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Review

Viruses in Extreme Environments

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Abstract

The tolerance limits of extremophiles in term of temperature, pH, salinity, desiccation, hydrostatic pressure, radiation, anaerobiosis far exceed what can support non extremophilic organisms. Like all other organisms, extremophiles serve as hosts for viral replication. Many lines of evidence suggest that viruses could no more be regarded as simple infectious “fragments of life” but on the contrary as one of the major components of the biosphere. The exploration of niches with seemingly harsh life conditions as hypersaline and soda lakes, Sahara desert, polar environments or hot acid springs and deep sea hydrothermal vents, permitted to track successfully the presence of viruses. Substantial populations of double-stranded DNA virus that can reach 10^9 particles per milliliter were recorded. All these viral communities, with genome size ranging from 14 to 80 kb, seem to be genetically distinct, suggesting specific niche adaptation. Nevertheless, at this stage of the knowledge, very little is known of their origin, activity, or importance to the *in situ* microbial dynamics. The continuous attempts to isolate and to study

viruses that thrive in extreme environments will be needed to address such questions. However, this topic appears to open a new window on an unexplored part of the viral world.

1. Introduction

Extremophiles include organisms from the three domains of life, Archaea, Bacteria and Eukarya, which thrive in extreme environments that are characterized by physico-chemical conditions close to the limit values in which an organism can live. As bacteria and archaea are almost omnipresent on the planet and have evolved for over 3.5 billion years, “extremophile” conjures up images of prokaryotes, especially from the domain Archaea. Although archaea are present in many moderate environments, they are still primarily considered extremists, flourishing in habitats that brave the physical limits for life, such as sulfur-rich hot acid springs and geysers, deep-sea environment and deep sea hydrothermal vents, hypersaline and soda lakes or strictly anoxic ecosystems (see review in Rothschild & Mancinelli 2001).

The specific feature of Extremophiles is their remarkable capabilities to adapt to extreme conditions in term of pH, salinity, desiccation, hydrostatic pressure, radiation, anaerobiosis that would be inevitably lethal for non extremophilic organisms. Extremophiles thrive at temperatures exceeding 80°C and even more than 100°C at hyperbaric pressure (extreme thermophiles in hydrothermal vents) while others live at subzero temperatures (psychrophiles in sea ice). Extreme piezophiles, which can withstand the enormous hydrostatic pressure associated with great depths, grow well in the deep sea and even in deep subsurface sediments as deep as 1000 meter below the seafloor (mbsf) under anaerobic conditions. Extremophiles are also able to cope with environments of very low water activity and develop well in desert or saturated brines (extreme halophiles) while acidophiles and alkalophiles live in extremely acid and alkaline waters at pH values below 2 and exceeding 10, respectively. In proportion to the multiplicity of physical and geochemical constraints in an extreme ecosystem, organisms that thrive under the seemingly harsh conditions are most of the times polyextremophiles.

Like all other organisms, extremophiles serve as hosts for viral replication. Viruses and virus-like elements (i.e. satellite virus, satellite RNA and viroids) are the smallest infectious biological entities (see http://www.ncbi.nlm.nih.gov/ICTVdb/origin2.htm). Since they are not autonomous - they depend on a cellular host for replication - viruses have been considered as not really alive for a long time. Many lines of evidence have definitely suggested that they could no more be regarded as simple infectious “fragments of life” but on the contrary as one of the major components of the biosphere, who have probably played a key role in the early cellular evolution and that have a profound influence on cellular life (e.g. genome plasticity, biochemical adaptations required to life in extreme environments).

Viruses exist wherever cellular life is found and span the three domains of life. But the extent of viral ubiquity and diversity still remains largely unknown. The recently accumulated knowledge on the number of viruses, from marine environments at least, shows that they probably encompassed all other forms of life in abundance on the earth and represent a vast reservoir of biodiversity (Fuhrman 1999; Wommack & Colwell 2000; Weinbauer 2004; Rohwer 2003; Suttle 2005; Edwards & Rohwer 2005; Breibart & Rohwer 2005). The universal tree of life can thus be considered as immersed into a virtual viral ocean (Bamford 2003). As new niches are explored for life, especially in extreme environments, presence of viruses is readily detected and an amazing number of (new) viruses is discovered.

The aim of this present mini-review, that was inspired following discussions at the workshop entitled “Investigating Life in Extreme Environments”, organised by the European

Science Foundation (Sant Feliu de Guixols, Spain, 5-8 November 2005), is to give a brief overview of the recent findings about viruses thriving in extreme conditions.

2. Extreme halophilic viruses

Liquid water is an absolute requirement for metabolic activity and growth. The high concentration of ions in hypersaline environments is one of the major factors affecting microbial activity because the dissolved substances make the water partly unavailable to microorganisms. Hypersaline habitats, which can vary considerably in ionic composition, are rather common in hot, dry areas throughout the world. Despite seemingly harsh conditions, these environments can be productive ecosystems where halophiles that include a range of organisms (archaea, green algae, cyanobacteria, bacteria) easily cope with osmotic stress and even can withstand in saturated NaCl (Madigan et al. 2003). If the first extremophilic and halophilic virus was discovered fortuitously in 1974, consistent reports on the occurrence of viruses in such extreme habitats raised in the early 1980s from halobacteria (Dyall-Smith et al. 2003).

2.1. Viruses in hypersaline environments

Assessing the viral abundance in the hypersaline Dead Sea where magnesium concentration exceeds 50%, quantities of virus-like particles that reach easily 10^7 particles ml^{-1} were reported (Oren et al. 1997). In their study of solar salterns, Guixa-Boixareu and co-workers (Guixa-Boixareu et al. 1996) showed that both virus-like particles abundance and diversity increased with salinity and reached about 10^9 virus particles ml^{-1} at salinities higher than 25%. Hypersaline environments are also important reservoirs of viruses that exhibit a large genomic diversity with genome sizes varying from 10 kb to 533 kb. (Sandaa et al. 2003). Pulsed-field electrophoresis analysis showed that the viral population structure vary along a salinity gradient from near seawater (40 ‰) to saturated sodium chloride brine (370 ‰). Populations of virus-like genome ranging in size from 32 kb to 340kb were preponderant within 40 ‰ to 220 ‰ salinity gradients, whereas ponds with salinity higher than 220 ‰ contained virus-like genomes with size ranging from 10 to 189 kb. As changes in the total prokaryotic community structure depending on salinity were also recorded, this suggests that viral populations have a dynamic, which probably depends on their hosts' ecology (Sandaa et al. 2003).

Considering the morphological diversity of viruses in hypersaline environments, direct observations with electronic microscope revealed a majority of lemon-shaped particles resembling the archaeal Fuselloviruses, while only some virus-like particles were of head-tail morphology (Oren et al. 1997). In other hand all halophilic viruses isolated from this type of habitat until now infect archaea, most of them (12/15) have a head-and-tail morphology (Fig. 1, Table 1) reminiscent of bacteriophages belonging to the three main families *Myoviridae*, S*iphoviridae* and *Podoviridae*, highlighting the remarkable morphological similarity between archaeal and bacterial tailed phages. Only three viruses exhibiting different morphotypes more closely related to those of hyperthermophilic archaeoviruses were also characterized. These haloviruses were the spindle-shaped His1 (Fig. 2), His2 which is pleomorphic and the spherical SH1 (Fig. 3). Such differences between direct observations and laboratory specimens suggested that characterized viruses probably did not reflect the real *in situ* morphological diversity. The bias resides perhaps in the fact that hosts cells easily isolated and cultivated in laboratory are not the dominant species of the natural haloarchaeal flora in hypersaline environments (Dyall-Smith et al. 2003).

All halophilic viruses described until now have genomes which consist of linear double-stranded DNA. Looking at the genome sequences, only little sequence similarity (less than 10%) with bacteria, bacteriophages and eukaryotic viruses were observed. This phenomenon could be partly due to isolation caused by such particular ecosystem (Dyall-Smith et al. 2003). However, there are also strong genetical relationships between different haloviruses, as shown by the haloviruses ϕCh1 and ϕH which share up to 97% nucleotide identity, while their hosts, isolated from distinct and geographically distant sites, are phylogenetically different (Klein et al. 2002, Tang et al. 2002). The haloviruses HF1 and HF2 also have genomes that share up to 99 % nucleotide identity in the first 60 % of their sequence. However, the remainder part shows a significant divergence (87 % identity) due to numerous base changes and insertion/deletion events. This significant shift in sequence similarity suggests a recent recombination event between either the two halovirus or with another HF-like halovirus. This recombination occurrence seems to be rather common among viruses from hypersaline waters (Tang et al., 2004, Bath et al. 2006).

2.2. Viruses in alkaline lakes

Even if the water chemistry of soda lakes is similar to hypersaline lakes, solar salt evaporation ponds and deep-sea hypersaline basins, alkaline lakes differ by the high levels of carbonate minerals in the surroundings rocks that maintain pH ranging between 10 and 12. In addition, Ca^{2+} and Mg^{2+}

are virtually absent because they precipitate out at high pH and carbonate concentrations (Madigan et al. 2003).

Bacterial abundances and seasonal changes in community composition were recorded in the past decades, but no previous reports on the occurrence of viruses merged before 2004 from such extreme habitat until Jiang and co-workers tackled the virus populations in Mono Lake, which is a large alkaline (pH~10), moderate hypersaline lake lying at the western edge of Great Basin in California (Jiang et al. 2004).

In this peculiar environment, viral abundance (from 1.10^8 to 1.10^9 ml^{-1}) is among the highest observed in any natural aquatic system examined so far. Pulse-field gel electrophoresis revealed length of dsDNA viral genomes ranges from 14 up to 400 kbp, with a majority between 30 kpb to 60 kbp and the analysis of band patterns highlighted at least three dominant clusters of populations defined on the similarities in the viral genome size distribution. Thus, deep-water viral community represents a distinct group from surface and mid-water viral communities suggesting a strong stratification of viral distribution between oxic and anoxic waters.

To date, only one lytic phage, named ϕMono1, has been isolated and partly characterized from this viral population (Table 1). Surprisingly, this virus strain infects a bacterial host, which is closely related to *Idiomarina baltica* previously isolated from surface water of the central Baltic Sea. Using ϕMono1 dsDNA genome as probe in hybridization experiments also revealed seasonal fluctuations in viral communities.

3. Viruses in deserts

In deserts, that are extremely dry and exposed to extremes of UV light irradiation and temperature variation, water is always a very limiting factor for life. Nonetheless, eukaryotic and prokaryotic microorganisms have adapted to these extreme conditions and have been found in hot desert such as the Atacama Desert of Chile (Evans & Johansen 1999).

A recent study, carried out on surface sands collected from 13 different locations in the Sahara Desert in Morocco and Tunisia, reported for the first time the presence of virus-like particles. These particles exhibit a great diversity of morphotypes representative of the three major bacteriophage families: *Myoviridae*, *Siphoviridae* and *Podoviridae* (Table 1). In addition, pulse-field gel electrophoresis of double-stranded DNA, extracted from the enriched bacteriophages preparations, suggests also a genetic diversity with the presence of at least four potential intact viral genomes ranging in size from 45 kpb to 270 kpb (Prigent et al. 2005).

4. Viruses in polar environments

Extreme cold environments such as high-altitude glaciers, polar permafrost, the Dry Valleys of Antarctica, which are the coldest and driest desert on the earth, as well as sea ice, also provide habitats from microbial life (Staley & Gosink 1999). Annual sea ice in the Arctic develops important and dynamic microbial communities (Grossi et al. 1984; Kottmeier et al. 1987; Smith et al. 1989). In Antarctica, microorganisms, including prokaryotes and microeukaryotes thrive in sea ice and cold water (Thomas & Dieckmann 2002). Several well-documented studies reported the presence of viruses and the relationship between viral and bacterial production in Arctic and Antarctic sea ice and in perennially ice-covered lakes located in Taylor Valley, Antarctica.

In Artic sea ice, viral abundance was recorded to be very high as showed by direct counts (9.10^{6} ml^{-1} to 3.10^{8} ml^{-1}). This value, which was 10- to 100-fold greater than the concentration of viruses in the underlying sea water ($1.1.10^{6}$ ml^{-1}), corresponded with the bacterial abundance in sea ice compared to the water column. Viral proliferation appeared to be enhanced in sea ice relative to open water. Moreover, the virus-to-bacteria ratios were among the highest reported in natural samples, providing the first account of viruses as a dynamic component of sea ice microbial communities (Maranger et al. 1994).

Three distinct phage-host systems (Table 1), which are highly dependent of low temperature conditions, were also isolated and characterized from samples of Artic sea ice collected in north-west of Svalbard. The hosts are psychrophilic bacteria whose closest relatives are *Shewanella frigidimarina*, *Flavobacterium hibernum* and *Colwellia psycherythrae*, respectively. The three phages, which are lytic and host-specific, showed an even more pronounced adaptation to cold temperatures than their hosts did. In fact, phage development was clearly restricted to a lower temperature maximum in comparison to the maximal growth temperature of the host bacterium. Transmission electron microscopy (TEM) observations revealed that these polar phages having a dsDNA genome are morphologically similar to the double-stranded DNA phage families *Siphoviridae* and *Myoviridae* and (Borriss et al. 2003).

Interestingly, samples of Ross Sea pack ice in Antarctic revealed that the range of total viral abundance was similar with the concentration found in Artic sea ice (between 5.2 10^6 ml^{-1} to 3.5 10^8 ml^{-1}). TEM observations showed that the viruses, which compose the population, are large, with 40% icosahedral, 37% spherical and 23% lumpy forms, and all of them likely infect microeukaryotes (Gowing 2003).

In Antarctic perennially ice-covered lakes, which are microbially dominated ecosystems, virus densities seemed to be less important than in sea ice with a maximum value that reached 3.4 10^7 ml^{-1}. Nevertheless, this virus abundance was higher than in other freshwater or marine systems and the viral population appeared to be highly active in the water column. Many of viruses were found to be large icosahedral specimens, morphologically similar to double-stranded DNA viruses isolated from temperate environments that infect photosynthetic and non-photosynthetic flagellates (Kepner et al. 1998).

5. Viruses in deep subsurface sediments

The deep subsurface biosphere is one of the least-understood habitats on Earth, even though the huge microbial biomass therein likely plays an important role on global biogeochemical cycles. Recently, the Ocean Drilling Program (ODP) revealed that chemolithotroph microbes thrive in anoxic reducing environments under oceans and continents to depths of >1000 m despite harsh conditions (i.e. high hydrostatic pressure, anaerobiosis and low concentration in organic nutrients). Prokaryotic biomass in deep marine sediments exceeds 10^5 microbial cells cm^{-3} even at depths close to 1,000 mbsf (Parkes et al. 1994, 2000).

Presence of viruses in buried marine sediments was investigated recently after drilling a hole at 228.7 meters below sea seafloor to a depth at 105.1 mbsf and 118.2 mbsf, near the west Canadian coast (Bird et al. 2001). Analyses revealed the existence of large amounts of viruses. Viral abundances appeared to follow bacterial numbers very closely with an average up to 10^9 g^{-1} of dry sediment at 105.1mbsf. Even if microbial communities seemed to be stratified in subseafloor sediments, nothing is known about the viral diversity and the interactions between viral and prokaryotic communities. Nonetheless, given the scarcity of eukaryotic bacterivores in deep marine sediments, the only source of mortality by external agents for the bacterial community lies in phage attack. Thus, considering bacterial and viral abundances being highly correlated, viruses appear to be potential actors of subsurface sediments biogeochemistry.

6. Viruses in extreme thermal environments

Live has also adapted to hot temperatures. Given that early branching organisms could have been hyperthermophiles among *Archaea* and thermophiles among *Bacteria*, looking for viruses in such extreme environment may provide interesting information about virus evolution in the early cellular life. The recent observations indicated that terrestrial and oceanic hydrothermal environments represent a bottomless reservoir of a truly remarkable morphological and genomic viral diversity.

6.1. Viruses from terrestrial hot springs

Early studies on viruses of hyperthermophiles were pioneered in the laboratory of Wolfram Zillig in the 1980s. A systematic screening of surface hot springs located in Japan, Iceland, New Zealand, Italy, Russia and the United States led to the isolation of an unprecedented diversity of new viruses (Fig. 4) (Rice et al. 2001 ; Rachel et al., 2002 ; Prangishvili and Garrett, 2005).

The vast majority of the hyperthermophilic viruses isolated from acidic or neutral hot springs (>80°C) were found to infect a broad spectrum of members of the extremely thermophilic *Crenarchaeaota*, including representatives of the genera *Sulfolobus*, *Thermoproteus*, *Acidianus, Pyrobaculum* (Table 1). Based on their exceptional morphology and genomic properties the crenarchaeal viruses were classified in 7 new families which include: lemon-shaped *Fuselloviridae*, filamentous *Lipothrixvidae*, stiff rod-shaped *Rudiviridae*, droplet-shaped *Guttaviridae*, spherical *Globuloviridae*, two tailed spindle-shaped *Bicaudaviridae* and bottle-shaped *Ampullaviridae*. The International Committee of Taxonomy of Viruses has already approved the first four families. The crenarchaeal viruses showed no clear similarities in their morphologies or at the genomic level to either bacterial or eukaryal viruses, except perhaps members of three viral families. The rod-shaped virions of the *Rudiviridae* and *Liphotrixviridae* resemble tobamoviruses and closteroviruses of vascular plants, respectively, while those of the *Globuloviridae* resemble that of viruses of the *Paramyxoviridae*, which infect vertebrates. The 25 hyperthermophilic viruses isolated so far exhibited double-stranded DNA genomes, linear or circular of 15 to 75 kb, most of them being sequenced and revealing an amazing diversity at the genomic level (Prangishvili et al. 2006). Few significant sequence matches were obtained with either bacterial or eukaryal genes and very few genes have been assigned functions. However, there is some evidence that a 37-kDa coat protein of the *Sulfolobus* turreted icosahedral virus (STIV) can generate a tertiary and quaternary structure similar to that of capsid proteins of bacterial and animal viruses, despite the lack of significance gene similarity. This suggests that some viruses may have a common ancestor that precedes the division into three domains of life (Rice et al. 2004; Khayat et al. 2005). The fact that for most of these viruses, analysis of their genomes showed little or no similarity to genes in the public databases suggests that all these newly discovered viruses employ novel biochemical mechanisms for viral functions.

All viruses of acidophilic hyperthermophiles (except TTV1 and ATV) are non lytic and persist in host cells in a stable state (pseudolysogeny or “carrier state”). It was hypothesized that such a survival strategy was beneficial for viruses, helping them to avoid direct exposure to the harsh conditions of the host habitat (Prangishvili and Garrett 2004, 2005).

However, hyperthermophilic viral populations, which can reach concentrations of a million viruses per milliliter, were also reported to be resistant to shifts to lower temperature in their natural ecosystem (Breitbart et al. 2004). Breitbart and co-workers showed that more than 75 % of phage particles collected from Californian hot springs remained physically intact when incubated on ice. Moreover, they are dynamic and actively produced *in situ* with a population turnover time of one or two days. As viruses are the only known microbial predators in this extreme environment, they exert likely an important influence on the microbial community via a high virus-mediated microbial mortality.

6.2. Viruses from deep sea hydrothermal vents

Deep-sea-vent areas are one of the most extreme habitats on Earth. They are characterized by high hydrostatic pressures, hot (400°C) to warm (10-30°C) temperatures and the hydrothermal fluids are acidic, reduced and enriched with chemicals including heavy metals, methane and hydrogen sulphide (Prieur 1997).

Recently, systematic searches carried out on samples collected in various geographically distant hydrothermal sites revealed high and unexpected abundance and diversity of viruses in deep-sea hydrothermal vents. Viral abundance was recorded to be high as showed by direct counts ($1.45.10^5$ to $9.9.10^7$ ml^{-1}). High viral abundance at active vents, relative to those in surrounding waters, indicated viral production and hence, virus mediated microbial mortality (Ortmann and Suttle 2005).

Considering the morphological diversity, direct observations with electronic microscope revealed a great morphological diversity. With the exception of the filamentous and rod-shaped morphotypes which are also known for the *Bacteria*, the morphologies seemed to be characteristic of archaeal viruses. Indeed, the lemon-shaped type prevailed and novel pleomorphic morphologies such as “spoon-shaped” and spindle particles with bipolar expansions were also discovered. The exotic morphological similarities exhibited by viruses from both deep-sea and terrestrial hot environments are very astonishing. For example, the presence of lemon-shaped viruses in diverse extreme environments (salterns, subsurface anaerobic sediments, acidic thermophilic continental solfatara and deep-sea vents) in addition to the fact that this morphotype has never been found among the *Bacteria* or *Eucarya* strengthens the idea of their specificity to the archaeal domain and probably reflects a deep evolutionary history within this domain (Geslin et al. 2003a).

One of these deep-sea hyperthermophilic viruses was successfully purified and was further characterized (Table 1). This virus, named PAV1, is lemon-shaped (120 nm x 80 nm) with a short tail terminated by fibers and infects the hyperthermophilic euryarchaeota *Pyrococcus abyssi*. PAV1 persists in the host strain in a stable carrier state. PAV1 genome consists of a double-stranded circular DNA of 18 kb, which is also present in high copy number in a free form in the host cytoplasm. Viral genome comparisons with all other archaeal, bacterial or eukaryal viruses do not reveal any significant similarity (Geslin et al. 2003b).

7. Concluding remarks

Despite the ubiquity of viruses, until recently relatively little was known about viruses in extreme environments because in many instances the extreme growth conditions required by extremophiles have precluded a search for viruses. However, over the past few years our knowledge of viruses in extreme environments considerably increased. Tracking viruses in ecological niches with seemingly harsh conditions has been successful and the presence of virus populations has been consistently detected in all the explored environments. All viral communities appeared to be substantially abundant to the populations rate that are often greater than in standard environments (e.g. 10^9 ml^{-1} in solar salterns, 3.5 10^8 ml^{-1} in Antarctic sea ice). All viruses isolated so far from extreme environments are double-stranded DNA viruses with moderate genomic complexity (the genome size range from 14 to 80 kb). It is conceivable that this very stable form of genome may be necessary to face harsh constraints of extreme habitats. It could also explain why no RNA virus has been isolated yet, especially from hot environments. However, PFGE analysis used to depict the viral community structure (e.g. in desert and hypersaline habitats environments) produces evidence of a more complex diversity with the recovering of uncharacterized large dsDNA viruses.

The viral communities seem also to be genetically distinct, suggesting specific niche adaptation and great diversity. Nevertheless, at this stage of the knowledge, little is known of their origin, activity, or importance to the *in situ* microbial dynamics and continuous attempts to isolate and to study viruses that thrive in extreme environments will be needed to address such questions. Moreover, several terrestrial extreme environments are still unexplored, e.g. evaporites, subglacial Antarctic lakes like Lake Vostok, where the DNA signature of a thermophilic bacteria (*Hydrogenophilus sp.*) has been detected (Bulat et al. 2004) or the stratosphere and its airborne biota.

Exploring the virus diversity in extreme environments, the description of an amazing number of new and extraordinary archaeal viruses isolated from terrestrial hot springs especially appears as a benchmark discovery that open a new window on an unexplored and very intriguing part of the viral world (Prangishvili et al. 2006).

More than 85% of the viral genomic sequences lack similarity to previously reported sequences. Thus, the genome of hyperthermophilic viruses and that of any other virus that thrives with extreme conditions probably contains an astronomical number of still unknown proteins. Although some of these proteins could be functional analogues of already known proteins, it would be not surprising to discover proteins encoding novel functions. This exceeds previous results from viral metagenomic analyses (68%) and reinforces the view that viruses represent by far the largest unexplored reservoir of genomic diversity on Earth (Edwards & Rohwer, 2005). This constitutes an important issue for further research aimed at understanding the origin of viruses and early life evolution but also for practical purposes such as identification of new enzymatic tools useful for the manipulation of DNA *à façon*.

Extremophiles are probably among the earliest forms of cellular life on Earth that still thrive in a wide range of extreme environments. Therefore, understanding their biology would allow developing hypotheses regarding the conditions required for the origination and early diversification of cellular life on Earth. Even if our perception of the existing viral diversity in extreme ecosystems is still scarce, the recent findings contribute to raise challenging questions about the role of viruses in the early cellular life.

Considering the last updated Forterre’s scenario (Forterre, 2006) which hypothesised that viruses have played a key role in both RNA-to-DNA transition and in emergence of the three cellular domains presently known, the research on viruses is entering a new exciting stage. The study of the biology and ecology of new viruses isolated from extremophile environments may shed light on the early biological processes as well as on viral evolution.

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Figures

Figure 1: Negative stain electron microscopy of head-and-tail halovirus HF2. Scale bar represents 100 nm.
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Figure 2: Negative stain electron microscopy of spindle-shaped halovirus His1. Scale bar represents 100 nm.
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Figure 3: Negative stain electron microscopy of spherical halovirus HS1. Scale bar represents 100 nm.
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Figure 4: Transmission electron microscopy of virus and virus-like particles isolated from Yellowstone National Park.

(A) SSV1 *Fusellovirus*, (B) SIRV *Rudivirus* and (C) SIFV *Lipothrixvirus* previously isolated from thermal area of Japan or Iceland. (D) SSV-like, (E) SIRV-like and (F) SIFV-like particle morphologies isolated from Yellowstone National Park thermal features. (G-I) Virus-like particles isolated from Yellowstone National Park thermal features. Bars indicate 100 nm.

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TABLE:

Table 1: Main features of the extremophilic viruses so far characterized in extreme environments.