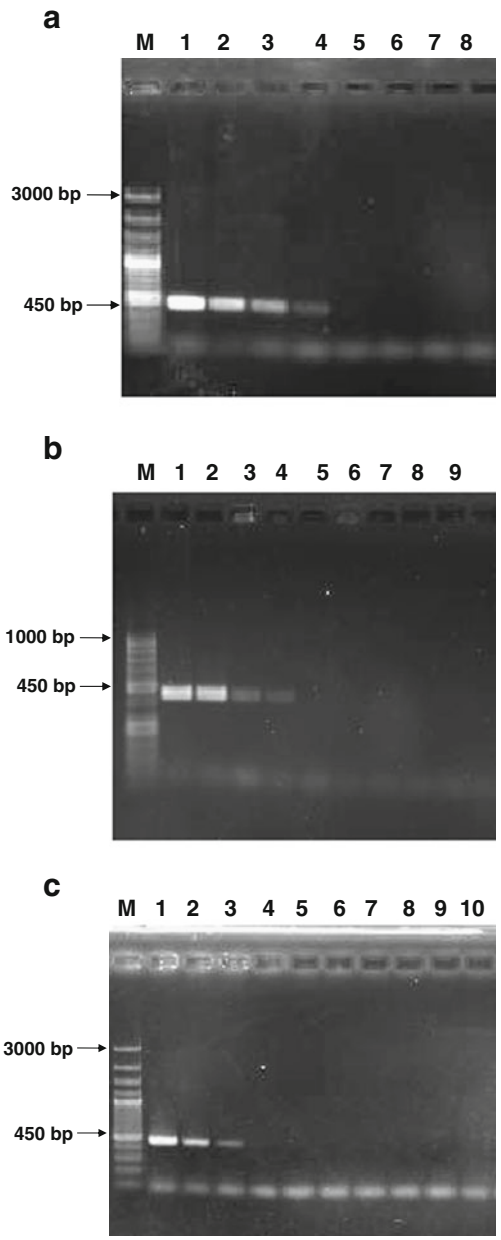

Fig. 6 The sensitivity of PCR for detection of **a** *V. parahaemolyticus* ATCC 33846 genomic DNA. *M* 100 bp marker; *1* positive control; *2* 22.5 ng test $tube^{-1}$; *3* 2.25 ng test $tube^{-1}$; *4* 225 pg test $tube^{-1}$; *5* 22.5 pg test $tube^{-1}$; *6* 2.25 pg test $tube^{-1}$; *7* 225 fg test $tube^{-1}$; *8* negative control; **b** *V. parahaemolyticus* ATCC 33846 in pure cultures. *M* 100 bp marker; *1* positive control; *2* 2.39×10^{5} CFU mL^{-1}; *3* 2.39×10^{4} CFU mL^{-1}; *4* 2.39×10^{3} CFU mL^{-1}; *5* 2.39×10^{2} CFU mL^{-1}; *6* 2.39×10^{1} CFU mL^{-1}; *7* 2.39 CFU mL^{-1}; *8* 2.39×10^{-1} CFU mL^{-1}; *9* negative control; and **c** *V. parahaemolyticus* ATCC 33846 in seeded shrimp samples. *M* 100 bp marker; *1* positive control; *2* 8.9×10^{6} CFU g^{-1}; *3* 8.9×10^{5} CFU g^{-1}; *4* 8.9×10^{4} CFU g^{-1}; *5* 8.9×10^{3} CFU g^{-1}; *6* 8.9×10^{2} CFU g^{-1}; *7* 8.9×10^{1} CFU g^{-1}; *8* 8.9×100 CFU g^{-1}; *9* 8.9×10^{-1} CFU g^{-1}; 10 negative control

DNA amplification (Hara-Kudo et al. 2005). However, the present study performed in seeded shrimp, which usually contain a variety of components, did not indicate that complex food compounds significantly inhibit the sensitivity of LAMP. This finding is similar to that in the previous studies which showed that the LAMP method was less affected by various components of the clinical samples than PCR (Kaneko et al. 2007). Recently, real-time PCR was also used to detect vibrios in seafood (Cañigral et al. 2010). The real-time PCR assay has many advantages over the conventional PCR, including rapidity, a low contamination rate, a higher sensitivity and easy standardization, but it requires fluorogenic primers and probes as well as expensive detection equipment. The high cost of the instruments required to perform the real-time assay restricts the use to laboratories with good financial resources. So the LAMP method is relatively desirable in the development of a diagnostic system.

It was observed that the result by LAMP detection was the same as the one by PCR amplification in the detection of natural seafood. DNA amplification-based techniques detected two more shares of positive samples than the culture method. In the study, the enrichment step before LAMP and PCR amplification was used, which excluded the possibility of detecting dead or damaged cells along the living cells. Compared the PCR and plate culture methods in the detection of *V. parahaemolyticus* in mussels and environmental samples, PCR after sample enrichment was more sensitive than the conventional culture method (Blanco-Abad et al. 2008). The same result occurred in detecting *V. parahaemolyticus* from seafood harvested along the southwest coast of India (Raghunath et al. 2008). Moreover, the LAMP assay was more sensitive than the PCR assay. The high sensitivity of LAMP assay and PCR was the reason why two more positive samples appeared.

In the present study, the LAMP assay developed and optimized is highly specific and sensitive for the detection and identification of *V. parahaemolyticus* both in culture isolates and in seafood samples. The assay performs under isothermal conditions with a single temperature step at 60°C for about 1 h and allows for one-step identification of *tlh* gene amplification without the use of a high-precision thermal cycler. Compared to conventional microbiological methods and PCR techniques, it is easier to perform, more effective, and lower in cost. Therefore, the LAMP assay is expected to provide a rapid and reliable alternative to the current *V. parahaemolyticus* detection methods by reducing the analysis time, cost, and labor. This is beneficial not only to the seafood industry but also to the consumer by ensuring the safety of the seafood provided for consumption.

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