Arch Virol (2002) 147: 2419–2429
DOI 10.1007/s00705-002-0895-2

Archives of Virology
Printed in Austria

Remarkable morphological diversity of viruses and virus-like particles in hot terrestrial environments

Brief Report

R. Rachel, M. Bettstetter, B. P. Hedlund, M. Häring, A. Kessler, K. O. Stetter, and **D. Prangishvili**

Lehrstuhl für Mikrobiologie und Archaeenzentrum,
Universität Regensburg, Germany

Received April 18, 2002; accepted July 25, 2002
Published online October 24, 2002

Summary. Electron microscopic studies of the viruses in two hot springs (85 °C, pH 1.5–2.0, and 75–93 °C, pH 6.5) in Yellowstone National Park revealed particles with twelve different morphotypes. This diversity encompassed known viruses of hyperthermophilic archaea, filamentous *Lipothrixviridae*, rod-shaped *Rudiviridae*, and spindle-shaped *Fuselloviridae*, and novel morphotypes previously not observed in nature. Two virus types resembled head-and-tail bacteriophages from the families *Siphoviridae* and *Podoviridae*, and constituted the first observation of these viruses in a hydrothermal environment. Viral hosts in the acidic spring were members of the hyperthermophilic archaeal genus *Acidianus*.

*

Viruses are probably the most abundant biological entities on the planet [for a review, see reference 52]. A comprehensive picture of their diversity should help to understand the role of viruses in microbial ecology and the entire ecosystem, as well as contribute to understanding the origin and evolution of viruses.

Only about a dozen viruses of hyperthermophilic organisms are among more than 5000 known viruses of prokaryotes [1]. They exhibit spindle-like, filamentous and rod shapes and have been assigned to three novel families: *Fuselloviridae*, *Lipothrixviridae*, and *Rudiviridae* [for reviews, see references 37, 38, 54, 56]. A fourth family, *Guttaviridae*, has been proposed, but not yet recognised for droplet-shaped particles which are densely covered with thin tail fibres [3]. Hosts of the viruses are different strains of hyperthermophilic archaea of the genera *Thermoproteus*, *Sulfolobus*, and *Acidianus*, isolated from hot springs in Japan, Iceland, and New Zealand. In addition to these viruses, icosahedral particles, with

and without projections, as well complex particles with a spindle-shaped central body and appendages at each end were shown to be produced by *Sulfolobus* strains recently enriched from hot pool samples from Yellowstone National Park, U.S.A. [42].

Here we present results of studies of viruses in samples from two hot springs in Yellowstone National Park, one acidic, with a pH of 2.0, 85 °C located at Crater Hills (44 ° 39′ 13.3″N and 110 ° 28′ 39.8″W), and another, neutral, Obsidian Pool, with a pH of 6.0, 75–93 °C (44° 36′ 35.4″N and 110° 26′ 20.6″W). Both samples were enriched prior to investigation. One enrichment culture, named CHE, was established by adding 1 ml of the sample from Crater Hills to 50 ml of an acidic medium (pH 3.0) previously developed for culturing *Sulfolobus* [55], in a long-necked Erlenmayer flask. The inoculated flask was incubated while shaking at 80 °C. Immediately after detection of cell growth (after 10 days incubation), cells were removed by a 5 min centrifugation at 5000 × g and the supernatant was filtered through a 0.2 μm filter (Acrodisc PF 0.8/0.2 μm, Pall Gelman Laboratory, Ann Arbor, MI, USA). The filtrate was subjected to ultracentrifugation for 2 hours at 38 000 rpm in a Beckman Coulter (Fullerton, CA, USA) SW41 rotor. Pelleted particles were suspended in 30 μl of sterile medium or distilled water, deposited on a carbon-coated copper grid and negatively stained with 2% uranyl acetate, pH 4.5. Samples were examined using a CM12 transmission electron microscope (TEM) (FEI, Eindhoven, The Netherlands) operated at 120 keV. The magnification was calibrated using catalase crystals, negatively stained with uranyl acetate [41]. All images were digitally recorded using a slow-scan CCD-camera that was connected to a PC running TVIPS software (TVIPS GmbH, Gauting, Germany).

The Obsidian Pool sample was used to inoculate an 800 ml chemostat culture, named OPE. Cells were enriched and maintained on Allen Medium (pH 6.5) [2] supplemented with 0.001% yeast extract, 0.005% peptone, 0.1 mM $CaSO_4 \times 2 \cdot H_2O$, 0.05 mM Na_2SO_4, 0.1 mM KNO_3 and 3 mM $Na_2S_2O_3 \times 5 \cdot H_2O$, which was continuously replaced (dilution rate of 12 ml h^{-1}). The chemostat was maintained under strictly anaerobic conditions at 85 °C and was flushed with N_2 and CO_2 (80/20 v/v, 20 ml min^{-1}). Viruses and virus-like particles (VLPs) in the OPE culture were examined as described above for the CHE culture.

Morphotypes of the new viruses and VLPs: We observed an unexpected and unprecedented viral diversity in both enrichment cultures, namely nine distinct virus and VLP morphotypes in the CHE culture, and at least five distinct morphotypes in the OPE culture. None of the particles seemed to predominate in any of the cultures.

In the CHE culture three virus morphotypes resembling known viruses of hyperthermophilic archaea were present. We observed: (i) rigid, helical rods of about 1030 × 23 nm (Fig. 1A), similar to rudiviruses SIRV1 and SIRV2 of *Sulfolobus* [35]; (ii) filamentous particles of 850–950 × 24 nm with elongated terminal structures of 100 × 8–13 nm often found to be attached to long, thin filaments (Fig. 1B,C); these particles resembled the lipothrixvirus TTV2 of *Thermoproteus* [27]; (iii) 80 × 60 nm spindle-shaped particles (Fig. 1D) similar

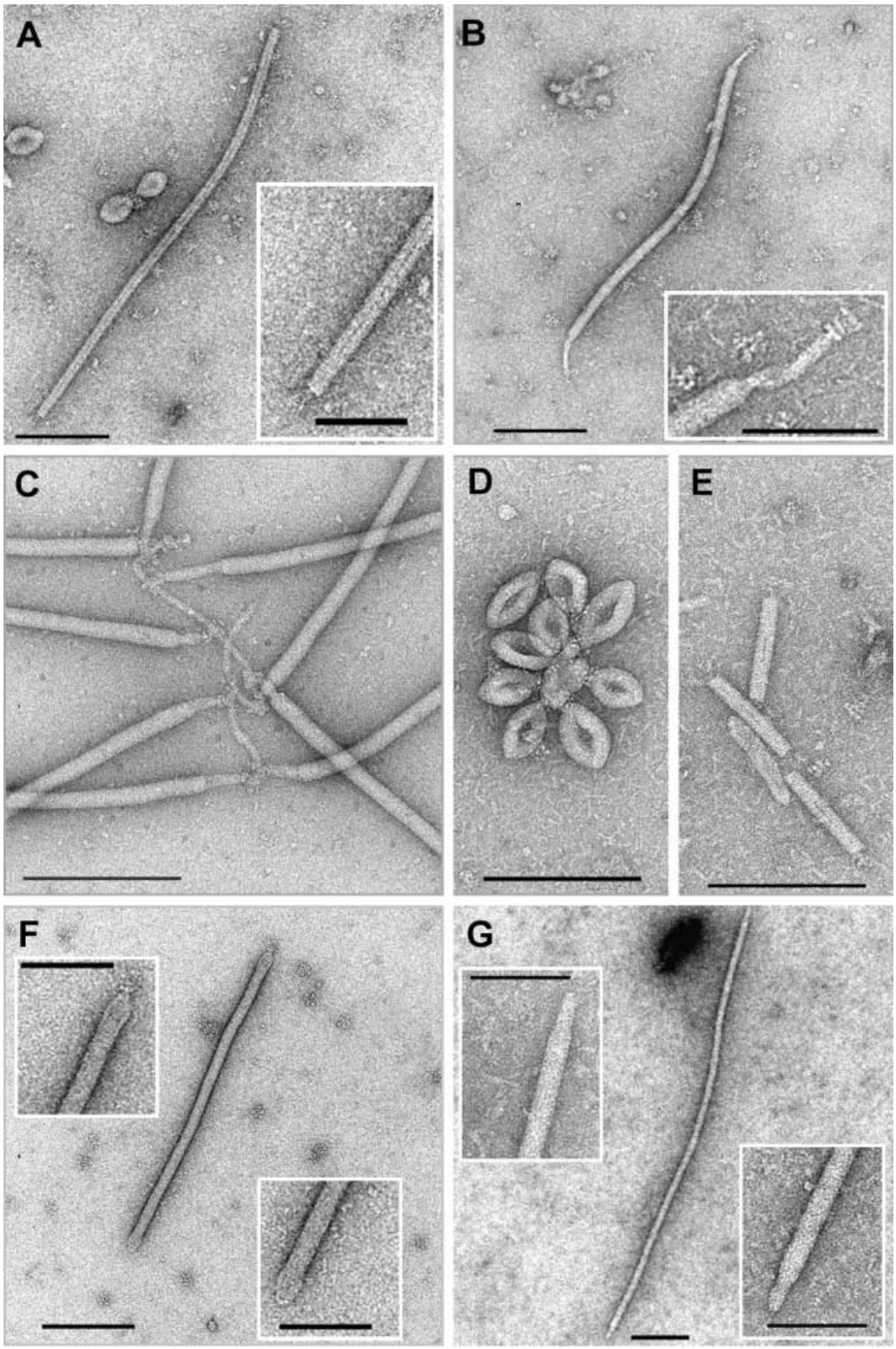
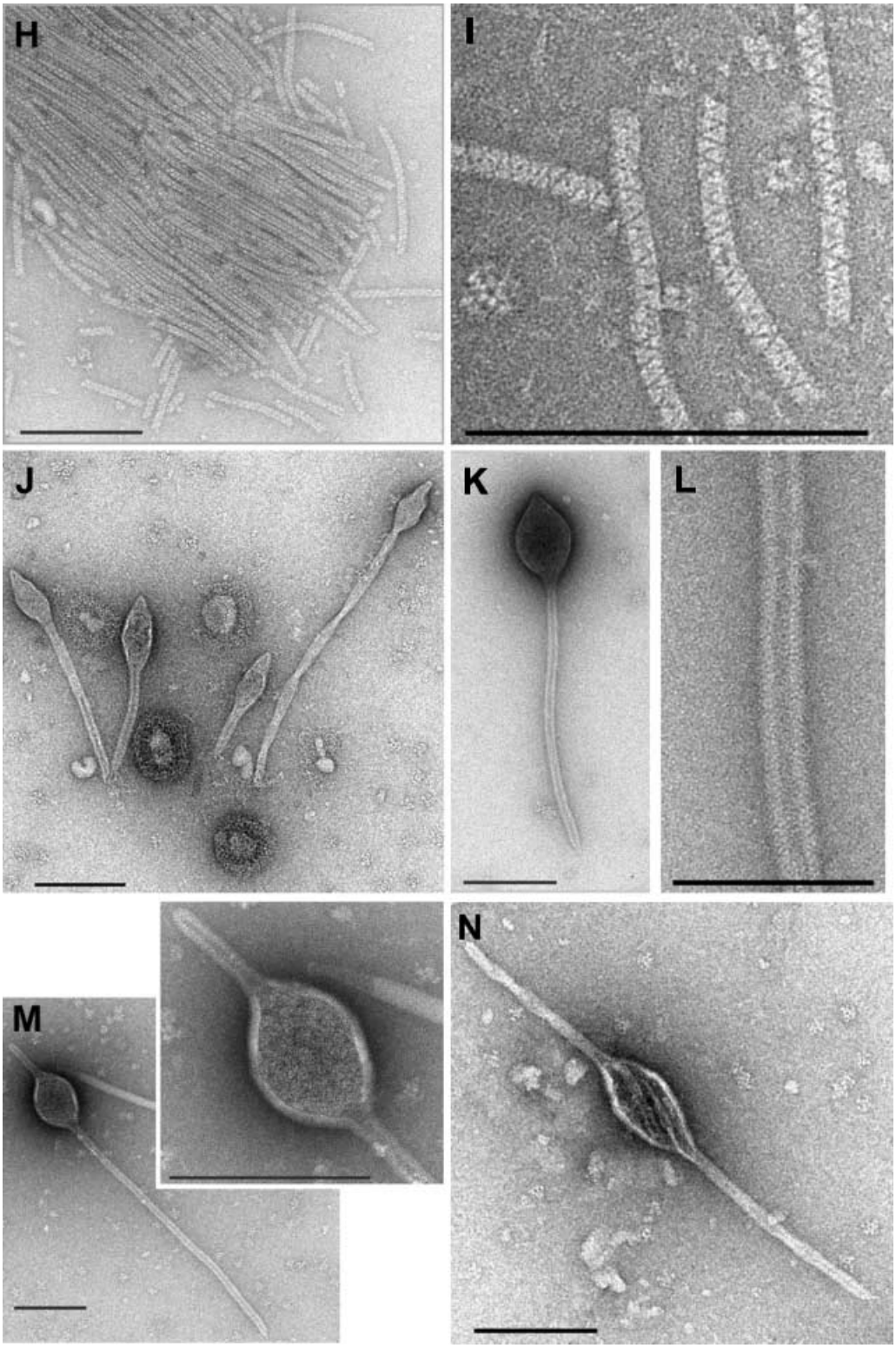


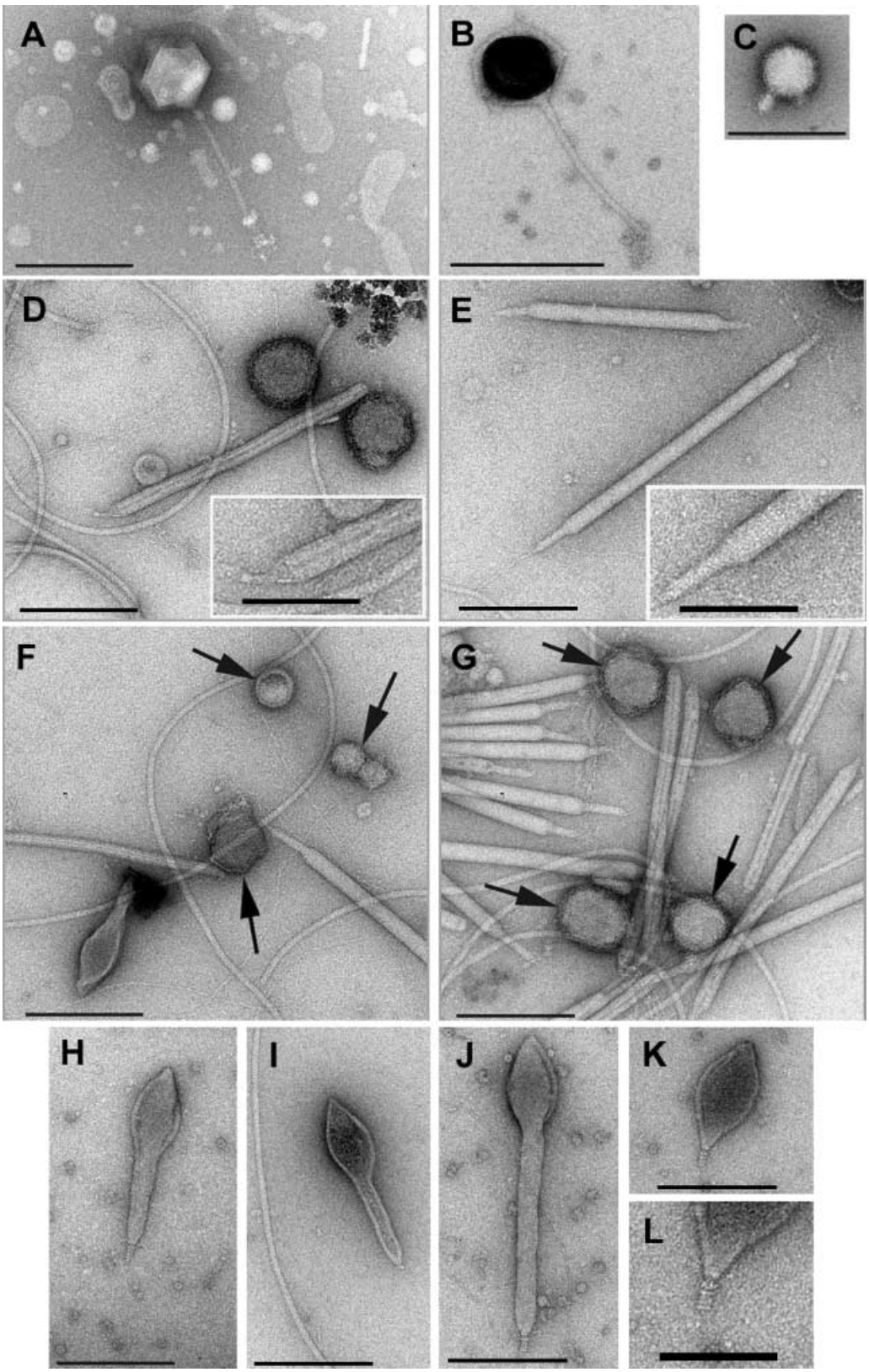
Fig. 1 (*continued*)


H
I
J
K
L
M
N

in size and shape to fuselloviruses SSV1, SSV2, and SSV3 of *Sulfolobus* [54]. Short, rigid, helical rods of approximately 140 × 23 nm, without appendages on the ends (Fig. 1E) were similar in size and shape to viruses of vascular plants, e.g., the Tobacco mosaic virus. Other particles did not resemble known viruses or VLPs. They comprised: (i) filamentous particles of 700–850 × 23 nm with bulbous ends (Fig. 1F), (ii) filamentous particles of about 1550 × 23 nm with rounded tips (Fig. 1G), (iii) zipper-like particles consisting of triangular subunits, 15 nm in width, with lengths of 100–200 nm (Fig. 1H,I), (iv) pleomorphic particles with arrow-like heads of 130–150 × 56–70 nm and helical tails of 260–760 × 23 nm (Fig. 1J), and (v) complex particles consisting of an approximately 180 × 125 nm ellipsoid body to which one or two appendages 24 nm in width and 100–620 nm in length were attached (Fig. 1K to N). High magnification (Fig. 1L) revealed a helical arrangement of subunits in these appendages.

In the OPE culture typical tailed bacteriophages were found. These consisted of icosahedral heads of 100–140 nm in diameter and tails which were 10 nm wide and either 220–280 nm long and flexible (Fig. 2A,B), or very short (Fig. 2C). The particles resembled representatives of the families *Siphoviridae* and *Podoviridae*, respectively. Until now, these generally ubiquitous viruses had never been detected in hot environments. Only two viral morphotypes found in the OPE culture were similar to known viruses of hyperthermophilic archaea. They included: (i) rod-shaped helical viruses of about 510 × 27 nm (Fig. 2D), similar to rudiviruses SIRV1 and SIRV2 of *Sulfolobus*, and (ii) filamentous particles of 250–520 × 30 nm with elongated terminal structures of approximately 35 × 15 nm (Fig. 2E), resembling lipothrixvirus TTV2 of *Thermoproteus*. Round particles found in the OPE culture were in two size classes, with approximate diameters of 115 nm and of 60–70 nm, respectively (Fig. 2F,G). These particles may be viruses; alternatively, they could be membrane vesicles similar to those produced by different strains of *Sulfolobus* [19, 36]. One type of VLP found in the OPE culture appeared to be morphologically new. They were pleomorphic arrow-shaped particles with heads of 150–200 × 75–100 nm and tails of 150–300 × 30 nm with clearly distinguishable terminal structures of 30 × 11 nm (Fig. 2H to L). The particles superficially resembled the particles shown in Figs. 1J and 1K, however,

Fig. 1. Transmission electron micrographs of viruses and VLPs found in an enrichment culture of a sample from the Crater Hills region in Yellowstone National Park (CHE). Samples were negatively stained using 2% uranyl acetate, pH 4.5. **A** a rod-shaped particle; inset: enlarged terminus; **B** filamentous particles with flexible, elongated terminal structures; inset: enlarged particle end, **C** often found attached to thin filaments; **D** spindle-shaped particles; **E** short rod-shaped helical particles; **F** filamentous particles with bulbous termini, both shown enlarged in the insets; **G** long filamentous particles with rounded ends, both shown enlarged in the insets; **H** aggregated zipper-shaped particles; **I** four zipper-shaped particles at high magnification; **J** pleomorphic particles with arrow-shaped heads and helical tails; **K**–**N** ellipsoid particles, **K** with one helical appendage, which is shown enlarged in **L**, **M** with two helical appendages; **N** with twisted head and two helical tails. Bars, 200 nm (100 nm for insets)


A
B
C
D
E
F
G
H
I
J
K
L

differed from them in the width and ultrastructure of the tail, and also by the presence of a specific terminal structure.

Hosts of viruses and VLPs: Several rounds of dilution of the CHE culture in fresh medium did not result in a substantial decrease in numbers of any of the VLPs, suggesting that the host strains were actively growing in the enrichment culture. Cells in the culture were morphologically homogenous irregular cocci of 0.5–1.5 μm in diameter. To identify hosts, DNA was extracted from cells collected from the growing culture, and 16S rDNA was amplified by the polymerase chain reaction (PCR) using the primers 519uF and 1406uR [15]. The PCR product was sequenced directly and yielded a single sequence 99% identical to those of the hyperthermophilic archaea *Acidianus ambivalens* and *A. infernus*. Although members of the genus *Sulfolobus* would be expected to grow under the conditions used for the CHE culture, PCR conducted with *Sulfolobus*-specific primers [42] failed to yield a product.

Fourteen single strains of *Acidianus* were isolated from the CHE culture, either by plating on colloidal sulfur-containing Gelrite (Kelco, San Diego) plates [as described in reference 55], or by using optical tweezers [as described in references 4 and 25]. The 16S rDNA sequence (positions 519–1406) from each isolate was 100% identical to the sequence from the enrichment culture DNA. One of these strains turned out to be a host for the filamentous virus shown in Fig. 1B,C and another was a host for two particles with distinct morphotypes shown in Fig. 1D and in Figs. 1H,I. It is important to note that neither of the VLPs detected in the CHE culture is morphologically identical to the one presumed *Acidianus* virus known, which was detected in a sample from Iceland [55].

In contrast to the CHE culture, many different bacteria and archaea were enriched in the OPE culture. This was expected since a variety of electron donors and acceptors were provided and because Obsidian Pool contains an extremely diverse community of prokaryotes [5, 26]. From this culture, we have been able to recover 16S rDNA sequences representing several known genera, which can be regarded as possible hosts. These were *Thermofilum*, *Thermoproteus*, *Thermosphaera* from the archaeal phylum Crenarchaeota, *Archaeoglobus* from the archaeal phylum Euryarchaeota, and *Thermus*, *Geothermobacterium*, and *Thermodesulfobacterium* from the domain Bacteria. In addition, sequences from noncultivated members of the Crenarchaeota, the "Korarchaeota" [5], the Nanoarchaeota [24] and the bacterial phylum Aquificae have been recovered.

Fig. 2. Transmission electron micrographs of viruses and VLPs found in an enrichment culture of a sample from Obsidian Pool in Yellowstone National Park (OPE). Samples were negatively stained using 2% uranyl acetate, pH 4.5. **A**,**B** head-and-tail viruses; **C** possible head-and-tail virus with short tail; **D** rod-shaped particle; inset: enlarged particle end; **E** filamentous particles with elongated terminal structures, shown enlarged in the inset; **F**,**G** spherical particles with different diameters; **H**–**K** pleomorphic particles with arrow-shaped heads and wide tails with specific terminal structures, enlarged in **L**. Bars, 200 nm (100 nm for insets)

Concluding remarks

Previously, different viruses and VLPs have been reported in extreme thermal environments in Yellowstone National Park [42]. However, their hosts are exclusively strains of the archaeal genus *Sulfolobus*. On the contrary, none of the viruses and VLPs described in the present communication are harboured by *Sulfolobus* strains, although four of them are morphologically similar to known *Sulfolobus* viruses. Hosts for at least three particles were shown to be strains of the archaeal genus *Acidianus*, and very likely, six other types of particles found in the CHE culture also have *Acidianus* hosts. Such diversity of unique virus types from a single host is remarkable. Moreover, there is no precedent for finding VLPs with nine different morphotypes at a single hydrothermal site.

The morphological diversity of viruses and VLPs in hot aquatic environments appears to exceed that in any freshwater, estuarine, or marine system with moderate or cold temperatures (not regarding viruses parasitizing native higher eukaryotes). According to numerous reports, native viruses and VLPs observed in water samples from the latter habitats, or their enrichments, are almost exclusively either tailed bacteriophage-like particles, or are tailless polyhedrons [7–11, 13, 14, 16–18, 21, 22, 28–32, 34, 39, 40, 43–53]. In addition to these morphotypes, VLPs with star or spindle shapes were found in hypersaline Dead Sea waters [33]. Spindle-shaped viruses were also found in saltern ponds and were shown to infect extremely halophilic archaea [6, 20].

All identified hosts of viruses from hot habitats are species of the hyperthermophilic genera *Sulfolobus*, *Thermoproteus* and *Acidianus* and belong to the archaeal phylum Crenarchaeota. Thus, at present, it is unclear whether the remarkable diversity of virus morphotypes is specific to hot environments, or is host-specific, that is, specific for crenarchaeotes. The latter case would mean that there is a dramatic difference in virus morphotypes between viruses infecting the two phyla of the archaeal domain, Euryarchaeota and Crenarchaeota. In contrast to the morphological variety of crenarchaeal viruses, all but two viruses of euryarchaeotes are typical head-and-tail phages, belonging to the bacteriophage families *Myoviridae* and *Siphoviridae* [reviewed in 1 and 38]. Moreover, crenarchaeotes are not restricted to high temperatures and are also abundant in some temperate environments [12, 23]. Further studies on virus ecology are required to confirm the observed differences in morphotypes of prokaryotic viruses in hot and moderate aquatic ecosystems, and to understand evolutionary implications of this observation.

Acknowledgments

We are deeply indebted to Mark Young for arranging a trip to Yellowstone National Park and providing the opportunity to sample the Crater Hills region. Kenneth Stedman and Francisco Roberto were helpful in sampling. Obsidian pool samples were obtained with the assistance from Karen Reinhart and Cheryl Jenkins under sampling permit 00115. The assistance of Daniela Naether and Peter Hummel in preparation and inspection of the TEM samples is acknowledged. B. P. H. was supported by the Alexander von Humboldt Foundation. Part of

this work was supported by the Deutsche Forschungs gemeinschaft (grant Pr 663/2-1) and the Fonds der Chemischen Industrie. D. P. is grateful to Rudolf K. Thauer for motivating discussions.

References

1. Ackermann H-W (2001) Frequency of morphological phage descriptions in the year 2000. Arch Virol 146: 843–857
2. Allen MB (1959) Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte. Arch Microbiol 32: 270–277
3. Arnold HP, Ziese U, Zillig W (2000) SNDV, a novel virus of the extremely thermophilic and acidophilic archaeon *Sulfolobus*. Virology 272: 409–416
4. Ashkin A, Dziedzic JM, Yamane T (1987) Optical trapping and manipulation of single cells using infrared laser beams. Nature 330: 769–771
5. Barns SM, Fundyga RE, Jeffries MW, Pace NR (1994) Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment. Proc Natl Acad Sci USA 91: 1609–1613
6. Bath C, Dyall-Smith ML (1998) His1, an archaeal virus of the *Fuselloviridae* family that infects *Haloarcula hispanica*. J Virol 72: 9392–9395
7. Bergh O, Børsheim KY, Bratbak G, Heldal M (1989) High abundance of viruses found in aquatic environments. Nature (Lond) 340: 467–468
8. Børsheim KY (1993) Native marine bacteriophages. FEMS Microbiol Ecol 102: 141–159
9. Børsheim KY, Bratbak G, Heldal M (1990) Enumeration and biomass estimation of planktonic bacteria and viruses by transmission electron microscopy. Appl Environ Microbiol 56: 352–356
10. Bratbak G, Heldal M, Norland S, Thingstad TF (1990) Viruses as partners in spring bloom microbial trophodynamics. Appl Environ Microbiol 56: 1400–1405
11. Cochlan WP, Wikner J, Steward GF, Smith DC, Azam F (1993) Spatial distribution of viruses, bacteria and chlorophyll alpha in neritic, oceanic and estuarine environments. Mar Ecol Prog Ser 92: 77–87
12. DeLong EF, Wu KY, Prezelin BB, Jovine RV (1994) High abundance of Archaea in Antarctic marine picoplankton. Nature 371: 695–697
13. Demuth J, Neve H, Witzel K (1993) Direct electron microscopy study on the morphological diversity of bacteriophage populations in Lake Plussee. Appl Environ Microbiol 59: 3378–3384
14. DePaola A, Motes ML, Suttle CA (1998) Phages infecting *Vibrio vulnificus* are abundant and diverse in oysters (*Crassostrea virginica*) collected from the Gulf of Mexico. Appl Environ Microbiol 64: 346–351
15. Eder W, Ludwig W, Huber R (1999) Novel 16S rRNA gene sequences retrieved from highly saline brine sediments of Kebrit Deep, Red Sea. Arch Microbiol 172: 213–218
16. Frank H, Moebus K (1987) An electron microscopic study of bacteriophages from marine waters. Helgol Meeresunters 41: 385–414
17. Fuhrman JA, Suttle CA (1993) Viruses in marine planktonic systems. Oceanography 6: 51–63
18. Garza DR, Suttle CA (1995) Large double-stranded DNA viruses which cause the lysis of a marine heterotrophic nanoflagellate (*Bodo* sp.) occur in natural marine communities. Aquat Micr Ecol 9: 203–210
19. Grimm R, Singh H, Rachel R, Typke D, Zillig W, Baumeister W (1998) Electron tomography of ice-embedded prokaryotic cells. Biophys J 74: 1031–1042

20. Guixa-Boixareu N, Calderón-Paz JI, Heldal M, Bratbak G, Pedrós-Alió C (1996) Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient. Aquat Microb Ecol 11: 215–227
21. Hara S, Terauchi K, Koike I (1991) Abundance of viruses in marine waters: assessment by epifluorescence transmission electron microscopy. Appl Environ Microbiol 57: 2731–2734
22. Hennes KP, Simon M (1995) Significance of bacteriophages for controlling bacterioplankton growth in a mesophilic lake. Appl Eviron Microbiol 61: 333–340
23. Hershberger KL, Barnes SM, Reysenbach A-L, Dawson SC, Pace NR (1996) Wide diversity of Crenarchaeota. Nature 384: 420
24. Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC, Stetter KO (2002) A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. Nature 417: 63–67
25. Huber R, Burggraf S, Meyer T, Barns SM, Rossnagel P, Stetter KO (1995) Isolation of a hyperthermophilic archaeum predicted by *in situ* RNA analysis. Nature 376: 57–58
26. Hugenholtz P, Pitulle C, Hershberger KL, Pace NR (1998) Novel division level bacterial diversity in a Yellowstone hot spring. J Bacteriol 180: 366–376
27. Janekovic D, Wunderl S, Holz I, Zillig W, Gierl A, Neumann H (1983) TTV1, TTV2, and TTV3, a family of viruses of the extremely thermophilic, anaerobic, sulfur-reducing archaebacterium *Thermoproteus tenax*. Mol Gen Genet 192: 39–45
28. Kepner RL, Wharton RA, Suttle CA (1998) Virus in Antarctic lakes. Limnol Oceanogr 43: 1754–1761
29. Maranger R, Bird DF (1995) Viral abundance in aquatic systems: a comparison between marine and fresh waters. Mar Ecol Prog Ser 121: 1–3
30. Maranger R, Bird DF, Juniper SK (1994) Viral and bacterial dynamics in Arctic sea ice during the spring algal bloom near Resolute, NWT Canada. Mar Ecol Prog Ser 111: 121–127
31. Mayer JA, Taylor FJR (1979) A virus which lyses the marine nanoflagellate *Micromonas pusilla*. Nature 281: 299–301
32. Müller DG, Stache B (1992) Worldwide occurence of virus infections in filamentous marine brown algae. Helgol Meerunters 46: 1–8
33. Oren A, Bratbak G, Heldal M (1997) Occurrence of virus-like particles in the Dead Sea. Extremophiles 1: 143–149
34. Pina S, Creus A, Gonzalez N, Girone R, Felip M, Sommaruga R (1998) Abundance, morphology and distribution of planktonic virus-like particles in two high-mountain lakes. J Plankton Res 20: 2413–2421
35. Prangishvili D, Arnold HP, Götz D, Ziese U, Holz I, Kristjansson JK, Zillig W (1999) A novel virus family, the *Rudiviridae*: Structure, virus-host interactions and genome variability of the *Sulfolobus* viruses SIRV1 and SIRV2. Genetics 152: 1387–1396
36. Prangishvili D, Holz I, Stieger E, Nickell S, Kristjansson JK, Zillig W (2000) Sulbolobicins, specific proteinaceous toxins produced by strains of the extremely thermophilic archaeal genus *Sulfolobus*. J Bacteriol 182: 2985–2988
37. Prangishvili D, Stedman KM, Zillig W (2001) Viruses of the extremely thermophilic archaeon *Sulfolobus*. Trends Microbiol 9: 39–42
38. Prangishvili D, Zillig W (2002) Viruses of the Archaea. In: Brenner S, Miller JH (eds), Encyclopedia of Genetics, vol. 4, Academic Press, San Diego, pp 2114–2116
39. Proctor LM, Fuhrman JA (1990) Natural mortality of marine bacteria and cyanobacteria. Nature 343: 60–62
40. Proctor LM, Fuhrman JA, Ledbetter MC (1988) Marine bacteriophages and bacterial mortality. Eos 69: 1111–1112

41. Reilein A (1998) Preparation of catalase crystals. http://www.itg.uiuc.edu/publications/techreports/98-009
42. Rice G, Stedman KM, Snyder J, Wiedenheft B, Brumfield S, Mc Dermott T, Young M (2001) Novel viruses from extreme thermal environments. Proc Natl Acad Sci USA 98: 13341–13345
43. Skotinski AH, Gibbs A, Wrigley NG (1976) Further studies on *Chara corrallina* virus. Virology 75: 457–468
44. Suttle CA (2000) Cyanophages and their role in the ecology of cyanobacteria. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Kluwer Academic Publishers, Amsterdam, pp 563–589
45. Suttle CA, Chan AM (1995) Viruses infecting the marine prymnesiophyte *Chrysochromulina* spp: isolation, preliminary characterization and natural abundance. Mar Ecol Prog Ser 118: 275–282
46. Torella F, Morita RY (1979) Evidence by electron micrographs for a high incidence of bacteriophage particles in the waters of Yaquina Bay, Oregon: ecological and taxonomical implications. Appl Environ Microbiol 37: 774–778
47. Valentine AF, Chen PK, Colwell RR, Chapman GB (1966) Structure of a marine bacteriophage as revealed by the negative-staining technique. J Bacteriol 91: 819–822
48. Van Etten JL, Lane LC, Meints RH (1991) Viruses and virus-like particles of eukaryotic algae. Microbiol Rev 55: 586–620
49. Weinbauer MG, Peduzzi P (1994) Frequency, size and distribution of bacteriophages in different marine bacterial morphotypes. Mar Ecol Prog Ser 108: 11–20
50. Wichels A, Biel SS, Gelderblom HR, Brinkhoff T, Muyzer G, Schütt C (1998) Bacteriophage diversity in the North Sea. Appl Environ Microbiol 64: 4128-4133
51. Wilhelm SW, Suttle CA (1999) Viruses and nutrient cycles in the sea. BioScience 49: 781–788
52. Wommack KE, Colwell RR (2000) Virioplankton. Micr Mol Biol Rev 64: 69–114
53. Wommack KE, Hill RT, Kesse M, Russek-Cohen E, Colwell RR (1992) Distribution of viruses in the Chesapeake Bay. Appl Environ Microbiol 58: 2965–2970
54. Zillig W, Arnold HP, Holz I, Prangishvili D, Schweier A, Stedman KM, She Q, Phan H, Garrett R, Kristjansson JK (1998) Genetic elements in the extremely thermophilic archaeon *Sulfolobus*. Extremophiles 2: 131–140
55. Zillig W, Kletzin A, Schleper C, Holz I, Janekovic D, Hain J, Lanzendörfer M, Kristjansson JK (1994) Screening for *Sulfolobales*, their plasmids and their viruses in Icelandic solfataras. System Appl Microbiol 16: 609–628
56. Zillig W, Prangishvili D, Schleper C, Elferink M, Holz I, Albers S, Janekovic D, Götz D (1996) Viruses, plasmids and other genetic elements of thermophilic and hyperthermophilic Archaea. FEMS Microbiol Rev 18: 225–236

Author's address: Dr. David Prangishvili, Lehrstuhl für Mikrobiologie, Universität Regensburg, Universitätsstrasse 31, D-93053 Regensburg, Germany; e-mail: david.prangishvili@biologie.uni-r.de