Bacteriophage isolation using enrichment cultures

Mathias Middelboe, Amy M. Chan, and Sif K. Bertelsen

Abstract

A generally more efficient way of isolating lytic phages from marine environments is by the use of enrichment cultures. In this approach, the prefiltered water sample that is to be screened for phages against a given target bacterium, is enriched with a bacterial growth medium and amended with that target bacteria (Eisenstark 1967; Carlson 2005). This allows any lytic phages present in the sample to infect the target bacteria and propagate in the cultures, and subsequently, be isolated and purified. The two main advantages of the enrichment approach are 1) that it allows for screening for phages in a much larger volume of sample (typically 25–50 mL, rather than 5–10 µL), thus increasing the probability of isolating rare phages, and 2) that it allows the combination of different target hosts (e.g., different strains of a specific bacteria of interest) in the same incubation, again increasing the possibility of phage isolation.

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Guidelines

The following is the standard procedure in our lab when searching for phages against specific target bacteria in environmental samples. The sample volume and number of host strains used may be varied according to the sample investigated and the purpose of the phage isolation.

We recommend that bacterial growth during incubation is examined by OD_{525} measurements, which can give an indication of whether bacterial growth is inhibited by phage lysis. If only a single target bacterial strain is inoculated in the enrichment culture, the presence of a lytic phage against that particular strain will often result in clearing of the culture. However, if the lytic potential of the phage is limited, and/or if several strains are used, phage lysis may be difficult to detect by visual inspection of the culture, as some strains may be resistant to infection by the present phages. In that case, even a small decrease in OD relative to the control culture may indicate the presence of lytic phages.

1. If phage production is detected (in fact, phage production may have occurred even if lysis is not detectable by reduced OD values, the culture is transferred to a 50-mL centrifuge tube and the bacteria are pelleted (10,000g, 10 min).
2. The supernatant is then sterile filtered (0.2 µm or 0.45 µm filtered) and kept at 4°C until further analysis. A few drops of chloroform will preserve the sample, however, it also introduces the risk of eliminating lipid-containing phages.
3. To verify the presence of lytic phages in the enrichment culture filtrates, 5□-10 µL aliquots of the filtrates are spotted on lawns of host bacteria as described above.

Cleared liquid cultures and clearing zones on plates of immobilized host bacteria may potentially contain several types of infective phages that were present in the original sample and which

propagated in the enrichment culture. Subsequent steps of isolation and purification are therefore necessary to obtain stocks of specific phages (see below).

A more general search for phages, which are lytic to cooccurring bacteria in the water sample, requires a different procedure. In that case, 25 mL unfiltered water sample is amended with 1 mL 10 growth medium and incubated overnight (or longer depending on incubation temperature and type of target bacteria). During this incubation, lytic phages will potentially propagate by infecting indigenous bacteria that are favored by the given substrate and incubation conditions. As for the incubations above, phages produced during incubation are obtained after centrifugation and sterile filtration.

Concomitant with the enrichment culture incubations, potential bacterial host cells are then isolated after spreading 100 µL subsamples of the original water sample on agar plates containing a growth medium that is similar to the medium in the enrichment cultures. Single colonies are picked and restreaked on new agar plates, and subsequently transferred to liquid medium. New phageâhost systems can then be obtained by spotting aliquots of filtered enrichment culture on lawns of bacterial isolates in soft agar, as above, and inspected for clearing zones.

Protocol

Step 1.
Potential host cells are grown overnight in liquid cultures containing a rich growth medium (e.g., MLB).

DURATION
18:00:00

Step 2.
Adjust to an optical density measured at 525 nm (OD_{525}) of 0.3–0.5.

Step 3.
Approximately 25 mL water sample is filtered (0.2 µm or 0.45 µm syringe filters) to minimize the risk of bacterial contamination of the enrichment cultures.

NOTES
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Note also here that the filtration may result in loss of a fraction of the phages present in the original sample.

Step 4.
Transfer filtered samples to triplicate sterile 100 mL culture flasks.

Step 5.
Add 3 mL 10× growth medium (i.e., 10 times concentrated medium).

Step 6.
Start enrichment cultures by adding 1 mL of each of the target host strains of interest (e.g., 1–6 different strains for each incubation) to the culture flask.

Step 7.
Incubate the cultures on a shaking table at a temperature and period that is appropriate for the host bacteria.

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Typically 1 - 5 days.

Step 8.

A control culture is established where the environmental sample is replaced by 25 mL artificial seawater (or 0.02 µm filtered water sample) to verify bacterial growth in absence of phages.