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Bacteriophage-Based Bioluminescent Bioreporter for the Detection of *Escherichia coli* O157:H7

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ABSTRACT

The rapid detection of pathogenic bacteria in food and water is vital for the prevention of foodborne illness. In this study, the *lux* reporter genes were used in a new bioassay that allows pathogen monitoring without multiple sample manipulations or the addition of exogenous substrate. A recombinant phage specific for *Escherichia coli* O157:H7 was constructed that, upon infection, catalyzes the synthesis of *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL). This phage PP01 derivative carries the *luxI* gene from *Vibrio fischeri* under the control of the phage promoter P_L. OHHL produced by infected *E. coli* O157:H7 induces bioluminescence in bioreporter cells carrying the *V. fischeri lux* operon. The ability of phage PP01-*luxI* to detect several strains of *E. coli* O157:H7 was confirmed in a 96-well plate assay. In this assay, *luxCDABE* bioreporter cells capable of detecting OHHL were mixed with phage PP01-*luxI* and *E. coli* O157:H7, and luminescence was monitored. Reporter phages induced light in bioreporter cells within 1 h when exposed to 10^4 CFU/ml of *E. coli* O157:H7 and were able to detect 10 CFU/ml in pure culture with a preincubation step (total detection time, 4 h). The detection method was also applied to contaminated apple juice and was able to detect 10^4 CFU/ml of *E. coli* O157:H7 in 2 h after a 6-h preincubation.

Rapid detection methods for *Escherichia coli* O157:H7 in food and water are necessary to detect both naturally occurring outbreaks and bioterrorist events. A variety of methods exist for the detection of *E. coli* O157:H7, but most are slow or require significant sample manipulation *(5, 6, 11, 18)*. Culture methods require skilled personnel, and results are not obtained for 24 to 48 h *(5, 18)*. Enzyme-linked immunosorbent assays and other immunological methods require staining and washing steps *(5, 6, 18)*. Rapid detection methods based on PCR are fast and reliable but cannot differentiate between live and dead bacterial cells and can be fairly complex *(5)*. Biosensor-based systems can be rapid and amenable to real-time monitoring, but presently, they cannot detect low concentrations of bacteria and have difficulty detecting bacteria in food matrices *(22, 25)*.

The specificity of bacteriophages for their bacterial hosts has long been used for the identification of bacterial pathogens. Phage lysis assays are commonly used for the identification and typing of bacteria (reviewed in Marks and Sharp *(13)*). In the past 20 years, the genetic manipulation of bacteriophages to make "reporter phages" with reporter genes, such as bacterial luciferase (*luxAB*), β-galactosidase (*lacZ*), and green fluorescent protein (*gfp*), has been documented *(3, 8, 9, 12, 24, 27, 28, 30)*. Phages are metabolically inert when outside their host, and so the reporter proteins encoded in these phages are not produced until the appropriate bacterium is infected. In the existing *lux*-based phage reporter systems, bacteria are detected following phage infection, an incubation and growth period, and the addition of an exogenous substrate *(3, 9, 12)*. While these methods are capable of the specific and sensitive detection of bacteria, they are not amenable to continuous monitoring because they require excessive sample manipulation.

Recently, a rapid GFP reporter phage detection system was described that can detect and differentiate live and dead *E. coli* O157:H7 cells *(1, 20)*. This GFP reporter was constructed in bacteriophage PP01, which was isolated from swine stools by Morita et al. *(16)*. Phage PP01 was found to infect a variety of *E. coli* O157:H7 strains (17 of 19 tested, including toxigenic and nontoxigenic) but none of the 14 non-O157:H7 *E. coli* strains examined *(16, 21)*. Other species of bacteria that have been tested, including *Salmonella enterica* serovar Typhimurium, *Pseudomonas fluorescens, Pseudomonas aeruginosa, Citrobacter freundii, Erwinia carotovora,* and *Yersinia enterocolitica,* have not shown susceptibility to the phage *(16, 21)*. This PP01-GFP reporter phage is rapid and specific, but like all GFP-based assays, it requires a high-energy light source for the excitation of GFP, which may limit its potential for use in real-time on-line industrial and environmental monitoring. In the present study, we describe a PP01 reporter phage that uses the *lux* reporter genes in a unique construct that permits detection without the addition of substrate and has the potential for use in real-time on-line monitoring.

The *lux* bioluminescence genes were isolated from the marine bacterium *Vibrio fischeri* and include a cassette of five genes (*luxCDABE*) whose products are necessary for the generation of light and two genes (*luxI* and *luxR*) that

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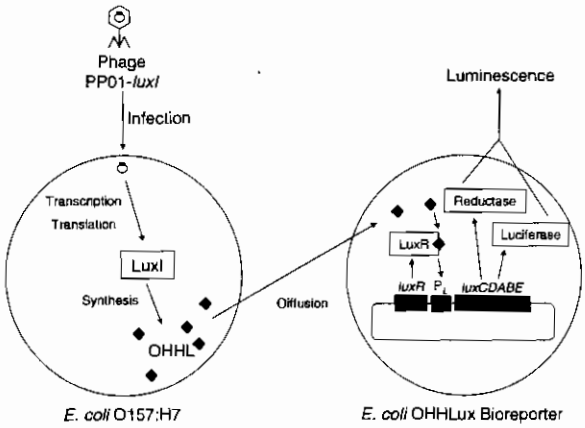

FIGURE 1. *Bacteriophage-based bioluminescent bioreporter for pathogen detection. Phage PP01-*luxI*, which carries the* luxI *gene under the control of a strong phage promoter, injects its genome into* E. coli *O157:H7. Upon infection,* luxI *is transcribed from the phage genome, and as a result,* N*-(3-oxohexanoyl)-*L*-homoserine lactone (OHHL) is produced. OHHL diffuses from the host cell and into a nearby bioreporter cell, where it binds to the transcriptional activator LuxR. The LuxR-OHHL complex interacts with* P_L *to activate the transcription of* luxCDABE, *resulting in the production of a detectable light signal.*

produce the components of a quorum sensing system that activates the transcription of the *luxCDABE* cassette *(14).* The luciferase genes (*luxAB*) encode the proteins responsible for generatiug bioluminescence, while the reductase (*luxC*), transferase (*luxD*), and synthetase (*luxE*) genes encode proteins that produce an aldehyde substrate required for the bioluminescence reaction. Bioluminescent bacteriophage-based bioreporters developed in the past carry the *luxAB* genes but lack the *luxCDE* genes *(3, 9, 12, 24, 28, 30).* Thus, once these bacteriophages infect their target cells, detection requires the addition of exogenous substrate. The entire *luxCDABE* cassette is too large to be accommodated by most phage genomes, and so to eliminate the need for exogenous substrate, an alternative approach is necessary.

The detection system described in the present study takes advantage of the *luxI-luxR* quorum sensing system. Quorum sensing systems are cell-to-cell communication networks based on the synthesis of diffusible autoinducer molecules (reviewed in Miller and Bassler *(15)*). *V. fischeri* expresses low levels of *luxI* and produces small amounts of the diffusible molecule *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL). When a high concentration of *V. fischeri* is present, the concentration of OHHL becomes high enough to bind to LuxR and activate the transcription of *luxCDABE* and *luxI*, causing the production of light. In the system used in this study, the complete *lux* cassette (*luxCDABE*), along with the transcriptional regulator *luxR*, is carried by a "bioreporter cell" that detects the diffusible OHHL produced by a target pathogen infected with a phage carrying the *luxI* gene (Fig. 1). The initial feasibility of such phage-based bioreporters was demonstrated with a phage lambda–*E. coli* K-12 model *(23).* In this study, *luxI* was integrated into phage PP01 for the detection of its host, *E. coli* O157:H7, in pure culture and in artificially contaminated apple juice.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. Bacteriophage PP01 was obtained from Hajime Unno (Tokyo Institute of Technology) and was stored in SM buffer (10 mM $MgSO_4$, 100 mM NaCl, 0.01% gelatin, and 50 mM Tris-HCl [pH 7.5]). The bacterial strains used in this study and their American Type Culture Collection (ATCC) designations were as follows: *E. coli* O157:H7 (ATCC 43888 [nontoxigenic], ATCC 43889, ATCC 43894, ATCC 43895, and ATCC 700927); *E. coli* serotypes O7 (23503), O55 (12014), and O126:K71 (12807); *E. coli* K-12 (strain 10798); *E. coli* DH1 (strain 33849); *E. coli* MM294 (strain 33625); and *Y. enterocolitica* (strain 23715). All strains were grown in Luria-Bertani medium (LB; 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per liter of H_2O [pH 7.0]). The bioreporter used in this study was *E. coli* OHHLux, which has been previously described *(23).* Briefly, the bioreporter cells carry *luxR* and *luxCDABE* under the control of the bidirectional *lux* promoter. Activation of the *lux* operon in these cells occurs in response to OHHL at 5 nM and higher.

Construction of the reporter phage. The methods used for the construction of the PP01-*luxI* reporter phage were based on those used by Oda et al. *(20)* to create a PP01-GFP phage. Construction of the *luxI*-carrying plasmid used for the recombination of PP01 entailed the fusion of the *V. fischeri luxI* gene (GenBank accession no. Y00509) upstream of the left arm promoter (P_L) of phage lambda in a pLEX vector (Invitrogen, Carlsbad, Calif.). Downstream of this fusion, an *rrnB* T_1T_2 transcriptional terminator from the pKK223-3 cloning vector (GenBank aecession no. M77749) was ligated. Finally, the *soc2, soc1,* and *soc* genes of phage PP01 (GenBank accession no. AY247798) were cloned and inserted upstream and downstream of the *luxI* construct to allow homologous recombination to oeeur between the plasmid and the phage. Each individual gene and stepwise fusions were initially constructed in pCR2.1- or pCR4-TOPO TA cloning vectors (Invitrogen). The DNA isolations were performed witb Wizard Minipreps, Midipreps, or Lambda Preps (Promega, Madison, Wis.) and purified when necessary with the Geneclean Spin Kit (Q-Biogene, Carlsbad, Calif.). The PCR reactions were carried out in an MJ Research DNA Engine tetrad (Waltham, Mass.) with Ready-To-Go PCR beads (Amersham Biosciences, Piscataway, N.J.). The DNA was sequenced at all steps with the ABI Big Dye Terminator Cycle Sequencing reaction kit on an ABI 3100 DNA Genetic Analyzer (Applied Biosystems, Foster City, Calif.).

The *luxI* gene was amplified by PCR from *V. fischeri* with forward primer 5′-CATATGACTATAATGATAAAAAAATCGG-3′ and reverse primer 5′-CATATGTTAATTTAAGACTGC-3′ to introduce the unique restriction site *NdeI* at both termini (underlined) and cloned into a pCR2.1-TOPO vector (Fig. 2A). The *luxI* gene was excised from the TOPO vector by *NdeI* restriction digestion and ligated into the *NdeI* inulticloning site of the pLEX vector, thereby placing *luxI* in frame with the Lambda P_L promoter (Fig. 2B). Directionality was confirmed by restriction digestion and sequencing.

The *rrnB* transcriptional terminator was amplified by PCR from pKK223-3 with forward primer 5′-ATCGATAAGAGTTTGT-AGAAACGC-3′ and reverse primer 5′-CTGTTTTGGCGGATG-3′ to introduce the restriction site *ClaI* at the 5′ end (underlined) and cloned into a pCR2.1-TOPO vector (Fig. 2C).

The P_L-*luxI* fusion was amplified by PCR from pLEX with

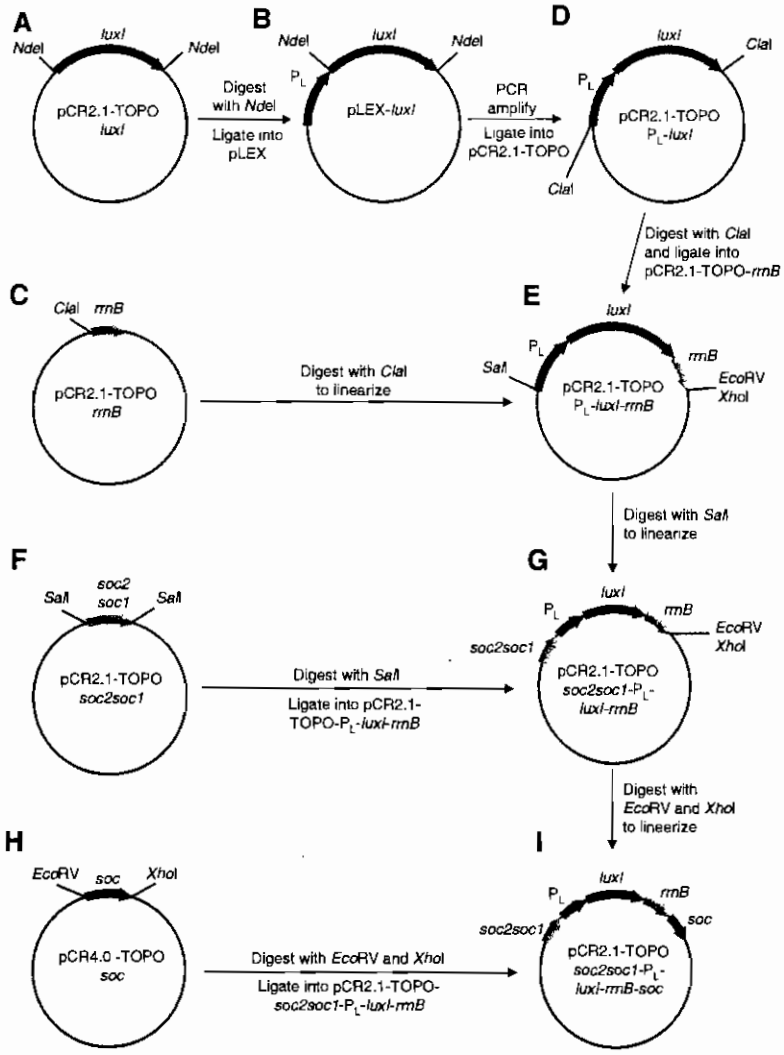

FIGURE 2. *Genetic construction of plasmid pCR2.1*-soc2soc1-P_L-luxI-rrnB-soc. *See "Materials and Methods" for details.*

forward primer 5′-ATCGATGTCGACTCTAGAGGATCC-3′ and reverse primer 5′-ATCGATATTCGAGCTCGGTACCATA-3′ containing the restrietion site *Cla*I (underlined) and cloned into a pCR2.1-TOPO vector (Fig. 2D). This vector was then digested with *Cla*I and ligated into the *Cla*I site of the *rrnB* TOPO vector described above to create a P_L-*luxI-rrnB* fnsion within a pCR2.1-TOPO vector (Fig. 2E). Directionality was again confirmed by restriction digestion and sequencing.

The *soc2soc1* genes of phage PP01 were amplified with forward primer 5′-GCGTCGACGAAGAAATCTTTAAACTTTATTATCTG-3′ and reverse primcr 5′-CAGTCGACTCTCCTTTTATTTAAATTACATGAC-3′ to iutrodnce the *Sal*I restriction site (underlined) and cloned into a pCR2.1-TOPO vector (Fig. 2F). The *soc2soc1* genes were excised from the TOPO vector by digestion with *Sal*I and ligated into the *Sal*I multicloning site of pCR2.1P_L-*luxI-rrnB* just upstream of P_L (Fig. 2G). Directionality was confirmed by restriction digestion and sequencing.

The *soc* gene of phage PP01 was amplified with forward primer 5′-AAGATATCCATGGCTAGTACTCGCGGTTA-3′ and reverse primer 5′-TCTCTCGAGGGTTTAATCCAACGATTTAACAT-3′ to introduce the *Eco*RV and *Xho*I restriction sites, respectively (nnderlined), and inserted into the TA cloning vector pCR4.0-TOPO vector (Fig. 2H). The *soc* gene was excised from the TOPO vector by digestion with *Eco*RV and *Xho*I and ligated into the *Eco*RV-*Xho*I multicloning site of pCR2.1-*soc2soc1*-P_L-*luxI-rrnB* just downstream of *rrnB* (Fig. 2I). Insertion was confirmed by restriction digestion and sequencing.

The completed construct pCR2.1-*soc2soc1*-P_L-*luxI-rrnB-soc* was electroporated into *E. coli* O157:H7 (ATCC 43888, nontoxigenic), and transformed cells were selected on LB plates containing 50 μg of kanamycin per ml. Log-phase cultures of *E. coli* O157:H7 containing pCR2.1-*soc2soc1*-P_L-*luxI-rrnB-soc* were infected with phage PP01 at a multiplicity of infection of 0.01 and 0.1 and incubated for 5 h at 37°C to allow a homologous recombination between the plasmid and the phage to occur (Fig. 3). Bacteria were lysed by the addition of chloroform and then incubated for 15 min at 37°C. Cell debris was pelleted, and phages were titered in top agar overlays.

Recombinant PP01-*luxI* phages were detected by a plaque hybridization assay with the Alkphos Direct Labeling and Detection System (Amersham Biosciences). A probe for *luxI* was obtained by amplifying a 1-kb portion of the *luxI* gene with the forward primer 5′-GGATCCCAAAAATACACTTGACCATT-3′ and the reverse primer 5′-GAGCTCTTATTTGATGCCTGGCAGTT-3′

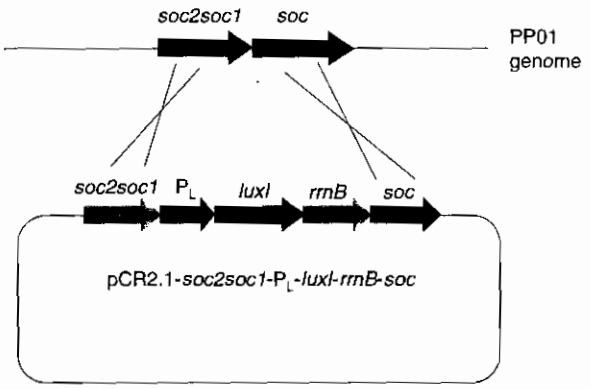

FIGURE 3. *Homologous recombination between plasmid pCR2.1*-soc2soc1-P_L-luxI-rrnB-soc *and phage PP01. Crossover events occur in the* soc2soc1 *and* soc *regions, resulting in the integration of* P_L-luxI-rrnB *into the phage PP01 genome.*

and was labeled according to the kit directions. Hybridization was performed according to the kit directions. Labeled plaques were visualized with a Molecular Dynamics Storm 840 phosphorescent imager (Molecular Dynamics, Union City, Calif.).

Phages that were labeled in the hybridization assay were propagated, and the presence of *luxI* in these phages was confirmed by PCR with the forward primer 5′-GCAATTCCATCGGAGGAGTA-3′ and the reverse primer 5′-GCACTCTGTTGACCAAGCAA-3′. One confirmed PP01-*luxI* phage clone was propagated and used for all subsequent experiments.

Detection of *E. coli* O157:H7 in pure culture. To detect *E. coli* O157:H7 in pure culture, the PP01-*luxI* reporter phage and *E. coli* OHHLux bioreporter cells were combined with a dilution series of *E. coli* O157:H7. Phage PP01-*luxI* was diluted to a concentration of 10^8 PFU/ml in SM buffer. The *E. coli* OHHLux bioreporter cells were grown overnight in LB at 28°C to an optical density at 600 nm (OD_{600}) of 0.35 (~1×10^8 CFU/ml). The volume and concentration of *E. coli* OHHLux used for the detection of OHHL in the phage bioreporter system were previously optimized *(23)*. Host *E. coli* O157:H7 strains (ATCC 43888, ATCC 43889, ATCC 43894, ATCC 43895, and ATCC 700927) were grown in LB at 37°C to an OD_{600} of 0.2 (~1×10^8 CFU/ml) and then serially diluted 1:10 to 10 CFU/ml in 10-ml aliquots of LB. Triplicate samples of each strain dilution were then distributed into a black 96-well microtiter plate (100 μl per well; Dynex Technologies, Chantilly, Va.). Next, PP01-*luxI* reporter phage cells (100 μl per well, ~1×10^7 PFU/ml final concentration) and *E. coli* OHHLux bioreporter cells (50 μl per well, ~2×10^7 CFU/ml final concentration) were added to all wells. Control wells included pairwise combinations of all constituents (target cells plus *E. coli* OHHLux, target cells plus PP01-*luxI* reporter phage, and *E. coli* OHHLux plus PP01-*luxI* reporter phage) as well as each constituent by itself at identical volumes and concentrations as for the test wells, and in triplicate. Plates were sealed with Breathe-Easy (Diversified Biotech, Boston, Mass.) membranes and monitored for bioluminescence at approximate 1-h intervals over a 22-h period in a Perkin-Elmer 1450 Microbeta Plus liquid scintillation counter (Perkin-Elmer, Norwalk, Conn.) at 30°C.

To increase the sensitivity of this assay, a preincubation step was added. Tubes from each *E. coli* O157:H7 dilution series above were incubated with shaking (200 rpm) for 3 h at 37°C to promote growth. After 3 h, 100-μl samples were removed from the preincubated dilution tubes and transferred to a microtiter plate as described above, with phage PP01-*luxI* and *E. coli* OHHLux bioreporters added to each well also as described above. The microtiter plate was sealed and monitored for bioluminescence as described above. A Student's *t* test was performed to determine the point at which the luminescence in the test wells significantly exceeded the luminescence in the control wells ($P < 0.05$).

Specificity of assay for *E. coli* O157:H7. The specificity of phage PP01 for *E. coli* O157:H7 is well established *(16, 17, 21)*, but the specificity of the recombinant PP01-*luxI* phage needed to be confirmed experimentally. Thus, we tested the PP01-*luxI* reporter phage assay on various other *E. coli* O-serotypes (O7, O55, and O126:K71); other *E. coli* strains, such as K-12, DH1, and MM294; and known OHHL-producing strains, such as *Y. enterocolitica (26)*, with nontoxigenic *E. coli* O157:H7 ATCC 43888 serving as a positive control. The selection of this particular set of target strains was based on previous PP01 susceptibility studies conducted by Morita et al. *(17)* and previous OHHL reporter specificity studies conducted in our own laboratory *(23)*. All strains were grown in LB to a population density of approximately 1×10^8 CFU/ml, whereupon triplicate 100-μl samples were transferred to individual wells in a black 96-well microtiter plate. Next, 100 μl of PP01-*luxI* reporter phage (~1×10^7 PFU/ml final concentration) and 50 μl of *E. coli* OHHLux bioreporter cells (~2×10^7 CFU/ml final concentration) were added to all wells. Controls were as described above. The plate was sealed and monitored for bioluminescence as described above.

Detection of *E. coli* O157:H7 (ATCC 43888) in apple juice. For these particular studies, only the nontoxigenic strain of *E. coli* O157:H7 was used (ATCC 43888). Phage PP01-*luxI* was diluted to a concentration of 10^8 PFU/ml in SM buffer. The *E. coli* OHHLux bioreporter cells were grown in LB at 28°C to an OD_{600} of 0.35. Nontoxigenic *E. coli* O157:H7 (ATCC 43888) was grown at 37°C in LB to an OD_{600} of 0.2 (~1×10^8 CFU/ml). The cells were pelleted by centrifugation (10 min at 3,000 × *g*) and suspended in an equal volume of commercial pasteurized apple juice. A 1:10 dilution series of this culture was made in apple juice down to ~1 CFU/ml. For detection, 0.5 ml of the artificially contaminated apple juice was added to 3 ml of LB in a culture tube and preincubated in a 40°C shaker-incubator for 1 to 6 h prior to detection. Pure apple juice (0.5 ml) was mixed with 3 ml of LB and incubated overnight as a control. One hundred–microliter samples of each *E. coli* O157:H7 dilution were then distributed into a black 96-well microtiter plate (100 μl per well). Next, 100 μl of PP01-*luxI* reporter phage (~1×10^7 PFU/ml final concentration) and 50 μl of *E. coli* OHHLux bioreporter cells (~2×10^7 CFU/ml final concentration) were added to all wells. Control wells, plate seals, and bioluminescence-monitoring parameters were as described above.

RESULTS

PP01-*luxI* reporter phage assay in pure culture. The ability of phage PP01-*luxI* to detect *E. coli* O157:H7 at concentrations ranging from 10^8 to 10 CFU/ml in LB was examined. Phage PP01-*luxI* was combined with *E. coli* OHHLux bioreporter cells in a dilution series of several individual *E. coli* O157:H7 strains (ATCC 43888, ATCC 43889, ATCC 43894, ATCC 43895, or ATCC 700927) in a 96-well plate and monitored for bioluminescence emission. At 10^4- to 10^8-CFU/ml concentrations, the assay could rapidly (<1 h) detect all *E. coli* O157:H7 strains without a preincubation step (Fig. 4). Concentrations below 10^4 CFU/

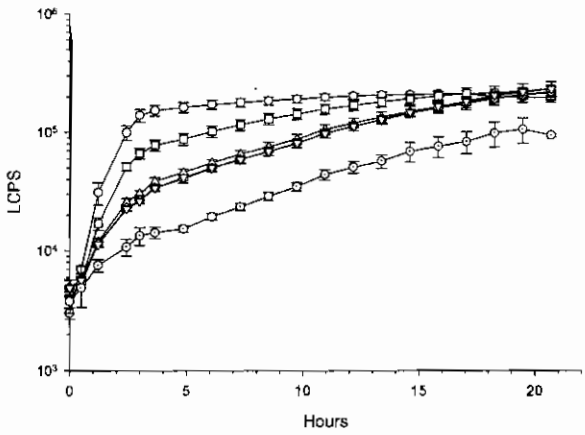

FIGURE 4. *Detection of* E. coli *O157:H7 strains without preincubation. Data represent the bioluminescent response overaged across the five strains tested (ATCC 43888, ATCC 43889, ATCC 43894, ATCC 43895, and ATCC 700927) (n = 15). The O157:H7 strains could be detected at 10^8 (○), 10^7 (□), 10^6 (△), 10^5 (◇), and 10^4 (▽) CFU/ml in 1 h or less. Control wells containing OHHLux bioreporters alone (⊙) establish background bioluminescence levels. Light productian is shown as luminescent counts per second (LCPS).*

ml did not significantly differ from OHHLux bioreporter background bioluminescence (data not shown). These lower dilution series tubes were therefore allowed to preincubate for 3 h at 37°C prior to assay initiation, which resulted in a detection limit for all O157:H7 strains of ~10 CFU/ml (initial concentration) in a total time (preincubation and assay) of approximately 4 h (Table 1).

Specificity of the PP01-*luxI* reporter phage assay. The specificity of phage PP01 for *E. coli* O157:H7 is well established *(16, 21)*, and the specificity of the OHHLux bioreporter cells used in this study has been previously described *(23)*. To demonstrate that our assay system did not deviate from the specificities of its components, we repeated the assays with various non-O157:H7 strains (*E. coli* serotypes O7 [23503], O55 [12014], and O126:K71 [12807], *E. coli* K-12 [10798], *E. coli* DH1 [33849], and *E. coli* MM294 [33625]) and the OHHL-producing strain *Y. enterocolitica* (23715). No significant bioluminescence was produced by any of the *E. coli* strains, signifying that the recombinant PP01 phage reporter maintains specificity for *E. coli* O157:H7 (Fig. 5). A significant bioluminescent response was observed in the presence of *Y. enterocolitica*, as was expected, since this strain naturally synthesizes OHHL and therefore triggers the OHHLux bioreporter *(23, 26)*.

PP01-*luxI* reporter phage assay to detect *E. coli* O157:H7 in apple juice. Outbreaks of *E. coli* O157:H7 infection have been linked to products such as unpasteurized apple juice *(2, 4)*. The ability to detect *E. coli* O157:H7 in apple juice was tested as a potential application for the PP01-*luxI* phage detection system. Apple juice was artificially contaminated with the nontoxigenic *E. coli* O157:H7 ATCC 43888 at concentrations ranging from 1 to 10^8 CFU/ml. An initial experiment showed that detection in undiluted apple juice was not possible, as no light was produced, even at the highest concentration of bacteria, even when the samples were brought to a neutral pH (data not shown). To circumvent this problem, samples were diluted in LB (0.5 ml of inoculated apple juice to 3 ml of LB) and grown at 40°C for 1 to 6 h prior to detection. The higher incubation temperature was used to favor the growth of *E.*

TABLE 1. *LCPS in pure culture averaged across all five* E. coli *O157:H7 strains tested*[a]

Initial concn of *E. coli* O157:H7 (CFU/ml)	Preincubation time (h)	Total assay time, including preincubation (h)	LCPS at time of detection
10^8	0	0.5	6,993 ± 130
10^7	0	1.0	16,889 ± 2,065
10^6	0	1.0	11,914 ± 718
10^5	0	1.0	11,417 ± 627
10^4	0	1.0	11,255 ± 599
10^3	3	4.0	14,011 ± 412
10^2	3	4.0	13,882 ± 357
10	3	4.0	13,796 ± 463
0 (control)	NA[b]	0.5–4.0	3,771 ± 763–4,286 ± 119

[a] Data are shown for first reading in which the averaged luminescent counts per second (LCPS) of the contaminated samples significantly exceeded the LCPS of the controls. Background luminescence slowly increases over time. Control values shown are from the control wells giving the highest luminescence at the time of detection.

[b] NA, not applicable.

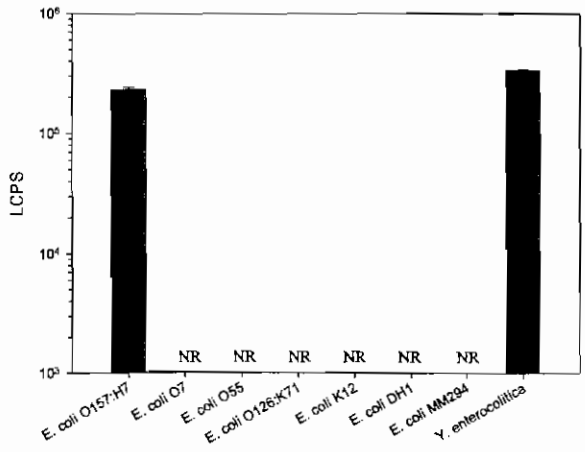

FIGURE 5. *Cross-reactivity of the PP01-*luxI *detection system. Potential host cells (10^8 CFU/ml, 100 μl per well) were mixed with phage PP01-*luxI *(10^8 PFU/nl, 100 μl per well) and* E. coli *OHHLux bioreporter cells (10^8 CFU/ml, 50 μl per well) in the wells of a 96-well plate. The nontoxigenic* E. coli *O157:H7 ATCC 43888 served as a positive control. Light production was monitored, and peak bioluminescence is shown as luminescent counts per second (LCPS). Data were normalized by subtracting the background luminescence of* E. coli *OHHLux bioreporter cells. NR, no response recorded above background. Error bars represent the standard deviation of three replicate wells.*

TABLE 2. *Detection of* E. coli *O157:H7 ATCC 43888 in apple juice*[a]

Initial concn of *E. coli* O157:H7 (CFU/ml)	Preincubation time (h)	Total assay time, including preincubation (h)	LCPS at time of detection
10^8	1	2.4	125,110 ± 12,448
10^7	2	4.5	203,026 ± 16,778
10^6	4	5.9	152,606 ± 14,281
10^5	5	5.9	337,780 ± 22,202
10^4	6	7.6	236,900 ± 64,701
0 (control)	NA[b]	2.4–7.6	12,836 ± 1739–44,843 ± 10,861

[a] Data are shown for first reading in which the luminescent counts per second (LCPS) of the contaminated sample significantly exceeded the LCPS of the controls. Background luminescence slowly increases over time. Control values shown are from the control wells giving the highest luminescenee at the time of detection.

[b] NA, not applicable.

coli O157:H7 over background microflora *(29)*. This method allowed the detection of *E. coli* O157:H7 at 10^8 CFU/ml in less than 3 h (1-h preincubation, 1.5-h assay) and down to 10^4 CFU/ml in less than 8 h (6-h preincubation, 1.5-h assay) (Table 2). No significant biolumimescence was observed when *E. coli* concentrations were below 10^4 CFU/ml.

DISCUSSION

E. coli O157:H7 is an ongoing threat because of naturally occurring foodborne outbreaks and its potential for use in bioterrorism. Rapid methods for the detection of *E. coli* O157:H7 in the product production line and in drinking and recreational water sources are needed to minimize the human health impact of this pathogen. While there are a wide variety of assays available for the detection of *E. coli* O157:H7, very few are amenable to adaptation for real-time use in production line and environmental monitoring. The GFP-PP01 phage assay introduced by Oda et al. *(20)* was a step toward this, but the required light source to excite GFP restriets its potential for use in miniaturized remote sensing devices. Detection methods based on the bioluminescent *lux* system can easily be integrated into an existing technology, the bioluminescent bioreporter integrated circuit *(19)*, for use in real-time remote monitoring.

As a first step toward developing a remote monitoring system, we have constructed a PP01-*luxI* phage that, in conjunction with bioreporter cells, is capable of specifically detecting *E. coli* O157:H7. Control assays demonstrated that *E. coli* OHHLux bioreporters do not produce significant light when alone or mixed with LB, phage PP01, or *E. coli* O157:H7. In pure culture, the phage reporter system is able to detect mid-level ($>10^4$) concentrations of nontoxigenic *E. coli* O157:H7 in approximately 1 h (Fig. 4). To detect lower concentrations (10 to 10^3 CFU/ml), a preincubation step is necessary, increasing the total assay time to 4 h. We confirmed that our phage bioreporter system does not cross-react with selected non-O157:H7 *E. coli* strains (Fig. 5). Only a subset of strains was examined, because the specificity of both components of this system has already been demonstrated *(16, 21, 23)*. A previously demonstrated characteristic of the assay is the potential for false-positive signaling due to resident nonreporter phage-related OHHL within the sample *(23)*. Thus, the presence of bacterial populations that naturally synthesize OHHL, such as *Y. enterocolitica,* will induce false-positive signaling (Fig. 5). Controls that contain the sample matrix with only the OHHLux bioreporter added would need to be prepared in order to identify intrinsic OHHL "contamination."

To demonstrate the feasibility of this detection system in a food sample matrix, *E. coli* O157:H7 ATCC 43888 was detected in apple juice. The detection of bacteria directly in apple juice was not possible, even when the pH of the apple juice was adjusted from its initial 3.75 to ~7.0. It appeared that the apple juice interfered with the growth and function of the bioreporter cells, as the background luminescence was decreased in these experiments. To avoid this problem without going through lengthy bacterial purification steps, a sample of the contaminated apple juice was diluted into LB prior to the detection assay. Dilution of the sample made a preincubation step necessary but still allowed the detection of high concentrations ($>10^8$) of *E. coli* O157:H7 in less than 3 h (Table 2). The detection limit in apple juice was higher than in LB, with no detectable luminescence produced in response to concentrations of *E. coli* below 10^4 CFU/ml. At 10^4 CFU/ml, detection was possible in less than 8 h (6-h preincubation, 1.5-h assay). Detection limits of 10 CFU/ml or lower are desirable in food matrix assays because the infectious dose of *E. coli* O157:H7 is believed to be 10 to 100 cells *(10)*. It is possible that a longer preincubation period would decrease the detection limit of this assay. We are now focusing on ways to improve the sensitivity and rapidity of this assay.

The bacteriophage-based bioluminescent bioreporter described in the present study represents a unique detection system that couples the specificity of the host-bacteriophage interaction with the *lux* quorum sensing and luminescence machinery. This bioreporter system has been shown in initial testing to detect *E. coli* O157:H7 from pure culture at mid-level concentrations (10^4 CFU/ml) without sample preincubation and at lower concentrations (down to 10 CFU/ml) after sample preincubation in a 96-well plate assay format. However, the clustering that occurs within the 10^4- through 10^6-CFU/ml dilution range (Fig. 4) indicates that the assay is not necessarily responding quantitatively to OHHL levels. A meticulous examination of phage, host cell, and bioreporter cell numbers and OHHL concentrations in relation to bioluminescent light output still needs to be conducted. Until this occurs, the assay remains more of a presence-and-absence indicator of *E. coli* O157:H7 presence, but its rapidity ensures timely preliminary recognition that can then be followed up with more established detection techniques.

While this assay falls short of the desired 10-CFU/ml detection limit in food matrices, its limit of detection is close to that of existing immunoassays and fluorescent phage-based assays *(5–7)*, and the sensitivity of this assay

may be increased through additional genetic modifications of the reporter phage. The assay demonstrated in this study is in a benchtop format, but its significance lies in its potential to be developed into a rapid, low-cost, low-energy, on-line monitoring system for the detection of *E. coli* O157:H7 in industrial and environmental settings.

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