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BACTERIOCIN PRODUCTION AND BACTERICIDAL ACTIVITY AGAINST CLINICAL PATHOGENS

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Abstract

Objectives: To Isolate the Lactic Acid Bacteria, Produce the bacteriocine and teste the antimicrobial activity.

Methods: Bacteriocin producing lactic acid bacteria (LAB) was isolated from raw milk and cheese samples using de Man, Rogosa and Sharpe (MRS) plate. The isolated LAB was identified by morphological and biochemical tests and confirmed as *Streptococcus sp.* and *Lactobacillus sp*. Supernatant of *Streptococcus* and *Lactobacillus* culture are partially purified and used for bactericidal activity test against clinical pathogens such as *Bacillus, Pseudomonas, Staphylococcus,* and *Salmonella*.

Results: The antimicrobial activity test performed and among the 4 pathogens *Lactobacillus* were effectively inhibited clinical pathogens and showed higher inhibitory activity against *Staphylococcus* and *Pseudomonas.* The *Streptococcus* and *Lactobacillus* were analyzed by SDS-PAGE (10%) polyacyrlamide slab to separate the proteins. The presence of protein band nearing the molecular weight around 75 kda confirmed the presence of bacteriocin.

Conclution: The bacteriocins produced from milk products is the very good probiotic natural food. The peculiar antimicrobial activity of *Lactobacillus* and *Streptococcus* can positively have influence on their use as appetiser cultures for modern fermented nutriments and natural antibiotics.

Keywords: Bacteriocin, Lactic acid bacteria, Bactericidal activity, *Streptococcus*, *Lactobacillus*,

Introduction

The different types of Antimicrobial compound are produced by Lactic acid bacteria [1]. The antimicrobial compounds are organic acids, diacetyl, hydrogen peroxide, reuterin and bacteriocin or bactericidal proteins through lactic fermentations [2, 3]. The gram positive bacteria such as lactic acid bacteria are mostly produced in the bacteriocins [4, 5]. LAB produces the antimicrobial compounds that compounds are prominence for the economic significant food, feed fermentation and preservation. Further metabolic end products, several LAB strains similarly produce antimicrobial proteinaceous compounds called bacteriocins, which bacteria kill closely related bacteria [6,7].

LAB producing bacteriocins having antimicrobial properties and it is natural peptides and proteins. The LAB producing bacteriocins potential applications is food and health care sectors have concerned the strong attentiveness of academia and the industry, resulting in an inspiring expanse of published investigation on their production, purification, genetics and applications [8,9,10]. Bacteriocins are high molecular weight antibacterial compounds and protein in nature. Bacteriocin is produced by certain bacterial strains. The bacteriocins are most extensively studied and the colicins are produced by *Escherichia coli* and positive other strains of the *Enterobacteriaceae* [11,12]. The bacteriocins produced inhibited food decomposition and pathogens such as S*taphylococcus aureus, Escherichia coli, Bacillus cereus, B. subtilis, Listeria monocytogenes* and *Clostridium perfringens* which are intractable to traditional food preservation [13,14].

In the present research, *Lactobacilli* and *Streptococcus* strains were used to produce effective bacteriocin against clinical pathogens *Bacillus, Pseudomonas, Staphylococcus* and *Salmonella*. The bacteriocin was partially purified and their antagonistic activity in the direction of gram positive and negative bacteria was performed. The Bacteriocin production and molecular weight of the protein was performed using SDS- PAGE.

Materials and Methods

Sample collection and isolation

The bacteria were isolated from raw milk and cheeses which obtained from local market in and around Madurai. The sample was inoculated in trypticase soy broth and incubated at 37^{o} C for 24 hrs for enrichment. The enriched samples serially diluted up to 10^{-7} and spread the MRS agar plate and incubated for 48 hrs at 37^{o}C. The colonies were randomly purified by sub cultured on MRS agar plate before characterization. The selected isolates were identified using Berge's Manual of determinative bacteriology [19].

Partial Purification of bacteriocin

The nutrient broth was seeded with 10% inoculums of isolated bacteria and incubated at 37^{o}C for 72 hrs at 150 rpm. Partial purification of the bacteriocin was completed by salt saturation method. The isolated bacterial culture was saturated with 70% ammonium sulphate and stored at 4^{o}C to precipitate out the proteins

and centrifuged after 24 hrs at 12,000 rpm for 30 min. after the certification pellet was deposited in the phosphate buffer of pH 7.0 at -4^{0}C. Due to impenetrability of partially purified bacteriocin in deionized water phosphate buffer, tris-HCL and triton x20, pellet was purified by frequent washing technique, conceited the dissolution of impurities in phosphate buffer. Thus giving it six times wash in the subsequent method. 1ml of partially purified bacteriocin was taken in Eppendorff tube and centrifuged at 12,000 rpm for 5 min. the pellet was washed with phosphate buffer five times, and centrifugation at 12,000 rpm for 5 min. the bacteriocin was finally dissolved in phosphate buffer supplemented with SDS (0.6% w/v) and the anti-microbial activity of crude bacteriocin was tested by well diffusion assy.

Antibacterial test of bacteriocin against clinical pathogens

Inhibitory effect of crude bacteriocin on test bacteria (clinical pathogen) was tested by agar well diffusion method. Muller Hinton II agar plates were cut the well with 8 mm diameter. The bottom of every well was protected with viscous agar. The agar was allowed to solidify to avoid spreading of the mixture and supernatant complete the base. The wells were packed with 20 µl of mixture, filtrate of supernatant and refrigerated for 2 hrs to allow for distribution of assessment substrate, followed by incubation for 24 hrs at 37^{o}C. The diameter of zone was then measured in milli meter (mm) values. The Bacteriocin molecular weight was estimated by SDS-PAGE.

Result

In this present work, the *Bacillus, Pseudomonas, Staphylococcus* and *Salmonella* were isolated from the clinical sample collected from the hospital environment. The pus, urine, sputum samples were obtained from Meenakshi Mission hospital Madurai. The pus, urine, sputum were subjected to direct microscopic cultural and biochemical examination. The biochemical characterizations of *Bacillus, Pseudomonas, Staphylococcus* and *Salmonella* were given Table 1.

Table 1 Biochemical Characterizations of Clinical Pathogens

S.No	Biochemical test	*Pseudomonas*	*Bacillus*	*Salmonella*	*Staphylococcus*
1	Gram's stain	Negative	positive	Negative	Positive
2	Indole	Negative	negative	Negative	Negative
3	Methyl Red	Negative	negative	Positive	Positive
4	Vogesproskauer	Negative	negative	Negative	Positive
5	Citrate	Negative	positive	Positive	Negative
6	Carbohydrate utilization	A^+/G^-	A^+/G^-	A^+/G^+	A^+/G^-
7	Catalase	Positive	positive	Negative	Positive

The bacteriocin producing bacteria was isolated from the raw milk and cheese collected in and around Madurai and the selected bacteria was identified as *Streptococcus* and *Lactobacillus* based on its physiological and biochemical characteristics. The *Streptococcus* was gram positive cocci and *Lactobacillus* was gram positive rod. The biochemical characterizations of *Streptococcus* and *Lactobacillus* was non motile, catalase negative, carbohydrate fermentation positive growth of 4% NaCl positive and H_2S production test positive (Table 2).

Table 2 Biochemical Characterizations of Bactericin Producing Isolates

S.No	Biochemical characters'	Isolate 1	Isolate 2
1.	Gram's Staining	Gram positive cocci	Gram positive rod
2.	Motility	Non motile	Non motile
3.	Catalase	Negative	Negative
4.	Carbohydrate fermentation	Positive	Positive
5.	Growth of 4% Nacl	Positive	Positive
6.	H_2S production	Positive	Positive

Antibacterial activity of bacteriocin producing *streptococcus* and *lactobacillus*

The inhibitory activity pattern of partially purified *Streptococcus* and *Lactobacillus* was tested against clinical pathogens. Invitro antibacterial activity of bactericin producing organisms such as *Streptococcus* and *Lactobacillus* was carried out by Kirby bauer method.

Four clinical pathogens such as *Bacillus, Pseudomonas, Staphylococcus* and *Salmonella* were used in this study. Among the four organism's *Bacillus* and *Staphylococcus*, were inhibited by *Streptococcus* which showed the zone of inhibition 5 mm and 4 mm in diameter (Table 3). *Pseudomonas* and *Salmonella* were not inhibited by *Streptococcus.* Another bacterial isolate *Lactobacillus* was effectively inhibited the clinical pathogens and showed higher inhibitory activity against *Staphylococcus* with the zone of inhibition 11 mm in diameter. *Lactobacillus* inhibited the growth of *Pseudomonas*, the gram negative bacteria, the zone of inhibition is 10 mm in diameter and there was activity against *Salmonella. Bacillus*, the gram positive bacteria were moderately inhibited by *Lactobacillus*, which revealed the zone of inhibition, is 7 mm in diameter (Table 3).

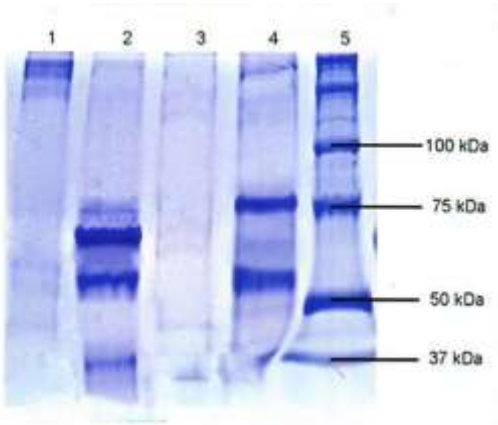
Table 3 Antimicrobial Activities: Zone of Inhibitions of *Streptococcus* and *Lactobacillus*

S.No	Organism	*Streptococcus* (mm)	*Lactobacillus* (mm)
1	*Bacillus*	4	7
2	*Staphylococcus*	5	11
3	*Pseudomonas*	No zone	10
4	*Salmonella*	No zone	No zone

Molecular weight determination by SDS-PAGE

The protein bacteriocin produced from *Streptococcus* and *Lactobacillus* was analyzed by SDS-PAGE (10%) polyacyrlamide slab. The presence of partially purified protein band nearing the molecular weight around 75 kda confirmed as presence of bacteriocin (figure.2).

Figure 2 SDS-Page analysis of partially purified bacteriocin from *Streptococcus* and *Lactobacillus*



2- *Streptococcus* 4- *Lactobacillus* 5- Marker

DISCUSSION

The present study highlights the separation, classification and activity of bacteriocin produced by *Streptococcus* and *Lactobacillus* from raw milk and cheese. It is high level of nutrient and organic substance. The bacterial isolate *Streptococcus* and *Lactobacillus* was tested for antibacterial activity against *Bacillus, Pseudomonas, Staphylococcus* and *Salmonella*. The maximum inhibitory activity was confirmed by *Lactobacillus* against *Bacillus, Staphylococcus,* and *Pseudomonas,* while the smallest activity was revealed by *Streptococcus* against *Pseudomonas* and *Salmonella*. The inhibitory result was confirmed by *Lactobacillus* and *Streptococcus* against these bacteria is an suggestion of control of antibacterial activity.

The lactic acid bacteria were well known for probiotics with advantageous possessions to human health. Their antimicrobial activity is one of the supreme essential probiotic characteristics. The production of antimicrobial substances by resident or transit lab micro flora, to remove the pathogen is under extensive study in recent time. Results also discovered the presence of the bacteriocin in the experimental organisms. Bacteriocins have been described to be inhibitory against some other bacteria [15]. Possession of bacteriocin by *Lactobacillus* and *Streptococcus is* an suggestion that the bacteria can be used as probiotic.

Partially purified bacteriocin from *Lactobacillus* and *Streptococcus* revealed that the homogeneity of three protein band because the protein is partially purified on 10% native PAGE. Its molecular weight was assessed at 75 kDa by SDS-PAGE. Similar results were reported by Ivanova *et al*., Karthikeyan and Santosh,

Rajaram *et al*. and Ogunshe *et al*. [15,16,17,18] The peculiar antimicrobial activity of *Lactobacillus* and *Streptococcus* can positively have influence on their use as appetiser cultures for modern fermented nutriments and natural antibiotics.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Authors declare no conflict of interest

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