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Increasing The Yield of Bacteriocin Produced by Lactobacillus Plantarum Using Suspension Culture

Esha Dwivedi[1*] and Lalit Kumar Singh

Department of Biochemical Engineering,

[*]**Corresponding author:** Esha Dwivedi, Department of Biochemical Engineering, India.

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Abstract

Bacteriocins a heterogenous group of antibacterial peptides produced by Lactobacillus sp are temperature resistant that substantially extend in their mode of action, molecular weight, genetic origin and shows vast variety spectrum. It has braking action against gram-positive bacteria. Bacteriocins can be codified as antibiotics, but they significantly different from each other. The major dissimilarity between bacteriocins and antibiotic is that bacteriocins encloses its activity to strain of species producing it or especially to the strain of same species producing it or especially to the strain of same species on the other hand antibiotic have wider activity spectrum. These antibacterial peptides are produced during the active phase of growth whereas antibiotics are generally secondary metabolites. Nowadays, between large numbers of Lactic acid bacteria, Lactobacilli has acquired special recognition due to bacteriocins production. In diary industry a group that occurs generally and has extensive experience in food for secured usage are called lactic acid bacteria.

Keywords: Bacteriocin, Antibiotic Targeting application, Secondary Metabolite

Introduction

An antimicrobial peptide with bactericidal or bacteriostatic effect are synthesized by ribosomes and its production is done by numerous species of bacteria. Majority of the gram-positive bacteria along with the lactic acid bacteria are considered to be producing bacteriocin [1]. Bacteriocin produced from lactic acid bacteria are a type of complexes that oppose intrinsically related gram-positive bacteria [2]. Because of their proteinaceous nature bacteriocins are presumed to be safe for humans as they degrade in the gastrointestinal tract [3]. A combination of other stress –inducing processes like electroporation, heating, high hydrostatic pressure, freezing, chelating agents with bacteriocin generated by lactic acid bacteria has been reported to be functional against gram-negative to gram-positive bacteria [4]. Therefore, in case of nisin and pediocin, bacteriocins turned out to be suggested and agreed as food preservatives and enhanced usage in veterinary and pharmaceutical uses [5]. The investigation of proteinaceous antimicrobial compounds from lactic acid bacteria was initiated when the discovery of nisin took place .But the recognition and characterization of bacteriocins ,a large number of chemical diverse gained attention in recent years. Inclusion of bacteriocins as a bio preservative ingredient has been studied to a great extent and seen to be worthwhile in controlling pathogens and spoilage microorganisms in model food systems [6]. Low proportional guanine and cytosine content in their DNA a group of gram-positive, nonsporulating, immotile,aerotolerant,rod and coccus shaped bacteria with the help of homofermentative pathways produce lactic acid. They generally lack catalase but the cultures grown on low sugar concentrations gives pseudocatalase activity. For the production on industrial scale of fermented meat, a cereal product that is the most widely used starter culture bacteria. The unique ability to degrade carbohydrates, fats and proteins in food make them so useful. They are helpful in absorption process of amino acids, essential minerals and vitamins, produces flavour and aroma and slows downs the food spoilage process. Because of their ability to improve microbiological quality along with foods safety it is considered as protective cultures [7].

Review Of Literature

High bacteriocin leads similarly the food fermentation processes induces [8-11]. Bacteriocins have been classified as antibiotics earlier but they differ. The activity of bacteriocin is restricted to the strain of the species fabricating it or to the strain of the same species. Whereas antibiotics have wide range of spectrum activity and are secondary metabolites contradictory to it bacteriocins are produced in the primary phase of the growth and synthesized ribosomally [12]. Undergoing posttranslational modification makes bacteriocins easily degradable by proteolytic enzymes specifically by proteases present in the mammalian gastrointestinal tract, making it safe for human intake. They have low molecular weight over 10 kDa.Bacteriocins also differ from antibiotics with reference to their applications, activity, mode of actions, synthesis and interaction [13]. Because of their several advantages in industrial fermentations lactic acid bacteria can be used in pharmaceutical, chemical and other industries. Structural gene of bacteriocin encipher a gene which contains a double-glycine leader sequence whose action are to

delay biological activism inside the producer and to allow transporter system for a recognition signal. The N-terminal leader sequence varies in length from 15 residues to approximately 30 residues [14]. Bacteria synthesized by multienzyme complexes, the peptide antibiotics like bacitracin and gramicidin have not been successful on showing extensive implementation in the care of the infectious diseases [15]. During the past decades the DNA sequence of an immense amount of bacteriocin genetic loci has been influenced. The organization of bacteriocin loci has been revealed to a great variation through these studies. The presence of a plasmid has often correlated with the bacteriocin production whereas chromosome fragments have been located. Physiological potentiality of inhibiting with the development on agar media of certain other, mainly encloses related bacteria first attracted the attention of researchers till the significant progress in bacteriocin research came from the investigating colicins. To improve food preservation the further prospect has offered the wide use of food grade organisms in food industry. Including both gram negative and gram positive bacteria's ,the anthrax persued to be one of the most prominent target diseases in previous observations and various adversary of the in vivo and in vitro growth .Ammonium sulphate precipitation is used for purifying bacteriocin of lactic acid before cation exchange or hydrophobic interaction chromatography.In order to reduce the ionic strength of bacteriocin solution dialysis or desalting is an important step before performing cation-exchange chromatography. Otherwise, diluting bacteriocin solution with a suitable buffer and distilled water can be a other approach. Concentration of bacteriocin solution can also be done with freeze drying. The volume and nature which includes starting material is to be obtained in the way to use appropriate method along with the apparatus available and procedure of purification sequence. It is very difficult to get concentrated source of bacteriocin hence, first concentration and purification step is important to obtain crude bacteriocin for its application. The major precaution to be kept in mind for purification is to maintain temperature as low as possible. By maintaining several criteria's some bacteriocin concentration and purification is done by using ultrafiltration membranes. However,to purify large amount of volume this technique is quiet inappropriate. These methods are said to be useful where they use concentration process like ammonium sulphate precipitation or fractional absorption through which initial large volumes can be decreased easily.

Material and Methods

The microorganism chosen for this study was Lactobacillus plantarum. It is a heterofermentaive LAB microorganisms. The isolation of these bacteria was done from fresh curd sample. For the isolation MRS (De Mann Rogosa Sharpe) media was used. The curd was diluted by adding distilled water followed by serial dilution for spreading on MRS agar plates. The colonies were visible after overnight incubation.

Modified MRS media is used for both inoculum preparation and as an culture media. For inoculum a single colony of a particular strain grown in MRS agar plate is inoculated into MRS broth culture with additional amount of glucose 2% and is made up to the final volume by using distilled water. Lactobacillus plant arum is a species of lactic acid bacteria that is known to produce various types of bacteriocins. Here are some general methods for the production and purification of bacteriocin produced by Lactobacillus plantarum.

Bacteriocin Production

Lactobacillus plantarum is grown in a suitable growth medium containing nutrients such as sugars, amino acids, and minerals. The culture is incubated under optimal growth conditions, such as a controlled temperature and pH, to promote the production of bacteriocin. The bacteriocin is typically secreted into the growth medium, which can be harvested for further purification.

Cell Separation

The bacterial cells are separated from the growth medium by centrifugation or filtration. The resulting cell-free supernatant contains the bacteriocin and can be used for further purification. Ammonium sulfate precipitation: The bacteriocin-containing supernatant is treated with ammonium sulfate, which causes the bacteriocin to precipitate out of solution. The precipitate is collected by centrifugation and can be dissolved in a small amount of buffer for further purification.

Chromatography

Various chromatographic techniques can be used for further purification of the bacteriocin, such as ion-exchange chromatography, size-exclusion chromatography, or reverse-phase chromatography. These techniques separate the bacteriocin from other proteins and contaminants based on their size, charge, and hydrophobicity.

Antimicrobial Activity Assay

The purified bacteriocin is tested for its antimicrobial activity against a range of bacteria, including pathogens. This can be done using methods such as the well diffusion assay, where the bacteriocin is added to wells made in agar plates containing the test bacteria. The zone of inhibition around the well indicates the antibacterial activity of the bacteriocin.

Overall, the production and purification of bacteriocin produced by Lactobacillus plantarum involves several steps, and the specific methods may vary depending on the bacteriocin type and application.

Conclusion

Increasing worries with respect to health has urged the production of foods without chemical preservatives and with the minimal processing. Although it is already known that foods with less processing or absence of preservative gives a chance to various harmful microorganisms to grow and replicate. Therefore to avoid such a scenario bacteriocin a natural biopreservative harmless to humans has taken charge as an alternative to chemical preservatives in foods.Bacteriocin a ribosomally produced with low molecular mass peptides outside the cytoplasmic membrane consists of bactericidal effect as mode of action. This effect generally affects the broad-spectrum gram pare immune to its own producing cells.

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