International Journal of Microbiological Research 9 (3): 76-80, 2018
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2018.76.80

Biopreservation, Promising Strategies to Improve the Safety and Shelf-Life of Foods: A Review

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Abstract: Biopreservation refers to 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 order to achieve improved food safety and to harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by combinations of innovative technologies that include biological antimicrobial systems such as lactic acid bacteria (LAB) and/or their metabolites. The antagonistic properties of LAB derive from competition for nutrients and the production of one or more antimicrobial active metabolites such as organic acids (lactic and acetic), hydrogen peroxide and antimicrobial peptides (bacteriocins). Nowadays the use of LAB bacteriocins is considered an integral part of hurdle technology. Their combined use allows most pathogenic and spoilage bacteria to be controlled and also extend their inhibitory activity spectrum to such intrinsically resistant organisms as the Gram-negative bacteria.

Key words: Bacteriocins · Biopreservation · Food Safety · Hurdle Technology

INTRODUCTION

Modern technologies in food processing and microbiological food safety standards have reduced but not eliminated the likelihood of food-related illness and product spoilage in industrialized countries. Food spoilage refers to the damage of the original nutritional value, texture, flavour of the food that eventually render food harmful to people and unsuitable to eat. The increasing consumption of pre-cooked food, prone to temperature abuse and the importation of raw foods from developing countries are among the main causes of this situation [1].

One of the concerns in food industry is the contamination by pathogens, which are frequent cause of food borne diseases. For instance, in the USA, acute gastroenteritis affects 250 to 350million people with more than 500 human deaths annually and approximately 22 to 30% of these cases are thought to be food borne diseases with the main foods implicated including meat, poultry, eggs, seafood and dairy products [2]. The bacterial pathogens that account for many of these cases include *Salmonella*, *Campylobacter jejuni*, *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium* [1].

Until now, approaches to seek improved food safety have relied on the search for more efficient chemical preservatives or on the application of more drastic physical treatments (e.g. high temperatures). Nevertheless, these types of solutions have many drawbacks: the proven toxicity of many of the commonest chemical preservatives (e.g. nitrites), the alteration of the organoleptic and nutritional properties of foods and especially recent consumer trends in purchasing and consumption, with demands for safe but minimally processed products without additives [3].

To harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by combinations of innovative technologies [4]. Among alternative food preservation technologies, particular attention has been paid to biopreservation to extent the shelf-life and to enhance the hygienic quality, minimizing the impact on the nutritional and

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organoleptic properties of perishable food products [5]. Biopreservation rationally exploits the antimicrobial potential of naturally occurring (micro-) organisms in food and/or their metabolites with a long history of safe use. This review will summarize basic knowledge and current applications of biopreservation, an ecological approach to improve the safety and shelf-life of foods. Based on this state-of-the-art, future trends and areas of research that deserve more attention will be also discussed.

Biopreservation: Definition and Concept: Biopreservation is the use of natural or controlled microbiota orantimicrobials as a way of preserving food and extending its shelf life [3]. According to Stiles [6] biopreservation can also be defined as the extension of shelf life and food safety by the use of natural or controlled microbiota and/or their antimicrobial compounds. One of the most common forms of food biopreservation is fermentation, a process based on the growth of microorganisms in foods, whether natural or added. It employs the breakdown of complex compounds, production of acids and alcohols, synthesis of Vitamin-B12, riboflavin and Vitamin-C precursor, ensures antifungal activity and improvement of organoleptic qualities such as, production of flavor and aroma compounds.These organisms mainly comprise lactic acid bacteria, which produce organic acids and other compounds that, in addition to antimicrobial properties, also confer unique flavours and textures to food products [7].

Traditionally, a great number of foods have been protected against spoiling by natural processes of fermentation. Currently, fermented foods are increasing in popularity (60% of the diet in industrialized countries) [8] and, to assure the homogeneity, quality and safety of products, they are produced by the intentional application in raw foods of different microbial systems (starter/protective cultures). Moreover, because of the improved organoleptic qualities of traditional fermented food, extensive research on its microbial biodiversity has been carried out with the goal of reproducing these qualities, which are attributed to native microbiota, in a controlled environment.

Methods of Biopreservation

Starter Cultures: The starter cultures of fermented foods can be defined as preparations of one or several systems of microorganisms that are applied to initiate the process of fermentation during food manufacture [9] fundamentally in the dairy industry and, currently, extended to other fermented foods such as meat, spirits, vegetable products and juices. The bacteria used are selected depending on food type with the aim of positively affecting the physical, chemical and biological composition of foods, providing attractive flavour properties for the consumer. To be used as starter cultures, microorganisms must fulfill the standards of GRAS status (Generally Recognized As Safe by people and the scientific community) and present no pathogenic or toxigenic potential. In addition, use must be standardized and reproducible [10].

Lactic acid bacteria: Lactic acid bacteria are important as natural biopreservatives which possess antagonistic properties against the spoilage bacteria and pathogens. The metabolites of LAB include acidic components like acetic acid, lactic acid, hydrogen peroxide and bacteriocins which are peptide in nature [11]. When the LAB competes for nutrients then they produce these metabolite components and the antimicrobial product, nisin which acts as a promising preservative for food [12]. The bacteriocins produced by LAB are also used in the Hurdle Technology. Using the bacteriocin producing LAB with other effective preservation techniques is effective in controlling the growth of spoilage microorganisms and inhibiting their generation, growth and bioactivity [13].

LAB bacteriocins: Bacteriocins are bacterial ribosomally synthesized peptides or proteins with antimicrobial activity. The antimicrobial ribosomally synthesized peptides produced by bacteria, including members of the LAB, are called bacteriocins. Such peptides are produced by many, if not all, bacterial species and kill closely related microorganisms [14]. Nowadays, the term bacteriocin is mostly used to describe the small, heat-stable cationic peptides synthesized by Gram positive bacteria, namely lactic acid bacteria (LAB), which display a wider spectrum of inhibition [11]. Since LAB have been traditionally associated to food and are regarded as safe, food biopreservation 72 has mostly focused on LAB bacteriocins.

The traditional role of LAB on food and feed fermentations is the main load- bearing pillar on which the use of bacteriocins in biopreservation relies. Examples of bacteriocin application in the production of primary food commodities are found in veterinary, agriculture and aquaculture. Nisin and lacticin have been incorporated into commercial prophylactic measures against mastitis. Bacteriocins have also been suggested as an alternative to antibiotic feeding and the use of bacteriocin producers able to colonize the gastrointestinal tract has successfully reduced the carriage of zoonotic pathogens [15, 16].

Besides food biopreservation, bacteriocins have been shown to accelerate cheese ripening by promoting the release of intracellular enzymes to the cheese matrix and a subsequent increase in the concentration of volatile and other compounds responsible of the sensory attributes of the matured cheese [17].

Applications of Bacteriocin-producing Lab in Food: The strategies for the application of LAB and/or bacteriocins in food are diverse which include inoculation of food with LAB (starter cultures or protective cultures) where bacteriocins are produced *in situ;* use of food previously fermented with the bacteriocin-producing strains as an ingredient in the food processing; and addition of purified or semi-purified bacteriocins [18]. Although most bacteriocins have been isolated from food-associated LAB, they are not necessarily effective in all food systems. However, several bacteriocins certainly do have potential in food applications when used under the proper conditions.

Application in Dairy Products: Nisin and/or nisin-producing strains have an effective impact against pathogenic bacteria such as *Clostridium botulinum* in cheese and against *L. monocytogenes*incheeses such as Camembert [3, 19].

Application in Meat Products: Nisin, enterocin AS-48, enterocins A and B, sakacin, leucocin A and especially pediocin PA-l/ach, alone or in combination with several physicochemical treatments, modified atmosphere packaging, high hydrostatic pressure, (HHP), heat and chemical preservatives are the most and commonly used bacteriocins in meat and meat products [20, 21].

Application in Seafood: The deterioration of fresh fish is generally caused by Gram-negative microorganisms; however, in vacuum-packed fresh fish and seafood, pathogenic organisms such as *Clostridium botulinum*and *L. monocytogenes*can also cause problems. The combination of nisin and Microgard reduced the total aerobic bacteria populations of fresh chilled salmon, increased its shelf-life and also reduced the growth of innoculated*L. monocytogenes*in frozenthawed salmon [22].

Hurdle Technology: The hurdle concept was introduced by Leistner in 1978 and stated that the microbial safety, stability, sensorial and nutritional qualities of foods are based on the application of combined preservative factors (called hurdles) that microorganisms present in the food are unable to overcome [23]. Thus, hurdle technology refers to the combination of different preservation methods and processes to inhibit microbial growth. An intelligent application of this technology requires a better understanding of the occurrence and interaction of different hurdles in foods as well as the physiological responses of microorganisms during food preservation. Using an adequate mix of hurdles is not only economically attractive; it also serves to improve not only microbial stability and safety, but also the sensory and nutritional qualities of a food [24].

This is a method of rendering the food to be free from contaminating and spoilage bacteria and pathogens by the combination of one or more methods. The pathogenic microorganisms have to pass through these individual approaches called “hurdles” for maintaining their activity in food products. Proper combination of hurdles will lead to destruction of the microbes and can prevent their further growth [13]. The propercombination of the hurdles ensures the microbial safety in food thereby maintaining its nutritional andorganoleptic parameters for consumer preference[25].

The hurdles include the properties like processing at high temperature, storage at low temperature, lowering pH (increasing acidity), water activity (aw) and/ or redox potential including the presence of biopreservatives or other preservative components in food products. The intensity of the hurdle is ascertained and controlled according to the type of spoilage microorganism(s) and regulated as per consumer safety and preference without sacrificing the quality and appearance of the final food product [12, 26, 27].

Applications of Hurdle Technology: In the past and often still today, hurdle technology has been applied empirically without knowledge of the governing principles in the preservation of a particular food. In industrialized countries, hurdle technology is of great interest in the food industry for extending the shelf life and safety of minimally processed foods, such as those that display low fat contents and/or salt [28]. Similarly, it is applied in fermented or refrigerated foods in which low temperature is often the only hurdle to be overcome (e.g. during distribution), which can lead to the alteration and intoxication of the foods.

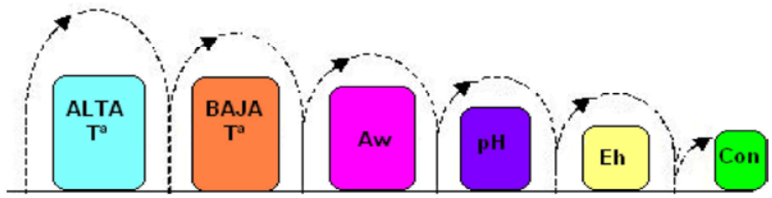

Fig. 1: Hurdle technology in food preservation (adapted from [3]

In developing countries, most foods are stored without refrigeration and are stabilized by the empiric use of hurdle technology. Several traditional foods have already been optimized by the intentional application of hurdles for safety and stability enhancement [29]. In addition, this technology is used for making new products and for reducing energy-consuming hurdles (e.g. refrigeration) or chemical preservatives (e.g. nitrites) [30]. The need to incorporate novel and effective combinations has spurred interest for natural and biological preservatives [3] such as LAB and their antimicrobial compounds.

Topics for the Future: Despite of the some research has been done to generating knowledge and increasing attention on bacteriocin and hurdle technology as food biopreservation to increase safety and shelf life of food, there are still several basic and applied issues that deserve further attention to fully exploit their antimicrobial potential in safety and shelf life of food. Special topics that need basic research for future may include:

- Resistant mechanism,
- New or enhanced antimicrobials and
- Safety concern which may emerge by applied of biopreservatives

CONCLUSION

Biopreservation is the method used for food preservation by using natural antimicrobials and microbiota thereby offers the potential to extend the storage life and food safety. The use of bacteriocins and/or bacteriocin-producing strains of LAB are of great interest as they are generally recognized as safe organisms and their antimicrobial products as biopreservatives. The beneficial products formed due to fermentation by the bacteria are used in this process to reduce the rate of food spoilage and to render the food free from pathogenic microorganisms and metabolites. Besides, using an adequate mix of hurdles is not only economically attractive; it also serves to improve microbial stability and safety, as well as the sensory and nutritional qualities of a food. The principle hurdles employed in food safety are temperature (higher or lower), water activity (aw), pH, redox potential (Eh), chemical preservatives, vacuum packaging, modified atmosphere, HHP, UV and competitive flora (LAB producing antimicrobial compounds). This process is gradually increasing in popularity for its ecologically benign approach. Therefore, based on the above conclusion the following suggestion is forwarded:

- it is desire to continue to expand our understanding of the influences that environmental factors have on the implantation and survival of bacteriocinogenic strains and the activity of their bacteriocins in order to quantitatively estimate their efficacy for future applications in food model systems and establish adequate means of application of these biopreservatives.

ACKNOWLEDGEMENT

We kindly admit those authors conducted research on the area of biopreservation, because their published manuscript serves as an input for this review paper.

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