The ISME Journal (2008) 2, 1112–1121

www.nature.com/ismej

ORIGINAL ARTICLE

Lysogenic virus–host interactions predominate at deep-sea diffuse-flow hydrothermal vents

Shannon J Williamson[1,3], S Craig Cary[1], Kurt E Williamson[2,3], Rebekah R Helton[2], Shellie R Bench[1,2,4], Danielle Winget[1] and K Eric Wommack[1,2]
[1]*University of Delaware, Graduate College of Marine Studies, Newark, DE, USA and* [2]*Department of Plant and Soil Sciences, University of Delaware, Newark, DE, USA*

The consequences of viral infection within microbial communities are dependent on the nature of the viral life cycle. Among the possible outcomes is the substantial influence of temperate viruses on the phenotypes of lysogenic prokaryotes through various forms of genetic exchange. To date, no marine microbial ecosystem has consistently shown a predisposition for containing significant numbers of inducible temperate viruses. Here, we show that deep-sea diffuse-flow hydrothermal vent waters display a consistently high incidence of lysogenic hosts and harbor substantial populations of temperate viruses. Genetic fingerprinting and initial metagenomic analyses indicate that temperate viruses in vent waters appear to be a less diverse subset of the larger virioplankton community and that these viral populations contain an extraordinarily high frequency of novel genes. Thus, it appears likely that temperate viruses are key players in the ecology of prokaryotes within the extreme geothermal ecosystems of the deep sea.
The ISME Journal (2008) **2,** 1112–1121; doi:10.1038/ismej.2008.73; published online 21 August 2008
Subject Category: microbial population and community ecology
Keywords: lysogeny; hydrothermal vent; metagenomics; virioplankton

Introduction

The microbial ecology of deep-sea hydrothermal vent environments has been the subject of intense scrutiny since their discovery in 1977 (Corliss *et al.*, 1979). In deep-sea vent environments, hydrothermal activity supports diverse chemosynthetic microbial communities, which form the foundation upon which vent macrofauna depend. Vent prokaryotes have developed unique adaptation strategies to cope with extreme physical–chemical conditions, believed to be representatives of the archaean earth (Kelley *et al.*, 2002). Significant research efforts have been aimed toward understanding the genetic diversity, metabolic capacity and physiological adaptations of vent prokaryotes (Van Dover, 2000; Kelley *et al.*, 2002; Hou *et al.*, 2004; Nakagawa *et al.*, 2004, 2005, 2007; Nercessian *et al.*, 2005; Vetriani *et al.*, 2005; Moussard *et al.*, 2006; Scott *et al.*, 2006; Sogin *et al.*, 2006; Huber *et al.*, 2007), yet nothing is known regarding the role that viruses play in shaping the population biology and ecology of these unique microbial communities. Only two studies have reported direct, cultivation-independent examinations of viruses within deep-sea hydrothermal vent environments (Wommack *et al.*, 2004; Ortmann and Suttle, 2005). These studies report primarily on the abundance of viruses and their potential influence on microbial mortality at hydrothermal vents, but the nature of virus–host interactions in these environments was not explored.

Both temperate and virulent bacteriophages (viruses that infect prokaryotes) can serve as vectors for the horizontal transmission of genes between bacterial species or even genera by means of transduction (see Ackermann and DuBow (1987) for review). Through the process of lysogeny, a temperate phage establishes a quasistable genetic relationship with its host cell that may benefit the host population through the expression of phage-encoded fitness-enhancing phenotypes (Levin and Lenski, 1983) (that is, lysogenic conversion). The prevalence of lysogenic prokaryotes has been assessed in a range of aquatic environments through prophage induction from whole microbial communities

Correspondence: KE Wommack, Department of Plant and Soil Sciences and College of Marine Studies, Delaware Biotechnology Institute, University of Delaware, 15 Innovation Way, Newark, DE 19711, USA.
E-mail: wommack@dbi.udel.edu
[3]Current address: J Craig Venter Institute, Microbial and Environmental Genomics, La Jolla, CA, USA.
[4]Current address: Ocean Sciences Department, University of California Santa Cruz, Santa Cruz, CA, USA.
Received 2 April 2008; revised 3 July 2008; accepted 3 July 2008; published online 21 August 2008

(Jiang and Paul, 1994, 1996; Weinbauer *et al.*, 2003). Detectible lysogeny occurs either sporadically or seasonally depending on the type of environment sampled (Wilcox and Fuhrman, 1994; Weinbauer and Suttle, 1999; McDaniel *et al.*, 2002; Williamson *et al.*, 2002; Williamson and Paul, 2004; Long *et al.*, 2007). However, no marine environment has consistently demonstrated detectible levels of lysogeny within prokaryotic hosts. The current consensus is that lysogeny is more prevalent under suboptimal conditions for host survival and growth.

In broader evolutionary terms, viruses have recently been posited as reservoirs for genetic information poised to enter the collection of 'active' genes within prokaryotic communities as environmental conditions demand (Goldenfeld and Woese, 2007). Thus, prophage induction through environmental stimuli may be a means to promote phage-mediated gene exchange and recombination within a prokaryotic community in response to compromising environmental conditions. If indeed inducible prophages serve in this capacity, then environments that pose significant physical and chemical challenges to sustaining life would be predicted to support a higher proportion of temperate prophage. To test this hypothesis, the frequency of inducible prophage was assessed within diffuse-flow waters of deep-sea hydrothermal vents, where cold seawater seeps into the subsurface and mixes with rising hydrothermal vent fluids, and across the overlying pelagic water column.

Materials and methods

Sample collection and processing
For each sampling location, 120 l of diffuse-flow hydrothermal vent water was collected using a large-volume water sampler (Wommack *et al.*, 2004). Hydrothermal venting areas, where diffuse-flow temperatures were between 20 and 70 °C, were targeted for collection. A CTD rosette was employed to collect approximately 100–120 l of water from discrete depths within the water column. Diffuse-flow and water column samples were concentrated by tangential flow filtration as described in Wommack *et al.* (2004).

Prophage induction experiments
Duplicate 20 ml prokaryote concentrate diffuse-flow vent and water column samples were either treated with mitomycin C (MC) (1.0 μg ml^{-1} final concentration) or left untreated as controls. All samples were incubated statically, in the dark, at environmentally relevant temperatures for 24 h, followed by fixation with glutaraldehyde (2% final concentration). Five millilitres of subsamples were flash-frozen in liquid nitrogen and stored at −80 °C before microscopic observation. Lysogenic fractions were calculated according to the 'burst size method' outlined in Williamson *et al.* (2002). The burst size used in all calculations was 50 virus particles per burst.

Randomly amplified polymorphic DNA PCR
Mitomycin C-treated and -untreated diffuse-flow prokaryote concentrates from vent sites Bio9 and M Vent were passed through 0.22-μm Sterivex filters (Millipore Corp., Billerica, MA, USA) to remove all the cells. Viruses were simultaneously washed three times with 1 × TE to remove all MC and glutaraldehyde and concentrated according to Wommack *et al.* (2004) before randomly amplified polymorphic DNA PCR (RAPD-PCR). One random 10-mer primer (5′-GGTCCCTGAC-3′) was used in all RAPD-PCRs. One microliter of viral concentrate (Bio9 = 6×10^7 viruses μl^{-1}; M Vent = 1×10^7 viruses per μl) was added to PCRs containing the following: 4.0 μM final concentration of the random primer, 0.16 mM final concentration of each nucleotide, 5.0 U Taq DNA polymerase (Fisher Scientific, Hampton, NH, USA) and 1.0 × final concentration PCR buffer. Thermocycler conditions were 10 min at 94 °C followed by 33 cycles of 30 s at 94 °C, 3 min at 35 °C and 1 min at 72 °C. The final cycle was followed by 10 min at 72 °C.

Clone libraries
Immediately following gel electrophoretic analysis, whole populations of RAPD-PCR products or single RAPD-PCR bands were purified using the QIAquick PCR Purification kit or the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA), respectively. Purified PCR amplicons were TOPO TA-cloned into the pCRII-TOPO vector (Invitrogen, Carlsbad, CA, USA) and transformed into chemically competent *Escherichia coli*. Transformations were spread onto Luria-Bertani plates containing 50 μg ml^{-1} kanamycin and treated with 40 mg ml^{-1} X-gal. Plates were incubated overnight at 37 °C. White colonies were streaked to isolation on Luria-Bertani plates containing 50 μg ml^{-1} kanamycin, then picked into 96-well microtiter plates containing selective Luria-Bertani media and incubated for 18 h at 37 °C. Sterile glycerol (15% final concentration) was added to each well, and the clones were stored at −80 °C until further processing.

For the shotgun metagenome library, 175 μl of diffuse-flow vent viral concentrates from three sites (Teca, BioVent and M Vent) were pooled and subsequently washed two times with 1 × TE to remove salts (Wommack *et al.*, 2004). The washed viral concentrate was added to a 2 ml Beckman micro-ultracentrifuge tube (Fullerton, CA, USA), and the final volume was brought up to 2.0 ml with the addition of 1 × TE. Viral particles were pelleted at 25 000 r.p.m. for 10 h at 10 °C. Pelleted viral particles were resuspended in a total volume of 180 μl 1 × TE and heated at 60 °C for 40 min to liberate viral DNA. The frozen denatured viral

suspension was sent to Lucigen (Middleton, WI, USA) for the construction of a linker-amplified shotgun library (LASL) according to Breitbart *et al*. (2002). Transformations and colony selection were conducted as described for the RAPD library. Plasmid DNA was amplified using TempliPhi DNA Sequencing Template Amplification Kit (Amersham Biosciences, Piscataway, NJ, USA) before restriction digestion and sequencing. The presence of insert was verified by digesting plasmid DNA with 1 U *Eco*RI in Buffer H (Promega, Madison, WI, USA) for 1 h at 37 °C. One hundred and sixty clones from the RAPD library containing various insert sizes and 96 clones from the metagenome library were sequenced at the University of Delaware's Sequencing and Genotyping Center on an ABI Prism 3100 Genetic Analyzer.

Statistical analysis: prophage induction experiments
Comparisons between viral abundance in independent treated ($n=2$) and control ($n=2$) samples were performed on log-transformed values using two-tailed *t*-tests for normally distributed data or the Mann–Whitney *U*-test for data that were not normally distributed. For 2002 samples, statistical tests were performed using fields of view data from microscope enumerations rather than replicate counts as $n=1$ for treatment and control incubations. Standard deviations are reported for ambient viral abundance data and standard errors are reported for secondary production data.

Epifluorescence microscopy
Enumeration of prokaryotic cells and virus-like particles were conducted according to Williamson *et al*. (2003) with the following modifications. Filtration volumes for all prokaryote concentrate samples were 4 ml. Duplicate filters per independent sample were analyzed by epifluorescence microscopy using an Olympus BX61 microscope (Olympus America Inc., Melville, NY, USA) with an fluorescein isothiocyanate excitation filter. Ten fields per sample were digitally photographed at $\times 1000$ magnification using a QCapture 2 camera (QImaging, British Columbia, Canada). Prokaryotic cells and virus-like particles were enumerated using the IP Lab (version 3.9) software (Scanalytics Inc., Fairfax, VA, USA).

Secondary production
Heterotrophic prokaryotic cells produced per hour were estimated by the leucine-incorporation method (Kirchman, 2001) with samples processed by centrifugation (Smith and Azam, 1992) in triplicate. ^{3}H leucine was added to a final concentration of 20 nM. Hydrothermal vent samples and deep-water column samples (2000, 1500 and 500 m) were incubated for 5 h, and shallow water samples (deep chlorophyll maximum and surface) were incubated for 1 h. All samples were incubated at environmentally relevant temperatures.

Cluster analysis of RAPD fingerprints
Bands within the digital image of the electrophoretic gel were identified using the software package Gel Compar (Applied Maths Inc., Austin, TX, USA). The position of bands within each sample RAPD-PCR fingerprint was normalized according to known bands within the pGEM molecular weight marker flanking the sample lanes. The position tolerance setting was 6.8% with an optimization setting of 0.79% and no change toward the ends of the fingerprint. These settings were based on an iterative optimization test of the data. A dendogram was constructed using the unweighted pair-group method using arithmetic averages (UPGMA) based on a Jaccard coefficient similarity matrix of the molecular fingerprints.

Sequence analysis
Randomly amplified polymorphic DNA sequences were trimmed for vector and quality using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI, USA). LASL DNA sequences were trimmed for vector and quality using PHRED (Ewing *et al*., 1998). Translating BLASTs (tblastx) were performed for the following databases: NCBI nt, NCBI env-nt as well as environmental viral metagenomes generated from the Chesapeake Bay (Bench *et al*., 2007), Delaware agricultural soil (Wommack *et al*., 2004), Mission Bay, CA water (Breitbart *et al*., 2002) and sediments (Breitbart *et al*., 2004), and human feces (Breitbart *et al*., 2003). Nucleotide BLASTs were performed for NCBI nr and env-nr databases. In the event in which an environmental sequence was the top BLAST hit, the full-length protein or DNA sequence was compared to the NCBI nt or nr database to further aid in the annotation of diffuse-flow viral sequences.

Accession numbers
The viral sequences reported in this paper have been submitted to GenBank with the following accession numbers: ED017435–ED017695 for the RAPD sequences and ED017696–ED017873 for the LASL sequences.

Results

Viral abundance
Diffuse-flow water samples collected from vents along the East Pacific Rise (9° 50′ N) using a large-volume water sampler exhibited enhanced levels of sulfide and dissolved iron, characteristic of hydrothermal vent water (Wommack *et al*., 2004). Average viral abundance within water column and

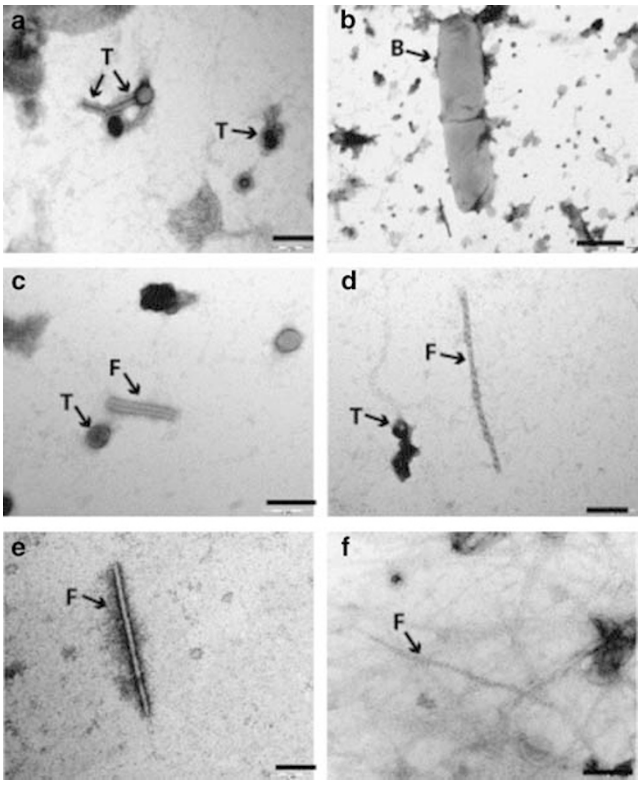

Figure 1 Transmission electron micrographs of viruses and bacteria from 9°N East Pacific Rise: (**a** and **b**) 2500 m water sample; (**c** and **d**) Bio9 vent; and (**e** and **f**) M and Q vents. Filamentous phage (F); tailed phage (T) and bacterium (B). Scale bars: (**a**, **c** and **d**) 100 nm; (**e**) 200 nm; (**b**) 500 nm.

diffuse-flow vent water samples were at least 10-fold higher than prokaryote abundance (shallow water: 190 ($\pm$60) $\times 10^5$ viruses and 9.0 ($\pm$3.5) $\times 10^5$ prokaryotes per ml; deep water: 21 ($\pm$22) $\times 10^5$ viruses and 1.8 ($\pm$0.6) $\times 10^5$ prokaryotes per ml; and vent diffuse-flow waters 54 ($\pm$75) $\times 10^5$ viruses and 1.8 ($\pm$9.2) $\times 10^5$ prokaryotes per ml). While sunlit surface waters displayed the highest viral abundances (170 ($\pm$10) $\times 10^5$ viruses per ml), diffuse-flow vent waters contained almost threefold more viruses than surrounding deep-sea water.

Prophage induction

Mitomycin C, a DNA mutagen, is an effective stimulator of prophage induction and has been used to assess the occurrence of lysogeny in numerous aquatic environments (Jiang and Paul, 1994, 1996; Weinbauer *et al.*, 2003; Williamson and Paul, 2004). Statistically significant increases in viral abundance over control values were observed for 7 of 10 diffuse-flow vent samples treated with MC. Examination of virus-like particles by transmission electron microscopy showed that MC-induced vent viruses were of similar size and morphology to known viruses (Figure 1). In contrast, only three of nine water column samples exhibited statistically significant increases in viral abundance upon treatment with

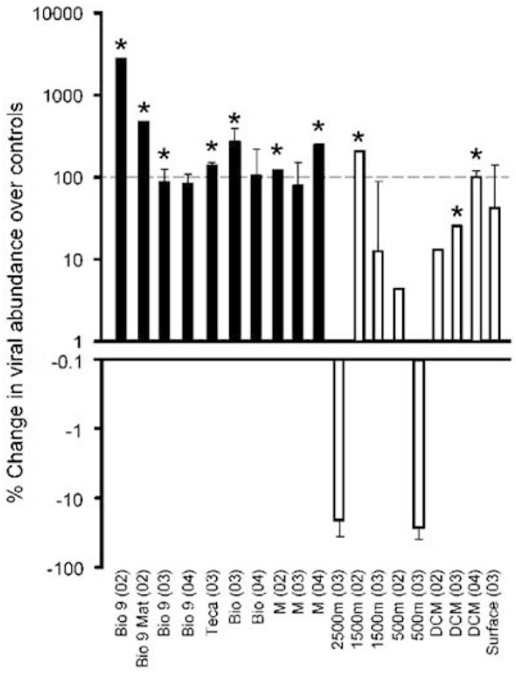

Figure 2 Percent change in viral abundance over untreated controls from prokaryotic concentrate diffuse-flow vent and water column samples. Error bars represent s.d. for '03 and '04 samples. Asterisks indicate statistical significance assuming equal variances at the 90 or 95% CI for the following samples: Bio9 '02 ($P=0.002$); Bio9 mat '02 ($P=0.0002$); Bio9 '03 ($P=0.03$); BioVent '03 ($P=0.06$); Teca '03 ($P=0.03$); M Vent '02 ($P=0.014$); M Vent '04 ($P=0.02$); 1500 m '02 ($P=2\times10^{-7}$); DCM '03 ($P=0.03$) and DCM '04 ($P=0.005$). With the exception of the Surface '03, Bio9 '02 and Bio9 mat '02 samples, all log-transformed data were normally distributed. DCM, deep chlorophyll maximum.

MC (Figure 2). In nearly every diffuse-flow sample induction experiment, viral abundance increased more than 100% within the MC treatment as compared to only two experiments among the water column samples. Deep-water samples (2500 and 500 m) exhibited large decreases in viral abundance with MC treatment (Figure 2).

Significant prophage induction in diffuse-flow waters was accompanied by low levels of secondary heterotrophic production in ambient raw water samples (Figure 3). Average ^{3}H leucine incorporation rates within diffuse-flow vent water (6.92 ($\pm$1.53) $\times 10^{-3}$ pmol leucine per h) were roughly equal to the average rates observed for deep water column samples (2500, 1500 and 500 m) (5.47 ($\pm$0.18) $\times 10^{-3}$ pmol leucine per h), but were threefold lower than shallow water samples (deep chlorophyll maximum and surface) (2.16 ($\pm$2.53) $\times 10^{-2}$ pmol leucine per h). Comparisons of secondary production rates within prokaryote concentrates showed that average rates for diffuse-flow vent water samples (11.2 ($\pm$0.22) $\times 10^{-2}$ pmol leucine per h) were threefold higher than those for deep water column samples (3.83 ($\pm$2.58) $\times 10^{-2}$ pmol leucine per h). This finding suggests that larger molecular weight growth substrates (that is, $>$30 kDa) may have been concentrated along with

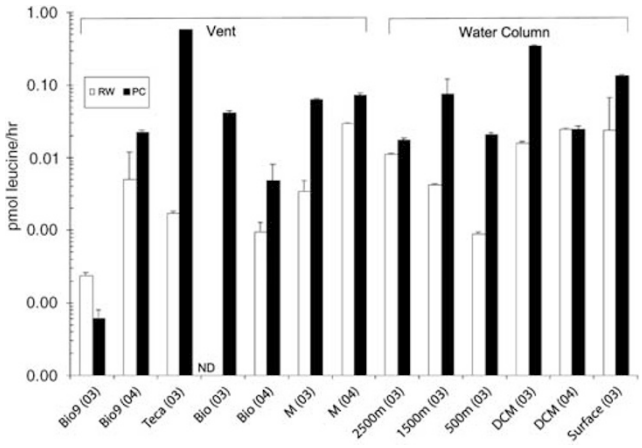

Figure 3 Secondary production in diffuse-flow vent and water column samples. White bars represent ambient raw water. Black bars represent prokaryote concentrates. ND is not determined. Error bars represent s.e.

Table 1 Inducible fraction of prokaryote concentrates

Sample	*Inducible fraction (%)*
Diffuse-flow	
Bio9 Vent '03	129.1 (±23.5)[a]
Bio9 Vent '04	5.4 (±3.9)
Teca Vent '03	2.7 (±.09)
BioVent '03	5.9 (±8.0)
BioVent '04	3.6 (±0.02)
M Vent '03	3.7 (±0.4)
M Vent '04	1.0 (±0.3)
Water column	
Surface '03	6.4 (±8.6)
DCM '03	3.1 (±0.7)
DCM '04	1.0 (±0.1)
500 m '03	−6.4 (±4.5)
1500 m '03	2.5 (±17.8)
2500 m '03	−12.5 (±8.9)

[a]s.d.

prokaryotic cells resulting in a stimulation of secondary heterotrophic production in these samples. Consequently, such metabolic stimulation of vent prokaryotes may have resulted in enhanced prophage induction upon treatment with MC.

The frequency of inducible cells within prokaryote concentrates (Williamson *et al.*, 2002) ranged from 1.0 (±0.3%) to 130 (±24%) in diffuse-flow samples and from −13 (±9.0%) to 6.4 (±8.6%) in water column samples (Table 1). Negative inducible fractions, resulting from decreases in viral abundance in MC-treated samples, were observed for two out of three deep-water column samples, indicating the lack of lysogens at these depths. In contrast, all diffuse-flow vent samples exhibited inducible fractions similar to the productive environments of the photic zone. Thus, the predisposition of deep-sea hydrothermal vent diffuse-flow water samples to produce significant numbers of viruses upon treatment with MC is a strong indication that these marine environments are unique with respect to the density of temperate phage and lysogenic prokaryotes they contain. As certain populations of lysogens may be insensitive to treatment with MC, the occurrence of lysogeny within diffuse-flow samples may be underestimated.

Diffuse-flow viral diversity: RAPD-PCR

Randomly amplified polymorphic DNA PCR banding patterns were generated from viral concentrates of diffuse-flow water samples and MC-treatment and control incubations at two distinct diffuse-flow hydrothermal vent environments (Bio9 and M Vent). Each RAPD-PCR banding pattern was unique suggesting that diffuse-flow vent environments support genetically distinct viral communities (Figure 4). Cluster analysis of RAPD banding patterns confirmed that the Bio9 control and diffuse-flow fingerprints were most similar to each other (56%) and they shared the least similarity with the induced fingerprint (39%) (Figure 4). A similar but less dramatic trend was seen in M Vent experiments. These levels of similarity are well below those of replicate reactions, which typically share >80% banding similarity for virioplankton assemblages (Winget and Wommack, 2008). As the number of RAPD-PCR fragments reflects the diversity of template sequences in the original sample (Franklin *et al.*, 1999), induced viruses from vent sites Bio9 (~20 bands) and M Vent (~10 bands) appear to be genotypically distinct less-diverse subsets of whole viral communities within diffuse-flow waters.

Metagenomic analysis of diffuse-flow viral communities

DNA sequences from nine RAPD-PCRs (258 sequences) and an LASL of diffuse-flow viral communities (176 sequences) averaged 488 bases in length and were analyzed through BLAST homology searches against seven databases (Altschul *et al.*, 1990) (Table 2). RAPD sequences were derived from viral concentrates of two diffuse-flow water samples; two treatment and control incubations each from vent sites Bio9 and M Vent; and a single band common to the three Bio9 viral fingerprints. Typically ~80% of sequences within a single RAPD-PCR band were identical indicating that most of the amplified DNA resulted from a single viral template (Figure 4).

Only ~25% of sequences had significant BLAST homology ($E \leqslant 0.001$) to known sequences within NCBI's non-redundant database (nr) (Table 2). This proportion is lower than the ~35% NCBI nr homolog rate seen in other Sanger read length viral metagenome libraries (Edwards and Rohwer, 2005). Inclusion of BLAST searches against environmental sequence databases (env-nt, env-nr and other viral metagenomes) increased overall BLAST homolog

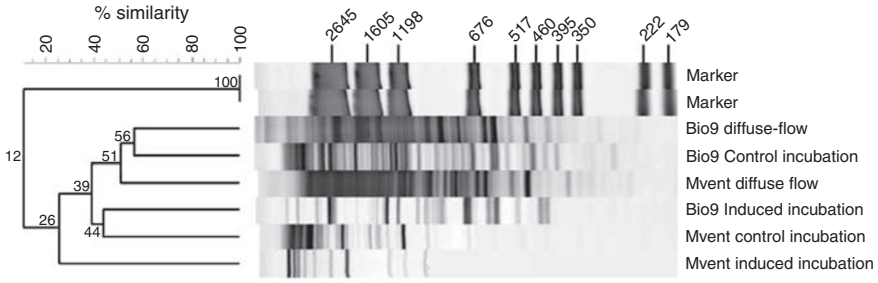

Figure 4 RAPD-PCR fingerprints of viral concentrates collected directly from diffuse-flow water, from control (no mitomycin C addition) or mitomycin C-treated diffuse-flow prokaryote concentrates. Size of marker bands are given in basepairs of DNA. Numbers at cladogram nodes indicate percent similarity. RAPD-PCR, randomly amplified polymorphic DNA PCR.

Table 2 Distribution of BLAST homologs to deep-sea hydrothermal vent viral sequences according to sequence database

Library (no. of sequences)		*Top BLAST hits by database*							*Sequences with a hit*	*Novel sequences*
		Vir DBs			*env-nt/nr*		*nt/nr*			
		Ches virioplk[a]	*Mtpk soil*[b]	*Other virioplk*[c]	*env-nt*	*env-nr*	*nt*	*nr*		
RAPD-1 (81)	Number of hits	4	1	5	29	26	23	26	36	45
	% of library	5%	1%	6%	36%	32%	28%	32%	44%	56%
	Log median *E*-score	−14.8	−4	−11	−25.2	−22.3	−17.7	−14.7		
RAPD-2 (178)	Number of hits	8	6	10	61	54	47	53	86	92
	% of library	4%	3%	6%	34%	30%	26%	30%	48%	52%
	Log median *E*-score	−8.4	−3.3	−5.5	−25	−16.7	−12.4	−14.7		
LASL-1 (178)	Number of hits	32	16	32	59	51	35	30	122	137
	% of library	18%	9%	18%	33%	29%	20%	17%	47%	53%
	Log median *E*-score	−12.6	−8.3	−9.3	−15.7	−12	−16	−13.5		
Totals (437)	Number of hits	44	23	47	149	131	105	109	213	224
	% of library	10%	5%	11%	34%	30%	24%	25%	49%	51%
	Log median *E*-score	−11.3	−6.7	−8.7	−22.3	−15.1	−15.7	−14.5		

Abbreviations: LASL, linker-amplified shotgun library; RAPD, randomly amplified polymorphic DNA PCR.
[a]Chesapeake Bay virioplankton.
[b]Matapeake soil viruses.
[c]Other marine virioplankton.

frequency to ~49%. In particular, searches against three viral metagenome Sanger libraries found an additional 13 homologs (7% of the library) within the LASL as compared to none within the RAPD libraries. Otherwise, per database BLAST hit frequencies between the three libraries were similar. Overall, the quality of BLAST alignments was greatest for environmental homologs as these hits tended to have the lowest median expectation score (Tables 2 and 3). Thus, a high proportion (51%) of viral metagenome sequences from diffuse-flow environments were completely novel, having no homology to any existing sequence data (Table2 and Figure 5). By comparison, similar exhaustive homology searches indicated that only 30% of the sequence information within a shotgun library of Chesapeake Bay virioplankton was novel (Bench *et al.*, 2007). The degree to which the 488 bp average read length of the vent viral libraries contributed to the high frequency of novel sequences is unknown. Recent *in silico* simulation studies have shown that the frequency of viral and microbial metagenome sequences with significant BLAST homology is sensitive to read length (Wommack *et al.*, 2008).

Finally, significant BLAST homologs of RAPD sequences were more frequently bacterial in origin, whereas three times as many significant viral homologs (for example, terminases and replication proteins) were identified among LASL sequences (Table 3). Library-specific biases in the frequency of viral hits likely reflect the fact that a transcription-free vector was used for the LASL that prevented the loss of clones due to modified bases and lethal genes (Edwards and Rohwer, 2005).

Discussion

In this first systematic investigation of virus–host interactions and extant genetic diversity within viruses of deep-sea hydrothermal vent ecosystems, the consistent detection of MC-inducible prophage

Table 3 Description of diffuse-flow hydrothermal vent viral sequences

Library type	*Location and sample*	*Total no. of sequences*	*Number (log median* E*-score) of significant BLAST homologs ($E \leqslant 10^{-3}$)*				
			Database			*Taxonomy of BLAST homologs*	
			GenBank		*Vir DB*	*Bacteria*	*Viruses*
			nt or nr	*env-nt or env-nr*			
RAPD	Bio9, DF	30	1 (−8.3)	0	4 (−3.0)	0	^{S}1 (−8.3)
	Bio9, Ind	40	10 (−7.3)	12 (−11.7)	10 (−5.0)	$^{\gamma}$2 (−5.7) ^{O}2 (−7.0)	^{P}1 (−5.6)
	Bio9, Contr	37	8 (−15.5)	2 (−7.3)	4 (−7.0)	$^{\alpha}$1 (−35.8)	0
	M Vent, DF	59	4 (−15.5)	21 (−25)	12 (−9.7)	$^{\alpha}$1 (−21.3) $^{\gamma}$1 (−3.0) ^{O}1 (−10.0)	^{P}1 (−24.0)
	M Vent, Ind	20	16 (−21.9)	16 (−41.6)	0	$^{\alpha}$1 (−7.7) $^{\beta}$4 (−33.8) $^{\gamma}$11 (−11.3)	0
	M Vent, Contr	34	29 (−17.5)	32 (−25.9)	4 (−8.3)	$^{\alpha}$5 (−15.6) $^{\beta}$5 (−25.6) $^{\gamma}$16 (−17.8) $^{\delta}$1 (−29.8)	0
RAPD (single band)	Bio9, DF	19	3 (−13.8)	0	0	0	0
	Bio9, Ind	18	10 (−7.8)	0	0	0	0
	Bio 9, Contr	4	4 (−7.6)	0	0	0	0
LASL	Mixed location, DF	176	42 (−15.8)	62 (−14.7)	55 (−11.0)	$^{\alpha}$1 (−19.6) $^{\beta}$2 (−4.3) $^{\gamma}$6 (−67.6) ^{O}6 (−6.3)	^{M}1 (−7.6) ^{S}1 (−10.0) ^{P}7 (−18.7) ^{U}2 (−4.3)
Totals		437	127	145	89	66	14

Abbreviations: α, alpha proteobacteria; β, beta proteobacteria; γ, gamma proteobacteria; δ, delta proteobacteria; Contr, control; DF, diffuse-flow; Ind, induced; LASL, linker-amplified shotgun library; M, Myoviridae; O, bacterial classification other than proteobacteria; P, Podoviridae; RAPD, randomly amplified polymorphic DNA PCRS, Siphoviridae; U, unclassified viral family.

within diffuse-flow prokaryote communities is a strong evidence for the fact that lysogenic virus–host interactions play a critical role in the ecology of prokaryotes in this extreme environment. In contrast, induction assays of samples from the water column demonstrated a mixed and inconsistent induction response similar to that documented for many other marine environments (Wilcox and Fuhrman, 1994; Weinbauer and Suttle, 1996, 1999; Cochran and Paul, 1998; McDaniel *et al.*, 2002; Ortmann *et al.*, 2002; Williamson *et al.*, 2002; Williamson and Paul, 2004). Thus, a predominance of inducible lysogens supports the hypothesis that challenging physical–chemical conditions select for prokaryotes harboring temperate phage. Moreover, a high density of lysogenic prokaryotes may be another characteristic differentiating deep-sea vent from other marine environments.

For extremeophillic prokaryotes, temperate phage may provide phenotypes that improve the fitness of lysogenic strains (Summit and Baross, 2001), whereas for phage, the lysogenic state may represent a means of avoiding the inactivating effects of this deep-sea extreme environment (Cottrell and Suttle, 1995). Prophage alteration of host phenotype can occur at the level of single-gene determinants (for example, virulence factors within bacterial pathogens Canchaya *et al.*, 2004) or more globally through altered gene regulation (Chen *et al.*, 2005). Whatever the mechanism may be, the observation of abundant populations of lysogens and temperate phage indicates that phage-mediated gene exchange and recombination may be critical to the survival and stability of prokaryotes within extreme environments.

The lack of prophage induction observed in most deep water samples overlying the EPR contrasts with the observations of abundant lysogens in Mediterranean deep waters (Weinbauer *et al.*, 2003). Thus, despite the relatively stable conditions of the deep sea, ecosystem-specific factors may influence the occurrence of lysogeny. In addition, the occurrence of lysogeny in the marine environment has been correlated to low levels of secondary production (McDaniel *et al.*, 2002; Williamson *et al.*, 2002; Weinbauer *et al.*, 2003), and our results indicate that secondary production rates are substantially lower in diffuse-flow vent ambient water as opposed to the euphotic zone (Figure 3). Together, these results suggest that temperate phage enters into lysogenic interactions with their hosts within hostile, low-productivity marine environments. As diffuse-flow vents also support diverse populations of chemoautotrophs, the relationship between lysogeny and rates of primary production warrants further attention.

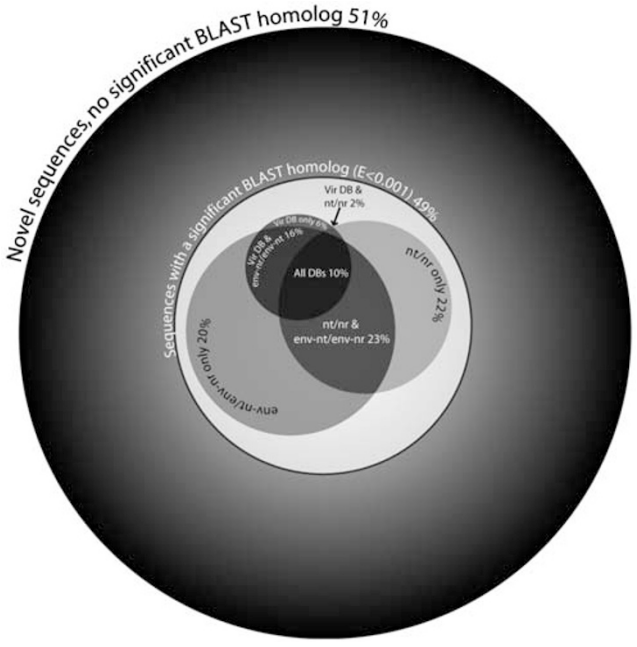

Figure 5 Venn diagram of BLAST search results by database and relationship of sequences with a significant BLAST homolog ($E<0.001$) to all sequences. Circle size represents the number of sequences within each category and are drawn to scale. Areas of circle intersections approximate the frequency of BLAST homologs across multiple databases. Outermost black circle represents all sequences, whereas the inner white circle represents all sequences with a significant BLAST homolog. The venn diagram contained within the white circle shows the frequency of sequences with BLAST homologs to three database types: nt/nr (GenBank nucleotide and GenBank non-redundant protein); env-nt/env-nr (GenBank environmental nucleotide and GenBank environmental non-redundant protein); and Vir DB (three viral metagenome libraries).

Preliminary evidence from RAPD fingerprints, TEM micrographs and metagenome sequence data indicates that like many other marine ecosystems (Wichels *et al.*, 1998; Wommack *et al.*, 1999; Short and Suttle, 2002; Edwards and Rohwer, 2005; Angly *et al.*, 2006; Culley *et al.*, 2006), the vents contain diverse viral communities. The unique metagenomic signature exhibited by diffuse-flow viruses may have developed in response to the environmental characteristics and pressures that define diffuse-flow hydrothermal vents. Furthermore, within a vent site, temperate phage comprises a less diverse subset of the larger virioplankton community, which may reflect the high host specificity of temperate phage combined with a low diversity of lysogenic hosts. However, local conditions within different vent sites appear to host genetically distinct prophage communities, so overall diversity of temperate prophage across a range of diffuse-flow environments may be high. This pattern of high local diversity supports recent work showing that diversity within discrete marine virioplankton assemblages rivals that seen across the global ocean (Angly *et al.*, 2006). Because phages are well-known vectors for lateral gene transfer, the observed site-specific nature of vent viral communities may facilitate gene transfer between hosts at one site while inhibiting genetic exchange between hosts at different sites; perhaps influencing the finely tuned adaptive nature of vent prokaryotes.

In-depth investigations of virus–host interactions within deep-sea ecosystems lead us to the conclusion that diffuse-flow hydrothermal vent environments are unique with respect to the magnitude of inducible lysogens and temperate phage that they consistently support. The lateral transfer of genes between organisms is a driving force in the evolution and adaptation of microbes within any environment. Phage-mediated gene transfer maybe particularly prevalent in extreme environments, such as hydrothermal vents, in which adaptive pressures are exceptionally high. These data lend strong support to the recently posited hypothesis that prophage induction facilitates the dispersal of host genes within a prokaryotic community in response to environmental triggers (Goldenfeld and Woese, 2007). How temperate phage specifically influences the adaptive phenotypes of vent prokaryotes is a subject of continued investigation.

Acknowledgements

We acknowledge the support of a University of Delaware Research Foundation grant to KEW, NSF Microbial Observatories award 0132070 to KEW and the NSF Alvinella Metagenome award to SSC. SJW, KW, RRH, SRB and SCC contributed to the field investigations and subsequent data collection. SRB and SJW conducted viral metagenome sequence analysis. KEW and SJW planned the experiments and contributed equally to the writing. DMW and SJW performed the statistical analyses.

References

Ackermann HW, DuBow MS. (1987). *Viruses of Prokaryotes: General Properties of Bacteriophages*. CRC Press, Inc: Boca Raton, Florida, 202pp.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. (1990). Basic local alignment search tool. *J Mol Biol* **215**: 403–410.

Angly FE, Felts B, Breitbart M, Salamon P, Edwards RA, Carlson C *et al.* (2006). The marine viromes of four oceanic regions. *PLoS Biol* **4**: e368.

Bench SR, Hanson TE, Williamson KE, Ghosh D, Radosovich M, Wang K *et al.* (2007). Metagenomic characterization of Chesapeake Bay virioplankton. *Appl Environ Microbiol* **73**: 7629–7641.

Breitbart M, Felts B, Kelley S, Mahaffy JM, Nulton J, Salamon P *et al.* (2004). Diversity and population structure of a near-shore marine-sediment viral community. *Proc R Soc Lond B Biol Sci* **271**: 565–574.

Breitbart M, Hewson I, Felts B, Mahaffy JM, Nulton J, Salamon P *et al.* (2003). Metagenomic analyses of an uncultured viral community from human feces. *J Bacteriol* **185**: 6220–6223.

Breitbart M, Salamon P, Andresen B, Mahaffy JM, Segall AM, Mead D *et al.* (2002). Genomic analysis of uncultured marine viral communities. *Proc Natl Acad Sci USA* **99**: 14250–14255.

Canchaya C, Fournous G, Brussow H. (2004). The impact of prophages on bacterial chromosomes. *Mol Microbiol* **53**: 9–18.

Chen Y, Golding I, Sawai S, Guo L, Cox EC. (2005). Population fitness and the regulation of *Escherichia coli* genes by bacterial viruses. *PLoS Biol* **3**: e229.

Cochran PK, Paul JH. (1998). Seasonal abundance of lysogenic bacteria in a subtropical estuary. *Appl Environ Microbiol* **64**: 2308.

Corliss JB, Dymond J, Gordon LI, Edmond JM, Herzen RPV, Ballard RD *et al.* (1979). Submarine Thermal Springs on the Galapagos Rift. *Science* **203**: 1073–1083.

Cottrell MT, Suttle CA. (1995). Dynamics of a lytic virus infecting the photosynthetic marine picoflagellate *Micromonas pusilla*. *Limnol Oceanogr* **40**: 730–739.

Culley AI, Lang AS, Suttle CA. (2006). Metagenomic analysis of coastal RNA virus communities. *Science* **312**: 1795–1798.

Edwards RA, Rohwer F. (2005). Viral metagenomics. *Nat Rev Microbiol* **3**: 504–510.

Ewing B, Hiller L, Wendl MC, Green P. (1998). Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. *Genome Res* **8**: 175–185.

Franklin RB, Taylor DR, Mills AL. (1999). Characterization of microbial communities using randomly amplified polymorphic DNA (RAPD). *J Microbiol Methods* **35**: 225–235.

Goldenfeld N, Woese C. (2007). Biology's next revolution. *Nature* **445**: 369.

Hou S, Saw JH, Lee KS, Freitas TA, Belisle C, Kawarabayasi Y *et al.* (2004). Genome sequence of the deep-sea gamma-proteobacterium *Idiomarina loihiensis* reveals amino acid fermentation as a source of carbon and energy. *Proc Natl Acad Sci USA* **101**: 18036–18041.

Huber JA, Mark Welch DB, Morrison HG, Huse SM, Neal PR, Butterfield DA *et al.* (2007). Microbial population structures in the deep marine biosphere. *Science* **318**: 97–100.

Jiang SC, Paul JH. (1994). Seasonal and diel abundance of viruses and occurence of lysogeny/bacteriocinogeny in the marine environment. *Mar Ecol Prog Ser* **104**: 163–172.

Jiang SC, Paul JH. (1996). Occurrence of lysogenic bacteria in marine microbial communities as determined by prophage induction. *Mar Ecol Prog Ser* **142**: 27–38.

Kelley DS, Baross JA, Delaney JR. (2002). Volcanoes, fluids, and life at mid-ocean ridge spreading centers. *Annu Rev Earth Planet Sci* **30**: 385–491.

Kirchman D. (2001). Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. *Methods Microbiol* **30**: 227–237.

Levin BR, Lenski RE. (1983). *Coevolution in Bacteria and their Viruses and Plasmids*. Sinauer: Sunderland, Mass.

Long A, McDaniel LD, Mobberley J, Paul JH. (2007). Comparison of lysogeny (prophage induction) in heterotrophic bacterial and *Synechococcus* populations in the Gulf of Mexico and Mississippi river plume. *ISME J* **2**: 132.

McDaniel L, Houchin LA, Williamson SJ, Paul JH. (2002). Lysogeny in marine *Synechococcus*. *Nature* **415**: 496.

Moussard H, Corre E, Cambon-Bonavita M-A, Fouquet Y, Jeanthon C. (2006). Novel uncultured Epsilonproteobacteria dominate a filamentous sulphur mat from the 13°N hydrothermal vent field, East Pacific Rise. *FEMS Microbiol Ecol* **58**: 449–463.

Nakagawa S, Takai K, Inagaki F, Hirayama H, Nunoura T, Horikoshi K *et al.* (2005). Distribution, phylogenetic diversity and physiological characteristics of epsilon-Proteobacteria in a deep-sea hydrothermal field. *Environ Microbiol* **7**: 1619–1632.

Nakagawa S, Takaki Y, Shimamura S, Reysenbach AL, Takai K, Horikoshi K. (2007). Deep-sea vent epsilonproteobacterial genomes provide insights into emergence of pathogens. *Proc Natl Acad Sci USA* **104**: 12146–12150.

Nakagawa T, Nakagawa S, Inagaki F, Takai K, Horikoshi K. (2004). Phylogenetic diversity of sulfate-reducing prokaryotes in active deep-sea hydrothermal vent chimney structures. *FEMS Microbiol Lett* **232**: 145–152.

Nercessian O, Bienvenu N, Moreira D, Prieur D, Jeanthon C. (2005). Diversity of functional genes of methanogens, methanotrophs and sulfate reducers in deep-sea hydrothermal environments. *Environ Microbiol* **7**: 118–132.

Ortmann AC, Lawrence JE, Suttle CA. (2002). Lysogeny and lytic viral production during a bloom of the cyanobacterium *Synechococcus* spp. *Microb Ecol* **43**: 225–231.

Ortmann AC, Suttle CA. (2005). High abundances of viruses in a deep-sea hydrothermal vent system indicates viral mediated microbial mortality. *Deep Sea Res Part I Oceanogr Res Pap* **52**: 1515–1527.

Scott KM, Sievert SM, Abril FN, Ball LA, Barrett CJ, Blake RA *et al.* (2006). The genome of deep-sea vent chemolithoautotroph *Thiomicrospira crunogena* XCL-2. *PLoS Biol* **4**: e383.

Short SM, Suttle CA. (2002). Sequence analysis of marine virus communities reveals that groups of related algal viruses are widely distributed in nature. *Appl Environ Microbiol* **68**: 1290–1296.

Smith DC, Azam F. (1992). A simple economical method for measuring bacterial protein synthesis rates in seawater using (super) ^{3}H-leucine. *Mar Microb Food Webs* **6**: 107–114.

Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR *et al.* (2006). Microbial diversity in the deep sea and the underexplored 'rare biosphere'. *Proc Natl Acad Sci USA* **103**: 12115–12120.

Summit M, Baross JA. (2001). A novel microbial habitat in the mid-ocean ridge subseafloor. *Proc Natl Acad Sci USA* **98**: 2158–2163.

Van Dover CL. (2000). *The Ecology of Deep-sea Hydrothermal Vents*. Princeton University Press: Princeton, New Jersey, USA

Vetriani C, Chew YS, Miller SM, Yagi J, Coombs J, Lutz RA *et al.* (2005). Mercury adaptation among bacteria from a deep-sea hydrothermal vent. *Appl Environ Microbiol* **71**: 220–226.

Weinbauer MG, Brettar I, Hofle MG. (2003). Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters. *Limnol Oceanogr* **48**: 1457–1465.

Weinbauer MG, Suttle CA. (1996). Potential significance of lysogeny to bacteriophage production and bacterial

mortality in coastal waters of the Gulf of Mexico. *Appl Environ Microbiol* **62**: 4374–4380.

Weinbauer MG, Suttle CA. (1999). Lysogeny and prophage induction in coastal and offshore bacterial communities. *Aquat Microb Ecol* **18**: 217–225.

Wichels A, Biel SS, Gelderblom HR, Brinkhoff T, Muyzer G, Schutt C. (1998). Bacteriophage diversity in the North Sea. *Appl Environ Microbiol* **64**: 4128–4133.

Wilcox RM, Fuhrman JA. (1994). Bacterial-viruses in coastal seawater—lytic rather than lysogenic production. *Mar Ecol Progr Sers* **114**: 35–45.

Williamson KE, Wommack KE, Radosevich M. (2003). Sampling natural viral communities from soil for culture-independent analyses. *Appl Environ Microbiol* **69**: 6628–6633.

Williamson SJ, Houchin LA, McDaniel L, Paul JH. (2002). Seasonal variation in lysogeny as depicted by prophage induction in Tampa Bay, Florida. *Appl Environ Microbiol* **68**: 4307–4314.

Williamson SJ, Paul JH. (2004). Nutrient stimulation of lytic phage production in bacterial populations of the Gulf of Mexico. *Aquat Microb Ecol* **36**: 9–17.

Winget DM, Wommack E. (2008). Randomly amplified polymorphic DNA (RAPD)-PCR as a tool for assessment of marine viral richness. *Appl Environ Microbiol* **74**: 2612–2618.

Wommack KE, Bhavsar J, Ravel J. (2008). Metagenomics: read length matters. *Appl Environ Microbiol* **74**: 1453–1463: AEM.02181-07.

Wommack KE, Ravel J, Hill RT, Colwell RR. (1999). Hybridization analysis of Chesapeake Bay virioplankton. *Appl Environ Microbiol* **65**: 241–250.

Wommack KE, Williamson SJ, Sundbergh A, Helton RR, Glazer BT, Portune K *et al.* (2004). An instrument for collecting discrete large-volume water samples suitable for ecological studies of microorganisms. *Deep Sea Res Part I Oceanogr Res Pap* **51**: 1781–1792.