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Recent approaches in food bio-preservation - a review

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Abstract

Bio-preservation is a technique of extending the shelf life of food by using natural or controlled microbiota or antimicrobials. The fermentation products as well as beneficial bacteria are generally selected in this process to control spoilage and render pathogen inactive. The special interest organism or central organism used for this purpose is lactic acid bacteria (LAB) and their metabolites. They are capable to exhibit antimicrobial properties and helpful in imparting unique flavour and texture to the food products. The major compounds produced by LAB are bacteriocin, organic acids and hydrogen peroxide. Bacteriocin is peptides or proteins with antimicrobial activity. On the basis of size, structure and post-translational modification, bacteriocin is divided into four different classes. Due to non-toxic, non-immunogenic, thermo-resistance characteristics and broad bactericidal activity, LAB bacteriocins are considered good bio-preservative agents. The most common LAB bacteriocin is nisin which has wider applications in food industry and has been Food and Drug Administration (FDA) approved. Nisin and other bacteriocin are being used in vegetables products, dairy and meat industries. Apart from LAB metabolites, bacteriophages and endolysins has promising role in food processing, preservation and safety. Bacteriocins and endolysins are more suitable for DNA shuffling and protein engineering to generate highly potent variants with expanded activity spectrum. Genetically modified bacteriophages may also be helpful in bio-preservation, however; their safety issues must be addressed properly before selection as bio-preservative agent.

Keywords: Bacteriocins, Bacteriophages, Bio-preservation, Endolysins, Lactic acid bacteria.

Introduction

The foods of animal origin are highly perishable due to high nutritional content, moisture and neutral pH. These foods require proper preservation to maintain quality and safety. Failing which leads to human illnesses and disease outbreaks. These food borne illnesses are serious and costly public health concern worldwide. So to maintain the quality and safety of foods various measures are generally adopted in food industry i.e. good manufacturing practices, good hygienic practices etc. But preservation of food by a suitable means is the key of food quality and safety. There are number of preservation techniques started from low temperature preservation like refrigeration, freezing etc. and thermal preservation techniques like pasteurization, sterilization and preservation using certain chemicals. Now a day modern preservation techniques like bio-preservation, irradiation and hurdle technologies are also common. In traditional preservation techniques food alters their status and loss some nutrients. So the modern techniques are more suitable to achieve the safety and quality of foods. It is also relevant in today world due to globalization of food market, introduction of novel foods, innovations of new technologies, demand of minimally processed products with ready-to-eat quality and longer shelf life. Among

all the preservation techniques adopted now bio-preservation is more reliable from 'farm to fork' concept. It is capable to enhance shelf life with great quality, hygienic status with minimal nutritional and organoleptic losses.

Bio-preservation is a technique of food preservation in which antimicrobial potential of naturally occurring organisms and their metabolites are exploited. It is capable to harmonize and rationale the necessary safety standards with traditional means of preservation and modern demand of the food safety and quality. The bio-preservation techniques of various foods are mainly relying on the quality of biological antimicrobial systems such as lactic acid bacteria (LAB) and/or their bacteriocins, bacteriophages and bacteriophage-encoded enzymes. They are widely used in food industry to get a typical texture and flavour of the food products. They are helpful in maintaining the quality and safety of foods and are common bio-preservatives in industrialized world.

Biological methods for food preservation

The use of microbiota and/or antimicrobials in shelf life enhancement of foods is a new branch of science. The fermentation is a typical example of this process in which microbes are grown naturally or by addition. Fermentation process produces numbers of beneficial

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products with the bacteria which helps in reduction of food spoilage and renders the food free from pathogenic microorganisms and metabolites (Ganguly, 2013). This process is gradually gaining popularity due to its ecologically benign approach. The main organism used for the purpose is LAB and their compounds like organic acids which are capable to exert antimicrobial properties as well as imparts unique flavour and texture to the food products (Lucera *et al.*, 2012). Number of food products is preserved by fermentation since time immemorial. In industrialized world about 60 percent food items are subjected to fermentation to assure homogeneity, quality, and safety of products. They prefer to use native microbiota under controlled environment to get typical texture and flavour for a specific product. The major use of microbiota in food industry is in dairy industry but they can also be exploited in meat and vegetable products. Bio-preservatives eligible for use in food must fall under the category of generally recognized as safe with no pathogenic and toxic effect on food. Biological agents used in food may be categorized as starter cultures and protective cultures. Starter cultures are generally used group of microorganisms which are used to initiate the fermentation and helps in production of certain compounds responsible for typical texture and flavouring compounds in fermented products. The protective culture is mainly used to control the antimicrobial activity and reduces the survival and growth of pathogenic micro-organisms in foods. However, combination of both starter and protective culture is generally preferred for use in food industry.

Natural antimicrobials for food bio-preservation

Natural antimicrobials for food bio-preservation are shown in Table 1.

Lactic acid bacteria (LAB)

LAB is generally includes *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Carnobacterium*, *Aerococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* genera of micro-organisms. All these micro-organisms are Gram-positive, nonmotile, non-sporeforming, rod- and coccus-shaped organisms and are capable to ferment carbohydrates. The chief product obtained after fermentation is lactic acid. Composition wise LAB contains low proportions of G+C in their DNA (<55%) which provides more thermostability to the organism. Physiologically LAB possess unique properties like resistance to bacteriophages, proteolytic activity, lactose and citrate fermentation, production of polysaccharides, high resistance to freezing and lyophilization, capacity for adhesion and colonization of the digestive mucosa, and production of antimicrobial substances.

The basic function of LAB is in food fermentation and recognized as GRAS (generally recognized as safe). LAB can be utilized in production of various fermented products of dairy, meat and vegetable. It helps in protection of nutritional qualities of raw material, enhances the shelf life of food and protects food against spoilage and pathogenic organisms.

Major compounds produced in LAB fermentation are organic acids, hydrogen peroxide and bacteriocins. Additionally LAB can be utilized in production of probiotics.

The genera *Lactobacillus* and *Bifidobacterium* are of major concern in this field and are mainly obtained from human gastrointestinal tracts. Other probiotic organisms may be summarized as *E. faecium*, *E. faecalis*, *S. thermophilus*, *L. lactis* subsp. *lactis*, *Le. mesenteroides*, and *P. acidilactici*.

Bacteriocins

Bacteriocins are bacterial ribosomally synthesized peptides or proteins with antimicrobial activity. Initially bacteriocins are mainly described to the colicins which are relatively large proteins of up to 80 kDa and were primarily obtained from *E. coli*. They were capable to kill very closely related bacteria upon binding to the inner membrane or other cytosolic targets (García-Bayona *et al.*, 2017).

However, today world is recognizing the bacteriocins as small, heat-stable cationic peptides synthesised by Gram positive bacteria called LAB and possess wider spectrum of inhibition (Mokoena, 2017). Traditionally LAB is regarded as safe for use in food so the food bio-preservation is mainly rely on the use of LAB bacteriocins. The classification of bacteriocins in major classes with their quality characteristics are listed in Table 2.

LAB bacteriocins

Bacteriocins produced by LAB which are capable to kill closely related micro-organisms. Bacteriocins produced by LAB are thermostable cationic molecules contains up to 60 amino acid residues and hydrophobic patches. The effectivity of LAB bacteriocin is mainly due to electrostatic interactions with negatively charged phosphate groups on target cell membranes through initial binding, forming pores and killing the cells leads to lethal damage and autolysin activation to digest the cellular wall. But by nature these bacteriocins are inactivated by proteases in the gastrointestinal tract. However, LAB bacteriocins are considered good bio-preservative agents due to non-toxic, non-immunogenic, thermo-resistance characteristics and broad bactericidal activity. These bacteriocins are most effective against Gram-positive bacteria and some, damaged, Gram-negative bacteria including various pathogens such as *L. monocytogenes*, *Bacillus cereus*, *S. aureus*, *Salmonella* etc.

Table 1. Natural antimicrobials for food bio-preservation.

	Antimicrobials	Source	Food bio-preservation
Antimicrobial substances derived from bacterial cell metabolism	Organic acids	Main end products of fermentation.	Decrease the pH of surrounding environment, creating a selective barrier against non-acidophiles. Lactic acid exerts antimicrobial effect by disruption of the cytoplasmic membrane and interference with membrane potential.
	CO ₂	Heterofermentative LAB produces CO ₂ as a byproduct of sugar fermentation.	Creation of an anaerobic environment and antagonistic effects specifically against aerobic bacteria and produce carbonic acid.
	Diacetyl (2,3-butanedione)	LAB as a by-product of metabolic activity	Antibacterial activity against <i>Listeria</i> , <i>Salmonella</i> , <i>Escherichia coli</i> , <i>Yersinia</i> , and <i>Aeromonas</i> .
	Hydrogen peroxide	Produces by LAB in presence of oxygen and action of flavoprotein oxidases or NADH peroxidase.	Antibacterial effect through oxidative damage of proteins and increase of membrane permeability.
	Reuterin	Low molecular- weight antimicrobial compound produced by <i>Lactobacillus reuteri</i> and some other LAB.	Antimicrobial activity against bacteria as well as yeasts and molds by inhibiting DNA synthesis.
	Reutericyclin	Reutericyclin-producing strains of LAB.	Reutericyclin acts as a proton ionophore and dissipation of the proton motive force against gram-positive bacteria including <i>Lactobacillus</i> spp., <i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>Enterococcus faecalis</i> , <i>Staphylococcus aureus</i> and <i>Listeria innocua</i> .
Antifungal compounds	Natamycin (pimaricin)	Produced from <i>Streptomyces natalensis</i>	Broad-spectrum antifungal biopreservative for foods and beverages by binding irreversibly to the cell membrane of fungi and causes membrane hyperpermeability leading to rapid leakage of essential ions and peptides and ultimately cell lysis.
Antimicrobials from Animal Sources	Lysozyme	Naturally found as part of defense system of living organisms.	Generally recognized as safe (GRAS) for direct addition to foods.
	Lactoferrin	Natural protein found in milk and other secretions.	Antimicrobial activity due to its iron-binding capacity and polycationic nature against a wide range of bacteria including foodborne pathogens like <i>Carnobacterium</i> , <i>Listeria monocytogenes</i> , <i>Escherichia coli</i> , <i>Klebsiella</i> and viruses (Lønnerdal, 2011; Gyawali and Ibrahim, 2014).
	Lactoperoxidase	Antimicrobial system that originated from milk.	Effective against gram-negative bacteria.
	Ovotransferrin	Produced by hydrolysis of natural proteins.	Inhibits bacterial growth due to iron deprivation.
	Protamine	Naturally present in spermatic cells of fish, birds and mammals.	Broad antimicrobial activity against gram-positive bacteria, gram-negative bacteria, and fungi. Used as preservative in wide variety of foods ranging from confection items to fruits and rice.
	Pleurocidin	Present in myeloid cells and mucosal tissues of many vertebrates and Invertebrates.	Antimicrobial activity against several foodborne bacteria such as <i>L. monocytogenes</i> and <i>E. coli</i> O157:H7, and pathogenic fungi.
	Chitosan	Polycationic biopolymer naturally present in exoskeletons of crustaceans and arthropods.	Considered as safe food additive and has antibacterial activity against both Gram-positive and Gram-negative bacteria such as <i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i> , <i>Bacillus cereus</i> , <i>E. coli</i> , <i>Shigella dysenteriae</i> , and <i>Salmonella typhimurium</i> (Gyawali and Ibrahim, 2014). Used in biodegradable edible coatings, singly or dosed with other antimicrobial substances (Elsabee <i>et al.</i> , 2013).
Antimicrobials derived from plants	Essential oils such as saponins, flavonoids, carvacrol, thymol, citral, eugenol, linalool, terpenes, and their precursors	Plant material like flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots.	The concentration of 0.05–0.1% of essential oils has demonstrated activity against pathogens, such as <i>Salmonella typhimurium</i> , <i>E. coli</i> O157:H7, <i>L. monocytogenes</i> , <i>B. cereus</i> and <i>S. aureus</i> in food systems.

Table 2. Classification of bacteriocins

Class	Nomenclature	Qualities	Examples	Reference
Class-I	Post-translationally modified peptides called Lantibiotics	Characterized by distinctive thioether-based intramolecular rings of lanthionine and β -methyl-lanthionine	Nisin, discovered in 1928, lactacin <i>L. lactis</i> , citolysin of <i>E. faecalis</i> , and lactacin of <i>L. lactis</i>	Xie and van der Donk, 2004
Class-II	Thermostable, non-modified non-lantibiotic linear peptides of <10 kDa. It is recognized as largest class among Gram positive bacteriocins and further divided into three subclasses based on distinctive N-terminal sequence. i. Class II-1-Pediocin-like bacteriocins ii. Class II-2 lacks leader peptide iii. Class II-3 other than above.	Characterized By short cationic peptides with high isoelectric points. It contains potent-antilisteria activity	Pediocin PA-1/AcH produced by <i>Pediococcus</i> , Enterocin EJ97 by <i>E. faecalis</i> and Enterocin L50A by <i>E. faecalis</i> .	Breukink <i>et al.</i> , 1999
Class-III	Comprises large (> 30 kDa) heat labile proteins like colicin- V and microcins.	Bacteriocins are Gram negative circular peptides characterized by a peptide bond between the C- and N-terminus. It possesses the bacteriolytic extracellular enzymes like hemolysins and muramidases which can mimic the physiological activities of bacteriocins.	Helveticin J of <i>L. helveticus</i> and bacteriocin Bc-48 of <i>E. faecalis</i> .	Wiedemann <i>et al.</i> , 2001
Class-IV	Circular peptides possess intriguing and novel type of antimicrobial substances produced not only by bacteria but also by plants and mammalian cells.	Characterized by a peptide bond between the C- and N-terminus are clustered. They are existed in form of head-to-tail peptide chain ligation, which makes thermomolecules with neither an origin nor an end.	Enterocin AS-48	Martínez <i>et al.</i> , 2008

The genetic manipulation in these bacteriocins is easy to perform due to presence of genetic determinants in plasmid thus variety of natural peptide analogues with desirable characteristics can be produced in shorter period of time. The most common LAB bacteriocin is nisin which has wider applications in food industry. Food and Drug Administration (FDA) has approved the use of nisin like bacteriocins produced from GRAS microorganism like *Lactococcus lactis* into various types of processed cheese, dairy products and canned foods. It is proven fact that Nisin is quite effective against Gram-positive spoilage microorganisms and food-borne pathogens such as *L. monocytogenes*.

Use of bacteriocins in food systems

The bacteriocins in general have broad spectrum activity against number of micro-organisms. The application of bacteriocins, particularly nisin, in food systems is influenced by inadequate physical conditions and chemical composition of food including

pH, temperature, nutrients, etc. It is also affected by spontaneous loss in production capacity, inactivation by phage of producing strain and antagonism effect of other microorganisms in foods. The negative factors in its effectivity include resistance against certain pathogens, biological activity interference by certain environmental conditions, higher retention of bacteriocin molecules like fat by food system components, inactivation by other additives, slower diffusion and solubility and/or irregular distribution of bacteriocin molecules in the meat matrix. The prerequisites for use of bacteriocins in food are its production from GRAS micro-organisms, high specific activity of bacteriocins, thermostability, capability to enhance quality and flavour. Bacteriocins must be safe to human health and bio-preservation must fulfill the GRAS regulations. The common bacteriocin named nisin was first time used in 50's to inhibit the outgrowth of *Clostridium tyrobutyricum* responsible for late

cheese blowing. However, the major role of bacteriocin is in inhibition of pathogenic and spoilage bacteria during food processing. Nisin has an immediate pH dependent bactericidal effect and it is further increases with decrease in pH values (Salem, 2012). The bactericidal effect is obtained by the effect of *Lactobacillus acidophilus* growth. Thus it can be interpreted that nisin and *L. acidophilus* is more effective together as compared to the single use.

The commonly used bacteriocins in foods like dairy, meat and vegetable products are lantibiotics nisin and lactacin, pediocin-like bacteriocins and, enterocin AS-48 etc. in foods bacteriocins can be applied in three forms i.e. *in situ* production by starter or protective cultures, as an ingredient of fermentation of a bacteriocinogenic strain and as an additive in a semi- or purified preparation. Among all *In situ* production is considered cost-effective. Nisin-producing dairy starters are generally designed to specifically inhibit *Staphylococcus aureus* in acid-coagulated cheeses and *C. tyrobutyricum* in semi-hard cheeses (Rilla *et al.*, 2003, 2004). Whereas, protective cultures is mainly used to enhance the hygienic quality of raw meat and fish products and do not contribute to sensory attributes of food (Devlieghere *et al.*, 2004). The use of bacteriocins as ingredients or additives requires low-cost food-grade media such as lactacin 3147 and the enterocin AS-48 can best produced in media containing whey (Ananou *et al.*, 2008). It is because whey as a substrate is an attractive option of recycles a by-side product of the dairy industry. Besides bio-preservation bacteriocins have been shown to accelerate cheese ripening by promoting the release of intracellular enzymes to the cheese matrix. Some volatile compounds are responsible for sensory attributes of the matured cheese. Bacteriocins also ensures homogenous fermented products (Ryan *et al.*, 2001) and bacteriocins based food grade markers (immunity proteins) offer the possibility to replace antibiotic selective markers for genetic engineering of food-related bacteria (Brede *et al.*, 2007). The list of commonly occurring bacteriocins and their possible uses are enumerated in Table 3.

A new bacteriocin named sonorensin belongs to heterocycloanthracin subfamily of bacteriocins is found effective against both active and non-multiplying cells of gram +ve as well as gram -ve bacteria. It is showing marked inhibition against biofilm of *Staphylococcus aureus*. The growth inhibition property of sonorensin is due to increased membrane permeability. Low Density Polyethylene (LDPE) film coated with sonorensin is proved to be effectively control growth of spoilage bacteria like *Listeria monocytogenes* and *Staphylococcus aureus* (Chopra *et al.*, 2015). It may be suggested as good alternative to current antibiotics and biopreservative mean for chicken meat and tomato samples.

Bacteriophages

Bacteriophages or phages are most abundantly found microorganisms on earth and wide spreaded on foods of various origins (Brüssow and Kutter, 2005). They are viruses that specifically infect and multiply in bacteria and are usually harmless to humans, animals, and plants. Most of the bacteriophages are belong to the *Caudovirales* order and conatins icosahedral head and a double-stranded 179 DNA tail.

Based on tail structures bacteriophages can be classified under three families: *Myoviridae* with contractile tail, *Siphoviridae* contains long non contractile tail and *Podoviridae* family having extremely short tail. Although some of the other bacteriophages are cubic, filamentous or pleomorphic phages with dsDNA, single-stranded DNA, double-stranded RNA or single-stranded RNA (Ackermann, 2007). They may further be classified as virulent and temperate depending on their life cycle. Virulent are generally following the lytic life cycle while temperate enters in lysogenic cycle by inserting their DNA into the bacterial chromosome (prophage). Virulent bacteriophages are capable to lyse the host bacterium and exerted antimicrobial activity (Sulakvelidze and Kutter, 2005; Hanlon, 2007).

Utilization of bacteriophages in food system

Bacteriophages can suitably be utilized in prevention or inhibition of colonization, control of diseases in livestock through phage therapy, decontamination of carcasses and other raw products such as fresh fruit and vegetables, and to disinfect equipment and contact surfaces by phage bio-sanitation and enhance the shelf life of manufactured foods by phage bio-control. Bacteriophages can be successfully utilized for reduction of pathogen carriage in livestock farming and after slaughter or milking. Bacteriophages are effective against *Salmonella* (Fiorentin *et al.*, 2005) and *Campylobacter* (Atterbury *et al.*, 2005) in chicken and pathogenic *E. coli* (Raya *et al.*, 2006) in ruminants. To achieve biosanitation reduction in biofilm can be obtained (Azeredo and Sutherland, 2008) by reducing or eradication of *S. aureus* nasal or skin colonisation in food handlers (Mann, 2008).

In bio-preservation, bacteriophages can work symbiotically with fermentation organisms during fermentation. It is quite effective method in preparation of food processing's and reduces effectively the like *Salmonella* in cheddar production (Modi *et al.*, 2001), *S. aureus* growth in curd manufacturing (García *et al.*, 2007; 2009) and complete eradication of *Listeria monocytogenes* during ripening, and storage of acid coagulated and semi-hard cheeses (Carlton *et al.*, 2005) and inhibition of *Enterobacter sakazakii* in reconstituted infant formula milk (Kim *et al.*, 2007) and *Salmonella typhimurium* on chicken frankfurters (Whichard *et al.*, 2003).

Table 3. Common bacteriocins and their possible uses in food industry.

	Bacteriocin	Food application
Dairy industry	Nisin	<i>Clostridium butulinum</i> in cheese, <i>L. monocytogenes</i> in cheeses such as Camembert, Ricotta and Manchego
	Pediocin AcH	Effective against milk and Cheddar and Munster cheeses against <i>L. monocytogenes</i> , <i>S. aureus</i> and <i>E. coli</i> O157:H7 lacticin against undesirable LAB. <i>L. monocytogenes</i> and <i>B. cereus</i> in Cheddar, Cottage cheese and yogurt and enterocin AS-48 against <i>B. cereus</i> , <i>S. aureus</i> and <i>L. monocytogenes</i> in milk and Manchego cheese.
Meat industry	Nisin, Enterocin AS-48, Enterocins A and B, Sakacin, Leucocin A and especially Pediocin PA-I/AcH alone or in combination with several physicochemical treatments like modified atmosphere packaging, high hydrostatic pressure (HHP), heat and chemical preservatives Bacteriocinogenic LAB	Quite effective against <i>L. monocytogenes</i> and other pathogens. Bio-protective cultures to protect pathogens in food processing.
	Pediocin PA-I/AcH	It is more suitable for use in meat and meat products than nisin but <i>P. acidilactici</i> is not an indigenous meat strain.
Vegetable products	Nisin	In tinned vegetables and fruit juices.
	Pediocin PA-I/AcH	In salad and fruit juices.
	Enterocin AS-48	Effective against <i>B. cereus</i> in rice and vegetables and against pathogens such as <i>E. coli</i> O157:H7, <i>S. aureus</i> and the spoilage bacterium <i>Alicyclobacillus acidoterrestris</i> .
Fish products	Combination of nisin and Microgard	Gram-negative microorganisms generally encountered in fresh chilled salmon and <i>L. monocytogenes</i> in frozen thawed salmon.
	Bacteriocins culture containing <i>Carnobacterium divergens</i> culture in combination with lactic acid, sodium chloride, and/or nisin	In inhibition of <i>L. monocytogenes</i> in rainbow trout.

Some of the commercially available phage preparations have got approval from FDA like *Listex* and *LMP 102* for ready-to-eat meat, anti-*E. coli* and anti-*Salmonella* phage-based product to treat live animals prior to slaughtering.

It also helps in detection of food borne pathogens in which phage specificity and the efficacy of host recognition is generally exploited.

Endolysins

Endolysins are also called phage lysins which are phage-encoded peptidoglycan hydrolases employed by the majority of bacteriophages to enzymatically degrade the peptidoglycan layer of the host bacterium. Bacteriophages are utilizing the endolysins at the end of their replication cycle to degrade the peptidoglycan of the bacterial host.

Endolysins are good antimicrobials because of absence of an outer membrane in the Gram-positive bacterial cell wall and its easy accessibility to peptidoglycan (Young *et al.*, 2005). Structurally most of the endolysins lack secretory signals and their access to peptidoglycan inside the cell is dependent on small hydrophobic proteins, named holins. Endolysins are mostly having narrow spectrum lytic activity and often restricted to phage host bacterial activity from which

they are originally derived. However, some exceptions are also existed like Enterococcal phage lysin which are capable to lyse enterococci as well as *Streptococcus pyogenes*, group B streptococci and *S. aureus* (Yoong *et al.*, 2004).

Role of Endolysins in food system

The role of endolysins in food bio-preservation is still in infancy. But there are number of endolysins are isolated to work against several zoonotic and food-borne pathogens. The good thing in this regard is the non-existence of resistance against endolysins till date. It is suggested by several scientists now that transgenic cows expressing endolysins have less chances of mastitis and *S. aureus* milk contamination (Donovan *et al.*, 2006). Similarly Staphylococcal endolysin is found capable to reduce *S. aureus* biofilms (Sass and Bierbaum, 2007). Endolysins are also having suggested roles in dairy processing particularly purified endolysins are found to destroy *S. aureus* in pasteurized milk.

Reports suggested that recombinant LAB are capable to release active *Listeria* endolysin but their antagonistic activity in milk still requires authentication (Turner *et al.*, 2007). It has also been indicated in food safety and has specificity against CBDs.

Bio-preservation in Indian context

In India use of fermented food and beverage with local food and crops as well as other biological resources is very common. Fermentation has been used since time immemorial in Indian subcontinent as the means of an effective and low cost means to preserve the food for quality and safety. The typical examples are idli, dosa, dhokla, khaman in south India, kinema in Eastern Himalayan region, jalebi in north India, sez in Uttaranchal. Fermented milk product like dahi is popular in almost whole India. Fermented fish products like Ngari and Hentak are famous in Manipur, tungtap in Meghalaya. Dahi is a very popular product produced by fermentation in India. During conversion of milk to dahi by culture bacteria several types of organic acids like lactic acid, acetic acids are produced. In addition, metabolites like lactocidin, nicin and acidophilin are also produced and exhibited antibacterial property. Though, the use of microbes in fermentation is unknowingly. So the scientific knowledge and validation need to be done to popularize the bio-preservation facts with traditional knowledge.

Conclusion

Overall bio-preservation of food by bacteriocins, bacteriophages and endolysins has promising role in food processing, preservation and food safety. Bacteriocins and endolysins are believed to have more suitability for DNA shuffling and protein engineering to generate highly potent variants with expanded activity spectrum. Genetically modified bacteriophages may also be helpful in bio-preservation but their safety issues must be addressed properly before selection as bio-preservative agent. The bacteriocins may be a good hurdle agent and successfully can be incorporated into packaging films and combined with modified atmosphere packaging (MAP). Similarly phages and endolysins have been successfully combined with nisin and high hydrostatic pressure enhances endolysin activity. Bacteriophages and endolysins has also been advocated as good disinfectants in food environments, including food handlers, but delivery strategies have to be implemented. So to attain bio-preservation in most holistic manner it is mandatory to prove the freedom from resistance particularly of bacteriocins and bacteriophages. However, endolysin has no such problem indicated till date. But phages mutate at frequencies significantly higher than that of bacteria. But new phage selection might easily overcome bacterial resistance. Lysogeny process is quite deteriorative because of it bacterial resistant becomes superinfection. Thus temperate phages should always be avoided. But on revolution in genetic technology and advancement in molecular techniques it is expected that bacteriocins, bacteriophages and endolysins will prove the future bio-preservative.

Conflict of interest

The authors declare that there is no conflict of interests.

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