Detection, partial purification and characterization of bacteriocin produced by Lactobacillus pentosus FPTLB13 isolated from freshwater fish

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RESEARCH ARTICLE

ABSTRACT

Lactobacillus pentosus FPTLB13 was isolated from fresh water fish, capable of producing bacteriocin that had broad spectrum of inhibition (6400 AU/ml) against *Lactobacillus casei*, Lactobacillus sakei, Pseudomonus aeruginosa, Escherichia coli, Staphylococcus aureus and *Enterococcus faecalis*. The antimicrobial activity was stable at 80°C for 15 min, and partly active at 121°C for 15 min. It remained active after incubation in pH range of 2-8 for 4 h at 37°C, but only partially inactive in media above pH 10. The bacteriocins produced by the test isolates maintained full stability after storage for 60 days at -20 °C; partial stability after storage for 120 days at 4 °C; while activity was not detected after storage for 60 to 120 days at 37 °C. Its active principle was proteinaceous in nature since the bacteriocin was inactivated by proteolytic enzymes, but not by other non-proteolytic enzymes. Mitomycin C and Ultra violet light did not affect the activity of the bacteriocin, while chloroform extraction completely destroyed their activity. The bacteriocin was resistant to treatment with SDS, Tween 20, Tween 80, urea and EDTA. Treatment with Triton X-100 reduced the activity of the bacteriocin and Triton X-114 had no effect on it. No bacteriocin adsorption was detected at pH 1 to 2, whereas 100% bacteriocin adsorption was found at pH 7.0. Based on Tricine SDS-PAGE the estimated molecular mass of bacteriocin was 3.0 kDa. No plasmid was isolated, suggesting that the genes encoding the bacteriocins were located on the genome.

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good safety is an important issue of international Γ concern. Prepacked food items available in food market contain variety of chemical preservatives, which may alter chemical constituents, nutritional and organoleptic qualities of food resulting in serious adverse effects on health (Messi et al., 2003). Thus, biopreservation of food has emerged as an attractive and safe approach among which, bacteriocins have received increasing attention due to their unique properties of inhibiting food-borne pathogens and spoilage causing microorganisms. Bacteriocins are proteinaceous in nature and are produced by various strains of bacteria, most importantly lactic acid bacteria. They usually exhibit antagonistic activity against microorganisms closely related to the bacteriocin producing microorganisms. Several of these bacteriocins are bactericidal against pathogenic and food spoilage bacteria (Caplice and Fitzgerald, 1999). In the present study, a bacteriocin

Chowdhury, S., Dora, K.C. and Banerjee, S.P. (2010). Detection, partial purification and characterization of bacteriocin produced by *Lactobacillus pentosus* FPTLB13 isolated from freshwater fish. *Asian J. Animal Sci.*, **5**(2): 174-180. produced from *Lactobacillus pentosus* FPTLB13, isolated from fresh water fish, was partially purified and characterized.

MATERIALS AND METHODS

Lactic acid bacteria screened for bacteriocin production and bacteriocin producing strain *Lactobacillus pentosus* FPTLB13 were isolated from gut of fresh water fish (*Cirrhinus mrigala*). Bacterial strains used as indicator organisms and their growth media are shown in Table 1 along with their specific growth medium.

Lactic acid bacteria, isolated from gut of *Cirrhinus mrigala* were cultured in MRS broth (HiMedia, India) and screened for bacteriocins production according to the method described by Van Reenen *et al.* (1998). The target strains and growth media are listed in Table 1. The antibacterial activity was expressed as arbitrary units per ml (AU/ml). One AU was defined as the reciprocal of the highest serial two fold dilution producing a distinct inhibition of the indicator lawn (Van Reenen *et al.*, 1998). Cell-free supernatants with antimicrobial activity were treated with proteinase K (1mg/ml) to determine if the activity is caused by the presence of bacteriocins.