Int. J. Food Ferment. Technol., **10**(1): 01-13, June 2020

DOI: 10.30954/2277-9396.01.2020.3

REVIEW PAPER

Lactic Acid Bacteria and their Metabolites as a Biopreservative in Fresh Produce

Ishwerpreet Kaur Jawanda*, Param Pal Sahota and Ritika Modi

Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India

*Corresponding author: ishwerpreet-mb@pau.edu

Paper No.: 1 **Received:** 19-03-2020 **Revised:** 17-05-2020 **Accepted:** 02-06-2020

ABSTRACT

Trends and globalized trade in fresh produce consumption is a challenge and an opportunity to the agri-food sector. An urgent pursuit to develop new efficient antimicrobial agents that show effectiveness across the distribution chain and food manufacturing units have flourished. Among the many strategies proposed for preserving minimally processed fruits and vegetables, bio-preservation is a promising tool. Therefore, the use of lactic acid bacteria and its metabolites (organic acids, hydrogen peroxide, antifungal peptides, and bacteriocins) appeal much interest during recent years. The adjuvant production of lactic acid during growth bestows LAB with notable selective advantages in the diverse ecological niches. Hence, their potential as preservatives in many food matrices appears to be enormous. Notably, authorized regulatory agencies are well for widespread commercial use, the agents remain small. Food bio-preservation is a novel method of preservation that is benignly an ecological approach with natural microflora and non-toxic biologically active compounds. In this review, we delineate several aspects of lactic acid bacteria, antimicrobial potential of potent metabolites, summarize the mechanisms of antimicrobial action and finally,recent potential applications in the curtailment of food borne pathogens.

Keywords: Antimicrobial, Bio-preservation, Fresh produce, Safety,Lactic acid Bacteria, Metabiotics.

Fresh produce is perceived by consumers to be wholesome, being a source of vitamins, nutrients, fibers, proteins, and antioxidants (Slavin and Lloyd, 2012). These "superfood" are a storehouse of vitality, which, with their delicate, subtle flavors and crisp textures, add to sensorial properties in salads.

Despite their host of nutritional advantages,the food-borne outbreaks have not waned even in the 21st century. Leafy greens like cabbage, lettuce, spinach that are potent vehicles for transmission of human pathogens were traditionally associated with foods of animal origin (Berger *et al.* 2010). Fresh produce is classified as "most perishable" goods in the market such as leaves (kale, lettuce), roots (beet, carrot), tubers (potato), and stems (celery). The exposure of contamination by bacteria, yeasts, molds also increases as the pH is generally found in range of (5.0-6.5) makes fresh produce as favorable habitats.

Three main factors contribute to the burden of reported cases. Foremost, the globalization pathway of the food and nutrition industry has undoubtedly contributed to the outbreaks across the continents, leading to infection by the same contaminated produce (Godfray *et al*. 2010). Second, to increase the food supply chain, the changes in the horticultural practices have burgeoned the risk of transmission of pathogens and even cross-contamination. Besides, advancement in the microbial detection, identification

How to cite this article: Jawanda, I.K., Sahota, P.P. and Modi, R. (2020). Lactic Acid Bacteria and their Metabolites as a Biopreservative in Fresh Produce. *Int. J. Food Ferment. Technol.*, **10**(1): 01-13.

Source of Support: None; **Conflict of Interest:** None

methods, bio-surveillance techniques (PCR panels, antigen-based assay) have contributed to the increase in the reported cases (Mangal *et al.* 2016).

Reducing the impact of spoilage micro-organisms becomes even more thought-provoking as consumer demands for fresh produce without chemical additives is increasing (Sharma and Joshi, 2019). The quest for new interventions in substitution to chemical additives is pivotal to ensure food quality and safety. Hence, food bio-preservation is a novel method of preservation, which is benignly an ecological approach with natural microflora and non-toxic biologically active compounds. Furthermore, as they entail natural cycles with minimum environmental impact, this fits in well with the notion of sustainable agriculture.

LACTIC ACID BACTERIA AND BIOPRESERVATION

Biopreservation employing probiotic Lactic acid Bacteria (LAB) implicates the antimicrobial complex composed of these non-pathogenic microorganisms; either by themselves or as a combination with their metabolites (bacteriocins) to enhance the microbial food safety due to their antagonistic properties against other undesired pathogenic bacteria (*Listeria*, *Clostridium*, *Staphylococcus*, and *Bacillus* spp.) (Arqués *et al.* 2015). Their potential as preservatives to enhance the shelf-life in many food grids turns out to be immense. Indeed, they are present naturally in food products and often provide strong competence by producing a broad array of antimicrobial metabolites such as active antimicrobials (organic acids, hydrogen peroxide, diacetyl, acetoin, reuterin, reutericyclin, antifungal peptides, and bacteriocins) (Collins *et al.* 2010). These protective cultures are live micro-organisms deliberately added to foods to control their bacteriological status without hindering the organoleptic properties.

The lactic acid bacteria aremicro-aerophilic bacilli/cocci, Gram-positive, non-sporulating, principally of the genera *Lactobacillus, Leuconostoc, Lactococcus, Pediococcus* and, *Streptococcus* (Bintsis, 2018). As the probiotic hypothesis states, their adherence to epithelial cells lead to colonization of the intestine, thereby entailing a fierce barrier against undesirable bacteria (Campana, 2017). Hence, these renowned starter cultures not only furnish"unique"flavor and aroma to fermented products but as wellin extending the shelf-life of the product by conversion of lactose to lactic acid (Khan *et al*. 2010). This controlled *in-situ* acidification contributes crucially to biopreservation.

Hence, the functional metabolites secreted by probiotics, designated as "metabiotics," "postbiotics," provide the advantage of being a safer and probably more effective strategy (Shaikh *et al*. 2017; Shenderov, 2013). With a determined chemical structure, these are the structural components of probiotic microorganisms and their metabolites. Metabolites of LAB comprises microbial cells, constituents and metabolites; bacteriocins (Table 1) and other low molecular weight (LMW) antimicrobial molecules, short-chain fatty acids,organic acids, polysaccharides, peptidoglycans, enzymes, antioxidants, peptides with various activities, amino acids and other (Shaikh *et al*. 2017; Shenderov, 2013).

Hence, the burgeoning of pathogenic bacteria resistant to antibiotics has led to an intensive investigation on probiotic LAB, and its metabiotics. Also, the early biotechnology tool development entailed the manipulation of the metabolic pathways to improve the efficiency of LAB as starters, adjunct cultures and probiotics. This review has been designed to delineate the antimicrobial possibility of potent metabolites produced by LAB. In this review, the recent literature on the potential of lactic acid bacteria as bioprotective culture in fresh produce, and their antimicrobial mechanism of action, potential applications in the inhibition of food-borne pathogens has been elucidated.

SOURCES AND ROUTES OF PATHOGEN CONTAMINATION IN FRESH-CUT PRODUCE

Fruits and vegetables are contaminated with pathogenic bacteria via myriad ways at various points through the pre-harvest and post-harvest steps (Fig. 1).

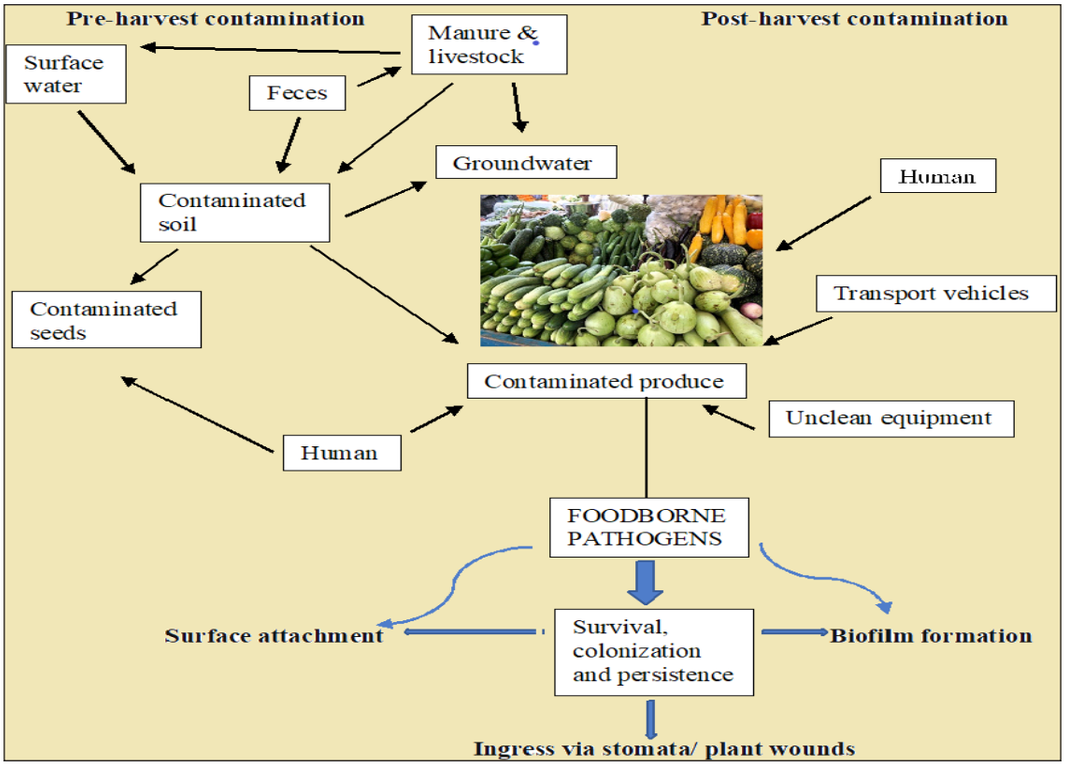

Fig. 1: Overview of pre-harvest and post-harvest contamination of produce

Studies underline that irrigation water,both method and timing of irrigations contributes to contamination of entero-pathogens (Olaimat and Holley, 2012). With spray irrigation, a higher risk of contamination has been observed as direct deposition onto the fresh produce occurs as compared to surface irrigation and drip irrigation. Studies show that on spray-irrigated lettuce leaves, *E. coli O157:H7*can prevail for 220 days. Furthermore, the internalization of *E. coli O157:H7* in leafy vegetables such as spinach and parsley are also observed (Erickson *et al*. 2014).

Numerous studies have examined the prevalence of fecal indicator organisms and specific food-borne pathogens in surface water and groundwater. A study by (Sood *et al.* 2017) observed that vegetable samples (n=420) growing around Buddha nallah, a natural stream of Sutlej (Punjab), have contaminated groundwater. Also, a high MPN index (upto 2400) was reported from water samples (Sahota *et al.* 2014), which accounted for high positive percentage of 98.5 (cucumber) and 91.2 (spinach).

Furthermore, increment in fresh produce contamination is caused by animal manure used as a soil amendment. Depending upon the soil characteristics (salinity, total nitrogen, etc.), humidity, and weather conditions, *E. coli O157:H7, Salmonella, Listeria monocytogenes, and Campylobacter jejuni* can survive around six months (Holley *et al.* 2006).

Several post-harvest unit operations cause inner tissue exposure to pathogens. Additionally, the washing/disinfectant/sanitizing steps furnish the fresh produce more prone to pathogen contamination. The release of nutrient exudate aids the proliferation of pathogenic bacteria as well as its attachment. The accelerated growth of *E. coli O157:H7* due to tissue damage in lettuce has been elucidated (Brandl *et al.* 2008). Notably, a distinct target for *Salmonella*'s proliferation are the cut leaf surfaces where

Escherichia coli O157:H7, Salmonella spp., and Listeria monocytogenes exist. A recent outbreak linked to pre-cut melons associated with *Salmonella* was reported (CDC 2019).

NEED FOR BIOPRESERVATION

In context to modern masses, consumeristic trends, and food safety legislation, food preservation poses a significant challenge. From resistance of food pathogens to current preservatives and conscious consumers seeking quality-rich, preservative-free foods with extended shelf-life has inflicted the need for safer alternatives.

To accentuate the shelf life of minimally processed fresh produce, many chemical, physical,and biological treatments have been suggested. To date, chlorine as a 'food sanitizer' for assessing fresh-cut fruits and vegetables (including sprouts) is a predominant treatment. However, it has limited antimicrobial activity as only 1–2 logarithmic reductions in pathogenic flora occur and it further deteriorates its organoleptic properties. The production of carcinogenic halogenated compounds (trihalomethanes, haloacetic acids) referred to as DBPs (Disinfection byproducts) from chlorine poses significant human health risks (Gadelha *et al*. 2019). This calls into question the use of chlorine as 'food sanitizer.' Physical alternatives, such as ionizing radiation, refrigeration, modification of atmosphere (MAP), pulsed-light, ozone and high hydrostatic pressure (HHP), etc. (Rico *et al*. 2007), causes an alteration in their delicate, subtle flavors and crisp textures.

Hence, to harmonize the imperative demands of the consumer and to balance the quality and safety of fresh produce, biopreservation is a promising tool. The novel isolates of LAB are the central pillar of this intervention and a key to reducing chemical additives. Interestingly, the microbial antagonism has been used in food processing grids to ameliorate fresh products, focusing as a natural means to control the shelf-life.

Notably, the application of LAB antimicrobial peptides i.e., bacteriocins as preservatives is not equally flourishing as their insight has dramatically surged during the last 30 years. The narrow inhibition spectrum, restrictive regulation concerning food additives, and efficacy of food constituents explain the lack of general industrial operation of bacteriocins. Nisin produced by *Lactococcus lactis* is in extensive use in over 48 countries as assessed by the Food and Drug Administration (FDA) (Ross *et al*. 2002). Hence, the influx of bacteriocins in the food grid by in situ production applying live LAB, the so-called 'protective bio-cultures,' is the need of the hour.

SELECTION CRITERIA OF BIOPROTECTIVE CULTURE

The norms for being protective and 'ideal' with high potential for application in foods (Holzafpel *et al.* 1995) are summarized as:

- Reputed Generally recognized as safe (GRAS);
- Survivability during product manufacturing and distribution;
- Low-temperature storage (refrigerator) and pH tolerant;
- No effect on intrinsic sensory factors (flavor, aroma, texture);
- Wide antimicrobial spectrum(inhibit the pathogenic flora in the food);
- Function as 'indicator' under abuse conditions
- Non-toxic to human life; and
- Noinfluence on other intrinsic attributes.

Lactic Acid Bacteria

Lactic acid bacteria (LAB) have traditionally been associated with human culture and well-being throughout history. The preservative effect is due to probiotic LABs in combination with their metabiotics. The method of glucose fermentation under standard conditions is an important aspect that differentiates the LAB genera. Active functional metabolites, like"organic acids (lactic, acetic, methanoic, propionic, and butanoic acids) step up the action by reduction of the pH of the media, and substances such

as (Refer Fig. 2). Notably, various other mechanisms being suspected to be convoluted in the inactivation of the pathogens are discussed further in the review.

Fig. 2: Antimicrobial substances of Lactic acid bacteria

While in homofermentative bacteria, lactic acid is the end product through the "Embde–Meyerhof–Parnas (EMP) pathway" (Fig. 3a), heterofermentative bacteria (Fig. 3c) produce equimolar quantities of lactic acid, carbon dioxide, and ethanol or acetate through the "phospo-ketolase pathway" (Mozzi, 2016). Additionally, other interesting metabolites as antibacterial compounds (e.g., bacteriocins) (Hugenholtz *et al.* 2002), aroma compounds (diacetyl, acetoin, etc.) (Fig. 3b), vitamins, exopolysaccharides (EPS), low-calorie sugars (e.g., mannitol), short-chain fatty acids, and γ-aminobutyric acid (GABA) are also produced' (Stiles, 1996).

Moreover, LAB survives under cold storage temperatures and used directly as food additives, as their fermentation products or purified metabolites instead. Studies show that LAB reduces cholesterol (Jeun *et al.* 2010), increase the nutritional value of food, control intestinal infections and improve digestion because LABs produce lactase in the digestive tract of humans and animals.

One important characteristic of LAB is its ability to produce antimicrobial compounds bacteriocins that inhibit the growth of pathogenic micro-organisms;therefore, it can be used as

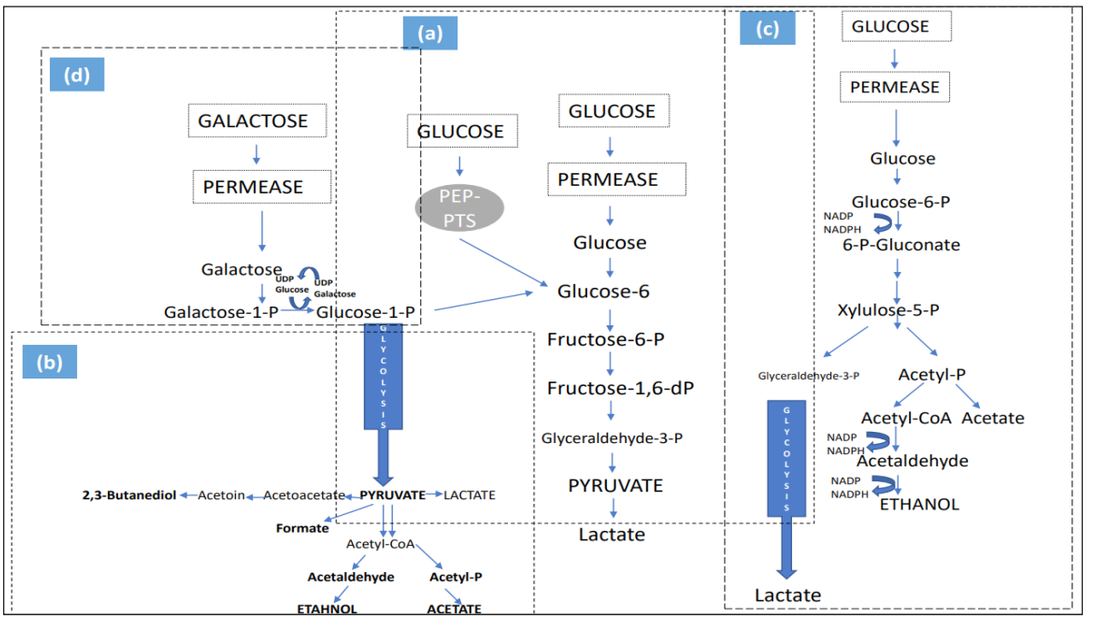

Fig. 3: Pathways of glucose metabolism. **(a)** Homofermentative pathway. **(b)** Mixed-acid metabolism. **(c)** Heterofermentative pathway. (d) Leloir pathway. (**Source:** Adapted from Mozzi *et al.* 2015: Biotechnology of LAB)

bio-preservatives. There are several classes of bacteriocins i.e., simple peptides or proteins, and others contain lipid molecules. They act as bactericidal or bacteriostatic agents against other bacteria. Being degraded by proteolytic enzymes, they hinder the growth of microorganisms that are phylogenetically close to bacteria that produced bacteriocins. The bacteriocins produced by LAB can inhibit the growth of pathogenic microbes and those involved in decomposition such as *Bacillus cereus, Clostridium botulinum, Clostridium perfringens, Listeria monocytogenes,* and *Staphylococcus aureus*.

The application of bacteriocins in food does not affect the taste and appearance of the product. Bacteriocins produced by LAB can be utilized in the form of supernatant, partially purified, or more wholly purified products. Bacteriocins are commonly used in the food industry, especially in fermented foods, to inhibit the growth of bacterial contaminants. The antimicrobial compounds may affect bacterial metabolism and toxin production (Rolfe 2000).

Mechanisms of Action: Protective lactic acid bacteria exercise their antibacterial activity majorly by three mechanisms: displacement/exclusion (extinction), competition for space, and nutrients and production of a wide range of antimicrobial metabolites (Fig. 4).

Displacement/exclusion: The probiotic LABs adhere firmly to surfaces,displace pre-adhered pathogenic flora and survive there for a prolonged period.

Competition for nutrients and space: Microorganisms populating a given environment must contend for nutrients, space, and other resources. Prior research substantiates that microbes have derived a large competency for phenotypic variation, therefore letting them to secrete a variety of metabolites and molecules (e.g., proteases and siderophores) outcompeting their neighbors. Bacterial growth may be deterred by limited amounts of minerals, amino acids, or sugars despite its nutrient richness. The Jameson effect (concurrent deceleration of all microbial populations) and the Lotka-Volterra competition are two models of growth competition for bacterial. In context, lactic acid bacteria outcompete *Listeria* for essential nutrientsas elucidated in meat products (Cornu *et al.* 2011).

ANTIMICROBIAL MECHANISMS OF LAB/ PRODUCTION OF METABOLITES

The plethora of combined mechanisms by several metabolites produced during the fermentative pathways result in antimicrobial activity (Caplice and Fitzgerald, 1999).

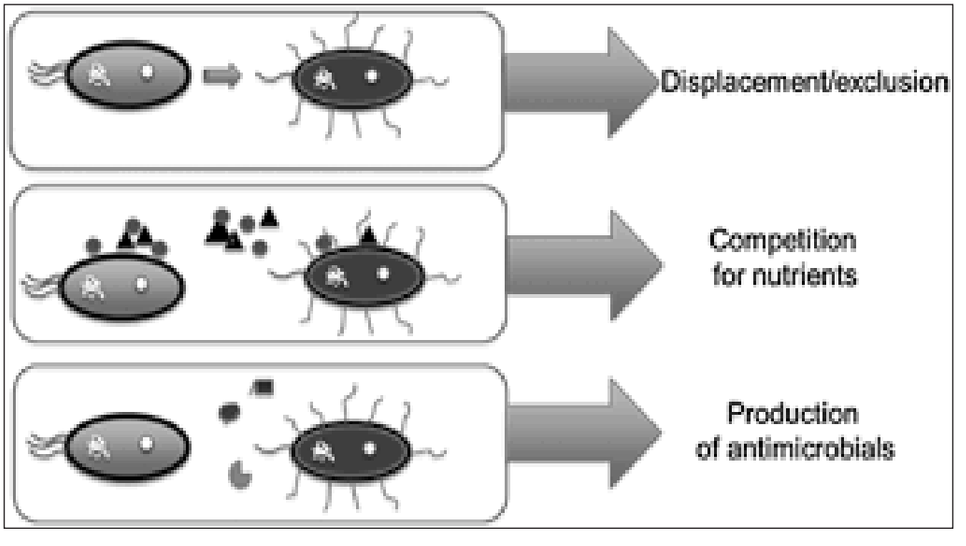

Fig. 4: Three main mechanisms process of antimicrobial activity (**Source:** Campana 2017/ Creative Commons CC BY *Copyright © 2017, Springer Nature*)

Organic Acid

An antagonistic effect on the pathogenic microflora is exhibited by acid production, such as cytoplasmic acidification. The mechanism of inhibition of the active transport, uncoupling of energy production, the modifying of their membrane potential has been elucidated (Cleveland *et al*. 2001).According to Ciarlo *et al*. (2016), the organic acids as lactic, acetic, and propionic acids are six-carbon compounds that accentuate the host from microbial infections. Interestingly, organic acid are treated as best antimicrobials against *Salmonella* (Mani-López *et al*. 2012).

Hydrogen peroxide

Over a wide range of temperature, pH, and variety of carbon and nitrogen sources, H_2O_2 is produced by LAB. Studies prove that strains that produce H_2O_2 inhibit the growth of pathogenic psychrotrophic bacteria at low temperatures(Reis *et al.* 2012). H_2O_2 shows a bactericidal effect against emerging foodborne pathogens such as *Aeromonas hydrophila* (Ito *et al*. 2003). Absence of catalase enzyme results in hydrogen peroxide (H_2O_2) accumulation, which inhibits pathogens and implicates LAB as bioprotective (Caplice and Fitzgerald, 1999).

Carbon dioxide

Carbon dioxide production occurs through the hetero-fermentative pathway. At higher concentrations, it retards bacterial growth and popularly used in MAP (Modified Atmospheric Packaging) and, thus, useful in extending the shelf life of perishable foods (Daniels *et al*. 1984). An anaerobic micro-environment is created, which is toxic to some aerobic bacteria (Cleveland *et al*. 2001).

Diacetyl (2,3-butanedione)

Diacetyl, an aromatic compound with a butteryodor, is produced by *Streptococcus*, *Leuconostoc*, *Lactobacillus*, Pediococcus. By interference with arginine utilization in the periplasmic space, diacetyl inhibits *Listeria, Salmonella, Yersinia, E. coli,* and *Aeromonas* (Ammor *et al*. 2006).

Bacteriocins

Bacteriocins are divided into four groups (Table 1). The four recognized classes are:

Class 1 includes Lantibiotics: Peptides containing modified amino acids; Nisin is the best-characterized bacteriocin formed by *Lactococcus lactis* strains. A few LAB strains produce protein compounds with a significant antimicrobial effect, which are

Table 1: Classification of bacteriocins

Class	Subclass	Description	Examples
I Lantibiotics, Heat stable	**Ia**	Cationic and hydrophobic peptides	Nisin
	Ib	Globular peptides	Mersacidin, Cinamycin
	Ic	Non-active lantibiotics	Sap B
II Small, heat- stable, non-lanthionine, membrane-active peptides	**IIa**	Pediocin like bacteriocin	Pediocin PA1, Sakacin A
	IIb	Two component bacteriocin	Lactococcins G and F
	IIc	Multicomponent	Enterocin EJ97
III Large heat-labile proteins	**IIIa**	Bacteriolytic	Enterolysin A
	IIIb	Non-bacteriolytic	
IV Heat stable, circular peptides		An undefined mixture of proteins, lipids, and carbohydrates	AS-48, gassericin A, acidocin B

Source: *(Adapted from Verma et al. 2014: Encyclopedia of food microbiology).*

Table 2: Studies with LAB and biopreservation of fresh produce

Food Matrix	Lactic acid bacteria	Target pathogen	Process duration	Effect	References
Fresh-cut pear	*Lactobacillus rhamnosus* GG	*Salmonella spp*. and *L. monocytogenes*	Nine days at 5◦C	Reduction (approx. 1.8 log units) in *L. monocytogenes*	Iglesias *et al.* 2018
Mixed salads	*Lactobacillus casei* IMPCLC34	*Aeromonas hydrophila, Salmonella spp., Staphylococcus spp., Listeria spp.*	Six days	Reduction in target pathogens except *Listeria spp.*	Vescovo *et al.* 1996
Fresh-cut cantaloupe/ melons	*Lactobacillus plantarum B2, Lactobacillus fermentum PBCC11.5*	*L. monocytogenes*	Eleven days	Reduction of *L. monocytogenes*	Russo *et al.* 2015
Fresh-cut fruit mixture	*Lactobacillus pentosus* MS031 (pentocin MS1 and pentocin MS2)	*E. coli, S. aureus, L. monocytogenes*	Ten days	96.3% reduction of *L. monocytogenes*	Yi *et al.* 2020
Fresh leafy greens (parsley, lettuce, spinach, etc.)	*Pediococcus pentosaceus* Pediocin DT016	*L. monocytogenes*	Fifteen days	Significant inhibition *L. monocytogenes* by a difference of 1.4 log/CFU	Ramos *et al*. 2020
Bananas	Enterocin KT2W2G-cinnamon oil combination at 6:4	*Klebsiella variicola, Lactococcus lactis subsp. Lactis, K. pneumoniae, E. faecalis*	—	Inhibition of pathogenic bacteria	Issouffou *et al*. 2018
Mungbean sprouts	*Enterococcus mundtii*	*L. monocytogenes*	Dipped for 30 mins	2 log reduction (CFU/g) of *L. monocytogenes*	Bennik *et al.* 1999
Cabbage	Crude bacteriocin extracts (*Lactobacillus* A, B, C)	*S. aureus, E. coli, Salmonella, Pseudomonas spp., Shigella spp.*	Three days	Inhibition zone diameter of (19 mm) *S. aureus and E. coli, Shigella spp.* (10 mm)	Orji *et al.* 2020
Potatoes	Nisin-formic acid combination	*Bacillus subtilis*	Ten days	Inactivation of the *Bacillus subtilis*	Ajingi *et al.* 2020
Fresh strawberries, tomatoes, and mushrooms	Bacteriocin, producing by *Pediococcus* spp.	*E. coli* and *Shigella spp.*	Fifteen days	Increased shelf-life and enhanced microbiological quality	Skariyachan *et al.* 2019

bacteriocins. Highly specific, like lactococcins, have a wide antimicrobial spectrum, like *Nisin*. The mechanism of action is the disruption of membrane function by pore formation in the bacterial cell membrane (Reis *et al*. 2012).

Class 2. The small non-lanthionine bacteriocins. Class II comprises minimally modified, heat-stable bacteriocins. The anti-listerial bacteriocin pediocin is a member of this group. Class IIa includes Pediocin like *Listeria* active peptides with a consensus N-terminal sequence Tyr-Gly-Asn-GlyVal and two cysteines forming a S-S bridge in the N- terminal half of the peptide (Eijsink *et al.* 1998).

Class 3. The larger, heat-labile bacteriocins. Inactivated at 60–100 °C (10–15 min), which includes helveticins V, helveticins J, acidofilicin A, and lactacins A and B.

Class 4. Large complex bacteriocins are carrying lipid or carbohydrate moieties.

Notably, Classes, I and II are the focal points for most of the probiotic research.

APPLICATION OF PROTECTIVE CULTURES IN FOOD PRESERVATION

Over the years, anextensive use of bioprotective agents/ cultures as a 'green benefit' has been extended. The probiotic LAB and its metaboliteshave shown evidential antibacterial activity *E. coli, L. monocytogenes, Shigella sonnei, and Salmonella typhimurium*. For instance, the shelf-life of fresh produce and salad dressings is upto a couple of weeks but with fresh-cut produce only for 4-10 days under refrigerated conditions. The use of LAB as a biopreservation approach can be enhanced in combination with other types of methods. For example, low oxygen Modified Atmosphere Packaging (MAP) is a well-proclaimed way enforced for fresh produce storage for spanning their period of validity (Oliveira *et al.* 2015). One method employed by Dong *et al*. (2020) for reducing pathogenic *L. monocytogenes* in cabbages was an aggregation of *Lactobacillus plantarum subsp. plantarum CICC 6257* with low oxygen MAP.

Notably, *Lactobacillus casei* IMPC LC34 synergistically with culture filtrate and lactic acid was used against pathogens in RTU salad vegetables. A reduction of 5 log CFU/g in total mesophilic bacteria count was reported with a 3% culture to permeate after storage of 6 days at 8°C (Torriani *et al.* 1997). In another study, using agar spot assay, *Leuconostoc spp., Lactobacillus plantarum, Weissella spp.* and *Lactococcus lactis*were inoculated on wounded Golden Delicious apples and also on Iceberg lettuce leaves. Consequently, the reduction of *S. Typhimurium* and *E. coli* by 1 to 2 log CFU/wound or g,and total inhibition of *L. monocytogenes* was reported (Corbo *et al.* 2015).

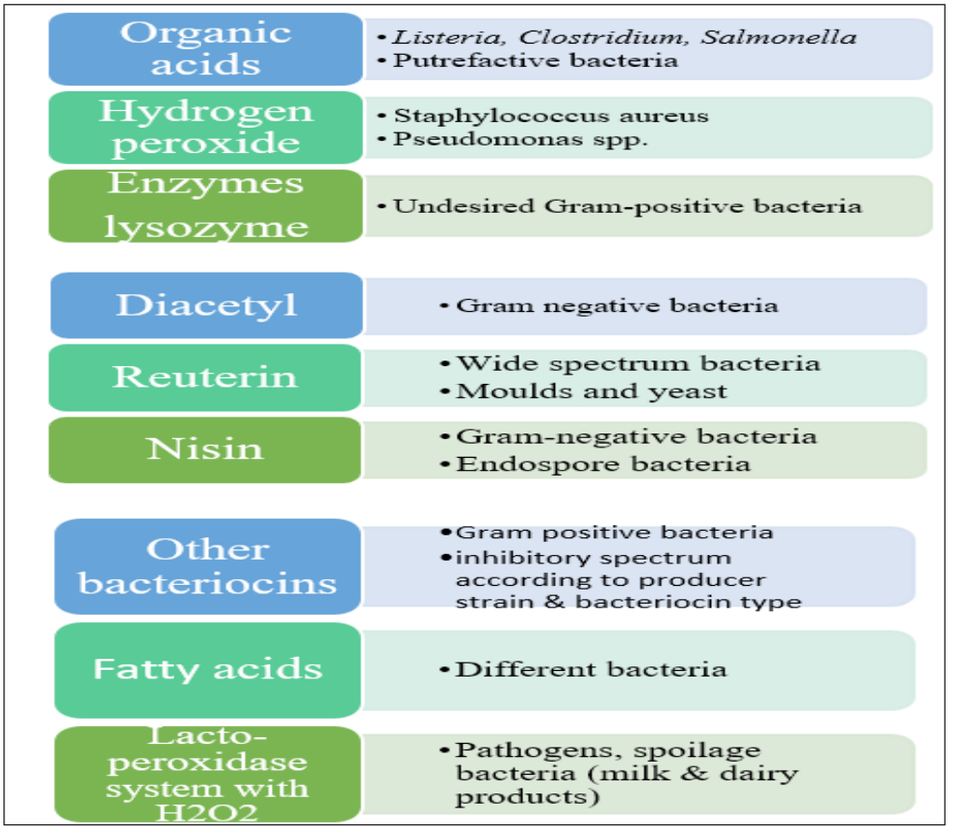

Fig. 5. Metabolic products of lactic acid bacteria with antimicrobial properties. (***Source:*** Modified from Holzapfel *et al.* 1995)

In a more recent study, Li *et al*. (2020) concede that with a suspension of *L. plantarum* sprayed on fresh lotus roots, a transformation of 84.17% catechin after contact time with plant skin for 30 h was reported. The study was conducted to evaluate the post-harvest factors of lotus roots and limit the oxidation of phenolic compounds that leads to enzymatic browning reaction. Notably, texture such as hardness and cohesiveness improved. Thus, contributing to the efficacy of lactic acid bacteria as an approach to extend the shelf life of fresh lotus roots. "Postbiotics/ Metabiotics" also cause the reduction of foodborne pathogens in the fruit juices (Tenea & Barrigas, 2018).

Furthermore, the presence of competitive flora, especially LAB, and growth characteristics of *L. monocytogenes,* was evaluated on fresh-cut salads and the commonly used ingredients alongside them. Most products did not show any presence of *L. monocytogenes* greater than 3.4 log CFU/g. Interestingly, only the Galia melon exceeded the mean population of 3.4 log CFU/g, which is the main ingredient in fruit salads, indicates the root cause of *L. monocytogenes* contamination. The effect of native competitive microflora, especially LAB, was reported to inhibit the *L. monocytogenes* some components of fruit salads, such as non-pasteurized potatoes, white cabbage, and mango (Lokerese *et al*. 2016).

A more recent study by Ramos *et al*. (2020) emphasized on an alternative approach to examine the bacteriocinogenic LAB, *Pediococcus pentosaceus DT016,* against *L. monocytogenes* to maintain the safety of fresh vegetables. Interestingly, a significantly low number of pathogens ($p < 0.01$) in vegetables inoculated with *P. pentosaceus DT016* was detected and a difference of 1.4 log CFU/ g was substantiated in the two. Antagonistic effect of *Pseudomonas graminis* CPA-7 was successfully established against two prevalent pathogens (*Salmonella spp.* and *L. monocytogenes*) in fresh-cut apples, peaches (Alegre *et al.* 2013) and, melons (Abadias *et al*. 2014).

In another study, *Enterococcus mundtii* and *Pediococcus parvulus* were evaluated for their potential to inhibit *L. monocytogenes* on refrigerated mung bean sprouts under modified atmosphere packaging (MAP). These two strains can produce a bacteriocin (pediocin-like mundticin). Notably, *E. mundtii* was favored as a higher maximum growth rate was exhibited compared to *P. parvulus* strains at CO_2 concentrations (20%) suitable for refrigerated storage (4 to 8°C) of vegetables under MAP. Subsequently, the *E. mundtii* strain was then evaluated further for in situ control of *L. monocytogenes* on mung bean sprouts stored under specific conditions 1.5% of O_2, 20% of CO_2, 78.5% of N2 at 8°C. The application of pure mundticinwas reported to be effective at a concentration of 200 AU/ ml as a coating/washing with an alginate film against *L. monocytogenes* (Bennik *et al.* 1999).

A cyclic antimicrobial peptide producing strain *Enterococcus faecalis* A-48 was evaluated as a protective culture against *B. cereus*. A reduction of 1.0–1.5 log units in viable cell count was observed after the treatment at 6°C but not at other storage temperatures. Furthermore, inhibition by co-inoculation with the AS-48 producer strain *E. faecalis* A-48-32 on soybean sprouts showed bacteriocin production at 15 and 22 °C. In both cases, bacteriocin activity was observed up to 72 hours of storage but not after more extended periods. This study proved that combination treatment should be preferred under temperature-abuse conditions (CoboMolinos *et al.* 2008).

ADVANTAGES AND LIMITATIONS

Lactic acid bacteria being a food preservative of natural origin makes it the most beneficial approach for minimizing the impact of pathogens on food. It further endows low economic losses by enhancing the fresh-cut produce's shelf-life. Also, it is labelled with GRAS status and already predominate the microbial flora of many fermented foods making it considerable for biopreservation. Another advantage of their use is that they can be either used by 'incorporation during production or by dipping, surface coating, or spraying of finished products' (Ghanbari *et al*. 2014). These cultures can have both quantitative and qualitative effects on the microflora. Bioprotective cultures can also show adverse effects on end-product quality, notably by inhibiting/ competiting with

starter cultures essential for fermentation (Oumer *et al*. 2001). Besides, activity of the antimicrobial compounds produced by protective cultures in situ can be lost due to interaction with food components such as nitrites, lipids, and proteolytic enzymes. Inhibition is generally limited to specific pathogens; although, a few bacteriocins have broad spectra of activity. As most foodborne pathogens are typically Gram-negative bacteria, the problem can be due to the resistant nature of gram-negative bacteria to bacteriocins of LAB. At the same time, some bacteria can show resistance to specific bacteriocins through prolonged exposure to bacteriocins (Bastos *et al*. 2015).

CONCLUSION

Protective cultures of LAB and metabolites produced, are pivotal for biocontrol, as their potential inhibitory efficacy against pathogens is well documented, without altering the organoleptic properties of fresh produce. Interestingly, primary metabolites, bacteriocins, in situ after injection to the fresh produce, prove to be reliable and environmentally friendly. Furthermore, characteristics of the strain culture in use, their commercial viability, the application dose, and the compound mechanisms of action needs modulation. As metabolites through various means impart a plethora of health benefits, this urges the need to deeply study the mechanisms of action at the molecular level to claim the benefits. The condition of the hour is to evaluate the effectiveness of bio-preservative on these complex food matrices having varied components. In this context, a plethora of antimicrobial metabolites of LAB can offer us good benefits in abundance and a promising access to the streaming food-related issues.

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