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Phage Transduction: A Method to Transfer Ampicillin Resistance from Donor to Recipient *E. coli*

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Overview

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Transduction is a form of genetic exchange between bacteria that utilizes bacteriophages, or phages, a class of virus that infects exclusively prokaryotic organisms. This form of DNA transfer, from one bacterium to another by way of a phage, was discovered in 1951 by Norton Zinder and Joshua Ledererg, who termed the process "transduction" (1). Bacteriophages were first discovered in 1915 by British bacteriologist Frederick Twort, then independently discovered again in 1917 by French-Canadian microbiologist Felix d'Herelle (2). Since then, the structure and function of these phages have been widely characterized (3), dividing these phages into two classes. The first of these classes are the lytic phages which upon infection multiply within the host bacterium, disrupting the bacterial metabolism, lysing the cell, and releasing progeny phage (4). As a result of this anti-bacterial activity and the increasing prevalence of antibiotic-resistant bacteria, these lytic phages have recently proved useful as a substitute treatment for antibiotics. The second of these classes are the lysogenic phages which can either multiply within the host via the lytic cycle or enter a quiescent state in which their genome is integrated into that of the host (Figure 1), a process known as lysogeny, with the ability for phage production to be induced in multiple later generations (4).

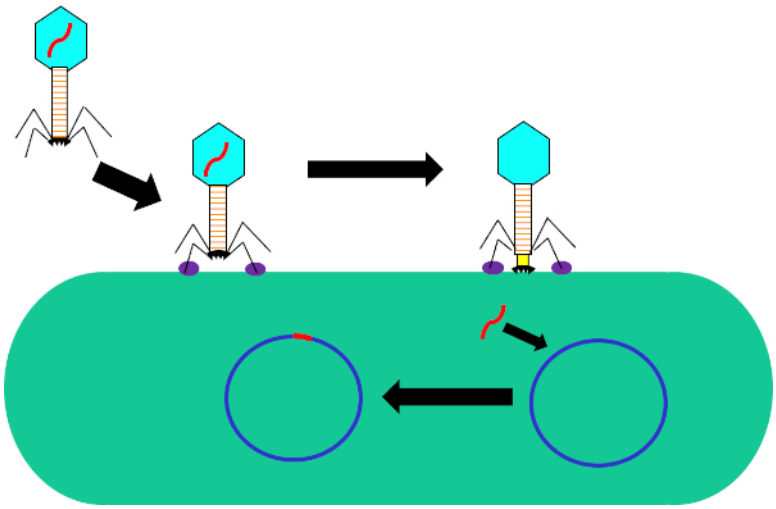

Figure 1: Infection of host cell by bacteriophage. Adsorption by the phage to the bacterial cell wall via interactions between the tail fibers and receptor (purple). Once on the cell surface, the phage is irreversibly attached to the bacterial cell using the base plate (black) which is moved to the cell wall by the contractile sheath (yellow). Phage genome (red) then enters the cell and integrates into the host cell genome.

While bacterial transduction is a naturally occurring process, using modern technology it has been manipulated for the transfer of genes into bacteria in the laboratory setting. By inserting genes of interest into the genome of a lysogenic phage, such as phage, one is able to transfer these genes into the genomes of bacteria and consequently express them within these cells. While other methods of gene transfer, such as transformation, use a plasmid for gene transfer and expression, the insertion of the phage genome into that of the recipient bacterium not only has the potential to confer new traits to this bacterium, but also allows for naturally occurring mutations and other factors of the cellular environment to alter the function of the transferred gene.

Compared to other methods of horizontal gene transfer, such as conjugation, transduction is fairly flexible in the criteria required for donor and recipient cells. Any genetic element that can fit inside the genome of the phage being used can be transferred from any strain of donor bacteria to any strain of recipient bacteria as long as both are permissive to the phage, requiring the expression of necessary phage receptors on the cell surfaces. Once this gene is moved out of the donor genome and packaged into the phage, it can be transferred to the recipient. Following transduction, it is necessary to select for recipient bacteria that contain the gene of interest have to be selected for. This could be done by use of a genetic marker, such as a FLAG-tag or polyhistidine-tag, to mark the gene of interest, or the intrinsic function of the gene, in the case of genes that encode for antibiotic resistance. Additionally, PCR could be used to further confirm successful transduction. By using primers for a region within the gene of interest and comparing signal to a positive control, bacteria that has the gene of interest, and a negative control, bacteria that underwent the same steps as the transduction reaction with no phage. While bacterial transduction is a useful tool in molecular biology, it has and continues to play an important role in the evolution of bacteria, particularly with regard to the recent rise of antibiotic resistance.

In this experiment, bacterial transduction was used to transfer the gene encoding for resistance to the antibiotic ampicillin from the W3110 strain of *E.coli* to the J53 strain via the P1 bacteriophage (5). This experiment consisted of two main steps. First, the preparation of P1 phage containing the ampicillin resistance gene from the donor strain. Second, the transfer of this gene to the recipient strain by transduction with the P1 phage (Figure 1). Once carried out, the successful transfer of the ampicillin resistance gene could be determined by qPCR (Figure 2).

If transduction was successful, the J53 strain of *E. coli* would be resistant to ampicillin, and the gene conferring this resistance detectable by qPCR. If unsuccessful, there would be no detection of the ampicillin resistance gene and ampicillin would still function as an effective antibiotic against the J53 strain.

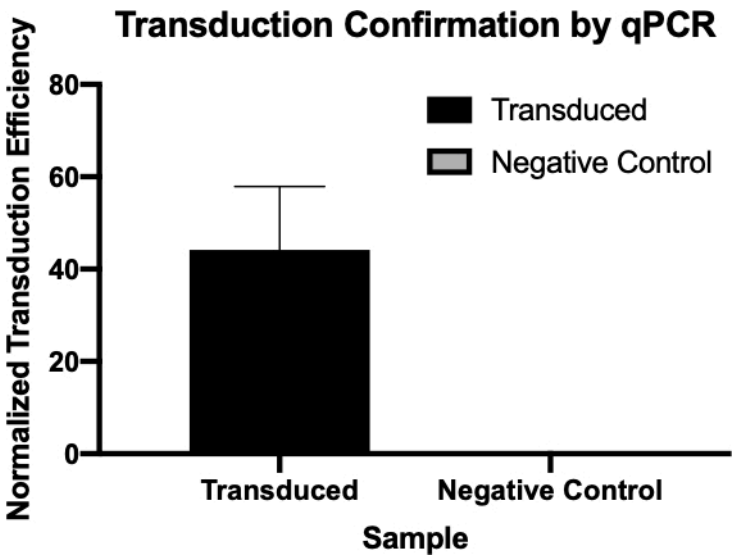

Figure 2: The confirmation of successful transduction by qPCR. By comparing the C_q values detected for the gene of interest from the transduction reaction and the negative control reaction, and normalizing these values against a housekeeping gene, one was able to confirm that bacterial transduction was successful.

Procedure

1. Set-up

1. Before commencing any work involving microbes, sterilize the workspace using 70% ethanol. Always use the necessary PPE (lab coat and gloves).
2. Ensure that LB agar plates with 1x ampicillin, commercially available P1 phage lysate solution, chloroform, 1 M sodium citrate, glycerol, and a box of pre-sterilized plastic pipette tips and cell spreaders are close at hand.
3. Prepare sterile LB by autoclaving and use it to make three 1 mL aliquots of LB salt solution.
    1. mM $MgCl_2$ (952.11-2.3803 µg), 5 mM $CaCl_2$ (11.098 mg), 0.1-0.2% glucose (100-200 µg)
    2. mM $MgSO_4$ (12.0366 mg), 5 mM $CaCl_2$ (11.098 mg)
    3. mM sodium citrate (25.806 mg)
4. Once finished, sterilize all surfaces as well as gloves with 70% ethanol and wash hands.

2. Protocol

1. **Donor Phage Lysate Preparation**
    1. Prepare a 1 mL culture of donor W3110 strain *E. coli* in LB with 1x ampicillin grown overnight at 37 °C with aeration and shaking at 220 rpm.
    2. Dilute this overnight culture 1:100 in 1 mL of fresh LB supplemented with 10-25 mM $MgCl_2$, 5 mM $CaCl_2$, and 0.1-0.2% glucose.
    3. Grow this bacterial dilution at 37 °C for 2 hours with aeration and shaking at 220 rpm.
    4. Once these cells have reached early logarithmic growth phase (noticeable growth and slight turbidity), add 40 µL of commercially available P1 phage and leave at 37 °C with aeration and shaking at 220 rpm.
        1. Before phage addition, the measured optical density at 600 nm of these cells should be approximately 0.4 (6).
    5. Monitor cells for 1-3 hours until the culture has lysed.
        1. Lysis will result in increased cellular debris as well as a notable decrease in turbidity (i.e. cells will be considered lysed once one is able to see through the culture).
    6. Add several drops (50-100 µL) of chloroform to the lysate and mix by vortex.
        1. Chloroform sterilizes the phage lysate by killing any remaining donor cells, leaving only phage and increasing the titer of this lysate.
    7. Centrifuge lysate at 14,000 rpm for 2 minutes to remove debris and transfer supernatant to a fresh tube.
    8. Add a few drops of chloroform and store at 4 °C for no more than one day.
2. **Transduction**

1. Prepare a 1 mL culture of recipient J53 strain *E. coli* grown overnight in LB at 37 °C with aeration and shaking at 220 rpm.
2. Transfer 100 µL of donor phage lysate (2.1) into a 1.5 mL microfuge tube and incubate with cap open at 37 °C for 30 minutes.
    1. This incubation allows for any remaining chloroform in the P1 lysate solution to evaporate before being added to the recipient cells.
3. Gently pellet recipient strain cells by centrifugation at 6,000 rpm for 5 minutes.
4. Resuspend these cells in 300 µL of fresh LB containing 100 mM $MgSO_4$ and 5 mM $CaCl_2$. (P1 phage requires calcium to be infectious).
5. Set up two reactions using the recipient bacteria cells and prepared donor phage lysate: 1) transduction reaction combining 100 µL recipient J53 strain *E. coli* and 100 µL donor phage lysate, and 2) negative control combining 100 µL recipient J53 strain *E. coli* and 100 µL of LB containing 100 mM $MgSO_4$ and 5 mM $CaCl_2$.
6. Incubate at 37 °C for 30 minutes with shaking at 220 rpm.
7. Add 1 mL LB and 200 µL 1M sodium citrate (pH=5.5) and incubate for 1 hour at 37 °C with shaking at 220 rpm.
    1. Citrate is used to reduce the infectivity of P1 by chelating with calcium, preventing the lysis of recipient bacteria.
    2. Incubation of this solution allows for the expression of the ampicillin resistance marker.
8. Pellet cells by centrifugation at 6,000 rpm for 5 minutes.
9. Resuspend cell pellets in 100 µL of LB with 100 mM sodium citrate (pH 5.5). Vortex and plate entire solution for both reactions on two LB agar plates.
    1. LB plate should have 1x Amp for the transduced sample and no Amp for the negative control.
    2. P1 phage contamination on this plate requires re-streaking before freezer stocks can be prepared.
    3. If phage is not removed, cultures grown from these colonies will not grow unless in the presence of a calcium chelator.
10. Pick about 3-4 colonies from both plates and streak again on two LB agar plates spread with 100 µL of 1 M sodium citrate (pH=5.5).
    1. LB plate should have 1x Amp for the transduced sample and no Amp for the negative control.
11. Incubate the plates at 37 °C overnight to allow colonies free of phage to grow.
12. Pick colonies from both plates and use them to grow overnight cultures in 5 mL of LB at 37 °C with aeration and shaking at 220 rpm.
13. Isolate DNA from these cultures by DNA miniprep using 4.5 mL of the total culture volume.
    1. Elute DNA using 35 µL of nuclease-free water.
    2. Measure the resulting concentration by Nanodrop. Pure DNA will generate an absorbance ratio ($A_{260/280}$) of approximately 1.8.
14. Use the remaining 0.5 mL of each culture to prepare 1 mL glycerol stocks by making a 1:1 mixture of 100% glycerol and bacterial culture.
15. Store bacterial glycerol stocks at -80 °C.

3. Data Analysis and Results

1. Confirmation of transduction by qPCR
    1. Prepare two qPCR master mixes for six qPCR reactions, three using qPCR primers for the ampicillin resistance gene, and the other three using qPCR primers for a housekeeping gene (14.5 µL per reaction): 12.5 µL qPCR buffer mix + 1 µL forward primer + 1 µL reverse primer.
        1. In this experiment, we used SYBR Green master mix.
        2. Housekeeping gene primers were designed to amplify a segment of DNA within the bacterial gene encoding for DNA gyrase B (7).
    2. For each qPCR reaction, combine 100 µg of DNA from each reaction (10.5 µL) with 14.5 µL of qPCR master mix.
    3. Using a qPCR machine and the thermocycling protocol listed in Table 1, amplification was measured for the ampicillin resistance and housekeeping genes for all six reactions.
    4. Cq values generated by qPCR were used to calculate the normalized transduction efficiency of the ampicillin resistance gene (Figure 3), confirming the successful transduction of the ampicillin resistance gene.
        1. The Cq value, or cycle quantification value, of a sample is the earliest PCR cycle number at which a signal exceeded the background threshold is detected. Low Cq values correspond to more target sequence, vice versa.
        2. Normalized transduction efficiency of a gene within a sample can be calculated using these Cq values by subtracting the value of the housekeeping gene from that of the target gene, generating a ΔCq value, which can be used to calculate normalized transduction efficiency by $2^{(-\Delta Cq)}$.

	Temperature	**Time**
Denaturation	94 °C	2 min
40 cycles:		
Denaturation	94 °C	15 sec
Annealing, extension, and fluorescence read	60 °C or 5 °C below lowest primer T_m	1 min

Table 1: qPCR Thermocycling Protocol

Applications and Summary

The transfer of genes to and from bacteria by bacteriophage, while a natural process, has proved extremely useful for a multitude of research purposes. While other methods of gene transfer such as transformation and conjugation are possible, transduction uniquely uses bacteriophages; not only allowing for gene integration into the host genome, but also for gene delivery to multiple bacteria that are not susceptible to other methods. This process, while especially useful in the laboratory, has also been used in the recently emerging field of gene therapy, more specifically in alternative gene therapy, a therapeutic strategy that utilizes bacteria to deliver therapeutics to target tissues, many of which are not susceptible to other delivery methods and have much clinical relevance (8,9).

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