



Review Article

Volume 14 Issue 4 - March 2018
DOI: 10.19080/ARTOAJ.2018.14.555927

Agri Res & Tech: Open Access J

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Induction of Bacteriocins from Lactic Acid Bacteria; a Strategy to Improve the Safety of Fresh Fruits and Vegetables



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Submission: January 31, 2018; Published: March 13, 2018

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Abstract

Foodborne outbreaks related to the consumption of contaminated fresh fruits and vegetables occur recurrently, despite the implementation of good agricultural and good manufacturing practices, control measures and strict regulations to ensure food safety. Therefore alternative technologies to effectively prevent Foodborne illnesses are necessary. Many lactic acid bacteria exert antagonistic activity against important Foodborne pathogens by producing bacteriocins; toxic compounds of proteic nature that can selectively stop the growth of sensitive bacteria by causing membrane damage or by inhibiting the expression of fundamental genes. Some bacteriocins have already been used in the food industry as natural antimicrobials to prevent the spoilage of food products. This mini-review addresses some strategies for the control of pathogens in fresh fruits and vegetables through the use of bacteriocin-based technologies. The production and incorporation of bacteriocins during pre- and post-harvest handling of fruits and vegetables may be an ecological approach to ameliorate the quality and safety of fresh horticultural products.

Keywords: Foodborne diseases; Food safety; Bacterial antagonism; Bacteriocinogenic; Co-culture; Oligosaccharide

Lactic Acid Bacteria for the Potential Control of Foodborne Diseases

Foodborne diseases (FBD) are a worldwide common and expensive health problem, involving recurrent outbreaks that have even caused the death of consumers. The Food and Drug Administration (FDA), along with the Centers for Disease Control and Prevention are constantly investigating outbreaks related to the consumption of contaminated food in the USA, where enteropathogenic bacteria such as *Salmonella* spp. and *Escherichia coli* O157:H7 are the most frequently found causal agents. For instance, so far in January 2018, the FDA has reported two multistate salmonellosis outbreaks related to the consumption of fresh and frozen fruits and vegetables. Even when the FDA Food Safety Modernization Act, among other issues, establishes requirements for the preventive control of FBD, such as HACCP verification programs, it is estimated that every year there are almost half a million deaths associated with these diseases [1]. Due to this high incidence, novel alternatives are required to reduce the FBD cases and, consequently, the mortality associated with this type of diseases. In addition to the implementation of preventive measures, in case of food contamination during the cultivation, harvesting and processing chain, strong control methods need to be developed to avoid the growth and spread of microbial pathogens, without affecting

the quality and acceptance of the food products. A promising strategy is the use of innocuous bacteria with antagonistic activity against FBD causing pathogens. Bacterial antagonism is basically a term used to describe the process by which the growth and proliferation of certain bacteria are inhibited by competing species, often by the production of toxic metabolites that may even be lethal. A well-known group of non-pathogenic bacteria with antagonistic activity against pathogens are Lactic Acid Bacteria (LAB); a very heterogeneous group of Gram-positive bacteria, characterized by the production of lactic acid as the main fermentation product. LAB have been ancestrally used for the production of dairy products and, some strains are producers of potent antagonistic substances such as hydrogen peroxide, short-chain fatty acids, antimicrobial peptides, and bacteriocins. Therefore, the use of their antagonistic metabolites, at adequate proportions, is not likely to pose a health concern, but on the contrary, they could be key components for controlling pathogens and ensuring food safety [2].

Bacteriocins Produced by LAB and Their Mode of Action

Nowadays, nisin, a bacteriocin produced by *Lactococcus lactis*, is generally recognized as safe (GRAS) by the FDA [3]

and is currently being used as a preservative agent in the food industry to prevent the growth of *Listeria monocytogenes*. The commercialization of nisin since the 1950s, triggered the research interest to isolate new bacteriocins from different sources so that by the 1990s, there was a variety of bacteriocins with different activity spectra, some of which are still in the process of seeking approval for use as food additive. Bacteriocins are ribosomally synthesized peptides that when secreted act selectively on other bacteria, permeabilizing its membrane and potentially leading to cell death [4]. Bacteriocins are classified as antimicrobial compounds, similar to the roles played by defensins (produced by mammals) and thionines (produced by plants) [5]. Among the large number of bacteriocins studied so far, LAB are the most frequent producers, including some strains that are able to synthesize up to three bacteriocins with different characteristics [6]. While some bacteriocins act against a highly specific target, some others are known to harm both Gram-positive and Gram-negative bacteria [7-10]. The reason of such particularities, along with the regulatory mechanism comprising their production and processing have not been fully elucidated yet, but may vary according to the features of the target bacterial surface and the bacteriocin structural characteristics [8,11]. Some bacteriocins are synthesized as precursors that require a post-translational processing such as glycosylation or hydrolysis in specific signaling sequences [12].

The most recent classification for bacteriocins produced by LAB was proposed by Alvarez-Sieiro et al. [13] as follows:

Class I

Proteins with a molecular weight less than 10kDa with post-translational modifications. This class includes those proteins that are prone to undergo some modifications through their biosynthesis, due to the presence of a signal peptide sequence that will allow the recognition, transport, and maintaining of the inactive peptide. These bacteriocins are also characterized for its thermostability.

Class II

Proteins with a molecular weight less than 10 kDa without post-translational modifications. This class consists of proteins that do not have unusual modifications and do not require any effector for transport. Similar to class I, these bacteriocins are also thermostable.

Class III

Proteins with a molecular weight greater than 10kDa without post-translational modifications. These may exert a lytic and non-lytic mechanism of action. Unlike bacteriocins of class I and II, those of class III are thermolabile.

The integrative analysis of common molecular elements found in diverse antagonistic bacterial species has allowed the formulation of a general model integrated by genes and proteins involved in the biosynthesis, modification, secretion, and

immunity of bacteriocins [8,14]. These elements are regularly present in the same operon coding for the bacteriocin. The production of these antimicrobial molecules in LAB is often under the control of a three-component signal transduction system comprising an inductive factor, a transmembrane protein histidine kinase, and a response regulator. Briefly, this system detects the inducer stimulus and transmits the signal into the cell through phosphorylation-dephosphorylation relays, culminating in the transcription of target genes required for the processing and secretion of bacteriocins (reviewed by [15]).

The mode of action of bacteriocins is one of the mechanisms about bacterial antagonism that has received more attention, mainly due to their similarity to the function of antibiotics. Concisely, bacteriocins can cause the formation of pores in the membrane of sensitive bacteria, which alters cell permeability and decompensates the electrochemical homeostasis. Once inside the target cells, these molecules can bind nucleic acids to prevent gene expression and to interrupt cell biosynthesis [5,6].

Strategies for the induction of bacteriocins

The production of bacteriocins allows the optimization of nutrient resources by inhibiting the growth of competing species. The mechanisms triggering the synthesis of bacteriocins are still being explored and vary according to the type of producer strain. Some bacterial species follow a constitutive production mode, while a quorum sensing regulation has also been proposed [16]. The constitutive production of bacteriocins often starts toward the end of the log phase and beginning of the stationary phase of growth; however in some species it may start earlier, closer to the middle part of the exponential phase, as in the production of pediocin AcH by *Pediococcus acidilactici* H [17]. The quorum sensing-controlled production of bacteriocins is related to the bacterial population and to cell-cell communication mediated mainly by small signaling peptides [18].

The pH and culture medium composition, particularly the type and concentration of carbon source, has a marked impact in the production of bacteriocins [19,20], which may be due to the ability to tune the allocation of nutrients for either growth or for bacteriocins synthesis based on environmental cues [16]. The production of bacteriocins by the LAB *Lactobacillus curvatus* (Arla-10), *Enterococcus faecium* (JFR-1), *Lactobacillus paracasei* ssp. *paracasei* (JFR-5) and *Streptococcus thermophilus* (TSB-8) was significantly higher in MRS broth than in BHI broth [19]. These broths differ in the complexity of carbon and nitrogen sources.

Different types of oligosaccharides with varying chemical composition and degree of polymerization, such as inulin derived fructooligosaccharides (FOS) [20], galactooligosaccharides produced from lactose [21], and xylooligosaccharides obtained from wood xylans [22] induce the growth and antagonistic activity of LAB by promoting metabolic adjustments that enhance the production of antimicrobial compounds. Particularly, FOS supplementation enhanced the spectrum and

antimicrobial activity of bacteriocins produced by *Lactobacillus* strains (*L. plantarum*, *L. casei*, and *L. brevis*) isolated from corn ensilage and molasses against FBD-related pathogens [20]. Likewise, supplementation of MRS with the di- and tri-saccharides lactulose and raffinose, respectively, increased the bacteriocinogenic activity of *L. paracasei* CMGB16 against *E. coli* [23]. Interestingly, supplementation with the disaccharide trehalose was reported to promote the growth of *L. lactis* ssp. *lactis* C101910 and *Lactococcus* sp. GM005 and to augment the production of bacteriocins even to a greater extent than FOS [24]. In recent years, some pectic derived oligosaccharides (POS) have begun to be proposed as potential prebiotic substances, due to their capacity to stimulate the growth of probiotic LAB [25,26]. Recent findings in our research group indicate that supplementation of LAB cultures with oligogalacturonides (the most abundant type of POS), with a degree of polymerization from 3 to 20, enhances their antagonistic activity against FBD-related pathogens. Therefore, oligogalacturonides may become promising inductors for the production of bacteriocin-like inhibitory substances.

The induction of bacteriocin synthesis by co-culture of the producer LAB strain with competitor species has lately demonstrated to be an effective strategy to maintain their ability to produce these antimicrobial compounds [18]. Additionally, co-culture methods have proved to enhance the antimicrobial activity of LAB and to increase the production of bacteriocins in comparison with monocultures, in a strain-specific manner [27]. The ability to induce the synthesis of bacteriocins in a different bacterial species may be developed by either related or unrelated microorganisms, however, the relationship between bacteriocin-inducing and bacteriocin-producing strains is not well understood yet [18]. Also, inductor strains may or not be susceptible to the bacteriocins synthesized by producer LAB strains. On the contrary, some reports indicated that co-culturing bacteriocin-producing LAB strains with known inductor strains either did not increase the production of bacteriocins [28] or suppressed their production [29]; overall indicating that the co-culture-inducible synthesis of bacteriocins is likely to be strain specific. Among the LAB bacteriocins successfully induced through co-culture strategies so far are lactacin B, kimchicin G7, paracin 1.7, and plantaricins A, NC8 and MG (Reviewed by [18]), gassericin E [30], and pediocin [31].

Role of bacteriocins produced by LAB in the development of innovative safety strategies for fresh vegetable products

Several bacteriocins have already been used as natural antimicrobials to prevent spoilage of diverse food products and simultaneously reduce the concentration of synthetic additives. Although there are many bacteriocins produced by antagonistic LAB strains isolated from vegetable food products (Reviewed by [32]), the most studied food matrices with incorporated bacteriocins have been meat- and dairy-based products. The activity of bacteriocins on fruits and vegetables seems to vary

according to the type of food product. The bacteriocin enterocin 416K1 (produced by *Enterococcus casseliflavus* IM 416K1) was able to completely kill *L. monocytogenes* from contaminated processed apple and grapes within 8 h posttreatment; however, no considerable inhibition of this pathogen was observed for processed pineapple and melon fruits [33]. Likewise, enterocin AS-48, produced by *Enterococcus faecalis* A-48-32, inhibited *L. monocytogenes* growth in whole raspberries and sliced strawberries and blackberries stored at low temperatures [34].

Bacteriocins share important features that allow them to be used in the food industry; they are resistant to surfactants, active in a wide pH range and they are often thermostable [13]. Also, a prerequisite prior to the application of newly discovered bacteriocins in food products intended for human consumption is their sensitivity to digestive proteases. Purified bacteriocins may be added directly to the food matrix as a food additive or applied as coatings using a carrier matrix; alternatively, if bacteriocins are produced naturally by food-grade bacteria, the producer strain may be considered GRAS and can be inoculated for the production of fermented food [35].

Considering fresh fruits and vegetables are natural reservoirs of LAB, the screening of epiphytic bacteriocinogenic strains isolated from their surfaces would provide novel bacteriocin-producing strains adapted to the same environmental conditions used for the growth and storage of fresh horticultural products. The incorporation of bacteriocins-based technologies to avoid the proliferation of pathogens in these type of fresh foods may be achieved through either one of the following strategies or their combination:

- A. The application of prebiotic oligosaccharides on the surface of fruits and vegetables, for inducing the production of bacteriocins by epiphytic LAB;
- B. The inoculation of these products with GRAS bacteriocin-producing LAB;
- C. The application of active antimicrobial films or coatings containing commercially available bacteriocins.

In summary, the use of bacteriocins on fresh horticultural products may be an ecological alternative to combat pathogens and ameliorate the incidence of FBD. The production of bacteriocins in sufficient amounts is a challenge that may be addressed through the use of inductor substances or by co-culture strategies with inductor strains. Future research should highlight the importance of screening for novel bacteriocin-producing LAB strains isolated from unusual sources and under variable growing conditions, which may also lead to the discovery of new bacteriocins with a different spectrum or to the enhancement of their antibacterial activity.

Conclusion

The production of bacteriocins by lactic acid bacteria and their subsequent incorporation during pre- and post-harvest

handling of fruits and vegetables could improve the quality and safety of fresh horticultural products in an ecological manner. The induction of bacteriocin synthesis by adopting the strategies addressed hereby would play a crucial role in the effectiveness of this approach.

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DOI: [10.19080/ARTOAJ.2018.14.555927](https://doi.org/10.19080/ARTOAJ.2018.14.555927)

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