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Isolation and Screening of bacteriocin producing lactic acid bacteria from milk and milk products

*V. Sumathi, D. Reetha**
Dept of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai nagar-608002, Tamil nadu, India

**Corresponding Author, Email: drjeban@rediffmail.com*

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Abstract

One of the most important groups of acid producing bacteria in food industry is the lactic acid bacteria,(LAB). LAB are widespread in nature and predominate in microflora of milk and its products LAB are known to produce bacteriocin and have great potential in food bio preservatives. In the present study a total of 160 strains of LAB were isolated from eight different milk and milk products collected from Dairy Farm, Annamalai University. They were screened for the production of bacteriocin active against *Listeria monocytogenes*. Among the 160 strains 22 strains were observed as positive bacteriocin producers. The highest percent (25.0%) of bacteriocin producers was recorded in butter followed by yoghurt (20%), curd (18.75%) buffalo milk (16.66%), Goat milk (13.88%) Cheese (11.11%) cow milk (10%) and dairy milk (6.25%). The bacteriocin producers were identified upto generic level as *Enterococcus, Lactococcus, Lactobacillus* and Streptococcus. There was a striking predominance of the genus Enterococcus (55%) followed by *Lactococcus* (22%) in the milk and milk products. The spectrum of inhibitory activity of the bacteriocin producers against Gram positive and Gram negative pathogens were tested and observed that, activity is restricted to Gram positive organisms, but *Lactobacillus* and *Streptococcus* were active against both Gram positive and Gram negative organisms. The bacteriocins produced by LAB are to be characterized in the further studies.

1. Introduction

Microorganisms 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 are used in making starter culture for dairy products.

Lactic acid bacteria are a group of related bacteria that produce lactic acid as a result of carbohydrate fermentation. These microbes are broadly used by us in the production of fermented food products, such as Yogurt (*Streptococcus Spp.* and *Lactobacillus Spp.*), Cheeses (*Lactococcus Spp.*), *Sauerkraut* (*Leuconostoc Spp.*) and Sausage.

Indigenous micro flora of lactic acid Bacteria play an important role in milk and food products. These acid bacteria are a group of gram positive, non-sporing, non-respiring cocci or rods which produce lactic acid as the major end product during the fermentation. Some of the metabolites of these bacteria have an antimicrobial effect against many food spoilage and pathogenic bacteria, include lactic acid, diacetyls, hydrogen peroxide and proteinaceous substances bacteriocins (1,2).

LAB are known for their production of antimicrobial compounds, including bacteriocins or bacteriocin – like peptides. Bacterocins of LAB are defined as ribosomally synthesized proteins or protein complexes usually antagonistic to genetically closely related organisms (3,4).

Listeria monocytogenes is one of the pathogens of concern to food safety due its widespread present in the environment, its high virulence and its resistance in stressful conditions (5).

L. Monocytogenes has been shown to be sensitive to many bacteriocins produced by LAB. In particular, all bacteriocins of the lantibiotics and the subclass II a are, by definition, inhibitory to *L. monocytogenes* (6,7). Therefore, use of these antimicrobial substances or their producer strains as a biological means to enhance the control of specific food borne pathogens is worthwhile considering and hence, search for new

bacteria cin producing LAB potentially useful to food preservation should continue.

This study aimed to isolate as large as possible number of bacteriocin producing LAB active against *L. monocytogenes* from milk and milk products and to screen the inhibitory effect against certain food borne pathogens.

2. Materials and Methods

2.1. Collection of samples

Four different milk samples namely dairy milk, cow milk, Buffalo milk, and Goat milk and four different milk products viz, butter, curd, yoghurt and cheese were collected from the Dairy farm of Annamalai university, Annamalai nagar, Tamilnadu, India. The samples were aseptically collected and brought to the laboratory of Department of Agriculture Microbiology, Annamalai University, via ice box for the isolation of bacteriocin producing lactic acid bacteria and further screening of them.

2.2. Strains and culture conditions

The indicator organism used in the present study, *L. monocytogenes* ATCC 7644, and the food borne pathogen of Gram positive the and Gram negative (*Bacillus cereus*, *Staphylococcus aureus*, *E.coli, Salmonella typhi* and *Enterococcus cloacae*) were obtained from the Department of clinical microbiology, Rajah muthiah medical college, Annamalai university. All the above test organisms and the lactic acid bacterial strains isolated in the present study were maintained in MRS Agar at 4°C and subcultured every three weeks.

2.3. Isolation of bacteriocin producing lactic acid bacteria

The bacteriocin producing LAB were isolated from the above collected milk and milk products. Ten fold dilutions of each sample was done with sterile water and were plated on MRS (de Rogosa and sharpe) medium (8) and then incubated for 24 hrs at 30C, After incubation, colonies were selected, grown in MRS broth. All the strains were subjected to gram staining and catalase production test. The Gram positive and catalase negative strains were selected and maintained for further studies. The strains were tested for their bacteriocin producing ability against *Listeria monocytogenes* (ATCC 7644) by the well diffusion assay described by (9). The bacteriocin positive colonies of significant diameter of zone of inhibition against the indicator organism were recorded and identified upto generic level, according to Bergey's manual of determinative bacteriology (1994).

2.4. Detection of inhibitory activity of bacteriocin producers

Cell free culture supernatants obtained from the bacteriocin producers viz, *Lactococcus*, *Lactobacillus*, *Streptococcus* & *Enterococcus* by centrifugation of cultures at 8,000 g at 4°C for 10 min were adjusted to pH 6 with 1N NaoH, filtered through 0.45 μm pore size filters and stored at -20°C until use.

The antimicrobial activity of the supernatant was determined by the well diffusion method (10).

The supernatant (100 μl) was placed in wells (8mm in diameter) cut in MRS plates (20ml) seeded (1% v/v) with the Gram positive (L. *monocytogenes, Bacillus cereus*, *Staphylococcus aureus*) and Gram negative (*E. coli, Salmonella typhi, Enterococcus cloacae)* organisms. The plates were incubated at 37°C for 24 hrs. the diameter of the zones of growth inhibition were then measured.

3. Results and Discussion

3.1. Isolation of bacteriocin producers

A total of 160 lactic acid bacterial strains were isolated from four different milk samples (dairy milk, cow milk, buffalo milk, and Goat milk) and four different milk products (curd, butter, yoghurt and cheese). All the 160 strains were tested for their bacteriocin producing ability against the indicator organism *L. monocytogenes* and the percent of positive strains of bacteriocin producers were given in Table-1.

Table 1. Frequency of bacteriocins Producing lactic acid bacteria Isolated from milk and milk products

Sl. No	Source	Total isolates of LAB	No. of positive Bacteriocin Producers	% of B+ isolates
1	Dairy milk (Annamalainagar)	32	2	6.25
2	Cow milk	20	2	10.00
3	Buffalo milk	24	4	16.66
4	Goat milk	36	5	13.88
5	Curd	16	3	18.75
6	Butter	8	2	25.00
7	Cheese	9	1	11.11
8	Yoghurt	15	3	20.00
	Total	160	22	13.75%

The results showed that among the 160 isolates about 70% (112 isolates) were isolated from the milk samples and the remaining 30% (48 isolates) were isolated from the samples of milk products. Out of 160 isolates of LAB only 22 isolates (13.75%) were observed as the positive for bacteriocin production against *L. monocytogenes*. Among the different samples tested, Butter recorded the highest percent of bacteriocin producers (25.0%) followed by yoghurt (20%), curd (18.75) Buffalo milk (16.66) Goat milk

(13.88%) Cheese (11.11%) Cow milk (10.0%) and dairy milk (6.25%).

3.2. Identification of bacteriocin producers

All the 22 bacteriocin producers were studied for their morphological and certain biochemical characteristics and identified upto generic level, according to Bergey's manual of determinative bacteriology as, *Lactococcus Sp.* (5 strains) *Lactobacillus sp.* (3 strains), *Enterococcus sp.* 12 strains) and *Streptococcus Sp.* (2 strains). The results showed that a striking predominance of the genus *Enterococcus* (55%) followed by *Lactococcus* (22%).

3.3. Inhibitory activity of bacteriocin producers

The spectrum of inhibitory activity of identified bacteriocin producers were tested by well diffusion method, against Gram positive and Gram negative organisms and the results are given in Table-2. The results showed that the spectrum of inhibitory activity was restricted to Gram positive organisms both by *Lactococcus* and *Enterococcus*, where as the *Lactobacillus* and *Streptococcus* were able to inhibit both Gram negative and Gram Positive organisms. The results of the present study confirms the high incidence of *Enterococcus* in milk and milk products, reported in other studies (11).

Table 2. Spectrum of inhibitory activity of bacteriocins producers

LAB	Gram Positive pathogens			Gram Negative pathogens		
	Listeria monocytogenes	*Bacillus cereus*	*Staphylococcus aureus*	*Salmonella typhi*	*Enterobacter cloacae*	*E.Coli*
Lactococcus sp.	+++	+	-	-	-	-
Lactobacillus sp.	++++	+	++	+++	+++	+++
Enterococcus sp.	+++	++	++	-	-	-
Streptococcus sp.	+++	++	++	++	++	-

++++-inhibition zone more than 25 mm
+++-Zone between 18-20 mm
++-Zone between 12-16 mm
+-Less than 7 mm
--No inhibition zone

According to Klaenhammer (3) 99% of all bacteria may make at least one bacteriocin. It has been extensively reported that the environmental factors including stressful conditions influence the magnitude of bacteriocin production to overcome the competitive strains living in the same environment (12). The characterization of the bacteriocins produced by the LAB to be done in the further studies.

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