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Development of a specific cytolethal distending toxin (*cdt*) gene (*Eacdt*)–based PCR assay for the detection of *Escherichia albertii*

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ABSTRACT

Many *Escherichia albertii* isolates, an emerging pathogen of human and birds, might have been misidentified due to the difficulty of differentiating this bacterium from *Escherichia coli* and *Shigella* spp. by routine biochemical tests, resulting in underestimation of *E. albertii* infections. We have developed a polymerase chain reaction (PCR) assay that targets *E. albertii* cytolethal distending toxin (*Eacdt*) genes, which include the genes previously identified as *Escherichia coli cdt-II*. This assay could generate a single 449-bp PCR product in each of 67 confirmed *E. albertii* strains but failed to produce PCR product from any of the tested non–*E. albertii* enteric strains belonging to 37 different species, indicating 100% sensitivity and specificity of the PCR assay. The detection limit was 10 CFU per PCR tube and could detect 10^5 CFU *E. albertii* per gram of spiked healthy human stool. The *Eacdt* gene-based PCR could be useful for simple, rapid, and accurate detection and identification of *E. albertii*.

1. Introduction

Escherichia albertii is a Gram-negative facultative anaerobic bacterium, which is a newly described, emerging human and avian pathogen (Huys et al., 2003; Oaks et al., 2010). This bacterium was initially isolated from the stool of a 9-month-old girl with diarrhea in Bangladesh and described as an *eae* gene-positive *Hafnia alvei* isolate by routine biochemical tests (Albert et al., 1991). Subsequently, extensive characterization of these *H. alvei* strains revealed that these strains belonged to the genus *Escherichia* and constituted a new taxon for which *E. albertii* was proposed (Huys et al., 2003; Janda et al., 1999).

E. albertii is a close relative of enteropathogenic *Escherichia coli* (EPEC), particularly atypical EPEC (aEPEC), which can form attaching and effacing lesions on intestinal mucosa. In both bacteria, the *eae* (*E. coli* attaching and effacing) gene encoding intimin, a 94-kDa outer membrane protein, mediating intimate attachment of bacteria to epithelial cells, was detected. In addition to the *eae* gene, *E. albertii* harbored *cdt* genes that encode cytolethal distending toxin (CDT). CDT comprises 3 different subunits: CdtA, CdtB, and CdtC. Even though the importance of CDT in disease is not yet fully understood, CDT was shown to be associated with persistent colonization and invasion by the bacteria, which, in turn, affect the severity of the resulting diseases (Fox et al., 2004; Ge et al., 2005, 2007; McAuley et al., 2007; Pandey et al., 2003; Young et al., 2004). In addition, certain strains of *E. albertii* carry genes encoding Shiga toxin 2 or its variants (Stx2a and Stx2f) (Brandel et al., 2015; Hinenoya et al., 2017a, 2019; Murakami et al., 2014; Ooka et al., 2012), which are a primary virulence factor of Shiga toxin–producing *E. coli* (STEC). STEC are responsible for severe diseases such as hemorrhagic colitis, hemolytic–uremic syndrome, and neurological disorder (Rivas et al., 2014).

After the discovery of CDT in *E. coli* (Johnson and Lior, 1987), CDT has been identified in several Gram-negative pathogenic bacteria including *Aggregatibacter actinomycetemcomitans*, *Campylobacter* spp., *Haemophilus ducreyi*, *Providencia alcalifaciens*, *Shigella* spp., and *E. albertii* (Asakura et al., 2007; Hinenoya et al., 2009; Hyma et al., 2005; Shima et al., 2012; Yamasaki et al., 2006; Samosornsuk et al., 2015). Interestingly, several *Campylobacter* spp. including *C. jejuni*, *C. coli*, and *C. fetus* were found to carry species-specific *cdt* genes, and

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this finding led to the development of *cdt* gene-based multiplex polymerase chain reaction (PCR) and PCR–restriction fragment length polymorphism, by which *Campylobacters* could be easily and rapidly detected and identified at the species level (Asakura et al., 2008; Kamei et al., 2014). In *E. coli*, although 5 subtypes of CDT (EcCDT-I through EcCDT-V) have been reported so far (Yamasaki et al., 2006), our previous studies showed that *Eccdt-II* gene-positive *E. coli* (CTEC-II) are indeed *E. albertii*, not *E. coli* (Hinenoya et al., 2014, 2017b). Furthermore, all reclassified *E. albertii*, which were previously designated EPEC, contained the *cdt* genes exhibiting high sequence similarity to *Eccdt-II* and *E. albertii cdt* (*Eacdt*) genes (Hinenoya et al., 2019). Other reports have also found *cdt* genes in all *E. albertii* except a strain isolated from an asymptomatic cat (Oaks et al., 2010, Ooka et al., 2012, 2015). Since *E. albertii* is difficult to distinguish from *E. coli* and *Shigella* spp. by routine biochemical tests, various genetic methods such as multilocus sequence (MLS) analysis have been utilized to identify *E. albertii* precisely. However, MLS analysis is time-consuming and laborious since 7 housekeeping genes have to be amplified by PCR and sequenced, followed by phylogenetic analysis of the concatenated sequences. Therefore, in this study, we attempted to develop an *Eacdt* gene-based PCR for simple, rapid, and accurate detection of *E. albertii*. The sensitivity and specificity of the PCR assay were evaluated using pure bacterial cultures and spiked samples, and the results were also compared with other *E. albertii*–specific PCR assays reported previously.

2. Materials and methods

2.1. Bacterial culture, growth conditions, and DNA extraction

Detailed information of bacterial strains used in this study is listed in Table S1. *E. albertii*, *E. coli*, *Escherichia hermanii*, *Shigella* spp., *Providencia* spp., *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella* spp., *Proteus mirabilis*, *Salmonella enterica*, *Vibrio cholerae*, and *Wolinella succinogenes* were grown in Luria–Bertani (LB) broth or Tryptic Soy Broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 37 °C for 18 h. *Vibrio parahaemolyticus* was grown in LB broth containing 3% sodium chloride at 37 °C for 18 h. *Arcobacter* spp., *Helicobacter* spp., and *Campylobacter* spp. except *C. concisus*, *C. curvus*, and *C. hominis* were grown on blood agar [blood base agar no. 2 (Thermo Fisher Scientific Inc., Waltham, MA) supplemented with 5% (*v*/v) defibrinated horse blood (Nippon Bio-Supp. Center, Tokyo, Japan)] under a microaerobic condition (5% O_2, 7.5% CO_2, 7.5% H_2, 80% N_2) at 37 °C for 2 days or more. *C. concisus*, *C. curvus*, and *C. hominis* were grown on blood agar containing 6% formate and fumarate at 37 °C for 48 h or more under an anaerobic condition (80% N_2, 10% CO_2, 10% H_2). *Klebsiella variicola* strain JCM12419 was purchased from the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan.

DNA template for PCR was prepared by boiling method as described previously (Hoshino et al., 1998) with slight modifications. Briefly, bacterial liquid culture at midexponential phase or bacterial suspension prepared from bacteria grown on appropriate agar plates was diluted 10 times with TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0), boiled for 10 min, kept on ice for 5 min, and centrifuged at 15,000 *g* for 5 min at 4 °C. The resulting supernatant was subjected to PCR assays as described below.

2.2. PCR

PCRs were carried out by Thermal cyclers of GeneAmp PCR System 9700 or Veriti (Thermo Fisher Scientific Inc.) using *TaKaRa Taq*™ (Takara Bio Inc., Shiga, Japan) and oligonucleotide primers prepared by GeneDesign, Inc. (Osaka, Japan). PCR mixture contained 1 μL of DNA template, 0.5 μM each of forward and reverse primers, 0.2 mM each of dNTP, and 0.5 U of r*Taq* DNA polymerase and its buffer (1×) in a 20-μL reaction volume. PCR products were analyzed by electrophoresis in 2% agarose gel (Promega, Fitchburg, WI, USA) and visualized with UV light after staining with 1.0 μg/mL of ethidium bromide (Sigma-Aldrich Co., St. Louis, MO, USA). Images were captured on a ChemiDoc imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). *E. albertii* strain LMG20976^T and *E. coli* strain C600 were used as a positive and negative control, respectively.

Eacdt gene-specific PCR developed in this study was performed with a primer set, EaCDTsp-F2 and EaCDTsp-R2. Specificity and sensitivity of the PCR were evaluated with 65 *E. albertii* and 143 other bacteria. Other *E. albertii*–specific PCRs developed by Hyma et al. (2005), Ooka et al. (2015), and Lindsey et al. (2017) were also performed for comparison with primer sets of clpX_28F, clpX_411R, lysP_107F, lysP_358R, mdh_50F and mdh_164R; E_al_OF and E_al_OR; and EC_F, EC_R, EA_F, EA_R, EF_F and EF_R, respectively. Primer sequences and PCR conditions are summarized in Table 1.

2.3. Detection limit of the Eacdt gene-specific PCR

Early logarithmic phase of *E. albertii* strain LMG20976^T in LB broth was 10-fold serially diluted with sterile Dulbecco's phosphate buffered saline (pH 7.4). Each dilution was spread on LB agar to determine viable bacterial cell count and also used to prepare DNA template by the boiling method as described above. One microliter aliquot of the DNA template was subjected to the *Eacdt* gene-specific PCR.

2.4. Spiking stool with E. albertii

A stool specimen was obtained from a healthy person. His stool culture was negative for bacterial pathogens including *eae*-gene positive bacteria and *S. boydii*. The stool (0.2 g) was mixed with 10 μL of *E. albertii* LMG20976^T fresh culture which was serially diluted from 10^8 to 10^3 CFU/mL. DNA was isolated from the spiked stools by the QIAamp® DNA Stool Mini Kit (QIAGEN, Venlo, Netherlands) following a protocol for pathogen detection and subjected to the *Eacdt* gene-specific PCR.

2.5. MLS analysis

MLS analysis was performed as described previously (Hinenoya et al., 2017b) according to the protocol of the University of Warwick (Wirth et al., 2006). Briefly, 7 housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *purA*, *mdh*, and *recA*) were amplified by PCR and sequenced using BigDye® Terminator v1.1 and ABI 3130 Genetic Analyzer (Thermo Fisher Scientific, Inc.). The loci were concatenated into a 3423-bp sequence in the above order. A neighbor-joining tree was constructed using MEGA6 software with default setting and boot-strapping 1000 times. The reference strains included were randomly selected from our previous study (Hinenoya et al., 2019).

2.6. Serotyping

S. boydii strains AQ7324 and AA17401 were examined by slide agglutination test if they were serotype 13 or not with the corresponding rabbit antisera (Denka Seiken Co., Ltd., Tokyo, Japan) as described previously (Hinenoya et al., 2009).

2.7. Nucleotide sequences accession numbers

All nucleotide sequences obtained in this study were deposited into the DDBJ (DNA Data Bank of Japan) database. The accession numbers are LC424386-LC424399 (for the 7 housekeeping genes [*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, *recA*] used for MLS analysis) and LC424400 (for nucleotide sequence of PCR product from *E. albertii* strain AA17401).

Table 1
Primers and PCR conditions used in this study.

Primer	Sequence (5′–3′)	Target gene or locus	Amplicon (bp)	PCR conditions (30 cycles)						Reference
				Denaturing		Annealing		Extension		
EaCDTsp-F2	GCTTAACTGGATGATTCTTG	*Eacdt*	449	94 °C	30 s	50 °C	30 s	72 °C	30 s	This study
EaCDTsp-R2	CTATTTCCCATCCAATAGTCT									
E_al_OF	GGTCCATAATGAATCTGACTGA	Part of	846	94 °C	30 s	54 °C	30 s	72 °C	60 s	Ooka et al., 2015
E_al_OR	CCATATGACAGGCGTAATTGAT	*yejH* and *yejK*								
clpX_28F	TGGGCTCGAGTTGGGCA	*clpX*	384	94 °C	30 s	58 °C	60 s	72 °C	60 s	Hyma et al., 2005
clpX_411R	TCCTGCTGCGGATGTTTACG									
lysP_107F	GGGCGCTGCTTTCATATATTCTT	*lysP*	252							
lysP_358R	TCCAGATCCAACCGGGAGTATCAGGA									
mdh_50F	CTGGAAGGCGCAGATGTGGTACTGATT	*mdh*	115							
mdh_164R	CTTGCTGAACCAGATTCTTCACAATACCG									
EC_F	CCAGGCAAAGAGTTTATGTTGA	*cdgR*	212	94 °C	30 s	57 °C	60 s	72 °C	30 s	Lindsey et al., 2017
EC_R	GCTATTTCCTGCCGATAAGAGA									
EA_F	GTAAATAATGCTGGTCAGACGTTA	Part of	393							
EA_R	AGTGTAGAGTATATTGGCAACTTC	*cbl* and *nac*								
EF_F	AGATTCACGTAAGCTGTTACCTT	Part of	575							
EF_R	CGTCTGATGAAAGATTTGGGAAG	hypothetical protein								

3. Results

3.1. Evaluation of Eacdt gene-specific PCR

A primer pair, EaCDTsp-F2 and EaCDTsp-R2, was newly designed in this study from the conserved region of the *Eacdt* genes. Expected size of amplicon (449 bp) was obtained by PCR using the primer pair when *E. albertii* strain LMG20976^T was provided as the template. *E. coli* strain C600, which was used as a negative control, did not yield the 449-bp amplicon (Fig. 1). The PCR product was confirmed to be a part of *Eacdt* genes by sequencing. Sensitivity and specificity of the *Eacdt* gene-specific PCR were evaluated with additional 64 *E. albertii* strains and other species of bacteria listed in Tables 2 and S1. A specific PCR product (449 bp) was obtained from all the *E. albertii* strains in addition to the type strain LMG20976^T, indicating that the sensitivity was 100% (65/65). No PCR product was obtained from any of the tested non–*E. albertii* strains except the 2 *S. boydii* strains that were positive for the *Eacdt* gene-specific PCR. MLS analysis revealed that these 2 *S. boydii* strains belonged to a distinct lineage of *E. albertii*, indicating that both these strains are indeed *E. albertii*, not *S. boydii* (Fig. S1). Furthermore, the serotype of these 2 strains was determined to be 13, which is identical to *E. albertii* strains reidentified from *S. boydii* by Hyma et al. (2005). Thus, the specificity of the *Eacdt* gene-specific PCR was also 100%.

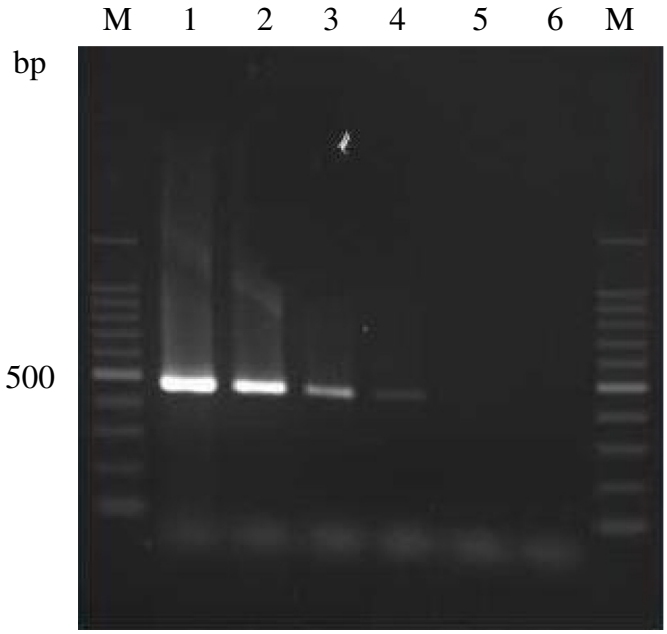

Fig. 1. Detection limit of *Escherichia albertii* by *Eacdt* gene-based PCR assay. M, 100-bp DNA ladder (Takara Bio Inc., Shiga, Japan); lane 1, 10^4 CFU per tube of *E. albertii* strain LMG20976^T; 2, 10^3 CFU; 3, 10^2 CFU; 4, 10^1 CFU; 5, 10^0 CFU; 6, *E. coli* strain C600 (negative control). Five microliters each of PCR products was analyzed by 2% agarose gel.

3.2. Detection limit of E. albertii in pure culture and stool specimen

The detection limit of the PCR was evaluated with template DNA prepared from pure culture in early logarithmic phase of a representative *E. albertii* strain and determined to be 10 CFU in a PCR tube (Fig. 1). To examine the detection limit of the *Eacdt* gene-specific PCR for *E. albertii* in stool specimens, a stool specimen, in which *E. albertii* was not detected by the PCR, was spiked with various numbers of *E. albertii* strain LMG20976^T cells. Thus, the detection limit of the *Eacdt* gene-specific PCR was determined to be ~10^5 CFU per gram stool (Fig. 2).

3.3. Comparison of the sensitivity between Eacdt gene-specific PCR assay and other E. albertii-specific PCR assays

Using 67 *E. albertii* strains including the 2 newly reidentified strains from *S. boydii*, sensitivity of various *E. albertii*-specific PCR assays developed by Hyma et al. (2005), Ooka et al. (2015), and Lindsey et al. (2017) (tentatively named as Hyma-PCR, Ooka-PCR, and Lindsey-PCR, respectively) was examined and compared with that of the *Eacdt* gene-specific PCR assay (Tables 2 and S1). Representative gel picture of the PCR products is shown in Fig. 3. The Hyma-PCR could identify all the 67 strains as *E. albertii*. The sensitivity of the Ooka-PCR was 98.5% (66/67 strains tested) as PCR yielded a larger amplicon of ~1.6 kb from strain AA17401, although the expected size of amplicon of 846 bp was obtained from the remaining 66 strains (Fig. 3). Sequencing of the PCR products revealed that a ~0.8-kb IS element belonging to IS1 family was found in the targeted region of the strain AA17401. Sensitivity of the Lindsey-PCR was also 98.5% (66/67): no amplicon was obtained in the strain P6796 by PCR assay (Fig. 3).

4. Discussion

Recently, outbreaks of food poisoning due to *E. albertii* have been increasingly recognized particularly in Japan, viz., Fukuoka in 2003, Oita in 2005, Kumamoto in 2011 and 2013, Hiroshima in 2015, Okinawa and Shizuoka in 2016, and Tochigi in 2017 (NIID, 2016). In addition, *E. albertii* has been isolated from healthy and diseased birds (Oaks

Table 2
Evaluation of *Eacdt* gene-based PCR assay.

Bacterial species	*n*[a]	Eacdt-PCR[b]	Hyma-PCR[c]	Ooka-PCR[d]	Lindsey-PCR[e]
E. albertii	65	65	65	65	64
Shigella boydii (SbCDT)[g]	2	2	2	1	2
S. boydii	63	0	ND	ND	ND
S. dysenteriae	3	0	ND	ND	ND
S. flexnerii	3	0	ND	ND	ND
S. sonnei	3	0	ND	ND	ND
E. coli (EcCDT-I)	2	0	ND[f]	ND	ND
E. coli (EcCDT-III)	4	0	ND	ND	ND
E. coli (EcCDT-IV)	2	0	ND	ND	ND
E. coli (EcCDT-V)	4	0	ND	ND	ND
E. coli (EcCDT-III/EcCDT-V)	1	0	ND	ND	ND
E. coli (*cdt* gene-negative)	10	0	ND	ND	ND
E. hermanii	2	0	ND	ND	ND
Providencia alcalifaciens (PaCDT)	4	0	ND	ND	ND
P. alcalifaciens (*cdt* gene-negative)	5	0	ND	ND	ND
P. heimbachae	1	0	ND	ND	ND
P. rettgeri	1	0	ND	ND	ND
P. rustigianii (PrCDT)	1	0	ND	ND	ND
P. rustigianii (*cdt* gene-negative)	1	0	ND	ND	ND
P. stuartii	1	0	ND	ND	ND
Proteus mirabilis	1	0	ND	ND	ND
Citrobacter freundii	1	0	ND	ND	ND
Enterobacter cloacae	1	0	ND	ND	ND
Klebsiella oxytoca	1	0	ND	ND	ND
K. pneumoniae	1	0	ND	ND	ND
K. variicola	1	0	clpX/mdh[h]	0	0
Salmonella enterica	3	0	clpX/mdh	0	0
	2	0	clpX/lysP[h]	0	0
	1	0	mdh[h]	0	0
Arcobacter butzleri	1	0	ND	ND	ND
A. skirrowii	1	0	ND	ND	ND
Campylobacter coli	1	0	ND	ND	ND
C. concisus	1	0	ND	ND	ND
C. curvus	1	0	ND	ND	ND
C. fetus	1	0	ND	ND	ND
C. helveticus	1	0	ND	ND	ND
C. hominis	1	0	ND	ND	ND
C. hyointestinalis	2	0	ND	ND	ND
C. jejuni	1	0	ND	ND	ND
C. lari	1	0	ND	ND	ND
C. mucosalis	1	0	ND	ND	ND
C. upsaliensis	1	0	ND	ND	ND
Helicobacter cinaedi	1	0	ND	ND	ND
H. fenneliae	1	0	ND	ND	ND
H. hepaticus	1	0	ND	ND	ND
H. pyroli	2	0	ND	ND	ND
Vibrio cholerae	1	0	ND	ND	ND
V. parahaemolyticus	1	0	ND	ND	ND
Woinella succinogenes	1	0	ND	ND	ND

[a] Number of strains tested in this study.
[b] *Eacdt* gene-based PCR developed in this study.
[c] Developed by Hyma et al. (2005).
[d] Developed by Ooka et al. (2015).
[e] Developed by Lindsey et al. (2017).
[f] Reidentified as *E. albertii* in this study.
[g] Not done.
[h] Amplified gene fragments were described.

et al., 2010; Ooka et al., 2012), indicating that *E. albertii* could be an emerging zoonotic pathogen. It has also been reported that some *E. albertii* strains produced Stx2, which may cause life-threatening complications including hemolytic uremic syndrome in humans (Brandel et al., 2015; Hinenoya et al., 2017a, 2019; Murakami et al., 2014; Ooka et al., 2012).

Due to its phylogenetic relatedness with other members of the family *Enterobacteriaceae*, a substantial portion of *E. albertii* isolates has been mischaracterized as EPEC, EHEC, and likely CTEC-II (Oaks et al., 2010; Ooka 2012; Hinenoya et al., 2017b). Furthermore, in the 'National Enteric Disease Surveillance: *Shigella* Surveillance Overview' in 2011, the Centers for Disease Control and Prevention also reported that *S. boydii* serotype 13 is regarded as *E. albertii* (https://www.cdc.gov/ncezid/dfwed/PDFs/shigella-annual-report-2011-508c.pdf). Indeed, 2 *S. boydii* strains were also reidentified as *E. albertii* in this study, and both of them were confirmed as serotype 13. MLS analysis is a reliable genetic method by which *E. albertii* can be clearly differentiated from *E. coli* and *Shigella* spp., but it is not practically helpful in terms of time, cost, and labor including the prerequisite of bacterial isolation. Therefore, it was difficult to assess the real burden of *E. albertii*–related infectious disease. Although 3 PCR assays targeting *E. albertii*-specific genetic loci have been reported (Hyma et al., 2005; Ooka et al., 2015; Lindsey et al., 2017), their sensitivities and specificities needed more evaluations because they were developed based on genetic properties of a limited number of *E. albertii* strains. In the current study, we have developed an *Eacdt* gene-specific PCR assay as a simple, rapid, and reliable means to detect *E. albertii*, with the sensitivity and specificity of the bioassay being 100%.

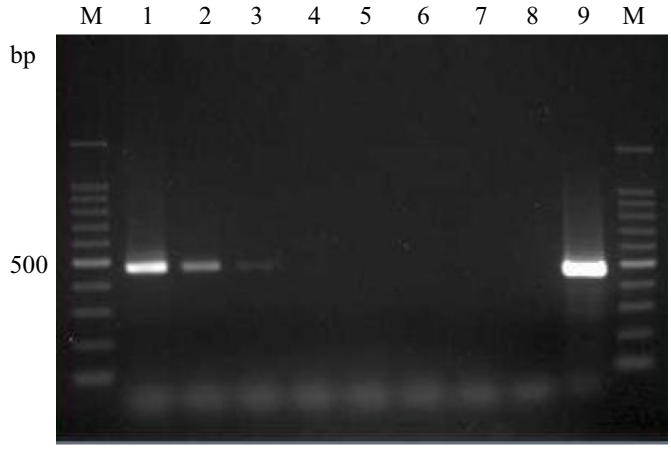

Fig. 2. Spike experiment to evaluate detection limit of *E. albertii* by *Eacdt* gene-specific PCR. Healthy human stool (0.2 g) was spiked with *E. albertii* strain LMG20976^T of 1.9×10^6 to 1.9×10^1 CFU, respectively. DNA was extracted and subjected to *Eacdt* gene-specific PCR. M, 100-bp DNA ladder (Takara Bio Inc.); lane 1, 10^6 CFU of *E. albertii* strain LMG20976^T was spiked to 0.2 g stool (equivalent to ~10^7 CFU/g); 2, 10^5 CFU (~10^6 CFU/g); 3, 10^4 CFU (~10^5 CFU/g); 4, 10^3 CFU (~10^4 CFU/g); 5, 10^2 CFU (~10^3 CFU/g); 6, 10^1 CFU (~10^2 CFU/g); 7, nonspiked; 8, *E. coli* strain C600 (negative control); 9, *E. albertii* strain LMG20976^T (10^4 CFU/PCR tube, positive control). Five microliters each of PCR products was analyzed by 2% agarose gel.

Under the evaluation with the *E. albertii* and non–*E. albertii* strains used in this study, the sensitivity of the *Eacdt* gene-specific PCR assay and the Hyma-PCR assay was 100%, followed by 98% for the Ooka-PCR assay and the Lindsey-PCR assay (Table 2). Recently, Maeda et al. (2014) reported nonspecific amplification from enrichment culture of food samples by the Hyma-PCR assay, in which the sequences of the *lysP* (~87%) and *mdh* (~86%) gene products were most homologous to those of *K. variicola*. Additionally, nonspecific amplification of *lysP* and *mdh* genes was also shown in *Morganella morganii* and in *C. freundii*, *K. oxytoca*, *S.* Braenderup, and *S.* Typhimurium, respectively. Our study also demonstrated such nonspecific amplifications in *K. variicola* and *Salmonella enterica* strains (Table 2), suggesting less specificity of the Hyma-PCR. Previous epidemiological studies (Oaks et al., 2010; Hinenoya et al., 2014, 2017a, 2017b, 2019) revealed that all the *E. albertii* isolates carried *cdt* genes. Furthermore, Ooka et al. (2015) showed that *Eacdt* genes are located in chromosomal DNA but not in mobile genetic elements such as prophages and transposons. Our previous study showed that *Eacdt* genes are highly conserved and easily distinguished from other *cdt* genes in nucleotide sequences (Hinenoya et al., 2017b). These observations suggest that *Eacdt* genes could be stable and uniformly present in all *E. albertii* strains as species-specific genes. However, of all *E. albertii* strains isolated to date, only CB10113, an atypical *E. albertii* strain isolated from an asymptomatic cat, was reported that it does not contain *cdt* genes due to a large deletion (~85 kb) in genome (Ooka et al., 2015). Irrespective of possession of *Eacdt* genes, *E. albertii* should be precisely identified to species level in order to perform systematic epidemiological study of *E. albertii* and assess virulence properly. It is obvious that all the *E. albertii* PCR assays including the *Eacdt* gene-specific PCR assay have their own limitations, and at least 2 PCR assays, most probably *Eacdt* gene-specific PCR assay and either of the other 3 assays (Hyma et al., 2005; Lindsey et al., 2017; Ooka et al., 2015), should be used for accurate identification of *E. albertii*.

The *Eacdt* gene-specific PCR showed a low detection limit of 10 CFU per PCR tube of *E. albertii*. Furthermore, after spiking stool, as low as ~10^5 CFU of *E. albertii* could be detected in 1 g of stool. In addition, *Eacdt* genes could be amplified in the DNA template of stool specimens of diarrheal patients from which *E. albertii* could be isolated, while no amplicon was obtained from any of the specimens from which *E. albertii* could not be detected (data not shown). Thus, the *Eacdt* gene-specific PCR assay could be used as a simple and rapid test for detection of *E. albertii* in clinical laboratories.

In summary, an *Eacdt* gene-specific PCR assay for both detection and identification of *E. albertii* was established. This PCR assay could be one of the choices for detection and identification of *E. albertii* not only in clinical laboratories but in epidemiological studies to identify the reservoirs and modes of infection. Further studies using unidentified strains, clinical specimens, and environmental samples should validate *Eacdt* gene-specific PCR assay for a rapid and reliable identification of *E. albertii*.

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Conflict of interest

None to declare.

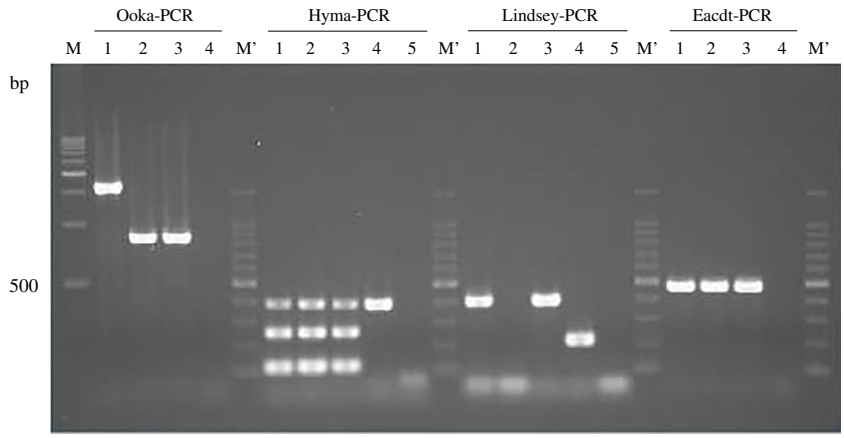

Fig. 3. Representative result of *Escherichia albertii* identification PCRs. Representative result of PCR products of *E. albertii*–specific PCRs developed by Ooka et al. (Ooka-PCR), Hyma et al. (Hyma-PCR), Lindsey et al. (Lindsey-PCR), and *Eacdt* gene-specific PCR (Eacdt-PCR), respectively, is shown. M, 500-bp DNA ladder; M', 100-bp DNA ladder (Takara Bio Inc.); 1, reidentified *E. albertii* strain AA17401; 2, *E. albertii* strain P6796; 3, *E. albertii* strain LMG20976^T (positive control); 4, *E. coli* strain C600 (negative control in Ooka-PCR and Eacdt-PCR); 5, distilled water (negative control in Hyma-PCR).

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diagmicrobio.2019.04.018.

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