

EFFECT OF ULTRAVIOLET LIGHT AND POLLUTANTS ON SURVIVAL OF BACTERIOPHAGE ISOLATES FROM ENVIRONMENTAL SAMPLES

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ABSTRACT

Twenty bacteriophages were isolated from Pavana river water and sewage samples in Pune. Out of all the isolates two bacteriophages were characterized and identified as coliphage (Leviviridae) and staphylophage (Podoviridae). The effect of ultraviolet light and environmental pollutants (detergents and heavy metals) was evaluated on survival of them. The results indicated that, coliphage was more resistant to ultraviolet light than that of staphylophage. Besides, the results obtained from effect of detergents on survival of isolates indicated that coliphage was relatively more resistant against all the detergents other than SDS, however, response of the isolates against SDS was identical. In addition, response of the isolates against heavy metals indicated that MPIC value of Cu was relatively high. While, MPICs of Cd and Hg were relatively low and the MPIC value of Zn for coliphages was high than for staphylophage. Therefore, based on foregoing evidence the present study interpreted that probably coliphage is relatively more resistant to radiation and environmental pollutants than staphylophage, which is related to their genome and structures.

INTRODUCTION

Bacteriophage is a virus that infects bacteria, which can either instantly kill a bacterial cell or integrate its DNA into the host bacterial chromosome (Madigan et al. 1997). Nowadays, use of bacteriophages is quite common for different purposes such as an indicator (Hilton & Stozky 1973, Grabow et al. 1987, Kennedy et al. 1985, Borrego et al. 1987 and Morinigo et al. 1992), phage typing (Sakamoto et al. 1975, O'Brien et al. 1999, O'Neill et al. 2001, Zadoks et al. 2002, Gustafson et al. 2003), food preservatives and decontaminants (Greer 1986, Greer & Dilts 1990, 2002, Leverentz et al. 2001, 2003), transducer (Zinder & Lederberg 1952, Campbell 1976, Schicklmaier & Schmieger 1995), and therapeutic agents (Bruynoghe & Maisin 1921, d' Herelle 1926, Soothill 1994, Levin & Bull 1996, Weber-Dabrowska et al. 2000, Ahmad 2002). However, some scientists believe that bacteriophages could be considered as faecal indicators instead of bacterial indicators but we believe that irradiation and environmental pollutants could affect survival of these indicators. Hence, the present study was conducted to evaluate effect of irradiation and environmental pollutants on survival of bacteriophage isolates from environmental samples.

MATERIALS AND METHODS

Sampling sites and sampling: One hundred and five river water samples were collected from eight sampling sites along the river Pavana. Thirty-five and forty sewage samples were collected from Pashan village and Shivaji market (Camp area) respectively. The methods for isolation of bacteriophages from the sources were overlay technique (Adam 1959) and modified enrichment technique (Cappuccino & Sherman 1999). Twenty bacteriophages were isolated from different sources, and out

of that two were characterized (Ackermann 1992).

Preparation of lysate: It was prepared by adding 0.1 mL of *E. coli* phage (Ecp) and *S. aureus* phage (Sap) respectively to 0.1 mL (10^6 /mL) overnight growth of *E. coli* and *S. aureus*. Then 0.1 mL of each mixture was spread onto the surface of nutrient agar plates. The plates were incubated at 37°C and 27°C respectively for Ecp and Sap (optimum temperature). After 24hrs the plates were checked for confluent lysis. The surface of each plate with confluent lysis was flooded with 5 mL nutrient broth and kept at room temperature for 30 min. The lysate from each plate was harvested by sterile glass rod and collected into sterile tube. The tubes were centrifuged at 3000 rpm for 10 min. The supernatant was passed through a sterile membrane filter of pore size 0.45µm (Sartorius A. G. Goethingen, Germany). The filtrate was used as lysate.

Effect of UV on survival of bacteriophage isolates: One mL supernatant of each phage was diluted in phosphate buffer saline (PBS) (0.1 M, pH 7.4) to give 10^6 pfu/mL (Iosson 1982). The phage suspensions (10 mL) were transferred into sterile glass petri dishes, and exposed to UV at varied time intervals (0, 20, 40, 60, 90, 120, 150, 180 sec). At each time interval, 0.1 mL of the irradiated phage suspension was withdrawn and diluted in PBS to 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} dilutions. Then 0.1 mL of each dilution was mixed with 0.1 mL of the target bacterium (1.5×10^8 cfu/mL) and plated. The plates were incubated at temperatures, 37°C and 27°C for Ecp and Sap respectively. The plates were checked for formation of plaques at time intervals of 6, 12, 18 and 24hrs. The plaques from each plate were recorded and the plaque forming units per mL at each UV dose were determined.

Effect of detergents on survival of phages: In the present study MIC values of the detergents and metals against the target bacterium were determined and then different concentrations of detergents and metals were prepared below the MIC values of detergents and metals against the target bacterium. Hence, minimal phage inactivating concentration (MPIC) of detergents was determined by adding, 1mL of different concentrations of detergents to 1mL phage lysate (10^6 pfu/mL Ecp and Sap). The mixtures were kept for 1 hour at room temperature. Overnight growth (0.1 mL) of each bacterial host was spread onto the surface of nutrient agar medium. Then 10 µl aliquot from each detergent dilution was withdrawn and dropped onto the surface of nutrient agar medium seeded with respective host. The plates were incubated at 37°C and 27°C for Ecp and Sap respectively. After 18 hrs MPIC of each detergent was determined as minimal concentration, which prevented plaque formation by bacteriophage. Sub- minimal phage inactivating concentration (SPIC) was the concentration below MPIC value, which was evaluated at one-hour contact period for each detergent. Plaque forming units were counted and recorded at each SPIC value.

Detergents used in this study were:

Anionic-SDS.

Noionic-Tween-80, Triton X 100.

Cationic- Cetrimide.

Effect of metals on survival of phages: Minimal phage inactivating concentration (MPIC) of each metal was determined by adding 1mL of respective concentration (0.1-0.001 M) of metal to 1mL of phage lysate (10^6 pfu/mL Ecp and Sap). The mixtures were kept for 1hour at room temperature. Then, 10µl of each dilution was withdrawn and dropped onto the surface of nutrient agar medium spread with overnight growth (0.1 mL) of respective bacterial host. The plates were incubated at 37°C and 27°C for Ecp and Sap respectively. After 18hrs MPIC of each metal was determined as minimal concentration, which prevented plaque formation by bacteriophage. Sub-minimal phage inactivating

concentration (SPIC) of each metal was the concentration below its MPIC value. Plaque forming units were determined at SPIC value of each metal.

RESULTS AND DISCUSSION

The results obtained from effect of UV light on survival of bacteriophage isolates are shown in Table 1. The data indicated that the survival of Ecp and Sap reduced with increase in UV exposure. In addition, these data showed that Ecp is relatively more resistant to UV light than Sap. It is because the survival of Sap is nil at 180 sec, while that of Ecp is 0.1%. The Fig 1. represents graphical representation of the data on effect of UV on survival of bacteriophage isolates, which is related to the irradiation time and surviving fraction (\log_{10} pf/p₀) of the phage population. The rate of inactivation of Sap is relatively more than that of Ecp, indicating its more susceptibility to UV light.

The results on MPIC of detergents against bacteriophage isolates are presented in Table 2. The response of Ecp and Sap to SDS was identical, however, Sap was relatively more sensitive than Ecp to the detergents tested. These results were supported by number of plaque forming units obtained at SPIC values of detergents. As seen in the Table 3 the percentage survival of Ecp was relatively more. The statistical analysis of the data ($F < 0.05$) confirmed that the percentage survival of Ecp was more than that of Sap. These results also indicated that effect of cetrimide was relatively high while that of

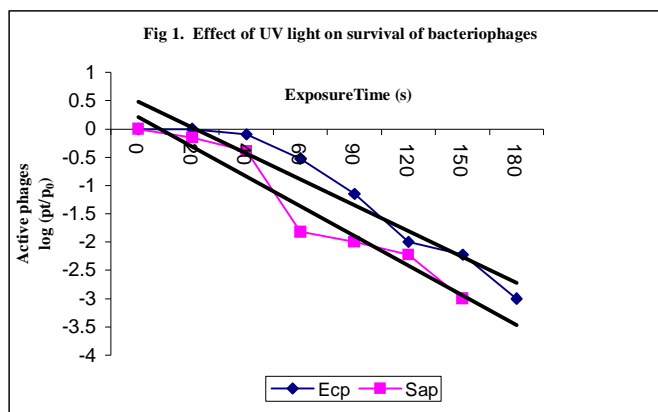


Table 1: Effect of ultraviolet light on survival of bacteriophage isolates.

UV exposure (s)	% Survival* of	
	Ecp	Sap
0	100	100
20	100	70
40	80	40
60	30	1.5
90	7	1
120	1	0.6
150	0.6	0.1
180	0.1	0

*pfu at 0 time exposure were 10⁵/mL

Table 2: MPIC* values of detergents against Ecp and Sap isolates.

Detergents	MPIC ($\mu\text{g/mL}$) against	
	Ecp	Sap
SDS	10^3	10^3
Triton $\times 100$	10^3	10^2
Cetrimide	7.7×10^2	7.7×10^1
Tween 80	1.08×10^5	1.08×10^3

MPIC*: Minimal phage inactivating concentration

Table 3: Effect of SPIC* values of detergents on survival of bacteriophage isolates.

Detergents	% Survival of	
	Ecp	Sap
SDS	0.84	0.70
Triton $\times 100$	0.80	0.07
Cetrimide	0.52	0.05
Tween 80	0.64	0.06

*SPIC: Sub-minimal phage inactivating concentration; The pfus without detergents, 5×10^5 /mL

Table 4: MPIC* values of metals against Ecp and Sap phage isolates.

Phage isolate	MPIC (mM) of			
	Cd	Cu	Hg	Zn
Ecp	1	10	1	10
Sap	1	10	1	1

MPIC*: Minimal phage inactivating concentration

Table 5: Effect of SPIC* values of metals on survival of bacteriophage isolates.

Metals	% Survival of	
	Ecp	Sap
Cd	0.09	0.08
Cu	0.15	0.12
Hg	0.06	0.06
Zn	0.14	0.07

*SPIC: Subminimal phage inactivating concentration; The pfus without metal, 5×10^5 /mL

SDS was relatively low on inactivation of viruses.

The results obtained from MPIC of heavy metals against bacteriophage isolates are shown in Table 4. As seen in this table MPIC of Cu was relatively high. While, MPICs of Cd and Hg were relatively low, the MPIC value of Zn for Ecp is higher than for Sap indicating high sensitivity of Sap.

The Table 5 represents effect of heavy metals (SPIC values) on percentage survival of bacteriophages. As seen in this table, the sensitivity of Ecp and Sap to Cu is almost same as indicated by their survival. However, the Sap is relatively more sensitive than Ecp to Zn as indicated by its significant reduction in survival. The percentage survival of Ecp and Sap was similar with Cd, Cu and Hg.

Ultraviolet light is one of the environmental factors, which was evaluated on survival of bacteriophage isolates. Lowff (1953) believed that starvation and solar UV light radiation have deleterious effect on bacteriophage replication and some environmental stresses will act to override the negative effects of starvation on bacteriophage replication. In addition, Iosson (1982) reported that prime effect of ultraviolet light of wavelength (254 nm) on suspension of bacteriophage was nucleic acid.

Besides, various pilot and full-scale studies suggest that UV is more effective for the inactivation of viruses than chlorine based disinfectants (Chen & Kuo 1993, Chester & Jacangelo 1993). In the present study, effect of UV light on survival of bacteriophage isolates was evaluated at different time intervals. The results obtained from this study indicated that coliphage (Ecp) was relatively more resistant to UV light. This can be due to type of nucleic acid of Ecp, which may be different from that of Sap. It is because the findings indicated presence of RNA in Ecp and DNA in case of Sap. Therefore, on the basis of mechanism of effect of UV light on living organisms it can be concluded that RNA should be resistant to UV light than DNA. It is obvious that, prime effect of UV light on nucleic acid is thymine dimer formation, which blocks normal replication of nucleic acids (Setlow et al. 1963), while thymine base is absent in RNA. In addition, Iosson (1982) believed that RNA phages, which are icosahedral are much less sensitive than filamentous DNA phages. Besides, MS2 phage, which is RNA phage, was proposed as an indicator of higher resistance to UV than the human enteric viruses (Wilson & Gerba 1992).

On the other hand, the results obtained from effect of detergents on survival of bacteriophage isolates indicated that effect of anionic detergents (SDS) on Ecp and Sap was similar, however, effect of non-ionic (Tween 80 and Triton X100) and cationic detergents (Cetrimide) on survival of Ecp and Sap was different. Jarrell & Kropinski (1976) reported that the titre of pseudophage at 37°C in the presence and absence of SDS and sodium deoxycholate was similar. On the contrary, our results indicated that all the detergents, which were used in the study, at different concentrations had effect on survival of the phage isolates. However, SDS had relatively less adverse effect and cetrimide had relatively high adverse effect on survival of bacteriophage isolates. In support of the data, we have to consider the mechanism of effect of detergents on living organisms. It is because the prime effect of detergents on living organisms is protein as well as microbial membrane (Prescott et al. 1999). Although, lipids have not been found as composition of the bacteriophage isolates, proteins were considered as main component of their structure. Therefore, it is obvious that detergents could affect proteins of the bacteriophage. The varied effects of the detergents on the bacteriophage isolates must be due to varied charges elicited on the bacteriophage proteins. It was because of the rate of charge neutralization of bacteriophage proteins resulted in adsorption of the phages to host and, therefore, it could affect survival of the phages.

In addition, effect of metals against bacteriophage isolates indicated that MPIC of Cu, Cd and Hg was similar, however, varied effects have been observed on survival of bacteriophage isolates of Zn. In general, pfu/mL of Ecp in presence of metals was more than Sap. Therefore, it can be concluded that Ecp is relatively more resistant to heavy metals.

For many years the ions of heavy metals such as mercury, silver, arsenic, zinc and copper were used as germicides. Heavy metals combine with proteins, often with their sulfhydryl groups, and inactivate them.

They may also precipitate cell proteins (Prescott et al. 1999). The findings of Mitchell (1971) suggest the role of heavy metals in virus inactivation in seawater. Besides, metallic ions have already been shown to be involved in phage multiplication (Andrews & Elford 1932, Wahl 1946, Rountree

1947, Dolby & Czekalowski 1949, Adams 1949). In addition, the results of Spizizen et al. (1951) indicated that sulphhydryl compounds inhibit multiplication of coliphage T2r+ by interfering in some way with a delicate ionic balance in the growth system. All salts have a certain amount of germicidal action depending on their concentration; salts of heavy metals have a greater action. The salts of silver, copper and mercury are used as disinfectants. They are protein coagulants and have the capacity to combine with free sulphhydryl groups of cell enzyme, when used at appropriate concentrations (Ananthanarayan & Paniker 1996). Hence, based on foregoing evidence heavy metals have antiviral properties. In general, although coliphage (Ecp) was relatively more resistant to ultraviolet light and environmental pollutants, the entrance of detergents and heavy metals in the aquatic environment could affect survival of viral indicator such as coliphage as well as staphylophage. To interpret these observations, the genome and structure of bacteriophages must be considered as a target in further studies.

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