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Note

The Rapid Detection of *Salmonella* from Food Samples By Loop-Mediated Isothermal Amplification (LAMP)

SHIGEKO UEDA* AND YOSHIHIRO KUWABARA

Laboratory of Hygiene, Kagawa Nutrition University
3-9-21, Chiyoda, Sakado-shi, Saitama 350-0214, Japan

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Loop-mediated isothermal amplification (LAMP) assay was applied to the detection of *Salmonella* in food and human materials. It was possible for the assay to detect *Salmonella* within 60min. All of 54 serovars of *Salmonella* tested were amplified, but all bacteria tested other than *Salmonella* were not. The LAMP assay could detect 10^2 cfu/ml levels of *Salmonella*. The specificity was similar to that of a PCR assay, but the sensitivity of LAMP was considered to be greater. Thus, the LAMP assay was confirmed to be a rapid, specific and sensitive detection method for *Salmonella*.

Key words：*Salmonella*/LAMP/PCR/Food.

Salmonella is one of the most common causal agents of foodborne infections in Japan. Because *Salmonella* contamination levels in foods are generally low compared to clinical specimens, it has been necessary to use the culture enrichment method to test food samples and more than three days are required for its detection and identification (Hara-Kudo et al., 2005). For public health and the food industry, rapid, sensitive and specific methods to detect *Salmonella* in food are required. Of several molecular genetic methods, the polymerase chain reaction (PCR) assay is the most widely used for specific amplification of a target gene, and it has been reported to be able to rapidly detect target pathogens (Bej et al., 1990; Chiu and Ou, 1996; Golsteyn-Thomas et al., 1991). Recently, LAMP that amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions has been developed as a novel nucleic acid amplification method (Enosawa et al.,2003; Mori et al.,2001; Notomi et al.,2000). This method relies on an auto-cycling strand displacement DNA synthesis performed by the *Bst* polymerase large fragment, and is different from PCR in that four or six different primers perform the amplification of the target gene, and the amplification uses a constant temperature between 60 and 65℃ for about 60 min. The amplification products have variously sized structures consisting of alternate inverted repeats of the target sequence on the same strand in large amounts. The target gene is simply detected by the increase in the turbidity, as the reaction produces the precipitate correlated with the amount of target DNA synthesized. In this study, we evaluated the specificity and sensitivity of a LAMP assay for the rapid detection of *Salmonella*.

The specificity of the LAMP assay was tested by using 54 serovars of *Salmonella* spp., 4 gram-negative species including *Citrobacter freundii, Escherichia coli, Yersinia enterocolitica* and *Vibrio parahaemolyticus*, and 6 gram-positive species including *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Bacillus licheniformis, Clostridium botulinum, Clostridium perfringens* and *Listeria monocytogenes. Salmonella* spp. and *C. freundii* were cultured on DHL agar medium (Nissui). *E. coli* O157 was cultured on Rainbow agar medium (Biolog). Other *E. coli* serovars and strains were cultured on EMB agar medium (Nissui). *Y. enterocolitica* was cultured on CIN agar medium (Oxoid), *V. parahaemolyticus* on TCBS agar medium (Nissui), *L. monocytogenes* on Oxford base agar medium

*Corresponding author. Tel/Fax：+81-49-282-3717.

(Nissui), *B. cereus* on NGKG agar medium (Nissui), and *C. botulinum* and *C. perfringens* were on CW agar medium (Eiken). After each strain was incubated at 37℃ for 18h, a typical colony on the selective agar medium was used for the LAMP and PCR assays. To evaluate the sensitivity of the LAMP assay, *S*. Enteritidis was inoculated into liquid samples and 10% suspensions of solid samples at concentrations of 10^0 - 10^3 cfu/ml. The food samples were egg, milk, yogurt, omelette, hamburger, raw pork, beef and chicken, roast beef, lettuce, vegetable salad, watermelon, apple juice, spinach saute and cake. Also, the blood and faeces of a healthy human subject were used. The enrichment media containing Brain heart infusion broth (BBL), Trypticase broth (BBL) and EEM broth (Nissui) were utilized as references.

The LAMP reaction was performed using a Loopamp DNA kit (Eiken Chem. Co., Ltd.). A colony on the selective agar media or 50 μl of the sample suspension was added to 50 μl of the extraction solution (pH12.5) (Eiken Chem. Co., Ltd.) in a microcentrifuge tube and heated at 100℃ for 5 min to extract DNA. The samples were centrifuged at 2,000g for 1 min, and the supernatant was transferred to a new microcentrifuge tube and used as the template DNA solution for the LAMP assay. A 20 μl of 'Reaction Mix. Sal' containing the primers for *Salmonella* detection and 1 μl of *Bst* DNA polymerase were mixed in another tube. A 20 μl aliquot of the mixture was transferred and 5 μl of the template DNA solution was added to a reaction tube. The reaction components were mixed in the tube, incubated at 65℃ for 60 min and then heated to 80℃ for 2 min to terminate the reaction. *Salmonella* DNA preparation extracted from *S*.Enteritidis was used as a positive control and the DNA-omitted reaction mixture was used as a negative control. The amplification of the gene was confirmed by real-time monitoring of the increase of turbidity produced by magnesium pyrophosphate during the reaction (Mori et al., 2001) by using a loopamp realtime tubidimeter (Teramecs, Kyoto), which sequentially measured the absorbance of the reaction mixture at 650 nm. The LAMP assay was able to detect *Salmonella* within 60 min.

PCR was performed with a kit for detection of *Salmonella invA* gene (Takara). The PCR mixture (100 μl) in a reaction tube contained 10 μl of template DNA, 8 μl of 2.5 mM dNTP mixture, 1 μl of primer-1, 1 μl of primer-2, 2.5 U of *Taq* DNA polymerase, 10 μl of 10×PCR buffer and 69.5 μl of double-distilled water. The PCR reaction was performed with a DNA thermal cycler (Biocraft, type QTP-1) and was subjected to 35 cycles consisting of 1 min at 94℃ for denaturation, 1 min at 55℃ for annealing and 1min at 72℃ for extension. A 10 μl aliquot of the PCR products was subjected to electrophoresis on a 2%(w/v) agarose (Sigma) gel in Tris-acetate-EDTA buffer (Ueda et al., 2000).

The specificity of LAMP was examined by carrying out reactions with DNA from various *Salmonella* spp. and other gram-negative and gram-positive bacteria. *S*. Enteritidis and *S*. Typhimurium gave positive reactions, but bacterial strains other than *Salmonella* did not (Table 1). Furthermore, it was confirmed that all of 54 serovars of *Salmonella* were confirmed to give positive results, and other gram-negative bacteria gave negative results in the LAMP assay (Table 2). The PCR method also showed positive results for *Salmonella*, but showed negative results for all other

TABLE 1. Specificity of the LAMP method for the detection of *Salmonella* spp.

Bacterial species/serovar/strain	Result
Salmonella Enteritidis	+
Salmonella Typhimurium	+
Escherichia coli	
O127:H21 (EPEC[1])	−
O124:HNM (EIEC[2])	−
ST and LT producer (ETEC[3])	−
O157:H7 (VT1 and VT2 producer) (EHEC[4])	−
O157:H7 (VT2 producer) (EHEC[4])	−
O157:H7 (non-VT producer) (ATCC43888)	−
O111:NM (VT1 and VT2 producer) (EHEC[4])	−
O26:NM (VT1 and VT2 producer) (EHEC[4])	−
strain V517	−
strain IFO3301	−
type Ⅰ from food	−
Citrobacter freundii	
strain 1	−
strain 2	−
strain 3	−
strain 4	−
strain 5	−
strain 6	−
strain 7	−
strain 8	−
strain 9	−
strain 10	−
Yersinia enterocolitica	−
Vibrio parahaemolyticus	−
Staphylococcus aureus (8 strains)	−
Bacillus cereus (3 strains)	−
Bacillus subtilis	−
Bacillus licheniformis (2 strains)	−
Clostridium botulinum	−
Clostridium perfringens	−
Listeria monocytogenes	−

1) Enteropathogenic *E. coli*; 2) Enteroinvasive *E. coli*; 3) Enterotoxigenic *E. coli*; 4) Enterohemorrhagic *E. coli*

TABLE 2. Species specificity of LAMP and PCR method for *Salmonella* serovars.

Salmonella species/serovar	Result		*Salmonella* species/serovar	Result	
	LAMP	PCR		LAMP	PCR
Agona	+	+	Montevideo	+	+
Anatum	+	+	Muenchen	+	+
Bareilly	+	+	Muenster	+	+
Blockley	+	+	Nagoya	+	+
Braenderup	+	+	Narashino	+	+
Brandenburg	+	+	Newport	+	+
Cerro	+	+	Ohio	+	+
Chailey	+	+	Oranieburg	+	+
Chester	+	+	Othmarschen	+	+
Chincol	+	+	Paratyphi A	+	+
Derby	+	+	Paratyphi B	+	+
Djugu	+	+	Potsdam	+	+
Dublin	+	+	Saintpaul	+	+
Enteritidis	+	+	Sandow	+	+
Hadar	+	+	Schwarzengrund	+	+
Haifa	+	+	Senftenberg	+	+
Hartford	+	+	Singapore	+	+
Heiderberg	+	+	Stanley	+	+
Holcomb	+	+	Stanleyville	+	+
Infantis	+	+	Tennessee	+	+
Isangi	+	+	Thompson	+	+
Litchfield	+	+	Typhi	+	+
Liverpool	+	+	Typhimurium	+	+
Livingstone	+	+	Uppsala	+	+
London	+	+	Virchow	+	+
Manhatten	+	+	Weltevreden	+	+
Mbandaka	+	+	Zanzibar	+	+

bacteria other than *Salmonella* (Table 2). Thus, the specificity of the LAMP assay was as high as that for PCR, as described by Hara-Kudo et al. (2005). Also, the LAMP assay was shown to be able to detect many pathogenic microorganisms specifically by using the primers designed for the respective target genes (Furuhata et al., 2005; Horisaka et al., 2004; Yoda et al., 2007).

The sensitivity of the LAMP assay for *Salmonella* was examined by determining the detection limit from 3 different liquid media and 10% suspensions of different food samples and human materials inoculated with various levels of *Salmonella* Enteritidis cells. Hara-Kudo et al. (2005) described the detection limit of LAMP assay as 370 to 434 cells/ml of the concentration. In this study, the LAMP assay was able to detect the target gene from all food and human materials contaminated with 10^2 cfu/ml of *Salmonella*. However, it was suggested that even levels less than 10^2 cfu/ml were detectable depending on the material suspensions. Ueda et al. (2000) reported that the detection limit of PCR was 10^3 to 10^5 cfu/ml of *Salmonella* cells. Furthermore, if the *Salmonella* contamination level in the sample was estimated to be below the detection limit, it was found to be detected by using the appropriate enrichment preculuture for several hours. Horisaka et al. (2004) also described that LAMP was 100 times more sensitive than PCR. Notomi et al. (2000) reported that the sensitivity of LAMP was not influenced by the copresence of nontarget DNA in samples and Enosawa et al. (2003) reported that LAMP was not inhibited by blood serum and plasma heparin, which are known to inhibit PCR.

This study demonstrated that the LAMP assay was effective in detecting *Salmonella* rapidly, specifically and with high sensitivity.

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