Investigation of Isolation, Production And Characterization of Bacteriocin Producing Lactic Acid Bacteria

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Abstract- *In the present investigation, totally 13 colonies were randomly picked from the curd sample. The bacteriocin producing strains were identified as Lactobacillus sp., (LAB 1), Leuconostoc sp., (LAB 2), Streptococcus sp., (LAB 3), based on physiological and biochemical characteristics. The culture filtrated from Lactobacillus sp., exhibited antibacterial activity against three indicate test strains The test isolate was grown in MRS medium under optimized conditions. Bacteriocin activity of the test isolate was determined by agar well diffusion assay against E.coli, Salmonella typhi ,Klebsiella pneumonia, The maximum zone of inhibition was observed in organism,E.coli (13mm), Klebsiella pneumoniae(10mm), Salmonella typhi (9mm), respectively. The optimum temperature of Lactobacillussp.,in MRS medium was 37ºC highest viable cell count of Lactobacillusat 37ºC reached. Bacteriocin production was maximized when Lactobacillus sp., was grown in controlled pH fermentation medium of 6.5. Antibacterial substance as bio preservatives in curd. Because of strong inhibition of this potent bacteriocin against challenging pathogens. Bactericidal be used natural preservative to enhance the different processed curd products.*

Keywords- Curd, Lactic Acid Bacteria, MRS agar, Bacteriocin, E.coli, Salmonella typhi, Klebsiella pneumoniae.

I. INTRODUCTION

Microorganisms produce an extraordinary array of microbial defence systems. These include classical antibiotic, metabolic by products, lytic agents, numerousProteins, exotoxins and bacteriocins gene-coding antimicrobial peptides, have been observed in every living organism and these produce by bacteria are termed bacteriocins. These potential chemotherapeutic agents are defined by their bactericidal or bacteriostatic action on strains closely related to the produce bacteria.

LAB has been used as probiotics to manage intestinal disorders. Lactose intolerance, acute gastroenteritis constipation and inflammatory bowel disease. Bacteriocins use can reduce on farm use of traditional antibiotics. LAB is known for their production of antimicrobial compounds, including bacteriocins or like peptides, Bacteriocins of LAB are defined as ribosomally synthesized proteins complexes usually antagonistic to genetically related organisms.

Bacteriocins use can reduce on farm use of traditional antibiotics.Bacteriocins are proteinaceous or peptide toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strains, they are similar to yeast and paramecium killing factors andare structurally functionally, and ecologically diverse. Bacteriocins were first discovered by Andre Gratia in 1925involved in the process of searching for ways to kill bacteria, which also resulted in the development of antibiotics and the discovered of bacteriophage , all within a span of a few years. He called his first discovery a Colicine because it killed *E.coli.* In thepresentinvestigation,**i**solation, production and characterization of bacteriocin producing Lactic acid bacteriafrom curd sample

II. MATERIALS AND METHODS

Isolation and Identification of Lactic Acid Bacteria

For isolation of LAB 1g of curd sample was suspended in 9ml of sterile distilled water and shaken vigorously for two minutes. Then were isolated the suspended curd sample is serially diluted. The dilution of 10^5,$10^6$and 10^7 are inoculated on Mann Rogosa Sharpe (MRS)agar plates. The plates were incubated at 37ºC for 2-3days.After incubation all the colonies were purified by single colony isolation after re-streaking on MRS medium (Holt 1994).

Characterization of LAB

Overnight- incubated cultures of bacteriocin producing isolates were Gram stained and examined microscopically for morphology and phenotype. Catalase test

was performed by adding few drops of 3% hydrogen peroxide to a test-tube containing 24h –old culture of each isolate.

Isolation of bacteriocin producing isolates

Lactic acid bacteria were isolated from the wide of sample like curd by appropriate dilutions,plated on MRS agar and incubated anaerobically at 37° C for 28 hours. Well isolated cultureswere picked up and transferred to MRS broth for enrichment of LAB.

Production of crude Bacteriocin

The isolated strain was grown in MRS broth seeded with 5% inoculums of overnight culture and maintained an aerobically at 30ºCfor 48 hours. After the incubation cells were removed from the growth medium by centrifugation (5000 rpm for 15 min, 4ºC) the cellfree supernatant was adjusted to pH 6.0 and it was used as crude bacteriocin (Ogunbanwo*et al.,* 2003).

III. CHARACTERIZATION OF BACTERIOCINS

Test Bacterial Pathogens

The culture of pathogenic strains *E.coli, Salmonella thypi*and *Klebsiella pneumonia,*were obtained from Idhaya Culture Collection Centre, Idhaya College for women Kumbakonam. Culture used in this study was transferred twice the specific medium before use.

Bacteriocin assay

The isolates from the curd sample were tested for their ability to produce bacteriocin. The isolates maintained in MRS agar were propagated in MRS broth and incubated at 37ºC for48 hrs cells were separated by centrifugation at 5000rpm for 10 min. The pH of the cell free supernatant was adjusted to 50 with 2N NaoH , cell free supernatant was passed through 0.22µm membrane filter andevaluated for antimicrobial activity by agar well diffusion method (Sarikaya , *et al*.,2004).

Antibacterial activity of Bacteriocins by agar well diffusion method

The enriched broth was screened for antibacterial agar well diffusion method (Aslim, *et al.,* 2004).In this method, the supernatant of bacterial isolates were take about 20ul was suspected in the agar well against the indicator organism such as *E.coli, Salmonella typhi, Klebsiella pneumoniae*. Petridishes with nutrient agar that were previously inoculated with 0.1 ml of individual test bacteria were poured and solidified petridishes were stored for 2 hrs at 4ºCwells of mm diameter were made and filled with 200µl of bacteriocin . The inoculated plates were kept at 4ºCfor 24 hrs. Incubated at 37ºC for 24 hrs.Inhibition zones around the wells were measured.

IV. RESULT AND DISCUSSION

Isolation and Identification of Lactic acid Bacteria

A total of 13 colonies were Randomly picked from the curd sample. The bacteriocin producing strains was isolated from curd sample. The strains were identified as *Lactobacillus* sp.,(LAB 1), *Leuconostoc* sp., (LAB 2), *Streptococcus*sp.,(LAB 3), based on in physiological and biochemical characteristics (Plate -2; Table 1&2). In the previous study isolated from curd and cucumber sample were found to be gram –positive and catalase –negative, but only isolate showing potent antibacterial activity against L. Diacetylactis were chosen for further characterization morphologically, the cells of 3 isolate the Coccus type and arranged either in pairs or tetrads. Their colonies on MRS agar were circular,low convex with entire margin and cream coloured. The cells of remaining 9 rod shaped isolates were arranged in pairs or chains .their colonies on MRS agar were found to be circular, low convex with entire margin and non – pigmented. (Mahantesh *et al.*,2010).

Rajaram*et al.*,(2010) investigated, the isolation, characterization and activity of bacteriocin produced by *L.lactis* from marine environment .it is rich in nutrient and organic matter. To state that the isolate *L.lactis* was tested for antibacterial activity against gram-positive and gram-negative bacteria such as *Lactobacillus* sp., *Lleuconostoc* sp., *and Streptococcus* sp., Lactic acid bacteria the highest inhibitory activity was demonstrated against *Leuconostoc* sp. and *Streptococcus* sp.,the inhibitory effect demonstrated by *Lactobacillus*sp., these bacteria is an indication of possession of antibacterial activity.

Plate-1 Isolation of LAB from curd sample (Master plates)

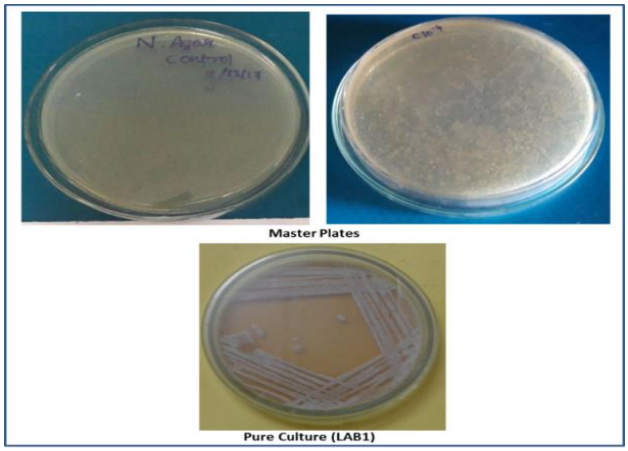


Table-1 Morphological Characterization of Lactic Acid Bacteria

S.No	Morphological characterization	LAB 1	LAB 2	LAB 3
1	Gram staining	Gram(+)	Gram(-)	Gram(+)
2	Motility test	Non - motile	Non-motile	Non-motile
3	Size	Large	Small	Large
4	Shape	Rods	Cocci	Cocci
5	Margins	Irregular	Irregular	Entire
6	Surface texture	Smooth	Smooth	Smooth
7	Consistency	Mucoid	Moist	Moist
8	Pigmentation	White	Yellow	White

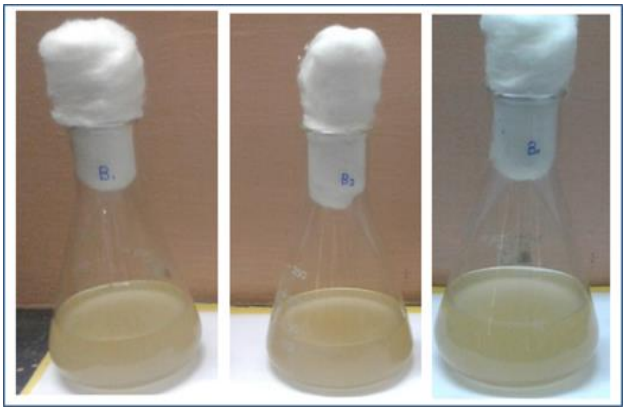
S.No.	Biochemical Test	LAB 1	LAB 2	LAB 3
1.	Indole Production	-	+	-
2.	Methyl Red	+	+	+
3.	Vogesproskauer	-	-	-
4.	Catale test	-	+	+
5.	Oxitase test	-	-	
6.	Citrate Utilization	-	-	+
7.	Urease	-	-	+
8.	Carbohydrate fermentation	+	+	-
9.	Nitrate reduction	+	-	+
10.	TSI	-	-	-

(+) Positive; (-) Negative

Bacteriocin production

The bacteriocin assay and the strains showing a relative positive result and the diameters were measured as described in plate-2. Measurement biomass and bacteriocin production and shown in result showed that *Lactobacillus* sp., produce bacteriocin in MRS broth. The strain exhibited a good bacteriocin production. The bacteriocin production was higher during the stationary phase of the growth of the organism where maximum biomass occurred 24 hrs.Bacteriocin production was influenced when incubated in different enzymes amylase, DNAase, RNA ase, and lipase resulted in greater bacteriocin production proteinase K and pepsin were strongly inhibited bacteriocin production .this is in contrast to results obtained byIvanova*et al*., 2000.

Plate-2 Bacteriocin production from LAB



Antibacterial activity of Bacteriocin

Antibacterial activity of partially purified bactriocin against test indicators was contacted in petriplate the selected bacterial pathogen namely organism,*E.coli, Salmonella typhi*and*Klebsiella pneumonia.* The maximum zone of inhibition was observed in organism, *E.coli* (13mm),*Klebsiella pneumonia* (10mm), *Salmonella typhi* (9mm), respectively(Table- 3; Fig.-1). This study related to Lactic acid bacteria isolate from curd sample products includes *Lactobacillus, Leuconostoc,* and *Streptococcus*. All LAB were subjected to inhibitory activity test using agar well diffusion method.(Girum*et al.,* 2005) observed varying degree of inhibition on various food borne pathogens by the culture filtrate of LAB. In this study essentially it is noted that bacteriocin has a lytic bactericidal mode of action. (Nagalakshmi*et al.,* 2013).

Table- 3 Antibacterial activity of Bacteriocin

S.No	Human Pathogenic Bacteria	Bacteriocin Activity Zone of Inhibition (mm)
1.	*E.coli*	14
2.	*Salmonella typhi*	12
3.	*Klebsiellapneumoniae*	9

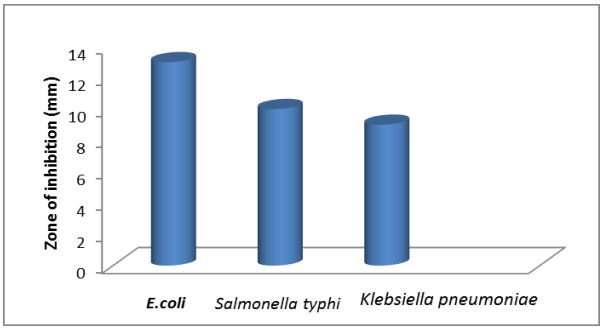


Fig-1 Antibacterial activity of Bacteriocin

Bacteriocin is a bacterial substance, biological protein moiety and a bactericidal mode of action against the homologies chemical analysis indicated that some bacteriocin are quite complex molecules, lipid and carbohydrate components in addition to protein or simple proteins (Karthikeyan*et al.*,2009).

V. CONCLUSION

The Isolated identified Bacteriocin production strains, *Lactobacillus*sp., *Leuconostoc* sp., *Streptococcus* sp., from curd sample. The culture filtrate *Lactobacillus,*sp.,exhibited antibacterial activity against 3 indicator test strains, bacteriocin activity of the isolate was determined by agar well diffusion assay against *E.coli*, *Salmonella*sp., *Klebsiella pneumoniae.,*The results revealed that *E.coli* was least sensitive to crude bacteriocinsupernatent of *Lactobacillus* sp., as compared to other indicator strains.Bacteriocins are bacterially produced peptides that are active against other bacteria and against which the producer has a specific immunity mechanism. They are produced by all major lineages of bacteria and archaea and constitute a heterogeneous group of peptides with respect to size, structure, mode of action, antimicrobial potency, immunity mechanisms and target cell receptors. We conclude with suggestions for future work and the possible ways in which bacteriocins could potentially be applied to enhance health.

VI. ACKNOWLEDGEMENT

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