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Identification of Lactic Acid Bacteria from Fermented Indian Food and Study its Physical and Cultural Parameters on Bacteriocins Produced By Them

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Abstract-In various South Indian fermented food lactic acid bacteria plays important role. The starter cultures in food is Lactic acid bacteria (LAB) and are known to produce antimicrobial substances such as bacteriocins, having great potential as food bio preservatives. Idli and Dosa are fermented food prepared by the people in South India. Bacteriocin producing LAB were isolated from dosa batter and idli batter and identified as species of Lactobacillus. Evaluation of culture supernatant of the isolates for their antimicrobial activity against pathogens like Staphylococcus aureus md Pseudomonas sp. The stability of the bacteriocins was tested at different temperatures and pH. Isolates produced bacteriocins were stable at temperatures ranging between 30 to 80°C and over a wide range of pH from 2 to 10. The molecular weight of partially purified bacteriocin was analyzed by SDS-PAGE. The range of molecular weights between 16 to 48 kDa.

Keywords: LAB, pathogens,bacteriocin

I. INTRODUCTION

Fermentation is one of the oldest forms of food preservation in the world. Fermented dairy products and their microbial and functional characteristics have been widely studied. In India, a wide variety of traditional fermented foods made from ingredients like milk, cereals, pulses and vegetables. Isolation and screening of microorganisms from naturally occurring processes have always been the most powerful means for obtaining useful cultures for scientific and commercial purposes [1] Idli and dosa are very widely used fermented foods of South India. It is prepared from rice and black gram mungo (Phaseolus mungo), a legume. The ingredients are carefully washed, soaked in water separately, then ground, mixed, and finally allowed to ferment overnight. When the batter has been raised sufficiently, it is cooked by steaming and served hot. The product has a very soft and spongy texture and a desirably sour flavor and taste. During lactic acid fermentation these bacteria not only have their effect on food and flavour but they are also known to produce and excrete compounds with antimicrobial activity, such as bacteriocins. Bacteriocins of LAB are considered as safe natural preservatives or biopreservatives, as it is assumed that they are degraded by the proteases in gastrointestinal tract [2]. The inhibitory spectrum of some bacteriocins also includes food spoilage and/or food-borne pathogenic microorganisms [3]. The discovery of nisin, the first bacteriocin used on a commercial scale as a food preservative dates back to the first half of last century but research on bacteriocins of LAB has expanded in the last two decades, searching for novel bacteriocin producing strains from dairy, meat and plant products, as well as traditional fermented products. Many bacteriocins have been isolated and characterized [2].

Earlier, we have reported the characterization of LAB isolates, a bacillus from idli and dosa better and another from vegetable pickle as well as characterization of the partially purified bacteriocins from the same [4]. Present investigation reports on the isolation and identification of lab from fermented Indian food and study its physical and cultural parameters on bacteriocins produced by them.

II. MATERIALS AND METHODS

A. Chemicals

All chemicals were obtained either from SD Fine Chemicals, India while the proteolytic enzymes, molecular weight markers

and bacteriological media were obtained from Sigma, USA and Hi-Media, India respectively.

B. Isolation Of LAB

From dosa and idli batter LAB were isolated by appropriate serial dilutions, and were plated on MRS (de Mann Rogosa Sharpe) agar by pour plate and incubated anaerobically at 37 ^{o} C for 2-3 days for the colonies to develop. Following incubation 10 colonies from each sample were randomly selected from MRS agar plate. They were propagated twice and streaked on MRS agar to check the purity of the isolates and then stored in MRS soft agar (0.5 %) overlaid with 50 % glycerol at -20 ^{o} C. Pure colonies were again cultured on MRS agar slants. The isolates maintained in frozen stocks were propagated twice in MRS broth (duplicate) and used for further study.

C. Antimicrobial Activity And Bioassay

The antimicrobial activity of these isolates were studied by disc diffusion procedure (Tadese et al., 2005). A loopful of each of the LAB isolates from MRS agar slants was inoculated in the tubes having 10ml sterile MRS broth. Broth cultures were incubated at 37 ^{o} C for 2 days. To obtain culture free supernatant after incubation, the culture were centrifuged at rpm 5000 for 40 min at 4 $^{o}_{C}$ .The pH of the CFS was adjusted to 7 with 1M NaOH(Sharpe et al., 1979). Control for each tube was prepared using un-inoculated MRS broth. Sterile cotton swabs were dipped into the culture of test microorganisms and inoculated by swapping over entire surface of Muller Hinton agar plates, which were preset. Whatman filter paper was used to prepare sterile filter disc of 6mm. each disc was impregnant with the culture supernatant. Then these disc were air dried and placed in plate for 15 min. After 1 day of incubation at 37 ^{o} C each plate were examined for zone of inhibition. The diameter of inhibitory zone were measured.

D. Identification Of Lactic Acid Bacteria

Using biochemical method the selected isolates were identified. According to Bergey,s manual of Determinative bacteriology identification of isolates was performed.

E. Effect Of Heat Treatment

The culture free supernatant were exposed to various heat treatments. The culture supernatants were incubated for 30, 60, 90,120 ^{o} C for 15 min.

F. Effect Of pH

The pH of culture supernatant was adjusted in the range of 2 to 12 with 1M NaOH(Hernandez et al.,2005). After incubation for 1 hour and before plating the pH treated sample were neutralized.

G. Molecular Weight Determination

The isolates were grown in MRS broth at 37 ^{o} C for 2 days. After incubation, the culture were centrifuged after which bacteriocins were precipitated from the supernatant with 45% saturated ammonium sulphate (Aktypis et al., 1998) and kept overnight at -20 ^{o} C for precipitation. After precipitation the supernatant was centrifuged and formed pellets which were stored in phosphate buffer. The molecular weight of bacteriocins were determined using SDS - PAGE. Molecular markers 6.5 to 175kDa was used. After electrophoresis the gel was stained with coomassie brilliant blue.

III. RESULTS

Total of 20 bacterial strains isolated from 2 types of food. Gram staining support the characterization of lacto bacilli. Out of 30 strains 5 strains found to be gram positive. Among these 5 strains three c1 c2 and e1 from idli batter and f2 and f3 were obtained from dosa batter. The antimicrobial activity of 5 isolates of LAB and their degree of inhibition against test pathogen were studied. From 5 LAB the culture supernatant of 4 isolates yielded zone of inhibition. No zone of inhibition against pseudomonas.

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Table1- Zone of inhibition for 4 bacteriocin producing isolates against *S.aureus* and *Pseudomonas*

Test	C2	E1	F2	F3
Motility	-	-	-	-
Catalase	-	-	-	-
Growth at 15 c	+	+	+	+
Growth in 45c	-	+	-	+
Growth in 4% nacl	+	+	+	+
Growth in 6.5% nacl	-	+	-	+
Growth in milk with 0.1% methylene blue	+	+	+	+
Growth in milk with 0.3% methylene blue	+	+	+	+
Growth in presence of 0.3% sodium Deoxycholate	+	+	+	+
Growth in presence of 0.5% sodium Deoxycholate	+	++	+	++
Growth in presence of 1.0% sodium Deoxycholate	+/+	+/+	+/+	+/+
Sugar fermentation				
Sucrose	+/-	+/+	+/-	+/+
Maltose	+/+	+/-	+/+	+/-
Mannitol	+/-	+/-	+/-	+/-
Lactose	+/+	+/+	+/+	+/+
Fructose	+/-	+/-	+/-	+/-
Glucose				

Table2- Biochemical characterization of bacteriocin producing LAB isolates

The result of different biochemical test for the 4 bacteriocin producing LAB strains is showed in table 2. The result showed that strain c2 and f2 were of *L.plantarum* and strain E1 and f3 belonged to *L.fermentum*

Pathogens	C2	E1	F2	F3
S.aureus	*12*	*9*	*12*	*9*
Pseudomonas	-	-	-	-

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Table 3- Effect of temperature and pH on the zones of inhibition (in mm) of bacteriocins

Temperature ^{0}C …… pH……

Isolates Pathogen	30	50	80	2	4	6	8	10	12
C2	12.00±0.00	11.8±0.25	10.25±0.35	9.25±0.35	11.3±0.350	12.0±0.00	11.80±0.35	9.25±0.35	-
E1	9.00±0.00	9.0±0.00	9.25±0.35	9.00±0.00	9.00±0.00	9.5±0.70	9.25±0.35	9.00±0.00	-
F2	12.00±0.00	12.00±0.00	11.3±0.350	9.00±0.00	11.3±0.350	12.00±0.00	11.3±0.350	9.00±0.00	-
F3	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00	9.50±0.70	9.00±0.00	-

The effect of temperature and pH on inhibitory activity of bacteriocins produced by various LAB isolates have been showed on table 3. The antimicrobial substance produced by the isolates was stable during heat treatment at 30, 50 and 80^{0}C for 30 min.

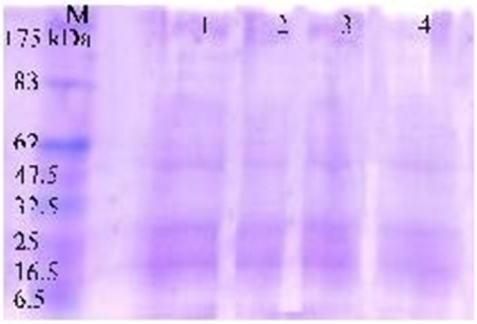

Fig1-Protein pattern after SDS-PAGE

The apparent molecular weight of bacteriocin were analysed in Fig 1.Coomassie Brilliant Blue stained gel showed several bands. Protein patterns of SDS-PAGE ammonium sulphate precipitates of whole cell free extracts of LAB isolates. Numbers at the top of the gel indicate the protein profile of C2, E1, F2 and F3. M is the protein marker.

IV. DISCUSSION

In this study strains were isolated from fermented food like idli and dosa batter. Only those strains were isolated producing antimicrobial compounds. The result obtained in our study regarding the production of antimicrobial compounds against human pathogens in complete agreement with the work done by other workers (Tadese *et al*., 2005). They found varying degree of inhibition using various indicator microorganism although, inhibitory substances produced by lactic acid bacterial strain act differently on different indicator strains (Savadogo *et al.,* 2004*).* The production of organic acid and hydrogen peroxide by lactobacilli was reported to inhibit both gram positive and gram negative bacteria (Olasupo *et al*., 19997). Pseudomonas not showing sensitivity because the antimicrobial compounds of LAB isolates may be attributed to resistance of gram negative bacteria due to nature of their cell wall. Bacteriocins produced by *Pediococcus acidilactic* interacts with lipoteichoeic acid that are absent in gram negative bacteria (Bhunia et al., 1991).Because of heat stability at 80^{0}C for 30 min, it can be use as food additive. Our results are similar with previous work results that the activity of bacteriocin produced by lactobacilli was completely lost at 121^{0}C for 15 min (Hernnadez *et al*., 2005).

For the growth and metabolism there is minimal, maximal and optimal pH for every microorganism. The important part of this study was the effect of pH on antimicrobial compounds produced by our isolates. Regarding the pH Tolerant

bacteriocins, the results obtained in our study are consistent with other reports (Karaoglu *et al*., 2003). the pH tolerance is an extremely important feature , since the isolates have the ability to survive, grow and produce their antimicrobials both under acidic and alkaline conditions.

The molecular weight determination was done by SDS-PAGE . present result agrees with those obtained from other bacteriocins such as pediocin , where the molecular weight estimated to be 16.5 kDa(Gonzalez and Kunka ,1987). The unique properties of bacteriocins revealed due to characterization. It emphasize on their application in food industry as biological control of spoilage and pathogenic microoraganisms.

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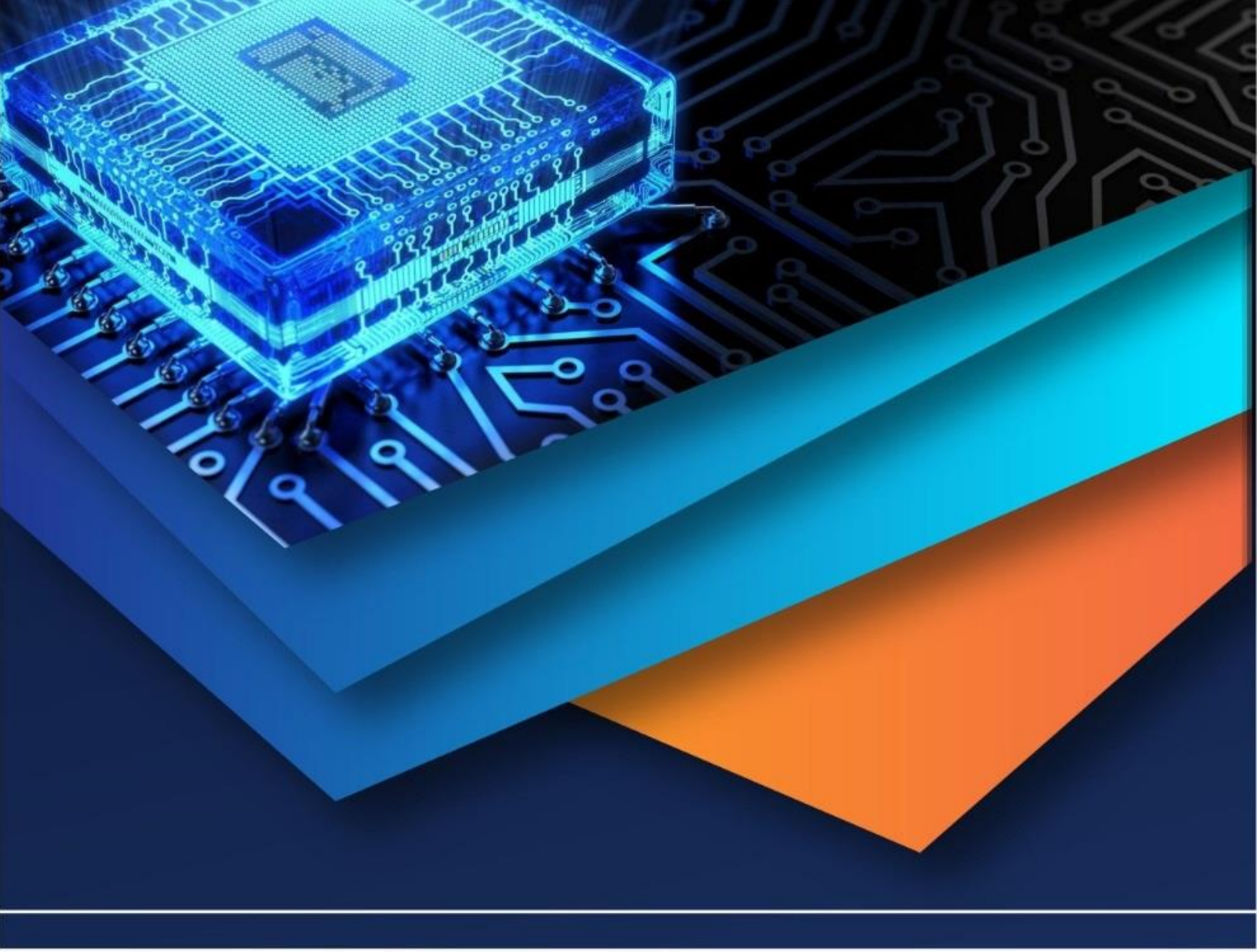

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