Short Communication

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EFFECT OF DILUTION, TEMPERATURE AND pH ON THE LYSIS ACTIVITY OF T4 PHAGE AGAINST E.COLI BL21

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ABSTRACT

The effect of various "range" of dilution, temperature and pH on T4 bacteriophage lytic activity against *Escherichia coli* had been characterized. Results showed that T4 bacteriophage did lysis from 10^{-1} to 10^{-7} dilutions, while no lysis at dilutions 10^{-8} to 10^{-10}. The yield of T4 bacteriophage is highly dependent upon temperature. Low temperatures of 4°C did not permit T4 bacteriophage to perform lysis on host. While at 15°C, 25°C and 30°C there has been lysis but with little delay. Similarly at thermophilic temperature 41°C T4 bacteriophage developed and performed lysis on its host but at temperature regimes of 45°C, 55°C and 70°C, the T4 bacteriophage was completely inactive. The ideal temperature for T4 bacteriolytic activity was 37°C. pH of media was also found affecting virus survival indirectly by influencing extent of virus adsorption to other particles and surfaces and T4 bacteriophage was stable in pH range from 4 to 10. Our study demonstrates that apart from ideal values of respective parameters previously been demonstrated, the lytic activity of T4 bacteriophage is maximal at various other values of temperature, pH and dilution, which can broaden the spectrum of application of phage therapy.

Key words: T4 Phage, E coli, Dilution, Temperature, pH

INTRODUCTION

In most of the developing countries *Escherichia coli* (*E. coli*) is the cause of one third cases of diarrhoea in children (Gomez-Duarte *et al*., 2013) and also in people travelling to developing countries *E. coli* causes traveler's diarrhoea (Ahmed *et al*., 2013). Also in farm animals and birds *E. coli* is linked with diarrhoea. *E. coli* has wide spectrum of diseases than any other bacterial specie due to its flexible genetic nature (Kaper *et al*., 2004).The emergence of *E. coli* O:157 as a main pathogen of food is a evidence of its malleable nature. Furthermore, Shigella specie, which is an significant cause of dysentery, is considered taxonomically as subspecie of *E. coli* (Brussow, 2005).

Meanwhile specific treatment and preventive measures for *E. coli* diarrhoea are lacking, however, presently the soul of treatment is oral rehydration (Bhandari *et al*., 2008). This comparatively cheap and simple measure has saved many lives, but by this way the natural course of disease remains unchanged because oral rehydration does not have antibacterial property. Community sanitation and water programs can improve the quality of drinking water and can prevent the spread of *E. coli* as it has a orofeacal route of transmission, but these measures are quiet expensive for developing and threshold countries (Brussow, 2005).

Therefore, there is a good historical background to consider phage therapy as a treatment for *E. coli* infection and phage therapy is not a fresh idea because phage therapy was used for approximately a century as antimicrobial agents (Monk *et al*., 2010). In 1921, phages promoted their use as a treatment for bacterial diarrhoea (Summers, 2001). Many phage based therapy has been sold by pharmaceutical companies (Housby and Mann, 2009). During World War 2, phages had been used by respective armies against dysentery and US army has also focused research on it (Hausler, 2007). Moreover, there is a report by using phage against *E. coli* in veterinary (Brussow, 2005) and now phage therapy is back in news (Merril *et al*., 2003).

These studies have made possible the availability of abundant and useful background information on the basic biology and genetics of *E. coli* and bacteriophage T4 (Clokie *et al*., 2011). Other characteristics that help the *E. coli* and bacteriophage T4 for use in the laboratory as a preferred system for conducting experimental evolution are their comparatively short time of generation, large sizes of population and relative ease in culturing (Bohannan and Lenski, 2000).

It is very important to note that bacteriophages have served as a vital tool for understanding molecular biology besides serving as potential treatment agent to combat bacterial infection. Another important aspect that favored the pathogenic study of bacteriophages in the present study is their presence as abundant entities in the biosphere. This eventually paved the way for bacteriophages in playing pivotal role in microbial evolution and pathogenesis (Ackermann, 2007). Moreover, a number of phages have been isolated from the *E. coli.* The T4 bacteriophage family is one of the best characterized groups of *E. coli* phage (Miller *et al*.,

2003). The effect of various dilutions, temperatures and pH on the lysis activity of T4 bacteriophage against *E. coli* BL21 was checked and this is the first report on the lysis activity of T4 bacteriophage in these above conditions.

MATERIALS AND METHODS

Bacteria, Phage and Culture Media: The *E. coli* BL21 strain was used as the primary host for lysis activity of the bacteriophage named Escherichia coli bacteriophage (ATCC11303-B4). The *E. coli* BL21 was obtained from the American Type Culture Collection (ATCC). All bacterial stock cultures prepared/obtained were stored at -80 ^{0}C in Luria-Bertani broth (Oxiod) containing 50% (v/v) glycerol. All frozen stock cultures were activated on LB agar plates before trail. For looking at the effect of different temperature, pH and dilution regimes, a single colony of *E. coli* BL21 was isolated from LB plate and inoculating in to LB broth at a 37°C until the OD_{600} reached 0.8. Bacteria and phages were propagated in LB broth.

LB medium consisted of tryptone (10g), yeast extract (5g) and sodium chloride (10g) per 1,000 (pH 7) (Sezonov *et al*., 2007). For phage plaque formation, LB-based solid medium containing 1.5 and 0.5% agar was used for the lower and upper layer, respectively (Sambrook and Russell, 2001).

The effect of temperature and pH on the lysis activity of T4 bacteriophage (10^9 pfu/ml) against *E. coli* BL21 was checked by plaque count assays and the MOI was 3. The different pH of media was obtained by using HCl and NaOH. To check the effect of dilution on the lysis activity of T4 bacteriophage against *E. coli* BL21, the T4 bacteriophage was diluted in LB broth through a serial dilution and then the lysis activity was checked against *E. coli* BL21.

Plaque count assays: The progress of lysis was recorded by plaque count assays to check the effect of different temperature, pH and dilution on the lysis activity of T4 bacteriophage against *E. coli* BL 21 similar to that of Adams (1959).

Plating of phage: For experiment 0.2 ml of *E. coli* was taken and added 0.1 ml of T4 bacteriophage to suspension. The suspension was then added to the soft agar and poured onto base plate. Agar tube was rolled between palms to mix for 2 or 3 seconds, and quickly poured onto agar surface of warm base plate. In order to disperse soft agar over the surface of the base plate agar they were gently moved in the pattern of figure eight. Soft agar was allowed to harden and then Incubated at 37°C.

RESULTS AND DISCUSSION

Molecular mechanisms of bacteriophage lysis have been studied quite extensively for the bacteriophage control of bacterial virulence in animals and human (Young *et al*., 2000). It has been found/concluded that lysis of the bacterial host is the final event in the infection cycle of a lytic bacteriophage (Wang *et al.,* 2000). The result showed that T4 bacteriophage did lysis from 10^{-1} to 10^{-7} dilutions while from 10^{-8} to 10^{-10} dilutions T4 bacteriophage had no lysis activity as shown in Table -1. It means that at highest dilutions, T4 bacteriophage fails to do lysis. Lysis can be produced by phage dilution up to a certain point; as Worley-Morse *et al*. (2014) reported that bacteriophage concentration is very important for lysis activity.

Temperature is one of the most important environmental factor that strongly affects many aspects of the biological systems. One of the important characteristic of the temperature, as environmental factor, is its fluctuation over a wide range of spatial and temporal scales that makes possible as well as limits existence of life in different niches. Influence of temperature upon the biological system is very vivid and it has been observed that evolution of phenotypic traits, species distributions, and extinctions in many cases can be traced to changes in temperature regimes (Vale *et al.*, 2008). Present study results are in confirmation with the above findings as during the experiment it was observed that yield of T4 bacteriophage was highly temperature dependent. The T4 bacteriophage was unable to develop and perform lysis on *E.coli* BL21 at 4°C, while on 15°C, 25°C and 30°C, the activity was carried out but in a little bit delayed manner (Table -1). These findings of the study are in line with those of Groman (1962), who observed that lysis by phage was delayed if the incubation temperature was below 37°C. Similarly, this study showed that at thermophilic temperature 41°C, T4 bacteriophage developed and performed lysis on his host bacteria (Table -1) and support the results of Pollard and Woodyatt (1964), who reported that bacteriophage developed at 41.2°C. While, temperature regimes of 45°C, 55°C and 70°C proved as limiting factor and caused the actual inactivation of the T4 bacteriophage (Table -1). Study results regarding the inactivation are in confirmation with those observed by Basdew and Laing (2014) who reported that increase in temperature decreases virus survival and activity. In the same way, findings by Pope *et al.* (2004) that indicate an increase in bacteriophage yield till 30°C and 39°C corroborates the present study results which revealed that 37°C was ideal temperature for bacteriolytic activity of T4 bacteriophage against *E. coli* BL21 (Table -1).

Present study findings regarding exertion of indirect influence on the survival of virus by pH of water media through affecting the extent of virus adsorption to

other particles and surfaces confirm the results obtained by the Gerba (1984). Similarly the findings of the present study regarding the stability had shown by T4 bacteriophage at different pH regimes ranging from 4 to 10 (Table -1). pH finding of the study confirmed Langlet *et al*. (2007) results which indicated that virus exhibited stability at wide range of pH regimes.

Table 1. Effect of dilution, temperature and pH on T4 phage lysis activity

	Condition	Lysis
Dilution	10^{-1}	Lysis
	10^{-2}	Lysis
	10^{-3}	Lysis
	10^{-4}	Lysis
	10^{-5}	Lysis
	10^{-6}	Lysis
	10^{-7}	Lysis
	10^{-8}	No Lysis
	10^{-9}	No Lysis
	10^{-10}	No Lysis
Temperature	4°C	No Lysis
	15°C	Lysis
	25°C	Lysis
	30°C	Lysis
	37°C	Lysis
	41°C	Lysis
	45°C	No Lysis and Inactivation
	55°C	No Lysis and Inactivation
	70°C	No Lysis and Inactivation
pH	pH 4	Lysis
	pH 5	Lysis
	pH 6	Lysis
	pH 7	Lysis
	pH 8	Lysis
	pH 9	Lysis
	pH 10	Lysis

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