Chapter 6

SUMMARY AND CONCLUSION

A vibriophage ΦMV-5 was isolated from the water sample of the Mangalavanam mangrove of Kerala using the host Vibrio sp.MV-5, which was first isolated from the same location, and later found to resemble Vibrio vulnificus on the basis of partial 16s RNA sequence.

The phage ΦMV-5 was purified, concentrated and stored. Some of the structural, physicochemical and genomic characteristics of the phage was studied. Transmission electron microscopic studies suggest that the vibriophage ΦMV-5 is filamentous in nature. They appear to be single long filaments that are approximately 1.1 μm in length and 0.03 μm in diameter and are considered to be inovirus-like bacteriophages. The phage also showed a pointed head and a blunt tail characteristic of all filamentous phages.

The protein profile was characterized by reductive and non-reductive SDS-PAGE. The optimal multiplicity of infection was observed to be 5 PFU/ml.

The parameters effecting phage multiplication were calculated from the one-step growth curve and the latent period for the phage ΦMV-5 was found to be about 30 minutes and the rise period was 50 minutes. The burst size was 60 phages per cell. The phage showed 100% adsorption within 30 minutes of incubation with the host cells.

The studies conducted on the effect exerted by various factors like CaCl₂, temperature, pH, sugars and NaCl, on phage viability revealed that 10 mM CaCl₂
was optimum for the propagation of the phage. The phage showed temperature tolerance up to 70°C. There was a decrease in the PFU for 50, 60 and 70°C as time of temperature treatment was extended. This also indicated that the phage can tolerate temperatures up to 70°C. Phage titers decreased below the detection limit at 80°C, 90°C and 100°C even within 15 seconds.

The phage was viable over a pH range from 5 to 11 at 37°C. But, the viral suspension was completely inactivated at pH 2-4 after 30 min, indicating high sensitivity to lower pH. All the sugars tested drastically affected phage viability - inhibition ranging from 80% to 100% was observed, with glucoseamine completely inhibiting the phage particles.

It was also noted that the phage \( \Phi MV-5 \) was tolerant up to 3 M NaCl, with maximum PFU at 0.25 M NaCl. Beyond this level, the phage titer value decreased.

The effect of pH, temperature and NaCl on the process of adsorption of the phage \( \Phi MV-5 \) with the host was also studied. The maximum adsorption percentage was seen at pH 7, temperature range from 30°C to 40°C, and NaCl concentration from 0.25 to 1 M.

The cumulative effect of all the parameters optimized during the course of this study resulted in the dramatic increase of the burst size from 60 to 121. Mitomycin C led to increase in the burst size indicating that all the prophages can be induced to lysis with this DNA damaging agent of the host.

The result of the broth clearing experiment also pointed towards the filamentous nature of the phage \( \Phi MV-5 \). The phage \( \Phi MV-5 \) was found to have a broad host range.
The DNA stained green following acridine orange staining, indicating its double stranded nature. The phage DNA was resistant to the restriction enzymes Eco RI, Bam HI, Bgl II, Hind III, Not I, Pst I and Sau 3AI. The major coat protein gene was studied using a set of primer designed. The partial sequence obtained showed similarity to five vibriophages already reported.

The phage ΦMV-5 showed the presence of five of the six virulence genes screened for, which included tcpA, toxR, ace, zot, ctxA and sxt. These virulence genes are characteristic of vibrios, especially that of V. cholerae and it was found that except for the ctxA, phage possessed all the other virulence factors. The presence of these virulence factors was also checked in the host and it was observed that except ace and ctxA all genes were present in the Vibrio sp. MV-5, found to resemble V. vulnificus. Hence it is assumed that this may be due to the presence of phage genome in lysogenic association with the host DNA.
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Conclusion

The temperate, filamentous phage \( \Phi \text{MV-5} \) isolated from Mangalavanam mangrove of Kochi, using the environmental strain of \textit{Vibrio} sp. MV-5 shares many similar properties with other marine phage isolates, while also remaining unique. The study has revealed that the interaction of temperate phages and the microbial population in the marine environment may contribute significantly to microbial genetic diversity and composition by conversion and transduction and which requires greater study.

Prophages contribute a substantial share of the mobile DNA of their bacterial hosts and seem to influence the short-term evolution of pathogenic bacteria. Automated methods for systematic investigation of prophages and other mobile DNA elements in the available bacterial genome sequences will be necessary to understand their role in bacterial genome evolution. In the past, phages were mainly investigated as the simplest model systems in molecular biology. Now it is increasingly realized that phage research will be instrumental in the understanding of bacterial abundance in the environment. One can predict that phage research will impact diverse areas such as geochemistry and medicine. Success will largely depend on integrative multidisciplinary approaches in this field. Clearly, further studies are required to understand how vibriophages interact with \textit{Vibrios} to promote this organism’s acquisition of the critical genes which alter its virulence or adaptation to its environmental niche.

It is evident from this study and comparison with those reports cited above that vibriophage \( \Phi \text{MV-5} \) is a previously unreported bacteriophage. It is recommended that the minimum requirement for reporting a new phage should be novel morphological markers and a description of host range, both of which have been achieved in this study. The detailed description of physicochemical properties are also an advantage for further comparisons (Ackermann et al., 1978).