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1 Review

2 Viruses in Extreme Environments

3  
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1 Key words: bacteriophages, deep sea subsurface  
2 environment, deserts, extreme environments, hot springs,  
3 hydrothermal vents, hypersaline habitats, polar ecosystems,  
4 viral diversity, viral abundance.

## 6 **Abstract**

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

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

## 4 5 **1. Introduction**

6  
7 Extremophiles include organisms from the three domains of  
8 life, Archaea, Bacteria and Eukarya, which thrive in extreme  
9 environments that are characterized by physico-chemical  
10 conditions close to the limit values in which an organism can  
11 live. As bacteria and archaea are almost omnipresent on the  
12 planet and have evolved for over 3.5 billion years,  
13 “extremophile” conjures up images of prokaryotes, especially  
14 from the domain Archaea. Although archaea are present in  
15 many moderate environments, they are still primarily  
16 considered extremists, flourishing in habitats that brave the  
17 physical limits for life, such as sulfur-rich hot acid springs  
18 and geysers, deep-sea environment and deep sea  
19 hydrothermal vents, hypersaline and soda lakes or strictly  
20 anoxic ecosystems (see review in Rothschild & Mancinelli  
21 2001).

22 The specific feature of Extremophiles is their remarkable  
23 capabilities to adapt to extreme conditions in term of pH,  
24 salinity, desiccation, hydrostatic pressure, radiation,  
25 anaerobiosis that would be inevitably lethal for non

1 extremophilic organisms. Extremophiles thrive at  
2 temperatures exceeding 80°C and even more than 100°C at  
3 hyperbaric pressure (extreme thermophiles in hydrothermal  
4 vents) while others live at subzero temperatures  
5 (psychrophiles in sea ice). Extreme piezophiles, which can  
6 withstand the enormous hydrostatic pressure associated with  
7 great depths, grow well in the deep sea and even in deep  
8 subsurface sediments as deep as 1000 meter below the  
9 seafloor (mbsf) under anaerobic conditions. Extremophiles  
10 are also able to cope with environments of very low water  
11 activity and develop well in desert or saturated brines  
12 (extreme halophiles) while acidophiles and alkalophiles live  
13 in extremely acid and alkaline waters at pH values below 2  
14 and exceeding 10, respectively. In proportion to the  
15 multiplicity of physical and geochemical constraints in an  
16 extreme ecosystem, organisms that thrive under the  
17 seemingly harsh conditions are most of the times  
18 polyextremophiles.

19 Like all other organisms, extremophiles serve as hosts for  
20 viral replication. Viruses and virus-like elements (i.e. satellite  
21 virus, satellite RNA and viroids) are the smallest infectious  
22 biological entities (see  
23 <http://www.ncbi.nlm.nih.gov/ICTVdb/origin2.htm>). Since  
24 they are not autonomous - they depend on a cellular host for  
25 replication - viruses have been considered as not really alive

1 for a long time. Many lines of evidence have definitely  
2 suggested that they could no more be regarded as simple  
3 infectious “fragments of life” but on the contrary as one of  
4 the major components of the biosphere, who have probably  
5 played a key role in the early cellular evolution and that have  
6 a profound influence on cellular life (e.g. genome plasticity,  
7 biochemical adaptations required to life in extreme  
8 environments).

9 Viruses exist wherever cellular life is found and span the  
10 three domains of life. But the extent of viral ubiquity and  
11 diversity still remains largely unknown. The recently  
12 accumulated knowledge on the number of viruses, from  
13 marine environments at least, shows that they probably  
14 encompassed all other forms of life in abundance on the earth  
15 and represent a vast reservoir of biodiversity (Fuhrman 1999;  
16 Wommack & Colwell 2000; Weinbauer 2004; Rohwer 2003;  
17 Suttle 2005; Edwards & Rohwer 2005; Breibart & Rohwer  
18 2005). The universal tree of life can thus be considered as  
19 immersed into a virtual viral ocean (Bamford 2003). As new  
20 niches are explored for life, especially in extreme  
21 environments, presence of viruses is readily detected and an  
22 amazing number of (new) viruses is discovered.

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

1 Science Foundation (Sant Feliu de Guixols, Spain, 5-8  
2 November 2005), is to give a brief overview of the recent  
3 findings about viruses thriving in extreme conditions.  
4

## 5 **2. Extreme halophilic viruses**

6

7 Liquid water is an absolute requirement for metabolic  
8 activity and growth. The high concentration of ions in  
9 hypersaline environments is one of the major factors  
10 affecting microbial activity because the dissolved substances  
11 make the water partly unavailable to microorganisms.  
12 Hypersaline habitats, which can vary considerably in ionic  
13 composition, are rather common in hot, dry areas throughout  
14 the world. Despite seemingly harsh conditions, these  
15 environments can be productive ecosystems where halophiles  
16 that include a range of organisms (archaea, green algae,  
17 cyanobacteria, bacteria) easily cope with osmotic stress and  
18 even can withstand in saturated NaCl (Madigan et al. 2003).  
19 If the first extremophilic and halophilic virus was discovered  
20 fortuitously in 1974, consistent reports on the occurrence of  
21 viruses in such extreme habitats raised in the early 1980s  
22 from halobacteria (Dyall-Smith et al. 2003).  
23

### 24 *2.1. Viruses in hypersaline environments*

25

1           Assessing the viral abundance in the hypersaline Dead Sea  
2           where magnesium concentration exceeds 50%, quantities of  
3           virus-like particles that reach easily  $10^7$  particles  $\text{ml}^{-1}$  were  
4           reported (Oren et al. 1997). In their study of solar salterns,  
5           Guixa-Boixareu and co-workers (Guixa-Boixareu et al. 1996)  
6           showed that both virus-like particles abundance and diversity  
7           increased with salinity and reached about  $10^9$  virus particles  
8            $\text{ml}^{-1}$  at salinities higher than 25%. Hypersaline environments  
9           are also important reservoirs of viruses that exhibit a large  
10          genomic diversity with genome sizes varying from 10 kb to  
11          533 kb. (Sandaa et al. 2003). Pulsed-field electrophoresis  
12          analysis showed that the viral population structure vary along  
13          a salinity gradient from near seawater (40 ‰) to saturated  
14          sodium chloride brine (370 ‰). Populations of virus-like  
15          genome ranging in size from 32 kb to 340kb were  
16          preponderant within 40 ‰ to 220 ‰ salinity gradients,  
17          whereas ponds with salinity higher than 220 ‰ contained  
18          virus-like genomes with size ranging from 10 to 189 kb. As  
19          changes in the total prokaryotic community structure  
20          depending on salinity were also recorded, this suggests that  
21          viral populations have a dynamic, which probably depends  
22          on their hosts' ecology (Sandaa et al. 2003).

23                 Considering the morphological diversity of viruses in  
24                 hypersaline environments, direct observations with electronic  
25                 microscope revealed a majority of lemon-shaped particles



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

21 All halophilic viruses described until now have genomes  
22 which consist of linear double-stranded DNA. Looking at the  
23 genome sequences, only little sequence similarity (less than  
24 10%) with bacteria, bacteriophages and eukaryotic viruses  
25 were observed. This phenomenon could be partly due to

1 isolation caused by such particular ecosystem (Dyall-Smith et  
2 al. 2003). However, there are also strong genetical  
3 relationships between different haloviruses, as shown by the  
4 haloviruses  $\phi$ Ch1 and  $\phi$ H which share up to 97% nucleotide  
5 identity, while their hosts, isolated from distinct and  
6 geographically distant sites, are phylogenetically different  
7 (Klein et al. 2002, Tang et al. 2002). The haloviruses HF1 and  
8 HF2 also have genomes that share up to 99 % nucleotide  
9 identity in the first 60 % of their sequence. However, the  
10 remainder part shows a significant divergence (87 % identity)  
11 due to numerous base changes and insertion/deletion events.  
12 This significant shift in sequence similarity suggests a recent  
13 recombination event between either the two halovirus or with  
14 another HF-like halovirus. This recombination occurrence  
15 seems to be rather common among viruses from hypersaline  
16 waters (Tang et al., 2004, Bath et al. 2006).

## 17 18 *2.2. Viruses in alkaline lakes*

19  
20 Even if the water chemistry of soda lakes is similar to  
21 hypersaline lakes, solar salt evaporation ponds and deep-sea  
22 hypersaline basins, alkaline lakes differ by the high levels of  
23 carbonate minerals in the surroundings rocks that maintain  
24 pH ranging between 10 and 12. In addition,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$

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

3 Bacterial abundances and seasonal changes in community  
4 composition were recorded in the past decades, but no  
5 previous reports on the occurrence of viruses merged before  
6 2004 from such extreme habitat until Jiang and co-workers  
7 tackled the virus populations in Mono Lake, which is a large  
8 alkaline (pH~10), moderate hypersaline lake lying at the  
9 western edge of Great Basin in California (Jiang et al. 2004).

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

22 To date, only one lytic phage, named  $\phi$ Mono1, has been  
23 isolated and partly characterized from this viral population  
24 (Table 1). Surprisingly, this virus strain infects a bacterial  
25 host, which is closely related to *Idiomarina baltica*

1 previously isolated from surface water of the central Baltic  
2 Sea. Using  $\phi$ Mono1 dsDNA genome as probe in  
3 hybridization experiments also revealed seasonal fluctuations  
4 in viral communities.

### 6 **3. Viruses in deserts**

7  
8 In deserts, that are extremely dry and exposed to extremes of  
9 UV light irradiation and temperature variation, water is  
10 always a very limiting factor for life. Nonetheless, eukaryotic  
11 and prokaryotic microorganisms have adapted to these  
12 extreme conditions and have been found in hot desert such as  
13 the Atacama Desert of Chile (Evans & Johansen 1999).

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

25

#### 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 for 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 Arctic sea ice, viral abundance was recorded to be very high as shown by direct counts ( $9 \cdot 10^6 \text{ ml}^{-1}$  to  $3 \cdot 10^8 \text{ ml}^{-1}$ ). This value, which was 10- to 100-fold greater than the concentration of viruses in the underlying sea water ( $1.1 \cdot 10^6 \text{ ml}^{-1}$ ), 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

1 dynamic component of sea ice microbial communities  
2 (Maranger et al. 1994).

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

19 Interestingly, samples of Ross Sea pack ice in Antarctic  
20 revealed that the range of total viral abundance was similar  
21 with the concentration found in Arctic sea ice (between  $5.2$   
22  $10^6 \text{ ml}^{-1}$  to  $3.5 \cdot 10^8 \text{ ml}^{-1}$ ). TEM observations showed that the  
23 viruses, which compose the population, are large, with 40%  
24 icosahedral, 37% spherical and 23% lumpy forms, and all of  
25 them likely infect microeukaryotes (Gowing 2003).

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

## 13           **5. Viruses in deep subsurface sediments**

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

1            Presence of viruses in buried marine sediments was  
2 investigated recently after drilling a hole at 228.7 meters  
3 below sea seafloor to a depth at 105.1 mbsf and 118.2 mbsf,  
4 near the west Canadian coast (Bird et al. 2001). Analyses  
5 revealed the existence of large amounts of viruses. Viral  
6 abundances appeared to follow bacterial numbers very  
7 closely with an average up to  $10^9$  g<sup>-1</sup> of dry sediment at  
8 105.1mbsf. Even if microbial communities seemed to be  
9 stratified in subseafloor sediments, nothing is known about  
10 the viral diversity and the interactions between viral and  
11 prokaryotic communities. Nonetheless, given the scarcity of  
12 eukaryotic bacterivores in deep marine sediments, the only  
13 source of mortality by external agents for the bacterial  
14 community lies in phage attack. Thus, considering bacterial  
15 and viral abundances being highly correlated, viruses appear  
16 to be potential actors of subsurface sediments  
17 biogeochemistry.

## 18 19 **6. Viruses in extreme thermal environments**

20  
21 Live has also adapted to hot temperatures. Given that early  
22 branching organisms could have been hyperthermophiles  
23 among *Archaea* and thermophiles among *Bacteria*, looking  
24 for viruses in such extreme environment may provide  
25 interesting information about virus evolution in the early



1 cellular life. The recent observations indicated that terrestrial  
2 and oceanic hydrothermal environments represent a  
3 bottomless reservoir of a truly remarkable morphological and  
4 genomic viral diversity.

#### 6 *6.1. Viruses from terrestrial hot springs*

7  
8 Early studies on viruses of hyperthermophiles were pioneered  
9 in the laboratory of Wolfram Zillig in the 1980s. A  
10 systematic screening of surface hot springs located in Japan,  
11 Iceland, New Zealand, Italy, Russia and the United States led  
12 to the isolation of an unprecedented diversity of new viruses  
13 (Fig. 4) (Rice et al. 2001 ; Rachel et al., 2002 ; Prangishvili  
14 and Garrett, 2005).

15 The vast majority of the hyperthermophilic viruses  
16 isolated from acidic or neutral hot springs (>80°C) were  
17 found to infect a broad spectrum of members of the  
18 extremely thermophilic *Crenarchaeaota*, including  
19 representatives of the genera *Sulfolobus*, *Thermoproteus*,  
20 *Acidianus*, *Pyrobaculum* (Table 1). Based on their  
21 exceptional morphology and genomic properties the  
22 crenarchaeal viruses were classified in 7 new families which  
23 include: lemon-shaped *Fuselloviridae*, filamentous  
24 *Lipothrixviridae*, stiff rod-shaped *Rudiviridae*, droplet-shaped  
25 *Guttaviridae*, spherical *Globuloviridae*, two tailed spindle-

1           shaped *Bicaudaviridae* and bottle-shaped *Ampullaviridae*.  
2           The International Committee of Taxonomy of Viruses has  
3           already approved the first four families. The crenarchaeal  
4           viruses showed no clear similarities in their morphologies or  
5           at the genomic level to either bacterial or eukaryal viruses,  
6           except perhaps members of three viral families. The rod-  
7           shaped virions of the *Rudiviridae* and *Liphotrixviridae*  
8           resemble tobamoviruses and closteroviruses of vascular  
9           plants, respectively, while those of the *Globuloviridae*  
10          resemble that of viruses of the *Paramyxoviridae*, which infect  
11          vertebrates. The 25 hyperthermophilic viruses isolated so far  
12          exhibited double-stranded DNA genomes, linear or circular  
13          of 15 to 75 kb, most of them being sequenced and revealing  
14          an amazing diversity at the genomic level (Prangishvili et al.  
15          2006). Few significant sequence matches were obtained with  
16          either bacterial or eukaryal genes and very few genes have  
17          been assigned functions. However, there is some evidence  
18          that a 37-kDa coat protein of the *Sulfolobus* turreted  
19          icosahedral virus (STIV) can generate a tertiary and  
20          quaternary structure similar to that of capsid proteins of  
21          bacterial and animal viruses, despite the lack of significance  
22          gene similarity. This suggests that some viruses may have a  
23          common ancestor that precedes the division into three  
24          domains of life (Rice et al. 2004; Khayat et al. 2005). The  
25          fact that for most of these viruses, analysis of their genomes

1 showed little or no similarity to genes in the public databases  
2 suggests that all these newly discovered viruses employ  
3 novel biochemical mechanisms for viral functions.

4 All viruses of acidophilic hyperthermophiles (except  
5 TTV1 and ATV) are non lytic and persist in host cells in a  
6 stable state (pseudolysogeny or “carrier state”). It was  
7 hypothesized that such a survival strategy was beneficial for  
8 viruses, helping them to avoid direct exposure to the harsh  
9 conditions of the host habitat (Prangishvili and Garrett 2004,  
10 2005).

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

## 23 24 *6.2. Viruses from deep sea hydrothermal vents*

25

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

7 Recently, systematic searches carried out on samples  
8 collected in various geographically distant hydrothermal sites  
9 revealed high and unexpected abundance and diversity of  
10 viruses in deep-sea hydrothermal vents. Viral abundance was  
11 recorded to be high as showed by direct counts ( $1.45 \cdot 10^5$  to  
12  $9.9 \cdot 10^7$  ml<sup>-1</sup>). High viral abundance at active vents, relative to  
13 those in surrounding waters, indicated viral production and  
14 hence, virus mediated microbial mortality (Ortmann and  
15 Suttle 2005).

16 Considering the morphological diversity, direct  
17 observations with electronic microscope revealed a great  
18 morphological diversity. With the exception of the  
19 filamentous and rod-shaped morphotypes which are also  
20 known for the *Bacteria*, the morphologies seemed to be  
21 characteristic of archaeal viruses. Indeed, the lemon-shaped  
22 type prevailed and novel pleomorphic morphologies such as  
23 “spoon-shaped” and spindle particles with bipolar expansions  
24 were also discovered. The exotic morphological similarities  
25 exhibited by viruses from both deep-sea and terrestrial hot

1 environments are very astonishing. For example, the presence  
2 of lemon-shaped viruses in diverse extreme environments  
3 (salterns, subsurface anaerobic sediments, acidic  
4 thermophilic continental solfatara and deep-sea vents) in  
5 addition to the fact that this morphotype has never been  
6 found among the *Bacteria* or *Eucarya* strengthens the idea of  
7 their specificity to the archaeal domain and probably reflects  
8 a deep evolutionary history within this domain (Geslin et al.  
9 2003a).

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

## 21 22 7. Concluding remarks

23  
24 Despite the ubiquity of viruses, until recently relatively  
25 little was known about viruses in extreme environments

1 because in many instances the extreme growth conditions  
2 required by extremophiles have precluded a search for  
3 viruses. However, over the past few years our knowledge of  
4 viruses in extreme environments considerably increased.  
5 Tracking viruses in ecological niches with seemingly harsh  
6 conditions has been successful and the presence of virus  
7 populations has been consistently detected in all the explored  
8 environments. All viral communities appeared to be  
9 substantially abundant to the populations rate that are often  
10 greater than in standard environments (e.g.  $10^9$  ml<sup>-1</sup> in solar  
11 saltens,  $3.5 \cdot 10^8$  ml<sup>-1</sup> in Antarctic sea ice). All viruses isolated  
12 so far from extreme environments are double-stranded DNA  
13 viruses with moderate genomic complexity (the genome size  
14 range from 14 to 80 kb). It is conceivable that this very stable  
15 form of genome may be necessary to face harsh constraints  
16 of extreme habitats. It could also explain why no RNA virus  
17 has been isolated yet, especially from hot environments.  
18 However, PFGE analysis used to depict the viral community  
19 structure (e.g. in desert and hypersaline habitats  
20 environments) produces evidence of a more complex  
21 diversity with the recovering of uncharacterized large dsDNA  
22 viruses.  
23 The viral communities seem also to be genetically distinct,  
24 suggesting specific niche adaptation and great diversity.  
25 Nevertheless, at this stage of the knowledge, little is known

1 of their origin, activity, or importance to the *in situ* microbial  
2 dynamics and continuous attempts to isolate and to study  
3 viruses that thrive in extreme environments will be needed to  
4 address such questions. Moreover, several terrestrial extreme  
5 environments are still unexplored, e.g. evaporites, subglacial  
6 Antarctic lakes like Lake Vostok, where the DNA signature  
7 of a thermophilic bacteria (*Hydrogenophilus sp.*) has been  
8 detected (Bulat et al. 2004) or the stratosphere and its  
9 airborne biota.

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

16 More than 85% of the viral genomic sequences lack  
17 similarity to previously reported sequences. Thus, the  
18 genome of hyperthermophilic viruses and that of any other  
19 virus that thrives with extreme conditions probably contains  
20 an astronomical number of still unknown proteins. Although  
21 some of these proteins could be functional analogues of  
22 already known proteins, it would be not surprising to  
23 discover proteins encoding novel functions. This exceeds  
24 previous results from viral metagenomic analyses (68%) and  
25 reinforces the view that viruses represent by far the largest

1 unexplored reservoir of genomic diversity on Earth (Edwards  
2 & Rohwer, 2005). This constitutes an important issue for  
3 further research aimed at understanding the origin of viruses  
4 and early life evolution but also for practical purposes such  
5 as identification of new enzymatic tools useful for the  
6 manipulation of DNA *à façon*.

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

17 Considering the last updated Forterre's scenario (Forterre,  
18 2006) which hypothesised that viruses have played a key role  
19 in both RNA-to-DNA transition and in emergence of the  
20 three cellular domains presently known, the research on  
21 viruses is entering a new exciting stage. The study of the  
22 biology and ecology of new viruses isolated from  
23 extremophile environments may shed light on the early  
24 biological processes as well as on viral evolution.

25



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1                   **Figures**

2  
3                   **Figure 1:** Negative stain electron microscopy of head-and-  
4 tail halovirus HF2. Scale bar represents 100 nm.

5                   Reprinted from *Research in Microbiology*, vol. 154, Dyall-  
6 Smith M, Tang SL, Bath C, “Haloarchaeal viruses: how  
7 diverse are they?”, 309-313, Copyright (2003), with  
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9  
10                  **Figure 2:** Negative stain electron microscopy of spindle-  
11 shaped halovirus His1. Scale bar represents 100 nm.

12                  Reprinted from *Research in Microbiology*, vol. 154, Dyall-  
13 Smith M, Tang SL, Bath C, “Haloarchaeal viruses: how  
14 diverse are they?”, 309-313, Copyright (2003), with  
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16  
17                  **Figure 3:** Negative stain electron microscopy of spherical  
18 halovirus HS1. Scale bar represents 100 nm.

19                  Reprinted from *Research in Microbiology*, vol. 154, Dyall-  
20 Smith M, Tang SL, Bath C, “Haloarchaeal viruses: how  
21 diverse are they?”, 309-313, Copyright (2003), with  
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23  
24                  **Figure 4:** Transmission electron microscopy of virus and  
25 virus-like particles isolated from Yellowstone National Park.

1 (A) SSV1 *Fusellovirus*, (B) SIRV *Rudivirus* and (C) SIFV  
2 *Lipothrixvirus* previously isolated from thermal area of Japan  
3 or Iceland. (D) SSV-like, (E) SIRV-like and (F) SIFV-like  
4 particle morphologies isolated from Yellowstone National  
5 Park thermal features. (G-I) Virus-like particles isolated from  
6 Yellowstone National Park thermal features. Bars indicate  
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8 Reprinted from PNAS, vol. 98, Rice G., Stedman K, Snyder  
9 J, Wiedenheft B, Willits D, Brumfield S, McDermott T,  
10 Young MJ, “Viruses from extreme thermal environments.”,  
11 13341-13345, Copyright (2001), with permission from  
12 National Academy of Sciences, U.S.A.

13  
14 **TABLE:**

15  
16 **Table 1:** Main features of the extremophilic viruses so far  
17 characterized in extreme environments.