Environmental Biology

INTER-INSTITUTIONAL POSTGRADUATE PROGRAM

Bacteria-Phage Interactions in the Marine Antarctic Environment

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Abstract

Bacteriophages are key players in marine ecosystems, shaping microbial communities, biogeochemical cycles, and genetic exchange. Despite their global significance, viral communities in the Southern Ocean remain understudied. This study reports the first isolation and characterization of five novel Antarctic marine phages with diverse morphotypes (Myovirus, Siphovirus, and Podovirus). Their host range varied, with Pseudoalteromonas sp. and Psychrobacter sp. being the most abundant hosts. Higher temperatures negatively affected the infectivity of one phage, while others remained stable. Whole-genome analysis revealed that two phages were temperate, while host genome analysis identified genes associated with cold adaptation and a prophage in one bacterial genome. These findings enhance our understanding of viral-host dynamics in extreme environments, highlighting the need for further exploration of Antarctic phage diversity.

Introduction

Bacteriophages (phages) are viruses that infect bacteria and are the most abundant biological entities on Earth (~10^{31} particles). They play key roles in shaping microbial communities, driving genetic exchange, and regulating biogeochemical cycles. In marine environments, phages contribute to bacterial mortality (20–40% daily), nutrient recycling, and the viral shunt, which redirects organic matter into microbial loops instead of higher trophic levels. In polar regions, where grazers are less abundant, phages serve as primary regulators of bacterial populations. Despite their ecological importance, Antarctic marine phages remain largely unexplored, with most existing studies relying on metagenomics. To date, no phage-host isolations from Antarctic seawater have been reported. This study aims to isolate and characterize Antarctic marine phages for the first time, investigating their genetic diversity, morphology, host interactions, and environmental adaptability.

Table 1. Summary of Antarctic phages isolated during this study. Phages GA2 and GA3 and well as GA4 and GA5 are the same phages.

Phages	Isolation (base/ method)	Morphotype	Genome length (bp)	GC content (%)	Total ORFs	ORFs with known function	Phage lifestyle	Bacterial host
GA1	Prat 6 (0m) / Direct spot	Myovirus	-	-	-	-	-	P10.17 Pseudoalteromonas
GA2	Prat 6 (25m) Enrichment 27	Siphovirus	102,923	40.0	165	57	lytic	P6.97 Pseudoalteromonas
GA4	Prat 6 (25m) Enrichment 26	Podovirus	40,017	43.9	75	35	temperate	P6.122.1 Psychrobacter
GA6	Prat 7 (0m) Enrichment 37	Podovirus	43,434	42.6	63	30	lytic	P7.9 Psychrobacter
GA7	Prat 6 (25m) Enrichment 28	-	41,026	41.9	67	34	temperate	P6.111 Pseudoalteromonas

Materials and Methods

Seawater samples were collected near Greenwich Island, Antarctic Peninsula, during the 59th Chilean Antarctic Expedition (February 2023). Bacterial isolates were cultivated on three different media (LB, R2A, Marine) at 4°C and 20°C to ensure diversity. Flocculates containing phages were generated using iron particles.

During my master thesis, Phage isolation was performed using the enrichment method, followed by spot tests to detect phages. Phage morphology was examined via transmission electron microscopy (TEM), and DNA was extracted using a phenol-chloroform protocol. Whole-genome sequencing was conducted for both phages and bacterial hosts, with bioinformatic analyses for genome assembly, annotation, and phylogeny. Host range was assessed using spot assays on 78 bacterial isolates and phage infectivity at different temperatures (4, 8, 13, and 25°C) was evaluated through optical density measurements over 24 hours.

Results

Five distinct novel marine phages were isolated from Antarctic seawater, infecting Pseudoalteromonas and Psychrobacter species. Morphological analysis identified Myovirus, Siphovirus, and Podovirus morphotypes. Host range assays revealed varying infectivity, with some phages exhibiting broad host specificity, while others were highly specific. The bacterial isolates identified as phage hosts were classified as *Pseudoalteromonas* sp., *Psychrobacter* sp., and *Pantoea agglomerans* based on 16S rRNA gene sequencing. Temperature assays demonstrated that infectivity was generally enhanced at higher temperatures, except for phage GA2, which lost activity at 25°C. Genome sequencing confirmed the presence of two temperate phages, and revealed interesting functional gene annotations like anti-CRISPR proteins, while genome annotations of the bacterial hosts revealed adaptations to cold environments, as well as a prophage in a hosts genome.

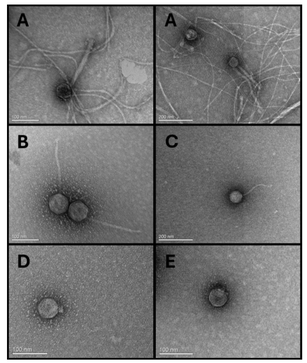

Figure 1. Digital migrographs of phages obtained with Transmission electron microscopy (TEM). Each letter represents a different phage (A) GA1, (B) GA2), (C) GA3), (D) GA4), (E) GA6.

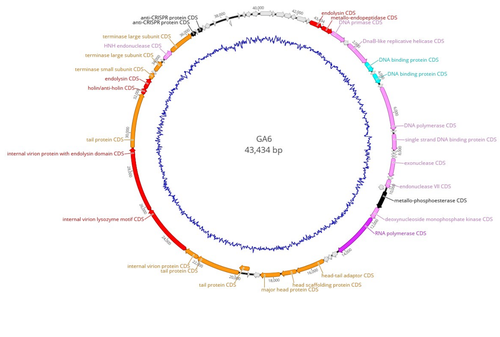

Figure 2. Functional annotation of the phage GA6 genome represented in a circular diagram.

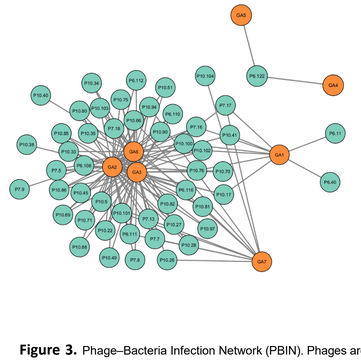

Figure 4. The infectivity of phages GA1, GA2, GA3, GA4, GA5, GA6 and GA7 at four different temperatures (4, 8, 13, and 25°C). Different letters show statistical differences ($p < 0.05$)

Discussion

The genome analysis of the isolated phages confirmed their novelty. Similar to previous studies in the Southern Ocean, which reported only a few cold-adaptive viral genes (Heinrichs et al., 2024), our analysis did not reveal specific genes linked to cold adaptation. However, the bacterial hosts contained cold shock proteins and heat-inducible chaperones, consistent with past research. Notably, we identified two anti-CRISPR proteins in phage GA6, which could have potential biotechnological applications in overcoming CRISPR-resistant bacteria (Stanley & Maxwell, 2018). Additionally, two phages (GA4 and GA7) carried integrase and excisionase genes, indicating temperate lifestyles, supporting Antarctic studies that report high lysogeny during winter and spring when bacterial abundance is low (Anesio & Bellas, 2011). Furthermore, a prophage identified in *Pseudoalteromonas* sp. exhibited similarity to temperate *Pseudoalteromonas* phages from the Arctic, aligning with metagenomic findings suggesting some phages may have bipolar distributions (Liu et al., 2023). These results highlight both the uniqueness and conserved evolutionary patterns of Antarctic phages and their bacterial hosts, reinforcing the need for further studies on polar viral ecology.

Conclusions

In this study, we isolated and characterized Antarctic phages from seawater, identifying five distinct phages and their bacterial hosts. Whole genome sequencing, host range experiments, and temperature assays revealed diverse morphologies, host specificities, and temperature responses, confirming the discovery of novel viral entities. Given the Southern Ocean's unexplored viral diversity, our findings highlight the need for further studies integrating metagenomics with traditional isolation methods. Our next step is to compare these results with similar experiments conducted in Chile using samples from the Yelcho base during the 59th Chilean Antarctic Expedition.

References

1. Anesio, A. M., & Bellas, C. M. (2011). Are low temperature habitats hot spots of microbial evolution driven by viruses? In Trends in Microbiology (Vol. 19, Issue 2, pp. 52–57). Elsevier Ltd. https://doi.org/10.1016/j.tim.2010.11.002
2. Dion, M. B., Oechslin, F., & Moineau, S. (2020). Phage diversity, genomics and phylogeny. In Nature Reviews Microbiology (Vol. 18, Issue 3, pp. 125–138). Nature Research. https://doi.org/10.1038/s41579-019-0311-5
3. Heinrichs, M. E., Piedade, G. J., Popa, O., Sommers, P., Trubl, G., Weissenbach, J., & Rahlff, J. (2024). Breaking the Ice: A Review of Phages in Polar Ecosystems (pp. 31–71). https://doi.org/10.1007/978-1-0716-3549-0_3
4. Liu, Z., Jiang, W., Kim, C., Peng, X., Fan, C., Wu, Y., Xie, Z., & Peng, F. (2023). A Pseudomonas Lysogenic Bacteriophage Crossing the Antarctic and Arctic, Representing a New Genus of Autographiviridae. International Journal of Molecular Sciences, 24(8). https://doi.org/10.3390/ijms24087662
5. Stanley, S. Y., & Maxwell, K. L. (2018). Phage-Encoded Anti-CRISPR Defenses. Annual Review of Genetics, 52(1), 445–464. https://doi.org/10.1146/annurev-genet-120417-031321
6. Suttle, C. A. (2005). Viruses in the sea. In Nature (Vol. 437, Issue 7057, pp. 356–361). Nature Publishing Group. https://doi.org/10.1038/nature04160
7. Suttle, C. A. (2007). Marine viruses - Major players in the global ecosystem. Nature Reviews Microbiology, 5(10), 801–812. https://doi.org/10.1038/nrmicro1750
8. Walker, J. M. (2009). Bacteriophages (M. R. J. Clokie & A. M. Kropinski, Eds.; Vol. 501). Humana Press. https://doi.org/10.1007/978-1-60327-164-6
9. Weitz, J. S., Poisot, T., Meyer, J. R., Flores, C. O., Valverde, S., Sullivan, M. B., & Hochberg, M. E. (2013). Phage-bacteria infection networks. In Trends in Microbiology (Vol. 21, Issue 2, pp. 82–91). https://doi.org/10.1016/j.tim.2012.11.003

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