Sensitive and rapid detection of Vero toxin-producing *Escherichia coli* using loop-mediated isothermal amplification

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A loop-mediated isothermal amplification (LAMP) assay was developed to detect Vero toxin (VT)-producing *Escherichia coli* rapidly (within 60 min). The 24 strains of VT-producing *E. coli* were successfully amplified, but 6 strains of non-VT-producing *E. coli* and 46 bacterial species other than *E. coli* were not. The sensitivity of the LAMP assay was found to be > 0.7 c.f.u. per test using serogroups O157, O26 and O111 of VT-producing *E. coli*; this sensitivity is greater than that obtained by PCR assay. Furthermore, the LAMP assay was examined for its ability to detect VT-producing *E. coli* in food because of the difficulty of detection in food samples. The recovery of VT-producing *E. coli* by LAMP assay from beef and radish sprouts inoculated with the pathogen was high, similar to that obtained using culture methods with direct plating and/or plating after immunomagnetic separation. Although PCR assay was unable to recover VT-producing *E. coli* from half of the radish samples, LAMP assay was successful in most samples. In addition, VT-producing *E. coli* was successfully detected in cultures of the beef samples by LAMP assay, but not by the culture method. The LAMP products in naturally contaminated beef samples were analysed to confirm the specific amplification of the VT-encoding gene, and were found to show a specific ladder band pattern on agarose gel after electrophoresis. Additionally the sequences of the LAMP products coincided well with the expected sequences of the VT-encoding gene. These results indicate that the proposed LAMP assay is a rapid, specific and sensitive method of detecting the VT-producing *E. coli*.

INTRODUCTION

Sensitive and rapid detection methods for Vero toxin (VT)-producing *Escherichia coli* are required. VT-producing *E. coli* has many serotypes, although O157 predominates. Thus, the early detection of VT is important to save both time and cost. The present culture methods require more than 3 days, and rapid detection methods like enzyme immunoassay require a high population of the target pathogen (Bennett *et al.*, 1996; Chapman *et al.*, 2001). A PCR assay was developed to rapidly detect target pathogens in enrichment cultures; however, the assay generally requires electrophoresis to detect the amplicon, which takes several hours. Recently, real-time PCR assays have been applied to detect food-borne pathogens, but such assays requires an expensive thermal cycler with a fluorescence detector (Mullah *et al.*, 1998).

Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method that relies on an auto-cycling strand displacement DNA synthesis performed by the *Bst* DNA polymerase large fragment (Nagamine *et al.*, 2001, 2002; Notomi *et al.*, 2000). LAMP is different from PCR in that four or six primers perform the amplification of the target gene, the amplification uses a single temperature step at 60–65 °C for about 60 min, and the amplification products have many types of structures in large amounts. Thus, LAMP is faster and easier to perform than PCR, as well as being more specific. Furthermore, gel electrophoresis is not needed, because the LAMP products can be detected indirectly by the turbidity that arises due a large amount of by-product, pyrophosphate ion, being produced, yielding an insoluble white precipitate of magnesium pyrophosphate in the reaction mixture (Mori *et al.*, 2001). Since the increase in the turbidity of the reaction mixture according to the production of precipitate correlates with the amount of DNA synthesized, real-time monitoring of the LAMP reaction can be achieved by real-time measurement of turbidity.

VT is classified into two types: VT1 and VT2 (Jackson *et al.*, 1987), and VT1- and VT2-producing *E. coli* are regarded as pathogens. VT1 is identical with Shiga toxin. VT2 differs from VT1 in its physico-chemical, immunological and molecular genetic properties. Several variants of VT, especially VT2, have been reported. Sensitive detection methods for VT must target VT1 and VT2, including VT2vha, VT2vhb (Ito *et al.*, 1990, 1991) and VT2vp1 (Lin *et al.*, 1993; Weinstein *et al.*, 1988). In the present study, we developed a LAMP assay to detect VT1 and/or VT2-producing *E. coli* rapidly and sensitively.

METHODS

Bacterial strains. Is this present study, 24 strains of VT–producing *E. coli*, 6 strains of non-VT-producing *E. coli* and 46 bacterial species other than *E. coli* (Table 1) were tested using the LAMP assay to show specificity. *Vibrio* spp. were cultured on trypticase soy agar (TSA) medium (Difco) containing 3 % NaCl for 18 h at 37 °C. The other bacteria were cultured on brain heart infusion agar (Difco) at 37 °C for 18 h. Colonies were suspended to 2 McFarland standard turbidity in 5 ml PBS. The bacterial suspension was diluted in PBS to 10^6 c.f.u. ml^{-1}. The dilutions were used for the LAMP assay.

Cultures of five strains of VT-producing *E. coli* (nos. 157–891, ATCC43890, 157–120, BFR 26015 and 97353) were tested to investigate the sensitivity of the LAMP and PCR assays. They were cultured in tryptic soy broth (TSB; Difco) at 37 °C for 18 h and serially diluted tenfold in PBS to 10^{-3}–10^{-7} dilutions. Each dilution was used for both LAMP and PCR assays. To determine the inoculum size, 0.1 ml each dilution of VT–producing *E. coli* culture was plated onto TSA in duplicate. After incubation at 37 °C for 18 h, the number of colonies was counted.

Food samples. Ground beef and radish sprouts were purchased from retail shops in Tokyo for inoculation with VT-producing *E. coli*. The aerobic bacterial counts in the ground beef and radish sprouts were 5.3 and 8.2 log c.f.u. g^{-1}, respectively.

LAMP assay. In the present study, five primers (two inner primers, two outer primers and one loop primer) targeting the *VT1* gene and six primers (two inner primers, two outer primers and two loop primers) targeting the genes of *VT2*, *VT2vha*, *VT2vhb* and *VT2vp1* (Table 2) were used for the LAMP reactions (Notomi *et al.*, 2000); the domains targeted by these primers are shown in Fig. 1. The LAMP reaction was performed using a Loopamp DNA amplification kit (Eiken Chemical). Each 50 µl bacterial dilution or culture sample was added to 42 µl extraction solution (50 mM NaOH, pH 12.5) (Beige *et al.*, 1995) in order to extract DNA before heating at 100 °C for 5 min. After flash heating, the samples were added to 8 µl 1 M Tris/HCl (pH 7.0) and centrifuged (Tomy) at 2000 *g*, and the supernatant was transferred to a new micro-tube and used as the template DNA solution for the LAMP assay. The LAMP reaction mixture contained the primers for VT detection (20 µl), *Bst* polymerase (1 µl) and template DNA solution (5 µl). The reaction components were mixed in a tube, incubated at 65 °C for 60 min using a Loopamp LA-200 (Teramecs) and then heated to 80 °C for 2 min to terminate the reaction. LAMP amplification was detected as a value of turbidity at 650 nm using a Loopamp LA-200 in real-time. In addition, turbidity produced by the by-product magnesium pyrophosphate was noted visually. VT-producing *E. coli* DNA extracted from a suspension of the pathogen by heating at 95 °C for 10 min was used as a positive control each time.

Aliquots of 1 µl LAMP products were electrophoresed in 2 % agarose gel. After staining with ethidium bromide, the products were detected under UV light.

PCR assay. The bacterial dilutions in PBS of five strains of VT-producing *E. coli*, described above, were heated at 95 °C for 10 min, and centrifuged at 10 000 *g* for 5 min. The supernatant was transferred to a new micro-tube as the template DNA solution for PCR. PCR targeting VT-encoding genes was performed as follows. Primer sets EVC-1 and EVC-2 (0.5 µl each) (Takara Bio), dNTP mixture (4 µl), 10× Taq buffer (5 µl), Takara Taq (0.25 µl), template DNA solution (2.5 µl) and distilled water (37.25 µl) were mixed in a reaction tube. The reaction was performed at 94 °C for 1 min for denaturing, at 55 °C for 1 min for annealing and at 72 °C for 1 min for extension using a thermal cycler (ABI7000, Applied BioSystems). After 35 cycles of the reaction, followed by heating to 72 °C for 10 min, the PCR products were subjected to electrophoresis in 3 % agarose gel. After staining with ethidium bromide, the PCR product (171 bp) was detected under UV light.

Recovery of VT-producing *E. coli* from food inoculated with the pathogens. For inoculation, serogroups O157 (no. 157-212) and O26 (no. BFR 26015) of VT-producing *E. coli* were cultured in TSB at 37 °C for 18 h. Cultures were diluted in PBS to a 10^{-6} dilution, and 0.1 ml was inoculated into each 25 g of ground beef and radish sprouts. The food samples were homogenized in 225 ml modified EC including novobiocin (mEC+n) broth as an effective enrichment broth for serogroups O157 and O26 (Hara-Kudo *et al.*, 1999, 2000; Okrend *et al.*, 1990) with a Stomacher (model 400; AJ Seward) prior to incubation, and then incubated statically at 42 °C for 18 h.

The cultures of food inoculated with serogroup O157 were plated onto each of two plates of sorbitol MacConkey agar (Oxoid) supplemented with cefixime (0.05 mg l^{-1}) and potassium tellurite (2.5 mg l^{-1}) (CT-SMAC) and CHROMagar O157 (CHROMagar) directly, or after immunomagnetic separation (IMS) performed according to the manufacturer's instructions with Dynabeads anti-*E. coli* O157 (Dynal). At least three colonies suspected to be O157 were tested for agglutination using an *E. coli* O157 : H7 UNI latex kit (Unipath Oxoid). Acid production from lactose, acid non-production from cellobiose and non-fluorescence under UV light were observed in cellobiose-lactose-indole-β-D-glucuronidase (CLIG) agar (Kyokuto).

The cultures of food inoculated with serogroup O26 were plated onto each of two plates of rhamnose MacConkey agar (MacConkey agar with lactose replaced by rhamnose) (Hiramatsu *et al.*, 2002) supplemented with cefixime (0.05 mg l^{-1}) and potassium tellurite (2.5 mg l^{-1}) (CT-RMAC) and Rx ViO26 medium (Eiken Chemical) directly, or after IMS performed according to the manufacturer's instructions using immunomagnetic beads coated with anti-O26 antibody (Denka Seiken). At least three colonies suspected to be O26 were tested for agglutination using the *E. coli* O26 antiserum (Denka Seiken).

For PCR assay, 0.1 ml mEC+n culture was transferred to a tube and centrifuged at 10 000 *g* for 10 min. After removing the supernatant, 0.1 ml sterilized distilled water was added to the tube and the mixture was heated at 95 °C for 5 min. After centrifuging at 10 000 *g* for 10 min, the supernatant was used as template for the PCR described above.

For the LAMP assay, 50 µl culture was added to 50 µl extraction solution to extract DNA, and the LAMP reaction was then carried out as described above.

Analysis of LAMP products. Using meat obtained in an inspection carried out by the local government of Saitama prefecture,

Table 1. Specificity of the LAMP assay

Species	Strain no.	Serotype	Production of VT*		LAMP
			VT1	VT2	
Escherichia coli	157-1	O157:H7	+	+	+
	157-10	O157:H7	+	+	+
	157-18	O157:H7	+	+	+
	157-42	O157:H7	+	+	+
	157-61	O157:H7	+	+	+
	157-137	O157:H7	+	+	+
	157-457	O157:H7	+	+	+
	157-23	O157:H7	+	+	+
	157-212	O157:H7	+	+	+
	157-487	O157:H7	+	+	+
	157-891	O157:H7	+	+	+
	ATCC 43890	O157:H7	+	−	+
	157-132	O157:H7	−	+	+
	157-126	O157:H7	−	+	+
	157-298	O157:H-	−	+	+
	157-120	O157:H7	−	+	+
	157-174	O157:H7	−	+	+
	KE 135	O157:H-	−	−	−
	BFR 26001	O26:H11	+	−	+
	BFR 26002	O26:H-	+	−	+
	BFR 26009	O26:H11	+	−	+
	BFR 26015	O26:H11	+	+	+
	BFR 26016	O26:H11	+	+	+
	97109	O111:H-	−	−	−
	97353	O111:H-	+	−	+
	BFR 1	O101:H9	−	−	−
	BFR 2	O145:H-	−	−	−
	BFR 3	O145:H16	−	−	−
	BFR 4	O113:H21	−	+	+
	JCM 1649		−	−	−
Citrobacter freundii	JCM 1657				−
Citrobacter koseri	EKN34				−
Edwardsiella tarda	EKN639				−
Enterobacter aerogenes	JCM 1235				−
Enterobacter cloacae	ATCC 13047				−
Enterococcus avium	JCM 8722				−
Enterococcus cecorum	JCM 8724				−
Enterococcus durans	JCM 8725				−
Enterococcus faecalis	JCM 5803				−
Enterococcus pseudoavium	JCM 8732				−
Escherichia fergusonii	ATCC 35469				−
Escherichia hermannii	ATCC 37650				−
Hafnia alvei	JCM 1666				−
Klebsiella oxytoca	EKN406				−
Klebsiella pneumoniae subsp. *pneumoniae*	JCM 1662				−
Lactococcus lactis subsp. *lactis*	JCM 5805				−
Lactococcus raffinolactis	JCM 5706				−
Leuconostoc gelidum	JCM 9697				−
Leuconostoc mesenteroides subsp. *dextranicum*	JCM 9700				−
Listeria monocytogenes	LIS1				−
Micrococcus luteus	JCM 1464				−
Morganella morganii	EKN183				−
Proteus mirabilis	JCM 1669				−

Table 1. cont.

Species	Strain no.	Serotype	Production of VT*		LAMP
			VT1	VT2	
Proteus vulgaris	EKN184				−
Providencia alcalifaciens	EKN182				−
Providencia rettgeri	EKN4088				−
Providencia stuartii	EKN4092				−
Pseudomonas aeruginosa	ATCC 27853				−
Salmonella enteritidis	ATCC 13076				−
Salmonella typhimurium	ATCC 17311				−
Serratia marcescens	EKN233				−
Staphylococcus aureus	ATCC 25923				−
Staphylococcus capitis subsp. *capitis*	JCM 2420				−
Staphylococcus epidermidis	JCM 2414				−
Staphylococcus haemolyticus	JCM 2416				−
Staphylococcus intermedius	JCM 2422				−
Staphylococcus warneri	JCM 2415				−
Staphylococcus xylosus	JCM 2418				−
Streptococcus equinus	JCM 5802				−
Streptococcus pyogenes	JCM 5674				−
Vibrio mimicus	ATCC 33653				−
Vibrio parahaemolyticus	ATCC 17802				−
Vibrio vulnificus	IFO 15645				−
Yersinia enterocolitica	EKN823				−
Yersinia pseudotuberculosis	YER2				−

*Determined by a reversed passive latex agglutination assay.

Japan, 25 g surface portions of beef loaf samples were incubated in mEC + n at 42 °C for 18 h. The food cultures were plated onto each of two plates of CT-SMAC and CHROMagar O157 directly, or after IMS with Dynabeads anti-*E. coli* O157. The culture (1 ml) was tested by the PCR and LAMP assays described above. In four samples, the VT-encoding gene was detected in the enrichment culture by LAMP assay, although VT-producing *E. coli* O157 was not isolated. The enrichment cultures of four purchased beef samples (samples 03-150, 03-156, 03-266 and 03-304) were analysed by LAMP assay. The LAMP products from the samples were purified using a QIAquick PCR purification kit (Qiagen) and sequenced directly with the Vt1F2Cy5 (5′-CAT TCG TTG ACT TCT TAT CTG G-3′) and Vt2F2Cy5 (5′-CAC TCA CTG GTT TCA TCA TAT CTG G-3′) primers for *VT1* and *VT2* using an ABI 310 genetic analyser (Applied Biosystems) with a BigDye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems). The PCR amplification products were

Table 2. LAMP primers

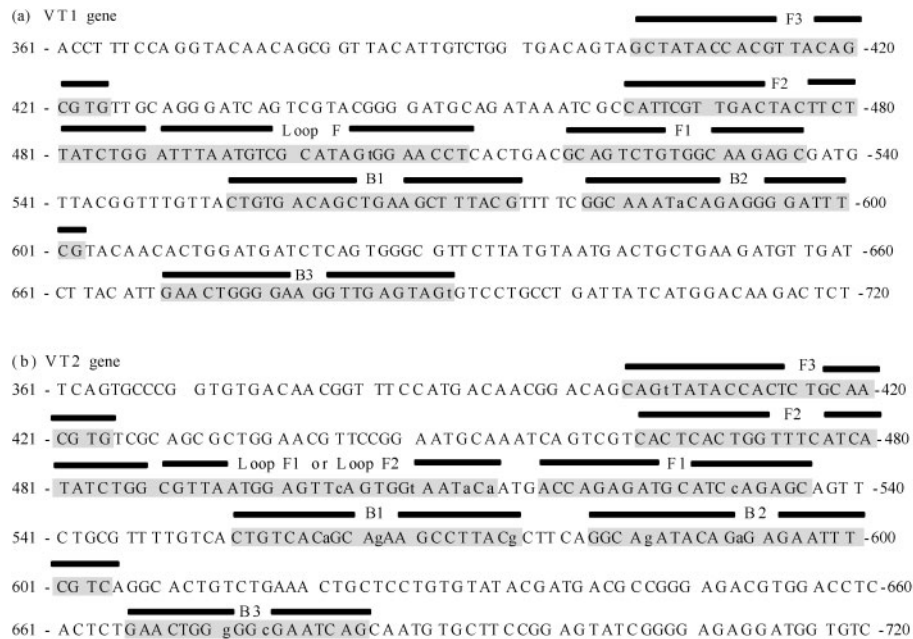| Primer	Sequence (5′-3′)
VT1	
FIP	GCTCTTGCCACAGACTGCACATTCGTTGACTACTTCTTATCTGG
BIP	CTGTGACAGCTGAAGCTTTACGCGAAATCCCCTCTGAATTTGCC
F3	GCTATACCACGTTACAGCGTG
B3	ACTACTCAACCTTCCCCAGTTC
Loop F	AGGTTCCGCTATGCGACATTAAAT
VT2	
FIP	GCTCTTGATGCATCTCTGGTACACTCACTGGTTTCATCATATCTGG
BIP	CTGTCACAGCAGAAGCCTTACGGACGAAATTCTCCCTGTATCTGCC
F3	CAGTTATACCACTCTGCAACGTG
B3	CTGATTCGCCGCCAGTTC
Loop F1	TGTATTACCACTGAACTCCATTAACG
Loop F2	GGCATTTCCACTAAACTCCATTAACG

Fig. 1. Nucleotide sequences of targets for primers in the LAMP assay of VT-encoding *gene of E. coli*. (a) *VT1* gene: a multiple alignment was determined by analyses of various *VT1* sequences from the GenBank database (accession no. BA000007). (b) *VT2* gene: a multiple alignment was determined by analyses of various *VT2* sequences from the GenBank database (accession no. AE005174). Small letters indicate variant bases.

purified using a Montage PCR centrifugal filter device (Millipore) according to the manufacturer's instructions. The purified DNA was sequenced using an ABI310 Genetic Analyser (Applied Biosystems) with a Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems).

Aliquots 1 μl LAMP products were electrophoresed in 2 % agarose gel. After staining with ethidium bromide, the products were detected under UV light.

RESULTS

Specificity of the LAMP assay

Twenty-four strains of VT-producing *E. coli*, including either VT1- or VT2-producing *E. coli* or both were positive in the LAMP assay (Table 1). The serogroups were O157, O26, O111 and O145, and the serogroup did not affect the assay. Six strains of non-VT-producing *E. coli*, including serogroup O157, were negative in the assay. Additionally, 46 species of non-VT-producing bacteria other than *E. coli* were not detected.

Sensitivity of the LAMP assay

The sensitivities of the LAMP and PCR assays were tested on five strains of VT-producing *E. coli* at various cell densities. In the LAMP assay, all strains were detected in the 10^{-6} dilution (0.7–2.2 cells per test tube) (Table 3). In the PCR assay, 10^{-6} dilutions of all strains, 10^{-5} dilutions of two strains and 10^{-4} dilutions of one strain were negative, even

though they had been positive in the LAMP assay. The sensitivity of the LAMP assay was higher than that of the PCR assay for all strains tested in the present study.

Recovery of VT-producing *E. coli* from food samples inoculated with the pathogen

The inoculation levels of serogroups O157 and O26 were 8.9 and 5.9 c.f.u. per 25 g of ground beef, and 36.8 and 20.8 c.f.u. per 25 g of radish sprouts, respectively (Table 4).

In the ground beef samples, serogroup O157 was detected perfectly by all methods (Table 4). Serogroup O26 was also perfectly detected by the PCR and LAMP assays, and the IMS culture method. However, the culture method with direct plating failed to detect serogroup O26 in one of ten samples. In the radish sprout samples, the PCR and LAMP assays, and the culture method using plating onto CT-SMAC, were successful in detecting serogroup O157 in all samples (Table 4). However, the detection ratio of plating onto CHROMagar O157 was less than that of plating onto CT-SMAC. Serogroup O26 was detected in nine of ten samples using the LAMP assay and culture with IMS, and in five of ten samples with PCR assay.

Analysis of LAMP products of naturally contaminated beef samples

Fig. 2 shows the results of the LAMP assay by three detection methods of LAMP products in four samples of beef culture and a strain culture. The cultures of the samples, but not that

of the negative control, showed an increase in turbidity, which occurred over time (Fig. 2a). Visible turbidity was detected in the test tube (Fig. 2b). The amplicons in the tubes were analysed by agarose gel electrophoresis, and the ladder patterns of four samples showed specific amplification of the target sequence (Fig. 2c). Additionally, the LAMP products were analysed by sequencing. In the LAMP products for the *VT1* gene, sequences from a part of the F1 region to a part of the B1 region were mostly homologous to the expected sequences (Fig. 3a). In the LAMP products for the *VT2* gene, sequences from a part of the F1 region to a part of B2 were also mostly homologous to the expected sequences (Fig. 3b).

DISCUSSION

The specificity of LAMP was confirmed for VT1- and VT2-producing *E. coli* of various serogroups (Table 2). In the present study, a loop F primer designed for *VT2* contained two types. Six strains of VT2- but not VT1-producing *E. coli* showed a positive reaction. To confirm the sensitivity of LAMP, serogroups O157, O26 and O111, as three predominant serogroups in many countries including Japan (National Institute of Infectious Diseases & Infectious Diseases Control Division, Ministry of Health and Welfare of Japan, 2005), were tested at various concentrations. The minimum dilution for the detection of five strains of three serogroups was 10^{-6}. However, the concentrations of the *E. coli* cells were different among strains. These results demonstrate that the detection limit of the LAMP assay is between 0.7 and 2.2 cells per test tube (Table 3). The *E. coli* concentration of the sample was 140–440 cells ml^{-1}. Because the contamination level of VT-producing *E. coli* in food is usually low, enrichment is necessary to detect it. After enrichment, the population of VT-producing *E. coli* should reach the minimum level detectable by the LAMP assay. In the detection of VT-producing *E. coli* by PCR, the PCR sensitivity found in the present study was similar (Table 3) to that obtained in previous studies (10–17 cells per test tube) (Kawasaki *et al.*, 2005; Read *et al.*, 1992), though the conditions of the PCR assay differed to some extent. Because the DNA extraction conditions were different between the PCR and LAMP assays, these conditions might affect the sensitivity. In an elementary experiment, the sensitivity of PCR assay using DNA extracted by heat treatment at 100 °C for 5 min with alkaline extraction solution and at 95 °C for 5 min without extraction solution was compared. There was no difference in a test for cells without food culture or with sprouts culture.

Thus, in comparison with other assays, it was suggested that the LAMP assay is more sensitive because the amplification of the target gene using LAMP is faster and greater in yield.
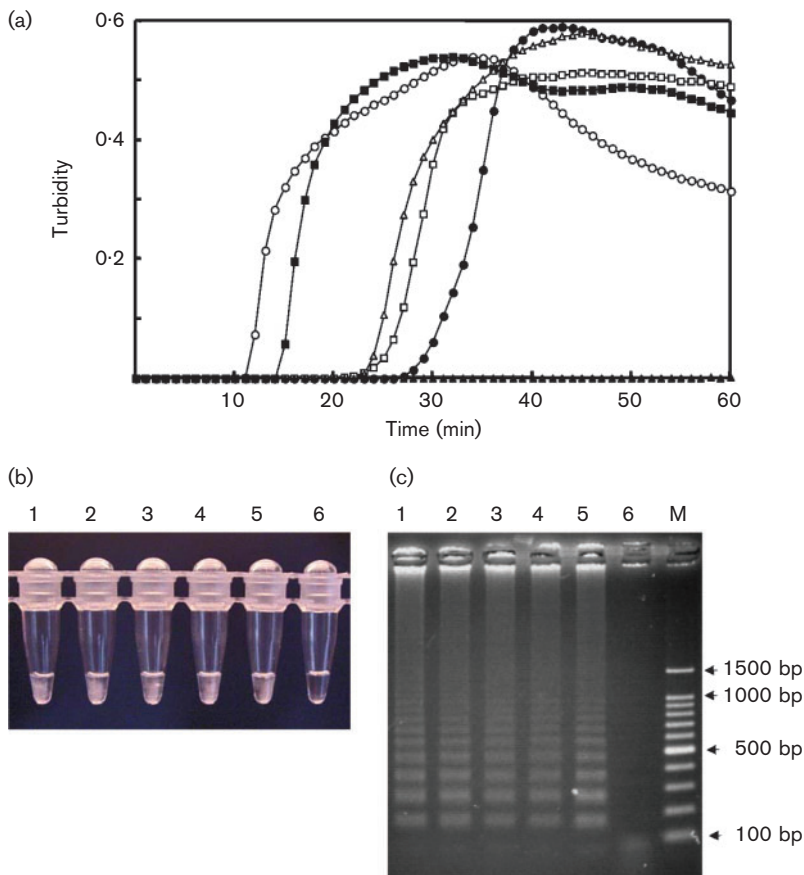

Fig. 2. The detection of VT-producing *E. coli* in naturally contaminated beef samples by turbidity and agarose electrophoresis in the LAMP assay. (a) Enterohaemorrhagic *E. coli* (EHEC) detection by real-time turbidity at 650 nm. Cell suspension of strain no. 157-212 (○) was used as a positive control, the samples were cultures in mEC+n of naturally contaminated beef samples 03-150 (●), 03-156 (□), 03-266 (■) and 03-304 (△), a negative control was used (▲). (b) Visual EHEC detection by observation of turbidity: tube 1, strain no. 157-212; tube 2, beef sample 03-150; tube 3, beef sample 03-156; tube 4, beef sample 03-266; tube 5, beef sample 03-304; tube 6, negative control. (c) EHEC detection by agarose gel electrophoresis: lane 1, strain no. 157-212; lane 2, beef sample 03-150; lane 3, beef sample 03-156; lane 4, beef sample 03-266; lane 5, beef sample 03-304; lane 6, negative control; lane M, 100 bp ladder size markers (Takara).
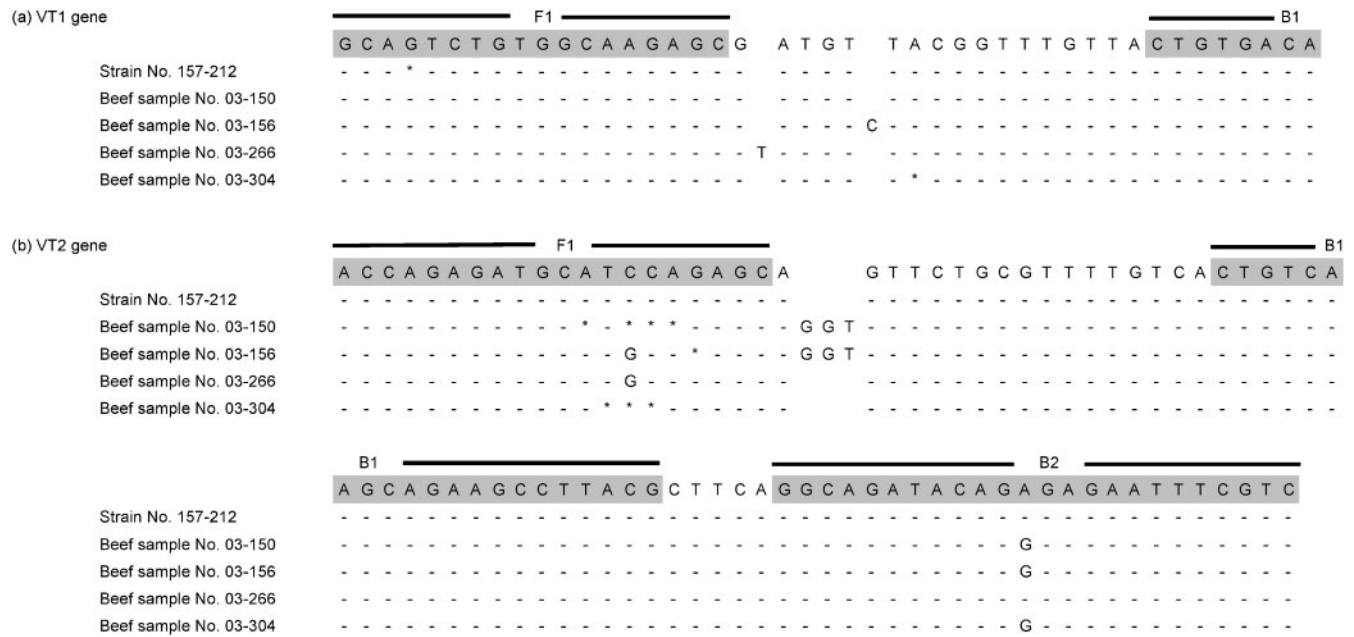
Fig. 3. Analysis of the sequences of products of LAMP for VT-producing *E. coli* in naturally contaminated beef samples: (a) *VT1* gene, (b) *VT2* gene. The cultures of four samples and a control strain (no. 157-212) were tested. Identical (-) and missing (*) nucleotides compared with the expected sequences are indicated.

To confirm the LAMP amplification of the target, an examination of the ladder patterns by agarose gel electrophoresis is useful. Furthermore, the sequences of the product are useful to confirm the PCR amplification of the target. Notomi *et al.* (2000) confirmed the structures of the LAMP amplified products for HBs regions of hepatitis virus B by cloning and sequencing. The results of the present analysis show that the sequences of LAMP products from naturally contaminated beef samples coincide well with the expected sequences.

Table 3. Sensitivity of the LAMP assay in enterohaemorrhagic *E. coli*

Strain no.	Serogroup	VT		Dilution of cultures for assay			
				10^{-3}	10^{-4}	10^{-5}	10^{-6}
157-891	O157	VT1 and VT2	Cell number in test tube	1113	111.3	11.1	1.1
			LAMP assay				
			PCR assay			—	—
ATCC 43890	O157	VT1	Cell number in test tube	800	80	8.0	0.8
			LAMP assay				
			PCR assay			—	—
157-120	O157	VT2	Cell number in test tube	1338	133.8	13.4	1.3
			LAMP assay				
			PCR assay				—
BFR 26015	O26	VT1 and VT2	Cell number in test tube	675	67.5	6.8	0.7
			LAMP assay				
			PCR assay				—
97353	O111	VT1	Cell number in test tube	2225	222.5	22.3	2.2
			LAMP assay				
			PCR assay				—

Table 4. Recovery of enterohaemorrhagic *E. coli* O157 and O26 by the culture method, and detection by PCR and LAMP assays

Ten samples were tested in each inoculation experiment. Five samples of each food were tested to confirm no natural contamination with enterohaemorrhagic *E. coli* O157 or O26. Inoculation level (c.f.u. per 25 g): 8.9 of O157 : H7 and 5.9 of O26 in ground beef; 36.8 of O157 : H7 and 20.8 of O26 in radish sprouts.

Serogroup	Detection method	IMS	Agar medium	Food	
				Ground beef	Radish sprouts
O157	Culture method	−	CT-SMAC	10	10
		−	CHROMagar O157	10	3
		+	CT-SMAC	10	10
		+	CHROMagar O157	10	7
	PCR assay			10	10
	LAMP assay			10	10
O26	Culture method	−	CT-RMAC	9	8
		−	RX O26	9	8
		+	CT-RMAC	10	9
		+	RX O26	10	9
	PCR assay			10	5
	LAMP assay			10	9

VT-producing *E. coli* is known to include many serogroups (Bettelheim, 2000) and new serogroups might be added in the future. In fact, 1 % of isolates of VT-producing *E. coli* in Japan are currently untypable using commercially available antisera (National Institute of Infectious Diseases & Infectious Diseases Control Division, Ministry of Health and Welfare of Japan, 2004). Methods of detecting VT-producing *E. coli* were developed using culture and molecular techniques. The isolation of VT-producing *E. coli* is essential for the typing of strains and identification of the cause of food-borne infections. Effective media to isolate serogroup O157 are available worldwide, but there are few effective media for other VT-producing *E. coli* (Bettelheim, 1995; Hara-Kudo *et al.*, 2002).

The present study demonstrates that the LAMP assay is effective in detecting VT-producing *E. coli* rapidly and with high sensitivity; we thus recommend it as an effective and time-saving method. Firstly the presence of the VT-encoding gene is tested in enrichment culture with LAMP assays. Immediately after the result is obtained, VT-encoding gene positive culture is plated onto agar media for serogroup O157 and other serogroups of *E. coli*. In addition, IMS using beads coated with O157 and other O antibodies improves the isolation of VT-producing *E. coli* from VT-encoding gene positive culture. After overnight incubation, colonies suspected to be serogroup O157 and other serogroups of *E. coli* on each medium are tested for the VT-encoding gene using the LAMP assay. Finally, the serogroups of all VT-encoding gene positive colonies are determined. This procedure improves public health, including food safety.

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