Obtaining pure phage stock

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Abstract

Clearing zones on lawns of host bacteria potentially contain all lytic phages against the given host that were present in the original water sample. Little is still known about the occurrence and diversity of phages infecting specific hosts, but generally isolation procedures as those described above may reveal multiple phages against specific target bacteria (e.g., Comeau et al. 2006; Holmfeldt et al. 2007; Stenholm et al. 2008). It is therefore necessary to further isolate the phages to obtain specific stocks of single phages.

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Guidelines

If the relative abundance of individual phages in a phage assemblage obtained from a single clearing zone vary significantly (more than 10-100-fold), it will be very difficult to isolate the least abundant types as they would be diluted out in the attempt to obtain single plaques on the plates. Consequently, the method selects for isolation of the dominant fraction of lytic phages against a certain host bacterium at the time of sampling.

Protocol

Step 1.
Transfer phages from the clearing zone on the plate to 1 mL phage buffer or sterile sea water in a sterile tube by scraping off the surface layer of the soft agar containing the phages using a sterile loop.

NOTES
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Alternatively, use a Pasteur pipette to harvest a plug of the soft agar.

Step 2.
Allow the phages to diffuse into the medium overnight at 4°C.

DURATION
18:00:00

Step 3.
Vortex the tube and centrifuge the sample (10,000 x g, 10 min) to remove bacteria and agar.

DURATION
00:10:00

Step 4.
Transfer the supernatant to a new tube. This sample will typically contain 10^6–10^8 phages mL^{-1}.

Step 5.
To isolate single phages, dilutions of this concentrate should be done, followed by plaque assay, and subsequent isolation of phages from single plaques.

NOTES

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Different plaque morphologies may be selected as an indication of the presence of different phages. Again, the phages are transferred to 1 mL phage buffer, vortexed, and centrifuged, and subsequently, the supernatant containing the phages are transferred to a new tube.

Step 6.
Usually, this procedure is repeated 3 times to dilute out any contaminant phage associated with the phage of interest and increase the probability that only one specific phage is present in the final phage stock.

Step 7.
In the end, the phage concentrate is 0.2 µm filtered and kept in the fridge.

NOTES

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If the phage is insensitive to chloroform, preservation with a few drops of chloroform will prolong the life span of the phage stock. A viability test should, however, be carried out before adding chloroform to the sample. Stocks of specific phages in a buffer can remain infective for years.