Prophage Induction in Natural Populations of Heterotrophic Bacteria

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Abstract

This protocol gives two methods for prophage induction in heterotrophic bacterioplankton; one with no viral reduction and the other with viral reduction (see guidelines).

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

Natural populations of heterotrophic bacteria, no viral reduction

Materials

-Freshly filtered (0.02 µm; Whatman Anodisc) formaldehyde solution (formalin; 37%);
-Mitomycin C (Sigma; 1 mg/mL stock solution, dissolved in deionized water [DI]);
-Materials for epifluorescence or flow cytometry enumeration of viral particles;
-Electron microscopy–grade glutaraldehyde (Sigma Aldrich).

Prophage induction- Procedure in "steps"

Natural populations of heterotrophic bacteria, viral reduction

Materials

-Freshly filtered (0.02 µm) formaldehyde;
-Mitomycin C (Sigma);
-Materials for epifluorescence or flow cytometry enumeration of viral particles;
-Cartridge (30- or 100-kDa cutoff) to make virus-free water;
-Filtration (0.2 µm pore size) to reduce viral abundance (see also Weinbauer et al., this volume).

Prophage induction—The rationale of the virus reduction approach is to avoid new infection by reducing the number of viruses and, thus, the encounter rates with hosts (Weinbauer and Suttle 1996). This can be accomplished by several methods (see Weinbauer et al., this volume). Prokaryotic cells with reduced viral abundance (25–50 mL) are incubated at in situ temperature in triplicates with or without inducing agent C (see also above). Samples for enumeration of prokaryotes and viruses are taken periodically and fixed as described above. Calculation of induced viral production and the

percentage of cells containing a prophage (% lysogens) is calculated as described above.

References

Chen, F., J. R. Lu, B. J. Binder, Y. C. Liu, and R. E. Hodson. 2001. Application of digital image analysis and flow cytometry to enumerate marine viruses stained with SYBR Gold. Appl. Environ. Microbiol. 67:539-545.

Danovaro, R., and M. Middelboe. 2010. Separation of free virus particles from sediments in aquatic systems, p. 74-81. In S. W. Wilhelm, M. G. Weinbauer, and C. A. Suttle [eds.], Manual of Aquatic Viral Ecology. ASLO.

Jiang, S. C., and J. H. Paul. 1996. The abundance of lysogenic bacteria in marine microbial communities as determined by prophage induction. Mar. Ecol. Prog. Ser. 142:27-38.

Wommack, K. E., and R. R. Colwell. 2000. Virioplankton: Viruses in aquatic ecosystems. Microbiol. Mol. Biol. Rev. 64:69-114.

Protocol

Natural populations of heterotrophic bacteria, no viral reduction

Step 1.

For unconcentrated seawater samples, add 25 mL each to a control or treatment, 50-mL sterile, conical centrifuge tubes.

ANNOTATIONS

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If many inducing agents are to be investigated, increase the number of treatment tubes accordingly.

Natural populations of heterotrophic bacteria, no viral reduction

Step 2.

Take an additional sample (25 mL) and fix with 1% 0.02-µm filtered formalin.

Natural populations of heterotrophic bacteria, no viral reduction

Step 3.

For the treatment samples, add 1 µg/mL mitomycin C (or 0.5 µg/mL in oligotrophic environments).

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If other mutagens are to be used, it is a good idea to include a mitomycin C treatment as a positive control. Mutagens can be added at any concentration desired, but this can be limited by the solubility of the mutagen (e.g., polynuclear aromatic hydrocarbons; Jiang and Paul 1996).

Natural populations of heterotrophic bacteria, no viral reduction

Step 4.

The samples are incubated for 16–24 h at room temperature and fixed with either 2% glutaraldehyde (for TEM), 1% formalin (epifluorescence microscopy), or 1% formalin/0.5% glutaraldehyde (flow cytometry [FCM]).

DURATION

24:00:00

Natural populations of heterotrophic bacteria, no viral reduction

Step 5.

Samples for enumeration by epifluorescence microscopy should be counted within 24 h of collection or stored as frozen slides stained with SYBR Gold.

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Chen et al. 2001; see Danovaro and Middelboe 2010, this volume.

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Step 6.

Count both bacteria and viruses in control and treated samples.

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For induction to have occurred, viral counts in the treatment must exceed those in the control (i.e., be statistically different).

Natural populations of heterotrophic bacteria, no viral reduction

Step 7.

Calculate the % lysogenic bacteria as follows:

% lysogens = $[(VDC_T - VDC_C)/B_z]/BDC_{T=o'}$

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where VDC_T is the viral direct counts (in viruses/mL) in the treatment, VDC_C is the viral direct counts in the control, B_Z is the average burst size, and $BDC_{T=0}$ is the bacterial counts at the set up of the experiment (T = 0).

Natural populations of heterotrophic bacteria, no viral reduction

Step 8.

The average burst size can be derived by TEM observation of bacterial bursts (i.e., when viruses become visible in the cell at the end of the latent period; Ackermann/Heldal, this volume).

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We have found an average for our samples from the Gulf of Mexico of 30, whereas taking an average of the literature from a recent review (Wommack and Colwell 2000) indicates a value of 53.5 ± 48.