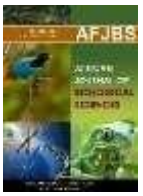
https://doi.org/10.48047/AFJBS.6.4.2024.768-776

African Journal of Biological Sciences



Effect Of Process Parameters On Bacteriocin Extraction From Lactic Acid Bacteria (LAB) Isolated From Fermented Sugarcane Juice Samples Collected From Local Vendors Of Jammu (J&K) Region

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Abstract
In today's times, when different varieties of food and food ingredients are in the market and the same are contributing in major part of the economy, with respect to it, foodborne diseases (FBDs) also on the other side are emerging as global concerns. Even with the adoption of contemporary methods for food preservation, foodborne illness continues to rise and is a major cause of death, particularly in nations lacking adequate procedures for monitoring food safety. Every year, almost one-third of the world'spopulation contracts food-borne illnesses because of eating tainted or intoxicating foodssuch as meat, poultry, canned goods, and fermented dairy goods. The fermentation of milk is caused by various strains of lactic acid bacteria (LAB), which are generally classified as probiotics. These strains are isolated from yoghurt and produce a variety of other antimicrobial compounds, including ethanol, hydrogen peroxide, diacetyl, fatty acids, reuterin (3-hydroxypropionaldehyde), and bacteriocins. These bacteria have antagonistic action against a variety of infections, which enhances health. Yogurt is thought to be a source of Lactobacillus bacteria, which fights pathogens that cause stomach infections and certain digestive diseases. Yogurt is thought to be a beneficial probiotic diet. To identify a new strain of Lactobacillus bacteria with the greatest efficacy,several strains of the bacterium have been isolated from yogurt around the globe. The present study utilizes the isolation and screening of Lactobacillus strains from sugarcane juice collected from different vendors using MRSA broth and MRSA agar. The studies revealed the isolation of 450 isolates from 50 samples of 10 days stored sugarcane juice kept in controlled conditions. The most common species that produce antimicrobials were shown to be Lactobacillus salivarius, Lactobacillus gasseri, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus paracasei, Lactobacillus crispatus and Enterococcus spp. These strains are further utilized for production of bacteriocins. The dominant isolates were screened for bacteriocin production characteristics. The generation of bacteriocin from the earlier mentioned isolates was also assessed following48 hours of incubation at various temperatures (25, 30, 35, 40, and 45 °C). The results of the study suggest that the favorable range of temperature for bacteriocin production was 35-42^{0}C. The predominant isolates were further stored as glycerol stocks andmaintained further for future use. The bacteriocins were evaluated for antimicrobial activity against the different food borne pathogens. The antimicrobial activity was alsoinfluenced by pH and temperature variations.

Key words: Food borne diseases, food preservation, probiotics, antimicrobial activity, bacteriocins, food borne pathogens, Lactic acid bacteria

INTRODUCTION

Bacteriocins are the normal peptides emitted by many kinds of microscopic organisms to kill different microorganisms. This permits them to acquire an upper hand in their field, dispensing with contenders and acquiring assets. These peptides are orchestrated inside ribosomes, yet some are widely post-translationally altered. Lactic corrosive microscopic organisms (LAB) are a distinct group of microbes that, as a major byproduct of starch maturation, create lactic acid [1-3]. Lactic acid bacteria are used in many different types of matured foods and play a major role in food handling and appropriate maturity. Lactic acid bacteria apply powerful opposing movement against numerous food debasing microorganisms through the development of natural acids, diacetyl, hydrogen peroxide, inhibitory chemicals, and bacteriocins [4-6]. Species of Lactic acid bacteria having a place with the genera Lactococcus, Lactobacillus, Pediococcus, Streptococcus, Oenococcus, Leuconostoc, Enterococcus, and Carnobacterium generate a wide range of bacteriocins, most of which are segregable into a small number of groups. One of the most promising natural foods bio preservatives are these fungi that produce bacteriocin [8]. Lactic acid bacteria bacteriocins are classified into three primary classes according to their structural, physicochemical, and molecular properties [9-11]. Class 1 bacteriocins are anti-toxins. Little, cationic, hydrophobic, thermostable peptides containing strange post-translationally framed amino acids (e.g., the thioether amino acids lanthionine and additionally 3-methyllanthionine). The tiny cationic, water-phobic bacteriocins belong to the second class. The second class bacteriocins is little cationic, water-phobic, thermostable peptides which remain unaltered after translation, save for the cleavage of the pioneer peptide that originates from prebacteriocin. It belongs to 2a subclass, or pediocin-like bacteriocins, and has the consensus sequence Tyr-Gly-Asn-Gly-Val at its N-terminus, along with strong antilisterial action. There are three subclasses within this class. Subclass 2b or bacteriocin. Full activity requires two polypeptide chains. Bacteriocins not having a place with subclass 2c or different subgroups. Class 3 bacteriocins are a large group of heat-labile, hydrophilic proteins. Antimicrobial peptides (AMPs) are universal regular anti-infection agents delivered by an assortment of microbial, plant, bug, and mammalian cells. As of late, AMPs certainly stand out as new antibacterial specialists to battle unsafe organisms, particularly those that are impervious to ordinary anti-microbials. While looking for new antibacterial specialists, they can act as layouts for new medication plans [12-14]. Based on their various structural properties, nAMPs are divided into various groups. These AMPs are delivered by microorganisms to kill or hinder different microbes and are known as bacteriocins [15-16]. The research is concentrating on the screening and isolation of Lactobacillus sp strains found in sample of sugarcane juice collected from local vendors in the Jammu region (J&K) because the antimicrobial peptides are significant and can be isolated from a variety of Lactobacillus sp strains.

MATERIALS AND METHODS

Isolation and Screening of Lactic acid bacteria

The seclusion and examination of LAB from various 15 sugarcane juice tests gathered from neighborhood merchants in Jammu (J&K) region in new cleaned bottles. To allow for efficient fermentation within the samples, they were kept at room temperature (32^0 C) for ten days. The disengagement and screening system began following 48 hours of maturation.

Separation of Lactic acid bacteria (LAB): Biochemical portrayal of bacteriocin producing LAB

The bacteriocin creating LAB were disengaged from aged sugar stick juice tests. Since samples underwent aseptically gauging, the material was dissolved in 100 milliliters of pure, somewhat developed ocean water to produce a 1:10 weakening. This allowed the example to be weakened successively, spread out over certain plates, and then formed into a 10-overlap sequential weakening. The MRS agar (Merck, Germany) was then used to subfine a volume of 0.1 mL from each sample to confine lactic corrosive microorganisms. The strain was cultured on MRS agar plates and kept for 48 hours at 37 °C in an anaerobic jar. Separate communities with common LAB features were taken from every plate and sent to MRS Inclines. Conventional methods of morphological, physiological, and biochemical aspects up to the genome level were used to identify the chosen strain. 16S rRNA quality sequencing enabled the sub-atomic distinguishing confirmation of the separation. [17-24].

Effect of growth conditions on bacteriocin production

The effect of various growth conditions such as pH, temperature, and incubation time to produce bacteriocin was done with MRS broth. Following manufacturing concoction of bacteriocin and MRS broth (1%, v/v) was inoculated with an overnight prepared inoculum and incubated at various ranges of temperature (25, 30, 35, 40 and 45 °C), pH (4.5, 5.0, 5.5, 6.0 and 6.5) and incubation time for 48 hours (expect for incubation time effect). Now, the examination for bacteriocin production (AU/mL) is done. [25, 26].

Production of bacteriocin from Lactic acid bacteria

The Prominent strains were grown in MRS broth (Hi Media Laboratory, Pvt Ltd. India) at suitable (pH 6) having 1 % inoculum, grown at optimum cultural prerequisite for about 48 hours. Following the incubation phase, the microbial cells were separated from the growth medium (MRS Broth) with centrifugation technique which runs at 15000 rpm for 15 minutes, 4 °C. The collected cell-free supernatant was utilized as crude bacteriocin after being pH-6.5-adjusted with 1 mol/L NaOH. In detail, after adding 400 g of ammonium sulphate per litre of culture supernatant, the mixture was allowed to settle for a full day at 4 °C. After 20 minutes of centrifugation at 6,000 rpm, the protein precipitates were collected then liquefied in fifty millilitres of 20 mmol/L sodium phosphate buffer (pH 6.0). And additionally, a diethyl aminoethyl-cellulose column (1.5 x 40.0 cm) that had been equilibrated with 0.1 mol/L Tris-HCl buffer (pH 9.0), was also applied. NaCl (0-1 mol/L) linear salt gradient was used to elute the sample. After being combined, all the active components were concentrated using ammonium sulphate, positioned upon to Sephadex G-75 column and eluted using the the identical buffer at a 0.5 mL/min flow rate. Next, the eluted fractions' bacteriocin activity was examined. (Buchanan et al. 1997; Collins et al. 1999; Elmafa et al. 2001). To create the crude bacteriocin needed for additional testing, the active portions of the various bacteriocins were combined and concentrated.

Evaluation of antimicrobial activity of bacteriocin against different pathogenic strains

LAB consortium bacteriocin's antibacterial activity was assessed using the agar well diffusion method [27]. The study was performed against S. aureus, Salmonella Abony, E. coli, Aspergillus Niger and Aspergillus flavus. The results showed that, the bacteriocin solution possessed antimicrobial activity against all the strains studied in varied range at 100 µg/ml with diameter of zone of inhibition observed in 15 – 30 mm. The crude bacteriocin extract treated with different temperature and pH values. The unrefined bacteriocin extract was given distinct treatment at

temperature values ranging from 25, 30, 35, 40 and 450C and the antimicrobial activity against the respective strains was determined. The pH of the crude bacteriocins samples viz. pH, 4.5, 5.0, 5.5, 6.0 and 6.5 was also varied and the antimicrobial activity of such samples were also determined against the mentioned pathogenic strains.

RESULTS AND DISCUSSION

In the present investigation, the studies revealed the isolation of 450 isolates from 50 samples of 10 days stored sugarcane juice kept in controlled conditions. The predominant strains were identified as Lactobacillus salivarius, Lactobacillus gasseri, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus paracasei, Lactobacillus crispatus and Enterococcus spp. These strains are further utilized for production of bacteriocins. The dominant isolates were screened for bacteriocin production characteristic (Table 1; Figure 1; Figure 2 (a) & (b); Figure 3). The bacteriocin production from the said isolates was also checked after incubation at varying temperatures such as 25, 30, 35, 40 and 45 °C with incubation time for 48. The results of the study suggest that the favorable range of temperature for bacteriocin production was 35-42^{0}C (Table 2). The results of the study correlate to the previous findings [28-30], but the present study revealed that effective bacteriocin concentration by the respective isolated strains is produced at more than 35^{0}C temperature at pH 6-6.5. Further, the lactic acid bacteria producing bacteriocins were grown in a selective medium to prepare the consortia. The bacteriocin samples from Lactic acid bacterial consortia showed significant antimicrobial activity against all the pathogens studied (Table 3; Figure 4). The results of the study of effect of temperature and pH on bacteriocins results in reduced or no antimicrobial activity against the pathogens studied (Table 4 and Table 5).

CONCLUSION

The results of the study conclude that, Lactic acid bacteria (LAB) isolated and screened from fermented sugarcane juice are having significant capabilities for production of bacteriocin which gets dominantly produced at the range of 35^{0}C-42^{0}C via Lactic acid bacteria at pH, 6-6.5. Thus, the study concludes that, natural fermentation process of sugar can yield antimicrobial compounds like bacteriocin of industrial significance which may lead to development of natural preservatives for food. The optimal pH and temperature as revealed in the study should be maintained effective for utilization and getting more effectiveness of bacteriocins as per antimicrobial point of view.

CONFLICTS OF INTEREST

There are no conflicts of interest, according to the authors.

Figure 1: Isolated cultures of Lactic acid bacteria (LAB) from fermented sugarcane juice samples

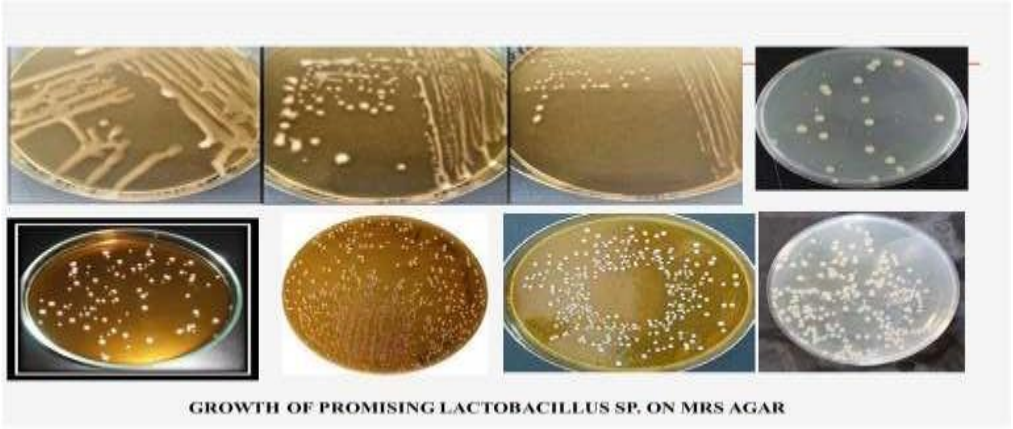


Figure 2 (a): Growth of colonies of Lactic acid bacteria (LAB) on MRS agar

Figure 2 (b): Growth of colonies of Lactic acid bacteria (LAB) on MRS agar

Table 1: Diversity of Lactic acid bacteria (LAB) isolates from sugarcane juice samples

Sugarcane juice samples	Total number of microbes isolated	Lactic acid bacteria (LAB) isolated	Dominant isolates producing Bacteriocin
50	723	450	07

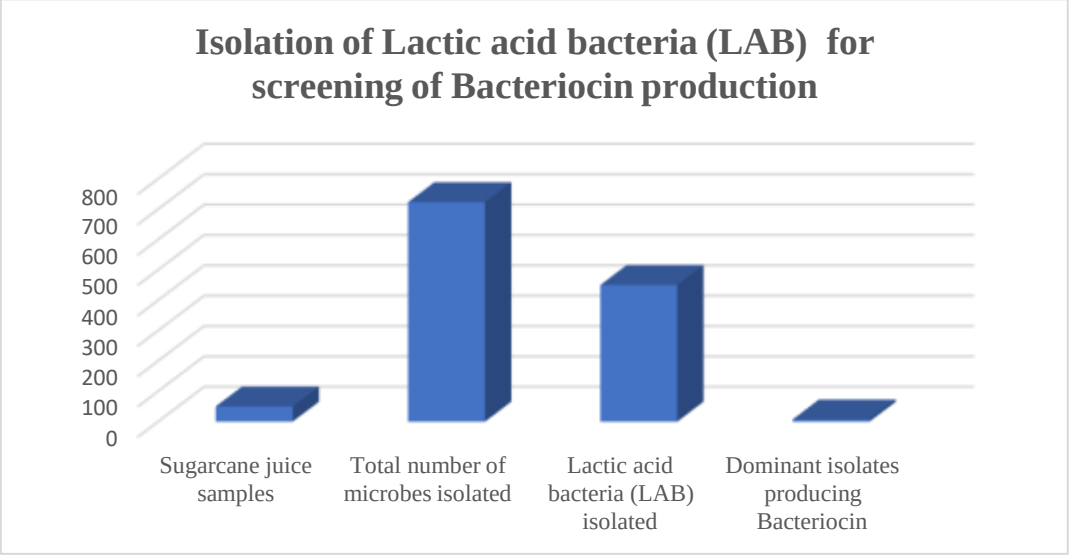


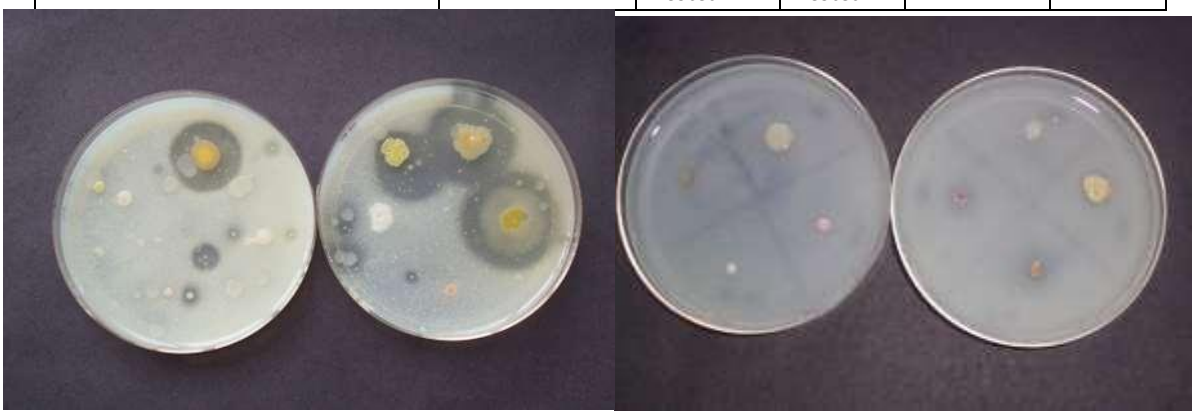
Figure 3: Diversity of Lactic acid bacteria (LAB) isolates from sugarcane juice samples

Table 2: Diversity of Lactic acid bacteria (LAB) isolates from sugarcane juice samples

Lactic acid bacteria (LAB) isolates	Bacteriocin production	Average range of temperature (^{0}C)
07	Significant	35–42^{0}C

Table 3: Antimicrobial activity of crude bacteriocin against pathogenic strains

Samples/Positive Control	Antimicrobial activity of crude bacteriocin- Diameter of zone of inhibition (mm)				
Crude Bacteriocin sample produced from Lactic acid bacteria (LAB) consortia	Staphylococcus aureus	Salmonella Abony	Escherichia coli	Aspergillus Niger	Aspergillus flavus
	27.0	25.0	28.0	26.0	25.0
Azithromycin (1mg/ml)	35.0	32.0	35.0	Not Tested	Not Tested
Fluconazole (1mg/ml)	Not Tested	Not Tested	Not Tested	28.0	25.0



(A) (B)

(C) (D) (E)

Figure 4: Antimicrobial activity of bacteriocin extracted from Lactic acid bacteria (LAB consortia)- (A) E. coli; (B) Salmonella Abony; (C) Staphylococcus aureus; (D) Aspergillus Niger; (E) Aspergillus flavus.

Table 4: Effect of variation of temperature treatments on crude bacteriocin against pathogenic strains

Temperature (^{0}C) values	Antimicrobial activity of crude bacteriocin - Diameter of zone of inhibition (mm)				
	Staphylococcus aureus	Salmonella abony	Escherichia coli	Aspergillus niger	Aspergillus flavus
25^{0}C	20.0	18.0	22.0	21.0	20.0
30^{0}C	21.0	20.0	23.0	21.0	21.0
35^{0}C	27.0	25.0	28.0	26.0	25.0
40^{0}C	23.0	23.0	21.0	22.0	22.0
45^{0}C	22.0	23.0	16.0	18.0	18.0

Table 5: Effect of variation of pH treatments on crude bacteriocin against pathogenic strains

pH values	Antimicrobial activity of crude bacteriocin - Diameter of zone of inhibition (mm)				
	Staphylococcus aureus	Salmonella abony	Escherichia coli	Aspergillus niger	Aspergillus flavus
4.5	18.0	18.0	11.0	12.0	0.0
5.0	18.0	22.0	18.0	21.0	0.0
5.5	20.0	22.0	25.0	22.0	21.0
6.0	26.0	27.0	28.0	27.0	24.0
6.5	27.0	25.0	28.0	26.0	25.0

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