

## REVIEW ARTICLE

# Biopreservation: bacteriocins and lactic acid bacteria

Deepali Chittora, Bhanu Raj Meena, Tripta Jain, Kanika Sharma

Microbial Research Laboratory, Department of Botany, University College of Science, Mohanlal Sukhadia University, Udaipur, Rajasthan, 313001

Received: 08.03.2022

Accepted: 12.04.2022

## ABSTRACT

Bacteriocinogenic lactic acid bacteria are used in biopreservation of food products. Biopreservation is the new and innovative approach to control the food spoilage is growing significance for several industries and consumers. Lactic acid bacteria are used in biopreservation because of their food-grade and generally recognized as safe (GRAS) status. So, the LABs can be helpful to control the frequent growth of pathogens and spoiling microorganisms in food and feed products. Reported literature showing that maximum number of LABs has been isolated from fermented food and dairy products. These isolates may have biopreservation ability, probiotic properties, fermentation ability and antimicrobial activity. Antimicrobial activity is due to production of metabolites such as organic acid, antifungal peptide and bacteriocins. The cell free supernatant of LABs can be purified by ammonium sulfate precipitation method to collect the bacteriocins. These bacteriocins may have antimicrobial activity tested against food contaminating microbes. Later on purification and characterization are important step to identify the bacteriocins to conclude the molecular composition and molecular weight. Several purification strategies have been used for purification of bacteriocins from complex cultivation broth such as salting-out, solvent extraction, ultrafiltration, adsorption-desorption, ion-exchange, and size exclusion chromatography. The characterization of bacteriocins has been done by LC-MS, Mass spectroscopy, FTIR, MALDI-TOF and SDS PAGE. These purified bacteriocins can be recognized as safe and good solution to control the microbial food spoilage at commercial level..

**Keywords:** Lactic acid bacteria, bacteriocins, food preservation, fungi, biopreservation, spoilage, purification

**Citation:** Chittora, D., Meena, B. R., Jain, T., and Sharma, K. 2022. Biopreservation: bacteriocins and lactic acid bacteria. *Journal of Postharvest Technology*, **10**(2): 1-15.

## INTRODUCTION

Microbial spoilage of foodstuffs is a common and global problem. About 5 to 10% of the world's food production has been lost due to fungal deterioration (Rouse et al., 2008; Gwiazdowska et al., 2016). In Western Europe more than 200 million of annual economic loss has been recorded due to mould spoilage of food products (Zhao, 2011; Saranraj and Geetha, 2012). About 25% of global food production has been lost due to microbial spoilage during post-harvest period (Gram and Dalgaard, 2002). Mainly, this problem has been linking with food contamination by fungi and their mycotoxins which creates potential health

\* For correspondence: D. Chittora (Email: [deepalichittora@yahoo.com](mailto:deepalichittora@yahoo.com))

hazard to consumers. The inhibition of fungal and yeast growth in food is remains a big challenge for food industries. Several food safety standards and modern preservation techniques have been used to reduce the food spoilage and related diseases. Still food borne diseases has been occurred due to spoilage of food during storage, packaging and transportation (Schnurer and Magnusson, 2005).

So, strict rules and suitable strategies are immediately needed for resolving this problem. Several food preservation techniques have been used as antidote to food spoilage (Adebayo and Aderiye, 2010). Food preservation methods such as chemical or physical treatments (heat, UV radiation etc.) have been used for prevention of microbial growth in food. The development of resistance in microbes towards chemical preservatives is the major drawback of continuous use of chemicals, (Kinay et al., 2007). This would change the organoleptic and nutritional properties of food. Complement consumer demands for safe and minimally processed food without additives, this method should be replaced by safe and ecofriendly biopreservatives. The use of non-pathogenic microorganisms and their metabolites to improve microbiological safety and extend the shelf life of foods is defined as “biopreservation” (Adebayo and Aderiye, 2010).

One of the most common forms of biopreservation is fermentation, where beneficial microorganisms are grow on food under controlled condition and preventing the growth of food spoiling microbes. Several researches in food sciences are concentrated on identification and development of protective bacterial cultures with antimicrobial effects against known pathogens and spoilage organisms. At present lactic acid bacteria have a great potential to control microbial spoilage for being used as a biopreservative (Gerez et al., 2009). Lactic acid bacteria are broadly used in the fermentation of extensive variety of food products and identified for their preservative and therapeutic effects (Chittora and Sharma, 2018).

LABs are appeared as a good alternative of chemical and physical methods of preservation to control and eliminate the contamination of fungi and bacteria (Schnurer and Magnusson, 2005; Dalie et al., 2010). Lactic acid bacteria (LAB) are particular concern as biopreservation organisms because of their food grade and their GRAS (Generally recognized as safe) status (Strom et al., 2005; Gerez et al., 2009). The biopreservative property of LABs is due to production of antimicrobial metabolites, such as organic acids, hydrogen peroxide, diacetyl, inhibitory enzymes and bacteriocins (Piard and Desmazeaud, 1991). Amongst these antimicrobial metabolites, bacteriocin is the concentrated object has been found as a potentially safe class of biopreservative. Bacteriocins are antimicrobial peptides produce during the primary phase of bacterial growth. Most of the bacteriocins have bacteriocidal activity against gram-positive bacteria but few reports are found against fungi (Klaenhammer, 1993; Nissen-Meyer and Nes, 1997).

Literature revealed information presented that few of the bacteriocins have an ability to inhibit the fungal mycelial growth and spore germination (Aruna and Madhuri, 2015). There are some examples of antifungal effect of antimicrobial compounds such as, bacteriocins of lactic acid bacteria (LAB) have been found to be effective against *Penicillium sp.*, *Aspergillus sp.* and *Fusarium sp.* (Tumbariski et al., 2018). Proteinaceous compound of *Lactobacillus coryniformis* subsp. *coryniformis* strain Si3 had antifungal effect against several moulds and yeasts (Magnusson and Schnurer, 2001; Siedler et al., 2019).

Hence, the major aim of this review is to study the antimicrobial potential of LAB, especially bacteriocins. They can be used as biopreservatives, in order to control and eliminate the growth of food spoilage microbes such as fungi and bacteria. They give aroma and specific organoleptic characteristics to the food products and maintaining the nutritive quality and improving the shelf life of food.

## Lactic acid bacteria

Lactic acid bacteria (LAB) are member of phylogenetically distinct divisions of gram-positive, coccus or rod shape, aerotolerant and catalase negative bacteria. They are nitrate reductase negative except with *Lactobacillus plantarum*. They are chemotrophic because they ferment carbohydrates for energy and produce lactic acid (Salminen and Von Wright, 2004; Axelsson, 2004; Holzapfel and Wood, 2012). They metabolize carbohydrate by homo or heterofermentative pathways. They can tolerate low pH, high salt concentration and perform therapeutic role in the intestinal tract (Pelinescu et al., 2009; Kumar et al., 2014; Bartowsky, 2019). LABs have ability to breakdown complex metabolites for their survival in human and animal intestinal tract (Piard and Desmazeaud, 1991). They produce a variety of antimicrobial metabolites, such as formic acid, propionic acid, lactic acid, acetic acids, pyrrolidone-5-carboxylic acid, diketopiperazines, 3- phenyllactate, Diacetyl, Reactive oxygen species hydrogen peroxide, Bacteriocin, diacetyl and reuterin (Trias et al., 2008; Mozzi et al., 2016; Bartowsky, 2019; Gänzle and Salovaara, 2019). Their mechanism of action is difficult to reveal due to complex and synergistic interactions between different compounds (Niku-Paavola et al., 1999; Piard and Desmazeaud, 1991). Fig 1: Metabolites produced by Lactic acid bacteria.

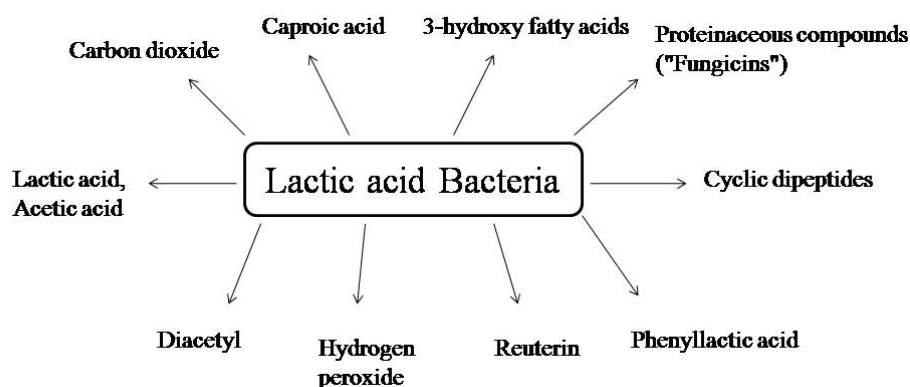


Fig 1: Metabolites produced by Lactic acid bacteria

## Distribution and sources of LABs

Lactic acid bacteria are the largest genus abundant in carbohydrate-rich substances. LAB is generally associated with habitats rich in nutrient such as curd, milk, butter, milkpeda, ghee, cheese, meat, beverages and vegetables etc. (Arokiyamy and Sivakumar, 2011; Tserovska et al., 2002; Chen et al., 2003; Sood et al., 2013; Bhuiyan et al., 2017; Hawaz, 2014). They are widespread in nature found in soil, vegetables, meat, milk, fermented dairy products and the human body. *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus bulgaricus* (*L. bulgaricus*) are the two bacteria required to make yogurt. Commercial yogurts contain *Lactobacillus acidophilus* (*L. acidophilus*) and *Bifidobacterium bifidus* (*B. bifidus*). *Lactobacillus casei* (*L. casei*) is frequently found in cheeses. The majority of *Lactobacillus* species have been isolated from the gastrointestinal tract of some of the vertebrates. The second largest genus i.e. *Pediococcus* strains have been isolated from vegetables (Sathe et al., 2007), sourdough (Hassan and Bullerman, 2008), fermented beverages, grass silage (Magnusson, 2003) or milk (Delavenne et al., 2012) and their fermentation products (Todorov, 2009; Djadouni et al., 2012). Whereas, *Leuconostoc* have been isolated from chilled meats or clinical sources, although they are also obtained from plant material, fermented dairy products and wines (Rajilic-Stojanovic et al., 2014; Goldstein et al., 2015). *Lactococcus* species have

abundantly found in dairy products e.g. sour milk; they are also isolated from plant material. LABs are also found in decomposing plant material and fruits, fermented meat and fish, cereals, beets, pickled vegetables, potatoes, sourdough, silages, fermented beverages, juices, fish and other marine products, sewage and cavities of some vertebrates (Lee et al., 2009; Liu et al., 2014). *Lactobacillus plantarum* LP 31 strains has been found in dry-fermented sausage, marine water and living decomposing marine organisms (Ishikawa et al., 2003; Itoi et al., 2008; Muller et al., 2009; Pasteris et al., 2006). In humans, they are particularly resided in the oral cavity, ileum, colon, vagina and human breast milk (Coeuret et al., 2003; Sieladie et al., 2011; Singh et al., 2014). Lactic acid bacteria (LAB) are also found in fermented cocoa for producing a high quality taste and flavor chocolates (Ayertey et al., 2017). List of Sources of Lactic acid bacteria given in (Table 1).

**Table 1: Sources of Lactic acid bacteria**

Sources	Isolated lactic acid Bacteria
Camel's milk	<i>Lactobacillus lactis</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus paracasei</i>
Yak milk	<i>Lactobacillus plantarum</i> , <i>Lactobacillus pentosus</i> , <i>Lactobacillus paraplantarum</i> , <i>Enterococcus lactis</i> and <i>Enterococcus faecium</i>
Goat's milk	<i>Lactobacillus plantarum</i> and <i>Pediococcus acidilactici</i>
Cow's milk	<i>Lactobacillus helveticus</i>
Whey	<i>Lactobacillus plantarum</i> and <i>Lactobacillus fermentum</i>
Dairy products	<i>Streptococcus thermophilus</i> and <i>Lactobacillus plantarum</i>
Cheeses	<i>Lactobacillus plantarum</i> , <i>Lactobacillus helveticus</i> , <i>Lactobacillus lactis</i>
Tibetan kefir grain	<i>Lactobacilluskefiranoferiensis</i>
Dry-fermented sausage	<i>Lactobacillus plantarum</i>
Sourdough	<i>Lactobacillus sanfrancisco</i>
Beer	<i>Lactobacillus plantarum</i> VTT E-78076
Green Gram	<i>Pediococcus sp.</i> , <i>Enterococcus</i> , <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Leuconostoc</i> , <i>Pediococcus</i> , <i>Weissella</i> , etc.
Fermented Garlic	<i>Weissella (W.) cibaria</i> and <i>Leuconostoc (Le.) citreum</i> , <i>Lactobacillus sakei</i>
Fruit juices	<i>Lactobacillus plantarum</i> and <i>Leuconostoc species</i>
Buffalo milk	<i>Lactobacillus fermentum</i> , <i>Lactobacillus sp. G1</i> and <i>Lactococcus sp.</i>
Shrikhand	<i>Lactobacillus acidophilus</i> <i>Lactobacillus sporogens</i> , <i>Lactobacillus rhamnosus</i>
Idli batter	<i>Lactobacillus plantarum</i> and <i>Lactobacillus lactis</i> , <i>Lactobacillus mesenteroides</i> and <i>Streptococcus faecalis</i>
Curd	<i>Lactobacillus spp.</i> , <i>Lactococcus</i> , <i>Leuconostoc</i>

**References:** (Gänzle and Salovaara, 2019), (Bhuiyan et al., 2017), (Chittora and Sharma, 2018).

### LABs used as biopreservatives

At large scale food production, consumers need high value, preservative-free, harmless and slightly processed food with extended shelf-life. These requirements have been fulfill by the use of LABs and their metabolites for maintaining safety standards and reducing the risk associated with food (Gaenzle, 2015). Lactic acid bacteria and their metabolite inhibit the

growth of food spoilage microorganisms and sustaining the flavor, texture and nutritive quality of feed and food products (Carr et al., 2002; Siedler et al., 2019; Gaenzle, 2015). They show beneficial effect on the health of neonate and adults.

LAB is employing from ancient traditions in food and feed for positive health effects. They are included in diet of many developing countries consists of fermented foods. They are used as starter cultures for the development of dairy products such as yoghurt, butter milk, cottage cheeses, hard cheeses and soft cheeses etc. (Carr et al., 2002). The coexistence of LAB and fungi in food is vital for the success of biotechnological applications. The combination of LAB:yeast is used for sourdough bread production.

“Biopreservation refers to extended shelf life and enhanced safety of food by using natural or added microflora or their antimicrobial metabolites” (Ross et al., 2002; Mozzi et al., 2016). LABs are employing from ancient traditions in food and feed for positive health effects. They are well known as starter cultures for the development of dairy products such as yoghurt, butter milk, cottage cheeses, hard cheeses and soft cheeses etc. (Carr et al., 2002). The coexistence of LAB and fungi in food is also vital for the success of biotechnological applications. The combine ratio of LAB and yeast has been used for sourdough bread production. They are included in 25% of the European diet and 60% of the diet in many developing countries consists of fermented foods (Chittora et al., 2019).

Several traditional techniques such as physical and chemical methods have been used for prevention of food spoilage by microorganisms. Physical methods such as drying, freeze-drying, cold storage, modified atmosphere storage, heat treatments (Prokopov et al., 2007) and chemical methods includes acetic acid, lactic acid, propionic acid, sorbic acid and benzoic acids, natamycin, and sodium benzoate have been used for the preservation of food products (Nielsen and de Boer, 2001). Chemicals are used for control of food spoilage by inhibiting the growth and germination of both bacterial and fungal spores. But, some of the microbes developed a resistance towards chemicals (Sanglard, 2002; Siedler et al., 2019). Hence, LABs and their metabolites are appearing as a potentially good alternative of chemical method for maintaining the nutritive quality and improving shelf life of food products (Dalie et al., 2010; Schnurer and Magnusson, 2005; Shehata et al., 2019).

Following approaches are commonly used in the application of bacteriocins for biopreservation of food products(Schillinger et al., 1996) These are:

1. Inoculation of food with LAB that produce bacteriocin while growing within the products. The ability of the LAB to grow and produce bacteriocin in the products is crucial for their incorporation.
2. Addition of purified or partially purified bacteriocins as food additives.
3. Use of product previously fermented with a bacteriocin producing strain as an ingredient of food product.

## **BACTERIOCIN**

Bacteriocins are group of potent antimicrobial peptides produced by some microorganisms including Gram-positive lactic acid bacteria, primarily active against closely related organisms. Colicins produced by *Escherichia coli* are the first bacteriocins discovered by Gratia in 1925. Bacteriocins are potent antimicrobial peptide produced during the primary phase of bacterial growth (Belguesmia et al., 2010; Chikindas et al., 2018). Bacteriocins are small cationic molecules of about 30–60 amino acids, forming amphiphilic helices and stable at 100°C for 10 min and they differ in spectrum of activity, mode of action, molecular weight (MW), genetic origin and biochemical properties. Bacteriocin-producing LAB strains protect themselves from

their own toxins by the expression of a specific immunity protein, encoded in the bacteriocin operon (Moll et al., 1999). They are primarily active against closely related gram-positive bacteria such as *Bacillus*, *Clostridium*, *Staphylococcus* and *Listeria* (Klaenhammer, 1993; Nissen-Meyer and Nes, 1997; Alvarez-Sieiro et al., 2016). List of some Lactic acid bacteria and their bacteriocins given in (Table 2).

**Table 2: List of Lactic acid bacteria and their bacteriocins**

Producing organisms (LABs)	Bacteriocin
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Nisin, Lacticin 481
<i>Bacillus subtilis</i> 168	Subtilosin A
<i>Streptococcus pyrogenes</i>	Streptolysin S
<i>Bacillus subtilis</i> 168	sublancin 168
<i>Leuconostoc carnosum</i> 4010	Leucocin C
<i>Pediococcus acidilactici</i>	Pediocin
<i>Lactococcus lactis</i>	Lactococcin G
<i>Lactococcus</i> sp. QU 12	Lactocyclicin Q
<i>Lactococcus</i> sp. QU 5	Lacticin Q
<i>Lactococcus lactis</i> subsp. <i>ceremoris</i> LMG 2130	Lactococcin A
<i>Enterococcus faecalis</i> LMG 2333	Enterolysin A
<i>Lactobacillus helveticus</i> 481	Helveticin J
<i>Lactobacillus plantarum</i>	Plantaricin ST31
<i>Lactococcus lactis</i>	Nisin Z
<i>Enterococcus faecalis</i> subsp. <i>liquefaciens</i>	Enterocin AS48
<i>Lactobacillus plantarum</i> C19	Plantaricin C19
<i>Pediococcus acidilactici</i> M	Pediocin ACM
<i>Lactobacillus acidophilus</i> DSM 20079	Acidocin D20079
<i>Lactococcus lactis</i> QU 5	Lacticin Q
<i>Lactobacillus amylovorus</i>	Lactobin A
<i>Carnobacterium divergens</i> M35	Divergicin M35
<i>Enterococcus faecium</i> CRL 35	Enterocin CRL35
<i>Lactococcus lactis</i> subsp. <i>lactis</i> B14	Bozacin 14
References: (Piard et al., 1993), (Elegado et al., 1997), (Tahiri et al., 2004), (Deraz et al., 2005), (Nielsen and de Boer, 2000).	

Several industrially important bacteriocins of LAB are approved by FDA, using as biopreservatives (Such as nisin and pediocin). They have broad spectrum of inhibition against bacteria, fungi and other microbes. LABs and their metabolites are natural biopreservatives in various food products that may also help to combat human pathogens.

Bacteriocin producing LAB has been associated with food to maintain their nutritive value (De Vuyst and Vandamme, 1994b; Soomro, Masud, and Anwaar, 2002). Bacteriocins are generally active against pathogenic bacteria but little evidence about their antifungal activity. *Lactobacillus coryniformis* subsp. *coryniformis* strain Si3 producing bacteriocins like proteinaceous

compound is found to be effective against several moulds and yeasts (Magnusson and Schnurer, 2001). This peptide is small (approximately 3 kDa), heat stable, and active in the pH range 3-6 and totally inactivated by enzymatic treatment such as proteinase K (Magnusson and Schnurer, 2001; Nes and Holo, 2000). Amylovorin L471 of *Lactobacillus amylovorus*, Lactocin S of *Lactobacillus sakei* and TV35b of *L. pentosus* are examples of antifungal peptide (Magnusson and Schnurer, 2001). *Lactobacillus pentosus* is producing a TV35b proteinaceous compound, which shows antifungal activity against *Candida albicans* via inhibition of pseudohyphae formation. The production and purification of the antifungal peptide followed the similar kinetics as the bacteriocins (Magnusson and Schnurer, 2001; Mataragas et al., 2002; Zacharof MP and Lovitt, 2012). Hence, antifungal peptides of LABs may also be considered as Bacteriocins.

#### CLASSIFICATION SCHEME FOR BACTERIOCINS (Cotter et al., 2005)

Klaenhammer classified the bacteriocins of LAB into four major classes (Klaenhammer, 1993; Kemperman et al., 2003; Jeevaratnam et al., 2005; Abanoz and Kunduhoglu, 2018). It has recently been anticipated that circular bacteriocins should be regarded as class V bacteriocins (Kemperman et al., 2003). Proposed "Universal" Bacteriocin Classification Scheme of Cotter et al., 2006 mention in (Fig. 2).

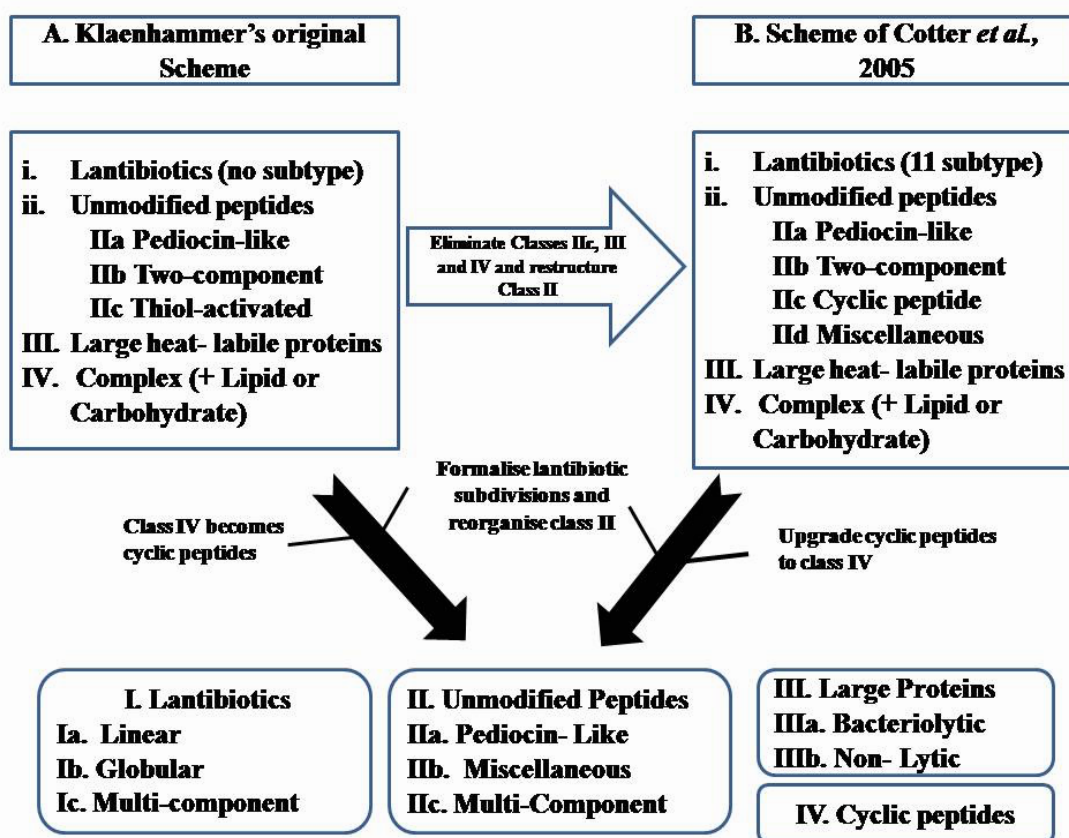


Figure 2: Proposed "Universal" Bacteriocin Classification Scheme (Cotter et al., 2006)

### **Klaenhammer Classification**

**Class I** – This class includes small membrane-active peptides with molecular weight <5 kDa. It contains the unusual amino acids lanthionine and b-methyl lanthionine, e.g., nisin, lactacin 481, carnocin UI49, lactocin S. They have two groups:

**Ia** Lantibiotics - presence of post-translationally modified amino acids viz. Lanthionine,  $\beta$ -methyllanthionine etc. eg. Nisin.

**Ib** Lantibiotics - globular proteins with no net charge or a net -ve charge eg. Mersacidin.

**Class II** – This class includes small heat-stable, nonlanthionine containing membrane active peptides (>10 kDa). The mature forms of bacteriocins have amphiphilic helices with varying amounts of hydrophobicity and  $\beta$ -sheet structure. They have moderate (100°C) to high (121°C) heat stability. These include small heat stable non-lantibiotic peptides and further being classified into three sub groups: IIa, IIb and IIc. Subgroups that can be defined within the class II bacteriocins following are:

**IIa-** These are Listeria active peptides containing a consensus sequence of Y-G-N-G-X-C at their N-terminals. eg. Pediocin PA-1, Pediocin AcH, Leucocin, Curvacin etc.

**IIb-** These groups have two different proteinaceous monomers join to form a poration complex. eg. Lactococcin G, Lactacin F etc.

**IIc-** These are thiol-activated peptides in which Cys and Ser residues are post-translationally modified to thiazole and oxazole, respectively. eg. Lactococcin(Cotter et al., 2005).

**Class III-** These include large heat labile proteins eg. Lactacin A and Lactacin B, Pediocin A.

**Class IV-** These are complex proteins i.e. proteins that are complexed with other chemical moieties e.g. Carbohydrates or lipids. Which are required for their activity. e.g. Plantaricin S, Leuconocin S and Pediocin SJ-1 etc.

### **Revised Classification**

They divide the bacteriocins into three distinct categories: the lanthionine-containing Lantibiotics (Class I) and the non-lanthionine-containing bacteriocins (Class II), while moving the large and heat-labile murein hydrolases (formerly Class III bacteriocins) to a separate designation called 'Bacteriolysins'.

### **Methods of purification**

Several strategies are used for isolation and purification of bacteriocins from complex cultivation broths to get final products. Biotechnological procedures including salting-out, solvent extraction, ultrafiltration, and adsorption-desorption, ion-exchange, and size exclusion chromatography etc. are among the most usual methods for purification.

Purification process starts with the concentration of bacteriocins from the culture supernatant using diatomite calcium silicate (Coventry et al., 1996) or ammonium sulfate precipitation etc. (Yang et al., 1992; Guyonnet et al., 2000; An et al., 2017).



Thereafter, succeeding steps using preparative isoelectric focusing and multiple chromatographic separations, including cation exchange, gel filtration, hydrophobic interaction and reverse-phase liquid chromatography are necessary to achieve significant purification of bacteriocins. Following different strategies have been used for the purification of various classes of bacteriocins.

### Characterization of bacteriocins

Fourier Transform Infra-Red (FTIR) has been used to finding out vibrating bonds and functional groups present in bacteriocins. FTIR analysis is also used for the identification of organic, inorganic, and polymeric materials utilizing infrared light for scanning the samples. Alterations in the attribute pattern of absorption bands clearly indicate a change in the material composition. FTIR is useful in identifying and characterizing unknown materials, detecting contaminants in a material, finding additives, and identifying the chemical composition and oxidation. Fourier transform infrared spectroscopy (FTIR) is the technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid and gas.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS PAGE) is a technique used for the separation of proteins on the basis of their molecular weight. It is the technique widely used in forensics, genetics, biotechnology and molecular biology to separate the protein molecules based on their electrophoretic mobility or molecular weight (Schagger, 2006). This is the preferred electrophoretic system for the resolution of proteins smaller than 30 kDa.

Liquid chromatography or mass spectrometry (LC/MS) is the new system for rapid finding and identification of bacteriocins. Biological analysis and characterization of small molecular weight class of bacteriocins is an analytical challenge (Nandakumar et al., 2000; Mugochoi et al., 2001). In the past, several studies have used mass spectrometry based methods such as matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS; Rose et al., 1999 ; Hindré et al., 2003) and, lately, electrospray ionization-liquid chromatography-mass spectrometry (ESI-LC-MS; Zendo et al., 2008; Malini and Janakiraman, 2012) for the detection and determination of molecular mass of bacteriocins. MALDI-TOF has been used for determination of bacteriocins in the active fractions obtained during the purification of Bacteriocins. MALDI-TOF MS was first recognized in 1988 as a method of examining large molecules. MALDI-TOF MS is essential tool in the analysis of molecular mass of biopolymers, including peptides and proteins.

**Table 3:** Name of bacteriocins and their purification method.

Name of bacteriocins	Purification method	References
Plantaricin ST31	Ammonium sulfate precipitation - Sep-pack C18, cartridge – RP-HPLC, Cation exchange Chromatography	(Guyonnet et al., 2000)
Nisin Z	Expanded bed, Ion-exchange chromatography	(Elegado et al., 1997)
Enterocin AS48	Cation exchange – Reverse Phase Chromatography	(Cheigh et al., 2004)
Lactobin A	Ammonium sulfate precipitation - Chloroform-methanol extraction - RP-FPLC	(Alvarez-Sieiro et al., 2016)

Acidocin D20079	Ammonium sulphate precipitation - Cation exchange chromatography - Octyl Sepharose column.	(Coeuret et al., 2003)
Pediocin PA-1 Lactococcin B	Etanol precipitation - Isoelectric focusing - Ultrafiltration	(Deraz et al., 2005)
Plantaricin C19	Adsorption/release – RP-HPLC	(Ogunbanwo et al., 2003)
Lacticin Q	Acetone precipitation - Cation exchange chromatography – RP-HPLC	(Tahiri et al., 2004)

The quantitative proteomics approach using liquid chromatography (LC) for peptide separation, followed by tandem mass spectrometry (MS/MS) and bioinformatics analysis will allow more sensitive detection, sequence information, and relative quantitation of low-abundant, small-molecular-weight proteins or peptides in a complex sample (Aebersold and Mann, 2003; Nesvizhskii, 2007). Recently, electron-transfer dissociation (ETD) has developed as an alternative peptide-fragmentation method and has shown to generate more complete series of ions and more extensive sequence information. Name of bacteriocins and their purification methods given in (Table 3).

## CONCLUSION

Lactic acid bacteria are the advance group of bacteria. They are used in biopreservation of food products. They have broad spectrum of inhibition due to their antimicrobial potential. Antimicrobial activity of LABs is due to production of variety of metabolites such as organic acids and antimicrobial peptides etc. The major focus of this review is to study the antimicrobial potential of LABs and their metabolites in food preservation. Bacteriocins are the key metabolite of present study for biopreservation of food products. So, the bacteriocins producing LABs has been selected in several food processing industry is to obtain chemical free, contamination free and safe food products. Hence, it can be concluding that lactic acid bacteria and bacteriocins hold a potential for extension of shelf-life and improvement of microbiological safety in food industry.

## REFERENCES

- Abriouel, H., Valdivia, E., Martinez-Bueno, M., Maqueda, M. and Galvez, A. 2003. *Journal of Microbiological Methods*, 55: 599.
- Adebayo, C.O. and Aderiye, B.I. 2010. Antifungal activity of bacteriocins of lactic acid bacteria from some Nigerian fermented foods, *Research journal of microbiology*, 5: 1070-1082,
- Alvarez-Sieiro, P., Montalbán-López, M., Mu, D. and Kuipers OP. 2016. Bacteriocins of lactic acid bacteria: extending the family, *Applied microbiology and biotechnology*, 100: 2939-2951.
- An, Y., Wang, Y., Liang, X., Yi, H., Zuo, Z., Xu, X. and Han, X. 2017. Purification and partial characterization of M1-UVs300, a novel bacteriocin produced by *Lactobacillus plantarum* isolated from fermented sausage. *Food Control*. 81: 211-217.
- Arokiyarny, A. and Sivakumar, P.K. 2011. Antibacterial activity of Bacteriocin producing *Lactobacillus spp.* isolated from traditional milk products. *Current Botany*, 2: 05-08.

- Aruna, G. and Sai, J.M. 2016. Antifungal Activity of Bacteriocin Produced by Lactic Acid Bacteria from Fermented Green Gram. *International Journal of Science and Research*, 5: 1827-1831.
- Axelsson, L. 2004. Lactic acid bacteria: classification and physiology. *Food Science and Technology-New York-Marcel Dekker*, 139: 1-66.
- Ayertey, E., Hilary, D.Z. and Betty, O.N. 2017. Screening, isolation and characterization of lactic acid bacteria strains in fermenting cocoa heaps from the Eastern Region of Ghana. *Scientect Journal of Life Sciences*, 1: 1-7.
- Bartowsky, E. 2019. Lactic Acid Bacteria (LAB). *Microbiological and Functional Aspects*, 287:
- Bhuiyan, R., Shill, S., Islam, A. and Chakraborty, S. 2017. Screening of *Lactobacillus* spp. from raw goat milk showing probiotic activities against pathogenic bacteria. *African Journal of Microbiology Research*, 11: 620-625,
- Carr, F.J., Chill, D. and Maida, N. 2002. The lactic acid bacteria: a literature survey. *Critical Reviews in Microbiology*, 28: 281–370.
- Cheigh, C.I., Kook, M.C., Kim, S.B., Hong, Y.H. and Pyun, Y.R. 2004. Simple one-step purification of nisin Z from unclarified culture broth of *Lactococcus lactis subsp lactis* A164 using expanded bed ion exchange chromatography. *Biotechnology Letters*, 26: 1341-1345.
- Chen, H. and Hoover, D.G. 2003. Bacteriocin and their food application. *Comprehensive Rev. in Food Science and Technology*, 2: 82-100.
- Coeuret, V., Dubernet, S. and Bernardeau, M. 2003. Isolation, characterization and identification of Lactobacilli focusing mainly on cheeses and other dairy products. *Le Lait*, 83: 269-306.
- Dalie, D.K.D., Deschamps, A.M. and Richard-Forget, F. 2010a. Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review *Food Control*, 21: 370–380.
- Delavenne, E., Mounier, J., Deniel, F., Barbier, G. and Le Blay, G. 2012. Biodiversity of antifungal lactic acid bacteria isolated from raw milk samples from cow, ewe and goat over one-year period. *International journal of Food Microbiology*, 155: 185-90.
- Deraz, S.F., Karlsson, E.N., Hedstrom, M., Andersson, M.M. and Mattiasson, B. 2005. Purification and characterisation of acidocin D20079, a bacteriocin produced by *Lactobacillus acidophilus* DSM 20079. *Journal of Biotechnology*, 117: 343-354.
- Chittora, D., Mittholiya, S., Meena B.R. and Sharma, K. 2019. Screening of Antimycotic Activity of Bacteriocin Producing Lactic Acid Bacteria against Food Spoilage Fungi. *International Journal of Pharmacy and Biological Sciences-IJPBSTM*, 9 (1): 250-258.

- Chittora, D. and Sharma, K. 2018. Isolation of Bacteriocin producing lactic acid bacteria from buffalo milk. *World journal of Pharmaceutical Research*, 7 (5): 277–288.
- Djadouni, F. and Kihal, M. 2012. Antimicrobial activity of lactic acid bacteria and the spectrum of their biopeptides against spoiling germs in foods. *Brazilian Archives of Biology and Technology*, 55: 435–443.
- Elegado, F.B., Kim, W.J. and Kwon, D.Y. 1997. Rapid purification, partial characterization, and antimicrobial spectrum of the bacteriocin, Pediocin AcM, from *Pediococcus acidilactici* M. *International Journal of Food Microbiology*, 37: 1-11.
- Gänzle, M.G. and Salovaara, H. 2019. Lactic Acid Bacteria. *Microbiological and Functional Aspects*, 2: 199.
- Gerez, C.L., Torino, M.I., Rollan, G., and de Valdez, G.F. 2009. Prevention of bread mould spoilage by using lactic acid bacteria with antifungal properties. *Food Control*, 20: 144–148.
- Goldstein, E.J.C., Tyrrell, K.L. and Citron, D.M. 2015. Lactobacillus species: Taxonomic complexity and controversial susceptibilities. *Clinical Infectious Diseases*, 60: S98–S107,
- Guyonnet, D., Fremaux, C., Cenatiempo, Y. and Berjeaud, J.M. 2000. Method for rapid purification of class IIa bacteriocins and comparison of their activities. *Applied and Environmental Microbiology*, 66: 1744-1748.
- Gwiazdowski, R. 2016. Ocena mozliwosci zastosowania bakterii *Propionibacterium freudenreichii* ssp. *shermanii* P4 oraz *Lactobacillus plantarum* L2 w ochronie rzepaku ozimego przed chorobami. *Rozprawy Naukowe Instytutu Ochrony Roślin Państwowego Instytutu Badawczego, Zeszyt*, 31: 124.
- Hassan, Y.I. and Bullerman, L.B. 2008. Antifungal activity of *Lactobacillus paracasei* ssp. *tolerans* isolated from a sourdough bread culture. *International Journal of Food Microbiology*, 121: 112– 115.
- Holzappel, W.H.N. and Wood, B.J.B. *The genera of lactic acid bacteria (Vol. 2)*. Springer Science and Business Media, 2012.
- Ishikawa, M., Nakajima, K., Yanagi, M., Yamamoto, Y., Yamasato, K. 2003. *Marinilactibacillus psychrotolerans* gen. nov., sp. nov., a halophilic and alkaliphilic marine lactic acid bacterium isolated from marine organisms in temperate and subtropical areas of Japan. *International Journal of Systematic and Evolutionary Microbiology*, 53.
- Joerger, M.C. and Klaenhammer, T.R. 1986. Characterization and purification of helveticin J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus* 481. *Journal of Bacteriology*, 167: 439-446.
- Kinay, P., Mansourc, M.F., Gablerb, F.M., Margosanc, D.A. and Smilanick, J.L. 2007. Characterization of fungicide-resistant isolates of *Penicillium digitatum* collected in California. *Crop Protection*, 26: 647–656.
- Klaenhammer, T.R. 1993. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology*, 12: 39–85.

- Kumar, R., Pandey, S., Kapoor, P., Awasthi, S. and Bhatnagar, T. 2014. Isolation and characterization of endemic strains of *Lactobacillus* sp. and evaluation of their probiotic activity. *International Journal of Current Microbiology and Applied Sciences*, 3: 907-916.
- Lee, C.H. and Campbell-Platt, G. 2009. In food science and technology. *Food biotechnology*, 85–113.
- Liu, W., Pang, H., Zhang, H. and Cai, Y. 2014. Biodiversity of lactic acid bacteria. In *Lactic Acid Bacteria*; Zhang Y, Cai Y, Eds.; Springer Science + Business Media: Dordrecht, The Netherlands.
- Magnusson, J. and Schnurer, J. 2001. *Lactobacillus coryniformis subsp. coryniformis* strain Si3 produces a broad-spectrum proteinaceous antifungal compound. *Applied Environmental Microbiology*, 67: 1-5.
- Magnusson, J. 2003. Antifungal activity of lactic acid bacteria (Ph.D. thesis). Swedish University of Agricultural Sciences, Agraria 397, Uppsala, Sweden.
- Mataragas, M., Drosinos, E.H. and Metaxopoulos, J. 2003. Antagonistic activity of Lactic acid bacteria against *Listeria monocytogenes* in sliced cooked cured pork shoulder stored under vacuum or modified atmosphere at 4±2°C. *Food Microbiology*, 20: 259-256.
- Moreno, I., Lerayer, A.S.L., Baldini, V.L.S. and Leitao, M.F. 2000. Characterization of bacteriocins produced by *Lactococcus lactis* strains. *Brazilian Journal of Microbiology*, 31: 184-192.
- Mozzi, F., Raya, R.R., Vignolo, G.M. and Love, J.C. 2016. *Biotechnology of Lactic Acid Bacteria—Novel Applications 2e*.
- Muller, D.M., Carrasco, M.S., Tonarelli, G.G. and Simonetta, A.C. 2009. Characterization and purification of a new bacteriocin with a broad inhibitory spectrum produced by *Lactobacillus plantarum* LP 31 strain isolated from dry-fermented sausage. *Journal of Applied Microbiology*, 106: 2031-2040.
- Nes, I.F. and Holo, H. 2000. Class II antimicrobial peptides from lactic acid bacteria. *Biopolymers*, 55: 50–61.
- Nielsen, P.V. and de Boer, E. 2000. Food preservatives against fungi. In Samson RA, Hoekstra ES, Frisvad JC, and Filtenborg O (Eds.), *Introduction to food- and airborne fungi Utrecht: Centraal Bureau voor Schimmelcultures*, 357–363.
- Niku-Paavola, M.L., Laitila, A., Mattila-Sandholm, T., and Haikara, A. 1999. New types of antimicrobials produced by *Lactobacillus plantarum*. *Journal of Applied Microbiology*, 86: 29–35.
- Nissen-Meyer, J. and Nes, I.F. 1997. Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis, and mechanism of action. *Archives of Microbiology*, 167: 67–77.
- Pelinescu, D.R., Sasarman, E., Chifiriuc, M.C., Stoica, I., Nohita, A.M., Avram, I., Serbancea, F. and Dimov, T.V. 2009. Isolation and identification of some *Lactobacillus* and *Enterococcus* strains by a polyphasic taxonomical approach. *Romanian Biotechnological Letters*.14: 4225-4233.


- Piard JC and Desmazeaud M. Inhibiting factors produced by lactic acid bacteria. 1991.1. Oxygen metabolites and catabolism end-products. *Le Lait*, 71: 525–541.
- Rajilic-Stojanovic, M. and de Vos, W.M. 2014. The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiology Review*, 38: 996–1047.
- Ross, P.R., Morgan, S., and Hill, C. 2002. Preservation and fermentation: past, present and future. *International Journal of Food Microbiology*. 79: 3–16.
- Rouse, S., Harnett, D., Vaughan, A., and Van Sinderen, D. 2008. Lactic acid bacteria with potential to eliminate fungal spoilage in foods. *Journal of Applied Microbiology*. 104: 915–923.
- Sathe, S.J., Nawani, N.N., Dhakephalkar, P.K., and Kapadnis, B.P. 2007. Antifungal lactic acid bacteria with potential to prolong shelf-life of fresh vegetables. *Journal of Applied Microbiology*. 103: 2622–2628.
- Schnurer, J. and Magnusson, J. 2005. Antifungal lactic acid bacteria as biopreservatives. *Trends in Food Science and Technology*, 16: 70–78.
- Siedler, S., Balti, R. and Neves. A.R. 2019. Bioprotective mechanisms of lactic acid bacteria against fungal spoilage of food. *Current opinion in biotechnology*, 56: 138-146.
- Sieladie, D.V., Zambou, N.F., Kaktcham, P.M., Cresci, A. and Fonteh, F. 2011. Probiotic properties of lactobacilli strains isolated from raw cow milk in the western highlands of Cameroon. *Dunarea de Jos*, 9: 12-28.
- Singh, A.K., Pandey, A., Sharma, M., Singh, K. and Singh, A. 2014. probiotic activities of lactic acid bacteria isolated from human breast milk. *Journal of Biological Engineering Research and Review*, 1(2): 07-12.
- Sood, S.K., Vijay Simha, B., Kumariya, R., Garsa, A.K., Mehla, J., Meena, S. and Lather P. 2013. Highly specific culture-independent detection of YGNGV motif-containing pediocin-producing strains. *Probiotics and Antimicrobial Proteins*, 5: 37–42.
- Strom, K., Schnurer, J., and Petter, M. 2005. Co-cultivation of antifungal *Lactobacillus plantarum* MiLAB 393 and *Aspergillus nidulans*, evaluation of effects on fungal growth and protein expression. *FEMS Microbiology Letters*. 246: 119–124.
- Tahiri, I., Desbiens, M., Benech, R., Kheadr, E., Lacroix, C., Thibault, S., Ouellet, D. and Fliss, I. 2004. *International Journal of Food Microbiology*, 97: 123.
- Todorov, S.D. 2009. Bacteriocins from *Lactobacillus plantarum* production genetic organization. *Brazilian Journal of Microbiology*, 40: 209–221.
- Trias, R., Baneras, L., Badosa, E., and Montesinos, E. 2008a. Bioprotection of Golden Delicious apples and Iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. *International Journal of Food Microbiology*, 123: 50–60.

Yang, R., Johnson, M.C. and Ray, B. 1992. Novel method to extract large amounts of bacteriocins from lactic acid bacteria. *Applied Environmental Microbiology*, 58: 3355-3359.

Zacharof, M.P. and Lovitt, R.W. 2012. Bacteriocins produced by lactic acid bacteria: A review article. *APCBEE Procedia*, 2: 50–56.



© The Author(s)

This is an  Open Access article licensed under a Creative Commons license: Attribution 4.0 International (CC-BY).