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REVIEW ARTICLE

BIOPROTECTIVE CULTURES FOR USE IN DAIRY PRODUCTS

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ABSTRACT

With ever expanding population, food safety and maintenance of its quality remains one of the biggest challenges for agriculture and food processing sector in the present time. Its high time to look for new antimicrobial agents and their implementation through better technologies to control microbial spoilage and foodborne diseases. Bio-protection of food products especially fermented ones, via lactic and propionic acid bacteria has gained much attention in the last few decades. Recent research has shown the anti-fungal potential of lactic acid bacteria, and propionibacteria for the protection of fermented dairy products such as yogurt, cheese and sour cream. *In-situ* antagonistic activity of these bioprotective cultures in food matrix itself has tremendous capacity for reducing the wastage of fermented dairy products. Deep and extensive studies have shown that metabolites of LAB and propionibacteria act in synergy to inhibit fungal contamination in fermented dairy products. However, availability of such cultures or their metabolites at commercial scale, play a critical role in their widespread use at different stages of food chain. In this review, recent literature on the potential of lactic acid bacteria and propionic acid bacteria as bioprotective culture, their mechanism of action has been summarized. Recent applications of bioprotective cultures in dairy products and constraints in this field, have also been discussed.

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INTRODUCTION

According to the United Nations' Food and Agricultural Organization (FAO), approximately one third of the of food produced in the world, is wasted each year due to undesirable microorganism and lack of proper storage facility. However, the true scale of food wastage has not been realized and its impacts have not been well understood until now. UNEP Food Waste Index Report estimates that in 2019, around 931 million tonnes of food waste were generated, 61% of which came from households, 26% from food service and 13% from retail. Also, 8-10% of global greenhouse gas emissions are directly or indirectly associated with food that is not consumed (United Nations Environment Programme, 2021). Finding ways to reduce food waste can provide multi-faceted benefits for people, environment and earth. Oilseeds, meat and dairy products contribute 20% towards the global quantitative food waste per year (www.unep.org).

As far as the dairy sector is concerned, in past also, high income countries witnessed their highest levels of losses and waste during the consumption phase, with estimated losses and waste at 7 percent of produce purchased. Four percent losses have been observed in the agricultural production phase with minimal losses and waste between the farm and retail. Middle income countries, however, see the highest levels of losses during agricultural production (over 20%), primarily due to poor cattle management and milking practices. Five percent and higher losses have been observed during processing, distribution and consumption losses are also, reflecting technological and cold chain deficiencies, leading to losses and shorter storage and shelf-lives of products. Low-income countries reported their highest losses levels during processing, due to primitive technologies and poor hygiene. Apart from food waste due to spoilage, foodborne diseases (FBD) are a significant impediment to the socioeconomic development worldwide.

In the developing countries, food safety remains a marginalized policy objective and FBD present a constant threat to public health. There is lack of accurate data on the global burden, and cost of FBD (Devleesschauwer et al., 2018). Foodborne Disease Burden Epidemiology Reference Group (FERG) established by WHO estimates global and regional burdens of foodborne disease. Global Disease Burden report 2019 estimated 33 million disability adjusted life years (DALY) (WHO, 2019). In the last two decades, concerns over food security and safety have increased due to their dramatic impact on human health and well-being. Developing newer ways to sustainably supply healthy food to the growing population of the world, is one of the big challenges of 21st century. In this regard, newer effective alternatives to chemical additives should be searched. The lactic acid bacteria (LAB) group play an important role in the preservation of fermented foods and development of organoleptic attributes (Saeed and Salam, 2013). LAB has a long history of use and are Generally Recognized As Safe (GRAS), they are used as bioprotective cultures to extend the shelf life and ensure the safety of many foods (Castellano et al., 2008). LAB are also responsible for the development of desirable rheological properties, flavour formation and production of other high-value compounds (Garnier et al., 2018). Bioprotective cultures are a solution for reducing global food waste in Dairy Sector. Food processors face a major challenge, with consumers demanding safe foods with a long shelf-life, but also expressing a preference for minimally processed products, less severely damaged by heat and freezing and not containing chemical preservatives. In this paper, recent developments in identification and newer strategies to use bioprotective cultures in dairy industry and their mechanism of action has been discussed. Also, the potential advantages and limitations of using LAB and PAB as bioprotective culture has been reviewed.

Bioprotective cultures: Preservation of foods using antagonistic microorganisms or their antimicrobial metabolites has been termed as bio-protection or bio-preservation. Bioprotective culture can be defined as "live microorganisms that are added deliberately to foods to control its bacteriological status without changing its technological and sensory qualities." Bioprotective cultures are preparations consisting of live microorganisms in pure form or their concentrates that are added to foods with the aim of reducing risks by pathogenic or toxigenic microorganisms" (Vogel et al. 2011). The main objectives of bio-protection are to extend storage life, to enhance food safety, and to improve sensory qualities. LAB have a major potential for use in bio-preservation because they are safe for consumption, and during storage they naturally dominate the microbiota of many foods (Ben Said et al., 2019). Due to their typical association with food fermentations and their long tradition as food-grade bacteria, LAB has been granted GRAS status. In the first instance, these cultures should be considered as additional safety factor that can improve the microbiological quality and safety of food. Their implementation should support good manufacturing practices, thereby reducing risks of growth and survival of pathogens and spoilage organisms. In addition, under abuse conditions of temperature, handling, etc., their metabolic activities (e.g., acid or gas production) may serve as an indicator of microbial risk (Tabanelli et al., 2020).

Desirable qualities of bioprotective cultures: An excellent dairy starter culture is the one which is able to produce lactic acid at a faster rate and optimum amount of flavour and aroma compounds. Starter culture brings about the desirable texture in the product consistently whereas a bioprotective culture is expected to produce antimicrobial metabolites for pathogen inhibition until the product expires. It also has to adapt well to the ecological niche of food products (Ben Said et al., 2019). The selection criteria for a bacterium to be used as a bioprotective culture for food fermentation are:

No health risks

- No production of toxins
- No production of biogenic amines or other metabolites detrimental to health
- Non-pathogenic

Bring about beneficial effects in product

- Adaptation to particular food matrix
- Product specific pathogen inhibition
- Survivability during product manufacturing and storage and distribution
- Ability to grow during storage at refrigerator temperature
- Reliability of consistent protective activity
- Competitiveness against autochthonous organisms
- Specific enzymatic activities, e.g., cheese (catalase)
- No negative (sensory) effects on product under GMP (e.g., no production of acid, gas, slime, etc. depending on product type)

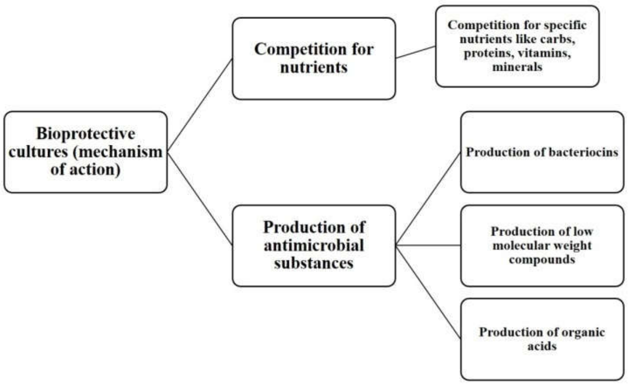

Figure 1. Mechanism of action of bioprotective cultures

Mechanism of action: Lactic acid bacteria and propionic acid bacteria exhibit protective or inhibitory effect against other microorganisms as a result of the competition for nutrients and/or of the production of bacteriocins or other antagonistic compounds such as organic acids, hydrogen peroxide, enzymes and phenyl lactic acid or other low molecular weight compounds (Fig. 1). These attributes are the result of the active metabolism of the selected culture in a particular food system and competitive exclusion of undesirable microorganisms for nutrients and by production of inhibitory metabolites (Ben Said et al., 2019). A distinction can be made between starter cultures and protective cultures in which metabolic activity (acid production, protein hydrolysis) and antimicrobial action constitute the major objectives, respectively. Metabolites produced by LAB and PAB responsible for protective activities has been discussed in the section below.

Table 1. **List of bacteriocins produced by bioprotective cultures**

Producer organism	Bacteriocins	Properties/ inhibitory spectrum
Lactococcus lactis subsp. *lactis* ATCC 11454	Nisin	Lantibiotic, broad spectrum, plasmid mediated, bactericidal, produced late in the growth cycle
Lactobacillus curvatus CRL705	Lactocin	*Listeria innocua* and *Brochothrixthermosphacta*
Carnobacteriumdivergens M35	Divergicin M35	*Listeria monocytogenes* and *Staphylococcus aureus*
*Pediococcuspentosaceus*FBB61	Pediocin A	Broad spectrum
*Pediococusacidilactici*H	Pediocin AcH	Broad spectrum
Pediococcusacidilactici	Pediocin PA-1	*Listeria* spp.
*Leuconostocgelidum*UAL187	Leucocin	Broad spectrum, plasmid mediated, bacteriostatic, produced early in the growth cycle
L. helveticus 481	Helveticin J	Narrow spectrum, chromosomally mediated
*Carnobacteriumpiscicola*LV17	Carnobacteriocin	Bactericidal, narrow spectrum, plasmid mediated

Bacteriocin: Bacteriocins produced by LAB received considerable attention owing to their potential for industrial applications in food bio-preservatives (Goyal et al., 2018). Bacteriocins are small, ribosomally synthesized peptides produced by bacteria, that are active against other bacteria and against which the producer has a specific immunity mechanism (Cotter et al., 2013). Bacteriocins are a heterogeneous group of antimicrobial peptides which are classified into two groups. First group that undergo significant post-translational small modifications (class I) and second unmodified peptides (class II). Bacteriocins of LAB kill or inhibit the growth of other bacteria, either in the same species (narrow spectrum of activity) or across genera (broad spectrum of activity). Bacteriocins that are produced by LAB Bacteriocins produced by LAB are small, ribosomally synthesized, antimicrobial peptides or proteins that possess activity towards closely related Gram-positive bacteria, whereas producer cells are immune to their own bacteriocins (Cotter and Ross, 2005). The first report about bacteriocin was made by Rogers (1928) who showed the antagonistic activity for *Lactococcus lactis* against *L. delbrueckii* ssp. *bulgaricus*. The substance was determined to be a polypeptide and named nisin (Rogers, 1928). Its antibacterial spectrum includes inhibition of streptococci, staphylococci*, Bacillus* spp.*,* clostridia and lactobacilli (Rogers, 1928). In general, bacteriocins are cationic peptides that display hydrophobic or amphiphilic properties and the bacterial membrane is in most cases the target for their activity (Cotter et al., 2013). Examples of a few bacteriocins have been enlisted in Table 1. Nisin is the first bacteriocin that was approved by USFDA as an antimicrobial additive. Currently, it is currently used in over 50 countries to improve food safety and extend product shelf life (Egan et al., 2016). A digital database, known as BACTIBASE, has been compiled using all the relevant information like physico-chemical properties, structure and inhibitory spectrum of bacteriocins produced by all Gram-positive and Gram-negative bacteria (Hammami et al., 2010). BAGEL3 is another data mining tool for identification of more (novel) classed of post-translationally modified peptides (van Heel et al., 2013). For detection of tailoring enzymes in RiPP clusters, antiSMASH (antibiotics and secondary metabolite analysis shell) is another widely used tool (Blin et., 2021).

Organic acids: The main organic acids produced by LAB are lactic acid and acetic acid besides some other acids depending on the strain of LAB (Hladíková et al., 2012). PAB mainly produce propionic acid by fermenting carbohydrates. Species or strain, culture composition and growth conditions are responsible for quantitative difference in the amount of organic acid produced. These acids diffuse through the membrane of the target organisms in their hydrophobic undissociated form and then reduce the cytoplasmic pH and stop metabolic activities (Ben Said et al., 2019). Higher acidity leads to reduction in the activity of acid-sensitive enzymes and damages proteins and DNA (van de Guchte et al., 2002). Secretion of the organic acids in LAB, is generally regarded as the primary antimicrobial effect. The inhibition activity of LAB to the growth of pathogenic bacteria especially Gram-negative psychrotrophs is most likely due to the production of organic acids and bacteriocin. In a recent study, propionic and acetic acids from *Propionibacterium jensenii*, and lactic and acetic acids from *L. rhamnosus* has been validated to exert major contribution towards antifungal activity besides some free fatty acids and volatile compounds (Garnier et al., 2020).

Carbon dioxide: Carbon dioxide is one of products produced by hetero-fermenters LAB. The activity of CO_2 is due to two factors firstly, it creates anaerobic condition and replaces the existent molecular oxygen in the products and secondly, CO_2 has antimicrobial activity and this activity is important in the vegetable fermentation to prevent the growth of spoilage fungi Common fruit spoilage organisms such as *Botrytis*, *Rhizopus* and *Penicillium* are not inhibited by 10% CO_2 but concentrations between 20-50% have strong antifungal activity (Lee et al., 2016).

Hydrogen peroxide: Hydrogen peroxide (H_2O_2) is produced by most of the LAB when oxygen is available. Mode of action of hydrogen peroxide is well studied. Hydrogen peroxide acts by strong oxidizing effect on the bacterial cell, and to the destruction of basic molecular structures of cellular proteins (Reis et al., 2012). Hydrogen peroxide acts as a precursor for the production of free radicals such as superoxide (O_2^-) and hydroxyl (OH^-) radicals, which results in bactericidal effects due to DNA damage. Inhibition of food borne pathogens and food spoilage bacteria by LAB has been credited at least in part to the activity of H_2O_2 (Enitan et al., 2011).

Reuterin: Reuterin (3-hydroxy propionaldehyde or 3-HPA) is a broad-spectrum antimicrobial substance produced by certain strains of *Limosilactobacillus reuteri*. When excess amount of glycerol is present in the medium, reuterin is produced by *L. reuteri* strains as an intermediate step in the conversion of glycerol to 1,3-propanediol (Luthi-Peng et al., 2002). This reaction is a coenzyme-B_{12}-dependent reaction catalyzed by the enzyme glycerol dehydratase. Reuterin is active against many kinds of microorganisms including Gram-positive and Gram-negative bacteria, viruses and fungi. Antifungal activity was shown against species of *Candida, Torulopsis,*

Table 2. Antifungal compounds from protective cultures and their range of antifungal spectrum

S. No.	LAB or PAB	Spoilage and pathogenic fungi	Remarks	Antifungal compound	Reference
1	*Lactobacillus rhamnosus* MDC 9661	*Penicillium aurantioviolaceum* and *Mucor plumbeus*	Armenian dairy products (matsoun, sour cream and different types of cheeses)	Proteinaceous nature associated with bacterial cell wall	Bazukyan et. al., 2018
2	Heterofermentative lactobacilli (*L. plantarum, L. paraplantarum* and *L. pentosus*)	*Penicillium candidum* and *Debaromyceshansenii*	Feta cheese	Substances of proteinaceous nature, organic acid, hydrogen peroxide	Voulgari et al., 2010
3	*Levilactobacilllus brevis*	*Aspergillus* niger, *Aspergillus* flavus, *Trichoderma* viride, *Fusarium graminearum*	Traditional Bulgarian dairy product "Katak"	Organic acids and ethanol	Tropcheva et al., 2014
4	*Lactobacillus plantarum* L244 and *Lactobacillus harbinensis* L172	*P. commune, M. racemosus* and *Rhodotorula mucilaginosa*	Delay in growth of fungi by 2-24 days in sour cream was observed	Acetic acid and lactic acid, phenyllactic acid, acetic acid, hydroxyphenyllactic acid, 3-phenylpropanoic, 5-oxopyrrolidine-2-carboxylic acid, 3-(4-hydroxyphenyl) propanoic acid, diacetyl and acetoin	Salas et al., 2018
5	*Lactobacillus plantarum* L244 and *Lactobacillus rhamnosus* CIRM-BIA1113	*P. commune, M. racemosus* and *R. mucilaginosa*	Delay in growth of fungi by 1 days in semi-hard cheese was observed	Acetic acid and lactic acid, phenyllactic acid, acetic acid, hydroxyphenyllactic acid, 3-phenylpropanoic, 5-oxopyrrolidine-2-carboxylic acid, 3-(4-hydroxyphenyl) propanoic acid, diacetyl and acetoin	Salas et al., 2018
6	*L. plantarum* N7	*Pichia pastoris* D3, *Aspergillus niger* D1, *Geotrichum candidum* N1, *Kluyveromyces marxianus* W1, and *Penicillium chrysogenum* B1	Yogurt	Not specified	Xu et al., 2021
7	*Lactobacillus harbinensis*	*Debaryomyces hansenii, Kluyveromyces lactis, Kluyveromyces marxianus, Penicillium brevicompactum, Rhodotorula mucilaginosa* and *Yarrowia lipolytica*	Milk and yogurt	Not specified	Delavenne et al., 2013
8	*Lacticaseibacillus paracasei* DGCC2132	*Penicillium* sp.	Yogurt	Diacetyl as major antifungal compounds	Aunsbjerg et al., 2015
9	*Lactiplantibacillus plantarum*	*Penicillium solitum, Aspergillus versicolor, Cladosporium herbarum*	Cottage cheese	Not specified	Cheong et al., 2014
10	*Lacticaseibacillus rhmnosus* A238 alone and in combination with *Bifidobacterium animalis* ssp. *lactis* A026	*Penicillium chrysogenum*	Solidified dairy matrix and cheese	Secondary metabolites and/or competition for nutrients	Fernandez et al., 2017

Table 3. List of commercially available bioprotective cultures for dairy products

Sr. no.	**Commercial Name**	**Constituent protective bacteria**	**Spectrum of activity**	**Applicable foods**	**Format available**	**Manufacturing Company**	**Country**
1	Holdbac™ LC	*Lactiplantibacillus plantarum*	*Leuconostoc,* heterofermenative lactobacilli, enterococci,	Cheddar and other hard and semi hard cheese verities	Freeze dried and frozen	DuPont, Danisco	Denmark
2	Holdbac YM-B LYO	*Lacticaseibacillus rhamnosus, Propionibacterium freudenreichii* ssp. *shermani*	Yeast and mold, Heterofermentative lactic acid bacteria	Yogurt, quark, Mozzarella, Feta, Large-eyed cheese & Emmental	Freeze dried and frozen	DuPont, Danisco	Denmark
	Holdbac YM-C LYO	*Lacticaseibacillus paracasei, Propionibacterium freudenreichii* subsp. *shermani*					
3	Holdbac LC LYO	*Lacticaseibacillus rhamnosus*	*Leuconostoc*, heterofermentative lactobacilli and enterococci	Anti-late blowing of cheese (Feta, Swiss)	Freeze dried and frozen	DuPont, Danisco	Denmark
4	Holdbac*Listeria* LYO	*L. plantarum*	*Listeria* spp.	Cheese (Anti – *Listeria*)	Freeze dried and frozen	DuPont, Danisco	Denmark

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5	PAL LC 705 D 2U, PAL LC 705 D 10U, PAL LC 705 D 50U	*Lacticaseibacillus rhamnosus*	Yeast (Y) and mold (M)Heterofermentative lactic acid bacteria	Fermented products, Emmental/Swiss cheese	-	LaboratoiresStanda	FRANCE
6	PAL BIOPROTECT D 2U, PAL BIOPROTECT D 10U	*Lacticaseibacillus rhamnosus, Propionibacterium freudenreichii* spp. *shermanii*	Yeast and molds, *Leuconostoc, Enterococcus* spp.	Fermented products, Emmental/Swiss, Feta cheese	-	LaboratoiresStanda	FRANCE
7	BioSafe®BS 10	*Lactococcus lactis subsp. lactis*	*Clostridium tyrobotyricum*	Cottage cheese	DVS culture	CHR Hansen	Denmark
8	HURDLES4TM (LPR A)	*Lacticaseibacillus rhamnosus, Lactobacillus plantarum*	*Clostridium tyrobutyricum*	Emmental/Swiss	-	Choosheen	Ireland/Australia
9	HURDLES4™ (LR B and LPR A)	*Lacticaseibacillus rhamnosus* and *Lactobacillus plantarum*	Yeast and mold	Yogurt and fermented milks	--	Choosheen	Ireland/Australia
10	Sacco Lyofast LPRA	*Lacticaseibacillus rhamnosus* and *lactobacillus plantarum*	*Clostridium tyrobutyricum,* yeasts and molds	Cheese and fermented milk products	Freeze-dried culture	Anchar Limited	New Zealand
11	Bioprofit	*Lacticaseibacillus rhamnosus* LC705 and *Propionibacterium freudenreichii* ssp. *shermanii* JS	Yeasts, molds and *Bacillus* spp.	Yoghurt	Culture concentrate	Valio Ltd	Finland
12	NSLAB cultures (LR B)	*Lacticaseibacillus rhamnosus*	Yeast and Molds, Propionic acid bacteria and hetero fermentative lactic acid bacteria	Dairy Products	-	CLERICI-SACCO	Italy
13	NSLAB cultures (LP AL)	*L. plantarum*	*Listeria* spp.	Dairy Products	-	CLERICI-SACCO	Italy
14	NSLAB cultures (LPR A)	*L. plantarum, L. rhamnosus*	Yeast and molds	Dairy Products	-	CLERICI-SACCO	
15	NSLAB cultures (FPR 2)	*E. faecium, L. plantarum, L. rhamnosus*	Yeast and molds	Dairy Products	-	CLERICI-SACCO	Italy
16	MicroGaurd™ (Wesman Foods, Inc., Beaverton, Oreg., USA)	*P. freudenreichii* ssp. *shermanii*	Yeast and molds	Fermented products (yogurt, Cheese)	-	Wesman Foods	USA
17	Bovamine® Meat Cultures	*Lactobacillus acidophilis* (NP35, NP51), *Lactobacillus lactis* NP7, *Pediococcus acidilactici* NP3	*E. coli* O157:H7, *Salmonella, Listeria*	Cultured milk products, Sour cream, Yogurt, Cottage cheese, Cheddar Cheese	-	Texas Tech University	USA
18	FreshQ Cultures	*Lacticaseibacillus paracasei* and *Lacticaseibacillus rhamnosus* strains	Yeast and molds	Yogurt, Sour cream, Quark, Kefir, Tvorog, White cheese, Cottage cheese, Pasta Filata	Freeze dried	CHR Hansen, Hørsholm	Denmark
19	YO-PROX	*Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium lactis.*	Yeast and molds	Yogurt, fermented milk, cheese	Freeze dried	Bioprox	France

Saccharomyces, Aspergillus and *Fusarium* (Arqués et al., 2004). Reuterin exerts its antimicrobial action by reacting with thiol groups and primary amines thereby inactivating proteins and small molecules (Vollenweider & Lacroix, 2004).

Phenyllactic acid: Phenyllactic acid is special organic acid produced by certain LAB and shows antifungal activity (Gerez et al., 2009). Phenyllactic acid isolated from LAB has broad spectrum range to inhibit pathogenic bacteria and fungi (Svanström et al., 2013). Cortés-Zavaleta et al., 2014 observed that LAB two strains *L. acidophilus* ATCC-4495 and *L. plantarum* NRRL B-4496, produced phenyllactate and exhibited antifungal activity against *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Penicillium expansum*, and *Aspergillus flavus.* PLA production was reported to be in the range from 0.021 to 0.275 mM. Supplementation of medium with phenylalanine, phenylpyruvic acid and glucose have been shown to enhance the PLA production (Mu et al., 2012).

Antifungal activity of LAB and propionibacteria: Traditional fermented dairy and non-dairy products have been used as the isolation source of cultures with protective attributes (Yazgan et al., 2021). Recently several researchers have given importance to the antifungal activity of LAB and PAB for their use as bioprotective culture in low acid foods such as yogurt, sour cream, cheese, sourdough bread etc (Garnier et al., 2018, 2019; Leyva Salas et al., 2017; Varsha & Nampoothiri, 2016). Fermented dairy foods have extended shelf life in comparison to the ingredients, they are made from, due to biopreservative effect of metabolites produced by LAB & PAB during fermentation. Low acid foods especially fermented dairy products such yogurt, curd, sour cream, low acid fermented dietary supplement and/or probiotic drinks are prone to be contaminated with acid-tolerant fungi. The most frequently occurring fungi in spoiled dairy products are *Mucor, Cladosporium, Penicillium, Meyerozyma, Rhodotorula, Candida, Yarrowia* spp. (Garnier, Valence, Pawtowski, et al., 2017).

In a recent study by Xu et al., 2021, *Lactiplantibacillus plantarum* N7 was found to exhibit antifungal activity against *Pichia pastoris* D3, *Aspergillus niger* D1, *Geotrichum candidum* N1, *Kluyveromyces marxianus* W1, and *Penicillium chrysogenum* B1 in a challenge test in yogurt. Further, a whey beverage was also prepared with this bioprotective culture and *in situ* tests also showed that the *L. plantarum* N7 could slow fungal growth. Bioprotective cultures have been reported to produce a wide range of antifungal substances such as organic acids, low molecular weight bioactive compounds, proteinaceous compounds hydroxyl fatty acids as shown in Table 2. Levya Salas et al. (2019) conducted a study to elucidate the mode of action of antifungal bioprotective cultures in four dairy products. A total of 56 antifungal compounds were detected through chromatographic techniques (LC and GC, GC–MS, LC-QToF). Out of these, 33 antifungal compounds were present in significantly higher amounts when compared to the control in any one of the dairy products which were inoculated with a potential antifungal bioprotective culture. However, all of these compounds were present at concentrations below their minimum inhibitory concentration against a particular fungal strain. These results have substantiated the fact that antifungal compounds secreted by LAB & propionibacteria in dairy products act synergistically to inhibit fungal spoilers. The common antifungal compounds were acetic acid, lactic acid, phenyllactic acid, hydroxyphenyllactic acid, 3-phenylpropanoic, 5-oxopyrrolidine-2-carboxylic acid, 3-(4-hydroxyphenyl) propanoic acid, diacetyl and acetoin (Leyva Salas et al., 2019). A peptide of 9-amino acids derived from αs_2-casein of milk was also detected in the fermentate derived from a bioprotective culture *L. rhmnosus* CIRM-BIA1113 active against *Mucor racemosus* and *Rhodotorula mucilaginosa. In-situ* production of certain antifungal compounds and their synergistic action is the basis of protection against fungi. The antifungal compounds in the fermentates derived from food grade bioprotective cultures can also be used as antifungal ingredients for dairy products.

Limitations: With increasing demand from consumers as well as legislative bodies for minimally processed foods free from preservatives, finding ways to bio-preserve foods has become the one of the leading research areas in food processing sector (Garnier et al., 2018). Bioprotective cultures have tremendous potential to reduce food wastage and find their application in various types of foods. In dairy products such as yogurt, soft-, semi soft- and hard cheeses, sour cream, these cultures are used to control bacterial and fungal spoilers both. In certain types of cheeses, especially cheeses made with raw milk or milk given lower heat treatment, *Listeria* contamination is a problem, wherein anti-*Listeria* bioprotective cultures are sought. Despite the use of preventive approach during manufacturing, fungal spoilage of low acid dairy products is a major concern for dairy industry. The importance of LAB and propionibacteria for its antifungal attributes, has been reinforced in recent times to reduce food wastage in dairy sector (Garnier et al., 2018, 2020; Garnier, Valence, Pawtowski, et al., 2017; Leyva Salas et al., 2019; Salas et al., 2018). Though, several food grade cultures with antibacterial and antifungal activity have been reported in the literature, only a few could reach to a research stage where they could be commercialized. Table 3 enlists bioprotective cultures that are commercially available for use in dairy products. The reason why promising candidates with bioprotective abilities could not reach to commercialization stage can be a number of constraints that might have impaired the process. The first constraint is related with the adaptation of the bioprotective culture in the final food matrix. Several researchers have reported that lesser number of bioprotective bacterial candidates were able to show the equivalent impact in the food intended for final use, when compared to the *in vitro* testing using the laboratory medium with almost defined composition (Delavenne et al., 2013). The difference in the observed activity depends upon several others factors such as contaminant load, concentration of the culture used, complex interaction with the biomolecules in the food, consistency of the food matrix and competition for nutrients. This problem can be averted by using targeted food matrix as screening medium or by using ingredients from the targeted food matrix or semi-synthetic media. In this way, one can discover cultures that are compatible with the starter culture in case of the fermented food and also know about the effect of bioprotective culture on the organoleptic properties of the product. Second constraint is the need of a rigorous safety assessment of the bioprotective cultures. Bioprotective culture intended for direct use in food or as an ingredient or cell free supernatant must be assessed for its safety i.e it should be of GRAS status and identified up to species level. The strain should not possess unusual acquired antibiotic resistance, pathogenicity factors or should not produce other toxic compounds like toxins, biogenic amines and allergens. The culture should be assessed for its stability and concentration in its selling form up to the end of its shelf life (Leyva Salas et al., 2017).

Applications of bioprotective cultures in dairy foods: A novel application of protective cultures is their use in dairy and food products to inhibit the growth of pathogenic microorganisms (*Vibrio, Listeria monocytogenes* and histamine producing bacteria) and spoiling microbiota including yeast and molds to enhance the safety and shelf life of the food. Industrial starters like SafePro® (CHR Hansen, DK) and HOLDBAC^{TM} (Danisco, DK) find several applications in efficient spoilage and pathogen prevention in fermented dairy and food products. It is used for growth control of yeasts and moulds and some heterofermentative lactic bacteria in fresh fermented foods, for growth control of *Leuconostoc*, heterofermentative *Lactobacilli* and *Enterococci* in hard and semi-hard cheese and for growth control of *Listeria* in soft and smear cheese, fermented dairy and food products. MicroGard^{TM} is a bioprotective pasteurized fermentate obtained by fermenting skim milk by *Propionibacterium freudenreichii* spp. *shermanii* and its protective action has been associated with diacetyl, propionic, acetic and lactic acid and probably due to a heat stable peptide. It inhibits Gram-negative bacteria such as *Pseudomonas, Salmonella* and *Yersinia* as well as yeasts and moulds. It has been approved by the FDA for use especially in Cottage cheese and fruit flavoured yoghurt. Another commercial product is Bioprofit^{TM} (Valio, Helsinki, Finland) which contains *Lactobacillus rhamnosus* LC705 and *Propionibacterium freudenreichii* JJ. The product is reported to inhibit yeasts and moulds in dairy products and *Bacillus* spp. in sourdough bread. ALTA^{TM} 2341 (Quest International, USA) is produced from *Pediococcus acidilactici* fermentation and has to rely on the inhibitory effects of natural metabolites, including organic acids and the bacteriocin pediocin. When used can serve as an effective barrier to help control the development of *Listeria* in dairy products. ALC 01 (Niebüll, Germany) is also a patented antilisteral culture developed especially for soft cheese production. Its protective activity is due to pediocin generated by *Lactobacillus plantarum*. It inhibits the growth of *Listeria* on the surface of artificially and/or naturally contaminated Munster cheese after spray treatment. FARGO^{TM} 23 (Quest International, USA) contains the same metabolites as for ALTA^{TM} 2341, but live culture producing pediocin is present in greater quantity. In France, it is added to raw milk intended for raw milk cheese production (López-Cuellar et al., 2016).

Conclusion and future perspectives

In recent years, preservative free foods have been the choice of masses and there has been growing interest in the use of bacteria and/or their products (fermentates or cell free supernatant) as natural preservation agents. Protective cultures are used in the controlled microflora applications while conserving the quality and safety of the product. The potential application of protective cultures as consumer-friendly bio-preservation is important. Acceptance of protective cultures applications will depend on careful selection and application of suitable protective cultures. Efforts are required to facilitate extensive and deep studies on a particular strain or a combination of strains in targeted food matrix and their commercialization. Also, other crucial aspects in the commercialization of bioprotective culture such as their safety assessment, effect on the organoleptic attributes of the dairy products, and stability up to the end of shelf life should be evaluated prior to marketing. Bioprotective cultures can be used as one of the components of hurdle technology to avoid bacterial and fungal spoilage in the product and good manufacturing practices should be encouraged. More research is needed on the usage possibilities of these bioprotective cultures and their metabolites for specific target products in dairy industry.

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