Food Bio-Preservation: An Overview with Particular Attention to *Lactobacillus plantarum*

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ABSTRACT

Food bio-preservation has gained considerable attention during the last decade. It is considered a promising alternative to classical means of food preservations and meets current consumer demands for the consumption of safe, nutritious, and chemical-free products. The main techniques of food bio-preservation, include the application of bacteriocins, bacteriophages, endolysins, and protective cultures, which compose mainly of lactic acid bacteria. To date, the use of lactic acid bacteria (as protective cultures) or their metabolites in foods is known as the main acceptable tool of food bio-preservation. This review focus on the concept of food bio-preservation and its techniques, and the role of lactic acid bacteria and their antimicrobials in food bio-preservation. Among lactic acid bacteria, particular attention is given to *Lactobacillus plantarum*, a versatile species with important antimicrobial activity. This species is known to produce numerous bacteriocins and antifungal-active compounds. Also, *Lactobacillus plantarum* is extensively used in the production of many fermented foods either as a starter culture and probiotic microorganism.

Keywords: ***Bio-preservation, bacterioncin, LAB, L. plantarum, Protective culture.***

Food bio-preservation

Currently, food preservation and safety are the main preoccupation of consumer, and food producer. Food preservation is largely related to the quality and safety of the food product. It is a very crucial issue to prevent foodborne illnesses, which is serious and costly. Food preservation aims to maintain the safety and overall quality of the product (organoleptic and nutritional), reduce the wastage of excess food, extend shelf-life of the product, and preserve the food materials during transportation (Devi *et al.*, 2015).

There are several methods for food preservation, including physical techniques such as conservation at low temperature (refrigeration, and, freezing), and application of thermal treatments (pasteurization, boiling, and sterilization,……). Foods are also preserved by adding some chemical preservatives (sodium chloride, sodium nitrite, benzoates, sorbates…).

These traditional preservation techniques may represent health hazards, alter the sensory of food and lead to the loss of some nutritive elements. So, the bio-preservation techniques look to be more convenient to improve food quality and safety. Moreover, bio-preservation could extend food shelf life with good hygienic status and minimal adverse effects on nutritional and organoleptic aspects. To date, there is increasing interest to replace traditional food preservation means by combinations of innovative technologies that include biological antimicrobial systems such as LABs or their metabolites (Nath *et al.*, 2014). Indeed, finding natural antimicrobial agents with significant antimicrobial activity has received considerable attention to enhance the quality and shelf-life of a food product. The application of LABs and/or their metabolites to extend the shelf life of foods is known as food bio-preservation (Lin & Pan, 2017). Besides the application of LAB and their metabolites, food bio-preservation techniques are also relying on the application of bacteriophages and bacteriophage-encoded enzymes (e.g., endolysins). These applications are extremely necessary to reduce or prevent the use of chemical preservatives or severe thermal treatments, both of which can adversely affect the safety or quality of food products.

Bio-preservation techniques

Bacteriophages

Bacteriophages, true parasites, are viruses that can multiply only inside the bacterial cells. They are not able to infect or cause damage to human and animal cells (Singh, 2018). Bacteriophages are predominating microorganisms and wide-spread on foods (Brüssow & Kutter, 2005). The majority of the bacteriophages are composed of a head and a double-stranded 179 DNA tail. Based on tail structures, bacteriophages can be classified mainly into three groups as 1) contractile tail, 2) long non-contractile tail and 3) extremely short tail (Ackermann, 2007). Bacteriophages are classified as virulent or temperate viruses. Virulent bacteriophages have lytic life cycle while temperate viruses have a lysogenic cycle by integrating their DNA into the bacterial chromosome (prophage). Virulent bacteriophages are capable to lyse the host bacterium and exerted antimicrobial activity (Hanlon, 2007).

In the field of food preservation, bacteriophages could be used effectively to prevent contamination or colonization of pathogens on food surfaces (e.g., decontamination of carcasses, fresh, fruits, and vegetables). Bacteriophages could be successfully used to control *Salmonella* sp., *Campylobacter* sp. and *Escherichia coli* in poultry and ruminants (Atterbury *et al.*, 2005; Raya *et al.*, 2006). In dairy products, bacteriophages appeared to be efficient to suppress *Salmonella, Staphylococcus aureus,* and *Listeria monocytogenes* in different cheese varieties (Kim *et al.*, 2007). Some bacteriophages-based products are approved by the US food and drug administration (FDA) and launched for commercial applications (Singh, 2018). For example, products named Listex® and LMP 102® are applied for ready-to-eat meat, as well as products containing anti-*E. coli* and anti-*Salmonella* phage to treat live animals before slaughtering (Moye *et al.*, 2018). Further research is still needed to overcome many limitations of phage applications in the food industry including the rise of resistance and intolerance to environmental conditions.

Endolysins

Endolysins, phage lysins, are phage-encoded peptidoglycan hydrolases employed by the majority of bacteriophages to enzymatically degrade the peptidoglycan layer of the host bacterium. Bacteriophages use the endolysins at the end of their replication cycle to degrade the peptidoglycan of the bacterial host (Oliveira *et al.*, 2013). Endolysins have a high antimicrobial activity particularly against Gram-positive bacteria (Young *et al.*, 2005). Indeed, endolysins have a narrow spectrum of lytic activity and are often restricted to phage host bacterial activity from which they are originally derived (Yoong *et al.*, 2004). To date, development of resistance against endolysins has never been reported. Endolysins have several potential applications, including treatment of mastitis and prevention of *S. aureus* and *Listeria* sp. biofilms (Turner *et al.*, 2007). Endolysin treatment represents a novel and promising strategy for controlling antibiotic-resistant bacteria, which are a frequently encountered problem in the sector of the food industry (Chang, 2020).

Protective cultures

The food fermentation process is a typical example of the biological preservation of foods. It leads to the formation of a large number of bacterial cells and the accumulation of beneficial metabolites, which can potentially reduce the incidence of food spoilage and pathogenic microorganisms (Ganguly, 2013). Biological preservatives eligible for application in food must be recognized as a safe, non-pathogenic, non-toxic producer and culturable in food. The biological agents usually used in food bio-preservation are a starter and/or protective cultures. The application of starter cultures is mainly to initiate fermentation, produce organic acids and generate flavour and aroma compounds responsible for typical organoleptic characteristics of the fermented product. However, the protective cultures, due to their antimicrobial activity, are generally used to control growth and reduce the survival of many pathogenic and spoilage-causative microorganisms in foods. In practice, a combination of both cultures is generally recommended for application in the food industry.

Lactic acid bacteria (LABs)

Lactic acid bacteria (LABs) are recognized as GRAS (generally recognized as safe) and have been used in food fermentation for centuries. LABs are intensively used in the production of a wide variety of fermented dairy, meat, and vegetable products. Lactic acid bacteria are a group of Gram-positive bacteria, nonmotile, non-spore-forming, rod- or cocci-shaped microorganisms and generally characterized by fermenting carbohydrates to mainly produce lactic acid. It is a heterogeneous microbial group that contains 10 bacterial species, including *Lactococcus, Streptococcus, Pediococcus, Lactobacillus, Leuconostoc, Enterococcus, Carnobacterium, Oenococcus, Aerococcus, Tetragenococcus, Vagococcus*, and *Weisella*. These microorganisms contain low G+C ratio (<55%) in their DNA which provides more thermostability to the cells. LABs can possess very interesting characteristics, such as the ability to resist freezing and freeze-drying, ferment citrate, produce exo-polysaccharides and antimicrobial substances, in addition to their resistance to stressful conditions and ability to colonize the digestive tract. (Singh, 2018).

Fermentation by LABs has been shown to control and suppress a wide variety of both food spoilage and pathogenic microorganisms (Sharma *et al.*, 2012). Food fermentation is the oldest approach of food bio-preservation that has been proven to be efficient to improve food safety and quality. LABs have the ability to extend the shelf life of fermented foods has been attributed to their capacity to produce various inhibitory compounds, including organic acids (lactate, acetate, propionate, etc….), diacetyl, hydrogen peroxide, and many antifungal active molecules, including free fatty acids and phenyl lactic acid (Steele *et al.*, 2013). Also, they can produce inhibitory peptides known as bacteriocins to inhibit pathogenic bacteria and bacteria responsible for food spoilage. The inhibitory effects of these substances have not yet been characterized and are believed to be achieved through a range of different mechanisms (Liu *et al.*, 2011).

Antimicrobials produced by LABs

The ability of LABs to inhibit other microorganisms is taken as the basis of bio-preservation to enhance the safety and quality of food products. LABs culture with potential inhibitory activity is known as protective culture. The antimicrobials produced by LABs are generally recognized as safe and food-grade molecules and widely accepted for food preservation (Messaoudi *et al.*, 2013).

Organic acids

Food fermentation by LABs results in significant production of organic acids, as a by-product of their metabolism, which exerts antimicrobial activity (Ross *et al.*, 2002). Lactate, acetate, and propionate are the most common organic acids produced by LABs. Organic acids are implicated centuries in food preservation and they have proven the efficiency to extend the shelf-life many food products. In addition, they are considered normal constituents of fermented products, where they contribute to the sensory characteristics of these products. Organic acids play important role in the preservation of many types of food, such as fermented dairy vegetables and meat products. Production of organic acid is the principal way by which LABs can compete and inhibit other competitors. The organic acids can acidify the surrounding environment to inhibit the growth of many pathogens (Akbar & Anal, 2014).

Among organic acids, lactate acid is the abundant product acid produced by LABs during the homo-fermentation of hexoses. It is also produced by hetero-fermentation along with acetate, ethanol, and carbon dioxide (CO_2) (Ross *et al.*, 2002). Lactate is found in many foods either as a product of *in situ* microbial fermentation (cheeses and yoghurt) or as a food additive; acidulate, in a wide variety of processed foods (soups, mayonnaise, and processed eggs (Datta & Henry, 2006).

The inhibitory effect of lactate is attributed to the diffusion of its non-dissociated form leading to acidification of cytoplasm and failure of proton motive forces. This affects the pH gradient of the cell membrane and decreases the available energy for cells to grow (Wee *et al.*, 2006). When present in a mixture with other organic acids, lactate contributes to reduce the acidity, while acetate acid and propionate act as antimicrobial agents by interfering cell membrane maintenance potential of the target cells (Ross *et al.*, 2002).

The inhibitory effect of lactic acid compared with other short-chain organic acids, including formic, acetic, and propionic acids was investigated at an equal molarity basis. The inhibition was affected by pH and atmosphere conditions. At pH of 5.8 versus *Yersinia enterocolitica*, the inhibitory effect order was formate > acetate > propionate > lactate, whereas, at lower pH of 3.90, the inhibitory effect became formate > lactate > acetate > propionate, respectively. This may indicate that the antibacterial activity of short-chain organic acids is molecular size-dependent. However, lactate is enhanced under anaerobic conditions compared to aerobic ones meanwhile, the *Y. enterocolitica* was more tolerant to formate and acetate when cultivated anaerobically (El-Ziney *et al.*, 1997).

Lactate has a significant antifungal activity, which can be improved when mixed with acetate. Pelaez *et al.* (2012) reported that the mixtures of lactate and acetate had a synergistic effect to inhabit toxigenic strains of *Aspergillus flavus*.

Acetate is used in many food products as an aroma compound and as an antimicrobial that has abroad spectrum of inhibitory activity against many bacterial species (either Gram-positive or –negative), yeasts, and molds. Acetate has stronger antimicrobial activity compared to lactate (Malti & Amarouch, 2008). Depending on its concentration, acetate can exert bacteriostatic or bactericidal effects. The bacteriostatic activity appears at low concentrations (≤ 0.15%), but at concentrations above 0.3 % the bactericidal effect is present (Reis *et al.*, 2012). The antimicrobial effect of acetate is attributed to the un-dissociated molecule (Reis *et al.*, 2012).

Basically, propionic acid-producing bacteria (*Propionibacterium* sp.) have the ability to convert lactate generate large amounts of propionate in addition to acetate and CO_2 (Suomalainen *et al.*, 1999). However, hetero-fermentative LABs are able to produce little amounts of propionic acid. Due to its antifungal activity, propionic acid could be used as food preservative. In addition, it has strong activity to to reduce the viability of many bacterial species, including both Gram-positive and Gram-negative bacteria (Reis *et al.*, 2012). Propionate could be used to control molds and yeasts in cheeses, butter, bakery products, and some fresh fruit (Ray, 2004). Its inhibitory effect depends on the decrease in pH caused by lactic acid. To exert its inhibitory effect propionate interacts with cell membranes to neutralize the electrochemical proton gradient leading to reduction of amino acid uptake and consequently the fungal growth.

Bacteriocins

Bacteriocins are ribosomally-synthesized peptides that have an antibacterial activity against closely related bacteria (Jack *et al.*, 1995). They are produced both by Gram-positive (*Streptococcus, Lactococcus*, etc..) and Gram-negative bacteria (*Pseudomonas*, *Escherichia coli, Klebsiella* etc..).

The majority of bacteriocins produced by LABs are selectively active against closely related species while Nisin®, exceptionally, has antimicrobial activity against many Gram-positive bacteria, including *Bacillus sp.,* and *Clostridium botulinum* (Hurst, 1981). Bacteriocins produced by LABs are classified into 4 classes (Klaenhammer, 1993) as shown in Table (1). This classification includes:

Class I (lantibiotic bacteriocins): this class includes small peptides (> 4 kDa) that contain unusual amino acid residues (lanthionine and β-methyl lanthionine) as well as dehydrated amino acids, (Xie & van der Donk, 2004).

Class II (non-lantibiotic bacteriocins): this class includes heat-stable small bacteriocins (4–6 kDa). Bacteriocins belonging to this class do not contain unusual amino acids in their composition (Liu *et al.*, 2011). This class could also be divided into tree subclasses, including pediocin-like bacteriocins (IIa), two-peptide bacteriocins (IIb), and circular bacteriocins (IIc) (Cotter *et al.,* 2005), and

Class III: this group includes non-bacteriocin lytic proteins (bacteriolysins), which have large molecular mass (> 10 kDa), and heat-labile peptides (Wiedemann *et al.*, 2001).

Class-IV circular peptides: this class contains antimicrobial bacteriocins produced by LABs, plants, and mammalian cells (Burgos *et al.*, 2014; Golneshin *et al.*, 2020).

Among bacteriocins, purified nisin, to our

Table 1: Classification of bacteriocins

Classes	Characterization	Bacterioicn/producer organism	Reference
I-Lantibiotics	• They contain thioether-intramolecular rings of lanthionine and β-methyl-lanthionine	• Nisin/*lactococcus lactis* subsp. *diacetylactis*	Xie and van der Donk (2004)
II-Non-lantibiotics	• They are linear short cationic peptides (<10 kDa) with high isoelectric points. • They are divided into three subclasses (a, b, and c).	• Pediocin PA-1 & AcH/ pediococci. • Enterocin EJ97/ *Enterococcus faecalis* • Enterocin L50A/ *Enterococcus faecalis*	Papagianni & Anastasiadou(2009)
III-large bacteriocins	• They are large (> 30 KDa) heat-labile proteins. • They contain a peptide bond between the C- and N-terminus.	• Helveticin J/ *Lactobacillus helveticus* • Bacteriocin Bc-48 /*Enterococcus faecalis*	Wiedemann *et al.* (2001)
IV – cyclic bacteriocins	• They are circular peptides produced by bacteria, plants, and mammalian cells. • They contain a peptide bond between the C- and N-terminus.	• Enterocin AS-48 /Enterococcus faecalis • Plantacyclin B21AG / *Lactobacillus plantarum* B21	Burgos *et al.* (2014) Golneshin *et al.* (2020)

knowledge, is the only bacteriocin licensed for addition to foods (Schillinger *et al.*, 1996). Another interesting antimicrobial peptide that belongs to Class IIa bacteriocin is pediocin PA-1. Pediocin PA-1 is not approved as pure substance by FDA however, one commercial crude fermentation product distributed as Alta 2341 (Quest International) (Lopez-Cuellar *et al.*, 2016) or CHOOZIT™ FLAV 43 (Danisco) which is lyophilized pediocin producing cultures (Papagianni & Anastasiadou, 2009). MicroGARD® fermentates are skim milk fermented by LABs contains potent inhibitors toward Gram-negative and Gram-positive bacteria and yeast and fungi (DUPONT) and approved by FDA (Makhal *et al.*, 2015).

Bacteriocins produced by LABs have several advantages that make them promising for food preservation. This because LABs are implicated in food fermentation for centuries and have a proven history of safety, thereby their metabolites are generally recognized as safe. Also, none of the LABs bacteriocins have shown a toxic effect against eukaryotic cells. Also, the proteinaceous nature of bacteriocins ensures their safety as they become inactive by digestive proteases (Drider *et al.*, 2006).

Bacteriocins can exert a bactericidal effect at the membrane level, which is effective to inhibit antibiotic-resistant microorganisms (Thomas *et al.*, 2000). The thermal and pH stability of bacteriocins make them a promising candidate to be used as a food preservative. The genetic determinants responsible for bacteriocin production are usually located on plasmids, which could be transferred from donor to recipient cells leading to improve the characteristics of bacteriocin-producing strain. The application of bacteriocins as a food preservative has many aspects, including (1) reduction of the incidence of food poisoning, cross-contamination, and the use of chemical preservatives, and (2) improve quality and extend the shelf-life of food products (Gálvez *et al.*, 2007). There are different ways to apply bacteriocins in foods, including the application of producer strains, the addition of free or encapsulated (Gálvez *et al.*, 2007).

The mechanism of bacteriocins action is principally attributed to the formation of a pore in the bacterial cell membrane (Sahl & Bierbaum, 1998), leading to the efflux of small molecules such as amino acids, potassium ions, and ATP (Lins *et al.*, 1999). Other mechanisms have been proposed to describe the mechanism of bacteriocins action on the bacterial cells. Bacteriocins could induce the activity of cell-wall acting enzymes (amidases) through the release of the enzymes from their inhibitors (teichoic and teichuronic acids) leading to bacterial autolysis (Severina *et al.*, 1998). Bacteriocins may also act on specific intracellular targets, including induction of proteolytic enzyme production, metabolic disorder, and reduction of respiratory function (Sahl & Bierbaum, 1998). This may explain the formation of lysis vesicles previously reported by Benech *et al.* (2002) in cells of *Listeria innocua* and *Lactobacillus casei subsp. casei* treated with nisin Z.

LABs-producing bacetriocins and their metabolites are of particular interest to the food industry. For the bio-preservation of foods, LABs-producing bacteriocins and their metabolites could be applied to foods through three approaches. First is the direct application of LABs-producing bacteriocins, which produce the bacteriocin into foods during food processing. The second approach is the direct addition of the purified or crude bacteriocins onto the food product and lastly is applied to a previously fermented product from a bacteriocin-producing strain (Lin & Pan, 2017).

Reuterin

Reuterin (3-HPA) has an effective wide spectrum inhibitory action against Gram-positive and -negative bacteria, yeast, moulds, viruses, and protozoa (El-Ziney *et al.*, 2000). It is known that Gram-negative bacteria are more susceptible to reuterin than Gram-positive. Reuterin is produced by some LABs, particularly *Lactobacillus reuteri*, during the anaerobic fermentation of the glycerol (Axelsson *et al.*, 1989). It can also be produced by *L. coryniformis, L. brevis, L. collinoides,* and *L. buchneri* (Nakanishi *et al.*, 2002). Reuterin was successfully able to inhibit *Escherichia coli* O157:H7 and *Listeria monocytogenes* in milk and cheese (El-Ziney & Debevere, 1998) and extend the shelf-life of fermented milk products, without drastically affecting their quality parameters (Ortiz-Rivera *et al.*, 2016).

Reuterin-producing LABs showed antifungal activity against many yeasts and moulds, including members belong to species *Candida, Saccharomyces, Torulopsis, Aspergillus,* and *Fusarium* (Chung *et al.*, 1989). The addition of glycerol to the growth medium of these LABs induces their antifungal activity (Magnusson & Schnurer, 2001).

Hydrogen peroxide (H_2O_2)

Some LABs produce H_2O_2 during their growth that can improve self-life and inhibit the growth of many pathogenic microorganisms in food (Dahiya & Speck, 1968). Hydrogen peroxide-producing LABs are able to inhibit the growth of many psychrotrophic microorganisms at refrigeration temperatures. The formation of H_2O_2 is taken place through the oxidization of lactatic acid. Lactobacilli implicated in meat fermentation such as *L. plantarum, L. sakei*, and *L. pentosus* have heme-dependent catalase activity, which adversely affects the organoleptic properties by increasing the rancidity, due to fat oxidation, and discoloration of the final products (Ammor & Mayo, 2007).

Hydrogen peroxide exerts strong bactericidal and fungicidal effects (Brul & Coote, 1999). Biologically, H_2O_2 is a component of the lactoperoxidase system, found in milk, in addition to thiocyanate. This system has strong antimicrobial activity against bacteria and fungi. It is primarily active against microorganisms that produce H_2O_2 (Seifua *et al.*, 2005). Many LABs especially those that belong to lactobacilli produce sufficient H_2O_2 to activate the lactoperoxidase system (Wolfson & Sumner, 1993). As H_2O_2 is not normally detected in raw milk (FAO, 1999), it is permitted to add H_2O_2 at a concentration of 100–800 ppm (Luck, 1962) for the preservation of raw milk in the absence of refrigeration. Thus, H_2O_2 may be added or generated by the addition of sodium percarbonate or glucose oxidase to activate the lactoperoxidase system (Kussendrager & van Hooijdonk, 2000).

Carbon dioxide (CO_2)

During the hetero-fermentation of hexoses, LABs can produce carbon dioxide as lactic acid. Hetero-fermenters LABs can produce large quantities of CO_2. The antimicrobial activity of CO_2 is attributed to its ability to create anaerobic conditions and replace the oxygen in the food products. This ability could be used to prevent the growth of spoilage microorganisms, including bacteria and fungi, in foods (Lindgren & Dobrogosz, 1990). The antibacterial activity of CO2 is attributed to its capacity to create anaerobic environment and its ability to act as a potential antimicrobial agent. Indeed, a lower concentration of carbon dioxide can stimulate the growth of some microorganisms, but a higher concentration prevents the growth of many of them (Borneman *et al.*, 2012). Gram-negative bacteria are more sensitive to carbon dioxide as compared to Gram-positive bacteria (Akbar & Anal, 2011). Carbon hydroxide are extensively used in food preservation through modified atmosphere packaging.

Diacetyl

Diacetyl production is produced by many genera of LABs, including lactococci, lactobacilli, and *Leuconostoc* (Clark *et al.*, 2015). It is produced mainly through citrate metabolism (Malti & Amarouch, 2008). Diacetyl is active mainly against Gram-negative bacteria through its reaction with some amino acids, particularly arginine, during utilization of amino acids (Malti & Amarouch, 2008). It is considered one of the main aroma components in dairy products. Diacetyl concentration varies widely among dairy products. Its concentration in butter ranges from 0.4 to 4.0 ppm (Chrysan, 2005) and yoghurt may contain higher levels up to 16 ppm (Güler & Gürsoy-Balci, 2011). The American Food and Drug Administration (FDA) considers diacetyl to have GRAS status (Birkenhauer & Oliver 2003). Diacetyl is lethal against Gram-negative bacteria, inhibitory for Gram-positive bacteria but ineffective against clostridia even under anaerobic conditions (Jay, 1982).

Antifungal compounds

Spoilage of food by molds and yeasts (known collectively as fungi) is responsible for considerable economic losses and food waste. These microorganisms have important proteolytic and lipolytic activities, and could potentially deteriorate the sensorial quality of many food products (Pitt & Hocking, 2009). The annual fungal attacks on wheat, maize, and rice have been estimated to cause an economical loss of $60 billion in crops worldwide (Varsha & Nampoothiri, 2016). Microbial spoilage of foods with molds may pose health threats to humans and animals due to poisonous compounds (mycotoxins) produced by many fungal species (Milicevic *et al.*, 2016). Many chemical preservatives (e.g. potassium sorbate, sulfur dioxide, and calcium propionate) are usually added to food to control yeasts and molds. The majority of these chemical preservatives have limited efficiency and may adversely affect the overall product quality (Bata & Lasztity, 1999). Thus, the use of LABs and their antifungal metabolites offers the best alternative to chemical preservatives. In general, LABs are considered "Green preservatives" due to their safety and ability to inhibit fungal growth in food without causing adverse effects on the sensorial quality and nutritional value of food products (Pawlowska *et al.*, 2012).

Kim (2005) reported that five strains of LABs, isolated from Kimchi product, had a wide antifungal activity against strong activity against *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium commune* and *Fusarium moniliforme).* The isolates were further identified as *Lactobacillus sakei, Lactococcus lactis* subsp. *lactis*, *Lactobacillus pentosus*, *Lactobacillus casei*, and *Lactobacillus cruvatus*. Probiotic strains with potential antifungal activity were used in bread fermentation with yeast to challenge the growth and aflatoxin production by *Aspergillus parasiticus* and *Penicillium expansum* (Saladino *et al.*, 2016). The probiotic strains could reduce the production of aflatoxin(s) by 84.1 to 99.9% and extend the shelf-life of bread by about 3-4 days. In an attempt to develop antifungal cultures to be used for the bio-preservation of dairy products Salas *et al.* (2018) screened 32 strains of LABs and propionibacteria for their antifungal activity against *Penicillium commune, Mucor racemosus, Galactomyces geotrichum,* and *Yarrowia lipolytica*. Two antifungal cultures containing *L. plantarum* L244 along with *L. harbinensis* L172 or *L. rhamnosus* CIRM-BIA1113 could effectively delay the growth of *M. racemosus*, *P. commune,* and *Rhodotorula mucilaginosa* without effect on organoleptic properties of sour cream. Both *Pediococcus pentosaceus* KTU05-10, and *Pediococcus acidilactici* KTU05-7 have strong antifungal activity. They were able to suppress growth of *Fusarium poae*, and *Fusarium culmorum* (Juodeikienea *et al.*, 2018). Also, these microorganisms could reduce the formation of deoxynivalenol, zearalenone, T-2 and HT-2 toxins by 23, 34, 58, and 73% respectively. In addition, treatment of wheat grains with permeate previously fermented with *P. acidilactici* KTU05-7 and *P. pentosaceus* KTU05-10 strains resulted in increased germination of wheat grains by 9.5 and 7.9%, respectively.

Earlier studies on antifungal activity of LABs indicated the ability of *Lactobacillus plantarum* to inhibit fungi is attributed to its ability to produce phenylacetic acid and 4-hydro phenyl lactic (Lavermicocca *et al.*, 2000). Also, bacteriocin-like substances and compounds with low molecular weight that can be produced by *L. plantarum, L. pentosus* and *L. coryniformis* have been shown to exert antifungal activity (Magnusson *et al.*, 2003). In general, the antifungal metabolites produced by LABs include organic acids as phenylacetic, hydroxyphenyllactic, benzoic acids, fatty acids, volatile compounds (such as diacetyl, acetoin), cyclic dipeptides, hydrogen peroxide, reuterin, and/or proteinaceous compounds (Salas *et al.*, 2017).

Lactobacillus plantarum protective action

Importance of *L. plantarum*

The species *Lactobacillus plantarum* is one of the most important members of the genus *Lactobacillus*. This species is extensively implicated in the food industry either as a starter culture or probiotic microorganism. *L. plantarum* is a versatile species with important and diverse characteristics. The species can be found in many raw and fermented food products (Guidone *et al.*, 2014). *L. plantarum* has been shown to have a crucial role in the flavor development and texture of a wide variety of fermented foods, including dairy products, fermented meat and fermented vegetables (Adesulu-Dahunsi *et al.*, 2017). Also, *L. plantarum* is known to be a good source of many enzymes, including ester hydrolases (Kim *et al.*, 2017), lipase (Uppada *et al.*, 2017), lactate dehydrogenase (Krishnan *et al.*, 2000), and α-Amylase (Panda & Ray, 2008). *L. plantarum* has been shown to produce a wide range of proteolytic enzymes with potential application in dairy sector (Tchorbanov *et al.*, 2011).

In addition, *L. plantarum* has the ability to enhance the nutritional quality and vitamin contents of many food products (Swain & Ray, 2016; Panda *et al.*, 2017). It could increase the vitamin concentrations like folate, riboflavin, vitamin B12 in yogurt (Li *et al.*, 2017). It can also produce large amounts of vitamin B12 in fermented foods (Arena *et al.*, 2014).

L. plantarum has been shown to have significant effects on the flavor and texture development in many fermented foods (Adesulu-Dahunsi *et al.*, 2017). Several strains of *L. plantarum* have been shown to improve the overall quality of many fermented foods, including taste and aroma enhancement, nutritional attributes and health-promoting activities (Lee *et al.*, 2016).

L. plantarum is characterized by its ability to produce antimicrobial cyclic dipeptides. *L. plantarum* strain LBP-K10 has been shown to produce antimicrobial cyclic dipeptides active against multidrug-resistant bacteria, pathogenic fungi, and influenza A virus Kwak *et al.* (2017).

Some strains of *L. plantarum* have been shown to produce exopolysaccharides (EPS) with potential application in the food industry. EPS are biopolymers with a high molecular weight that produced extracellularly by some microorganisms (bacteria, fungi, and algae) and considered food-grade biopolymers (Zhou *et al.*, 2016). Numerous strains of EPS-producing *L. plantarum* have been isolated from fermented foods (yoghurt, cheese, fermented meat, etc…….). *L. plantarum* KF5, isolated from Tibet kefir, could produce EPS consisted of glucose and mannose (Wang *et al.*, 2010). *L. plantarum* strain C88 (isolated from tofu) has been shown to produce exo-polysaccharide composed of glucose and galactose at molar ration of 2:1, respectively and a molecular mass of 1.1×10^6 Da (Zhang *et al.*, 2013). Another strain of *L. plantarum* 7081, isolated from Chinese fermented product, has been shown to produce EPS composed of glucose, mannose, and galactose with a molecular mass of 203 kDa (Wang *et al.*, 2015). Gangoiti *et al.* (2017) isolated an EPS producer strain, known as *L. plantarum* CIDCA 8327, from kefir. The EPS produced by this strain have promising functional properties to improve physical characteristics of functional foods. The strain *L. plantarum* K041 described as high EPS producer was isolated from traditional Chinese pickles (An *et al.*, 2017).

In addition to its antimicrobial activity, *L. plantarum* is considered a probiotic promising candidate. This microorganism has potential antioxidant, antimutagenic, and immune-stimulating activities. There is increased attention to apply *L. plantarum* in medical sectors for the treatment of Alzheimer's, Parkinson's, diabetes, cardiovascular diseases, cancer, obesity, hypercholesterolemia, hypertension, urinogenital complications, gastrointestinal disorder, and liver disease (Murofushi *et al.*, 2015).

Antimicrobial activity of *L. plantarum*

Bacteriocins production

L. plantarum has been shown to produce a wide variety of bacteriocins known as plantricins (Table 2). Indeed, *L. plantarum* can produce bacteriocins of high and broad activity targeting many pathogenic microorganisms such as *Listeria monocytogenes*, *Staphylococcus aureus*, and *Aeromonas hydrophila* (Bernbom *et al.*, 2006; Messi *et al.*, 2001). Plantaricins, common bacteriocins produced by *L. plantarum*, are two peptides of bacteriocins. *L. plantarum* MBSa4, isolated from fermented sausage, has been shown to produce bacteriocin known as M1-UVs300 (Barbosa *et al.*, 2016). The bacteriocin-M1- UVs300 was described as heat resistant and active against Gram-positive and Gram-negative bacteria over a range of pH (2–8) (An *et al.*, 2017). This bacteriocin has also shown antagonistic properties to fungi (Barbosa *et al.*, 2016).

L. plantarum NTU 102, isolated from homemade Korean pickled cabbage, has shown an antimicrobial activity against *Vibrio parahaemolyticus* (Lin & Pan, 2017). This antimicrobial activity has been shown to decrease by the proteolytic enzymes (e.g., trypsin, pepsin, and proteinase K). Interestingly, antibacterial activities of bacteriocin produced by strain *L. plantarum* NTU102 remained constant within a pH range from 1.0 to 4.0 but disappeared at pH > 5.0. The bacteriocin also lost its inhibitory effect after heating at 121°C/15 min. It has been recommended to use this strain as biopreservative to control *Vibrio parahaemolyticus* in food. *L. plantarum* B21, isolated from Vietnamese sausage, displayed antimicrobial activity against *Clostridium perfringens*, and *Listeria monocytogenes* (Golneshin *et al.*, 2020). This activity was attributed to its ability to produce a thermostable circular peptide (bacteriocin) known as plantacyclin B21AG. The peptide (5668 Da) demonstrated resistance to a wide range of pH and proteolytic enzymes.

Antifungal activity

The antifungal activity exerted by *L. plantarum* is well documented and many studies have reported on its role in inhibiting the growth of a wide variety of moulds and yeasts (Crowley *et al.*, 2013). Among 897 isolates of LABs, originated from fruits, vegetables, and herbs, 12 strains belonging to *L. plantarum* were found to have strong antifungal activity (Cheong *et al.*, 2014). The strain *L. plantarum* YML007, isolated from kimchi, has a strong antifungal effect against *Aspergillus flavus*, *A. oryzae*, and *Fusarium oxysporum* (Rather *et al.*, 2013). The antifungal activity of this strain was attributed to its ability to produce a novel protein of 1.256 kDa. Numerous strains of *L. plantarum* have been shown the ability to produce antifungal bioactive peptides active against *Aspergillus parasiticus* and *P. expansum* (Luz *et al.*, 2017). Also, Russo *et al.* (2017) screened 88 strains of *L. plantarum* for

Table 2. Common plantricins produce by *Lactobacillus plantarum*

Types of plantricins	Strains	Source of Isolate	Molecular mass (kDa)	Target organism(s)	Reference
Plantaricin T	*L. plantarum* LPCO10	Fermented green olive	ND	*Clostridium tyrobutyricum Enterococcus faecalis*	Jiménez-Díaz *et al.* (1993)
Plantacin 154	*L. plantarum* LTF 154	Fermented sausage	≤ 3.0	*Bacillus* spp., *Staphylococcus aureus*, *Salmonella typhimurium*	Kanatani & Oshimura (1994)
Plantaricin 149	*L. plantarum* NRIC 149	Pineapple	2.2	Listeria monocytogenes, Staphylococcus aureus	Kato *et al.* (1994)
Plantaricin F	*L. plantarum* BF001	Spoiled catfish fillets	0.4-6.7	*Staphylococcus aureus, Salmonella typhimurium, Listeria monocytogenes*,	Fricourt *et al.* (1994)
Plantaricin C	*L. plantarum* LL441	Cabrales cheese	3.5	Listeria monocytogenes	González *et al.* (1994)
Plantaricin LC74	*L. plantarum* LC74	Crude goat's milk	≤ 5	*L. plantarum* DSM20174 *Leuconostoc paramesenteroides* DSM 20288	Rekhif *et al.* (1994)
Plantaricin C	*L. plantarum* LL441	Cabrales cheese	3.5	Listeria monocytogenes	González *et al.* (1994)
Plantaricin SA6	*L. plantarum* SA6	Fermented sausage	3.4	L. plantarum, Lactobacillus brevis, Listeria grayi	Rekhif *et al.* (1995)
Plantaricin S	*L. plantarum* LPCO10	Fermented green olive	2.5	Clostridia, Propionibacteria, and *Enterococcus faecalis*	Jimenez *et al.* (1995)
Plantaricin UG1	*L. plantarum* UG1	Dry sausage	3.0 -10.0	*Listeria monocytogenes* LMG10470	Enan *et al.* (1996)
Plantaricin 423	*L. plantarum* 423	Sorghum beer	3.5	*Bacillus cereus, Clostridium sporogenes,* and *Enterococcus faecalis,*	Van Reenen *et al.* (1998)
Plantaricin 35d	*L. plantarum* 35d	Italian sausages	4.5	inhibitory activity against pathogenic bacteria,	Messi *et al.* (2001)
Plantaricin W	*L. plantarum* LMG 2379	Wine	2.3	*Listeria monocytogenes Bacillus cereus*, and *Staphylococcus aureus*	Holo *et al.* (2001)
Plantaricin C19	*L. plantarum* C19	Fermented cucumbers	3.8	Listeria monocytogenes	Atrih *et al.* (2001)
Plantaricin JK	*L. plantarum* C١١	Fermented cucumbers	3.4-3.7	*L. plantarum* 965	Diep *et al.* (2003)
Plantaricin ST28MS	*L. plantarum* ST28MS	Molasses	ND	*Lactobacillus casei, Staphylococcus aureus,* and *Enterococcus faecalis*	Todorov, & Dicks (2005)
Plantaricin - 163	*L. plantarum* 163	Chinese fermented vegetables	3.5	*Staphylococcus aureus, Listeria monocytogenes,*	Hu *et al.* (2013)
Plantaricin ZJ008	*L. plantarum* ZJ008	Fresh milk	1,3	*Micrococcus luteus, Staphylococcus aureus*	Zhu *et al.* (2014)
Plantaricin ZJ5	*L. plantarum* ZJ5	fermented mustard	4.5	Staphylococcus aureus, Listeria monocytogenes	Song *et al.* (2014)
Plantaricin Y	*L. plantarum* 510	Japanese white wine grape	4.2	Listeria monocytogenes	Chen *et al.* (2014)
Plantaricin ST8SH	*L. plantarum* ST8SH	Salami	ND	*L. monocytogenes* ScottA and *E. faecalis* ATCC	Todorov *et al.* (2016)
Plantaricin JLA-9	*L. plantarum* JLA-9	Fermented cabbage	< 1.0	*Staphylococcus aureus Micrococcus luteus*	Zhao *et al.* (2016)
Plantaricin K25	*L. plantarum* K25	Kimchi	1.7	*Bacillus cereus* ATCC 14579, *Listeria monocytogenes* NCTC 10890	Wen *et al.* (2016)
Plantaricin DL3	*L. plantarum* DL3	Chinese fermented cabbage	3.5	Listeria monocytogenes, Pseudomonas aeruginosa	Lv *et al.*, (2018a)
Plantaricin JY22	*L. plantarum* JY22	Golden carp intestine	4.1	*Bacillus cereus* CMCC 63301	Xinran *et al.* (2018)
Plantacyclin B21AG	*L. plantarum* B21	Vietnamese sausage	5.7	*Listeria monocytogenes Clostridium perfringens*	Golneshin *et al.* (2020)

their antifungal activity against *Aspergillus flavus* , *Aspergillus niger*, *Penicillium expansum*, *Penicillium chrysogenum*, *Penicillium roqueforti*, *Fusarium culmorum*, and *Cladosporium* spp. The author reported a significant phenotypic heterogeneity among the antifungal activity trait. Among tested fungal species, *A. niger, A. flavus*, *P. roqueforti*, and *Cladosporium* spp. Exhibited strong resistance toward the antifungal metabolites produced by *L. plantarum*. Approximately, 60 to 80% of *L. plantarum* strains were unable to affect growth of tested moulds. However, 75% of the *L. plantarum* strains showed a strong inhibitory effect against *F. culmorum*, *P. expansum*, and *P. chrysogenum* (Russo *et al.*, 2017). Phenotypic variation corresponded to ten different genotypes that were associated with the ability of *L. plantarum* to produce antifungal metabolites (**Dong** *et al.***, 2017**). Indeed, numerous studies have been reported on the antifungal activity of *L. plantarum* (Muhialdin *et al.*, 2016; Lv *et al.*, 2018b; Quattrini *et al.*, 2018).

Antifungal activities of *L. plantarum* strains have been attributed to the ability to produce phenyl lactic acid, cyclic dipeptides, fatty acids, and organic acids (Dong *et al.*, 2017). Gupta and Srivastava (2014) studied the antifungal peptides produced by *L. plantarum* (LR14) against spoilage fungi (*Mucor racemosus, Aspergillus niger, Rhizopus stolonifer* and *Penicillium chrysogenum*). The peptides delayed the hyphal growth and spore germination of all tested moulds. Besides, *L. plantarum* showed a high ability to bind mycotoxins (Dong *et al.*, 2017).

CONCLUSION

The species *L. plantarum* is a significant member of the genus *Lactobacillus*. It has potential application in the food industry either as a starter culture or a candidate microorganism with potential probiotic activity. Members of the species have important technological and probiotic properties and can be integrated into many fermented food products. They have wide the ability to produce a wide range of antimicrobials to control food spoilage and pathogenic microorganisms. Also, *L. plantarum* is considered an excellent candidate for the sector of food bio-preservation. In this concept, studies are needed to characterize the microbial ecosystem of Egyptian foods to elucidate the role of *L. plantarum* in food technology and preservation.

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الحفظ الحيوي للأغذية:
نظرة شاملة على الاهتمام بـالنوع *Lactobacillus plantarum*

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لقد حظى الحفظ الحيوي للأغذية باهتمام كبير خلال العقد الماضي. و يعتبر الحفظ الحيوي بديلاً واعدًا للطرق التقليدية المستخدمة فى حفظ الطعام كما أنه يلبي متطلبات المستهلكين الحالية لاستهلاك منتجات آمنة ومغذية وخالية من المواد الكيميائية. تشمل التقنيات الرئيسية للحفظ الحيوي للأغذية استخدام البكتريوفاج و الإندوليزين والمزارع البكتيرية الوقائية التي تتكون أساسًا من بكتيريا حمض اللاكتيك. و حتى الآن ، يعتبر أستخدام المزارع الوقائية و نواتج أيض بكتيريا حمض اللاكتيك في الأغذية على أنها الأداة الرئيسية المقبولة للحفظ الحيوي للأغذية. و يركز هذا الاستعراض المرجعى على مفهوم الحفظ الحيوي للأغذية وتقنياتها و بكتيريا حمض اللاكتيك ومضادات الميكروبات التى تنتجها. من بين بكتيريا حمض اللاكتيك، تم إيلاء اهتمام خاص لـلنوع *Lactobacillus plantarum* ، وهو نوع من بكتيريا حمض اللاكتيك متعدد الاستخدامات و له نشاط مهم فى تثبيط نمو الميكروبات المرضية وتلك المسببة لفساد الأغذية.