Isolation of temperate phages by plaque agar overlay

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Abstract

This protocol gives a method for isolating temperate phages from marine viral concentrations. This protocol uses the conventional plaque agar overlay and looking for turbid or haloed plaques, a hallmark of temperate phages.

Paul, J. H., and M. Weinbauer. 2010. Detection of lysogeny in marine environments, p. 30–33. In S. W. Wilhelm, M. G. Weinbauer, and C. A. Suttle [eds.], Manual of Aquatic Viral Ecology. ASLO.

Please see the full chapter for additional details.

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Guidelines

Overview

The isolation of temperate phages requires a cultivatable host and a source of concentrated viruses. For example, we isolated a pseudotemperate phage (φHSIC) and its host from the same bacterial/viral concentrate (Jiang et al. 1998) that we obtained in the Sand Island Channel, Oahu, HI, USA. The host was first isolated by standard isolation streaking on marine agar using inoculum from a microbial population concentrate. This protocol uses the conventional plaque agar overlay and looking for turbid or haloed plaques, a hallmark of temperate phages.

Materials

- Standard marine agar (1.5%) plates (i.e., Zobell 2216 or ASWJP);
- Sterile marine broth;
- Sterile marine soft agar (1%), 3 mL per 15-mL tube;
- Water bath;
- Marine host bacterial culture in exponential growth, 20–50 mL;
- Viral concentrate.

Protocol

Step 1.

Soft agar overlay tubes are melted in boiling water and placed in the 47°C water bath.

ANNOTATIONS
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The host bacterium should be growing exponentially (this can be verified by A_{600} measurements of

about 0.4–0.6).

Step 2.
One tube of soft agar is removed from the water bath.

ANNOTATIONS

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The agar should have cooled to 47°C

Step 3.
1.0 mL host culture and either 1.0 or 0.1 mL viral concentrate is added.

Step 4.
The contents of the tube is mixed well by rolling back and forth between two hands, and the tube contents are immediately emptied onto an agar plate.

Step 5.
The top agar is gently spread over the agar surface by sliding the plate on the bench surface using a circular motion.

Step 6.
The top agar is allowed to harden by not disturbing the plates for 30 min.

DURATION

00:30:00

Step 7.
The plates are incubated (top agar side down) overnight to 48 h.

DURATION

48:00:00

Step 8.
Temperate phage plaques will appear as turbid or cloudy plaques, whereas purely lytic phage will appear as sharply defined, clear plaques.

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Plaques may appear haloed (clear area with a larger turbid halo) and are often the result of pseudotemperate phages. Turbid plaques can be picked and replaqued to purify the temperate phage (three replaquings are recommended). It may also be possible to isolate the lysogenized host by carefully picking the turbid plaque and using isolation streaking on marine agar plates. The putative lysogen can be checked for harboring a prophage by mitomycin C induction.