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Swot up of antimicrobial protein produced bacteria from ruminant mammal milk and its ramification on *Pseudomonas* sp, *Staphylococcus* sp. and *Salmonella* sp.

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***Abstract*— Bacteriocin are proteins which are produced by bacteria of one strain but it is toxic to the other strain of related species. Bacteriocin of LAB (Lactic Acid Bacteria) is exceptional vitality for the dairy industry and is successfully looked for their application in milk products, taking into account their hostile impacts against sustenance borne pathogens. This study demonstrates the isolation of bacteriocin producing from the goat raw milk sample and it is described by physiological and the biochemical tests. Three sequesters of bacteriocin creating LAB were isolated from goat milk. The culture supernatants of the three segregates were surveyed for their antimicrobial activity against food destroying organisms, for example *Pseudomonas sp*, *Staphylococcus aureus* and *Salmonella typhi*. The distances across of the inhibitory zone keep running between 9-12 mm. This bacteriocin may have potential use as bio preservatives and may help in enhancing the gut environment by battling a few pathogenic microorganisms.**

Index Terms : Bacteriocin, LAB, Pseudomonas sp, Staphylococcus aureus and Salmonella typhi.

I. INTRODUCTION

Lactic acid bacteria have a potential for use in bio preservation since they are safe to consume and amid capacity they actually rule the microflora of numerous nourishments. In addition, some LAB show powerful antimicrobial activities as little, heat-stable, ribosomally synthesized antimicrobial peptides called bacteriocins. These have a bactericidal and bacteriostatic mode of action, for the most part hindering microorganisms that are firmly identified with the creating strain. In milk, brined vegetables, numerous cereal items and meat with included carbohydrate, the growth of lactic acid bacteria deliver new product. In crude meats and fish that are chill put away under vacuum or in a domain with raised carbon-di-oxide concentration, the lactic acid bacteria, turn into the prevailing populace and save the meat with a concealed fermentation. There is a developing consumer interest for processed food products containing lower levels or no chemical preservatives prompting indigenous exploration concentrates on in the field of screening of bacteriocin as sustenance additives.

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II . MATERIALS AND METHODS

A. SAMPLE COLLECTION

Goat milk samples were collected using sterile container and transported to the laboratory using ice box from Namakkal District.

B . ISOLATION OF LACTIC ACID BACTERIA

LAB were isolated from milk sample of dilution 10^{-1} to 10^{-7} and were placed on skimmed milk agar with bromocresol purple as a pH indicator and incubated at 37°C for 24 hours. Then the isolated colonies were sub cultured in the nutrient agar slants.

C. SCREENING OF BACTERIOCIN PRODUCERS

Active bacteriocin producer was isolated from the acid forming colonies and were inoculated into MRS broth followed by MHA containing the lawn of test culture *Bacillus coagulans* which was the assay media. Colonies forming the zone of inhibition were selected and inoculated into the MRS broth and identified by morphological and biochemical characteristics.

D. MORPHOLOGICAL IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION

The techniques that have been used for the morphological identification and biochemical characterization includes Gram staining, Indole Production Test, Methyl Red (MR) test, Voges-Proskauer (VP) test, Citrate utilization test, Catalase activity for H_2O_2 production, Oxidase test, Starch hydrolysis test, and Sugar fermentation test.

E. *DETERMINATION OF ACTIVITY OF CAP AGAINST FOOD BORNE PATHOGENS*

a. Preparation of Crude Antimicrobial Protein (CAP):

The selected colonies were inoculated in MRS broth and incubated for 16 hrs to reach the optical density 1.086. Then the broth cultures were centrifuged at 10,000rpm for 10 mins. The supernatant was collected to get cell free extract.

And this crude extract was used to study their antimicrobial activity against food borne pathogens.

b. Antimicrobial Activity of CAP against food borne pathogens

Sterile cotton swab was dipped into the broth cultures of *Pseudomonas* sp, *Staphylococcus aureus and Salmonella typhi* were streaked separately on Muller Hinton Agar plates. Wells were made on the MHA plates and filled with CAP of three strains, about 25, 50, 75 and 100µl concentration. After incubation colonies forming the largest zone of inhibition were selected for further study.

III.RESULTS

A. ISOLATION AND SCREENING OF BACTERIOSIN PRODUCING MICROBE

Five acid producing colonies from skimmed milk agar plates were placed on lawn of *Bacillus coagulans*. With reference to the Tab. I, among the five strains, strain 3 & 5 produced inhibition zone.

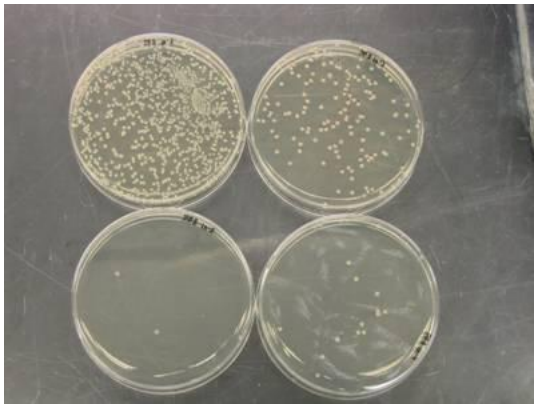


Fig 1: Isolation of bacterial colonies using nutrient agar medium by spread plate technique

Fig 2: Strain 3 on skim milk agar

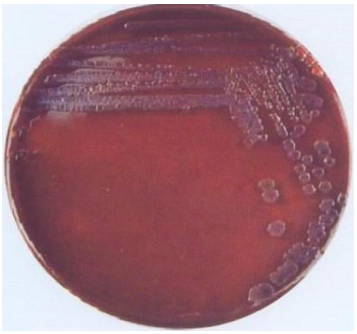


Fig 3: Strain 5 on skim milk agar

Tab. I: Screening of bacteriosin producing bacteria using *Bacillus coagulans*

S.NO	PRESENCEOR ABSENCE OF ZONE OF INHIBITON
Isolate 1	_
Isolate 2	_
Isolate 3	+
Isolate 4	_
Isolate 5	+

B. *MORPHOLOGICAL IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION*

The zone forming colonies were identified as rod shaped, gram negative and motile organisms.

Tab. II. Morphological identification

TESTS	MORPHOLOGY
Gram's Staining	Pink rods
Motility Test	motile

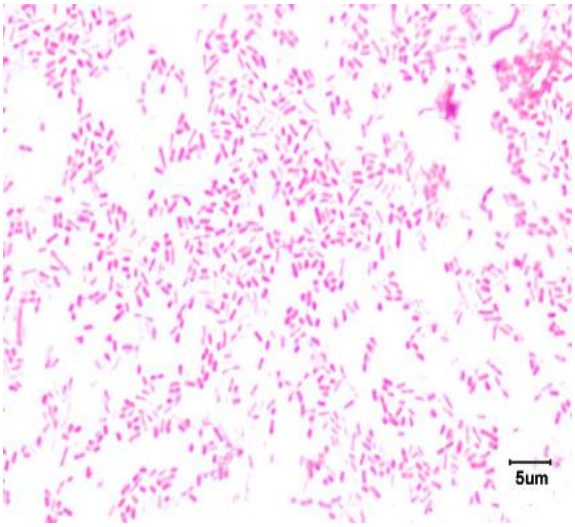
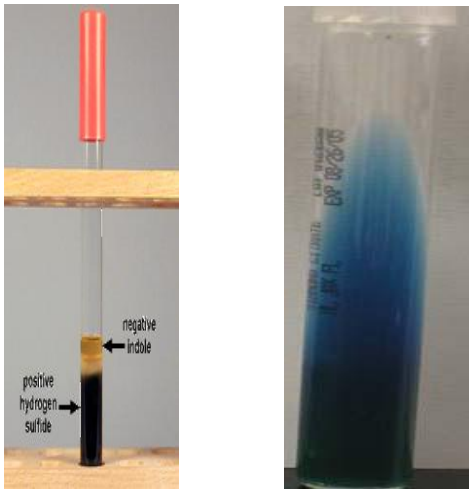


Fig 4: Gram's staining

Tab.III. Biochemical characterization

S.NO	BIOCHEMICAL TESTS	RESULTS	
		STRAIN 3	STRAIN 5
1	Indole	_	_
2	Methyl Red	_	_
3	Voges Proskauer	+	+
4	Citrate	+	+
5	Catalase Enzyme	_	_
6	Oxidase	_	_
7	Lipase	_	_
8	Cellulose	+	+
9	Glucose	+	+
10	Sucrose	+	+
11	Lactose	+	+
12	Mannitol	+	+
13	Urea hydrolysis	+	_
14	H_2S test	_	_
15	Grams	- rods	- rods



(a) (b)

Fig 5: (a) Indole test (b) Citrate utilization test

C. *ANTIMICROBIAL ACTIVITY OF CAP AGAINST FOOD BORNE PATHOGENS*

The CAP of 2 isolates was inoculated into the plates inoculated with *Pseudomonas* sp, *Staphylococcus aureus* and *Salmonella typhi*.

Tab. IV: Antagonistic effect of cap

Tab. IV (a): Strain 3

Species	Conc.& inhibition			
	25µl	50 µl	75 µl	100 µl
***Pseudomonas* sp.**	10	12	13	14
Staphylococcus aureus	10	11	12	13
Salmonella typhi	-	7	8	9

Tab. IV (b): Strain 5

Species	Conc.& inhibition			
	25µl	50 µl	75 µl	100 µl
***Pseudomonas* sp.**	17	18	20	20
Staphylococcus aureus	11	13	14	11
Salmonella typhi	13	14	17	17

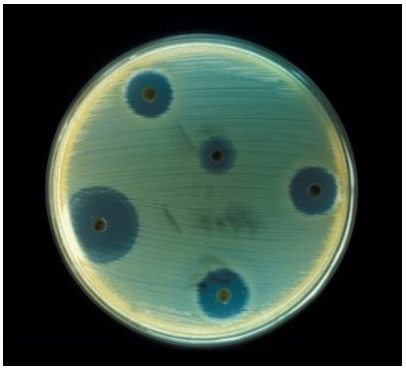


Fig 6 : Zone of inhibition against *Staphylococcus aureus*

Fig 7 : Zone of inhibition against *Salmonella tyhpi*

IV. DISCUSSION

Bacteriocin producing strains may be used as protective cultures to improve the microbial safety of foods and also in preservation of fermented foods by the inhibition of food spoiling bacteria such as *Pseudomonas sp, Staphylococcus aureus, Salmonella* sp and *Listeria monocytogens* etc. (Antonio Galvez *et al*., 2007).

The antagonistic activity of CAP against food borne pathogens, isolated from spoiled goat milk, was determined by Well diffusion assay. The milk borne pathogens were isolated using skim milk agar and nutrient agar plates and they were identified by Gram's staining and biochemical tests including IMViC, TSI, oxidase, catalase, coagulase and carbohydrate fermentation tests (sucrose, glucose, mannitol and lactose), nitrate reduction test, starch hydrolysis test, urease test, Gelatin hydrolysis and lipase test.

Five acid producing colonies were placed on Muller Hinton Agar (MHA) plates containing lawn of *Bacillus coagulans*. Among these, strain 3 and 5 produced highest inhibition zone and were selected.

The bacteriocin producing bacteria were identified as rod shaped Gram negative bacteria and their CAP has considerable antimicrobial activity against food borne pathogens like *Pseudomonas sp*, *Staphylococcus aureus and Salmonella typhi.*

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