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RESEARCH ARTICLE

ISOLATION OF BACTERIOPHAGE AGAINST *STAPHYLOCOCCUS AUREUS* CAUSING MASTITIS

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ABSTRACT

Some endemic diseases are associated with the dairy production these are called production diseases. One of them is mastitis. It is the most expensive disease that causes large economic effects. It is the most expensive diseases on dairy farms. Due to chronic nature of these diseases economic damage is spread out and economic damage of certain factors such as milk production decreases cannot seen directly. Mastitis caused by *Staphylococcus aureus* is a major concern to the dairy industry due to its resistance to antibiotic treatment. In future the phage therapy may replace antibiotic treatment for combating the infection caused by *Staphylococcus aureus* because phages are more useful in the study of fundamental aspects of molecular biology and in the diagnostic laboratory for the identification of pathogenic bacteria. In the present research bacteriophage was isolated against *Staphylococcus aureus* from the sewage water. The isolated bacteriophage showed lytic activity against these bacteria.so it signifies the underlying potential of bacteriophage therapy.

INTRODUCTION

Mastitis is a multi-etiological complex disease and defined as "Inflammation of parenchyma of mammary glands and is characterized by bacteriological, physical and usually chemical changes in milk and pathological changes in glandular tissue" (Sudhan and Sharma 2010). It is considered as the most frequent production disease in the developing countries (Rajala-Schultz, Gröhn *et al*. 1999; Seegers, Fourichon *et al*. 2003). The world wide cause of mastitis is *Staphylococcus aureus*. It is responsible for the culling of the cows and major financial loses in dairy farming. We know that all the strains of *Staphylococcus aureus* don't behave in the similar way towards causing infection.(Green and Bradley 2004; Han, Kim *et al*. 2013) *Staphylococcus aureus*has ability to evade and affect the host immune system. The pathogenic role of secretory and surface associatedfactors is not completely established. (Green and Bradley 2004). *Staphylococcus aureus* resposible for causing mastitus tends to attain resistance agianstcommenly used antibiotics. Among the methods alternative to antibiotics and chemotherapeutics for combating bacterial infections, therapy using bacteriophages is frequently mentioned (Rose 1996; Smith, Pearson *et al*. 1999; Thacker 2003; Dixon 2004; Levin and Bull 2004; Thiel 2004).Lytic phages are similar to antibiotics in that they have remarkable antibacterial activity (Harper and Enright 2011).However, therapeutic phages have some at least theoretical advantages over antibiotics, and phages have been reported to be moreeffective than antibiotics in treating certain infections in human (Kochetkova, Mamontov *et al*. 1988) and experimentally infected animals (Sulakvelidze, Alavidze *et al*. 2001).

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The phages are highly specific and so the changes of any further infections or side effects arealmost nil while the antibiotics destroy the bacteria whether they are cause of infection or not so many chances are there for secondary infections to evolveStill the identification of the bacteria is the first need if the phages have to be applied for therapeutic purpose while antibiotics need not any highly investigated pathogens to be administered (Costantini, Eliceiri *et al*. 2012)

MATERIALS

The composition of media used was in gram/liter unless otherwise specified according to the requirement. Sterilization was done by autoclaving at 121^0C and 151b./ $inch^2$ for 15 minutes. Solution was filtered by using syringe filter of 0.2 µm. 0.1 M HCl and 0.1 M NaOH were used to adjust the pH of media. All glass-ware was washed and cleaned with detergent and then sterilized in autoclave, dried variably at 60-100^0C.

Glass ware used

Incubater	At 37^0C
Test tubes	20 ml
Ependorf	1.5 ml
Micropipette	1000 µl, 500 µl, 100 µl
Micro tips	1000 µl, 500 µl, 100 µl
Centrifuge	11000 G
Filter paper assembly	0.45µl
Flask	25ml

Chemicals and media

2 X L-Broth

Table 2.2. To prepare 2 XL-broth add 40 gram in 1000 ml of distilled water and autoclaved it.

Serial No.		Components	Gms/L
1	1.	Tryptone	20
2	1.	Yeast Extract	10
3	2.	NaCl	10

Fresh culture of *staphylococcus aureus*

Strain.	*staphylococcus aureus*

Chloroform

Chloroform	Pure

Poly ethylene glycol

10 % poly ethylene glycol is prepared by adding 10 ml in 90 ml of autoclaved water.

Table 10 % Poly ethylene glycol.

Poly ethylene glycol	Distilled water
10 ml	90 ml

1 M NaCl solution

1 molar NaCl is prepared by adding 58g in 1000 ml autoclaved water.

Table 1. M NaCl

NaCl	Distilled water
58g	1000ml

Phosphate buffer saline

Concentrated phosphate buffer slime is used.

Phosphate buffer sline	Pure

L-Agar

35 gram L-agar is dissolved in 1000 ml of distilled water and autoclaved it.

Table L – Agar 1000 ml.

S. No.	Components	gms/L
1	Tryptone	10.0
2	Yeast Extract	5.0
3	NaCl	5.0
4	Agar	15.0

METHODS

Sampling

Sampling was done from sewage and the liquid manure from cowsheds and main drain of township.

Host Bacterial Strains

This study included different bacterial strains that are *Staphylococcus aureus.* As the host strains for the isolation of bacteriophages against them was kindly provided by Dr. Noman from department of microbiology and molecular genetics university of Punjab.

Enrichment of Bacteriophages: (Blackman)

Take10 ml of centrifuged sewage sample Mix it with 10 ml of 2X l-Broth. Add 100 µl of Bacterial fresh culture and incubating with shaking at 37°C for 24 hours to enrich host bacteria-specific bacteriophages. Add few drops of chloroform and leave the mixture for 15 min. Centrifuge at 11000 G for 5 min.Take supernatant and add 10% polyethylene glycol along with 1 molar NaCl. And sample was incubatedfor overnight at 4°C.Centrifuge at 11000 G for 20 min. Take pallet wasresuspended with 1 ml phosphate Buffer slime and filter it with the help of 0.45µ filter. Isolation and purification of Bacteriophage: (Brown and Faulkner 1975). Take enrichment collected from the above procedure. Add Bacterial culture. Incubated at 37°C for 15 min. Pour on to the solid agar plate and incubated at 37°C for 16-20 hour. Examine the plaque formation.Separate out the plaque with pasture pipette. Drop in to the fresh L-Broth having few drops of chloroform and save at 4°C.

RESULTS

Host bacterial strains

Two host bacterial strains of *staphaureus* were streaked and incubated at 37 C for 24 hr these bacterial strains served as host against the isolated bacteriophage in all experiments.

Isolation, Enrichment and purification of bacteriophage

A bacteriophage was isolated and purified from the enrichment against host. It was isolated from the main sewage drain of township. The plaque morphology is shown in the figure 3.1.after incubation of over layered plates at 37 C for 24 hr.
Mastitis cause considerable changes in milk as shown in the table(Khan and Khan 2006).

Table. Difference between normal Milk and Mastitis Milk

Constituent	Normal milk	Mastitis milk with high SCC
Fat	3.5	3.2
Lactose	4.9	4.4
Total protein	3.61	3.56
Total casein	2.8	2.3
Whey protein	0.8	1.3
Serum albumin	0.02	0.07
Lactoferrin	0.02	0.1
Immunoglobulin	0.1	0.60
Sodium	0.057	0.105
Chloride	0.091	0.147

Mastitis causes many changes in the milk are as follow:

- Casein which is major milk protein and high nutritional quality decreases due to which the concentration of whey

protein increased and this protein adversely affects the quality of dairy products such as cheese.

- Due to increased vascular permeability serum albumin, immunoglobulins, transferrin and other serum proteins passes into the milk (Sharif and Muhammad 2009).
- Due to higher SCC (somatic cell count) concentration of serum albumin and immunoglobulin are increased which reduce the heat stability of milk.
- There is decreased absorption of calcium from blood into milk as a result impaired coagulation(Haenlein, Schultz *et al*. 1973).

DISCUSSION

Mastitis has been one of the costly disease of dairy animals and is responsible for significant lose in term of animals as well as money. Many pathogens have been found to be associated with causing this disease but staphylococcus aureus considered one of the most important pathogen. Causing mastitis. Infection with staphylococcus aureus is the major concern because staphylococcus aureus tend to develop resistance against commonly used antibiotics. So alternative therapy for this bacterial infection is need of the need of the hour. So a promising alternative treatment against bovine mastitis is in the form of phage therapy (Han, Kim *et al*. 2013) (Kwiatek, Parasion *et al*. 2012) (Garcia, Madera *et al*. 2009). It has already been used against many pathogens such as Ecoli (Dąbrowska, Skaradziński *et al*. 2010)(Matsuzaki, Yasuda *et al*. 2003). But the staphylococcus aureus specific phages have not been worked upon extensively. Some of the phageshave been isolated against xome staphylococcus aureus assosiated disease but not against mastitis. (Capparelli, Parlato *et al*. 2007),(Dąbrowska, Skaradziński *et al*. 2010; Kwiatek, Parasion *et al*. 2012). In current study we have isolated phages against staphylococcus aureus causing mastitis.phages were isolated from sewage water and method use for this isolation was double layer agar plate method. Waste water ftom local farm in Lahore was use for screening of staphylococcus aureus. Specific phages isolated phages have lytic activity against pathogenic staphylococcus aureus which were isolated from milk of infected cow. This phage did not infected microbe other than staphylococcus aureus e.gEcoli and klebsellaEcoli etc. This phage was produced at large scale for further experimental work.

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