

IDENTIFICATION AND CHARACTERIZATION OF MYCOBACTERIOPHAGE FROM MARINE WATER

Submitted in partial fulfillment of the requirements for the award of Master of
Technology degree in Biotechnology

by

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO & CHEMICAL ENGINEERING**

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

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This is to certify that this Project Report is the Bonafide work of **RAMYA. N (41810016)** who carried out the project entitled "**IDENTIFICATION AND CHARACTERIZATION OF MYCOBACTERIOPHAGE FROM MARINE WATER**" under our supervision from January 2023 to May 2023

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ABSTRACT

Bacteriophages or Phages are ubiquitous in marine water. In this study, we isolated the Mycobacteriophage from a Marine water sample. Mycobacteriophage is a virus that infects the mycobacterium bacterial species. Phages are visible to identify in the double-layer agar (DLA) method. For isolating the Mycobacteriophages, Mycobacterium Smegmatis culture was used for plaque formation. The plaques confirmed the presence of Mycobacteriophage. High titer phage lysate was prepared from plaque grown plate. It was characterized to identify the size and shape of phage by Transmission Electron Microscopy images. The size of Mycobacteriophage ranges between 50-60 nm. Further, it will be sequenced for species identification.

Keywords : Mycobacteriophages, Plaques, TEM, double-layer agar, High titer phage lysate.

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LIST OF ABBREVIATION

7 H11 MB agar	-	7 H11 Middlebrook Agar
7H9 MB agar	-	7H9 Middlebrook broth
DLA	-	Double layer Agar
HTL	-	High titer Lysate
ADC	-	Albumin bovine serum, Dextrose, Catalase
nm	-	Nanometer
g	-	gram
ml	-	milliliter
l	-	liter

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CHAPTER 1

INTRODUCTION

Bacteriophages are ubiquitous and the most abundant biological entities on earth. They are also known as phage or viruses that replicate and infect only bacterial cells. Bacteriophages are discovered by Frederick Twort and Felix d'Herelle in 1915. He introduced phage therapy for multi-drug resistant bacterial infections. Phages do not cause major drawbacks and its eco-friendly to use, Phages have the ability to act directly on the cell wall to produce lytic enzymes. The bacteriophage which infects the mycobacterium species is known as Mycobacteriophage. Mycobacteriophage were first isolated in 1940. Thus, Mycobacteriophages are used to treat disease and infections caused by mycobacterium. Mycobacteriophages are mostly isolated from human infections, sewage, soil compost, animal waste. It is challenging and difficult to isolate the phages from environmental sample than clinical samples. For last two decades the scientists and researchers were started to concentrated on bacteriophages. Mycobacteriophage are double-stranded DNA phage with tail. Till date there is no RNA or single-stranded DNA phages were isolated from Mycobacteriophage, For Mycobacteriophage isolation most probably Mycobacterium Smegmatis is used as host for plaque formation because *Mycobacterium Smegmatis* is a rapid growth organisms compared to other mycobacterial species. Immense efforts have been concentrated to understand the role of viruses in diversity, horizontal gene transfer, the structure of the community, and consistent impacts on microbial abundance and mortality. The recent development of several unidentified viruses resulted from genomic and metagenomic sequencing of viral isolates. Moreover, it began to scratch the surface of marine viral sequence diversity hence 65% to 95% of marine viral metagenomic sequences are not identical to previously identified sequences, compared to 10% for cellular metagenomic surveys. Investigating the varied host and environmental elements that could form viromes as a whole is hindered by the fact that studies of viral evolution have typically been restricted to single viruses and/or single hosts.

CHAPTER 2

LITERATURE SURVEY

2.1 Diversity of Viruses

The marine virus has consistently concentrated on two ranges, viruses as pathogens infecting aquatic organisms and marine food chain driven by phage thus dominant for global biogeochemistry and host progression. The diversity of marine viruses and viral communities is the focus in one area. Yet viruses defy traditional concepts to know that viral genetic diversity is very substantial. Immense efforts have been concentrated to understand the role of viruses in diversity, horizontal gene transfer, the structure of a community, and consistent impacts on microbial abundance and mortality (Breitbart et al. 2007). The size of marine viruses ranges from 3000 nt to 1,290,000 bp.

Therefore, some diversity sense of this can be gained through quantitative examination of the genome length distribution in a sample (Steward, Culley, and Wood-Charlson 2013). Investigating the varied host and environmental elements that could form viromes as a whole is hindered by the fact that studies of viral evolution have typically been restricted to single viruses and/or single hosts. Fortunately, with the introduction of NGS, particularly whole RNA sequencing, this is changing. Aspects of host biology can significantly influence viral diversity, and as a result, may potentially be important drivers of virus emergence, according to metagenomic-based research in particular (Geoghegan et al. 2021). In accession to virulence genes, unexpected metabolic and functional pathways are committed with marine virome genes. Comparative studies between microbiomes and viromes show that respiration gene frequency is subsided in viromes, while nucleic-acid gene metabolism is more abundant (Rohwer and Thurber 2009a). Hydrothermal vents (where cold seawater is mixed with warm seawater) of viral particles are a higher diffuse flow than seawater on the surface. In the vent system, inorganic chemicals (calcium, sulfide, iron, and salt) of microbial adaption through high concentration, high pressure, and high temperature under deep-sea which could be gene stores for viral communities (Williamson et al. 2008).

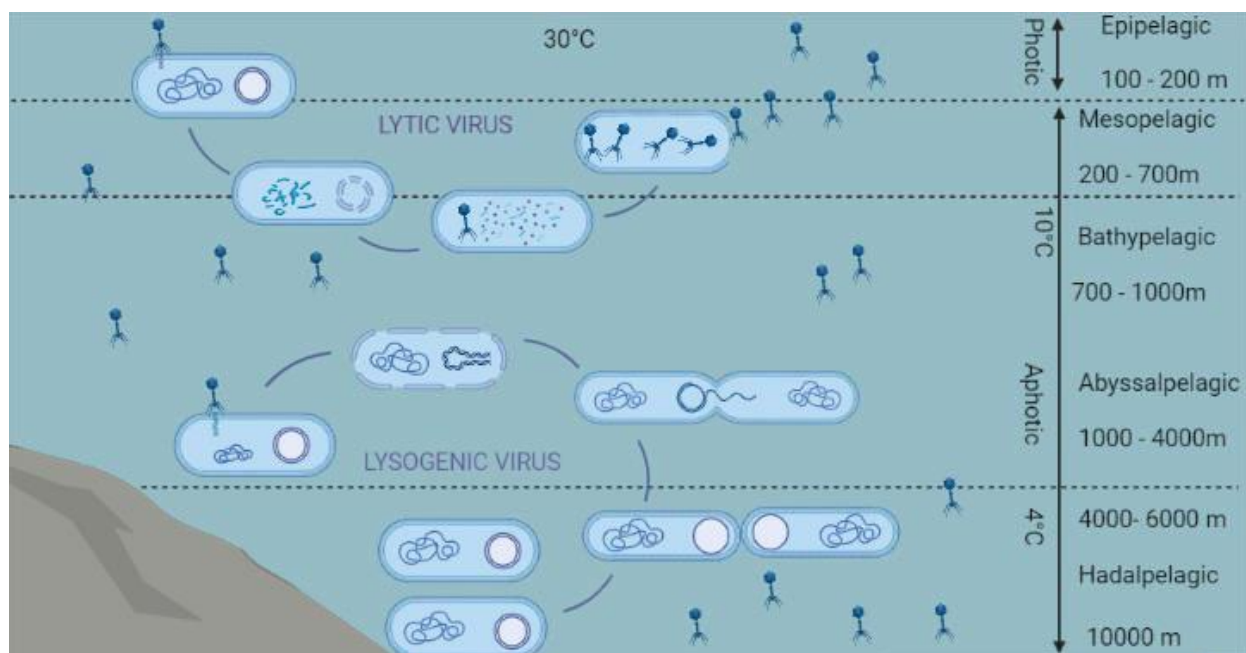


Fig 2: Viral abundance in Marine Ocean

The lytic cycle and the Lysogenic cycle play a vital role in the marine ocean. This process produces viruses, it begins through the adsorption of a virus on the surface of microbes present in marine water. It will infect and penetrate the cell to release viral particles. The lytic cycle will lyse the cell by the lytic phage to release more infected viruses but in the lysogenic cycle, the temperate phage will transform the genetic material to produce multiples of infected bacterial cells. Thus, the process mechanism enumerated the viral abundance. Lytic phages (virulent) are produced in the epipelagic zone which is in the photic condition in a marine ecosystem. Lysogenic phages (temperate) are produced by ultraviolet radiation, temperature, climatic changes, and heavy metals in the deep sea which is in aphotic condition.

The recent development of several unidentified viruses resulted from genomic and metagenomic sequencing of viral isolates. Some marine viruses affect protists that have no visible resemblance and existing sequence, whereas 60% to 80% of open reading frames from cultured genome phage show no similarity sequence in Genbank. Moreover, it began to scratch the surface of marine viral sequence diversity hence 65%

to 95% of marine viral metagenomic sequences are not identical to previously identified sequences, compared to 10% for cellular metagenomic surveys (Breitbart et al. 2007).

Generalized transducing agents (GTAs), phage, and eukaryotic viruses are examples of marine viruses and viral-like organisms that may affect host cells in a variety of ways. A phage may either destroy its bacterial host cell by lysing it or transmit genetic material from one host to another or from its genome by using its accessory genes to express. Because viruses lack a generally conserved gene like the ribosomal DNA genes in cellular organisms, and because the majority of viral hosts are challenging to cultivate, diversity has proven difficult to detect. Whole viral communities have been isolated and analyzed using pulsed-field gel electrophoresis or shotgun sequencing to get around these challenges (Rohwer and Thurber 2009a). In the earlier 1980s, the viral abundance is estimated through a culture-based method for transformation where the virus magnitude is higher compared to culture-based methods. Consequently, researchers initiate to find the relationship ratio between hosts and viruses and termed as 'virus to bacterium ratio' (VBR) or 'virus to microbial ratio' (VMR) from fresh water and marine water (Breitbart 2012).

The virus and microbes abundance is relatively influenced by environmental factors where microbial abundance is higher than viruses-induced mortality which is considered to be more important at the eutrophic site (Wigington et al. 2016). Viral abundance has been monitored at different depths (Fig: 2). According to the statistics, the viruses are highly abundant in the euphotic zone and the virus level is exponentially reduced as the depth and density increase (Tucker et al. 2011a). Mostly, in the coastal environment, the viral abundance is higher compared to offshore waters (Marchant et al. 2000). The virus-to-bacteria ratio and the degree of lysogeny are detected to determine the nature of marine viral communities through seasonal variations in viral abundance, will be higher in autumn and summer than in winter. More research has been undergone for years on seasonal changes to determine marine communities and variability and also it is monitored yearly (Breitbart 2012).

2.2 Bacteriophage

Bacteriophages are ubiquitous and the most abundant biological entities on earth. They are also known as phage viruses that replicate and infect only bacterial cells. Further studies have been undergone on the interactions of marine bacteriophage to examine the controlling bacterial populations and variation through the role of bacteriophage (Miller 2001). Phages do not cause major drawbacks and it's eco-friendly to use, Phages can act directly on the cell wall to produce lytic enzymes.

Bacteriophages particularly sense the specific components to target bacterial cell walls like lipopolysaccharide (gram-negative) or peptidoglycan (gram-positive), pili, flagella, and capsular polysaccharides (Ramos-Vivas et al. 2021). The phage can be determined through the double-layer agar method which produces plaque formation. The phage size largely determines its rate of diffusion through the medium, and large plaques are produced through small phages which means not complete. The correlation between plaque morphology and the structure of phages is difficult to identify (Alonso, Rodríguez, and Borrego 2002).

The bacteriophages are used as enzymes for assertive in various fields such as agriculture, medicine, and veterinary science. Also, ligase, recombinase, nuclease, viral DNA and RNA polymerase, and other enzymes have become classic tools in molecular biologists' toolboxes and had immense success in biotechnological applications (Tucker et al. 2011b). Such multi-drug-resistant bacteria and microbial diseases are controlled by bacteriophage therapy. Also used as a mechanism for antibiotic resistance to infections and pathogens (Kalatzis et al. 2016). Various studies have discovered that after one or two "cycles" of coevolution (during which the host develops resistance and the virus evolves to overcome that resistance), a bacterial strain develops resistance that the virus cannot defeat. The phage's inability to produce host-range mutants indicates that antagonistic coevolution, at least on an ecological time scale, is unlikely to be significant in natural systems. Coevolution ever tested before this study has been observed in *E. coli*, indirectly in Archaea and *P. fluorescens* (Brockhurst et al. 2007).

2.3 Morphology Studies

For the past two decades, direct viral count methods have been obtained from transmission electron microscopy to flow cytometry and epifluorescence microscopy to

detect the virus from thousands of samples throughout the oceans. The early enumeration methods are the double-agar-layer (DAL) method or transmission electron microscopy (TEM) and/or epifluorescence microscopy (EFM) (Pratama and Van Elsas 2018). The initial discovery of virus detection using various organisms including transmission electron microscopy (TEM), PCR-based analyses, DNA in situ hybridization, immunohistochemistry, flow cytometry, next-generation sequencing (NGS), and metagenomic analyses (Ambalavanan et al. 2021). Epifluorescence microscopy and Flow cytometry were performed to detect the virus present in the sample. Epifluorescence microscopy visualizes the double-stranded DNA which are prokaryotic viruses (Bachy et al. 2021). SYBR green and SYBR gold fluorescent dyes firmly bind to double-stranded DNA and RNA are extremely sensitive staining of virions (Pratama and Van Elsas 2018). SYBR Green I is a highly sensitive nucleic acid stain that was performed to detect and enumerate the marine virus.

Flow cytometry analyses used fluorescence to visualize the virus detection such as Green fluorescence (520/35 nm band-pass), red fluorescence (692/40 nm band-pass), side scatters, and forward scatter were normalized with 0.75 μm YG fluorescent beads (Bachy et al. 2021). Flow Cytometry (FCM) analysis improving its high efficiency and rapidity for viral counting through the operator's skill level is better than transmission electron microscopy (TEM) or epifluorescence microscopy (EFM). Hence researchers preferred to use TEM for the plaque-forming unit (PFU) counts for the enumeration of phages and viral diversity (Pratama and Van Elsas 2018). Microscopy would only be able to distinguish between one virus pool and two or even three virus populations when using the AFC procedure on natural samples. Several highly fluorescent nucleic acid-specific dyes have also been used to stain the cultures of several other marine virus families, including *Baculoviridae*, *Herpesviridae*, *Myoviridae*, *Phycodnaviridae*, *Picornaviridae*, *Podoviridae*, *Retroviridae*, and *Siphoviridae*. SYBR Green I produces the highest fluorescence, making it possible to identify DNA viruses with genome sizes between 48.5 and 300 kb (kilobases). Small genome-sized RNA viruses (7.4-14.5 kb) are currently beyond the scope of AFC's capability (Burkill and Gallienne 2001).

Viruses are difficult to culture, and to overcome these difficulties viral communities were isolated and analyzed by pulsed-field gel electrophoresis or shotgun sequencing. More

than 5000 viral genotypes or species in 100 liters of seawater and nearly 1 million species in 1kg of marine sediments show the virus-diverse rise of marine viral metagenomics through shotgun sequencing (Rohwer and Thurber 2009a). Lectin and nucleic staining for combined confocal laser-scanning microscopy were performed to demonstrate high viral abundance in the ocean (Weinbauer et al. 2009). 99% of bacteria cannot be isolated using standard techniques even though it provides phage ecology for significant insight. Moreover, some bacteria are dominant and they cannot produce referable plaques on bacterial lawns. Bacterial diversity can be determined via amplification and 16s rDNA sequencing. The phage genome does not have a single genetic element that can be used in a similar manner (Mizuno et al. 2013; Rohwer and Thurber 2009b). The small size of the virus and low DNA virus are also important barriers to molecular and microscopic studies. Earlier studies of viral communities determined the advanced understanding of viral diversity. It includes the pulse-field gel electrophoresis method to separate viruses according to the genome size, “signature genes” of amplification among virus groups, metagenomic sequencing, and randomly amplified polymorphic DNA PCR. The above-mentioned methodology revealed and demonstrated the novel viral abundance and diversity.

Whole viral communities have been isolated and analyzed using pulsed-field gel electrophoresis or shotgun sequencing to get around these issues. Pulsed-field gel electrophoresis (PFGE) has been used to investigate naturally occurring viral assemblages because conventional electrophoresis can only resolve DNA that is less than 20,000 – 30,000 bp in length. With this method, viral genome sizes in seawater can range from about 20,000 to >500,000 bp (Steward, Culley, and Wood-Charlson 2013). Marine viral metagenomics, which emerged as a result of shotgun sequencing, has demonstrated that viruses are incredibly varied, with more than 5,000 viral genotypes or species present in every 100 liters of seawater and up to 1 million species per kilo of marine sediment (Rohwer and Thurber 2009a).

2.4 Classification of Virus

The Virus can be either DNA or RNA virus which is classified into seven groups, such as double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), positive-sense single-stranded RNA,

negative-sense single-stranded RNA, and double-stranded DNA reverse transcriptase, it is précised to orders, families, genera, and species (Steward, Culley, and Wood- Charlson 2013) which has been detailed in **Table:1**. Recent studies examine the taxonomic analysis of viral populations, 97.84% of double-stranded DNA (dsDNA) viruses and 0.16% of single-stranded RNA viruses were detected.

The order *Caudovirales*, which contains the families *Myoviridae* (26.11%), *Podoviridae* (20.94%), and *Siphoviridae* (11.07%), was home to the majority of the viral populations (average, 59.22% [range from 45.68 to 69.51%]). It's interesting to note that the Nucleocytoplasmic large DNA viruses (NCLDV) family members *Phycodnaviridae* (16.65%), *Mimiviridae* (8.71%), and *Pandoraviridae* (2.60%) contain large viruses that infect eukaryotes (Gao et al. 2022).

2.4.1 DNA Virus

DNA viruses that attack archaea that survive in extremely hot geothermal habitats exhibit remarkable morphological variation. Along with typical spherical and filamentous viruses, it also contains viruses with various and peculiar forms that resemble bottles, droplets, coils, and spindles. The majority of these viruses develop by creating a nucleoprotein filament from their genetic DNA and capsid proteins, which are then condensed into a variety of shaped bodies. In the instance of the *Portogloboviridae* family's *Sulfolobus* polyhedral virus 1 (SPV1), such a nucleoprotein filament is compressed into the globular center, which is further enclosed by an outer polyhedral capsid (Liu et al. 2017). The *Tectiviridae*, *Corticoviridae*, *Sphaerolipoviridae*, and *Turriviridae* families of icosahedral bacterial and archaeal viruses with an inner lipid envelope, which the virion mimics, but in which DNA is present in the naked form (Rissanen et al. 2013).

Research on the ecology and abundance of viruses in seawater has mainly focused on viruses that infect microbes by double-stranded DNA (ds) for many years (Miranda et al. 2016). Large DNA viruses known as iridoviruses can attack cold-blooded mammals as well as invertebrates, crabs, and mollusks. *Ranavirus*, *Lymphocystivirus*, *Megalocytivirus*, *Iridovirus*, and *Chloriridovirus* are the five families that make up the family *Iridoviridae* at the moment. *Ranaviruses* have garnered the most interest among these genera because of their effect on the ecological variety of the world and their

contribution to significant financial losses in the aquaculture business. Studies have only been conducted in nonhost cells, even though the early stages of *ranavirus* infection, particularly virus entrance, have been examined (Wang et al. 2014). Nearly 200 proteins have been found in virions of *pandoraviruses* and *pithoviruses*, which are quite prevalent in large viruses.

Several capsid and structural proteins are involved in the formation of the virion itself, but these proteins also play a range of functions in transcription, translation, protein processing, and resilience to redox stress. These frequently include the components of DNA and RNA polymerase, ribonucleotide reductase, an enzyme for capping mRNA, superoxide dismutase, and a diversity of proteins whose functions are unknown (Aylward and Moniruzzaman 2022). Unexpected proteins that are packed in the virions of other giant viruses have been discovered recently. For example, one research found that *pandoraviruses* contain enzymes that are homologous to the components of the TCA cycle, and another found that *marseillevirus* virions contain histones that are virally encoded. A crucial part of infection in some big DNA viruses is the packing of multiple proteins into the virion (Liu et al. 2021).

2.4.2 RNA Virus

Single-stranded RNA (ssRNA) and double-stranded RNA (dsRNA)-containing RNA viruses are two subgroups of RNA viruses. The ssRNA viruses can be further split into types that incorporate both positive and negative-sense (coding and noncoding) RNA molecules into their viral particles (Lang et al. 2009). In the ocean, However, the variety and abundance of RNA viruses are still mainly ambiguous. Based on the relative quantification of RNA and DNA from purified viral particles and extrapolations of genome sizes, the few estimates of marine RNA virus prevalence indicate that up to 50% of the viral particles in seawater are RNA viruses (Steward, Culley, and Wood-Charlson 2013). A comprehensive meta-transcriptomics study of possible vertebrate-associated RNA viruses was conducted in more than 186 host species, reflecting the great variety found in the phylum Chordata (Stanojević et al. 2020a). Early studies on marine algal viruses focused primarily on those with DNA genomes, but most study indicates that RNA viruses, particularly those with positive-sense, single-stranded RNA (ssRNA) genomes,

are common in equatorial and moderate coastal waters (Miranda et al. 2016). All of these investigations point to the presence of a diverse array of positive-polarity single-stranded RNA (ssRNA) and double-stranded RNA (dsRNA) viruses in the marine RNA virosphere, with an apparent majority of viruses that infect eukaryotes (Culley 2018). Thorough analyses of marine RNA viral diversity on a worldwide scale are currently not accessible and are not included in the study due to methodological constraints (Gregory et al. 2019). The recently discovered viral domain known as *Riboviria* includes all RNA viruses. RNA viruses with reverse transcriptase (RT) make up the kingdom *Pararnavirinae*, while RNA viruses with RNA-dependent RNA polymerase (RdRp) make up the kingdom *Orthornavirinae*. Based on RNA-dependent RNA polymerase's phylogenetic study, the kingdom *Orthornavirinae* divides into five groups (Kolundžija, Cheng, and Lauro 2022). In recent research, the researchers discovered several domains (such as 255 DEAD-like helicase C) that had not previously been seen (Zhang et al. 2022). Interestingly, some giant phages' capsids have also been found to contain RNA polymerase components, proving that eukaryotic viruses are not the only ones that can experience this phenomenon (Ceyssens et al. 2014).

Table 1: Classification of Marine Viruses and taxonomical information

Nucleic Acid	Genome type	Order	Family	Size	Shape	Host	Reference
DNA	Double-Stranded DNA	<i>Rowavirales</i>	<i>Adenoviridae</i>	90 - 100nm	Icosahedral	Vertebrates	(Mukhopadhyaya et al. 2019)
		<i>Herpesvirales</i>	<i>Herpesviridae</i>	100 - 110 nm	Icosahedral, Enveloped	Fish, Corals, mammals, mollusks, and bivalve	(Mukhopadhyaya et al. 2019)
		<i>Pimascovirales</i>	<i>Iridoviridae</i>	120 - 200 nm	Icosahedral Symmetry	Fish, Mollusks	(Wang et al. 2014)
		<i>Pimascovirales</i>	<i>Marseilleviridae</i>	190 - 250 nm	Icosahedral	Protist, algae	(Colson et al. 2011)
		<i>Zurhausenvirales</i>	<i>Papillomaviridae</i>	40 - 55 nm	Round, Icosahedral	Marine crustaceans	(Bossart 2007)
		<i>Sepolyvirales</i>	<i>Polyomaviridae</i>	40 - 50 nm	Icosahedral	Marine mammals	(Anthony et al. 2013)
		<i>Chitovirales</i>	<i>Poxviridae</i>	240 - 300 nm	Brick or Oval shape	Steller sea lions, seals	(Bracht et al. 2006)
		<i>Lefavirales</i>	<i>Baculoviridae</i>	40 - 60 nm	Rod-shaped, enveloped	Crustaceans	(Mukhopadhyaya et al. 2019)

		<i>Algavirales</i>	<i>Phycodnaviridae</i>	100 - 220 nm	Icosahedral capsid, enveloped	Algae	(Ambalavanan et al. 2021)
		<i>Incertae sedis</i>	<i>Nudiviridae</i>	96 - 232 kbp	Rod-shaped	Aquatic crustaceans	(Petersen et al. 2022)
		<i>Incertae sedis</i>	<i>Nimaviridae</i>	65 - 70 nm	Oval or Ellipse, enveloped	Marine crustaceans	(Mukhopadhyaya et al. 2019)
		<i>Caudovirales</i>	<i>Mycoviridae</i>	50 - 110 nm	Icosahedral with contractile tail	Bacteria	(Proctor 1997)
		<i>Caudovirales</i>	<i>Podoviridae</i>	130 - 200 nm	Icosahedral	Bacteria	(Proctor 1997)
		<i>Caudovirales</i>	<i>Siphoviridae</i>	60 nm	Icosahedral with noncontractile tail	Bacteria	(Proctor 1997)
		<i>Ligamenvirales</i>	<i>Lipothirixviridae</i>	400nm	Thick rod with lipid coat	Archea	(Prangishvili, Forterre, and Garrett 2006)
		<i>Incertae sedis</i>	<i>Corticoviridae</i>	60 - 75 nm	Icosahedral with spike	Bacteria	(Ambalavanan et al. 2021)
		<i>Incertae sedis</i>	<i>Mimiviridae</i>	650 nm	Icosahedral with projection	Marine fish, algae	(Monier et al. 2008)
		<i>Incertae sedis</i>	<i>Totiviridae</i>	30 - 45 nm	Round, Icosahedral	Protist	(Ambalavanan et al. 2021)
		<i>Incertae</i>	<i>Tectiviridae</i>	60 nm	Icosahedral with	Bacteria	(Ambalavanan et al. 2021)

		<i>sedis</i>			noncontractile tail		al. 2021)
			<i>Plasmoviridae</i>	50 - 125 nm	Lipid-coated, Icosahedral	Archea	(Proctor 1997)
	Single-Stranded DNA	<i>Incertae sedis</i>	<i>Anelloviridae</i>	30 - 50 nm	Icosahedral, circular	Vertebrates	(Mukhopadhyaya et al. 2019)
		<i>Circulivirales</i>	<i>Circoviridae</i>	15 - 25 nm	Icosahedral, Non-enveloped	Vertebrates	(Ambalavanan et al. 2021)
		<i>Piccovirales</i>	<i>Parvoviridae</i>	23 - 28 nm	Icosahedral, linear segment	Vertebrates, Invertebrates	(Ambalavanan et al. 2021)
		<i>Geplafuvirales</i>	<i>Genomoviridae</i>	20-22 nm	Icosahedral	Unidentified	(Smith et al. 2021)
		<i>Recrevirales</i>	<i>Redondoviridae</i>	20 - 22 nm	Unidentified	Vertebrates	(Tu et al. 2021)
		<i>Cretevirales</i>	<i>Smacoviridae</i>	Unidentified	Unidentified	Unidentified	(Wang et al. 2014)
		<i>Polivirales</i>	<i>Bidnaviridae</i>	25 nm	Icosahedral, Non-enveloped	Marine sediments	(Koonin, Krupovic, and Yutin 2015)
		<i>Baphyvirales</i>	<i>Bacilladnaviridae</i>	34 nm	Icosahedral, Non-enveloped	Diatoms	(Wang et al. 2014)
		<i>Incertae sedis</i>	<i>Geminiviridae</i>	22 x 38	Icosahedral	Protozoa	(Wang et al. 2014)
		<i>Incertae</i>	<i>Nanoviridae</i>	18 - 20	Icosahedral	Protozoa	(Petersen et al.

		<i>sedis</i>		nm			2022)
		<i>Incertae sedis</i>	<i>Inoviridae</i>	80 - 1950 nm	Filamentous	Bacteria	(Ambalavanan et al. 2021)
		<i>Incertae sedis</i>	<i>Plectroviridae</i>	15 x 200 - 400	Rod-shaped	Bacteria	(Gunathilaka 2014)
		<i>Incertae sedis</i>	<i>Microviridae</i>	25 - 27 nm	Icosahedral	Bacteria	(Ambalavanan et al. 2021)
		<i>Bunyavirales</i>	<i>Peribunyaviridae</i>	80 - 120 nm	Round, Enveloped	Crustaceans	(Bojko et al. 2019)
		<i>Mononegavirales</i>	<i>Paramyxoviridae</i>	60 - 30 x 1000	Enveloped	Mammals	(Ambalavanan et al. 2021)
RNA	Double-Stranded RNA	<i>Durnavirales</i>	<i>Picobirnaviridae</i>	34 - 41 nm	Icosahedral capsid	Marine mammals	(Proctor 1997)
		<i>Reovirales</i>	<i>Reoviridae</i>	60 - 80 nm	Icosahedral	Algae	(Wiik-Nielsen et al. 2012)
		<i>Incertae sedis</i>	<i>Totiviridae</i>	30 - 40 nm	Icosahedral, Round	Protists, Shrimp, macroalgae	(Rousvoal et al. 2016)
		<i>Incertae sedis</i>	<i>Birnaviridae</i>	65 nm	Non-enveloped single-shelled	Fish, Mollusks	(Isshiki et al. 2004)
		<i>Incertae sedis</i>	<i>Cystoviridae</i>	60 - 75 nm	Icosahedral with lipid coat	Bacteria	(Krishnamurthy et al. 2016)

	Single-stranded RNA	<i>Picornavirales</i>	<i>Calciviridae</i>	27 - 40 nm	Icosahedral, Non-enveloped	Fish, mammals	(Smith, Skilling, and Latham 1981)
		<i>Stellavirales</i>	<i>Astroviridae</i>	41 nm	Spherical	Marine water, mammals	(Bosch et al. 1997)
		<i>Martellivirales</i>	<i>Virgaviridae</i>	20 nm	Rod-shaped	Arthropods	(Stanojević et al. 2020b)
		<i>Picornavirales</i>	<i>Picornaviridae</i>	22 - 30 nm	Icosahedral, round	Algae, crustaceans	(Ambalavanan et al. 2021)
		<i>Ortervirales</i>	<i>Retroviridae</i>	100 - 200 nm	Spherical to pleomorphic	Fish	(Aiewsakun and Katzourakis 2017)
		<i>Martellivirales</i>	<i>Togaviridae</i>	60 - 70 nm	Icosahedral, round	Fish	(Taksdal and Sindre 2016)
		<i>Mononegavirales</i>	<i>Rhabdoviridae</i>	70 nm	Bullet-shaped or bacilliform	Fish	(Kim et al. 2005)
		<i>Articulavirales</i>	<i>Orthomyxoviridae</i>	80 - 120 nm	Spherical with spikes	Fish, Mammals, seabirds	(Van Campen and Early 2001)
		<i>Nidovirales</i>	<i>Coronaviridae</i>	200 x 42	Rod-shaped with projections	Crustaceans, Fish, Seabirds	(Ambalavanan et al. 2021)
		<i>Nidovirales</i>	<i>Dicistroviridae</i>	30 nm	Icosahedral, Round	Crustaceans	(Ambalavanan et al. 2021)
		<i>Picornavirales</i>	<i>Marnaviridae</i>	25 nm	Icosahedral, Round	Algae	(Ambalavanan et al. 2021)

		<i>Incertaesedi s</i>	<i>Leviviridae</i>	26 nm	Icosahedral, Round	Bacteria	(Krishnamurthy et al. 2016)
		<i>Incertaesedi s</i>	<i>Microviridae</i>	25 - 27 nm	Icosahedral with spikes	Bacteria	(Kirchberger, Martinez, and Ochman 2022)
		<i>Incertaesedi s</i>	<i>Nodaviridae</i>	30 nm	Icosahedral, Round	Fish	(Munday and Nakai 1997)

2.5 Phenotypic and Genotypic Virus

Previous studies have confirmed that the virus placed in order virales based on the organism the virus infects were adverse biological entities that have both phenotypic and genotypic diversity (Steward, Culley, Mueller, et al. 2013). Several genes have been identified and isolated in both virus and host to control interaction for phenotype and genotype genomic analysis. In each chemostat, they detected 4 and 13 newly evolved viral phenotypes which differed in host range and viral resistance evolved newly from 4 to 11 *Synechococcus* (Marston et al. 2012). According to advances, ocean communities are made up of several bacterial groups arranged into genotypic (and phenotypic) clusters of related species. Although there are similarities between these patterns and metazoan communities, the underlying mechanisms for microbial communities may be very different (Polz et al. 2006). It has been suggested that marine viruses are the main source of novel genes in the water and that known viruses may not be indicative of real viromes (Kristensen et al. 2010). A fundamental aim of biology is to comprehend how genotypes lead to phenotypes. As difficult as this process is when considering cellular life, viruses make it far more difficult. A virus's distinct entity vanishes during reproduction and is replaced by a metabolic mixture of the virus and the host (DeLong et al. 2022).

2.6 Eukaryotic and Prokaryotic Viruses

The two main predators of prokaryotes in marine waters are phage and protozoa (nanoflagellates). Bacterial populations can be controlled in general or in highly particular ways in soil and marine habitats. For instance, it is known that the virus SF-9 exclusively targets *Shigelladysenteriae* Type 1. The potential of phage in the biological management of the fish disease has also been investigated. In this instance, *Pseudomonas plecoglossicida*, the bacterium that causes the bacterial hemorrhagic ascites disease in cultured ayu fish (*Plecoglossus altivelis*), is controlled using two distinct phages (Pepper and Gentry 2015). The collection of known host genes is carried out and transferred to a lesser degree to eukaryotic viruses and phages. The negative effects of viruses are concentrated mostly on modifying their host's physiology. Viral infections enhance the unexpected and potentially positive ways

for their host evolution, distribution, metabolism, and immunity (Rohwer and Thurber 2009a). Eukaryotic viruses with a large double-stranded DNA genome, ranging from 100 kb to 1.26 Mb, known as nucleocytoplasmic big DNA viruses, infect eukaryotes. They can influence the metabolism of the algal host. It is well known that certain of them influence the population dynamics of bloom-forming algae in marine settings (Vincent et al. 2022). From 2.3 to 6.3 million detect various prokaryotic and viral DNA samples generated via high-throughput sequences. Found 1.1 to 5.6 million detect for various samples to read quality and length control. Denovo assemble using CLC genomic workbench for filtered paired-end ends, resulting in 29,362 contigs with 1000bp of minimum length.

2.7 Lytic and Lysogenic Cycle

Viruses infect bacteria through a lytic and lysogenic cycle. Lytic and Lysogenic cycles are essential for virus-host interaction (Chen et al. 2021). Lysogenic and lytic are two different ways that viruses can live. Until recently, it was believed that most viruses from surface waters selected a lytic life strategy, while those from deep-sea settings with little organic matter and little microbial activity preferred a lysogenic life strategy. The lysogenic life strategy is a superior adaption to harsh and energy-scarce deep-sea settings. However, there is still a lot unknown about viral life in deep water (Gao et al. 2022). By inducing the lytic cycle, which results in bacterial cell lysis, phages directly control bacterial populations. Bacterial populations might considerably expand in the absence of phage as a regulator (Pepper and Gentry 2015). The majority of the host's transcription and translation is stopped by the phage during the lytic cycle. Phages must continue photosynthesis throughout the infection cycle to maintain the proton motive force necessary to lyse the host. The D1 proteins, which are encoded by the cyanophage, are produced during the infection cycle to counteract the decrease in host gene expression brought on by the virus (Rohwer and Thurber 2009a). In the deep ocean, heterotroph is dominant to a lytic virus for ecology (Tschitschko et al. 2016). In the lysogenic cycle, DNA interacts with the host cell to attach the phage genome to assimilate into the host cell to retain the signal to transform a lytic cell. In the lytic cycle, the virus attached and penetrates to produce virions (Abdulrahman Ashy and Agustí 2020). Through the viral shunt, viral lysis facilitates the exchange of organic matter

between the living biomass and the dissolved organic carbon pool. It is believed that the viral shunt releases close to 10 billion tonnes of carbon every day, and this process is crucial to the cycling of nutrients that drives ocean production (Coutinho et al. 2017).

Bacteriophages are lytic and lyse the bacterial host after replication, the bacteriophage integrates into the bacterial chromosome as a prophage while others enter a lysogenic life cycle. In the lysogenic process, the host and temperate phages have stable interaction, either integrating into the bacterial chromosome or via plasmid maintenance. In lysogen, the phage genome is maintained as a “silent” form of infection till prophage can be triggered to become lytic thus the process is termed induction. Prophage is induced and initiates lytic replication in response to environmental factors and signals (Secor and Dandekar 2020) & (Breitbart 2012). In the ocean, the lytic process remains more essential than the lysogenic process but certain studies found cultural marine bacteria in a high proportion of prophages. Half of the isolated bacteria are regulated through chemical induction from prophages containing a diverse range of marine environments (Breitbart 2012). Bacterial colonies increased in phage production through laboratory methods because the particles bind with bacteria present in higher cell-specific than free-living bacteria and higher density. During initial lysis, these particles were recolonized by bacterial strains for additional phage resistance to enhance particles and affect host dynamics (Tomaru and Nagasaki 2007).

2.8 Meta – OMICS

Meta- Omics is the statistical data that provides biological studies such as the genome, proteome, transcriptome, or metabolome. Meta-omics is an advanced method based on algorithmic analyses. This improved the merit studies in marine virome to detect specific problems which have a unique effect. (Pratama and Van Elsas 2018, Mukhopadhyaya et al. 2019)

2.8.1 Metagenomics

Metagenomic data analyze hundreds and thousands of viral genotypes in the marine oceans (Breitbart et al. 2007). The International Committee on Taxonomy of Viruses (ICTV) has historically required extensive data on host range, replication cycle, and the structure and properties of viral particles in order to describe and classify a new group

of viruses. The samples considered to be viromes may not be primarily of viral origin and may instead be contaminated with non-viral DNA, which would point to a significant flaw in the current metagenomic methods (Kristensen et al. 2010). High-throughput sequencing and metagenomic methods have, however, fundamentally altered the field of virology, with many more viruses being known simply from sequence data that have been experimentally characterized. A workshop was held to create a new framework for virus taxonomy in the metagenomics era because most viruses are now found through metagenomics and lack a direct correlation with biological agents. Sequence data prepare a wealth of information that can be used for taxonomy, including evolutionary relationships, overall genome organization, characteristics of genome expression, genome replication strategy, and the presence or absence of various distinctive motifs and local and global composition (Simmonds et al. n.d.). The taxonomic distribution of the sparse quantity of identifiable reads in these metagenomes indicates that bacteriophages predominate in the reservoir of DNA-containing viruses in saltwater (Miranda et al. 2016).

2.8.2 Transcriptomics

As a method to reveal viral activity and variety in various ecological settings, meta transcriptomics hasn't received much attention yet. One of the few investigations found gene expressions suggestive of both lytic and lysogenic virus activities in the sub-seafloor sediments. In recent research, metagenomics was used in conjunction with available metatranscriptomics data to identify the expression of viral auxiliary metabolic genes (AMGs), which may be involved in modifying host sulfur metabolism upon infection (Moniruzzaman et al. 2017). The first line of evidence can be easily and affordably provided by demonstrating active transcription of an HGT-acquired gene using either gene-specific reverse transcriptase-PCR or transcriptomics, both of which are now quite routine methods. Even in animals with limited genetic resources, transcriptional methods can be used. In 2019, researchers discovered a relationship between Si stress and the early, active, and lytic phases of viral infection using metatranscriptomic analysis of cell-associated diatom viruses and focused quantification of extracellular viruses. Si stress was found to increase virus-induced mortality in colonies of the bloom-forming diatom *Chaetoceros tenuissimus* (Kranzler et al. 2019).

CHAPTER 3

AIM AND OBJECTIVE

3.1 AIM

To isolate and characterize Mycobacteriophage from the marine water sample.

3.2 OBJECTIVES

- Collection of water sample from marine source.
- Isolation of Mycobacteriophage using double-layer agar method.
- Structural identification is confirmed by Transmission Electron Microscopy.

CHAPTER 4

MATERIALS AND METHODS

4.1 SAMPLE COLLECTION

Water sample is collected from the surface area of Kovalam Beach, Chennai. The sample was filtered through 0.45 syringe filter to remove the bacteria and other contaminants from water then the sample is filtered through 0.22 syringe filter to filter the phages. The sample is stored in a sterile container at 4°C.

MEDIA PREPARATION

ADC ENRICHMENT (100 ml)

Bovine Serum Albumin	-	5g
Glucose	-	2g
Nacl	-	0.85g
Distilled Water	-	100ml

7H9 MIDDLEBROOK BROTH (1000 ml)

7H9 Middlebrook broth	-	4.7g
Distilled water	-	900 ml
Glycerol	-	5 ml
ADC enrichment	-	100 ml

7H11 MIDDLEBROOK AGAR (1000 ml)

7H11 Middlebrook Agar	-	4.7g
Calcium Chloride	-	0.011g
Distilled water	-	900 ml

Glycerol	- 5 ml
ADC enrichment	- 100 ml

0.7 % SOFT AGAR PREPARATION (1000 ml)

7H9 Middlebrook broth	- 4.7g
Agar-Agar	- 7g
Calcium Chloride	- 0.011g
Distilled water	- 900 ml

4.2 BACTERIAL STRAIN

Mycobacterium Smegmatis culture were used for isolating Mycobacteriophage. 10µl of *Mycobacterium Smegmatis* was inoculated on fresh 7H9 Middlebrook broth with supplement of ADC . The culture was incubated at 37°C for overnight. Grown culture was used as host for Mycobacteriophage.

4.3 PHAGE ENRICHMENT METHOD

20 ml of fresh 3-fold 7H9 Middlebrook broth is prepared in conical flask. Added 20 ml of filtered sample into broth and 1ml of *Mycobacterium Smegmatis* culture is added. Kept in shaker at 37°C for 5 hrs (OD value should be 0.8). OD value should be make to 0.8 untill the culture should grow. Then the sample is centrifuged at 4000 rpm for 5 minutes. The supernatant is filtered through 0.45 µm and 0.22 µm. The sample is prepared.

4.4 DOUBLE-LAYER AGAR METHOD

7H11 Middlebrook Agar plates were prepared and leave it for 30 mins to solidify. Add 10µl of *Mycobacterium Smegmatis* culture and 90µl of filtered water sample into the test tube. Leave the test tube for 10 to 20 mins. 0.7% of soft agar were taken for 3ml and 1ml of ADC enrichment into test tube. Mix all the composition and pour into the 7H11

Middlebrook Agar plate and swirl the plate to spread the soft agar. Allow the plate to get solidify and kept at 37°C for 12 to 18 hrs.

4.5 PLAQUE FORMATION

Incubated plates were observed for plaque formation. Plaques were formed on double layer agar plate. The above mentioned double layer plating was repeated for confirmation. Count the number of plaques formed in each plate. Calculated the plaque forming units for incubated plates.

4.6 SERIAL DILUTION

The sample was serially diluted by adding 100µl of sample is mixed into 900µl of phage buffer (10⁻¹, 10⁻², 10⁻¹⁰). 10 l of *M. Smegmatis* culture and 90µl of sample was inoculated and plated on Double layer Agar plate.

4.7 SPOT TEST

Visible plaque were selected for spot test. 10µl of Phage buffer is taken, then it kept on the single plaque and squeezed 7 to 10 times to remove the plaque from grown plate. The sample is spotted on double layer agar plate which is inoculated with *M. Smegmatis*. Spot test is used to identify and confirm the plaques.

4.8 ULTRACENTRIFUGE

Plaques were squeezed 7 to 10 times phage buffer on specific plaque and transfer into 1ml of phage buffer. Leave the sample for overnight incubation at 37°C. Then, it is Ultracentrifuge at 65000g for 2 hours at 4°C. The pellet is resuspend with phage buffer and store at 4°C.

4.9 HIGH TITER PHAGE TITRATE

5ml of phage buffer is add on the grown plaque plate. Leave the plates for overnight at 4°C. The plates were kept at room temperature for 1 hour. The plaques are diffused into buffer. The phage buffer were collected from the plate and filtered through 0.22 m syringe filter. Stored at 4°C.

4.10 TRANSMISSION ELECTRON MICROSCOPY

10 µl of high titer phage lysate is added on copper grid for 1 minute. Remove the sample using filter paper. Add 10 µl of 2 % uranyl acetate for 1 minute. Then remove the stain using filter paper and allow the grid to air dry. Sample grid is observed at 100kV with magnification.

4.11 EXTRACTION OF DNA

High titer lysate sample is extracted for DNA extraction. Transfer 1 ml of high titer lysate into 2ml eppendorf. Add 12.5 µl of 1M MgCl₂ and mix gently. Then add 1µl of RNase and vortex the sample and incubate for 30 mins at room temperature. Reagents such as 40µl of 0.5 M EDTA, 5µl of proteinase K and 50µl of 10% of SDS. Vortex the mixture and incubate at 55°C for 60 minutes. Transfer 500µl of mixture into two tubes and add equal volume Phenol:Chloroform:Isopropanol (P:C:I). Centrifuge at 8000rpm for 10 minutes and repeat until white interphase is gone. Then DNA precipitation is followed by adding 1 ml of 95% ethanol and 50µl of 3M sodium acetate, kept on ice for 5 minutes. Cotton ball like precipitate is formed then the sample is centrifuged at 8000rpm for 20 minutes. Carefully remove the supernatant, resuspend the pellet using sterile water. DNA sample is stored at 4°C for short term and -20°C for long term.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 SAMPLE COLLECTION

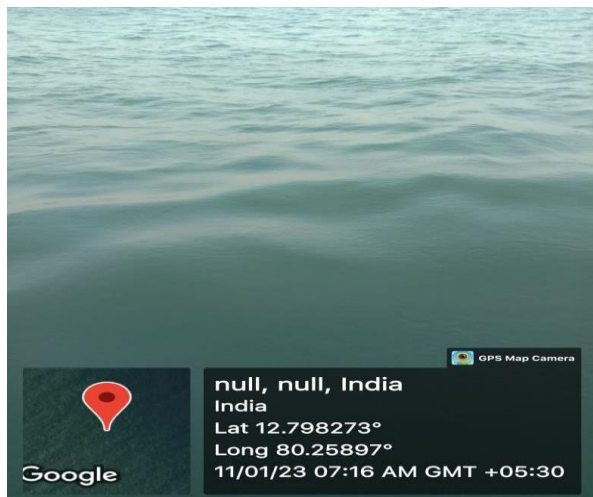


Fig 5.1.1 Sample collection

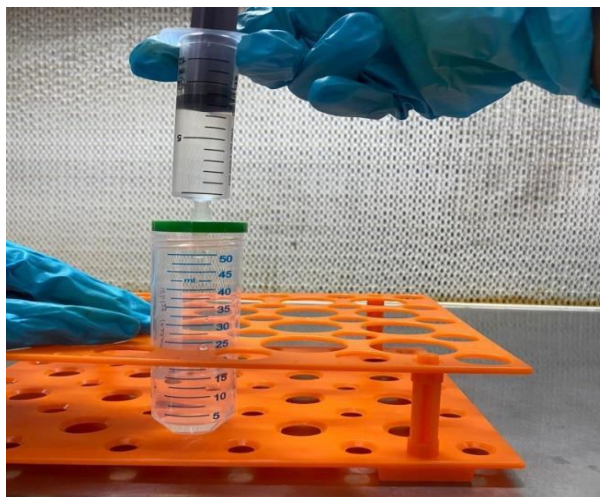


Fig 5.1.2 Sample filtration

Water sample is collected from Kovalam Beach, Chennai (Latitude 12.798273 and Longitude 80.25897). The filtered sample was maintained and stored at 4°C.

5.2 MYCOBACTERIUM STRAIN



Fig 5.2.1 M.Smegmatis streaked plate

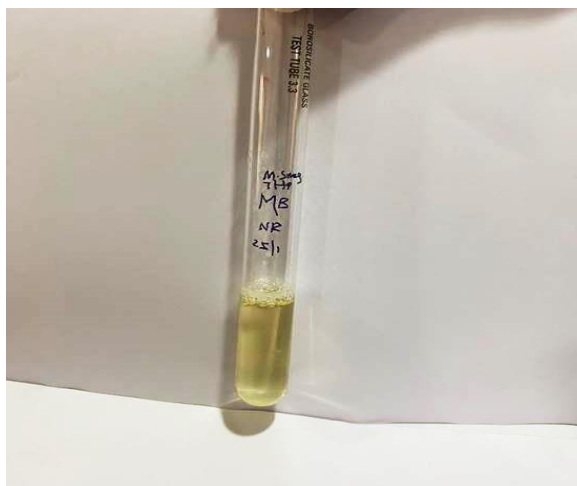


Fig 5.2.2 M.Smegmatis inoculated broth

M. Smegmatis culture is inoculated on 7H9 Middlebrook broth and quadrantly streaked on 7H11 Middlebrook Agar plate.

5.3 PHAGE ENRICHMENT SAMPLE



Fig 5.3.1 Phage enrichment inoculated plate

50 µl of Phage enrichment sample and 50 µl of *M. Smegmatis* is inoculated into 0.7% of soft agar. Poured on prepared 7H11 Middlebrook agar plate. There is no plaque formation on the plate.

5.4 DOUBLE-LAYER AGAR PLATE

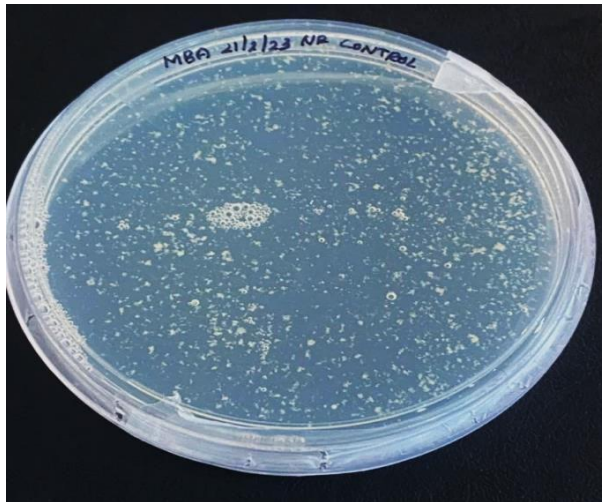


FIG 5.4.1 Control double-layer plate

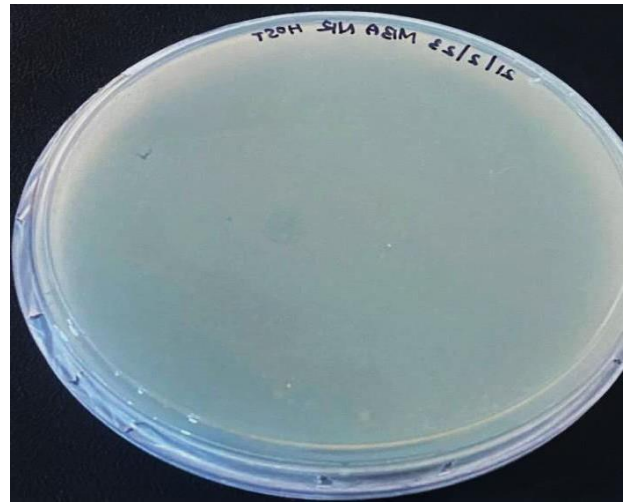


Fig 5.4.2 *M.Smegmatis* inoculated plate

Mycobacterium Smegmatis culture is inoculated into 0.7% of soft agar. Poured on prepared 7H11 Middlebrook agar plate. Fig 5.3.2 shows the bacterial lawn on Double layer agar plate.

5.5 PLAQUE FORMATION

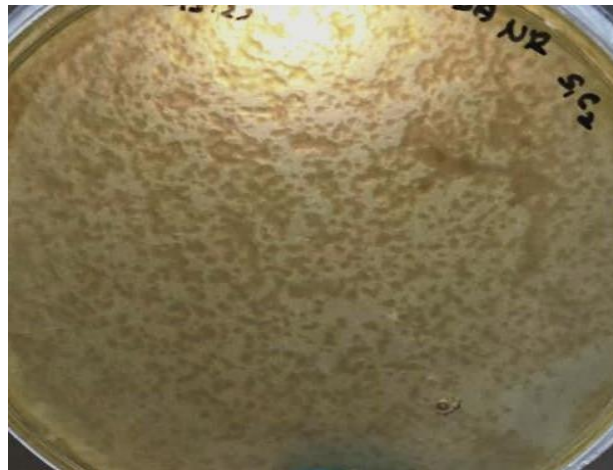


Fig 5.5.1 Plaque formation on Double-layer agar plate

50 μ l of Filtered water sample and 50 μ l of *M.Smegmatis* culture were inoculated into 0.7% of soft agar. Poured on prepared 7H11 Middlebrook agar plate.and kept at 37°C for 12 to 18 hrs to observe the plaque formation.

5.6 SERIAL DILUTION

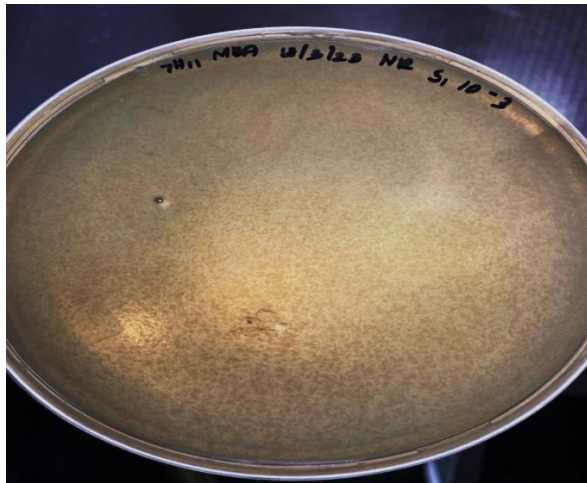


Fig 5.6.1 Serially diluted sample 10⁻³



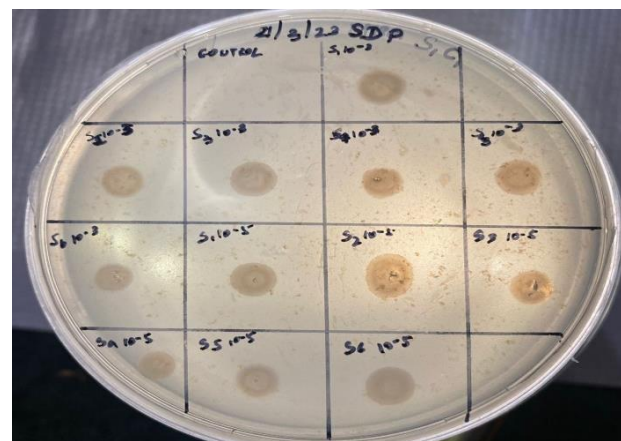
5.6.2 Serially diluted sample 10⁻⁵

90 μ l of Filtered water sample and 10 μ l of *M. Smegmatis* culture were inoculated into 0.7% of soft agar. Poured on prepared 7H11 Middlebrook agar plate. and kept at 37°C Plates were observed after 18 hours of incubation. Plaques were formed on plate Plaques were differentiated and counts were less according to the serial dilution method.

5.7 SPOT TEST



Fig 5.7.1 Plaque formation



5.7.2 No plaques were formed

Plates were observed after 18 hours of incubation. Plaques were formed on serial diluted samples. No plaques were formed on plate (Fig 5.6.2). Sample S₁C₂ were shown a plaque formation on (Fig 5.6.1).

5.8 ULTRACENTRIFUGE

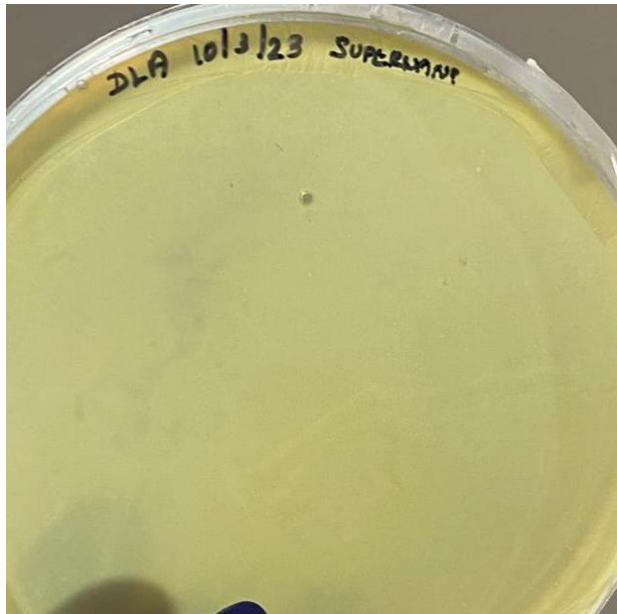


Fig 5.8.a Supernatant sample

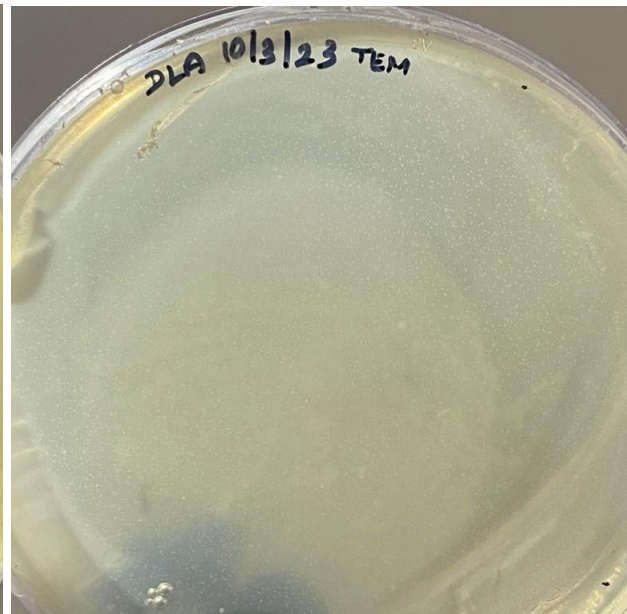


Fig 5.8.b Resuspended sample

The Ultracentrifuged sample is used for TEM analysis. Supernat sample and resuspended sample were inoculated on double-layer agar plate. No plaques were formed in Ultracentrifuged sample.

5.9 HIGH TITER PHAGE LYSATE



FIG 5.9 High Titer Lysate Plate.

High titer phage lysate sample were inoculated and plated on Double Layer plate. The plaques were formed on double-layer agar plate.

5.10 TRANSMISSION ELECTRON MICROSCOPY

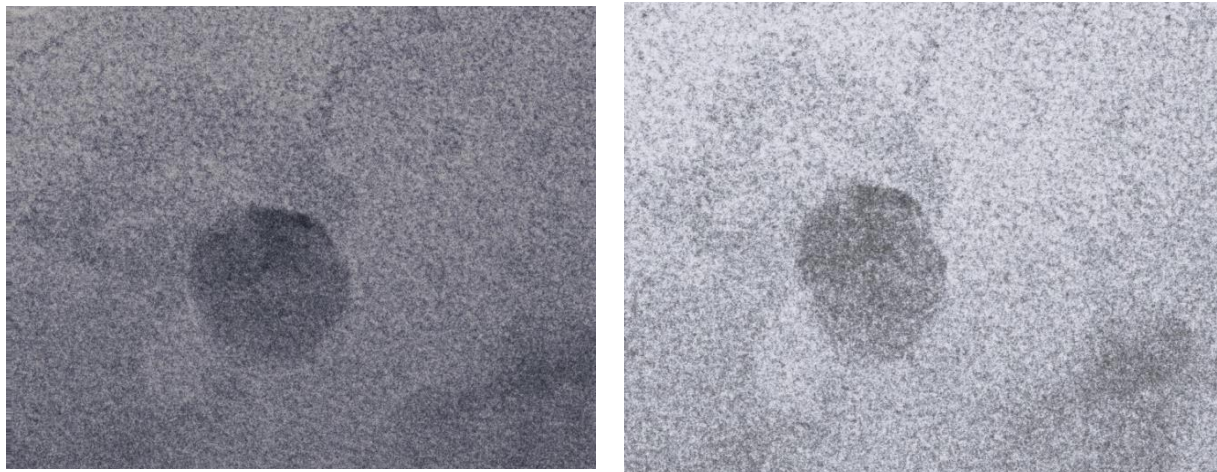
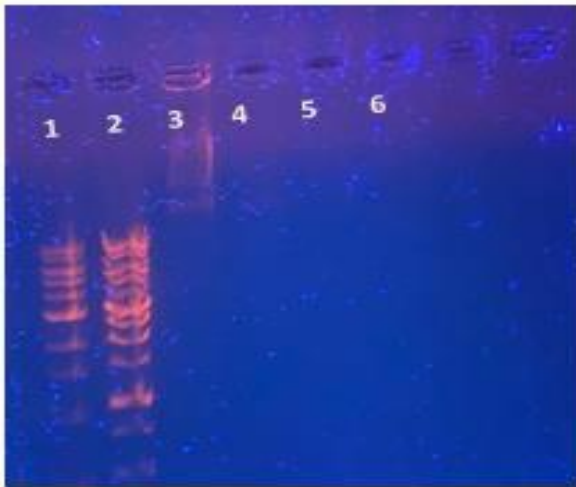


Fig 5.10.1 Mycobacteriophage image in TEM analysis.

Sample were prepared on Carbon grid and placed inside the Transmission electron microscopy. The Mycobacteriophage image were observed at 200kV. Scale marker is 100nm,.

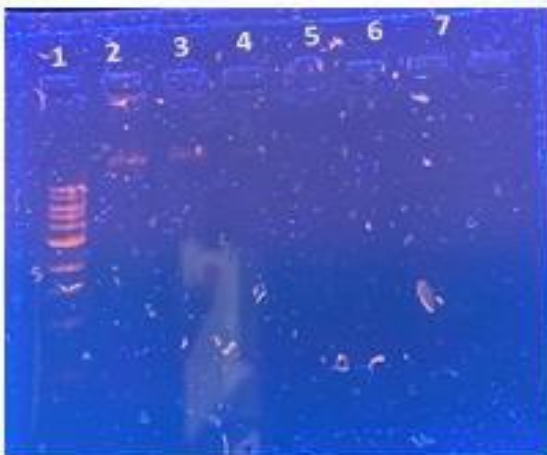
5.11 EXTRACTION OF DNA



Band observed on Lane 3(Sample 1)

LANE	SAMPLE	VOLUME
1	1 kb DNA ladder	5 μ l
2	1 kb DNA ladder	5 μ l
3	Sample 1	8 μ l (S1)+ 2 μ l (dye) (10 μ l)
4	Sample 3	8 μ l (S3) + 2 μ l (dye) (10 μ l)
5	Sample 5	8 μ l (S5) + 2 μ l (dye) (10 μ l)
6	Sample 6	8 μ l (S6) + 2 μ l (dye) (10 μ l)

Fig 5.11.1 The samples extracted without adding DNase and RNase were run in AGE.



Band observed on Lane 2 (Sample 1) and Lane 3 (Sample 2)

LANE	SAMPLE	VOLUME
1	Marker	5 μ l
2	Sample 1	8 μ l + 2 μ l (10 μ l)
3	Sample 2	8 μ l + 2 μ l (10 μ l)
4	Sample 3	8 μ l + 2 μ l (10 μ l)
5	Sample 4	8 μ l + 2 μ l (10 μ l)
6	Sample 5	8 μ l + 2 μ l (10 μ l)
7	Sample 6	8 μ l + 2 μ l (10 μ l)

Fig 5.11.2 The samples extracted using RNase were run in AGE.

The DNA band confirms the sample contains DNA. Mycobacteriophage is the double-stranded DNA. It has been confirmed using Agarose Gel Electrophoresis by visualizing DNA band.

CHAPTER 6

SUMMARY AND CONCLUSION

Mycobacteriophage is the most challenging among the other bacteriophages by its functions. Mycobacteriophage can be used to treat mycobacterial infections. Isolating Mycobacteriophage from marine water is very difficult due to temperature changes and environmental adaptability. Numerous Phages were present in the marine ocean according to its deep water. Two types of phages can be isolated, they are lytic phages and lysogenic phages. Bacteriophages can be used as phage therapy treatment which is an alternative to antibiotics and it is eco-friendly to nature. Specific target to kill the mycobacterial infections does not cause any damage or harm to living organisms. New entomological research has shown amazing tripartite symbioses between bacteria, phages, and insects. In such ecosystems, viruses regulate the number of bacterial symbionts. As a result, the host's physiology and ecosystem are altered. These tripartite symbioses between the phage, microbe, and eukaryote provide an example of the subtleties of viral ecology that have up until now gone unnoticed. Many research and drug were introduced for mycobacterial infections according to its mutations still there are mysteries to cure and predict the mutations. Mycobacteriophage therapy can be used to treat mycobacterial infections. It can be used to treat mutated infections. There is still a lot of fascinating biology to be uncovered. Viral infections have a variety of unanticipated and possibly advantageous effects on their hosts' metabolism, immunity, dispersion, and evolution. We predict that future research on marine viral infections will be focused on specific areas. An effort is needed to find and assess the viruses that infect marine species. Studies on these marine viruses will provide intriguing and significant information.

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