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Development of multiplex loop-mediated isothermal amplification for three foodborne pathogens

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Abstract

Staphylococcus aureus, *Salmonella*, and *Shigella* are three major foodborne pathogenic microorganisms that cause global public health problems. We developed a multiplex loop-mediated isothermal amplification (mLAMP) assay for simultaneous detection of *S. aureus nuc*, *Salmonella fimY*, and *Shigella ipaH* in fresh fruit juice using three sets of primers. In addition, three different restriction enzyme cleavage sites were designed in each forward inner primer (FIP), namely, *Xho*I in *nuc* FIP, *Kpn*I in *fimY* FIP, and *BamH*I in *ipaH* FIP. DNA was amplified using the LAMP assay at 64 °C for 50 min followed by endonuclease restriction digestion to separate the LAMP products of three pathogens. The minimum amount of genomic DNA of *S. aureus*, *Salmonella*, and *Shigella* that could be detected by mLAMP was 100 fg/25 µL, whereas for mPCR, it was 1 pg/25 µL. The artificially contaminated juice can also be detected by mLAMP after enrichment, which had the limit of detection (LOD) of 2 CFU/10 mL. In conclusion, the mLAMP developed in this study could be potentially used in the detection of *S. aureus, Salmonella*, and *Shigella* in food, particularly as a primary screening method in developing areas.

Keywords: multiplex LAMP; *Staphylococcus aureus*; *Salmonella*; *Shigella*.

Practical Application: Detection of foodborne pathogens.

1 Introduction

According to the World Health Organization (2015), unsafe food causes over 200 human diseases due to harmful bacteria, viruses, parasites or chemical substances, ranging from diarrhoea to cancers, and foodborne and waterborne diseases kill about two million people each year. Foodborne pathogens can cause a wide range of diseases including severe diarrhea and severe infections. *Staphylococcus aureus*, *Salmonella enterica*, and *Shigella* are three major foodborne pathogenic microorganisms that cause public health problems around the world. It is estimated that non-typhoidal *S. enterica* causes 93.8 million cases of acute gastroenteritis and 155,000 deaths every year around the world, of which 85% are foodborne (Food and Agriculture Organization of the United Nations, 2016). *Shigellosis* has high morbidity and mortality, particularly in resource-poor countries, where 167 million cases of diarrhea and over a million deaths occur annually (Von Seidlein et al., 2006). These three pathogens are responsible for 26.71% of bacteria foodborne events in China (Liu et al., 2016).

Based on the public health issue and economic losses caused by *S. aureus, Salmonella* and *Shigella*, these three pathogens should be monitored in foods (World Health Organization, 2003, 2015). The gold standard for *S. aureus, Salmonella* and *Shigella* testing is culture-based assays, which typically involve culture, identification, biochemical and serological detection. However, these assays are relatively complicated and time-consuming. Therefore, more rapid and simple methods are warranted, mainly includes immunology and molecular biology (Amani et al., 2015; Chen et al., 2015; Maerle et al., 2014; Nouri et al., 2018; Shao et al., 2011).

Loop-mediated isothermal amplification (LAMP) is a simple, rapid, sensitive, and specific method for the detection of pathogens that was developed by Notomi et al. (2000). Since it was developed, LAMP was used for detection and identification of viruses, bacteria and parasites (Huy et al., 2012; Imai et al., 2006; Shao et al., 2011; Sun et al., 2017). Compared to other detection methods, one of the most significant advantages of LAMP is its low cost, as this implies that it has a huge potential application value for pathogen detection. The purpose of this study was to develop an efficient and inexpensive assay for the detection of *S. aureus, Salmonella*, and *Shigella*, and assessed the application potential of mLAMP in food. This is the first time that mLAMP has been utilized in the simultaneous detection of *S. aureus*, *Salmonella*, and *Shigella* in juice using a single reaction.

2 Materials and methods

2.1 Bacterial strains and culture conditions

Seventeen standard strains were used for specificity and sensitivity testing in this study (Table 1). The culture medium of *S. aureus*, *Salmonella*, *Shigella*, and *Escherichia coli* was nutrient agar (NA), whereas *Listeria monocytogenes* was cultured in brain heart infusion (BHI). All strains were cultured at 37 °C.

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2.2 DNA extraction

Genomic DNA was extracted from each bacterial strain after overnight growth using a QIAGEN DNA extraction kit (DNeasy kit, QIAGEN, German). The concentration of the extracted DNA was determined at A260/280 by spectrophotometer (Shimadzu, UV-1700) and stored under at -20 °C.

2.3 Primer design

The sequences of the *S. aureus nuc* gene, *S. enterica fimY* gene, and *Shigella ipaH* gene were downloaded from NCBI and used in designing primers using Primer Explorer 5. Primer sequences were verified with BLAST® after design. Three sets of primers were used in the LAMP assays, and each set was used to amplify 17 DNA sequences of *S. aureus, Salmonella,* and *Shigella* (Table 1). *Xho*I was introduced into *nuc* FIP (Forward Inner Primer), *Kpn*I was introduced into *fimY* FIP and *BamH*I was introduced into *ipaH* FIP (Table 2). Three sets of primers were used in this study (Table 2).

Table 1. The 17 bacterial strains used in this study.

No.	Bacterial species	Strain serial number	Origin
1	*Staphylococcus aureus*	10384	China Center of Industrial Culture Collection, CICC
2	*S. aureus*	21600	
3	*S. aureus*	21601	
4	*S. aureus*	23656	
5	*Salmonella*	21482	
6	*Salmonella*	21484	
7	*Salmonella*	21493	
8	*Salmonella*	21513	
9	*Shigella*	21534	
10	*Shigella*	21535	
11	*Shigella*	21680	
12	*Escherichia coli*	10389	
13	*E. coli*	10667	
14	*E. coli*	21530	
15	*L. monocytogenes*	21633	
16	*L. monocytogenes*	21635	
17	*L. monocytogenes*	23929	

2.4 LAMP assay

Each LAMP reaction contained the following: 12.5 µL of a 2× Master Mix (WarmStart LAMP Kit, NEB, USA), 5 pmol each of F3 and B3, 40 pmol each of FIP and BIP (backward inner primer), 1 µL of DNA, and H_2O to a final reaction volume of 25 µL. LAMP reactions were performed under the isothermal condition of 64 °C for 50 min and then 80 °C for 5 min to terminate the reaction.

For multiplex LAMP (mLAMP), the following reaction componets were employed: 12.5 µL of a 2× Master Mix, 1.67 pmol each of *nuc*, *fimY*, and *ipaH* F3 and B3, 13.3 pmol each of *nuc*, *fimY*, and *ipaH* FIP and BIP, 1 µL each of DNA, and H_2O to a final reaction volume of 25 µL. Each assay was repeated three times.

2.5 mPCR assay

Multiplex PCR (mPCR) was conducted in parallel with the mLAMP assay. Each mPCR consisted of the following: 12.5 µL of a Taq premix (Premix Taq, TakaRa, China), 1.67 pmol of each *nuc*, *fimY*, and *ipaH* PCR primers, 1 µL each of *S. aureus, Salmonella, Shigella* DNA template, and H_2O to a final reaction volume of 25 µL. mPCR was performed using the following conditions: 94 °C for 3 min; followed by 30 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 20 s; and a final extension at 72 °C for 5 min. Each assay was repeated three times.

Table 2. Primers used in this study.

Primer name	Sequence (5'→3')
LAMP primers	
nuc F3	AAAAGATGGTAGAAAATGCHAAG
nuc B3	TGTTCATGTGTATTGTTAGGTT
nuc FIP	ACGCTAAGCCACGTCCATATTCTCGAG(*Xho*I)AAAATTGAAGTCGAGTTTGACA
nuc BIP	TATGCTGATGGAAAAATGGTAAACGTAAACATAAGCAACTTTAGCCAAG
fimY F3	AGAAAGCTTTGCCTGTGG
fimY B3	WAACCTCGCTTATCGGAA
fimY FIP	AGCAAAGCGTACCTTATCATCGGGTACC(*Kpn*I)GTTAAGGAGGGTGATAAGTTG
fimY BIP	GACGTGCTATTTCTTTTAAAGAGGCAGCTTTAGCCGTACTGAC
ipaH F3	GCTGGAAAAACTCAGTGCCT
ipaH B3	GGAACATTTCCCTGCCCA
ipaH FIP	CGACACGGTCCTCACAGCTCGGATCC(*BamH*I)TTCGACAGCAGTCTTTCGC
ipaH BIP	ATCTCCGGAAAACCCTCCTGGTAGCGCCGGTATCATTATCGA
PCR primers	
nuc F3	AAAAGATGGTAGAAAATGCHAAG
nuc B3	TGTTCATGTGTATTGTTAGGTT
fimY F1	AAGGAGGGTGATAAGTTGTTT
fimY B1	AGCCGTACTGACTGGTTGA
ipaH F1	CGCGCTCACATGGAACAA
ipaH B1	AGTTTCTCTGCGAGCATGG

2.6 Restriction endonuclease analysis

The LAMP products were digested by restriction endonucleases *Xho*I, *Kpn*I, and/or *BamH*I at 37 °C for 1 h. Each 30-μL restriction endonuclease system consisted of 3 μL of the restriction enzyme buffer, 1 μL of each enzyme, 3 μL of the LAMP product, and H_2O.

2.7 Specificity and sensitivity testing

The specificity of the LAMP assays used in this study was tested on 17 strains (Table 1), which included five foodborne pathogens. The specificity of the restriction endonuclease reactions was assessed by cross enzyme digestion. The LAMP products of *S. aureus*, *Salmonella,* and *Shigella* were digested by *Xho*I, *Kpn*I, and *BamH*I separately.

To evaluate the sensitivity of the mLAMP assays, serial 10-fold dilutions of the DNA templates were used.

2.8 mLAMP for the simultaneous detection of S. aureus, Salmonella, and Shigella in artificially contaminated fresh fruit juice

The present study assessed the application potential of mLAMP in food. Artificially contaminated fresh fruit juices were prepared as described by Shao et al. and Garrido with modifications (Garrido-Maestu et al., 2017; Shao et al., 2011). The juices used in this study were purchased from a local supermarket. *S. aureus*, *S. enterica*, and *Shigella* were incubated in nutrient broth (NB) at 37 °C for shaking overnight. The concentration of three pathogens were determined by CFU (colony forming unit) counting, meanwhile OD_{600} was measured. Bacterium was diluted in NB, final inoculum concentration was adjusted to 100 CFU/mL, 50 CFU/mL, 10 CFU/mL, 5 CFU/mL, 2 CFU/mL, 1 CFU/mL. 3 mL of bacteria (1 mL of each) were added to 10 mL of juice, and mixed with 87 mL of NB. This matrix was incubated for 18 h at 37 °C. After incubation, 1 mL of matrix was taken and DNA was extracted following the DNA extraction above. Each inoculum concentration was repeated three times.

2.9 Product testing

The LAMP, PCR, and digestion products were analyzed by electrophoresis on a 2% agarose gel containing 0.5 μg/mL Andy Safe (Applied Bioprobes, USA).

3 Results

3.1 The specificity of LAMP assays and restriction endonuclease digestions

Multiplex LAMP was used to simultaneously detect *S. aureus*, *Salmonella*, and *Shigella*. Three sets of primers corresponding to the *nuc* of *S. aureus,* the *fimY* of *Salmonella*, and the *ipaH* of *Shigella* were designed (Table 2). Figure 1 shows that all three sets of primers were specific to their target templates, and no amplified DNA bands were detected after gel electrophoresis with non-target DNA (Figure 1).

In this study, *S. aureus*, Salmonella, and Shigella were detected by single-tube mLAMP assay, and restriction endonuclease digestion was used to analyze the mLAMP products due to

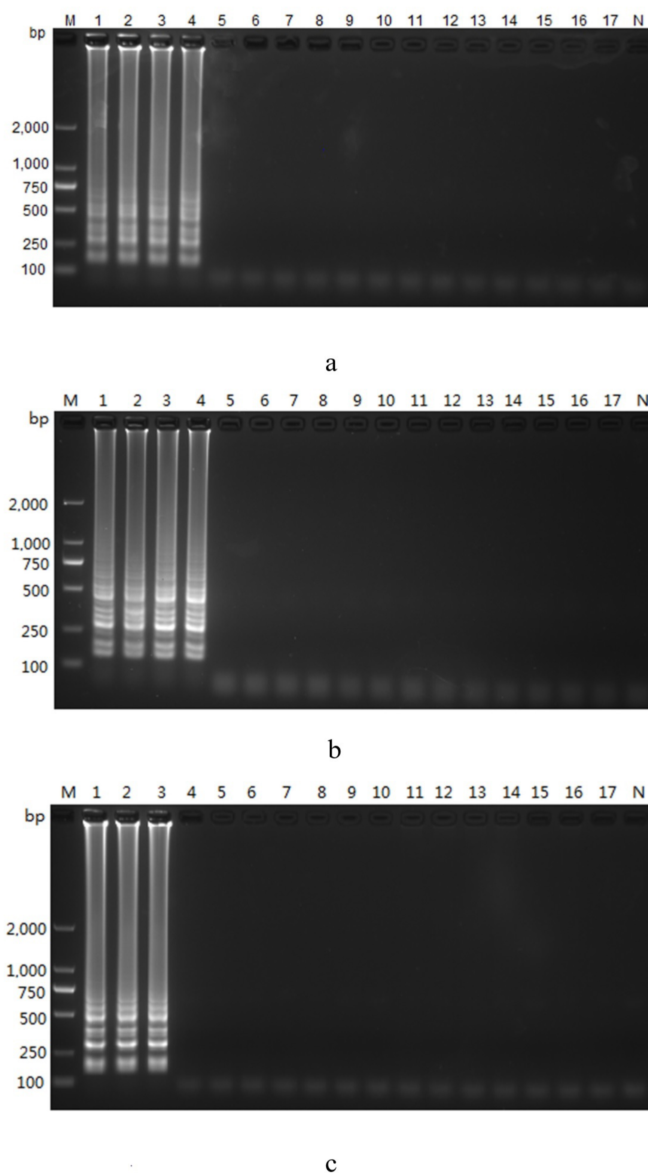

Figure 1. Specificity of LAMP assays. Lanes: M, 2,000-bp DNA marker; 12~14, 3 *Escherichia coli* strains; 15~17, 3 *Listeria monocytogenes* strains; N, negative control; (a) 1~4, 4 *Staphylococcus aureus* strains; 5~8, 4 *Salmonella* strains; 9~11 3 Shigella strains; (b) Lanes: 1~4, 4 *Salmonella* strains; 5~8, 4 *Staphylococcus aureus* strains; 9~11 3 *Shigella* strains; (c) Lane: 1~3, 3 *Shigella* strains; 4~7, 4 *Staphylococcus aureus* strains; 8~11, 4 *Salmonella* strains. Source: by Cong XU.

the different enzyme cut sites which introduced in three FIPs. Specific enzyme digestions were important in discriminating different templates. Figure 2 shows that the LAMP products of *S. aureus*, *S. enterica*, and *Shigella* were only digested by *Xho*I, *Kpn*I, and *BamH*I, respectively, as expected (Figure 2).

3.2 mLAMP assay and multiplex restrict endonuclease digestion

Three sets of primers and *S. aureus*, *Salmonella,* and *Shigella* DNA templates were added into one tube, where DNA was amplified; then the mLAMP products were analyzed by

enzyme digestion. The DNA bands showed that the majority of the mLAMP products were digested by the three enzymes, whereas only a part of products were digested by *Xho*I, *Kpn*I, or *BamH*I (Figure 3), meaning that there were three kinds of products in the mLAMP products and indicating that *S. aureus*, *S. enterica*, and *Shigella* DNA were all amplified in a single-vessel mLAMP system.

3.3 The sensitivity of mLAMP and mPCR assays

In this study, the sensitivity of the mLAMP assay was compared to the mPCR assay. Three pairs of primers were used in mPCR. Three pairs of primers were used in mPCR, namely, *nuc* F3/B3, *fimY* F1/B1, and *ipaH* F1/B1 were listed in Table 2. Figure 3 shows that a 10-fold dilution of DNA templates ranging from 1 ng to 1 fg per 25 μL was used to test the sensitivity of the mLAMP and mPCR assays. The DNA ladder patterns using 1 ng to 100 fg of DNA were clearly visible, indicating that the limit of detection (LOD) of mLAMP was 100 fg/25 μL, whereas that of mPCR was 1 pg/25 μL for target fragments of 181 bp, 126 bp, and 216 bp, as shown in the 1 ng to 1 pg lanes (Figure 4). Therefore, the mLAMP assay was 10 times more sensitive than the mPCR assay.

3.4 mLAMP assay for artificially contaminated juice

To assess the applicability of mLAMP in practical testing, *S. aureus*, *Salmonella*, and *Shigella* were simultaneously inoculated into juice. *S. aureus, Salmonella*, and *Shigella* were detected by mLAMP when the initial concentration was >2 CFU/10 mL

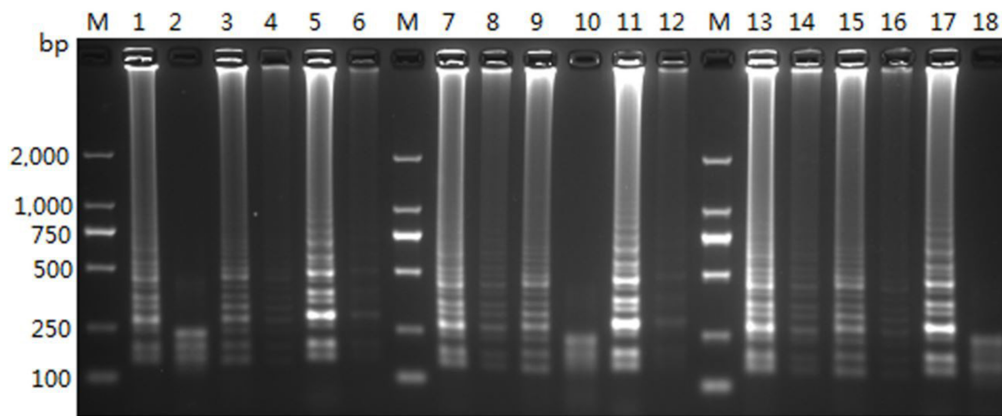

Figure 2. Specificity of restriction endonuclease reactions. Lanes: M, 2,000-bpDNA marker; 1,7,13, LAMP products of *Staphylococcus aureus*; 3, 9, and 15, LAMP products of *Salmonella*; 5, 11, and 17, LAMP product of *Shigella*; 2, 4, and 6, LAMP products of *S. aureus, Salmonella*, and *Shigella* digested by *Xho*I; 8, 10, and 12, LAMP products of *S. aureus, Salmonella*, and *Shigella* digested by *Kpn*I; 6, 12, and 18, LAMP products of *S. aureus, Salmonella*, and *Shigella* digested by *BamH*I. Source: by Cong XU.

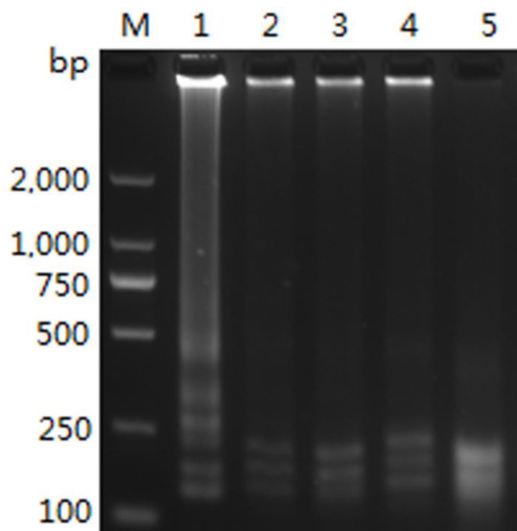

Figure 3. mLAMP assay and restriction endonuclease digestion. Lanes: M, 2,000-bp DNA marker; 1, mLAMP products of *Staphylococcus aureus, Salmonella*, and *Shigella*; 2~4, mLAMP products of *S. aureus, Salmonella*, and *Shigella* were separately digested by *Xho*I, *Kpn*I, and *BamH*I separately; 5, mLAMP products of *S. aureus, Salmonella,* and *Shigella* were digested by *Xho*I, *Kpn*I, and *BamH*I. Source: by Cong XU.

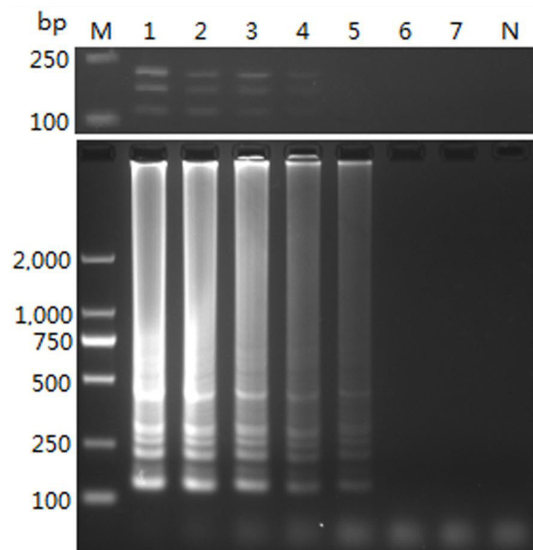

Figure 4. Sensitivity of mLAMP and mPCR assays. Upper panel: mPCR assay; lower panel: mLAMP assay. Lanes: M, 2,000-bp DNA marker; 1~7, *Staphylococcus aureus*, *Salmonella*, and *Shigella* DNA content was 1 ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg, and 1 fg; N, negative control. Source: by Cong XU.

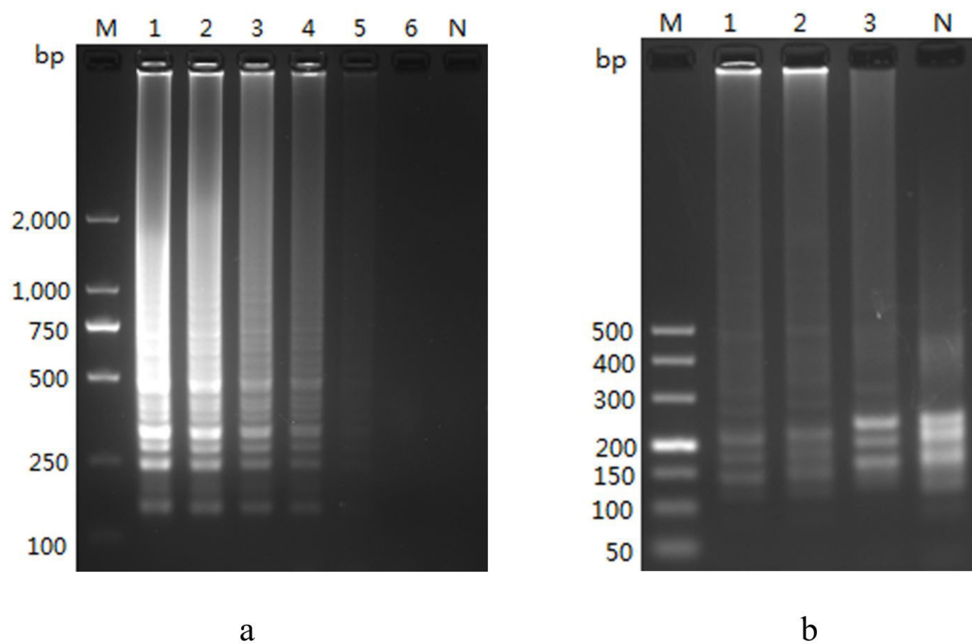

Figure 5. Sensitivity of mLAMP assays for artificially contaminated juice. Lanes: M, 2,000-bp DNA marker; (a) 1~6, the numbers of *S. aureus*, *Salmonella*, and *Shigella* in artificially contaminated juice sample were 100, 10, 8, 5, 2, and 1 CFU/mL; N, negative control; (b) 1~3, mLAMP products of *S. aureus, Salmonella,* and *Shigella* were separately digested by *Xho*I, *Kpn*I, and *BamH*I separately; 4, mLAMP products of *S. aureus*, *Salmonella*, and *Shigella* were digested by *Xho*I, *Kpn*I, and *BamH*I. Source: by Cong XU.

in the contaminated juice (Figure 5a). Furthermore, mLAMP products were digested by *Xho*I, *Kpn*I, and *BamH*I, respectively, to distinguish the products of the three pathogens (Figure 5b).

4 Discussion

S. aureus, *Salmonella*, and *Shigella* are three important foodborne pathogens that are largely responsible for foodborne illnesses occurring around the world (Wang et al., 2007; World Health Organization, 2015), particularly in developing countries (Von Seidlein et al., 2006). An effective and rapid monitoring mean is thus essential for the control of these three bacterial infections.

LAMP has become a powerful tool to detect foodborne pathogens since it was developed (Maerle et al., 2014; Wang et al., 2007, 2008). Yang et al. (2011) previously reported that the sensitivity of LAMP in detecting *S. aureus* was 1.25 CFU per reaction tube and 10.3 CFU per reaction in the artificial contamination test. Garrido-Maestu et al. (2017) reported systematic loop-mediated isothermal amplification assays were able to detect <10 cfu/25 g food samples. Shao et al. (2011) established mLAMP to simultaneously detect *Salmonella spp.* and *Shigella spp.* in milk, and their detection limits were 100 fg DNA/ tube with genomic DNA and initial inoculation levels of 5 CFU/10 mL. Wang et al. (2015) reported a multiple endonuclease restriction real-time LAMP technology for simultaneously differentiating *Shigella spp.* and *Salmonella spp.* using 62.5 and 125 fg DNA per tube, respectively.

In the present study, restriction enzyme cleavage sites were designed in each FIP primer, namely, *Xho*I in *nuc* FIP, *Kpn*I in *fimY* FIP, and *BamH*I in *ipaH* FIP. After mLAMP, restriction digestion was performed to distinguish *S. aureus, Salmonella*, and *Shigella* or a DNA mixture of any of the three pathogenic bacteria. Both LMAP and restriction digestion were pathogen-specific. The mLAMP LOD for each pathogen were 100 fg DNA/25 µL which is identical to Wang et al. (2015), and 2 CFU/10 mL of initial inoculation in juice. The sensitivity of mLAMP was 10-fold higher than mPCR, which is the same as that of previous reports (Shao et al., 2011; Wang et al., 2015). Furthermore, in this study, mLAMP can be completed within 20 h (including culture, DNA extraction, LAMP, enzyme digestion and gel analysis) and is thus superior to traditional methods that are performed within the range of five to seven day (based on the methods protocols). In addition, mLAMP and endonuclease restriction only require a heating block, which is a common laboratory equipment. This study could be applied on preliminary detection of *S. aureus*, *Salmonella*, and *Shigella* when SYBR GREEN I is added in the reaction system (data not shown). For as long as any kinds of the above-mentioned bacteria exist in the sample, they could be detected by direct observation, with no need for electrophoresis and enzyme reaction in the preliminary estimation. Restriction enzyme digestion could be further applied in order to determine the species of the pathogenic bacterium.

In conclusion, this study provides a rapid, specific, sensitive, and low-cost assay for the simultaneous detection of *S. aureus, Salmonella*, and *Shigella*. The detection of these three bacterial pathogens in juice indicates that mLAMP could be potentially used in batches food screening in basic and field laboratories.

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References

Amani, J., Ahmadpour, A., Imani Fooladi, A. A., & Nazarian, S. (2015). Detection of *E. coli* O157:H7 and *Shigella dysenteriae* toxins in clinical samples by PCR-ELISA. *The Brazilian Journal of Infectious Diseases*, 19(3), 278-284. http://dx.doi.org/10.1016/j.bjid.2015.02.008. PMid:25911087.

Chen, Z., Zhang, K., Yin, H., Li, Q., Wang, L., & Liu, Z. (2015). Detection of *Salmonella* and several common *Salmonella* serotypes in food by loop-mediated isothermal amplification method. *Food Science and Human Wellness*, 4(2), 75-79. http://dx.doi.org/10.1016/j.fshw.2015.05.001.

Food and Agriculture Organization of the United Nations – FAO. World Health Organization – WHO. (2016). *Interventions for the control of non-typhoidal Salmonella spp. in beef and pork*. Rome. Retrieved from http://www.who.int/foodsafety/publications/mra_30/en/

Garrido-Maestu, A., Fuciños, P., Azinheiro, S., Carvalho, J., & Prado, M. (2017). Systematic loop-mediated isothermal amplification assays for rapid detection and characterization of *Salmonella* spp., Enteritidis and Typhimurium in food samples. *Food Control*, 80, 297-306. http://dx.doi.org/10.1016/j.foodcont.2017.05.011.

Huy, N. T., Hang, L. T. T., Boamah, D., Lan, N. T. P., Van Thanh, P., Watanabe, K., Huong, V. T., Kikuchi, M., Ariyoshi, K., Morita, K., & Hirayama, K. (2012). Development of a single-tube loop-mediated isothermal amplification assay for detection of four pathogens of bacterial meningitis. *FEMS Microbiology Letters*, 337(1), 25-30. http://dx.doi.org/10.1111/1574-6968.12002. PMid:22946506.

Imai, M., Ninomiya, A., Minekawa, H., Notomi, T., Ishizaki, T., Tashiro, M., & Odagiri, T. (2006). Development of H5-RT-LAMP (loop-mediated isothermal amplification) system for rapid diagnosis of H5 avian influenza virus infection. *Vaccine*, 24(44-46), 6679-6682. http://dx.doi.org/10.1016/j.vaccine.2006.05.046. PMid:16797110.

Liu, Z. T., Zeng, J. H., Juan-Juan, L. I., Wan, Q. Q., Wan, R., Si-Yang, Y. U., et al.(2016). Analysis of foodborne disease outbreak incidents in schools in Yunnan Province between 2010 and 2015. *Zhongguo Shipin Weisheng Zazhi*, 28, 730-734.

Maerle, A. V., Riazantsev, D. I., Dmitrenko, O. A., Petrova, E. I., Komaleva, R. L., Sergeev, I. V., Trofimov, D. Iu., & Zavriev, S. K. (2014). Detection of *Staphylococcus aureus* toxins using immuno-PCR. *Bioorganicheskaia Khimiia*, 40(5), 571-577. PMid:25895352.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, 28(12), E63. http://dx.doi.org/10.1093/nar/28.12.e63. PMid:10871386.

Nouri, A., Ahari, H., & Shahbazzadeh, D. (2018). Designing a direct ELISA kit for the detection of Staphylococcus aureus enterotoxin A in raw milk samples. *International Journal of Biological Macromolecules*, 107(Pt B), 1732-1737. http://dx.doi.org/10.1016/j.ijbiomac.2017.10.052. PMid:29030180.

Shao, Y., Zhu, S., Jin, C., & Chen, F. (2011). Development of multiplex loop-mediated isothermal amplification-RFLP (mLAMP-RFLP) to detect *Salmonella spp*. and *Shigella spp*. in milk. *International Journal of Food Microbiology*, 148(2), 75-79. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.05.004. PMid:21652102.

Sun, X., Ji, Y., Liu, X., Xiang, M., He, G., Xie, L., Suo, J. X., & Suo, X. (2017). Improvement and evaluation of loop-mediated isothermal amplification for rapid detection of toxoplasma gondii infection in human blood samples. *PLoS One*, 12(1), e0169125. http://dx.doi.org/10.1371/journal.pone.0169125. PMid:28056092.

Von Seidlein, L., Kim, D. R., Ali, M., Lee, H., Wang, X., Thiem, V. D., Canh, D. G., Chaicumpa, W., Agtini, M. D., Hossain, A., Bhutta, Z. A., Mason, C., Sethabutr, O., Talukder, K., Nair, G. B., Deen, J. L., Kotloff, K., & Clemens, J. (2006). A multicentre study of Shigella diarrhoea in six Asian countries: disease burden, clinical manifestations, and microbiology. *PLoS Medicine*, 3(9), 1556-1569. http://dx.doi.org/10.1371/journal.pmed.0030353. PMid:16968124.

Wang, L., Shi, L., Alam, M. J., Geng, Y., & Li, L. (2008). Specific and rapid detection of foodborne Salmonella by loop-mediated isothermal amplification method. *Food Research International*, 41(1), 69-74. http://dx.doi.org/10.1016/j.foodres.2007.09.005.

Wang, S., Duan, H., Zhang, W., & Li, J.-W. (2007). Analysis of bacterial foodborne disease outbreaks in China between 1994 and 2005. *FEMS Immunology and Medical Microbiology*, 51(1), 8-13. http://dx.doi.org/10.1111/j.1574-695X.2007.00305.x. PMid:17666075.

Wang, Y., Wang, Y., Luo, L., Liu, D., Luo, X., Xu, Y., Hu, S., Niu, L., Xu, J., & Ye, C. (2015). Rapid and sensitive detection of *Shigella spp*. and *Salmonella spp*. by multiple endonuclease restriction real-time loop-mediated isothermal amplification technique. *Frontiers in Microbiology*, 6, 6. http://dx.doi.org/10.3389/fmicb.2015.01400. PMid:26697000.

World Health Organization – WHO. Food and Agriculture Organization of the United Nations – FAO. (2003). *Assuring food safety and quality: guidelines for strengthening national food control systems*. Geneva: WHO.

World Health Organization – WHO. (2015). *World Health Day 2015: food safety*. Geneva: WHO. Retrieved from http://www.who.int/campaigns/world-health-day/2015/en/

Yang, H., Ma, X., Zhang, X., Wang, Y., & Zhang, W. (2011). Development and evaluation of a loop-mediated isothermal amplification assay for the rapid detection of *Staphylococcus aureus* in food. *European Food Research and Technology*, 232(5), 769-776. http://dx.doi.org/10.1007/s00217-011-1442-8.