

Characterization and *In Vitro* Application of *Lactobacillus plantarum* Bacteriocin as Biopreservative

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ABSTRACT

Food preservation is the major fear to the consumer due to the life threatening food borne diseases that caused a serious side effects on human health. *Listeria monocytogenes* is the greatest threat to food industry specifically meat and meat products. Bio preservation is a safer approach used to increase the shelf life of food products as compared to physical and chemical approaches of food preservation. In this scientific approach, bacteriocin (bac-ib45) produced by *Lactobacillus plantarum* was subjected to purification, characterization and applied as a bio preservative to protect the refrigerated meat sample from *L. monocytogenes*. The unique and stable properties, broad antimicrobial potential with bactericidal mechanism of action suggest its application for the preservation of food at 4°C.

Keywords: Antibacterial protein; Bacteriocin; Bio preservation; Cytotoxicity; Thermostable protein.

INTRODUCTION

Listeria monocytogenes is the pathogenic bacteria that can cause life threatening diseases when neglected. It is a facultative anaerobic bacterium, capable of surviving in the presence or absence of oxygen and at low temperature. These properties of Listeria make it an important microorganism that involved in the spoilage of food. Several studies reported the use of bacteria as well as their metabolic compounds for the preservation of foods from pathogenic microbes. Among them, Bacteriocins are highly specific and efficient antibacterial proteins. These antimicrobial proteins are becoming more important due to their dual ability of using not only for clinical purposes but also for food industry. In food industries, LAB (lactic acid bacteria) bacteriocins received extra importance due to their safe status for human animal and plants. In the past few years, investigations on LAB bacteriocins have been increased. In different food products the biopreservative proficiency of many bacteriocins had been demonstrated earlier (Sohaib and Jamil, 2017; Kang *et al.* 2017; Stempel *et al.* 2015). Keeping all the aforementioned considerations in view the aim of the proposed research is to produced, purified and characterized bacteriocin from lactic acid bacteria and applied as bio preservative.

OBJECTIVES

The objectives of the proposed study are:

- Production and purification of bacteriocin from *Lactobacillus plantarum* KIBGE-IB45.
- Determination of antimicrobial spectrum, mode of action and cytotoxicity of bacteriocin specifically against Listeria pathogen.
- Characterization and In vitro application of bacteriocin to enhance the stability of meat at refrigeration.

METHODOLOGY

Screening and Production of Bacteriocin

Various microorganisms were screened for the production of bacteriocin. Lactic acid bacteria that were capable of producing bacteriocin against most of the tested strains was identified AS *Lactobacillus plantarum* using 16SrDNA sequence analysis and selected for the production of bacteriocin at 35°C in MRS medium.

Purification and Characterization of Bacteriocin

Bacteriocin produced by *Lactobacillus plantarum* KIBGE-IB45 was purified using sepharose CL 6b column. After purification antimicrobial spectrum was determined using AWD (agar well diffusion) method (Ansari *et al.*, 2018). Bacteriocin was characterized in terms of thermal stability, pH stability, amino acid analysis, Fourier transform infrared (FTIR) spectroscopy and effect of different compounds on bacteriocin.

Mode/mechanism of Action

Mode of action of bac-ib45 was determined using time killing method and confirmed by electron microscopy using SEM.

In Vitro Application

For in *vitro* application of bacteriocin cytotoxic effect of bacteriocin was determined on NIH-3T3 cell lines using MTS method. Furthermore, bacteriocin was also applied as biopreservative to enhance the shelf-life of meat at refrigeration. Different groups were established and effect of bacteriocin on meat to control the growth of *L. monocytogens* was investigated using plate count method.

CONCLUSIONS/RESULTS

Bacteriocin of *L. plantarum* KIBGE IB45 was found to be active against numerous multi-drug resistant & food-borne pathogen especially *L. monocytogens*. In-vitro cytotoxicity showed that Bac-ib45 was non cytotoxic protein (Ibrahim *et al.*, 2019). The results of current investigation revealed that bacteriocin was approximately 42 fold purified with the molecular weight of approximately 20 kDa assessed by Tricin SDS PAGE. This bacteriocin is enormously stable to high temperature and variation in pH (Table 1). Purified bacteriocin showed extensive anti-microbial activity against most of the tested multi-drug resistant and food-borne bacterial and fungal strains (Figure 1). Bac-ib45 showed stability against other tested organic and inorganic compounds such as metallic ions, surfactant and solvents. However, bacteriocin from *L. plantarum* showed sensitivity towards proteolysis enzymes. The time killing method of this peptide and electron microscopic study ratified the bactericidal mode against *L. monocytogens*. Bio preservation analysis revealed that bacteriocin was capable of preserving meat sample up to 14 days and increase the shelf life of meat at 4°C. This unique bacteriocin having broad inhibitory potential and killing mode against microbes can be used for the preservation of food products and for other clinical purposes.

Table 1. Effect of various temperature on activity of bac-ib45 after 3 hours of incubation.

Temperature °C	Residual Activity %
Control	100
50	90
60	85
70	80
80	80
90	70
100	60



Figure 1. Antibacterial performance of bac-ib45 against *L. monocytogenes* ATCC7644.

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