Isolation of cyanophages by liquid enrichment assay

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Abstract

Another convenient way to screen larger sample volumes is to perform liquid enrichment cultures (Suttle 1993). By this approach, larger volumes of water samples can be screened for cyanophages, thus enabling detection of “rare” viruses. Similar to the use of enrichment cultures for bacteriophages, the disadvantages include the fact that lysis of the host cells are not always obvious, especially if the initial titer is low or if the host culture is not clonal or unialgal.

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Guidelines

If low titers are expected, the viruses in the sample can be concentrated via TFF to make a virus concentrate (VC) (Suttle et al. 1991; Wommack et al. 2010, this volume). To increase the detection limit, several different VCs can be combined and added to the same culture. Another convenient way to screen larger sample volumes is to perform liquid enrichment cultures (Suttle 1993). By this approach, larger volumes of water samples can be screened for cyanophages, thus enabling detection of □□rare□□ viruses. Similar to the use of enrichment cultures for bacteriophages, the disadvantages include the fact that lysis of the host cells are not always obvious, especially if the initial titer is low or if the host culture is not clonal or unialgal. Also, more steps are required to dilute out nonreplicating viruses to obtain pure clonal isolates. Because it is an end-point dilution assay, only the most abundant phages will be isolated. The principle is the same as for the isolation of bacteriophages mentioned earlier except that the sample volumes screened are in the order of liters instead of milliliters.

Protocol

Step 1.
Prefilter at least 3 L water sample through a glass fiber filter.

NOTES
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(e.g., Advantec type GC50, Whatman type GF/C, or Gelman type A/E)

Step 2.
Filter sample through 0.2 µm or 0.45 µm low protein binding PVDF filter.

NOTES
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(e.g., Millipore Durapore filter)

Step 3.
Dispense the filtered water samples (e.g., 0.5 L or more) into culture vessels, e.g., 1 L or larger Erlenmeyer flasks.

Step 4.
Add nutrients to the filtered water to support growth of the target cells.

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For example, F/2 nutrients for marine cyanobacteria or BG-11 nutrients (Rippka et al. 1979) for freshwater cyanobacteria.

Step 5.
Seed the filtered water with 1% to 10% v/v of host culture.

NOTES
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Target cells must be in exponential growth to avoid loss of potential viral infection (e.g., viruses adsorbed to dead or dying cells will not cause infection and subsequent production of progeny virus).

Step 6.
As a control, replace the filtered environmental sample with virus-free (0.02 µm filtered or heat-killed) water sample.

NOTES
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The volume of this culture need not be as large as the experimental flasks. This control is to make sure that there is not anything in the water sample that would inhibit growth of the target cells (e.g., chemical inhibition).

Step 7.
Incubate the flasks at the temperature and light conditions appropriate for the cyanobacteria and look for signs of lysis.

NOTES
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This could take 2 to 3 weeks, depending on the growth rate of the host as well as the initial titer of cyanophages. It is recommended that the in vivo chlorophyll fluorescence be monitored regularly. A small decrease in relative fluorescence could indicate the presence of a lytic virus.

Step 8.
Remove an aliquot of the enrichment culture, and pellet remaining cells by centrifugation (e.g., 20 minutes at 6000g).

DURATION
00:20:00

Step 9.
Filter the supernatant through a 0.22 µm or 0.45 µm PVDF filter.

Step 10.
Store the lysate at 4°C until further analysis.

Step 11.
Verify the presence of lytic phages by liquid assay (or plaque assay).

Step 12.
To propagate/amplify the lytic agent, the liquid bioassay is repeated using the putative lytic agent as

the test sample.

PROTOCOL

. Propagation/Amplification of Lytic Agent

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Step 12.1.
Set up bioassay using 5 mL or larger culture tubes in triplicates.

Step 12.2.
Add between 5 to 50 µL of each sample below to target cells in log phase.

NOTES

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a. Whole lysate (unfiltered).
b. Filtered lysate (0.22 or 0.45 µm)
c. Negative control (no addition, or use filtered media)

Step 12.3.
Monitor in vivo chlorophyll fluorescence for about 1 week, look for decrease in relative fluorescence compared with control cultures.

Step 12.4.
If the cultures lyse, then the lytic agent is most probably a virus.

Step 12.5.
Propagate the lytic agent several times to dilute out nonreplicating viruses.

Step 12.6.
Filter the lysate and use it to obtain pure clonal stocks.