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Characterization of Bacteriocin Produced by *Lactobacillus delbreukii* Isolated from Yoghurt.

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ABSTRACT

The present study was aimed at characterization of bacteriocin produced by *Lactobacillus delbreukii* isolated from yoghurt. Bacteriocin was produced by growing the lactobacillus strain in MRS broth (pH-6.0) seeded with 5% inoculum of overnight culture and maintained anaerobically at 30ºC for 48 h. Cells were removed from the growth medium by centrifugation and the cell-free supernatant was used as bacteriocin. Well diffusion assay was done against indicator organism, E.coli by treating the bacteriocin with different reagents, and untreated bacteriocin was used as control. Addition of enzymes, amylase and lipase showed slightly positive effect on bacteriocin production, while Proteinase K and pepsin inhibited bacteriocin production. Among detergents, Sodium dodecyl sulphate (SDS), Tween 80 and Tritone X-100 stimulated bacteriocin production and was inhibited by EDTA and urea. On addition of NaCl to MRS medium, 1% NaCl increased bacteriocin production while it was strongly inhibited at 4% NaCl. Bacteriocin production was increased in MRS medium containing 1% glucose and 1% peptone as carbon and nitrogen sources. The bacteriocin was purified by ammonium sulphate precipitation, followed by dialysis. The protein content was determined by Lowry method and was found to be 198 mg for culture filtrate and 27 mg after dialysis.

Key words: Yoghurt, bacteriocin, indicator organism, lactic acid bacteria, *Lactobacillus delbreukii*.

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INTRODUCTION

Bacteriocins are antimicrobial peptides or proteins produced by different microorganisms, including food-grade microorganisms such as lactic acid bacteria [1-3]. They are ribosomally synthesized peptides of 30 to less than 60 amino acids, with a narrow to wide antibacterial spectrum against gram positive bacteria [4]. Lactic acid bacterial bacteriocins are defined into four distinct classes: *Class I*, lantibiotics, are small (<5 kDa) peptides containing the unusual amino acids lanthionine (Lan), -methyllanthionine (MeLan), dehydroalanine, and dehydrobutyrine; *Class II*, small (<10-kDa), relatively heat-stable, non-lanthionine-containing membrane-active peptides, subdivided into *Listeria*-active peptides with the N-terminal consensus sequence-Tyr-Gly-Asn-Gly-Val-Xaa-Cys- (Class IIa), poration complexes requiring two different peptides for activity (Class IIb), and thiol-activated peptides requiring reduced cysteine residues for activity (Class IIc); *Class III*, large (>30-kDa), heat-labile proteins; and *Class IV*, complex bacteriocins that contain essential lipid or carbohydrate moieties in addition to protein [5]. Bacteriocins have been produced and characterized from lactobacillus sp isolated from different environments, several food products of dairy industry and even from vegetables and fruit pulps, [6-15]. Partial characterization of a bacteriocin produced by *Lactobacillus delbrueckii* subsp. lactis UO004, was done, which is an intestinal isolate with probiotic potential. It was found to be a hydrophobic, heat-stable polypeptide, and also stable and active over a wide pH range [16].

The objectives of this study were to isolate *Lactobacillus delbreukii* from yoghurt, and to characterize the bacteriocin produced from it.

MATERIALS AND METHODS

Isolation and Identification of Lactic Acid Bacteria

Lactic acid bacteria were isolated from samples of fresh yoghurt collected from the commercial market. Yoghurt samples were serially diluted in peptone medium (10^{-1} – 10^{-6}), incubated at 23ºC for 30 min and then plated onto De Man Rogosa Sharpe (MRS) medium [17, 18]. The plates were incubated at 37ºC for 24-72 hrs. Isolated colonies with typical characteristics namely off white, raised, spherical small with entire margins were picked from each plate and transferred to MRS broth for further analysis. The strain identification was done using the standard morphological, physiological and biochemical assays [19,20]. The identified genus *Lactobacillus* was further classified to the species level based on their ability to ferment sugars [21].

Agar-Well Diffusion Assay

The *Lactobacillus delbreukii* strains that were selected were grown in MRS broth at 37ºC for 48 hrs. Cells were separated by centrifugation at 5000 rpm for 10 min at room temperature. Aliquots (50 µl) of the cell culture were placed in 4-mm-diameter wells that had been cut in nutrient agar plates previously swabbed with the indicator organism. After overnight incubation, the diameters of the zones of growth inhibition were measured. Antimicrobial activity was expressed in arbitrary units (AU/ml). One AU was defined as the reciprocal of the highest level of dilution resulting in a clear zone of growth inhibition [22].

Effect of NaCl on Bacteriocin Production

MRS broth with 0, 1%, 3% and 4% NaCl was sterilized by autoclaving and were inoculated with10% of the overnight bacteriocin producing culture and incubated at 37°C for 24 hrs. Bacteriocin activity was assayed by inoculating the culture supernatant against indicator organism [23].

Effect of Enzymes and Detergents

The sensitivity of the active substance to enzymes was tested on cell-free supernatant (pH 6.0) of 24 h cultures incubated at 30ºC and were treated for 2 h with 1.0 mg final concentration of the following enzymes: proteinase K, pepsin and lipase (all from Hi Media Laboratory Pvt Ltd. India). The surfactants tested were sodium dodecyl sulphate (SDS), Tween 80, Tritone X-100, EDTA and urea at final concentration 1% (all from Hi Media Laboratory Pvt. Ltd. India). Control consisted of active supernatant. All samples and control were incubated at 30ºC for 5 h and tested for activity by well diffusion assay.

Sensitivity to Chloroform

The culture supernatant was mixed with an equal volume of chloroform and kept at room temperature for 4 hrs before antimicrobial activity testing [24].

Effect 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%) and lactose (1%) while nitrogen sources were tryptone (1 %) and peptone (1%) [25].

Purification of Bacteriocin

The crude bacteriocin was precipitated with 80% ammonium sulphate saturation. The precipitate was dialysed against 20 mM potassium phosphate buffer (pH 7.0) for 12 h at 4ºC.

Determination of Protein

Protein concentration of the bacteriocin in supernatant was determined by the Lowry method [26], using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

The present investigation highlights the characterization of bacteriocin produced from *L.delbreukii* isolated and identified from yoghurt samples. The morphological characters of raw milk, natural whey starter and cheese were studied [27]. It was stated that Lactobacilli are generally isolated on rich media such as MRS which is routinely used for the isolation and counting of Lactobacilli for most fermented food products [17].

The six strains LB-01 to LB-06 were subjected to well diffusion assay against indicator organism, E.coli. Strain LB-03 showed the highest zone of inhibition (Table-1) and was selected to produce bacteriocin which was further characterized by treating with different reagents. The effect of NaCl on the production of the bacteriocin was studied. 1% NaCl increased the production of bacteriocins in the strain while it was reduced at 3% NaCl concentration and was almost lost at 4% NaCl concentration (Table-2; Fig-1). The influence of culture medium components on the production of bacteriocin was investigated. The result of this study revealed an increase in bacteriocin production in MRS medium containing 1% glucose and 1% peptone to normal MRS medium which was found to be optimum (Table-2).

The effect of enzymes like proteinase K, pepsin and lipase on bacteriocin production was also investigated (Table-3). In the presence of lipase there was positive effect of bacteriocin production. Proteinase K and pepsin strongly inhibited bacteriocin production. Table-3; Fig-2, shows the effect of detergents and chelating agents like sodium dodecyl sulphate (SDS), Tween 80, Triton X-100, EDTA and urea on bacteriocin production. Sodium dodecyl sulphate (SDS), Tween 80 and Triton X-100 could stimulate the bacteriocin production. In contrast, it was strongly inhibited by EDTA and urea (Table-3). The strain was sensitive to chloroform. Variation in the concentration of constituents might have an influence on the amount of bacteriocin produced [15].

In the purification process the proteins were concentrated by 80% ammonium sulphate precipitation followed by dialysis. Protein content was determined by Lowry method [26], and was found to be 198 mg for culture filtrate and 27 mg after dialysis Table-4.

Table 1: Zone of inhibition of *L. delbreukii* against indicator organism, *E.coli.*

Sl.No	Strain code	Zone of inhibition (in cm)
1	LB-01	1.2
2	LB-02	1.5
3	LB-03	2.2
4	LB-04	1.5
5	LB-05	1.4
6	LB-06	1.7

Table 2: Zone of inhibition on treating with NaCl, and carbon and nitrogen sources.

Sl.No	Treatment	Zone of inhibition (in cm)
1	control	1.6
2	NaCl 1%	1.5
3	NaCl 3%	1.3
4	NaCl 4%	0.9
5	Glucose 1%	1.5
6	Lactose 1%	0.6
7	Tryptone 1%	1.2
8	Peptone 1%	1.3

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Table 3: Zone of inhibition on treating with chloroform, enzymes and detergents.

Sl.No	Treatment	Zone of inhibition (in cm)
1	control	1.6
2	Chloroform	1.0
3	Proteinase-k	0.9
4	Pepsin	0.8
5	Lipase	1.6
6	SDS	1.6
7	Tween 80	1.5
8	Triton X-100	1.5
9	EDTA	1.2
10	Urea	0.7

Table 4: Protein concentration of bacteriocin from *Lactobacillus delbreukii*.

Purification stage	Volume (ml)	Total protein (mg)
Culture filtrate (supernatant)	100	198
Ammonium sulphate precipitation, (80% saturation) and dialysis	25	27

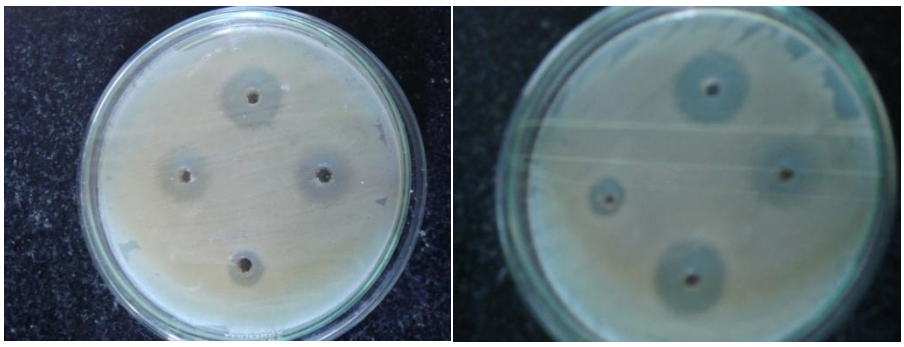

Figure-1: Effect of different concentrations of NaCl and, carbon and nitrogen source on bacteriocin production

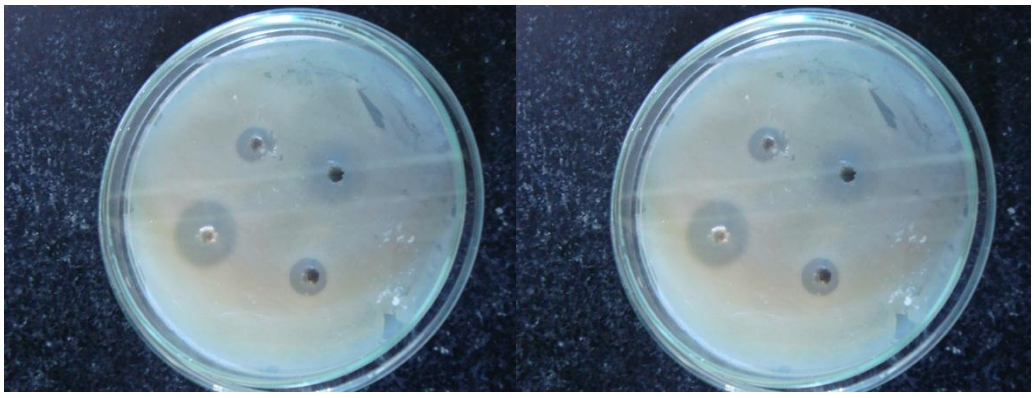

Figure-2: Zone of inhibition formed by bacteriocin treated with enzymes and detergents

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