Chapter 5

DISCUSSION

Vibriophages are phages infecting *Vibrios* and they are well documented. The earliest records of the isolation of bacteriophages appeared in the 1920s. As typical viruses, phages infect suitable bacterial host cells for proliferation (Josephsen and Neve, 1998). Phages have been isolated from the environment in which host bacterial strains generally survive and can be found in sewage, faeces, soil, and water (Chakrabarti *et al.*, 2000). In most of the earlier studies, the phage was isolated from the same sample as that from which the host bacterium was isolated. In the present study too, a filamentous vibriophage ΦMV-5 was isolated from the Mangalavanam mangrove, Kochi using a host *Vibrio* sp. MV-5, which was also isolated from the same location.

The host for phage ΦMV-5, *Vibrio* sp. MV-5 isolated from the Mangalavanam mangrove closely resembles *V. vulnificus* based on partial 16S rRNA gene analysis. *Vibrio vulnificus* is a naturally occurring estuarine bacteria (DePaola *et al.*, 1994) capable of causing primary septicemia or gastroenteritis after ingestion and secondary septicemia through skin lesions in individuals with underlying chronic diseases. It is abundant in a variety of estuarine habitats, and highest levels have been reported in the intestines of finfish (DePaola *et al.*, 1997). They have also been reported from mangroves throughout the world.

The plaque formed by a particular phage is one of the many important parameters for characterization of the phage. A lot can be determined about a bacteriophage from looking at its plaque size and shape. There are two known forms of phage reproduction, lytic and lysogenic. In a lytic cycle, a bacteriophage
infects a host bacterium and converts it into a "phage factory". The bacteriophages inside eventually destroy or lyse the bacterium and burst out as many as 50 to 200 new phages. Larger plaques mean that closer to 200 phages are produced every time the process occurs or that the phage has had more time on the plate to consume the bacteria. Smaller plaques mean the opposite. A clear plaque is strong evidence of a lytic phage. In the lytic cycle, the host bacterium is eventually destroyed, so there is nothing visible in that area.

The plaques produced by the vibriophage ΦMV-5 were characteristically turbid, which is the typical plaque morphology of temperate phages (Jiang et al., 1998). The turbidity in a turbid plaque arises from the growth of surviving bacteria within the zone of lysis. In a lysogenic cycle, the infecting phage mixes some of its DNA with that of the host bacterium, and becomes immune to any other phage of the same type. These are known as temperate phages, which use both a lytic and lysogenic life cycle. They create turbid plaques because many of the bacteria are not destroyed, just genetically different. If a plaque has both a lytic and lysogenic area, it is an indication of the phage having both lytic and lysogenic life cycles. It also suggests that the phage is temperate.

Phage ΦMV-5 showed characteristic small turbid plaques of the size of a pinhead, but with smooth outline, typical of plaques exhibited by filamentous phages. Phages lytic to *V. vulnificus* have been discovered in the estuarine water samples of Louisiana which included four morphological groups. The plaques were reported to be minute (1 mm or less) with fuzzy outlines and complete lysis was not evident (Pellon *et al.*, 1995). A diverse group of *V. vulnificus* phages, reported to be abundant in Gulf Coast oysters throughout the year, where suggested to be an important agent in the control of microbial populations in estuarine and coastal environments. (DePaola *et al.*, 1997). There are reports of temperate phages for *V. vulnificus*, which showed no evidence of lysis (Marco-Noales *et al.*, 2004).
Previous reports have indicated that lysogenic phages are abundant among marine bacterial isolates (Jiang and Paul, 1994), suggesting the temperate nature of many marine bacteriophages. However, isolation of temperate phages from marine environments was rare. Out of the 300 marine phage isolates only 29 were found to be temperate. However, this phage collection was obtained by liquid nutrient enrichment isolation method which favors isolation of lytic phages (Moebus, 1980). The same approach was used in the present study also. The phage was isolated using the soft agar overlay method used for the isolation of lytic phages.

As observed under the Transmission Electron Microscope (TEM), phage ΦMV-5 morphology was found to be typical that of a filamentous phage. Each phage particle consisted of single, long filament that was approximately 1.1 μm in length and 0.03 μm in diameter. The phage also shows a pointed head and a blunt tail, characteristic of all filamentous phages, and may be considered to be an inovirus-like bacteriophage.

Filamentous phages constitute a large family of bacterial viruses that infect many gram-negative bacteria. Their defining characteristic is a circular, ss DNA genome encased in a long somewhat flexible tube composed of thousands of copies of a single major coat protein, with a few minor proteins at the tips. The genome is small-a dozen or fewer closely packed genes and an intergenic region that contains sequences necessary for DNA replication and encapsidation. Unlike most bacterial viruses, filamentous phages are produced and secreted from infected bacteria without cell killing or lysis.

The ends of the phage particle ΦMV-5 were clearly distinguishable in electron micrographs. There is a pointed head and a blunt edge. All filamentous phages that have been characterized use pili, which are long and slender cell surface appendages that resemble the phage themselves as receptors. Many
vibriophages reported are found to have similar filamentous nature. EM of phage 493 of *V. cholerae* O139 revealed the filamentous nature of the particle with an estimated width of 2.6 nm and variable length. In some preparations, one end of the phage appeared to carry slender fibers. The phage was resistant to pH values of 2.8-11.8 and to heat treatment up to 60°C for 10 min, but it was totally inactivated at 75°C and by chloroform treatment (Jouravleva *et al.*, 1998).

*Vibrio cholerae* is known to be a host to a variety of bacteriophages (vibriophages), which include virulent phages as well as temperate phages, represented by the kappa-type phages produced by most strains of the El Tor biotype (Guidolin and Manning, 1987; Takeya, 1974). Another group includes the filamentous phages, which have a single-stranded DNA (ssDNA) genome (Campos *et al.*, 2003a; Ehara *et al.*, 1997; Ikema and Honma, 1998; Kar *et al.*, 1996; Waldor and Mekalanos, 1996). Several of the *V. cholerae*-specific filamentous phages have been implicated in virulence gene transfer among *V. cholerae* strains (Campos *et al.*, 2003b; Davis and Waldor, 2003; Waldor and Mekalanos, 1996). Filamentous phages of *V. cholerae* have also been found to be distinct from the well-characterized filamentous coliphages, in that some of these phages can form lysogens (Campos *et al.*, 2003a; Kar *et al.*, 1996; Waldor and Mekalanos, 1996). The most remarkable of these phages is CTXΦ (Waldor and Mekalanos, 1996), which exists as a prophage in toxigenic *V. cholerae* and encodes the cholera toxin (CT).

The vibriophage ΦMV-5 isolated was concentrated using polyethylene glycol (PEG). It is a high-molecular-weight, water-soluble polymer, and has been used to concentrate viruses from aqueous suspensions. This method is termed as a phase separation rather than a precipitation (Yamamoto *et al.*, 1970) and was initially used in combination with NaCl and another polymer, dextran sulfate (DS), to concentrate preparations of poliovirus (Norrby and Albertssson, 1960).
bacteriophage, adenovirus etc. Subsequent studies indicated that PEG is equally effective when used in combination with only NaCl to concentrate bacteriophage (Yamamoto et al., 1970).

The concentrations of PEG and NaCl were varied to determine the optimum conditions for the recovery of infectious ΦMV-5 in the pellets and the 20% conc. of PEG 6000 was found to be the optimum for the phase separation of this phage. The purpose of this study was to determine the optimum conditions, in terms of the properties and relative recovery of virus particles, for concentrating the phage. The results demonstrated that although maximum recovery was obtained with 40% PEG 6000 for ΦMV-5, maximum purity was obtained at 20%.

Although PEG precipitation could be used to concentrate the bacteriophage, it was reported that the bacteriophage concentrated using PEG 6000, were more often broken into head and tail components and would obviously not be viable for phage VHML infecting V. harveyi (Oakey and Owens, 2000). But, in the present study, viable phage particles with maximum purity could be retrieved through PEG precipitation. This showed the greater stability of the phage ΦMV-5.

The protein profiles of the ΦMV-5 showed a single band with a molecular weight of ~45 kDa suggesting that there is only one major protein. Invariably, the major protein is the Major Coat Protein (mcp).

SDS-PAGE of Vibrio cholerae typing phage e5 had displayed 13 structural polypeptides with molecular sizes ranging from 21.5 kDa to 90 kDa. The major component had a molecular size of 50 kDa (Basu et al., 1993).
In the present study, the focus was also on the interaction of the vibriophage with its host cell. The influence of physicochemical parameters on phage viability and adsorption to sensitive cells were especially investigated. The phage ΦMV-5 was also characterized for its propagation and adsorption parameters.

The first step in the growth of bacteriophage is its attachment to susceptible bacteria. The rate of this attachment can be readily measured by centrifuging the bacteria out of a suspension containing phage, at various times, and determining the amount of phage which remains unattached in the supernatant. Phage cannot multiply except when attached to bacteria, therefore, the rate of attachment may under certain conditions, limit the rate of growth (Ellis and Delbruck, 1939).

The MOI resulting in highest phage titer under standard conditions was considered as the optimal MOI and used in subsequent large scale phage production. From the data obtained, the optimal multiplicity of infection was observed to be 5 PFU/ml.

Previous research revealed the potential importance of latent period, burst size, and the ability to produce lysogens as “strategies” by which a phage might optimize its ability to survive in nature (Abedon, 1989; Stewart and Levin, 1984; Wang et al., 1996). The infection cycle of ΦMV-5 was characterized for their phage multiplication parameters: latent period was found to be 30 minutes and the rise period was 50 minutes. From the calculations, it was found that the burst size was 60 phages per cell. On comparison it was found that the burst size and latent time values varied from that previously reported for vibriophages. An earlier report involving a survey of 52 cultured marine phages, found that there is great variation
in the burst size; the average marine phage burst size was 185, and burst sizes range from 5 to 610 (Borsheim, 1993; Jiang et al., 1998).

In phage e5, a Vibrio El Tor typing phage, growth was characterized by a latent period of 15 min, a rise period of 13 min and a burst size of around 100 pfu per cell (Basu et al., 1993). Corresponding values for Ph-1, the only other cholera phage belonging to the same family and species were 50 min and $10^3$ pfu per cell (Mukherjee, 1978).

The latent periods of phages nt-1 and nt-6, isolated from a salt marsh, are 50 and 60 min respectively, under optimal conditions. However, the latent periods were shown an increase to 170 and 120 min respectively, when the temperature was 10°C below optimal (Zachary, 1978). A phage infecting Vibrio fischeri MJ-1 had a latent period of 25 min (Levisohn et al., 1987), while the latent periods for two bacteriophages isolated from the North Sea were 150 and 180 min (Chen et al., 1966). The latent periods of phage $\Phi$MV-5 are comparable to those previously reported for other vibriophages.

The phage latent period is typically under the control of a phage protein complex known as a holin. Holins restrain the activity of cell-wall-digesting endolysins, and mutations in holin genes can significantly modify the timing of host cell lysis (Young et al., 2000). Increasing rates of phage exponential growth—larger burst sizes, shorter generation times, or, for well-mixed cultures (Yin and McCaskill, 1992), faster phage adsorption—should lead to faster phage-mediated exploitation of host populations. Both burst size and the phage generation time, however, are controlled by the phage latent period, with greater burst sizes associated with longer latent periods but shorter generation times associated with shorter latent periods. This conflict between burst size enlargement and generation time reduction complicates phage latent-period optimization (Abedon et al., 2001).
In order to design adsorption assays, the stability of phages was examined during 30 min at 37°C. ΦMV-5 phage showed maximum adsorption at 30 min.

The influence of physical parameters on the process of adsorption was also studied in addition to propagation factors. The lytic cycle of a phage begins with its adsorption on the cell wall receptors of a sensitive bacterial host, a highly specific event (Quiberoni et al., 2004a). The maximum burst size will be obtained only under conditions of maximum adsorption. Therefore, the effect of temperature, Ca²⁺, pH and NaCl on the adsorption process was also studied. Regarding the effect of temperature on the adsorption process, the results were highly dependent on the system studied.

There are some recognized factors affecting the phage adsorption process, such as the presence of Ca²⁺ ions, the physiological state of bacterial cells, pH and temperature. The role of inorganic cations like Ca²⁺ and Mg²⁺ is noteworthy and it was observed that 10 mM CaCl₂ was optimum for the propagation of the ΦMV-5 phage. Beyond this concentration also, the effect was the same. 1 mM CaCl₂ showed a reduced effect than 10 mM CaCl₂ on adsorption, but enhanced final PFU and faster adsorption as compared to the control. These results obtained for ΦMV-5 phage in the present study, showed that Ca²⁻ was not indispensable either for the adsorption or for the completion of the lytic cycle, although lysis was faster in the presence of the cation. Regarding the effect of calcium, similar results were obtained for some Streptococcus thermophilus and Lact. Delbrueckii subsp. Bulgaricus phages (Binetti et al., 2002; Quiberoni et al., 2004b). The requirement for Ca²⁻ (or Mg²⁺) not only stabilizes the coiled DNA inside the phage capsid, but also greatly improves the adsorption rate, and controls the penetration efficiency of phage DNA into the bacterial cells (Se’chaud et al., 1988). Calcium ions were required for the penetration of phage genomes into the host cells of Lactobacillus casei (Watanabe and Takesue, 1972).
Phage ΦMV-5 was shown to tolerate temperatures up to 70°C and was rapidly inactivated at temperatures above 70°C. Usually vibriophages are associated with high temperature tolerance since they are present in higher numbers during summer season.

Thermal inactivation profiles of phage have been quite extensively studied for vibriophages (Mukherjee, 1978; Yamamoto et al., 1970). The half life of thermal inactivation of e5 at 60°C was found to be 12.5 min (Basu et al., 1993) compared to 2.5 min of that found for Ph-1 (Mukherjee, 1978).

The phage ΦMV-5 was viable over a wide pH range of 5 to 11 at 37°C. The viral suspension was completely inactivated after 30 min at pH 2-4 indicating high sensitivity to lower pH. Salinity, pH and temperature are factors that play an important role in the inactivation of *Vibrio vulnificus* and *Vibrio parahaemolyticus* bacteriophages. The optimum pH for *vibrio* bacteriophages was around a neutral pH of 6 to 8. Most proteins are stable at around neutral pH. However, at extreme pH values some proteins begin to swell and unfold (Fennema, 1996). Bacteriophages are composed of a core of nucleic acid covered by a protein coat. A phage capable of infecting a bacterium attaches to a receptor site on the cell surface then uses lysozyme that is located in the phage tail to weaken the bacterial cell wall. A phage tail fiber injects the DNA through the weakened wall into the bacterial cell. This process is very important for phage reproduction. However, pH can interfere with lysozyme enzyme or protein coat thus preventing phage to attach to receptor sites of the host cell (Leverentz et al., 2001; Leverentz et al., 2004).

Phages adsorb to specific receptor sites on the bacterial cell wall. In gram-negative bacteria the receptors have been identified as protein and lipopolysaccharide components of the outer membrane layer surrounding the peptidoglycan. A particular phage or group of phages will adsorb to a specific site,
and different phages adsorb to different sites. Thus, on the surface of a given bacterial cell a variety of receptors are present, each type being present in large numbers (Chakrabarti et al., 2000).

The effect of sugars on the viability of phage \( \Phi MV-5 \) was studied using several sugars. All the sugars were found to exhibit a very high rate of inhibition on the phage ranging between 80 to 100%. 100% phage viability was lost with glucoseamine. This actually points towards the role of sugars in the process of adsorption and infection of \( \Phi MV-5 \). These sugars may be acting as a part of the receptors which help in the process of their infection into the host cell.

Significant inhibition of phage was observed when it was incubated with sugars. Most of the sugars tested are known to be a part of bacterial cell wall. This fact suggests that these may be an essential component of phage receptor structures or that their conformation is recognized by phages. For \textit{Lactobacillus casei} phages, it was reported that the receptor was the rhamnose of the cell wall polysaccharide (Watanabe et al., 1993).

There was 10% adsorption of phage \( \Phi MV-5 \) to its host at 0.01M NaCl. This was followed by 65%, 60%, 61% and 60% for NaCl concentrations from 0.25 to 1M NaCl.

In some lysogenic systems the spontaneous rate of phage production cannot be modified. In other systems production of phage can be induced at will by the action of some physical and chemical agents. Inducing agents do not unmask a masked phage particle. Lysogeny is perpetuated in the form of prophage. By definition, as long as the prophage remains prophage, it is unable to develop into phage. Therefore in order that a lysogenic bacterium may be able to produce phage, the prophage must lose its nature of prophage. Inducing agents seem to act
in altering the chromosome-prophage equilibrium. This could be achieved by an alteration of the chromosome, an alteration of the prophage, or a modification of the environment of the chromosome-prophage system (Lwoff, 1953). The effect of inducing agents would be to increase the probability of a change of the bacterial chromosome, the change being responsible for the detachment of the prophage.

The effect of a DNA damaging agent, Mitomycin C could induce the phage \( \Phi MV-5 \) and the titer was found to be \( \sim 250\% \) higher after induction. Typically, chemical means or UV C radiation (UV-C) is used to damage DNA or inhibit DNA replication; this activates DNA repair mechanisms, including the RecA protein which cleaves a repressor and induces prophages to enter the lytic cycle. One of the most effective and widely used inducing agents is mitomycin C (Ackermann and DuBow, 1987). It has been used by a number of investigators to examine lysogeny in marine viral communities and isolates (Jiang and Paul, 1994; Jiang and Paul, 1995; Tapper and Hicks, 1994). Although some prophages cannot be induced by mitomycin C or UV-C (Calendar, 1970), there is no evidence that such lysogenic associations occur in high relative abundance in the sea or elsewhere. There are several reports which confirms the inducing capacity of Mitomycin C. The increased titer of phages may be because all lysogenic prophages are induced to become lytic. (Jiang and Paul, 1995; Oakey and Owens, 2000; Tapper and Hicks, 1994; Weinbauer and Suttle, 1996) Hence, in the absence of data which indicate otherwise, it is reasonable to assume that mitomycin C and UV-C are also effective inducing agents for marine lysogens.

Broth clearance experiment was used to confirm the nature of the phage. It was observed that no significant lysis occurred in the culture. Active phages could be isolated from the broth upon filtration. This indicates that the phage is filamentous, and therefore may not cause complete lysis of the bacterial host. The filamentous nature of \( \Phi MV-5 \) was also confirmed by TEM. Considering the
growth parameters obtained from the one-step growth curves, it was noteworthy that latent and burst times obtained were shorter than those determined for the other systems studied. This fact also adds to the lack of clearing of the broth.

The host specificity of phage ΦMV-5 involved using 225 strains of environmental Vibrios and 7 clinical strains. Phage ΦMV-5 showed specificity to only 22 environmental strains and none at all towards the clinical isolates. Phage ΦMV-5 could effectively exhibit the lysogenic and lytic cycle characteristic of filamentous phages in the environmental isolates of Vibrio cholerae, V. furnissii, V. harveyi, V. alginolyticus, V. parahaemolyticus etc. This suggested that the phage has a broad host range within the Vibrio group.

Experimental evidence of phages exerting a strong selective pressure on microbial populations comes from host-range analysis of phage isolates and the observation that very closely related bacterial species and even strains of the same species are infected by different phages (Moebus, 1991; Suttle and Chan, 1993; Waterbury and Valois, 1993). Of the many vibriophages described, phage KVP40 differed in having a broad host range; it has been reported to infect eight Vibrio species, including Vibrio cholerae and Vibrio parahaemolyticus, the non-pathogenic species Vibrio natriegens, and Photobacterium leiognathi (Matsuzaki et al., 1992). The presence of alternative hosts is a mechanism to tide over unfavourable seasons. Similar results are obtained for a number of marine phages. Seasonal shifts in patterns of host range of phages during winter and the persistence of these viruses in oysters during winter supported by hosts other than V. parahaemolyticus was reported by Comeau et al (2005). These results imply that there can be strong coupling between phage and host populations that occur in different environments and that a broad host range can explain the occurrence of phage populations even during periods when their hosts are apparently absent.
Broad-host-range (polyvalent) phages are common among marine temperate phages. Indeed, some observations suggest that phages isolated from nutrient-poor marine environments showed a trend towards increased polyvalency, possibly representing an adaptation to low host cell concentrations (Chibani-Chennoufi et al., 2004). The polyvalent phages infecting different genera in the Enterobacteriaceae must be regarded with some caution because this family is such a closely related bacterial group. Even data on marine phages indicate that most of them are host species specific, many even show strain specificity. Polyvalence was more prevalent in cyanophages, but fluorescence-labeled cyanophages attached specifically only to their host and not other bacteria of the natural consortium (Hennes et al., 1995). Data from the ocean showed that polyvalency was correlated with phage morphology. Phages isolated from high-light-adapted Prochlorococcus hosts yielded exclusively Podoviridae that were strain specific (Sullivan et al., 2003). In contrast, low-light-adapted Prochlorococcus hosts yielded Myoviridae that also infected Synechococcus spp., a phylogenetically related cyanobacterium. Similarly, Synechococcus-infecting Myoviridae also cross-infected Prochlorococcus spp., lending some support to the polyvalency concept in the marine environment. Also, in other environments, Myoviridae showed a broader host range than Siphoviridae and Podoviridae (Chibani-Chennoufi et al., 2004).

A detailed understanding of the biology of vibriophages represents an alternative to study the genetics of these species, as phage genomes can be exploited to develop genetic tools for biotechnological applications. Furthermore, knowledge on environmental factors that influence their binding to sensitive cells is very important to develop strategies for their industrial applications and use in phage therapy.
Chapter 5

The phage ΦMV-5 DNA stained green with acridine orange, suggesting the double stranded nature of its DNA, as against orange for single strandedness. This may turn out to be a unique report since all the filamentous phages of Vibrios are known to be single stranded DNA molecule. ΦMV-5 DNA was not digested by the restriction enzymes Eco RI, Bam HI, Bgl II, Hind III, Not I, Pst I and Sau 3A I and therefore may be resistant to all these enzymes. It is observed that most of the vibriophages are resistant to restriction enzymes (Sen and Ghosh, 2005). Similar results of resistance were also observed for phage ε5 (Basu et al., 1993). The nucleic acid type and gross morphology are the most important properties for phage description and classification and less emphasis should be placed on molecular weight and restriction endonuclease patterns (Ackermann and DuBow, 1987). Other reports have shown that none of the phage DNAs isolated could be digested by BamHI and most were found to be resistant to restriction digestion by XbaI, KpnI, SalI, PstI, SacI, and Smal, but the reason for this resistance was not yet determined (Jiang et al., 1998).

One reason for this resistance may be that phages often modify their genomic DNA to avoid host restriction systems or to target their DNA for activity by specialized phage-encoded enzymes. V. parahaemolyticus Φ TB16T and Φ TB16C could not be cloned using standard approaches (e.g. enzymatic digestion and cloning) (Rohwer et al., 2001).

In order to study the relationship of the major coat protein of phage ΦMV-5 with other phages, a set of primers were designed and a small portion of the mcp gene was amplified, sequenced and compared with other reported phages. The BLAST results, showed that phage ΦMV-5 gave a hit to five reported Vibriophages VpV262, VP2, VP5, Φ JL001 and KVP40. It was not possible to compare with V. vulnificus phage sequences and other filamentous phage mcps.
since reports on gene sequences of these organisms are currently not available on
the NCBI database.

Mcps of morphologically similar phages of vibrios and enterobacteria have
been fairly conserved during the course of evolution of the phages and their host
bacteria (Matsuzaki et al., 1998). Conservation of Mcps was also recognized by
(Monod et al., 1997) in classical T-even and certain “pseudo-T-even” coliphages.

A major goal of phage genomic sequencing projects should be to provide
the information necessary to classify marine phage into guilds that reflect their
biology (Paul et al., 2002). Current phage taxonomy relies on the morphological
characteristics of the free phage particle as established by the International
Committee on Taxonomy of Viruses (ICTV) (Murphy et al., 1995). The ICTV
classification, however, provides very little information about the ecological niches
or lifestyles of phage. Additionally, the ICTV system does not have sufficient
resolution to address phage biodiversity questions, nor will it be useful for
analyzing uncultured marine phage or prophage genomes. In response to these
shortcomings, numerous groups are actively constructing phage taxonomical
systems based on completed genomic sequences (Lawrence et al., 2002; Rohwer
and Edwards, 2002). These systems will help classify marine phages into families
that provide information about their lifecycles and ecological roles, as well as
identify phage types that deserve more detailed analyses.

Both the phage ΦMV-5 DNA and its host DNA were screened for the
presence of virulence genes using six primers including tcpA, toxR, ace, zot, ctxA
and sxt employing PCR. Toxin co-regulated pili are the sites of attachment for the
temperate phage, CTXΦ in V. cholerae. Its expression is co-regulated by the
expression of cholera toxin genes ctxA and ctxB (Mukhopadhyay et al., 2001).
Whole pili structure are coded by many genes and tcpA codes for the A subunit of
a cluster of proteins making toxin-co-regulated pili. This gene is directly responsible for virulence in *Vibrio cholerae*. The presence of this gene was surprising in the phage $\Phi$MV-5 since the host was not *V. cholerae*. The indication of this gene in the host *Vibrio* sp. MV-5 may be because of the lysogenisation of the phage DNA.

There was also positive amplification for *tcpA* and *zot* in the phage $\Phi$MV-5 genome and in the host genome. These toxin genes are all key virulence factors of *Vibrio cholerae*. Other significant virulence factors like the *ace* and *ctx A* did not yield any amplification in the host indicating their absence. *ace* was amplified in the phage $\Phi$MV-5.

*toxR* code for a transmembrane transcription controlling protein. Expression of more than seventeen virulence genes is under the co-ordinate control of the ToxR protein. ToxR is a transmembrane protein and it binds to and activates the promoter of the operon encoding cholera toxin. ToxR controls transcription of toxT, whose product in turn is directly responsible for activation of several virulence genes under ToxR control (Victor *et al.*, 1991). Many environmental signals act via the ToxR system. The presence of ToxR was also revealed by the PCR amplification in both phage $\Phi$MV-5 and host system.

*Sxt*, an integrative conjugative element of around 100kb size, was originally reported from *V. cholerae* (Waldor and Mekalanos, 1996). The SXT element has an integrase gene and many other genes of unknown function (John *et al.*, 2002). SXT integrase, *int* from *V. cholerae* is about 1200bp long. This was the gene that was targeted in order to check for the presence of SXT element in the host and the phage. Both the phage $\Phi$MV-5 as well as its host were shown to reveal the presence of *sxt*. 

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Within the last few years, there has been a major increase in the genetic analysis of *V. cholerae* and its phages. Much of this effort has been associated with the acceptance of the need for a live oral vaccine against cholera (Guidolin and Manning, 1987). The importance of other vibriophages also is gaining more attention.

The phage ΦMV-5 was found to have broad host range infecting 7 different species of *Vibrios* including *Vibrio cholerae*, *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyi*, *V. vulnificus*, and *V. furnissi* which do not harbor *tcpA*, *toxR*, *ace*, *ctxA*, *zot* and *sxt* except *V. cholerae* which harbored *toxR* and *sxt*. The specific host, *V. vulnificus* MV-5, of phage ΦMV-5 showed the presence of *tcpA*, *toxR*, *zot* and *sxt*. The presence of these genes in the host may be because of the lysogenic association with the phage. Since the phage can lysogenize a number of species within the *Vibrio* group, it may act as a potential source of horizontal gene transfer among these environmental bacterial species and may lead to their evolution into potential pathogens.