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review

Antimicrobial Activity – The Most Important Property of Probiotic and Starter Lactic Acid Bacteria

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Summary

The antimicrobial activity of industrially important lactic acid bacteria as starter cultures and probiotic bacteria is the main subject of this review. This activity has been attributed to the production of metabolites such as organic acids (lactic and acetic acid), hydrogen peroxide, ethanol, diacetyl, acetaldehyde, acetoine, carbon dioxide, reuterin, reutericyclin and bacteriocins. The potential of using bacteriocins of lactic acid bacteria, primarily used as biopreservatives, represents a perspective, alternative antimicrobial strategy for continuously increasing problem with antibiotic resistance. Another strategy in resolving this problem is an application of probiotics for different gastrointestinal and urogenital infection therapies.

Key words: antimicrobial activity, bacteriocins, lactic acid bacteria, probiotics, starter cultures

Introduction

Lactic acid bacteria (LAB) are Gram-positive, non-spore forming, catalase-negative bacteria that are devoid of cytochromes and are of nonaerobic habit but are aero-tolerant, fastidious, acid tolerant and strictly fermentative; lactic acid is the major end-product of sugar fermentation (*1*). They are the most widely used bacteria as starter cultures for the industrial processing of fermented dairy, meat, vegetable and cereal products. Despite the starter culture addition, non-starter lactic acid bacteria (NSLAB), originating from the raw material and environment, grow out during fermentation and may reach higher numbers than the starters. Reduction of pH and conversion of sugars to organic acids are the primary preserving actions that these bacteria provide to fermented food. However, many kinds of food are still fermented naturally, without the use of starter cultures, by autochthonous lactic acid bacteria, which form the characteristic properties of the products. These natural isolates of lactic acid bacteria from spontaneous fermentations could be used as specific starter cultures or as adjunct strains, after phenotypic and genotypic characterisation, and they represent a possible source of potentially new antimicrobial metabolites (*2–4*). In addition, the application of lactic acid bacteria and their antimicrobial metabolites in the prevention of food spoilage and the extension of the shelf life of food that is ready to eat, fresh-tasting, nutrient and vitamin rich, minimally processed and biopreserved are the major challenges for the current food industry (*5*). The use of bacteriocin-producing lactic acid bacteria as protective strains or bacteriocins in form of purified or concentrated compounds as biopreservatives to control undesirable bacteria remains a primary focus of researches related to food safety and quality (*6*).

In the concept of functional food, especially in dairy industry, there is an increasing interest for probiotic products that contain lactic acid bacteria of intestinal

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origin. Probiotic lactic acid bacterial strains must be chosen according to accurate selection criteria in order to survive the transition through gastrointestinal tract and preferably colonize the intestinal tract for a sufficiently long period to achieve the desired healthy effect (*7*). One of the most important properties of probiotics is protection against pathogens in the intestinal tract of the host. The role of antimicrobial compounds produced by probiotic strains as prophylactic agents against enteric infections is crucial and well documented (*8–10*).

The antimicrobial activity of starter cultures and probiotic bacteria has been attributed to the production of metabolites such as organic acids (lactic and acetic acid), hydrogen peroxide, ethanol, diacetyl, acetaldehyde, other low molecular mass compounds with antimicrobial activity and bacteriocins (*11,12*). Industrial potential of antimicrobials from lactic acid bacteria is illustrated in Fig. 1.

Antimicrobials from Lactic Acid Bacteria

Antimicrobial substances produced by lactic acid bacteria can be divided into two main groups: low molecular mass substances with molecular mass <1000 Da and high molecular mass substances with molecular mass >1000 Da, such as bacteriocins. All non-bacteriocin antimicrobial substances from LAB are of low molecular mass (*13*).

Low molecular mass antimicrobials

The metabolites of LAB with antimicrobial activity are accumulated in their environment at the levels and proportions that depend on the species of LAB and chemical composition of the growth media. Fermentation of hexoses by lactic acid bacteria is characterized by homofermentative production of lactic acid or by heterofermentative production of equimolar amounts of lactate, acetate/ethanol and carbon dioxide. Pentoses are fermented by many heterofermentative and homofermentative LAB in the same way since phosphoketolase of homofermentative LAB is generally inducible by pentoses. Fermentation of pentoses yields the equimolar amounts of lactic and acetic acid.

Most of heterofermentative species have flavoprotein oxidases, which catalyse the reduction of oxygen, resulting in the accumulation of hydrogen peroxide. During heterofermentations, products such as formic acid, acetoin, acetaldehyde and diacetyl, which possess antimicrobial activity, can be accumulated. Malic, lactic and citric acid can be further metabolised to other antimicrobial products such as acetic acid, formic acid and CO_2 (*14*). The main low molecular mass metabolites of LAB and their antimicrobial spectra are shown in Table 1 (*11,14–19*).

Organic acids

The most important and best characterised antimicrobials produced by LAB are lactic and acetic acid. The amount and type of acids produced during fermentation influence the subsequent microbial activity in the fermented material. Acetic acid, for example, is more antagonistic against yeasts compared to lactic acid. Some oxidative yeasts are able to utilize organic acids as a carbon and energy source and consequently cause spoilage through deacidification in fermented, especially plant material where they are naturally present (*20*). The inhibitory effect of organic acids is mainly caused by undissociated form of the molecule, which diffuses across

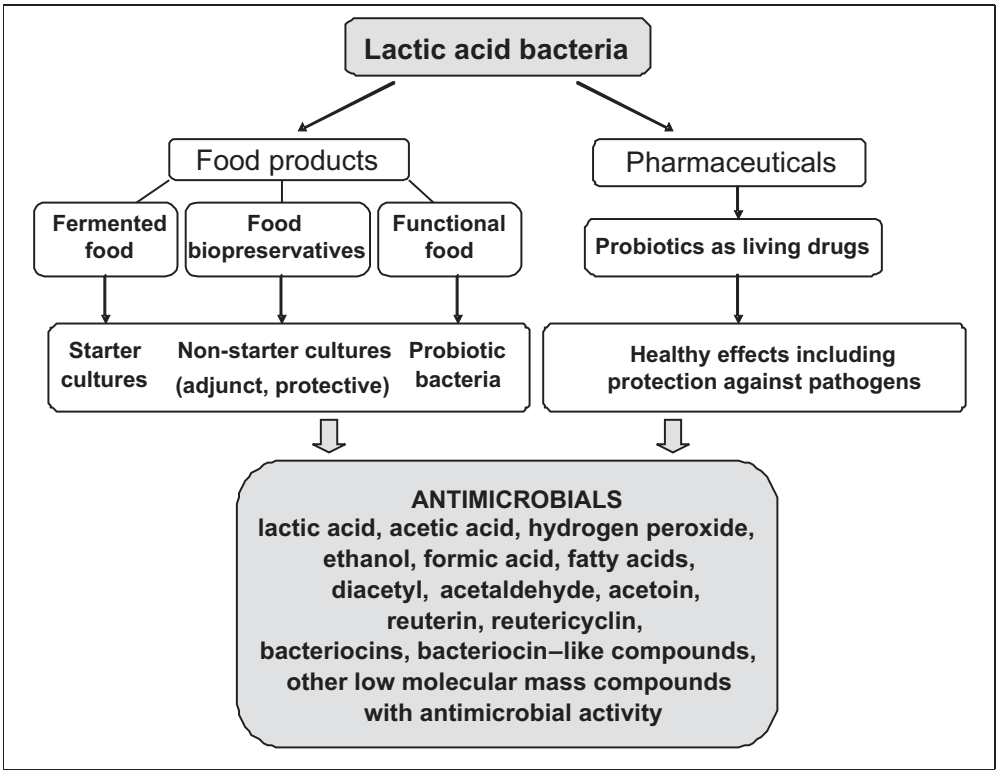

Fig. 1. Industrial potential of antimicrobials from lactic acid bacteria

Table 1. Low molecular mass antimicrobial metabolites of lactic acid bacteria (*11,14–19*)

Compound	Microorganisms producers	Antimicrobial spectrum
lactic acid	all lactic acid bacteria	yeasts Gram-positive bacteria Gram-negative bacteria
acetic acid	heterofermentative lactic acid bacteria	yeasts Gram-positive bacteria Gram-negative bacteria
diacetyl acetaldehyde acetoin	variety of genera of lactic acid bacteria including: *Lactococcus*, *Leuconostoc*, *Lactobacillus* and *Pediococcus*	yeasts Gram-positive bacteria Gram-negative bacteria
hydrogen peroxide	all lactic acid bacteria	yeasts Gram-positive bacteria Gram-negative bacteria
carbon dioxide	heterofermentative lactic acid bacteria	most of the taxonomic groups of microorganisms
reuterin	*Lactobacillus reuteri*	fungi, protozoa, Gram-positive and Gram-negative bacteria
reutericyclin	*Lactobacillus reuteri*	Gram-positive bacteria
cyclic dipeptides	*Lactobacillus plantarum* *Lactobacillus pentosus*	fungi
3-phenyllactic acid 4-hydroxyphenyllactic acid	*Lactobacillus plantarum*, *Lactobacillus alimentarius*, *Lactobacillus rhamnosus*, *Lactobacillus sanfranciscensis*, *Lactobacillus hilgardii*, *Leuconostoc citreum*, *Lactobacillus brevis*, *Lactobacillus acidophilus*, *Leuconostoc mesenteroides*	fungi
3-hydroxy fatty acids	*Lactobacillus plantarum*	fungi
benzoic acid methylhydantoin mevalonolactone	*Lactobacillus plantarum*	fungi Gram-negative bacteria

the cell membrane towards the more alkaline cytosol and interferes with essential metabolic functions. The toxic effects of lactic and acetic acid include the reduction of intracellular pH and dissipation of the membrane potential (*15,19*).

Hydrogen peroxide

Antimicrobial activity of hydrogen peroxide is attributed to its strong oxidizing effect on the bacterial cell and to the destruction of basic molecular structures of cell proteins (*14*). In raw milk, hydrogen peroxide produced by lactic acid bacteria can, after being catalysed by lactoperoxidase, oxidise endogenous thiocyanate. The oxidized intermediary products are toxic to different bacteria (*16*). Hydrogen peroxide production has been considered as the main metabolite of LAB that could protect against urogenital infections, especially in the case of bacterial vaginosis (*21*).

Diacetyl, acetaldehyde and acetoin

Heterofermentative LAB produce active acetaldehyde by decarboxylation of pyruvate. This product then condenses with pyruvate, forming α-acetolactate and it is converted by α-acetolactate synthases to diacetyl. The product of decarboxylation of α-acetolactate and reduction of diacetyl is acetoin (*13,22*). Diacetyl (2,3-butanedione) is best known for the buttery aroma that it imparts to fermented dairy products, but this property as well as high concentration needed to provide preservation of food limit the use of diacetyl as food preservative. Similarly, an acetaldehyde, usually present in fermented dairy products in concentrations smaller than necessary for inhibition of undesired microorganisms, also plays a role in controlling the growth of contaminants, together with other antimicrobial metabolites of lactic acid bacteria (*11*).

Carbon dioxide

The influence of carbon dioxide on product preservation is twofold. Namely, except for its own antimicrobial activity, it creates an anaerobic environment by replacing the existent molecular oxygen. The antifungal activity of CO_2 is due to the inhibition of enzymatic decarboxylations and to its accumulation in the membrane lipid bilayer resulting in dysfunction in permeability (*14*).

Reuterin and reutericyclin

Selected isolates of *Lactobacillus reuteri* produce two compounds, reuterin and reutericyclin, both active towards Gram-positive bacteria. Reutericyclin is a tetramic acid derivative and reuterin is a mixture of monomeric, hydrated monomeric and cyclic dimeric forms of β-hydroxypropionaldehyde with a broader spectrum of inhibitory activity, including Gram-negative bacteria, fungi and protozoa (*23–25*).

Other low molecular mass antimicrobials

Other low molecular mass compounds with antimicrobial activity against Gram-positive and Gram-nega-

tive bacteria, moulds and yeasts have been described, including antifungal cyclic dipeptides, phenyllactic acid, 4-hydroxyphenyllactic acid and 3-hydroxy fatty acids (*26–28*). Niku-Paavola *et al.* (*29*) discovered new types of antimicrobial compounds produced by *Lactobacillus plantarum* (benzoic acid, methylhydantoin and mevalonolactone) active against fungi and some Gram-negative bacteria.

Bacteriocins of lactic acid bacteria

Some of LAB produce bacteriocins, antibacterial proteinaceous substances with bactericidal activity against related species (narrow spectrum) or across genera (broad spectrum of activity) (*30,31*). Bacteriocin biosynthesis is a desirable characteristic for strain selection as it serves as an important mechanism of pathogen exclusion in fermented foods as well as in the gastrointestinal environment.

Bacteriocins are ribosomally synthesized peptides or proteins with antimicrobial activity produced by many Gram-positive and Gram-negative bacteria; however, those produced by food grade LAB have received considerable attention due to their potential application in food industry as natural preservatives (biopreservatives). LAB bacteriocins are small antimicrobial peptides or proteins that possess activity towards closely related Gram-positive bacteria, whereas producer cells are immune to their own bacteriocins (*32–34*). There are several proposed bacteriocin classifications divided into 3 or 4 classes: (*i*) lantibiotics or small, heat-stable, lanthionine-containing, single- and two-peptide bacteriocins (class I), whose biologically inactive prepeptides are subjected to extensive post-translational modification; (*ii*) small, heat-stable, non-lanthionine-containing bacteriocins (class II), including pediocins like or *Listeria*-active bacteriocins (class IIa), two-peptide bacteriocins (class IIb) and circular bacteriocins (class IIc); and (*iii*) bacteriolysins or large, heat-labile, lytic proteins, often murein hydrolases (class III) (*31,32, 35*). Some authors (*36,37*) also proposed (*iv*) class IV bacteriocins that require non-proteinaceous moieties (lipid, carbohydrate) for their activity (Table 2, *31,35–37*).

Lantibiotics are small (<5 kDa) peptides containing unusual amino acids lanthionine, dehydroalanine, α-dehydroalanine and dehydrobutirine. According to their chemical structures and mode of action, they are subdivided into type A and type B lantibiotics (*34,38,39*). Type A lantibiotics are elongated amphiphilic lantibiotics, like nisin, with a net positive charge, which are active through the formation of pores in bacterial membranes, leading to the dissipation of membrane potential. Type B lantibiotics are smaller globular peptides, like mersacidin, which have negative or no net charge, and act through the inhibition of specific enzymes (*31,34,40*).

Class II encompasses the more common non-lanthionine-containing bacteriocins, which are non-modified, small (<10 kDa), heat stable peptides. Representatives belonging to this heterogeneous group of bacteriocins are divided into 3 subgroups. Class IIa includes pediocin-like peptides having an N-terminal consensus sequence –Tyr-Gly-Asn-Gly-Val-Xaa-Cys. Pediocin-like peptides have attracted much attention due to their specific activity against food pathogen *Listeria monocytogenes* (*41*). Class IIb contains bacteriocins requiring two different peptides for their activity, and class IIc contains the remaining peptides of the class, including *sec*-dependent bacteriocins (*34*). Class III bacteriocins (bacteriolysins) are large (>30 kDa), heat-labile antimicrobial proteins not as well characterised, whose mechanism of action is distinct in function as they lyse the sensitive cells by catalysing cell-wall hydrolysis (*31*). Only four LAB bacteriolysins have been genetically characterised so far (*42–44*), although the non-LAB bacteriolysins have been identified.

Table 2. Classification, major characteristics and some examples of bacteriocins (*31,35–37*)

Classification	Major characteristics	Examples
Class I		
lantibiotics/lanthionine-containing bacteriocins subdivided into:	small (<5 kDa) membrane-active peptides containing unusual amino acids	type A: nisin, lactocin S, lacticin 481
type A lantibiotics	– elongated peptides with a net positive charge	type B: mersacidin
type B lantibiotics	– smaller globular peptide with negative or no net charge	
Class II		
non-lathionine-containing bacteriocins subdivided into:	heterogeneous class of small (<10 kDa) heat-stable post-translation unmodified non-lantibiotics	IIa: pediocin PA1, sakacin A, sakacin P, leucocin A, curvacin A
subclass IIa	IIa: pediocin-like	IIb: lactococcin G, lactococcin M, lactacin F, plantaricin A
subclass IIb	IIb: two-peptide	
subclass IIc	IIc: with wide range of effects on membrane permeability and cell wall formation	IIc: acidocin B, enterocin P, enterocin B, reuterin 6
Class III		
bacteriolysins	large (>30 kDa) heat-labile antimicrobial proteins	lysostaphin, enterolysin A, helveticin J, helveticin V-1829
	complex proteins with domain-type structure that function through the lyses of sensitive cells by catalysing cell wall hydrolysis	
Class IV		
	complex bacteriocins carrying lipid or carbohydrate moieties	plantaricin S, leuconocin S, lactocin 27, pediocin SJ1

Class IV of complex bacteriocins that require non-proteinaceous moieties like carbohydrate or lipid for their activity has also been suggested by some authors (*36*); however, bacteriocins in this class have not been characterised convincingly, hence definition of this class requires additional characterisation (*31,34*).

Mode of bacteriocin action

Bacteriocins that are produced by LAB can be of broad or narrow spectrum, but in general, the activity is directed against low G+C Gram-positive species (*31*). The antibacterial spectrum includes spoilage organisms and foodborne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus*. Wide ranges of mode of action have been described for bacteriocins, such as enzyme activity modulation, inhibition of outgrowth of spores and formation of pores in cell membrane. Most bacteriocins interact with anionic lipids that are abundantly present in the membranes, and consequently initiate the formation of pores in the membranes of susceptible cells (*34,38*). However, generalised membrane disruption models cannot adequately describe the mode of action of bacteriocins. Rather, specific targets seem to be involved in pore formation and other activities. For the nisin and epidermin family of lantibiotics, the membrane-bound cell wall precursor lipid II has been identified as target (*45*). Most of class II bacteriocins dissipate the proton motive force (PMF) of the target cell *via* pore formation (*46*). The subclass IIa bacteriocin activity depends on a mannose permease of the phosphotransferase system (PTS) as a specific target. The subclass IIb bacteriocins (two-component) also induce dissipation of the PMF by forming cation- or anion-specific pores; specific targets have not yet been identified. Finally, subclass IIc comprises miscellaneous peptides with various modes of action such as membrane permeabilisation, specific inhibition of septum formation and pheromone activity (*31*).

Resistance and immunity to bacteriocins

Bacteriocin producer has developed protection mechanisms against its own bacteriocin. Two distinct systems of bacteriocin immunity in the producing cell have been identified. Protection can be mediated by dedicated immunity protein and/or a specialised ABC-transporter system involving two or three subunits that probably pump the bacteriocin through the producer membrane. These two immunity systems can work synergistically to protect the producing cells from their own bacteriocin (*47*). In the case of lantibiotic immunity, *e.g.* protein LanI, which is most likely localised at the cytoplasmic membrane, probably confers immunity to the producer cell by preventing pore formation by the bacteriocin. Related ABC-transporter system LanEFG probably acts by excreting bacteriocins that were inserted into the membrane back to the extracellular microenvironment and thus keeping bacteriocin concentration in the membrane under a critical level (*35,38*). Regulation of bacteriocin production and immunity is most frequently mediated through two-component signal-transduction systems, often as part of the quorum-sensing mechanism (*48*).

Bacteriocin-Producing Starter and Non-Starter Lactic Acid Bacteria in Food Industry

Besides the well-known biopreservative effects of antimicrobial metabolites of lactic acid bacteria such as lactic acid, acetic acid, hydrogen peroxide and diacetyl, bacteriocins have the most immediate potential in food application as biopreservatives and they can be readily introduced into food without any concentration or purification (*31*). Since lactic acid bacteria are generally regarded as safe (GRAS) according to the FDA, they could be used in food production and food biopreservation.

Bacteriocin-producing starter cultures

The main antimicrobial effect of starter LAB, responsible for biopreservation, is the rate of acidification, but in slightly acidified products or to eliminate undesirable microorganisms that display acid tolerance, such as *Listeria monocytogenes*, the bacteriocinogenic activity could play a crucial role. The use of bacteriocin-producing starter cultures may not only contribute to food safety, but also prevent the growth of undesirable autochthonous lactic acid bacteria that produce off-flavour. This property may improve the competitiveness of the starter cultures and lead to a more controlled and standardized fermentation process as it has been shown in sourdough, fermented sausage, fermented vegetables and olives, and cheese production (*25,49–51*).

Bacteriocin-producing adjunct cultures

Bacteriocin producers can be delivered to a food product as an adjunct culture, together with the starter culture. In this case, the ability of starter adjunct to grow and produce bacteriocin in the product is crucial for its successful use. The bacteriocin-producing adjunct cultures are mostly isolated from raw milk, vegetables, cereals and other natural sources of lactic acid bacteria that are believed to contain strains essential not only for the characteristic flavour of traditional fermented products, but also with promising and useful properties such as bacteriocinogenic activity, which will make them applicable as starters. For example, *Lactococcus lactis* strain, which produces both nisin and lacticin 481, isolated from raw ewe's milk, might be used as adjunct culture to the commercial starter in the manufacture of dairy products to inhibit or destroy undesired microorganisms (*52*). Adjunct culture does not need to contribute to the flavour but it is important that the starter culture is resistant to bacteriocin produced by the adjunct culture. One of the exceptions is the controlled lysis of starter culture during cheese manufacture caused by bacteriocin-producing strain, with the aim to release intracellular enzymes, needed for accelerated ripening and improvement of product flavour (*31,53*).

Bacteriocin-producing protective cultures

Bacteriocinogenic protective cultures alone can be used to inhibit spoilage and pathogenic bacteria during the shelf life of non-fermented foods by producing bacteriocin *in situ* or previously cultured in growth medium and after that applied as an ingredient in food processing. Two preparations are already present on the

market: ALTA™ 2341, containing pediocin PA1 produced by *Pediococcus acidilactici*, and Microgard™, a commercially available fermented milk product containing antimicrobial metabolites. In the literature different milk-based preparations such as lacticin 3147 are described (*54*). The addition of purified or semi-purified bacteriocins as food preservatives requires approval from legislative point of view. There is also a problem of costly production because of low production rates, instability and expensive downstream processing of bacteriocins. If immobilized or microencapsulated bacteriocin or bacteriocinogenic strain is applied on the food surface, much lower concentration is needed compared to the application in the whole food volume (*5,55*). Other advantages of immobilized bacteriocins are the possibility of gradient-dependent, continuous supply of bacteriocin and the protection against food components and enzymatic inactivation. The use of antimicrobial films containing immobilized bacteriocins for the development of antimicrobial packaging is a recently developed technique (*56,57*).

Use of bacteriocins in combination with other antimicrobial factors

The antimicrobial spectra and activity of bacteriocins can be extended through the synergy between different antimicrobial factors such as inorganic salts (especially sodium chloride), organic acids and their salts, chelating agents (such as EDTA), essential oils and their active components, phenolic compounds, as well as other natural antimicrobials. Application of bacteriocins together with different physicochemical treatments, like heat treatment, modified atmosphere packaging, high hydrostatic pressure, pulsed electric field, pulsed magnetic field and gamma irradiation, has received great attention in recent years (*5,34,58,59*). The effectiveness of bacteriocins in combination with hurdle technology will depend on the type of food and its natural microflora. Thus with acidification of the food acidotolerant bacteria may be selected, while heat treatment may favour bacterial endospores, but in combination with bacteriocins higher sensitization may be achieved after optimization of doses and conditions. Furthermore, the Gram-negative bacteria could become sensitive to bacteriocin activity upon exposure to hurdles such as chelating agents that destabilize the bacterial outer membrane (*5,60–62*).

Application of nisin, the most famous bacteriocin, in food industry

So far, nisin is the only bacteriocin licensed as food preservative (E234). Commercial production of nisin by *Lactococcus lactis* ssp. *lactis* began in England in 1953, and international acceptance of nisin was given in 1969 by the Joint Food and Agriculture Organisation/World Health Organization (FAO/WHO) (*63*). In 1988, it was approved by the US Food and Drug Administration (US FDA) for use in pasteurized, processed cheese spreads and since then, as a food additive in over 50 countries (*31*). Nowadays, the most established available form of nisin for use as a food preservative is Nisaplin™. Applications of nisin have been developed for processed cheese, dairy desserts, milk, fermented beverages, bacon, frankfurters and fish, often in combination with hurdle technologies to achieve better inhibitory effect (*11,54,64–66*). Its use extends shelf life of the food by inhibition of Gram-positive spoilage bacteria such as *Listeria*, *Staphylococcus* and *Mycobacterium*, and spore-forming bacteria *Bacillus* and *Clostridium* (*67–71*). The spores of these bacteria are more sensitive to nisin than their vegetative cells, so nisin is often applied in heat-processed food such as canned vegetables. The spectrum of its activity can be successfully broadened when it is applied in combination with chelating agent such as EDTA (*72,73*). Very few variants of six naturally occurring nisin molecules are described with enhanced activity against Gram-positive pathogens (*74*).

The Role of Antimicrobial Activity of Probiotic Lactic Acid Bacteria in Prevention and Treatment of Infections

The widespread use of antibiotics in treatment of infections resulted in increased number of antibiotic resistant bacteria, fewer treatment options and most antibiotics ineffective (*75*). Alternative antimicrobial strategies in the treatment and prevention of gastrointestinal infections are the application of probiotics and their antimicrobial metabolites such as bacteriocins. Probiotic is a mono- or mixed culture of live microorganisms which, applied to animal or man, affect beneficially the host by improving the properties of the indigenous microflora, according to broader Fuller's definition (*76*), proposed by Havenaar and Huis in't Veld (*77*). Recently, probiotics have been defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (*63*). Probiotics are largely administered through functional foods and as dietary supplements (pharmaceuticals) or biotherapeutics (approved drugs with important therapeutic applications) (*78*). Lactic acid bacteria are the most important probiotic microorganisms because they are autochthonous in the human gastrointestinal tract of healthy people (*79,80*). A considerable number of health benefits have been postulated as a result of the probiotic intake, including modification of gut microflora, prevention of pathogen colonisation, stimulation of gut immunity, reduction in inflammatory reactions, prevention of colon cancer, alleviation of lactose intolerance, lowering of serum cholesterol and reduction of food allergies (*7,81*). Each property is strain-dependent, and must be confirmed by *in vitro* experiments, animal experiments and clinical trials. Mode of action of probiotics includes antagonistic effects against pathogenic microorganisms in intestinal tract (embracing multiple mechanisms for preventing infection), alteration of microbial metabolism in the intestinal tract, stimulation of immunity and increase of nutritional value of food.

Much of the benefit derived from probiotic LAB is a consequence of their ability to acidify the intestine by producing the lactic acid and thus create a hostile environment for pathogens. Besides lactic acid, probiotic bacteria can also produce antimicrobials such as hydrogen peroxide, bacteriocins, short-chain fatty acids such as acetic, propionic and butyric acid, rendering vital nutrients unavailable to pathogens and altering the redox potential of the intestinal environment. There is also

considerable evidence that deconjugation of conjugated bile salts in the intestine is the mechanism of the resistance of probiotic bacteria to high concentration of bile salts in small intestine, which are inhibitory for Gram-positive bacteria, but have little effect against Gram-negative bacteria. However, upon deconjugation, the free bile acids are more toxic for both, Gram-positive and Gram-negative microorganisms, which is also one of the antagonistic mechanisms against pathogens in the intestine (*82–85*).

Another line of probiotic defence against infection in the intestinal tract is the enhancement of intestinal barrier function by the promotion of mucin production and by colonisation resistance mechanism, which prevents colonisation of the intestine by pathogens (*10*). Colonization resistance is apparent in two major regions of the intestinal habitat: the luminal contents and the mucosal surfaces. In the luminal contents, the most important resistance mechanism is the production of antagonistic metabolites by probiotic or autochthonous beneficial bacteria that suppress multiplication of pathogens. Competition for nutrients present in limited quantities in the intestine is another mechanism that regulates populations of the established intestinal microflora. At the mucosal surfaces, the resistance mechanism of prime importance is the occupation of adhesive sites. Although the composition of the intestinal microflora is rather stable in healthy individuals and described mechanisms effectively impede colonization by pathogens, these harmful microorganisms become impaired when intestinal microflora is disturbed by endogenous and exogenous stress factors (*7,86*).

Adherence factors on the surface of probiotic cells, mostly proteins or polysaccharides, may promote pathogen exclusion, mucosal integrity and host immunomodulation. Comparative genome analyses confirmed the role of mucus-binding proteins (Mub) in intestinal mucus adherence of *Lactobacillus* strains isolated from the intestine. Namely, the MUB domains found exclusively in intestinal lactobacilli suggest that these proteins mediate specific interactions or functions between these microbes and their hosts (*87,88*). Cell surface structures such as teichoic acids, lipoteichoic acids and surface layer proteins (S-layers) have also been reported as important for probiotic adhesion and immunomodulation. S-layer proteins from different strains of *L. acidophilus*, *L. helveticus*, *L. brevis*, *L. kefir* and *L. crispatus* have been shown to be involved in mediating adhesion to different host surfaces (*89–92*). Additionally, some of them are found to prevent adhesion of the foodborne pathogens, such as *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, to cultured intestinal epithelial cell lines, to frozen sections of intestinal tissue, as well as to intestinal mucus and uroepithelial cells (*84,93–97*). Surface proteins have also been characterised as key factors involved in immunomodulation (*98–100*). Not only LAB themselves were reported to activate immune cells and to confer enhanced protection against enteropathogens (*61,101–104*). Non-bacterial fractions of fermented milk, containing bacterial metabolites produced during fermentation by LAB, were effective in induction of different cytokine patterns and enhanced protection against enteropathogens in mice (*105–107*).

The mechanisms behind the prevention of gastrointestinal and urinary tract infections by probiotic bacteria have been elucidated in animal, but also in human studies, confirming enhancement of immune responses and production of antimicrobial substances (*11,97,108–111*). However, there is increasing clinical evidence that probiotics are effective not only in the treatment and prevention of gastrointestinal diseases, but also in chronic liver disease, multiple organ dysfunction syndrome and autoimmune disease (*87,112–114*).

Probiotic Lactic Acid Bacteria and Bacteriocins in Human and Veterinary Medicine

The overuse and misuse of antibiotics in humans, veterinary and agriculture practices causes the spread of numbers of community- and hospital-acquired infections produced by bacterial strains resistant to single and multiple antimicrobial drugs (*115*). This situation and the already imposed prohibitions of the use of antibiotics as growth promoters for farm animals have drawn attention to possible alternatives. The application of probiotic cells and competitive exclusion preparations of lactic acid bacteria in human and veterinary prophylactic and curing therapy is one of the alternatives. The administration of bacteria that produce antimicrobial substances, especially bacteriocins, is more cost-effective approach than the application of pure antimicrobials. There are numerous probiotic products on the market with various health claims, mostly for treatment of antibiotic or travel diarrhoea and for the balance of intestinal or vaginal microflora of humans. All of these products are considered food supplements, not drugs. However, a new generation of probiotics, considered living drugs, will be tailor-made for different gastrointestinal and urogenital disease therapies or as delivery systems for vaccines, immunoglobulins and other protein-based therapies. Some of the finished and ongoing studies, based on genomic, proteomic and metabolomic research, are promising in evaluation of specific probiotics as drugs for prevention and treatment of infections and other diseases (*21,74,78,87,97,113*).

There are also a lot of nutritional additives with probiotic bacteria for farm animals already in use or have been proposed as means to reduce or eliminate pathogens or as a means to improve growth and feed conversion (*116*). In addition, agents such as bacteriocins have been studied or proposed as potential human and animal therapeutics because they are considered more natural than the currently used antibiotics and are produced by GRAS lactic acid bacteria. Although Gram-negative bacteria do not represent target cells for bacteriocins, additions of chelating agents such as EDTA and detergents such as Tween 80 can broaden their antimicrobial spectrum (*117*). The best studied lantibiotic, nisin, has a potential in treatment of peptic ulcer disease by inhibiting *Helicobacter pylori* (*118*). The possible therapeutic use of nisin was proposed by Brumfitt *et al*. (*119*) in combination with peptidoglycan-modulating antibiotics and confirmed its activity against MRSA (methicillin-resistant *Staphylococcus aureus*) and VRE (vancomycin-resistant enterococci). Giacometti *et al*. (*120*) proposed the use of nisin in combination with polymycin E or clari-

thromycin against infection caused by *Pseudomonas aeruginosa*. After the treatment of catheters and tracheotomy tubes with nisin, Gram-positive bacteria were inhibited (*121*), while Severina *et al.* (*122*) obtained the inhibition of multidrug resistant pathogens such as *Staphylococcus* and *Streptococcus* strains by nisin. Development of novel bacteriocin-based drugs, aimed at potential target cells, both prokaryotic and eukaryotic, offers the possibility to design improved antibiotics with refined characteristics (*123*). The company Biosynexus (Gaithersburg, MD, USA) has developed a topical antibiotic preparation with nisin as one of the active components for skin infection treatment. Furthermore, the company also has a product containing bacteriolysin lysostaphin, a large bacteriolytic protein active against antibiotic-sensitive as well as antibiotic-resistant *Staphylococcus aureus*. Both products are focused on the prevention and treatment of multidrug--resistant staphylococcal infections in humans. There are also two commercial preparations of nisin for veterinary medicine, effective in the prevention and treatment of mastitis, produced by ImmuCell Corporation (Portland, ME, USA), Wipe Out® and Mast Out® (*124*). The efficacy of nisin Z in the treatment of bovine subclinical mastitis, especially against drug-resistant *S. aureus*-caused intramammary infections, was evaluated by Wu *et al.* (*125*). Furthermore, two-peptide bacteriocin lacticin 3147, produced by *Lactococcus lactis*, showed potential in the prevention of infectious diseases such as bovine mastitis caused by staphylococci and streptococci (*126*,*127*). This bacteriocin is also used as an active agent in Pfizer Animal Health (Pfizer Inc, New York, NY, USA) product under the name Orbeseal Teat Sealant for the prevention of intramammary infections throughout the dry cow period (*128*). Furthermore, there is an example how pathogenic bacteriocin-producing strain *Streptococcus mutans*, a causative of dental caries, could be used for the therapy after genetic modification in order to lose its pathogenicity and ability to produce lactic acid, but still can colonize the oral cavity and produce mutacin, a bacteriocin active against pathogenic strains (*129*). Nowadays, protein engineering enables improved stability and solubility of nisin at physiological pH, important for its use in human therapy (*130*). The Nisin-Controled Expression (NICE) system in *Lactococcus lactis* is one of the best characterized expression systems that can be upregulated more than 1000-fold with the addition of nisin (*131*). Induction with NICE system could be used for the expression of heterologous proteins in *Lactococcus lactis*. Good example is the expression of E7 antigen from human papilloma virus type-16 (HPV-16) on the cell wall of *Lactococcus lactis* (*132*). The capacity of different strains of LAB to produce heterologous antigens, either intracellularly, extracellularly or cell wall-attached, has been clearly demonstrated and makes them potential candidates for the development of new safe mucosal vaccines. Among them, *Lactococcus lactis*, as the model lactic acid bacterium, is the most frequently used as live vaccine delivery vector (*131*). Current methodologies and techniques for genetic manipulations of LAB allow progress and development of novel vaccine production and types of vaccinations using LAB and their bacteriocin expression systems.

Concluding Remarks

The potential of using bacteriocins of lactic acid bacteria, primarily used as biopreservatives, represents a perspective, alternative antimicrobial strategy against the continuously increasing problem of antibiotic resistance. Another strategy in resolving this problem is an application of probiotics in prophylaxis and therapy of different gastrointestinal and urogenital infections. Characterization of lactic acid bacteria and their beneficial mechanisms allows progress in their use in the food industry and their potential in promoting human and animal health and nutrition.

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