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***Review Article** (Open Access)*

SUSTAINABLE SOLUTIONS FOR FISH PRESERVATION: BACTERIOCINS AS ECO-FRIENDLY ALTERNATIVES

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ABSTRACT

The persistent challenge posed by post-harvest microbial fish spoilage is currently suppressing the pivotal role of the fisheries industry in global nutrition and economy. The conventional methods of preserving food, despite their widespread uses, possess inherent disadvantages. Thus, there is elevating interest in biopreservation, specifically the application of bacteriocins derived from lactic acid bacteria (LAB) and other biological sources, as a forward-looking, eco-friendly alternative.

Addressing the pitfalls of conventional techniques, the review paper explores the potential of bacteriocins in mitigating spoilage risks. Notable bacteriocins, such as Nisin, Pediocins, Lacticin NK24 and Enterocin CD1, are spotlighted for their efficacy in extending fish product shelf life. The synergistic integration of bacteriocins with diverse preservation methods is discussed, offering promising avenues in terms of efficacy and sustainability. The paper also emphasizes on the necessity of ongoing investigation to discover new bacteriocins, elucidate their interactions with innovative methods of preservation, and maintain the sensory qualities of entire fish. This review aims to aid in the development of fish preservation paradigms, in line with changing consumer desires for natural, minimally processed goods, and rigorous quality control procedures.

***Keywords:** Biopreservation, Fish, Bacteriocins, Nisin, Hurdle Technology, Lactic Acid Bacteria*

INTRODUCTION

The fisheries industry plays a significant role in providing dietary proteins, micronutrients, minerals and fatty acids to communities across globe, contributing significantly to both nutrition fulfilment and economic upswing (Smith *et al.*, 2010). However, the fishery sector encounters a considerable obstacle in the form of post-harvest fish loss, wherein spoilage contributes a notable proportion (Adewolu and Adoti, 2010; Ghaly *et al.*, 2010; Kumolu-Johnson and Ndimele, 2011; Kruijssen *et al.*, 2020).

Degradation of fish quality due to spoilage occurs through various mechanisms, but microbial spoilage being a major contributor, leading to the generation of undesirable metabolites and off-flavors (Gram and Dalgaard, 2002). The perishable nature of fish products, coupled with insufficient preservation infrastructure in developing nations and climatic variations worldwide, accelerates the rate of deterioration process within hours (Gram and Dalgaard, 2002; Baptista *et al.*, 2020). Furthermore, spoilage due to microbes produces a considerable risk to food safety, as pathogenic organisms like, *Salmonella, Shigella, Staphylococcus, Listeria* and *Clostridium* can cause food-borne diseases (Gram and Huss HH, 1996; Gram and Dalgaard, 2002; Olatunde and Benjakul, 2018; Dehghani *et al.*, 2018).

Conventional fish preservation processes, for example chilling, freezing, drying, smoking, brining, fermentation, canning and chemical substancess, have been applied to extend the shelf life of fish products (Akinola *et al.*, 2006; Bate and Bendall, 2010). However, these methods come with drawbacks such as protein denaturation, nutrient loss, texture damage and the potential growth of spoilage bacteria (Neumeyer *et al.* 1997; D'Amico *et al.*, 2006; Alizadeh *et al.*, 2007; Mojisola, 2014; Abraha *et al.*, 2018; Mahmud *et al.*, 2018). In addition, the application of synthetic preservatives and chemical additives creates concerns about allergies, behavioral changes, and potential carcinogenic effects (UNEP and OECD, 2002: DJC, 2009; U.S. National Library of Medicine, 2010; Sambu *et al.*, 2022).

In response to these challenges, there is an elevating interest in innovative modern approaches to fulfil consumer demands for natural, fresh fish with minimal chemical additives. One innovative promising way is the application of antimicrobial substances of biological origin, including plant extracts, animal-derived enzymes, organic acids, probiotics, and/or their bacteriocins, to counter the economic losses related with inferior quality fish (Gálvez *et al.*, 1990; Kumar *et al.*, 2011; Ghanbari *et al.*, 2013; Hassoun and Çoban, 2017; Mei *et al.*, 2019).

The main intent of this review paper is to evaluate the limitations of traditional fish preservation methods that are generally employed and emphasizes the requirement of adopting bacteriocin-based biopreservation as a novel and effective approach. By examining scientific data, this paper highlights areas where insufficient knowledge is present and emphasizes the need for further research to establish quality control processes in the fishery sector.

BEYOND TRADITION: A CRITICAL ANALYSIS OF FISH PRESERVATION METHODS AND BIOPRESERVATION SOLUTIONS

The main objective that works behind fish preservation is to maintain the freshness of post-harvested fish, by delaying the putrefaction process. Several popular preservation methods, including drying, smoking, freezing, chilling, brining, fermentation, and canning, have been used to extend the shelf life of fish and fish products (Akinola *et al.*, 2006; Bate and Bendall, 2010). Furthermore, fisheries industry often employ chemical substances aimed at controlling moisture, enzymes, oxidative reactions, and microbial spoilage (UNEP and OECD, 2002: DJC, 2009; U.S. National Library of Medicine, 2010; Sambu *et al.*, 2022). Despite their widespread use, traditional processing techniques in the fishing industry possess certain drawbacks (Bate and Bendall, 2010) (table-1):

Table 1: Unveiling Limitations: Traditional Fish Preservation Techniques and reported Drawbacks.

Traditional Processing Techniques	**Drawbacks**	**References**
Chilling	Denaturation of protein, mechanical damage, and texture loss. Psychrophilic microorganisms and spores survive freezing temperatures. Freezing ineffective against oxidative and enzymatic spoilage, leading to ice crystal formation, textural damage, and membrane disruption with subsequent oxidation	Neumeyer *et al.*, 1997; D'Amico *et al.*, 2006; Alizadeh *et al.*, 2007
Drying	Reduction in weight, nutritive value, and indigestible flesh. High-temperature drying triggers lipid oxidation, resulting in off-flavor in fish products.	Mahmud *et al.*, 2018
Salt Preserving	Excessive salt creates an environment for salt-tolerant bacteria, leading to "pink eye" spoilage .	Bate and Bendall, 2010
Smoking	Tough texture, unwanted color shift, degradation of heat-sensitive nutrients, and denaturation of proteins and loss of amino acids. Potential health hazards due to carcinogenic substances from inadequately selected wood. Accelerates rancidity of fat and reduces digestibility of fat products.	Mojisola, 2014; Abraha *et al.*, 2018
Canning	Alteration of nutritional composition, denaturation of proteins induced by heat during canning, compromising	Abraha *et al.*, 2018

	heat-sensitive vitamins. Sterilization process may compromise integrity of vitamins like thiamine, riboflavin, and niacin.	
Irradiation	Ineffectual in eliminating bacterial spores. High doses induce undesirable sensory changes like off-flavors in fish products.	Sikorski and Sun, 1994
Chemical Additives	Allergies, attention disorders, behavioral changes and cancer risks during prolonged exposure. Sodium nitrate and sodium nitrite preservatives have been related with the production of nitrosamines, well-known carcinogens, raising concerns about their potential association with an increased risk of certain cancers. butylatedhydroxytoluene (BHT) and butylated hydroxyanisole (BHA), another two well-known preservatives, are responsible for skin allergies and chronic liver, thyroid, and kidney ailments during long-term usage. Propyl gallate, use to counter foodborne microbial growth, has been associated with adverse health effects, including prostate inflammation and the development of tumors in the brain, pancreas and thyroid.	Heijden *et al.*, 1986; Chung *et al.*, 1993; DJC, 2009; U.S. National Library of Medicine, 2010; Sambu *et al.*, 2022; UNEP and OECD, 2002
High-Pressure Treatment	Undesirable outcomes such as lipid oxidation, color changes, and texture hardening in fish.	Ashie *et al.*, 1996; Angsupanich and Ledward, 1998; Chevalier *et al.*, 2001; Sequeira-Munoz *et al.*, 2006;
Ozone	Disadvantageous due to unstable nature, promoting surface oxidation, and potential harm in high concentrations to human health.	Gonçalves, 2009

These drawbacks and limitations related with conventional fish preservation methods emphasize the imperative for alternative techniques, to address safety concerns and enhance efficacy. Because for example in the USA, approximate 22% to 30% of acute gastroenteritis incidents are attributed to food-borne diseases, with a heightened risk due to the growing trend in consumption of precooked seafood prone to temperature abuse (Mead *et al.*, 1999). Moreover, importing raw seafood from developing nations has led to outbreaks of foodborne diseases (Rodgers, 2001; McCabe-Sellers and Beattie, 2004). Thus, Challenges arise due to toxicity of chemical preservatives, alterations in the organoleptic and nutritional properties of fish due to physical treatment; rise of antibiotic-resistant microbes and the increasing consumer preference for minimally processed foods underscore the urgency for an alternative solution, namely "Biopreservation" (Feldhusen, 2000: De Martinis *et al.*, 2001; Devlieghere *et al.*, 2004; Kumar and Schweiser, 2005; Fisher *et al.*, 2005; Gálvez *et al.*, 2007; Dortu and Thonart, 2009; Zhou *et al.*, 2010; Pilet and Leroi, 2011). The utilization of natural, eco-friendly biological preservatives against bacteria and fungi, replacing chemical counterparts, represents a modern paradigm in food preservation (Tsai *et al.*, 2002; Burt, 2004; Bakkali *et al.*, 2008). Various antimicrobial substances originated from microbes, plant essential oils, tea polyphenols, rosemary extract and animal-derived chitosan are promising candidates for biopreservation (Tsai *et al.*, 2002; Burt, 2004: Bakkali *et al.*, 2008;). Among them, Lactic acid bacteria (LAB) or their metabolites, generally recognized as safe (GRAS), contain enough potential in combating spoilage bacteria and food-borne pathogens during storage (O'sullivan *et al.*, 2002; Hugas *et al.*, 2002; Ross *et al.*, 2002). LAB showcases its efficacy in food preservation through the secretion of inhibitory antimicrobial products, like bacteriocins and bacteriocin-like inhibitory substances (BLIS) (Gálvez *et al.*, 2010). The appealing characteristics of bacteriocins, including their proteinaceous nature, non-toxicity, non-immunogenicity, thermotolerant property, wider bactericidal activity make them well-suited and safe for fish preservation (Nath *et al.*, 2014). These attributes ensure lower risks of harmful by-products upon consumption and enable the maintenance of antimicrobial activity even after exposure to pasteurization and sterilization processes. Bacteriocins, with their diverse antimicrobial activity, offer a promising and innovative answer, gaining enough interest in recent times for their potential as biopreservatives in food.

DIVERSE BACTERIOCINS BY LAB FOR BIOPRESERVATION: A COMPREHENSIVE OVERVIEW

LAB with Generally Recognized as Safe (GRAS) status perform a important part in food fermentation, notably as starter cultures in the manufacture of dairy, meat, and vegetable products (Ray, 1992). Their considerable contribution lies in preserving the nutritional values of raw foods, lengthening shelf life and suppressing spoilage and pathogenic microbes. This preservation is gained either due to nutrient competition or because of the production of inhibitory substances s such as organic acids, hydrogen peroxide and bacteriocins (Ray, 1992). Bacteriocins, either chromosomally or plasmid-coded, ribosomally synthesized antimicrobial peptides by bacteria, including LAB, can be bactericidal, eliminating specific microorganisms, or bacteriostatic, inhibiting their growth (Gillor *et al.*, 2008; Reis *et al.*, 2012; Singh and Ghosh, 2012). Typically, LAB bacteriocins are thermostable cationic molecules with up to 60 amino acid residues and hydrophobic patches (Mokoena, 2017; Chikindas *et al.*, 2017). Their mode of action icludess electrostatic interactions with negatively charged phosphate groups on target cell membranes, leading to initial binding, pore formation, cell death and autolysin activation for cellular wall digestion (Gálvez *et al.*, 1990; Abee, 1995). This diverse group of peptides varies in size, structure, mode of action, antimicrobial potency, immunity mechanisms and target cell receptors, being produced by various bacterial lineages and archaea (Gillor *et al.*, 2008). Thus, there is numerous varities of bacteriocins produced by different LAB and can be classified on the basis of their biochemical and genetic characteristics (Klaenhammer, 1993; Cotter *et al.*, 2005; Balciunas *et al.*, 2013) (table-2):

Table 2: Classification and Characteristics of LAB Bacteriocins

Classification	Characteristics	Example
Class I (Lantibiotics)	• Post-translationally modified, containing lanthionine and B-methyllanthionine. • Members are stable against heat and acids. • Subdivided into Type A and Type B. • Type A: Linear, larger (21 to 38 amino acids). Kills target cell by depolarizing the cytoplasmic membrane. • Type B: More globular, smaller (up to 19 amino acids), with leader peptides cleaved by an ABC-transporter.	Nisin (Type A), Mersacidin (Type B)
Class IIa	• Small, heat-stable, • non-lanthionine-containing, • not post-translationally modified. Positively charged at neutral pH. • strong inhibitory activity against Listeria	Pediocin PA-1, Pediocin AcH, Sakacins A and P, Leucocin A, Enterocins A and P, Carnobacteriocin
Class IIb	• Complementary activity of two peptides.	Lacticin F,

	Formation of pores in target cell membranes leading to cell death.	Lactococcin G
Class IIc	• Cyclic bacteriocins with covalently linked N- and C-termini. • Cationic and hydrophobic. • Disrupt target-cell membrane and proton motive force.	Subtilisin A
Class IId	• Includes bacteriocins requiring lipid or carbohydrate moieties for activity.	Lactocin A, Lactocin B
Class III	• Heat-labile peptide antibiotics (>30 kDa) produced by Lactobacillus. • Involve catalyzing cell wall hydrolysis.	Helveticin J, Lactacin B
Class IV (Cyclic Peptides)	• Circular antibacterial peptides with head-to-tail peptide chain ligation. • Limited information about structure and function.	Leuconocin S, Lactocin 27

INNOVATIVE APPLICATIONS OF BACTERIOCINS WITH SYNERGISTIC APPROACHES: REVOLUTIONIZING FISH PRESERVATION

Food safety by the application of bacteriocins can be done through three main approaches: the inclusion of a purified or semi-purified bacteriocin preparation as a food ingredient, the integration of an ingredient previously fermented with a bacteriocin-producing strain, or the substitution of a starter culture in fermented foods with a bacteriocin-producing culture, allowing for in situ production of the bacteriocin (Deegan *et al.*, 2006).

Nisin, Class I bacteriocins, originated from Lactococcus lactis subsp., has achieved overall acceptance for commercial use in approximately 50 countries being effective in preventing microbial spoilage (Gharsallaoui *et al.*, 2016; Chaves *et al.*, 2017). Nisin Z, a notale variance of Nisin, proved to enhance solubility and diffusion characteristics, playing a pivotal role in the preservation of fish (Arauz *et al.*, 2009). Sofra *et al*., (2018), reported that the inclusion of nisin (2 × 10^4 IU/100 g) in an osmotic solution efficiently suppress spoilage in tuna slices, extending the shelf life to 51 days at 5°C. Another study demonstrated that a concentration of 1000 IU/g of Nisin a significant reduction in the *Listeria* population over the storage duration (Abdollahzadeh *et al.*, 2014). Furthermore, Nisin exhibited a beneficial impact on the color stability of rainbow trout during storage, attributed to the formation of hydrophobic bonds between carotenoids and the apolar fraction of nisin (Chaves *et al.*, 2017).

Pediocins, classified as Class II bacteriocins, exhibit surprising efficacy compared to nisin in eliminating *Listeria monocytogenes* from refrigerated fish (Yin *et al.*, 2007). Furthermore, pediocins exhibit potential activity across a broad pH and temperature range, making them valuable biopreservatives for fish (Porto *et al.*, 2017; Papagianni and Anastasiadou, 2009). An investigation conducted by Yin *et al.*, (2007), into the biopreservative effectiveness of pediocin ACCEL on refrigerated fresh fish fillets. Pediocin ACCEL showcased superior effectiveness compared to nisin in suppressing the growth of *Listeria monocytogenes* during the refrigeration of fish. The studies highlighted that *Pediococcus acidilactici* ALP57, isolated from non-fermented shellfish such as oysters, mussels and clams, could synthesize pediocin bac ALP57. This bacteriocin, approximately 6.5 kDa in size with a concentration of 12,800 AU/mL, demonstrated antimicrobial activity against *Listeria monocytogenes* ESB54, particularly during its exponential growth phase (Pinto *et al.*, 2009).

Lacticin NK24 from *Lactococcus lactis*, alongside nisin, demonstrated the ability to impede microbial growth on packaged fresh oysters, significantly preserving chemical quality and extending shelf life (Kim *et al.*, 2002). Lacticin, distinct from nisin, exhibits different target specificity and greater effectiveness (Garcia-Cayuela *et al.*, 2017).

Enterocin CD1, produced by *Enterococcus faecalis* CD1, showcased positive results in high-value marine cod (*Epinephelus diacanthus*) fish fillets. The treatment effectively lowered total viable count and spoilage bacteria during a 28-day storage period at 4°C, showing variations in sensory aspects compared to the control (Sarika *et al.*, 2017).

Additionally, antibacterial synergy was observed when Nisin, Pediocin 34, and Enterocin FH99 bacteriocins were combined against *Listeria monocytogenes* ATCC 53135, surpassing their individual effects (Kaur *et al.*, 2013). In a study by Einarsson and Lauzo, (1995), the efficacy of nisin Z and crude bavaricin A on extending the shelf life of brined shrimp was assessed.

Several bacteriocins, including Pentocin JL-1, have demonstrated efficacy against multidrug-resistant (MDR) bacterial pathogens too, particularly MDR *Staphylococcus aureus* (Cui *et al.*, 2012; Gabrielsen *et al.*, 2014; Perez *et al.*, 2014; Jiang *et al.*, 2017; Newstead *et al.*, 2020). Klebicins, another group of bacteriocins, have proven activity against MDR and carbapenem-resistant *Klebsiella* species (Denkovskienė *et al.*, 2019). These findings underscore the potential of bacteriocins in addressing challenges posed by microbial resistance and their diverse applications in food preservation.

Bacteriocins of LAB have few documented efficacy against distantly-related bacteria, particularly gram-negative microbes (Johnson *et al.*, 2017; Chatterjee and Sanyal, 2023). However, combined treatments that involve bacteriocins and specific hurdles can enhance their effectiveness, even against resistant gram-negative bacteria (Ananou *et al.*, 2007). Relying solely on a bacteriocin in food may not guarantee satisfactory safety, especially concerning gram-negative pathogenic bacteria protected by an outer membrane. The synergistic action of multiple preservation methods or at the very least, their combined application may offer greater protection than individual methods, thereby improving the safety and quality of food (Martinez and De Martins, 2005). Some notable examples are presented in table 3:

Table 3: Examples of Combined Treatments Enhancing Bacteriocin Efficacy in Fish Preservation

Treatment Description	**Outcomes**	**References**
Metal Chelators or Physical Methods with Bacteriocins	Enhanced control of *Escherichia coli* O157:H7, Salmonella growth	Ananou *et al.*, 2007
Nisin, Lactic Acid, Sodium Chloride in Rainbow Trout	Improved preservation without specified microbe targets	Nykänen *et al.*, 2000
Nisin, Carbon Dioxide, Low Temperature in Cold-Smoked Salmon	Inhibition of *Listeria monocytogenes* growth	Nilsson *et al.*, 1997
Nisin, Sodium Lactate, or Their Combination in Vacuum-Packed Cold-Smoked Rainbow Trout	Inhibition of *Listeria monocytogenes*, Mesophilic aerobic bacteria	Niskanen and Nurmi, 2000
Nisin and Microgard in Fresh Chilled Salmon	Extended shelf-life without specified microbe targets	Zuckerman and Ben Avraham, 2002

Lactoperoxidase System and Nisin in Fresh and Ice-Stored Sardines	Inhibition of bacterial growth	Elotmani and Assobhei, 2004
Chitosan (1%) with Nisin (0.6%) in Large Yellow Croaker During Refrigerated Storage	Enhanced quality preservation during storage	Hui *et al.*, 2016
Irradiation, Vacuum Packaging, High-Pressure Processing with Nisin	Extended shelf life	Behnam *et al.*, 2015; Kakatkar *et al.*, 2017; Lebow *et al.*, 2017
Radiation and Nisin in Seer Fish Steak During Chilled Storage	Extended shelf life without specified microbe targets	Kakatkar *et al.*, 2017
Osmotic Pre-Treatment with Nisin, Vacuum Packing, and Storage in Tuna	Significant extension in shelf life	Sofra *et al.*, 2018
Nisin with Natural Antioxidants (Rosemary Extract, Essential Oil, Tea Polyphenols) in Fresh Fish	Maintenance or improvement of sensory qualities, delayed chemical modifications, and inhibition of microbial spoilage	Gao *et al.*, 2014; Yang *et al.*, 2017; Ju *et al.*, 2017; Roomiani *et al.*, 2017;
Bacteriocins with Physical or Chemical Preservatives to Enhance Efficacy Against Gram-Negative Bacteria	Improved efficacy against Gram-negative bacteria	Abriouel *et al.*, 1998; Zhang and Mustapha, 1999; Garriga *et al.*, 2002; Ananou *et al.*, 2004; Ananou *et al.*, 2005;

The efficacy of bacteriocin activity is profoundly influenced by both the physical conditions and chemical composition inherent in the food matrix (Lahiri *et al.*, 2022). Ghalfi *et al.*, (2006), explored different strategies for application of bacteriocin-producing strain *Lactobacillus curvatus* CWBI-B28, which include in situ production, spraying with partially purified bacteriocin, packaging in bacteriocin-coated plastic film and immobilization onto the producer cell. The use of nisin in combination with stress factors like edible films and antimicrobial agents such as essential oils, sodium lactate, or chitosan has been reported, impacting the activity of bacteriocins (Ye *et al.*, 2008; Ekhtiarzadeh *et al.*, 2012; Schelegueda *et al.*, 2012; Resa *et al.*, 2016). Successful coating of bacteriocins onto packaging materials has been achieved, such as nisin/methylcellulose coatings for polyethylene films and nisin/EDTA/citric solutions coated onto PVC, nylon and LLDPE films. Additionally, corn zein films containing nisin have been explored as an effective packaging solution (Natrajan and Sheldon, 2000; Hoffman et al., 2001: Appendini and Hotchkiss, 2002;). Biodegradable packaging material impregnated with bacteriocin 7293 from *Weissella hellenica* showed antimicrobial activity against both Gram-positive and Gram-negative bacteria in pangasius fish fillets (Woraprayote *et al.,* 2017). Bacteriocin (EFL4) obtained from *Enterococcus faeca*lis L04, applied as a coating on ready-to-eat salmon fillets, exhibited antimicrobial activity against Staphylococcus putrefaciens and total viable counts, reducing muscle degradation and amine production (Mei *et al.,* 2020).

CONCLUSION

The critical challenges faced by the fisheries industry, due to degradation of fish quality due to spoilage, accelerated by microbial activities and the limitations of traditional preservation methods, despite their widespread use, poses significant risks to both food safety and the economic sustainability of the industry. The shift toward non-traditional preservation methods, combining bacteriocins and other natural preservatives, opens new avenues for enhancing the nutritional and sensory aspects of fish products. Thus emergence and the application of bacteriocins-based biopreservation derived from LAB, stands out as a promising and innovative solution to address these challenges. Bacteriocins offer a natural, eco-friendly alternative with diverse antimicrobial activities, making them suitable for fish preservation. The promising outcomes in various studies with bacteriocins, such as Nisin, Pediocins, Lacticin NK24, and Enterocin CD1, in extending the shelf life of fish products has been demonstrated.

Moreover, the review emphasizes the need for further research in exploring novel bacteriocins from different strains and their combination with innovative, mild preservation techniques. The two-fold criteria of economic attractiveness and preservation of safety, sensory, and nutritional qualities in fish products must be met for the successful implementation of these strategies. Importantly, the application of bacteriocins in whole fish preservation remains an underexplored area and future research should focus on preserving the organoleptic properties of whole fish. Continued research endeavors are essential to establish robust quality control processes and meet evolving consumer preferences in the dynamic field of fish preservation.

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