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An Insight into the Fabrication of Anti-Microbial Packaging Materials for Food Preservation Using Bacteriocin Isolated From Lactobacillus

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ABSTRACT
Lactobacillus bacteriocin antimicrobial packaging is also an alternative for addressing the growing demand to have safe minimally processed and preservative free foods. Bacteriocins are ribosomally produced antimicrobial peptides synthesized by lactic acid bacteria and which prevent a wide range of Gram- positive and a few Gram-negative pathogenic microorganisms. They are appropriate in food applications because of their GRAS status. Recent progress is incorporation of bacteriocins in biodegradable and edible films such as protein-based polysaccharide-based materials such as starch as well as chitosan and nanocomposites. These active systems of packaging release or immobilize bacteriocins at food surfaces inhibiting microbial growth and increase shelf life without quality loss. The nisin and pediocin addition has shown a tremendous decrease in the pathogens including Listeria monocytogenes Escherichia coli and Staphylococcus aureus. Lactobacillus bacteriocins exhibit stability under a broad pH and temperature parameter and are synergistic with other antimicrobials. Biodegradable polymers are environment friendly due to the minimisation of plastic waste. Nanotechnology also comes in handy in release and efficacy regulation. Overall, this is a decent sustainable solution to food preservation and needs additional optimization before it can be used in large scale. The second stream of research should establish new combinations of biopolymers scalability regulatory approval and real time validation into various food systems across the globe which should be adopted.

Keywords: Antimicrobial packaging, Bacteriocins, Lactobacillus, Food preservation, Biodegradable films, Edible coatings.

I. INTRODUCTION

Food preservation has been an extremely grave concern in the food industry that aims at conserving the quality, safety and shelf life of food. The level of food safety could never be overestimated because the rate of population growth is constantly rising, and the demand of ready-to-eat and minimally processed food grows. These traditional methods of preservation such as refrigeration, drying, salting, and chemically-based preservatives have been very popular. They are also characterized by two shortcomings that include loss of nutrients, sensory property and health risks that may be caused by synthetic additives [2].

The consumers have shown tremendous inclinations in the consumption of natural, safe and environment friendly food material within the past few years. It has resulted in the use of new preservatives and this has minimized the use of chemical preservatives. Antimicrobial packaging is one of such promising methods, in which the active ingredients are incorporated in the packaging medium, such that to prevent the development of the spoilage and pathogenic microorganisms [5,8]. The antimicrobial packaging is also able to respond to the food environment as opposed to the traditional packaging which only gives passive protection to the food safety and shelf life.

The next technology that is currently being developed is antimicrobial packaging that aims at enhancing the preservation of foods by adding antimicrobial preservatives during food packaging. These are agents which are incorporated into food or those on the surface that they act on the microorganisms [4]. This may be useful in the prevention of contamination of microbes, and preservation of sensory and nutritional food products. The antimicrobial agents that are tested to be utilized in packaging have also included organic acids, essential oils, enzymes and bacteriocins. Bacteriocins have also received particular interest among them because of their specificity, safety and efficacy [10].

There are two types of antimicrobial packaging systems i.e. migratory and non-migratory systems. In the migratory system, the antimicrobial agents are released slowly into the food and on the packaging surface in the non-migratory system. The selection of suitable antimicrobial agents and packaging materials are usually the determinants of these systems.

The first one is the antibacterial peptide secreted by lactic acid bacteria (LAB) as bacteriocins. Their capability to inhibit closely related species of bacteria and some food-borne diseases are one of their properties [15]. As a rule, bacteriocins are considered to be safe to use in food. They are mostly used by rupturing the cell membrane of potential microorganisms that result in cellular leakage and cell death [13,42]. They also disrupt the DNA replication and protein synthesis in a few instances.

Depending on their activity and structure, bacteriocins can be divided into numerous groups. A well-researched example of bacteriocins is nisin, which is an equivalent antimicrobial to conventional preservatives, but with a superior safety profile as well [16]. In the fermented food, lactic acid bacteria, in particular, a genus Lactobacillus, produces bacteriocins in great amounts. These bacteria are also known as probiotics and the bacteriocins have the general antimicrobial properties that can be applied in food preservation [17].

Lactobacilluscasei is one of the important species that are capable of producing powerful bacteriocins. It has been mentioned to be effective in the purpose of inhibiting pathogens like Escherichia coli, Staphylococcus aureus and Listeria monocytogenes [1]. Moreover, the Lactobacillus species bacteriocins are not sensitive to temperature or pH range, thus, make them useful in various food systems [19].

The antimicrobial packaging systems have also enhanced their performance due to nanotechnology. Nanocomposites improve antimicrobial property, mechanical strength and resistance of the packaging material [34]. They are also able to control the release of bacteriocins thereby prolonging the actions of antimicrobials. Integration of nanotechnology and biodegradable materials has been a significant revolution to the food packaging industry not only in the issues of the environment but also attributes [32].

Although bacteriocins have good potential in antimicrobial packaging, it is still faced with a series of challenges. The greatest problem is that the bacteriocin activity is lost during the processing and storage. The variables that may affect their stability are temperature, pH, and contact with packaging material [20]. The other problem is the ability to distribute bacteriocins uniformly throughout the packaging medium because uneven distribution of bacteriocins may decrease the antimicrobial effect.

Then it is succeeded by lack of economic and technological capability to enhance production and commercialisation of the bacteriocin based packaging systems. It ought to make safety, regulatory and consistency in their performance a success in food industry [50].

The main objective of the research is to come up with antimicrobial packaging surfaces using bacteriocins generated by the Lactobacillus species. The research is aimed at purifying and characterizing bacteriocins, production of biodegradable packaging films and testing of their antimicrobial activity. There will also be consideration on the use of these materials in food preservation in the paper. Bacteriocin based antimicrobial packaging is also appropriate and sustainable solution to food preservation as it could help in minimizing the use of chemical preservatives besides improving food safety.

II. LITERATURE SURVEY

2.1 Antimicrobial packaging

The increasing demand of long shelf life and improved safety has led to the utilization of new technologies in food preservation as an alternative to the old ones. The use of antimicrobial packaging whereby active agent is added to the packaging mechanism is also a promising technology in packaging. The antimicrobial packaging compared to the traditional type of packaging, which is a passive barrier, reacts actively with food to avoid microbial growth [5,2] as shown in Figure 1. It has been demonstrated that antimicrobial packaging has a negative impact on contamination and positively influences shelf life by suppressing microorganisms of spoilage and pathogens [4]. It is especially relevant to food products that are minimally processed and are more likely to contain microbes [8]. The technologies of bioactive packaging also contribute to the quality and safety of food and enhance their shelf life [3]. Additionally, the bio-based antimicrobial packaging is a green alternative to the synthetic preservatives [9].

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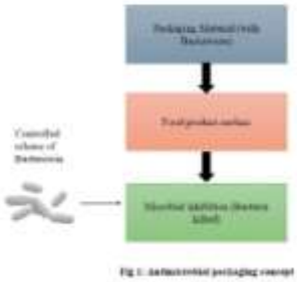

Figure1: Antimicrobial packaging concept

2.2 Structure and Function of Bacteriocin

Bacteriocins are antimicrobial peptide toxins, which are produced by bacteria, particularly lactic acid bacteria. These are produced through ribosomal synthesis and possess bactericidal effect against species which are very closely related and certain pathogens [15]. They contribute a lot towards competition in microbes through a natural defensemechanism [13]. They are mainly responsible for the interference of the cell membrane and leakage of cell contents [42]. Bacteriocins classification is done according to their structure and mode of action [14] as shown in Figure 2. They are preserved at a wide range of environmental conditions, and this allows them to be used in the food preservation process [16].

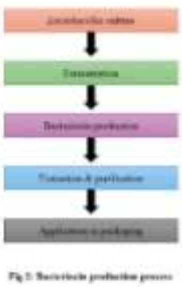

Figure2: Bacteriocin production process

2.3 Packaging Antimicrobial Agents

Some of the antimicrobial agents that are used in packaging systems include organic acids, essential oils, enzymes, and bacteriocins. Essential oils have proven to possess significant anti-microbial properties against food microbes [6]. Of these agents, bacteriocins are favoured the most, because they are specific, safe and compatible with food systems [10] as shown in Figure3. Natural antimicrobial films are also acceptable to the consumers than the synthetic additives [7].

Figure 3: Incorporation into packaging film

2.4 Lactobacillus and Lactic Acid Bacteria

The bacteria that are widely used in fermenting food are the lactic acid bacteria because they have the capacity of synthesizing antimicrobial compounds like bacteriocins. They find application in preservation of food and in prolonging food shelf-life [17]. The Lactobacillus species are some of the most important producers of bacteriocin. The bacteria also produce antimicrobial compounds that prevent microbes and improve intestinal wellbeing [18]. Their bacteriocins are very promising in their applications in the food preservation [19], manufacture and application of their bacteriocins has been extensively studied [20].

2.5 Edible films and coatings

The use of edible films and coatings is the other visible feature of antimicrobial package. The edible films that have been coated with the antimicrobials can be utilized to reduce food spoilage and extend the shelf life [28]. One more research which has shown higher antimicrobial activity is application of bacteriocin impregnated chitosan films [29]. The protein films on the other hand have also been demonstrated as a good delivery system of antimicrobial agents [30]. These findings demonstrate that edible films have a lot of potential in the use of bacteriocin in preservation of food.

The rising popularity of biodegradable packaging materials has been brought about by the environmental issue. Starch and chitosan are the biopolymers that have been extensively utilized in antimicrobial packaging systems [31]. The use of biodegradable antimicrobial films has already been applied in the preservation of food [33], and starch-based films have also been reported to be applicable in the packaging industry [39]. Chitosan also has antimicrobial properties and thus it is a good material to use in packaging [40].

Antimicrobial packaging systems have also been improved through nanotechnology. It enhances the antimicrobial capability and functioning of the packaging [34]. Nanocomposites are better in antimicrobial release and antimicrobial barrier properties [32] and their benefits in packaging have been extensively described [35].

Bacteriocins mostly affect gram-positive bacteria, and it affects the cell membrane [41]. They cause holes in the membrane causing the cell to die [42]. They are very efficient in food preservation as their production and use in biotechnology have been widely investigated [43]. Among others, bacteriocins have been used in various food systems [44] and one of them has been seen to have been used in dairy food systems where they have been seen to greatly enhance shelf life [46]. They also deal with foodborne pathogens [47] and their application in packaging is also surveyed well [50].

Although these are benefits, there are a number of challenges. The stability problem, as well as the problems of the production is also very high [20], and the even distribution of the packaging material is also important. Large-scale commercialization and production have not been studied [50].

The effectiveness is also dependent on the response of the bacteriocin to the food matrices. Bacteriocin may react with food components and lower their antimicrobial potential, particularly in complex food substances like dairy and meat foods [10].

It is also another factor that is essential in ensuring release of the antimicrobial agents is regulated. The rate of release of the bacteriocins by the packaging materials determines the level of efficiency. The diffusion is also kept at a low level, and this ensures prolonged antimicrobial effect [24]. Such factors as polymer composition, film thickness and environmental conditions influence the release mechanisms.

Bacteriocins are used together with other antimicrobial agents and enhance their efficacy. It has been found to have synergistic effects when used together with essential oils, organic acids and enzymes [6]. These combinations enhance the antimicrobial spectrum and lower the concentration level of the one-agent used.

Antimicrobial packaging is also done with probiotics. This issue of lactic acid bacteria has also led to bacteriocins and other bioactive compounds which are known to improve food safety and food preservation [18]. This two-fold purpose enhances food safety as well as functional attributes.

Another area of significant concern in the context of carrying out antimicrobial packaging research is the lack of standardization of the testing methods and characteristics of the material. The nature of experimental conditions, microbial strains and packaging material can influence the outcome of studies differently. The standardized protocols are required to measure the efficacies of the antimicrobials, the mechanical properties, and even the safety to be reproducible and to be reliable. Table 1 illustrates the summary of the role of bacteriocin in antimicrobial packaging.

2.6 Mechanism of Bacteriocin action

The action of bacteriocins on the cell membrane of the target microorganisms is considered to be the main cause of antimicrobial effect of bacteriocins. They are also important in the action against Gram-positive bacteria [41]. The bacteriocins penetrate the cell membrane forming pores that cause leakage of cellular contents and eventually death of the cell occurs [42]. They are also used in preservation of food which is promoted by their production and application in biotechnology [43].

Bacteriocins have also found extensive application as food preservation systems [44], such as the dairy industry, where they are incorporated as food preservatives to increase shelf life [46]. They also can be used to control foodborne pathogens [47], and their use in commercial packaging has been proved [50].

Nonetheless, there are still a number of challenges. Stability and production limits are also significant setbacks [20], and also the difficulties in the consistency of the distribution of the packaging materials. Commercialization on a large scale needs more research and development [50].

The antimicrobial efficacy can be influenced by the effects of interactions between bacteriocin-food matrix. Adsorption of bacteriocins to food substances could decrease their availability and functionality [10].

Controlled release mechanisms are required in long-term antimicrobial activity. Diffusion release systems are also said to be effective in long-term [24] as shown in Figure 4.

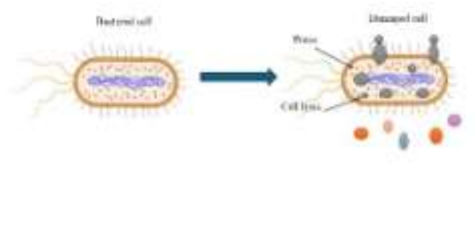

Figure4: Mechanism of Bacteriocin action

2.7 Future problems andCommercialization of Bacteriocins

The potential of bacteriocins is faced by a number of challenges to their large-scale production and commercialization. These severe drawbacks are high cost of production, purification problems and storage stability [43]. The effect on consistency of industry is also variable with regard to bacteriocin production of different Lactobacillus strains.

Another important factor is regulatory approval Strict safety tests are to be performed before commercialization including toxicity, allergenicity and migration tests [50]. Certain bacteriocins such as nisin are considered to be safe in particular but the new systems take a very long time in their approval.

The biopolymer in engineering has enhanced the antimicrobial packaging materials. Natural polymers like chitosan and starch which alternated are good in mechanical properties and antimicrobial properties [33]. Multibiopoly composite films are also more sustainable and useful.

A good way of minimizing microbial loading is bacteriocin packaging and it prolongs shelf life without compromising quality of food [23]. The systems are very valued in maintenance of nutritional and sensory properties.

The latest technologies like smart packaging and sensors will be generated and will transform antimicrobial packaging. With such systems, food quality can be monitored in real time and so can microbial contamination [34]. Combination with bacteriocins may result in more convenient and safer preservation techniques as shown in Table 1.

Sustainability is one of the motivators in the packaging innovation. Antimicrobial material made of a biodegradable material reduces the environmental effect and supports the principles of a circular economy [31]. Renewable resources are also used to increase sustainability.

Nonetheless, there is the problem of standardization being wanting in the testing processes. Changes in experimental conditions and materials might have an effect on reproducibility. There is need to have standard protocols so that thereis reliability in evaluation of antimicrobial efficacy and material properties [36].

Table 1 Summary of the role of bacteriocin in antimicrobial packaging

S. No	STUDY FOCUS	KEY FINDINGS	REFERENCE
1	Bacteriocin-based packaging using Lactobacillus casei	Demonstrated effective antimicrobial activity and improved food shelf life	[1]
2	Review of antimicrobial packaging	Highlighted importance of active packaging in food safety	[2]
3	Antimicrobial packaging system	Defined concepts and applications of active packaging	[5]
4	Bacteriocins in food preservation	Confirmed Bacteriocin as natural food preservatives	[10]
5	Mechanism of Bacteriocins	Explained membrane disruption as main antimicrobial action	[13]
6	Safety of Bacteriocins	Established bacteriocins as safe (GRAS) antimicrobial agents	[15]
7	Incorporation into packaging	Demonstrated controlled release of Bacteriocins in films	[24]
8	Edible Antimicrobial films	Showed effectiveness of films in extending shelf life	[28]

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9	Biopolymer packaging	Highlighted bio degradable alternatives to plastics	[31]
10	Nanotechnology in packaging	Improved stability and controlled release of antimicrobials	[34]
11	Bacteriocin packaging application	Identify challenges in commercialization and scalability	[50]

III. CONCLUSION

The fact that the Lactobacillus secreted bacteriocins are applied to the antimicrobial food wrappings is an important innovation in the food preservation. Based on the literature under discussion, antimicrobial packaging is superior to the former preservation method since it is a dynamic deterrent of the proliferation of the spoilage and pathogen organisms [2,5]. In order to increase the safety of food, natural antimicrobial agents may be incorporated as part of the food packaging systems though at the same time it also reacts to the demands of consumers who want food products that have minimum additives and no food additives.The application of bacteriocins has been introduced in the sunshine as a possible bio-preservative due to its specificity, safety and a wide range of foodborne microorganisms [15,10]. The Lactobacillus species, especially Lactobacillus casei has demonstrated a massive potential in the secretion of a stable and efficient bacteriocins that may be applied in the packaging of food [1,19]. The introduction of bacteriocin in foods as food films and food coats has been observed to enhance massively the foods and shelf life of the foods. The use of protein, starch and chitosan-based films as carriers of bacteriocins has been found since they enable the release of the latter in a regulated way and increases the antimicrobial activity [24,28,29].In addition, the problem of synthetic plastics and antimicrobial packaging on the environment can be surmounted using biodegradable material and, simultaneously, they can become functioning and sustainable [31,33]. Nanotechnology has also managed to enhance bacteriocin-based packaging by enhancing stability, mechanical properties and release profile of antimicrobial agents [34,32]. The innovations are applied in the development of the next generation of the packaging materials which are environment friendly, efficient and safe.Although such benefits exist, there are still certain issues such as: retention of bacteriocins at the processing grade, consistency of distribution in the packaging container and cost-effective mass production [20,50]. Regulatory acceptance and customer acceptance, too, are highly critical aspects that ought to be put into consideration in the effective commercialization of such technologies.To sum up, Lactobacillus based bacteriocin-based antimicrobial packaging is a promising and sustainable food preservation. It enhances food safety, shelf life and environmental effect. The further research should be focused on optimization of the processes of formulations, improvement of their stability and scalability and translation of the laboratory research to the industry.

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