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ANTIMICROBIAL PROPERTIES OF PROBIOTIC *LACTOBACILLUS CASEI* (DM 60) AGAINST SELECTED PATHOGENS

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Abstract: The antimicrobial properties of probiotic *Lactobacillus casei* (DM 60) isolated from dairy products were studied using the agar well diffusion assay. Bacteriocin producing *Lactobacillus* spp isolated from yoghurt, showed wide range of antimicrobial activity against some major food borne pathogens.
Keywords: *Lactobacillus casei*, antimicrobial compound (bacteriocin).

Introduction

Lactobacilli belong to the group of lactic acid bacteria (LAB), that have several distinguished abilities such as production of lactic acid, enzymes such as β-Galactosidase and natural antimicrobial substances called bacteriocins. Bacteriocins are bacterially produced peptides that are active against other bacteria and against which the producer has a specific immunity mechanism (Cotter and Ross 2005). They are produced by all major lineages of bacteria and archaea and constitute a heterogeneous group of peptides with respect to size, structure, mode of action, antimicrobial potency, immunity mechanisms and target cell receptors (Gillor et al 2008). Bacteriocins are biologically active peptides produced by several bacterial species especially by *Lactobacillus* spp and are active against both gram positive and gram negative pathogenic bacteria (Bhattacharya, 2010; Kaur et al 2012). Bacteriocins are emerging such as use in functional foods and use in human therapy as an alternative to antibiotics. Bacteriocins antimicrobial properties are aimed at stimulating the immune system. It can be used as an aid treatment of gastrointestinal and urinary tract diseases (Benkerroum et al 2007; Shelar et al 2012).

Materials and Methods

Bacteriocin activity assay

Bacterial strain *Lactobacillus casei* (DM 60) was used in this study. It was grown in MRS broth at 30°C for 24h. Crude cell-free supernatant of *Lactobacillus casei* (DM 60) was

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collected by centrifugation at 8000 rpm for 20 min at 4 °C. The cell-free supernatant was adjusted to pH 6.0 using 1N NaOH and it was used as crude bacteriocin. Antibacterial activity of the culture supernatant of the isolates was determined by well diffusion assay. Antibacterial property of *Lactobacillus* strain was evaluated against different pathogens such as, *E. coli* ATCC 29522, *Bacillus cereus* ATCC 10702, *Staphylococcus aureus* subsp. *aureus* ATCC 29213 and *Staphylococcus aureus* MTCC 902. All Pathogenic isolates were lawn cultured over MHA plates and six millimeter diameter wells were punched in the plates and filled with 200 µl of supernatant. After incubation of the plates at 30°C for 24 h, diameters of zone of inhibition were measured (Kaushik, 2009).

Effect of various factors on production of bacteriocin

To determine the effect of various factors on antimicrobial activity of potent probiotic strain, it was allowed to grow in various parameters such as temperatures, pH and NaCl concentrations separately. It was incubation at different temperature (4, 30, 37 and 45 °C), at different pH (3, 4.5, 5.5, 6.5 7, 7.5 and 8.5) and different concentrations of NaCl (1-4%). The same well diffusion method was followed (Roos et al 2005).

Sensitivity of bacteriocin to enzymes, pH, temperature

200 µl of crude supernatant of *Lactobacillus casei* (DM 60) was treated with Proteinase K (Sigma) and lysozyme (Sigma) at 0.1 mg/ml and 1mg/ml final concentrations, and incubated at 37°C for 2 h. Enzymes reactions were terminated by boiling for 5 min. To test the pH stability, the bacteriocin was incubated at 37°C for 2 h at pH 2.0 to 10.0 (at increments of one pH unit). The effect of temperature on the bacteriocin was tested by heating the crude bacteriocin at 100°C for 60 and 120 min (Tiwari and Srivastava 2008).

Results and Discussion

Lactobacillus casei (DM 60) was found to be bacteriocin producer. The strain supernatant exhibited antimicrobial activity against all test pathogens. The diameter of inhibition zones in well diffusion method was greater than 10 mm. Complete inactivation was observed when supernatant of was treated by Proteinase K, thus confirming its proteinaceous nature. The antimicrobial substance produced by the tested isolate remained fully stable after heat treatment from 60 to 100°C for 60 min, lower pH values (pH 5 and below) and active in 7% salt concentration. In the present study, it could be concluded that the potent probiotic antimicrobial isolate (DM60) was able to show activity at 30 and 37 °C, at pH range of 5.5 and 6.5 and 1 to 2% NaCl concentration.

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