

A Characterization and Evaluation of Antibacterial Efficacy of Bacteriocin Produced by *Bacillus subtilis* Isolated from Raw Milk

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Abstract

This study presents Bacteriocin (antimicrobial compound) produced from *Bacillus subtilis* isolated from unpasteurized milk. The antibiotic susceptibility tests for *B. subtilis* isolated from raw milk against various antibiotics demonstrated high sensitivity to Tetracycline, Moderate and less sensitivity towards Streptomycin and Chloramphenicol respectively while being resistant to Penicillin. Culture filtrate containing Bacteriocin showed thermostability (up to 100 °C), expressed a pH tolerance (2.0-10.0) and emerged resistance towards trypsin and pepsin. Partial purification of bacteriocin was performed using ammonium sulphate precipitation and then purified by passing into a column of Silica gel. In SDS-PAGE analysis, purified bacteriocin isolated from *Bacillus subtilis* showed the molecular weight to be approximately 3.4 KDa. The isolate Bacteriocin was tested finally for antibacterial activity against *E. coli*, associated with food borne illnesses. The zone of inhibition clearly proved that Bacteriocin has inhibitory effect on the food pathogen *E. coli*, thus confirming its bactericidal action in food.

Keywords: Antibacterial Agent, Bacteriocin, Bacteriocidal, Biopreservative, *Bacillus subtilis*, *E. coli*, *Micrococcus luteus*

1. Introduction

Majority of bacteria produce antimicrobial compounds such as broad spectrum classical antibiotics, metabolic products like organic acids and lytic agents such as lysozyme, besides several types of protein exotoxin and bacteriocins, that are biologically active compounds with bactericidal action. Bacteriocin (antimicrobial peptides) family is the most abundant and diverse group of bacterial defence systems. Bacteriocins are peptides produced by microbes that are usually active against bacteria closely similar to the producer organism [5]. Some bacteriocins are active against pathogenic and food spoilage bacteria and much research has focused on their potential as antimicrobials in food preservation. Contrary to the currently used antibiotics, bacteriocins are generally recognized to be more natural and hence they are believed to have been present in many of foods eaten since ancient times [1].

Though a great deal of research work is carried out on the bacteriocins produced by lactic acid bacteria they have the disadvantage that they are unsuitable for use as probiotics in the agricultural industry since they are sensitive to heat and effect of salts.

Similar to LAB, some candidates of *Bacillus* species such as *B. subtilis*, *B. licheniformis* are Generally Considered as Safe (GRAS) bacteria [7]. *Bacillus subtilis* is well known to produce many antibiotic and antimicrobial compounds including the bacteriocins [8]. In particular, bacteriocins from *Bacillus subtilis* are found to exhibit antimicrobial activity against several pathogenic organisms like *Micrococcus luteus* and *Escherichia coli*. This distinctive property of *Bacillus subtilis* enhances its utilization as a probiotics and hence used up in bio preservation.

Therefore, in the present study, our primary objective was to evaluate the practical use and application of *Bacillus* bacteriocins in the preservation of food with an

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