PHYSICOCHEMICAL CHARACTERIZATIONS OF PHAGE LYTIC TO *STAPHYLOCOCCUS AUREUS* FROM MASTITIS, SEWAGE WATER AND FARM YARD SLURRY OF LIVESTOCK

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ABSTRACT

The study was performed to isolate and characterize the lytic phage Staphylococcus aureus, from the farm yard resource of Cattle, Buffaloe and Poultry in all 50 samples were obtained and 2 phages were recovered. They were characterized on the basis of plaque morphology, host range determination, effect of pH and temperature on phages against Staphylococcus aureus,. This preliminary study could be useful to search potential candidate phage for treatment and control of Bovine mastitis.

Key words: Isolation, Bacteriophage, Staphylococcus aureus, and Characterization

INTRODUCTION

Phage therapy is gaining important once due to emergence of bacterial resistant to antibiotic. Phages can be used to treat pathogenic bacteria, as an alternative approach to control infectious bacterial disease. Phages can be used as therapeutic or prophylactic agents against bacterial pathogens like Staphylococcus infection in mice (Rawat and Verma et al. (2007)) and Pastuerellosis infection in chicken (Durairajan et al., 2012) Bacteriophages specific to Staphylococcus aureus from mastitis have been isolated by several investigators. However, many of the fundamental properties of this group of phages are yet to be determined. The objective of this study was to gain a more precise understanding of the Staphylococcus phages, by studying their biological, biochemical and physical characteristics.

MATERIALS AND METHODS

Bacterial culture

Initially Staphylococcus aureus, were grown on Typticase soy broth. A standard culture was prepared by harvesting the growth from slant of Nutrient agar (48 hr) and adding it to broth and final concentration determined by viable count method Rawat and Verma et al. (2007)such standard culture were used in all experiments.

Phage isolation and enrichment

Isolation of Phage was carried out according to method describe by Rawat and Verma et al. (2007) with some modification. In brief, sewage sample were collected from livestock farms. After centrifugation (5000 × g for 15 min) of sewage, 50 ml of supernatant was added in 10X

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concentrated 50 ml NZCYM broth, incubated for 18 hrs at 37˚C and filtered through 0.22µm pore size syringe filter. The filtrates were tested for the presence of phage.

Phage detection

Detection of phages was carried out as per test described by Eisentark (1967). Initially, soft agar in 3ml (NZCYM broth with 0.7% agar) was seeded with 0.1 ml culture (108 cfu /ml), mixed and poured on Nutrient agar plate. After solidification, 3µl of filtrate was spotted on the lawn of Staphylococcus aureus. After drying, the plate was incubated at 37˚C for 48 hr. A clear zone in the plate, resulting from the lysis of host cell, indicated the presence of phage.

Purification and preparation of stock phage suspension

Spotted area which showed the presence of plaque was selected for purification of phage. A small amount of semisolid agar from such area was removed by wire loop and shaken in 1 ml NZCYM broth. 0.1 ml of this broth was then incorporated into semisolid NZCYM (0.75 %) agar and overlay along with seed organism. Phage preparations were obtained by harvesting from agar overlay plates by eluting in SM media at 4˚C followed by centrifugation at 8000×g for 15min. The resultant supernatant fluid was sterilized by passage through a Millipore HA filter pad and stored at 4˚C.

Working stock of isolated phage were prepared by seeding 500ml flask containing 100ml of NZCYM medium with 0.5 ml suspension of Staphylococcus aureus harvested from 48 hr Staphylococcus aureus agar slope into sterile PBS (pH 6.2). Isolated phage suspension added in this culture which would give 0.01 multiplicity of infection (MOI) After incubation for 48 hr at 37˚C culture were centrifuged at 10000g for 10 min. Supernatant fluid was then sterilized by passage through membrane filter (0.22µ, Millipore filter corp, Bereford mass., U.S.A.) and kept at 4˚C. Titer of these phage stock was determined by double overlay technique.

Host range of phage

To identify the host range of phage, a 30µl of phage stock was spotted on spread lawn culture of Staphylococcus aureus from different field isolates of Staphylococcus aureus. After spot had dried, the plates were incubated at 37˚c for 48 h.

Concentration of phage lysate

Filtered phage lysate was treated with DNAse I, RNAse A, each to a final concentration of 1 µl/ml at room temperature for 30 min. Polyethylene glycol 8000 and NaCl were added to a final concentration of 10% (w/v) and 1 M, respectively. After gentle mixing, the phage preparation was incubated overnight at 4˚C. The phage were pelleted by centrifugation at 10000 X g for 20 min, then resuspended in 5 ml of 10mM Tris –HCl buffer (pH 7.4) .

Phage DNA extraction

Bacteriophage DNA was isolated using Lambda DNA extraction kit (Qiaegen, USA) as per the direction given by manufacturer.

Phage adsorption

This experiment was carried out as described by Rawat and Verma (2007). A host strain culture in NZCYM was infected by phage suspension to give an MOI of 0.01, and incubated at 37˚C. Aliquots of 0.5 ml were taken at an interval of 2 min during a total period of 30 min and immediately placed in ice. After centrifugation at 12000g for 5 min, supernatant was titrated for unabsorbed phages by the double –layer agar plate method. NZCYM broth containing only phage was used as a control.

Thermal sensitivity test

Initially 900µl of sterile deionized water was preheated to a desirable temperature, ranging from 40-80˚C. 100µl of phage solution (107pfu/ml) was added to preheated water after heating to assigned temperature for 30 min, the solution was placed in an ice warm both. Surviving phage titer was assayed by the double layer method in triplicate manner.

pH sensitivity test

The phage at final concentration of 106 pfu/ml in PBS (pH 7.4) was adjusted in step of 1 pH unit from pH 2 to 12 using HCl or NaOH and incubated overnight (12 hr) at 37oC. After readjustment to pH 7, the double layer method was performed to determine phage titre.

Chloroform sensitivity test

100µl of 10% chloroform was added in 900µl of phage suspension and kept for 30 min. Further double layer method was performed to determine the titer.

Electron Microscopy

Phage suspension (108pfu/ml) was mixed with 2.5% of gluteraldehyde and kept for 1 hr at 4˚C .Then 25µl of of fixed phage was added to suface of grid and left for 2 min. The phage was stained with 0.5% uranyl acetate for 2 min and then inspected with a Electron microscope

RESULTS AND DISCUSSION

Phages are generally found that environment where chances of host bacteria are more R (Verma et al., 2013). Phage lytic to Staphylococcus aureus is present in environment such as sewage and museums culture. Therefore, in the present study, the lytic phage specific to Staphylococcus aureus was isolated from farm sewage water.

In the phage therapy, phages with broad host range are more useful than narrow host range since they can inhibit several strains of bacterial pathogen (Rawat and Verma, 2007). Phage is lytic to most species of Staphylococcus aureus; it can be useful to bovine caprine, swine and Poultry for phage therapy against Staphylococcus aureus infection

This study showed that the phage tolerated to relatively high temperature ranging from 50˚C to 80˚C and a broad range of pH. Phage was more susceptible to acidic environment than alkaline as phage was often sensitive to protein denaturation in an acidic environment. Furthermore, phage was stable and survived pH between 5 to 10. The results are consistent with previous observation made that most phages were able to survive well over a wide range of pH. These characteristic may be useful for the application of the phage in different environment like acidic, alkaline and fibrinous content of mastitis milk.

Additionally Phages is sensitive to chloroform as per the previous finding of Staphylococcus aureus but toluene significantly did not affect the phage. It can used in phage preparation to check the growth of contaminating bacteria.

Since phage contains tail and it fell into the order caudovirales. Phage possess an icosahedral head and a long tail. Adsorption of phage to bacteria was very slow and it require further study to determine the growth parameter of phage (lytic cycle, latent period and burst size). It might be due to narrow specificity of receptor of phage attachment or it may be due standardizations of media composition (Brinley-Morgan et al., 1990). Therefore phage has a potential to be used as a biological disinfectant to control Staphylococcus aureus in the environment or in septic wound and Staphylococcal dermatitis.

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Figure: 1

Stability of phage treated with different pH at 37˚C for 12 hr. % stability calculated with reference Pfu/ml at pH 7. Stability: (N/No) x 100, where N is the number of phage viable after 12 hr of incubation and No is the initial number of phages. Values are means of 3 determinations

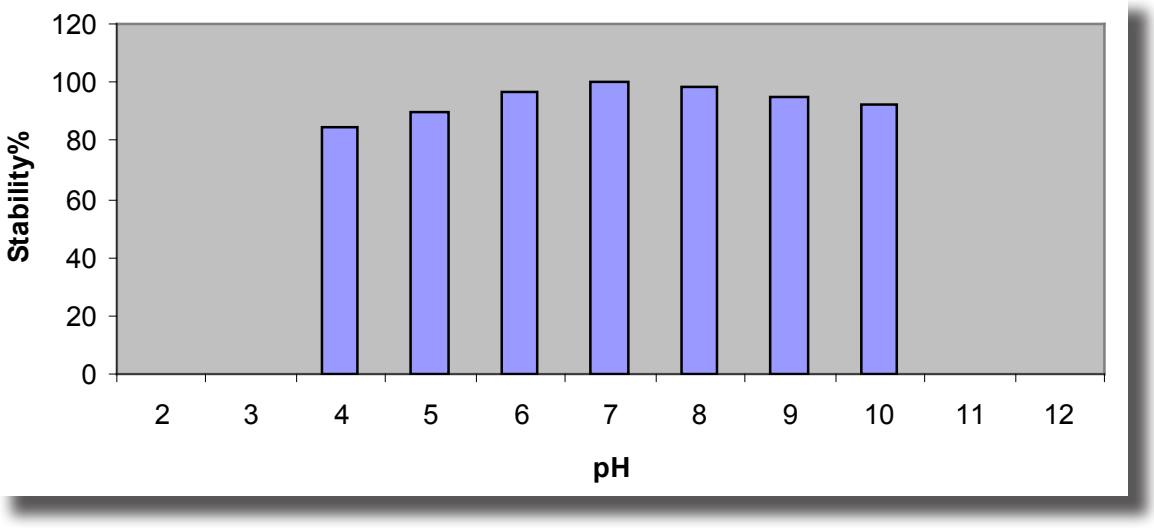

Figure: 2

Effect of temperature on stability of phage after 1 hr incubation. %Stability:(N/No)x 100, where N is the number of viable phage after 1hr of incubation and No is the initial number of phage ;% stability with reference pfu/ml at 37˚C.Value are the means of 3 determinations

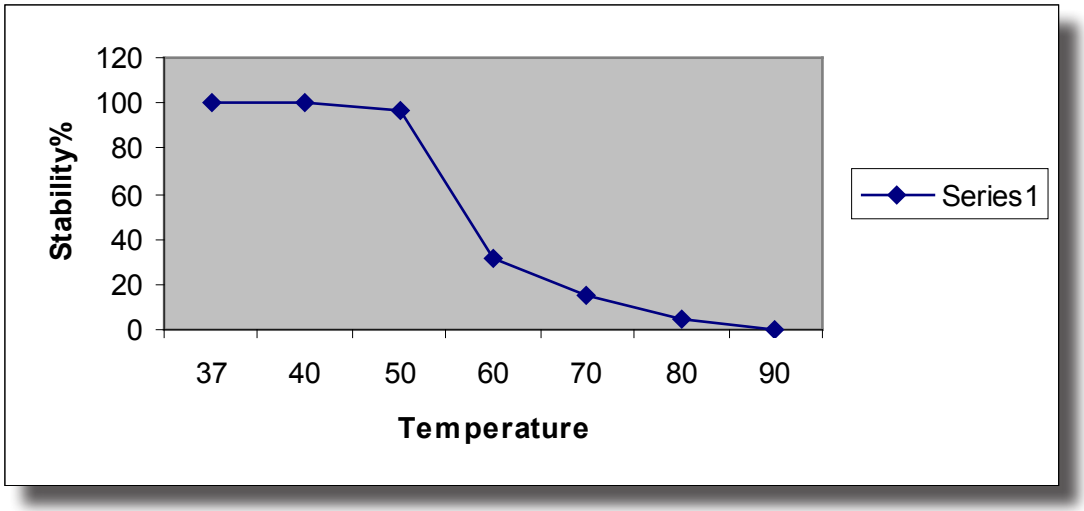

Figure: 3

Adsorption of phage on Staphylococcus aureus. Values are mean of 3 pfu