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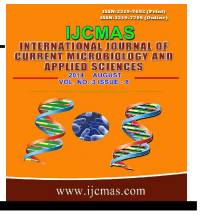

Original Research Article

Screening and Evaluation of Antibacterial Activity of Bacteriocin Producing Lab against some Selected Bacteria Causing Food Spoilage

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Keywords

Bio-preservatives, Fermented foods, Lactic acid bacteria, Lactobacillus, bacteriocins, probiotics.

A B S T R A C T

Bacteriocins are ribosomally synthesized antimicrobial peptides that are active against other bacteria either of the same species (narrow spectrum), or across genera (broad spectrum). The biopreservation of foods using bacteriocinogenic lactic acid bacteria (LAB) isolated directly from foods was considered as an innovative approach. The objectives of this study were to isolate and identify bacteriocin producing LAB from various food samples such as curd, milk and probiotics and to evaluate their antimicrobial effects on selected spoilage and pathogenic microorganisms *in vitro*. LAB were isolated using Nutrient agar and MRS media. The agar diffusion bioassay was used to screen for bacteriocin producing LAB. Antimicrobial activity of broth and cell supernatant was tested against selected pathogens. Only *Staphylococcus aureus* was inhibited by isolates (from milk and curd sample) producing bacteriocin. *Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae* were inhibited by probiotic. Bacteriocin could potentially be used as food preservatives.

Introduction

Technologies on preservation of food products help in maintaining its nutritional values and ensures its safety issues. These are the current areas of food research. For the preservation of food products many chemicals are being used for the activation of food borne pathogens. Chemical preservatives have undesirable side effects like alteration in the constituents, nutritional, and organoleptic properties of the food and toxic effects on human health. Despite all these measures, food borne diseases by pathogenic bacteria do occur frequently.

The ever increasing demands of ready to eat food products without chemical preservation have led to development of new techniques of using biopreservatives. Biopreservatives extends the storage time of high quality foodstuff without using chemical preservatives. Hence with the increasing demand for biopreservatives, bacteriocin has caught the attention of food researchers in industries. [19] Lactic acid bacteria (LAB) produce lactic acid either through homofermentive or heterofermentive pathway and are wide spread in nature and

also found in human digestive system. *Lactobacilli* are considered as beneficial bacteria because they have their ability to break down proteins, carbohydrates & fats in food and help in absorption of necessary elements and nutrients such as minerals, amino acids and vitamins required for the survival of humans and other animals. Genetic and biochemical analysis of this LAB revealed that they produce a variety of antimicrobial substances with potential importance for food fermentation and preservation. Apart from the metabolic end products, some strains also secrete antimicrobial substances known as bacteriocin.

Bacteriocin and the organisms that produce them have potential in the food and feed industry as well as in the pharmaceutical industry as a source of probiotics. [2,12] The probiotic bacteria used in commercial products today are mainly members of the genera *Lactobacillus* and *Bifidobacterium*[1,4,5].The lactic acid bacteria(LAB) are comprised of at least ten genera: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, tetragenococcus and Vagococcus.* [17,18] Most representatives of these group have been consumed for thousands of years, do not pose any health risk to humans and are designated as GRAS organisms (generally recognized as safe). [8]The search for new bacteriocin producing lactic acid bacteria bacteria is of great significance because of their potential use in fermented food and feed industries.

LAB exerts a strong antagonistic activity against many food spoilage organisms and food borne pathogens. [13] Lactic acid bacteria are known to inhibit bacterial pathogens which disturb the normal microbial flora of gastrointestinal tract disturbing the human health. Lab bacteria have a probiotic effect on human health. [11,16] Bacterial pathogens like E.coli, Streptococcus, Salmonella are known to be inhibited by probiotics. It also lead to decrease in toxicity and increase in the amount of nutrient production. [7]

The aim of the study was to isolate bacteriocin producing bacteria from fermented foods (probiotic drinks, curd and milk) by centrifugation, and to evaluate antimicrobial activity of bacteriocins by Agar well diffusion method.

Materials and Methods

The research work for the present investigation has been carried from June 2013 to April 2014.

Isolation of Bacteriocin producing LAB

Sample like curd, milk & probiotic were collected from different sources. 1ml of milk samples and 1gm of curd samples were inoculated in 50ml of MRS broth separately in an aseptic condition. Similarly transfer 1gm of probiotic powder into another flask containing 50ml MRS broth and incubate each flask at 37^0 c for 24hrs.

Strain isolation

After sufficient growth was observed in MRS broth 1 ml of each culture from the sample flask was inoculated in 9 ml of normal saline solution. After homogenization serial dilution were prepared with sterile 0.85% (w/v) sodium chloride upto 10^{-7} and were plated on MRS agar plates. The plates were incubated at temperature 37˚C for 24 h. Different colonies were then sub-cultured to purity in MRS broth. Stock cultures were maintained in glycerol stocks at -20˚C.

Identification of isolates

The identification was done mainly on the basis of morphological, biochemical & cultural characteristics. Morphology was examined by Gram staining [12], motility was observed by hanging drop method. Biochemical characterization was done by sugar fermentation test, Indole, MR-VP & citrate utilization test and catalase test. Cultural characterization based on colony characteristics.

Test culture preparation

The pathogenic bacterial cultures of *Escherichia coli (ATCC No. 10799), Bacillus subtilis (ATCC No. 6633) Staphylococcus aureus (NCIM No. 2021), Klebsiella pneumoniae(NCIM No.2707), Pseudomonas aeruginosa(NCIM No.2037)* were procured from NCL, Pune were used as test organisms for evaluating the antimicrobial activity.

Antimicrobial activity

Gram positive homofermentive catalase negative selected isolates were tested for their antimicrobial activity against *E.coli, B.subtilis S.aureus, K.pneumoniae, P.aeruginosa.* Agar well diffusion assay was performed for analyzing the inhibitory potential against food borne pathogenic test organisms. The isolates showing the wide spectrum of inhibition were selected for further analysis.

Detection of inhibitory activity of bacteriocin by Agar well diffusion assay

The 1ml of culture was taken and was centrifuged in the eppendroffs at 10,000 rpm for 30min at 4^0C. The culture supernatant was neutralized with 1N NaOH. Then 80 µL of broth & culture supernatant of it was added in to the wells of MRS plates seeded with *E.coli, S. aureus, Bacillus subtilis, K. pneumoniae, P. aeruginosa* and *Lactobacillus lactis* (NCIM 2370) as indicator organisms. Then the plates were observed for zone of inhibition. The isolates showing the zone of inhibition were selected.

Results and Discussion

Lactobacillus colonies from milk (3 samples)& curd (3 samples) with typical characteristics namely white, small with entire margin were picked & transferred to nutrient broth which was then subjected to classification based on morphological & biochemical characters. All strains reacted positively to Gram staining under a light microscope. *Lactobacilli* are generally long rods sometimes they are coccoid. *Lactobacillus* do not posses flagella, indole is not produced, acidic & non acidic end products are not produced, citrate not utilized & are catalase negative. All isolates from curd & milk were found to ferment glucose & lactose was also fermented except curd sample 2. Only sample 2 (of curd & milk) ferment mannitol (Table 1,2).

In agar well diffusion assay, the culture broth & supernatant obtained from bateriocins producers strains were tested for antimicrobial activity against the test organisms. Bacteriocins obtained from the isolates of curd (sample 3) and milk (sample 3) showed inhibitory activity against *S .aureus* only (Table 4, 5), bacteriocins produced from probiotic samples showed inhibitory activity against *E.coli, S. aureus and K .pneumoniae* & resistant against *P. aeruginosa* (Table 3).

Lactic acid bacteria are widespread in nature and also found in human digestive system. *Lactobacilli* are considered as beneficial

bacteria, they exert a strong antagonistic activity against many food-contaminating microorganisms. [14] Certain *Lactobacilli* synthesize antimicrobial compounds that are related to the bacteriocins family [3,6]. The probiotic bacteria used in commercial products today are mainly members of the genera *Lactobaccilus* and *Bifidobacterium*[10].

Further, the bacteriocins produced by *Lactobacillus* species isolated from various samples were checked for their antimicrobial activity against *E.coli, S.aureus, K.pneumoniae* and *P.aeruginosa*. From this it was found that bacteriocins from probiotic seem to be most active against *E.coli, S.aureus* and *K.pneumoniae*. The antimicrobial agent from isolated *Lactobacilli* of milk and curd demonstrated a strong antimicrobial activity against *S.aureus.*

From the results it was found that, the bacteriocins are bit tightly attached to the bacteria and therefore showing the zone of inhibition even in cell free supernatant. The zone of inhibition was seen quite more in broth.

The similar antibacterial effect of probiotic was described by Narender et al in 2010 [9]. They showed that, the bacteriocin are bit tightly attached to the cells and so showing the zone of inhibition even in cell free supernatant. They also reported that the zone of inhibition was quite more in broth as compared to CFS.

The inhibitory spectrum of bacteriocin produced by different species of *lactobacilli* varies greatly. Most bacteriocin inhibit only closely related gram positive bacteria, where as others are active against a broad spectrum of gram positive and gram negative bacteria. Svetlana L, Seatovic *et al* [15] examined the effect of the bacteriocins from *L.plantarum* G2 on other microorganisms. They also tested, the neutralized cell-free supernatant for antimicrobial activity against Gram-positive and Gram-negative bacteria by agar well diffusion test and showed that, the bacteriocin was active against all the tested strains of the genera *Lactobacillus*: *L.acidophilus, L.rhamnosus, L.leishmany, L.plantarum* G_1 and *L.casei* G_3 and also inhibited the growth of the pathogenic bacteria *S.aureus* and *S.abony*.

Raja Narender. B *et a.* [9] carried out the Isolation and characterization of bacteriocins from fermented Foods and probiotics. In their experiment they found that the bacteriocins are a bit tightly attached to the cells and so showing the zones of inhibition even in cell free extract. The zone of inhibition was quiet more in broth in both Meat and Probiotic drink.

Organic acids and/or H2O2 produced by LAB had strong antimicrobial effects on all microorganisms. Bacteriocinogenic LAB present in curd, milk, and probiotics may have potential to be used as biopreservatives in foods. LAB can produce antagonistic compound that vary in their spectra of activity. Over all bacteriocins produced by *Lactobacillus* species isolated from milk and curd showed narrow spectrum activity. The bacteriocin produced by *Lactobacillus* species isolated from probiotic showed good activity against both Gram- positive and Gram- negative food spoilage and pathogenic bacteria. The isolated LABs exhibiting excellent probiotic characteristics can be used in controlling and improvement of intestinal microbial flora and thus can contribute in health benefits to consumers. This makes the bacteriocins producing lactic acid bacteria a good antagonistic agent for exploitation in the field of biotechnology.

Table.1 Morphological & Biochemical characterization of *Lactobacillus* isolated from Milk and Curd

Sr. No.	Tests	Milk Sample			Curd Sample		
		Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
1	Morphology	Coccobacilli	Rod	Rod	Rod	Rod	Rod
2	Gram reaction	+	+	+	+	+	+
3	Motility	-	-	-	-	-	-
4	Indole	-	-	-	-	-	-
5	MR	-	-	-	-	-	-
6	VP	-	-	-	-	-	-
7	Citrate	-	-	-	-	-	-
8	Catalase	-	-	-	-	-	-
9	Glucose	+	+	+	+	+	+
10	Lactose	+	+	+	+	-	+
11	Mannitol	-	+	-	-	+	-

Table.2 Antimicrobial activity of Probiotic against test organisms

Test organisms	Probiotic	
	Broth	CFS
E.coli	SI (14mm)	SI (13mm)
S.aureus sps	VSI (18mm)	VSI (16mm)
Klebsiella sps	SI (12mm)	SI (10mm)
Pseudomonas sps	NI	NI

Diameter of well: 0.5cm

Table.3 Antimicrobial activity of bacteriocin (from milk) against test organisms

Test organisms	Milk	
	Broth	CFS
E.coli	NI	NI
S.aureus sps	VSI (16mm)	VSI (15mm)
Klebsiella sps	NI	NI
Pseudomonas sps	NI	NI

Diameter of well: 0.5cm

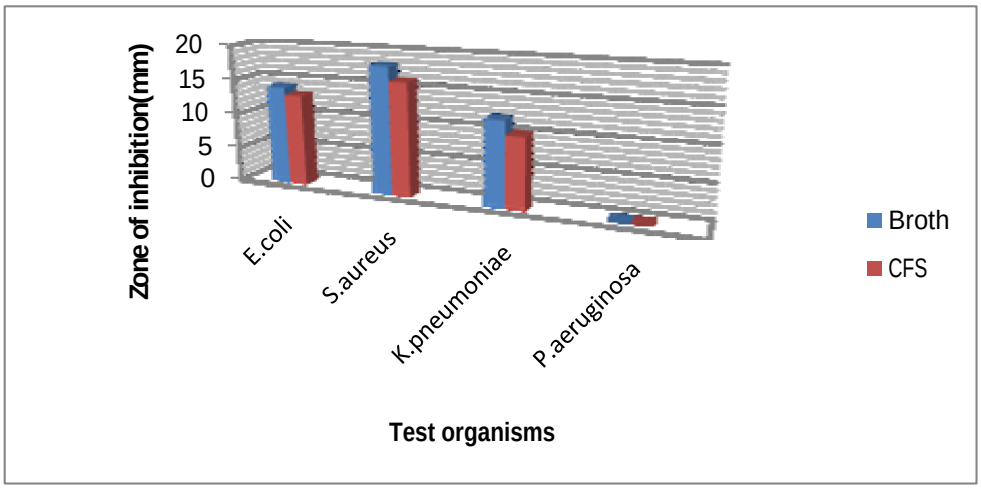
Table.4 Antimicrobial activity of bacteriocin (from curd) against test organisms

Test organisms	Curd	
	Broth	**CFS**
E.coli	NI	NI
S.aureus sps	VSI (17mm)	VSI (14mm)
Klebsiella sps	NI	NI
Pseudomonas sps	NI	NI

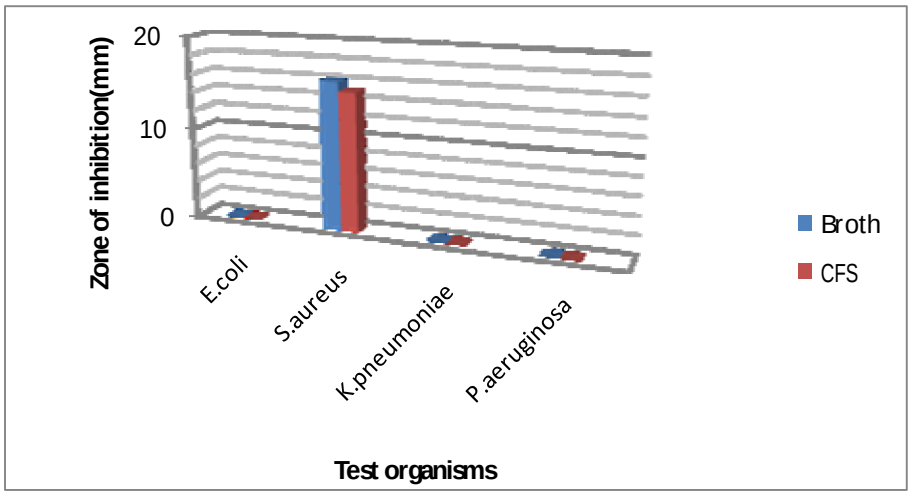
Diameter of well: 0.5cm

Degree of inhibition: -SI: Strong inhibition zone (10-14 mm); **VSI:** Very strong inhibition (15-18 mm); **NI:** No inhibition

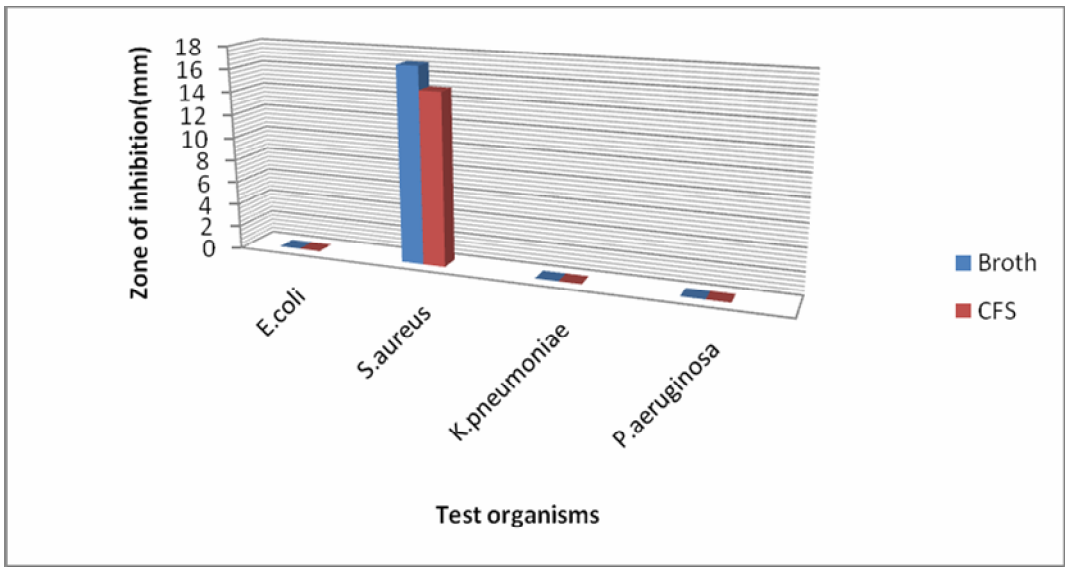
Graph.1 Antimicrobial activity of Probiotic against test organisms



Graph.2 Antimicrobial activity of Bacteriocin (from milk) against test organisms



Graph.3 Antimicrobial activity of Bacteriocin (from curd) against test organisms



In this study, essentially it is noted that bacteriocins has a lytic bactericidal mode of action which can prove helpful in combating food borne pathogens and help in maintain food quality in dairy industries. *Lactobacilli* from milk, curd & probiotic that inhibited certain pathogenic organisms by producing bacteriocin may be beneficial for a probiotic culture to be triumphant in colonizing & to contend with pathogens. Further studies on purification of proteins and its characterization in this concern are recommended.

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References

1. Bonaparte C, Reuter G.(1997). "Bifidobacteria in commercial dairy products: which species are used?" Microecol Ther,26, 181-98.]
2. Benkerroum, N. et al., 2007. " Screening for Lactic Acid producing Lactic Acid Bacteria from various Moroccan food products and partial characterization of putative bacteriocins". Biotechnology, 6: 481-488.
3. Diep D B, Haverstein L S, Nes I F. (1996). " Charecterization of the locus responsible for the bacteriocin production in Lactobacillus planatarum C11". J. Bacteriol. 178, 4472-4483.
4. Holzapfel W H, Schillinger U, Du Toi M, Dicks L. (1997). "Systematics of probiotics lactic acid bacteria with references to modern phenotypic and genomic methods". Microecol Ther,26, 1-10.
5. Huis in't Veld JHJ, Havnaar R.(1997). " Selections criteria and application of probiotics microorganisms in man and animal". Microecol Ther,26,43-58.
6. Jack R W. Tagg J R, Ray B. (1995). "Bacteiocin of gram positive bacteria". Microbiol. 59, 171-200.

7. Lash, B. W., et al., 2005. "Detection and partial characterization of a broad-range bacteriocin produced by L. Planatrum (ATCC 8014)''. Food Microbiol., 22:199-204.
8. Nakamura T., Yamazaki N., Taniguchi H. and Fujimura S " Production, purification, and properties of a bacteriocin from Staphylococcus aureus isolated form saliva". Infection and Immunity, 39(2): 609-614, (1983).
9. Raja Narendra B et al., "Isolation and characterization of Bacteriocin from Fermented Foods and Probiotics". Int. J. Pharma and Bio Sci. (1)3, ISSN 0975-6299.
10. Reuter G.(1997). "Present and future of probiotics in Germany and in Central Europe" Biosci. Microflora, 16, 43-51.
11. Sawa, N., et al 2009. "Identification and characterization of lactocyclicin Q, a novel cyclic bacteriocin produced by Lactococcus sp. strain QU 12". Applied Environ. Microbiol., 75: 1552-1558.
12. Saxelin, M., et al., 2005. "Probiotic and other functional microbes: From markets to mechanisms". Curr. Opin. Biotechnol., 16: 204-211.
13. Sridhar Rao PN: Department of Microbiology, JJMMC, DAVANGERE. / www.microrao.com
14. Stoyanova, L.G., et al., 2007. " A comprision of the properties of bacteriocin formed by Leuconostoc lactis isolated from raw cattle milk and its preliminary optimization for the bacteriocin production". Res . J. Microbiol., 43:604-610.
15. Svetlana L et al., " The partial characterization of the Antibacterial peptide bacteriocins G2 produced by the probiotic bacteria Lactobacillus planatarum G2". J. Serbian Chemical society, 76(5), 699-707, JSCS-4151.
16. Thakur, R.L. and R. Utpal, 2009. "Antibacterial activity of Leuconostoc lactis isolated from raw cattle milk and its preliminary optimization for the bacteriocin production". Res.J. Microbiol., 4:122-131.
17. Thapa, N., et al., 2004. "Microbiol diversity in ngari, hentak and tungtap, fermented fish products of North-East India". World J. Microbiol. Biotechnol., 20:599-607.
18. V. Bali et al., "Isolation Screening and evaluation of antimicrobial activity of potential bacteriocin producing lactic acid Bacteria isolate". Microbiology Journal,2011 ISSN 2153-0696/DOI:10.3923/mj.2011. Academic Journal Inc.
19. Yildirim, M., 2001. "Purification of buchnericin LB produced by Lactobacillus buchneri LB". Turk. J. Biol., 25:59-65.