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THE USE OF LACTIC ACID BACTERIA AND THEIR METABOLITES TO IMPROVE THE SHELF LIFE OF PERISHABLE FRUITS AND VEGETABLES

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Abstract

As a result of biochemical transformation and microbiological contamination that can occur during storing and commercialization, freshly picked fruits and vegetables tend to be very perishable products, thus having a shortened shelf life. Lactic acid bacteria (LAB) are microorganisms that play a crucial role in a wide variety of fermentation processes. Using microorganisms Generally recognized as safe (GRAS), LAB, including their metabolites, is proven to be a promising alternative of food preservation by respecting food safety measures in a natural way. Such metabolites produced and released by probiotic lactic bacteria strains are known as postbiotics. Vegetables and fruits can benefit from the antagonistic activity of postbiotics due to their content of bioactive products, such as short-chain fatty acids, antimicrobial peptides, organic acids, carbohydrates, vitamins and enzymes. This review is focused on the advances of the sustainable biopreservation products to extend the shelf life of perishable fruits and leafy vegetables, by the use of synergic postbiotics from LAB strains.

Key words: *contamination, fruits, vegetables, lactic acid bacteria, postbiotics, shelf-life.*

INTRODUCTION

Consumers concern regarding safe and healthy food has driven the food industry to research new technologies and alternatives to enhance the quality of and reliability on food by using few chemical substances (Alegre et al., 2013). Using GRAS (Generally Recognized As Safe) microorganisms, like lactic acid bacteria (LAB) and/or their naturally occurring metabolites, is proven to be a promising alternative for maintaining food safety and is also perceived by costumers as a natural preservation method. Fruits and vegetables are a good source of vitamins and minerals, without which the human body could not maintain an adequate health state and could not fight diseases. Fruits, as a food source, are extremely delectable, have a low-calorie intake and a low quantity of fats, are facile to prepare, and also from the point of view of their impact on human health, they play a crucial role by preventing a plethora of diseases by consumption.

Fruits and vegetables contain pectin, cellulose, oils, healthy fats and proteins. As a result of biochemical transformation and microbiological contamination that can occur during storage and commercialization, freshly picked fruits and vegetables tend to be very perishable products thus having a shortened shelf life. Using preservation means to almost eliminate food losses and to enhance the quality and safety of food has been always a staple in food industry; however, contemporary consumers usually develop antipathy against food additives, seeing them as unhealthy, although they do not understand the action mechanism and how they affect health (Bearth & Hartmann, 2017). All these alterations can lead to a significant degradation of organoleptic characteristics, such as color, aroma and taste, which leads to economic losses (De Corato, 2020).

Because of these reasons, recent researches are focused on developing products with reduced contents of additives or using natural ingredients to assure the quality and safety and also meet the consumer requirements (Guimarães et al., 2020). In this regard, natural antimicrobial agents (AA) gained important attention from researchers, allowing manufacturers to replace synthetic additives whilst following food safety measures and help develop healthier food.

According to the scientific literature, as shown in Table 1, fruits and vegetables are classified into four categories based on their perishability.

Table 1. Fresh fruits and vegetables categorized by perishability (source: Ahmad et al., 2017)

Group	Vegetables	Fruits
Extremely perishable	Endives, early potatoes, spring onion and garlic, mushrooms, orache spinach, lettuce, parsley, garden	Blueberries, strawberries, gooseberries, blackcurrant, blackberries, raspberries, mulberry, dates, wild strawberry
Very perishable	Okra, cucumbers, cauliflower, leeks zucchini, green beans, green peas, cabbage, asparagus,	Apricots, cherries, quince, peaches, plum, grapes,sour cherries
Perishable	Potatoes, aubergines, bell peppers, artichoke, carrots, tomatoes	Apples, pears, cantaloupe, watermelon, bananas
Less perishable	Potatoes, onions, garlic, kohlrabi, horseradish, leek, root vegetables, red cabbage, white cabbage, beetroot	Apples, pears, chestnuts, oranges, lemons

LACTIC ACID BACTERIA

LAB are Gram-positive, nonrespiring and non-motile microorganisms with a variable optimum pH, between 5.5 and 5.8, that helps extend the shelf life of products by inhibiting the growth of the alteration microorganisms and improving their organoleptic properties (Jiang et al., 2021). Generally, LAB are referred to as probiotics due to their capacity to resist in the gastro-intestinal tract, or to produce exopolysaccharides, biological preservatives and bacteriocins.

As shown in Figure 1, LABs are a key component in various parts of the food industry (bread manufacturing, cheese industry, beverages, other fermented food, etc.) Fermentation is an efficient process that conserves energy and increases the shelf life of food products (Capozzi et al., 2017).

The LABs are microorganisms that perform various fermentation processes, and are used as primary or secondary starter cultures. They have antimicrobial properties and can extend the shelf life of fruits and vegetables (Ranadheera et al., 2019). The bioactive agents produced by these microorganisms are known as postbiotics, having, for instance, antimicrobial properties (Barros et al., 2020).

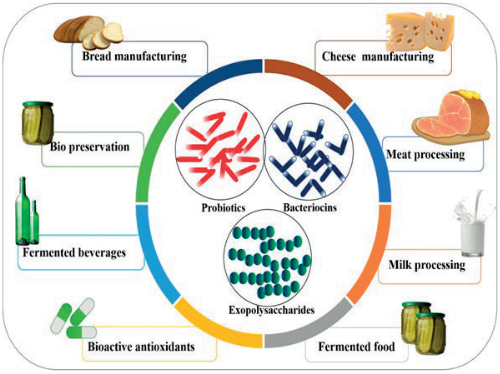

Figure 1. Lactic acid bacteria and their application (source: Raj et al., 2021)

Using LABs in food biopreservation is considered a promising technique to prevent pathogenic microorganisms' development, like *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Salmonella enterica* Serovar *Typhimurium, Proteus vulgaris Bacillus cereus*, but also the spoilage molds, such as *Aspergillus* spp., *Penicillium* spp., *Botrytis cinerea.* The antimicrobial capabilities of LABs are linked to various actions by the production of antimicrobial compounds such as bacteriocins, hydrogen peroxide, organic acids, etc. (Ramos et al., 2020).

LAB as PROBIOTIC

The innovations made in the last years in functional foods generated a wide variety of bioactive compounds that positively influence health, like probiotics, postbiotics, prebiotics or natural antioxidants, peptides, etc. (Fernandes et al., 2019).

In 2002, the United Nations' Food and Agriculture Organization and the World Health Organization defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Studies in this area showed that multiple LAB strains can be considered probiotics, and their postbiotic compounds frequently equally present health benefits to the consumer (Aguilar-Toalá et al., 2018). Some advantages of adding probiotics to food products are the interaction with pathogenic bacteria, due to the fact that probiotics can inhibit the growth of pathogenic agents by producing antimicrobial substances (Mousavi et al., 2020). The mix of probiotics with fruits and vegetables can deliver both prebiotics and alimentary fibers needed by the human body, proving to be an important way of developing the probiotic industry in the future. The aforementioned combination has diverse forms and can be easily incorporated into the products of vegetables and fruits. The most used probiotic bacteria employed in the food industry belong to the genera *Lactoabcillus* spp. and *Bifidobacterium* spp. (Table 2), but much more other species were subject of researches (Table 3). For instance, the effect of washing fresh-cut lettuce with *Lactococcus lactis* solutions has been assessed to analyse the survival of *Listeria monocytogenes*.

Table 2. The most used probiotics species in the food industry (source: Žuntar et al., 2020)

Lactobacillus spp.	*Bifidobacterium* spp.	Other spp.
L. lactis	*B. lactis*	*Enterococcs faecalis*
L. fermentum	*B. infantis*	*Escherichia coli*
L. acidophilus	*B. breve*	*Bacilus cereus*
L. brevis	*B. longum*	*Enterococcus faecium*
L. delbrueckii	*B. bifidum*	*Pediococcus acidilactici*
L. paracasei	*B. animalis*	*Leuconostoc mesenteroides*
L. helveticus		*Bacilus coagulans*
L. bulgaricus		
L. plantarum		
L. casei		
L. rhamnosus		

The viability of this pathogen decreased by 1.2-1.6 log units instantly after washing. After washing the vegetables (green asparagus and beans) with *Enterococcus faecium*, cells of *Bacillus cereus* decreased by instantly 1.0-1.5 log unit (Borges et al., 2018). Alegre et al., 2013 discovered that some probiotics inactivated by heat can maintain important cellular structures and can exercise biologic activity in the host body. Direct use of probiotics and selected LABs in food packaging systems was researched and now it is known usually as food probiotic packaging (Espitia et al., 2016; Odila et al., 2016). Incorporation of bacterial cells in the packaging material can improve the antimicrobial potential of the whole package by developing and by freeing antimicrobial substances or even by the competition between the probiotics with spoilage microorganisms and pathogenic agents from the food surface (Motalebi et al., 2020).

POSTBIOTIC

The postbiotic term is relatively new and there is no generally recognized definition yet. Langella and Martin in 2019 defined postbiotics as viable bacterias products or metabolic products from microorganisms that exercise biologic activity inside the host. Consequently, this definition can be extended to soluble food grade bioactive factors produced by microorganisms while growing and fermenting in complex microbiological culture media, cellular compounds and substances produced by the action of the microorganism on food ingredients.

The word "postbiotic" is used now to refer to bioactive metabolites, such as organic acids, short chain fatty acids, carbohydrates, antimicrobial peptides, enzymes, cofactors, immune signalling compounds, and complex agents (Moradi et al., 2019; Rajakovich & Balskus, 2019).

The efficiency of postbiotics in food systems depends on the LAB strain from which the postbiotic is produced, the targeted microorganism/contaminant, concentration, the application type, as well as the food type. Fruits and vegetables can benefit from the antagonistic activity of postbiotics. They can be used as washing solutions, as disinfection agents for fresh products industry. The direct addition of bacteriocin can be used in vegetable and fruit wash pre-treatment in order to inhibit the growth of microbial pathogens.

Postbiotics can also restrict the growth of foodborne pathogenic agents from fruits and vegetables (Tenea & Barrigas, 2018). Alvarez et al. (2021), demonstrated the effectiveness of a *Lactobacillus plantarum* strain incorporated into an edible coating based on exopolysaccharide from *Weissella confusa* on the physicochemical and microbiological quality of cherry tomato; the coating managed to inhibit the griwth of *Fusarium* spp. and *Rhizopus stolonifera.* In a study by De Simone et al., 2021, cell-free supernatants of *L. plantarum*, managed to inhibit the growth of

B. cinerea by 14 days compared to the control samples.. Duran et al. (2016) incorporated natamycin (antifungal medication) and commercially nisin (a bacteriocin produce by *Lactococcus lactis*) in a chitosan edible coating which extended the shelf life of strawberries, decreasing the aerobic mesophilic bacteria, yeast and mold levels. In this regard, combining postbiotics with different natural antimicrobial agents (AA) can increase the overall biological activity.

The outcome of a recent study by Pique et al. from 2019, underlines that postbiotics have many pharmacodynamic characteristics on living bacteria. It was proved that bacteriocins can interact with many compounds present in the food matrix, such as enzymes, proteins and carbohydrates (Silva et al., 2018), which may lead to the inhibition of their active postbiotic potential.

Further, will be described the most important characteristics of some of the LABs' postbiotics: bacteriocins, organic acids, exopolysaccharides and oxygen peroxide. To the present day, the majority of reports focused on the potential use of postbiotic metabolites, as bacteriocins in biopreservation of different food products and EPS.

Table 3. Lactic acid bacteria used for the inhibition of different pathogens in agro-food products (source: Mostafidi et al., 2020)

Product	Probiotic	Pathogen
asparagus, broccoli, cauliflower, celery, lettuce, spinach	*L. mezenteroid*	*Aeromonas* spp.
alfalfa sprouts, carrot, lettuce, onions sprouts, parsley, radish	*Enterococcus mundtii*	*Staphylococcus aureus*
potatoes, green onions, spinach, lettuce, peppers, parsley, mushrooms	*Leuconostoc,* *L. mezenteroides*	*Campylobacter jejuni*
cabbage, coriander, apples	*Enterococcus mundtii*	*E. coli*
bean sprouts, cabbage, chicory cucumber, eggplant, lettuce, potatoes, radish, salad, vegetables, tomato	*E. faecium* *Pediococcus pentosaceus* *L. plantarum*	*Listeria monocytogenes* *Bacillus cereus*
salad, cauliflower, eggplant, endive, parsley, ananas, peppern, strawberries, tomatoes, melons	*Enterococcus mundtii* *L. mesenteroides* *L. plantarum*	*Salmonella enterica* Serovar *Typhimurium*

BACTERIOCINS

Bacteriocins are synthetized mainly by LABs, which possess a wide range of inhibitory activity and are molecules with low mass. They are thermoresistant antibacterial peptides and are synthetized in the ribosome. An important fact is, that bacteriocins are well known to not alter food products sensorial and nutritional properties, being postbiotics with great potential in food industry applications (O'Bryan et al., 2018, Rohani et al., 2011). Together with the aforementioned substances, some extracellular biologically active agents with antibacterial activity, namely exopolysaccharides (EPS), gained more and more ground in food related processes (Mbye et al., 2020).

Antimicrobial metabolites are narrow range compounds and pathogenic agents treated with some of them, including bacteriocins, can develop immunity despite the fact that the costs of isolation and purification of bacteriocins is high (Kumariya et al., 2019). Regarding the case of the postbiotic mix, food can take advantage of the wide range antimicrobial activity, as well as of the synergic activities between the organic acids and other metabolites, besides the heightened thermostability of the postbiotic mix.

LAB produce a series of extracellular antimicrobial substances which not only restricts pathogenic microorganisms, but also the ones that cause food alteration. *In situ* production of antimicrobial agents by protective LAB made their exploitation in means of food preservation more accessible and compatible (Hussein et al., 2018). Keeping in mind that many microorganisms produce bacteriocins, the ones produced by LABs attracted the attention most because of their extended uses in food processing and food fermentation as natural biopreservatives. For instance, these were widely used in food conservation, such as in cheese, meat, vegetable and fruit preservation (Drider et al., 2016). Using bacteriocins in the food sector can lower the need of chemical preservatives or thermic treatments. Bacteriocins can be used conjunctively with other traditional treatments as part of the technology chain (Favaro et al., 2015). Using bacteriocins as an alternative to traditional preservation of fruits and vegetables is still under study, even though they have known preservation properties. Until the present day, only nisin (sold under the name Nisaplin) and pediocin (sold under the name Micocin) were approved the for use as food additives. Bacteriocin, enterocin, and nisin were commonly used in food factories to prevent product spoilage (Johnson et al., 2018).

Bacteriocins' classification was under revision many times. Recent classification organizes bacteriocins in three major groups based on their physiochemical and structural properties (Zacharof et al., 2012).

In the first class, bacteriocins, or lantibiotics, are small peptides, thermostable, which are largely modified during the post-translational phase and are made up of characteristic polycyclic amino acids (Kaur et al., 2015). In the second class the bacteriocins are also small thermostable peptides, but without lantinoin, such as pediocin and sakacin. They are mainly active against *Listeria monocytogenes* (Kaur et al., 2015).

Bacteriocins from the third class are thermolabile proteins with heavy molecular mass. Table 4 describes bacteriocins which have been tested for preservation of fruit products.

Nisin is the most researched and characterized bacteriocin for the preservation of fruit products. It is a first class bacteriocin produced by *Lactococcus lactis* strains. Nisin has inhibitory properties against foodborne enteropathogens, including *Clostridium difficile* (Martinez et al., 2016, Le Lay et al., 2015). The use of nisin in certain food is authorized as preservative additive (E-234) in EU (Allendeat et al., 2007). Nisin is effective against *Staphylococcus aureus*, *Clostridium difficile, Bacillus subtilis, Listeria monocytogenes*, *Alicyclobacillus acidoterrestris*, and even against some gram-negative bacteria (Barbosa et al., 2017). This bacteriocin inhibited the vegetative growth and sporulation of *Bacillus acidoterrestris* in apples, grapes and orange juice (Barbosa et al., 2017). Komitopoulou et al., 1999 in their research showed that by adding nisin in fruit juices inhibited *Alicyclobacillus acidoterrestris* development at all temperatures, storage conditions and storage time.

Pediocin is produced by *Pediococcus* spp., maintains stability in a wide pH range (3-9) and during high thermic processing techniques (65-121ºC). It has antibacterial activity against many foodborne pathogens, such as *C. perifringens* and *L. monocytogenes*, also against gram-positive bacteria that infects food (Barbosa et al., 2017).

This kind of bacteriocin was widely studied for its use in food preservation, mainly for animal food products, but recent reports reveal that pediocin can also be used in fruit products (Narsaiah et al., 2015).

Enterocin is a cyclic peptide produced by *Enterococcus faecalis*. It has a broad spectrum concerning both gram-positive and Gram-negative bacteria; it is thermostable and resists in high pH conditions. Enterocin is stable at heating temperature od 90ºC for 120 minutes and storage at 4ºC for 6 months (Barbosa et al., 2017). Molinos et al., 2008 showed that this bacteriocin has the potential to be used in fruit and vegetable conservation against *Listeria monocytogenes*.

Table 4. Bacteriocins that have been tested in the preservation of fruit products (source: Barbosa et al., 2017)

Bacteriocin	**Class**	**Producer strain**	**Antimicrobial activity**	**Applied product**
Nisin	I	*Lactococcus lactis sup lactis*	*A. acidoterrestris*	Fruit juices, mango pulp,
Bificin	I	*Bifidobacterium animalis*	*Alicyclobacillus acidoterrestris*	Fruit juices
Bovicin	I	*Streptococcus bovis*	*Clostridium tyrobutyricum, Bacillus cereus, A. acidoterrestris*	Mango pulp
Pediocin	II	*Pediococcus pentosaceus*	*Mesophilic bacteria* and *fungi*	Minimally processed papaya
Enterocin	II	*Enterococcus casseliflavus*	*Listeria monocytogenes*	Ready to eat fruits

Bificin is produced by *Bifidobacterium* spp. and thrives in lower pH ranges and higher temperatures. This characteristics are crucial for a bacteriocin to be used in fruit product preservation (Pei et al., 2013). Bificin presented antimicrobial activity against many strains of *S. aureus* and *E. faecium* (Pei et al., 2014). It could also be used in the edible coatings or in the plastic foil matrix both used in packaging minimal processed fruits.

EXOPOLYSACCHARIDE (EPS)

EPS produced by LAB are a diverse group of polysaccharides produced by many species, and are another useful characteristic of LAB. They can improve the stability and organoleptic characteristics of food (Michalak., 2018). The most prominent EPS producing lactic acid bacteria are *Weissella, Lactococcus, Pediococcus, Lactobacillus, Streptococcus* and *Bifidobacterium* sp.

EPS are classified in heteropolysaccharides produced intracellularly from several monosaccharides and homopolysaccharides which are produced in the extracellular medium. Homopolysaccharides can be characterized depending on the type of monosaccharide present in their structure. For instance, dextran is the only component that contains glucose. These are produced by *Streptococcus*, *Lactobacillus, Oenococcus, Weissella* and *Leuconostoc* (Farinazzo et al., 2020).

Heteropolysaccharides consist of repeated units with different degrees of polymerization and are composed of two to eight different monosaccharides such as fructose, rhamnose, glucose or galactose. Heteropolysaccharides are produced by members of the genera *Bifidobacterium, Lactococcus* and *Lactobacillus* (Ripari et al., 2019).

The technofunctional role of EPS in food is related to their ability to act like hydrocolloids and retain moisture in the product (Mende et al.,2016).

ORGANIC ACIDS

The goal of the LAB is to accelerate the production of organic acids in food by introducing them during the cell cycle. In fermented food, the souring effect is caused by the fermentation of carbohydrates to lactic and acetic acids. Treating fruits with organic acids can reduce the number of microorganisms in them. This method is usually used for treating fruits and vegetables perishable like strawberries, raspberries, blueberries and cantaloupes (Linares et al., 2018). Organic acids such as acid ascorbic and citric, malic, tartaric are commonly acids found in fruits and vegetables and can be used as preservatives.

Due to the decrease in environmental pH, organic acids reduce microbial loads and interfere with the cell's internal pH. They also affect the metabolic enzymes and protein synthesis (Corbo et al., 2015).

The effects of malic and citric acids on metabolism are minimal in peaches. In contrast, decreased malic acid contents have no significant effects on grapes (Famiani et al., 2016 a,b).

HYDROGEN PEROXIDE (H_2O_2)

When oxygen is present, LAB can produce hydrogen peroxide. This chemical is produced by the action of flavoproteinases or nicotinamide adenine dinucleotide peroxidase (NADH). Hydrogen peroxide is a precursor to the production of free radicals, which can be bactericidal by damaging DNA. It has been found that LAB that producing H_2O_2 can inhibit the growth of pathogenic *Clostridium botulinum, Listeria monocytogenes* bacteria and interfere with the development of food preservatives (Ben at al., 2019).

PEPTIDES

LAB can also produce antimicrobial peptides in addition to the well-known hydrogen peroxide and organic acids. (Muhialdin et al., 2016). Peptides are heat resistant and have low acidity, so they can also be used as preservatives in food. Peptides have multiple actions, such as degradation of the microbial membrane and inhibition of macromolecule synthesis (Waghu et al., 2020). A high antimicrobial activity has been attributed to LAB derived peptides against many pathogens *Escherichia coli*, *Salmonella enteritidis* and *Listeria monocytogenes* (Siroli et al., 2015).

The peptides obtained from the fermentation of vegetable performed by *Lactobacillus* *plantarum* showed advantageous properties s (Jakubczyket al., 2017)

Brittany Forkus et al. (2017) used antimicrobial peptides produced by *Escherichia coli* and it was found that the inhibit the growth of *Salmonella enterica* by damaging the cell wall structure.

CONCLUSIONS

Aside from enhancing the functional features of fermented food, the selection of new autochthonus LAB is also important to improve the sensory quality of the finished product.

The composition and quality of the exopolysaccharides and other postbiotic compounds produced by these microorganisms can help improve the taste and texture of the perishable fruits and vegetables.

This paper highlights potential alternative methods, detrimental to the traditional ones of fruit and vegetable products preservation. New research is requested to find optimal mixture of such postbiotic products, of different LAB origins, to be used for new minimal processing technology for fruits and vegetables with short shelf-life.

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