Electron Microscopy of Haloviruses

Kenneth M. Stedman, Kate Porter, and Mike L. Dyall-Smith

Abstract

This is a protocol from:

Stedman, K. M., K. Porter, and M. L. Dyall-Smith. 2010. Chapter 6: The isolation of viruses infecting Archaea. Manual of Aquatic Viral Ecology. Waco, TX:American Society of Limnology and Oceanography. doi:10.4319/mave.2010.978-0-9845591-0-7

Please see the published manuscript for additional information.

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Guidelines

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Standard negative stain EM works best on samples with low salt concentrations, but many haloviruses are stable only at high salt concentrations. If one uses high salt preparations, the salts can crystallize on the grid, occluding the particles and heating up the specimen. One way to overcome the problem is to first fix the sample using gluteraldehyde. Another problem is poor adsorption to plastic-coated grids (e.g., formvar). Pretreatment of the grids with poly-L-lysine can alleviate this issue.

The method for examining haloviruses was adapted from that described by Tarasov et al. (2000).

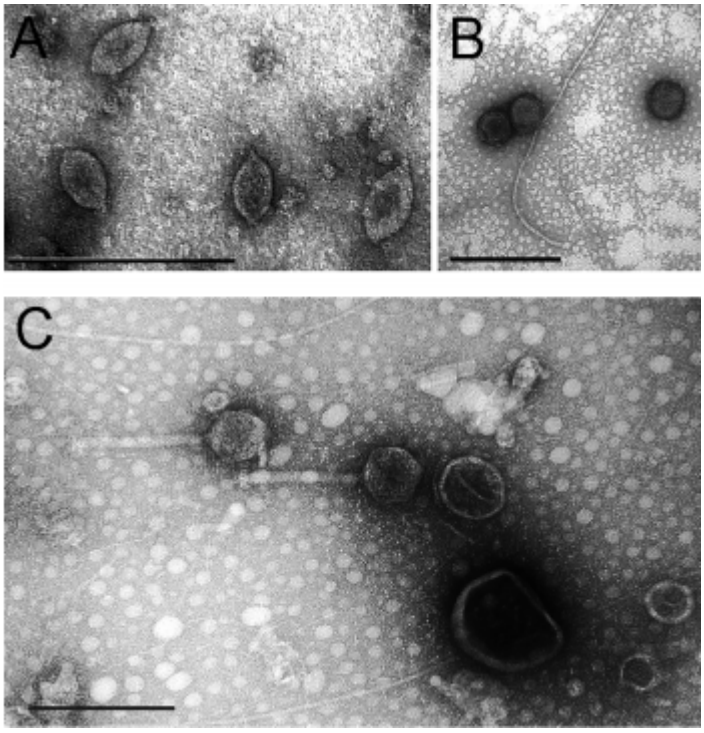

Fig. 3: Electron micrographs of haloviruses and VLPs.
(A) Spindle-shaped particles of His1 virus (host is *Har. hispanica*).
(B) Spherical particles of SH1 virus. Also seen is a flagellar filament from the host (*Har. hispanica*).
(C) Head-tail VLPs, and other structures, seen in a natural hypersaline water sample (Serpentine lake, Rottnest Island, Western Australia). All scale bars 200 nm. Negative stain with uranyl acetate.

Protocol

Step 1.
A sample of virus is placed on a sterile surface and the grid, plastic-coated side down, placed on the droplet for 1.5–2 min.

DURATION
00:02:00

Step 2.
The grid is then placed, for 1–1.5 min, on a drop of freshly filtered 2% w/v uranyl acetate and excess stain removed with filter paper.

DURATION
00:01:30

Step 3.
After air drying, grids may be examined by transmission electron microscopy, as described in guidelines (Fig. 3A, B, and C in guidelines).

NOTES
Ken Stedman 17 Dec 2015
For more information see guidelines.