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Review Article

Bacterial viruses in marine environment and their ecological role and bioprospecting potential: a review

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A B S T R A C T

Bacterial viruses have great potential for their applications especially in phage therapy, nanotherapy, phage display, food decontamination, surface disinfection and bio-detection. However, marine forms are least understood. It is also important to improve the methods of production of marine bacteriophages in large-scale level, and their purification and recovery processes. Advanced studies on the engineering of marine phage production and downstream purification processes are almost non-existent. Marine bacterial viruses are among the greatest genetic resources on the biosphere and they deserve a special attention for their bioprospecting potential and ecological role in marine environment.

Introduction

Viruses are the sub-microscopic forms of life, attacking all organisms on the earth. The viruses that specifically affect bacteria are termed as bacterial viruses or bacteriophages or bacteria-eaters (Topley and Wilson, 1929). The bacteriophages were first identified by Frederick Twort (1915) and Felix d'Herelle (1917) (Duckworth, 1976). They are unique to have bacterial host-specificity. Viruses of unknown hosts are termed as virus-like particles (VLPs) which are the most abundant constituents of all the aquatic ecosystems including the ocean (Fuhrman, 1999). Since the oceans are the world's largest biosphere, marine viruses are the most abundant biological entities on the planet. The estimated overall abundance of marine viruses in the world's oceans is on the order of 10^{30} (Suttle, 2005; 2007), a value that exceeds by ten times of the abundance of prokaryotes (Suttle, 2005). Marine viruses store 200 million tons ($2x10^{11}$/g) of total carbon equivalent of 75 million blue whales. If all the marine virus particles are placed end to end they will span about 10 million light years (100 times the distance across) our own galaxy (Suttle, 2005). However, marine viruses in general and bacterial viruses in particular have been little studied. Hence, this review describes the current knowledge about the marine bacterial viruses for their ecological significance and

biotechnological prospects and it also highlights some strengths and limitations of the methods commonly used for viral analysis.

Research on bacterial viruses in marine environment

Bacterial viruses or bacteriophages in seawater were first observed in the first half of the last century (Kriss and Rukina, 1947). In 1979, Marine viral particles were discovered to be abundant and morphologically similar to phage (Torrella and Morita, 1979), and the phage from marine bacteria were soon cultured (Moebus, 1980). In the 1990s, genetic diversity of the marine phage and eukaryotic viruses and their importance in the ecology of the marine plankton community were known. Further studies demonstrated the contribution of viruses and protists to the global biogeochemical cycling arising from the lysis of plankton (Fuhrman and Noble, 1995; Gobler et al., 1997). The first marine viral genomes were sequenced (Rohwer etal., 2000) and subsequently genomics and metagenomics were studied to characterize the diversity of both RNA viruses (Culley et al., 2007) and DNA viruses (Comeau et al., 2006) in seawater, along with their effects on host physiology and ecology (Paul et al., 2005). The milestones in the findings of marine virology are depicted in table 1.

Occurrence and distribution of bacterial viruses

The tailed bacteriophages appear to dominate marine ecosystems in number and diversity. In particular phages with contractile tails (such as myoviruses and T4-like viruses) or long flexible tails (such as siphoviruses and lambda-like viruses) are predominant (Sullivan et al., 2003). The average virus is about one-hundredth the size of the average bacterium. Most viruses that have been studied have a diameter between 10 and 300 nanometres (Mann, 2005). Although viruses from the marine environment have been isolated and enumerated, there is little information on the abundance or global distribution of specific phage types (Zachary, 1974). The frequent isolation of bacteriophages in marine sediments against many different bacterial genera reflects the complex and extensive nature of the microbial flora in the marine environment (Stevenson and Albright, 1972).

Viral abundance, also reported as number of virus-like particles and it typically ranges from 10^5 to 10^8 particles per milliliter of surface waters of the marine environment (Danovaro et al., 2003). The viral abundances in surface sediments at all depths down to abyssal sediments exceed those in the water column reaching values of 10^8–10^9 viral particles per litre (Siem- Jørgensen et al., 2008). Moreover, high viral abundances have also been reported in subsurface sediment (Bird *et al*., 2001).

The highest VLP abundances are present in tropical seawater near mangrove forests (2.2×10^6– 1.2×10^7 VLPs ml^{-1}) and in samples collected during the rainy season, whereas abundances are low above oceanic reefs (1.5 to 4.3×10^6 VLPs ml^{-1}). The viral communities are abundant in mangrove habitats as compared to other biotopes for the reason that the mangroves are dynamic detritus-based systems, rich in both prokaryotic and eukaryotic organisms in particular microorganisms. This vast abundance of microorganisms in the mangrove ecosystem requires a strong viral community to control the bacterial population and functions within the

Table.1 Milestone findings in marine virological research

Year	Mile stones in marine virology	Reference
1946	Marine viruses discovered	Zobell (1946); Spencer (1955)
1947	Bacteriophages discovered in sea	Kriss and Rukina (1947)
1979	Viruses detected by flow cytometry	Hercher *et al*., (1979)
1980	Marine phage culture	Moebus (1980)
1986	Abundance of viral particles in seawater	Torrella and Morita (1979)
1990	Viral role to biogeochemical cycling	Weinbauer *et al*., (2002)
1990	Viral decay rates measured	Heldel and Bratbak (1991)
1995	Role of virus and protists in plankton mortality	Wommack *et al*., (1999); Gobler *et al*., (1997)
1998	SYBR Green method for marine viral counts	Noble and Fuhrman (1998)
1999	First marine virus sequenced (PM2 Phage genome)	Mannisto *et al*., (1999)
2000	*Roseobacter* SIO1 genome sequenced	Rohwer *et al*., (2000)
2000	PFGE used to compare viral consortia	Steward (2001)
2002	First marine viral genome sequenced	Rohwer *et al*., (2000)
2003	Photosynthetic genes found in cyanophages	Millard *et al*., (2004); Sullivan *et al*., (2005)
2004	High abundance of temperate phages in seawater	Chen *et al*., (2006)
2005	First molecular characterization of temperate phages	Goh *et al*., (2005)
2007	Global marine virus explored	Breitbart *et al.,* (2007)
2006	DNA virome sequenced	Comeau *et al.,* (2006)
2007	RNA virome sequenced	Culley *et al.,* (2007)

Table.2 The distribution and abundance of marine viral particles per litre

Location	Viral abundance (virus particles/L)	Reference
Long Island Sound	1×10^{11}	Proctor and Fuhrman (1990)
Caribbean Sea	$1.9\text{-}4.8 \times 10^{9}$	Proctor and Fuhrman (1990)
Chesapeake Bay	$2.6\text{-}14 \times 10^{9}$	Wommack *et al*., (1992)
Southern California Bight	$0.3\text{-}52 \times 10^{9}$	Cochlan *et al*., (1993)
Norwegian coast	$4\text{-}9 \times 10^{10}$	Bratbak *et al*., (1996)
Bermuda	$4.2\text{-}5 \times 10^{8}$	Jiang and Paul (1996)
Bering and Chukchi Seas	$2.5\text{-}35 \times 10^{9}$	Steward *et al*., (1996)
Western Gulf of Mexico		
Offshore	$3\text{-}4 \times 10^{8}$	Weinbauer and Suttle (1997)
Coastal	$1.5\text{-}28.3 \times 10^{10}$	Weinbauer and Suttle (1997)
Santa Monica Bay	1×10^{10}	Noble and Fuhrman (1997)
San Diego, California	2×10^{9}	Sano *et al.,* (2004)
Lake Geneva	$1.5\text{-}3 \times 10^{10}$	Duhamel and Jacquet (2006)
Cochin Backwaters, India	3.9×10^{10}	Parvathi *et al*., (2011)
Zuary estuary, Goa, India	$1.0\text{-}2.6 \times 10^{10}$	Mitbavkar *et al.,* (2011)

ecosystem. However, the viral studies in mangrove ecosystems are extremely limited (Haq and Kathiresan, 2011). Distribution and abundance of marine viruses reported from different locations in the world are shown in table 2.

Ecological role of bacterial viruses

In the sea, abundant occurrence of viruses plays significant roles to: (1) control the microbial species diversity, (2) exchange the genetic material among marine bacteria following viral attack, (3) help in the process of marine food chain, and to (4) disseminate the toxins by killing bacteria, such as cholera toxin. This leads to outbreak of cholera disease in human and it has close correlation with sea surface temperature brought out by global warming (Proctor and Fuhrman, 1990).

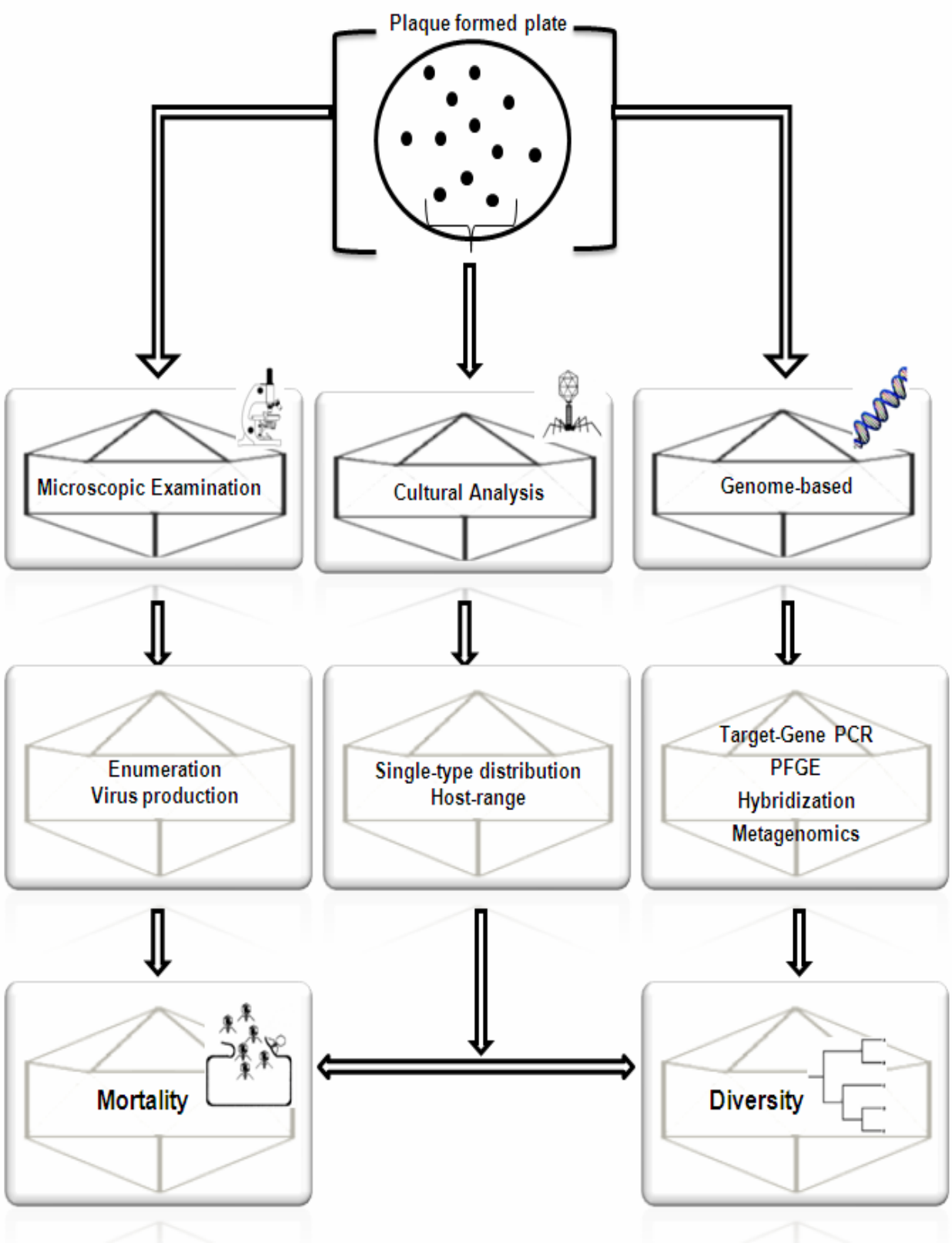
Viral lysis may also be a mechanism of controlling the bacterial community composition (Wommack *et al*., 1999). Viral infection may cause about 60% in heterotrophic marine bacteria in coastal and offshore environments (Proctor and Fuhrman, 1990). Thus the role of viruses into the microbial food web has refined our understanding of the ecological and biogeochemical role of microorganisms in the ocean (Weinbauer *et al*., 2002). They appear to influence biogeochemical cycles globally, provide and regulate microbial biodiversity, carbon cycle through marine food webs, and are essential in preventing bacterial over-populations (Waldor *et al.*, 2005). The bacterial viruses have the remarkable ability to manipulate the life histories and evolution of their bacterial hosts in the long past. In the evolution, viruses are an important means of horizontal gene transfer, which increases genetic diversity (Haq and Kathiresan, 2011).

Marine viruses may play an important role in the carbon cycle by increasing the efficiency of the biological pump which is diagrammatically represented in fig. 1.

Bacteriophages are important components of oceanic food webs principally because of their ability to kill bacteria, thereby releasing dissolved organic matter, nutrient recycling (Middelboe *et al*., 1996), and the pathways of organic carbon utilization, with cascade effects on marine microbial food webs and organic matter cycling (Fuhrman and Noble, 1995). The bacterial viruses release from bacterial host the unstable compounds, such as amino acids and nucleic acids, which are recycled, near the surface, whereas more indigestible carbon-rich material is exported to deeper waters. Thus, the material that is exported to deeper waters by the 'viral shunt' is highly carbon rich as compared to the material from which it is derived. This will increase the efficiency of the biological pump (Suttle, 2007). About one-quarter of the organic carbon in the sea flows through the viral shunt (Wilhelm and Suttle, 1999). Heterotrophic bacteria represent 40-70% of the living carbon in the photic zone of surface waters (Fuhrman *et al.,* 1989). If deeper waters are included, heterotrophic bacteria become even more significant contributors to overall biomass.

Virus makes the flow of carbon and nutrients from secondary consumers (upward-black arrows) by destroying their host bacterial cells, which release their contents into the pool of dissolved organic matter (DOM) in the marine environment (grey arrows). DOM is used as a nutrient source by bacteria, plankton and other primary producers present in the aquatic environment thereby transferring them into the food web. Secondary production

Figure.1 General Methods used to analyze marine phages webs



in many aquatic regions may exceed primary production (Sorokin, 1971); this imbalance is due in part to the recycling of carbon through the "microbial loop" (Azam *et al*., 1983). This process results in reuse of carbon derived from photosynthesis several times as it passes through the food web (Cole *et al.,* 1982).

Marine bacteriophage constitutes an important part of deep sea. They range between $5x10^{12}$ and $1x10^{13}$ phage per square metre in deep sea sediments and their abundance closely correlates with the number of host prokaryotes present in the sediments. They are responsible for the death of 80% of the prokaryotes found in the sediments, and almost all of these deaths are caused by cell lysis. The bacterial phages therefore, play an important part in shifting nutrients from living forms into dissolved organic matter and detritus (Danovaro et al., 2008; Wommack and Colwell, 2000).

Isolation and characterization of bacterial viruses

Zobell (1946) has isolated phages from seawater of the littoral zone, but not beyond the zone. The phages isolated by Kriss and Rukina (1947) from the Black seawater and mud samples are found active against terrestrial bacterial species such as *Bacillus subtilis* and *Micrococcus albus,* whereas the phages isolated by Smith and Krueger (1954) against a marine vibrio from marine mud in San Francisco is not strictly of marine origin. In the earlier studies phages isolated from marine environment against different bacterial species are tabulated in table 3.

Detection and enumeration of bacterial viruses

The available methods for the determination of virus abundance in aquatic environments include counting by transmission electron microscopy (TEM) (Paul *et al.,* 1993), flow cytometry (Marie *et al*., 1999) and by epifluorescence microscopy (EFM) (Drake *et al*., 1998). EFM is reported to be up to seven times more efficient than TEM for counting viruses (Weinbauer and Suttle, 1997). The EFM also allows an accurate and easily performed enumeration, avoiding the use of expensive and bulky equipment (Fuhrman, 1999). The methods that are commonly used for detecting phages are given in table 4. The general methods of analyzing bacteriophages are depicted in fig. 2.

Potential applications of marine bacterial viruses

Interest in bacterial viruses is increasing due to their applications in phage therapy (Housby and Mann, 2009), detection and diagnostics (Shen *et al*., 2009), bacterial infection treatment (Wall *et al*., 2010) and recombinant protein production (Oh *et al.*, 2007). The bacterial viruses have been identified as important tools in many aspects of nano-medicine (Villaverde, 2010).

However, most of these works are confined to bacterial viruses of terrestrial origin. Marine bacteriophages have received only little attention. There is a possibility for exploring the potential of marine cyanophages to be used to prevent or reverse eutrophication. Kurtboke (2005) have developed an improved technique that involves the exploitation of marine actinophages as a tool to reduce the numbers of common marine bacteria, which impedes the growth of rare actinomycetes on isolation plates.

Table.3 Isolation of marine bacteriophages from different marine biotope

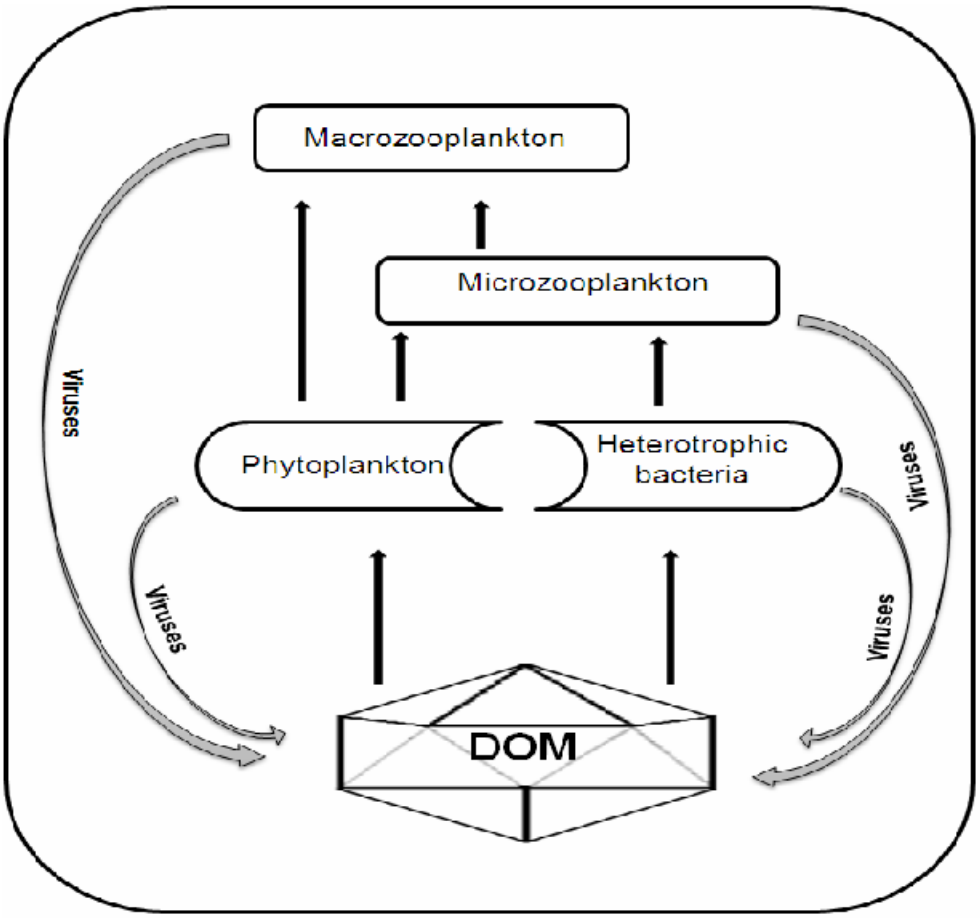
Sl. No.	Phages	Host	Source of bacteriophages	Available information	Reference
1	Cyanophages	*Synechococcus* sp.,	Coastal waters from Bermuda, Mass and United Kingdom	Myovridae and Styloviridae	Wilson *et al.*, (1993)
2	Marine phages	*Vibrio parahaernolyticus*	Tampa Bay (Florida. USA),	Isolates are all Myoviridae	Kellogg *et al*., (1995)
3	Lysogens or Bacteriocinogens	*Vibrio* spp., *Vibrio harveyi*	Marine environment	Lysogenic Phage production	Jiang and Paul (1998)
4	Myovirus-like (VHML) Temperate phages	*Vibrio harveyi*	Moribund prawn larvae in tropical Australia	Concentration of phage particle and nucleic acid extraction were efficient	Oakey and Owens (2000)
5	Siphoviridae-like phage (VHS1) Temperate Bacteriophages	*Vibrio harveyi*	Shrimp culture ponds in Thailand	Belong to Siphoviridae family	Park *et al*., (2000)
6	Marine actinophages	Actinomycetes	Mangrove muds and sediments	selective isolation and taxonomy of rare actinomycetes	Kurtboke (2005)
7	MS2 Coliphage	*E.coli* C-3000	Sea foods	Used PEG 8000 precipitation method	Venkatesan *et al*., (2008)

Table.4 Summary of direct method of analyzing viral-like particles derived from different locations.

Approach(es)	Sampled locations	References
Direct counts, TEM	Key Largo, FL, USA	Paul *et al.,* (1993)
EFM and TEM	Pier of Marine Science Institute, Port Aransas, Texas, USA	Weinbauer and Suttle (1997)
Direct counts (AODC), TEM, EFM	Santa Monica Pier, CA, USA, and Denmark	Noble and Fuhrman (1998)
Digital Image Analysis and FCM	Georgia Coastal Rivers	Chen *et al*., (2001)
PCR marker gene analysis	Florida Keys, FL, USA	Lipp *et al.*, (2002)
PCR marker gene analysis	Hawaii, USA	Culley and Steward (2007)
Direct counts, viral metagenomics	Line Islands, Kingdom of Kirabati	Dinsdale *et al.,* (2008)
RAPD-PCR	Chesapeake bay	Helton and Wommack (2009)
RT-PCR	Tianjin coast, Bohai Bay, China	Zhang *et al*., (2010)
FCM	Zuary estuary, Goa, India	Mitbavkar *et al*., (2011)
FCM	Indian oil Corporation Ltd Jetty, Cochin, India	Parvathi *et al*., (2011)

(EFM=Epifluorescence Microscope; AODC=Acridine Orange Direct Counts; TEM=Transmission Electron Microscope; FCM=Flow Cytometry; RT-PCR=Reverse Transcriptase Polymerase Chain Reaction)

Figure.2 The role of virus in marine food



Phage therapy is the recent development in the field of phage research due primarily to the increasing incidence of antibiotic-resistant bacteria and the lack of development of new types of antibiotics to control infections caused by these antibiotic-resistant organisms (Cerveny *et al.*, 2002). The therapeutic uses of phages in humans have been recently reviewed by Alisky *et al.*, (1998); the overall reported success rate for phage therapy is found to be in the range of 80-95%. Phage therapy has been applied to a variety of infections like bacterial dysentery, wound infections, gastrointestinal tract infections, infections of skin nasal mucosa and gastrointestinal tract infections (Mathur *et al*., 2003).

In nanomedicines, viral nanoparticles (VNPs) are particularly valuable because they are not only biocompatible but also biodegradable, and also they are non-infectious and non- hazardous to humans and other mammals (Kaiser *et al*., 2007). The basic VNP structure is without nucleic acid but with only protein coat and this can be 'programmed' in a number of ways so that the internal cavity can be filled with drug molecules, imaging reagents, quantum dots and other nanoparticles, whereas the external surface can be attached with targeting ligands to allow cell-specific delivery of drugs (Pokorski and Steinmetz, 2011).

The potential of bacteriophages to control infectious diseases in fishes is known (Vinod *et al*., 2006). Karunasagar *et al*., (2007) have isolated lytic bacteriophages against *V. harveyi* and proved that the bacteriophage treatment at 2×10^6 pfu ml^{-1} level results in over 85% survival of *Penaeus monodon* larvae suggesting that bacteriophage therapy will be an effective alternative to antibiotics in shrimp hatcheries since there is a ban on use of most antibiotics in aquaculture.

Phage display is a very powerful technique for obtaining libraries containing millions or even billions of different peptides or proteins. Phage display (Smith, 1985) has been used for affinity screening of combinatorial peptide libraries to identify ligands for peptide receptors, define epitopes for monoclonal antibodies, select enzyme substrates (Kay *et al.*, 1996), and screen cloned antibody repertoires (Griffiths and Duncan, 1998).

Concluding remarks

Bacterial virus in particular marine forms have a great potential for their applications especially in phage therapy, nanotherapy, phage display, food decontamination, surface disinfection and bio-detection. However, it is a matter of importance to improve the methods of production of marine bacteriophages in large-scale level, and their purification and recovery processes. Advanced studies on the engineering of marine phage production and downstream purification processes are almost non-existent. Marine bacterial viruses deserve a special attention for their bioprospecting potential and ecological role in marine environment.

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