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Bacteriophage Transduction in *Staphylococcus epidermidis*

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Abstract

The genetic manipulation of *Staphylococcus epidermidis* for molecular experimentation has long been an area of difficulty. Many of the traditional laboratory techniques for strain construction are laborious and hampered by poor efficiency. The ability to move chromosomal genetic markers and plasmids using bacteriophage transduction has greatly increased the speed and ease of *S. epidermidis* studies. These molecular genetic advances have advanced the *S. epidermidis* research field beyond a select few genetically tractable strains and facilitated investigations of clinically relevant isolates.

Keywords

Staphylococcus epidermidis; bacteriophage; transduction; bacteriophage 71

1. Introduction

Transduction is the process by which DNA is transferred from one bacterium to another by bacterial viruses known as bacteriophage. Norton Zinder and Joshua Lederberg discovered this process in 1951 (1). Bacteriophage reproduction relies on replicational, transcriptional, and translation machinery of the host bacterial cell to make new virions. During this process, the packaging of bacteriophage DNA is a relatively low fidelity event and pieces of bacterial chromosome may become mistakenly packaged into the bacteriophage capsid. The lytic cycle leads to a bloom of new bacteriophage particles, which are released by lysis of the host cell (2,3). Research methods for bacterial strain construction have taken advantage of the accidental bacterial DNA packaging into the bacteriophage to manipulate recipient strains by selection of antibiotic resistance markers or other genetic determinants.

Generalized transduction is the process by which any bacterial gene may be transferred to another bacterium via a bacteriophage. This is in contrast to specialized transduction, which is restricted on the DNA the bacteriophage can move. Generalized transduction occurs by a headful packaging mechanism, followed by infection of the recipient bacterial host and recombination onto the chromosome. The use of transduction in molecular microbiology labs rely on lytic bacteriophages, as opposed to a lysogenic cycle where the bacteriophage DNA integrates into a specific site in the host chromosome and remains dormant. During the

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lytic cycle of infection, the virus takes control of the bacterial cell's machinery to replicate its own DNA and produce more viral particles. In headful packaging, the bacteriophage fill their capsid with viral genetic material, but the low frequency accidental packing of bacterial plasmid or chromosomal DNA into the viral capsid sets the stage for downstream generalized transduction. Following another round of lytic infection, the bacteriophage infect new recipient bacteria and inject the foreign DNA (viral and bacterial) into the recipient cells. In this transduction event, the transferred bacterial DNA can integrate into the recipient bacterium's genome through homologous recombination or recircularize into a replicating plasmid (3). The final result is the successful transduction of bacterial genetic information from one strain to another, which is especially important as a mechanism through which antibiotic-resistance genes are exchanged between bacteria (4,2). Pathogenicity islands are also known to horizontally transfer to new strains using this mechanism (5).

Transduction has been adapted as a laboratory method for transferring genetic material to manipulate *S. epidermidis*. The isolation and first use of bacteriophage 71 traces back to early work on bacteriophage-typing staphylococci by Baird-Parker in the early 1960's (6,7). Dietrich Mack and colleagues adapted bacteriophage 71 (8) for generalized transduction in *S. epidermidis* in 1998 (9). In order to improve transduction of chromosomal markers, bacteriophage 71 lysates were UV-irradiated (10,11). The addition of bacteriophage transduction to the molecular "toolbox" for *S. epidermidis* has enhanced and accelerated discoveries in clinical isolates. In this article, we outline a straightforward method for generalized transduction of chromosomal markers and plasmids in *S. epidermidis* using bacteriophage 71.

2. Materials

13×100 mm Tryptic soy agar (TSA)/Brain Heart Infusion (BHI) slants

Tryptic soy broth (TSB) + 5 mM $CaCl_2$

Petri plates

15 mL Falcon tubes (BD Biosciences) or equivalent

TSA (1.5% agar) + 5 mM $CaCl_2$

TSA (1.5% agar) + 500 mg/L NaCitrate + antibiotic of choice

Soft Agar TSA (0.5% agar) + 5 mM $CaCl_2$

0.5 M $CaCl_2$

0.02 M NaCitrate

Antibiotic of choice; typical antibiotics and concentrations include erythromycin 10 μg/mL, chloramphenicol 10 μg/mL, trimethoprim 10 μg/mL, tetracycline 2–10 μg/mL, kanamycin 50 μg/mL.

Equipment

Centrifuge

Incubators – Static and shaking

Water Bath 50°C

3 Methods

3.1 Bacteriophage 71 Propagation

Transduction of plasmid and chromosomal markers of interest requires a phage titer of approximately 10^{10} pfu/ml. As phage titers gradually decrease during storage at 4°C, it is appropriate to propagate phage 71 to acquire a high titer (10^{10}) before the transducing lysate is generated.

1. Grow *S. epidermidis* propagation strain on 13×100 mm TSA slant overnight at 37°C. Ensure that a plasmid free strain of bacteriophage 71 susceptible *S. epidermidis* is used. Strain 1457 is recommended as it is both a good recipient and propagation strain for phage 71 (12) and allows for optimal phage titers (10^{10} PFU -Plaque Forming Units; ***see*** note 1).
2. Resuspend *S. epidermidis* propagation strain in 1 mL TSB + 5 mM $CaCl_2$ (***see*** note 2).
3. Add 4 mL TSA soft agar to 10 Falcon tubes (15 mL). Hold in 50°C water bath to prevent agar from solidifying.
4. Serially dilute bacteriophage 71 stock 10 fold to 10^{-10} in TSB + 5 mM $CaCl_2$.
5. Combine 10 μl *S. epidermidis* cells and 100 μl bacteriophage dilution to soft agar. Gently mix (do not vortex) and pour onto TSA + 5 mM $CaCl_2$ plates. Repeat for all 10 bacteriophage dilutions. Fresh TSA plates work best to prevent soft agar from drying out during phage propagation.
6. Incubate overnight (plates right side up) at 37°C. Do not invert plates to make sure soft agar is maintained on the agar surface.

3.2 Harvest Bacteriophage and Titer Determination

1. Select up to three plates for bacteriophage harvest. Optimal plates will show near confluent lysis and minimal bacterial growth (Ideally the 10^{-3}–10^{-5} plates, but this depends upon the original titer of bacteriophage 71 stock).
2. Add 3 mL TSB to plates. Harvest bacteriophage by breaking up and scraping off soft agar with a plate spreader. Transfer resulting agar/TSB mixture to a 50 mL tube.

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[1]A rigorous examination of *S. epidermidis* strains that are susceptible to phage 71 has not been performed. If using *S. epidermidis* strains other than 1457, check susceptibility of each strain to phage 71 by first streaking each strain to be tested on TSA containing 5mM $CaCl_2$. 10μl of phage 71 stock (10^{10} pfu/ml) is then spotted on the plate in the first quadrant and allowed to dry. The plate is incubated at 37°C for 24 hours; an area of lysis will be evident in *S. epidermidis* phage 71 susceptible strains.

[2]Calcium chloride ($CaCl_2$) is added to the media to facilitate bacteriophage 71 attachment to *S. epidermidis*. The addition of NaCitrate chelates the calcium, halting the bacteriophage infectious cycle and preventing reinfection.

3. Break up agar as much as possible by gently pipetting up and down to facilitate the release of bacteriophage particles. Avoid bubbles, vortexing, and sonication as they mechanically sheer bacteriophage tails.
4. Centrifuge 10 min at 10,000 × g.
5. Filter supernatant through 0.45 μm filter.
6. Store bacteriophage at 4°C.
7. The titer of the resulting bacteriophage lysate should be determined by repeating the experiment outlined in 3.1; optimal bacteriophage titer should be approximately 10^{10} pfu/ml. In some cases, when the original bacteriophage 71 stock titer was low, multiple propagation experiments may be required to acquire a titer of 10^{10} pfu/ml.

3.3 Preparation of Transducing Lysate

1. Repeat bacteriophage propagation and harvest protocol (3.1 and 3.2) using *S. epidermidis* strain of interest (either plasmid or chromosomal marker). Note that overnight growth may require 30°C if using temperature sensitive plasmid (i.e. $pE194_{ts}$-derived). 10^{10} pfu/ml of the transducing lysate should be achieved to ensure an appropriate transduction frequency (~10^{-8})

3.4 Transduction

1. Grow the strain to be transduction recipient overnight on a 13×100mm TSA slant.
2. Resuspend recipient strain in 1 mL TSB + 5 mM $CaCl_2$.
3. Add 500 μL of the recipient strain supension to a 50 mL tube.
4. Add 1.5 mL TSB + 5 mM $CaCl_2$.
5. Add 500 μL bacteriophage 71 transducing lysate (10^{10} pfu/ml) to tube (***see*** note 3).
6. Shake at 225 RPM for exactly 20 min at 37°C (30°C if transducing a temperature sensitive plasmid).
7. Add 1 mL cold (4°C) 0.02 M NaCitrate.
8. Centrifuge at 3000 RPM, 4°C for 10 min.
9. Resuspend pellet in 1 mL cold (4°C) 0.02 M NaCitrate.
10. Plate 100 mL of transduced cells each to TSA + 500 mg/L NaCitrate + antibiotic plates.
11. Incubate at 37°C (30°C if transducing a temperature sensitive plasmid).
12. Pick single colonies to streak for isolation on the TSA + 500 mg/L NaCitrate + antibiotic plates (***see*** note 4)

[3]As a negative control, perform the transduction experiments using all components except the bacteriophage to control for contaminated phage lysate.
[4]NaCitrate is required in these plates to chelate residual calcium in the TSA. Although individual transductant colonies are picked, the titer of bacteriophage 71 on the plate is so high that phage are typically transferred to subsequent TSA plates resulting in partial lysis of colony growth if NaCitrate is not added.

13. Confirm movement of plasmid/chromosomal marker by standard methods including plasmid analysis, PCR or Southern Blot.

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