Bacteriophage isolation by spotting on target host cells

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Abstract

Phage lysis of host bacteria can be visualized by plaque formation on lawns of host cells in soft agar overlayed on agar plates (Adams, 1959; Sambrook et al. 1989). This principle can be used for the detection and subsequent isolation of specific lytic phages in environmental samples (e.g., Carlson 2005). Spotting environmental samples on lawns of a target host cell would thus reveal the presence of a lytic phage for that particular host cell in the given sample.

Citation: Mathias Middelboe, Amy M. Chan, and Sif K. Bertelsen Bacteriophage isolation by spotting on target host cells. **protocols.io**
dx.doi.org/10.17504/protocols.io.dps5nd
Published: 08 Feb 2016

Guidelines

The main advantage of this procedure is that presence of lytic viruses is visually apparent as clearing zones on host bacterial lawns, and that subsequent isolation of phages is therefore fast, as the phages can be isolated directly from the plaques.

Protocol

Step 1.
The host cells are grown overnight in liquid cultures containing an appropriate growth medium for the organism.

NOTES

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(i.e., a medium that will yield a visible lawn of bacteria in soft agar when plated on an agar plate)

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Rich media such as Luria Broth (10 g tryptone, 5 g yeast extract, 10 g NaCl in 1 L distilled water) or MLB (0.5 g Casamino acids, 0.5 g peptone, 0.5 g yeast extract, 3 mL glycerol in 800 mL prefiltered [GF/C] seawater and 200 mL distilled water) can be used to culture many varieties of marine bacteria.

Step 2.
Measure optical density spectrophotometrically at 525 nm (OD_{525}) and adjust OD to 0.3–0.5 with growth medium.

NOTES

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This ensures sufficient bacterial density as well as the capacity for further bacterial growth during the following plate incubation.

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Step 3.
Soft agar (0.5 to 0.6% agar in media of choice) is melted in a water bath or a microwave oven.

Step 4.
Aliquot 4mL of soft agar into sterile tubes and keep just above solidification temperature until use.

NOTES

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The solidification temperature of soft agar depends on the agar or agarose used and is about 45°C for common agar. If the host bacteria cannot survive exposure to 45°C soft agar, low-melting-point agaroses are available for a range of lower temperatures

Step 5.
200–300 µL bacterial culture is added to the 4 mL tubes with melted soft agar.

Step 6.
The bacteria-soft agar mixture is then vortexed and immediately poured onto an agar plate with an agar that supports growth of the host bacterium.

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(e.g., Zobell agar [5 g tryptone, 1 g yeast extract, 15 g agar in 800 mL GF/C filtered sea water and 200 mL distilled water])

Step 7.
Distribute bacteria-soft agar mixture evenly on the plate, which is placed on a flat surface.

Step 8.
When the soft agar containing the target bacteria has solidified, triplicate aliquots of 5–10 µL of each of the environmental water samples from which phages should be isolated are spotted on top of the soft agar.

NOTES

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Before spotting, the samples should be filtered (e.g., 0.2 µm or 0.45 µm syringe filters) or centrifuged (e.g., 10,000g, 10 min) to remove bacteria.

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These procedures would minimize bacterial contamination in the spotting zone, which may hide clearing zones. As mentioned above, filtration and centrifugation may also remove a fraction of the phages in the sample, and thus reduce the chance of finding lytic phages against the target bacteria.

Step 9.
As a negative control 5–10 µL phage buffer (e.g., SM buffer: 450 mM NaCl, 50 mM $MgSO_4$, 50 mM Tris, 0.01 % Gelatin, pH = 8) or 0.02 µm filtered sample water is spotted in triplicate on the soft agar.

NOTES

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If the abundance of specific phages is expected to be low, the phages can be concentrated by various procedures prior to spotting on the target bacteria. Concentrating procedures are described elsewhere in this special issue (Wommack et al. 2010, this volume).

Step 10.
The plates are incubated for 1–3 d depending on the growth rate of the bacteria, and the presence of lytic phages in the sample is detected as a clearing zone (plaque) in the spotted area of the lawn of bacteria that develops over time on the plate.

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A single or a few phages added will result in only small plaques in the zone, whereas many phages in the spotted sample will yield a large clearing zone.

Step 11.

If clearing zones appear in the spotted area, this indicates the presence of lytic phages, which can be isolated and purified

NOTES

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To confirm that the clearing is due to phage lysis and not some other growth-inhibiting factor in the original sample, a dilution series of the sample can be performed and spotted on the target bacteria. Diluted sufficiently, phages would appear as single plaques in the spotted zone rather than a gradual reduction in growth inhibition as would be the case if the clearing was caused by some chemical factor. Alternatively, heat-killed (e.g., 90°C for 5 min) or 0.02 µm filtered controls can be used to verify that a clearing zone is caused by a biological component and not a chemical.

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Presence of phages can also be verified by SYBR staining and subsequent detection by epifluorescence microscopy (Noble and Fuhrman 1998; Suttle and Fuhrman 2010, this volume).

Step 12.

Once detected as clearings in the spotting zone, phages are further isolated and purified from the plates.

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