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Article

Biopreservative effect of bacteriocin in fruit juice preservation

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Abstract: This study aimed to evaluate the biopreservation activity of bacteriocin against some spoilage organisms. This bacteriocin was produced by lactic acid bacteria (LAB) isolated from dahi samples. Man Rogosa Sharpe (MRS) broth and agar were used for the isolation of LAB from the dahi. Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of other pathogenic and spoilage bacteria. Many lactic acid bacteria produce a high diversity of different bacteriocins. This study was conducted to produce the bacteriocin which produced by *Lactobacillus* spp. from dahi sample. Dahi samples were collected from different retailer shops of Chittagong Metropolitan Area (CMA). *Lactobacillus* spp. was isolated from these dahi samples by using MRS broth (Hi-Media, India) at 37ºC for 48 h. The crude bacteriocin was produced and purified by ammonium sulphate precipitation. The adversary properties of bacteriocin were perused by agar well diffusion method. The durability of bacteriocin was studied in different temperature and pH. The bacteriocin was stable at 37ºC and acidic pH. The antimicrobial activity of the isolates was done by growth inhibition test against some selected pathogenic microorganisms likely *Escherichia coli, Pseudomonas aeroginosa, Salmonella paratyphi.* The result showed that the isolates of *Lactobacillus* spp. inhibited the growth of these pathogenic organisms. By this study the antibacterial efficacy of bacteriocin was determined for fresh orange juice. The biopreservative effect of bacteriocin was observed on the basis of low colony counts in total plate count agar (TPA). The study result showed that bacteriocin producing *Lactobacillus* spp. can be successfully used as biopreservative for fruit juice preservation.

Keywords: bacteriocin; antibacterial; LAB

1. Introduction

Bacteriocins are bacterial ribosomally synthesized peptides or proteins with antimicrobial activity (Nes *et al*., 2007). Huge amount of chemical compounds are generally used to inhibit the pathogens and spoilage organisms associated with foods so as to preserve the foods for long time (Bali *et al*., 2011). Various strains of lactic acid bacteria (LAB) associated with food systems produce bacteriocin which is proteinaceous substance that exhibit bactericidal activity against closely related organisms (Schillinger, 1989). The bacteriocin producing lactic acid bacteria (LAB) are generally isolated from food products like fermented dairy and meat products (Torodov *et al*., 2007). Nowadays, the term bacteriocin is mostly used to describe the small, heat-stable cationic peptides

synthesized by Gram positive bacteria, namely lactic acid bacteria (LAB), which display a wider spectrum of inhibition (Vij *et al*., 2011).

Lactic acid bacteria (*Lactobacillus* spp.) are reported to have inhibitory activity against common human pathogens (Servin, 2004). Lactic acid bacteria (LAB) are able to produce antimicrobial substances such as bacteriocins which have great potential to be used in therapeutics and as food bio-preservatives (Arokiyamary, 2012).

Bacteriocins are normally not termed antibiotics in order to avoid confusion, which can potentially illicit allergic reactions in humans and other medical problems (Rawal *et al*., 2013). In recent years, bacteriocins are considered as an important attention for their possible biopreservative activity in foods (O'sullivan *et al*., 2002). The use of non-pathogenic microorganisms and/or their metabolites to improve microbiological safety and enhance the durability of foods is defined as biopreservation (Denkova, 2012). Antagonistic properties of lactic acid bacteria (LAB) related to their safe history of use in traditional food fermented products make them very attractive to be used as biopreservatives (Jeevaratnam *et al*., 2005).

2. Materials and Methods

2.1. Sample collection

Dahi samples were collected aseptically from different retailer shops and straightly transported to laboratory at 4ºC for the isolation of lactic acid bacteria.

2.2. Isolation and identification of bacteria

Under the laboratory facilities, the small representative parts from each dahi were inoculated into MRS broth (Hi-Media, India) and kept for incubation at 37ºC for 48 hrs. By using MRS agar the pure culture of LAB was prepared (Figure 1). Furthermore this bacterial isolate was also cultured with anaerobic jar by using MRS agar also and incubated in carbon dioxide incubator at 37ºC for 48 hrs. Then the isolates were examined microscopically by Gram's staining (Figure 1). The isolated strain was inoculated into sugar broth to investigate the fermentation ability of the isolated organisms in different sugars. The activity of catalase enzyme was examined by rubbing the colonies of isolate with 3% hydrogen peroxide (H_2O_2) and the oxidase test was accomplished by using 1% solution of tetramethyl-p-phenylenediamine hydrochloride. According to biochemical test results, all the isolates belonged to *Lactobacillus* spp. according to Bergey's Manual of Determinative Bacteriology (Bergey, 1934).

2.3. Production of bacteriocin

The isolate was cultured in MRS broth and incubated at 37ºC for 48 hrs. After the incubation period, the broth culture of the isolates was centrifuged at 5000 X g for 10 minutes. The cells were settled down and the cell free supernatant was collected and used as crude bacteriocin.

2.4. Screening of the produced bacteriocin for antimicrobial activity by agar well diffusion method

Under aerobic condition, the antimicrobial activity of bacteriocin against some pathogenic organisms was examined by using well diffusion method. In perform growth inhibition by agar well diffusion method, Muller Hinton plates were heavily seeded uniformly with the test organisms. The restrictive activity of bacteriocin against some selected pathogenic microorganisms (*Escheriachia coli*, *Pseudomonas aeroginosa* and *Salmonella paratyphi*) was tested on Muller-Hinton agar (Figure 2). In Muller-Hinton agar plate wells were made by gel cutter at sterile condition and 150 µL of cell free supernatant (crude bacteriocin) was added to each of the wells. Then the plates were incubated at 37ºC for 48 hrs for further examination. The antimicrobial activity of bacteriocin was determined by measuring diameter of the zone of inhibition around the wells (Table 2).

2.5. Partial purification of bacteriocin

The crude bacteriocin was treated with ammonium sulphate solution of different concentrations stirred well. Then the treated bacteriocin was kept undisturbed at 4ºC for overnight. Precipitates were collected by centrifugation at 10000 X g for 10 minutes. Then the precipitate was dissolved in 0.5 M potassium phosphate buffer.

2.6. Characterization of bacteriocin

2.6.1. Effect of heat

Same volume (5 ml) of bacteriocin was taken into different test tubes and heated at 30, 50, 70, 90 and 100ºC for about 15 minutes. Then the treated bacteriocin was examined for antimicrobial activity.

2.6.2. Effect of pH
The purified bacteriocin was adjusted to pH 3, 5, 7, 9 and11 with hydrochloric acid (HCl) and sodium hydroxide (NaOH), incubated for 4 hrs at room temperature and similarly assayed for antimicrobial activity.

2.6.3. Effect of enzyme
The purified bacteriocin was treated with proteolytic enzyme (papain). The bacteriocin with enzyme (test) and bacteriocin without enzyme were taken into two separate test tubes and incubated at 37ºC for 2 hrs. Then the test and control were heated at 100ºC for 4 minutes to deactivate the enzyme activity. The treated bacteriocin was then examined for antimicrobial activity by using well diffusion method.

2.7. Biopreservative effect of bacteriocin on orange juice
The prepared orange juice was serially diluted at 10^{-6}. A portion (control) of diluted sample was examined initially for total plate count and the plates were incubated at 37ºC for 24 hrs. The other portion of diluted juice was added with 5% purified bacteriocin and refrigerated. Then the colony count of both the test (with bacteriocin) and the control (without bacteriocin) were recorded and compared.

3. Results
3.1. Isolation and identification of bacteria
The bacteriocin producing lactic acid bacteria was isolated from the dahi samples. This bacterial strain was identified as *Lactobacillus* spp. according to its physiological and biochemical characteristics (Table 1).

3.2. Bacteriocin production
The bacteriocin producing isolated organism was grown in MRS broth at 37ºC for 24 hrs. After incubation, the broth was centrifuged at 5000 X g for 15 minutes and the settled cell portion was separated out. The crude bacteriocin was found as supernatant which was used to estimate the antimicrobial activity.

3.3. Inhibitory activity
The inhibitory activity of the bacteriocin produced by the isolated strain was determined by using agar well diffusion method against some selected pathogenic organisms likely *Escherichia coli, Pseudomonas aeroginosa, Salmonella paratyphi*. The produced bacteriocin showed inhibitory activity against *Escherichia coli, Pseudomonas aeroginosa,* and *Salmonella paratyphi* (Figure 3).

3.4. Effect of heat and pH on bacteriocin
The activity of bacteriocin from the isolate was examined with different temperature (30, 50, 70, 90 and 100 ºC) and pH (3, 5, 7 and 9). The maximum zone of inhibition was evaluated at temperature 30 ºC and pH 3.

3.5. Effect of enzyme on bacteriocin
The activity of bacteriocin was completely inhibited by the proteolytic enzyme (papain). This indicated that the isolated bacteriocin is proteinaceous in nature.

3.6. Biopreservative effect of bacteriocin
The preservative effect of produced bacteriocin was tested with fresh orange juice. In the test (Juice with bacteriocin) the maximum reduction of colony was observed at 5% concentration of bacteriocin and in control (Juice without bacteriocin) no population reduction was observed. Furthermore the results indicated that the microbial count drastically decreased in orange juice.

Table 1. Biochemical reactions by the isolates.

Sample Code	Gram Staining	Motility Test	Indole Test	Methyl Red Test	VP Test	Oxidase Test	Catalase Test	Urease Test	Citrate Utilization Test
DH1	+	-	-	-	-	-	-	-	-
DH2	+	-	-	-	-	-	-	-	-
DH3	+	-	-	-	-	-	-	-	-
DH4	+	-	-	-	-	+	-	+	+
DH5	+	-	-	-	-	-	-	-	-

(+ = positive; - = negative; VP = Voges-Proskaur)

(a) (b)

Figure 1. (a) Isolates grown on MRS agar; (b) Microscopic view of isolates when Gram stained.

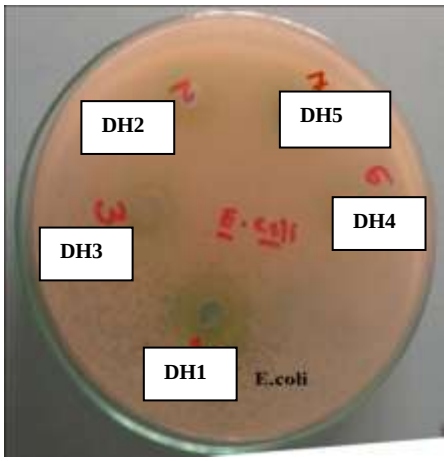

Figure 2. Antibacterial activity of bacteriocin.

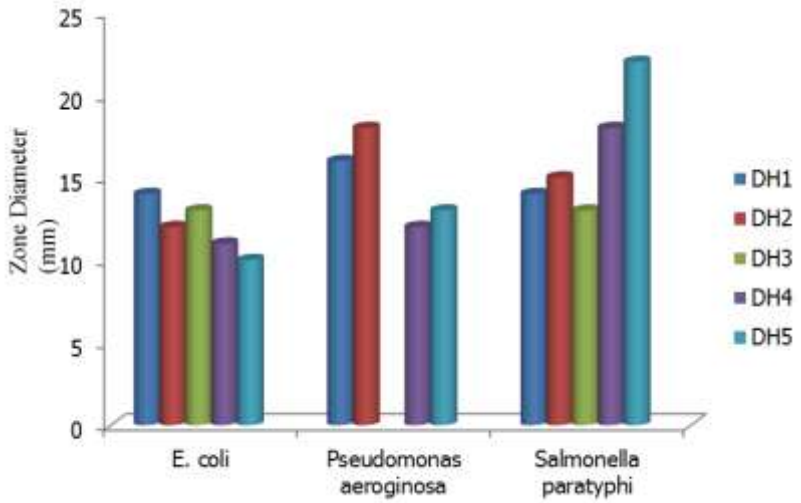

Figure 3. Graphical representation of antimicrobial activity of bacteriocin.

Table 2. Antimicrobial activity of bacteriocin against pathogenic organisms.

Isolates	Pathogenic bacteria	Zone of inhibition (mm)
DH1	*Escheriachia coli*	14
	Pseudomonas aeroginosa	16
	Salmonella paratyphi	14
DH2	*Escheriachia coli*	12
	Pseudomonas aeroginosa	18
	Salmonella paratyphi	15
DH3	*Escheriachia coli*	13
	Pseudomonas aeroginosa	-
	Salmonella paratyphi	13
DH4	*Escheriachia coli*	11
	Pseudomonas aeroginosa	12
	Salmonella paratyphi	18
DH5	*Escheriachia coli*	10
	Pseudomonas aeroginosa	13
	Salmonella paratyphi	22

4. Discussion

This study highlights the isolation, characterization and activity of bacteriocin produced by *Lactobacillus* spp. Dahi was found as a good source of lactic acid forming bacteria (LAB). The biochemical characterization of the isolated strain from dahi indicated that it was *Lactobacillus* spp. This isolated strain was examined with three different pathogenic organisms which are commonly present in food and can cause illnesses in human. The selected pathogenic organisms were *Escherichia coli, Pseudomonas aeroginosa, and Salmonella paratyphi*. The result showed that the growth of these pathogenic organisms was retarded by the bacteriocin which was produced by *Lactobacillus* spp. By this present study the biopreservation activity of bacteriocin was examined for orange juice. The lower colony count of organisms was found in test (with 5% bacteriocin) in total plate count agar. This study revealed that the bacteriocin which is produced from the isolated organisms from fermented dahi posses a wide spectrum of inhibitory activity against pathogenic organisms. Klaenhammer (1993) reported that bacteriocins are antimicrobial agents produced by bacteria which are active against closely related bacteria. The natural food preservative was used in 1931 which was nisin. The food and drug administration (FDA) approved nisin to be use in pasteurized processed cheese in 1988 reported by Rossland *et al*. (2005). As *Lactobacillus* spp. produces bacteriocin like nisin the present study also has the potential to improve the biopreservation system.

5. Conclusions

Now-a-days, lactic acid bacteria (LAB) avowed as safe and LAB can produce bacteriocins which may be considered as a good solution to the problem of resurgence of resistant strains to antibiotics. Consumer attention is needed to the existence of natural substances that can protect against food-borne related illness. Since bacteriocins are considered natural substances, they might have a proper adoption from consumers who start to demand more natural and safe food products. The isolated *Lactobacillus* spp. can produce bacteriocins which retard the growth of a number of pathogenic organisms. This produced bacteriocin may be considered potential to be used as natural biopreservative as well as antibacterial agent in food preservation.

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Conflict of interest

None to declare.

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