Isolation and morphological characterization of a *Vibrio gigantis* bacteriophage

Marin ORDULJ[1]*, Ivica ŠAMANIĆ[2] Andrea BOŽIDAR[1], Ivana BOČINA[2], Nikolina BAUMGARTNER[1], Maja KRŽELJ[1]
[1]University of Split, University Department of Marine Studies, Ruđera Boškovića 37, Split, Croatia
[2]Faculty of science, University of Split, Ruđera Boškovića 33, Split, Croatia
***mordulj@more.unist.hr**

Introduction

Vibrio gigantis has been isolated from various marine species, including aquatic invertebrates and finfish. It is considered an emerging bacterial pathogen that may threaten European seabass production (Yilmaz *et al*., 2023). The aim of the research is to find a potential candidate for phage therapy against potential vibriosis caused by *V. gigantis* in aquaculture as phage therapy has been regarded as a promising alternative to antibiotics for the biocontrol of infectious diseases (Letchumanan *et al*., 2016; Cai *et al*., 2023).

Workflow

Enrichment culture | Double Agar Overlay Assay | Phage isolation and stock preparation | Concentrated *V. gigantis* bacteriophage | Double Agar Overlay assay of concetrated *V. gigantis* bacteriophage

Materials and methods

- Seawater was filtered (10 µm, 3 µm, 0.2 µm) to remove large particles. 740 ml of filtered seawater was mixed with 27 ml Marine broth medium (Difco, 2216) to reach a final concentration of 0.3x 2216 broth. Afterward, 27 ml of an overnight culture of *Vibrio gigantis* was added. The mixture was incubated for 4 days at 20°C with shaking (100 rpm).
- Spot tests confirmed phage enrichment, showing lysis zones. Phage dilutions were plated, and three plaques were selected for further propagation. The phage stock was prepared by centrifuging at 10,000g for 10 minutes at 4°C, followed by PEG6000 treatment and another centrifugation to concentrate the phage.
- Phage morphology was observed using transmission electron microscopy. The phage was adsorbed onto a copper grid, stained with 2% uranileacetate, air-dried, and visualized with a JEM JEOL 1400 Flash microscope at 100 kV.

Results

The myovirus-like bacteriophage was successfully isolated. Its morphology includes an icosahedral capsid measuring approximately **68.87 ± 4.658 nm** in diameter, coupled with a contractile tail, resulting in a total length of **215.87 ± 9.8 nm** and a width of **20.29 ± 0.5 nm**. These dimensions not only provide insights into the physical structure of the phage but also offer valuable clues regarding its interactions with host bacteria. The larger size of the capsid and tail suggests potential implications for infectivity and host range, indicating a unique adaptability that warrants further investigation.

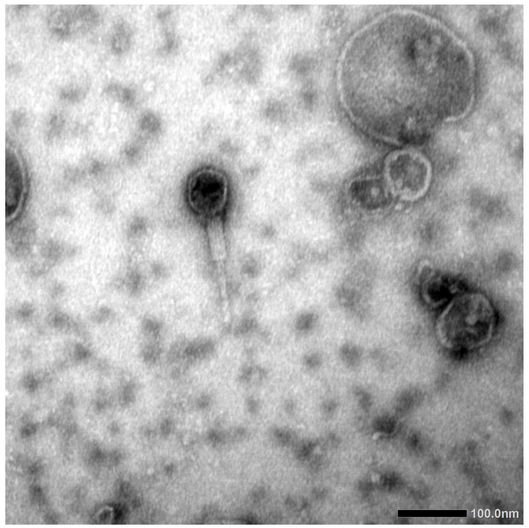

Figure 1. Isolated bacteriophage of *V. gigantis* captured using transmission electron microscope JEM JEOL 1400 Flash

Conclusion

The enrichment culture method is a reliable tool for the isolation of *V. gigantis* bacteriophage.

The phage dimensions provide insights into the physical structure of the phage which could help in understanding its interactions with the host bacteria.

Bacteriophage targeting *V. gigantis* could offer a realm of possibilities for aquaculture and public health as a natural alternative to antibiotics, potentially reducing antibiotic resistance concerns and promoting sustainable aquaculture practices.

References

Yilmaz S, Karataş S, Steinum TM, Gürkan M, Yilmaz DK, Abdel-Latif HMR. Isolation, Identification, and Pathogenicity of Vibrio gigantis Retrieved from European Seabass (Dicentrarchus labrax) Farmed in Türkiye. Animals (Basel). 2023 Nov 20;13(22):3580. doi: 10.3390/ani13223580. PMID: 38003197; PMCID: PMC10668765.

Cai L, Tian Y, Li Z, Yang Y, Ai C and Zhang R (2023) A broad-host-range lytic phage vB_VhaS-R18L as a candidate against vibriosis. Front. Microbiol. 14:1191157. doi: 10.3389/fmicb.2023.1191157

Letchumanan V, Chan KG, Pusparajah P, Saokaew S, Duangjai A, Goh BH, Ab Mutalib NS, Lee LH. Insights into Bacteriophage Application in Controlling Vibrio Species. Front Microbiol. 2016 Jul 19;7:1114. doi: 10.3389/fmicb.2016.01114. PMID: 27486446; PMCID: PMC4949243.

1st International Congress for Sustainable Ecosystems in the Mediterranean Area
October 2-3, 2024.
Split, Croatia