

## Synergistic Effect of Bacteriocin obtained from Fermented Goat and Cow Milk against Pathogens (*E. coli*, *Staphylococcus* and *Pseudomonas*)

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All the bacterial disorders are treated with antibiotics. Drug resistance and recurrence of infection are still a major problem (Joart 1978, Reid 2001). Synergistic effect of bacteriocin obtained from fermented milk against pathogens is the criteria of the study. In the fermentation of goat and cow milk by *Lactobacillus* and *Streptococci* sp the pH decreased rapidly from 6.2 to 3.6 in 25 hours. The pathogens *E.coli*, *Staphylococcus* sp and *Pseudomonas* sp were highly inhibited by fermented goat milk rather than the fermented cow milk. The pathogens *E.coli*, *Staphylococcus* sp and *Pseudomonas* sp were highly inhibited by fermented goat milk rather than the fermented cow milk. The partially purified bacteriocin expressed very high inhibitory activity towards *E.coli* (30 mm and 28 mm). It exhibited moderate activity towards *Staphylococcus* sp and *Pseudomonas* sp. One single protein band was obtained in the gel, indicating that bacteriocin was properly purified. The molecular weight of the bacteriocin was determined as 45,000 Da.

**Key words:** Bacteriocin, Sodium Dodecyl sulfate – Polyacrylamide gel electrophoresis, Urinary tract infection, Bovine serum albumin.

Our study focuses on purification and characterization of bacteriocin obtained from fermented goat and cow milk and analysis of its antibacterial activity against pathogens isolated from pathogens. Urinary tract infection (UTI) afflict an estimated of one billion people in the world annually (Reid and Bruce, 2001). It is caused by *Escherichia coli*. Staphylococcal infection causes food infection, nosocomial infection, skin infections and UTI (Cookson, 1998). *Pseudomonas aeruginosa* causes infantile

diarrhea and sepsis. Bacteriocin isolated from fermented goat and cow milk poses synergistic effect towards these pathogens.

### MATERIAL AND METHODS

*E.coli* was isolated from urethra of patients on Eosin methylene blue agar (EMB). *Pseudomonas* was isolated from stool sample on nutrient agar. *Staphylococcus* was isolated from pus sample on Mannitol salt agar (MSA). *E.coli* and *Pseudomonas* sp were identified by gram staining and biochemical tests. *Staphylococcus* was identified by gram staining and catalase and coagulase tests.

#### Fermentation of goat and cow milk

Commercially available cow and goat milk were sterilised and inoculated with *Lactobacillus* and *Streptococcus* sp. The milk samples were kept for incubation at 37 °C for 25 hours.

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### Analysis during fermentation

pH values of the fermenting milk samples were determined after every 5 hours till 25 th hour.

### Degree of inhibition

24 hour old culture of *E.coli*, *Staphylococcus* and *Pseudomonas* was prepared. It was serially diluted. From 10<sup>-6</sup> dilution 0.1 ml of the inoculum of *E.coli*, *Staphylococcus* and *Pseudomonas* was spread on the surface of EMB, MSA, and NA. 0.1 ml of the fermented milk was spread evenly on the above plate with a glass spreader. The plates were incubated at 37°C for 48 hours. The colony forming units (CFU/ml) was calculated. The inhibition of fermented goat and cow milk was observed.

### Partial purification of bacteriocin from fermented goat and cow milk

The fermented goat and cow milk was partially purified by salt saturation method (Benovit, Lebrihi *et al.*, 1977). The fermented goat and cow milk was saturated with 70% ammonium sulfate and stored at 4°C to precipitate out the protein. After 24 hours it was centrifuged at 20,000Xg at 4°C for 30 minutes. The pellet was washed 6 times with phosphate buffer. It was finally dissolved in phosphate buffer containing SDS (60% W/V).

### Inhibitory activity of bacteriocin by well diffusion assay

Muller Hinton agar plates were prepared and wells were cut on it. The plates were swabbed with test strains *E.coli*, *Staphylococcus* and *Pseudomonas sp.* 100 ml of purified bacteriocin from goat and cow milk was added to the wells. The plates were incubated at 37 °C for 24 hours.

### Purification of bacteriocin

### Sodium Dodecyl sulfate – Polyacrylamide gel electrophoresis (SDS - PAGE)

SDS – PAGE was conducted as described by Laemmli, U.K., 1970. Gels consisting of 12% separating gel and 5% stacking gel that were 0.75 mm thick, 5cm long and 9 cm wide was prepared

for the electrophoresis. Molecular size standards used were Bovine serum albumin (80,000Da), Ovalbumin (45,000Da), Lysozyme (18,500Da). The protein samples with bromothymol blue were loaded along with the standard protein markers into the respective wells. The gel was electrophoresized at 50-200 mv for 3-4 hours. After electrophoresis the gel was placed in destaining solution and washed with distilled water to remove excessive stain. The proteins fractionated into bands were observed and their molecular weights were determined by comparing the protein bands with the standard markers.

## RESULTS

### Colony Morphology and gram staining results of the pathogens

The colony appeared small and smooth with greenish metallic sheen on EMB agar medium. They revealed the presence of gram negative rods. Large, opaque green pigmented colonies appeared on NA. They revealed the presence of gram negative rods. Yellow colonies were observed on MSA. They revealed the presence of gram positive cocci in clusters.

### Effect of pH

In the fermentation of goat and cow milk by *Lactobacillus* and *Streptococci sp* the pH decreased rapidly from 6.2 to 3.6 in 25 hours (Fig. 1 & 2).

### Inhibition of *E.coli*, *Staphylococcus* and *Pseudomonas* by the fermented milk

The pathogens *E.coli*, *Staphylococcus sp* and *Pseudomonas sp* were highly inhibited by fermented goat milk rather than the fermented cow milk.

### Inhibitory activity of bacteriocin by well diffusion assay

The partially purified bacteriocin expressed very high inhibitory activity towards *E. coli* (30 mm and 28 mm). It exhibited moderate

**Table 1.** Showing Biochemical Characters result on *E.coli* and *Pseudomonas sp*

Biochemical test	Indole	Methyl red	Voges proskauer	Citrate	Catalase	Oxidase
<i>E.coli</i>	+	+	-	-	-	+
<i>Pseudomonas sp</i>	-	-	-	-	+	+

+ Positive; - Negative

**Table 2.** Showing Biochemical characters of *Staphylococcus* sp

Biochemical tests	Results
Catalase	Positive
Oxidase	Negative
Coagulase	Positive

activity towards *Staphylococcus* sp and *Pseudomonas* sp (Table 3). Bacteriocin isolated from fermented goat milk exhibited high inhibitory activity.

#### Purification of bacteriocin

#### Sodium Dodecyl sulfate – Polyacrylamide gel electrophoresis (SDS - PAGE)

One single protein band was seen in the gel, indicating that bacteriocin was properly

**Table 3.** Showing the inhibitory activity of bacteriocin towards pathogens

Test Samples	Zone of Inhibition (mm) Pathogens tested		
	<i>E. coli</i>	<i>Staphylococcus</i> sp	<i>Pseudomonas</i> sp
Fermented goat milk	30	11	6
Fermented cow milk	28	9	4

purified. The molecular weight of the bacteriocin was determined as 45,000 Da.

The pH of fermented milk decreased rapidly from 6.2 to 3.6 in 25 hours. The fermented milk exhibited inhibitory effect on the growth of *E. coli*, *staphylococcus* sp and *pseudomonas* sp. The precipitated bacteriocin produced inhibitory zone of 30 mm against *E. coli*, 11 mm against *Staphylococcus* sp and 6mm against *pseudomonas* sp. The bacteriocin produced a single band when run on SDS – PAGE, which indicates that it is pure. The molecular weight of bacteriocin was determined as 45,000 Da.

#### CONCLUSION

The bacteriocin precipitated from fermented goat milk exhibits high antagonistic and immunological properties against pathogens like *E.coli*, *Staphylococcus* sp and *Pseudomonas* sp than those of cow milk (Park 1994; Haenlein 2004). The pathogens are generally resolved by antibiotic treatment. However drug resistance is a major problem. Hence use of the naturally produced Bacteriocin from goat milk, reduces the risk of antibiotic resistance and also avoids side

effects. Hence bacteriocin is of high therapeutic value to mankind.

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