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Original Article

Isolation and Identification of *Escherichia albertii* from a Patient in an Outbreak of Gastroenteritis

Takayuki Konno*, Jun Yatsuyanagi, Shiho Takahashi, Yuko Kumagai, Eriko Wada, Machiko Chiba, and Shioko Saito

Health and Hygiene Division, Akita Prefectural Research Center for Public Health and Environment, Akita 010-0874, Japan

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SUMMARY: A microbial strain harboring the *eae* gene, which is known as the virulence gene of enteropathogenic *Escherichia coli* (EPEC) and most enterohemorrhagic *E. coli*, was isolated from a patient in a gastroenteritis outbreak that occurred in 22 patients in Akita Prefecture, Japan, in November 2011. The biochemical characteristics of the isolate were more similar to those of a novel *Escherichia* sp., *E. albertii* than *E. coli*. Partial 16S rRNA gene sequences of the isolate were identical to those of a certain *E. albertii* strain, but also showed a high degree of similarity to those of *E. coli* strains. Finally, we identified this isolate as *E. albertii* by performing PCR analysis that targeted the *uidA*, *lysP*, *mdh*, and *cdtB* genes in addition to *stx* and *eae* genes to differentiate between the EPEC and *E. albertii* strains.

INTRODUCTION

Escherichia albertii has been reported to be a novel *Escherichia* spp. and a potential diarrheagenic pathogen in humans (1,2). *E. albertii* was originally described as an unusual strain of *Hafnia alvei* that harbors the *eae* gene and encodes intimin, which is associated with the attaching-effacing phenotype of enteropathogenic *Escherichia coli* (EPEC) and most enterohemorrhagic *E. coli* (EHEC), but subsequent phenotypic and genetic studies suggested that this *H. alvei*-like strain does not belong to the genus *Hafnia* (1,3–5). Further studies by Huys et al. led to the characterization of these *H. alvei*-like strains as a new *Escherichia* spp., *E. albertii*, on the basis of DNA-DNA hybridization results and 16S rRNA gene sequence data (2).

In addition to intimin, cytolethal distending toxin (CDT) has been reported as a putative virulence factor in *E. albertii* (6). CDT was first identified in *E. coli* O128, which was isolated from the stool of a child who aged less than 2 years and showing gastroenteritis (7), and the toxin was detected in several pathogenic bacteria, such as *Shigella dysenteriae* (8), *Campylobacter* spp. (9), *Haemophilus ducreyi* (10), and *Actinobacillus actinomycetemcomitans* (11). In total, 3 genes, including *cdtA*, *cdtB*, and *cdtC*, were associated with cytotoxic activity (12–14); in particular, *cdtB* was associated with encoding the enzymatically active subunit (12,15).

E. albertii caused diarrheal diseases in children with symptoms of vomiting, mild dehydration, fever, and abdominal distention (1,16). Sharma et al. described that *E. albertii* could possibly be one of the factors responsible for the estimated 62,000,000 cases of foodborne illnesses and 3,200 deaths with an unknown etiological origin in the United States (17,18). However, isolation of *E. albertii* was rarely reported in Japan, and therefore, its prevalence and possible pathogenic role are currently unknown.

In November 2011, a gastroenteritis outbreak occurred in 22 patients in Akita Prefecture, Japan. Here, we report that an EPEC-like strain harboring the *eae* gene was isolated from a patient with norovirus infection in this outbreak. We identified the isolate using biochemical tests and performed genetic identification on the basis of 16S rRNA gene sequencing and PCR analysis targeting the *stx*, *eae*, *uidA*, *lysP*, *mdh*, and *cdtB* genes.

MATERIALS AND METHODS

Isolation of bacteria: Stool samples of 19 patients and 16 restaurant staff members, and 10 environmental samples from the restaurant were examined to detect etiological agents, including diarrheagenic *E. coli* (DEC), *Salmonella*, *Shigella*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Campylobacter*, *Vibrio*, and norovirus, by standard culture procedures and PCR methods.

To detect DEC, we inoculated the fecal samples of the patient onto DHL agar plate and incubated the plate at 37°C for 24 h. Isolated colonies were studied to classify the isolates as DEC by employing PCR method targeting the major virulence genes of DEC, such as *stx* for Shiga toxin-producing (STEC) or EHEC, *eae* for EPEC and most of EHEC, *aggR* and *astA* for enteroaggregative *E. coli* (EAggEC), *elt* and *est* for enterotoxigenic *E. coli* (ETEC), and *invE* for enteroinvasive *E. coli* (EIEC), as described previously with slight modification (19–22). The biochemical characteristics of the isolate were tested for its ability to produce indole and lysine decarboxylase, fermentation of lactose and xylose, and

*Corresponding author: Mailing address: Akita Prefectural Research Center for Public Health and Environment, 6-6 Sensyu kubota-machi, Akita 010-0874, Japan. Tel: +81-18-832-5005, Fax: +81-18-832-5938, E-mail: konno-takayuki@pref.akita.lg.jp

Table 1. Primer sequences used in PCR analysis for differentiating *E. albertii* from EPEC

Target	Property	Sequence (5′–3′)	Annealing temp.	Reference
stx	Shiga toxin	GAGCGAAATAATTTATATGTG	55	(40)
		TGATGATGGCAATTCAGTAT		
eae	intimine	CAGGATCGCCTTTTTTATACG	55	This study
		CTCTGCAGATTAACCTCTGC		(41)
uidA	beta-D-glucuronidase	TCAGCGCGAAGTCTTTATACC	55	This study
		CGTCGGTAATCACCATTCCC		
lysP	lysine-specific permease	GGGCGCTGCTTTCATATATTCTT	65	(25)
		TCCAGATCCAACCGGGAGTATCAGGA		
mdh	malate dehydrogenase	CTGGAAGGCGCAGATGTGGTACTGATT	65	(25)
		CTTGCTGAACCAGATTCTTCACAATACCG		
cdtB	cytolethal distending toxin	GAAAGTAAATGGAATATAAATGTCCG	55	(35)
		AAATCACCAAGAATCATCCAGTTA		

motility.

16S rRNA gene sequencing: The forward primer (5′-GGATCCAGACTTTGATYMTGGCTCAG-3′) and the reverse primer (5′-CCGTCAATTCCTTTRAGTTT-3′) were used to amplify bacterial 16S rRNA genes by PCR under the following conditions: 94°C for 5 min; 25 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s; and 72°C for 2 min (23,24). Amplified DNA fragments of about 900 bp were separated on a 2% (wt/vol) agarose gel. The gel was stained with ethidium bromide and visualized on a UV transilluminator, and PCR amplicons were purified using a QIAquick Gel Extraction Kit (QIAGEN, Tokyo, Japan), according to the manufacturer's instructions. DNA sequences of the PCR amplicons were determined using the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Tokyo, Japan) on an ABI-3130 apparatus (Applied Biosystems). A sequence similarity search was performed using an online-system with BLASTN.2.2.24 (http://blast.ddbj.nig.ac.jp/top-j.html).

PCR analysis to differentiate *E. albertii* from EPEC: To identify the EPEC strain, a set of primers targeting *uidA*, which is the specific housekeeping gene in the *E. coli* lineage, was used in addition to *stx* and *eae* genes. Two sets of primers targeting *lysP* and *mdh* genes, which were designed to detect *E. albertii* lineage-specific genetic polymorphisms (25), were used to identify the *E. albertii* strain. The *cdtB* gene as a putative virulence gene was also amplified. The primer sequences and annealing conditions used for detecting these genes are summarized in Table 1. Briefly, the PCR stages were denaturation at 94°C for 2 min, followed by 25 amplification cycles (94°C for 30 s, annealing for 30 s, 72°C for 45 s), and a final extension cycle (72°C for 2 min).

Analysis of CDT type: CDT typing was performed as per the method described by Kim et al. (26), with the following modification: the *cdt* type II R2 primer (5′-TTTGTGTTGCCGCCGCTGGTG-3′) and *cdt* type III R2 primer (5′-TTTGTGTCGGTGCAGCAGGGA-3′) were used for PCR analysis instead of reverse primers for *cdt* type II and *cdt* type III.

RESULTS AND DISCUSSION

In the gastroenteritis outbreak that occurred in Akita Prefecture, Japan, in November 2011, we detected EPEC and norovirus in 1 and 2 patients, respectively.

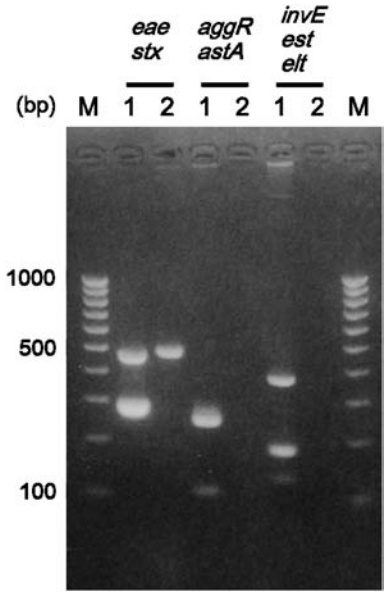

Fig. 1. PCR-based detection of the major virulence genes of DEC. The size of PCR amplicons of *stx*, *eae*, *aggR*, *astA*, *elt*, *est*, and *invE* are 289 bp, 479 bp, 254 bp, 106 bp, 130 bp, 186 bp, and 382 bp, respectively. Lanes: M, 100-bp size ladder (Bio-Rad, Tokyo, Japan); 1, Positive control (EHEC O157:H7 EDL931 for *stx* and *eae* [provided by Tokyo Metropolitan Research Laboratory of Public Health, Tokyo, Japan], EAggEC 17-2 for *aggR* and *astA* [kindly gifted from James B. Kaper, University of Maryland School of Medicine, Baltimore, Md., USA], ETEC JCM-8364 for *elt*, ETEC JCM-8365 for *est*, and EIEC JCM-8363 for *invE* [provided by RIKEN, Kanagawa, Japan]); 2, EC15062.

Furthermore, one strain, designated EC15062, was also isolated from a patient infected with norovirus by standard culture procedures for DEC. EC15062 was positive for *eae*, but negative for *stx*, *aggR*, *astA*, *elt*, *est*, and *invE*, which are the major virulence genes of DEC (Fig. 1). Therefore, we suspected that EC15062 was an EPEC strain. However, the *eae* gene is present not only in EPEC and most of EHEC, but also in other bacterial species, such as *E. albertii* and *Citrobacter rodentium* (formerly *C. freundii* biotype 4280) (1,27,28); therefore, it was neccesary to confirm whether EC15062 is an *E. coli* strain or not.

EC15062 produced indole and lysine decarboxylase; but it did not ferment lactose and xylose and was non-

Table 2. Comparison of biochemical characteristics between *E. coli*, *E. albertii*, and EC15062

	E. coli[1)]	*E. coli* inactive[1)]	*E. albertii*[2)]	EC15062
Motility	+	−	−	−
Indole production	98%	80%	−	+
Lysin decarboxylase	90%	40%	+	+
Lactose fermentation	95%	25%	−	−
Xylose fermentation	95%	70%	−	−

[1)]: Source: Farmer et al. (42).
[2)]: Source: Abbott et al. (39).

motile at 35°C, indicating that the biochemical characteristics of EC15062 were similar to those of *E. albertii* rather than *E. coli* (Table 2). Although the indole-production characteristics of EC15062 were different from those of *E. albertii* isolated from Bangladeshi children, this difference did not eliminate the possibility that EC15062 was an *E. albertii* strain, since in a study by Oaks et al., indole production was seen in the *E. albertii* strains isolated from birds (29).

The partial 16S rRNA sequences of EC15062 (823 nt) were identical to those of an *E. albertii* strain (accession no. HM194877), which was isolated from a teal, and showed 99.9% (822 of 823 nt) similarity to the sequences in *E. coli* strains. These findings suggested that the similarity analysis of 16S rRNA gene sequences between the strains was insufficient to differentiate *E. albertii* from *E. coli*.

The *uidA* gene has been often used for the identification of *E. coli* strains in previous studies since *uidA* is a specific housekeeping gene in *E. coli* and *Shigella* spp. (30–33). On the other hand, Hyma et al. reported a PCR method targeting conserved housekeeping genes, including *clpX*, *lysP*, and *mdh*, to identify *E. albertii* strains (25). Primers of *lysP* and *mdh* have been designed on the basis of *E. albertii*-specific nucleotide polymorphisms (25). We examined the presence of *uidA*, *lysP*, and *mdh*, in addition to *stx* and *eae*, to determine whether EC15062 is EPEC or *E. albertii* strain by comparative PCR analysis with a reference strain of EPEC, termed E2348/69 (Fig. 2). We confirmed that both the EPEC strain and EC15062 were negative for *stx*, but were positive for *eae* (same results as in Fig. 1). In contrast to the EPEC strain, EC15062 was negative for *uidA*, and positive for both *lysP* and *mdh*. On the basis of these results, EC15062 was identified as *E. albertii*.

We detected *cdtB*, a putative virulence gene of *E. albertii* in EC15062 (Fig. 2). Furthermore, we examined the CDT type of EC15062 by PCR analysis designed to classify *E. coli* CDTs. In *E. coli*, CDTs are classified into 5 types, on the basis of differences in the sequences of *cdt* (34–36). As shown in Fig. 3, EC15062 was positive for *cdt* type III and *cdt* type V. These results are consistent with those obtained by Hyma et al., who reported that the *cdt* sequences of *E. albertii* lineage were most similar to those of *cdt* type V and *cdt* type III (25). Hyma et al. suggested that there was no significant association between the presence of CDT and diarrhea (25). Okuda et al., however, showed direct experimental evidence for the role of CDT in diarrhea by using an animal model with a recombinant *E. coli* cloned with *S.*

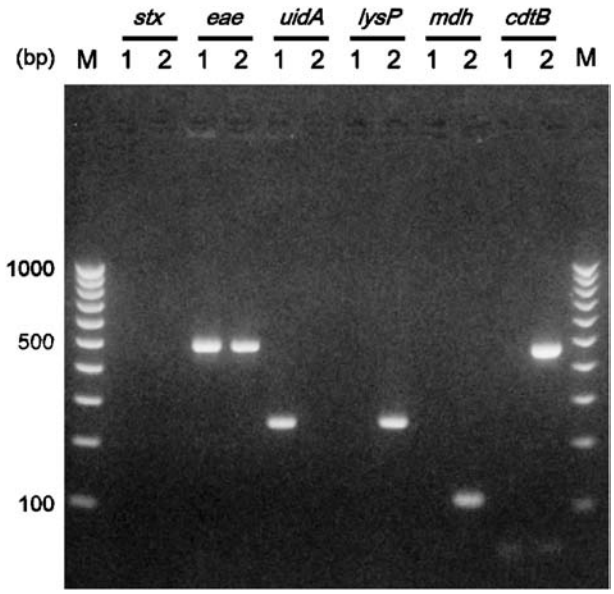

Fig. 2. Comparison of the presence of the virulence genes and housekeeping genes between EPEC and EC15062. The size of PCR amplicons of *stx*, *eae*, *uidA*, *lysP*, *mdh*, and *cdtB* are 518 bp, 479 bp, 248 bp, 252 bp, 115 bp, and 466 bp, respectively. Lanes: M, 100-bp size ladder (Bio-Rad); 1, EPEC (E2348/69, kindly gifted from James B. Kaper); 2, EC15062.

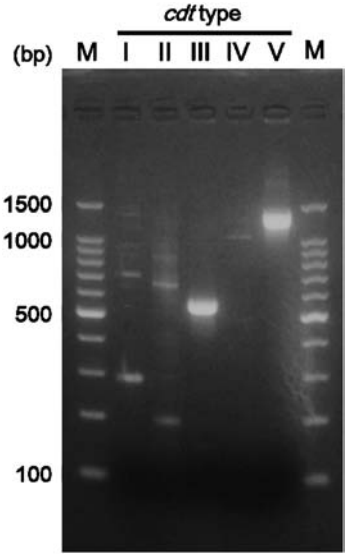

Fig. 3. CDT typing of EC15062. The size of PCR amplicons of *cdt* type I, II, III, IV, and V are 411 bp, 556 bp, 555 bp, 350 bp, and 1363 bp, respectively. Lanes: M, 100-bp size ladder (Takara, Shiga, Japan).

dysenteriae cdt operon (37). In *Campylobacter* spp., CDT was associated with the property of invasiveness (38). Although further studies are required to warrant the role of CDT in the diarrheagenicity of *E. albertii*, the *cdtB* gene may be one of the important virulence markers in *E. albertii*.

Huys et al. reported that *E. albertii* was associated with diarrhea in Bangladeshi children (2), but there is limited epidemiological data suggesting that *E. albertii* was the etiological agent of diarrheal outbreaks. In this study, we isolated *E. albertii* strain from a patient of the

gastroenteritis outbreak, which occurred in Akita Prefecture, Japan. To the best of our knowledge, this is the first report that describes the isolation of *E. albertii* from a diarrheal patient in Japan. We, however, could not determine whether *E. albertii* was the etiological agent in our case, because the patient was co-infected with norovirus, and only 1 patient was positive for *E. albertii* in this case.

The prevalence, epidemiology, and clinical relevance of *E. albertii* still remain unclear, in part, because *E. albertii* is either likely to remain often unidentified or be misidentified by routine biochemical identification methods employed in clinical laboratories (39). In the present study, we could successfully identify the strain as *E. albertii* by using PCR analysis to differentiate between *E. albertii* and EPEC, while the similarity analysis of 16S rRNA gene sequences was insufficient to identify and conclude the isolate as *E. albertii*. Our results suggested that the optimal method to isolate *E. albertii* strains was to detect the presence of *eae* on selective agar plates for *E. coli*, select lactose and xylose nonfermenting and nonmotile colonies, and differentiate *E. albertii* from EPEC by the PCR analysis. Isolation of a large number of *E. albertii* strains and proper identification of these isolates as *E. albertii* will be highly important to further elucidate the significance of *E. albertii* as a diarrheagenic pathogen.

Conflict of interest None to declare.

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