Viruses from Sulfolobus and close relatives

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

Abstract

The following methods are basically method “A” of Zillig et al. (1994) and were described recently in detail by Prangishvili (2006). These methods consist of enrichment cultures followed by host isolation and screening for virus production in both these isolates and enrichment cultures. These techniques are very similar to those used for bacteriophages, with the major exception being the extreme growth conditions (80˚C, pH 3).

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.

Citation: Kenneth M. Stedman, Kate Porter, and Mike L. Dyall-Smith Viruses from Sulfolobus and close relatives. **protocols.io**
dx.doi.org/10.17504/protocols.io.dpb5im
Published: 24 Oct 2015

Guidelines

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Alternative sample collection—If the laboratory is relatively close to the sampling location, water and sediment samples are collected as above, but instead of an anaerobic tube, a sterile screw-cap vial or centrifuge bottle is completely filled so that very little air is present. Samples can then be transported at ambient temperature and should be enriched within 8–10 h of collection (Rice et al. 2001).

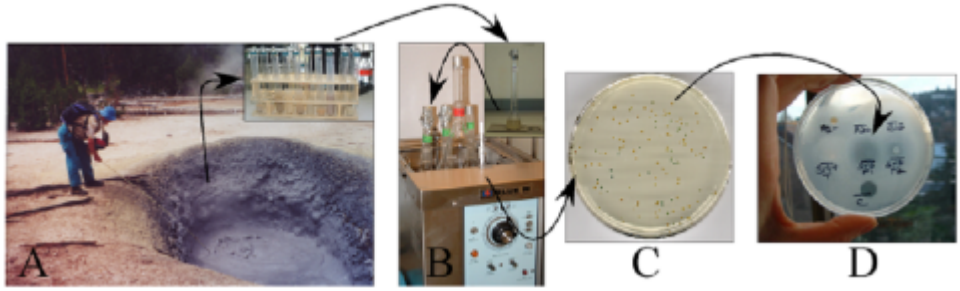

Fig. 1: Pictorial overview of isolation of *Sulfolobus* viruses.
(A) Wolfram Zillig sampling at a typical *Sulfolobus*-containing pool in Yellowstone National Park, USA, September 2000 (inset shows anaerobic tubes with samples).
(B) 80°C incubator with long-necked growth flasks (detail in inset).
(C) Singlecolony isolates of *Sulfolobus solfataricus* on a Gelrite® plate. This plate contains a mixture of *S. solfataricus* containing (blue colonies) and lacking (brown) a vector expressing the *lacS* gene from *S. solfataricus* and was sprayed with 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-gal) (see Jonuscheit et al. 2003).
(D) Lawn of *S.solfataricus* strain P1 with halos of growth inhibition due to virus production by 2-µl spots of virus-infected strains. Spots labeled SV2P1 and SV2P2 are from *S. solfataricus* strains P1 and P2 infected with SSV-I2 respectively (Stedman et al. 2003). Spot labeled C is a detergent-positive control. Spot labeled P2- is uninfected *S. solfataricus* strain P2 as a negative control.

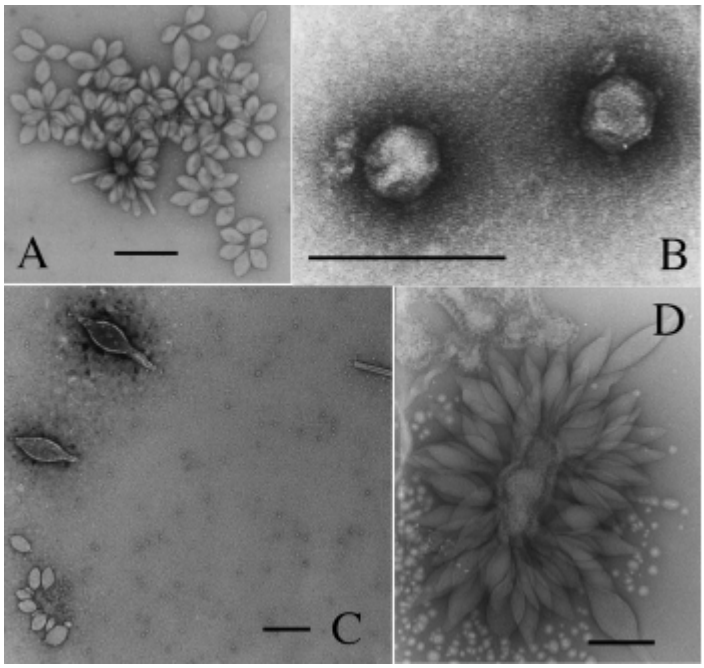

Fig. 2: TEM of *Sulfolobus* viruses and VLPs.
(A) *Sulfolobus* spindle-shaped virus SSV-I2 particles.
(B) *Sulfolobus* turreted icosahedral virus (STIV).
(C) Three different VLPs from an enrichment culture from Amphitheater Springs, Yellowstone National Park, USA. Note end of a *Sulfolobus islandicus* rod-shaped virus (SIRV)-like particle in upper right of image).
(D) Virus-like particles from Amphitheater Springs. All scale bars 200 nm. Negative stain with uranyl acetate.

Protocol

Preparation of anaerobic tubes for sample transport

Step 1.
For each sample to be collected, one anaerobic collection vessel (Fig 1A in guidelines) is prepared.

Preparation of anaerobic tubes for sample transport

Step 2.
A small amount (ca. 50–220 mg) of elemental sulfur (e.g., Riedel-deHaën) is placed in an anaerobic

tube.

Preparation of anaerobic tubes for sample transport

Step 3.

0.1 mL of a 2% resazurin solution and 0.1 mL of water saturated with H_2S are added.

NOTES

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A fresh Na_2S solution can also be used for reduction, but with less success; Stedman, unpublished.

Preparation of anaerobic tubes for sample transport

Step 4.

The air in the tube is displaced with CO_2 and N_2 by the Hungate technique and the tube is stoppered (Hungate et al. 1966).

Preparation of anaerobic tubes for sample transport

Step 5.

A cap is placed on the tube and the assemblage autoclaved.

NOTES

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A gas phase of 160 kPa of CO_2 and 1 kPa of H_2S has also been used successfully (Prangishvili 2006).

Sample collection and transportation for thermoacidophilic Archaea

Step 6.

Liquid and wet sediment samples are collected from turbid terrestrial hot springs with high temperature > 70˚C and low pH < 4.

NOTES

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The pH is often tested with pH paper because it is less susceptible to temperature changes than most pH electrodes.

Sample collection and transportation for thermoacidophilic Archaea

Step 7.

Samples are collected in sterile 50-mL conical flasks at the end of an extendible pole with a clamp (see Fig. 1A in guidelines).

Sample collection and transportation for thermoacidophilic Archaea

Step 8.

After most of the sediment is allowed to settle, the pH of the liquid is carefully adjusted to ca. 5.5 with solid $CaCO_3$ by slow addition and stirring.

Sample collection and transportation for thermoacidophilic Archaea

Step 9.

Once the pH is adjusted the sample is transferred to a pre-prepared anaerobic tube using a syringe (see above and Fig. 1A in guidelines).

Sample collection and transportation for thermoacidophilic Archaea

Step 10.

If the resazurin indicator changes to pink, drops of H_2S-saturated water are added until the sample clears.

Sample collection and transportation for thermoacidophilic Archaea

Step 11.

Samples can be maintained for up to 2 weeks at room temperature before enrichment.

NOTES

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For alternative sample collection, see guidelines.

Enrichment culture for host and virus isolation

Step 12.

Samples collected either in anaerobic tubes or filled centrifuge tubes are diluted 1:50 or 1:100 in *Sulfolobus* growth medium* (Zillig et al. 1994).

NOTES

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*Growth medium containing either yeast extract (0.1% w/v) and sucrose (0.2% w/v) as carbon sources or Tryptone (0.2% w/v) in longnecked Erlenmeyer flasks (see Fig. 1B in guidelines).

Enrichment culture for host and virus isolation

Step 13.

Incubate samples at 80˚C with shaking (150 rpm) for up to 2 weeks.

Enrichment culture for host and virus isolation

Step 14.

The salts in Sulfolobus growth medium are, per liter:

3 g $(NH_4)_2SO_4$	0.5 g $K_2HPO_4 \times 3\ H_2O$
0.1 g KCl	0.5 g $MgSO_4 \times 7\ H_2O$
0.01 g $Ca(NO_3)_2 \times 4\ H_2O$	1.8 mg $MnCl_2 \times 4\ H_2O$
4.5 mg $Na_2B_4O_7 \times 10\ H_2O$	0.22 mg $ZnSO_4 \times 7\ H_2O$
0.05 mg $CuCl_2 \times 2\ H_2O$	0.03 mg $Na_2MoO_4 \times 2\ H_2O$
0.03 mg $VOSO_4 \times 5\ H_2O$	0.01 mg $CoSO_4 \times 7\ H_2O$

Enrichment culture for host and virus isolation

Step 15.

The medium was buffered with 0.7 g glycine per liter and the pH was adjusted to pH 3–3.5 with 1:2 diluted sulfuric acid.

Enrichment culture for host and virus isolation

Step 16.

For long-term 80˚C growth, our favorite bath liquid is PEG 400, which is a noncorrosive, nontoxic, water soluble compound that does not evaporate (see Fig. 1B in guidelines).

NOTES

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Mineral oil and water can be used as bath liquid but are suboptimal due to cleanup and evaporation, respectively.

Enrichment culture for host and virus isolation

Step 17.

When growth is detected by either an increase in turbidity or production of a characteristic “damp sock” odor (W. Zillig pers. comm.), samples are plated on Gelrite® plates (see below and Fig. lC in guidelines), rediluted 1:50, and screened for VLP production by a spot-onlawn assay (see below and Fig. 1D in guidelines) or electron microscopy (see below and Fig. 2 in guidelines).

Enrichment culture for host and virus isolation

Step 18.

The second round of enrichment culture is also plated and screened for virus production.

Plating on Gelrite plates for host and virus isolation

Step 19.

Plates are made by slowly adding 6–10 grams/L Gelrite (Kelco) to Sulfolobus media (see above) and boiling until dissolved.

NOTES

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Gelrite, a xanthan gum, is used instead of agar because set Gelrite plates remain solid up to 90˚C.

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Alternatively, a 2 × Gelrite concentrate (12–20 g/L) is made in water, melted by boiling, and added to an equal volume of 2 × concentrated Sulfolobus medium (Grogan 1989).

Plating on Gelrite plates for host and virus isolation

Step 20.

Calcium ($Ca(NO_3)_2$) and magnesium ($MgCl_2$) are added to a final concentration of 1.5 and 5 mM, respectively, to stabilize the gel.

Plating on Gelrite plates for host and virus isolation

Step 21.

Before the gel solidifies, ca. 25 mL is poured into standard (90 mm) Petri plates with cams.

Plating on Gelrite plates for host and virus isolation

Step 22.

After the Gelrite solidifies, plates can be stored at 4˚C indefinitely.

Plating on Gelrite plates for host and virus isolation

Step 23.

Approximately 0.1 mL, from undiluted to 10^{-3}, of enrichment cultures are spread on Gelrite plates in the presence or absence of 0.5 mL 0.2% Gelrite dissolved in Sulfolobus medium.

Plating on Gelrite plates for host and virus isolation

Step 24.

Plates are incubated inverted in airtight moist containers at 75–80˚C for approximately 1 week before colonies appear (Fig. 1C in guidelines).

NOTES

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Multiple wet paper towels and a 90-mm Petri dish filled with water at the bottom of a sealable container (e.g., Tupperware®) is sufficient.