Microorganisms Improving Food Quality and Safety

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Abstract

Food quality and safety depends upon many factors, including various microbial properties. Microorganisms have been used for the production as well as for the quality and safety of the various food items. Antimicrobial and other properties of some selected microorganisms are being used to prevent food spoilage and food preservation. Some of the recent examples are use of bacteriocins and probiotics in food or by increasing the shelf life of food items by using microbial interactions or by reducing the pathogenic microorganisms by competitive microorganism. So the potential of microorganisms can be used as a tool for upgrading food safety and quality. The present work provides the summary on the use of various microbial systems, their modes of action, and application in various types of food systems.

Keywords

Bacteria · Bacteriocin · Food quality · Food safety · Probiotics

4.1 Introduction

Foodborne diseases are globally spread, and they are the main concern in today's era for creating health and economic problems. There has been much advancement in the field food industries related to processing, production, and packaging related to meet the changing requirements of changing society and our food habits. These have been the major health issue in developed as well as developing countries. This problem becomes more important due to the evolution of the microbes and their

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microbial environments, which plays a crucial role in the foodborne diseases. Food safety is a never-ending issue.

With the revolution in area of food sanitation and hygiene, there has been increase in the use of thermally pasteurized milk products which has led to improvements in food safety. There is also increase in the sophistication of new technologies which have contributed to advances in microbiological food safety with bringing healthier quality of foods and superior nutritional value.

4.2 Microorganism in Improving Food Quality and Safety

Food quality along with food safety has become a major concern in the food business since evolving food-associated pathogens have been associated with human infections and diseases. The contamination of bacteria in ready-to-eat products is of emerging concern to human health. This gives emphasis to the importance of developing active packaging, which prevents the growth and spread of food-associated pathogenic microorganisms. There has been surge in the consumer demand for reduced food additives and, mainly, chemical preservatives. So, there is currently extreme need for carrying out research in food applications of biopreservation and the natural antimicrobials.

Among various microorganisms, lactic acid bacteria provide an edge as they are observed as GRAS (generally recognized as safe). They produce various organic acids, enzymes, hydrogen peroxide, lytic agents, and antimicrobial peptides as well as bacteriocins which inhibit the growth of various microorganisms. More than 100 species of the *Lactobacillus* genus are used commercially as probiotics.

4.3 Use of Bacteriocin as Biopreservative

Among antimicrobial agents produced by bacteria, bacteriocins have acknowledged a significant attention to be used in food preservation. Bacteriocins are antimicrobial peptides produced in ribosomes by many bacteria. There have been various studies conducted on the role of bacteriocins in food preservation synthesized by lactic acid bacteria (LAB). These antimicrobial peptides inhibit the growth of several pathogenic bacteria such as *Bacillus cereus, Listeria monocytogenes*, and *Clostridium botulinum* by challenging their need for nutrients or producing different antimicrobial substances like bacteriocins, acetic acid, and lactic acid.

The means of action of bacteriocins could be bactericidal, with or without lysis of cell, or it may be bacteriostatic. They are often confused with antibiotics, but the major difference is in the bacteriocins that restrict their activity to particular species. Some of the major differences are given in Table 4.1.

Table 4.1 Comparison between bacteriocins and antibiotics

Characteristics	Bacteriocins	Antibiotics
Area of use	Food	Clinical
Synthesis	Ribosomal	Secondary
Range of activity	Narrow	Broad
Immunity to host cell	Yes	No
Mechanism of target cell	Usually adaptation affecting cell	Usually genetically transferable
Resistance or tolerance	Membrane composition	Determinant affecting different sites depending on the mode of action
Interaction requirements	Docking molecules are required sometimes	Specific target required
Mode of action	Mainly cause formation of pores, but in a few cases possibly cell wall biosynthesis	Cell membrane or intracellular targets
Toxicity/side effects	Unknown	Yes

Adapted from Cleveland et al. (2001)

4.4 Classification of Bacteriocins

The universally accepted classification of bacteriocins is still not there (Cleveland et al. 2001; Garcia et al. 2010; Zacharof and Lovitt 2012). Heng and Tagg in 2006 proposed the classification of bacteriocins by taking the nature of colicins into account. Therefore, they have grouped bacteriocins into four main classes. Majority of class Ia, II, and IV bacteriocins obtained from lactic acid bacteria have been applied in food biopreservation.

Class I (lantibiotics): They are posttranslationally modified peptides; they have varying lengths of amino acids from 19 to more than 50, typical thioether-based intramolecular rings of lanthionine and b-methyl-lanthionine. Class I bacteriocins are divided into two more categories, namely, class Ia and class Ib.

Class Ia bacteriocins: Composed of cationic and hydrophobic peptides; they are flexible in their structure contrasting to the rigid structure of class Ib and form pores in target membranes.

Class Ib bacteriocins: The structure of these peptides is globular in nature, with no net charge or a net negative charge. The antimicrobial activity of these bacteriocins is characterized by preventing the synthesis of specific enzymes (Cleveland et al. 2001; Garcia et al. 2010; Zacharof and Lovitt 2012).

Class II includes small (< 10 KDa) heat-stable, membrane-active peptides and is identified as the broad group of Gram-positive bacteriocins. Generally, they are short peptides with cationic nature and high isoelectric points. They are categorized into three subgroups.

Class IIa comprises pediocin-like *Listeria* active peptides with a conserved N-terminal sequence Tyr–Gly–Asn–Gly–Val and two cysteines forming an S–S bridge in the N-terminal half of the peptide.

Class IIb is a complex of two different peptides for its activity. Both peptides have different sequences in their primary amino acid. However, both peptides are encoded by their own adjoining genes, and solely one immunity gene is needed.

Class IIc includes those bacteriocins which are secreted by bacterium in the growth medium with general secretory pathway.

Class III comprises of large (> 30 KDa) heat-labile proteins which have uncertain potential of being used as food biopreservatives. Only two Gram-negative bacteriocins, colicin V and microcins, also fall in this class.

Class IV includes complex bacteriocins which include peptides distinguished by a peptidyl bond connecting the C- and N-termini that are clustered and essential carbohydrate and lipid moieties.

4.5 Commercially Available Bacteriocins

Bacteriocins are the preservatives synthesized by bacteria and have antibiotic attributes that are not the same as therapeutic antibiotics. Nisin and pediocin PA-1 are the only bacteriocins which are produced commercially. Bacteriocins were not used in food products until 1951. The search for new types of bacteriocins has been motivated in order to maintain the FDA's zero tolerance policy toward contamination of food by *Listeria monocytogenes*, a pathogenic bacterium common in the environment (Settanni and Corsetti 2008).

4.5.1 Nisin

Nisin is a class Ia bacteriocin or lantibiotic and is produced mainly by *Lactococcus lactis* subsp. *lactis*. It was developed in the early 1960s and is one of the most characterized and researched and also the most commercially important of all the bacteriocins. It is recognized to be safe by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives and has been licensed as a food preservative (E234). It has a molecular mass of 3510 daltons, and it is a 34-amino-acid-long, posttranslational modified polypeptide. Nisaplin™ is the most commercially available form of nisin from Danisco's for food preservative uses (Deegan et al. 2006). Eight different forms of nisin have been discovered and characterized by O'Connor et al., viz., nisins A, Z, F, and Q produced by *Lactococcus lactis* and nisins U, U2, P, and H produced by some *Streptococcus* strains. It has a wide range of activity against various lactic acid bacteria and other Gram-positive bacteria. It is also effective against heat-resistant bacterial spores of

Clostridium botulinum and against foodborne pathogens such as *L. monocytogenes, S. aureus,* or *B. cereus*. Its activity can be enhanced by using chelating agents (such as EDTA), sublethal heat, osmotic shock, and freezing, as these processes make the cell wall of Gram-negative microorganisms more permeable, which further makes them susceptible to the nisin (Galvez et al. 2007; Lucera et al. 2012).

4.5.2 Pediocin

Pediocin PA-1 is the class IIa bacteriocins and is produced by *Pediococcus acidilactici*, and it is marketed as Alta 2341™ or Microgard™. The pediocin PA-1 inhibited the growth of *L. monocytogenes* in ready-to-eat meat products (Deegan et al. 2006; Rodríguez et al. 2002). This bacteriocin has antimicrobial activity against a wide spectrum of Gram-positive bacteria such as *L. monocytogenes* and *S. aureus* and also against Gram-negative bacteria such as *Pseudomonas* and *Escherichia coli,* which are considered to be responsible for food spoilage or foodborne diseases. Pediocin is stable in a range of aqueous solutions and pH and resistant to heating and freezing. Recently, Verma et al. have produced the semi-purified pediocin which was effective in reducing the number of *S. aureus* and also increased the shelf life of buffalo milk.

4.6 Food Systems Application of Bacteriocins as Biopreservative

LAB and their bacteriocins have been taken up by humans from the past many years. The bacteriocins are proteinaceous in nature assumed to be destroyed by digestive proteases, with no adverse effect on the gut microbes, normal pH, and heat tolerance. Moreover, they are used as starter cultures for fermented foods. Three basic methods adopted for applying bacteriocins in food are:

1. Directly applied as purified and semi-purified preparation
2. The inoculation of lactic acid bacteria (LAB) that will synthesize bacteriocin in the food product
3. The use of an ingredient previously fermented with bacteriocin-producing bacteria (Garcia et al. 2010)

4.6.1 Milk and Milk Products

The spoilage of milk and its products is mainly caused by Gram-negative bacteria which are aerobic psychrotrophic in nature, heterofermentative lactobacilli, yeasts, molds, and spore-forming bacteria (Aneja et al. 2008; Ledenbach and Marshall 2009). LAB has a prolonged and harmless history to be used as preservatives in dairy fermentations where they are generally used as starter cultures, chiefly in the production of cheese. Those cultures which produce bacteriocin during cheese manufacture have been related to stop the growth of *Clostridium* which causes gas blowing in Swiss. Nisin-producing lactococci were found effective against clostridial spoilage when applied in these situations. So these primal examples explained the use of nisin-producing strains for inhibition of *Clostridium* sp., but nisin decreases the starter performance and ripening of cheese which diminished its use as biopreservative in cheese making. However, nisin-producing strains have shown slower rates of acid development and limited proteolytic activity and have the ability to ferment sucrose. Also, they have been reported as more sensitive to bacteriophage, which is an important consideration in commercial-scale cheese manufacture. The main aim of nisin is to provide protection against contamination with *L. monocytogenes* owing to create a problem during cheese manufacture and ripening. Many researchers have also carried out research on other bacteriocins as possible biopreservatives in various dairy products to control various pathogenic and spoilage microorganisms.

4.6.2 Meat and Meat Products

Fresh meat and fermented meat products make a perfect environment for the growth of pathogenic and spoilage microorganisms. The microorganisms associated with meat products generally belong to Enterobacteriaceae family, lactic acid bacteria, *Brochothrix thermosphacta*, and pseudomonads. The spoilage of refrigerated meat and meat products is caused by proliferation of *Listeria monocytogenes* during storage (Nychas et al. 2008). Nitrite is frequently used to stabilize red meat color and to prevent the food spoilage and poisoning organisms such as *C. botulinum* in curing meats. However, the adverse health effect of nitrite such as reaction with secondary amines in meats to form carcinogenic nitrosamines has encouraged the researchers to find the potential of using bacteriocins as an alternative to nitrite.

The mainly studied bacteriocins in meat and meat products are nisin, enterocin AS-48, enterocins A and B, sakacin, leucocin A, and especially pediocin PA-l/AcH. They have also been tested alone or in combination with various physicochemical treatments, modified atmosphere packaging, high hydrostatic pressure (HHP), heat, and chemical preservatives, as an additional hurdle to control the growth of *L. monocytogenes* and other pathogens (Ananou et al. 2007).

4.6.3 Fruits and Vegetables

Fruits and vegetables have short life span due to their high moisture content. After harvesting, their quality also decreases due to microbial growth, environmental factors, and maturity. They are made up of polysaccharide such as cellulose, hemicelluloses, and pectin. Spoilage microorganisms, particularly fungi, produce extracellular pectinases and hemicelluloses that can degrade these polysaccharides and cause spoilage in fruits and vegetables (Barth et al. 2009). LAB is also used as starter culture in various fermented vegetables, including table olives (*Enterococcus faecium* BFE 900, *Lactobacillus plantarum* LPC010), sauerkrauts (*Leuconostoc mesenteroides* NCK293, *L. lactis*), and pickles (Settanni and Corsetti 2008). Along with fermentation, starter cultures produce an extensive range of antimicrobial and proteinaceous substances which can inhibit the growth of flora in food products (Ross et al. 2002). The antimicrobial activity of bacteriocin has also been reported in literature against pathogenic microorganisms in fruits and vegetables. The effectiveness of nisin has been studied in combination with sodium lactate, EDTA, and potassium sorbate against pathogenic microorganisms in fresh cut fruits and found to be effective in reductions of 1 and 1.4 log cfu/g in the *Salmonella* population in fresh cut cantaloupe (Raybaudi-Massilia et al. 2009).

4.6.4 Seafood Products

The microflora on seafood constitutes microorganisms existing on the live animal and the microorganisms contaminating during processing. Only few microorganisms survive and proliferate under the product-specific conditions during storage, for example, *Vibrio* sp., *Aeromonas* sp., *Shewanella* sp., *Photobacterium phosphoreum*, and *Pseudomonas* cause fish-like smell in seafood (Gram 2009; Chahad et al. 2012). Many pathogenic microorganisms such as *Aeromonas, Clostridium botulinum*, *C. perfringens*, *Escherichia coli*, *L. monocytogenes*, *Salmonella*, *Staphylococcus aureus, Vibrio parahaemolyticus,* and *V. cholerae* serovar O1 and O139 have also been reported from seafood products (Ghanbari et al. 2013). Although successful studies in marine products have been carried out to prevent the growth of *Listeria* sp. by different species of bacteriocin-producing LAB, mainly from the *Carnobacterium* genus (Katla et al. 2001; Brillet et al. 2005; Vescovo et al. 2006; Pinto et al. 2009).

Antimicrobial Packaging Film The bacteriocins can be used in the active biopolymer films for applying in food packaging. Over-conventional synthetic packaging edible films provide several advantages. They exhibit biodecomposibility and are considered environmentally friendly by consumers, so they present as interesting substitute to prevent microorganism in foods.

To retard moisture, oxygen, aroma, and solute transport, the abilities of edible films or coatings have been examined. This is moreover improved by film-carrying food additives such as antioxidants, antimicrobials, flavors, and colorants. To enhance antimicrobial activity of bacteriocins, films should be used instead of being incorporated directly to the products. Many researchers have developed the antimicrobial films using other semi-purified bacteriocins. Out of numerous bacteriocins, enterocins produced by *Enterococcus faecium* have been determined to be effective for managing the growth of *L. monocytogenes* in meat products (Ananou et al. 2005). Looking into the advantage of adding bacteriocins in biofilms, various biofilms have been developed by various researchers.

4.7 Conclusion

With the growing awareness and demand of chemicals or additive free foods of consumers, there is a demand of new alternative methods of improving food quality and safety. To increase the shelf life and safety of dairy products, bacteriocins can be used as a beneficial approach. Bacteriocins are assorted groups of antimicrobial peptides/proteins which have a large spectrum of applications in foods and can be used against a wide range of bacteria with narrow specificity, although the efficiency of bacteriocins is little due to various reasons like food components, their solubility, and their degradation. If LAB-synthesized bacteriocins are used proportional to that present in cultured foods, then they can be termed as consumable. They can be combined with other technologies for food preservation. More research is needed to discover more bacteriocins which can be commercially used and have GRAS status.

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