Isolation of bacteriophages against *Salmonella* Enteritidis and their partial characterization

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ABSTRACT

Salmonellosis is a very important disease affecting both humans and animals. With the onset of drug resistance in *Salmonella*, use of phages for therapy seems to be an alternative approach in combating this problem. In the present study, sixteen phages against *Salmonella* Enteritidis were isolated from a total of 84 dairy and poultry sewage samples and upon characterization of phages at fixed pH 7 and different temperatures, it was found that the phages could survive at varied temperature conditions and were also sustaining at pH 7.

Keywords: Salmonella, bacteriophage, temperature sensitivity, pH

Salmonella is a major cause of enteric disease in animals and human beings (Domniguez *et al*., 2002). Ever since its discovery, it is a big problem affecting various species of animals and birds. Bacterial food poisoning is a major worldwide health problem (Mead *et al*., 1999 and Stark, 2009). Although, it may be caused by various food-borne pathogens but *Salmonella* remains the leading source throughout the world (Gast, 1997). *Salmonella* Enteritidis (*S*. Enteritidis) and *S*. Typhimurium are the most frequently identified *Salmonella* causing diseases in humans and animals. Though, various antibiotics have been used to combat the disease but due to development of multiple drug resistance it some-time results in the treatment failure (Singh *et al*., 2009). Phages are the organisms which are present in the nature and which kill the bacteria. The use of host specific phages is being promoted these days as an alternative and cost effective approach for controlling zoonotic bacteria (Sulkvelidze and Barrow, 2005). Since, phages are present in nature and face lot of adverse environmental conditions, thus, in the present study, the effect of physical parameters on phage were evaluated.

MATERIALS AND METHODS

Isolation of Bacteriophage against Salmonella Enteritidis

Bacteriophages were isolated using agar overlay technique. In brief, the sewage samples were collected and brought to the phage laboratory and isolation was done as per the protocol of Adam (1959) and Chandra *et al*. (2011).

Plaque forming unit plaque-forming unit (pfu) count

The phages were serially diluted 10 fold in PBS (pH 7.4). Equal quantity of each phage dilution (100 ul) and fresh exponential 6h growth of *Salmonella* Enteritidis were mixed in a soft NZ amines Casamino acids Yeast extract Magnesium Sulphate (NZCYM) (0.75%) agar using vortex mixer at 45°C and were poured onto a fresh NZCYM (1.5% agar) plate. After solidification the plates were incubated at 37°C for 12 h to observe and count plaques.

Effect of temperature

A 5ml phage preparation was subjected to various temperature conditions i.e. (-20 °C, 20 °C and 50 °C) individually. 500 µl was aspirated after 0, 4, 8, 24 and 48 h from the individual vial and pfu count was performed as per the above mentioned procedure.

Effect of pH

The effect of pH 7 on the phage survivability was observed. 100µl of the purified phage preparation was mixed with 900µl of suspension medium (SM) buffer and the pH was adjusted and incubated at 37^{0}C. 500 µl was aspirated after 0, 4, 8, 24 and 48 h from the individual vial and pfu count was performed as per the procedure mentioned above.

RESULTS AND DISCUSSION

Isolation of bacteriophage against Salmonella Enteritidis

In the present study a total of 84 sewage samples were processed for the isolation of phages. Out of 84 sewage samples 16 phages are found against *Salmonella* Enteritidis. It has been observed from the earlier studied that for almost all the bacteria there exists a phage corresponding to that bacterium and so phages offer a potential for targeted biological control of bacterial pathogens in human, animal, and plant diseases (Lederberg, 1996; Schuch *et al*., 2002; Xie *et al*. 2005; Chandra *et al*., 2011). Thus, the findings of our study are in corroboration of earlier studies where they too isolated bacteria from the environmental samples substantiating that there exists a phage for almost all the bacterium.

In the present study out of these 16 phages isolated only 10 were characterized further.

Effect of temperature

The isolated phage when exposed at -20°C revealed that, the numbers of phages were maintained constant except for the phages 5, 6 and 8 where there was a reduction in number of phages (Table 1). The phages when exposed at 20°C revealed that there was no reduction in any of the phage throughout the period of the study indicating this temperature to be suitable for phages (Table 2). The phages were exposed at 50^0C and their numbers remained unaffected except for phages 3, 4, 5, 6, 8 and 9 where there was a reduction in the number when compared from the initial number indicating that the phages could withstand a higher temperature too (Table 3) . These results are in corroboration with the findings of Chandra et al. (2012) where they also reported that phages could withstand a temperature of 50°C, similar to ours findings.

Table 1: Effect of temperature (-20°C) on phage count (10^6) at various time intervals

Phage No.	0 hrs	4 hrs	8 hrs	24 hrs	48 hrs
1.	>100	>100	>100	>100	>100
2.	>100	>100	>100	>100	>100
3.	>100	>100	>100	70	30
4.	>100	>100	>100	>100	>100
5.	>100	>100	12	40	40
6.	>100	50	50	15	11
7.	>100	>100	>100	>100	>100
8.	>100	80	40	20	20
9.	>100	>100	>100	>100	>100
10.	>100	>100	>100	>100	>100

Table 2: Effect of temperature (20°C) on phage count (10^6) at various time intervals

Phage No.	0 hrs	4 hrs	8 hrs	24 hrs	48 hrs
1.	>100	>100	>100	>100	>100
2.	>100	>100	>100	>100	>100
3.	>100	>100	>100	>100	>100
4.	>100	>100	>100	>100	>100
5.	>100	>100	>100	>100	>100
6.	>100	>100	>100	>100	>100
7.	>100	>100	>100	>100	>100
8.	>100	>100	>100	>100	>100
9.	>100	>100	>100	>100	>100
10.	>100	>100	>100	>100	>100

Effect of pH

Since the optimal pH for the human being and animal is neutral thus, in the present study we evaluated whether there could be any change in the number of phages

when exposed at this particular pH in vitro. In the present study we found that phages could survive at pH 7 without reduction in their phage count. Except for the phages 5 and 8 rest all the phages maintained their number when compared from initial count (Table 4). The results of the study are similar to the earlier findings, where no significant change in the phage number was observed when phages were exposed at pH 6 and 8 (Chandra *et al*., 2012).

Table 3: Effect of temperature (50°C) on phage count (10^6) at various time intervals

Phage No.	0 hrs	4 hrs	8 hrs	24 hrs	48 hrs
1.	>100	>100	>100	>100	>100
2.	>100	>100	>100	>100	>100
3.	>100	>100	>100	72	30
4.	>100	>100	>100	40	19
5.	>100	>100	>100	>100	26
6.	>100	>100	>100	40	30
7.	>100	>100	>100	25	8
8.	50	28	36	14	3
9.	>100	>100	>100	24	15
10.	>100	>100	>100	>100	>100

Table 4: Effect of pH 7 on phage count (10^6) at various time intervals

Phage No.	0 hrs	4 hrs	8 hrs	24 hrs	48 hrs
1.	>100	>100	>100	>100	>100
2.	>100	>100	>100	>100	>100
3.	>100	>100	>100	>100	>100
4.	>100	>100	>100	>100	>100
5.	>100	>100	>100	65	50
6.	>100	>100	>100	>100	>100
7.	>100	>100	>100	>100	>100
8.	>100	77	40	100	28
9.	>100	>100	>100	>100	>100
10.	>100	>100	>100	>100	>100

Thus, it could be concluded from the study that phages could be isolated against S. *Enteritidis* from the dairy and poultry sewage and the phages could withstand various temperature conditions without reduction in their numbers.

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