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Research Article

SCREENING, PRODUCTION AND ANTIBACTERIAL ACTIVITY OF BACTERIOCIN FROM *Lactobacillus* spp.

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ABSTRACT

The intent of the study is to determine the antimicrobial activity of *Lactobacillus* producing bacteriocin isolated from samples like dairy product (milk, curd), meat (mutton, chicken), sea food (fish, black prawns, white prawns), and alcoholic beverages (red wine, rose wine). The isolation was carried out by using de Man Rogosa Sharpe (MRS) agar medium. Total 55 isolates were obtained from 12 samples. The isolates from samples were confirmed as *Lactobacillus* spp. based on their morphological and biochemical characteristics. According to research work, 10 different isolates of *Lactobacillus* spp. were isolated from samples under study they are *Lactobacillus fermentum, Lactobacillus lactis, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus alimentarius, Lactobacillus buchneri, Lactobacillus sakei*, *Lactobacillus curvatus*, and *Lactobacillus farciminis*. There has been an explosion of basic and applied research on *Lactobacillus* spp. bacteriocins, primarily due to their potential application as biopreservatives in food and food products to inhibit the growth of food borne bacterial pathogens. Isolates were subjected to antibacterial activity test using agar well diffusion method. *Escherichia coli (*MTCC No.118), *Staphylococcus aureus* (MTCC No.737) and *Bacillus cereus* (MTCC No.1305) were found to be sensitive to bacteriocin produced by *Lactobacillus* spp. whereas, *Salmonella typhi (*MTCC No.733) was found to be resistant to bacteriocin. Therefore, it has a potential for application as a biopreservative in different food product as such or in combination with other preservation methods.

KEY WORDS: Bacteriocin, *Lactobacillus,* Antibacterial activity.

INTRODUCTION

Bacteriocins are a kind of ribosomally synthesized antimicrobial peptides [3], which can kill or inhibit bacterial strains closely-related or non-related to produced bacteria, but will not harm the bacteria themselves by specific immunity proteins. Bacteriocins become one of the weapons against microorganisms due to the specific characteristics of large diversity of structure and function, natural resource, and being stable to heat [4].

Many recent studies have purified and identified bacteriocin for application in food technology, which aims to extend food preservation time [1] [2], treat pathogen disease and cancer therapy, and maintain human health. Therefore, bacteriocins may become a potential drug candidate for replacing antibiotics in order to treat multiple drugs resistant pathogens in the future.

Bacteriocin is produce *by Bifidobacterium, Enterococcus faccium, Rastonia solanaccarum*, non-pathogenic *Escherichia coli*, yeast like *Saccharomyces boulardii* and Lactic acid bacteria. *Lactobacillus* is widely used in the food industry as starter culture for fermentation [5]. *Lactobacilli* have been used since decades against infectious diseases and have been extensively studied for

their ability to protect against pathogens. *Lactobacillus* bacteriocins are grouped as class-I bacteriocins (lantibiotics), class-II bacteriocins (heat-stable, non-lantibiotics), class-III bacteriocins (heat labile proteins with large molecular mass) and class-IV (hydrophobic and heat-stable proteins, associated with lipids or carbohydrates). The lantibiotics in general have wider spectrum of activity than the non-lantibiotics [3].

MATERIALS AND METHOD

Sampling

The dairy products [6] [13] like cow milk, buffalo milk, goat milk, curd from Botad, homemade curd, meat product [10] like chicken, mutton, sea foods [12] like fish, black prawns, white prawns, alcoholic beverages like red wine, rose wine samples were collected in sterile screw cap tubes.(Table.1)

Table 1: Sampling sites for samples

Isolate	Sites
C1	Curd from Pardi
PC1	Curd from Botad
M1	Buffalo milk from Pardi
CS1	Cow milk from Surat
G1	Goat milk from Olpad
OC1	Chicken from Olpad
OM1	Mutton from Olpad
F1	Fish from Olpad
BP1	Black prawns from Olpad
PP1	White prawns from Olpad
RS1	Rose wine from Sula
RD1	Red wine from Sula

Isolation of *Lactobacillus*

The collected samples were serially diluted (10^{-1} to 10^{-6}) in sterile distilled water. The diluted samples were plated (0.1 ml suspension) onto de Man Rogosa Sharpe (MRS) agar plates [9] and incubated at 37ºC for 48 hours. In a total of 55 different colonies initially observed.

Phenotypic Identification

The Morphological and colony characteristics were studied using de Man Rogosa Sharpe (MRS) agar plates [9]. The physiological characteristics of all the obtained isolates were studied. The biochemical characteristics (Indole, Catalase, Voges-Proskauer, Methyl Red, Citrate, Hydrogen sulphide production, Nitrate reduction, Gelatin hydrolysis test, Bile-Esculin) and sugar fermentation tests were also carried out using standard reference Bergey's Manual of Systematic Bacteriology [14].

Production of Bacteriocin

For the preparation of the inoculums, 10 ml of MRS broth was inoculated with 0.1 ml of freshly prepared culture of *Lactobacillus* spp. isolate and was incubated for 48 hours at 37ºC. From this pre-culture, 1 ml was added to 100 ml of MRS broth medium which after 48 hours of incubation at 37ºC, was used to inoculate the production media [16].

Antibacterial Activity against Indicator Bacteria

The recovered isolates were subjected for antibacterial activity by using agar well diffusion method [8] [10] [22]. The isolates and the indicator strains [*Escherichia coli (*MTCC No. 118), *Salmonella typhi (MTCC* No. 733), *Staphylococcus aureus* (MTCC No.737), *and Bacillus cereus (MTCC No.* 1305)] were grown in MRS broth and nutrient broth respectively [15]. The MRS broth were inoculated with 1 % (v/v) of the active culture of the recovered isolates and incubated at 37ºC for 48 hours. Cells were harvested by centrifugation at 6000 rpm for 15 minutes at 4 ºC and 50 µl supernatant were placed in 5mm diameter wells that had been cut in agar plates previously seeded with the indicator bacteria. The plates were incubated at 37ºC for 24 hours. After incubation, the diameter of zone of growth of inhibition was measured.

RESULTS

Isolation of *Lactobacillus* spp. was carried out by using de Man Rogosa Sharpe (MRS) agar medium.

Figure 1: de Man Rogosa Sharpe (MRS) agar medium

The total number of isolates were counted and further proceeded for their morphological and motility test. The colony characteristics of the obtained isolates were studied on de Man Rogosa Sharpe (MRS) agar plate, which gives white / creamy, circular, small / large colonies with entire margin after 48 hours of incubation (Fig 1). They were gram positive generally long rods sometimes they are short rods, coccoid and they were non motile. Following Table.2 shows result of various biochemical characteristics of the isolates.

Table 2: Biochemical characterization of the isolates.

Isolates	Biochemical Tests																				Probable Identity
	Tests								Carbohydrates												
	Indole	Catalase	Methyl Red	V-P Test	H_2S Production	Nitrate Reduction	Gelatin Hydrolysis	Bile-Esculin	Arabinose	Cellobiose	Lactose	Mannitol	Melibiose	Salicin	Sorbitol	Sucrose	Raffinose	Trehalose	Xylose		
C1	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	-	+	-		*L. lactis*
C2	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+		*L. fermentum*
C3	-	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+	+	-	+		*L. plantarum*
C4	-	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+	+	-	+		*L. plantarum*
C5	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	-	-		*L. sakei*
C6	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-	+		*L. buchneri*
C7	-	-	-	-	-	+	-	+	+	+	-	+	+	-	+	+	+	-	+		*L. plantarum*
C8	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-	+		*L. buchneri*
C9	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+		*L. fermentum*

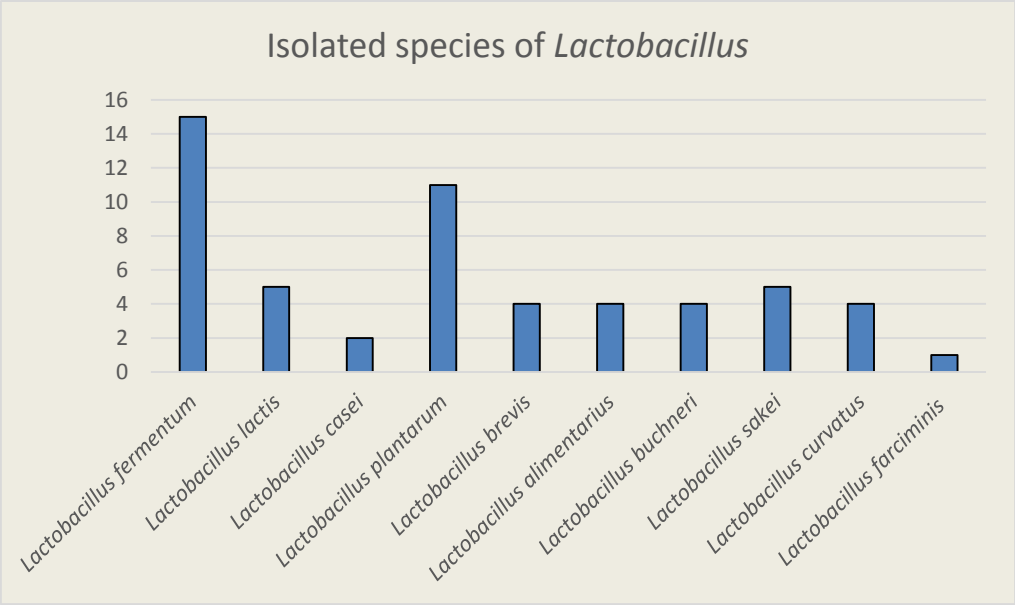
M1	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	-	+	-	*L. lactis*
M2	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	-	-	*L. sakei*
M3	-	-	-	-	-	+	-	+	+	+	-	+	+	-	+	+	+	-	+	*L. plantarum*
M4	-	-	-	-	-	+	-	+	+	+	-	+	+	-	+	+	+	-	+	*L. plantarum*
PC1	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
PC2	-	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+	+	-	+	*L. plantarum*
PC3	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-	+	*L. brevis*
PC4	-	-	-	-	-	+	-	+	+	+	-	+	+	-	+	+	+	-	+	*L. plantarum*
PC5	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-	+	*L. buchneri*
PC6	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	-	-	*L. sakei*
PC7	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	-	+	-	*L. lactis*
PC8	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	-	-	*L. sakei*
G1	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
G2	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
G3	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-	+	*L. brevis*
OM1	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	+	*L. alimentarius*
OM2	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	*L. sakei*
OM3	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	*L. curvatus*
OM4	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	+	*L. alimentarius*
OC1	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
OC2	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
OC3	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
OC4	-	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+	+	-	+	*L. plantarum*
CS1	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	-	+	-	*L. lactis*
CS2	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
CS3	-	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+	+	-	+	*L. plantarum*
CS4	-	-	-	-	-	-	-	+	-	+	-	+	+	-	+	+	-	+	-	*L. casei*
CS5	-	-	-	-	-	+	-	+	+	-	-	-	+	-	-	+	+	-	+	*L. brevis*
F1	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-	+	*L. brevis*
F2	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
F3	-	-	-	-	-	+	-	+	+	+	-	+	+	-	+	+	-	+	-	*L. casei*
F4	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
F5	-	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+	+	-	+	*L. plantarum*

F6	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	+	*L. alimentarius*
BP1	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	*L. curvatus*
BP2	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	+	-	-	+	*L. alimentarius*
BP3	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
BP4	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	*L. curvatus*
BP5	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
PP1	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	-	+	-	*L. lactis*
PP2	-	-	-	-	-	+	-	-	-	+	+	-	-	+	-	+	-	-	-	*L. farciminis*
PP3	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	*L. curvatus*
PP4	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	+	+	-	+	*L. plantarum*
RS1	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-	+	*L. buchneri*
RD1	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*
RD2	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	*L. fermentum*

'+': Positive, '-': Negative

By studying the morphology and biochemical characteristics, we found various species of *Lactobacillus* such as *Lactobacillus fermentum, Lactobacillus lactis, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus alimentarius, Lactobacillus buchneri, Lactobacillus sakei, Lactobacillus curvatus*, and *Lactobacillus farciminis*. The total number of isolates and its types are shown in fig 2.

Figure 2: Isolated species of Lactobacillus



Bacteriocin Assay

In total of 55 isolates, 24 isolates were able to produce bacteriocin. (6 from homemade curd, 2 from buffalo milk, 8 from town curd, 1 from cow milk, 1 from white prawns, 2 from black prawns , 2 from chicken and 2 from mutton).

Agar well Diffusion Assay

Bacteriocins obtained from the isolates showed inhibitory activity against *Escherichia coli* (MTCC No.118), *Staphylococcus aureus* (MTCC No.737) and *Bacillus cereus* (MTCC No.1305). *Salmonella typhi* (MTCC No.733) was resistant to bacteriocin producers. PC3, PC5, OC4, and OM4 showed very strong inhibition (Fig 3) against *Bacillus cereus* (MTCC No.1305) with zone of inhibition 20mm, 22mm, 19mm, and 18mm (Table No.3).

Figure 3: Antibacterial activity of Bacteriocin

Table 3: Inhibition of indicator bacteria by bacteriocin produced by isolates

ISOLATE NO.	INDICATOR STRAIN			
	***Bacillus cereus* (MTCC NO.1305)**	***Escherichia coli* (MTCC NO. 118)**	***Staphylococcus aureus* (MTCC NO.737)**	***Salmonella typhi* (MTCC NO.733)**
C1	15mm	-	12mm	-
C2	17mm	-	9mm	-
C3	12mm	-	15mm	-
C4	16mm	-	12mm	-
C5	19mm	-	-	-
C9	12mm	-	-	-
M1	15mm	-	-	-
M2	12mm	7mm	10mm	-
PC1	17mm	5mm	-	-
PC2	-	6mm	-	-
PC3	20mm	-	8mm	-
PC4	17mm	-	-	-
PC5	22mm	-	-	-
PC6	12mm	-	-	-

PC7	15mm	5mm	-	-
PC8	13mm	6mm	-	-
OM1	15mm	-	-	-
OM4	17mm	-	17mm	-
OC3	12mm	-	-	-
OC4	15mm	-	15mm	-
CS5	12mm	-	-	-
BP4	12mm	-	-	-
BP5	12mm	-	-	-
PP1	15mm	-	-	-

'-': No zone of inhibition

C6,C7, C8, M3, M4, G1, G2, G3, OM2, OM3, OC1, OC2, CS1, CS2, CS3, CS4, F1, F2, F3, F4, F5, F6, BP1, BP2, BP3, PP1, PP2, PP3, PP4, PP5, RS1 and RD2 were not able to produce inhibitory effect against indicator bacteria.

DISCUSSION

The study highlights the screening, production and antibacterial activity of bacteriocin from *Lactobacillus* spp. A total of 55 isolates were obtained from dairy products, meat, fish and wine samples. Isolates were confirmed as *Lactobacillus* spp. based on their morphological and biochemical characteristics. Kandler and Weiss (1986) have classified *Lactobacillus* isolates according to their morphology, physiology and biochemical test. *Lactobacillus plantarum* and *Lactobacillus fermentum* found in almost all samples. Singh and Rakesh Roshan Sharma (2009) stated that *Lactobacillus fermentum* and *Lactobacillus casei* were found in cow milk. Same results were found in our study. Among 55 isolates 24 isolates were able to produce bacteriocin. Isolates were show antibacterial activity against *Escherichia coli (*MTCC No.118), *Staphylococcus aureus* (MTCC No.737), and *Bacillus cereus* (MTCC No.1305) whereas; *Salmonella typhi* (MTCC No.733) was resistant to bacteriocin. Daeschel and Mc Kenney (1990) found the highest inhibitory activity by *Lactobacillus plantarum*. In red wine sample same result was not found in our study.

CONCLUSION

The study revealed that bacteriocin from *Lactobacillus* spp. isolated from different samples possesses a wide spectrum of inhibitory activity against *Staphylococcus aureus* (MTCC No.737), *Bacillus cereus* (MTCC No.1305) and *Escherichia coli (*MTCC No.118). Therefore, it has a potential for application as a biopreservative in different food products as such or in combination with other preservation methods.

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