



Biopotentials of Bacteriocin from *Lactococcus*.

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ABSTRACT

Bio preservation systems in foods are of increasing interest for industry and consumers. Bacteriocinogenic lactic acid bacteria and/or their isolated bacteriocins are considered safe additives (GRAS), useful to control the frequent development of pathogens and spoiling microorganisms in foods and feed. The spreading of bacterial antibiotic resistance and the demand for products with fewer chemicals create the necessity of exploring new alternatives, in order to reduce the abusive use of therapeutic antibiotics. In the present study, production and characterization of bacteriocin was carried out using lactic acid bacteria isolated from cheese. All morphological, physiological and biochemical tests showed that the bacterium is closely related to *Lactococcus* species. Bacteriocin production was achieved when the culture was grown on MRS medium. Partial purification of the isolated bacteriocin was carried out using ammonium sulphate precipitation. The molecular weight of the crude bacteriocin was found to be 4KDa. The bacteriocin was stable at higher temperatures. It showed good antimicrobial activity against *B.subtilis*, *E.coli* and *Pseudomonas aeruginosa*.

Keywords: Antibacterial activity, Bacteriocin, Biopreservatives, *Lactococcus*

INTRODUCTION

Microbes produce an extraordinary array of defense systems which include classical antibiotics, metabolic by-products and numerous types of protein exotoxins such as bacteriocins. Bacteriocins are protein or protein complexes that have antimicrobial activity against closely related species and various gram-positive bacteria including food spoilage bacteria and pathogens.^{1,2&3} Biopreservation systems such as bacteriocinogenic LAB cultures and/or their bacteriocins have received increasing attention, and new approaches to control pathogenic and spoilage microorganisms have been developed. Some lactic acid bacteria (LAB) demonstrated antagonism towards pathogenic and spoilage organisms.

Although bacteriocins are produced by many gram-positive and gram-negative species, those produced by LAB are of particular interest to the food industry, since these bacteria have generally been regarded as safe. Bacterial fermentation of perishable raw materials has been used for centuries to preserve the nutritive value of food and beverages over an extended shelf life⁴. Despite the several methods other than bacteriocins are employed for the preservation of food/beverages, an increasingly health conscious public may seek to avoid foods that have undergone extensive processing or which contain chemical preservatives.

The production of bacteriocins by LAB is advantageous for survival of the producing bacteria in a competitive ecological niche; therefore, they could be exploited by the food industry as a tool to control undesirable bacteria in a food-grade and natural manner, which is likely to be more acceptable to consumers.⁴ Lactic acid bacteria that

found to produce bacteriocins are classified into several genera including *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Pediococcus* and *Streptococcus*.⁵ The best-known bacteriocin is nisin produced by *Lactococcus lactis*. It is the only substance which has been approved for use as food additive in many countries.⁶ The use of preservatives in food industries involves the application of chemicals and substances that are hazardous to health. This can be overcome by the use of bacteriocins which can help to reduce the addition of chemical preservatives as well as the intensity of heat treatments, resulting in foods which are more naturally preserved and richer in organoleptic and nutritional properties. The present study aims to detect and investigate bacteriocins produced from Lactic acid-producing bacteria isolated from cheese.

MATERIALS AND METHODS

Isolation of Lactic Acid Bacteria from Cheese

One gram of fresh cheese (commercially available) was taken, homogenized and serially diluted. The sample was plated on De Man Rogosa and Sharpe agar (MRS) medium. It was then incubated at 37 °C for 48 hours. Bacterial colonies grown onto MRS agar were randomly selected and isolated to obtain pure cultures. The selected culture was propagated in MRS broth and it was grown at pH of 6.2 at 37 °C.

Characterization of the Isolated Strain

The isolates were identified and characterized based on morphological characteristics, staining and by following physiological and biochemical tests.



Morphological and Biochemical Assay

The form, elevation, size and pigmentation of the isolate were observed. Gram staining of the isolate was carried out. Indole production test, methyl red test, voges proskauer test, citrate utilization test, starch hydrolysis, carbohydrate fermentation, catalase test were carried out.⁸

Production and Purification of Bacteriocin.⁷

The culture was centrifuged at 10000 rpm for 20 minutes to obtain cell free supernatant. It was subjected to purification by precipitation. The relative solubility of proteins in various salt solutions is used as a basic fractionation technique. In general, proteins of higher molecular weight precipitate in lower concentrations of ammonium sulphate. Ammonium sulphate precipitation is widely used for the preparation of crude enzymes. 100 ml of bacterial culture was centrifuged at 10,000 rpm for 20 minutes. To the supernatant solid ammonium sulphate was added to achieve 80% saturation. The suspension was incubated overnight at 4 °C and agitated with a magnetic stirrer. The suspension was centrifuged at 10,000rpm for 20 minutes. The supernatant was discarded and the precipitate was mixed with minimum volume of PBS buffer. The sample was subjected for dialysis. The molecular weight of cure bacteriocin was determined using SDS-PAGE.

Antimicrobial assay

The antimicrobial activity was tested against *B.subtilis*, *E.coli* and *Pseudomonas aeruginosa*.

RESULTS AND DISCUSSION

Table 1: Identification and Characterization of Lactic Acid Bacteria

S. No	Characteristics	Lactic Acid Bacteria
1.	Colony Morphology	
	Form	Circular
	Elevation	Flat
	Size	Small
	Pigmentation	White
2.	Gram Staining	Positive
3.	Biochemical Assays	
	Indole Production	Negative
	Methyl Red test	Negative
	Voges Proskauer Test	Negative
	Citrate Utilization	Negative
	Starch Hydrolysis	Negative
	Catalase Test	Negative
	Carbohydrate Fermentation	Positive

Bacteriocin is a proteinaceous compound displaying bactericidal activity against many gram positive and gram negative bacteria. It plays an important role in food and in medical fields as bio preservative and therapeutic

agents. The production and characterization of bacteriocin was carried out using lactic acid bacteria isolated from cheese (Fig.1). All morphological, physiological and biochemical tests showed that the bacterium is closely related to *Lactococcus* species (Table.1, Fig.2). Bacteriocin production was achieved when the culture was grown on MRS medium. The molecular weight of the partially purified bacteriocin was found to be 4KDa. From different concentration of bacteriocin used against test organisms, it shown all the three were sensitive to bacteriocin activity. *Pseudomonas aeruginosa* did not show any antibacterial activity at 25% concentration of bacteriocin. But antibacterial activity was observed with 50%, 75% and 100% concentration (Fig.3). *B.subtilis* and *E.coli* was sensitive against all concentrations of bacteriocin and high antimicrobial activity was observed with 100% concentration. (Fig. 4).

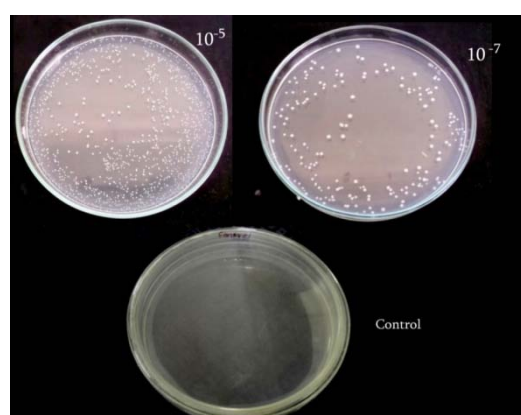


Figure 1: Isolation of Lactic Acid Bacteria from Cheese

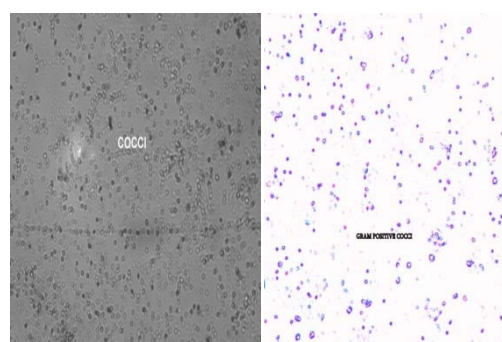


Figure 2: Gram's staining

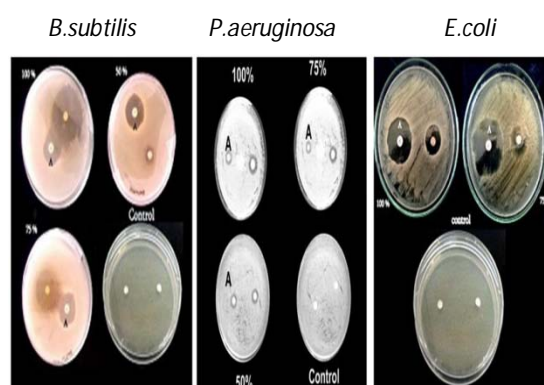


Figure 3: Antimicrobial Activity of Bacteriocin against Test Organisms

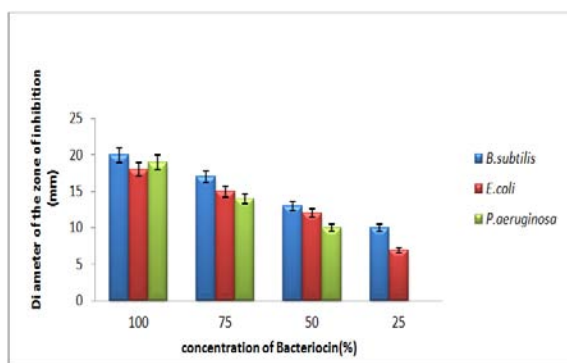


Figure 4: Antibacterial activity of Bacteriocin against test organisms

When bacteriocin was exposed to different pH (5, 6, 7, 8, 9), it showed higher bactericidal activity at pH 6, 7, 8 and loss of activity was observed with pH below 5 and above 9. From different temperatures (60 °C, 70 °C, 90 °C and 110 °C) tested, bactericidal activity was observed with temperatures 60, 70 and 90 °C. But no zone of inhibition was found at 110 °C (Fig. 5). Effective inhibitory spectrum of bacteriocin against different pH and temperatures, the activity was stable at pH 6, 7, 8 (Fig. 6) and temperature 60, 70 and 90 °C respectively. The bactericidal activity was found to decrease after treatment at temperature 110 °C for 10 minutes. Previous study also reported that Plantaricin A produced by *L. plantarum* was heat stable. Crude preparations of Lactacin B were stable at 100 °C for 10 minutes.⁹ Bacteriocin isolated from *L. acidophilus* IBB 801, designated as Acidophilin was heat stable (30min at 120 °C), and maintained in wide pH range.¹⁰

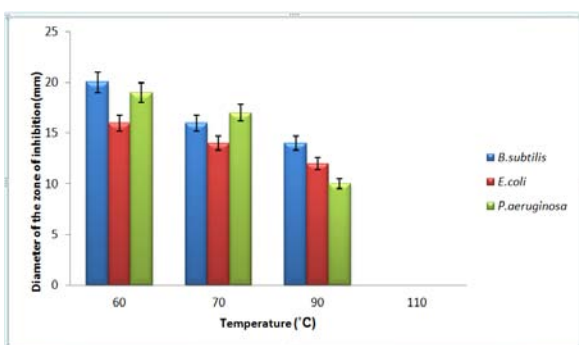


Figure 5: Effect of temperature on the antibacterial activity of Bacteriocin

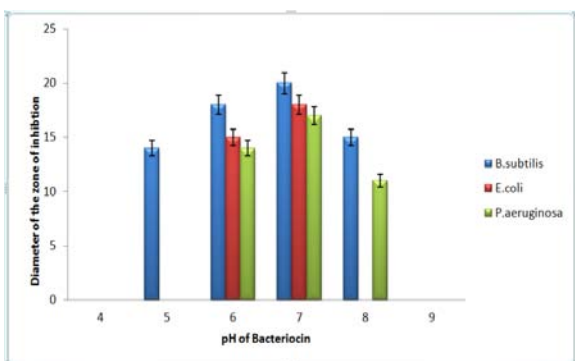


Figure 6: Effect of pH on the antibacterial activity of Bacteriocin

Bacteriocin produced by this lactic acid bacterium has some interesting characteristics that justify the study. It showed broad antibacterial spectrum. Results from these investigations clearly demonstrated that the inhibitory compound was heat stable. And therefore, special attention was focused on bacteriocin production. Bacteriocins of lactic acid bacteria are of particular interest because of their existing and potential applications as natural preservatives in food.¹¹ *Lactobacillus* strain LC109 isolated from a clinical sample was inhibitory against many species of Lactobacilli and other gram positive bacteria including *Listeria evanonii*, *Streptococcus agalactii* and *S. peyogenes*.¹²

Among three bacterial strains tested in the study, maximum inhibitory spectrum was observed in the case of *B. subtilis* than *Pseudomonas* and *E. coli*, with decrease in concentration of bacteriocin, the antimicrobial activity was found to decrease at 25% concentration. Bacteriocins are antagonistic proteins or peptides that show bactericidal activity against closely related species.¹³

Antibacterial activity of bacteriocin restricted to closely related species.¹⁴ Bacteriocin activities was stable at broad pH and temperature ranges.¹⁵ Bacteriocin production was favored under pH controlled conditions. Bacteriocin titers rapidly decreased, it may be due to strong adsorption of bacteriocin to the producing cells under less acidic condition. *L. acidophilus* 30S bacteriocin was active over a wide range of pH and was stable to various heat treatments. Strains of *L. acidophilus* as well as strain of *L. casei* and *L. plantarum* have been shown to survive at pH3 or less. The antimicrobial compound could be further explored by purification and characterization by ultra-filtration, High Performance Liquid Chromatography, Enzymatic treatments and stability assays.

CONCLUSION

Lactic acid bacteria have been recognized as safe, and bacteriocins produced by these microorganisms may be a good solution to the problem of resurgence of resistant strains to antibiotics. During the last decade, a large number of LAB bacteriocins have been identified, and in some cases, biochemically and genetically characterized. This explosion in bacteriocin research has been favored by the recognition of the role that these producing bacteria may play in the hygienic quality assurance of food and as feed supplements. However, to date only few bacteriocins are used as biopreservatives. This may be partially due to the fact that newly discovered broad-spectrum bacteriocins have yet to be fully characterized and officially approved. Further research is required to gain insight into the molecular mechanisms involved in bacteriocin production, immunity and mode of action, which is necessary for safe and effective exploitation of LAB-bacteriocins, either in the feed and food industry. There is a need to attract consumer attention to the existence of natural substances that can protect against food-borne related illness. The acceptance of probiotics

by the consumer was aided greatly when such bacteria were marketed as natural cultures that aid in digestion and health.

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