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Observation of virus-like particles in high temperature enrichment cultures from deep-sea hydrothermal vents

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Abstract

A systematic search was carried out on samples collected in various geographically distant hydrothermal sites located on the East Pacific Rise (EPR 9° N and 13° N) and Mid-Atlantic Ridge (MAR 36° N and 37° N) to investigate the diversity of virus-like particles (VLPs) from deep-sea vents. Eighty-nine positive enrichment cultures were obtained from one hundred and one crude samples at 85 °C. VLPs were detected by electron microscopy in fifteen different enrichments. Among the different morphotypes observed, the lemon-shaped type prevailed but rods and novel pleomorphic morphologies were also observed. Several observations strongly suggested that host strains of the novel VLPs belong to the hyperthermophilic euryarchaeal order Thermococcales.

Keywords: Virus-like particles; Deep-sea vents; Archaea; Hyperthermophile

1. Introduction

Viruses represent a huge reservoir of biodiversity and possibly the largest part of the biomass [15]. With about 5150 electron microscopic observations, viruses infecting prokaryotes (*Bacteria* and *Archaea*) constitute the largest of all viral groups [2]. However, our knowledge of viruses of *Archaea*, the third domain of life, is rather limited with less than 30 archaeal viruses well described to date [6,17,18]. The International Committee on Taxonomy of Viruses, ICTV, presently recognizes 3 families consisting exclusively of archaeal viruses infecting thermophilic *Crenarchaeota* isolated from hot terrestrial environments. These families, the filamentous *Lipothrixviridae* [4,8,18], the lemon-shaped *Fuselloviridae* [9,18] and the stiff rod-shaped *Rudiviridae* [10], were created to account for their unique features. One more crenarchaeal virus, SNDV a droplet-shaped virus, has been proposed to represent a fourth family, the *Guttaviridae* [5]. Recently, a number of viruses and virus-like particles (VLPs), some of them with novel morphotypes previously not observed in nature, were discovered from *Crenarchaeota* [11,14].

In the phylum *Euryarchaeota* about fifteen viruses have been isolated from extreme halophiles and methanogens. All but two possess the head-and-tail morphology, a feature that is typical of most bacterial viruses, and have been assigned to the families *Myoviridae* or *Siphoviridae* [1]. The two known exceptions are a virus-like particle isolated from *Methanococcus voltae* strain A3, VLPA3 [16], and the lytic virus His1 [6] which infects the extreme halophile *Haloarcula hispanica*, both showing a lemon-shaped morphology resembling SSV1 the type member of the *Fuselloviridae* [9]. Among the *Thermococcales*, the most important order (with currently 25 species described) of the phylum *Euryarchaeota*, no viruses have been yet reported. This order, composed of anaerobic heterotrophic sulfur-metabolizers, is one of the predominant groups of the hyperthermophilic microbial communities from deep-sea vents [7].

Here we present results of studies of VLPs in samples from deep-sea hyperthermophilic environments.

2. Materials and methods

2.1. Sampling locations

Samples were collected from deep-sea hydrothermal vents during oceanographic cruises organized over a three-

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year period in four different geographic sites: East Pacific Rise (EPR) 9° 50′ N, 104° 17′ W; 2500 m depth and 13° N, 104° W; 2600 m depth and Mid-Atlantic Ridge (MAR) 36° 16′ N, 33° 54′ W; 2400 m depth and 37° 50′ N, 31° 50′ W; 900 m depth (Table 1). Pieces of chimney, hydrothermal fluids and sediments were sampled by using manned and/or remote operated deep-sea submersibles. In situ growth chambers ("vent caps") and colonization modules were deployed on active chimneys for several days as described by Reysenbach et al. [13] and Alain et al. [3], respectively.

2.2. Sampling and enrichment culture conditions

Small amounts of crude samples (5 to 10 cm^3), were transferred to 50 ml sterile glass vials and flooded with a sterile solution of 3% (w/v) sea salts (Sigma). The vials were then closed tightly with butyl rubber stoppers (Bellco), pressurized with N_2 (100 kPa) and reduced by injection of a $Na_2S{\cdot}9H_2O$ solution (final concentration, 0.05% (wt/vol)) and stored at 4 °C until processing. Enrichment cultures were established anaerobically in a rich sulfur-containing medium, previously described by Ravot et al. [12]. Cultures, 50 ml of medium in a 100 ml serum bottle, were inoculated by adding 1.5 ml of a sample suspension described above and incubated at 85 °C with shaking (200 rpm) for different times ranging from 15 h (for fast growing cultures) to 14 days. Microbial growth was monitored by using a phase contrast microscope.

2.3. Detection of virus particles

Enrichment cultures scored positive were checked for the presence of virus particles by transmission electron microscopy as described by Rice et al. [14] with some modifications. 16 ml of culture (in late log phase or beginning of stationary phase) were centrifuged (3000 *g* for 10 min) and the supernatant was filtered through Acrodisc PF 0.8/0.2 µm filters (Pall Gelman Laboratory) to remove cells and mineral particles from the original crude sample. The filtrate was then subjected to ultracentrifugation at 100 000 *g* for 3 h in a Beckman SW rotor. Pelleted particles were suspended in 20 µl of TE pH 8 (10 mM Tris–Cl, 1 mM EDTA). A droplet of the suspension was spotted onto a carbon-coated copper grid for negative staining. The specimen was allowed to adsorb to the carbon layer for 2 min and excess liquid was removed with a piece of filter paper (Whatman). A droplet of an uranyl acetate solution (2%) was added to the carbon grid for 40 s and excess liquid was removed again. After the specimen was air-dried, it was examined using a Jeol electron microscope, JEM 100 CX II, operated at 80 keV.

3. Results and discussion

3.1. Morphotypes of the novel VLPs

From a total of 101 crude samples, 89 enrichment cultures, representing all of the geographically distributed sites were successfully established (Table 1).

Nine distinct virus morphotypes were detected in 15 of the 89 enrichment cultures, four of them containing multiple morphologies (Table 1, Fig. 1). Based on the morphology, these VLPs could be clustered in two major groups.

The first group contained rigid and flexible rod-shaped particles (Figs. 1A–1E). Short and thin rigid rods of approximately 66 × 8 nm (Fig. 1D) and long flexuous filaments of about 925 nm long and 25 nm in diameter (Fig. 1B), were detected in distinct enrichment cultures. Three different morphotypes were observed in the same enrichment culture including: (i) straight rods with apparent attachment fibers at one end of approximately 275 × 20 nm (Fig. 1A); (ii) particles of about 185 × 25 nm and characterized by an apparent axial canal resembling broken *Rudiviruses* SIRV1 and SIRV2 [10] (Fig. 1C); (iii) an intermediate morphology resembling a club: a more or less rigid rod larger at one end of approximately 260 × 20 nm (Fig. 1E). We do not know if the latter morphology corresponds to a new morphotype or to a deformed lemon-shaped type (see below) with elongated and flattened particles.

The second group included exclusively lemon-shaped particles. Two distinct subgroups could again be delineated based on the presence of a shorter or longer tail.

Subgroup I roughly corresponded to the lemon-shaped morphotype with VLPs very similar in shape to SSV1 [9], the virus type of the *Fuselloviridae* family and His1 [6]. The particles varied in length and width, from 100 × 40 nm for the smaller to 225 × 100 nm for the longer, and harbored a very short tail (Table 1; Figs. 1F and 1G). Sometimes these VLPs formed rosette-like structure similar to that observed with SSV1, where particles are clustered in a radial disposition attached by their tail to what is most likely residues of cell membrane (Fig. 1G). Several particles appeared to be flexible and some elongated forms were also observed with a flattened head and a long tail (Fig. 1H). The lemon-shaped morphotype appeared to be geographically widespread, as it has been observed in several cultures derived from samples collected at the East Pacific Rise and at the Mid-Atlantic Ridge sites.

In subgroup II, the main characteristic of the first morphotype is the presence of a well defined tail which is as long as (Fig. 1J) or longer than (Figs. 1I, 1K and 1L) the spindle-shaped head. These particles were observed only in enrichment cultures from samples of the Atlantic ocean sites. This tail could be rigid (Figs. 1I and 1J) or flexible (Figs. 1K and 1L). Particles with a rigid tail possessed a spindle-shaped head of about 100 × 50 nm prolonged by a short tail of 60–100 nm long (Fig. 1J), or a long tail of about 200 nm long (Fig. 1I), the latter morphology resem-

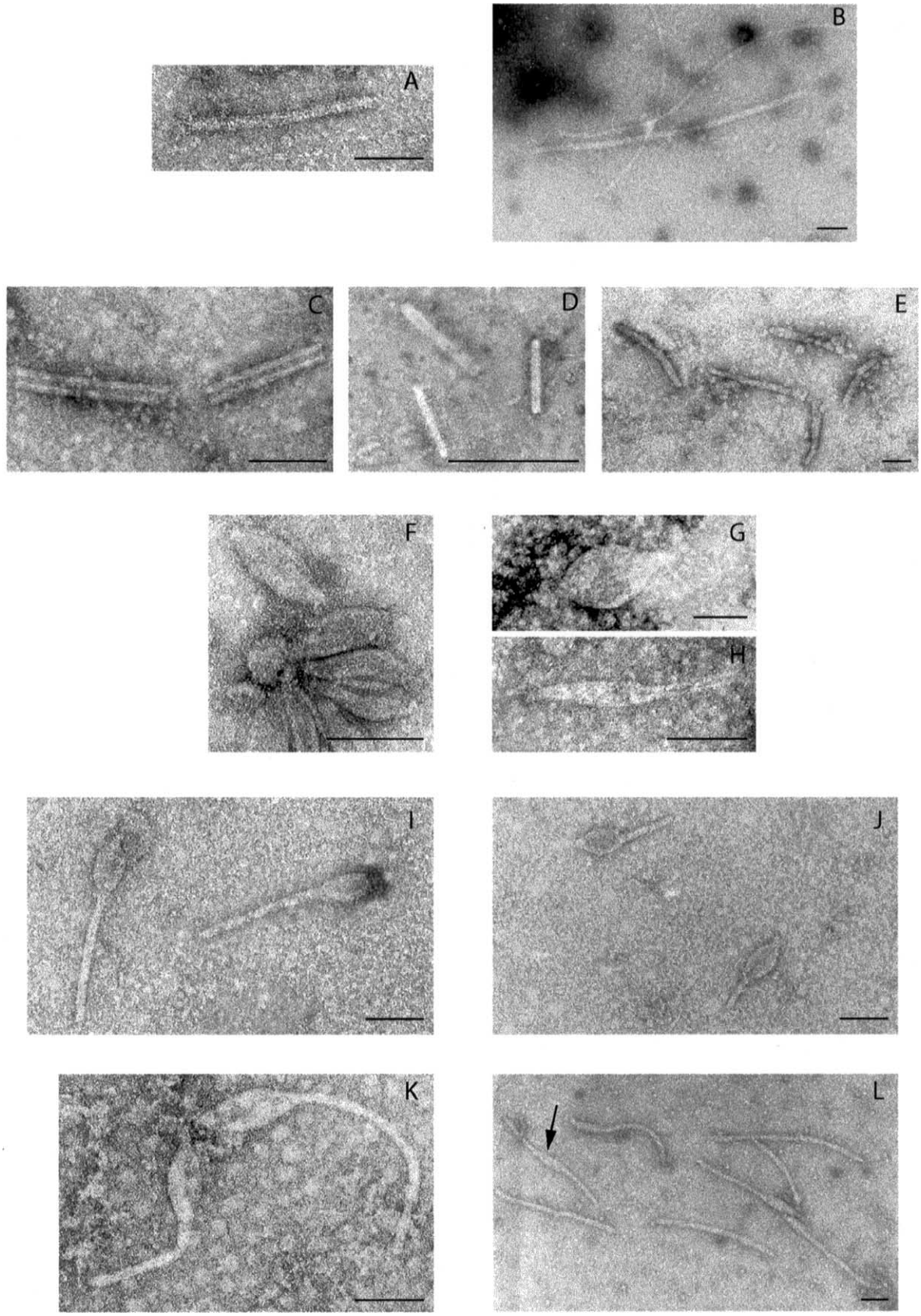

Fig. 1. Transmission electron micrographs, negatively stained, of VLPs detected from hot deep-sea hydrothermal vents. **A** to **E**: rod and filament VLPs. (In Fig. 1B, in addition to the virus-like particle, two flagella can be distinguished.) **F** to **H**: lemon-shaped VLPs. **I** to **L**: novel pleomorphic VLPs. Bar indicates 100 nm.

Table 1
Hydrothermal sites and VLPs features

Deep-sea hydrothermal sites (cruise)	Crude samples	Enrichment cultures established	Enrichment cultures containing VLPs	Sample types	Morphology	Approximate[a] dimensions L × l (nm)
East Pacific Rise, 13° N, 104° W (Amistad, 1999), 2600 m depth	42	35	AM17	Pieces of chimney	Lemon-shaped	100 × 40
						110 × 60
			AM03	Pieces of chimney	Lemon-shaped	115 × 50
East Pacific Rise, 9° 50′ N, 104° 17′ W (Extreme, 2001), 2500 m depth	36	35	Ext 12	Pieces of chimney	Lemon-shaped	160 × 50
			Ext 39	Colonization module	Lemon-shaped	225 × 100 **G**[b]
			Ext 37	Colonization module	Rod	66 × 8 **D**
			Ext 38	Colonization module	Club-shaped	250 × 20 **E**
Mid-Atlantic Ridge, 36° 16′ N, 33° 54′ W, Rainbow (Iris, 2001) 2400 m depth	21	17	IR02	Pieces of chimney	Lemon-shaped	100 × 40
			IR05	Pieces of chimney	Lemon-shaped	120 × 45 **F**
			IR06	Pieces of chimney	Lemon-shaped	275 × 25 **H**
			IR01ext	Pieces of chimney	Lemon-shaped	195 × 50 **J**
					Spoon-shaped	325 × 50 **I**
			IR08	Vent cap	Spoon-shaped	325 × 50 **I**
					“ Spoon-shaped” (flexible tail)	400 × 60 **K**
			IR04	Pieces of chimney	Spoon-shaped	430 × 25 **L**
					A symmetrical spindle shaped	430 × 25 **L**
			IR03	Sediment	Rod	80 × 15
			IR01int	Pieces of chimney	Rod	925 × 25 **B**
Mid-Atlantic Ridge, 37° 50′ N, 31° 50′ W, MenezGwen (Iris, 2001), 900 m depth	2	2	IR102	Pieces of chimney	Rod	185 × 25 **C**
					Rod	275 × 20 **A**
					Club-shaped	260 × 20 **E**

[a] Average of at least three determinations.
[b] Bold letters refer to morphotypes given in Fig. 1.

bling a spoon. Although the long tail particles were observed in several enrichment cultures (IR01ext, IR08; Table 1), the short tail particles were found in only one culture in association with the long tail type. This suggests that the short tail type may simply be an incomplete or broken version of the long tail type. Other particles with a spindle-shaped head possessed a flexible tail of about 280 nm (Fig. 1K).They may also be considered as a spoon-morphotype variant as they were always observed in association with particles harboring a rigid and long tail (Fig. 1I). The second morphotype of subgroup II corresponded to particles with a spindle-shaped center and appendages at each end of the central body. The dimensions of the central body were about 110 × 25 nm. Both appendages were approximately of 160 nm long. The overall tip-to-tip length was 430 nm (Fig. 1L, arrow). The latter morphology was usually observed in association with the spoon-shaped morphotype. Whether these two morphologies are different forms of a unique flexible particle or different particle types remains unknown. The spoon-shaped and the central body with long appendages are novel morphotypes never described for any known virus, but recently these particular VLPs were also detected in hot terrestrial springs at Yellowstone National Park [11,14].

3.2. Possible hosts of the VLPs

To ensure that the VLPs observed were actually produced in the enrichment cultures and not simply passaged from the original crude sample, subcultures were performed by dilution (1/20) of the cultures containing VLPs into fresh medium. After cell growth, VLPs were again observed for 8/15 subcultures and with the same abundance than as found in the respective primary culture, indicating that the host strains were actively growing. The lack of VLPs in the other subcultures may indicate that VLPs were not associated with cells growing in these cultures, but were only present in the original samples. Nevertheless, VLPs were present in subcultures originated from all kind of samples, and all the morphologies found in the primary cultures were again observed in subcultures.

Although we have not yet isolated the host strains of the VLPs several observations suggest that they belong to the Thermococcales order. The culture conditions chosen are routinely used in the laboratory for enrichment of anaerobic, neutrophilic, and heterotrophic sulfur-reducing hyperthermophiles from deep-sea hydrothermal vent samples. Such cultures are usually largely dominated by the fast-growing Thermococcales, since the incubation temperature (85 °C) allows growth of the most common genera *Thermococcus*

and *Pyrococcus* [7]. Examination by a phase-contrast microscope revealed only motile and non-motile cocci in the enrichment cultures, which strengthens the presumption of Thermococcales enrichment. In addition, among more than one-hundred colony-cloned isolates obtained from enrichment cultures of the samples collected at 13° N in the East Pacific Rise, all were found to be members of the Thermococcales order (mostly the *Thermococcus* genus) by 16S rDNA analysis (Forterre et al., unpublished data). Finally, we have recently characterized such a pleomorphic lemon-shaped virus infecting a strain of *Pyrococcus abyss*i isolated from a deep-sea vent in the Southwestern Pacific Ocean (C. Geslin et al., manuscript submitted [19]).

3.3. Concluding remarks

The present study reveals for the first time an unexpected morphological diversity of VLPs from deep-sea hydrothermal environments. With the exception of the filamentous rod-shaped particles which were also known for the *Bacteria* [1], the morphologies reported here seemed to be characteristic of archaeal viruses [11,14,18]. Lemon-shaped VLPs have previously been reported to be present in quite diverse environments including extreme ones such as acidic terrestrial solfatara (e.g., SSV1) [9] and salterns (e.g., His1) [6] but also in subsurface anaerobic sediments (VLPA3) [16]. The present study extends their distribution to another kind of extreme biotope, the deep-sea hydrothermal vents. More studies are needed for a better estimation of the total diversity of viruses or VLPs in deep-sea vent environments, and to describe novel virus genera and families.

The astonishing similarity found between VLP morphologies observed in both deep-sea and terrestrial hot environments may reflect the existence of a common archaeal virus ancestor, or may correspond to convergence selected by the extreme environmental conditions. Further studies will also be needed to address such fundamental questions on archaeal virus evolution.

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