Enumerating Viruses in Coral Mucus

Amandine Leruste, Thierry Bouvier and Yvan Bettarel


Published Ahead of Print 22 June 2012.

Updated information and services can be found at:
http://aem.asm.org/content/78/17/6377

**REFERENCES**

These include:

This article cites 15 articles, 5 of which can be accessed free at:
http://aem.asm.org/content/78/17/6377#ref-list-1

**CONTENT ALERTS**

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: http://journals.asm.org/site/misc/reprints.xhtml
To subscribe to another ASM Journal go to: http://journals.asm.org/site/subscriptions/
Enumerating Viruses in Coral Mucus

Amandine Leruste, Thierry Bouvier, and Yvan Bettarel
UMR 5119 ECOSYM—Université Montpellier 2-IRD-IFREMER-CNRS, Montpellier, France

The distribution of viruses inhabiting the coral mucus remains undetermined, as there is no suitable standardized procedure for their separation from this organic matrix, principally owing to its viscosity and autofluorescence. Seven protocols were tested, and the most efficient separations were obtained from a chemical treatment requiring potassium citrate.

Viruses have been probably the least studied among the biological entities that live in the mucus and form the coral holobiont (dinoflagellates, fungi, bacteria, archaea, cyanobacteria, etc.) (1, 4, 10, 11), until now. Speculation on their presence in coral lacks a scientific basis, and only a few scattered metagenomic studies have provided any new information (6, 12). It is now known that, although viruses can infect any of the microorganisms in the coral holobiont, they are mostly comprised of bacteriophages (6).

Moreover, there is no quantitative estimate of their density (per unit of mass of mucus or per unit area) in the literature, and their prevalence in the surface mucus layer is based only on qualitative observations (2, 9). A standard reliable method is thus required for estimating their abundance levels. In this study, seven previously described protocols were tested, and their suitability for counting viruses reliably from the coral mucus were compared.

The mucus was collected from six scleractinian species in the Cap d’Agde aquarium (France)—Fungia sp., Turbinaria sp., Alveopora sp., Favia sp., Lobophyllia sp., and Platygryra sp.—and the soft coral Heteroxenia sp. Briefly, corals were taken out of the water and exposed to air for 1 to 3 min (7). This stress caused the mucus to be secreted, forming long gel-like threads dripping from the coral surface that were directly collected in cryotubes, fixed in 900 μl of 1% trypsin incubation for 30 min at 37°C, followed by 1% trypsin incubation for 30 min at 37°C.

Mechanical method. The mechanical method was a homogenization method (6) based on a 1:10 dilution of the mucus after fixing (100 μl) in 0.02-μm-pore-size-filtered Milli-Q water, followed by 1 min of power homogenization (Polytron; PT 10 35 GT) at 5,000 rpm. A combination of this protocol and that based on the potassium citrate protocol was tested by diluting 100 μl of the mucus in 900 μl of 1% potassium citrate solution prior to homogenization.

Enzymatic method. The trypsin method (3) involved 100 μl of fixed mucus eluted into a solution of EDTA (10 mM, final concentration), vortexed for 1 min, and sonicated for 3 min, followed by 1% trypsin incubation for 30 min at 37°C.

Staining procedure. Viral abundances were determined in each mucus sample (treated and untreated) by using the standard technique with nucleic acid dye SYBR Gold and epifluorescence microscopy (8).

Statistical analyses. The treatment effectiveness (TE) was calculated based on the difference in viral abundances between the treated (T) and untreated control (C) mucus samples: TE (%) = ([T – C]/C) × 100. The significance of treatments and coral species on viral abundance was tested using multifactorial analysis of variance (ANOVA) and Tukey-Kramer mean post hoc comparison tests (alpha level = 0.05).

The reliability of each protocol was simply inferred from the levels of abundance detected for viruses, which are the net result of parameters such as brightness, contrast, background fluorescence, presence of remaining aggregates, staining quality, even distribution of particles on membrane, etc. Thus, the protocol resulting in the highest values was considered the most effective for separation and enumeration of viruses.

In this study, the 1% potassium citrate solution was the best overall eluent for the mucus samples, regardless of the coral species. Direct counts of viruses following their extraction with this compound ranged from 2.8 × 10⁷ VLP ml⁻¹ of mucus for Fungia sp. to 11.4 × 10⁷ VLP ml⁻¹ for Alveopora sp. (Fig. 1). The treatment effectiveness reached up to 252.3% and was significantly higher than that of the other chemical, mechanical, or enzymatic treatments (Tukey-Kramer, P < 0.05). Potassium citrate has been successfully used for more than 10 years to desorb viral particles.
from porous substrates, such as sediments and soils (13, 14). By raising the pH of a solution, it increases the electrostatic repulsion between the viruses and the substrate to which they are attached. Furthermore, unlike the other chemical eluents tested in this study, potassium citrate also greatly reduced the autofluorescence of the mucus, which made counting by epifluorescence much more reliable (Fig. 2). Last, the method is very fast, as the effect of the reagent is immediate and does not require any additional incubation. It is also inexpensive, as only a few milligrams are required of a stock at ca. 60 euros per 500 g of potassium citrate (VWR).

Unlike its remarkable efficiency for extracting prokaryotes from mucus (3), trypsin did not give the highest viral counts, possibly because this enzyme might also cause substantial damage to the viral capsid proteins. The homogenization method, previously used for DNA extraction in coral mucus, also resulted in very poor quality observations. This mechanical treatment is a powerful mucus liquefier, but the resulting spreading over the entire membrane surface has seemingly increased the background fluorescence of mucus, thus reducing the contrast and brightness of the stained viruses.

Our results were obtained from cultured corals rather than from in situ scleractinian colonies and may not be applicable to natural habitats. Nonetheless, this study shows that the citrate potassium method represents an interesting tool to investigate the dynamic processes of colonization and the spatial and temporal variability of the viral community abundances within the coral holobiont. It also might help to cope with several methodological barriers that prevent single-cell studies of these biological entities, including flow cytometry and electron microscopy.

ACKNOWLEDGMENTS

The research reported here was financially supported by the French EC2CO project CORINE and the Total Foundation.
REFERENCES


