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Role Of Biopreservation In Improving Food Safety And Storage

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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. *Bacillus spp.* have an antimicrobial action against Gram positive and Gram negative bacteria, as well as fungi, can therefore be used as a potential biopreservative in food processing due to its wide antimicrobial spectra. Bacteriocins are peptides or complex proteins biologically active with antimicrobial action against other bacteria, principally closely related species. Bacteriocins produced by lactic acid bacteria (LAB) have received particular attention in recent years due to their potential application in food industry as natural preservatives. Bacteriocin production in *Bacillus spp.* has been studied over the past few decades which include Subtilin from *B. subtilis*, Megacin from *B. megaterium* and Thermacin from *B. stearothermophilus*. Bio-preservation may be effectively used in combination with other preservative factors (called hurdles) to inhibit microbial growth and achieve food safety. 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.
Keywords **–** biopreservative, bacteriocin, bacillus, lactic acid bacteria

I. 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 precooked food especially seafood, prone to temperature abuse, and the import of raw seafood from developing countries results in outbreak of food borne illness [1]. One of the concerns in food industry is the contamination by pathogens, which are frequent cause of food borne diseases. In the USA, acute gastroenteritis affects 250 to 350 million 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]. Several bacterial pathogens including *Salmonella*, *Campylobacter jejuni*, *Escherichia coli 0157:H7*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium botulinum* are found associated with such outbreaks [1].

In order to achieve improved food safety against such pathogens, food industry makes use of chemical preservatives or physical treatments (e.g. high temperatures). These preservation techniques have many drawbacks which includes the proven toxicity of the chemical preservatives (e.g. nitrites), the alteration of the organoleptic and nutritional properties of foods, and especially recent consumer demands for safe but minimally processed products without additives. 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 [1].

The increasing demand for safe food has increased the interest in replacing chemical additives by natural products, without injuring the host or the environment. Biotechnology in the food-processing sector targets the selection, production and improvement of useful microorganisms and their products, as well as their technical application in food quality. The use of non-pathogenic microorganisms and/or their metabolites to improve microbiological safety and extend the shelf life of foods is defined as biopreservation [3]. Antagonistic properties of LAB allied to their safe history of use in traditional fermented food products make them very attractive to be used as biopreservatives [4].

II. BIO-PRESERVATION

The use of non-pathogenic microorganisms and/or their metabolites to improve microbiological

safety and extend the shelf life of foods is defined as biopreservation [1,3]. Bio-preservation refers to extended storage life and enhanced safety of foods using the natural microflora and (or) their antibacterial products. It can be defined as the extension of shelf life and food safety by the use of natural or controlled microbiota and/or their antimicrobial compounds [1,5]. 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. In fish processing, biopreservation is achieved by adding antimicrobials or by increasing the acidity of the fish muscle. Efforts have concentrated on identification and development of protective bacterial cultures with antimicrobial effects against known pathogens and spoilage organisms. Following compounds such as organic acids, bacteriocins, diacetyl and acetaldehyde, enzymes, CO_2, hydrogen peroxide etc. are contributing to antimicrobial activity by Microbiota.

III. BACTERIOCIN

Bacteriocins are peptides or complex proteins biologically active with antimicrobial action against other bacteria, principally closely related species. They are produced by bacteria and are normally not termed antibiotics in order to avoid confusion and concern with therapeutic antibiotics, which can potentially illicit allergic reactions in humans and other medical problems [6]. Bacteriocins differ from most therapeutic antibiotics in being proteinaceous agents that are rapidly digested by proteases in the human digestive tract. Since, bacteriocins are ribosomally synthesized; there exists a possibility of improving their characteristics to enhance their intensity and spectra of action [1,7]. Colicine was the first bacteriocin, discovered in 1925 by André Gratia and his workgroup [8].

Bacteriocin production could be considered as an advantage for food and feed producers since, in sufficient amounts, these peptides can kill or inhibit pathogenic bacteria that compete for the same ecological niche or nutrient pool. This role is supported by the fact that many bacteriocins have a narrow host range, and is likely to be most effective against related bacteria with nutritive demands for the same scarce resources [9].

IV. LAB BACTERIOCINS

A large number of new bacteriocins in lactic acid bacteria (LAB) have been characterized in recent years. Most of the new bacteriocins belong to the class II bacteriocins which are small (30–100 amino acids) heat-stable and commonly not post-translationally modified. While most bacteriocin producers synthesize only one bacteriocin, it has been shown that several LAB produce multiple bacteriocins (2–3 bacteriocins). The production of some class II bacteriocins (plantaricins of *Lactobacillus plantarum* C11 and sakacin P of *Lactobacillus sake*) have been shown to be transcriptionally regulated through a signal transduction system which consists of three components: an induction factor (IF), histidine protein kinase (HK) and a response regulator (RR).

Some bacteriocin-producing strains can be applied as protective cultures in a variety of food products and LAB bacteriocins possess many attractive characteristics that make them suitable candidates for use as food preservatives, such as:

- Protein nature, inactivation by proteolytic enzymes of gastrointestinal tract
- Non-toxic to laboratory animals tested and generally non-immunogenic
- Inactive against eukaryotic cells
- Generally thermo-resistant (can maintain antimicrobial activity after pasteurization and sterilization)
- Broad bactericidal activity affecting most of the Gram-positive bacteria and some, damaged, Gram-negative bacteria including various pathogens such as *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella*.

Genetic determinants generally located in plasmid, which facilitates genetic manipulation to increase the variety of natural peptide analogues with desirable characteristics [1].

In general, the following features should be considered when selecting bacteriocin-producing strains for food applications:

- The producing strain should preferably have GRAS (generally regarded as safe) status.
- Depending on the application, the bacteriocin should have a broad spectrum of inhibition that includes pathogens or else high specific activity.
- Thermostability.
- Beneficial effects and improved safety.
- No adverse effect on quality and flavour.
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V. BIOPRESERVATION WITH BACTERIOCIN: FIELD OF APPLICATION

Before bacteriocin can be applied in foods their cytolytic abilities should be assessed in detail. This is a very important issue since recently a cytolysin produced by *E. faecalis* was described that possesses both haemolytic and bacteriocin activities

[10]. Recombinant DNA technology is currently applied, to enhance production, to transfer bacteriocin genes to other species and for mutation and selection of bacteriocin variants with increased and/or broad activity spectra [11]. Continued study of the physical and chemical properties, mode of action and structure-function relationships of bacteriocins is necessary if their potential in food preservation is to be exploited. Further research into the synergistic reactions of these compounds and other natural preservatives, in combination with advanced technologies could result in replacement of chemical preservatives, or could allow less severe processing (eg. heat) treatments, while still maintaining adequate microbiological safety and quality in foods.

VI. CLASSIFICATION

On the basis of structure and mode of action bacteriocins are divided in 4 major groups [12].

Class I

- It is termed lantibiotics, constitue a group of small peptides that are characterized by their content of several unusual amino acids [13].
- They are small peptides that are differentiated from other bacteriocins by their content in dehydroamino acids and thioether amino acids. They include nisin, discovered in 1928 [14], lacticin 481 of *L. lactis* [15], citolysin of *E. faecalis* [10], and lacticin 3147 of *L. lactis* [16], among others.

Class II

- These are small, nonmodified, heat stable peptides [17].
- In general, they have an amphiphilic helical structure, which allows them to insert into the membrane of the target cell, leading to depolarisation and death.
- They comprises the (<10 kDa) thermostable non-lantibiotic linear peptides. They are divided into three subclasses on the basis of either a distinctive N-terminal sequence, the pediocin-like bacteriocins (class II.1) (e.g. pediocin PA-1/AcH produced by *Pediococcus* [18], the lack of leader peptide (class II.2) (e.g. enterocin EJ97 by *E. faecalis* [19], or neither of the above traits (class II.3)(e.g. enterocin L50A by *E. faecalis* [20].

Class III

- It is formerly termed bacteriolysins, large (> 30 KDa), heat-labile protein bacteriocins [21], such as helveticin J of *L. helveticus* [22] and bacteriocin Bc-48 of *E. faecalis* [23].
- It can function directly on the cell wall of Gram-positive targets, leading to death and lysis of the target cell.

Class IV

- It is presently reserved for cyclic bacteriocins composed not only from protein (also lipid or cidrate) [24].

Factors inhibiting bacteriocin production [25] include:

- inadequate physical conditions and chemical composition of food (pH, temperature, nutrients, etc.);
- spontaneous loss in production capacity;
- inactivation by phage of the producing strain;
- and antagonism effect of other microorganisms in foods.

The effectiveness of bacteriocin activity in food is negatively affected by:

- resistance development of pathogens to the bacteriocin;
- inadequate environmental conditions for the biological activity;
- higher retention of the bacteriocin molecules by food system components (e.g. fat);
- inactivation by other additives; slower diffusion and solubility and/or irregular distribution of bacteriocin molecules in the meat matrix [26].

Several factors, such as the presence of salts, other food ingredients, poor solubility and the uneven distribution of the bacteriocin, have all shown to effect the efficacy of bacteriocins in food [27].

VII. BACTERIOCINS OF VARIOUS GRAM POSITIVE BACTERIA

Although mast research has focused on antimicrobial agents produced by lactic acid bacteria, many bacteriocins of other Gram positive bacteria have been isolated and documented [28,29]. One such area of interest is the use of these bacteriocins to control the growth of undesirable microorganisms, particularly those of public health concern, e.g., *C. botulinum* and *L. monocytogenes*.

Bacteriocin production has been documented for a variety of Gram positive bacteria, including *Staphylococcus*, *Clostridium*, and *Bacillus spp*. Although the use of these bacteriocins may be precluded from foods because the producer strain may be a pathogen, recent developments in genetic engineering techniques have made the transfer of genes encoding for bacteriocin production from bath Gram positive and Gram negative bacteria to food grade microorganisms possible [30].

VIII. *BACILLUS* AS BIOPRESERVATIVE

Bacteriocin production in *Bacillus spp*. has been studied over the past few decades and several reports describe the production, isolation and characterization of bacteriocins from these species

[31,32,33], which include Subtilin from *B. subtilis*, Megacin from *B. megaterium* and Thermacin from *B. stearothermophilus* [34,35,36].

Subtilin, a cationic peptide produced by *B. subtilis*, having molecular mass of 3317 Da.; is a member of the group of bacteriocins known as the lantibiotics. The structure of subtilin was determined by Gross et al. (1973) [34].

Bacteriocin production by different strains of *B. stearothermophilus*, the main spoilage microorganisms of low acid canned products, was first described by Shafia (1966) [36]. He found that 12 out of 22 strains of *B. stearothermophilus* produced an inhibitory substance ta species of the same genus. The bacteriocin produced by these species was named as Thermacin.

More recently, Naclerio et al. (1993) [33] isolated the bacteriocin Gerein from *B. cereus*. The bacteriocin is relatively heat stable (75°C far 15 min), sensitive to proteolytic enzymes and has an apparent molecular mass of 9KDa.

Bacteriocins produced by *Bacillus spp*. could be alternatives to those produced by lactic acid bacteria for several reasons:

i. *Bacillus spp*., like lactic acid bacteria, have been used for hundreds of years in making food and various enzymes from *Bacillus* have been used intensively in food processing worldwide. No adverse effects have been demonstrated in humans from consuming foods made from *Bacillus spp*., and/or their products. Bacteriocins from these microorganisms would be safe for humans and would be no more of a risk than lactic acid bacteria.
ii. *Bacillus spp*. have an antimicrobial action against Gram positive and Gram negative bacteria, as well as fungi, and therefore have a greater antimicrobial spectra than lactic acid bacteria and their bacteriocins.
iii. The metabolic diversities of *Bacillus spp*. may result in bacteriocins with various properties such as inhibitory activity at alkaline, acidic condition or after thermal processing and would be suitable for food processing.
iv. The physiology/genetics of *Bacillus* are well understood, second only to those of *Escherichia coli*. Molecular biological techniques would provide safe/reliable tools for producing bacteriocins for the food industry.

IX. BIOPRESERVATION OF SEAFOOD PRODUCTS

Although bacteriocins are produced by many Gram-positive and Gram-negative species, those produced by LAB and now a days, *Bacillus sp*. are of particular interest to the food industry, since these bacteria have generally been regarded as safe. Among the lactic acid bacteria, a high diversity of bacteriocins is produced and several have been patented for their applications in foods. To date, the only commercially produced bacteriocins are the group of nisins produced by *Lactoccocus lactis*, and pediocin PA-1, produced by *Pediococcus acidilactici* [1,37].

Bacteriocins produced by lactic acid bacteria (LAB) have received particular attention in recent years due to their potential application in food industry as natural preservatives [38]. 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 [26]. Three approaches are commonly used in the application of bacteriocins for biopreservation of foods [1,26]:

1) Inoculation of food with LAB that produce bacteriocin in the products. The ability of the LAB to grow and produce bacteriocin in the products is crucial for its successful use.
2) Addition of purified or semi-purified bacteriocins as food preservatives.
3) Use of a product previously fermented with a bacteriocin producing strain as an ingredient in food processing.

The effectiveness of bacteriocins and protective cultures to control growth of *L. monocytogenes* in vacuum-packed cold smoked salmon has been demonstrated by several researchers. Katla et al, (2001) [39] examined the inhibitory effect of sakacin P and/or *L. sake* cultures (sakacin P producer) against *L. monocytogenes* in cold-smoked salmon. Nilsson et al, (1997) [40] showed that a non-bacteriocin-producing strain of *C. piscicola* was as effective as a bacteriocin-producing strain of *C. piscicola* in the inhibition of *L. monocytogenes* in vacuum-packed cold-smoked salmon. They suggested that the growth inhibition of *L. monocytogenes* was probably due to the competitive growth of *C. piscicola* that resulted in depletion of essential nutrients. The inhibitory effect of nisin in combination with carbon dioxide and low temperature on the survival of *L. monocytogenes* in cold-smoked salmon has also been investigated [40]. The effectiveness of nisin Z, carnocin UI49, and a preparation of crude bavaricin A on shelflife extension of brined shrimp was evaluated by Einarsson and Lauzon (1995) [41]. In a study using vacuum-packed cold-smoked rainbow trout, Niskänen et al, (2000) [42] examined the inhibition of *L. monocytogenes* and mesophilic aerobic bacteria by nisin, sodium lactate, or their combination.

X. CONCLUSION

Bio-preservation offers the potential to extend the storage life and food safety using the

natural microflora and (or) their antibacterial products. Shelf life of sea foods can be extended and safety ensured by the use of natural or controlled microbiota and/or their antimicrobial compounds. Bio-preservation may be effectively used in combination with other preservative factors (called hurdles) to inhibit microbial growth and achieve food safety [43]. 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) [1,43].

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