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Biopreservation of Fruits (Tomato and Banana) Using Bacteriocin Produced by *LACTOBACILLUS* SPP.

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Abstract: ***The study aims the isolation of Lactic acid bacteria and the extraction of Bacteriocin from the isolated Lactobacillus and use of Bacteriocin as the bio preservative for the fruits. Bio preservation is the use of biological microorganisms and their products for the preservation of foods. In this study Lactobacillus was isolated and the Bacteriocin, a peptide or complex protein produced by bacteria to inhibit the growth of similar or closely related bacterial species. Now-a-days Bacteriocins are widely used in food industries to preserve food. The Lactobacillus species was isolated from mother's breast milk and the Lactobacillus species was isolated and pure cultured. The Lactobacillus culture in MRS broth was centrifuged and supernatant was precipitated with ammonium sulfate. The precipitated supernatant was centrifuged and the pellet was stored in sodium phosphate buffer. The crude Bacteriocin extract from centrifugation was dried and purified form using chloroform-methanol extraction method. The antimicrobial activity of Bacteriocin was observed against pathogens like Escherichia coli, Bacillus, Salmonella, Serratia and Staphylococcus. In the antimicrobial activity, Bacteriocin does not exhibit any inhibitory activity against the pathogen Serratia, but it possess a fine inhibitory activity against other selected pathogens. The extracted Bacteriocin was applied in fruits (Tomatoes and Bananas) in different concentration (2 ml and 3 ml) and are daily observed along with the control. The result shows that the Bacteriocin applied in high concentration and low concentrations preserve the fruits (Tomatoes and Bananas) well than the control. Hence we conclude that Bacteriocin acts as a good bio preservative for food preservation.***

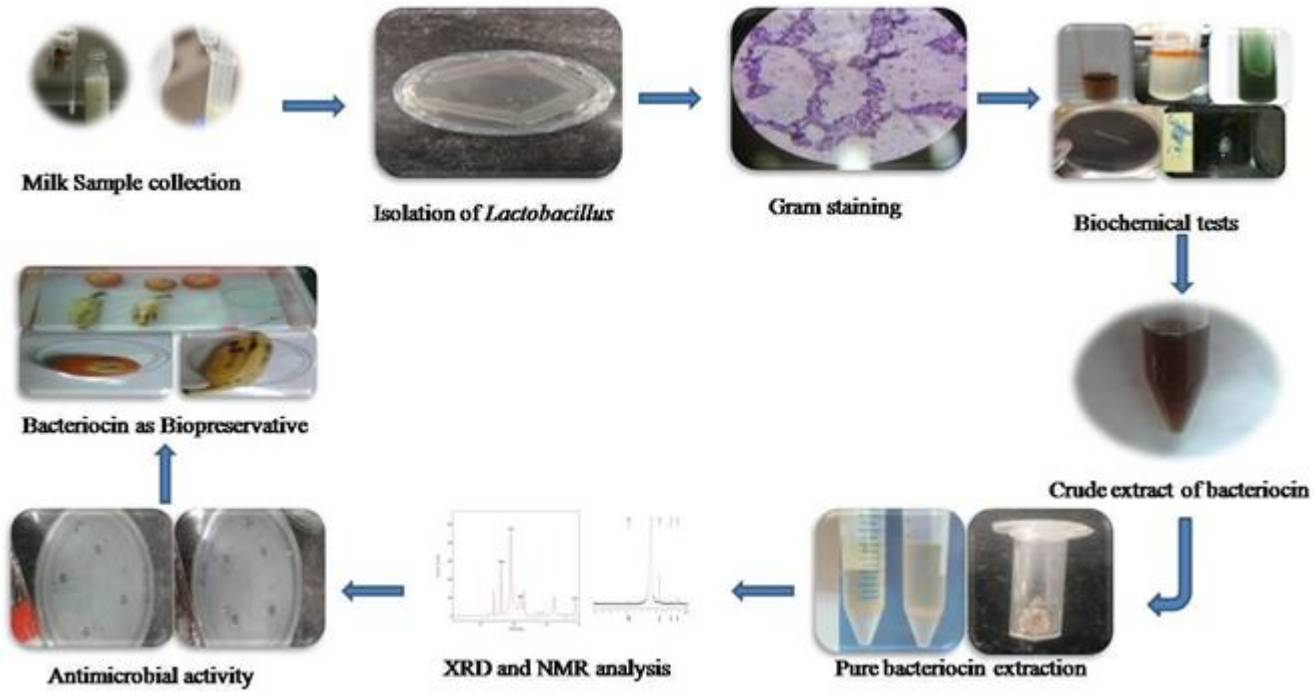
Keywords: Mother's breast milk, *Lactobacillus* spp., Bacteriocin, Preservation of fruits (Tomatoes and Bananas)

1. Introduction

Food is so important for the survival, food preservation is the oldest technique used by human beings to avoid spoilage. Food preservation refers to a technique which is used to prevent food from spoilage. Preservation is mainly done for some reasons like, to preserve the natural characteristics of food, to preserve the appearance of food, to increase the shelf value of food for storage. Different ways and means have been found and improved for the preservation purpose. Boiling, freezing & refrigeration, pasteurization, dehydration, pickling are the traditional methods for preserving food. Nuclear radiation is also being used now as a food preservation technique. Modified packaging techniques like vacuum packing and hypobaric packing also work as food preservation techniques. Food preservatives are categorized as natural, chemical and artificial. Natural food preservatives include sugar, salt, alcohol, vinegar etc. chemical reagents like benzoates (such as sodium benzoate, benzoic acid), nitrites (such as sodium nitrite), sulphides (such as sulfur dioxide), sorbates (such as sodium sorbate, potassium sorbate) are used as a food preservatives. Artificial food preservatives like antimicrobial agents, antioxidants, chelating agents. Preservatives are used to keep the food in fresh and stop the bacterial growth. But still there are certain preservatives especially chemical preservatives in foods are harmful if taken more than the prescribed limits. It will cause severe problems like allergies, skin rashes, asthma, kidney and liver damage, cancer, genetic defects etc. For these reasons researchers used microorganisms and their products for food preservation. Biopreservation, the term defined as the extension of shelf life and enhanced safety of foods by the use of natural or controlled microbiota and/or antimicrobial compounds, is an innocuous and ecological approach to the problem of food preservation and has gained increased attention in recent years. One of the common forms of food biopreservation is fermentation, a process based on the growth of microorganisms in foods, whether natural or added. These organisms mainly comprise lactic acid bacteria, which produce organic acids and other compounds that, in addition to antimicrobial properties, also confers unique flavors and textures to food products (Ananou and Maqueda et al., 2007).

2. Materials and Methods

The schematic representation of the standard methodology used in this study is given below



3. Result and Discussion

3.1 Isolation and Purification of Lactic Acid Bacteria

The human breast milk was collected from healthy mother from Kasturba hospital which is located in Chinalapatti, Dindigul, Tamil Nadu, India. The milk sample was used as an identification source. Lactic acid bacteria were isolated using MRS medium. The milky white colonies were obtained. The isolated colonies were catalase negative and Gram positive rod shaped. The isolated colonies were pure cultured using quadrant streak technique.

Plate 1: Isolated Pure Cultured Colonies

3.2 Identification of the Isolated *Lactobacillus*

The isolated colonies were confirmed as *Lactobacillus* Spp.. Using morphological, physiological and biochemical characteristics as described in the edition of "Bergy's Manual of Determinative Bacteriology". The morphological and physiological characteristics were identified by Gram staining, motility and pigmentation test. The biochemical characteristics were identified by indole test, MR-VP test, catalase test, citrate utilization test and starch hydrolysis test.

Table 1: Morphological and physical characteristics of isolated *Lactobacillus* spp.

Physical Characterization	Isolated *Lactobacillus* Spp.
Shape	Rod
Pigmentation	No pigmentation
Gram staining	+
Motility	-

(+) Positive; (-) Negative

Table 2: Biochemical Properties

Biochemical tests	Isolated *Lactobacillus* Spp.
Indole test	+
MR test	+
VP test	-
Catalase test	-
Citrate utilization test	-
Starch hydrolysis test	+

Production of Bacteriocin

The isolated *Lactobacillus* culture was inoculated in MRS broth and after 48 hours of incubation the broth was centrifuged and cell free supernatant was precipitated with ammonium sulfate and incubated for 24 hours in cold condition (4^{o} Celsius) and it was again centrifuged and the pellet was stored in sodium phosphate buffer with the pH of 6.The extract was considered as crude bacteriocin extract.

Plate 2: Crude bacteriocin extract

Antimicrobial Activity Test

Antimicrobial activity test was carried out to study the inhibition activity of crude bacteriocin extract against pathogens like *Salmonella*, *Serratia*, *Escherichia coli*, *Staphylococcus* and *Bacillus*. The antimicrobial activity of

crude bacteriocin against pathogens was determined by using Well diffusion method.

Plate 3: Antimicrobial Activity of Bacteriocin against *Bacillus* Spp.

Plate 4: Antimicrobial Activity of Bacteriocin against *Escherichia Coli*

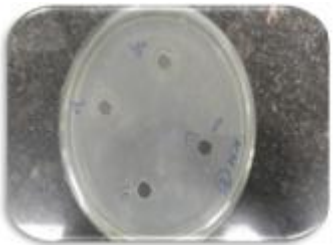


Plate 5: Antimicrobial Activity of Bacteriocin against *Staphylococcus* Spp.

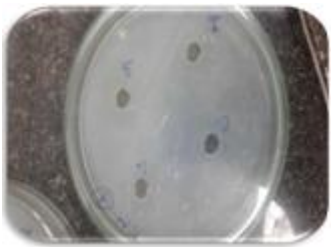


Plate 6: Antimicrobial Activity of Bacteriocin against *Salmonella* Spp.

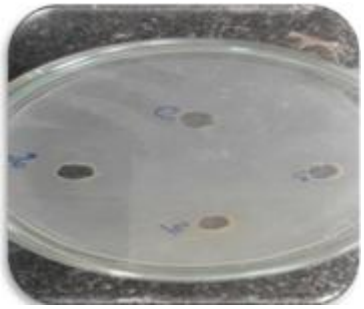


Plate 7: Antimicrobial Activity of Bacteriocin against *Serratia* Spp.

In the antimicrobial activity test, bacteriocin was quite active against *Bacillus, Escherichia coli, Salmonella, Staphylococcus* and it was not active against *Serratia*. The plot which was given above indicates the inhibition activity of bacteriocin against the selectedpathogens.

Pure Bacteriocin Extraction

The crude bacteriocin extract was further purified by the solvent extraction method called Chloroform-Methanol extraction method. This method is especially for precipitate protein. In this method, the crude bacteriocin extract was added with chloroform, methanol and distilled water and centrifuged at high-speed, the protein flakes were observed in the centrifuge tubes and the aqueous phase was removed, again some methanol was added and again centrifuged. The pellet obtained was vaporized and the powdered extract was stored in an eppendoff tube.

Plate 8: Pure Bacteriocin Extraction by Chloroform-Methanol Extraction Methods

Weight of Dry Bacteriocin Extract Calculation

Amount of crude bacteriocin extract
Weight of an empty eppendoff

NMR Analysis

The plot given above illustrates the chemical characteristics of dry bacteriocin using NMR analysis. Figure 6 shows 1H-NMR spectra of bacteriocin nisin protein. The peak at 1.261 corresponds to the terminal methyl groups (-CH_3) and the peak 2.119 corresponds to NH protons attached to another 2 protons with double bond is represent to NH_2. Moreover the protons attached with carbon (OCH_3 and CH_2) was featured the peak 3.602. In the amine groups of NH protons confirm the bacteriocin at the peak of 7.70 and 7.604. In addition, the peak at 4.701 corresponds to the unhydroxyl group of D_2O. So, presents of carbon and amine group of protons confirm the nisin structure of bacteriocin.

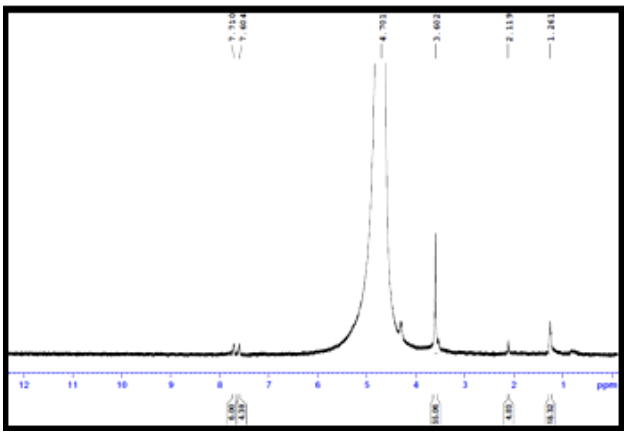


Figure 1: NMR spectrum of Bacteriocin

XRD Analysis

The plot given below illustrates the XRD analysis of dry bacteriocin. XRD analysis confirmed the crystalline nature of bacteriocin as it showed peaks. These peaks are the reflections of crystalline nature of bateriocin. XRD pattern also represents the face-centered cubic structure of bacteriocin.

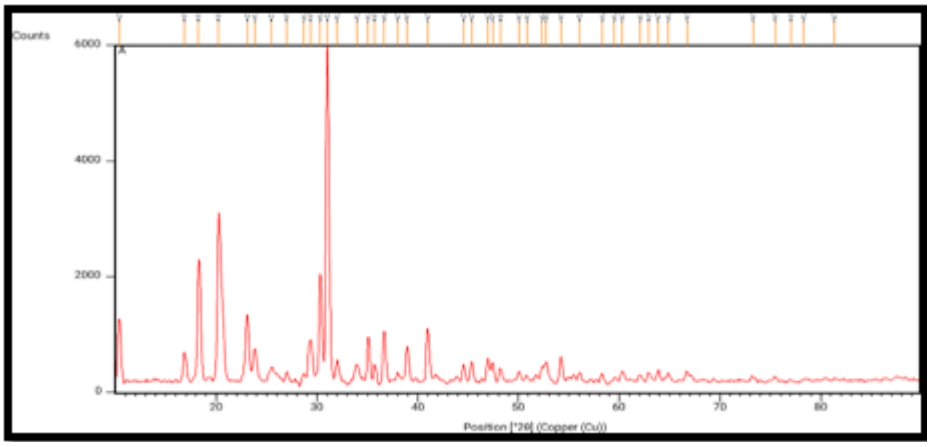


Figure 2: XRD spectrum of bacteriocin

A sharp and strong diffraction peak centered was appeared, which can be indexed into the reflection and closely matched the reported reference values of Joint Committee on Power Diffraction Standards (JCPDS pdf no: 87- 0720). The sharp peaks clearly indicate the cubic crystalline nature of the bacteriocin protein. In this study, crystalline size of the bacteriocin protein estimated by the Debye–Scherrer formula and the average size bacteriocin protein was found to be 62.30nm.

Bacteriocin Applied In Fruits as Biopreservative

Bacteriocin extract was sprayed in eatables like fruits i.e., Tomatoes and Bananas. The bacteriocin was applied in eatables as low concentration to high concentration. The eatables fruits as a control and the bio preservative sprayed fruits were compared with control. The results were observed daily.

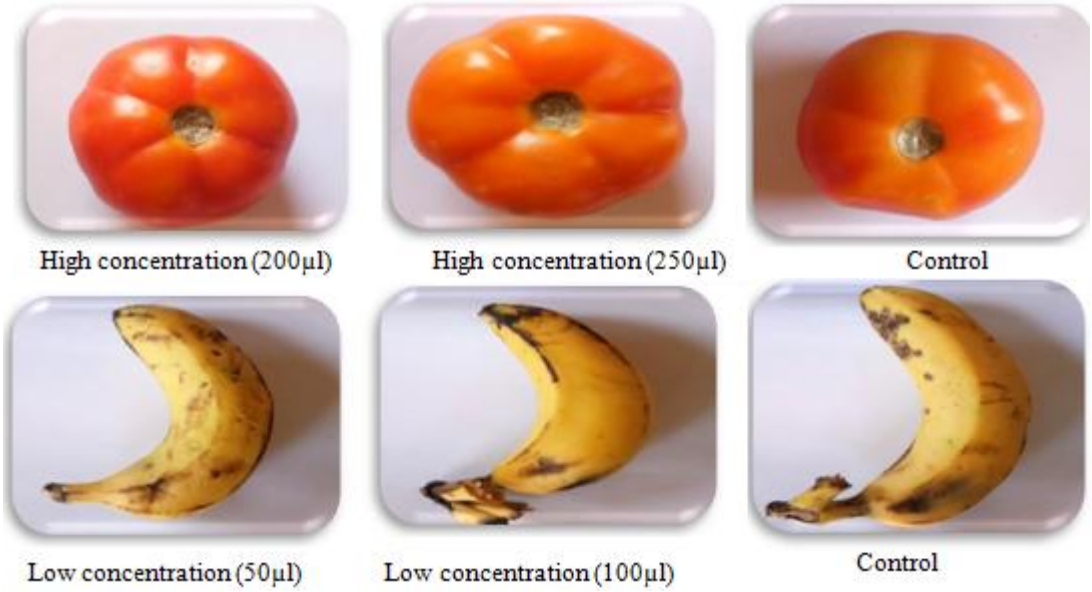


Plate 9: Fruits Selected For the Application of Biopreservative

Observation after 4 days

4. Discussion

All foods begin to deteriorate, or spoil as soon they are harvested or slaughtered. Most spoiling is caused by microorganisms such as bacteria or mold, or by chemical changes within the food itself due to enzyme action or oxidation. The purpose of food preservation is to stop or slow down the spoilage. Although many food preservation methods like Canning, Freezing, using chemicals like Benzoic acid etc. were employed to preserve foods. But these may cause some harmful effects to consumers. So that Biological aspects were followed in order to preserve food especially microorganisms and their products were used widely to preserve foods. It the use of antagonistic microorganisms or their metabolic products to inhibit or destroy undesired microorganisms. In foods to enhance food safety and extend shelf life. In this study, the metabolic product called Bacteriocins, a peptide or complex proteins which was extracted from lactic acid bacteria to preserve fruits like (Tomatoes and Bananas) from spoilage caused by microorganisms. For the bio preservation of fruits using Bacteriocins, Lactic acid bacteria was isolated from human breast milk which was collected from a healthy mother from Kasturba hospital, Chinnalapatti, Dindigul. Human breast milk is considered as one of the best source for the isolation of lactic acid bacteria. Other researchers used raw milk from cow and other sources for the Bacteriocin production. First of all Lactic acid bacteria from the mother's milk was isolated using MRS medium and the milky white colonies were obtained during incubation. Biochemical tests were carried out. The milky white colonies were pure cultured using quadrant streak technique. The culture was transferred into broth and was maintained in glycerol stocks. The same work was reported as collected milk samples were serially diluted in sterile distilled water and plated on sterile MRS agar (de Mann Rogosa Sharpe) plate and incubated in microaerophilic condition at 37° for 48 – 72 hours. The well isolated colonies were selected randomly and transferred in MRS broth. They were streaked on MRS agar to check the purity of the isolates and then stored in MRS soft agar (0.5%) overlaid with Glycerol at -20°C. The isolates were differentiated on the basis of morphological, and biochemical characteristics (Deshmukh and Thorat, 2013). Then the Bacteriocin was extracted from the from the *lactobacillus* culture in crude form by centrifugation and precipitation method using ammonium sulfate and similar work was done (Dan Xiao et al., 2010). Antimicrobial activity was done against some pathogens like *Bacillusn* Spp., *Escherichia coli*, *Staphylococcus*Spp.., *Serratia*Spp.., *Salmonella*Spp.. The zone of inhibition was observed from these observations we noted that extracted Bacteriocin was exhibit inhibitory effect against *Escherichia coli*, *Salmonella*Spp.., *Bacillus*Spp.., *Staphylococcus*Spp. and it was active against *Serratia*Spp.. This work was done in contrast to (Dan Xiao et al., 2010) were he reported *Escherichia coli* and *Bacillus*Spp. Are only activated against Bacteriocin. The pure form of Bacteriocin was extracted using solvent extraction method i.e. Chloroform – Methanol extraction method. The same work was reported as centrifuge was done to the crude extract with the addition of chloroform, methanol and distilled water. The powdered extract was stored in eppendoff (Dan Xiao, Michael Davidson., 2010).The dry Bacteriocin was used for the NMR, XRD analysis. The Bacteriocin was applied in eatables like fruits and the fruits were observed daily and they are compared with control. In some studies Bacteriocin was applied in fermented foods like Kimchi, Saurekrat for preservation (Luca settanni and Aldo Corsetti., 2008). The results were given on result section. These were all the work carried out in the study about bio preservation.

5. Conclusion

In this present study, *Lactobacillus* was isolated from mother's breast milk. Then the dried form of bacteriocin was recovered from the broth culture using Chloroform-Methanol extraction method. Then the antimicrobial activity of the extracted bacteriocin was observed against pathogens like *Bacillus*, *Escherichia coli*, *Staphylococcus*, *Salmonella* and *Serratia*. After that the bacteriocin was applied in fruits with different concentrations (5oµl, 100µl, 150µl, 200µl and 250µl). Use of chemical preservative preserve the fruits upto 6days but they exhibit toxic effect on the consumers. When compared the biopreservative with the chemical preservative, the biopreservative has the ebility to preserve food for 4 days but doesn't exhibit any toxic effects to consumers. Hence it showed that the biopreservative can have the ability to preserve food with no toxic effects and can use in food industries as food preservative.

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