Construction of mini-Tn10luxABcam/Ptac-ATS and its use for developing a bacteriophage that transduces bioluminescence to Escherichia coli O157:H7

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Abstract

Mini-Tn10luxABcam/Ptac-ATS was constructed in order to develop a luciferase-transducing bacteriophage for detecting Escherichia coli O157:H7. The transposon was designed to deliver a 3.6-kb insertion that confers n-decanal-dependent bioluminescence and resistance to chloramphenicol and was constructed using mini-Tn10cam/Ptac-ATS in the plasmid pNK2884 and luxAB from Vibrio harveyi. ΦV10, a temperate bacteriophage infecting common phage types of Escherichia coli O157:H7, was mutagenized as a prophage in E. coli O157:H7 strain R508. ΦV10::luxABcamA1-23 was rescued from the strain by propagating it on a strain lacking the bacteriophage and the vector containing the transposon. The bacteriophage transduced n-decanal-dependent bioluminescence to E. coli O157:H7 strain R508 that was measurable approximately 1 h post infection. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Bacterial luciferase is produced by a variety of marine bacteria including Vibrio harveyi [1]. The genes encoding luciferase (luxAB) have been cloned and expressed in a variety of bacteria, which become bioluminescent following exposure to an exogenous long-chain aldehyde, such as n-decanal, which is the substrate for the enzyme [2]. In 1987, Ulitzur and Khun constructed a derivative of bacteriophage lambda that contained the genes encoding bacterial luciferase [3]. It transduced n-decanal-dependent bioluminescence to Escherichia coli thereby permitting detection of the bacterium in pure culture, milk and urine. Since that time, bacterial luciferase genes have been inserted into bacteriophage A511 to produce a recombinant for detecting Listeria monocytogenes [4,5]. The firefly luciferase gene (luc) has also been used in this manner. It has been inserted into bacteriophage L5 to produce a recombinant for detecting Mycobacterium [6]. We are interested in developing a luciferase-transducing bacteriophage for detecting E. coli O157:H7, a cause of hemorrhagic colitis and the hemolytic uremic syndrome in humans [7].

In previous studies, luciferase-transducing bacteriophages were constructed from well-characterized bacteriophages by homologous recombination [4,6]. The bacteriophages used to phage-type E. coli O157:H7 may be suitable for development into reporter bacteriophages. Insertion of luciferase genes into these bacteriophages by homologous recombination is less practical because little is currently known about the genetics of these bacteriophages. In this regard transposon mutagenesis represents a more practical approach to developing such bacteriophages.

ΦV10 is a non-virulent bacteriophage used to phage-type E. coli O157:H7 [8]. It infects many phage types (PT) of E. coli O157:H7 including PT14, which is the most common in Canada. The PTs that are susceptible to infection with ΦV10 accounted for 64% of isolates typed during 1995 in Canada (R. Khakhria, personal communication). This report describes the construction of mini-Tn10luxABcam/Ptac-ATS and its use to generate a luciferase-transducing derivative of ΦV10.

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2. Materials and methods

2.1. Bacterial strains and culture media

The general cloning hosts *E. coli* DH5α and LE392 were used during construction of mini-Tn10luxABcam/Ptac-ATS. *E. coli* O157:H7 strain R508, which belongs to PT14, is sensitive to ΦV10, and was used during mutagenesis of ΦV10 and recovery of its mutants. These bacteria were grown in Luria-Bertani-Miller (LB) medium, which was supplemented with either ampicillin (Ap) or chloramphenicol (Cm) at concentrations of 100 μg ml⁻¹ and 10 μg ml⁻¹ respectively, as required. ΦV10 was a kind gift of R. Khakhria (Laboratory Center for Disease Control, Health Canada). It was propagated in *E. coli* O157:H7 strain R508 using the soft agar overlay technique [9].

2.2. Characterization of ΦV10

The morphology of ΦV10 was determined by electron microscopy and the size of its genome was estimated by restriction endonuclease mapping of purified bacteriophage DNA with XmnI [10].

2.3. Measurement of bioluminescence

Bioluminescent colonies on agar plates were visualized using a Biomedical Image Quantifier (BIQ) (Image Research Ltd., Cambridge, UK) 5 min after n-decanal was applied to the inner surface of the lid of petri plates. The light produced by bacteriophage-infected bacteria in broth culture was quantified in the same instrument 5 min after 5 μl of 5.8 mM n-decanal in 50% ethanol was added to 200 μl of culture in 96-well plates.

2.4. Transposon construction

The transposon mini-Tn10luxABcam/Ptac-ATS (Fig. 1) was constructed using mini-Tn10cam/Ptac-ATS in the plasmid pNK2884 (American Type Culture Collection, Manassas, VA, USA) and luxAB originally cloned from *Vibrio harveyi* [11] using standard methods [12]. pVhH (a gift of E. Meighen, University of Montreal) contains the complete coding sequence of luxAB on a 4.0-kb HindIII fragment of the lux operon of *V. harveyi* [11]. It does not contain the endogenous promoter controlling the expression of luxAB. A 2.1-kb amplicon containing the lux-AB gene was generated from pVhH by PCR using primers C (5'−GAGCATCTCCCAAAATAGGAGGATTGTTATG-3') and D (5'−GGCAAGCTCTGTACGGATGTTATTTGAC−3'). The primers were designed using publish

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**Fig. 1.** Structure of pHAL119 containing mini-Tn10luxABcam/Ptac-ATS. luxA, luxB: *V. harveyi* luciferase genes; bla: β-lactamase gene; cam: chloramphenicol acetyltransferase gene; ATS: altered target specificity transposase gene; IR: inverted repeat region.
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strain R508 in broth culture was examined by infecting E. coli décanal-dependent bioluminescence to U to induce the prophage. After 30 min incubation at 37°C, the culture was pelleted by centrifugation (6000 × g), resuspended in 2 ml of LB broth and incubated overnight at 37°C. The culture was filtered through a 0.8 µm – 0.2 µm sterile combination filter and the filtrate was stored at 4°C.

Mutant bacteriophage were isolated from the filtrate by using it to infect E. coli O157:H7 strain R508 at low multiplicity of infection as described above, with the exception that mixtures were dropped onto the surface of LB agar plates containing Cm. Isolates that were bioluminescent upon exposure to n-decanal, combined with Ap³ Cm³ resistance to infection with ΦV10, were assumed to contain mutant bacteriophages. Bacteriophages were prepared from these isolates by treatment with MTC as described above. Culture filtrates were titrated on E. coli O157:H7 strain R508 and the frequency and morphology of plaque formation, and the frequency of transduction of the phenotype of bioluminescence and Cm R were determined as described above. A single bacteriophage, sV10::luxABcamA1-23, was selected for further characterization. It was propagated directly from its E. coli O157:H7 lysogen by treatment with MTC as described above; the titers of such bacteriophage preparations were quantified as transducing units (TU) ml⁻¹.

The ability of ΦV10::luxABcamA1-23 to transduce n-decanal-dependent bioluminescence to E. coli O157:H7 strain R508 in broth culture was examined by infecting 1 × 10⁶ CFU with 2 × 10⁶ TU ΦV10::luxABcamA1-23 and observing light production over 5.5 h, and infecting 1 × 10⁶ CFU with 0–2 × 10⁶ TU ΦV10::luxABcamA1-23 and observing light production 2 h later. Infections were performed directly in 96-well plates which were incubated at 37°C without shaking.

3. Results

Electron microscopic examination of supernatants from ΦV10-infected E. coli O157:H7 strain R508 induced with MTC showed virions with isosahedral heads approximately 60 nm in diameter and short tails (not shown). Restriction digestion of purified ΦV10 DNA with XmnI yielded 11 DNA fragments ranging in size from 16.8 to 0.5 kb (not shown). The genome of the bacteriophage was calculated to be approximately 42 kb. Virions were not present in the supernatant from the parent uninfected E. coli O157:H7 strain R508 induced with MTC.

Mini-Tn10camPlac-ATS is a versatile transposon that may be easily derivatized because the genetic material between its inverted terminal repeats is situated on a BamHI fragment and the plasmid containing the transposon lacks other BamHI restriction endonuclease sites [15].

Mini-Tn10luxABcamPlac-ATS was constructed by inserting luxAB into mini-Tn10camPlac-ATS using a multi-step process designed to minimize the size of the resulting transposon. Initially, a DNA fragment containing the minimal coding sequence of luxAB and a ribosome binding site was amplified by PCR from pVhH and cloned as a BamHI-BglII fragment downstream of Plac in the vector pT7T3-18U creating pHAL100. Next, a DNA fragment containing Plac-luxAB was amplified by PCR from pHAL100 and cloned as a BglII fragment in the vector LITMUS28 creating pHAL108-2. This step was performed to construct a plasmid containing two fragments for making derivatives of mini-Tn10camPlac-ATS: a 2.1 kb BamHI-BglII fragment containing a promoterless luxAB; and a 2.3 kb BamHI fragment containing Plac-luxAB. To generate mini-Tn10luxABcamPlac-ATS, the promoterless luxAB was randomly cloned into BamHI-digested pNK2884 creating pHAL119. The structure of pHAL119 (Fig. 1) was determined by restriction endonuclease mapping and sequence analysis (data not shown).

pHAL119 was designed to deliver a transposon insertion of approximately 3.6 kb.

pHAL119 was introduced into E. coli O157:H7 strain R508 by transformation and the resulting strain was lysogenized with bacteriophage ΦV10. The prophage in the resulting strain was mutagenized by inducing the tac promoter controlling expression of the transposon transposase with IPTG then induced by treatment with MTC.

The culture filtrate from the induced strain contained 3.5 × 10⁸ pfu ml⁻¹ and transduced Ap³ and Cm³ at frequencies of 2 × 10⁴ ml⁻¹, and 6.5 × 10³ ml⁻¹, respectively. To rescue mutant bacteriophage, E. coli O157:H7 strain R508 was infected with the bacteriophage-containing filtrate, then plated onto agar containing Cm. Thirty-one Cm³ transductants were selected and four of these were Ap³ and resistant to superinfection with ΦV10 suggesting that they contained ΦV10 with a transposon insertion. Bacteriophages were induced from the four isolates and the resulting culture filtrates were titrated on E. coli O157:H7 strain R508. Bacteriophages from two of the isolates, named strains A1-23 and A1-26, formed pinpoint plaques, which contrasted with the larger plaques produced by wild-type ΦV10, and transduced n-decanal-dependent bioluminescence and Cm³ at frequencies of 5.5 × 10⁴ and 8.5 × 10³ ml⁻¹, respectively. Bacteriophages were not produced by the remaining two isolates, suggesting that the mutant bacteriophages were not viable.

For the bacteriophage from strains A1-23 and A1-26, the ratios of Cm³ transduction to plaque formation were 1.8 and 17, respectively. The bacteriophage from strain A1-23 produced transductants that were more bioluminescent than from A1-26, therefore it was decided to use this strain as the source of luxABcam-transducing bacteri-
phage in subsequent experiments. The bacteriophage produced by this strain was called $\Phi V10::luxABcam A1-23$.

Attempts to propagate $\Phi V10::luxABcam A1-23$ using the soft agar overlay technique were not successful. A possible explanation for this is that the transposon insertion resulted in formation of defective bacteriophage through loss of genetic material. This could be expected to occur since the transposon insertion represents about 8.6% of the bacteriophage genome. $\Phi V10::luxABcam A1-23$ was propagated from its lysogen by treatment of cultures with MTC. In this case replication may rely on complementation of a defective function by a cryptic bacteriophage in the host genome.

Luciferase and Cm$^R$ transduction frequencies obtained with filtrates from the induced lysogens were approximately $2 \times 10^8 \text{ ml}^{-1}$. The plaques formed by the bacteriophage were extremely small and difficult to count, therefore the titers of $\Phi V10::luxABcam A1-23$ preparations were reported as TU ml$^{-1}$.

The ability of $\Phi V10::luxABcam A1-23$ to transduce n-decanal-dependent bioluminescence to $E. coli$ O157:H7 strain R508 was examined in broth culture. In this matrix, bioluminescence above the luminometer background was easily measured 1 h after infecting the target organisms with the bacteriophage (Fig. 2). The maximum light output occurred at a multiplicity of infection of approximately one (Fig. 3).

4. Discussion

Mini-Tn10luxABcam/Ptac-ATS was designed to confer n-decanal-dependent bioluminescence provided the insertion into the bacteriophage occurred downstream of an active promoter. During selection of mutant bacteriophages this property would permit identification of mutants that were strongly bioluminescent as prophages, a property considered desirable since one would predict that strongly bioluminescent bacteriophages would be useful for detection of target organisms. The transposon was also designed to confer resistance to chloramphenicol, a marker permitting rescue of mutant bacteriophages. Additionally, the chloramphenicol resistance marker could simplify isolation of target organisms from complex samples.

Transposon mutagenesis of $\Phi V10$ with mini-Tn10luxABcam was easily performed and mutants were rapidly recovered by rescue in the parent strain. The bacteriophage isolated, $\Phi V10::luxABcam A1-23$, transduced n-decanal-dependent bioluminescence to $E. coli$ O157:H7 strain R508. Future investigations will examine the potential use of $\Phi V10::luxABcam A1-23$ for detecting pathogenic $E. coli$ O157:H7 in food.

Cells infected with $\Phi V10::luxABcam A1-23$ become bioluminescent and remain so. Since luxAB is promoterless in the transposon cassette, a host or bacteriophage promoter must be driving expression of luxAB in lysogens. In principle, luxAB could be subject to polar effects from an active host promoter or a bacteriophage promoter active during lysogeny, such as $P_{RE}$, which controls expression of cI, the lambda repressor [16].

The approach used in this report to generate a luciferase-transducing bacteriophage is generally applicable to other bacteriophage infecting Enterobacteriaceae. Indeed, it could be used to recover previously uncharacterized bacteriophages useful for detection of target organisms. For example, non-verotoxigenic serotype $E. coli$ O157

![Fig. 2. Time course for development of n-decanal-dependent bioluminescence by E. coli O157:H7 strain R508 infected with $\Phi V10::luxABcam A1-23$. Each data point represents the mean of duplicate determinations, which differed by less than 10%.](https://academic.oup.com/femsle/article-abstract/182/2/285/511073)

![Fig. 3. Effect of multiplicity of infection with $\Phi V10::luxABcam A1-23$ on n-decanal-dependent bioluminescence by E. coli O157:H7 strain R508. Each data point represents the mean of duplicate determinations, which differed by less than 10%.](https://academic.oup.com/femsle/article-abstract/182/2/285/511073)
strains could contain bacteriophages capable of infecting verotoxigenic strains of *E. coli* O157:H7. Attempting to isolate and characterize wild-type bacteriophages from these strains would be laborious and potentially non-productive. An alternative approach would be to transform these strains with pHAL119 and to perform transposon mutagenesis of resident prophages. The resulting mutant bacteriophages could then be isolated by rescue on the *E. coli* O157:H7 strains. This approach would permit direct and economical isolation of useful bacteriophages that transduce n-decanal-dependent bioluminescence to target organisms. Such bacteriophages could be used in a cocktail containing ΦV10::luxABcamA1-23 thereby permitting detection of a greater proportion of *E. coli* O157:H7.

References