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Lactic Acid Bacteria As Biological Preservative For Food

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Abstract

Fermentation of various food stuffs by lactic acid bacteria (LAB) is one of the oldest forms of biopreservation practiced by mankind. This review focused on Lactic acid bacteria isolated by screening method from traditional food products of African countries and their antimicrobial metabolites as natural preservatives to control the growth of spoilage and pathogenic bacteria in foods. Particularly in the use of various strains of lactic acid bacteria, one of their important attribute is their ability to produce antimicrobial compounds called bacteriocins. Some of these bacteriocins allow the inhibition of *Listeria monocytogenes*, a food-bome pathogen responsible for human listeriosis. However, food ingredients caused an inefficient action of bacteriocins produced by several lactic acid bacteria against *Listeria monocytogenes* achieving the phenomenon of *Listeria* growth rebound in bacteriocin-supplemented food models. This review discusses the potential of LAB and their principal antimicrobial peptides, bacteriocins in biological preservation of foods.

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Practical Applications

This work describes the preservative ability of lactic acid bacteria in food attributed to the production of antimicrobial metabolites including bacteriocins. This paper could be used in school research library for better knowledge of lactic acid bacteria.

1. Introduction

In spite of modern food processing and safety concepts currently used such as Hazard Analysis and Critical Control Points (HACCP) (**Liu et al., 2014**), Good Manufacturing Practice (GMP) (**Carpentie**r **et al., 2011**) and Good Agricultural Practice (GAP) (**Máthé, 2015**), the risk of serious illness to consumers is still significant (**Centers for Disease Control and Prevention (CDC) 2010; Lim et al., 2013**). In addition, increasing consumer demands for safe, healthy, fresh-tasting, minimally processed foods with a longer shelf life encourage food companies to apply novel hurdle strategies based on 'biopreservation'. This fact encourages the search for other alternatives, in particular those based on the use of biological agents known for their safety. Accordingly, the use of bacterial strains as the protective cultures seems to be a promising way to promote new categories of food such as the "bio-products", including natural compounds (organic acids, diacetyl, hydrogen peroxide…..) (**Han et al., 2014)** that may play preservative role. Lactic acid bacteria (LAB) are among the most well known and investigated producers of microbial antagonists. They are of particular interest in terms of the widespread occurrence of bacteriocins within the group and are also in wide use throughout the fermented dairy-, food-, and meat-processing industries. Their role in the preservation and flavor characteristics of foods has

been well documented (**Mills et al., 2011; Hassan et al., 2012; Kaban 2013; Kargozari et al., 2014**). However, it has been reported that bacteriocins produced by several lactic acid bacteria exert a transitory bactericidal effect against *L. monocytogenes*, often followed by re-growth of *Listeria* cells in bacteriocin-supplemented food models (**Guo et al., 2012; Chaalel et al., 2015; Rybalchenko et al., 2015**).This growth rebound might be due to factors that severely limit growth of bacteriocin-producing cells (e.g. restricted nutrient availability), to decreased bacteriocin action as a result of adsorption onto food particles, fats, and proteins, to the presence of a curing agent, to the emergence of bacteriocin-resistant cells, and/or to bacteriocin degradation by proteases of food and/or microbial origin (**Guo et al., 2012; Sofos et al., 2013 Eduardo et al., 2013; Schillinger, 2014**). To overcome this problem, introducing Plasmid-Mediated bacteriocin into a technological competent strain to produce more bacteriocins could be necessary. Elsewhere a recent trend exists in the construction by electroporation of the new strains from wild-type strains plasmids isolated from traditional products. Several *Lactobacillus* species have been successfully transformed for this purpose by electroporation (e.g. **Movahed & Li, 2011; Geng & Lu, 2013**; **Movahed & Li, 2013**).

Lactic Acid Bacteria and Their Uses in Food

Lactic acid bacteria are industrially important organisms recognized for their fermentative ability as well as their health and nutritional benefits (**Liu et al., 2011 ; Lahtinen et al., 2011; Li et al., 2012**). Species used for food fermentations belong to the genera *Lactococcus, Streptococcus, Pediococcus, Leuconostoc, Lactobacillus,* and the newly recognized *Carnobacterium* (**Bourdichon et al., 2012**). These organisms have been isolated from grains, green plants, dairy and meat products, fermenting vegetables, and the mucosal surfaces of animals (**Holzapfel &**

Wood, 2012; Smid et al., 2013). Once used to retard spoilage and preserve foods through natural fermentations, they have found commercial applications as starter cultures in the dairy, baking, meat, vegetable, and alcoholic beverages industries. They produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins or bactericidal proteins during lactic fermentations (**Liu et al., 2012; Biscola et al., 2013; Todorov et al., 2013; Han et al., 2014**). Not only are these components desirable for their effects on food taste, smell, color and texture, but they also inhibit undesirable microflora. LAB is generally employed because they significantly contribute to the flavor, texture and, in many cases, to the nutritional value of the food products (**Kaban 2013; Kargozari et al., 2014 ; Han et al., 2014**). LAB are used as natural or selected starters in food fermentations and exert the antimicrobial effect as a result of different metabolic processes (lactose metabolism, proteolytic enzymes, citrate uptake, bacteriophage resistance, bacteriocin production, polysaccharide biosynthesis, metal-ion resistance and antibiotic resistance) (**Deng et al., 2011; Gaggia et al., 2011; Dušková et al., 2012 Pothakos et al., 2014**). Lactic acid bacteria (LAB) play a key role in food fermentations where they not only contribute to the development of the desired sensory properties in the final product but also to their microbiological safety. LAB has a GRAS status (generally recognized as safe) and it has been estimated that 25% of the European diet and 60% of the diet in many developing countries consists of fermented foods (**Bourdichon et al., 2012**).

As mentioned above, LAB produces several natural antimicrobials, including bacteriocins. Bacteriocins produced by LAB are the subject of intense research because of their antibacterial activity against foodborne bacteria. Bacteriocin producing strain of LAB may be very important in competing with other organisms in the intestine. They consists of a biologically active protein moiety have a bactericidal mode of action and attach to specific cell receptors. Bacteriocins are

heterogeneous group of bacterial antagonists that vary considerably in molecular weight, biochemical properties, range of sensitive hosts and mode of action. **Reis et al. (2012)** define them as, protein or protein complexes with bactericidal activity directed against species that are usually closely related to the producer bacterium. Both Gram negative and Gram positive bacteria produce them. The Gram-negative bacteriocins are colicin, which are produced by strain of E.coli (**Budič et al., 2011**). These are large, complex proteins, 29-90 kDa, with characteristic structural domains involved in cell attachment, translocation and bactericidal activity. They bind to specific receptors on the outer membrane of the target cell. The bacteriocins produced by Gram-positive bacteria are small peptides 3-6 kDa, in size (**Messaoudi et al., 2012**), although there are exceptions (**Perez et al., 2014**). They fall with in two broad classes, viz the lantibiotics and the non- lantibiotic bacteriocins (**Perez et al., 2014**). Most of the Gram positive bacteriocins are membrane active compounds that increase the permeability of the cytoplasmic membrane (**Paiva et al., 2011; Cotter et al., 2013**). They often show a much broarder spectrum of bactericidal activity than the colicins. There is currently much interest in the application of bacteriocins in both food preservation and the inhibition of pathogenic bacteria (**Liu et al., 2012; Biscola et al., 2013; Todorov et al., 2013**). Most of the bacteriocins have been isolated from organisms involved in food fermentation. Bacteriocin production and resistance is considered as an important property in strains used as commercial inoculants to eliminate or reduce growth of undesirable or pathogenic organisms. At present four classes of LAB bacteriocins have been identified. Table 1 shows the classification and general characteristics of these bacteriocins.

Table 1. Classification and general characteristics of bacteriocins

Bacteriocin classes	Bacteriocin subclasses	Molecular mass	Characteristics of class/subclass	Bacteriocin
Class I	**A**	< 5 KDa	Lantibiotics	Nisin (**Capita et al., 2013**)
	B			Marsacydin alametycin (**Capita et al., 2013**)
Class II	**IIa**	< 10 KDa	Pediocin-like bacteriocins	Sakacin A, Sakacin P (**Hassan et al., 2012**)
	IIb		Two-peptides bacteriocins	Lactacin F (**Nissen-Meyer et al., 2010**)
	IIc		Sec-dependent bacteriocins	Carno-bacteriocin A (**Stoyanova et al., 2012**)
Class III		> 30 KDa	Heat-labile protein bacteriocins	Lactococcin B (**Cotter et al., 2013**)
Class IV		Large protein	Mixture of protein(s), lipid(s) and carbohydrate(s) in bacteriocin molecule	Leucoccin S, Mesenterocin 52 (**van Staden et al., 2011**)

Nisin, a class I bacteriocin which has demonstrated antilisterial activity, was the first bacteriocin to be characterized and is the only one approved worldwide for use in food applications. The inhibition of Listeria by nisin has been demonstrated in culture media as well as in different foods. As an illustration, cottage cheese, ricotta-type cheeses (**Mills et al., 2011**), fresh pork sausages (**Reis et al., 2012**), cold-salmon (**Montiel et al., 2014**), Turkish fermented sausages (sucuks) (**Kurt & Zorba, 2010**), raw and cooked pork meat (**Reis et al., 2012**), can be mentioned. Nisin is produced by *Lactobacillus lactis* subsp lactis strains, and is active against many gram positive and against some gram negative bacteria (**Yang et al., 2012**). Class II bacteriocins are relatively small cationic peptides (30- 100 amino acids) exhibiting a high degree of heat stability. The largest group of the class II bacteriocins which includes pediocin-like peptides, has attracted much of the attention due to their anti-Listeria activity. Pediocin PA-1 is the most extensively studied class IIa (or pediocin family) bacteriocin, due to its broad antibacterial activity, stability in foods, and potential for use as a food bio-preservative (**Hassan**

et al., 2012). The stability of pediocin PA-1 in foods such as cheese, frankfurters, Spanish dry fermented sausages and chicken sausage, has been demonstrated (**Nieto-Lozano et al., 2010**). Class III bacteriocins are Heat-labile protein and produced by members of the Lactobacillus genera. Helveticin J (**Hassan et al., 2012**) and Lactococcin B **(Cotter et al., 2013)** are the best known compound of this class. In addition to these three classes, there is a fourth group (Class IV) containing small cyclic cationic peptides, mixture of protein(s), lipid(s) and carbohydrate(s) molecule (**Rea et al., 2011; van Staden et al., 2011; Wenzel et al., 2014**).

The in situ production of bacteriocins may increase the competitiveness of the producer strain in the food matrix and contribute to the prevention of food spoilage (**Ravyts et al., 2012, Vignolo et al., 2012 and O'Shea et al., 2012; Cizeikiene et al., 2013**). For instance, bacteriocin-producing LAB can be used as an alternative to potassium nitrate to prevent late loss of cheese due to contamination by clostridia (**Carminati et al., 2015**). Several studies have indicated that LAB starter strains are able to produce their bacteriocins in food matrices and consequently display inhibitory activity towards sensitive food spoilage or pathogenic bacterial strains. The latter has been documented for fermented sausage (**Castro et al., 2011**; **Hwanhlem et al., 2011**), fermented vegetables and olives (**Settanni et al., 2010; Dal-Bello et al., 2012**), and dairy products (**Voulgari et al., 2010; Dal-Bello et al., 2012**). However, it has been reported that bacteriocins produced by several lactic acid bacteria exert a transitory bactericidal effect against *L. monocytogenes*, often followed by re-growth of *Listeria* cells in bacteriocin-supplemented food models (**Kouakou et al., 2010**). This growth rebound might be due to factors that severely limit growth of bacteriocin-producing cells (e.g. restricted nutrient availability), to decreased bacteriocin action as a result of adsorption onto food particles, fats, and proteins, to the presence

of a curing agent, to the emergence of bacteriocin-resistant cells, and/or to bacteriocin degradation by proteases of food and/or microbial origin (**Kouakou et al., 2010; Abo-Amer, 2011**). The desire to have more competitive strain to overcome this problem will be necessary. This will generate considerable interest in genetic transfer systems (**Kouakou et al., 2010**). Electroporation seems to be an efficient technique for transferring plasmid DNA isolated from certain bacteriocin producing strain into other technological competent lactic acid bacteria (LAB) strains . Electroporation refers to the process of subjecting living cells to a rapidly changing, high-strength electric field, thereby producing transient pores in their outer membranes facilitating diffusion and exchange of intracellular and extracellular components during the lifespan of the pore (**Ersus et al., 2010; Puértolas et al. 2012; Mahnič-Kalamiza et al., 2014**). However, the successful introduction of heterologous plasmid DNA into LAB is dependent upon the complexity of the strain (**Domínguez et al., 2010; Du et al., 2012**). So the advantage of this technique is that it is possible to vary the intensity of the electrical discharge to obtain adequate electric field permeabilising and facilitating diffusion and exchange of intracellular and extracellular components during the lifespan of the pore. For some bacteria, including some species of LAB, the failure to recover recombinants may be attributable to the possession of an active restriction-modification system by the host bacterium (**Du et al., 2012**).The restriction-modification status of the host strain might, therefore, have a major influence on the non-recovery of recombinants when Escherichia coli/Gram-positive shuttle plasmids isolated from strains of E. coli are used for electrotransformation

Selection and construction of suitable strains

Because of legislation reasons, the use of genetically modified organisms is forbidden (**Du, 2012**). Nevertheless, molecular biology offers immense perspectives for strain improvement. In

particular introducing a free Plasmid-Mediated bacteriocin into a technological competent strain to produce more bacteriocins (**Kouakou et al., 2010; Singh et al., 2011**). It should be noted that there are different methods of bacterial transformation. The discovery of natural gene transfer systems in bacteria has greatly facilitated the understanding of the genetics of microbial starter cultures and in some cases has been used for strain improvement. Genetic exchange in bacteria can occur naturally by three different mechanisms: transduction, conjugation, and transformation.

Transduction

Transduction involves genetic exchange mediated by a bacterial virus (bacteriophage). The bacteriophage acquires a portion of the chromosome or plasmid from the host strains and transfers it to a recipient during subsequent viral infection. Although transduction has been exploited for the development of a highly efficient gene transfer system in the gram-negative organism Escherichia coli, it has not been used extensively for improving microorganisms used in food fermentations. In general, transduction efficiencies are low and gene transfer is not always possible between unrelated strains, limiting the usefulness of the technique for strain improvement. In addition, bacteriophage have not been isolated and are not well characterized for most strains.

Conjugation

Conjugation, or bacterial mating, is a natural gene transfer system that requires close physical contact between donors and recipients and is responsible for the dissemination of plasmids in nature. Numerous genera of bacteria harbor plasmid DNA. In most cases, these plasmids are cryptic (the functions encoded are not known), but in some cases important metabolic traits are encoded by plasmid DNA. If these plasmids are also self-transmissible or mobilizable, they can be transferred to recipient strains. Once introduced into a new strain, the properties encoded by

the plasmid can be expressed in the recipient. The lactic acid bacteria naturally contain from one to more than ten distinct plasmids, and metabolically important traits, including lactose-fermenting ability, bacteriophage resistance, and bacteriocin production, have been linked to plasmid DNA. Conjugation has been used to transfer these plasmids into recipient strains for the construction of genetically improved commercial dairy starter cultures.

There are some limitations in the application of conjugation for strain improvement. To exploit the use of conjugative improvement requires an understanding of plasmid biology and, in many cases, few conjugative plasmids encoding genes of interest have been identified or sufficiently characterized. Conjugation efficiencies vary widely and not all strains are able to serve as recipients for conjugation. Moreover, there is no opportunity to expand the gene pool beyond those plasmids already present in the species.

Transformation

Certain microorganisms are able to take up naked DNA present in the surrounding medium. This process is called transformation and this gene transfer process is limited to strains that are naturally competent. Competence-dependent transformation is limited to a few, primarily pathogenic, genera, and has not been used extensively for genetic improvement of microbial starter cultures. For many species of bacteria, the thick peptidoglycan layer present in gram-positive cell walls is considered a potential barrier to DNA uptake. Methods have been developed for enzymatic removal of the cell wall to create protoplasts. In the presence of polyethylene glycol, DNA uptake by protoplasts is facilitated. If maintained under osmotically stabilized conditions, transformed protoplasts regenerate cell walls and express the transformed DNA. Protoplast transformation procedures have been developed for some of the lactic acid bacteria; however, the procedures are tedious and time-consuming, and frequently parameters must be

optimized for each strain. Transformation efficiencies are often low and highly variable, limiting the application of the technique for strain improvement.

Electroporation

The above mentioned gene transfer systems have become less popular since the advent of electroporation, a technique involving the application of high-voltage electric pulses of short duration to induce the formation of transient pores in cell walls and membranes. Under appropriate conditions, DNA present in the surrounding medium may enter through the pores. Electroporation is the method of choice for strains that are recalcitrant to other gene transfer techniques; although optimization of several parameters (e.g., cell preparation conditions, voltage and duration of the pulse, regeneration conditions, etc.) is still required.

Genetic engineering provides an alternative method for improving microbial starter cultures. This rapidly expanding area of technology provides methods for the isolation and transfer of single genes in a precise, controllable, and expedient manner. Genes that code for specific desirable traits can be derived from virtually any living organism (plant, animal, microbe, or virus). Genetic engineering is revolutionizing the science of strain improvement and is destined to have a major impact on the food fermentation industry.

Although much of the microbial genetic engineering research since the advent of recombinant DNA technology in the early 1970s has focused on the gram-negative bacterium *Escherichia coli*, significant progress has been made with the lactic acid bacteria and yeast. Appropriate hosts have been identified, multifunctional cloning vectors have been constructed, and reliable, high-efficiency gene transfer procedures have been developed. Further, the structural and functional properties, as well as the expression in host strains, of several important genes have been

reported. Engineered bacteria, yeast, and molds could also be used for the production of other products, including food additives and ingredients, processing aids such as enzymes, and pharmaceuticals.

Also, bioinformatics and comparative genomics approaches can provide strategies that lead to an improved functionality of food-grade microorganisms (**Bron et al., 2011**).

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