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ORIGINAL ARTICLE

Bacteriocin of *Lactobacillus plantarum* for Control of Bacterial Food Spoilage Agents

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ABSTRACT
Bacteriocins are antimicrobials produced by bacteria which are generally effective against closely related species. Lactobacillus plantarum MTCC 9503 was used as bacteriocin producer in the present study. Bacteriocin was prepared from different stages of growth of this organismand subjected to well diffusion assay. Lactobacillus plantarum produced 10,000 AU/ml of bacteriocin. Different concentrations of bacteriocin preparation ofL plantarum. were used against bacterial agents of food spoilage name Bacillus, Erwinia and Pseudomonas. Bacillus sp was inhibited at 4000 AU/ml of bacteriocin whereas the other two bacterial strains were inhibited at 5000 AU/ml.
Keywords- Bacteriocins, lactic acid bacteria, arbitrary units, microbial food spoilage.

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INTRODUCTION

Biopreservation refers to extended storage life and enhanced safety of foods using the natural microflora and their antibacterial products [1]. Lactic acid bacteria have a major potential for use in biopreservation because they are safe to consume and during storage they naturally dominate the microflora of many foods. Lactic acid bacteria are suitable as bacteriocin producers. They have been granted GRAS (generally regarded as safe) status. Bacteriocins are ribosomally synthesized proteins produced by them. They are anti-microbial in nature which can be exploited for food biopreservation. Bacteriocins can be used in two ways- addition of bacteriocin as a food additive, addition of a bacteriocin producer to the food. The only bacteriocin given GRAS status is Nisin [2] and is commercially available as Nisaplin (Danisco, Copenhagen, Denmark) [3]. It is routinely used especially in the production of dairy products. They are non-toxic to eukaryotic cells and hence pose no threat to human intestinal cells. Being proteinaceous in nature they are readily degraded by protelytic enzymes in human GI tract. They influence growth of food borne pathogens and also food spoilage organisms. They are not known to cause allergies. Being of LAB origin they are probiotic in nature and help in restoring the normal gut microflora.

MATERIAL AND METHODS

Microbial cultures used-*Lactobacillus plantarum* MTCC 9503 was used as a bacteriocin producer. *Lactobacillus brevis* MTCC 1750 was used as an indicator organism. The cultures were procured from Institute of Microbial Technology, Chandigarh. They were maintained on recommended MRS medium. They were subcultured every 28-30 days and the slants were checked for purity by staining. These cultures were stored at refrigeration temperature at all times.

Preparation of bacteriocin- Bacteriocins are generally produced towards the end of log phase or beginning of stationary phase.Growth stages of the bacteriocin producers were identified by plotting growth curve. To prepare partially purified bacteriocin by the method outlined in [4], cell free supernatant (CFS) was obtained by centrifuging the broth culture of different phases of growth at 10,000 rpm/20 min. pH of CFS was neutralized by addition of 0.1N NaOH. It obviates inhibition due to organic

acids which can give false positive results. It was followed by heating at 80^{0}C for 15 minutes to deactivate proteins which may accord a false positive result. It was then filter sterilization through 0.22 µm pore size cellulose acetate filter (Millipore). Bacteriocin was prepared from broth cultures from late log and early stationary phase.

Well diffusion assay-Bacteriocin activity was determined by the well diffusion method [5]. Plates containing solidified MRS agar (2% agar) were overlaid with soft MRS agar (0.8% agar). After solidification, the plates were inoculated with 0.1 ml of metabolically active culture of indicator strain. Wells were made in soft agar and 100µl of bacteriocin preparation was transferred into each well. Plates were incubated at 37°C for 24h and observed for presence/absence of zone of inhibition.

Quantification of bacteriocin-The bacteriocin was then quantified[6]. Serially ten-fold dilutions of the bacteriocin preparation was made and subjected to well diffusion assay. Arbitrary units (AU)is defined as the reciprocal of the highest dilution showing the minimum zone of inhibition of 2 mm[7]. It is expressed in arbitrary units (AU).

Assay of bacteriocin from *L plantarum* against food spoilage organisms- *Bacillus*sp, *Erwinia carotovora* MTCC 1428 and *Pseudomonas marginalis* MTCC 2578 were used as indicator organisms in this experiment. Metabolically active cultures of these organisms were prepared by inoculating and incubating them overnight in nutrient medium at their optimum temperature of growth. Well diffusion assay was performed using broth culture containing indicator cells equivalent to McFarland Standard 0.5. Different volumes of bacteriocin preparation were added to well which were made in the soft agar.

RESULTS AND DISCUSSION

Growth curve of bacteriocin producers was plotted and the various phases of growth were identified. No appreciable lag phase duration was observed. *L plantarum* MTCC 9503 exhibited log phase upto 9h, stationary phase upto 36h and decline phase upto 54h on incubation at optimum temperature of growth (Fig 1).

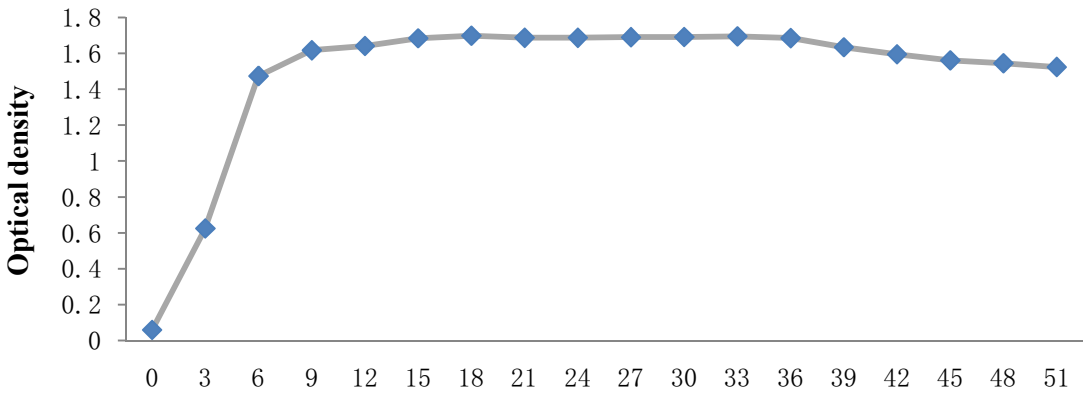

Fig 1- Growth stages of *Lactobacillus plantarum* MTCC 9503.

Bacteriocin prepared from different phases of growth was used for well diffusion assay using lactics as indicator organisms. Results are presented in Table 1.*L plantarum* MTCC 9503 produces maximum bacteriocin at early stationary growth phase. It shows maximum zone of inhibition (i.e. 1.85) after 12 h of incubation against *Lactobacillus brevis* MTCC 1750.

Table1: Well-diffusion assay with bacteriocins of *Lactobacillus plantarum*MTCC 9503.

S.No	Time period of incubation (h)	Zone of inhibition (cm)
1.	3 (Early Log Phase)	0.80
2.	6 (Mid Log Phse)	0.95
3.	9 (Late Log Phase)	1.08
4.	12 (Early Stationery Phase)	1.85
5.	18 (Mid Stationery Phase)	1.62

Results of quantification of bacteriocin are presented in Table 2.Bacteriocin preparationfrom*L plantarum* MTCC 9503exhibited zone of inhibition more than 2mm after diluting 10^{-3} times. It was calculated to contain 10,000 AU/ml.

Table 2: Quantification of bacteriocins of *Lactobacillus plantarum*MTCC 9503.

S.No	Dilution factor	Zone of inhibition (mm)
1.	Bacteriocin preparation	18
2.	10^{-1}	14
3.	10^{-2}	9
4.	10^{-3}	6
5.	10^{-4}	ND

In vitro antimicrobial spectrum against bacterial food spoilage agents considered in the present study are presented in Table 3. Different volumes of bacteriocin containing different concentration of it were added to wells prepared in soft agar. *Bacillus* was inhibited by 4000 AU/ml of bacteriocin whereas *Erwinia* and *Pseudomonas* is inhibited by 5000 AU/ml of bacteriocin preparation. One of the major drawbacks of bacteriocins is their narrow spectrum of activity. Produced by Gram positive lactics they are generally effective against other Gram positives more than Gram negatives. Minimum inhibitory concentration of bacteriocin against Gram positive.Plantaricin 35d produced by *Lactobacillus plantarum* is active against *Aeromonas hydrophila*[8]. Bacteriocin ST151BR is effective against *E coli* and is produced by *L pentosus*[9]. *E coli* is also susceptible to bacteriocin produced by *Lparacase*i subsp*. paracasei*[10]. *L plantarum* isolated from molasses produced bacteriocins effective against *E coli* and *Acinetobacter baumanii* and also some Gram positive bacteria [11]. Twoisolates, *Lactobacillus*spp, *Lplantarum* and *L lactis* from vegetable waste that produced a bacteriocin which inhibited the growth of *E coli* [12].

Table 3. Antimicrobial spectrum of *Lactobacillus plantarum*bacteriocin against bacterial agents of food spoilage.

S.No	Concentration of bacteriocin (AU)	Zone of inhibition (cm)		
		Bacillus	*E carotovora*	*P marginalis*
1	1000	ND	ND	ND
2	2000	ND	ND	ND
3	3000	ND	ND	ND
4	4000	6	ND	ND
5	5000	9	7	7

CONCLUSIONS

One of the major challenges before food industry today is to produce minimally processed foods as increased use of chemical preservatives have led to a spurt in incidences of food allergies. Biopreservation is a tool to meet this challenge. It is the use of biological agents to enhance the shelf life of foods. Lactic acid bacteria are widely regarded a probiotics. They also produce bacteriocins which being antimicrobials inhibit the growth of other organisms.*Lactobacillus plantarum*` bacteriocin has demonstrated efficacy against bacterial agents of food spoilage. It can be potentially exploited for processing of food.

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COMPETING INTEREST

The author declares that no competing interest exists.

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