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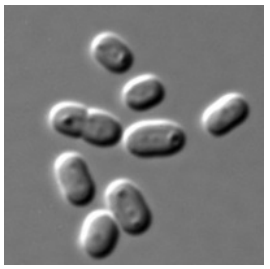
Prophage induction in marine Synechococcus



In 1 collection

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[1]Manual of Aquatic Viral Ecology

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Abstract

This protocol gives a method for prophage induction in marine *Synechococcus*.

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.

Guidelines

Materials

Sterile 96 well microtiter plates
Indicator host culture (i.e., Synechococcus WH7803)

References

Hurley, M. A., and M. E. Roscoe. 1983. Automated statistical analysis of microbial enumeration by dilution series. J. Appl. Bacteriol. 55:159-164.

Suttle, C. A., and A. M. Chan. 1994. Dynamics and distribution of cyanophages and their effect on marine Synechoccus spp. Appl. Environ. Microbiol. 60:3167-3174.

Weinbauer, M. G., and C. A. Suttle. 1996. Potential significance of lysogeny to bacteriophage production and bacterial mortality in coastal waters of the Gulf of Mexico. Appl. Environ. Microbiol. 62:4374-4380.

Before start

The samples for prophage induction are pretreated by the technique of viral reduction (Weinbauer and Suttle 1996).

Induction

1 Filter each sample through a 0.2-µm filter to a volume of approximately 5 mL to remove most of the ambient viruses.

2 Add virus-free (0.02-µm filtered) water prepared from the same sample and reduce the volume a second time.

3 Return the retentate to its original volume by adding virus-free seawater, dividing into aliquots, and incubating with and without inducing agent.

4 Amend treated samples with the inducing agent mitomycin C at a concentration of 1 µg/mL or with the inducing agent of choice.

Enumeration

5 Employ the most probable number (MPN) method to enumerate the cyanophage population (Suttle and Chan 1994).

6 Prepare a one- to five-dilution series of the environmental or prophage induction treatment sample using 96-well microtiter plates (Costar, Corning Inc.).

7 Freshly dilute a susceptible *Synechococcus* host 1:10 and place in each well.

8 Prepare control plates similarly using sterile SN media in the first column of wells.

9 Prepare three replicate treatment and control plates from each site.

10 Incubate the plates until good growth of the host organism is evident (10–14 days).

11 Score wells as positive for virus if lysis of the host organism is evident as a well clearing.

12 Calculate viral abundance for each plate using an MPN program (Hurley and Roscoe 1983).

Data analysis

13 Evaluate treatment and control cyanophage and *Synechococcus* counts by paired t test between samples using Minitab statistical software.

14 Perform comparison of induction results and environmental parameters using linear regression and X^2 analysis, also using Minitab.