FLVP assays

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Abstract

The following continued adaptation of Hennes et al. (1995) is a result of FLVP assay optimizations for the *Vibrio alginolyticus* PWH3a-phage P1 phage-host system (PHS) for use with SYBR Green I stain. Novices to microscopy would benefit from reading Wen et al. (2004) for details on preparing and storing slides using SYBR Green I stain. This protocol is a modification of the standard slide preparation techniques for bacteria and viruses in aquatic samples (see Suttle and Fuhrman 2010, this volume).

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Guidelines

Materials and Equipment:

• FLVP stock or 1:100 working stock (diluted in 0.02-µm filtered water or seawater medium)
• 1.5-mL microcentrifuge tubes
• appropriate host diluent (e.g., sterile seawater medium)
• 0.2-µm Anodisc (Whatman) and 0.45-µm cellulose (backing) filters
• microscope slides and coverslips (25 × 25 mm)
• mounting medium containing antifade (50:50 phosphatebuffered saline:glycerol with 0.1% p-phenylenediamine)
• epifluorescence microscope capable of 1000× magnification and equipped with a blue-light excitation filter (such as Olympus U-MWB2/U-MWIB2 filters).

Additional Experiments

FLVP microcosm experiments:

Seawater samples for the microcosm experiments were obtained from either a seawater holding tank at the University of British Columbia (49°16’N, 123°15′W) or a station in Vancouver Harbor (49°18’N, 123°06’W). All samples, before use in experiments, were screened for the endogenous presence of PWH3a using the FLVP assay.

In the first experiment, where the added (exogenous) bacterium did not dominate the system, PWH3a was added to a final concentration of 6.3×10^6 cells mL^{-1} in 500 mL of a natural background of prokaryotes at a final concentration of 2.1×10^6 cells mL^{-1} (~3:1 final ratio PWH3a:natural). The microcosm was enriched with MLB (10% final concentration) and incubated at 20°C with a 14:10 h light:dark cycle. Subsamples were taken at ~24-h intervals for prokaryotic cell counts (see Suttle and Fuhrman 2010, this volume) and FLVP assays. Shortly after the 72-h time point, PWH3a-P1 virus was added to the microcosm at a final concentration of ~10^5–10^6 viruses mL^{-1}.

In the second microcosm, where the added (exogenous) bacterium did dominate the system, the PWH3a final concentration was increased to 1.9×10^7 cells mL^{-1} in 50 mL of a further reduced natural prokaryotic background of 4.4×10^5 cells mL^{-1} (~40:1 final ratio PWH3a:natural). The microcosm was enriched as before and maintained at the same light level as the previous microcosm, yet the incubation was at the higher temperature of 30°C with aeration. Subsamples were taken as before, and infection of the microcosm was performed shortly after the 48-h time point with the same concentration of PWH3a-P1 virus as above.

FLVP receptor titration experiment:

Four flasks of 10 mL sterile MLB were inoculated with PWH3a at a final concentration of 1.4×10^6 cells mL^{-1}. FLVPs were added to three of the flasks at the following MOIs: 0 (uncoated control), 1000, and 10, to attempt to coat the PWH3a bacterium's cell surface receptors and render it resistant to subsequent infection. PWH3a-P1 virus was then added to the three flasks at an MOI of 0.01–0.1. The fourth flask did not contain any FLVPs or PWH3a-P1 and served as the control. Subsamples were taken from the four flasks at 2-h intervals for FLVP assays.

Protocol

Step 1.
Perform preparation of FLVPs as described in Viral and bacterial isolates, propagation and preparation of stocks.

Step 2.
Prepare a 10-fold dilution series of the culture or natural sample to be enumerated so that 10^5 cells mL^{-1} of the target (FLVP-specific species) will be obtained.

NOTES
Amy Chan 14 Oct 2015
This will result in a multiplicity of infection (MOI) of at least 3000 viruses per host cell (with an FLVP stock prepared as above from a $\geq 10^{10}$ viruses mL^{-1} initial phage stock).
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When mixing your host of choice with natural samples (e.g., for microcosm studies), also prepare a background control to check for FLVP attachment to natural cells.

Step 3.
Perform all subsequent steps under subdued light, since the stain will fade if exposed.

Step 4.
Add 0.1 mL FLVP working stock to 0.9 mL sample in a microcentrifuge tube for each slide to be prepared and vortex to mix.

NOTES
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Conversely, use 1 µL concentrated FLVP stock in 1 mL sample if not using the diluted working stock.

Step 5.
Allow up to 30 min for adsorption of FLVPs to target cells.

DURATION
00:30:00

NOTES
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Approximately 15 min was adequate for the PWH3a-P1 PHS, given that the adsorption kinetics of this phage are similar to typical coliphages such as T4; the time depends on the adsorption kinetics of your particular virus–host system and will have to be modified as such.

Step 6.
Filter each 1 mL sample onto a 0.2-µm Anodisc filter using a 0.45-µm HA filter for backing.

Step 7.
Pipette 10 µL mounting medium onto the surface of a slide.

Step 8.
Place the filter over the drop.

Step 9.
Pipette 10 µL mounting medium onto the surface of the filter.

Step 10.
Place a 25 × 25 mm coverslip over the filter.

NOTES
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(See "FLV tracer assays" for a discussion of mounting media.)

Step 11.
Observe the slides under blue-light excitation and count the cells with a fluorescent halo.

NOTES
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If a problem with nonspecific staining (excess, unwashed stain or leakage from FLVPs) occurs, it will be visible here as diffuse, whole-cell staining instead of the trademark halos.