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Abstract

A heat stable strain of *Halomonas aquamarina* was isolated from surface waters in the Gulf of Mexico that contains a temperate phage. To determine the impact of this phage on the metabolic lifestyle of its host, we have completely sequenced its genome and are performing experiments to determine the overall phage-host relationship. Shotgun libraries were constructed by linker amplification and sequenced. The phage genome is 40,350 bp long and is composed of at least 57 Open Reading Frames (ORFs), of which 22 remain completely unidentified (based on GenBank similarity). Several of the phage genes are similar to genes from the *Vibrio harveyi* myovirus-like temperate bacteriophage (VHML, 8 ORFs) and an uncharacterized phage from *Vibrio parahaemolyticus* strain 882 (VP882, 11 ORFs). Although no integrase was found, the *H. aquamarina* phage genome contained a putative *ci* repressor in a part of the genome that is in reverse orientation, typical of a lysogeny module, sequence data that has been confirmed by restriction enzyme analysis shows the absence of a *cos*-site. The genome of this phage was circularly permuted and contained a terminase, indicative of a *Pac*-site packaging mechanism. Interestingly, this prophage contains a protelomerase which may allow it to exist as a plasmid-like linear genome with telomeric ends. Studies are currently underway to determine if the prophage integrates into the host replicon or exists as a plasmid. As expected for a temperate phage, after a 24 hour prophage induction, with Mitomycin C, the strain produced more viral-like particles (3.73×10^{10} VLP ml⁻¹) than in the control (3.83×10^7 VLP ml⁻¹). Additionally, the induced viral concentration of this strain was higher at 8 hours after Mitomycin C (9.0×10^{10} VLP ml⁻¹) than after 24 hours. The *H. aquamarina* prophage is one of the few temperate marine phage genomes to be sequenced to date, and hopefully will yield information on lysogeny in the oceans.

Introduction

- H. aquamarina* is a gram negative halophilic γ -proteobacterium. It has been isolated from the water column around the world, including the brine-seawater interface of deep sea brine pools.
- Phages may be major vectors of gene transfer in the ocean.
- To date, few temperate marine phage genomes have been sequenced.
- Classic microbial techniques and genomics have increased the understanding of the host-phage lysogenic relationships.

Materials and Methods

- H. aquamarina* was isolated by a 80° C heat treatment during a 2001 cruise in the Gulf of Mexico.

Prophage induction

- To assess the time course of prophage induction, MitC was added to an actively growing culture at t=3 hrs. An untreated culture served as the control.
- Every 2 hrs after MitC addition, 5 mL aliquots were taken from each flask and serially diluted. Three 1 ml replicates filtered onto 0.22um Anodisc filters.
- Filters were stained with Sybr Gold stain and placed on slides.
- Using epifluorescent microscopy bacterial direct counts (BDC) were used to monitor bacterial growth and viral direct counts (VDC) monitored prophage induction.

H. aquamarina phage genome

- Phage lysate was prepared using large scale Mit C induction.
- The phage lysate was purified by PEG precipitation followed by a CsCl gradient.
- Phage DNA was formamide extracted.
- Linker amplified Shotgun Libraries (LASL) of the phage genome were created by Lucigen.
- Random clones from these libraries were sequenced using a ABI 3100 capillary sequencer (Applied Biosystems).
- The software program Sequencher (GeneCodes) was used to assemble contigs.
- Gaps between the contigs were resolved using multiplex PCR.
- Primer walking was used to sequence the ends of the phage genome.
- KODON (Applied Maths) was used to make genomic maps and perform gene comparisons.

- N15 is a 46,375 bp temperate phage of *Escherichia coli* (GI: 9630464) (Ravin, 00).

- VP882 is a uncharacterized phage infecting *Vibrio parahaemolyticus* strain 882, there are 2 partial sequences available in GenBank
 - 13,880 bp partial sequence (GI: 42718136).
 - 8,314 bp partial sequence (GI:42718150).

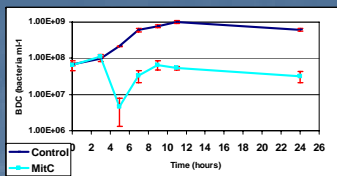


Fig 1. 24 hour MitC induction: Bacterial Growth
 MitC was added at t=3 hrs. Mean BDC for each time point was used to chart bacterial growth. Standard Deviation is represented by red bars

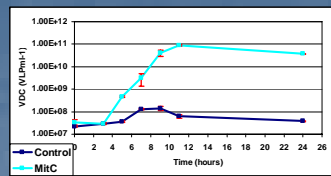


Fig 2. 24 hour MitC induction: Prophage Induction
 MitC was added at t=3 hrs. Mean VDC for each time point was used to chart viral induction. Standard Deviation is represented by red bars

ORF	Strand	Nucleotide Position	Best blastP hit	E value
2	+	1701-2117	Phage protein U-like [Pseudomonas fluorescens PFO-1]	8E-23
3	+	2101-2304	Phage tail X [Pseudomonas fluorescens PFO-1]	2E-10
4	+	2295-3323	Phage-related tail protein [Xanthomonas campestris pv. Campestris str. ATCC 33913]	4E-31
5	+	3449-4219	N6 adenine-specific DNA methyltransferase [Burkholderia amfarifaria AMMD]	5E-95
16	-	8041-8832	ParA plasmid partitioning protein [Pseudomonas alcaligenes]	5E-50
17	+	9191-10825	Protelomerase [Bacteriophage N15]	2E-56
19	-	11137-15234	Replication protein RepA [Bacteriophage VP882]	0
20	-	15480-16286	Repressor protein [Bacteriophage VP882]	6E-23
25	+	18006-18254	DnaK suppressor protein [Vibrio cholerae O395]	3e-4
26	+	18430-18900	Antiterminalin Q [Bacteriophage 82]	7E-10
33	+	21413-22009	Terminase small subunit [Escherichia coli O157:H7]	6e-4
34	+	22035-23981	Terminase large subunit [Synechococcus elongatus PC 6301]	5E-31
36	+	24612-26060	Portal protein [Xylella fastidiosa Ann-1]	1E-80
37	+	26077-27972	Major capsid protein [Xylella fastidiosa Ann-1]	3E-92
40	+	28566-29123	Minor tail protein [Wolbachia endosymbiont of Drosophila ananassae]	2E-13
42	+	29661-30295	Baseplate assembly protein [Pseudomonas fluorescens PFO-1]	5E-32
43	+	30287-30625	Baseplate protein [Xanthomonas oryzae pv. oryzae KACC10331]	7e-6
44	+	30526-31617	Baseplate assembly protein [Bacteriophage VP882]	7E-96
45	+	31566-32339	Phage tail protein [Pseudomonas fluorescens PFO-1]	4E-27
46	+	32340-32861	Tail fiber protein [Pseudomonas putida KT2440]	2E-21
49	+	33608-33958	Tail fiber protein [Yersinia pestis KIM]	5E-08
51	+	34771-35289	Tail fiber assembly protein [Pseudomonas fluorescens PFO-1]	5e-6
54	+	35850-37019	Tail sheath protein [Bacteriophage VP882]	2E-53
55	+	37020-37526	Tail tube protein [Bacteriophage VP882]	2E-36
57	+	38342-40534	Phage tail tape measure protein [Bacteriophage P27]	4E-44

Fig 3. *H. aquamarina* Phage draft 2 ORF List

Listed for each ORF with best match with a e value based on score by BLASTP search of NCBI database <http://www.ncbi.nlm.nih.gov/BLAST/>. ORFs with no significant hits or hits to hypothetical proteins were not included.

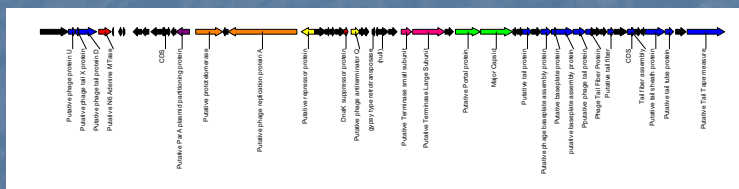


Fig 4. *H. aquamarina* Phage draft 2 Genomic Map

Kodon was used to construct this gene map.

Blue: Tail Orange: DNA replication Yellow: Lysogeny related Purple: Plasmid
 Green: Capsid Red: Nucleic acid metabolism Magenta: Packaging Black: Unknown

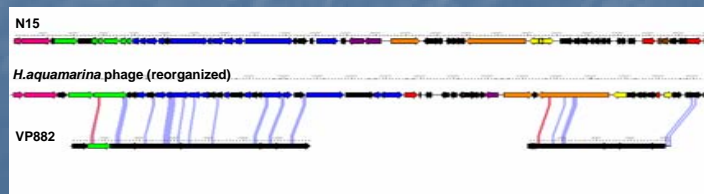


Fig 5. Rearranged *H. aquamarina* Phage Genomic Map with N15 and VP882 maps

Kodon was used to construct the gene maps and perform the gene comparisons of N15 and VP882 from Genbank files.

Blue: Tail Orange: DNA replication Yellow: Lysogeny related Purple: Plasmid
 Green: Capsid Red: Nucleic acid metabolism Magenta: Packaging Black: Unknown
 Brown: Lysis genes

Results and Discussion

Prophage induction

- Control treatment
 - Viral production (fig 2.) was greatest at 9 hrs (1.43×10^8 VLP ml⁻¹) then decreased at 24 hrs (3.83×10^7 VLP ml⁻¹).
- Mit C treatment
 - Prophage induction (fig 2.) was greatest at 8 hrs after Mit C addition (9×10^{10} VLP ml⁻¹), it decreased at 24 hrs (3.73×10^{10} VLP ml⁻¹).
 - Bacterial growth decreased with the addition of Mit C (fig 1).
 - The *H. aquamarina* phage behaved like a temperate phage when treated with the prophage inducing chemical, Mit C.

H. aquamarina phage genome

- Double stranded DNA genome.
- 40,350 bp and 57 ORFs (fig 3 & 4).
- Pac*-site packaging mechanism.
 - Sequence data came back on itself indicating a circularly permuted genome.
 - Absence of *cos*-site.
 - Terminase large and small subunits (ORF 33 & 34).
- Structural genes were located mostly on the right side.
 - 2 *capsid* proteins and 13 *tail* proteins.
 - This phage may be a Siphovirus based on the *tail* proteins.
- Possible lysogeny module with a *repressor* (ORF 20) in reverse orientation to the *antiterminalin* (ORF 26).
- Replication
 - May be similar to the DNA replication mechanism of the N15 phage.
 - RepA (ORF 19) which is a multi-domain protein similar to those that replicate plasmids replicating by the θ mechanism (del Solar, 98).
 - Protelomerase (ORF 17) resolves the ends of linear DNA into covalently closed hairpins (Ravin, 03).
- Does *H. aquamarina* phage exist as a plasmid?
 - No integrase gene was found
 - ParA plasmid partitioning protein (ORF 16) ensures daughter bacteria will contain the plasmid.
- Gene Organization Comparison (fig. 5)
 - Possible assembly error in draft 2 of *H. aquamarina* phage genome
 - The right arm of the genome from the *Terminase* on was moved the left
 - There were two areas of gene similarity between the *H. aquamarina* phage and VP882 genomes.
 - Structural module
 - Possible lysogeny module
 - There was no gene similarity between the *H. aquamarina* phage and N15 phage but there was organization homology between the two genomes.

Conclusions

- The *H. aquamarina* phage is one of the few temperate marine phage sequenced to date.
- Based on the genome, this phage may exist as a circular plasmid.
- The phage genome shared organization structure with other phages, supporting the modular theory of phage evolution.
- Further studies include resolving the genome assembly, determining morphology (TEM), and integration of phage genome.

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References

del Solar, G., et al. (1998). Microbiol. Mol. Bio. Rev. 62, 434-464.
 Ravin, V., et al. (2000). J. Mol. Biol. 299, 53-73.
 Ravin, V., (2003). FEMS Microbiol. Letters. 221, 1-6.