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RESEARCH ARTICLE

Lactic Acid Bacteria – A Potential Biopreservative In Sea Food Industry

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Abstract

Application of chemical preservatives and physical treatments like high temperatures are the common form of food preservation employed to achieve food safety against food borne pathogens like *Salmonella*, *Escherichia coli 0157:H7, Listeria monocytogenes, Staphylococcus aureus* and *Clostridium botulinum*.However, use of such treatments may have a toxic effect on food and alterthe organoleptic and nutritional properties, which are often unacceptable to the consumers. Present day consumer demands safe but minimally processed products without additives. Therefore, traditional preservation techniques are being replaced by combinations of innovative technologies that include biological antimicrobial systems such as use of non-pathogenic microorganisms and/or their metabolites, referred to as biopreservation. One such group of bacteria that has a potential for being used as a biopreservative is Lactic Acid Bacteria (LAB). In food systems, these bacteria are known to inhibit the growth of pathogenic and spoilage microorganisms, maintaining the nutritive quality and improving the shelf life of foods due to the production of inhibitor agents including organic acids, diacetyl, reuterin, hydrogen peroxide and bacteriocins. Bacteriocins are ribosomally synthesized peptides which are biologically active with antimicrobial actions against other bacteria. The use of bacteriocins combined with other preservation methods to create a series of hurdles during the manufacturing process has been recommended by several researchers to reduce food spoilage by microorganisms. Further exploration into the synergistic reactions of these compounds in combination with advanced technologies could result in replacement of chemical preservatives or severe processing treatments, while maintaining adequate microbiological safety and quality in seafood.

Introduction

Food safety standards and modern preservation techniques have diminished the likelihood of food related illness and product spoilage. However, 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. In Europe, morbidity from food borne illness is second only to respiratory diseases, with estimates of 50,000 to 300,000 cases of acute gastroenteritis per million populations every year (Ananouet al.,2007).Several bacterial pathogens including*Salmonella, Campylobacter jejuni, Escherichia coli 0157:H7, Listeria monocytogenes, Staphylococcus aureus* and *Clostridium botulinum* are found associated with such outbreaks.

In order to achieve improved food safety against such pathogens, food industry mostly relies on the application of chemical preservatives or more drastic physical treatments (e.g.high temperatures). These preservation techniqueshave many drawbacks which includes the proven toxicity ofmany of the commonest chemical preservatives (e.g. nitrites), the alteration of the organoleptic andnutritional 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 controllingmicrobial spoilage and safety hazards in foods are being replaced by combinations of innovativetechnologies that include biological antimicrobial systems such as lactic acid bacteria (LAB) and/or theirmetabolites.

1 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 (De Martinis et al, 2001). 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 (Stiles, 1996). One of the most common forms of food biopreservation is fermentation, where in natural or controlled condition microorganisms are promoted to grow on food. In seafood processing, biopreservation is achieved by adding antimicrobials or by increasing the acidity of the fish muscle. Researches in food sciences are now concentrated on identification and development of protective bacterial cultures with antimicrobial effects against known pathogens and spoilage organisms. One such group of bacteria that has a potential for being used as a biopreservative is Lactic Acid Bacteria (LAB).

2 LACTIC ACID BACTERIA AS A BIO-PRESERVATIVE

Lactic acid bacteria (LAB) are characterized as Gram-positive cocci or rods, non-aerobic but aerotolerant, able to ferment carbohydrates for energy and lactic acid production. The metabolic pathway from glucose may be homofermentative or heterofermentative. Lactic acid bacteria are also able to produce small organic substances that contribute with aroma and give specific organoleptic attributes to the products (Caplice and Fitzgerald, 1999). These microorganisms are found in several food products including milk, meat, fermented vegetables and beverages. In food systems, these bacteria are known to inhibit the growth of pathogenic and spoilage microorganisms, maintaining the nutritive quality and improving the shelf life of foods due to the production of inhibitor agents including organic acids (lactic and acetic acid), diacetyl, reuterin, hydrogen peroxide and bacteriocins (Lasagnoet al., 2002).They have also been used as flavour and texture producers.

Lactic acid bacteria include various major genera: *Lactobacillus, Lactococcus, Carnobacterium, Enterococcus, Lactosphaera, Leuconostoc, Melissococcus, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus and Weissella.* Other genera are*: Aerococcus, Microbacterium, Propionibacterium and Bifidobacterium* (Carr et al., 2002).

3 LAB BACTERIOCIN

Bacteriocins are ribosomally synthesized peptides which are biologically active with antimicrobial actions against other bacteria, principally closely related species. Bacteriocins differ from most antibiotics in being proteinaceous in nature and are rapidly digested by the enzyme proteases in the human intestinal tract. Since, bacteriocins are ribosomicallysynthesized; there exists a possibility of improving their characteristics to enhance their intensity and spectra of action (Saavedra et al., 2004). 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.

Thus, the use of bacteriocins has, in recent years, attracted considerable interest for use as biopreservatives in food, so as to invent an ever-increasing potential of these peptides.

Now-a-days cytolytic abilityof bacteriocins is a very important issue, since recently a cytolysin produced by *Enterococcus faecalis* was described that possesses both haemolytic and bacteriocin activities (Gillmore et al., 1990). 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 (Osmanagaoglu and Beyatli, 2001).

4 BIOPRESERVATION OF SEAFOOD PRODUCTS

Among theGram-positive and Gram-negative bacteriocin producers, LAB is 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 *Lactoccocuslactis*, and pediocin PA-1 produced by *Pediococcusacidilactici*(Schobitz et al., 1999).

Three approaches are commonly used in the application of bacteriocins for biopreservation of foods (Schillinger et al., 1996):

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 efficiency 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) demonstrated the inhibitory effect of sakacin P and/or *L. sake* cultures (sakacin P producer) against *L. monocytogenes* in cold-smoked salmon. Sakacin P had an initial inhibiting effect on growth of*L. monocytogenes*, while the cultures of *L. sake* had a bacteriostatic effect at 10°C for 4 week. When *L. sake* culture was added to salmon together with sakacin P, a bacteriocidal effect against *L. monocytogenes* was recorded. Nilsson et al. (1997) showed that a non-bacteriocin-producing strain of *Carnobacteriumpiscicola* was as effective as a bacteriocin-producing strain of *C. piscicola* in the inhibition of *L. monocytogenes* in vacuum-packed cold-smoked salmon. The growth inhibition was probably due to the competitive growth of *C. piscicola* that resulted in depletion of essential nutrients. Nilsson et al., (1997) reported that addition of nisin (500 or 1000 IU/g) to salmon inoculated with *L. monocytogenes* and stored at 5°C delayed, but did not prevent growth of *L. monocytogenes* in vacuum-packs. Numbers of *L. monocytogenes* increased to 10^8 CFU/g in vacuum packed salmon in 8 days, whereas CO_2 packing of cold-smoked salmon resulted in an 8-days lag phase for *L. monocytogenes* with numbers eventually reaching 10^6 CFU/g in 27 days. Addition of nisin to CO_2-packed cold-smoked salmon resulted in a 1- to 2-log10 reduction of*L. monocytogenes* followed by a lag phase of 8 and 20 days in salmon using 500 and 1000 IU nisin/g, respectively. The levels of *L. monocytogenes*remained below 10^3 CFU/g during 27 days of storage at both concentrations of nisin. The effectiveness of nisin Z, carnocin UI49, and a preparation of crude bavaricinA on shelf life extension of brined shrimp was evaluated by Einarsson and Lauzon (1995).Carnocin UI49 did not extend the shelf life compared to control (10-days shelflife), while bavaricin A resulted in a shelf life of 16 days. Nisin Z delivered a shelf life of 31 days. In a study using vacuum-packed cold-smoked rainbow trout, Niskanenand Nurmi (2000) examined the inhibition of *L. monocytogenes* and mesophilic aerobic bacteria by nisin, sodium lactate, or their combination. Trout samples were stored at 8°C for 17 days or at 3°C for 29 days. Both nisin and lactate inhibited the growth of *L. monocytogenes* in smoked fish, but the combination of the 2 compounds was even more effective. The combination of nisin and sodium lactate injected into smoked fish decreased the count of *L. monocytogenes* from 3.3 to1.8 $\log_{10}$CFU/g over 16 days of storage at 8°C. The level of *L. monocytogenes* remained almost constant (4.7 to 4.9 $\log_{10}$CFU/g) for 29 days at 3°C in the samples injected before smoking and which contained both nisin and sodium lactate.

5 HURDLE TECHNOLOGY

The hurdle concept,proposed by Leistner in 1978 (Leistner, 1978), stated 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. Thus, hurdle technology aims at 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 combination of hurdles is not only economically attractive; it also serves to improve microbial stability and safety, increase shelf life and ensures the sensory and nutritional qualities of a food.

The principle hurdles employed in food safety are temperature (higher or lower), water activity (a_w), pH, redox potential (Eh), chemical preservatives, vacuum packaging, modified atmosphere, high hydrostatic pressure (HHP), ultra violet (UV) and competitive flora (LAB producing antimicrobial compounds) (Ananouetal., 2007).

Table I: Use of LAB bacteriocins in Hurdle Technology(Ananou*et al,* 2007)

Bacteriocin	Other hurdles	Results
Nisin	HHP	Combination of HHP and nisin was effective to inactivate cheese indigenous microbiota. This combination was also effective against *S. carnosus*and *B. subtilis* spores, although a part of population survived the treatment
Nisin PediocinAcH	pH and low temperature	A significant reduction in *L. innocua* was observed with a combination of low pH 5.5 and nisin at 20 ºC. However, nisin-resistant cells regrew. Additional hurdles, such as refrigeration temperature, caused a dramatic reduction in population and allowed an increase of storage time to 10 days in liquid cheese whey.
	Pulsed electric fields (PEF)	The addition of nisin prior to PEF treatment increased the susceptibility of *L. innocua* to PEF treatment in whey.
	Sodium citrate and sodium lactate	The combination of low temperature, sodium lactate and/or sodium citrate with nisin controls *Arcobacterbutzleri* on chicken.
	HHP and high temperature	The combination of HHP, higher temperature, and pediocin acts synergistically, causing reduction of viability of *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, *Lb. sakei*, *Le. mesenteroides*
PediocinAcH Enterocins A and B	Sodium diacetate	Combination of pediocin and sodium diacetate works synergistically against *L. monocytogenes* at room and low temperature
	HHP	Enterocins A and B were used in combination with HHP to the enhancement of safety in cooked ham against *L. monocytogenes*. Pathogen counts were below detection limits at the end of storage.
Enterocin AS-48	Heat treatment	The efficacy of AS-48 against *S. aureus* was greatly enhanced by combination with a moderate heat treatment in milk.
	STPP, lactic, acetic and citric acids	The combination of AS-48 and STPP or lactate acts synergistically against *S. aureus*. The activity of AS-48 increases in the presence of organic acids at pH 4.5. The combination with lactate reduces *S. aureus* population by 6 log units under neutral pH.
	Mild heat treatment, OM-permeabilizing agents or acidic/alkaline pH	The antimicrobial activity of AS-48 against *E. coli* O157:H7 enhanced by combination with mild heat treatment, OM-permeabilizing agents (EDTA and STPP), or under acidic or alkaline conditions in buffer and in apple juice.

6 Applications Of LAB Bacteriocins In Hurdle Technology

The use of bacteriocins combined with other preservation methods to create a series of hurdles during the manufacturing process has been recommendedby several researchersto reduce food spoilage by microorganisms. In fact, it has been proven that the application of chemical preservatives, physical treatments (heat), or new mild non-thermal physical methods (pulsed electric field, HHP, vacuum, or modified atmosphere packaging), which increase the permeability of cell membranes, positively affects the activity of many bacteriocins (Garriga et al., 2002). Notably, combined treatments of bacteriocins with selected hurdles affecting outer-membrane (OM) permeability increase the effectiveness of some LAB bacteriocins against Gram-negative cells, which are generally resistant. The growth of Gram negative pathogens such as *E. coli* O157:H7 and *Salmonella* can also be controlled when metal chelators, such as EDTA, sodium tripolyphosphate(STPP) or physical methods such as heat and HHP, are used in combination with bacteriocins (Ananou et al, 2007).

7 Conclusion

The use of bacteriocin producing starter cultures as ingredients may not require special considerations in

many countries like USA provided the microorganism is GRAS. However, if a purified bacteriocin is used as a food preservative, the substance must be approved as GRAS. Further research is required to gain insight into the molecular mechanisms involved in bacteriocin production, immunity and mode of action, which is necessary for safe and effective exploitation of thebacteriocins. Moreover toxicological data and the fate of the molecule after ingestion are also required to establish the GRAS status.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 exploration 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 (e.g. heat) treatments, while still maintaining adequate microbiological safety and quality in foods.

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