Prophage induction in marine Synechococcus

John H. Paul and Markus Weinbauer

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.

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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).

Protocol

Induction

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

Induction

Step 2.

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

Induction

Step 3.

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

Induction

Step 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

Step 5.

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

Enumeration

Step 6.

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

Enumeration

Step 7.

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

ANNOTATIONS

VERVE Team 25 Aug 2015

Either Synechococcus isolate WH7803, our own isolate GM9901, or both.

Enumeration

Step 8.

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

Enumeration

Step 9.

Prepare three replicate treatment and control plates from each site.

Enumeration

Step 10.

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

Enumeration

Step 11.

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

Enumeration

Step 12.

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

Data analysis

Step 13.

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

Data analysis

Step 14.

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