

Studies On Some Properties Of Bacteriocins Produced By Lactobacillus Species Isolated From Agro-Based Waste

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Abstract

Lactic acid producing bacteria (LAB) were isolated from vegetable waste. These organisms were studied for bacteriocin production. Among the isolated cultures *Lactobacillus lactis* and *Lactobacillus plantarum* were potent producers of bacteriocins. Bacteriocin produced by these *Lactobacillus* species had a large spectrum of inhibition against food spoilage microorganisms and various related strains of lactic acid bacteria. The bacteriocin inhibited the growth of *Escherichia coli* but it showed least activity against *Candida albicans*. The antibacterial activity appeared to be pronounced between late logarithmic phase and early stationary phase. The bacteriocins were found to be heat stable. Supplementation with lactose, peptone and yeast extract enhanced the production of bacteriocin.

INTRODUCTION

Bacteriocins are highly specific antibacterial proteins produced by strains of bacteria active mainly against some other strains of same or related SPP (Gaur et al 2004). The bacteriocins produced by Lactic acid bacteria (LAB) are potent bio-preservative agents and the applications of these in food are currently the subject of extensive research. The search for new bacteriocins with a wider spectrum of activity and compatibility with different food system is being studied by some investigators.

Fruits and vegetable waste provide a good source for isolating LAB having antagonistic and probiotic properties. However, work on bacteriocinogenic LAB from agro-based waste has been limited. Therefore the present studies are carried out to examine LAB population isolated from damaged and spoiled vegetable wastes collected from local market yard. Bacteriocins produced by these organisms are characterized with respect to inhibition spectrum, effect of physical and chemical parameters and effect of cultural conditions on bacteriocin production.

MATERIALS AND METHODS

Vegetable waste containing spoiled cabbage and cucumber was collected from local market yard. It was then crushed, homogenized with kitchen blender, and inoculated in the deMan Rogosa and Sharpe (MRS) broth for enrichment of resident LAB. The tubes were incubated at 37°C for 24

hours.

SCREENING FOR BACTERIOCIN PRODUCERS

The enriched broth was serially diluted prior to being pour plated on MRS agar. The plates were incubated at 37°C for 16 hours till colonies appeared. The plates were then overlaid with MRS soft agar inoculated with indicator organisms isolated from raw milk. All the plates were incubated at 37°C for 24 hours. Colonies showing zone of inhibition were considered as potential bacteriocin producers.

IDENTIFICATION OF BACTERIOCIN PRODUCERS

The bacterial strains that were selected as potential bacteriocin producers were subjected to morphological, cultural and biochemical characterization according to Bergey's Manual of Determinative Bacteriology (1997).

BACTERIOCIN ASSAY

The strains that were selected as potential bacteriocin producers were grown in MRS broth at 37°C for 48 hours. Cells were separated by centrifugation at 5000 rpm for 10 minutes. The pH of the cell free supernatant was adjusted to 5.5 with sterile 0.2 N NaOH. Bacteriocin activity in the supernatant was then tested by agar well diffusion assay (Geis et al 1983). The antagonistic effects of the culture supernatants of bacteriocin producing LAB were tested on various Gram Positive and Gram Negative organisms listed

in Table - 2. MRS agar was used for lactic strains, nutrient agar for gram positive and gram negative organisms and Sabouraud Dextrose Agar was used for *Candida albicans*. All cultures were grown aerobically at 37°C for 48 hours. Inhibition zones around the wells were measured. The antimicrobial activity of the bacteriocin is defined as the reciprocal of the highest dilution showing inhibition of the indicator lawn and is expressed in activity units per ml (Nakamura et al 1983).

Figure 1

Table 2: Inhibition patterns of bacteriocin producer strains against different indicator organisms

Indicator strain	<i>L.lactis</i>	<i>L.plantarum</i>
<i>Micrococcus luteus</i>	+++	++
<i>Streptococcus epidermis</i>	++	-
<i>Staphylococcus aureus</i>	++	+
<i>Bacillus cereus</i>	+	+
<i>Bacillus subtilis</i>	+	-
<i>Bacillus megaterium</i>	++	+
<i>Escherichia coli</i> MTCC1588	++	++
<i>Escherichia coli</i> MTCC2939	+	++
<i>Lactobacillus acidophilus</i>	++	++
<i>Lactobacillus plantarum</i>	+	+
<i>Candida albicans</i>	-	-

Degree of Inhibition: + = Moderate inhibition zone 6-9mm
 ++ = Strong inhibition zone 10-14mm
 +++ = Very strong inhibition zone 15-18mm
 - = No inhibition zone.

STUDY OF PHYSICAL AND CHEMICAL PROPERTIES OF BACTERIOCIN

To test the heat resistance, 100 ml of culture supernatant was heated for 10 minutes at 60°C, 70°C, 80°C, 100°C and 121°C and residual bacteriocin activity was detected against indicator organism (Ogubanwo et al 2003). Sensitivity of bacteriocin to different pH values was tested by adjusting the pH of culture supernatant in the range of pH 4 to 9 and then kept at room temperature for 4 hours residual activity was determined against the indicator organism (Karao Glu et al 2003).

EFFECT OF PROTEOLYTIC ENZYME ON BACTERIOCIN ACTIVITY

Action of proteolytic enzyme was tested on culture supernatant by treatment with pepsin and trypsin each at a final concentration of 1mg per ml. It was then incubated at 37°C for 2 hours and residual activity of bacteriocin was assayed (Nakamura et al 1983).

EFFECT OF INCUBATION PERIOD ON BACTERIOCIN PRODUCTION

Active cultures of producer organisms (1 % v/v) were

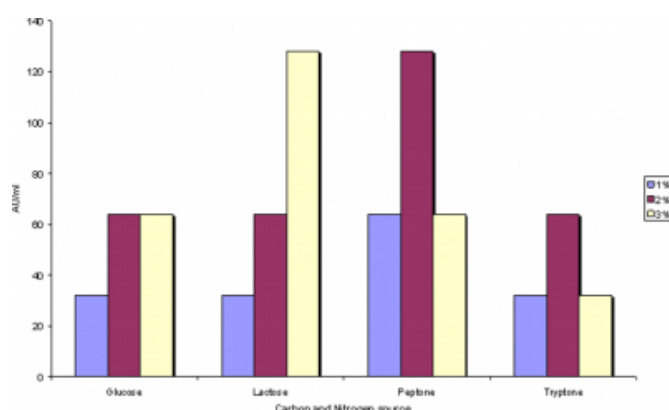
inoculated in 100 ml aliquots of sterile composed media. Inoculated flasks were incubated at 15, 24, 48, and 72 hours and at the end of each incubation period, bacteriocin activity was observed by inoculating culture supernatant against indicator organism.

EFFECT OF DIFFERENT CONCENTRATIONS OF CARBON AND NITROGEN SOURCE ON BACTERIOCIN PRODUCTION:

The effect of different concentrations of medium ingredients on bacteriocin production was evaluated using composed MRS medium. The carbon sources studied were glucose (1 – 4%) and lactose (1 – 4%) while nitrogen sources were tryptone (1 – 3%), peptone (1 – 3%) and yeast extract (0.5 – 2%).

Figure 2

Figure 1: Effect of concentration of Carbon and Nitrogen source on bacteriocin production



RESULTS AND DISCUSSION

DETECTION OF BACTERIOCIN PRODUCERS

Two isolates designated as P1 and P2 have shown clear zone of inhibition against the indicator organism and they were selected as potential bacteriocin producers. The isolates were identified as *Lactobacillus lactis* and *Lactobacillus plantarum* (Table -1). The culture supernatant obtained from both producer strains were tested for antibacterial activity against different indicator organisms (Table - 2). Both the producer organisms have wide antibacterial activity against both Gram positive and Gram negative bacteria. Largest zone of inhibition was observed against *Micrococcus lutae* MTCC 106. Therefore, *Micrococcus lutae* MTCC 106 was used as an indicator organism in further characterization. Inhibitory activity of bacteriocins was seen against *L. acidophilus*, *Lactobacillus plantarum* and *Escherichia coli*. However, none of the bacteriocins showed inhibitory activity

against *Candida albicans*.

Figure 3

Table 1: Biochemical characterization of bacteriocin producer

Biochemical Test	<i>L.lactis</i>	<i>L.plantarum</i>
Glucose	+	+
Lactose	+	+
Maltose	+	+
Mannose	+	+
Mannitol	-	+
Sucrose	-	+
Xylose	-	+
Catalase	-	-
Citrate	-	-
Growth on NaCl	-	-

+ = Positive test
- = Negative test

The effect of incubation period on bacteriocin production was also studied and it was observed that at the end of 48 hours, the activity was found to be maximum (Table - 3). Further studies were carried out at different temperatures and pH to test sensitivity of bacteriocin. The bacteriocins of both isolates were stable at 100°C for 10 minutes. Bacteriocin of *Lactobacillus lactis* retained its activity even at 121°C for 10 minutes (Table - 3). This indicated that bacteriocins produced by this LAB belonged to heat stable low molecular weight group of bacteriocins. Bacteriocin of *Lactobacillus lactis* was stable in acidic to neutral range i.e. from pH 4 to 7 but it became inactive in the alkaline range (Table - 3) whereas bacteriocins of *Lactobacillus plantarum* remained active only in the acidic range from pH 4 to 6. Both the bacteriocins were completely inactivated by trypsin and pepsin treatment.

Figure 4

Table 3: Effect of heat treatment, pH and proteolytic enzymes on bacteriocin

Bacteriocin producer strain	Resistance to heating (10min) Temp in °C				Sensitivity to different pH values						Sensitivity to Proteolytic enzyme	
	60	80	100	121	4	5	6	7	8	9	Pepsin	Trypsin
<i>L.lactis</i>	R	R	R	R	R	R	R	R	S	S	S	S
<i>L.plantarum</i>	R	R	R	S	R	R	R	S	S	S	S	S

R= Resistance: No change in Bacteriocins activity
S= Sensitive: Inhibition of Bacteriocins activity

The influence of culture medium components on the production of bacteriocin was investigated using *Micrococcus luteus* as an indicator organism. The results of our study revealed an increase in bacteriocin production in

MRS medium containing 3% lactose concentration and 2% peptone concentration. Lactose was found to be better carbon source than glucose. Thus, variation in the concentration of constituents might have an influence on the amount of bacteriocins produced. Ogunbanwo et al (2003) obtained maximum bacteriocin production by supplementing 1% glucose and 1% peptone to normal MRS media. However, it is observed from the present study that lactose and peptone gave better bacteriocin production.

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