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Bacteriocins from Lactic Acid Bacteria: Production, Purification, and Food Applications

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Key Words
Bacteriocins · Bacteriocins, food application · Bacteriocins, production · Bacteriocins, purification · Lactic acid bacteria · Lactic acid bacteria, antimicrobial potential

Abstract
In fermented foods, lactic acid bacteria (LAB) display numerous antimicrobial activities. This is mainly due to the production of organic acids, but also of other compounds, such as bacteriocins and antifungal peptides. Several bacteriocins with industrial potential have been purified and characterized. The kinetics of bacteriocin production by LAB in relation to process factors have been studied in detail through mathematical modeling and positive predictive microbiology. Application of bacteriocin-producing starter cultures in sourdough (to increase competitiveness), in fermented sausage (anti-listerial effect), and in cheese (anti-listerial and anti-clostridial effects), have been studied during in vitro laboratory fermentations as well as on pilot-scale level. The highly promising results of these studies underline the important role that functional, bacteriocinogenic LAB strains may play in the food industry as starter cultures, co-cultures, or bioprotective cultures, to improve food quality and safety. In addition, antimicrobial production by probiotic LAB might play a role during in vivo interactions occurring in the human gastrointestinal tract, hence contributing to gut health.

Introduction

Lactic acid bacteria (LAB) have a long history of application in fermented foods because of their beneficial influence on nutritional, organoleptic, and shelf-life characteristics [1, 2]. They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. In addition, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes is of importance. Whereas a food fermentation process with LAB is traditionally based on spontaneous fermentation or backslopping, industrial food fermentation is nowadays performed by the deliberate addition of LAB as starter cultures to the food matrix. This has been a breakthrough in the processing of fermented foods, resulting in a high degree of control over the fermentation process and standardization of the end products. Recently, the use of functional starter cultures, a novel generation of starter cultures that offers functionalities beyond acidification, is being explored [2–4]. For instance, LAB are capable of inhibiting various microorganisms in a food environment and display crucial antimicrobial properties with respect to food preservation and safety. In addition, it has been shown that some strains of LAB possess interesting health-promoting properties; one of the characteristics of these probiotics is the potential to combat gastrointestinal pathogenic bacteria such as *Helicobacter pylori*, *Escherichia*

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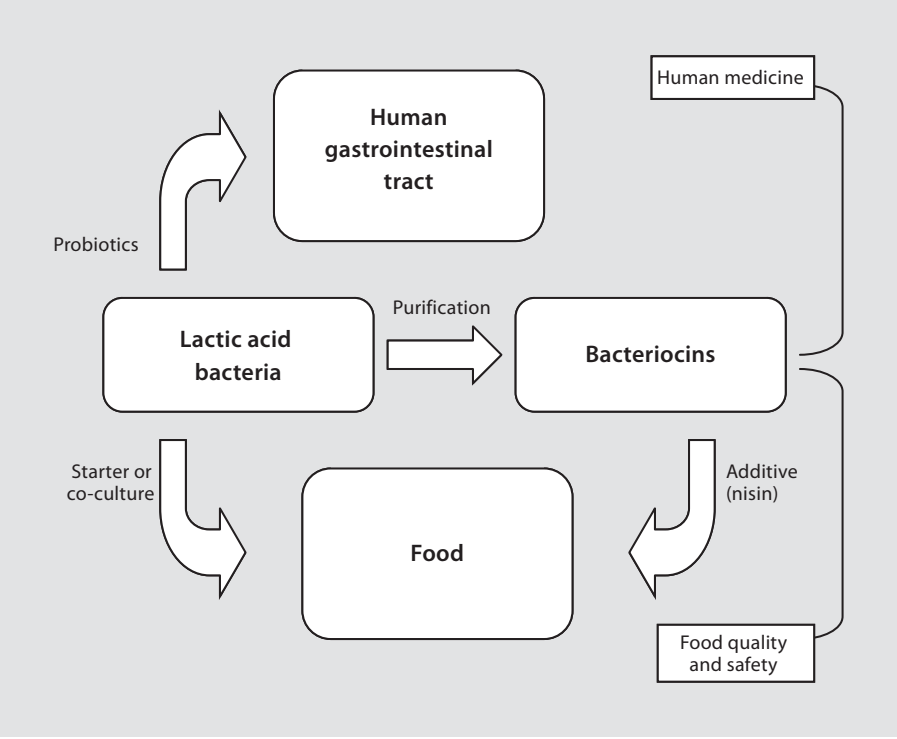

Fig. 1. Overview of the application potential of bacteriocin production by LAB in food quality and safety and in medicine, emphasizing their role as food ingredient and in the human gastrointestinal tract, respectively.

coli, and *Salmonella*. This paper focuses on the role of bacteriocins as fast-acting, antibacterial peptides in both food safety and gastrointestinal health (fig. 1).

Antimicrobial Potential of Lactic Acid Bacteria

LAB display a wide range of antimicrobial activities. Amongst these activities, the production of lactic acid and acetic acid is obviously the most important. However, certain strains of LAB are further known to produce bioactive molecules such as ethanol, formic acid, fatty acids, hydrogen peroxide, diacetyl, reuterin, and reutericyclin. Many strains also produce bacteriocins and bacteriocin-like molecules that display antibacterial activity [5]. Besides the production of bacteriocins, some LAB are able to synthesize other antimicrobial peptides that may also contribute to food preservation and safety. For instance, strains of *Lactobacillus plantarum*, isolated from sourdough and grass silage, display antifungal activity, due to the production of organic acids, other low-molecular-mass metabolites, and/or cyclic dipeptides [6–8]. It is not unlikely that additional, new antimicrobial peptides are to be discovered [9]. Although still in its infancy, there is good reason to believe that genomics will soon become an essential tool for exploring the antimicrobial potency of LAB [10]. Interestingly, bacteriocin screening programs have yielded, during the last decades, a large arsenal of bacteriocins with different properties, target species, and producer organisms [11].

Bacteriocins, a Class of Antibacterial Peptides for Promising Applications

Although bacteriocins may be found in many Gram-positive and Gram-negative bacteria [12], those produced by LAB have received particular attention in recent years due to their potential application in the food industry as natural preservatives [13]. Bacteriocins produced by LAB are small, ribosomally synthesized, antimicrobial peptides or proteins that possess activity towards closely related Gram-positive bacteria, whereas producer cells are immune to their own bacteriocin(s) [5, 11, 14]. The antibacterial spectrum frequently includes spoilage organisms and food-borne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus*. Besides their antimicrobial action towards undesirable bacteria, bacteriocins are believed to contribute to the competitiveness of the producer cells [15]. Activity against Gram-negative bac-

teria such as *E. coli* and *Salmonella* has been shown, but usually only when the integrity of the outer membrane has been compromised, for example after osmotic shock or low pH treatment, in the presence of a detergent or chelating agent, or after pulsed electric field or high-pressure treatment [16].

Among bacteriocins from LAB, distinction can be made between (i) lantibiotics or small, heat-stable, lanthionine-containing, single- and two-peptide bacteriocins (class I), whose inactive prepeptides are subject to extensive post-translational modification; (ii) peptide bacteriocins or small, heat-stable, non-lanthionine-containing bacteriocins (class II), including pediocin-like or *Listeria*-active bacteriocins (class IIa), two-peptide bacteriocins (class IIb), and circular bacteriocins (class IIc), and, arguably, (iii) bacteriolysins or large, heat-labile, lytic proteins, often murein hydrolases (class III) [14]. Extensive efforts have been made to resolve the relationship between structure and function for both class I and class II bacteriocins [17, 18]. The majority of the class I and class II bacteriocins are active in the nanomolar range, causing membrane permeabilization, leading to the dissipation of membrane potential and the leakage of ions, ATP, and other vital molecules from the target bacteria [14].

Production and Purification of Bacteriocins

Although bacteriocins can be produced in the food matrix during food fermentation, bacteriocins by LAB can be produced in much higher amounts during in vitro fermentations under optimal physical and chemical conditions [19]. The higher in vitro production is due to the absence of limiting factors, such as strong diffusion limitations, inactivation by proteases, and the adsorption to food particles [20]. However, even during controlled fermentor experiments, considerable differences in activity yields are obtained, and an influence of the environmental process conditions on the obtained bacteriocin activity can be seen. For instance, a decrease of pH results in a decreased adsorption of the bacteriocin molecules to the producer cells, and hence in an increased bioavailability [21, 22]. In addition, temperature and pH [23–25] as well as nutrient availability [26–28] seem to play a crucial role in bacteriocin production, whereas the presence of elevated amounts of sodium chloride usually decreases production levels [29, 30]. In general, the cultivation conditions directly affect bacteriocin production as such (specific bacteriocin production in particular) and, indirectly, through biomass production. This is to be explained by the fact that bacteriocin production is a growth-dependent physiological trait and hence follows primary metabolite kinetics [22–24].

Three major methods for the purification of bacteriocins by LAB to homogeneity can be distinguished. First, purification can be done by a conventional method that is based on a rather laborious series of subsequent steps of ammonium sulfate precipitation, ion exchange, hydrophobic interaction, gel filtration, and reversed-phase high-pressure liquid chromatography [31–33]. Second, a simple three-step protocol has been developed [34], including (i) ammonium sulfate precipitation, (ii) chloroform/methanol extraction/precipitation, and (iii) reversed-phase high-pressure liquid chromatography, the sole chromatographic step involved. Third, bacteriocins can be isolated through a unique unit operation, i.e. expanded bed adsorption, using a hydrophobic interaction gel, after maximizing the bioavailable bacteriocin titer through pH adjustment of the crude fermentation medium [35, 36]. Following the latter two methods, which are more rapid than the first conventional method and yet successful, several bacteriocins with interesting industrial potential have been purified, such as the class II bacteriocins amylovorin L (produced by *Lactobacillus amylovorus* DCE 471) and several enterocins (produced by the *Enterococcus faecium* RZS C5, RZS C13, and FAIR-E 406 strains), and the lantibiotic macedocin (produced by *Streptococcus macedonicus* ACA-DC 198) [34, 37–39].

Bacteriocin Production in Foods: Application Possibilities

Bacteriocins can be used as food additives. For instance, nisin is commercially made in a partially purified form [5, 18] and a marketed preparation with the pediocin PA-1 (AcH) producer is available [40]. As an alternative to the addition of bacteriocins to foods, bacteriocins may be produced directly in the food as a result of starter culture or co-culture activity [2]. Several studies have indeed indicated that LAB starter cultures or co-cultures are able to produce their bacteriocins in food matrices, and consequently display inhibitory activity towards sensitive food spoilage or pathogenic bacteria. The latter trait has mainly been documented for fermented sausage, fermented vegetables and olives, and dairy products [2, 13]. For instance, bacteriocin extraction has been demonstrated in the case of Cheddar cheese [41] and fermented

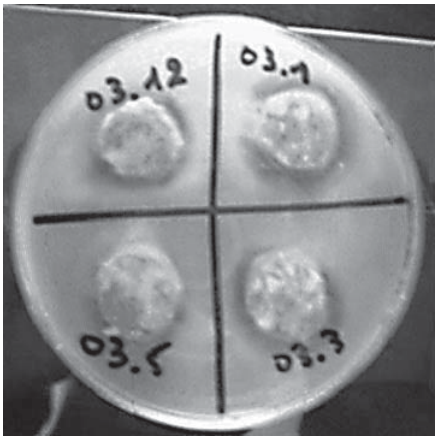

Fig. 2. In situ bacteriocin production by *Lactobacillus curvatus* LTH 1174, as demonstrated by the inhibition zones caused by pieces of Belgian-style fermented sausage on a bacteriocin-sensitive indicator layer containing *Listeria innocua* LMG 13568, in a direct diffusion assay. The pieces of meat were obtained after 1 (03.1), 3 (03.3), 5 (03.5), and 12 (03.12) days of fermentation. The fermented sausages were prepared with a commercial starter culture, supplemented with *L. curvatus* LTH 1174, at an inoculation level of 7.0 log colony-forming units per gram. Control sausages prepared with the same commercial starter culture, but without *L. curvatus* LTH 1174, did not yield inhibition zones.

Lactacin Fa	RNNWQTNVGGAVGSAMIGATVGGTICGPACAVAGAHYLPILWT**A**VTAATGGFGKIRK
Lactacin F	RNNWQTNVGGAVGSAMIGATVGGTICGPACAVAGAHYLPILWT**G**VTAATGGFGKIRK

Fig. 3. Comparison of the amino acid sequence of lactacin F (produced by *Lactobacillus johnsonii* VPI 11088) and lactacin Fa (produced by *L. johnsonii* La1).

sausage and sourdough [Foulquié Moreno MR, Leroy F, De Vuyst L, unpubl. results] (fig. 2). Because of the complexity of the food matrix and the difficulty of quantifying bacteriocin activities in foods, in vitro studies can be performed to simulate and study the in situ functionality of bacteriocinogenic starters [19, 42]. In this way, the kinetics of bacteriocin production by LAB strains in foods have been described in detail, amongst others through mathematical modeling and positive predictive microbiology [43]. Insights in the relationship between the food environment and kinetics of the starter culture have yielded valuable information about the in situ production of bacteriocins and its interactions with the target strains, which will be important if bacteriocins or bacteriocin-producing strains are to be increasingly used in food systems [19, 44, 45]. In particular, such information is essential when dealing with the potential problem of bacteriocin-resistant target bacteria [44, 45]. Application of bacteriocin-producing starter cultures in sourdough (to increase competitiveness and hence establish a desired microbial population), in fermented sausage (anti-listerial effect to meet the zero-tolerance policy in ready-to-eat foods), and in cheese (anti-listerial and anti-clostridial effects), have been studied during in vitro laboratory fermentations as well as on pilot-scale level [19, 41, 44–49]. Results of these studies were highly promising and underline the important role that functional, bacteriocinogenic strains of LAB may play in the food industry as starter cultures, co-cultures, or bioprotective cultures, to improve food quality and safety [2, 3, 13].

Bacteriocin Production by Probiotics

Probiotics are live microorganisms that, when consumed in an adequate amount as part of the food, confer a health benefit on the host [50]. An experimental focus on bacteriocin production by probiotic LAB strains has indicated that this potential might play a considerable role during in vivo interactions occurring in the human gastrointestinal tract, for instance towards *H. pylori* [4, 51–52]. Whereas bacteriocins in food are degraded by the proteolytic enzymes of the stomach, probiotic bacteria may lead to in situ production of bacteriocins in the gastrointestinal tract. Up to now, bacteriocins have been isolated from the commercial probiotic strains *Lactobacillus casei* Shirota and *Lactobacillus johnsonii* La1 [53]. The latter bacteriocin, referred to as lactacin Fa, is a two-peptide bacteriocin, which differs in one amino acid from the well-known bacteriocin lactacin F produced by *L. johnsonii* VPI 11088 (fig. 3). However, only activity towards Gram-positive indicator bacteria has been shown under

the conditions tested [51, 53]. In contrast, the inhibitory role of organic acids produced by probiotics towards Gram-negative pathogenic bacteria has been shown [9, 54].

Conclusions

Bacteriocins produced by LAB have the potential to cover a very broad field of application, including both the food industry and the medical sector. Concerning their use in food, bacteriocin-producing starter or co-cultures have been successfully applied in pilot-scale experiments (cheese, fermented sausage, sourdough, etc.), yielding food quality and food safety advantages. The current bottleneck hampering widespread industrial practice seems to be market implementation rather than scientific evidence or proof-of-concept. With respect to medical applications, antimicrobials produced by probiotic LAB might play a role during in vivo interactions occurring in the human gastrointestinal tract, hence contributing to gut health. Further research is needed to unravel the precise role of LAB bacteriocins in this process.

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