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PRODUCTION, PURIFICATION AND QUANTIFICATION OF BACTERIOCIN FROM *LACTOBACILLUS PLANTARUM* MTCC2621

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Abstract: **-** This study aimed to optimize the production of bacteriocin by Lactobacillus plantarum MTCC2621, followed by its purification and estimation of its protein content. Bacteriocins are antimicrobial peptides made by various bacteria, both Gram-positive and Gram-negative. Recently, bacteriocins produced by lactic acid bacteria (LAB) have gained attention because they can be used as natural food preservatives. The bacteriocin from L. plantarum has shown effectiveness against a wide range of foodborne pathogens. To optimize its production, the bacterium was grown in MRS broth with a pH of 6.0 at 37°C for 72 hours. The bacteriocin was then purified using 60% ammonium sulfate precipitation, and the protein content was measured using the Lowry method, resulting in 8.39±0.51 µg/ml of protein. Further research is needed to explore the stable production levels of bacteriocin under these conditions.

Keywords: Lactobacillus plantarum MTCC262, Bacteriocin, natural preservatives, antimicrobial

I. INTRODUCTION: -

Consumer demands for food items that are more naturally bio-preserved and use less chemicals to preserve food have led to the development of new fields for intensive bacteriocin research to understand the new range of antimicrobial compounds that might be used to combat foodborne infections. Food safety is becoming a major global problem, and application of bacteriocins to prevent food deterioration or pathogen development without seriously harming food itself is receiving a lot of attention (1,2). Furthermore, one of the main reasons for seeking the development of novel natural antibacterial chemicals like bacteriocins is the possibility that some food-borne organisms are resistant to the antibiotics now used to destroy dangerous germs. It is crucial to replace antibiotics and artificial and natural preservative agents in a variety of foods by using beneficial combinations of bacteria in bio-preservatives or by using them alone (3).
Numerous microbe species, including lactic acid bacteria (LAB), generate bacteriocins, which are low molecular, thermally stable, antimicrobial, and ribosome active peptides that have antimicrobial properties against food pathogenic bacteria and a certain class of fungus species (4, 5, 3,). Earlier, (6) identified bacteriocins to be narrow-spectrum bactericidal proteins to a strain or species that was extremely close to the one that produced the bacteriocins. The authors also proposed that a bacteriocin be created by a deadly biosynthetic process, which results in the death of the producer cell, conform to specific cell receptors, and be coded by plasmid-borne genetic determinants. Although these features are well-known and common to many bacteriocins, they are not all-inclusive. These are essentially complicated secondary proteins that are made by a wide range of bacteria and have the ability to hinder bacterial development (2). Since they have been unwittingly ingested through fermented foods for thousands of years, bacteriocins are naturally occurring antimicrobials that have been shown to revitalize attempts to identify new bacteriocins and produce sources, as well as for use as food bio-preservatives and other purposes (3, 7). Both related and unrelated bacterial strains can be killed or inhibited by bacteriocins, depending on which bacterium generates them. The fermenting or generating bacteria, however, won't be harmed by it since they often produce self-immunity proteins that shield them from being destroyed by their own bacteriocins. It is believed that

immunity proteins scavenge bacteriocins or engage in antagonistic competition for the bacteriocin receptor to protect generating cells (8, 2). Combining bacteriocins or bacteria with different action modes (hurdle) might increase their protective effects and decrease the occurrence of antimicrobial resistance (9). But the procedure of making bacteriocins is not simple. It is an extremely time-consuming procedure that calls for several meticulous rounds of microbial cell isolation, screening, and purification. Based on microbiological sources, several screening system types have been created (10). Nowadays, scientists and researchers have developed cutting-edge techniques for synthesizing bacteriocins from a variety of food-grade bacterial strains; nonetheless, complete purification of a bacteriocin from untreated fermentation is expensive (11). Therefore, it is rare to see refined bacteriocins used in commercial applications (12). The purification process of bacteriocins involves numerous processes, including salt precipitation, filtration, and chromatography, which multiply the concentration by almost perfect purity (13,14,10). Moreover, the many types of bacteriocins complicate the synthesis and purifying process. The majority of bacteria manufacture class I or class II amino acid sequences, based on their physicochemical characteristics, molecular weight, and other factors. The bacteriocins have been divided into three classes, according to a few studies. Class I and class II comprise heat stable bacteriocins with molecular weights under 10 kDa, whereas class III contains thermo-labile bacteriocins with molecular weights greater than 10 kDa (2). Most bacteriocins have tasteless, colourless, and odourless physical characteristics, which increase their acceptance in many applications. Additionally, bacteriocins are frequently a desirable option for food preservation and safety (7).

Research over the past two decades has looked at their possible use as food preservatives for milk and milk products, fruits, vegetables, meats, seafood, juices, and drinks (15,7). Of all the bacteriocins, nisin has undergone the most research and is currently GRAS-approved for use in food. Food safety experts are becoming more interested in the frequent usage and discovery of bacteriocins as a result of the rising need for natural food antimicrobials. Furthermore, by using them in minimally processed meals, harmful temperature-related treatments to nutrients are avoided (16,17). The purpose of the study was to investigate the process of bacteriocin isolation, purification, and quantification of bacteriocin from indicator organism.

II. METHODS AND MATERIALS

Selection of Indicator organism

The selected indicator organisms *Lactobacillus plantarum* MTCC2621 (18) was procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, Govt. of India. The cultures were sub cultured and stored in refrigerated conditions (4°C) for further work.

Growth and Media conditions

The de Man, Rogosa, and Sharpe (MRS) broth medium, nutrient broth medium and Muller– Hinton agar (Hi-Media Chemicals, India) were procured for production of bacteriocin and its assay along with the antibiotic susceptibility testing. Chemicals for analysis were procured from Hi-media and Merck, India.

Inoculum Preparation

Revive the MTCC culture by streaking method on MRS media plate. Transfer a single colony from the revived culture to MRS broth, incubated at 37ºC for 24 h in a shaker incubator.

Bacteriocin isolation

LAB isolates were inoculated into 50 mL of MRS broth medium as a starter culture and incubated at 37°C for 24 hours in a shaker incubator. The cell suspension was then inoculated in starter medium, and 15 mL of test bacterial isolates were transferred into 700 mL of MRS broth medium. This was incubated again at 37°C for 72 hours. The samples were taken and centrifuged at 4,500 rpm for 15 minutes at 4°C. The cell free supernatant was collected as a crude extract, were taken and followed for purification (19).

Purification of bacteriocin

The production of bacteriocin was followed by its purification. The cell-free supernatant (CFS) extracted from test bacteria, underwent partial purification through ammonium sulfate precipitation and dialysis. Initially, a 60% concentration of ammonium sulfate was added to 10 ml of CFS in sterile test tubes. This mixture was allowed to precipitate for 24 hours at 4°C, followed by centrifugation at 10,000 rpm for 20 minutes. The resulting precipitate was then re-suspended in 25 ml of 0.05 M potassium phosphate buffer and stirred at 4°C for another 24 hours. Subsequently, the suspension was dialyzed using a tubular cellulose membrane against 2000 ml of distilled water for 48 hours, with three changes of water. After dialysis, the purified sample was collected in sterile tubes and stored for further analysis (20).

Measurement of Crude Extract Bacteriocin

Total protein content estimation (by Lowry's Method); Blank, 0.2, 0.4, 0.6, 0.8, and 1ml of the working standard (BSA) stock solution (1mg/ml) was pipetted out in a series of test tubes and 0.05ml of bacteriocin were pipette out in other test tubes. Volume was made up to 1ml in all the test tubes with 1ml of water served

as blank. 1 ml protein solution and add 5 ml alkaline copper sulphate was added in all the test tubes including the blank. Mixed well and allowed to stand for 10 min. Then add 0.5 ml of Follin-ciocalteau solution was added, mixed well and incubated at room temperature in the dark for 30 min. Absorbance (A) at blue-purple colour formed, and result was recorded at OD_{660} nm. (Standard curve was prepared using BSA 1mg/ml) (21).

III. RESULTS AND DISCUSSION:-

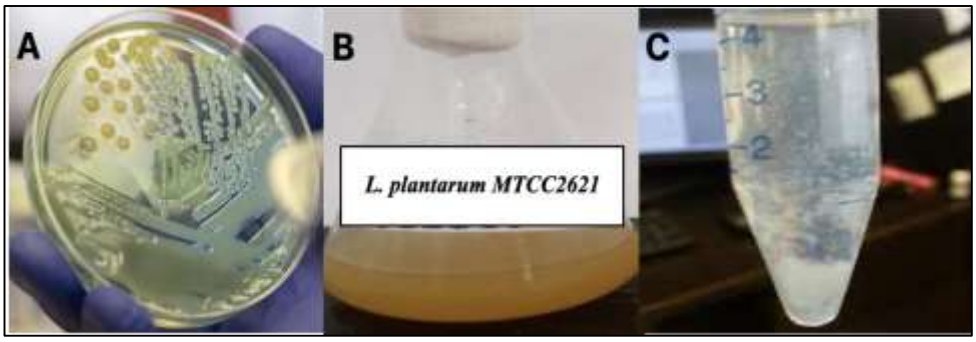

Figure 1. A. Revival of *Lactobacillus plantarum* MTCC2621, B. Bacteriocin islolation, C. Purification of bactreriocin in ammonium sulfate

Lactobacillus plantarum MTCC2621 was chosen for this study because it produces bacteriocin that is effective against many foodborne pathogens. The production of bacteriocin by this strain is a new discovery and has not been studied so far. The optimal protein concentration was found after 72 hours of incubation. This differs from another study where the best production time for bacteriocin by *L. plantarum* was at 48 hours(22).After producing bacteriocin with a broad antimicrobial range, it was partially purified using 60% ammonium sulfate precipitation. The protein content was then measured using the Lowry method, with UV spectrophotometry at 660 nm, and the results were reported as average values with mean ± SD from three measurements. The study found that *L. plantarum* MTCC 2621 produced bacteriocin with a protein content of 8.39±0.51 µg/ml after 72 hours of incubation. In contrast, another study reported a protein content of 0.93 mg/ml from *Lactobacillus* species after 36 hours(19), and yet another study found 25.3 mg/ml from *Lactobacillus viridescence* after 48 hours(23). Previous research showed that isolating SR21 (*Lactococcus lactis* spp. *Lactis 1*) required a protein level of around 0.0033 mg/ml, while isolating SR54 (*Lactobacillus brevis*) needed about 0.0034 mg/ml(24). Additionally, other research indicated that Amylosin 121 could be obtained using ammonium sulfate precipitation followed by dialysis(25). Further research is needed to explore the stable production levels of bacteriocin under optimum conditions.

IV. CONCLUSION:-

In conclusion, this study successfully demonstrated the production, purification, and quantification of bacteriocin from *Lactobacillus plantarum* MTCC2621. The bacteriocin exhibited significant antimicrobial activity against various food pathogens, underscoring its potential as a natural preservative in the food industry. The production process, optimized at 72 hours of incubation, yielded a protein content of 8.39±0.51 µg/ml, which was notably higher than what has been reported in other studies involving different strains of *Lactobacillus* sp. The partial purification method, involving ammonium sulfate precipitation followed by dialysis, effectively isolated the bacteriocin, confirming its proteinaceous nature. This research highlights the bacteriocin production and estimation of protein content in *L. plantarum* MTCC2621 in optimum condition.. Future studies should focus on scaling up production, assessing the bacteriocin's stability in various food matrices, and exploring its full spectrum of antimicrobial activity.

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