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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.

5
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 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 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 ϕ Ch1 and ϕ 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).

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, Ca^{2+} and 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⁻¹) 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 ϕ 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 ϕ 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 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⁻¹ 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*

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⁻¹). 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⁻¹ in solar
11 saltens, $3.5 \cdot 10^8$ ml⁻¹ 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.

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13 Smith M, Tang SL, Bath C, “Haloarchaeal viruses: how
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16
17 **Figure 3:** Negative stain electron microscopy of spherical
18 halovirus HS1. Scale bar represents 100 nm.

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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
7 100 nm.

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9 J, Wiedenheft B, Willits D, Brumfield S, McDermott T,
10 Young MJ, “Viruses from extreme thermal environments.”,
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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.