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Lactic Acid Bacteria and Their Bacteriocins: A Promising Approach to Seafood Biopreservation

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1. Introduction

The growing interest in a correct life style, including alimentation, and the parallel attention on food quality have contributed to orientate consumers towards fishery products which are considered safe, of high nutritional value and capable of influencing human health in a positive way [1]. The diverse nutrient composition of seafood makes it an ideal environment for the growth and propagation of spoilage micro-organisms and common food-borne pathogens [2]. It has been estimated that as much as 25% of all food produced is lost post-harvest owing to microbial activity [1,2]. It has been mentioned that as many as 30% of people in industrialized countries suffer from a food borne disease each year and in 2000 at least two million people died from diarrhoeal disease worldwide. It is clear that indigenous bacteria present in marine environment as well as the result of post contamination during process are responsible for many cases of illnesses [3,4]. In the last years, the traditional processes applied to seafood like salting, smoking and canning have decreased in favor of mild technologies involving lower salt content, lower cooking temperature and vacuum (VP) or modified atmosphere packing (MAP). The treatments are usually not sufficient to destroy microorganisms and in some cases psychrotolerant pathogenic and spoiling bacteria can develop during the extended shelf-life of these products [2,5]. As several of these products are eaten raw, it is therefore essential that adequate preservation technologies are applied to maintain its safety and quality. Among alternative food preservation technologies, particular attention has been paid to biopreservation to extend the shelf-life and to enhance the hygienic quality, minimizing the impact on the nutritional and organoleptic properties of perishable food products such as seafood [1,6]. Biological preservation refers to the use of a natural or controlled microflora and/or its antimicrobial metabolites to extend the shelf life and improve the safety of food. Lactic acid bacteria (LAB)

are particularly interesting candidates for this technique [1,2,6,7]. Indeed, they are frequently naturally present in food products and are often strong competitors, by producing a wide range of antimicrobial metabolites such as organic acids, diacetyl, acetoin, hydrogen peroxide, reuterin, reutericyclin, antifungal peptides, and bacteriocins [8-10]. Hence, the last two decades have seen intensive investigation on LAB and their metabolites to discover new LAB strains that can be used in food preservation [1,7,11-13].

2. Bacterial hazards associated with fish and fish products

From the viewpoint of microbiology, fish and related products are a risky foodstuff group. Pathogenic bacteria associated with seafood can be categorized into three general groups [14]: 1) Bacteria (indigenous bacteria) that belong to the natural microflora of fish (*Clostridium botulinum*, pathogenic *Vibrio* spp., *Aeromonas hydrophila*); 2) Enteric bacteria (non-indigenous bacteria) that are present due to faecal contamination (*Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli*, *Staphylococcus aureus*); and 3) bacterial contamination during processing, storage, or preparation for consumption (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium botulinum*, *Salmonella* spp.).

Vibrio parahaemolyticus has been isolated from sea and estuary waters on all continents with elevated sea water temperatures. *V. parahaemolyticus* is frequently isolated from fish, molluscs, and crustaceans throughout the year in tropical climates and during the summer months in cold or temperate climates [15]. Fish food associated with illnesses due to consumption of *V. parahaemolyticus* includes fish-balls, fried mackerel (*Scomber scombrus*), tuna (*Thunnus thynnus*), and sardines (*Sardina pilchardus*). These products include both raw and undercooked fish products and cooked products that have been substantially recontaminated [9,15]. The most affected by the pathogens are Japan, Taiwan, and other Asian coastal regions, though cases of disease have been described in many countries and on many continents [9,16]. Cases of diseases caused by *V. parahaemolyticus* are occasional in Europe. During 20 years, only two cases of gastroenteritis were recorded in Denmark. The interest in this organism has been widened by the finding that similar organisms, *V. alginolyticus* and group of F *Vibrio* sp. also cause serious disease in humans [17]. *V. cholerae* is often transmitted by water but fish or fish products that have been in contact with contaminated water or faeces from infected persons also frequently serve as a source of infection [1,9,19]. The organism would be killed by cooking and recent cases of cholera in South America have been associated with the uncooked fish marinade seviche (*Cilus gilberti*) [18].

E. coli is a classic example of enteric bacteria causing gastroenteritis. *E. coli* including other coliforms and bacteria as *Staphylococcus* spp. and sometimes enterococci are commonly used as indices of hazardous conditions during processing of fish. Such organisms should not be present on fresh-caught fish [9,20,21]. The contamination fish derived food with pathogenic *E. coli* probably occurs during handling of fish and during the production process [20,22]. An outbreak of diarrhoeal illness caused by ingestion of food contaminated with

enterotoxigenic *E. coli* was described in Japan [23]. The illness was strongly associated with eating tuna paste. Brazilian authors [24] isolated 18 enterotoxigenic strains of *E. coli* (ETEC) from 3 of 24 samples of fresh fish originating from Brazilian markets; 13 of them produced a thermolabile enterotoxin. Infection with verocytotoxin _ producing strains of *E. coli* (VTEC) after ingestion of fish was recorded in Belgium [25]. An outbreak caused by salted salmon roe contaminated, probably during the production process, with enterohaemorrhagic *E. coli* (EHEC) O157 occurred in Japan in 1998 [22]. The roe was stored frozen for 9 months but it appears that O157 could survive freezing and a high concentration of NaCl and retained its pathogenicity for humans [26].

Aeromonas spp. has been recognized as potential foodborne pathogens for more than 20 years. Aeromonads are ubiquitous in fresh water, fish and shellfish and also in meats and fresh vegetables [27]. The epidemiological results so far are, however, very questionable. The organism is very frequently present in many food products, including raw vegetables, and very rarely has a case been reported. Up to 8.1% of cases of acute enteric diseases in 458 patients in Russia were caused by *Aeromonas* spp. [28]. In this study, *Aeromonas* spp. isolates with the same pathogenicity factors were isolated from river water in the Volga Delta, from fish, raw meat, and from patients with diarrhoea. Most *Aeromonas* spp. isolates are psychrotrophic and can grow at refrigerator temperatures [29]. This could increase the hazard of food contamination, particularly where there is a possibility of cross-contamination with ready-to-eat food products.

Salmonella has been isolated from fish and fishery product, though it is not psychrotrophic or indigenous to the aquatic environment [30]. The relationship between fish and *Salmonella* has been described by several scientists; some believe that fish are possible carriers of *Salmonella* which are harbored in their intestines for relatively short periods of time and some believe that fish get actively infected by *Salmonella* [31]. Most outbreaks of food poisoning associated with fish derive from the consumption of raw or insufficiently heat treated fish and cross-contamination during processing and the U.S. Food and Drug Administration's (FDA) data showed that *Salmonella* was the most common contaminant of fish and fishery products [31]. The highest *Salmonella* incidence in fishery products was determined in Central Pacific and African countries while it was lower in Europe and including Russia, and North America [32]. The most common serovar found in the world was *S. sub* Weltevreden [30, 31]. In seafood the commonest serotype encountered was *S. sub* Worthington followed by *S. sub* Weltevreden.

Enterotoxins produced by *Staphylococcus aureus* are another serious cause of gastroenteritis after consumption of fish and related products. In 3 of 10 samples of fresh fish, higher counts of *Staph. aureus* were detected than permitted by Brazilian legislation [20, 33]. In the southern area of Brazil, *Staph. aureus* was isolated from 20% of 175 examined samples of fresh fish and fish fillets (*Cynoscion leiarchus*). *Staph. aureus* has also been detected during the process of drying and subsequent smoking of eels in Alaska in 1993 [34]. During the process, *S. aureus* populations increased to more than 10^5 CFU g⁻¹ of the analyzed sample, after 2 to 3 days of processing. Subsequent laboratory studies showed that a pellicle (a dried skin-like

surface) formed rapidly on the strips when there was rapid air circulation in the smokehouse and that bacteria embedded in/under the pellicle were able to grow even when heavy smoke deposition occurred.

In ready-to-eat products, cooking, preservation ingredients, and storage atmosphere inhibit the Gram-negative organisms, resulting in a longer shelf life. Such conditions favor the growth of psychotropic pathogens such as *Listeria monocytogenes*, allowing them to grow to dangerous levels [9,35,36]. *L. monocytogenes* is a serious threat to consumer health and safety and has been implicated in several deadly outbreaks around the world [1,2]. This organism is halotolerant (up to 28% w/v for short periods), resistant to freezing temperatures, can grow and multiply during refrigeration, where other competing organisms cannot, and is able to survive at low water activity (*aw*) [9,14,37]. *L. monocytogenes* is widely distributed in the general environment including fresh water, coastal water and live fish from these areas. Contamination or recontamination of seafood may also take place during processing [37-39]. Moreover, *L. monocytogenes* is a psychrotrophic pathogen with the ability to grow from under 0 to 45°C [40]. This ability to grow at storage temperatures means that this bacterium is the main hazard in this kind of product. The pathogenic bacteria *L. monocytogenes* may grow on fresh seafood. *Listeria* has been found in farmed rainbow trout [41]. The outbreak of listeriosis related to vacuum packed gravad and cold-smoked fish was described in at least eight human cases for 11 months in Sweden [42]. Cold-smoked and gravad rainbow trout (*Oncorhynchus mykiss*) and salmon (*Salmo salar*) have been focused on during recent years as potential sources of infection with *L. monocytogenes* and there are several report on isolation of this food borne pathogen from fish-processing plants environments [14,37,39,43-45]. Seafood treatment is necessary to prevent food-borne illness. However, the pervasive nature of *L. monocytogenes* makes it difficult for processors to fully eliminate the organism from the environment.

Development of new-generation foods, which are mildly processed, contain few or no preservatives, are packaged in vacuum or modified atmospheres to ensure long shelf life and rely primarily on refrigeration for preservation, has raised concerns of potential increases in botulism risk caused by psychrotrophic nonproteolytic group II *Clostridium botulinum* [46]. An average of 450 outbreaks of foodborne botulism with 930 cases have been reported annually worldwide [47]. The main habitat of clostridia is the soil but they are also found in sewage, rivers, lakes, sea water, fresh meat, and fish [48,49]. Most critical are the hygienic conditions for handling the product after smoking. There is a risk of botulism due to the growth of *C. botulinum* type E in smoked fish. The bacterium becomes a hazard when processing practices are insufficient to eliminate botulinal spores from raw fish, particularly improper thermal processing [21]. The growth of *C. botulinum* and toxin production then depends on appropriate conditions in food before eating: the temperature, oxygen level, water activity, pH, the presence of preservatives, and competing microflora [21]. A problem with *C. botulinum* has been encountered with some traditional fermented fish products. These rely on a combination of salt and reduced pH for their safety. If the product has insufficient salt, or fails to achieve a rapid pH drop to below 4.5, *C. botulinum* can grow. There was no evidence that the fish had been mishandled, but a low salt environment in the

viscera allowed the bacterium to multiply and to produce toxin. *C. perfringens*, an important cause of both food poisoning and non-food-borne diarrhoeas in humans, was found in a number of fish owing to contamination with sewage, which is the main source of this organism [21].

3. Biopreservation

Seafood products are known to be especially susceptible to both microbiological and biochemical spoilage pathways. The development of effective processing treatments to extend the shelf life of fresh fish products is a must [2]. Additionally, the consumers' demand for high-quality and minimally processed seafood has recently captivated great attention [5, 9]. However, an increase in foodborne illness outbreaks is concomitant with the increase in consumer demand for less processed foods [1]. These trends highlight the importance of studying new microbial stress factors to extend the shelf-life of foods. Until now, approaches to reduce the risk of outbreaks of food poisoning have relied on the search for addition of more efficient chemical preservatives or on the application of more drastic physical treatments such as heating, refrigeration, high hydrostatic pressure (HHP), ionising radiation, pulsed-light, ozone, ultrasound, etc [1,5,50]. In spite of some possible advantage, these types of treatments have many drawbacks and limitation in seafood products: the proven toxicity of many of the commonest chemical preservatives (e.g. nitrites) (3), the alteration of the organoleptic and nutritional properties of seafood by physical treatments due to their delicate nature (e.g. freezing damage, discolouration in case of HHP and ionising radiation) [50,51] and especially recent consumer trends in purchasing and consumption, with demands for healthy seafood products that have been subjected to less extreme treatments (less heat and chill damage), with lower levels of salts, fats, acids, and sugars and/or the complete or the partial removal of chemically synthesized additives [1,2,7]. To harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in seafood are being replaced by an alternative solution that is gaining more and more attention: "biopreservation technology" [2,9,13,52,53]. It consists in inoculating food with microorganisms, or their metabolites, selected for their antibacterial properties and may be an efficient way of extending shelf life and food safety through the inhibition of spoilage and pathogenic bacteria without altering the nutritional quality of raw materials and food products [54, 55].

Lactic acid bacteria (LAB) possess a major potential for use in biopreservation because they are safe to consume, and during storage they naturally dominate the microbiota of many foods. Certain LAB species and strains isolated from seafood have been shown to exert strong antagonistic activity against spoilage and pathogenic microorganisms such as *Listeria*, *Clostridium*, *Staphylococcus*, and *Bacillus* spp [56-58]. The antagonistic and inhibitory properties of LAB are due to the competition for nutrients and the production of one or more antimicrobially active metabolites such as organic acids (lactic and acetic acid), hydrogen peroxide, and antimicrobial peptides (bacteriocins) [10]. Certain LAB are able to grow at refrigeration temperatures and are tolerant to modified-atmosphere packaging, low

pH, high salt concentrations, and the presence of certain additives such as lactic acid, acetic acid, and ethanol. Because of these benefits, LAB can be used as protective cultures to restrict the growth of undesired organisms such as certain spoilage and pathogenic bacteria, with the subsequent benefits in terms of food safety [9,10,58]. Moreover, these microorganisms may have additional functional properties and, in some circumstances, they can be beneficial for the consumers [6]. LAB represent the microbial group most commonly used as protective cultures, as they are present in all fermented foods and have a long history of safe use [8]. Safety for the consumers is an aspect of great importance, in particular for some seafood products which are not cooked before consumptions, but also for other types of foods.

4. The role of lactic acid bacteria in biopreservation technology

4.1. Characterization and classification

Lactic acid bacteria (LAB) encompass a heterogeneous group of microorganisms having as a common metabolic property the production of lactic acid as the majority end - product from the fermentation of carbohydrates [59]. LAB are Gram (+), usually nonmotile, non - sporulating, catalase - negative, acid - tolerant, facultative anaerobic organisms and have less than 55 mol% G+C content in their DNA [60-62]. Except for a few species, LAB members are nonpathogenic organisms with a reputed generally recognized as safe status (GRAS). Taxonomic revisions of these genera and the description of new genera mean that LAB could, in their broad physiological definition, comprise around 20 genera [10]. However, from a practical, food-technology point of view, the following genera are considered the principal LAB: *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* [61]. The classification of lactic acid bacteria into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance [62, 63]. An important characteristic used in the differentiation of the LAB genera is the mode of glucose fermentation under standard conditions. In this regard, the accepted definition is that given by Hommes and Vogel [64]: obligately homofermentative LAB are able to ferment hexoses almost exclusively to lactic acid by the Embden–Meyerhof–Parnas (EMP) pathway while pentoses and gluconate are not fermented as they lack phosphoketolase; facultatively heterofermentative LAB degrade hexoses to lactic acid by the EMP pathway and are also able to degrade pentoses and often gluconate as they possess both aldolase and phosphoketolase; finally, obligately heterofermentative degrade hexoses by the phosphogluconate pathway producing lactate, ethanol or acetic acid and carbon dioxide; moreover, pentoses are fermented by this pathway [62]. Several strains of groups 1 and 2 and some of the hetero fermentative group 3 are either used in fermented foods, but group 3 are also commonly associated with food spoilage. (For a more detailed discussion concerning the metabolic pathways, see [59].

4.2. Antimicrobial components from LAB

4.2.1. Bacteriocins

Bacteriocins are ribosomally synthesized peptides, that exert their antimicrobial activity against either strains of the same species as the bacteriocin producer (narrow range), or to more distantly related species (broad range) [1,2,7]. It has been estimated that between 30% and 99% of all bacteria and archaea produce bacteriocins; their production by LAB is very significant from the point of view of their potential applications in food systems and thus, unsurprisingly, these have been most extensively investigated [6,10,12,60,65,66]. It has been noted that the activity of bacteriocins is frequently directed against bacteria that are related to the bacteriocin - producing strain or against bacteria found in similar environments [67]. It has also been noted that some bacteriocins can also play a role in cell signaling. Microorganisms that produce bacteriocins also possess immunity mechanisms to confer self - protection, that is, to protect bacteriocin producers from committing “suicide” [10,68,69]. Besides concern about antibiotic resistance, increasing consumer awareness of potential health risks associated with chemical preservatives has increased interest in bacteriocins. Bacteriocins are naturally produced so they are more easily accepted by consumers [54]. Bacteriocins are usually classified combining various criteria. The main ones being the producer bacterial family, their molecular weight and finally their amino acid sequence homologies and/or gene cluster organization [59,70]. Based on a relatively recent approach [69,71,72] bacteriocins produced by LAB have been categorized into two major classes: the lanthionine - containing bacteriocins or lantibiotics (class I) and the largely unmodified linear peptide antimicrobials (class II).

4.2.2. Organic acid production

An important role of meat LAB starter cultures is the rapid production of organic acids; this inhibits the growth of unwanted flora and enhances product safety and shelf life. The types and levels of organic acids produced during the fermentation process depend on the LAB strains present, the culture composition, and the growth conditions [74]. Fermentation of the carbohydrates, glucose, glycogen, glucose-6-phosphate and small amounts of ribose, in meat and meat products, produces organic acids by glycolysis (Embden-Meyerhof Parnas pathway, EMP pathway) or the Hexose Monophosphate, HMP pathway. L (+) lactic acid is more inhibitory than its D (-) counterpart [68]. The antimicrobial effect of organic acids lies in the reduction of pH, and in the action of undissociated acid molecules [75]. It has been proposed that low external pH causes acidification of the cytoplasm. The lipophilic nature of the undissociated acid allows it to diffuse across the cell membrane collapsing the electrochemical proton gradient. Alternatively, cell membrane permeability may be affected, disrupting substrate transport systems [72]. The LAB in particular are able to reduce the pH to levels where putrefactive (e.g. clostridia and pseudomonads), pathogenic (e.g. *Salmonella* s and *Listeria* spp.) and toxinogenic bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Clostridium botulinum*) will be either inhibited or killed [7]. Also, the undissociated acid, on account of its fat solubility, will diffuse into the bacterial cell, thereby reducing the intracellular pH and

slowing down metabolic activities, and in the case of Enterobacteriaceae such as *E. coli* inhibiting growth at around pH 5.1.

4.2.3. Other antimicrobials of LAB

Hydrogen peroxide is produced from lactate by LAB in the presence of oxygen as a result of the action of flavoprotein oxidases or nicotinamide adenine dinucleotide (NADH) peroxidase [76]. The antimicrobial effect of H₂O₂ may result from the oxidation of sulfhydryl groups causing denaturing of a number of enzymes, and from the peroxidation of membrane lipids thus increasing membrane permeability [8]. Most undesirable bacteria such as *Pseudomonas* spp. and *S. aureus* are many times sensitive to H₂O₂. Carbon dioxide (CO₂) is mainly produced by heterofermentative LAB. CO₂ plays a role in creating an anaerobic environment which inhibits enzymatic decarboxylations, and the accumulation of CO₂ in the membrane lipid bilayer may cause a dysfunction in permeability [8]. CO₂ can effectively inhibit the growth of many food spoilage microorganisms, especially Gram-negative psychrotrophic bacteria [77]. Diacetyl, an aroma component, is produced by strains within all genera of LAB by citrate fermentation. It is produced by heterofermentative lactic acid bacteria as a by-product along with lactate as the main product [8]. Diacetyl is a high value product and is extensively used in the dairy industry as a preferred flavour compound. Diacetyl also has antimicrobial properties. Diacetyl was found to be more active against gram-negative bacteria, yeasts, and molds than against gram-positive bacteria. Diacetyl is thought to react with the arginine-binding protein of gram-negative bacteria and thereby interfering with the utilization of this amino acid [78].

5. LAB in fish and fish products

LAB are not considered as genuine microflora of the aquatic environment, but certain genera, including *Carnobacterium*, *Lactobacillus*, *Enterococcus*, and *Lactococcus*, have been found in fresh and sea water fresh fish [61,63,79-83]. The number of lactobacilli in the gastrointestinal tract of Arctic char was smaller in those reared in sea water than in fresh water, while the number of *Leuconostoc* and enterococci remained the same [84]. It is well documented that lactobacilli are part, not dominant, of the native intestinal microbiota of Arctic charr (*Salvelinus alpinus* L.), Atlantic cod, Atlantic salmon (*Salmo salar* L.), and brown trout (*Salmo trutta*) [82,85]. Several studies have shown the presence of other lactic acid bacteria, specially carnobacteria such as *Carnobacterium maltaromaticum* and *Carnobacterium divergence* within the intestinal content of salmonid species like Arctic charr (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) [63,86-89], Atlantic cod [89], common wolffish (*Anarhichas lupus* L.) [85], brown trout [82] and also wild pike [63,82]. Bacteria of the genus *Enterococcus* have been isolated from the intestine of common carp (*Cyprinus carpio*) and brown trout [80,82].

LAB dominating in spoiled vacuum-packaged cold-smoked fish products include the genera of *Lactobacillus*, *Leuconostoc*, *Lactococcus* and *Carnobacterium* [9]. Magnússon & Traustadóttir

[91] reported the complete dominance of homofermentative lactobacilli in vacuum-packaged cold-smoked herring. In vacuum packaged cold-smoked salmon and herring, *Lactobacillus curvatus* has been found in majority together with lower numbers of *Lactobacillus sakei*, *Lactobacillus plantarum*, *Lactococcus* spp. and *Leuconostoc mesenteroides* [58,]. Paludan-Müller, Huss, & Gram [92] identified *Carnobacterium piscicola* as the dominant microorganism isolated from spoiled vacuum-packaged cold-smoked salmon. Leroi et al. [93] also isolated carnobacteria during the first stage of storage of vacuum-packaged cold-smoked salmon, whereas *Lactobacillus farciminis*, *Lactobacillus sakei*, and *Lactobacillus alimentarius* were isolated at advanced storage times. Other studies have also confirmed that most bacteria in vacuum-packaged “gravad” fish products stored at refrigeration temperatures are carnobacteria [94] and *L. sakei*, and to a lesser extent *Leuconostoc* spp., *L. curvatus*, and *Weissella viridescens* [95]. Gancel et al [90] have isolated 78 strains belonging to the genus *Lactobacillus* from fillets of vacuum packed smoked and salted herring (*Clupea harengus*). LAB has been found to occur in marinated herring, herring fillets and cured stockfish [58]. In marinated or dried fish, the lactic acid bacteria flora maybe quite diverse since the presence of *Lactobacilli* and *Pediococci* has been reported [90]. Thai fermented fishery products were screened for the presence of LAB by Ostergaard et al. [96]. LAB was found to occur in the low salted fermented products in the range of 10^7 - 10^9 cfu/g. The high salt product “hoi dornng” had a lower LAB count of 10^3 - 10^5 cfu/g. Olympia et al [97] have isolated 10^8 LAB/g from a Philippine low salt rice-fish product burong bangus. Several studies have been mentioned that some species of *Carnobacterium* such as *C. divergens* and *C. maltaromaticum* are present in seafood and are able to grow to high concentrations in different fresh and lightly preserved products such as modified atmosphere-packed (MAP) [98-100], chilled MAP [101,102], high-pressure processing treated seafood products [103] and vacuum-packed cold smoked or sugar-salted (“gravad”) seafood [53,93,95]. These studies clearly highlight the ability of LAB fish isolates to grow on different harsh condition rather than other organisms. Obviously many investigation have been shown that carnobacteria are common in chilled fresh and lightly preserved seafood, but at higher storage temperatures (15–25°C) other species could be dominate the spoilage microbial community of seafood.

6. Application of LAB in seafood

Treating catfish fillets with of 0.50% sodium acetate, 0.25% potassium sorbate with 2.50% lactic acid culture completely inhibited growth of Gram negative bacteria, improved catfish odor and appearance during 13 days storage [110]. Einarsson & Lauzon [111] treated shrimps with various bacteriocins from lactic acid bacteria and reported shelf life extension except carnocin UI49. Total mesophilic and psychotropic bacteria and MRS counts of the samples treated with carnocin UI49 were not different than those of controls at 4.5°C. In a study with five strains of lactic acid bacteria (four *Lactobacillus* and one *Carnobacterium*) on fermented salmon fillets, *L. sake* LAD and *L. alimentarius* BJ33 was regarded as suitable starters for fermentation of salmon fillets [112] based on starter growth (increase of more than 1log in 3 days) and acidification of muscle (e.g. pH reduction of approximately 0.7

units in 5 days) as well as sensory evaluation. Kislá & Ünlütürk [113] studied the microbial shelf life of rainbow trout treated with nisin-containing aqueous solution of *Lactococcus lactis* subsp. *lactis* NCFB 497 and lactic acid. They reported the dipping of rainbow trout fillets into a lactic culture did not prolonged the shelf life due to the low inoculum level and type of lactic culture used. Elotmani & Assobhei [114] evaluated the inhibition of the microbial flora of sardine by using nisin and a lactoperoxidase system (LP), observing the efficiency of the nisin-LP combination in inhibiting fish spoilage flora. In another study growth of *L. monocytogenes* was significantly inhibited ($P < 0.05$) by *L. sakei* Lb706 in rainbow trout fillets stored under vacuum at 4°C during 10 days of storage while bacteriocin negative Lb706-B did not affect the growth of *L. monocytogenes*. In the presence of the sakacin A-producing strain of *L. sakei* (Lb706), the growth of *L. monocytogenes* was significantly inhibited ($P < 0.05$) in the first 3 days of storage at 10°C, after which its count increased to 10^7 CFU g⁻¹ [115]. Altieri et al. [106] succeeded in inhibiting *Pseudomonas* spp. and *P. phosphoreum* in VP fresh plaice fillets at low temperatures by using a *Bifidobacterium bifidum* starter, and extending the shelf-life, especially under MAP. Bifidobacteria combined with sodium acetate (SA) extended refrigerated shelf-life of catfish fillets at 4°C [116]. The application of two *Lactobacillus sakei* CECT 4808 and *L. curvatus* CECT 904T protective cultures on refrigerated vacuum-packed rainbow trout (*Oncorhynchus mykiss*) fillets resulted in extension of shelf-life by 5 days by significantly improved in the counts of all microbiological spoilage indicator organisms (Enterobacteriaceae, *Pseudomonas* spp., H₂S-producing bacteria, yeasts and moulds) and also significantly improved in all examined chemical parameters and off-odour [117].

Under biopreservation, combined coating of *Lactobacillus casei* DSM 120011 and *Lactobacillus acidophilus* 1M in *Streptomyces* sp. NIOF metabolites, played effective role in lowering the biochemical and microbiological changes, extended shelf-life and safety of stored fish under low temperature as reported by Daboor & Ibrahim [118]. Tahiri et al. [119] suggest that selection of protective strains to improve the sensory quality of seafood products should focus on specific spoilage microorganism's inhibition. This approach was chosen by Matamoros et al., [120] who have isolated seven strains from various marine products on the basis of their activity against many spoiling and pathogenic, Gram positive and Gram negative marine bacteria. Among strains, two *Le. gelidum*, and two *Lc. piscium* demonstrated promising effect in delaying the spoilage of tropical shrimp and of VP CSS. However, no correlation with the classical quality indices measured was evidenced. A recent study demonstrated that this protective effect could be due to the inhibition of *B. thermosphacta* identified as one of the major spoiler organisms in cooked shrimp stored under MAP [121]. The inoculation of Tilapia (*Oreochromis niloticus*) fillets with *Lactobacillus casei* DSM 120011 and *Lactobacillus acidophilus* 1M at 2% concentration decreased both total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N) and thiobarbituric acid (TBA) values and improved the biochemical quality criteria, microbial aspects and safety of frozen fish fillets during 45 and 90 days storage. [122].

For Shirazinejad et al. [123] 2.0% lactic acid combined with nisin indicated the highest reduction in population of *Pseudomonas* spp. and H₂S producing bacteria during storage

time of Chilled Shrimp. Fall et al. [121] evidenced the in situ inhibition of *B. thermosphacta*, a major spoiling bacterium, by *L. piscium* that could explain the protective effect observed in shrimp. Additionally, those strains also showed an inhibitory effect on *L. monocytogenes* [124] and *Staph. aureus*. Recently, Sudalayandi & Manja [109] succeeded to preserve fresh fish through controlling spoilage bacteria and amines of Indian mackerel fish chunk for two days at 37°C by inoculating them with different strains of LAB such as *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Streptococcus thermophilus*, *Lactococcus lactis*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus helveticus*. Using bacteriocin-like metabolite producer and non-producer strains of *Pediococcus* spp. [125] only slightly improved sensory quality of Horse Mackerel during cold storage. It was concluded that *Pediococcus* strains used in this study were not proper for preserving horse mackerel fillets especially at low storage temperatures. EntP-producing enterococci isolated from farmed turbot, under a spray-dried format exhibited antilisterial, antistaphylococcal, and antibacilli activities in turbot fillets either vacuum-packaged or subjected to modified-atmosphere packaging [2].

LAB Protective cultures have not been applied in many other seafood products except for cold smoked salmon (CSS), as they are normally flora of such products at the end of storage, and *L. monocytogenes* control. The effectiveness of bacteriocins to control growth of *L. monocytogenes* in vacuum packed cold smoked salmon has also been demonstrated by several researchers. Among them, Sakacin P has been found to be very potent against *L. monocytogenes* and is one of the most extensively studied bacteriocins [126-131]. Leroi et al. [132] succeeded in increasing the sensory use-by-date of CSS slices by inoculating them with strains of *Carnobacterium* sp. However the results varied depending on the batch treated. Addition of nisin to CO₂ packed cold smoked salmon resulted in a 1 to 2 log₁₀ reduction of *L. monocytogenes* [11]. Using a strain of *C. maltaromaticum*, Paludan-Müller et al. [92] only slightly extended the shelf-life of smoked salmon. Budu-Amoako et al. [133] tested nisin combined with heat as anti Listerial treatment in cold- packed lobster meat, finding decimal reductions of inoculated *L. monocytogenes* of 3 to 5 logs, whereas heat or nisin alone resulted in decimal reductions of 1 to 3 logs.

Duffes et al. [65] isolated *C. divergens* and *C. maltaromaticum* strains that exhibited listericidal activity in a model experiment with cold-smoked fish. They found that *C. piscicola* V1 inhibited *L. monocytogenes* by the in situ production of bacteriocins in vacuum-packed cold-smoked salmon stored at 4°C and 8°C. In contrast, another related species, namely, *C. divergens* V41 and its divercin V41, only exhibited a bacteriostatic effect on the target microorganism. Two strains of *C. maltaromaticum* isolated from CSS demonstrated their efficiency to limit the growth of *L. monocytogenes* in VP CSS during 31 days of storage at 5°C [134]. In a study using vacuum-packed cold smoked rainbow trout, the combination of nisin and sodium lactate injected into smoked fish decreased the count of *L. monocytogenes* from 3.3 to 1.8 log₁₀ over 16 days of storage at 8°C [135]. Sakacin P was added to vacuum-packed cold smoked salmon, a lightly processed high-fat (15–20%) product, together with a sakacin P-producing *L. sakei* culture in order to study the effect on the growth of *L. monocytogenes*. In

this product, the combination of purified sakacin P and a live culture was found to be bactericidal against *L. monocytogenes*. The addition of sakacin P alone inhibited the growth of *L. monocytogenes* on this product for about 1 week [126]. Silva et al. [136] used a bacteriocin-producing *Carnobacterium* strain under a spray-dried format. This strain survived the process and retained antilisterial ability, although it lost activity against other Gram-positive targets such as *Staph. aureus*. Some authors have evaluated the antimicrobial activity of nisin combined with other bacteriocins. Bouttefroy & Milliere [137] tested combinations of nisin and curvaticin 13 produced by *L. curvatus* SB13 for preventing the regrowth of bacteriocin-resistant cells of *L. monocytogenes*, finding that this combination induced a greater inhibitory effect than the use of a single bacteriocin. Aasen et al. [131] studied the interactions of the bacteriocins sakacin P and nisin with food constituents in cold-smoked salmon, chicken cold cuts, and raw chicken. They stated that owing to the amphiphilic nature of these peptides, they can be adsorbed to food macromolecules and undergo proteolytic degradation, which may limit their use as preservation agents. More than 80% of the added sakacin P and nisin were rapidly adsorbed by proteins in the food matrix that had not been heat-treated, less than 1% of the total activity remaining after 1 week in cold-smoked salmon. In heat-treated foods, they found that, bacteriocin activity was stable for more than 4 weeks. No important differences were observed between sakacin P and nisin, but less nisin was adsorbed by muscle proteins at low pH. The growth of *L. monocytogenes* was completely inhibited for at least 3 weeks in both chicken cold cuts and cold-smoked salmon by the addition of sakacin P (3.5 µg), despite proteolytic degradation in the salmon.

In the presence of the bacteriocinogenic strain *C. maltaromaticum* CS526 isolated from surimi, the population of *L. monocytogenes* in CSS decreased from 10^3 to 50 CFU g⁻¹ after 7 days at 4°C [138]. This activity could be linked to the production of the bacteriocin piscicocin CS526, since a non-bacteriocin producing strain had a lower effect on the growth of the pathogenic bacteria [138, 139]. The growth of the protective *Carnobacterium* strains did not modify the sensory characteristic of the product. One of these strains showing the strongest inhibition activity produces a bacteriocin, named Carnobacteriocin B2 that was involved in the antilisterial activity [105]. Three strains of bacteriocin producing *Carnobacterium* have been tested with the agar diffusion test method against a wide collection of *L. monocytogenes* (51 strains) isolated from seafood. All of the *Listeria* strains were sensitive. The inhibition was confirmed in co-culture with a mix of *L. monocytogenes* strains in sterile CSS [140]. One of these strains, *C. divergens* V41 showed its ability to maintain *L. monocytogenes* at the initial inoculating level of 20 CFU g⁻¹ during 28 days of storage at 4°C and 8°C. The effect of this strain on sensory characteristics and physico-chemical parameters revealed that it did not spoiled the product [56].

A bacteriocinogenic strain of *L. sakei* isolated from CSS allowed a 4 log reduction of *Listeria innocua* after 14 days of storage at 4°C. A reduction of 2 log units after 24 h at 5°C was also demonstrated with that strain in CSS juice towards *L. monocytogenes* [141]. Mix of bacteriocin-producing LAB like *L. casei*, *L. plantarum* and *C. maltaromaticum* were successfully used to limit the growth of *L. innocua* in CSS [142]. *C. maltaromaticum* had no

effect on the inhibition of the Gram positive spoilage bacteria *B. thermosphacta* in cooked shrimps [143]. The anti-listerial activity of 3 LAB strains used individually or as co-cultures was assayed on cold-smoked salmon artificially contaminated with *L. innocua* and stored under vacuum at 4°C [142]. The association of *L. casei* T3 and *L. plantarum* PE2 was the most effective, probably due to a competition mechanism against the pathogen. In their study Tomé et al. [144] have also selected a strain of *Enterococcus faecium* among five bacteriocinogenic LAB strains for its ability to induce a decrease of the population of *L. innocua* inoculated in CSS. However in these studies the inhibition activities were not confirmed on *L. monocytogenes*. For Matamoros et al. [145] two LAB strains, *Lactococcus piscium* EU2241 and *Leuconostoc gelidum* EU2247 were efficient to limit the growth of both pathogenic bacteria *L. monocytogenes* and *S. aureus* in a challenge test in cooked shrimp stored under VP from 2 to 3 log CFU g⁻¹ units after 4 weeks at 8°C followed by 1 week at 20°C. The strain of *Leuconostoc* produced a bacteriocin-like compound but its activity was slight lower than the *Lactococcus* strain that was non-bacteriocinogenic. In another study, the application of *C. divergens* M35 towards *L. monocytogenes* in CSS resulted in a maximal decrease of 3.1 log CFU g⁻¹ of the pathogenic bacteria after 21 days of storage at 4°C whereas a non bacteriocinogenic strain had no effect [119].

7. Conclusion and future prospective

The presence of LAB in many processed seafood product is now well documented and the bio-protective potential of many strains and/or their bacteriocin has been highlighted in the last years. In situ production is readily cost-effective provided that the bacteriocin producers are technologically suitable. To date, only nisin and pediocin PA - 1 have been applied commercially in food applications where they are used to protect against spoilage and pathogenic organisms. However, other bacteriocins could be at least as effective for food processors as it is possible to apply them with hurdle approaches, particularly in light of consumer demands for minimally processed, safe, preservative - free foods. Control of pathogenic bacteria has widely focused on *L. monocytogenes* considered as the main risk in ready-to-eat seafood. However, in these minimally processed products, the new combination of hurdles can give selective advantages to enhance food safety and quality, particularly effective against other pathogenic bacteria like clostridia, vibrio or staphylococci. These goals can be facilitated through the incorporation of live bacteriocin - producing strain(s) or through the use of bacteriocins as concentrated preparations, either through direct addition to the seafood or in an immobilized form on packaging as well as in conjunction with other factors such as high pressure or pulse electric fields, to achieve more effective preservation of foods. The great results obtained with protective culture, bacteriocins for improving safety and quality of seafood products clearly indicate that the application of LAB protective culture and/or their bacteriocins in seafood product can suggest several important benefits; 1) extended shelf life of seafood during storage time, 2) decrease the risk for transmission of foodborne pathogens in lightly preserved seafood products, 3) ameliorate the economic losses due to seafood spoilage, 4) reduce the application of chemical preservatives and drastic physical treatments such as heating,

refrigeration, etc. causing better preservation nutritional quality of food, 5) good option for industry due to cost effective way and finally 6) a good response to consumer demands for minimally processed, safe, preservative - free foods. At present the new techniques and disciplines emerging in the post – genomic era, such as genomics, proteomics, metabolomics, and system biology, open new avenues for interpretation of biological data. In combination with classical and molecular techniques, these new methods will be invaluable in the rational optimization of LAB function in order to obtain safer traditional and new seafood products.

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