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The isolation of viruses infecting Archaea

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Abstract

A mere 50 viruses of Archaea have been reported to date; these have been investigated mostly by adapting methods used to isolate bacteriophages to the unique growth conditions of their archaeal hosts. The most numerous are viruses of thermophilic Archaea. These viruses have been discovered by screening enrichment cultures and novel isolates from environmental samples for their ability to form halos of growth inhibition, or by using electron microscopy to screen enrichment cultures for virus-like particles. Direct isolation without enrichment has not yet been successful for viruses of extreme thermophiles. On the other hand, most viruses of extreme halophiles, the second most numerous archaeal viruses, have been isolated directly from hypersaline environments. Detailed methods for the isolation of viruses of extremely thermoacidophilic Archaea and extremely halophilic Archaea are presented in this manuscript. These methods have been extremely effective in isolating novel viruses. However, Archaea comprise much more than extreme thermoacidophiles and extreme halophiles. Therefore a vast pool of archaeal viruses remain to be discovered, isolated, and characterized, particularly among the methanogens and marine Archaea. Some suggestions for expansion of the described methods are discussed. We hope these suggestions will provide an impetus for future work on these and other Archaeal viruses.

Introduction:

Archaea have been shown by molecular techniques to be widespread in many ecosystems (e.g., Chaban et al. 2006), but to date only about 50 viruses have been reported that infect this large and diverse group of organisms (Prangishvili et al. 2006). Most archaeal viruses have been isolated from either extreme thermoacidophiles or extreme halophiles (Prangishvili et al. 2006; Porter et al. 2007). This work was pioneered by the late Wolfram Zillig, but was not systematically addressed until the work of Prangishvili and Dyall-Smith, respectively. Early virus isolates of extreme halophiles (haloviruses) were of the head-and-tail type, the same morphology observed in more than 90% of described bacteriophages; more recent isolates have included representatives of spindle-shaped and spherical morphotypes (Porter et al. 2007; Pietila et al. 2009). In contrast, none of the viruses of extremely thermophilic crenarchaea are of the head-and-tail type, but show a fascinating variety of unique morphologies and genomes, indicating that we have only just begun to appreciate the diversity of archaeal viruses (Prangishvili et al. 2006).

Viruses in high temperature acidic environments are surprisingly low in abundance, commonly 10^3 per mL, as determined by either nucleic acid staining techniques (Ortmann et al. 2006; Prangishvili et al. 2006) or direct counting of virus-like particles (VLP) (Ortmann et al. 2006; Prangishvili et al. 2006). The reason for this is unknown. Therefore almost all of

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the viruses isolated from thermoacidophilic Archaea come from enrichment cultures of environmental samples.

Hypersaline waters are similar to marine ecosystems, with high VLP counts, commonly around 10^8 VLP per mL (Guixa-Boixareu et al. 1996; Oren et al. 1997; Diez et al. 2000; Pedros-Alio et al. 2000a; Danovaro et al. 2005; Bettarel et al. 2006). Despite the high virus levels, low cell growth rates, and frequent observations of VLPs inside cells, some studies predict that haloviruses are not major regulating factors of community size (Guixa-Boixareu et al. 1996; Pedros-Alio et al. 2000a; Pedros-Alio et al. 2000b). Although the viral role in microbial population control remains unclear, high virus numbers indicate that they should be readily isolable directly from water samples but, to date, only about 21 well-described haloviruses have been reported in the published literature (Pagaling et al. 2007; Porter et al. 2007; Pietila et al. 2009).

Methanogens are the first-identified and probably best-characterized members of the Archaea; however, reports of their viruses are surprisingly sparse in the literature, with only three different viruses described, one characterized in detail, and ten viruses or proviruses reported. Viruses of methanogenic Archaea have been isolated from anaerobic sludge digesters (Meile et al. 1989; Nolling et al. 1993) and found in supernatants of known cultures (Wood et al. 1989). It is unclear whether this lack of published viruses is due to the low abundance of viruses of Methanogens or insufficient screening. However, a recent bioinformatic analysis of the incomplete genome of *Methanococcus voltae* strain A3 indicated the presence of at least two different proviruses (Krupovic and Bamford 2008) and highlights the need for further study of viruses of methanogenic Archaea.

This manuscript gives methods for isolation of viruses of the thermoacidophilic archaeon *Sulfolobus* and close relatives and viruses from hypersaline waters. Methods used for the isolation of viruses of other thermophilic Archaea are also discussed. Similar methods have been used for isolation of the few viruses of methanogenic Archaea but are not discussed in detail here.

Materials and Procedures:

Viruses from Sulfolobus and close relatives—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).

Preparation of anaerobic tubes for sample transport—For each sample to be collected, one anaerobic collection vessel (Fig. 1A inset) is prepared. A small amount (ca. 50–220 mg) of elemental sulfur (e.g., Riedel-deHaën) is placed in an anaerobic tube, and 0.1 mL of a 2% resazurin solution and 0.1 mL of water saturated with H_2S are added (a fresh Na_2S solution can also be used for reduction, but with less success; Stedman, unpublished). 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). A cap is placed on the tube and the assemblage autoclaved. 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—Liquid and wet sediment samples are collected from turbid terrestrial hot springs with high temperature >70°C and low pH <4. The pH is often tested with pH paper because it is less susceptible to temperature changes than most pH electrodes. Samples are collected in sterile 50-mL conical flasks at the end of an extendible pole with a clamp (*see* Fig. 1A). 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. Once the pH is adjusted the sample is transferred to a pre-prepared anaerobic tube using a syringe (*see* above and Fig. 1A inset). If the resazurin indicator changes to pink, drops of H_2S-saturated water are added until the sample clears. Samples can be maintained for up to 2 weeks at room temperature before enrichment.

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).

Enrichment culture for host and virus isolation—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) containing either yeast extract (0.1% w/v) and sucrose (0.2% w/v) as carbon sources or Tryptone (0.2% w/v) in long-necked Erlenmeyer flasks (*see* Fig. 1B inset), and incubated at 80°C with shaking (150 rpm) for up to 2 weeks. 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 $CoSO_4 \times 7\ H_2O$. 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. 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); mineral oil and water can be used as bath liquid but are suboptimal due to cleanup and evaporation, respectively. 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), rediluted 1:50, and screened for VLP production by a spot-on-lawn assay (*see* below and Fig. 1D) or electron microscopy (*see* below and Fig. 2). The second round of enrichment culture is also plated and screened for virus production.

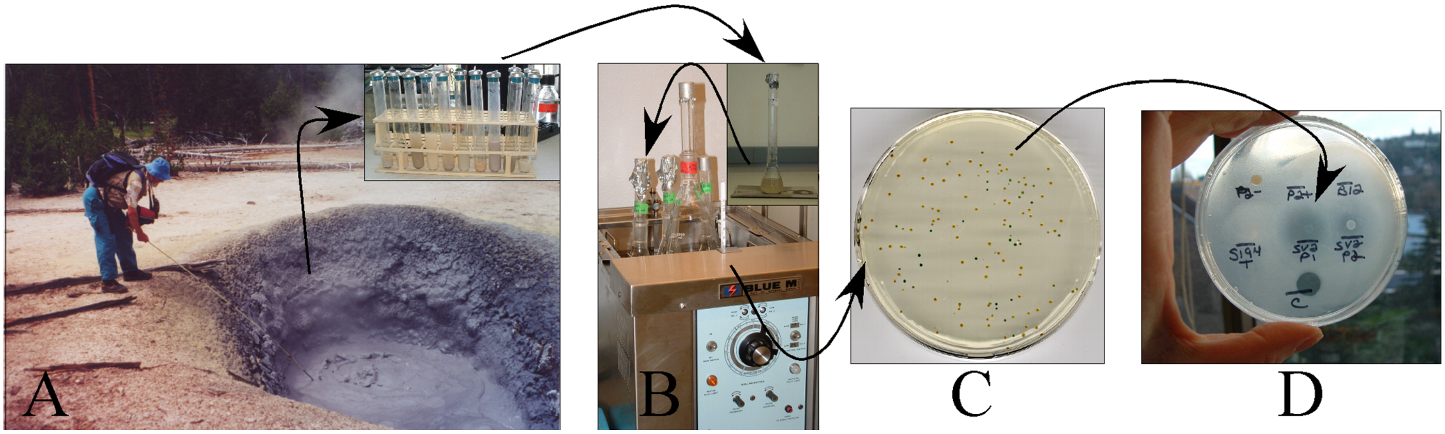

Fig. 1: Pictorial overview of isolation of *Sulfolobus* viruses. (A) Wolfram Zillig sampling at a typical *Sulfolobus*-containing pool in Yellowstone National Park, USA, September 2000 (inset shows anaerobic tubes with samples). (B) 80°C incubator with long-necked growth flasks (detail in inset). (C) Single-colony 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.

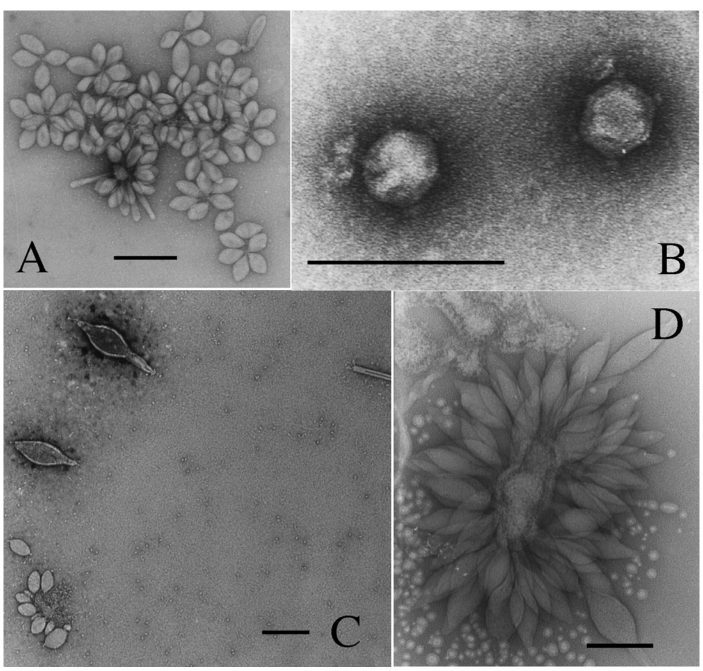

Fig. 2: TEM of *Sulfolobus* viruses and VLPs. (A) *Sulfolobus* spindle-shaped virus SSV-I2 particles. (B) *Sulfolobus* turreted icosahedral virus (STIV). (C) Three different VLPs from an enrichment culture from Amphitheater Springs, Yellowstone National Park, USA. Note end of a *Sulfolobus islandicus* 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.

Plating on Gelrite plates for host and virus isolation—Plates are made by slowly adding 6–10 grams/L Gelrite (Kelco) to Sulfolobus media (*see* above) and boiling until dissolved. Gelrite, a xanthan gum, is used instead of agar because set Gelrite plates remain solid up to 90°C. 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). 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. Before the gel solidifies, ca. 25 mL is poured into standard (90 mm) Petri plates with cams. After the Gelrite solidifies, plates can be stored at 4°C indefinitely. 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. Plates are incubated inverted in airtight moist containers at 75–80°C for approximately 1 week before colonies appear (Fig. 1C). 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.

Spot-on-lawn (halo) assay for screening enrichment cultures and isolates for viruses—This protocol is based on Schleper et al. (1992) as modified by Stedman et al. (2003). Gelrite plates are preincubated ca. 10 min at 80°C to dry, then 10 mL of Sulfolobus medium with ca. 0.2 % (w/v) Gelrite is boiled to dissolve the Gelrite. This "softlayer" is allowed to cool slightly (to ca. 80°C). Approximately 3 mL of softlayer are added to ca. 0.2 mL of exponentially growing host cells, generally *Sulfolobus solfataricus*, and spread on a plate by swirling. After the Gelrite solidifies, 1–2 µL of culture or supernatant to be screened is spotted on the plate. For a positive control, 1 µL of a 0.01% (v/v) Triton X-100 solution is spotted. Plates are incubated as above for 2–3 d and plates examined for clearing around spots (Fig. 1D).

Electron microscopy for virus identification and virus assemblage characterization—Generally, 5µL of an enrichment culture, or

0.2 µm filtered and centrifuged (10 min at 3000*g*) cell-free supernatant, is spotted onto carbon/formvar-coated electron microscope grids (Ted Pella or EM Sciences), allowed to absorb for 2 min, and then stained with 0.2% (w/v) uranyl-acetate for 15–30 s. Samples are examined by transmission electron microscopy (TEM), e.g., JEOL 100 cx, operated at 100 keV. VLPs can generally be discerned at ×16,000–20,000 magnification (Fig. 2). Generally this method is successful only if there is an indication for the presence of virus, for instance a halo on a lawn. Even when halos are formed, finding viruses by TEM can be challenging; often supernatants are concentrated 10- through 1000-fold by ultrafiltration or ultracentrifugation (Rice et al. 2001).

Viruses from hypersaline waters—Artificial salt water and medium MGM: Artificial salt water solutions are designed to mimic the natural concentrated brines where haloarchaea are found. The formulation used by M. Dyall-Smith (described in the online handbook, the *Halohandbook*, http://www.haloarchaea.com/resources/halohandbook/) is based on that described by Rodriguez-Valera et al. (Rodriguez-Valera et al. 1980; Torreblanca et al. 1986). Per liter, it contains 4 M NaCl, 150 mM $MgCl_2$, 150 mM $MgSO_4$, 90 mM KCl, 3.5 mM $CaCl_2$, adjusted to pH 7.5 using ca. 2 mL 1 M Tris-HCl (pH 7.5). At 30% w/v, the total salts are present in a much higher concentration than in seawater, but in approximately the same proportions. Adjustments of Mg^{2+}, pH, or other conditions may be necessary for specific haloarchaeal groups.

Modified growth medium (MGM) contains 5 g peptone and 1 g yeast extract per liter of salt water. The salt concentration is varied according to the host strain (*see* below), and is detailed in the *Halohandbook*.

Isolation of haloviruses from natural waters—Salt lake samples are collected from hypersaline waters, which typically range from 15% w/v total salt, up to saturation (ca. 35%). Samples are collected in sterile 5–10 mL vessels and may be stored for several weeks at room temperature. In the laboratory, cells and cellular debris are removed by centrifugation (5,000*g*, 10 min, room temperature). The supernatant is then screened directly for viruses by plaque assay. The use of chloroform is avoided, because it is known to have a detrimental affect on both phage-like and lipid-containing haloviruses. The choice of host strains depends on the experimental objectives, and includes well-characterized members of the Halobacteriaceae, such as *Hbt. salinarum* (host for ΦH and several others) or *Har. hispanica* (host for SH1, His1, His2, and others), or natural isolates from the same source, such as *Hrr. coriense* (host for HF2). To maximize isolation success, several hosts should be used for the same sample. The advantage of the use of characterized hosts is that methods for genetic manipulation are often established and their genome sequences have been determined.

Base and overlay plates (90 mm) are made with MGM (*see* above), solidified using 1.5% w/v agar. A range of salt water concentrations should be examined, because salt concentration seems to greatly affect the size and clarity of plaques. Using salt water concentrations that are 2% to 5% lower than the optimum for host growth commonly gives better plaques. Incubation temperature is also important, because some haloviruses plaque poorly or not at all at 37–42°C, whereas they form clear plaques at 30°C. Plates can be stored indefinitely at 4°C (wrapped in plastic to prevent dessication), but should be warmed to room temperature or warmer for use. For virus isolation, 100–500 µl of the cleared water sample is mixed with 150 µl of exponentially growing host cells. These may be characterized strains of haloarchaea, or natural isolates, for example, isolates from the natural water sample. Then, 3–4 mL of molten (50°C) top-layer MGM (with 0.7% w/v agar) is added, and the solution mixed gently and poured evenly over the plate. After setting on a level surface for 5–10 min, plates are incubated aerobically, inverted in airtight containers at 30°C and 37°C for 1–4 d, and checked every day for plaques.

Any visible plaques are picked using sterile glass Pasteur pipettes, or sterile plastic micropipette tips. These agar plugs are then transferred to tubes containing 500 µl of halovirus diluent (2.47 M NaCl, 90 mM $MgCl_2$, 90 mM $MgSO_4$, 60 mM KCl, 3 mM $CaCl_2$, 10 mM Tris-HCl pH 7.5), and vortexed to homogenize the sample. These suspensions are then replaqued on overlay plates to purify the isolates and to eliminate "false plaques" caused by artifacts in the agar overlay or contaminants in the water sample.

Isolation from lysogens—Several haloviruses have been isolated from laboratory strains of haloarchaea. Most were inadvertent discoveries, based on the spontaneous lysis of the host culture (e.g., ΦH, ΦCh1), or the detection of virus particles in purified preparations of flagella (Hs1). A more systematic approach would be to use induction by mitomycin C, and then to plaque cell supernatants on related (nonlysogenic) host strains. Indeed, this has been recently used to isolate a new halovirus, SNJ1 (Mei et al. 2007), from a strain of *Natrinema*, and could be used more widely.

Electron microscopy—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 following method for examining haloviruses was adapted from that described by Tarasov et al. (2000). 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. 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. After air drying, grids may be examined by transmission electron microscopy, as described above (Fig. 3A, B, and C).

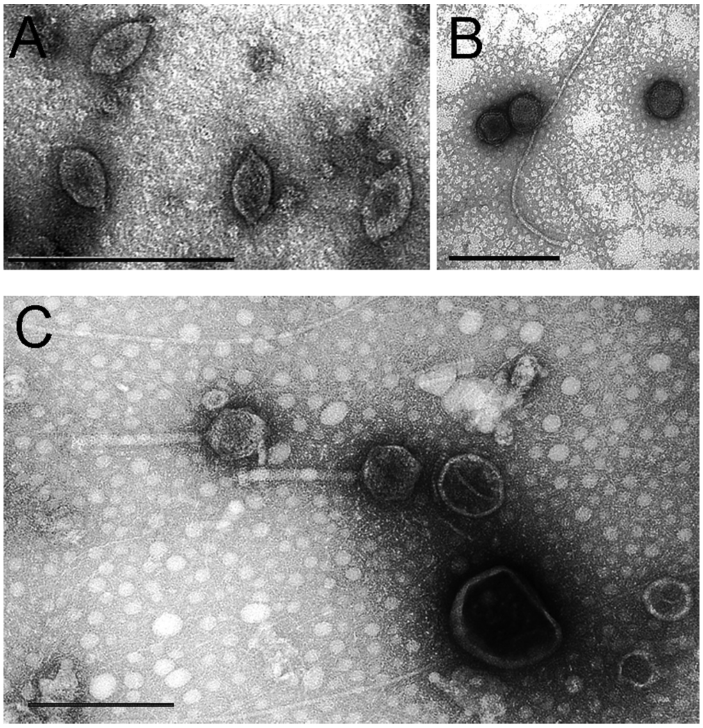

Fig. 3: Electron micrographs of haloviruses and VLPs. (A) Spindle-shaped particles of His1 virus (host is *Har. hispanica*). (B) Spherical particles of SH1 virus. Also seen is a flagellar filament from the host (*Har. hispanica*). (C) Head-tail VLPs, and other structures, seen in a natural hypersaline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

Assessment

Isolation of viruses of thermophilic Archaea—Use of the methods described above has been highly successful with the unprecedented discovery of three new virus families (*Fuselloviridae*, *Rudiviridae*, and *Lipothrixviridae*), one floating genus (*Guttaviridae*) and one proposed family (*Turriviridae*) of viruses just from *Sulfolobus* hosts (reviewed in Stedman et al. 2005). Approximately 10% of samples collected from Icelandic and other hot springs yielded viruses or other extrachromosomal elements on enrichment (Stedman et al. 2005). Anaerobic enrichment by Prangishvili and coworkers using otherwise similar procedures has allowed the isolation of three additional virus families (Prangishvili et al. 2006). A plethora of Fuselloviruses have also been isolated (Martin et al. 1984; Schleper et al. 1992; Arnold et al. 1999; Stedman et al. 2003; Wiedenheft et al. 2004; Stedman et al. 2006; Peng 2008) (Fig. 2A). Despite this success, eight new virus families each with a different morphotype, and on the order of 30 unique viruses, this is undoubtedly an undersampling of the diversity and prevalence of viruses in acidic hot springs, let alone in other environments. It is highly likely that these techniques and modifications thereof will allow the isolation of more and diverse viruses. The current limitation seems to be more lack of manpower than technique. Beyond manpower, more progress in host isolation and cultivation is likely to be the most critical step in allowing the discovery of more viruses.

Isolation of haloviruses—In the early days of halovirus research (1974–1993), deliberate virus isolation from natural hypersaline waters was uncommon. Major exceptions to this were the superb ecological studies of haloviruses reported by Daniels and Wais, who isolated *Halobacterium* species and their viruses from Jamaican salt lakes and noted the significant effect of salt concentration on virulence (Wais et al. 1975; Wais and Daniels 1985; Daniels and Wais 1990). Currently, there are only around 21 described haloviruses, of which 17 belong to the *Caudovirales*, 2 are members of the *Salterprovirus* group, and two are as yet unclassified (SH1, HRPV-1). The more recent isolates are morphologically and genetically more diverse (spindle and round morphotypes), and most were isolated directly from hypersaline water sources using methods described above (Porter et al. 2007). Currently, about 10 haloviruses are under active study (ΦCh1, BJ1, HF1, HF2, His1, His2, HRPV-1, SNJ1, and SH1), and these examples encompass the three known dominant morphotypes—head-and-tail, spindle-shaped and round—of haloviruses so far observed by direct EM of natural waters (Guixa-Boixareu et al. 1996; Oren et al. 1997; Diez et al. 2000; Santos et al. 2007). By negative stain TEM, HRPV-1 particles are reported to be pleomorphic (Pietila et al. 2009), but because this method often distorts particles (because of the low salt), it will be important to confirm this by cryo-EM. Filamentous VLPs, observed by F. Santos and colleagues (Santos et al. 2007) must be isolated to prove that they are not dissociated tail fragments from the head-and-tail VLPs. Nevertheless, because the cultivation barrier of haloarchaea has recently been overcome (Bolhuis et al. 2004; Burns et al. 2004a; Burns et al. 2004b), a better representation of the true viral population of salt lakes is now possible, and progress in field should improve dramatically.

Discussion

Isolation of viruses of thermophilic Archaea—Sulfolobus are often not the dominant organisms in hot springs with temperature >70°C and pH <4 (Snyder et al. 2004). Furthermore, *Sulfolobus* virus sequence diversity decreases with enrichment (Snyder et al. 2004). Therefore it is likely that the viruses reported to date are considerably fewer and less diverse than the viruses present in situ. Direct TEM imaging of concentrated samples from both acidic and neutral hot springs indicate that a number of viruses with novel morphology remain to be isolated. (Rice et al. 2001; Rachel et al. 2002).

Comparison of methods for isolation of haloviruses and viruses of thermophilic Archaea—The main difference between the two main techniques described here is due to the relative abundance of viruses and VLPs in the environments of the hosts. There are many more viruses in hypersaline environments than in thermoacidophilic ones. Therefore direct isolation has been successful for haloviruses, but not for viruses of thermoacidophiles. The conditions for host growth are also very different. Halovirus hosts grow at moderate temperatures, but at saturating salt conditions, whereas the thermoacidophiles grow only at temperatures greater than 70˚C, requiring the use of Gelrite for plates and lawns and the use of long-necked flasks and PEG400 bath fluid for liquid culture.

Isolation of viruses from other Archaea—The *Sulfolobales* are relatively well studied, but are only one relatively small group of Archaea (Huber and Stetter 2001). Of the other Archaea, only the viruses of extreme halophiles viruses have been studied in any depth (Porter et al. 2007). A few VLPs have been observed and one genome has been sequenced from enrichment cultures from deep-sea hydrothermal vent samples at very high temperatures (Geslin et al. 2003a; Geslin et al. 2003b; Geslin et al. 2007). Very little work has been done with methanogen viruses. Those that have been characterized appear to be more like bacterial Caudoviruses than the characterized viruses of thermoacidophilic Archaea. There are two exceptions, the VLP reported by Wood et al. (1989), and two possible proviruses in the *Methanococcus voltae* A3 draft genome sequence (Krupovic and Bamford, 2008). Nothing is known of viruses of the extremely abundant mesophilic Archaea that are present in soils and the oceans (reviewed in Chaban et al. 2006). The long-awaited isolation of one of the latter, *Nitrosopumilus maritimus* by Stahl and coworkers, should allow screening to take place (Konneke et al. 2005).

The genome sequences of many uncultured Archaea may provide clues from possible proviruses in their sequences that will allow the molecular screening of virus-sized samples from the oceans or soils without the need for cultivation of hosts, which is the critical bottleneck in the study of archaeal viruses. Additionally, metagenomic projects allow the identification of further viruses. For example, a halovirus-like sequence, EHP-1, has been recovered directly from a crystallizer pond, although the virus itself has yet to be isolated (Santos et al. 2007). Recently the genome of an apparently archaeal virus from an extremely acidic acid mine drainage site was cleverly determined by analysis of CRISPR sequences in a metagenome databank (Andersson and Banfield, 2008). The human gut metagenome project may also provide some clues to the presence of currently undetected viruses of Archaea. Methanogens are associated with gum disease (Lepp et al. 2004) and have long been known to be in human gut samples (e.g., Nottingham and Hungate 1968). Therefore, it is highly likely that their viruses are also present.

Clearly, there is a great deal remaining to be discovered in viruses of Archaea. Implementation and expansion of the methods described and proposed herein should greatly stimulate progress in the discovery, characterization and understanding of this understudied group of viruses.

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