

## Research Article

Isolation and Characterization of Bacteriocin Producing *Lactobacilli* from Dairy Butter Sample

M.Kannahi\*, N.Viji

PG and Research Department of Microbiology, Sengamala Thayyar Educational Trust women's College, Mannargudi, Tamilnadu, India.

\*Corresponding author's E-mail: kannahisri79@gmail.com

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## ABSTRACT

The present study was carried out with the lactic acid bacteria isolation and identification such as *Lactobacillus lactis*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus brevis* and *Lactobacillus fermentum* from butter sample. Five isolates of *Lactobacilli* supernatant was examined for acid and Bacteriocin production. The extracted compound showed antibacterial activity against the pathogenic microorganisms. *Lactobacillus fermentum* showed better antimicrobial activity on *Streptococcus pneumoniae* (32mm) followed by *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Klebsiella pneumoniae*. *Lactobacillus fermentum* had exhibited better antibacterial activity on *Streptococcus pneumoniae* (32mm) and *E.coli* (29mm). The results obtained from this study was the isolated Bacteriocin producing species of *Lactobacilli* play important role in the preservation of fermented food which is usually achieved by inhibition of contaminating spoilage bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus cereus*. This study revealed the possibility of using bacteriocin as food biopreservative to control food spoilage and pathogenic bacteria.

**Keywords:** Antimicrobial agent, Bacteriocin, *Lactobacillus* sps, Butter sample

## INTRODUCTION

Lactobacilli are important organisms recognized for their fermentative ability as well as their health and nutritional benefits.<sup>1</sup> They produce various compounds such as organic acids, diacetyl, hydrogen peroxide and Bacteriocin or bacterial proteins during lactic acid fermentations.<sup>2</sup> The use of lactic acid bacteria and their metabolites to improve microbiological safety and extend the shelf life of foods is defined as biopreservative. Bacteriocins are extra-cellularly released peptides or protein molecules, with a bactericidal or bacteriostatic mode of action against closely related species.

## Lactic Acid Bacteria

Lactic acid bacteria (LAB) are ubiquitous in nature and as a consequence are present as natural contaminants on a variety of food. Genera belong to the LAB family include *Lactococcus* sp., *Lactobacillus* sp, *Leuconostoc* sp, *Weissella* sp and *Pediococcus* sp., as well as *Streptococcus* sp and *Enterococcus* sp.<sup>3</sup>

Micro-organisms are important in dairy products. One of the most important groups of acid producing bacteria in the food industry is the Lactic Acid Bacteria (LAB) which is used in making starter culture for dairy products.

The proper selection and balance for starter culture is critical for the manufacture of fermented products of desirable texture and flavor. The microbiological quality of milk products is influenced by the initial flora of raw milk. It has also been recognized that Lactic Acid Bacteria (LAB) are capable of producing inhibitory substances other than organic acid (lactate and acetate) that are antagonistic

toward other microorganisms.<sup>4</sup> A number of different factors have been identified to contribute to the antimicrobial activity of LAB. Bacteriocins and their potential role as novel food preservatives have recently received greater attention as most of them are heat stable and amenable to proteolytic inactivation.<sup>5</sup>

*Lactobacillus*

They occur as single cells or loosely associated chains of bacteria. They are rarely motile and non-spore forming. They are Voges Proskauer and urease positive and it also produce acid on Triple sugar iron agar medium. It requires enriched media to grow and produce lactic acid as a major end product of sugar metabolism. *Lactobacilli* are commonly present in decomposing plant material, milk and other dairy products.

The objective of the present study was to isolate, characterize and identify the Bacteriocin *Lactobacilli* from butter sample. Total five bacterial isolates were isolated, out of this all the five isolated species were selected for further work, which shows antimicrobial activity the isolates were tested against with enteric and spoilage causing pathogens (*Lactobacillus lactis*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus brevis* and *Lactobacillus fermentum*).<sup>6</sup>

## MATERIALS AND METHODS

## Sample Collection

The Butter sample was collected from local market at Mannargudi in Thiruvavur District. The sample were collected and brought to the laboratory and kept in refrigerator at 4°C for the isolation of bacteriocin producing lactic acid bacteria.



## Total Cell Count

The most common method of enumerating the total microbial cells is the direct counting of cell suspension in a counting chamber of known volume using a microscope. One such counting chamber is Neubauer counting chamber. Another method involves an electric instrument, Coulter counter. In this instrument the number of cells is counted in directly by finding out the loss of conductance when the cells pass through a narrow orifice. A clean glass slide was marked as 1cm<sup>2</sup> area. 0.01ml of butter sample was spreaded and allows the slide to air dry. Then the slides are immersed in xylene solution to remove fat materials for 1 minute. The slides were washed and stained with methylene blue for 1-2 minutes. The slides were examined under oil immersion microscope and count the number of microorganisms in the field.<sup>7</sup>

## Isolation and Identification of Bacteriocin Producing lactic acid Bacteria

The dairy butter sample was inoculated into Man Rogosa Sharpe (MRS) broth After incubation, it was streaked in MRS agar at 37°C for 48hrs under anaerobic condition for isolation of Lactic acid bacteria. Well isolated colonies with typical characteristics namely pure white, small (2-3 mm diameter) with entire margins were picked from each plate and transferred to MRS broth. The lactic acid bacteria were identified on the basis of growth on selected MRS agar. Morphology, Gram staining and Biochemical tests were performed as a primary screening for identification of Lactic acid bacteria.<sup>8</sup>

Gram positive, rod shaped, non-spore forming and Citrate negative strain was selected for further studies. Further identification of the species of the lactobacilli were performed according to the biochemical test MR-VP, catalase test and growth on MRS broth as described in Bergey's Manual of systematic Bacteriology. The isolated lactobacilli were sub cultured and the purified cultures were maintained.

## Production of Bacteriocin

The isolated strains was propagated in MRS broth (1000 ml) seeded with 10% inoculum (10<sup>8</sup>CFU/ml) of overnight culture and incubated at 37°C for 48hrs. After incubation, the whole broth was centrifuged at 10,000 rpm for 15 min and the cell-free supernatant was used as crude bacteriocin.

These supernatant were tested for the retention of antibacterial activity against pathogenic microorganisms by well diffusion method.<sup>9</sup>

## Antibacterial Activity of the Bacteriocin Producing Isolates

One ml suspension from each isolate namely A, B, C, D and E was inoculated in 50ml sterile MRS broth flasks separately. These flasks were incubated at 37°C for 48

hrs, under anaerobic condition and uninoculated broth was used as a control.

Bacteriocin producing each isolate were used to study their antibacterial effects on different pathogenic bacteria namely *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumonia* and *Bacillus cereus*.

Antimicrobial activity of Bacteriocin against all above test microorganisms was determined by well diffusion method. Under aerobic conditions, Agar plates were seeded with test microorganisms after growing them in a nutrient broth and diluting appropriately. The inhibitory activity against all pathogenic microorganisms was tested on nutrient agar. Antibacterial activity of extracts was evaluated by agar well diffusion method after the medium was solidified, wells of 6mm were made in the plates with the help of a cork borer. Supernatant of the isolates was added into each well. After incubation, the antimicrobial activity was determined by measuring the diameter of the inhibition zone (mm) around the wells at 37°C for 24 hrs.<sup>10</sup>

## RESULTS AND DISCUSSION

### Collection of Sample

The present study, deals with bacteriocin producing strains were isolated and identified from Butter samples purchased from the local market at Mannargudi, Thiruvavur District. Using *Lactobacillus lactis* (A), *Lactobacillus bulgaricus* (B), *Lactobacillus plantarum* (C), *Lactobacillus brevis* (D) and *Lactobacillus fermentum* (E).

### Total bacterial count

The butter samples have high levels of the microbial quantity at different time intervals. The colony forming unit were recorded as *Bacillus megaterium* (10), *Escherichia coli* (29), *Streptococcus Pnuemoniae* (18), *Bacillus cereus* (26), *Proteus vulgaris* (21), *Staphylococcus aureus* (30), *Lactobacillus* (24), *Lactobacillus brevis* (20), *Salmonella typhi* (15) and *Klebsiella pnuemoniae* (12).

### Isolation and identification of bacteria

Serial dilution and plating method were used for the isolation of bacterial colonies. After incubation the white and pale white colour colonies were observed on nutrient agar plates. Five isolates of *Lactobacilli* supernatant was examined for acid & Bacteriocin production and performed inhibitory effects on the pathogenic microorganisms. The extracted compound showed antibacterial activity against pathogenic microorganisms (Table-1).

### Antibacterial activity of *Lactobacilli*

All the *Lactobacilli sp* were well performed for the antibacterial activity of tested pathogen, *Lactobacillus fermentum* showed better antimicrobial activity on *Streptococcus pneumoniae* (32mm) followed by



*Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Klebsiella pneumoniae*. *Lactococcus bulgaricus* have exhibited antibacterial activity on *Escherichia coli* (28mm) followed by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Bacillus cereus*. *Lactobacillus plantarum* have exhibited antibacterial activity on *Bacillus cereus* (27mm) followed by *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Streptococcus pneumoniae*. *Lactococcus lactis* showed that antibacterial activity on *Staphylococcus aureus* (25mm) followed by *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus cereus*. *Lactococcus brevis* have exhibited antibacterial activity on *Klebsiella pneumoniae* (20mm) followed by *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae* and *Bacillus cereus*.

The results of *Lactobacillus fermentum* was showed better antibacterial activity on *Streptococcus pneumoniae* (Table-2). Of these five isolates *Lactobacillus fermentum*.

Reported that the lactic acid bacteria originally isolated from raw goat's milk are probably the best candidates for improving the microbiological safety of dairy product because they are well adapted to the condition of milk and should be more competitive than lactic acid bacteria from other sources and the results from the present study suggest that the bacteriocin producing strain *Lactobacillus plantarum* could be used to improve the safety of traditional fermented foods of dairy origin were *Lactobacillus* commonly occur. The selective use of this bacteriogenic strain may improve the microbiological quality of such foods. Similar results have been observed in experiment related to inhibitory effect of bacteriocin produced by other *Lactobacillus* species.<sup>11</sup>

**Table 1:** Morphological and biochemical characters of bacterial isolates

S. No.	Name of the Test	(A)	(B)	(C)	(D)	(E)
1.	Gram staining	+	+	+	+	+
2.	Motility	Non motile	Non motile	Non motile	Non motile	Non motile
3.	Shape	Rod	Rod	Rod	Rod	Rod
4.	Indole test	-	-	-	-	-
5.	Methyl red test	+	+	-	-	+
6.	Voges-Proskaur test	-	-	+	-	-
7.	Citrate test	-	-	-	-	-
8.	Triple Sugar iron test	-	-	-	-	-
9.	Oxidase test	-	-	-	-	-
10.	Catalase test	-	-	-	+	-
11.	Carbohydrate fermentation test	-	-	-	-	-

(+) Positive  
(-) Negative

**Table 2:** Detection of antibacterial activity of the bacteriocin producing bacterial isolates

S. No.	Cultured broth	Zone of inhibition (mm) against the bacterial pathogens				
		<i>S.aureus</i>	<i>K.pneumoniae</i>	<i>B.cereus</i>	<i>E.coli</i>	<i>S.pneumoniae</i>
1	<i>Lactobacillus lactis</i>	25 mm	23 mm	20 mm	22 mm	24 mm
2	<i>Lactobacillus bulgaricus</i>	23 mm	21 mm	20 mm	28 mm	21 mm
3	<i>Lactobacillus plantarum</i>	15 mm	17 mm	18 mm	29 mm	/*ERav74/*-+ + + + , 78=wevbuuhhhgbbb=78+-Z-081
4	<i>Lactobacillus brevis</i>	21 mm	20 mm	16 mm	17 mm	19 mm
5	<i>Lactobacillus fermentum</i>	22 mm	21 mm	17 mm	29 mm	32 mm

A- *Lactobacillus lactis*  
B- *Lactobacillus bulgaricus*  
C- *Lactobacillus plantarum*  
D- *Lactobacillus brevis*  
E- *Lactobacillus fermentum*



## CONCLUSION

*Lactobacilli* are important organisms recognized for their fermentative ability as well as health and nutritional benefits. They produce various compounds such as organic acids, diacetyl, hydrogen peroxide and Bacteriocin or bacterial proteins during lactic acid fermentations. The present study was carried out with the lactic acid bacteria isolation and identification such as *Lactobacillus lactis*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus brevis* and *Lactobacillus fermentum* from butter sample.

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This study revealed the possibility of using bacteriocin as food biopreservative to control food spoilage and pathogenic bacteria.

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