A review on bacteriocins as food biopreservatives

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Abstract— Bacteriocins are ribosomally-synthesized peptides or proteins with antimicrobial activity, produced by different groups of bacteria. Many lactic acid bacteria (LAB) produce bacteriocins with rather broad spectra of inhibition. Several LAB bacteriocins offer potential applications in food preservation, and the use of bacteriocins in the food industry can help to reduce the addition of chemical preservatives as well as the intensity of heat treatments, resulting in foods which are more naturally preserved and richer in organoleptic and nutritional properties. These natural preservatives can control the growth of spoilage and pathogenic bacteria in foods. Bacteriocins can be added to foods in the form of concentrated preparations as food preservatives, shelf-life extenders, additives or ingredients, or they can be produced in situ by bacteriocinogenic starters, adjunct or protective cultures. Immobilized bacteriocins can also find application for development of bioactive food packaging. The effectiveness of bacteriocins is often dictated by environmental factors like pH, temperature, food composition and structure, as well as the food microbiota. Foods must be considered as complex ecosystems in which microbial interactions may have a great influence on the microbial balance and proliferation of beneficial or harmful bacteria.

Keywords-component; acid lactic bacteria; biopereservative; bacteriocins; antimicrobial activity;bacteriocin classifications

I. INTRODUCTION

In the production of food, it is crucial to take proper measures for ensuring its safety and stability during the shelf-life. Microbiological problems of today's food industry are: (i) the emergence of new pathogens and pathogens not previously associated with food consumption; (ii) the ability of microorganisms to adapt and change that has resulted in altered food safety hazards [1].

The empirical use of microorganisms and/or their natural products for the preservation of foods (biopreservation) has been a common practice in the history of mankind [2]. The lactic acid bacteria (LAB) are a group of Gram-positive bacteria with a variety of morphological, metabolic and physiological characteristics. They are included in the group of non-spore forming, non-respiring cocci or rods, catalase-negative, devoid of cytochromes; non-aerobic but aero-tolerant, fastidious, acid tolerant and strictly fermentative with lactic acid as the major end product during the fermentation of carbohydrates [3]. They are widely distributed in nature and have been isolated from grains, green plants, dairy and meat products, fermenting vegetables and mucosal surface of animals. LAB produce an array of antimicrobial substances (such as organic acids, diacetyl, acetoin, hydrogen peroxide, reuterin, reutericyclin, antifungal peptides, and bacteriocins [4, 5, 6, 7, 8].

Bacteriocins are ribosomally synthesized extracellulary released low molecular-mass peptides or proteins (usually 30–60 amino acids) which have a bactericidal or bacteriostatic effect on other bacteria [9, 10, 11].

The bacteriocins produced by LAB offer several desirable properties that make them suitable for food preservation: (i) are generally recognized as safe substances [9], (ii) are not active and nontoxic on eukaryotic cells, (iii) become inactivated by digestive proteases, having little influence on the gut microbiota, (iv) are usually pH and heat-tolerant, (v) they have a relatively broad antimicrobial spectrum, against many food-borne pathogenic and spoilage bacteria, (vi) they show a bactericidal mode of action, usually acting on the bacterial cytoplasmic membrane: no cross resistance with antibiotics, and (vii) their genetic determinants are usually plasmid-encoded, facilitating genetic manipulation [1].

The inhibitory spectrum of bacteriocins can be narrow and confined to closely related species, or it can be relatively broad, inhibiting a range of target organisms, including food-spoilage and pathogenic bacteria, such as *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium tyrobutyricum*, methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. In general, bacteriocins act mainly by pore formation in target cell membranes, or by inhibiting cell wall synthesis or enzyme activities in the cytosol (RNAse or DNAse).

Bacteriocins produced by lactic acid bacteria (LAB) are the most studied and promising bacteriocins produced by

Gram-positive bacteria, especially because many LAB are considered "generally recognized as safe" (GRAS). Many bacteriocin-producing LAB, including the genera *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc* and *Bifidobacterium*, have been isolated from different food matrices, such as fermented dairy products, vegetables, fruits, meat and fish and also from the human and animal gastrointestinal tract.

II. CLASSIFICATIONS OF BACTERIOCINS

Four general classes of antimicrobial peptides or proteins (bacteriocins) from LAB have been characterized to date [3]:

- Lantibiotics
- Small (≤13kDa) hydrophobic heat stable peptides
- Large (~30 kDa) heat-labile proteins
- Complex proteins that require additional carbohydrates or lipids moieties to attain antimicrobial activity.

A. Class I (Lantibiotics):

Lantibiotics are a family of membrane active peptides that contain the unusual thio-ether amino acids lanthionine and β-methyl lanthionine as well as other modified amino acids such as dehydrated serine and threonine [12]. Their distinguishing feature is the presence of post-translationally modified amino acid residues. The best example in this group is nisin produced by *Lactococcus lactis* subsp. *lactis*. Class I is being further subdivided into Ia and Ib. Class Ia bacteriocins, which include nisin, consist of cationic and hydrophobic peptides that form pores in the target membranes and have a flexible structure compared to the more rigid ones of class Ib. Class Ib bacteriocins, which are globular in nature, have no net negative charge [13].

B. Class II (small heat stable peptides):

They are bioactive peptides, which do not contain any modified amino acids residues such as lanthionine. They are also further subdivided into IIa and IIb, Class IIa includes pediocin-like bacteriocin having anti-listerial activity with a conserved N-terminal sequence Tyr-Gly-Asn-Gly-Val and two cysteines forming S-S bridge in the N-terminal half of the peptide. Bacteriocins composed of two different peptides comprised Class IIb, which need both peptides to be fully active. The primary amino acid sequences of the peptides are different. Though each one is encoded by its own adjacent genes, only one immunity gen is needed [14].

C. Class III (Large heat labile bacteriocins):

Heat labile proteins of large molecular weight include Helviticin-J, lactacins A and B. very little information is available on this group.

D. Class IV:

They include bacteriocins that form large complexes with other chemical moieties, carbohydrates or lipids required for activity. Presently, no such bacteriocins have been purified and it is believed that the reason is formation of complexes with other macromolecules in the crude extract due to their cationic and hydrophobic properties.

The majority of bacteriocins produced by bacteria associated with food belong to classes I and II.

III. MODE OF ACTION

Bacteriocins, particularly lantibiotics, inhibit target cells by forming pores in the membrane, depleting the transmembrane potential (Δψ) and /or the pH gradient, resulting in the leakage of cellular materials. Early studies suggest that in order for nisin to form pores, target cells require Δψ (inside negative) and ΔpH (inside alkaline). Bacteriocins are positively charged molecules with hydrophobic patches. Electrostatic interactions with negatively charged phosphate groups on target cell membranes are thought to contribute to the initial binding with the target membrane. It is likely that the hydrophobic portion inserts into the membrane, forming pores. There is debate over the types of pores formed by nisin, with most groups favoring the "barrel-stave" or "wedge" models. In the "barrel-stave" model, each nisin molecule orients itself perpendicular to the membrane, forming an ion channel that spans the membrane. According to the "wedge" model, after a critical number of nisin molecules associate with the membrane, they insert concurrently, forming a wedge [14]. Fig. 1 shows the effect of bacteriocin on a cell of pathogen bacteria [15].

IV. ADDITION OF BACTERIOCIN TO FOOD SYSTEMS

Foods can be supplemented with ex situ produced bacteriocin preparations, or by inoculation with the bacteriocin-producer strain under conditions that favour production of the bacteriocin in situ [16].

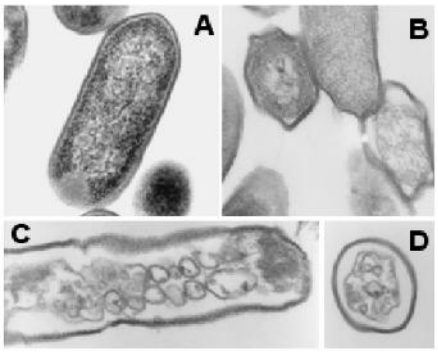

Figure 1. Example of damage caused by bacteriocin on *L. monocytogenes* CECT 4032 cells. **(A)** cells without enterocin AS-48; **(B)** cells treated with 0.1 µg/ml of AS-48 for 2 h; **(C** and **D)** cells treated with 3 µg/ml of enterocin AS-48 for 10 min (adapted from [15]).

In the first case, bacteriocin preparations obtained by cultivation of the producer strain in a fermentor at industrial scale followed by adequate recovery and processing can be added as partially purified or purified concentrates, which would require specific approval as preservatives from the legislative point of view. So far, nisin is the only bacteriocin licensed as a food preservative (E234).

Many preliminary studies on the activity of bacteriocins in vitro or in food systems are carried out with partially-purified preparations obtained from cultured broths. In most cases, a low concentration of bacteriocin is often recovered, which limits the efficacy of such preliminary tests.

Ex situ produced bacteriocins can also be added in the form of raw concentrates obtained by cultivation of the producer strain in a food-grade substrate (such as milk or whey). The resulting preparations may be regarded as food additives or ingredients from the legal point of view, since some of their components may play a recognized function in the food (such as increase in protein content or thickening). They also contain the cell-derived antimicrobial metabolites (such as lactic acid) and bacteriocins, affording an additional bioprotectant function [1].

Ex situ produced bacteriocins can also be applied in the form of immobilized preparations, in which the partially-purified bacteriocin or the concentrated cultured broth is bound to a carrier. The carrier acts as a reservoir and diffuser of the concentrated bacteriocin molecules to the food ensuring a gradient-dependent continuous supply of bacteriocin. The carrier may also protect the bacteriocin from inactivation by interaction with food components and enzymatic inactivation. Moreover, the localized application of bacteriocin molecules on the food surface requires much lower amounts of bacteriocin (compared to application in the whole food volume), decreasing the processing costs. A variety of methods have been proposed for bacteriocin immobilization, including adsorption to the producer cells [17], silica particles or corn starch powder [18], liposome encapsulation [19], and incorporation on gel coatings and films of different materials such as calcium alginate, gelatin, cellulose, soy protein, corn zein, collagen casings, polysaccharide based films, cellophane, silicon coatings, polyethylene, nylon or other polymer plastic films [20, 21, 22, 23, 24]. In most cases, immobilized bacteriocin preparations are applied on the surface of the processed food to avoid post-process contamination and surface proliferation of unwanted bacteria. A recent advance in this field is the use of immobilized bacteriocins in the development of antimicrobial packaging. A polyethylene film containing immobilized bacteriocin 32Y from *L. curvatus* reduced viable counts of *L. monocytogenes* during storage in the packaged pork steak and ground beef as well as in frankfurters [25, 26]. Similarly, a nisin containing cellophane coating reduced viable counts of total aerobic bacteria in fresh veal meat stored at 8 °C [23]. Therefore, the use of antimicrobial films containing bacteriocins can improve the quality and safety and prolong the shelf-life of food products.

In situ bacteriocin production offers several advantages compared to ex situ production regarding both legal aspects and costs. Lowering the costs of biopreservation processes may be highly attractive, especially for small economies and developing countries, where food safety may be seriously compromised [27].

The use of bacteriocinogenic cultures requires careful selection of strains that are well-adapted to the particular food environment in which they will be used and able to grow under the food processing and/or storage conditions and to produce enough bacteriocin amounts as to inhibit the target pathogenic or spoilage bacteria.

Bacteriocinogenic strains can be used either directly as starter cultures, as adjunct or co-cultures in combination with a starter culture, or as protective cultures (especially in the case of nonfermented foods). When used as a starter culture, the bacteriocinogenic strain must be able to carry out the desired fermentation process optimally besides being able to produce enough bacteriocin amounts to afford protection. In some cases, bacteriocin production may also serve to increase the implantation capacity, competitiveness and stability of the starter [28]. Adjunct cultures do not need to contribute to the fermentation, but they must not interfere with the primary function of the starter culture. For this reason, bacteriocin resistance of the starter culture may be a key factor. This may be achieved by selection of natural resistant mutants, by adaptation through repeated subcultivation with increasing bacteriocin concentrations, or by genetic modification. Nevertheless, sometimes this may not be necessary as the bacteriocin may just not be active on the starter culture (as may be the case of many of the bacteriocins that predominantly show antilisterial activity) or this may be much more tolerant to the bacteriocin than the target bacteria in the food system. Differences in inoculum density, a faster growth rate of the starter or a delayed bacteriocin production may also permit the starter to grow without interference from the bacteriocinogenic adjunct culture. As an example, inoculation of milk with an enterocin AS-48 producer enterococcal strain as adjunct culture in combination with a commercial starter culture for cheese manufacture had no effect on growth of the starter or the physicochemical properties of the produced cheese. At the same time, enough bacteriocin was produced in the cheese to ensure inhibition of *Bacillus cereus* [29].

Bacteriocinogenic protective cultures can be used to inhibit spoilage and pathogenic bacteria during the shelf life period of non-fermented foods. A protective culture may grow and produce bacteriocin during refrigeration storage of the food, and/or during temperature abuse conditions. In the first case, growth of the protective cultures must have a neutral impact on the physicochemical and organoleptic properties of the food, while under temperature abuse conditions the protective culture may even act as the

predominant spoiler, ensuring that pathogenic bacteria do not grow and that the spoiled food is not consumed [4].

V. Effectiveness of Bacteriocins in Food Systems

The application of bacteriocins, particularly nisin, in food systems has been extensively reviewed. It is now known that the production and activity of bacteriocins in foods can be influenced by many factors [15]:

- Factors negatively affecting production [14, 30] include: inadequate physical conditions and chemical composition of food (pH, temperature, nutrients, etc.); spontaneous loss in production capacity; inactivation by phage of the producing strain; and antagonism effect of other microorganisms in foods. Nisin, for example, is 228 times more soluble at pH 2 than at pH 8.
- The effectiveness of bacteriocin activity in food is negatively affected by: resistance development of pathogens to the bacteriocin; inadequate environmental conditions for the biological activity; higher retention of the bacteriocin molecules by food system components (e.g. fat); inactivation by other additives; slower diffusion and solubility and/or irregular distribution of bacteriocin molecules in the meat matrix [14, 16].

VI. Conclusions

The use of bacteriocins and/or bacteriocin-producing strains of LAB are of great interest as they are generally recognized as safe organisms and their antimicrobial products as biopreservatives. However, it is desirable to continue to expand our understanding of the influences that environmental factors have on the implantation and survival of bacteriocinogenic strains and the activity of their bacteriocins in order to quantitatively estimate their efficacy for future applications in food model systems and establish adequate means of application of these biopreservatives.

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