



SHORT RESEARCH ARTICLE

High efficiency generalized transduction in *Escherichia coli* O157:H7 [v1; ref status: indexed, <http://f1000r.es/8f>]

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Abstract

Genetic manipulation in enterohemorrhagic *E. coli* O157:H7 is currently restricted to recombineering, a method that utilizes the recombination system of bacteriophage lambda, to introduce gene replacements and base changes *inter alia* into the genome. Bacteriophage 933W is a prophage in *E. coli* O157:H7 strain EDL933, which encodes the genes (*stx2AB*) for the production of Shiga toxin which is the basis for the potentially fatal Hemolytic Uremic Syndrome in infected humans. We replaced the *stx2AB* genes with a kanamycin cassette using recombineering. After induction of the prophage by ultra-violet light, we found that bacteriophage lysates were capable of transducing to wildtype, point mutations in the lactose, arabinose and maltose genes. The lysates could also transduce tetracycline resistant cassettes. Bacteriophage 933W is also efficient at transducing markers in *E. coli* K-12. Co-transduction experiments indicated that the maximal amount of transferred DNA was likely the size of the bacteriophage genome, 61 kB. All tested transductants, in both *E. coli* K-12 and O157:H7, were kanamycin-sensitive indicating that the transducing particles contained host DNA.

Article Status Summary

Referee Responses

Referees	1	2	3
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- 1 **Joe Wade**, New York State Department of Health USA
- 2 **Donald Court**, Center for Cancer Research, National Cancer Institute USA
- 3 **Michael Donnenberg**, University of Maryland USA

Latest Comments

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 07 May 2013 (V1)

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Introduction

Enterohemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 is among the leading causes of food- and water-borne illnesses affecting humans in the U.S., Europe, and Japan (for review, see Donnenberg and Whittam (2001)¹, Kaper *et al.* (2004)² and Spears *et al.* (2006)³). These bacteria are highly infectious and ingestion of only 100–200 organisms is sufficient to trigger debilitating diarrheal disease. Although most EHEC infections resolve spontaneously after 5–10 days of abdominal cramping and bloody diarrhea, approximately 2–7% of cases progress to the potentially fatal hemolytic uremic syndrome due, in part, to the production of cytotoxic Shiga toxins which are capable of promoting kidney failure (Donnenberg and Whittam (2001)¹, Kaper *et al.* (2004)² and Spears *et al.* (2006)³). The genes for Shiga toxin (*stx2AB*) are located on a prophage in the late region between genes Q and S and are transcribed with the late region genes from promoter P_R' (Tyler *et al.* (2004)⁴).

The most widely used genetic manipulation in EHEC is recombineering, a method that utilizes the recombination system of bacteriophage lambda, to introduce gene replacements and base changes *inter alia* into the genome (Murphy (1998)⁵, Court *et al.* (2002)⁶). A method for using the generalized transducing bacteriophage P1 in EHEC has been reported previously but it requires the construction of galactose mutant derivatives of the recipient strain (Ho and Waldor (2007)⁷).

In EHEC strain EDL933, the *stx2AB* genes are located on prophage 933W, the genome size of which is 61 kB (Penna *et al.* (2001)⁸). The DNA sequence of both the prophage and free phage are known. During sequencing of the free phage genome, it was noticed that the ends of the genome could not be sequenced; the investigators interpreted this and other observations as indicating that the packaged chromosome might be terminally redundant (Plunkett *et al.* (2001)⁹). Because terminal redundancy is a shared feature among generalized transducing phages, this finding suggested that bacteriophage 933W might be capable of packaging host DNA and transducing genetic markers. We report here that bacteriophage 933W is capable of transducing genetic markers in unmodified EHEC and *E. coli* K-12 strains.

Methods

Bacterial strains

The EHEC strain used principally in this paper is KM80 (Carone *et al.* unpublished document), a derivative of EDL933 which lacks prophage 933W (obtained from Dr. K.C. Murphy). Derivatives of KM80 unable to utilize lactose (GM9289), arabinose (GM9290) or maltose (GM9291) were obtained after a 20 min exposure of a log phase culture in L broth to 40 µl ethyl methane sulfonate (Sigma-Aldrich) per ml at 37°C followed by a 1/100 dilution into broth. Aliquots of the saturated culture were plated on MacConkey Agar Base (Difco) containing lactose, arabinose or maltose to detect non-fermenting colonies. The *E. coli* K-12 strains used were AB1157 (*thr-1 araC14 leuB6(Am)Δ (gpt-proA)62 lacY1 tsx-33 supE44(AS) galK2(Oc) hisG4(Oc) rfbD1 mgl-51 rpoS396(Am) rpsL31(Str^R) kdgK51 xylA5 mtl-1 argE3(Oc) thi-1*) (Dewitt and Adelberg (1962)¹⁰) (obtained from Dr. E.A. Adelberg), MM294 (*endA1 hsdR17 supE44(AS) rfbD1 spoT1 thi-1*) (Meselson and Yuan (1968)¹¹) (obtained from Dr. M. Meselson), GM1731 (*cysG98::Tn5*)

(Shaw and Berg (1979)¹²) and GM1748 (*leu::Tn10*) (Kleckner *et al.* (1975)¹³). (Am=amber, AS=amber suppressor, Oc=ochre). Strains containing the Tn5 and Tn10 mutations were obtained from Drs. C. Berg and N. Kleckner respectively. The composition of L broth and minimal medium has been described previously (Nowosielska and Marinus (2008)¹⁴).

Bacteriophage construction, induction and transduction

Replacement of the *stx2AB* genes in EDL933 by recombineering as described by Murphy and Campellone (2003)¹⁵ was carried out to yield strain GM9251. In a polymerase chain reaction containing Herculase II DNA polymerase (Agilent Technologies), supplied buffer, 2 µl GM1731, and primers. ggctgtgctgctgattactcagccaaa-ggaacacctgtatatTATGGACAGCAAGCGAACCG and gattacacttgttaccacataccacgaatcaggttatgcctcaTCAGAAGAACTCGTCAA-GAAG (sequence in capital letters is of Tn5), a DNA fragment containing the kanamycin-resistance gene was synthesized. This fragment was electroporated into EDL933 containing pKM208, a thermosensitive plasmid bearing the bacteriophage lambda *exo* and *bet* genes (Murphy and Camellone (2003)¹⁵) followed by selection for kanamycin-resistant clones. These were tested further by the ability of the cells to produce infective centers on strain AB1157 (Dewitt and Adelberg (1962)¹⁰) and one of these was designated GM9251.

Prophage induction was accomplished by plating 10 µl of a standing overnight culture on solid medium and irradiating with 25 J/m² ultraviolet light followed by an overlay of 3.5 ml soft agar (0.3%) containing 300 µl of a standing overnight culture of the desired strain for bacteriophage propagation. Alternatively, the desired strain was mixed with dilutions of bacteriophage lysate at 37°C, incubated for 30 min, and incorporated in a soft agar layer on solid medium. In both induction and bacteriophage propagation, the plates were incubated at 37°C overnight. The next day, the soft agar layer was scraped off, and 4 ml of L broth or TM buffer (10 mM Tris-HCl, pH7.4, 10 mM MgSO₄) was added together with 0.15 ml chloroform. The mixture was vortexed and left at room temperature for 10 min followed by centrifugation in a microfuge at room temperature for 2 min at 10,000 rpm. The supernatant was either used immediately or mixed with an equal volume of 4 M ammonium sulfate. The bacteriophage suspension in ammonium sulfate is stable for at least one month at 4°C in the dark. During lysate preparation it is crucial that the temperature remain at or above 20°C. Lysates were titered on AB1157.

For transduction, the recipient strain was grown standing overnight in liquid medium at 37°C, centrifuged, and resuspended in fresh medium. The recipient strain (100 µl) was mixed with varying amounts of bacteriophage lysate and 400 µl fresh medium. For selection using auxotrophic markers, the mixture was spread on selective minimal medium without further incubation. For drug-resistance markers, the mixture was incubated at 37°C for 60 min before plating on antibiotic medium. The plates were incubated at 37°C.

A 933W lysogenic derivative of *E. coli* K-12 was constructed as follows. Dilutions of a GM9251 induced lysate were mixed with a standing overnight L broth culture of strain MM294 and incubated at 37°C for 60 min and portions added to L broth-kanamycin plates

and incubating them overnight at 37°C. Colonies were picked into L broth supplemented with kanamycin and grown overnight at 37°C. The broth cultures were diluted and 10 µl portions placed onto a soft agar (0.3%) layer containing 200 µl of a standing overnight culture of AB1157 on an L broth plate. After allowing the drops to dry the plate was incubated overnight at 37°C. A kanamycin-resistant lysogen that formed infective centers by this method was designated GM9255 and 933W phage induced from this lysogen was used in all experiments with *E. coli* K-12.

Results and discussion

Initial experiments with bacteriophage 933W *stx2AB::Kan* were confounded by its instability. It was found that the bacteriophage was cold-sensitive and would not form plaques on indicator strains at 30°C but could do so at 37°C. The lysates rapidly lost infective titer (>90%) upon incubation at 4°C overnight. The addition of 2 M ammonium sulfate to the lysates stabilized the titer.

The observation that the bacteriophage 933W chromosome had terminal redundancy suggested that, like bacteriophage P1, it should be capable of transducing genetic markers. Strain KM80, which is derived from EDL933 and is not lysogenic for 933W, was mutagenized with ethyl methane sulfonate and the lactose, arabinose and maltose non-fermenting derivatives were isolated. These derivatives were used as recipients for transduction and, after mixing with bacteriophage from strain GM9251, were plated on minimal medium containing the appropriate carbon source. The results in [Table 1](#) show that colonies were present on the plates with the mixture but not on plates with the recipient alone or the bacteriophage alone. Forty recombinants from each transduction were patched onto solid medium with kanamycin and all were sensitive.

A 933W *stx2AB::Kan* lysogen (GM9255) of *E. coli* K-12 strain MM294 was constructed and a lysate prepared on *E. coli* K-12 strain GM1748 (*leu::Tn10*). This lysate was used to transduce strain AB1157 (*Thr Ara*) to tetracycline-resistance (due to the *Tn10*). Of the 40 recombinants tested, 100% were *Ara*⁺ and 100% *Thr*⁺. Given that the gene order is *thr-ara-leu* and that the intervals between *thr* and *leu*, and *ara* and *leu*, are 80 and 14 kB respectively, the result indicates that cotransduction is possible for marker separated by 14 kB, but not 80 kB. This result is consistent with the 933W bacteriophage genome size of 61 kB. None of the 40 tetracycline-resistant recombinants were kanamycin-resistant, consistent with the transducing particles containing only host DNA.

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Table 1. Transduction in enterohemorrhagic *Escherichia coli*.

Selection	Transductants per 10 µl lysate			
	Plate 1	Plate 2	Plate 3	Average
Lac ⁺	130	128	134	131
Ara ⁺	72	71	73	73
Mal ⁺	89	88	88	88

Lac⁻ (GM9289), Ara⁻ (GM9290) and Mal⁻ (GM9291) derivatives of KM80 were transduced with a lysate prepared on GM9251. The number of transductants on each of three plates is shown followed by the average. There were no colonies on plates seeded with the recipient strain alone or from the GM9251 lysate.

Bacteriophage 933W is a member of a family of *stx*-encoding bacteriophages and these may also be capable of transduction. The annotation of a terminase in the 933W genome should be revised to a pacase to more clearly reflect the action of these enzymes. The ability of bacteriophage 933W to transduce genetic markers in EHEC and *E. coli* K-12 suggests that other related bacteria such as enteropathogenic *E. coli* or *Citrobacter rhodentium* may also be amenable to this type of genetic exchange. Only 933W non-lysogens have been used in the present study as recipients and it is not yet known if 933W lysogens can be transduced.

Conclusion

Bacteriophage 933W is capable of transducing genetic markers in EHEC and *E. coli* K-12.

Author contributions

MGM and AP conceived the study, designed and carried out the research and agreed to the final content, and wrote the paper.

Competing interests

No competing interests were disclosed.

Grant information

This work was supported by NIH grant GM063790 to MGM.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Current Referee Status:

Referee Responses for Version 1



Michael Donnenberg, School of Medicine, University of Maryland, Baltimore, MD, USA

Approved: 21 January 2013

Ref Report: 21 January 2013

The findings are novel and of potential interest in that generalized transduction, if confirmed in other pathogenic *E. coli* strains, could facilitate more rapid progress in understanding these infections. Such confirmation is necessary before its full impact can be appreciated.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.



Donald Court, Gene Regulation and Chromosome Biology Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, MD, USA

Approved: 16 January 2013

Ref Report: 16 January 2013

This paper describes methods used for *E. coli* O157:H7 (EHEC) to prepare phage lysates and to use those lysates for generalized transduction of bacterial markers, which is useful since P1 transduction in this strain requires the presence of a special gal mutant in any EHEC recipient. The general phage methods described are also useful. I have a few minor comments below to improve the paper.

- The authors point out that the kanR phage marker does not come along during the transduction event. See the last sentence of the abstract and the last sentence in the second paragraph of results. Both should say something to the effect that this indicates phage DNA is not involved instead of arguing that host DNA is. This is pretty obvious but I think the question is more whether there is some kind of host DNA pick into a phage genome.
- It would be informative if the authors can provide a frequency of generalized transductants per phage particle (as well as per 10 microliters). What is the frequency of lysogenization by this phage as measured by KanR? How does this transduction compare to a similar phage like P22.
- How frequently does the tet marker transduce the EHEC strain?

I mark this as approved for publication with some minor revisions as suggested.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.



Joe Wade, Wadsworth Center, New York State Department of Health, Albany, NY, USA

Approved with reservations: 14 January 2013

Ref Report: 14 January 2013

The authors describe a new transduction system for *E. coli* O157:H7, a strain that cannot be transduced using P1. Such a transducing phage would be a useful tool. The authors make a fairly convincing case that they have indeed isolated a transducing phage. However, there are some important, missing controls, and the efficiency of co-transduction of linked genes needs to be discussed in greater detail.

Specific comments:

1. Given that the impact of the transducing phage is likely to be limited to O157:H7 strains (i.e. not K-12), it is important to demonstrate transduction in O157:H7 more convincingly. Specifically, the authors should identify the specific mutation in the recipient strain and then show that this mutation is lost in the transductants. This will rule out spontaneous mutations as the cause of the phenotype. Alternatively, and perhaps more easily, the authors could attempt to transduce an antibiotic resistance gene, as they did for K-12.
2. The authors show that co-transduction occurs in K-12 between genes 14 kb apart. The frequency of co-transduction is 100% for 40 colonies tested. This is higher than I would have expected. Assuming random acquisition of host DNA by the phage, you would expect 8 or 9 colonies, on average, in which the two genes did not co-transduce. The probability of getting 40 out of 40 co-transductants would be $3e^{-5}$. This suggests that the phage does not package host DNA randomly. The authors should discuss the significance of this, especially in light of the utility of the phage for generalised transduction.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

1 Comment**Author Response**

Martin Marinus, Department of Biochemistry & Molecular Pharmacology , University of Massachusetts Medical School, USA

Posted: 07 May 2013

1. There were never any colonies on the control plates for the O157:H7 transductions for three different markers so we consider the possibility that the transductants are spontaneous revertants to be very low.
2. We agree with the reviewer on this point and it is very possible that the DNA is not packaged randomly.

Competing Interests: We have no competing interests

Article Comments

Comments for Version 1

Author Response

Martin Marinus, Department of Biochemistry & Molecular Pharmacology , University of Massachusetts Medical School, USA

Posted: 07 May 2013

We have now shown that a 933W lysogen can be transduced. Specifically, we transduced *E. coli* K-12 AB1157 and AB1157 (933W) strains to prototrophy for Leu+, Pro+, His+ and Arg+. The frequency of transduction of the lysogen was about 80% that of the non-lysogen for all markers.

Competing Interests: We have no competing interests
