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Sensitive detection of viable *Escherichia coli* O157:H7 from foods using a luciferase-reporter phage phiV10*lux*

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ABSTRACT

Escherichia coli O157:H7, a major foodborne pathogen, is a major public health concern associated with life-threatening diseases such as hemolytic uremic syndrome. To alleviate this burden, a sensitive and rapid system is required to detect this pathogen in various kinds of foods. Herein, we propose a phage-based pathogen detection method to replace laborious and time-consuming conventional methods. We engineered an *E. coli* O157:H7-specific phage phiV10 to rapidly and sensitively detect this notorious pathogen. The *luxCDABE* operon was introduced into the phiV10 genome and allowed the engineered phage phiV10*lux* to generate bioluminescence proportional to the number of viable *E. coli* O157:H7 cells without any substrate addition. The phage phiV10*lux* was able to detect at least 1 CFU/ml of *E. coli* O157:H7 in a pure culture within 40 min after 5 h of pre-incubation. In artificially contaminated romaine lettuce, apple juice (pH 3.51), and ground beef, the reporter phage could detect approximately 10 CFU/cm^2, 13 CFU/ml, and 17 CFU/g of *E. coli* O157:H7, respectively. Taken together, the constructed reporter phage phiV10*lux* could be applied as a powerful tool for rapid and sensitive detection of live *E. coli* O157:H7 in foods.

1. Introduction

Enterohemorrhagic *Escherichia coli* O157:H7 is a common foodborne pathogen that causes abdominal cramps and bloody diarrhea (Karch et al., 2005). *E. coli* O157:H7 can also lead to hemolytic uremic syndrome and hemorrhagic colitis as well as acute kidney injury and even death (Griffin and Tauxe, 1991). Foodborne outbreaks of *E. coli* O157:H7 are associated with consumption of contaminated foods such as fresh vegetables, acidic beverages, and ground beef (Chauret, 2011). Because ingestion of ~100 *E. coli* O157:H7 cells can cause symptoms (Tuttle et al., 1999), sensitive detection of the pathogens in foods is required. However, the culture-dependent conventional detection method is laborious and time-consuming; it takes 1–3 days by skillful operators to identify the cause of outbreaks (March and Ratnam, 1986; Waswa et al., 2007).

To overcome these drawbacks, much effort has gone into the development of a sensitive and rapid pathogen detection method. One promising alternative method is a bacteriophage-based detection system (Smartt et al., 2012). Phage-based systems can not only detect specific serotype strains, but also differentiate viable cells from nonviable cells. Reporter genes such as *gfp*, *lacZ*, and *lux* can be introduced into the bacteriophage genome to construct reporter phages (Goodridge and Griffiths, 2002; Oda et al., 2004; Waddell and Poppe, 2000). Among the reporter genes, luciferase gene *luxAB*, which originated from prokaryotes such as *Photorhabdus luminescens* and *Vibrio harveyi*, emits a more stable signal than other known reporter system. Because the bioluminescence released by the *luxAB* gene product is a physical signal and not a chemical signal, cells are not influenced by any accumulated signal products. However, the *luxAB*-based reporter system needs to be supplemented with substrate fatty aldehydes to generate bioluminescence. The requirement of substrate addition could make the method more laborious and susceptible to user error than substrate-independent methods (Smartt et al., 2012). Insertion of the whole *lux* operon, *luxCDABE*, into the phage genome can make the reporter phage an autonomous light-generating system by feeding the bioluminescent reaction with the *luxCDE*-produced aldehyde substrate

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(Franche et al., 2016; Kim et al., 2014).

The aim of this study was to develop a rapid and sensitive method for detecting *E. coli* O157:H7 from foods using a bacteriophage-based system to replace the conventional detection system. We manipulated the genome of *E. coli* O157:H7-specific phage phiV10 to harbor the *lux* operon, and were able to detect viable *E. coli* O157:H7 in real food matrices, including romaine lettuce, apple juice, and ground beef using this bioluminescent reporter phage phiV10*lux* without any extra substrates.

2. Materials and methods

2.1. Bacteria and bacteriophages

The bacteria strains used in this study are listed in Table S1. The bacteria strains were aerobically cultured at 37 °C overnight in Luria-Bertani (LB) broth. When necessary, antibiotics or chemicals were added to the media or agar at the following concentration: ampicillin (Ap, 50 μg/ml), chloramphenicol (Cm, 12.5 μg/ml), kanamycin (Km, 50 μg/ml), mitomycin C (1.0 μg/ml), and/or L-(+)-arabinose (final concentration, 50 mM).

Phage phiV10 was a prophage in the *E. coli* O157:H7 OE50 strain. To induce the wild-type (WT) or engineered phages, lysogenized *E. coli* O157:H7 OE50 was cultured to an optical density at 600 nm (OD_{600}) of 0.4, and the culture was treated with mitomycin C for 2 h. The induced phages were purified, propagated, and concentrated using CsCl gradient ultracentrifugation as previously described (Kim and Ryu, 2011).

2.2. Deletion of non-essential genes from the phiV10 genome

A one-step inactivation method was used to delete the specific phage genes (Datsenko and Wanner, 2000). Briefly, a kanamycin resistant (Km^R) cassette in plasmid pKD13 was amplified by polymerase chain reaction (PCR), wherein oligonucleotides had homologous sequences to the deletion target sites (Tables S2 and S3). An exponentially growing phiV10 lysogen harboring pKD46 was transformed with the amplified Km^R cassette after arabinose induction, resulting in a replacement of the deletion-target gene with the Km^R cassette. Finally, the Km^R cassette was removed using pCP20. The same method was sequentially applied to delete other genes. Deletion of target genes was confirmed by PCR and subsequent sequencing.

2.3. Plasmid construction for the lux operon insertion

To construct a suicide plasmid pDS132::*luxCDABE* for the integration of the *lux* operon into the phiV10 genome, we employed an isothermal assembly (Gibson et al., 2009) with the following four DNA fragments: SalI- and SacI-treated linearized plasmid pDS132, a PCR amplified *luxCDABE* operon from pBBR*lux* (Lenz et al., 2004), and PCR-amplified up- and downstream regions (~600 bp each) of the targeted insertion site. All DNA fragments had overlapped sequences (~30 bp) with the next fragment. The prepared four fragments were assembled into the pDS132::*luxCDABE* by incubating at 50 °C for 1 h in a reaction buffer (25% PEG-8000, 500 mM Tris-HCl pH 7.5, 50 mM $MgCl_2$, 50 mM DTT, 1 mM of dNTPs, and 5 mM NAD). The resultant plasmid was introduced into an *E. coli* DH5α λ *pir* strain.

2.4. Integration of the lux operon into the phage phiV10

To insert the *lux* operon into the mutated phage phiV10 [Δ*int* Δ*am* Δ*aip* Δ*oac*::*kan*], we used a double homologous recombination method with suicide plasmid pDS132 (Kim et al., 2014; Philippe et al., 2004). First, a suicide vector pDS132::*luxCDABE* in a conjugal donor *E. coli* S17-1 λ*pir* strain was transferred to recipient *Salmonella* cells harboring the mutated phiV10 as a prophage by conjugation overnight (Simon et al., 1983). Dependency on a pi protein for the plasmid replication resulted in the integration of the whole plasmid into the target site through primary recombination. Second, challenging of Km^R and Cm^R conjugants with sucrose (20% [w/v], final concentration) facilitated a secondary recombination, replacing the Km^R cassette with the *lux* operon. The resultant engineered reporter phage phiV10*lux* was obtained through a mitomycin C treatment.

2.5. Bacterial challenge assay

Exponentially growing *E. coli* O157:H7 OE50 culture (OD_{600} = 0.4) was infected with the WT phiV10 or phiV10*lux* at a multiplicity of infection of 1. Periodically, the OD_{600} of the culture was measured with spectrophotometry to check whether the bacterial growth was inhibited by the phages.

2.6. Bioluminescence assay

Prepared bacterial cultures or homogenized samples were infected with the engineered phage phiV10*lux* and continuously incubated at 37 °C for 40 min. Bioluminescence of each sample (200 μl) in a clean borosilicate glass tube (12 by 75 nm, Kimble chase, U.S.A.) was measured for 10 s of integration time using a luminometer (Lumat LB 9507; Berthold, Pforzheim, Germany). The bioluminescent intensity was displayed as relative luminescence units (RLU). Experiments were performed in triplicate and the results are expressed as the mean ± standard deviation (SD).

2.7. Viable cell detection assay

Exponentially growing *E. coli* O157:H7 cells were divided into two groups. One group was treated with 70% (v/v) ethanol at 37 °C and the other group was treated with Dulbecco's phosphate-buffered saline as a mock control. After 30 min, cells were washed with LB media three times, and subjected to the bioluminescence assay as described above.

2.8. Preparation of artificially contaminated foods with E. coli O157:H7

To artificially spike the foods with *E. coli* O157:H7, a culture of *E. coli* O157:H7 OE50 strain was grown overnight at 37 °C in LB broth and serially diluted with LB broth. Fresh romaine lettuce (*Lactuca sativa*) was purchased at a local grocery store (Seoul, South Korea) and cut into pieces approximately 2 cm by 5 cm using sterile scissors. The cut romaine lettuce pieces were placed on sterile aluminum foil in a biosafety cabinet. One-hundred microliters of prepared culture was spotted onto the surface of the romaine lettuce. After 2 h of drying in the biosafety cabinet at room temperature, the romaine lettuce pieces were homogenized in 90 ml LB broth for 2 min with a BagMixer 400 Laborator Blender (BangMixer, Interscience). The homogenized samples were plated on MacConkey sorbitol agar with cefixime, tellurite, and phenol red agar base with 1% sorbitol to count injured *E. coli* O157:H7 cells as well as normal cells. Portions of the remaining samples (5 ml) were pre-incubated at 37 °C for 5 h, then subjected to the bioluminescence assay as described above.

Apple juice (pH 3.51) was purchased at a local grocery store (Seoul, South Korea), portioned into 25-ml aliquots, spiked with 250 μl of prepared *E. coli* O157:H7 culture, and statically stored at 4 °C for 2 h. After the samples were homogenized in 225 ml LB broth with a homogenizer, *E. coli* O157:H7 cells were enumerated as described above. Similar to the romaine lettuce, remaining samples were also subjected to the bioluminescence assay after 5 h of pre-incubation.

Ground beef, which was purchased at a local grocery store (Seoul, South Korea), was weighed in 25 g and spiked with 250 μl of prepared *E. coli* O157:H7 culture. After 2 h of drying in the biosafety cabinet at room temperature, the samples were homogenized in 225 ml LB broth. Bacterial cell counting and bioluminescence assay were performed as described above for each sample.

3. Results

3.1. Construction of reporter phage phiV10lux harboring luxCDABE operon

To construct the reporter phage that could detect *E. coli* O157:H7, we inserted a bioluminescent reporter cassette *luxCDABE* operon into the genome of phage phiV10. The phage phiV10 is an *E. coli* O157:H7-specific temperate phage having 39,104-bp long dsDNA with 55 predicted genes (Perry et al., 2009). The genomic structure of phiV10 considerably resembles that of the *Salmonella enterica*-specific phage epsilon 15, placing phiV10 in the genus *Epsilon15virus* (Kropinski et al., 2007; Perry et al., 2009). Phylogenetic analysis of the phage's terminase large subunit revealed that phages in the genus *Epsilon15virus*, including phiV10, are predicted to utilize a headful packaging system during virion assembly (Kim et al., 2014), suggesting that phiV10 would experience a size limitation of the genome due to an increased genome size (~114.9%) after the insertion of the reporter *luxCDABE* operon (~5.9 kb). Previously, another member of the genus *Epsilon15virus*, *Salmonella*-specific phage SPC32H, was successfully engineered as the bioluminescent reporter phage by replacing several non-essential genes with the *luxCDABE* operon (Kim et al., 2014). Thus, we deleted whole genes or parts of genes that did not seem to be related with the phage's infectivity: integrase (*int*), adenine methylase (*am*), *O*-acetyltransferase (*oac*), and anti-immunity protein (*aip*) genes (Fig. 1). Indeed, the engineered phiV10 that lost these genes was able to infect *E. coli* O157:H7 and form plaques similar to the WT phage (data not shown). Consequently, approximately 3.0 kb was secured for the insertion of the *lux* operon.

The expression of reporter gene(s) is also an important issue for successful detection. Transcription of the *luxCDABE* operon from a strong promoter could ensure a high level of bioluminescence. Because phage-late genes encoding structural proteins are known to be strongly translated (Loessner and Scherer, 1995), we tested the expression level of gene *tsp* encoding a tail-spike protein. Strong expression from the promoter of *tsp* was confirmed using a β-galactosidase assay with a transcriptional *tsp-lacZ* fusion strain (data not shown), allowing us to target the area downstream of *tsp* as the *lux* operon insertion site (Fig. 1).

To introduce the *lux* operon, a phiV10 lysogen marked with a Km^R cassette at the target site was transformed with a suicide vector pDS132 harboring the *lux* operon flanked with up and downstream regions of the target site (Philippe et al., 2004). The Km^R cassette was replaced with the *lux* operon through sequential double homologous recombination, and this engineered sequence in the phage genome was confirmed by PCR and subsequent sequencing. The resultant engineered phage, phiV10 Δ*int* Δ*am* Δ*oac* Δ*aip* *tsp*::*luxCDABE*, was induced from the lysogen using mitomycin C treatment and designated phiV10*lux*.

Table 1
Host range of phiV10 and the engineered reporter phage phiV10*lux*.

Host	Plaques[a]	
	phiV10	phiV10*lux*
Escherichia coli		
O157:H7 OE50	CC	CC
O157:H7 ATCC 43895	CC	CC
O157:H7 ATCC 35150	CC	CC
O157:H7 2321	CC	CC
O157:H7 2324	CC	CC
O157:H7 3041	CC	CC
O157:H7 3046	CC	CC
O157:H7 ATCC 43880	C	C
O157:H7 86-24	C	C
O104:H4 NCCP 13721	–	–
O174 NCCP 15953	–	–
O91 NCCP 15957	–	–
O55 NCCP 15960	–	–
O21 NCCP 15962	–	–
MG1655	–	–
BL21	–	–
DH5α	–	–
DH10B	–	–
Salmonella typhimurium		
LT2	–	–
UK1	–	–
SL1344	–	–
ATCC 19586	–	–
ATCC 43147	–	–
Bacillus cereus		
KCTC 1094	–	–
Staphylococcus aureus		
RN4220	–	–

[a] CC, EOP range 0.1–1; C, EOP range 0.01–0.1; –, no plaque.

3.2. Comparison of WT phage phiV10 and engineered reporter phage phiV10lux

The genome of the constructed reporter phage phiV10*lux* was increased approximately 7.7% compared to that of the WT phage phiV10. To check whether the phage genome engineering described above altered the phage characteristics, we compared the host range and lysis activity of the WT phage and phiV10*lux*. Both the WT phage

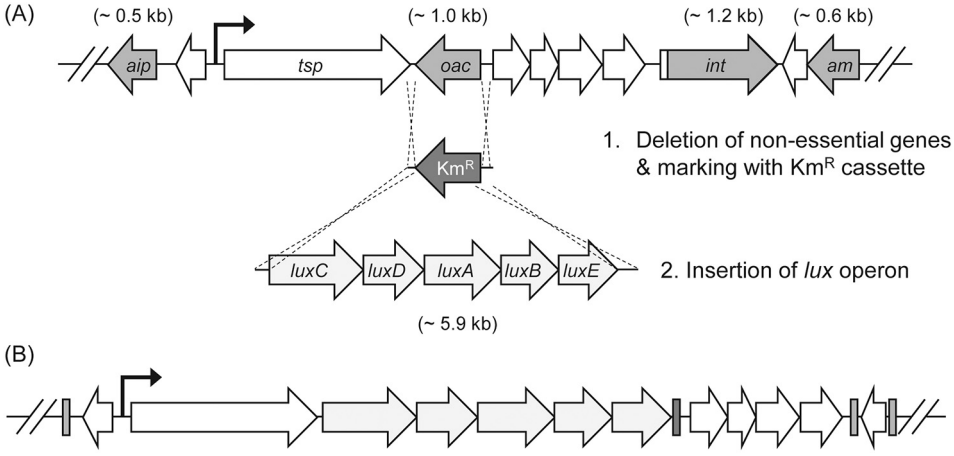

Fig. 1. Schematic representation of the phage phiV10 genome engineering. (A) Strategies for the genetic manipulation of phiV10. Non-essential genes (*i.e.*, *aip*, *oac*, *int*, *am*; gray arrows) were deleted or replaced with the kanamycin-resistant cassette (Km^R; dark gray arrow) through lambda red recombination. Then, the Km^R cassette was swapped with the *lux* operon (bright gray arrows) through double homologous recombination (details in Materials and methods). (B) Genome map of engineered phage phiV10*lux*. Putative promoter for tailspike gene (*tsp*) is represented by a black arrow.

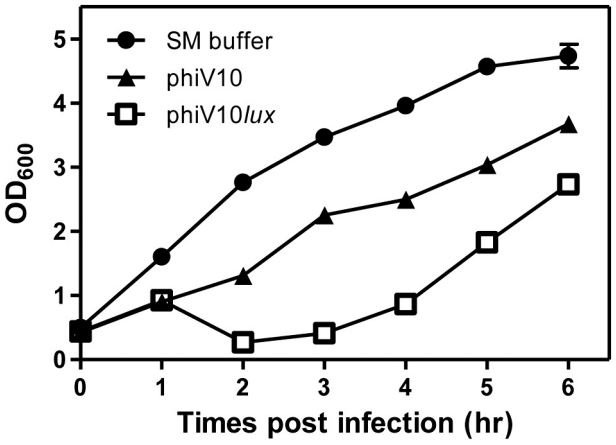

Fig. 2. Infectivity of phage phiV10 and phiV10*lux*. An *E. coli* O157:H7 culture was infected with the WT phage phiV10 or the engineered phage phiV10*lux* at an MOI of 1. *E. coli* O157:H7 culture treated with SM buffer was used as a negative control. PhiV10*lux* did not exhibit compromised infectivity, but rather showed stronger infectivity than phiV10. Because the integrase gene was deleted, phiV10*lux* could not opt for lysogenic development. Data are presented as mean (n = 3) ± SD.

and the engineered phage showed the same host specificity; they formed plaques with similar efficiency of plating on various *E. coli* O157:H7 strains, but no plaques were detected from the other O antigen serotypes of *E. coli*, *Salmonella* spp., and gram-positive bacteria tested (Table 1). In accordance with the previous studies, these results suggest the specificity of WT phiV10 and its derivative phiV10*lux* against a somatic antigen O157 of *E. coli* O157:H7 (Perry et al., 2009; Zhang et al., 2016).

In a bacterial challenge assay, the phage phiV10*lux* showed higher *E. coli* O157:H7 lysis activity than the WT phage phiV10 (Fig. 2). Deletion of the integrase gene probably prevented the phage from entering into the lysogenic cycle similar to the pseudo-lysogenic state (Cenens et al., 2013). Formation of relatively clear plaques by the engineered phage compared to the WT phage also supported the result of the bacterial challenge assay (data not shown). Taken together, the engineered phage phiV10*lux* did not have any different host recognition properties than the WT phage and was not compromised in the lysis activity against *E. coli* O157:H7 by genetic manipulations.

3.3. Optimization of conditions for rapid detection of E. coli O157:H7 using reporter phage phiV10lux

Infection of the constructed reporter phage phiV10*lux* transferred the *lux* operon to the target *E. coli* O157:H7 cells. After the transcription and translation of the *lux* operon using the host bacterial apparatuses, one of the gene products, luciferase, started to emit light signals. Because the light intensity could be influenced by the phage concentration, we infected *E. coli* O157:H7 with various concentrations of reporter phage and measured relative light units (RLUs) in various time intervals to optimize pathogen detection conditions (Fig. S1). Considering the limits of detection (LOD) and time required for detection, we decided to use 10^8 PFU/ml phages for the detection. In this condition, 10^3 CFU/ml of *E. coli* O157:H7 in LB culture could be detected within 40 min (Fig. S1A). In addition, the emitted bioluminescence was proportional to the number of live *E. coli* O157:H7 cells, enabling the prediction of *E. coli* O157:H7 cell numbers from the RLU values. Although 1 CFU/ml of *E. coli* O157:H7 could be detected within 7 h when 10^4 PFU/ml phage was used, the light signal was not proportional to the number of live *E. coli* O157:H7 cells (Fig. S1C), making it difficult to predict the number of cells. Because pathogenic *E. coli* O157:H7 can cause symptoms in humans with only a few cells (Tuttle et al., 1999), we tried to enhance the LOD by adding a pre-incubation period. After 5 h of pre-incubation, a single *E. coli* O157:H7 bacterium in a stationary state was enriched to 10^3 CFU/ml (Fig. S2). Consequently, the engineered phage phiV10*lux* was able to detect 1 CFU/ml *E. coli* O157:H7 within 40 min after the 5 h of pre-incubation (Fig. 3). Also, we could estimate the initial number of pathogenic *E. coli* cells because the light signal was proportional to the number of live bacteria cells.

3.4. Specific detection of viable E. coli O157:H7 using phiV10lux

One of the most important aspects of pathogen detection is specificity against the target bacteria. Because foods usually contain various strains of bacteria, the detection system should distinguish the target bacteria from other existing bacteria. To examine whether light emission by reporter phage phiV10*lux* was influenced by nonspecific bacteria, phiV10*lux* was applied to a bacterial culture containing *E. coli* O157:H7 and a non-pathogenic *E. coli* BL21 strain (Chart et al., 2000). As shown in Fig. 3, the bioluminescence emission was not different from that from a pure culture of *E. coli* O157:H7, suggesting that the presence of non-target bacteria did not affect the light emission.

Because the transferred *lux* genes from the reporter phage to the host bacteria are transcribed and translated by host bacterial machineries, bioluminescence signals could be emitted from viable cells only. Indeed, *E. coli* O157:H7 cells intended nonviable by 70% (v/v) ethanol treatment did not elicit meaningful bioluminescence after phiV10*lux* infection, while viable cells strongly did (Fig. 4). These results imply that the phage phiV10*lux*-based detection system is able to distinguish live *E. coli* O157:H7 from nonviable cells.

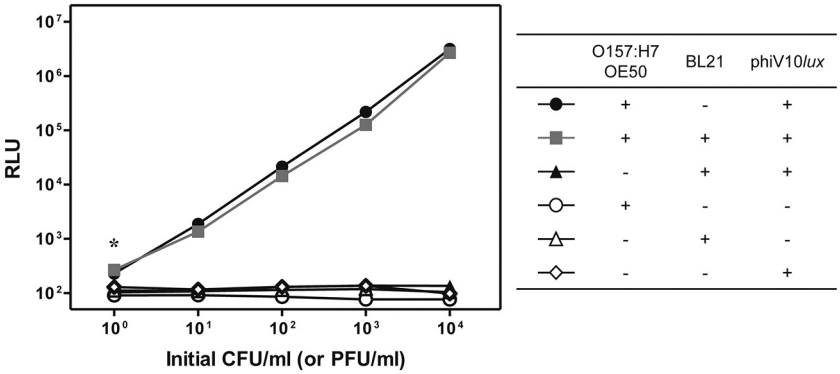

Fig. 3. Specific detection of *E. coli* O157:H7 by phiV10*lux*. The *E. coli* O157:H7 OE50 strain and/or the BL21 strain at a stationary phase were serially diluted and pre-incubated at 37 °C in LB broth for 5 h. The culture of each strain and the mixture of them were infected with the phage phiV10*lux* (10^8 PFU/ml) for 40 min. Bacterial cells without the phages or *vice versa* was used as a negative control. The bioluminescence was independently measured (n = 3) and the results are expressed as means. Groups containing both *E. coli* O157:H7 OE50 and phiV10*lux* showed significantly higher light signals than other groups (*, $P < 0.05$). Note that the number of *E. coli* O157:H7 OE50 cells only were considered in the x-axis for the group of O157:H7 OE50 + BL21 + phiV10*lux*.

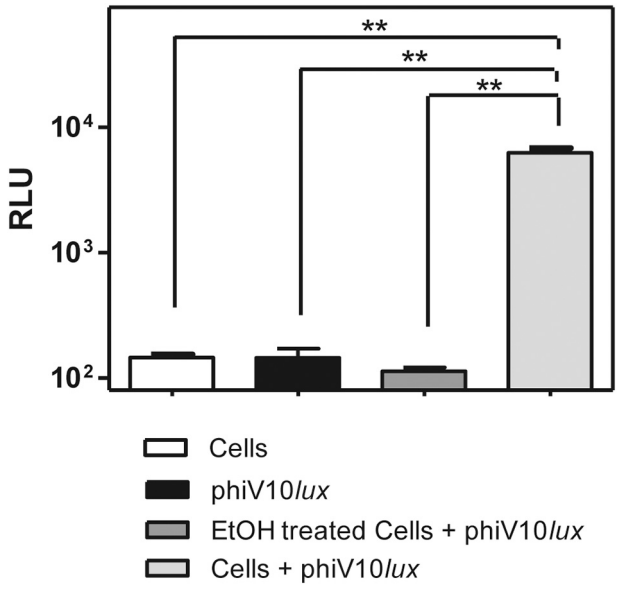

Fig. 4. Bioluminescence emission by phiV10*lux* is specific to viable *E. coli* O157:H7 cells. The exponentially growing *E. coli* O157:H7 culture was divided into two groups, and only one group was treated with 70% ethanol (EtOH) for 30 min. After washing with LB broth, each sample was infected with 10^8 PFU/ml of engineered reporter phages and incubated at 37 °C. Bioluminescence was measured after 40 min, post-infection. The mean values with SD from three independent experiments is shown (**, $P < 0.001$).

3.5. Detection of *E. coli* O157:H7 from artificially contaminated food samples

According to World Health Organization (WHO), primary sources of enterohemorrhagic *E. coli* outbreaks are minimally processed fresh vegetables, acidic juices, and ground beef. Thus, we tested *E. coli* O157:H7 detection ability of phiV10*lux* in three representative foods: romaine lettuce (*Lactuca sativa*) representing the fresh vegetables, apple juice (pH 3.5) representing the acidic juices, and ground beef. To mimic the pathogen-contaminated condition, the foods were artificially spiked with the *E. coli* O157:H7 culture. The preprocessed foods were homogenized using a stomacher, pre-incubated for 5 h and, subsequently, the reporter phage phiV10*lux* was mixed with the enriched samples for 40 min. The bioluminescence of the sample was proportional to the number of spiked *E. coli* O157:H7 cells (Fig. 5), and the bioluminescence were comparable to those of pure *E. coli* O157:H7 in same cell concentrations. The LOD of the phage phiV10*lux* was 10 CFU/cm^2 in romaine lettuce, 13.3 (± 4.7) CFU/ml in apple juice, and 16.7 (± 5.8) CFU/g in ground beef. Because the homogenized samples were diluted 10-fold during the procedure, we could not determine whether the reporter phage was able to reach the detection of a single *E. coli* O157:H7 cell in unit grams or milliliters of the foods. Taken together, the phage phiV10*lux*-based detection system detected *E. coli* O157:H7 in a real food matrix without being disturbed by various food components.

4. Discussion

Conventional methods for microorganism detection from food samples are labor-intensive and take a long time. Thus, alternative detection methods are required to improve the detection limit and reduce the detection time. Because *E. coli* O157:H7 has an extremely low infectious dose, the detection limit is important when considering alternative methods for pathogen detection. Typical rapid detection methods rely on capturing nucleic acids of the target pathogen using PCR, or enzyme linked immunosorbent assay (ELISA) using specific antibodies against the target organisms (Bonetta et al., 2016; Smartt and Ripp, 2011; Sunwoo et al., 2006). Although these methods are

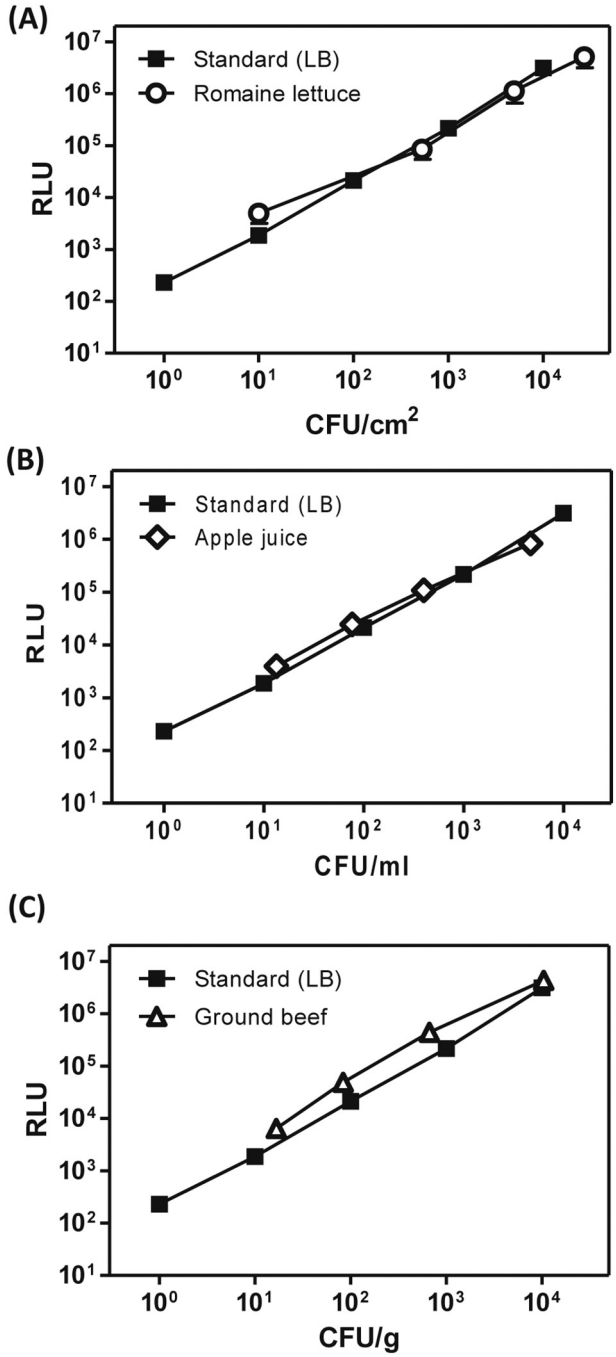

Fig. 5. Detection of *E. coli* O157:H7 in artificially contaminated foods. Various concentrations of *E. coli* O157:H7 culture in the stationary phase was inoculated in (A) romaine lettuce, (B) apple juice (pH 3.51), and (C) ground beef. After infection of phiV10*lux*, bioluminescence from each sample was measured (details in Materials and methods). Light signals from LB broth inoculated with *E. coli* O157:H7 were compared as a standard curve (black squares). Means ± SD from three independent experiments are illustrated. Limits of detection in this experiment were 10 CFU/cm^2 in romaine lettuce, 13.3 (± 4.7) CFU/ml in apple juice, and 16.7 (± 5.8) CFU/g in ground beef.

sensitive as well as rapid in specific pathogen detection, their major drawbacks are the inability to differentiate viable cells from nonviable cells and the inhibition by various food components (Rossen et al., 1992).

To overcome these drawbacks, we developed a phage-based detection system that can distinguish viable *E. coli* O157:H7 cells from nonviable cells rapidly and sensitively. The developed phage phiV10*lux* contains genes for bioluminescence, and thus, it could generate light signals by specifically infect the viable *E. coli* O157:H7 cells only

(Figs. 3 and 4). Within 40 min, we could detect 10^3 CFU/ml of *E. coli* O157:H7 cells in pure culture using the phiV10*lux* (Fig. S1).

One advantageous point of the phiV10*lux* over other bioluminescent phages is unnecessary of substrate addition for the pathogen determination. Due to the size limitation, typical bioluminescent reporter phages incorporate only the *luxAB* genes (~2.2 kb) instead of the whole *luxCDABE* operon (~5.9 kb), requiring the addition of substrates such as decanal to feed the bioluminescent reaction (Loessner et al., 1997; Waddell and Poppe, 2000). Recently, a small 516-bp gene *nluc* encoding a novel luciferase NonoLuc was also utilized to develop the bioluminescent reporter phage, but the light emitting still depends on the supplementation of imidazopyrazinone substrate (Zhang et al., 2016). Similar to a previous study on *Salmonella*-specific bioluminescent reporter phage SPC32H-CDABE (Kim et al., 2014), we could successfully integrate the entire *luxCDABE* operon into the phiV10 genome by deleting several non-essential phage genes: *aip*, *oac*, *int* and *am*. The fatty acid reductase encoded by *luxCDE* could autonomously provide the aldehyde substrates by recycling the reaction product fatty acids. This unnecessity of additional substrate allows repeated or continuous measurements of bioluminescence from a single sample, and also enables avoiding of potential user errors related with the substrate supplementation. However, enlarged genome size might cause problems regarding with genome packaging or genetic stability of the engineered phages (Roy and Cingolani, 2012). Therefore, detailed characterization on the phage genes and genome should precede the development of reporter phages to replace selected non-essential genes with the *lux* operon.

With a 5-hour pre-enrichment step, we could detect approximately 10 CFU/cm^2 (10 CFU/ml or 10 CFU/g) of *E. coli* O157:H7 from artificially contaminated real food matrices (Fig. 5). The levels of light signal were proportional to the number of spiked cells, implying that the number of contaminated *E. coli* O157:H7 cells in foods would be quantitatively predicted. Compared to conventional culture-dependent methods, which take a couple of days for pathogen determination and enumeration, the detection times of the phiV10*lux*-based method are significantly shorter. Thus, this method could more effectively prevent and/or control pathogen outbreaks. To further shorten the detection time, stronger promoter might be used to increase the expression level of the *lux* operon. An artificial constitutive promoter or strong bacterial promoter may be used with proper optimization instead of the phage's own promoter. Indeed, *E. coli* cells in water were detectable within just 1.5 h when the bacterial promoter P*rplU* was used to strongly express the *lux* operon in a bioluminescent reporter phage HK620::P*rplU-luxCDABE* (Franche et al., 2016).

Paradoxically, the high specificity of the phiV10*lux* against *E. coli* O157:H7 might be concerned as one of its drawbacks. Although *E. coli* O157:H7 is a typical causative serotype responsible for the complicated outbreaks of bloody diarrhea and hemolytic uremic syndrome, big six non-O157 shiga-like toxin producing *E. coli* (STEC) serotypes, including O26, O45, O103, O111, O121, and O145, have also threaten the food industries and health authorities (Wang et al., 2012). Because phage phiV10 recognize a somatic O157 antigen to infect the host bacteria, the non-O157 STECs including the big six cannot be detectable by the phiV10-derived phiV10*lux* (Zhang et al., 2016). Similar strategies applied to the phiV10*lux* as described here might be used to develop the reporter phage specific for each of these non-O157 STEC serogroups. Alternatively, engineering of the receptor binding proteins in the phage phiV10*lux* to recognize the non-O157 STEC, or *de novo* synthesis of the reporter phage genome could enable the detection of these notorious groups of pathogen (Ando et al., 2015).

Pathogen detection using the reporter phage is reliable, rapid, sensitive, user-friendly, and cost effective. In particular, bioluminescence reporter phages such as the phiV10*lux* are suitable for *in situ* monitoring of foods because the bioluminescence signal is not hindered by food components. Since unavailability of portable detection devices significantly delay the confirmation of foodborne pathogens (You et al., 2011), development of portable real-time luminometer is also required for a wide practical use of phiV10*lux* in the food industry. A smartphone-based biosensing technology could be applied to develop the portable, equipment-free, low-cost, and simply operating luminometeric system (Zarei, 2017). Taken together, the data presented here strongly support the potential of bioluminescent reporter phages as promising tools for ensuring food safety and improving public health. Further studies on the development of portable luminometer would open a new path for the implementation of bioluminescent reporter phage-based pathogen detections in the practical fields.

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Appendix A. Supplementary data

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